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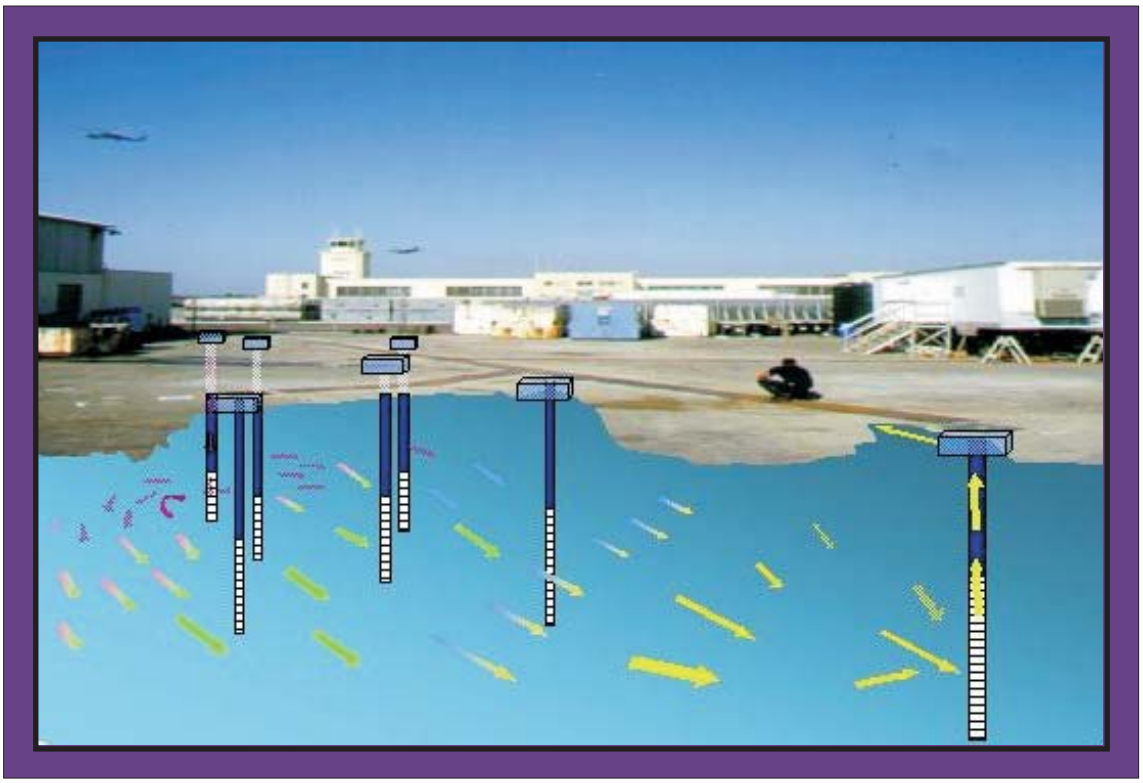
Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents



August 2004

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PRINCIPLES AND PRACTICES OF ENHANCED ANAEROBIC BIOREMEDIATION OF CHLORINATED SOLVENTS

August 2004

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ACRONYMS AND ABBREVIATIONS

µg/L	micrograms per liter
AFB	Air Force Base
AFCEE	Air Force Center for Environmental Excellence
AFRL	Air Force Research Laboratory
ASTM	American Society for Testing and Materials
atm	atmospheres
bgs	below ground surface
BOD	biological oxygen demand
^{o}C	degrees Celsius
CA	chloroethane
CAHs	chlorinated aliphatic hydrocarbons
CCAFS	Cape Canaveral Air Force Station
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CF	chloroform
CM	chloromethane
cm/sec	centimeters per second
CO_2	carbon dioxide
COD	chemical oxygen demand
CSM	conceptual site model
CT	carbon tetrachloride
DCA	dichloroethane
DCE	dichloroethene
DCM	dichloromethane
DNAPL	dense non-aqueous phase liquid
DO	dissolved oxygen
DOC	dissolved organic carbon
DoD	Department of Defense
Eh	hydrogen electrode
ESTCP	Environmental Security Technology Certification Program
Fe(II)	ferrous iron
Fe(III)	ferric iron
FeS	iron monosulfide
FeS_2	iron disulfide
f_{oc}	fraction of organic carbon
ft/day	feet per day
ft/yr	feet per year
gpm	gallons per minute
GSI	Groundwater Services, Inc.
H_2	molecular hydrogen
HCl	hydrochloric acid
HFCS	high-fructose corn syrup

ACRONYMS AND ABBREVIATIONS (Continued)

HRC®	Hydrogen Release Compound®
ITRC	Interstate Technology and Regulatory Council
lb/ft	pounds per foot
LEL	lower explosive limit
K_{oc}	partitioning coefficient between organic carbon and water
$K_s(H_2)$	Monod half-saturation constant
MAROS	Monitoring and Remedial Optimization System
MC	methylene chloride
MCL	maximum contaminant level
mg	milligram
mg/L	milligrams per liter
MNA	monitored natural attenuation
mol/L	moles per liter
MSDS	Material Safety Data Sheet
mV	millivolts
NFESC	Naval Facilities Engineering Service Center
nmol/L	nanomoles per liter
O&M	operation and maintenance
ORP	oxidation-reduction potential
Parsons	Parsons Corporation
PCA	tetrachloroethane
PCBs	polychlorinated biphenyls
PCE	tetrachloroethene (or perchloroethene)
PCR	polymerase chain reaction
PLFAs	phospholipids fatty acids
ppm	parts per million
psi	pounds per square inch
PVC	polyvinyl chloride
QA/QC	quality assurance / quality control
RABITT	Reductive Anaerobic Biological In-Situ Treatment Technology
RCRA	Resource Conservation and Recovery Act
rDNA	ribosomal deoxyribonucleic acid
redox	reduction-oxidation
ROI	radius of influence
RPM	restoration or remedial project manager
scfm	standard cubic feet per minute
SVE	soil vapor extraction
TCA	trichloroethane
TCB	trichlorobenzene
TCE	trichloroethene
TDS	total dissolved solids
TEAP	terminal electron accepting process

ACRONYMS AND ABBREVIATIONS (Continued)

TeCB	tetrachlorobenzene
TOC	total organic carbon
USACE	United States Army Corp of Engineers
USEPA	United States Environmental Protection Agency
VC	vinyl chloride
VFAs	volatile fatty acids
VOCs	volatile organic compounds

SECTION 1

STATE OF THE PRACTICE

1.1 PROBLEM STATEMENT

The Department of Defense (DoD) has identified hundreds of sites where groundwater is contaminated with chlorinated solvents; these represent one of the DoD's largest remediation liabilities. In addition to their use in many industrial processes, chlorinated solvents have historically been used for cleaning and degreasing such diverse products as aircraft engines, automobile parts, electronic components, and clothing in the military and commercial sectors. Chlorinated solvents were often released to the subsurface environment in waste water or in the form of dense non-aqueous phase liquids (DNAPLs). As a result of their physical and chemical properties, DNAPLs are difficult to remediate once they have migrated into groundwater aquifers.

Enhanced *in situ* anaerobic bioremediation can be an effective method of degrading various chlorinated solvents dissolved in groundwater, including chloroethenes, chloroethanes, and chloromethanes. Collectively, these compounds (some of which are degradation products of chlorinated solvents) are referred to as chlorinated aliphatic hydrocarbons (CAHs). Advantages of enhanced anaerobic bioremediation include complete mineralization of the contaminants *in situ* with little impact on infrastructure and relatively low cost compared to more active engineered remedial systems.

Enhanced* in situ *anaerobic bioremediation involves the delivery of an organic substrate into the subsurface for the purpose of stimulating microbial growth and development, creating an anaerobic groundwater treatment zone, and generating hydrogen through fermentation reactions.

This creates conditions conducive to anaerobic biodegradation of chlorinated solvents dissolved in groundwater. In some cases, organisms may need to be added, but only if the natural microbial population is incapable of performing the required transformations.

Numerous government entities, private industries, and university researchers have applied a variety of organic substrates to promote anaerobic reductive dechlorination of chlorinated solvents to innocuous end products. Large-scale anaerobic bioremediation projects have been initiated and are showing promising and even remarkable results. However, in light of the recent advances in the science and technology associated with enhanced anaerobic bioremediation, it is expected that research may increase not only the range of sites (e.g., DNAPL source areas) and contaminants amenable to this approach, but also will improve on the current practices in terms of the tools available to implement and monitor bioremediation.

Therefore, enhanced anaerobic bioremediation holds promise as a method to address remediation of chlorinated solvents in groundwater.

1.2 OBJECTIVE

The objective of this Principles and Practices document is to describe the state of the practice of enhanced anaerobic bioremediation. The scientific basis of enhanced anaerobic bioremediation is explained, and relevant site selection, design, and performance criteria for various engineered approaches in current practice are discussed. The practice of enhanced bioremediation of chlorinated solvents has developed rapidly over the last decade. This development should continue for the foreseeable future, and hopefully will lead to a body of information that will allow a more accurate and reliable comparison (and predictive capability) of the cost and performance of bioremediation alternatives relative to other remediation technologies than is available today.

Information provided in this document is intended to help restoration or remedial project managers (RPMs) make informed decisions about enhanced bioremediation as a remedial alternative, to select specific enhanced bioremediation approaches that are suitable for achieving remedial goals, and to track the cost and performance of enhanced bioremediation applications. Results and observations from enhanced bioremediation applications by the DoD will thereby contribute to the body of information available for improving the predictability of the cost and performance of enhanced bioremediation of chlorinated solvents in groundwater.

It should be noted that this document was written to help guide evaluation and application of enhanced anaerobic bioremediation; it is not intended as a critical evaluation of the process or as a strict protocol to implement the technology. Although enhanced anaerobic bioremediation has been applied at over 600 sites to date, it has yet to gain widespread acceptance as a proven technology, primarily due to a lack of consistency in achieving remedial objectives. It is clear that this process can enhance destruction of chlorinated solvents *in situ* at certain sites. However, there are likely many sites where conditions may limit or even preclude the use of enhanced bioremediation as part of the overall site remediation strategy. This document seeks to help the user identify these "red flag" conditions where enhanced bioremediation may not be usefully applied (see Appendix D for a discussion of application to several sites, including some where success was limited).

It is hoped that this document will allow the RPM and practitioner to better understand the process and only apply it where the probability of success is high. There are many sites where defensible data has been collected and published demonstrating enhancement of anaerobic biodegradation, and others where practitioners claim to have achieved site closure applying the process. However, the authors are not aware of any site where complete clean up or even site closure has been achieved for which quantitatively rigorous data has been published clearly demonstrating the site-wide clean up. This is not unusual; collection of data of this kind is expensive and can be difficult. The same is true of other *in situ* technologies such as *in situ* oxidation and *in situ* thermal treatment. Those considering enhanced anaerobic bioremediation must weigh the risk of failure in setting goals and in evaluating the process, versus the potential for enhanced bioremediation to effectively meet remedial objectives.

1.2.1 Intended Audience

This document is intended to provide DoD RPMs and their contractors with the information necessary to make informed decisions on using enhanced anaerobic bioremediation as a treatment technology for chlorinated solvents in groundwater. This document provides the RPM with the tools required to assess the application of enhanced anaerobic bioremediation at their sites and to identify optimum approaches, particularly when soliciting and reviewing enhanced bioremediation services.

1.2.2 Using the Principles and Practices Document

This Principles and Practices document is essentially divided into three parts, including an overview of enhanced anaerobic bioremediation (Section 1), a description of the science and principles of anaerobic biodegradation (Section 2), and the steps required to practice and evaluate the technology (Sections 3 through 6).

Section 1 introduces the reader to the document and provides a condensed overview of enhanced anaerobic bioremediation of chlorinated solvents. Section 2 provides a more detailed description of the "principles" of anaerobic biodegradation for those who desire more insight into the science of enhanced anaerobic bioremediation, including degradation processes and geochemical and microbial considerations.

There are three parts to this Principles and Practices of Enhanced Anaerobic Bioremediation document:

Part 1. Introduction and technology overview (Section 1)

Part 2. Principles of the science of enhanced bioremediation (Section 2)

Part 3. State of the practice of enhanced bioremediation (Sections 3 through 6)

Sections 3 through 6 summarize the "practice" of enhanced bioremediation. The reader who has sufficient knowledge of the science and wishes to screen the technology for applicability at a given site may go directly to Section 3, Preliminary Screening. The authors caution that use of the preliminary screening section does require some subjective judgment, and the user should first consider reading Section 2. Section 4 provides pre-design considerations and describes tools used to evaluate application of the technology once preliminary screening has been conducted, but before proceeding with system design. Section 5 provides design and engineering considerations, while Section 6 provides considerations for system monitoring and performance evaluation.

Section 7 contains references cited in the text of this document. Appendix A contains contact information for key project personnel involved in the generation of this document, including technical contributors and reviewers. Appendix B contains a sample contractual statement of work for RPMs who may need to solicit enhanced anaerobic bioremediation services. Appendix C provides a description of approximation techniques commonly used to determine substrate demand. Appendix D is an evaluation of alternative enhanced anaerobic bioremediation systems, while Appendix E contains example case studies for several substrate types.

1.3 ROADMAP TO ENHANCED *IN SITU* ANAEROBIC BIOREMEDIATION

Enhanced *in situ* anaerobic bioremediation has emerged in recent years as a remediation strategy for CAHs in groundwater. Advantages include complete mineralization of the contaminants *in situ* with little impact on infrastructure and relatively low cost compared to more active engineered remedial systems (e.g., groundwater extraction, permeable reactive iron barriers, or chemical oxidation).

There are many considerations to take into account when selecting and designing an enhanced bioremediation system. Enhanced anaerobic bioremediation as a remediation technology may not be appropriate at all sites due to the complexity of chlorinated solvent contaminant plumes (e.g., DNAPL source areas) and potential site-specific limitations (e.g., difficult hydrogeologic conditions). At some sites, it may have utility only when coupled with other remedial technologies. However, it is clear from the "success" stories described in this document that the technology holds promise when properly applied.

Enhanced anaerobic bioremediation may be appropriate at sites where:

- Site-specific data indicate that the contaminants present (including any toxic degradation products) can be readily degraded by native microbial populations under anaerobic conditions.

- Subsurface conditions (e.g., aquifer permeability) are conducive to adequate emplacement and distribution of a substrate, and creation of an *in situ* reactive zone conducive to anaerobic degradation of the targeted contaminants.

- A cost/benefit analysis indicates that the technology is cost-effective relative to other remedial measures (e.g., monitored natural attenuation [MNA], air sparging, groundwater extraction, permeable iron reactive barriers, or chemical oxidation).

A few conditions that may preclude the use of enhanced anaerobic bioremediation are listed below. "Red flags" are described in more detail in Section 3.3 (Site Screening Technical Considerations).

- Sites with impacted receptors, or with short travel time or distance to potential discharge and/or exposure points.

- Sites with inaccessible DNAPL sources.

- Difficult hydrogeologic conditions that may preclude cost-effective delivery of amendments, such as low permeability or a high degree of aquifer heterogeneity.

- Geochemical conditions (e.g., unusually low or high pH) that inhibit the growth and development of dechlorinating bacteria.

The intent of this Principles and Practices document is to provide a roadmap for appropriate and successful implementation of enhanced anaerobic bioremediation, while identifying "red flags" and avoiding "road blocks" that may limit success or lead to failure to achieve remedial goals. Figure 1.1 illustrates the steps involved in pursuing site closure using enhanced anaerobic bioremediation.

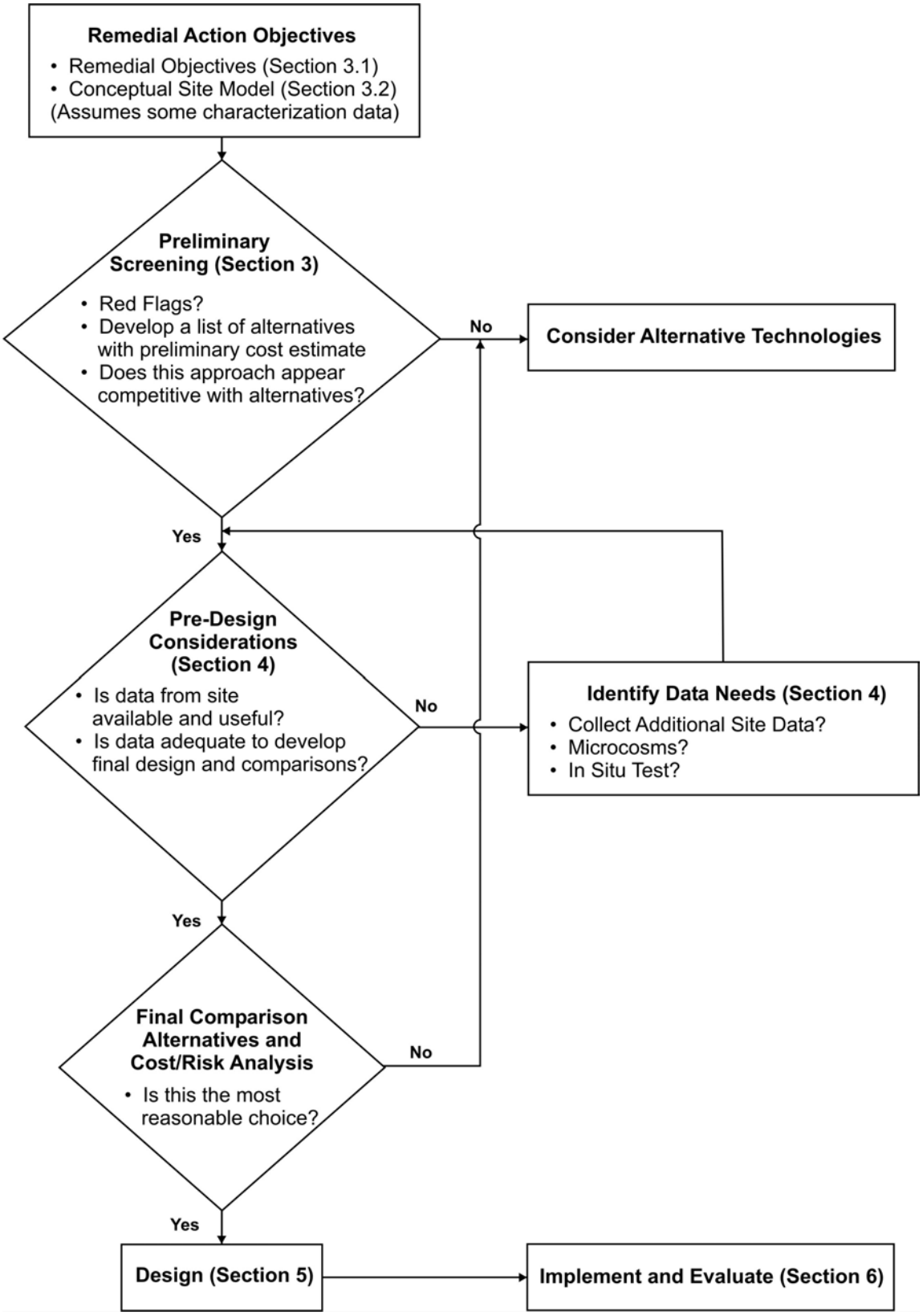

Figure 1.1 Enhanced Anaerobic Bioremediation Road Map

Development of remedial objectives, a conceptual site model (CSM), and preliminary screening (Section 3) are the first steps in evaluating the potential for applying enhanced anaerobic bioremediation for CAHs in groundwater. Development of a CSM (Figure 1.2) involves characterization of the nature of the release, the resulting contaminant plume, and site hydrogeology. In addition, an exposure pathway analysis is required to determine the level of risk posed by the contaminant release and to select and design an appropriate remedy. The physical and chemical characteristics of CAHs, whether in a DNAPL or aqueous phase, affect the fate and transport of these contaminants, and are also taken into account when developing the CSM.

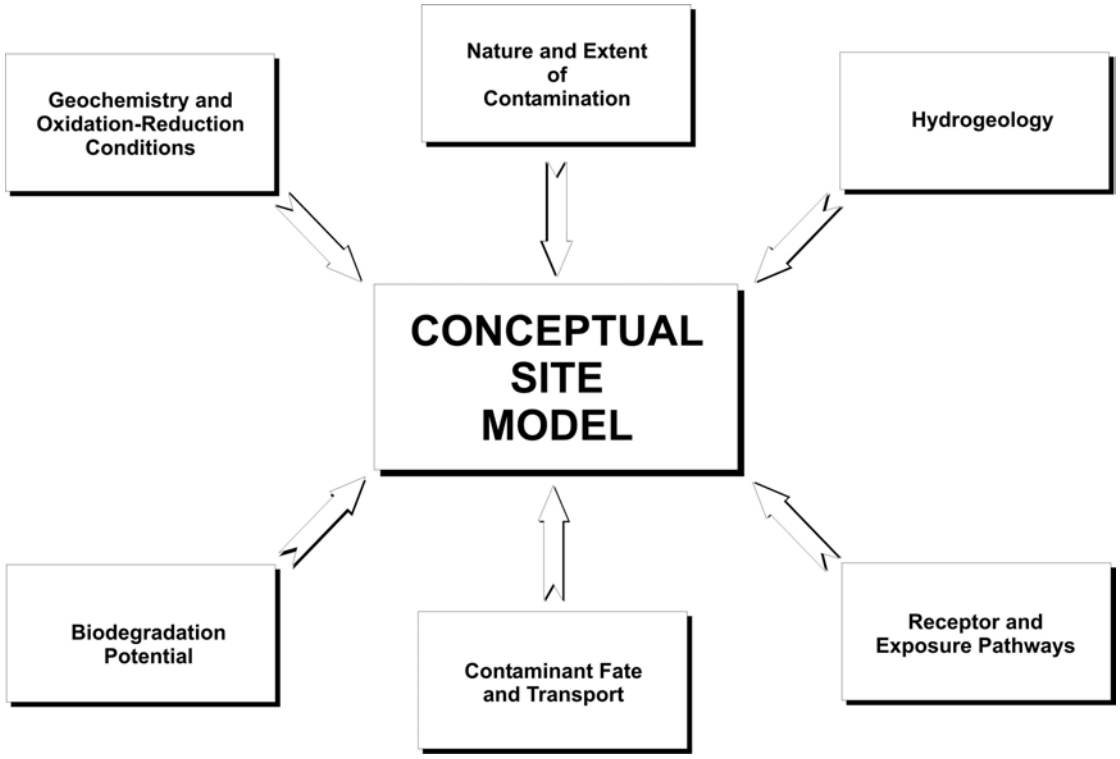

Figure 1.2 Elements of a Conceptual Site Model

Additional site characterization, laboratory microcosm studies, or small-scale field tests may be required as pre-design steps (Section 4) before a field-scale system can be designed and a cost calculated for comparison to other remedial technologies. If a determination is made to proceed with enhanced bioremediation, site-specific factors will continue to influence the design of the remedial system (Section 5) and the interpretation of performance results (Section 6).

1.4 TECHNOLOGY DESCRIPTION

Enhanced anaerobic bioremediation can be an effective method of degrading various forms of chlorinated compounds dissolved in groundwater. When anaerobic degradation of CAHs occurs naturally, it is considered a component of natural attenuation.

However, the site-specific conditions supporting natural degradation processes (biotic or abiotic) may not be optimal (e.g., organic carbon limited). Thus, the addition of an organic substrate to an aquifer has the potential to further stimulate microbial growth and development, creating an anaerobic environment in which rates of anaerobic degradation of CAHs may be enhanced. Therefore, a variety of organic substrates have been applied to the subsurface to promote anaerobic degradation of CAHs to innocuous end products. In some cases, microorganisms also may be added (bioaugmentation), but only if the natural microbial population is incapable of performing the required transformations.

Enhanced anaerobic bioremediation is not effective unless:

- **The contaminant is anaerobically degradable,**
- **Strongly reducing conditions can be generated and conditions for microbial growth are met,**
- **A microbial community capable of driving the process is present or can be introduced to the subsurface, and**
- **A fermentable carbon source can be successfully distributed throughout the subsurface treatment zone.**

1.4.1 Remedial Objectives and Regulatory Acceptance

In general, the remedial objective of enhanced anaerobic bioremediation is restoration of contaminated groundwater to pre-existing levels of beneficial use. In the case of drinking water aquifers, this is usually to federal or state established maximum contaminant levels (MCLs). In many cases, cleanup criteria may be less stringent if the impacted groundwater does not constitute a potable water supply. Exposure pathways such as surface water discharge or volatilization to soil vapor also may dictate cleanup criteria. Project- or site-specific remedial objectives may vary accordingly.

Regulatory acceptance of enhanced anaerobic bioremediation has evolved over the last several years. Enhanced bioremediation has been implemented under various federal programs, including the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and the Resource Conservation and Recovery Act (RCRA). The technology has been applied in over 32 states (Parsons, 2002a), including under the jurisdiction of regulatory agencies such as the California Regional Water Quality Control Board and the Florida Department of Environmental Protection. While the use of enhanced bioremediation has been approved by the United States Environmental Protection Agency (USEPA) and the majority of the states, it has yet to gain widespread acceptance as a proven technology, primarily due to a lack of consistency in achieving remedial objectives (see Section 3.1).

1.4.2 Applicable Contaminants (Chlorinated Solvents)

The most common chlorinated solvents released to the environment include tetrachloroethene (PCE, or perchloroethene), trichloroethene (TCE), trichloroethane (TCA), and carbon tetrachloride (CT). These chlorinated solvents are problematic because of their health hazards and their resistance to natural degradation processes.

Because these compounds exist in an oxidized state, they are generally not susceptible to aerobic oxidation processes (with the possible exception of cometabolism). However, oxidized compounds are susceptible to reduction under anaerobic conditions by either biotic (biological) or abiotic (chemical) processes. Enhanced anaerobic bioremediation is intended to exploit primarily biotic anaerobic processes to degrade CAHs in groundwater.

This Principles and Practices document addresses bioremediation of chlorinated solvents in groundwater, including chloroethenes, chloroethanes, and chloromethanes.

Collectively, these compounds (chlorinated solvent parent compounds and their chlorinated degradation products) are referred to as chlorinated aliphatic hydrocarbons (CAHs).

Other common groundwater contaminants that are subject to reduction reactions are also susceptible to enhanced anaerobic bioremediation. While not addressed in this document, constituents that can also potentially be treated with this approach include the following:

- Chlorobenzenes;
- Chlorinated pesticides (e.g., chlordane), polychlorinated biphenyls (PCBs), and chlorinated cyclic hydrocarbons (e.g., pentachlorophenol);
- Oxidizers such as perchlorate and chlorate;
- Explosive and ordnance compounds;
- Dissolved metals (e.g., hexavalent chromium); and
- Nitrate and sulfate.

Many of the techniques described in this document to create anaerobic reactive zones for chlorinated solvents may also be applicable to the design and implementation of enhanced anaerobic bioremediation systems for the constituents listed above.

1.4.3 Degradation Processes

There are many potential reactions that may degrade CAHs in the subsurface, under both aerobic and anaerobic conditions (Table 1.1). Not all CAHs are amenable to degradation by each of these processes. However, anaerobic biodegradation processes may potentially degrade all of the common chloroethenes, chloroethanes, and chloromethanes. A more detailed description of these degradation processes may be found in Section 2.1.

Anaerobic reductive dechlorination is the degradation process targeted by enhanced anaerobic bioremediation. Through addition of organic substrates to the subsurface, enhanced anaerobic bioremediation converts naturally aerobic or mildly anoxic aquifer zones to anaerobic and microbiologically diverse reactive zones, making them conducive to anaerobic degradation of CAHs.

Table 1.1 Potential Degradation Processes for CAHs

	Compound [a/]											
	Chloroethenes				Chloroethanes				Chloromethanes			
Degradation Process	**PCE**	**TCE**	**DCE**	**VC**	**PCA**	**TCA**	**DCA**	**CA**	**CT**	**CF**	**MC**	**CM**
Aerobic Oxidation	N	N	P	Y	N	N	Y	Y	N	N	Y	P
Aerobic Cometabolism	N	Y	Y	Y	P	Y	Y	Y	N	Y	Y	Y
Anaerobic Oxidation	N	N	P	Y	N	N	Y	P	N	N	Y	P
Direct Anaerobic Reductive Dechlorination	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Cometabolic Anaerobic Reduction	Y	Y	Y	Y	P	Y	Y	P	Y	Y	Y	P
Abiotic Transformation	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

Modified from ITRC (1998) using references listed in Table 2.1 in Section 2 of this document.

a/ PCE = tetrachloroethene, TCE = trichloroethene, DCE = dichloroethene, VC = vinyl chloride, PCA = tetrachloroethane, TCA = trichloroethane, DCA = dichloroethane, CA = chloroethane, CT = carbon tetrachloride, CF = chloroform, MC = methylene chloride, CM = chloromethane.

N = Not documented in the literature.

Y = Documented in the literature.

P = Potential for reaction to occur but not well documented in the literature.

Biodegradation of an organic substrate depletes the aquifer of dissolved oxygen (DO) and other terminal electron acceptors (e.g., nitrate or sulfate), and lowers the oxidation-reduction potential (ORP) of groundwater, thereby stimulating conditions conducive to anaerobic degradation processes. After DO is consumed, anaerobic microorganisms typically use native electron acceptors (as available) in the following order of preference: nitrate, manganese and ferric iron oxyhydroxides, sulfate, and finally carbon dioxide. Figure 1.3 illustrates a CAH plume where substrate has been injected into the source area. An anaerobic treatment area is created with the development of progressively more anaerobic zones closer to the source of organic carbon as electron acceptors are depleted. Anaerobic dechlorination has been demonstrated under nitrate, iron, and sulfate reducing conditions, but the most rapid biodegradation rates, affecting the widest range of CAHs, occur under methanogenic conditions (Bouwer, 1994).

1.4.4 Anaerobic Reductive Dechlorination

The following three general reactions may degrade CAHs by anaerobic reductive dechlorination:

- **Direct Anaerobic Reductive Dechlorination** is a biological reaction in which bacteria gain energy and grow as one or more chlorine atoms on a CAH molecule are replaced with hydrogen in an anaerobic environment. In this reaction, the chlorinated compound serves as the electron acceptor, and it appears that hydrogen serves as the

direct electron donor. Hydrogen used in this reaction is typically supplied by fermentation of organic substrates. This reaction may also be referred to as halorespiration or dehalorespiration (USEPA, 2000a).

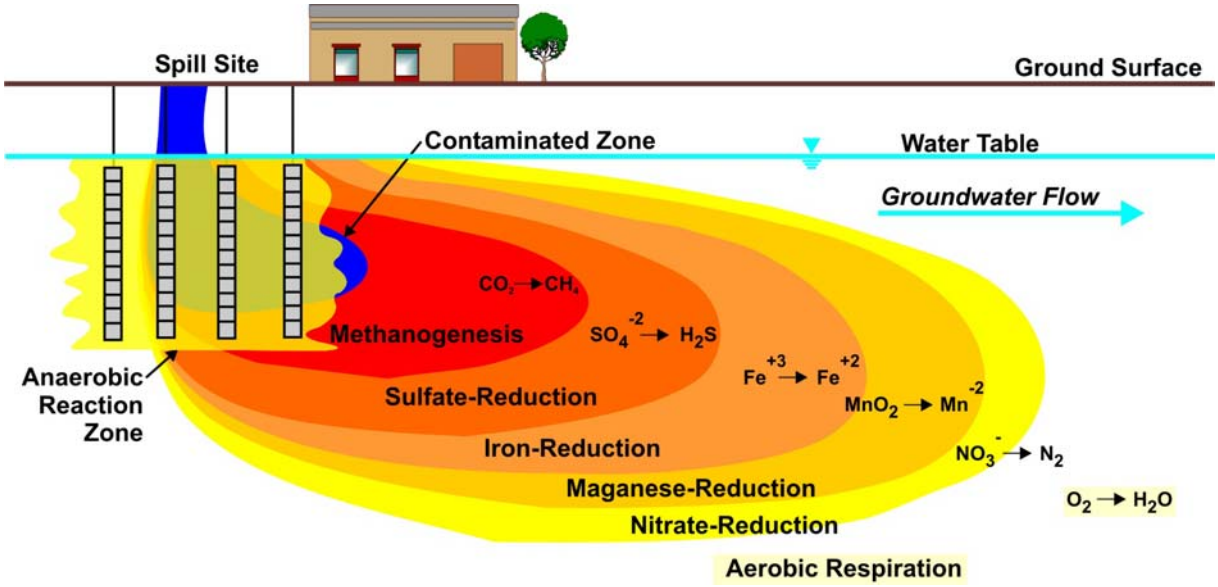

Figure 1.3 Reducing Zones Established Downgradient of Substrate Injection

- **Cometabolic Anaerobic Reductive Dechlorination** is a reaction in which a chlorinated compound is reduced by a non-specific enzyme or co-factor produced during microbial metabolism of another compound (i.e., the primary substrate) in an anaerobic environment. By definition, cometabolism of the chlorinated compound does not yield any energy or growth benefit for the microbe mediating the reaction (USEPA, 2000a). For the cometabolic process to be sustained, sufficient primary substrate is required to support growth of the transforming microorganisms.

- **Abiotic Reductive Dechlorination** is a chemical degradation reaction, not associated with biological activity, in which a chlorinated hydrocarbon is reduced by a reactive compound. Addition of an organic substrate and creation of an anaerobic environment may create reactive compounds, such as metal sulfides, that can degrade CAHs (e.g., Butler and Hayes, 1999; Lee and Batchelor, 2002). In this case, substrate addition may indirectly cause and sustain abiotic reductive dechlorination (Section 2.1).

In practice, it may not be possible to distinguish among these three different reactions at the field scale; all three reactions may be occurring. Enhanced bioremediation applications to date have targeted biotic dechlorination processes. As used in this document, *anaerobic dechlorination* includes the biotic processes of direct and cometabolic anaerobic reductive dechlorination and abiotic reductive dechlorination.

In general, biotic anaerobic reductive dechlorination occurs by sequential removal of chloride ions. The most thoroughly studied anaerobic dechlorination pathway is degradation

of PCE to TCE to *cis*-dichloroethene (DCE) to vinyl chloride (VC), and finally to ethene. Sequential transformation from PCE to TCE to the DCE isomers (*cis*-DCE or *trans*-DCE) to VC to ethene is illustrated in Figure 1.4.

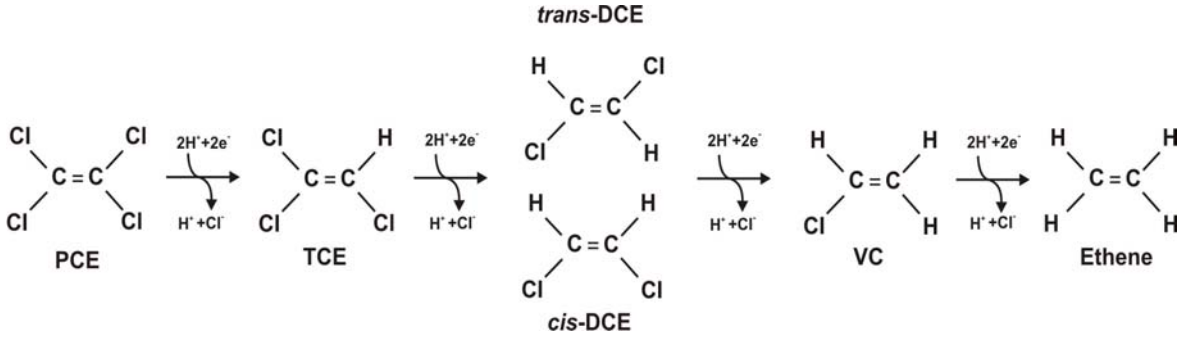

Figure 1.4 Sequential Reduction of PCE to Ethene by Anaerobic Reductive Dechlorination

In this reaction, hydrogen is the electron donor, which is oxidized. The chlorinated ethene molecule is the electron acceptor, which is reduced. While other fermentation products (e.g., acetate) may serve as an electron donor, hydrogen appears to be the most important electron donor for anaerobic dechlorination of CAHs (Maymo-Gatell et al., 1997; Fennell and Gossett, 1998).

Similar to the chloroethenes, the common chloroethanes and chloromethanes may be transformed sequentially by anaerobic dechlorination as follows:

- **Chloroethanes:** 1,1,1-TCA to 1,1-dichloroethane (DCA) to chloroethane (CA) to ethane.
- **Chloromethanes**: CT to chloroform (CF) to methylene chloride (MC) to chloromethane (CM) to methane.

Anaerobic dechlorination of CAHs depends on many environmental factors (e.g., anaerobic conditions, presence of fermentable substrates, and appropriate microbial populations). Anaerobic dechlorination also affects each of the chlorinated compounds differently. For example, of the chloroethenes, PCE and TCE are the most susceptible to anaerobic dechlorination because they are the most oxidized. Conversely, VC may degrade at lower reaction rates because it is the least oxidized of these compounds. Therefore, the potential exists for VC to accumulate in a treatment system when the rate at which it is generated is greater than the rate at which it degraded. This is a common concern because VC is considered more toxic than the other chlorinated ethenes. However, there are other degradation pathways for VC (Table 1.1), and the formation and persistence of large VC plumes (i.e., larger than the footprint of the initial CAH plume) is rarely observed in practice.

1.4.5 Molecular Hydrogen as a Direct Electron Donor

Researchers have recognized the role of hydrogen as a direct electron donor in the anaerobic dechlorination of CAHs (Holliger et al., 1993; Gossett and Zinder, 1996; Smatlak et al., 1996; Ballapragada et al., 1997). Laboratory cultures used to study direct anaerobic

reductive dechlorination are typically mixed cultures, with at least two distinct strains of bacteria: one strain ferments the organic substrate to produce hydrogen, and another strain uses the hydrogen as an electron donor for anaerobic dechlorination.

Hydrogen is generated by fermentation of non-chlorinated organic substrates, including naturally occurring organic carbon, accidental releases of anthropogenic carbon (fuel), or introduced substrates such as carbohydrates (sugars), alcohols, and low-molecular-weight fatty acids. As hydrogen is produced by fermentative organisms, it is rapidly consumed by other bacteria, including denitrifiers, iron-reducers, sulfate-reducers, methanogens, and dechlorinating microorganisms. Section 2.1 includes examples of biodegradation reactions that utilize hydrogen as an electron donor for reduction of native electron acceptors and CAHs. The production of hydrogen through fermentation does not, by itself, guarantee that hydrogen will be available for reductive dechlorination of CAHs. For anaerobic reductive dechlorination to occur, dechlorinators must successfully compete against other microorganisms that also utilize hydrogen.

1.4.6 Microbiology of Anaerobic Reductive Dechlorination

Current literature suggests that anaerobic reductive dechlorination of CAHs is carried out by a relatively few metabolic classifications of bacteria. These groups, which may behave very differently from one another, include methanogens, sulfate-reducing bacteria, and dechlorinating bacteria. The classifications and strains of bacteria that can reduce PCE and TCE to *cis*-DCE appear to be ubiquitous in the subsurface environment.

Some dechlorinators sequentially dechlorinate PCE to TCE, some to *cis*-DCE, and some to VC. (He et al., 2003a, 2003b). Complete dechlorination of PCE to ethene by a single species has only been demonstrated in the laboratory for *Dehalococcoides ethenogenes*. *Dehalococcoides ethenogenes* appear to be common, but not ubiquitous, in the environment (Hendrickson et al., 2002a; He et al., 2003a). Therefore, microorganisms that may facilitate dechlorination of DCE and VC to ethene may not be as prevalent at those capable of dechlorination PCE and TCE to *cis*-DCE.

But in nature, anaerobic dechlorination is typically carried out by mixed cultures of dechlorinators (Bradley, 2003). Flynn et al. (2000) demonstrated complete dechlorination of PCE to ethene with a mixed culture that did not contain the *Dehalococcoides* species, and found that at least two populations of dechlorinators were responsible for the sequential dechlorination of PCE to ethene observed. This suggests that mixtures of differing dechlorinating strains can achieve complete dechlorination without reliance on any one specific strain of bacteria. In addition, other degradation pathways exist for the less chlorinated compounds such as DCE and VC in both aerobic and anaerobic environments, which also may achieve the desired degradation endpoint.

1.5 APPLICATION OF ENHANCED ANAEROBIC BIOREMEDIATION

Application of enhanced anaerobic bioremediation starts with a review of site-specific conditions and evaluation of remedial objectives to determine if this remedial approach is appropriate for a site (refer to the Road Map in Figure 1.1). Once enhanced bioremediation is selected as a remedial alternative, design criteria for implementation are developed including selection of a substrate and system configuration. The following subsections describe some

common technology screening criteria, substrate alternatives, and system configurations used for enhanced anaerobic bioremediation. More detailed information can be found in Section 3 (Preliminary Screening), Section 4 (Pre-Design), and Section 5 (System Design and Engineering).

1.5.1 Technology Screening

The addition of an organic substrate to the subsurface to stimulate and enhance the anaerobic dechlorination process *in situ* has been explored at many sites. Enhanced anaerobic bioremediation has been applied under a broad range of site conditions, including the following:

Site-specific conditions must be reviewed prior to selecting enhanced anaerobic bioremediation as a remedial alternative.

It must be feasible to effectively distribute an organic substrate and induce strongly reducing conditions in the subsurface.

- **Hydrogeologic Settings.** Enhanced anaerobic bioremediation has been applied in a variety of hydrogeologic settings, from low permeability silts and clays to high permeability alluvial sand and gravel deposits to fractured bedrock. Enhanced bioremediation has been applied at depths up to 400 feet below ground surface (bgs) and with groundwater velocities ranging from a few feet per year to several feet per day. However, there are limits to applying the technology in settings with the extremes of very high and very low rates of groundwater flow. It may be impractical to maintain reducing conditions in high flow settings, due to the magnitude of groundwater and native electron acceptor flux. On the other hand, it may be difficult to inject substrates into tight formations, and under low flow settings mixing of substrate with groundwater due to advection and dispersion may be limited.

- **Contaminant Levels and Distribution.** The technology has typically been applied to groundwater plumes with concentrations of CAHs ranging from 0.01 to 100 milligrams per liter (mg/L). Sites with indications of residual or sorbed DNAPL (dissolved CAH concentrations in excess of 100 mg/L) also have been successfully treated. However, it may not be realistic to expect rapid remediation of source areas with DNAPL pools.

- **Geochemical Conditions.** During anaerobic dechlorination, CAHs function as electron acceptors in competition with naturally occurring (inorganic) electron acceptors. For example, a high rate of groundwater flow coupled with high concentrations of DO may create an oxygen electron acceptor demand that cannot practically be overcome with substrate addition.

In some cases, adverse site conditions can be mitigated with proper system design. For example, recirculation systems may be used to impose a hydraulic gradient and enhance groundwater flow at sites with very low natural hydraulic gradients. However, when pumping of significant quantities of groundwater is required, the technology may not be cost competitive with pump and treat; this becomes a site-specific issue. Once enhanced bioremediation has been selected as an appropriate technology, there are several substrate alternatives and system configurations to consider.

1.5.2 Substrate (Electron Donor) Alternatives

There are many organic substrates which can be naturally degraded and fermented in the subsurface that result in the generation of hydrogen. Examples of easily fermentable organic substrates include alcohols, low-molecular-weight fatty acids (e.g., lactate), carbohydrates (e.g., sugars), vegetable oils, and plant debris (e.g., mulch). The substrates most commonly added for enhanced anaerobic bioremediation include lactate, molasses, Hydrogen Release Compound (HRC®), and vegetable oils. Substrates used less frequently include ethanol, methanol, benzoate, butyrate, high-fructose corn syrup (HFCS), whey, bark mulch and compost, chitin, and gaseous hydrogen.

Table 1.2 summarizes the attributes of several substrate types. These substrates are classified here as soluble substrates, viscous fluids and low viscosity fluids, solid substrates, and experimental substrates. The physical nature of the substrate dictates the frequency of addition, the addition technique, and potential system configurations.

The selected organic substrate should be suitable for the biogeochemical and hydrodynamic character of the aquifer to be treated. A common goal is to minimize overall project cost by minimizing the number of required injection points, the number of injection events, and substrate cost (Harkness, 2000). The physical and chemical characteristics of the substrate (e.g., phase and solubility) may make certain substrates more suitable than others in particular applications. Furthermore, combinations of various substrates are becoming more common. For example, an easily distributed and rapidly degraded soluble substrate such as lactate may be combined with a slow-release substrate such as vegetable oil. HRC® is also available from the manufacturer in both a fast acting primer and a longer lasting HRC-X™ product.

The following paragraphs summarize each of the general substrate types and also describe some common substrate amendments/nutrients and bioaugmentation cultures. Further discussion of substrate and amendment alternatives can be found in Section 5.

Soluble Substrates. Substrates applied as a dissolved or "aqueous" phase offer the greatest potential for uniform distribution throughout the aquifer matrix relative to substrates applied as a viscous fluid or solid phase. Molasses and lactate are the most common substrates applied in an aqueous phase. Soluble substrates travel with advective groundwater flow, and are typically applied in a continuous or periodic (pulsed) mode to maintain a specified reactive treatment zone.

Viscous Fluids. Slow-release, viscous fluid substrates include HRC® and neat vegetable oils (Section 5.5.4). These substrates are intended to be long-lasting, where a single or limited number of injections are sufficient for site remediation. They are intended to be relatively immobile in the subsurface, and rely on advection and dispersion of soluble compounds (lactic acid for HRC®, metabolic acids generated by degradation of vegetable oil) for effective delivery throughout the aquifer matrix.

Table 1.2 Substrates Used for Enhanced Anaerobic Bioremediation

Substrate	Typical Delivery Techniques	Form of Application	Frequency of Injection
Soluble Substrates			
Lactate and Butyrate	Injection wells or circulation systems	Acids or salts diluted in water	Continuous to monthly
Methanol and Ethanol	Injection wells or circulation systems	Diluted in water	Continuous to monthly
Sodium Benzoate	Injection wells or circulation systems	Dissolved in water	Continuous to monthly
Molasses, High Fructose Corn Syrup	Injection wells	Dissolved in water	Continuous to monthly
Viscous Fluid Substrates			
HRC® or HRC-X™	Direct injection	Straight injection	Annually to bi-annually for HRC® (typical); Every 3 to 4 years for HRC-X™; potential for one-time application
Vegetable Oils	Direct injection or injection wells	Straight oil injection with water push, or high oil:water content (>20 percent oil) emulsions	One-time application (typical)
Low-Viscosity Fluid Substrates			
Vegetable Oil Emulsions	Direct injection or injection wells	Low oil content (<10 percent) microemulsions suspended in water	Every 2 to 3 years (typical); potential for one-time application
Solid Substrates			
Mulch and Compost	Trenching or excavation	Trenches, excavations, or surface amendments	One-time application (typical)
Experimental (few applications)			
Whey (soluble)	Direct injection or injection wells	Dissolved in water or slurry	Monthly to annually
Chitin (solid)	Trenching or injection of a chitin slurry	Solid or slurry	Annually to biannually; potential for one-time application
Hydrogen (gas)	Biosparging wells	Gas injection	Pulsed injection (daily to weekly)
Humic Acids (electron shuttles)	Direct injection or injection wells	Dissolved in water	Unknown; potentially semi-annually to annually

Low Viscosity Fluids. Vegetable oil emulsions have been developed in an effort to improve the distribution of substrate in the subsurface while still providing a long-lasting source of organic carbon. Microemulsions consisting of 5 to 10 percent vegetable oil in water by volume are relatively low-viscosity mixtures (e.g., non-dairy creamers like Coffee Mate®) compared to the viscous fluids described above. The use of microemulsions is the result of lessons learned in early vegetable oil field trials in which high injection backpressures, limited radii of influence (ROI), and reductions in hydraulic conductivity were observed using coarse viscous emulsions or neat vegetable oil (Section 5.5.4.3).

Solid Substrates. Solid phase substrates include mulch and compost. Mulch is generally obtained from shredding and chipping of tree and shrub trimmings and is primarily composed

of cellulose and lignin. Often "green" plant material or compost is incorporated to provide a source of nitrogen for microbial growth and as a source of more readily degraded organic carbon. Degradation of the substrate by microbial processes in the subsurface provides a number of breakdown products, including metabolic and humic acids, which act as secondary fermentable substrates. Solid substrates are typically placed in trenches or in excavations as backfill in a one-time event using conventional construction techniques.

Experimental Substrates. Experimental substrates are those selected for use as organic substrates, but for which few field applications have been conducted and whose performance is currently being evaluated. These include chitin, whey, and hydrogen gas. Other potential substrates that have been identified, but have yet to be demonstrated at the field scale, include milk, lactose (milk sugar), flour, tetrabutyl orthosilicate, and oleate (Yu and Semprini, 2002; Yang and McCarty, 2000a). Biomass produced by microbial growth also has been shown in the laboratory to be a suitable secondary substrate for anaerobic reductive dechlorination (Yang and McCarty, 2000a), and may extend effective treatment times beyond the depletion of the primary substrate.

Nutrients and Amendments. Under natural conditions, the aquifer may contain suitable amounts of trace nutrients for microbial growth; however, the nutritional demand imposed by rapid microbial growth in response to addition of an organic substrate may exceed the capacity of the aquifer system (Chamberlain, 2003). Substrate amendments may be used to provide additional nutrients for microbial growth. Substrate nutritional amendments generally include nitrogen, phosphorous, and yeast extracts.

In addition, fermentation of complex substrates to metabolic acids and hydrochloric acid (HCl) during anaerobic dechlorination may decrease the pH significantly in low-alkalinity systems. Lowering of pH to below 5 or 4 standard units may inhibit growth of sulfate-reducers, methanogens, and some dechlorinating microbes (Maillacheruvu and Parkin, 1996). Therefore, pH buffer amendments such as sodium bicarbonate may be required in groundwater systems with insufficient natural buffering capability.

Bioaugmentation. In many cases, the sole use of an organic substrate is sufficient to stimulate anaerobic reductive dechlorination (i.e., biostimulation). However, bioaugmentation may be considered at a site when an appropriate population of dechlorinating microorganisms is not present or sufficiently active to stimulate complete anaerobic reductive dechlorination of the CAH constituents present. To date, experience with bioaugmentation is limited, and there is some disagreement among practitioners as to its benefits. Bioaugmentation involves the injection of a microbial amendment comprised of non-native organisms known to carry dechlorination of the targeted CAHs to completion. For example, the presence of *Dehalococcoides*-related microorganisms has been linked to complete dechlorination of PCE to ethene in the field (Major et al., 2001; Hendrickson et al., 2002a; Lendvay et al., 2003). Commercial bioaugmentation products that contain these microorganisms are available.

1.5.3 System Configurations

Enhanced *in situ* anaerobic bioremediation can be implemented to provide source area or dissolved plume treatment or containment, or a combination of source area and dissolved plume remediation can be used. Enhanced bioremediation and conventional source treatment

or containment approaches (e.g., chemical oxidation or groundwater extraction) will be subject to the same difficulties associated with mass transfer limitations of a continuing source and preferential flow paths in heterogeneous formations. The single largest difference between conventional remedial technologies and enhanced bioremediation may be that enhanced bioremediation, if properly implemented, can maintain effectiveness over a longer period of time at a lower overall cost. This may make enhanced bioremediation an effective remedial approach due to the substantial challenges associated with significant CAH source mass removal. Typical system configurations and associated remedial action objectives that engineered anaerobic bioremediation may be used to address include the following:

- **Source Zone Treatment:** Remediation of source zones where good substrate/contaminant contact is possible.
- **Plume Containment using a Biologically Reactive Barrier:** Reduction of mass flux from a source zone or across a specified boundary.
- **Plume-Wide Restoration:** Total treatment of an entire dissolved plume.

In some cases, several approaches may be combined. For example, a source area may be targeted for remediation using a grid configuration, combined with a linear barrier configuration upgradient from a downgradient point of compliance (Figure 1.5).

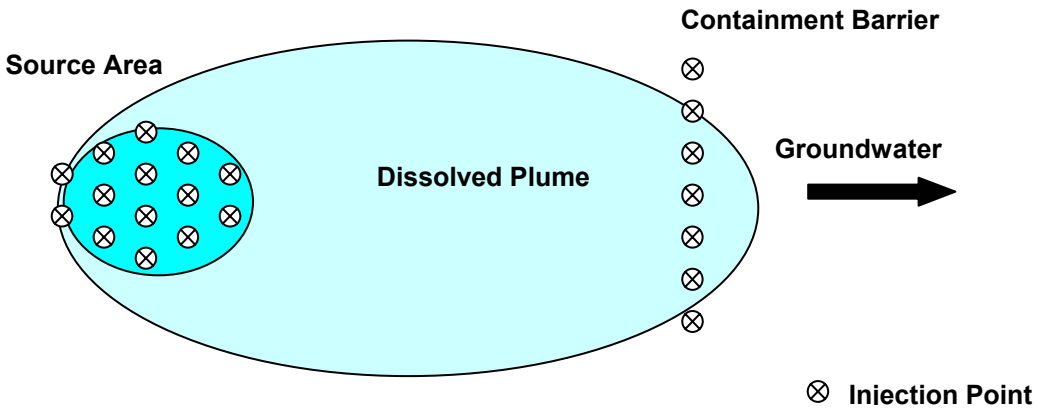

Figure 1.5 Schematic of Source Area and Biobarrier Injection Configurations

The appropriate application of enhanced anaerobic bioremediation will be site-specific and based on a strategy that takes into account final remedial objectives, feasibility of the application, and regulatory issues. Ultimately however, there will be an economic limit to the size of a plume that can be treated with a complete plume-wide application of enhanced bioremediation. For plume sizes greater than 10 to 20 acres, use of containment strategies combined with other remedial approaches may be more feasible.

Source Zone Treatment

Enhanced anaerobic bioremediation has been used to address source zones either to limit mass flux from the source zone or to accelerate source mass removal. Mass flux reduction is achieved by stimulating biodegradation in the dissolved phase, reducing contaminant mass

available to migrate downgradient. Source mass removal is achieved by accelerating DNAPL dissolution and then stimulating biodegradation of the dissolved contaminants. It should be recognized that many practitioners currently believe that not all CAH DNAPL source zones can be economically or feasibly cleaned up (e.g., Interstate Technology and Regulatory Council [ITRC], 2002; USEPA, 2003). Anaerobic dechlorination is a process that takes place in the aqueous phase and does not directly attack DNAPL mass. Therefore, enhanced bioremediation may be limited in its ability to rapidly treat DNAPL source zone areas.

On the other hand, treatment to reduce mass flux and to perhaps increase the rate of dissolution and treatment as compared to natural attenuation or groundwater extraction may be more achievable. Enhanced bioremediation of DNAPL sources is being researched and may someday be a proven and feasible long-term remedial alternative. The potential for enhanced dissolution or desorption using organic substrates is discussed in Section 2.3. Alternatively, injection of a low solubility, persistent carbon source such as vegetable oil into a source area may serve to reduce mass flux and to effectively sequester the source due to partitioning and lowering of hydraulic conductivity. However, while degradation of dissolved constituents may be stimulated, this may not accelerate destruction of DNAPL or sorbed source mass.

Plume Containment using Biologically Enhanced Barrier Systems

For large plumes having poorly defined, widely distributed, or inaccessible source areas, enhanced bioremediation systems may be configured as permeable reactive barriers (biobarriers) to intercept and treat a contaminant plume. For example, biobarriers may be employed at a property boundary or upgradient from a point of regulatory compliance to prevent plume migration to potential receptors. Biobarriers typically consist of either rows of substrate injection wells or a solid-substrate trench located perpendicular to the direction of groundwater flow.

Passive biobarriers typically use slow-release, long-lasting substrates (e.g., HRC®, vegetable oils, or mulch) that can be either injected or otherwise placed in a trench, and that are designed to remain in place for long periods to maintain the reaction zone. Contaminant mass is delivered to the treatment zone via natural groundwater flow. Capital and operating costs for a passive biobarrier configuration are typically lower than for plume-wide configurations because of a limited treatment area. However, life-cycle costs could be significant if the source of the CAHs upgradient of the biobarrier is not addressed.

Semi-passive or active biobarriers are similar to passive biobarriers except that a soluble substrate is typically injected periodically (semi-passive) or via a recirculation system (active). Soluble substrates migrate with groundwater flow, are depleted more rapidly, and require frequent addition. However, these systems offer the advantage of being able to adjust the rate or type of substrate loading over time, and soluble substrates may be easier to distribute throughout larger volumes of the contaminant plume. Recirculation can improve substrate distribution, contaminant/substrate mixing, and retention time for treatment; but the overall groundwater flux downgradient of the system does not change.

Plume-Wide Restoration

Enhanced bioremediation systems may be configured to treat dissolved CAHs across an entire contaminant plume. Creating an anaerobic reaction zone across broad areas of a plume is an aggressive approach that may reduce the overall timeframe for remediation. Plume-wide delivery systems will typically be configured as a large injection grid, or a recirculation well field may be employed to increase the effective area of substrate distribution. Higher initial capital and operating costs of recirculation systems may be offset by shorter remedial timeframes with lower monitoring and total long-term operating costs. However, plume-wide applications where substrate is delivered to the entire plume may be cost prohibitive for very large plumes or cost inefficient for low-level contaminant plumes.

At sites where larger plumes are present (greater than several acres), or the depth of the plume makes installing injection wells difficult and expensive, multiple treatment lines can be established perpendicular to the direction of groundwater flow, typically separated by 6 to 12 months of groundwater travel time. A recirculation approach may not be practical or cost effective at a large scale due to the large volumes of groundwater to be processed and ineffective *in situ* mixing in heterogeneous environments.

There is some controversy as to the cost effectiveness of using enhanced anaerobic bioremediation for plume-wide restoration. For any kind of recirculating system, groundwater pumping rates may have to be similar to pump and treat methods; the cost of enhanced bioremediation must be carefully compared to pump and treat. If substrate addition is done by some kind of multiple point injection relying on natural groundwater flow for dispersion, this may require very close spacing of injection points and or it may not result in good mixing of substrate and CAHs *in situ*.

1.5.4 Delivery Options

Common substrate delivery options include direct injection or recirculation of fluid substrates, or emplacement of solid substrates in biowall trenches (Section 5.4). Where direct-push methods can be used, substrate may be injected directly through the probe rods. This is a common approach for both slow-release and soluble substrates. Otherwise, injection wells are used. Soluble substrates may be injected in batch mode, or in the case of frequent injections, the use of automatic injection systems may be warranted.

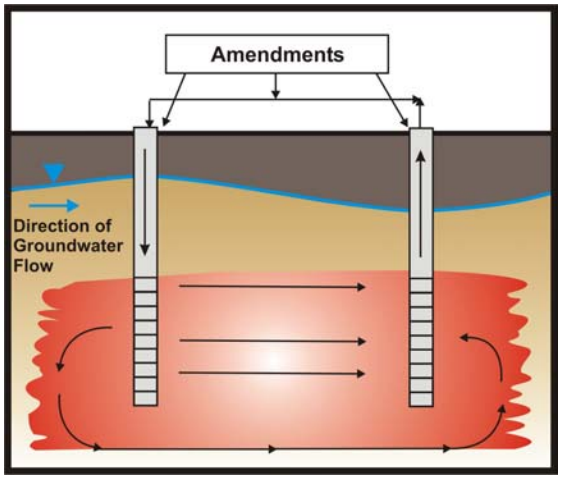

Figure 1.6 Schematic of a Horizontal Recirculation System

Recirculation systems may also be employed for distribution of soluble substrates. Figure 1.6 is an example of a horizontal circulation system. Recirculation may be continuous or in a pulsed mode. Substrates are added to the groundwater as it is reinjected into the treatment zone. Recirculation systems may be effective for difficult hydrogeological conditions. For example, recirculation may be used to effectively mix substrate and contaminated groundwater at sites with very low hydraulic gradients and low rates of groundwater flow. Delivery options are discussed in greater detail in Section 5.4.

1.6 ALTERNATIVE REMEDIAL STRATEGIES

Enhanced anaerobic bioremediation can be applied to achieve source reduction or plume-wide treatment, and it may be possible to complete the remedy in as little as 2 or 3 years. But for difficult sites (e.g., DNAPL source areas), it may be advantageous to combine enhanced anaerobic bioremediation with other remedial strategies or measures.

Strategies or measures that can be used in combination with an enhanced bioremediation application to either expedite treatment or to achieve site closure at lower life-cycle cost include the following:

- **Monitored Natural Attenuation**: MNA can often be employed as a polishing technique after enhanced bioremediation or to address large areas of low-level contamination that cannot be cost-effectively remediated with enhanced anaerobic bioremediation. For very large, dilute plumes at some DoD sites, this may be the only feasible and cost-effective approach.

- **Supplementary Engineered Remedial Measures**: Aggressive source reduction measures (e.g., soil vapor extraction (SVE), excavation, chemical oxidation, thermal technologies) may be used to quickly reduce contaminant source mass flux when a large percentage (greater than 95 percent) of the source mass can be effectively removed by these technologies. Use of enhanced bioremediation as a polishing step following source reduction may facilitate more rapid attainment of remedial endpoints. Some source removal methods (chemical oxidation or thermal treatment) may adversely alter the subsurface environment for application of enhanced bioremediation. However, this option is being considered as a potential remedy.

- **Maximizing Mass Removal with Ongoing Treatment Techniques**: Many sites have inefficient long-term pumping systems in place for hydraulic containment and/or mass removal. These systems are typically diffusion-limited, and often exhibit asymptotic mass removal rates. An enhanced bioremediation approach may be used in conjunction with an ongoing pumping system to expedite mass removal in source areas while pumping maintains containment of the contaminant plume.

1.7 ADVANTAGES AND LIMITATIONS OF ENHANCED ANAEROBIC BIOREMEDIATION

When selecting enhanced anaerobic bioremediation relative to other technologies, the RPM should evaluate both the advantages and limitations of this approach as described below.

Advantages of enhanced anaerobic bioremediation include the potential for complete destruction of dissolved CAH mass* in situ *with little impact on site infrastructure, lower capitol and maintenance costs relative to other highly engineered remedial technologies, and potential application to a wide variety of contaminants.

1.7.1 Advantages of Enhanced Anaerobic Bioremediation

Remediation of CAHs in the subsurface is difficult and sometimes technically infeasible due to aquifer heterogeneity and the density and hydrophobic nature of chlorinated solvent DNAPLs. Highly engineered remedial techniques such as pump-and-treat are costly due to inherent mass transfer limitations, capital expenditures, the need for treatment of secondary waste streams, energy consumption, and long-term operation and maintenance (O&M) requirements. Conversely, enhanced *in situ* anaerobic bioremediation may in some cases offer the following advantages:

- **Lower Capital and Maintenance Costs:** Lower capital costs often are realized because substrate addition can be easily accomplished using conventional well installations or by use of direct-push technology. Soluble substrates or soluble/fermentation products of slow-release substrates can potentially migrate into and disperse within heterogeneous lithologies via advection and diffusion. Systems used to mix and inject substrates can be readily designed and installed by environmental engineers, and O&M is generally routine.

- **Destruction of Contaminants *In Situ:*** CAHs that are treated have the potential of being completely mineralized or destroyed. Destruction of contaminants *in situ* is highly beneficial because contaminant mass is not transferred to another phase, there is no secondary waste stream to treat, potential risks related to exposure during remediation are limited, and there is minimal impact on site infrastructure. The biologically mediated reactions involved can generally be driven by indigenous microorganisms that are already resident in the groundwater.

- **Interphase Mass Transfer:** It appears that the enhanced anaerobic process may increase the rate of DNAPL source zone dissolution. This has sparked interest in enhanced bioremediation as a more efficient and expeditious method for remediating CAH source areas where remediation has been dissolution limited (see Section 2.3 for further discussion and Table 2.6 for a list of CAH compound physical and chemical properties).

- **Potential Application to a Variety of Contaminants:** In addition to CAHs, the technology may be applicable to a variety of other contaminants (see Section 1.4.2). Enhanced anaerobic bioremediation has the potential to treat any contaminant that can be made less toxic or less mobile through reduction reactions.

- **Treatment Train Options:** Enhanced anaerobic bioremediation can be used in tandem with existing or alternative remediation systems to optimize performance. (e.g., source removal via excavation or vapor extraction). Alternatively, anaerobic bioremediation systems may be coupled with downgradient aerobic reaction zones (e.g., air sparging trench) to degrade dechlorination products such as *cis*-DCE or VC that are amenable to degradation by oxidation processes.

1.7.2 Potential Limitations of Enhanced Anaerobic Bioremediation

Implementation of enhanced anaerobic bioremediation involves injection of a substrate that causes profound changes to the subsurface environment, and the degree of success may be subject to hydrogeological, geochemical, and biological limitations. Some of these problems also affect other remedial techniques and are not necessarily unique to enhanced anaerobic bioremediation. Several issues that should be considered prior to applying enhanced anaerobic bioremediation include, but are not limited to, the following:

If not carefully designed and implemented, disadvantages of enhanced anaerobic bioremediation may include longer timeframes for remediation, incomplete degradation of CAH parent compounds, adverse impacts to secondary water quality, and generation of volatile or noxious gases.

- **Site-Specific Limitations.** Site-specific limitations may include low permeability or a high degree of heterogeneity that limits the ability to effectively distribute the substrate throughout the aquifer. The depth to which enhanced bioremediation can be applied is a function of drilling capabilities and cost, and not necessarily a limitation of the bioremediation process. Other site-specific limitations may include high levels or influx of competing electron acceptors (e.g., DO, nitrate, or sulfate); inhibitory geochemical conditions (e.g., pH); or lack of appropriate microbial communities or species. As a result, degradation may be limited.

- **Timeframe for Remediation.** Enhanced bioremediation via anaerobic dechlorination is not an instantaneous process. The time required to develop the appropriate environmental conditions and to grow a microbial population capable of complete degradation may be on the order of several months to years at many sites. Therefore, the technology may require prolonged process monitoring and system maintenance.

- **Remediation of DNAPL Sources.** While anaerobic dechlorination has been shown to be a viable remedial approach for dissolved contaminant mass, and perhaps for limiting mass flux from or containing DNAPL source zones, it is not yet a proven technology for reducing significant DNAPL mass in source zones.

- **Incomplete Degradation Pathways and *cis*-DCE Stall.** Microbial populations capable of anaerobic dechlorination of the highly chlorinated compounds (e.g., PCE and TCE to *cis*-DCE) are thought to be more or less ubiquitous in the subsurface environment. However, the ability of these dechlorinators to compete with other native microbial populations or to complete the degradation of these compounds to innocuous end products may be an issue at some sites.

- **Secondary Degradation of Water Quality.** While anaerobic dechlorination may be effective in degrading chlorinated solvents, secondary degradation of groundwater quality may occur. Degradation reactions or excessive changes in groundwater pH and reduction-oxidation (redox) conditions may lead to solubilization of metals (e.g., iron, manganese, and potentially arsenic), formation of undesirable fermentation products (e.g., aldehydes and ketones), and other potential impacts to secondary water quality (e.g., total dissolved solids). Many of these changes are not easily reversed, and in the case of a slow-release carbon source it may take many years for the effects of the substrate addition to diminish. These issues should be considered during technology screening (Section 3.4.1).

- **Generation of Volatile Byproducts and Noxious Gases.** Stimulating biodegradation also may enhance generation of volatile byproducts and noxious gases (e.g., VC, methane, or hydrogen sulfide) that may degrade groundwater quality and/or accumulate in the vadose zone.

While these concerns and potential limitations should be considered when evaluating enhanced anaerobic bioremediation, many of them can be mitigated or compensated for by understanding the biogeochemical and hydrogeologic conditions of the aquifer system and using an appropriate design.

1.8 SUMMARY

Enhanced anaerobic bioremediation is a promising technology for the *in situ* remediation of CAHs in groundwater, which has been and is being applied at many sites. There are many substrate alternatives and system configurations that can be employed to stimulate anaerobic reductive dechlorination of CAHs. This principles and practices document is intended to provide RPMs with the information necessary to assess the application of enhanced anaerobic bioremediation at their sites and to identify optimum approaches, particularly when soliciting and reviewing enhanced bioremediation services.

A road map for implementing enhanced anaerobic bioremediation (figure 1.1) begins with characterization of a site, development of a CSM, and defining remedial objectives. Preliminary screening and evaluation of existing data is required to determine if enhanced anaerobic bioremediation is a suitable remedy for a specific site (Section 3). Often additional data collection or pre-design testing (Section 4) are required prior to a final decision as to whether enhanced anaerobic bioremediation is the most reasonable choice of a remedy compared to alternative technologies. Common attributes of system design (Section 5) and the implementation and evaluation of enhanced anaerobic bioremediation (Section 6) are described in this document to assist the RPM in assessing applications of this technology.

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SECTION 2

MICROBIOLOGICAL AND GEOCHEMICAL CONSIDERATIONS

Enhanced anaerobic bioremediation can be an effective method of degrading various forms of CAHs dissolved in groundwater. The most common CAHs released to the environment include PCE, TCE, TCA, and CT. Because these compounds are in an oxidized state, they are generally not susceptible to aerobic oxidation processes (with the possible exception of cometabolism). However, oxidized CAHs are susceptible to reduction under anaerobic conditions by either biotic (biological) or abiotic (chemical) processes. Enhanced anaerobic bioremediation is intended to stimulate and exploit biotic anaerobic processes to degrade chlorinated solvents in groundwater.

Enhanced anaerobic bioremediation is not effective unless:

- **The contaminant is anaerobically degradable,**
- **Strongly reducing conditions can be generated and other environmental conditions for microbial growth are met,**
- **A microbial community capable of driving the process is present or can be introduced to the subsurface, and**
- **A fermentable carbon source can be successfully distributed throughout the subsurface treatment zone.**

For enhanced anaerobic bioremediation to be effective, the contaminants and dechlorination products must be anaerobically degradable, strongly anaerobic conditions must be achieved, and environmental conditions for microbial growth must be met. Not only does this require the presence of a microbial community capable of driving the appropriate degradation processes, but the organic substrate used to stimulate anaerobic biodegradation processes must be uniformly added to the reaction zone and mixed with contaminated groundwater at appropriate concentrations.

This section describes the microbiological and geochemical conditions that must be achieved to successfully implement engineered anaerobic biodegradation of chlorinated solvents. Section 2.1 describes microbial processes and degradation pathways for chlorinated solvents. Because enhanced anaerobic bioremediation specifically targets biological anaerobic reductive dechlorination, Section 2.2 describes the microbial communities required for complete dechlorination to occur. Finally, Section 2.3 describes biological and chemical processes by which enhanced bioremediation may enhance the transfer of contaminant mass from DNAPL or sorbed phases to the dissolved phase, where it is subject to anaerobic biodegradation processes.

2.1 MICROBIAL PROCESSES AND DEGRADATION PATHWAYS

The study of the natural attenuation of chlorinated solvents has led to many discoveries as to how these contaminants are degraded in the subsurface. Understanding these processes and the pathways by which chlorinated solvents are degraded is essential to the application of engineered anaerobic bioremediation. Under some conditions, these processes may be sufficient to protect human health and the environment without the need for enhancement. ***A natural attenuation assessment should be conducted prior to considering the need for enhanced bioremediation.*** To date, successful enhanced bioremediation has simply been done through gaining an understanding of these naturally occurring attenuation processes and altering the environment to further stimulate them. This has resulted in many practitioners referring to enhanced bioremediation processes as enhanced natural attenuation.

2.1.1 Degradation Processes for Chlorinated Solvents

There are several potential reactions that may degrade CAHs in the subsurface, but not all CAHs are amenable to degradation by each of these processes (Table 1.1). For example, PCE is not amenable to any process of aerobic degradation, while TCE may only be degraded by aerobic cometabolism that typically requires addition of a substrate in the presence of oxygen. However, anaerobic biodegradation processes may potentially degrade not only PCE and TCE, but all of the common chloroethenes, chloroethanes, and chloromethanes. Table 2.1 further describes these potential degradation processes.

Enhanced anaerobic bioremediation seeks to exploit anaerobic biodegradation processes to completely degrade chlorinated solvents to innocuous end products. This approach involves the addition of organic substrates to the subsurface to create anaerobic and microbiologically diverse reactive zones that are conducive to the anaerobic degradation of CAHs. The degradation processes and the conditions under which they occur are described in the following subsections.

2.1.2 Anaerobic Reductive Dechlorination

The process of microbially facilitated anaerobic dechlorination has been well documented, and discussion of the overall process can be found widely in the literature (for example, see Wiedemeier et al. [1999] and USEPA [1998a and 2000a]). Anaerobic dechlorination of CAHs depends on many environmental factors including strongly anaerobic conditions, presence of fermentable substrates, generation of molecular hydrogen, and appropriate microbial populations to facilitate the reactions.

As listed in Tables 1.1 and 2.1, the three general reactions that may degrade CAHs by anaerobic reductive dechlorination include the following:

- **Direct Anaerobic Reductive Dechlorination** is a biological reaction in which bacteria gain energy and grow as one or more chlorine atoms on a CAH molecule are replaced with hydrogen in an anaerobic environment. In this reaction, the chlorinated compound serves as the electron acceptor and hydrogen serves as the direct electron donor. Hydrogen used in this reaction is typically supplied by fermentation of organic

substrates. This reaction may also be referred to as halorespiration or dehalorespiration (USEPA, 2000a).

Table 2.1 Description of Degradation Processes for CAHs

Degradation Process	Reaction Process	Alternate Process Terminology	Example References
Aerobic Oxidation	Compound is oxidized (used as an electron donor). Yields energy to the microorganism facilitating the reaction.	Hydroxylation, Epoxidation	Bradley and Chapelle, 2000; Tandoi et al., 2001; Hage and Hartmans, 1999
Aerobic Cometabolism	Compound is oxidized by an enzyme or co-factor produced during microbial metabolism of another compound.	--	McCarty et al., 1998; Hopkins and McCarty, 1995; McCarty and Semprini, 1994
Anaerobic Oxidation	Compound is oxidized (used as an electron donor) by electron acceptors other than oxygen. Yields energy to the microorganism facilitating the reaction.	--	Bradley and Chapelle, 1997; Bradley et al., 1998a, 1998b, and 1998c; Dijk et al., 2000
Direct Anaerobic Reductive Dechlorination	Compound is reduced (used as an electron acceptor). Yields energy to the microorganism facilitating the reaction.	Halorespiration, Dehalorespiration	Maymo-Gatell et al., 1999; Fennell and Gossett, 1998; He et al., 2003b
Cometabolic Anaerobic Reductive Dechlorination	Compound is reduced by an enzyme or co-factor produced during microbial metabolism of another compound.	Anaerobic Cometabolism	Maymo-Gatell et al., 2001; McCarty and Semprini, 1994; Rheinhard et al., 1990
Abiotic Transformation	Compound is reduced by chemical reactions. For example, degradation by iron monosulfides and other reactive inorganic compounds.	Abiotic Reductive Dechlorination, Hydrolysis, Dehydrochlorination, Elimination, Hydrogenolysis, Dichloroelimination	Lee and Batchelor, 2002; Butler and Hayes, 1999; Vogel and McCarty, 1987; Adrians et al., 2001; Gander et al., 2002; Ferrey et al., 2004

- **Cometabolic Anaerobic Reductive Dechlorination** is a reaction in which a chlorinated compound is reduced by a non-specific enzyme or co-factor produced during microbial metabolism of another compound (i.e., the primary substrate) in an anaerobic environment. By definition, cometabolism of the chlorinated compound does not yield any energy or growth benefit for the microbe mediating the reaction (USEPA, 2000a). For the cometabolic process to be sustained, sufficient primary substrate is required to support growth of the transforming microorganisms.

- **Abiotic Reductive Dechlorination** is a chemical degradation reaction not associated with biological activity where a chlorinated hydrocarbon is reduced by a reactive compound (Vogel et al., 1987). For example, abiotic transformation of CT, TCA,

PCE, TCE, and *cis*-DCE by metal sulfides has been investigated using pyrite (Weerasooriya and Dharmasena, 2001; Kriegman-King and Reinhard, 1994), troilite (Sivavec and Horney, 1997), mackinawite (Butler and Hayes, 1999 and 2000), and magnetite (Ferrey et al. 2004). In this case, substrate addition may indirectly cause and sustain abiotic reductive dechlorination.

In practice, it may not be possible to distinguish between the three different reactions listed above at the field scale. As used in this document, *anaerobic dechlorination* includes the biotic processes of direct and cometabolic anaerobic reductive dechlorination, and abiotic reductive dechlorination.

Anaerobic reductive dechlorination of CAHs using hydrogen as an electron donor are typically based on the following two half reactions:

(1) $H_2 \Rightarrow 2H^+ + 2e^-$

(2) $2e^- + H^+ + R\text{-}C\text{-}Cl \Rightarrow R\text{-}C\text{-}H + Cl^-$

These half reactions can be combined and balanced to produce the following generalized complete reaction:

(3) $H_2 + R\text{-}C\text{-}Cl \Rightarrow R\text{-}C\text{-}H + H^+ + Cl^-$

where C-Cl represents a carbon-chloride bond in a chlorinated molecule, C-H represents a carbon-hydrogen bond, and R represents the remainder of the molecule. In these reactions, two electrons are transferred with molecular hydrogen (H_2) as the electron donor (which is oxidized) and the chlorinated molecule (R-C-Cl) as the electron acceptor (which is reduced).

Although fermentation products (e.g., acetate) other than hydrogen have been identified as direct electron donors, several pure microbial cultures isolated to date require hydrogen as the electron donor for complete dechlorination of PCE to ethene (Maymo-Gatell et al., 1997; Fennell and Gossett, 1998). Therefore, it appears hydrogen may be the most important electron donor for anaerobic dechlorination.

In general, anaerobic dechlorination occurs by sequential removal of a chloride ion. For example, the chlorinated ethenes are transformed sequentially from PCE to TCE to the DCE isomers (*cis*-DCE or *trans*-DCE) to VC to ethene. This process of sequential dechlorination is illustrated in Figure 1.3.

Similarly, the common chloroethanes and chloromethanes may be transformed sequentially by anaerobic dechlorination as follows:

Chloroethanes: 1,1,1-TCA to 1,1-DCA to CA to ethane.

Chloromethanes: CT to CF to MC to CM to methane.

Anaerobic dechlorination of CAHs is associated with the generation of dechlorination products and chloride ions, and affects each of the chlorinated compounds differently. For example, of the chlorinated ethenes, PCE and TCE are the most susceptible to anaerobic

dechlorination because they are the most oxidized (i.e., they have a higher redox potential). They also yield more energy on their complete dechlorination to ethene. Conversely, *cis*-DCE and VC may degrade at lower reaction rates because they are the least oxidized of the chlorinated ethenes (i.e., they yield less energy during reductive reactions). Therefore, the potential exists for *cis*-DCE and VC to accumulate in a treatment system when the rate at which they are generated is greater than the rate at which they are degraded. This is a common concern for VC because it is considered more toxic than the other chlorinated ethenes. However, there are other degradation pathways for VC (see Table 1.1), and in the experience of the authors (e.g., Parsons, 2002a) the formation and persistence of large VC plumes (i.e., larger than the footprint of the initial CAH plume) is rarely observed in practice.

Similar analogies may be drawn for the chlorinated ethanes and chlorinated methanes, where potential accumulation of intermediate dechlorination products may occur. ***In general, the degradation pathways and microbiology of anaerobic dechlorination of chloroethanes and chloromethanes are less well studied than for the chlorinated ethenes.*** This is primarily because they occur less commonly as contaminants in groundwater.

2.1.3 Native Electron Acceptors and Oxidation-Reduction Conditions

Native electron acceptors compete with anaerobic dechlorination of CAHs, and anaerobic dechlorination will only occur under the appropriate geochemical conditions. After depletion of DO, anaerobic microbes will use nitrate as a terminal electron acceptor, followed by manganese (IV), iron (III), sulfate, and finally carbon dioxide (methanogenesis). Figure 2.1 illustrates the relative redox potential for which common reduction half reactions for native electron acceptors occur.

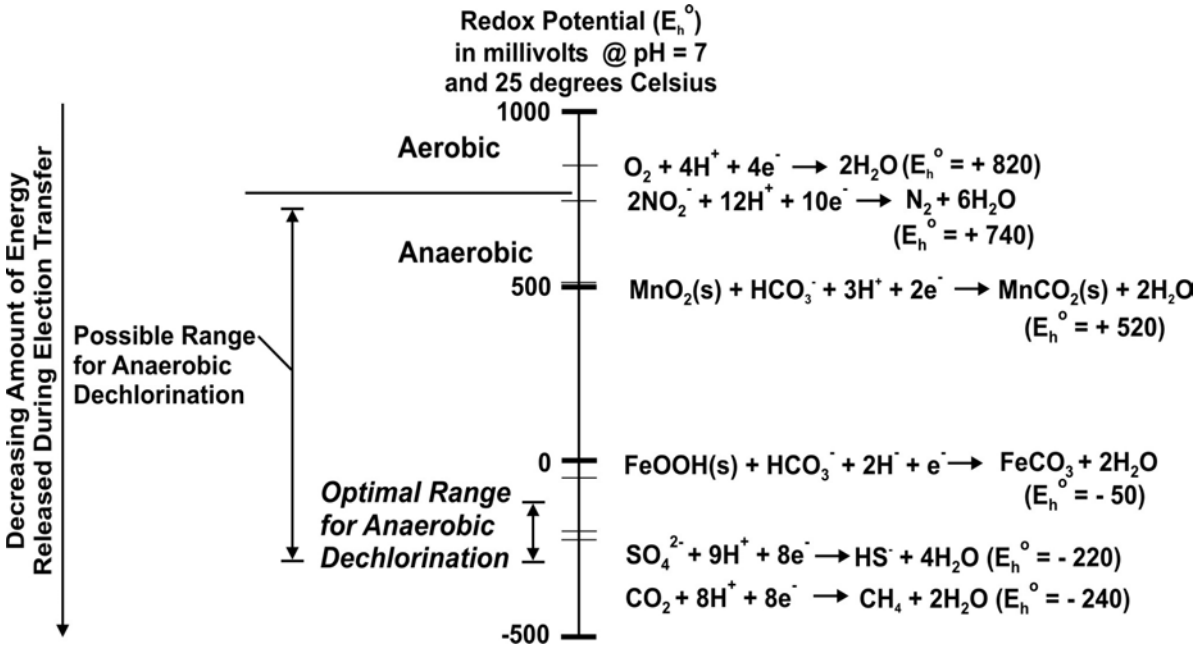


Figure 2.1 Oxidation-Reduction Potentials for Various Electron-Accepting Processes (modified from Bouwer, 1994)

The range of estimated relative redox potentials for reduction half reactions of chlorinated ethenes ranges from approximately 580 millivolts (mV) for PCE to TCE down to 360 mV for *cis*-DCE to VC in aqueous solution at a pH of 7 and a temperature of 25 Celsius (^{o}C) (Vogel et al., 1987). Redox potentials for reduction of chloroethanes (from 570 mV for TCA to DCA down to 350 mV for CA to ethane) and chloromethanes (from 670 mV for CT to CF down to 470 for CM to methane) are similar in range (Vogel et al., 1987). This range of redox potentials suggest that anaerobic reductive dechlorination may occur in the range of nitrate reduction to iron reduction (Figure 2.1). However, it appears that the most rapid and complete anaerobic dechlorination of CAHs occurs under the highly reducing conditions of sulfate reduction to methanogenesis (Bouwer, 1994). Therefore, as each sequential terminal electron accepting process (TEAP) drives the ORP of groundwater downward, anaerobic dechlorination will occur more readily.

Prevailing redox conditions are largely a result of the relative amount of organic carbon (electron donor) and electron acceptors present. Thus, DO, nitrate, and bioavailable iron must be depleted before sulfate-reducing or methanogenic conditions can be induced. In general, USEPA (1998a) suggests that DO less than 0.5 mg/L, nitrate less than 1.0 mg/L, sulfate less than 20 mg/L, and total organic carbon (TOC) greater than 20 mg/L are favorable for anaerobic dechlorination of CAHs. In addition, ferrous iron and methane concentrations greater than 1 mg/L and 0.5 mg/L, respectively, are indicative of favorable reducing conditions.

More reduced conditions are required as the oxidation state of the compound is lowered (i.e., from PCE and TCE to DCE and VC). For example, anaerobic dechlorination of PCE and TCE to DCE may readily occur under iron-reducing conditions, but this redox condition may not be optimal for further degradation of DCE to VC and ethene. As another example, dechlorinating microorganisms may preferentially degrade PCE and TCE to the exclusion of DCE because they gain more energy from dechlorination of the more highly chlorinated CAHs. Thus, dechlorination of DCE may not proceed until PCE and TCE are depleted.

The highest rates and greatest extent of anaerobic dechlorination occurs under sulfate-reducing and methanogenic condition.

Sufficient organic carbon must be present in order to deplete native inorganic electron acceptor, including DO, nitrate, bioavailable iron and manganese, and sulfate.

As a result, it is common for incomplete dechlorination to occur due when insufficient substrate loading leads to insufficiently reducing conditions. Considerations for substrate loading rates are discussed in Section 5.5 and Appendix C.

2.1.4 Fermentation Reactions and Molecular Hydrogen

Researchers have recognized the role of hydrogen as the direct electron donor in the anaerobic dechlorination of CAHs (Holliger et al., 1993; Gossett and Zinder, 1996; Smatlak et al., 1996; Ballapragada et al., 1997; Cupples et al., 2003). Laboratory cultures used to study direct anaerobic reductive dechlorination are typically mixed cultures, with at least two distinct strains of bacteria. One strain ferments the organic substrate to produce hydrogen, and another strain uses the hydrogen as an electron donor for anaerobic dechlorination. Other

direct electron donors also may be used for anaerobic dechlorination, including acetate (He et al., 2002). However, many researchers believe that molecular hydrogen is the most important electron donor for anaerobic dechlorination of CAHs. The following sections describe the fermentation reactions that produce molecular hydrogen and how hydrogen is utilized as an electron donor.

2.1.4.1 Fermentation

Fermentation is a balanced redox reaction in which different portions of a single substrate are oxidized and reduced, yielding energy. Fermentation does not require an external electron acceptor, such as oxygen. Rather, the organic molecule itself serves as both the electron donor and electron acceptor. Fermentation yields substantially less energy per unit of substrate compared to oxidation reactions, which utilize an external electron acceptor; thus, fermentation generally occurs when these external electron acceptors are depleted. Bacterial fermentation can be divided into two categories:

- **Primary Fermentation**: The fermentation of primary substrates such as sugars and amino acids yields acetate, formate, carbon dioxide (CO_2) and hydrogen (H_2), but also yields ethanol, lactate, propionate, and butyrate.

- **Secondary Fermentation:** The fermentation of primary fermentation products such as ethanol, lactate, propionate, and butyrate yields acetate, formate, H_2, and CO_2. Bacteria that carry out secondary fermentation reactions are called obligate proton reducers because the reactions must produce hydrogen to balance the oxidation of the carbon substrates. These secondary fermentation reactions are energetically favorable only if hydrogen concentrations are relatively low (10^{-2} to 10^{-4} atmospheres [atm] or 8,000 nanomoles per liter [nmol/L] to 80 nmol/L, depending on the fermentation substrate). Thus, these secondary fermentation reactions occur only when the produced hydrogen is used by other bacteria, such as methanogens or dechlorinators. The process by which hydrogen is produced by one strain of bacteria and used by another is called interspecies hydrogen transfer.

There are many carbon substrates that are naturally fermented at chlorinated solvent sites and that result in the generation of hydrogen. Examples of easily fermentable organics include carbohydrates (sugars), alcohols, low-molecular-weight fatty acids, and vegetable oils. The purpose of adding an organic substrate to the subsurface is to provide sufficient organic carbon to overcome native electron acceptor demand and be fermented to produce hydrogen for anaerobic dechlorination.

2.1.4.2 Donor-Specific Fermentation Reactions

Fermentation reactions with complex substrates can be highly variable and subject to site-specific conditions. Fermentation reactions for simpler substrates such as lactate have been determined by laboratory research and are easier to describe. For example, Martin et al. (2001) describe two degradation pathways for lactate. The first pathway produces acetate and hydrogen, and the second pathway produces propionate and acetate in a ratio of 2:1. While many fermentation reactions have been described for simple substrates in the laboratory, it is much more difficult to extrapolate these reactions to field conditions or to determine exact fermentation reactions for more complex substrates.

The Reductive Anaerobic Biological *In Situ* Treatment Technology (RABITT) protocol (Morse et al., 1998; Air Force Research Laboratory [AFRL] et al., 2001) attempts to exploit these reactions with carefully controlled microcosms and small scale pilot tests. For example, at Naval Air Station Alameda, California, the authors found that sodium benzoate did not promote dechlorination in microcosm studies. Butyrate was found to be superior to lactate because sulfate reducers (the site has elevated levels of sulfate) could rapidly consume lactate, but apparently not butyrate. Thus, when butyrate was fed, sulfate-reduction did not inhibit utilization of the substrate to promote anaerobic dechlorination.

2.1.4.3 Molecular Hydrogen as an Electron Donor

Hydrogen is generated by fermentation of non-chlorinated organic substrates, including naturally occurring organic carbon, accidental releases of anthropogenic carbon (fuel), or introduced substrates such as carbohydrates (sugars), alcohols, and low-molecular-weight fatty acids. As an example, lactate in the form of sodium lactate (a stable lactate salt solid) is commonly used as a substrate for enhanced anaerobic bioremediation. When added to the subsurface, sodium lactate disassociates in groundwater to form lactate and a sodium ion as follows:

(4) $C_3H_5NaO_3 \Rightarrow C_3H_5O_3^- + Na^+$

The lactate molecule may then be fermented, potentially by more than one process. For example, it may be fermented to acetate in the following fermentation reaction:

(5) $C_3H_5O_3^- + 2H_2O \Rightarrow C_2H_3O_2^-$ (acetate) + HCO_3^- (bicarbonate ion) + $H^+ + 2H_2$

Furthermore, the bicarbonate ion and a hydrogen ion may form carbon dioxide and water:

(6) $HCO_3^- + H^+ \Rightarrow CO_2^- + H_2O$

Combining equations (5), (6), and (7), the fermentation of lactate to acetate and hydrogen can be written as the following balanced fermentation reaction:

(7) $C_3H_5O_3^- + 2H_2O \Rightarrow C_2H_3O_2^- + CO_2^- + H_2O + 2H_2$

Thus, the fermentation of a single molecule of lactate to acetate produces two molecules of molecular hydrogen. The acetate produced in this reaction may be used directly as an electron donor for reduction reactions or may be further fermented to produce hydrogen. Table 2.2 lists a few examples of some other fermentation reactions where the substrate (electron donor) is fermented to produce hydrogen.

As hydrogen is produced by fermentative organisms, it is rapidly consumed by other bacteria, including denitrifiers, iron-reducers, sulfate-reducers, methanogens, and dechlorinating microorganisms. As an example, consider the reduction of PCE. First, molecular hydrogen disassociates in the following half reaction:

(8) $H_2 \Rightarrow 2H^+ + 2e^-$

Table 2.2 Examples of Fermentation Half Reactions Using Organic Substrates as an Electron Donor to Yield Hydrogen

Electron Donor	Electron-Donor (Oxidation) Reaction
Ethanol	$C_2H_6O + H_20 \Rightarrow C_2H_3O_2^- + H^+ + 2H_2$ *ethanol fermentation to acetate*
Methanol	$CH_4O + 2H_20 \Rightarrow CO_2^- + H_2O + 3H_2$ *methanol fermentation*
Acetate	$C_2H_3O_2^- + 4H_20 \Rightarrow 2CO_2^- + 2H_2O + 4H_2$ *acetate fermentation*
Butyrate	$C_4H_7O_2^- + 2H_20 \Rightarrow 2C_2H_3O_2^- + H^+ + 2H_2$ *butyrate fermentation to acetate*
Propionate	$C_3H_5O_2^- + 3H_20 \Rightarrow C_2H_3O_2^- + CO_2^- + H_2O + 3H_2$ *propionate fermentation to acetate*
Lactate	$C_3H_5O_3^- + 2H_2O + \Rightarrow C_2H_3O_2^- + CO2^- + H_2O + 2H_2$ *lactate fermentation to acetate*

Note: Fermentation reactions from Fennel and Gossett (1998) and He et al. (2002).

Then, PCE is reduced to TCE by the substitution of a chloride ion with a hydrogen ion and the transfer of two electrons:

(9) C_2Cl_4 (PCE) + $2H^+ + 2e^- \Rightarrow C_2HCl_3$ (TCE) + $H^+ + Cl^-$

Combining and balancing equations (9) and (10), the dechlorination of PCE using hydrogen as the electron donor can be written as follows:

(10) $H_2 + C_2Cl_4 \Rightarrow C_2HCl_3 + H^+ + Cl^-$

Table 2.3 lists a few examples of some common half reactions that utilize hydrogen as an electron donor for reduction of native electron acceptors and CAHs. The production of hydrogen through fermentation does not, by itself, guarantee that hydrogen will be available for reductive dechlorination of CAHs. For anaerobic reductive dechlorination to occur, dechlorinators must successfully compete against the other microorganisms that also utilize hydrogen.

Smatlak et al. (1996) suggest that the competition for hydrogen is controlled primarily by the Monod half-saturation constant $K_s(H_2)$, which is the concentration at which a specific strain of bacteria can utilize hydrogen at half the maximum utilization rate. Smatlak et al. (1996) measured $K_s(H_2)$ values for dechlorinators and methanogens of 100 nmol/L and 1,000 nmol/L, respectively. Based on this result, they suggested that dechlorinators would successfully compete for hydrogen only at very low hydrogen concentrations. This implies that the selection of an organic substrate whose fermentation results in a slow, steady, and low-level release of hydrogen (electron donor) over time could maximize dechlorination potential while minimizing methanogenic competition for the available hydrogen.

Table 2.3 Examples of Half Reactions Using Hydrogen as the Electron Donor

Electron Acceptor	Electron-Acceptor (Reduction) Half Reaction
Oxygen	$2H_2 + O_2 \Rightarrow 2H_2O$ *aerobic respiration*
Ferric Iron	$e^- + 3H^+ + FeOOH \Rightarrow Fe^{2+} + 2H_2O$ *"ferric oxyhydroxide" dissolution/reduction*
Sulfate	$4H_2 + H^+ + SO^{2-}_4 \Rightarrow HS^- + 4H_2O$ *sulfate reduction*
Carbon Dioxide	$4H_2 + CO_{2,g} \Rightarrow CH_{4,g} + 2H_2O$ *methanogenesis*
PCE	$H_2 + C_2Cl_4 \Rightarrow C_2HCl_3 + HCl$ *PCE reductive dechlorination*
TCE	$H_2 + C_2HCl_3 \Rightarrow C_2H_2Cl_2 + HCl$ *TCE reductive dechlorination*
DCE	$H_2 + C_2H_2Cl_2 \Rightarrow C_2H_3Cl + HCl$ *cis-1,2-DCE reductive dechlorination*
VC	$H_2 + C_2H_3Cl \Rightarrow C_2H_4 + HCl$ *VC reductive dechlorination*

Ballapragada et al. (1997) point out that competition for hydrogen also depends on additional factors, including the bacterial growth rate (relative cell yields), temperature (higher temperatures (35 ^oC) favor methanogens), and maximum hydrogen utilization rate. While they concluded that dechlorinating bacteria may out-compete methanogens for hydrogen utilization at low hydrogen concentrations ($K_s(H_2)$ values of 9 to 21 nmol/L), they also concluded that dechlorinators can compete successfully with methanogens up to a hydrogen partial pressure of 100 parts per million (ppm), or 50 nmol/L. Because hydrogen concentrations seldom exceed 50 nmol/L in methanogenic environments, dechlorinators should normally have an advantage. Cupples et al. (2003) investigated the effect of limiting both electron donor (hydrogen) and electron acceptor (*cis*-DCE and VC) substrates on reaction kinetics using bacterium strain VS (shown to metabolize both *cis*-DCE and VC). Based on experimental data, the authors calculated a $K_s(H_2)$ value of 7 ± 2 nmol/L, which is similar to that found by Ballapragada et al. (1997).

These studies suggest that attempts to limit hydrogen concentrations to reduce competition for hydrogen (e.g., by methanogenesis) and increase substrate utilization are unnecessary and may result in significant portions of the treatment zone remaining insufficiently reducing for complete dechlorination to occur. This may result in sites "stalling" at intermediate dechlorination byproducts such as *cis*-DCE or VC. Even though a large percentage of substrate added to the subsurface may be utilized for sulfate reduction or methanogenesis, the stoichiometric relationships for the direct anaerobic dechlorination of CAHs are relatively favorable (Section 2.1.4.4). High rates of anaerobic dechlorination and mass destruction may be achieved even with relatively low substrate utilization rates. Conversely, caution should be used to avoid adding too much substrate to the subsurface because other conditions may develop, such as degradation of secondary water quality or adverse changes in pH.

Hydrogen concentrations also are indicative of the dominant TEAP occurring in groundwater (Lovely et al., 1994; Chapelle et al., 1995). Table 2.4 lists the hydrogen concentrations within which each electron-accepting process is favored. For the most rapid

and extensive reductive dechlorination to occur, redox conditions should be in the sulfate reducing to methanogenic range. ***Yang and McCarty (1998) report that the optimal concentrations of hydrogen for anaerobic dechlorination to occur range from 2 nmol/L (mid-range of sulfate reduction) to 11 nmol/L (mid-range of methanogenesis).***

Table 2.4 Range of Hydrogen Concentrations for a Given Terminal Electron-Accepting Process

TERMINAL ELECTRON-ACCEPTING PROCESS	DISSOLVED HYDROGEN CONCENTRATION		
	(nmol/L)	(atm)*	(ug/L)
Denitrification and Manganese Reduction	< 0.1	$< 1.3 \times 10^{-7}$	$< 2.0 \times 10^{-4}$
Iron (III) Reduction	0.2 to 0.8	$0.26 - 1.0 \times 10^{-6}$	$0.4 - 1.6 \times 10^{-3}$
Sulfate Reduction	1 to 4	$1.3 - 5.0 \times 10^{-6}$	$2.0 - 8.0 \times 10^{-3}$
Methanogenesis	5 to 20	$63 - 250 \times 10^{-6}$	$1.0 – 4.0 \times 10^{-2}$
Optimum for Anaerobic Reductive Dechlorination	**2 to 1l**	**$2.6 - 125 \times 10^{-6}$**	**$4.0 \times 10^{-3} – 2.2 \times 10^{-2}$**

Adapted from Lovley et al., 1994; Chapelle et al., 1995; and Yang and McCarty, 1998

* In gas phase in equilibrium with water containing dissolved hydrogen.

Biodegradation at higher hydrogen partial pressures may require more electron donor, as a larger portion of available hydrogen would be used by methanogenic bacteria. However, this is compensated for by higher rates of dechlorination under methanogenic conditions and by providing a sufficient amount of organic substrate. In practice, the amount of substrate added and hydrogen produced does not appear to be detrimental to anaerobic dechlorination of CAHs.

2.1.4.4 Stoichiometric Relationships

As mentioned earlier, the generation of hydrogen *in situ* does not guarantee that it will be used solely for anaerobic reductive dechlorination. Thus, a direct stoichiometric relationship does not exist between hydrogen and CAH degradation in the subsurface or laboratory environment. However, even though the efficiency of utilization of hydrogen for reductive dechlorination is often estimated to be relatively low, the stoichiometric relationships for the direct anaerobic dechlorination of CAHs are relatively favorable.

For example, on a mass basis, 1 milligram (mg) of H_2 will dechlorinate the following mass of chlorinated ethenes, assuming 100 percent utilization of H_2 by the dechlorinating microorganisms (Gossett and Zinder, 1996):

- 21 mg of PCE to ethene
- 22 mg of TCE to ethene
- 24 mg of DCE to ethene
- 31 mg of VC to ethene

Thus, the observed presence of sulfate reducing and methanogenic processes may be compatible with a significant degree of anaerobic dechlorination and mass destruction.

2.1.5 Alternate Degradation Processes

Multiple degradation pathways exist for CAHs in both aerobic and anaerobic environments (Table 1.1). Microorganisms capable of anaerobic dechlorination of CAHs (e.g., *cis*-DCE and VC) may not be ubiquitous or sufficiently abundant to be effective in meeting remedial objectives. However, there are other degradation pathways that may occur for these compounds.

Some of these alternative processes do not produce dechlorination products (such as VC or ethene), and thus may be difficult to discern or quantify in the field. ***If measurable degradation of more highly chlorinated ethenes occurs without evidence of VC or ethene production, then these processes may be sufficient to achieve remedial endpoints.*** A lack of VC or ethene does not, by itself, provide adequate justification for bioaugmentation if degradation of contaminant mass (e.g., oxidation of VC) is otherwise being achieved at acceptable rates.

2.1.5.1 Oxidative Pathways

Lesser chlorinated dechlorination products such as VC may degrade by oxidative pathways. Aerobic oxidation of VC generally occurs at a higher rate than anaerobic reductive dechlorination. Anaerobic oxidation of VC also may occur under mildly reducing conditions such as iron- and manganese-reduction (Bradley et al., 1998a and 1998b). While oxidative pathways are not specifically targeted during enhanced anaerobic bioremediation, they may be important outside the anaerobic reaction zone in downgradient areas where groundwater geochemical conditions return to a natural state (redox recovery zone).

An example of a half reaction for the oxidation of VC is shown in the following equation:

(11) $4H_2O + C_2H_3Cl\ (VC) \Rightarrow 2CO_2 + 10H^+ + 10e^- + H^+ + Cl^-$

In this case VC is en electron donor, yielding 10 electrons. This reaction is coupled to the reduction of oxygen (electron acceptor) as in the following half reaction:

(12) $4e^- + 4H^+ + O_2 \Rightarrow 2H_2O$

Oxidative pathways may be exploited in sequential anaerobic/aerobic systems where higher chlorinated compounds are degraded by anaerobic dechlorination, and lesser chlorinated compounds such as VC are aerobically degraded in a downgradient redox recovery zone or engineered oxidation system (e.g., air sparging trench). In addition, more oxic groundwater zones provide for the precipitation of dissolved ions (e.g., ferrous iron or manganese) or biogenic gases (e.g., methane or hydrogen sulfide) produced in anaerobic treatment zones. This will improve the aesthetic qualities (i.e., taste and odor) of the groundwater.

Oxidation pathways for VC are faster than anaerobic reductive dechlorination. For aquifers that are naturally aerobic, plumes of VC migrating from the anaerobic reaction zone are rarely observed.

In some cases, a combination of an anaerobic reaction zone followed by an aerobic oxidation zone may be highly effective for treating chlorinated ethenes.

Aerobic biodegradation of *cis*-DCE in the absence of primary substrates in a pure-culture, laboratory setting has been reported by Coleman et al. (2002); however, it is less clear how significant this mechanism is for removal of DCE in the environment. Aerobic transformations of *cis*-DCE investigated under SERDP Project CU-1167 (personal communication with Dr. Frank Löffler) observed that aerobic degradation of *cis*-DCE did not occur except under cometabolic conditions in the presence of VC, ethene, or methane. This suggests that aerobic biodegradation of *cis*-DCE in the environment may not be significant at many sites.

2.1.5.2 Abiotic Pathways

A number of abiotic processes may degrade CAHs, under both aerobic and anaerobic conditions. Abiotic pathways may include hydrolysis, elimination, dehydrohalogenation, hydrogenolysis, dichloroelimination, and abiotic reductive dechlorination by a variety of reactive compounds (Table 2.1).

Hydrolysis is a substitution reaction in which an organic molecule reacts with water or a component ion of water, and a halogen substituent (e.g., chloride ions in CAH compounds) is replaced with a hydroxyl (OH^-) group. This reaction often produces alcohols as products. For example, CA may undergo hydrolysis to ethanol (Vogel et al., 1987).

Dehydrohalogenation is an elimination reaction involving halogenated alkanes (e.g., chloroethanes) in which a halogen is removed from one carbon atom, followed by subsequent removal of a hydrogen atom from an adjacent carbon atom. In this two-step process, an alkene (e.g., chloroethenes) is produced. For example, CA may be transformed to VC (Jeffers et al., 1989).

Hydrogenolysis refers to the replacement of a chlorine atom (or other halogen) by a hydrogen atom in a process that may be either biotic or abiotic in nature. Dichloroelimination is the removal of two chlorines by a hydrogen atom accompanied by the formation of a double carbon-carbon bond.

Some abiotic process are not driven by redox processes (e.g., hydrolysis and dehydrohalogenation), while other abiotic processes may be stimulated indirectly under the anaerobic conditions induced by addition of the substrate (e.g., abiotic reductive dechlorination by reactive metal sulfides). Hydrogenolysis and dichloroelimination generally do not occur in the absence of biological activity, even if the activity is indirectly responsible for the reaction. Therefore, it is not clear whether these reactions are truly abiotic, or if they occur in a manner similar to cometabolism (Wiedemeier et al., 1999).

Many abiotic transformations of CAHs occur at rates that are too slow to have significance in environmental restoration of groundwater. Notable exceptions include hydrolysis and elimination of 1,1,1-TCA, and hydrolysis of CA and CM. Abiotic degradation of 1,1,1-TCA occurs by hydrolysis to acetic acid and elimination to 1,1-DCE. McCarty (1996) estimated that 80 percent of 1,1,1-TCA transformed by abiotic processes is converted to acetic acid and 20 percent to 1,1-DCE. 1,1-DCE is considered more toxic than 1,1,1-TCA, but also is subject to anaerobic dechlorination. Degradation rates for 1,1,1-TCA by hydrolysis has been reported with half-lives on the order of 1 to 3 years (Jeffers et al., 1989; Vogel and McCarty, 1987).

CA and CM are also subject to relatively rapid degradation by hydrolysis, with a reported half-life of 0.12 years for hydrolysis of CA to ethanol (Vogel et al., 1987).

There appears to be a broad spectrum of metal containing minerals that may cause abiotic dechlorination of CAHs (Lee and Batchelor, 2003). Some of these minerals are metal oxides or are reduced species. For example, reduction of sulfate produces hydrogen sulfide, which in turn may react with iron minerals (i.e., iron oxide/hydroxides) to form iron monosulfide precipitates (FeS). With time, iron monosulfide will react with elemental sulfur to form iron disulfide (FeS_2). However, iron monosulfide will also rapidly react with oxidized compounds such as PCE and TCE to form acetylene (Butler and Hayes, 1999). It is notable that the major reaction product of the reaction of PCE or TCE with FeS is acetylene, and not intermediate dechlorination products such as DCE or VC.

Site-specific concentrations of reduced minerals that are reactive with CAHs may be elevated due to addition of organic substrates. For example, the presence of organic carbon, iron, and sulfate alone will typically result in the formation of reactive iron sulfides (e.g. pyrite, troilite, or mackinawite) due to the biological processes of iron and sulfate reduction (e.g., Lee and Batchelor, 2002, Butler and Hayes, 1999; Weerasooriya and Dharmasena, 2001). Other minerals of interest include, but are not limited to: goethite, magnetite, and green rust with respect to their capacities to support abiotic reductive dechlorination (e.g., Ferrey et al., 2004; Sivavec and Horney, 1997).

The formation of these reactive minerals is of interest in that it may enhance overall contaminant destruction. The minerals and associated abiotic degradation may persist even if subsurface conditions are not sufficiently anaerobic to sustain rapid anaerobic biodegradation. Conversely, if organic carbon is depleted and native electron acceptor influx is high, these reactive minerals may be transformed to less reactive mineral forms (e.g., FeS is oxidized to a ferric state).

The occurrence of abiotic reductive dechlorination may be pronounced for enhanced bioremediation applications in high sulfate (>100 mg/L) and high iron (e.g., >20 mg/L of ferrous iron produced) environments (Air Force Center for Environmental Excellence [AFCEE], 2003). Because addition of an organic substrate may indirectly stimulate this process, practitioners should evaluate the potential for these reactions to occur in these environments.

2.1.6 Relative Rates of Degradation

Anaerobic dechlorination is usually more rapid for highly chlorinated (more oxidized) compounds than for compounds that are less chlorinated (Vogel and McCarty, 1985; Vogel and McCarty, 1987; Bouwer, 1994**).** Figure 2.2 qualitatively shows the reaction rate and required conditions for anaerobic dechlorination of PCE to ethene.

PCE and TCE usually degrade faster than *cis*-DCE and VC by direct anaerobic reductive dechlorination in most anaerobic environments. VC (with a single chlorine atom) will degrade under sulfate-reducing and methanogenic conditions, and usually has a slow dechlorination rate relative to other chlorinated ethenes. Similar reaction summaries are shown for the TCA to ethane and CT to methane breakdown sequences on Figures 2.3 and 2.4.

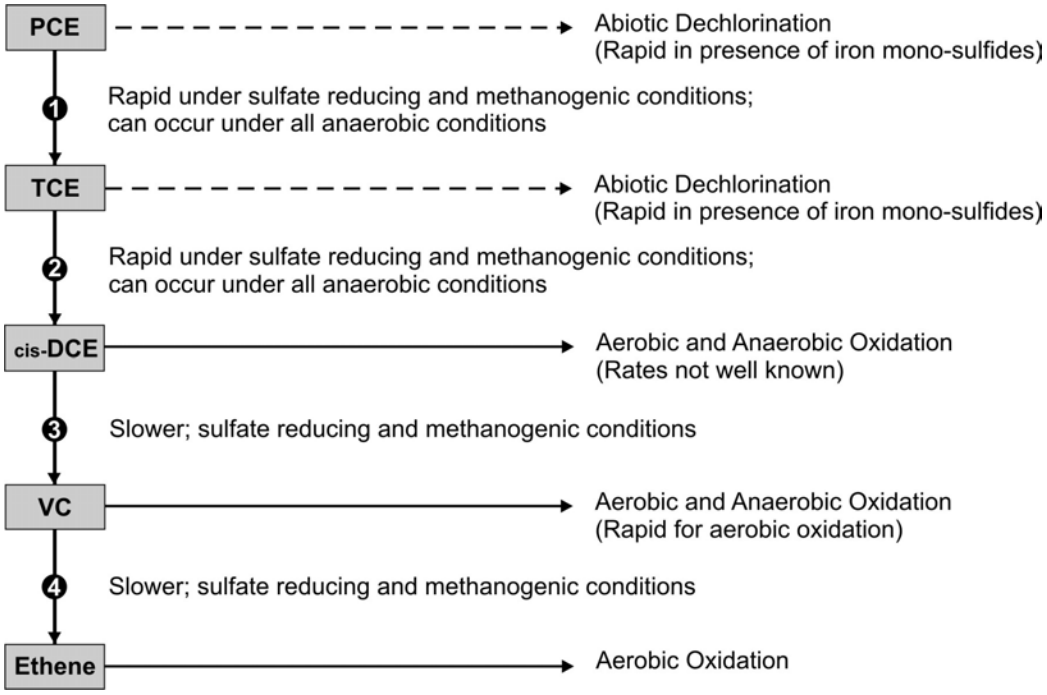


Figure 2.2 Reaction Sequence and Relative Rates of Degradation for Chlorinated Ethenes (modified from Wiedemeier et al., 1999)

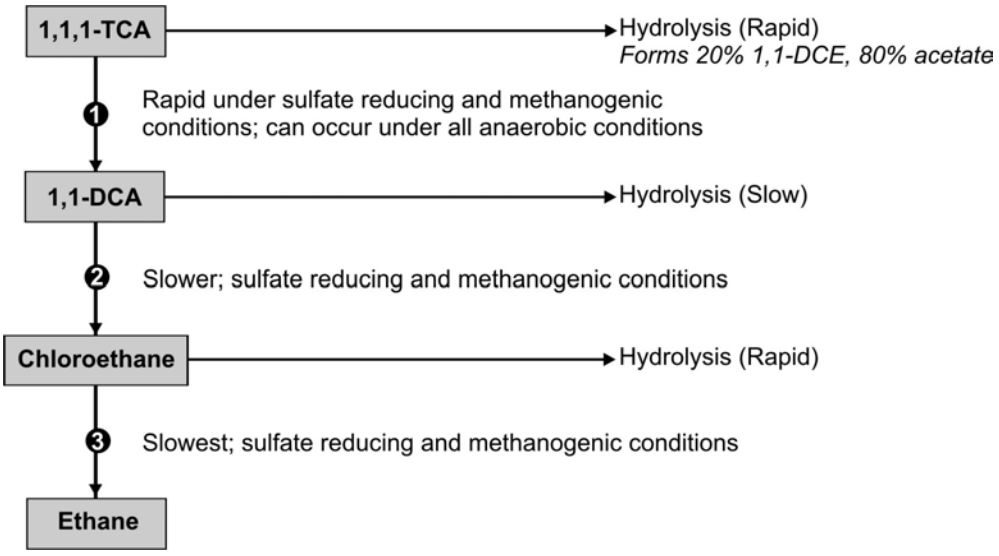


Figure 2.3 Reaction Sequence and Relative Rates of Degradation for Chlorinated Ethanes (modified from Wiedemeier et al., 1999)

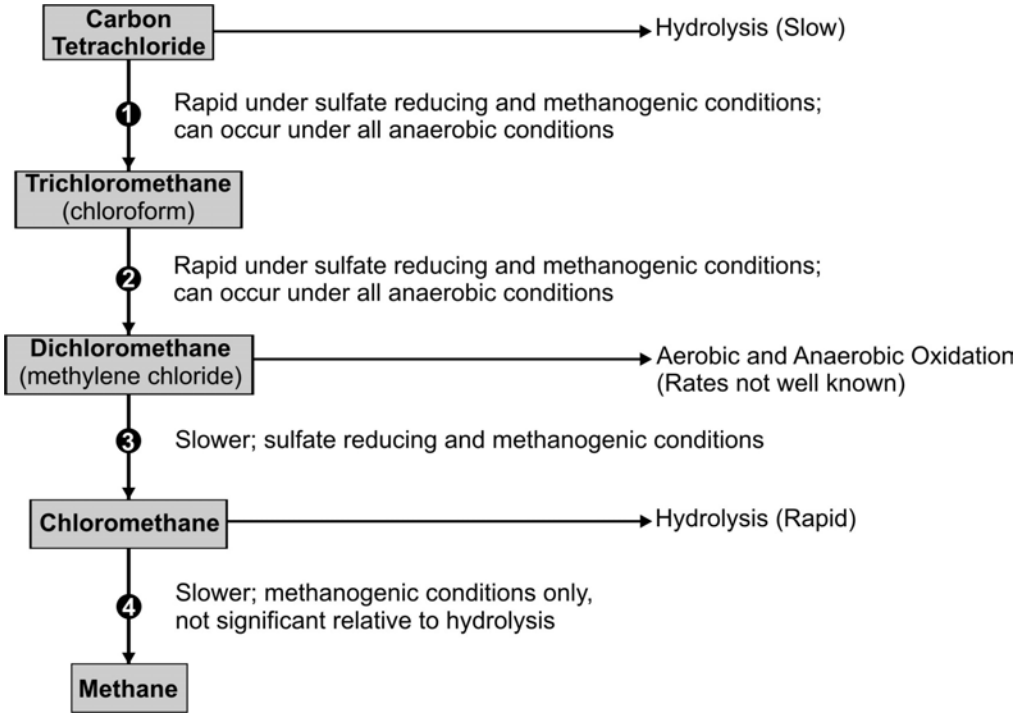


Figure 2.4 Reaction Sequence and Relative Rates of Degradation for Chlorinated Methanes (modified from Wiedemeier et al., 1999)

At many chlorinated ethene sites, concentrations of *cis*-DCE are often higher than any of the parent chlorinated ethenes. The accumulation of *cis*-DCE may be due to either slower rates of DCE dechlorination, or a lack of organisms that can reduce *cis*-DCE all the way to ethene (Gossett and Zinder, 1996). Although many researchers have commented that anaerobic dechlorination may result in the accumulation of *cis*-DCE and VC (e.g., Bradley and Chapelle, 1997; Weaver et al., 1995), VC accumulation appears to be much less pronounced than *cis*-DCE accumulation at many field sites. This may occur because the VC in many CAH plumes can migrate to zones that support oxidation of this compound, either aerobically or anaerobically. Therefore, the prevailing geochemical and redox conditions will have a profound impact as to what extent degradation of CAHs will occur.

In summary, a change in parent compound to dechlorination product ratios is a line of evidence identifying that degradation is occurring. However, a more important consideration is whether there is an unacceptable expansion of the dechlorination product groundwater plume. Significant changes in the ratios of parent to dechlorination product compounds have been seen to occur over periods of up to 24 months following substrate addition. A site-specific determination should be completed to determine what is acceptable or unacceptable from a risk management perspective.

2.2 MICROBIAL COMMUNITIES REQUIRED FOR ANAEROBIC REDUCTIVE DECHLORINATION

Current literature suggests that anaerobic reductive dechlorination is carried out by only a few metabolic classifications of bacteria, including methanogens, sulfate-reducing bacteria,

and dechlorinating bacteria. Anaerobic bacteria such as methanogens and sulfate-reducing bacteria are assumed to be ubiquitous in the subsurface environment (Chapelle, 1993). Even in aerobic environments, anaerobic micro-environments may provide for the survival of obligately anaerobic bacteria. Some types of sulfate-reducing bacteria can form spores under adverse conditions that germinate on the establishment of suitable growth conditions. These microorganisms, along with a variety of dechlorinating microorganisms, have been shown to be capable of dechlorinating PCE and TCE to *cis*-DCE. In particular, cultures containing *Desulfitobacterium*, *Dehalobacter restrictus*, *Desulfuromonas*, *Dehalospirillum multivorans,* and *Dehalococcoides* are known to be capable of dechlorinating PCE and TCE to *cis*-DCE (Scholz-Muramatsu et al., 1995; Gerritse et al., 1996; Krumholz, 1997; Maymo-Gatell et al., 1997; Holliger et al., 1998; Löffler et al., 2000). ***In practice, microorganisms capable of degrading PCE and TCE to cis-DCE should be considered ubiquitous in the subsurface environment***.

A more important consideration is the distribution of microorganisms that can degrade *cis*-DCE and VC to ethene, as well as those microorganisms capable of anaerobic dechlorination of the chloroethanes and chloromethanes. For example, dechlorination of *cis*-DCE and VC to ethene appears to be limited only to a few species of dechlorinating bacteria, which may not be ubiquitous in the environment (He et al., 2003a). Researchers have observed a correlation between the persistence of *cis*-DCE or VC and the absence of the *Dehalococcoides* group (Fennell et al., 2001; Hendrickson et al., 2002a). The known *Dehalococcoides* species can be divided into sequence groups and sub-groups based on *Dehalococcoides* 16S ribosomal deoxyribonucleic acid (rDNA) gene sequences, including the Ethenogenes group and the Alameda group. The *Dehalococcoides* group contains strains that are capable of dechlorination of a variety of different CAHs with varying degrees of specificity and efficiency (see GeoSyntec (2004) for a summary of *Dehalococcoides* dechlorinating capabilities). To date, complete sequential dechlorination of PCE to ethene by a single *Dehalococcoides* species has only been demonstrated for the species *Dehalococcoides ethenogenes* (Maymo-Gatell et al., 1999).

However, Flynn et al. (2000) demonstrated complete dechlorination of PCE to ethene with a mixed culture that did not contain the *Dehalococcoides* species. Rather, the mixed culture was capable of complete dechlorination by a combination of non-*Dehalococcoides* bacteria that used different portions of the reduction sequence. This suggests that mixtures of differing dechlorinating strains can achieve complete dechlorination without reliance on any one specific strain of bacteria. Because of the high diversity in natural microbial populations, caution is advised when citing the necessity of *Dehalococcoides* to achieve complete dechlorination of CAHs.

2.2.1 The Role and Occurrence of *Dehalococcoides* Microorganisms

Hendrickson et al. (2002a) performed a field study to evaluate how widely distributed *Dehalococcoides* strains were in the environment and to determine their association with dechlorination at chloroethene-contaminated sites. In the field study, at least one *Dehalococcoides* population was identified at 21 sites where complete dechlorination to ethene has been observed (Hendrickson et al., 2002a). Their findings suggested that, while *Dehalococcoides* organisms are widely distributed, they are not ubiquitous in the environment.

Hendrickson et al. (2002a) further showed that *Dehalococcoides* comprises metabolically and phylogenetically distinct subgroups. It is becoming evident that different strains of *Dehalococcoides* species can only degrade certain CAHs. For example, *Dehalococcoides* strain 195 (grouped with the Cornell subgroup) directly dechlorinates *cis*-DCE to VC, but can only co-metabolize VC to ethene, a relatively slower process (Maymo-Gatell et al., 1995 and 2001). However, this strain can also utilize 1,1-DCE, 1,2-DCA, and 1,2-dibromomethane.

In nature, anaerobic reductive dechlorination is carried out by mixed cultures of organisms, which may collectively effect complete dechlorination of CAHs to innocuous end products.

The practitioner should be careful not to exclude enhanced anaerobic bioremediation simply because* Dehalococcoides *species have not been detected.

As another example, *Dehalococcoide*s strain CDBD1 (Pinellas subgroup) dechlorinates 1,2,3-trichlorobenzene (1,2,3-trichlorobenzene [TCB]), 1,2,4-TCB, 1,2,3,4-tetrachlorobenzene (1,2,3,4-tetrachlorobenzene [TeCB]), and 1,2,3,5-TeCB to dichlorobenzenes; and 1,2,4,5-TeCB to 1,3,5-TCB; but cannot dechlorinate PCE or TCE to *cis*-DCE, VC, or ethene (Adrian et al., 2000).

He et al. (2003a, 2003b) have recently isolated a *Dehalococcoides* strain (BAV1, Pinellas subgroup) from the Bachman Road site in Michigan that is capable of utilizing VC as a metabolic electron acceptor using acetate as the electron donor. Cupples et al. (2003) also describe a mixed culture containing *Dehalococcoides* strain VS (Victoria subgroup) that is also capable of metabolizing *cis*-DCE and VC using hydrogen as an electron donor.

To detect *Dehalococcoides*-related species, samples are analyzed for 16S rDNA sequences specific to the genus. While this analysis is selective for *Dehalococcoides*-related species, it currently cannot differentiate among the *Dehalococcoides* strains. Therefore, current 16S rDNA gene-based approaches are inadequate for determining *Dehalococcoides* strains with different dechlorination characteristics. In practice, the mere presence of *Dehalococcoides* strains is not sufficient to guarantee complete or efficient degradation of chlorinated ethenes to ethene. Improved molecular probes and genetic screening techniques are being developed to overcome the current limitations of the 16S rDNA method. Quantitative real-time polymerase chain reaction (PCR) analyses are being developed for commercial use, while other researchers are focusing on compound specific reductase gene probes for strain identification (see Section 4.5 for further discussion of molecular screening techniques).

At sites where appropriate indigenous *Dehalococcoides* populations are present, properly designed biostimulation approaches have the potential to achieve complete dechlorination of PCE and TCE to ethene. However, at some sites *Dehalococcoides* species may be difficult to detect, missing the appropriate strains, or exist at population densities that cannot be stimulated by substrate addition alone. Some studies have established that bioaugmentation was useful to achieve complete dechlorination when *Dehalococcoides* strains were not present (Ellis et al., 2000; Major et al., 2002).

As mentioned previously, caution is advised when citing the necessity of *Dehalococcoides* to achieve complete dechlorination of chlorinated ethenes. Many of these *Dehalococcoides*-

related strains have only recently (within the last couple years) been isolated, and future research will likely isolate many additional strains with differing potential for degrading CAHs. Other organisms besides *Dehalococcoides* may be capable of converting *cis*-DCE and VC to ethene, but have not been isolated to date.

Perhaps more important is that field applications of substrate addition stimulate mixed cultures with a multitude of bacterial species that can potentially use various, overlapping compounds in the sequence of parent to dechlorination products. In some cases, alternate degradation processes (e.g., anaerobic oxidation or abiotic reaction) may be capable of degrading *cis*-DCE and VC to innocuous end products (i.e., acetylene and carbon dioxide). The ability for the microbial community at a site to completely dechlorinate chlorinated ethenes is often not apparent until biostimulation is applied either through field tests or carefully constructed microcosms (Section 4.3). Analysis for *Dehalococcoide*s may have better application as a diagnostic tool when complete dechlorination of chloroethenes is not observed.

2.2.2 Microbial Ecology

Natural aquifer systems are complex, dynamic ecosystems populated by broad and diverse populations of microorganisms. The composition and activity of microbial communities in the subsurface shifts continuously in response to environmental changes, including aquifer chemistry and the availability of organic substrates and nutrients. Addition of an organic substrate causes profound changes in the microbial ecology of an aquifer system. These changes are intended to stimulate a predictable progression in the shift towards anaerobic microbial populations capable of anaerobic dechlorination of CAHs. This progression inherently assumes that a succession of microbial species will compete for available resources within the aquifer system under the prevailing geochemical conditions.

The ability to engineer and manipulate this progression is the cornerstone to successful application of enhanced anaerobic bioremediation. The practitioner of enhanced bioremediation should understand that ecological succession depends on geochemical and microbial characteristics of the aquifer system that are difficult to discern, and the ability to uniformly add an organic substrate for microbial growth and development. In general, an ecological succession will proceed from aerobic microorganisms through nitrate-reducers, manganese-reducers, iron-reducers, sulfate-reducers, and finally methanogens. Dechlorinating bacteria that utilize CAHs as electron acceptors will be most active in the range of sulfate reduction to methanogenesis. Each step in this succession will only proceed in the presence of sufficient organic substrate for growth and when the supply of each successive electron acceptor is depleted.

The shift towards anaerobic populations capable of anaerobic dechlorination and their growth to levels that effect extensive dechlorination of CAHs in groundwater is referred to as "lag phase" or "acclimation period," which may be on the order of several weeks to 1 or 2 years during application of enhanced bioremediation.

2.2.3 Occurrence and Site-Specific Variability

Heterogeneities in the distribution of substrate, native (inorganic) electron acceptors, and microbial population density and type will result in ecological succession that varies in both

time and space throughout the treated aquifer system. Due to the dynamic properties of natural systems, enhanced biodegradation activities may require modification if there is a failure to reach an ecological endpoint where rapid and complete anaerobic dechlorination of CAHs occurs.

The spatial and temporal variability in reducing conditions due to fuel releases is well documented in the literature (e.g., AFCEE, 1995; Wiedemeier et al., 1999), and similar spatial and temporal variations in reducing conditions are observed during enhanced bioremediation as well (Suthersan et al., 2002). Pfiffner et al. (2000) collected multiple soil samples from the same depth from a site at Dover Air Force Base (AFB), Delaware. By performing phospholipid fatty acid (PLFA) analyses, Pfiffner et al. (2000) found shifts in gram-positive and gram-negative communities, and that these shifts correlated to changes in grain size. Furthermore, microbial counts decreased with higher clay content in the sediments. They concluded that the spatial structure of subsurface microbial communities can be dependent on the spatial distribution of key physical and chemical properties of the soil matrix. Therefore, microbial heterogeneity can be important in evaluating site conditions and the response to biostimulation.

Addition of an organic substrate is intended to optimize geochemical conditions for anaerobic dechlorination. Inducing uniform geochemical conditions across the entire contaminant plume likely cannot be achieved in practice, as zones of differing redox conditions will occur both spatially and temporally due to varying concentrations of substrate and electron acceptors, and due to aquifer heterogeneity. To account for aquifer system heterogeneity, most systems are designed to achieve a quasi-equilibrium through repeated injection of substrate or the use of long-lasting substrates to maintain highly reducing conditions across the target treatment zone. Design of an enhanced anaerobic bioremediation system must consider the impacts of aquifer heterogeneity and account for these heterogeneities to the extent practicable.

2.2.4 Environmental Requirements

In addition to carbon substrates and appropriate reducing conditions, microbial consortia may require additional nutrients and trace metals for population growth. For example, highly enriched cultures have been found to require the addition of vitamin B_{12} and sludge supernatant to sustain dechlorination (Maymo-Gatell et al., 1995). Nutritional factors may also be provided by other members of a diverse microbial consortium (Morse et al., 1998). Thus, stimulating a diverse microbial population is likely to be more advantageous than attempting to selectively stimulate individual species. Under natural conditions, the aquifer may contain suitable amounts of trace nutrients for microbial growth; however, the nutritional demand imposed by rapid microbial growth in response to addition of a carbon substrate may exceed the capacity of the aquifer system (Chamberlain, 2003). Therefore, substrate amendments may be used to provide sufficient nutrients for microbial growth. Substrate nutritional amendments that have been used in a limited number of applications include nitrogen and phosphorous, yeast extracts, and vitamin B_{12}.

While microbial populations can endure a wide range of pH, a pH close to neutral (6 to 8) is the most conducive to the growth and proliferation of healthy and diverse microbial populations necessary for anaerobic dechlorination. Many practitioners believe that anaerobic dechlorination is pH limited, based on the knowledge that many laboratory cultures

are healthier (grow more rapidly) under neutral pH conditions. Fermentation of complex substrates to metabolic acids and HCl during dechlorination may decrease the pH significantly in low-alkalinity systems. Low groundwater pH (<5) may encourage unfavorable fermentation reactions. Sites with pH outside the range of 5 to 9 may require more thorough biological screening (e.g., microcosm studies).

2.2.5 Reasons for Apparent/Actual DCE Stall or Slowdown

cis-DCE or VC stall is an informal term typically used to describe chlorinated ethene sites that exhibit sequential anaerobic dechlorination of PCE and TCE to *cis*-DCE or VC, but where the degradation of *cis*-DCE or VC stalls out (i.e., the *cis*-DCE or VC plumes do not appear to be converting to VC or ethene). This stall condition, which is observed at some, but not all, PCE/TCE sites, has been ascribed to a variety of factors, including the following:

- Lack of the necessary microbiological communities required to degrade *cis*-DCE to VC.

- Conditions sufficiently anaerobic to support the conversion of TCE to *cis*-DCE, but not sufficiently anaerobic (i.e., sulfate-reducing to methanogenic) to support the conversion of *cis*-DCE to VC via anaerobic dechlorination. This may simply be due to a lack of sufficient electron donor. Some practitioners have intentionally limited substrate addition in an effort to control hydrogen concentrations to achieve higher substrate utilization rates; this may in fact result in portions of the site stalling at *cis*-DCE or VC.

- A temporary shift in the ratio of parent CAHs to dechlorination products due to kinetic disparity, where parent compounds degrade at a faster rate than dechlorination products and concentrations of dechlorination products increase (apparent stall). As parent CAHs are depleted over time, degradation of dechlorination products may be sufficient to reduce concentrations and the reverse the apparent stall.

- Elevated levels of bioavailable iron in the soil matrix that inhibits degradation of *cis*-DCE (Evans and Koenigsberg, 2001; Koenigsberg et al., 2002).

While the cause of *cis*-DCE stall is still being evaluated by a number of researchers, the main implication is that at some (but not all) chlorinated ethene sites, *cis*-DCE plumes are expanding and are not being controlled. In other cases, site closure cannot be obtained due to the persistence of DCE in the treatment zone.

Microcosms may be a useful tool to diagnose whether *cis*-DCE stall is due to microbial insufficiency, and whether bioaugmentation can potentially be used to expedite complete dechlorination (Section 4.3). Substrate loading and geochemical conditions can be carefully controlled in microcosms. In microcosms constructed of native soil and groundwater where complete dechlorination was not observed, rapid and complete dechlorination stimulated by addition of a bioaugmentation culture may indicate that the cause of *cis*-DCE stall is due to microbial insufficiency rather than geochemical causes. Such a finding in a microcosm does not always indicate that a stall will persist in the field, since results can be influenced by a variety of systematic sampling problems, such as variability in distribution. For example, if DCE dechlorinators are not homogenously distributed in the environment, the soils collected

for the microcosms may or may not contain them. Microcosm results must be carefully evaluated and validated using field data.

2.2.6 Bioaugmentation

Bioaugmentation involves the injection of a microbial amendment comprised of non-native organisms known to carry dechlorination of the targeted chlorinated compounds to completion (GeoSyntec Consultants, 2004). Bioaugmentation may be used at a site when the presence of an appropriate population of microbial dechlorinators is not present or sufficiently active to stimulate complete dechlorination. To date, experience with bioaugmentation is limited, and there is some disagreement among practitioners as to its benefits.

In some cases, bioaugmentation with microorganisms known to degrade the contaminants present may be necessary if the natural microbial population is incapable of performing the required transformations.

Bioaugmentation has been demonstrated in a limited number of carefully controlled field studies, but there are a number of site-specific conditions that may limit or make the application of bioaugmentation difficult.

A common and reasonable practice is to do a cost/benefit analysis before proceeding with bioaugmentation. It is clear that bioaugmentation is not necessary at many sites, but it also appears that it has been beneficial at some sites. The practitioner should consider the cost of bioaugmentation and weigh that against the risks of proceeding without bioaugmentation. It is possible that the cost of bioaugmentation will be less than the cost of conducting testing to evaluate its necessity. The question of time is also important. If achieving complete dechlorination over a longer period, on the order of a year or more, is acceptable, then it may make sense to start the process without bioaugmentation. If there is more urgency and cost is less of a concern, then it could be reasonable to bioaugment from startup.

For chloroethenes, the presence of *Dehalococcoides*-related microorganisms has been linked to complete dechlorination of PCE to ethene in the field (Major et al., 2001; Hendrickson et al., 2002a). Commercial bioaugmentation products are now available based on these microorganisms. Dybas et al. (2002) describe the use of bioaugmentation using *Pseudomonas stutzeri* strain KC to degrade CT in a full-scale biocurtain at the Schoolcraft site in Michigan. In this case, the dechlorinating microorganism was isolated from another site where CT transformation was observed and grown onsite to quantities sufficient for field application.

Difficulties or limitations in applying bioaugmentation may be attributed to biotic and abiotic stresses, including limitations of nutrients and growth factors in an uncontrolled environment, suppression by competing native microbial populations, metabolism of other non-targeted compounds, inability to distribute the culture uniformly throughout the treatment zone, and inhibitory geochemical conditions such as pH, redox, temperature, and salinity (Suthersan, 2001). Nonetheless, bioaugmentation has been used with some success (Henssen et al., 2001; Major et al., 2001; Appendix E.9 and E.13).

Deciding if and when to implement bioaugmentation is discussed in more detail in Section 4.6. The increased cost of using bioaugmentation as compared to implementation of biostimulation alone should be carefully considered.

2.3 EFFECTS OF SUBSTRATE ADDITION ON DNAPL AND SORBED CONTAMINANT MASS

It appears that application of enhanced bioremediation in a source area may result in some enhanced dissolution from DNAPL or enhanced desorption of CAH mass sorbed to the soil matrix (Sorenson, 2003b; Carr et al., 2000; Cope and Hughes, 2001). This may increase the effectiveness of enhanced bioremediation to treat DNAPL sources by enhancing the mass transfer of CAH mass to the aqueous phase, where it is subject to biodegradation processes. The physical and chemical properties of CAHs affect many of these processes, and a summary of CAH properties are listed on Table 2.5. The extent to which this phenomenon occurs or can be engineered is limited and may not be significant at many sites.

Enhanced bioremediation in source areas may mobilize contaminant mass by displacement or potentially by enhanced dissolution or desorption. The effects of enhanced dissolution and desorption at the field scale are not well documented, but may be beneficial by transferring contaminant mass to the dissolved phase, in which it is subject to biodegradation processes.

During system design or pilot testing for source area applications, the practitioner should evaluate the potential for mobilization of contaminant mass and include contingencies, as appropriate.

Enhanced dissolution or desorption may occur from several processes, including increasing concentration gradients, creating more soluble dechlorination compounds, and possibly affecting interfacial tension. Degradation of aqueous phase CAHs increases the concentration gradient between groundwater and DNAPL, which may increase the rate of dissolution from the DNAPL (Sorenson, 2003b). Carr et al. (2000) conducted abiotic and biotic laboratory studies in continuous-flow stirred-tank reactors with a model DNAPL containing PCE and tridecane. Comparison of the biotic and abiotic reactors indicated a 14-fold increase in biotic PCE removal rates from the DNAPL due to dechlorination of the PCE and enhanced dissolution relative to the abiotic reactor results. Cope and Hughes (2001) conducted similar studies in upflow columns containing glass beads, and found that dechlorination in the biotic columns resulted in an increase in PCE removal by up to a factor of 16 relative to the effects of dissolution alone in the abiotic columns. Furthermore, they found that removal of total chlorinated ethenes in the biotic columns was enhanced by a factor of 5.0 to 6.5 over mass removal in the abiotic columns that resulted from dissolution alone.

Less chlorinated compounds are more soluble and less hydrophobic. For example, in the dechlorination sequence of PCE to TCE to DCE to VC, solubility goes from 150 mg/L for PCE to 1,100 mg/L for VC (Table 2.5). The organic carbon partition coefficients (K_{oc}), which defines the distribution of CAH mass between the sorbed and aqueous phases, also decreases as the level of chlorination decreases. As anaerobic dechlorination proceeds, each successive dechlorination product is more soluble and less susceptible to adsorption than the previous compounds in the sequence. This tendency may result in an increase in aqueous-phase concentrations of less-chlorinated dechlorination products (Payne et al., 2001; Sorenson, 2003b). However, the significance of this intuitive observation has not been quantified.

Table 2.5 Characteristics of Chlorinated Aliphatic Hydrocarbons and Dechlorination Products

Compound	Molecular Formula	Molecular Weight (g/mol)[a/]	Density (g/mL @ approx. 20 to 25 °C)[b/]	Henry's Law Constant (atm-m^3/mol)[e/]	Solubility (mg/L @ approx. 20 to 25 °C)[c/]	Vapor Pressure (mm Hg @ 20 °C)[d/]	Octanol/Water Partition Coefficient (log Kow)[f/]	Octanol/Carbon Partition Coefficient (log Koc)[g/]
Chloroethenes								
Tetrachloroethene (PCE)	C_2Cl_4	165.8 (1)	1.62 (1)	0.0132 (2)	150 (3)	14.0 (3)	2.53 (4)	2.42 (5)
Trichloroethene (TCE)	C_2HCl_3	131.4 (1)	1.46 (1)	0.0072 (2)	1,100 (3)	60.0 (3)	2.42 (4)	2.03 (5)
cis-1,2- Dichloroethene (*cis*-DCE)	$C_2H_2Cl_2$	96.94 (1)	1.28 (1)	0.0030 (2)	3,500 (3)	200 (6)	0.70	1.65 (7)
trans-1,2- Dichloroethene (*trans*-DCE)	$C_2H_2Cl_2$	96.94 (1)	1.26 (1)	0.0073 (2)	6,300 (4)	340 (6)	2.06 (7)	1.77 (5)
1,1-Dichloroethene (1,1-DCE)	$C_2H_2Cl_2$	96.94 (1)	1.22 (1)	0.021 (2)	2,250 (5)	500 (3)	2.13 (4)	1.81 (5)
Vinyl Chloride (VC)	C_2H_3Cl	62.51 (1)	Gas	0.218 (2)	1,100 (3)	2,660 (3)	0.60 (4)	1.23 (5)
Ethene	C_2H_4	28.05 (1)	Gas	8.60 (7)	131 (7)	30,800 (7)	1.13 (8)	2.48 (7)
Chloroethanes								
1,1,1-Trichloroethane (1,1,1-TCA)	$C_2H_3Cl_3$	133.4 (1)	1.34 (1)	0.0133 (2)	4,400 (3)	100 (3)	2.47 (4)	2.02 (5)
1,1,2-Trichloroethane (1,1,2-TCA)	$C_2H_3Cl_3$	133.4 (1)	1.44 (1)	0.0012 (7)	4,500 (3)	19 (3)	2.18 (4)	1.75 (5)
1,1-Dichloroethane (1,1-DCA)	$C_2H_4Cl_2$	98.96 (1)	1.18 (1)	0.0043 (2)	5,500 (3)	180 (3)	1.78 (4)	1.48 (5)
1,2-Dichloroethane (1,2-DCA)	$C_2H_4Cl_2$	98.96 (1)	1.24 (1)	0.00098 (6)	8,690 (3)	61 (3)	1.48 (4)	1.28 (5)
Chloroethane (CA)	C_2H_5Cl	64.51 (1)	Gas	0.0094 (2)	5,740 (3)	1,010 (3)	1.43 (4)	1.42 (7)
Ethane	C_2H_6	30.07 (1)	Gas	19.2 (7)	60.4 (3)	29,300 (3)	1.81 (8)	2.66 (7)

(continued)

Table 2.5 Characteristics of Chlorinated Aliphatic Hydrocarbons and Dechlorination End Products (continued)

Compound	Molecular Formula	Molecular Weight (g/mol)[a/]	Density (g/mL @ approx. 20 to 25 °C)[b/]	Henry's Law Constant (atm-m^3/mol)[e/]	Solubility (mg/L @ approx. 20 to 25 °C)[c/]	Vapor Pressure (mm Hg @ 20 °C)[d/]	Octanol/Water Partition Coefficient (log Kow)[f/]	Octanol/Carbon Partition Coefficient (log Koc)[g/]
Chloromethanes								
Tetrachloromethane/ Carbon Tetrachloride (CT)	CCl_4	153.8 (1)	1.58 (1)	0.0232 (4)	786 (4)	90 (3)	2.73 (4)	2.62 (4)
Trichloromethane/ Chloroform (CF)	$CHCl_3$	119.4 (1)	1.48 (1)	0.00367 (2)	8,000 (3)	160 (3)	3.98 (4)	1.45 (9)
Dichloromethane (DCM)/ Methylene Chloride (MC)	CH_2Cl_2	84.93 (1)	1.33 (1)	0.00244 (4)	19,400 (4)	380 (4)	1.25 (4)	1.44 (4)
Chloromethane (CM)/ Methyl Chloride	CH_3Cl_1	50.48 (4)	Gas	0.00882 (2)	6,500 (4)	4,310 (4)	0.91 (4)	1.40 (4)
Methane	CH_4	16.04 (1)	Gas	18.3 (7)	24 (3)	20,800 (7)	1.09 (8)	2.88 (7)

[a/] g/mol = grams per mole.

[b/] g/ml = grams per milliliter; °C = degrees Celsius.

[c/] mg/L = milligrams per liter.

[d/] mm Hg = vapor pressure measured as millimeters of mercury.

[e/] atm-m^3/mol = atmospheres-cubic meter per mole.

[f/] log Kow = log of octanol/water partition coefficient (dissolution coefficient).

[g/] log Koc = log of octanol/carbon coefficient (soil sorption coefficient).

References:

(1) Weast, R.C., M.J. Astle, and W.H. Beyer (eds.). 1989. *CRC Handbook of Chemistry and Physics*. 75th ed. Boca Raton, FL: CRC Press. 75th ed.

(2) Gossett, J.M. 1987. Measurement of Henry's Law Constants for C1 and C2 Chlorinated Hydrocarbons. *Environmental Science & Technology*, Vol. 21(2):202-208.

(3) Verschueren, K. 1983. *Handbook of Environmental Data on Organic Chemicals*. 2nd ed. New York: Van Nostrand Reinhold.

(4) Montgomery, J.H. 1996. *Groundwater Chemicals Desk Reference*. 2nd ed. Chelsea, MI: Lewis.

(5) Montgomery, J.H., and L.M. Welkom. 1990. *Groundwater Chemicals Desk Reference*. Chelsea, MI: Lewis.

(6) Howard, P.H., G.W. Sage, W.F. Jarvis, and D.A. Gray. 1990. *Handbook of Environmental Fate and Exposure Data for Organic Chemicals, Vol. II – Solvents*. Chelsea, MI: Lewis.

(7) Estimated using Lyman, W.J., W.F. Reehl, and D.H. Rosenblatt. 1990. *Handbook of Chemical Property Estimation Methods*. Washington, DC: American Chemical Society.

(8) Hansch, C, A. Leo, and D. Hoekman. 1995. *Exploring QSAR – Hydrophobic, Electronic, and Steric Constants*. Washington, DC: American Chemical Society.

(9) Grathwohl, P. 1990. Influence of Organic Matter from Soils and Sediments from Various Origins on the Sorption of Some Chlorinated Aliphatic Hydrocarbons. *Environmental Science & Technology,* Vol. 24:1687-1693.

Organic substrates added to enhance biodegradation or fermentation products such as organic acids or alcohols may lower the interfacial tension between DNAPL and groundwater. The higher the interfacial tension between two liquids (i.e., water and DNAPL), the less likely one is to dissolve into the other, and the more difficult it is for one to migrate through the other in the subsurface (Sorenson, 2003b). A lowering of interfacial tension may increase the mobility of the DNAPL and increase the potential for dissolution into groundwater. Interfacial tension changes are, in large part, a function of the specific substrate added to stimulate biodegradation. For example, Sorenson (2003b) found that sodium lactate dissolved in water lowered the interfacial tension of a TCE DNAPL in water by 26 to 47 percent, depending on the concentration of the sodium lactate. Pfeiffer (2003) similarly found that soybean oil lowered the interfacial tension of TCE DNAPL in water on the order of 13 to 39 percent.

Other processes such as organic carbon flooding and production of biosurfactants have been postulated that may potentially increase the mass transfer of contaminant mass from the sorbed phase to the dissolved phase (Payne et al., 2001). However, it has not been demonstrated whether these effects are significant at the field scale.

Payne et al. (2001) report increases in total dissolved concentrations of chlorinated ethenes ranging from 6 to over 20 times initial concentrations for two sites in carbonate aquifers where molasses was injected. Sorenson (2003b) reports that the effects of enhanced mass transfer resulted in a 23 fold increase in TCE concentrations due to injection of sodium lactate at the Test Area North Site in Idaho. ***However, the effectiveness of enhanced mass transfer due to anaerobic bioremediation in the field is still not well understood, and may be less pronounced for other sites.***

Despite the technical basis for this phenomenon and its reported occurrence at a limited number of sites, its real significance and the potential to engineer it are not fully understood and may be limited. Research continues on the ability of enhanced anaerobic bioremediation to facilitate mass transfer of remediation of DNAPL source areas (McMaster et al., 2004; Morrill et al., 2004).

This same phenomenon has the potential to cause a mobilization of the source mass and dechlorination products. The RPM needs to be aware that increases in dissolved CAH concentrations and contaminant mobility are possible, especially early on in the process. The extent to which this happens is not fully known or predictable, but many practitioners believe this is an important phenomenon. Often the effect is temporary, but RPMs and their contractors should be prepared to account for its occurrence. During pilot testing or system design, the possibility of physical displacement of groundwater, enhanced dissolution, and/or desorption should be carefully evaluated and incorporated into contingency plans.

SECTION 3

PRELIMINARY SCREENING

Enhanced anaerobic bioremediation has been applied at sites having a variety of hydrogeologic and biogeochemical conditions, and can be a cost-effective remedy in many environmental settings. However, there are conditions that may limit the success of adding an organic substrate to stimulate anaerobic reductive dechlorination, and for which alternative technologies may be better suited. Therefore, preliminary screening of a site is required prior to selecting enhanced bioremediation as a suitable remedy (Figure 3.1). This section describes conditions suitable for application of enhanced anaerobic bioremediation and those conditions that should trigger consideration of alternative technologies.

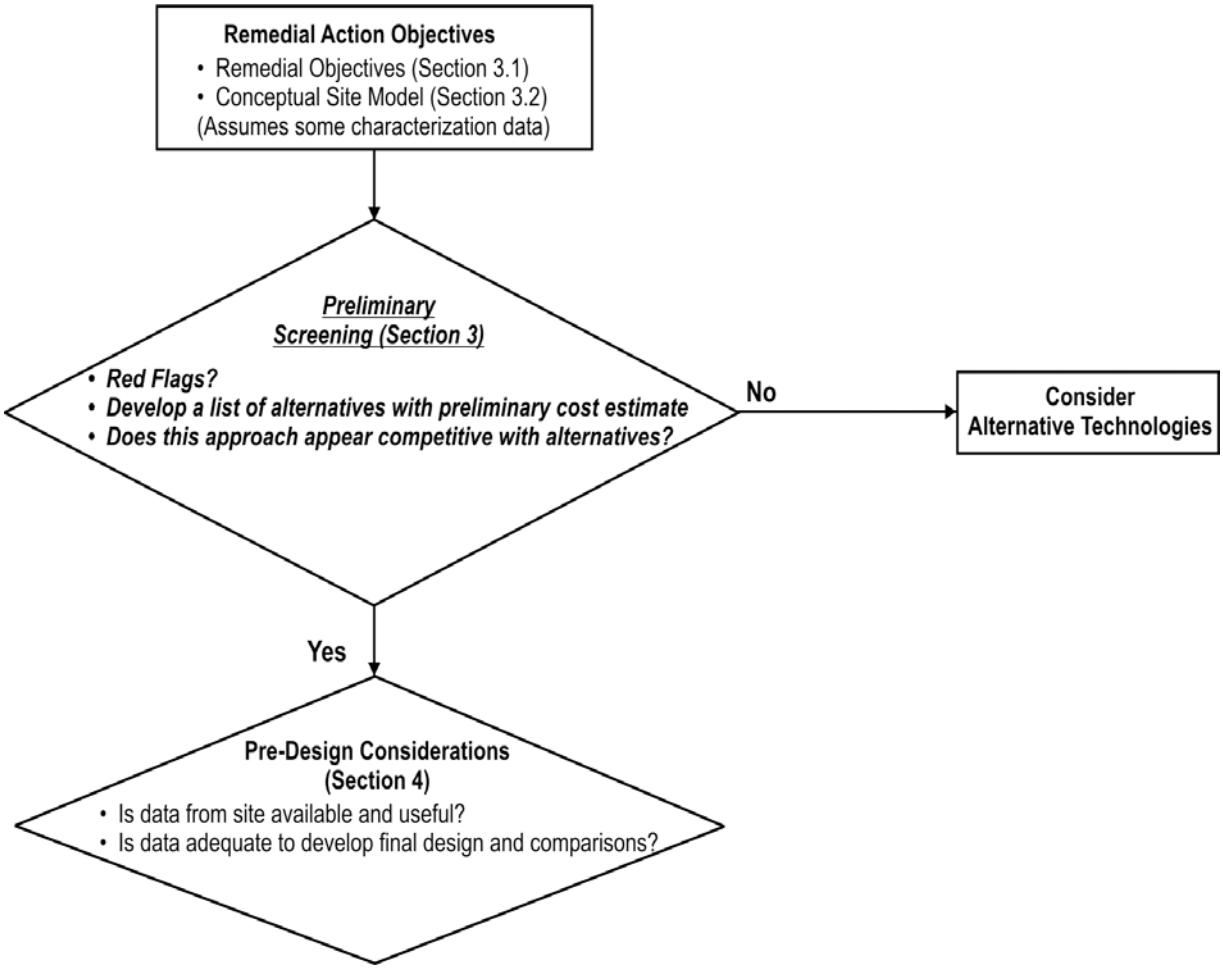


Figure 3.1 Preliminary Screening on the Enhanced Bioremediation Roadmap

Site screening criteria are primarily technical in nature and include contaminant type and distribution, site hydrogeology, geochemistry, and microbiology. Other site screening criteria relate to the ability to achieve remedial objectives, to regulatory concerns associated with changes in secondary water quality brought about by substrate addition, and to issues related to site infrastructure, utilities, and land use. The following sections describe typical remedial objectives and regulatory considerations that drive selection of the enhanced bioremediation alternative, development of a CSM that can be used to determine the suitability of a site for application of the technology, and other technical and pragmatic considerations for preliminary site screening.

While enhanced anaerobic bioremediation can be applied to a variety of site conditions, not all sites are suitable for the technology.

Preliminary screening is the first step to determine whether enhanced anaerobic bioremediation is an effective remedial strategy for your site.

3.1 REMEDIAL OBJECTIVES AND REGULATORY CONSIDERATIONS FOR ENHANCED ANAEROBIC BIOREMEDIATION

Prior to initiating an enhanced bioremediation study, remedial objectives should be established and potential regulatory considerations reviewed.

3.1.1 Remedial Objectives

Remedial objectives and performance metrics are driven by regulatory compliance requirements. To design a successful enhanced anaerobic bioremediation application, the regulatory framework should be reviewed and compliance standards and remedial endpoints clearly identified. The ability of enhanced anaerobic bioremediation to achieve drinking water MCLs in some settings has been demonstrated, but cannot be assumed to be possible at all sites. The use of less stringent, risk-based remedial goals may be more appropriate and achievable than default drinking water standards.

Enhanced bioremediation is necessarily limited in its ability to treat DNAPL source zone areas due to many of the same factors (e.g. mass transfer limitations or heterogeneity) that affect conventional technologies. Aggressive and geochemically compatible source zone treatment may be considered prior to applying enhanced anaerobic bioremediation (Stroo et al., 2003). Enhanced bioremediation may also be impractical for very large groundwater plumes on the order of tens of acres due to the shear volume of groundwater to be treated.

Typical remedial action objectives that engineered anaerobic bioremediation may be used to address include the following:

- Destruction of contaminant mass in source zones where effective substrate/contaminant contact is possible.
- Reduction of CAH concentrations in a dissolved plume to below regulatory criteria.
- Reduction of mass flux from a source zone or across some containment boundary.

- Enhancement of already occurring natural attenuation to reduce monitoring timeframes.

- Cost-effective and continuous treatment over relatively long remediation timeframes due to inabilities to substantially remediate the contaminant source(s).

Performance objectives based on dissolved contaminant concentrations alone should be used with caution. A significant amount (usually the majority) of contaminant mass in an aquifer system may be present as DNAPL or sorbed to the aquifer matrix. Due to the effects of dissolution and desorption of this contaminant mass, aqueous-phase concentrations alone may not accurately reflect the amount of mass being destroyed if there is continued mass transfer from DNAPL or sorbed mass to the aqueous phase.

3.1.2 Regulatory Considerations

Regulations that are potentially applicable to the use of enhanced *in situ* anaerobic bioremediation are similar to those for other *in situ* remediation technologies, but the injection of organic substrates and the resulting changes in groundwater conditions present unique challenges and concerns. Special regulatory considerations include the following:

- Substrates introduced into the subsurface should not include any known hazardous wastes. USEPA approval of acceptable materials for *in situ* bioremediation is discussed in a December 27, 2000, memorandum, "Applicability of RCRA Section 3020 to In-Situ Treatment of Ground Water" (USEPA, 2000b).

- Many states regulate the injection of materials into the subsurface, and may require an underground injection control permit as mandated by the Safe Drinking Water Act. Historic applications approved by other state or federal agencies should be referenced to facilitate acceptance of enhanced anaerobic bioremediation.

- The potential for production of toxic intermediate degradation byproducts, degradation of secondary drinking water quality, and production of noxious gases should be carefully assessed if potential exposure pathways exist.

When applying innovative technologies, the level of interaction with the regulatory community may need to be higher than with traditional remedial technologies. As the number of enhanced bioremediation applications grows, and the regulatory community becomes more familiar with the technology, it will be easier to gain their approval. Nonetheless, technical issues will remain to be addressed on a site-by-site basis.

A review of state policies on enhanced anaerobic bioremediation was conducted by the ITRC (1998). A typical regulatory concern is generation of VC in the reaction zone, which is an unavoidable result of sequential dechlorination of chloroethenes. While of concern to the regulatory community, VC generation should be acceptable if adequate degradation of VC can be accomplished. This requires establishment of a sufficient anaerobic reactive zone to allow depletion of parent compounds and complete sequential dechlorination of VC. Alternatively, degradation of VC may be accomplished by aerobic degradation processes in a downgradient redox recovery zone.

Underground injection control permits include information regarding the chemical nature of the substrate solution and address potential concerns with water quality resulting from the injection process. Underground injection control permits for injection of food-grade or common commercial substrates are generally waived or implemented with minimal paperwork (for example, permitting by rule). Re-injection of contaminated groundwater amended with a substrate has also been approved by the USEPA (2000b) for RCRA sites. Use of this USEPA document and reference to historical applications is generally sufficient to gain approval for re-injection of contaminated groundwater for recirculation systems or to use native groundwater for substrate preparation (e.g., dilution water or water for emulsions).

The potential for adverse impacts to water quality due to application of enhanced anaerobic bioremediation presents a greater challenge; it needs to be identified and addressed during design and in consultation with applicable regulatory agencies. Impacts on secondary drinking water quality and generation of toxic dechlorination products (e.g., VC) are generally temporal and limited to the immediate treatment area. Nonetheless, the potential exists for migration of adversely impacted groundwater or soil vapor, and these issues are typically addressed through additional monitoring (see discussion in Section 3.3).

3.2 CONCEPTUAL SITE MODELS

Development of a CSM and an understanding of the natural processes that are being stimulated ultimately guides the site selection and system design process. Guidance on developing CSMs and evaluating MNA can be found in various publications including USEPA (1998a), National Academy of Sciences (2000), and ITRC (1999).

An assessment of degradation potential is primarily based on a review of site-specific data on electron donors, electron acceptors, metabolic byproducts, geochemical indicators, contaminant trends, and hydrogeology. Other, less common means of assessing degradation potential such as field tests, laboratory microcosm studies, and microbiological analyses are described in Section 4. A CSM also summarizes the fate and transport of contaminants, migration pathways, exposure mechanisms, and potential receptors (Figure 3.2). Analysis of contaminant concentration trends can be used to determine whether an ongoing source of CAHs exists at a site, and whether natural attenuation processes are sufficient to control contaminant plume migration. In many cases, MNA alone may be an adequate and acceptable strategy for managing risks. Even in such cases, the use of enhanced anaerobic bioremediation may be appropriate to reduce life-cycle monitoring costs.

For enhanced bioremediation, the CSM must include a description and an evaluation of site-specific geologic features that will affect the method(s) of substrate emplacement. Given that underground injection is a common method of substrate distribution, careful attention should be placed on the presence and location of preferential flow paths versus the location of the contaminant mass. Even with diligent design measures, injected fluids will follow the paths of least resistance. If contaminants are localized in these more permeable zones, then conventional injection approaches are likely to achieve an acceptable substrate distribution.

However, if the CSM includes downward migration of contaminant mass into low permeability lithologies underlain by higher permeability strata, then the injected substrate may preferentially flow into the more permeable, but less contaminated, soil strata. A heterogeneous lithology is not a reason to abandon *in situ* bioremediation; it is an important factor that often defines the success or failure of any remedial action.

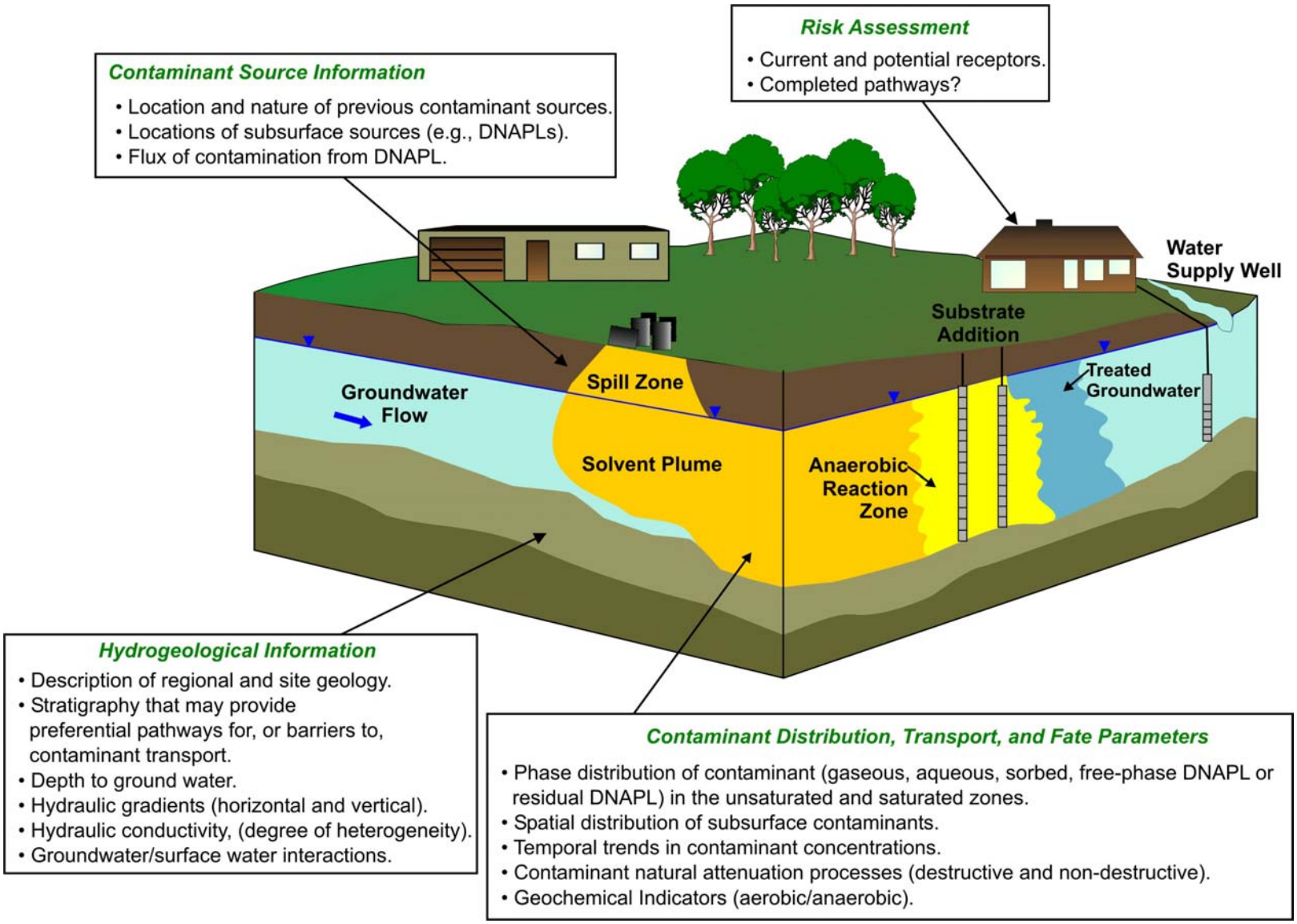


Figure 3.2 Elements of a Conceptual Site Model

With respect to emplacement of solid substrates via trenching, the presence of underground utilities, consolidated materials, rubble or cobbles, and the ability to reach the target depth (with or without benching) should be included in the CSM.

The following subsections describe a classification system for CAH plumes that is useful for evaluating the fate of CAHS at a site and the potential for stimulating anaerobic biodegradation processes.

3.2.1 Classification System for Chlorinated Solvent Plumes

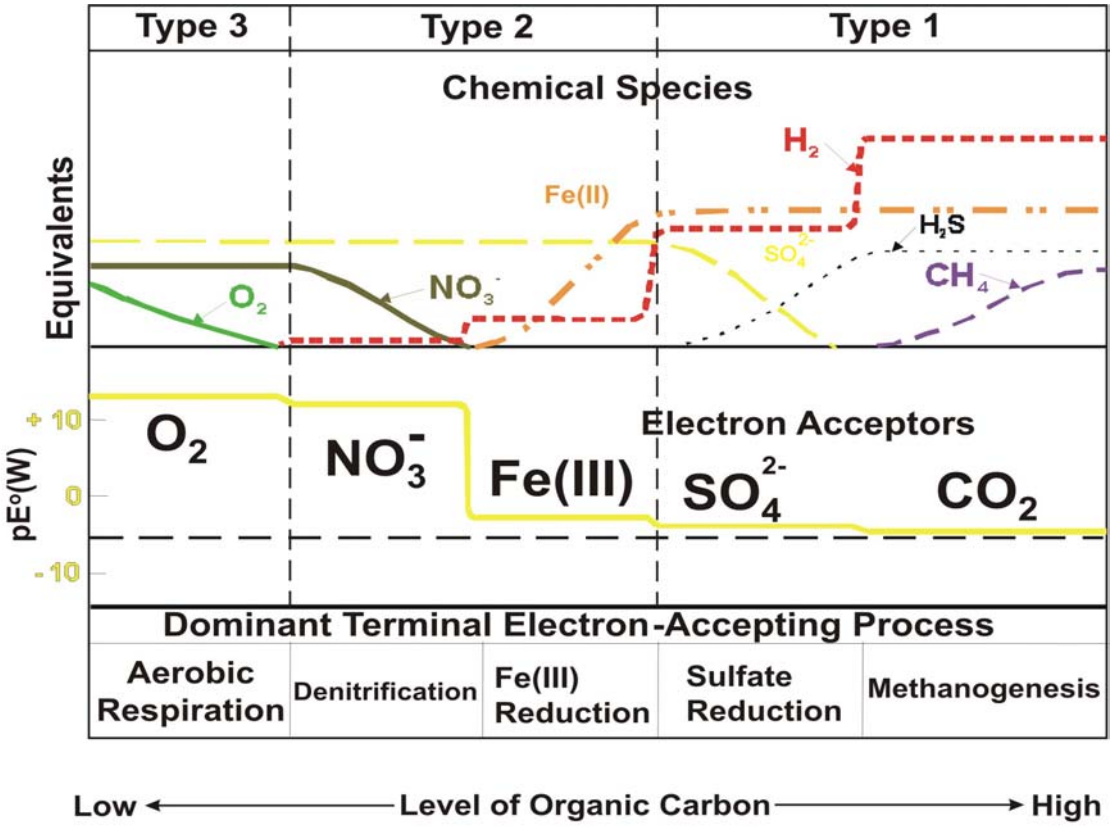
Chlorinated solvent plumes can exhibit different types of behavior, depending primarily on the amount of biologically available organic carbon (electron donor) in the aquifer and the distribution and type of electron acceptors being used by native microbial populations. Because the prevailing redox conditions influence the rate and extent of anaerobic dechlorination of CAHs, it is useful to classify chlorinated solvent plumes according to the prevailing redox conditions and resulting potential for dechlorination to occur.

Wiedemeier et al. (1996) proposed a classification system for chlorinated solvent plumes based on the amount and origin of fermentation substrates that produce the hydrogen that drives anaerobic dechlorination. The classification scheme presented in these sections follows Wiedemeier's original definition with only slight modification. Under the definition used here, the classification depends on relative amount of organic substrate available (regardless of origin) and the redox conditions that predominate within the aquifer system. The relative amount of organic substrate is emphasized because enhanced anaerobic bioremediation modifies this parameter to achieve redox conditions that are optimal for anaerobic dechlorination to occur. The three different types of plume behavior summarized below can be used to delineate zones of differing anaerobic biodegradation potential within a chlorinated solvent plume. Figure 3.3 illustrates the geochemical characteristics of the three types of environments.

3.2.1.1 Type 1 Environment: Groundwater Systems that are Highly Anaerobic due to High Levels of Organic Carbon

Type 1 environments occur in hydrogeologic settings that have relatively high organic carbon concentrations. Highly anaerobic conditions are typical at sites contaminated with fuel hydrocarbons, landfill leachate, or other anthropogenic carbon because these organics exert a tremendous electron-acceptor demand on the system. Anaerobic conditions also may result from the fermentation of naturally occurring organic material. However, with few exceptions (e.g., wetlands), most natural aquifers do not contain sufficient natural organic matter to generate the highly reducing conditions in which sulfate reduction and methanogenesis predominate.

The geochemistry of groundwater in a Type 1 environment is characterized by very low concentrations of DO (less than 0.5 mg/L), nitrate, and sulfate; and elevated concentrations of ferrous iron [Fe(II)] and methane. The presence of methane confirms that fermentation has been occurring at the site. If measured, hydrogen concentrations are typically greater than 1 to 2 nmol/L. Importantly, a Type 1 environment may result in the rapid and extensive dechlorination of the more highly chlorinated solvents such as PCE, TCE, CT, and TCA.



Modified from: Bouwer and McCarty, 1984

Figure 3.3 Geochemical Characteristics of Three Types of Chlorinated Solvent Plumes

3.2.1.2 Type 2 Environment: Systems that are Mildly Anaerobic due to Moderate Levels of Organic Carbon

Type 2 environments occur in hydrogeologic settings that have relatively moderate organic carbon concentrations. Prevailing redox conditions in a Type 2 environment are mildly anaerobic, with the primary redox reactions being nitrate, manganese, and iron reduction. Type 2 environments are differentiated from Type 1 environments in that the levels of organic carbon are not sufficient to induce widespread sulfate reduction and methanogenesis. Many aquifers are naturally Type 2.

This differentiation is important because a Type 2 environment generally results in slower dechlorination of the highly chlorinated CAHs and incomplete dechlorination of lesser-chlorinated CAHs (e.g., *cis*-DCE) compared to a Type 1 environment. Dechlorination products may tend to accumulate in a Type 2 environment. However, given sufficient organic loading by substrate addition, this environment may be modified to a Type 1 environment resulting in rapid and complete degradation of dechlorination products. If it appears in a Type 2 plume that there is insufficient carbon to completely degrade the CAH plume, or if biodegradation rates are not sufficient to meet remedial objectives, then application of enhanced anaerobic bioremediation may be ideal.

3.2.1.3 Type 3 Environment: Aerobic Systems with Low Levels of Organic Carbon

A Type 3 environment is characterized by a well-oxygenated groundwater system with little or no organic matter. Concentrations of DO typically are greater than 1.0 mg/L. In such an environment, anaerobic dechlorination will not occur, and highly chlorinated CAHs such as PCE, TCE, TCA, and CT will not degrade by biological processes. In this environment, very long dissolved-phase plumes are more likely to form. However, less-chlorinated CAHs such as VC (and possibly DCE) can be rapidly oxidized under these conditions. A Type 3 environment is often found in sediments having an inherently low organic carbon content and where no anthropogenic carbon has been released.

The Type 3 environment may be a challenge for enhanced anaerobic bioremediation, primarily due to a lack of an anaerobic microbial population. DO concentrations greater than 1.0 mg/L are generally toxic to anaerobic dechlorinating species, and it is logical to assume that these bacteria may only be present in small quantities in a dormant state. However, given the degree of microbial heterogeneity and presence of anaerobic "micro-environments" observed at many sites, there is a strong possibility that anaerobic conditions can be induced within a reasonable time at Type 3 sites.

3.2.1.4 Mixed Environments and Sequential Anaerobic/Aerobic Degradation

The scenario targeted by enhanced anaerobic bioremediation involves a reaction zone in which all chlorinated compounds are dechlorinated under strongly reducing (Type 1) conditions. The following sequence of reactions occurs under these conditions:

$$\text{PCE} \rightarrow \text{TCE} \rightarrow \text{DCE} \rightarrow \text{VC} \rightarrow \text{Ethene}$$

In practice, DCE and VC may accumulate if conditions are not sufficiently reducing (i.e., electron donor limited), there is not an appropriate microbial consortium present to degrade these compounds, or if they degrade more slowly than PCE and TCE (i.e., kinetic disparity).

However, a chlorinated solvent plume can exhibit all three types of behavior in different portions of the plume. For example, Wiedemeier et al. (1996) describe a plume at Plattsburgh AFB, New York, that exhibits Type 1 behavior in the source area and Type 3 behavior downgradient from the source. This fortuitous scenario involves dechlorination of PCE, TCE, and DCE, with accumulation of VC near the source or treatment area (Type 1 behavior) and oxidation of VC (Type 3 behavior) either aerobically or via iron reduction further downgradient. VC is oxidized to carbon dioxide in this type of plume and does not accumulate. The following sequence of reactions occurs in a plume that exhibits this type of mixed behavior.

$$\text{PCE} \rightarrow \text{TCE} \rightarrow \text{DCE} \rightarrow \text{VC} \rightarrow \text{Carbon Dioxide}$$

Note that ethene is not produced during this reaction, and that VC is typically removed from the system much faster than it is via anaerobic dechlorination.

Enhanced bioremediation systems may be designed to take advantage of mixed reaction zones. A strategy using sequential anaerobic/aerobic degradation may be employed where

more highly chlorinated compounds (e.g., PCE and TCE) are dechlorinated in an anaerobic reaction zone, and less chlorinated compounds (e.g., DCE and VC) are degraded by oxidation processes in a downgradient (natural or engineered) aerobic redox recovery zone.

3.3 SITE SCREENING TECHNICAL CONSIDERATIONS

There are a number of technical considerations that need to be evaluated in screening a site for application of enhanced anaerobic bioremediation. In general, these considerations fall into the following categories:

- Contaminant type and distribution,
- Microbiology,
- Hydrogeology, and
- Groundwater geochemistry.

Essentially, the purpose of substrate addition is to create a Type 1 environment. The type of environment and prevailing geochemistry present at a site should be taken into account when evaluating implementation of enhanced anaerobic bioremediation. For example, it is likely that a Type 3 environment will require the injection of a greater amount of substrate and require a longer lag time for acclimation and growth of anaerobic dechlorinating microbial populations. However, many site geochemical conditions that are not conducive to the growth and development of anaerobic microorganisms can be overcome by substrate addition.

In general, anaerobic or borderline aerobic/anaerobic sites that have insufficient organic carbon can be most easily and rapidly treated using enhanced anaerobic bioremediation. Typically, some dechlorination products (such as *cis*-DCE) are present at these types of sites, but the rate and extent of degradation is insufficient to drive the process to completion. Aerobic (Type 3) sites present a greater challenge in evaluating the potential for enhanced anaerobic bioremediation, but it has been clearly demonstrated that anaerobic dechlorination can be stimulated at these sites given sufficient amounts of substrate and time for succession, acclimation, and growth of dechlorinating bacteria.

Scoring systems used for natural attenuation studies (USEPA, 1998a) and enhanced bioremediation using the RABITT protocol (Morse et al., 1998) have been developed for evaluating the potential for anaerobic dechlorination. While it is useful to evaluate the parameters listed in these scoring systems, no single parameter can indicate the potential for successful application of enhanced bioremediation, and many undesirable conditions may be modified by addition of sufficient organic substrate. Table 3.1 summarizes some common criteria used to determine the suitability of a site for implementing enhanced anaerobic bioremediation. These are general guidelines only, and there may be notable exceptions to most all of the criteria. These criteria are discussed in further detail in the following subsections.

Table 3.1 Suitability of Site Characteristics for Enhanced Anaerobic Bioremediation

Site Characteristic	Suitable for Enhanced Bioremediation	Suitability Uncertain	Suitability Unclear - Possible Red Flag - Requires Further Evaluation
DNAPL Presence	Residual DNAPL or sorbed sources.	Poorly defined sources may require additional characterization.	May not be appropriate for aggressive treatment of pools of DNAPL.
Plume Size	Small, a few acres or less.	Medium to large, a few acres plus. May require concurrent technology.	Large plumes of many acres. May require concurrent technology.
On or Near Site Infrastructure	The risk of vapor intrusion from contaminants or biogenic gases is deemed acceptable.	Target treatment zone in close proximity to sensitive infrastructure.	Target treatment zone in an area where known vapor intrusion or high methane problems exist.
Evidence of Anaerobic Dechlorination	Slow or stalled dechlorination (see Table 3.2)	Limited evidence of anaerobic dechlorination.	No evidence of any degradation.
Depth	<50 feet to water	>100 feet to groundwater	Deep groundwater and deep contamination.
Hydraulic Conductivity	> 1 ft/day (>3 x 10^{-4} cm/sec)	0.01 to 1 ft/day (3 x 10^{-6} to 3 x 10^{-4} cm/sec)	<0.01 ft/day (<3 x 10^{-6} cm/sec)
Groundwater Velocity	30 ft/yr to 5 ft/day	10 ft/yr to 30 ft/yr, 5 ft/day to 10 ft/day	< 10 ft/yr, > 10 ft/day
pH	6.0 – 8.0	5.0 to 6.0, 8.0 to 9.0	< 5.0, > 9.0
Sulfate Concentration	< 500 ppm	500 to 5,000 ppm (with caution)	>5,000 ppm or presence of mineral gypsum may not be suitable

ft/day = feet per day; ft/yr = feet per year; cm/sec = centimeters per second; mg/L = milligrams per liter.

3.3.1 Contaminant Distribution

Enhanced anaerobic bioremediation takes advantage of natural processes that may already be contributing to the degradation of CAHs. The presence of degradation products that indicate that anaerobic dechlorination of CAHs is occurring, or has occurred, naturally is a favorable indicator. Conversely, the lack of any dechlorination products is a "red flag" that either enhanced bioremediation may not be a suitable approach or that further evaluation is required.

The release of CAHs is often associated with release of other potential electron donors such as fuels or landfill leachate. A review of historical records may indicate that anaerobic dechlorination occurred in the past, but that the system has stalled (e.g., at *cis*-DCE) once the initial electron donor supply was depleted. In this case, complete and rapid degradation can often be restored by substrate addition.

Enhanced anaerobic bioremediation has been successfully applied to a few sites with residual or sorbed DNAPL. Application to sites with large quantities of free-phase DNAPL has yet to be proven effective, and in these instances enhanced anaerobic bioremediation may be more suitable to reduce source mass or as a polishing step following application of more aggressive source removal technologies (Stroo et al., 2003). Highly elevated concentrations of solvents may act as toxic inhibitors to biodegradation, especially for sites where the release is relatively recent (e.g., within 1 to 3 years). However, dechlorinating bacteria (at least for chloroethenes) are known to be tolerant of concentrations nearing solubility limits (Yang and McCarty, 2000b).

Successful site closures to date (involving enhanced anaerobic bioremediation) typically have involved relatively small- to moderate-size plumes associated with small commercial operations such as dry cleaners (e.g., Appendix E.2). Within the DoD, it is not unusual to have large plumes (several thousands of feet in length) associated with multiple sources and long periods of industrial operation. An area-wide treatment using enhanced anaerobic bioremediation may simply not be economical where treatment areas exceed tens to hundreds of acres. In addition, the relationship of the plume and treatment area to site infrastructure may require special consideration of potential vapor intrusion risks.

3.3.2 Microbiology

Enhanced anaerobic bioremediation of CAHs is targeted at stimulating microbially mediated anaerobic reductive dechlorination. The success of the technology largely depends on the presence of appropriate dechlorinating bacteria and the ability to stimulate sufficient growth and activity to degrade contaminants to the extent (and at a rate) that meets the intended remedial objectives. Incomplete dechlorination (e.g., *cis*-DCE or VC stall) due to insufficiently reducing conditions or lack of appropriate dechlorinating populations are common microbial issues when applying enhanced anaerobic bioremediation. ***Determining the potential for complete anaerobic dechlorination using substrate addition is perhaps the most difficult question to answer in the site screening process***. Table 3.2 lists considerations and red flags for screening sites with chlorinated ethenes. A similar approach could be used for chloroethanes and chloromethanes.

Initially, a site can fall into one of three microbiological categories:

1. Sites where appropriate dechlorinating microorganisms are present, geochemical conditions are appropriate for their growth, and sequential dechlorination products (e.g., VC and ethene) are observed.

2. Sites where appropriate dechlorinating microorganisms are present, but at insufficient quantity or level of activity for complete sequential dechlorination to innocuous end products.

3. Sites where appropriate dechlorinating microorganisms are completely absent (rare).

In the first case listed above, biostimulation alone can be applied with a high degree of confidence. In the second case listed above, biostimulation alone may or may not be successful. It may be difficult to distinguish the second case from the third case, because detection and identification of appropriate microbial species in these systems is problematic.

Table 3.2 Considerations and Red Flags for Preliminary Screening of Sites with PCE and TCE

	Site Classification		
Conditions	**Type 1**	**Type 2**	**Type 3**
No *cis*-DCE or other dechlorination products	***Red Flag***. Lack of any dechlorination products suggests the aquifer is sterile. Enhanced bioremediation not recommended.	***Possible Red Flag***. Lack of any dechlorination products may be due to substrate limitations. Additional site evaluation (e.g., pilot test or microcosm test) recommended (Section 4).	***Red Flag***. Potential for complete anaerobic dechlorination cannot be determined. Additional site evaluation (e.g., pilot test or microcosm test) recommended (Section 4).
cis-DCE present, but not VC or ethene	Marginally suitable for enhanced bioremediation. Lack of VC or ethene under Type 1 conditions requires further evaluation (Section 4).	Suitable for enhanced bioremediation. Evaluate potential for complete anaerobic dechlorination (Section 4) and proceed with caution.	Presence of *cis*-DCE under Type 3 conditions may be a result of limited dechlorination at the source or in more anaerobic microenvironments. Requires further evaluation (Section 4).
VC and ethene present	Suitable for enhanced bioremediation. Consider MNA alternative first.	Suitable for enhanced bioremediation. Consider MNA alternative and whether system may become carbon limited in the absence of substrate addition.	VC and ethene should not be present under Type 3 conditions, although this may sometimes occur as the result of locally reducing conditions created by the NAPL mix. For example, if the material released contained biodegradable oils, it is possible that some anaerobic dechlorination will take place, even in an aerobic aquifer.

Without evidence of even limited degradation (i.e., no degradation past *cis*-DCE), confidence in the potential to stimulate complete dechlorination by biostimulation alone is unknown, even though appropriate geochemical conditions may be readily achieved with substrate addition. Because anaerobic dechlorination has been stimulated at Type 2 and Type 3 sites, it may be appropriate to simply observe whether biogeochemical conditions for stimulating anaerobic dechlorination can be induced at these sites via field tests. However, sites exhibiting marginal biogeochemical conditions may benefit from further site evaluation using microcosm or small pilot tests combined with the use of microbial screening techniques (Section 4).

3.3.3 Hydrogeology

The uncertainty in characterizing subsurface hydrogeology complicates all *in situ* treatment technologies, and must be considered during the site selection and design process. Inadequate characterization of the site hydrogeology can lead to remedial system failure. However, in many cases, the system can be designed to mitigate difficult hydrogeologic conditions. Difficult hydrogeologic conditions that may preclude cost-effective delivery of amendments include excessive groundwater flow velocity, low permeability, high levels of aquifer heterogeneity, or excessive depth to groundwater (i.e., high drilling costs). RPMs

should note that many of the conditions that are problematic for enhanced *in situ* bioremediation are also problematic for competing technologies, and any decision to use a given remedial technology should be made considering the potential costs and risks of other options.

Depth to Groundwater. Depth to water and the vertical thickness of the plume primarily impact the capital cost of drilling and delivering the substrate to the intended treatment zone. The capital expense of installing multiple injection wells in deep settings (e.g., greater than 100 feet bgs), or across thick formations needs to be compared to the costs associated with competing technologies. There are practical limits (perhaps 15 to 20 feet) to the maximum length of well screen across which a substrate can be uniformly injected; therefore, large saturated thicknesses may require multiple vertical injection points.

Hydraulic Conductivity. Hydraulic conductivity is a primary factor in effective distribution of substrate in the subsurface. In general, hydraulic conductivities greater than 1 foot per day (ft/day), or approximately 3×10^{-4} centimeters per second (cm/sec), are suitable for injection of dissolved substrates (Suthersan et al., 2002; Morse et al., 1998). It is generally infeasible to effectively distribute substrates in zones having a hydraulic conductivity less than 0.01 ft/day (3×10^{-6} cm/sec). Alternate injection techniques such as hydraulic fracturing may be used in some cases, but the timeframe for remediation may still be many years as remediation of the entire aquifer volume will likely be diffusion-limited.

Groundwater Flow. Groundwater velocity, flow direction, and horizontal and vertical gradients will impact the effectiveness of substrate addition. Most applications rely to some extent on advective groundwater flow or recirculation to distribute substrate uniformly throughout the intended treatment zone. Excessively high rates of groundwater flow (greater than 5 to 10 ft/day) may require large amounts of substrate to overcome a large influx of native electron acceptors migrating into the reactive zone. It may be impractical to maintain sufficiently reducing conditions in high-flow aquifers. Cross-gradient distribution of soluble substrates in high-flow regimes also may be limited by lower transverse dispersion. Where rates of groundwater flow are very low (less than 10 to 30 feet per year [ft/yr]), closer injection well spacing will be required and the timeframe for remediation may be extended due to reduced mixing of substrate and contaminant mass.

3.3.4 Groundwater Geochemistry

Redox processes in natural systems are rarely in equilibrium, and the predominant electron acceptor being utilized by microbial populations to derive energy often varies in zones across the site. Addition of an organic substrate is intended to consume native electron acceptors and to maintain optimal conditions for high rates of anaerobic dechlorination. Excessive levels of competing electron acceptors (e.g., DO, bioavailable iron, and sulfate) may limit the effectiveness of substrate addition. Groundwater geochemical characteristics across the site should be reviewed to identify any undesirable conditions.

Dissolved Oxygen and Oxidation-Reduction Potential. Background levels of DO and values of ORP are an indicator of the pre-injection redox conditions that must be lowered to achieve efficient dechlorination. In general, elevated levels of DO and nitrate in most aquifer systems can be overcome by providing adequate organic substrate. However, the problem may be compounded by other factors such as high rates of groundwater flow.

Bioavailable Iron. High levels of bioavailable ferric iron (as iron oxide or iron hydroxide minerals) may inhibit microbial anaerobic dechlorination in a manner similar to other competing electron acceptors. In particular, it has been theorized that the free energy associated with electron transfer during reduction of bioavailable iron by iron-reducing bacteria is greater than that associated with the reduction of *cis*-DCE. Therefore, anaerobic dechlorination of *cis*-DCE to VC may potentially be inhibited in the presence of relatively high levels of bioavailable iron because iron-reduction is more energetically favorable (Evans and Koenigsberg, 2001; Wilson et al., 2003). This may be a temporal phenomenon until the bioavailable iron is depleted; the concentrations or levels of bioavailable iron that may inhibit anaerobic dechlorination have not been well documented or defined. Because bioavailable iron cannot be determined from groundwater sampling alone, this parameter is frequently underestimated.

Sulfate/Sulfides. Existing guidance documents tend to suggest that, while CAH dechlorination under sulfate reducing conditions is feasible, high sulfate levels are problematic for CAH bioremediation. The anaerobic dechlorination scoring matrix in the USEPA (1998a) protocol results in a lower score (lower potential for anaerobic dechlorination) if sulfate exceeds 20 mg/L; similar cautions are provided by Morse et al. (1998). High sulfate levels may lower the efficiency at which substrate is used for anaerobic dechlorination.

However, there is ample evidence in the literature for dechlorination of a variety of CAHs at sites containing elevated dissolved sulfate levels (ITRC, 1998; Devlin and Muller, 1999; Appendix E.6). ARCADIS (Suthersan et al., 2002) reports successful application of enhanced anaerobic bioremediation at sites containing up to 500 to 700 mg/L of sulfate. Complete anaerobic dechlorination has been stimulated at several high-sulfate Air Force sites including Altus AFB, Oklahoma (sulfate up to 2,600 mg/L) and Travis AFB, California (sulfate up to 5,400 mg/L). Therefore, the presence of high sulfate concentrations does not necessarily preclude effective application of this technology.

Excessive levels of sulfides produced by reduction of sulfate may potentially inhibit anaerobic dechlorination. Elevated levels of dissolved sulfides or hydrogen sulfide have been shown to inhibit sulfate reducing bacteria and methanogens, as well as some fermentation reactions that produce hydrogen (e.g., Maillacheruvu and Parkin, 1996). The levels of sulfide that may potentially inhibit dechlorinating microorganisms (and whether these levels are commonly encountered in the field) are not well documented. In general, dissolved sulfide and hydrogen sulfide are rapidly co-precipitated with ferrous iron (a byproduct of ferric iron reduction), but this may not be sufficient to reduce sulfide levels at high sulfate/low iron sites, where there is insufficient iron to react with the sulfides.

pH and Alkalinity. A pH close to neutral (i.e., 6 to 8) is the most conducive to the proliferation of healthy, diverse microbial populations. Low pH conditions (<5) are detrimental to sulfate-reducing, methanogenic, and dechlorinating bacteria. Fermentative organisms favor lower pH conditions, and therefore will out-compete sulfate-reducing and methanogenic bacteria in more acidic environments; this can result in the formation of undesirable byproducts of fermentation, such as ketones, alcohols, and aldehydes. In such cases, pH buffering, typically using common basic salts such as sodium bicarbonate, may be used during implementation to raise and/or neutralize pH against further decreases. Sites with

pH outside of the 5 to 9 range may require more thorough biological screening (e.g., using microcosm studies) to evaluate the effect of pH manipulation on the existing dechlorinating microbial populations. In practice, care must be taken in evaluating site-specific behavior. For example, if groundwater pH is below 5 but complete dechlorination is observed in the field, then it may be clear that the local microbial population has adapted to low pH conditions.

Aquifer systems with lower buffering capacities are more susceptible to decreases in pH. Alkalinity is a general indicator of the buffering capacity of an aquifer system. However, because of the importance of the aquifer solids in establishing buffering capacity, groundwater alkalinity may underestimate the true buffering capacity. From a practical standpoint, alkalinities greater than 300 mg/L are generally sufficient to buffer against adverse pH changes. Alkalinity less than 100 mg/L is cause for concern, and pH should be monitored carefully.

Lowering of pH and problems with adequate buffering are more likely to occur where organic acids (e.g., lactic acid), organic acid salts (e.g., sodium lactate), or soluble sugars (e.g., HFCS or molasses) are used. Substrate selection, substrate loading rate, and the addition of buffering reagents should be carefully evaluated at sites with low alkalinity or in response to field observations of excessive drop in pH.

3.4 POTENTIAL ADVERSE IMPACTS

Application of enhanced anaerobic bioremediation can cause profound changes in the distribution of contaminants and the geochemistry of the treated aquifer. The potential for adverse impacts should be considered during the site screening process. While some site conditions may exacerbate these adverse impacts, in most cases they can be mitigated by design alternatives. This requires an understanding of the biogeochemical and hydrogeologic conditions of the aquifer system to be treated, and of the potential impacts that may occur.

3.4.1 Water Quality

Several changes in water quality may occur during anaerobic bioremediation. These changes occur primarily within the anaerobic treatment zone and may be of concern if drinking water aquifers are present and primary/secondary drinking water standards are enforced. These changes, which can affect the ability to meet remedial goals, include the following:

- Mobilization of metals or CAH mass or production of intermediate CAH byproducts (e.g., VC) for which drinking water standards (e.g., MCLs) exist; and

- Degradation of water quality such that non-CAH byproducts of anaerobic biodegradation (e.g., biological oxygen demand [BOD], taste and odor) impact water quality.

3.4.1.1 Mobilization of CAHs

Several processes may occur during application of enhanced anaerobic bioremediation that may mobilize CAH mass. Physical displacement of the dissolved plume and free-phase

DNAPL may occur during substrate injection. Processes that facilitate dissolution of DNAPL or desorption of sorbed CAH mass may also occur (Section 2.3). In general, transfer of CAH mass to the dissolved phase is beneficial, as this mass is available for biodegradation. However, many practitioners report an initial increase in dissolved concentrations may occur before degradation is enhanced to rates that prevent migration of this additional dissolved mass downgradient of the treatment zone. Therefore, the potential for an initial increase in CAH concentrations downgradient of the treatment area must be considered in regards to possible off-site migration or migration towards sensitive receptors.

Although in practice this is not often a problem, practitioners are wise to consider it. This concern can be mitigated by downgradient monitoring and development of a contingency plan for either containment or additional treatment. Suthersan et al. (2002) propose an "outside-in" approach for treating source areas, in which a reactive zone is first established downgradient of the source area to capture any mobilized contaminant mass before active treatment of the source is initiated. This approach should also facilitate the mixing of contaminants and substrate, and address the potential displacement of dissolved contaminant mass due to injection processes.

The production of toxic intermediate byproducts is also a common concern (e.g., the sequential dechlorination of chlorinated ethenes yielding VC). An evaluation of the potential for complete dechlorination to occur (Section 4.1) is recommended as part of the enhanced bioremediation site screening process. However, in most cases, VC will degrade via anaerobic dechlorination or other processes such as anaerobic or aerobic oxidation, and production of VC is usually considered to be only a temporary phenomenon limited to the vicinity of the reaction zone. Monitoring for intermediate dechlorination products is required to ensure that this is the case.

3.4.1.2 Secondary Water Quality Issues

The term "secondary water quality" is used in this document to refer to water-quality issues or concerns, apart from the primary contaminants being treated, that result from the substrate addition. Degradation of secondary water quality can occur as a result of mobilization of formerly insoluble forms of metals that occur naturally in the aquifer matrix. Other secondary water quality parameters that may be degraded include chemical oxygen demand (COD), BOD, total dissolved solids (TDS), and sulfides that affect taste and odor. These parameters should be monitored if regulated at the site. Table 3.3 lists some of the common parameters monitored during enhanced bioremediation and associated federal water quality standards. This list is not inclusive, as many states enforce additional water quality standards.

In general, the reduced groundwater environment induced by substrate addition may increase the mobility of some naturally occurring (but regulated) metals in the reactive zone (e.g., iron, manganese, and arsenic). This is not always problematic: in some cases migration of metals such as arsenic may be retarded by adsorption to the aquifer matrix. Additionally, the mobilized inorganics may be precipitated/immobilized downgradient of the reactive zone when the conditions return to a more oxidizing state. COD, BOD, TDS, and sulfides that affect taste and odor are necessarily elevated in the anaerobic reactive zone due to biodegradation of the substrate. Generation of reduced sulfur compounds (e.g., thiols or

mercaptans) or alcohols (e.g., 2-butanol or isopropanol) may occur under extreme fermentation conditions.

Table 3.3 Water Quality Parameters Subject to Regulatory Compliance at Enhanced Anaerobic Bioremediation Sites

Compound or Element	Molecular Formula	USEPA MCL (mg/L)[a/]	USEPA Secondary Standard[b/] (mg/L)
Chloroethenes			
Tetrachloroethene (PCE)	C_2Cl_4	0.005	--
Trichloroethene (TCE)	C_2HCl_3	0.005	--
cis-1,2-dichloroethene (*cis*-DCE)	$C_2H_2Cl_2$	0.070	--
trans-1,2-dichloroethene (*trans*-DCE)	$C_2H_2Cl_2$	0.100	--
1,1-dichloroethene (1,1-DCE)	$C_2H_2Cl_2$	0.007	--
Vinyl chloride (VC)	C_2H_3Cl	0.002	--
Chloroethanes			
1,1,1-trichloroethane (1,1,1-TCA)	$C_2H_3CL_3$	0.200	--
1,1,2-trichloroethane (1,1,2-TCA)	$C_2H_3CL_3$	0.005	--
1,2-dichloroethane (1,2-DCA)	$C_2H_4Cl_2$	0.005	--
Chloromethanes			
Carbon tetrachloride (CT)	CCl_4	0.005	--
Chloroform (CF)	$CHCl_3$	0.1 [c/]	--
Dichloromethane (DCM) (or methylene chloride [MC])	CH_2CL_2	0.005	--
Total trihalomethanes (includes CF)	--	0.080	--
General Water Quality Parameters			
Nitrate (as nitrogen)	NO_3^-	10	--
Nitrite (as nitrogen)	NO_2^-	1.0	--
pH	--	--	<6.5, >8.5
Chloride [d/]	Cl^-	--	250
Total dissolved solids (TDS) [d/]	--	--	500
Metals			
Arsenic [d/]	As	0.01	--
Selenium	Se	0.05	--
Iron [d/]	Fe	--	0.3
Manganese [d/]	Mn	--	0.05

[a/] USEPA MCL = USEPA Maximum Contaminant Level; mg/L = milligrams per liter.

[b/] National secondary drinking water regulations are non-enforceable guidelines. However, states may choose to adopt them as enforceable standards.

[c/] Tentative MCL (pending).

[d/] These are compounds or elements that in some cases may increase in concentrations as the result of anaerobic bioremediation

A nearby and geochemically similar groundwater plume contaminated with fuel hydrocarbons is one model to estimate the dimensions of the potential zone of secondary groundwater quality impact. If such site exists, it would be beneficial to the enhanced bioremediation design team to review available site data to determine the potential effects of substrate addition (in this case relative to fuel hydrocarbons) on groundwater quality.

3.4.2 Generation of Volatile Byproducts and Noxious Gases

Stimulating biodegradation also may enhance generation of volatile byproducts and noxious gases (e.g., VC, methane, and hydrogen sulfide) that may degrade groundwater quality and/or accumulate in the vadose zone. In addition, these gases can accumulate within subsurface structures (e.g., basements, utility corridors) in the immediate vicinity of a treatment zone. Evaluation of the potential for gas generation can be performed during engineering design of an individual system. Factors to be considered include depth to the zone of interest, potential concentrations and volumes of gases that may be produced, potential pathways for vapor migration, proximity of structures and underground utility corridors, and potential receptors such as building occupants.

Passive diffusion of these gases to the atmosphere and *in situ* degradation during transport may be sufficient to mitigate any safety concerns. However, vapor-phase concentrations of these compounds should be monitored when a potential concern exists to ensure that safe conditions are maintained. Standard industry health and safety practices should be followed during operation and monitoring of enhanced anaerobic bioremediation systems. Monitoring of potentially explosive gases should be considered, for public safety as well as the safety of the field staff. If required, venting of subsurface gases can be performed to protect against exposure or accumulation. While this issue is not considered a major impediment to implementation of enhanced anaerobic bioremediation, mitigation measures may be needed in some cases.

3.5 PROCEEDING WITH ENHANCED ANAEROBIC BIOREMEDIATION

If preliminary screening for enhanced bioremediation indicates it is a potential remedial strategy, the practitioner or environmental manager should consider whether it is the most reasonable approach. This should include a cost comparison to alternative technologies such as MNA, excavation, groundwater extraction, chemical oxidation, air sparging, and thermal- or resistivity-enhanced extraction. Enhanced bioremediation will likely be cost competitive in most cases. In some cases, a combination of technologies may be the most cost-effective approach.

It may be difficult to determine the potential for enhanced anaerobic bioremediation to stimulate complete anaerobic dechlorination and to meet remedial objectives during the preliminary screening process described in this section. However, proceeding directly to design and implementation of enhanced bioremediation may involve a significant risk that the approach will not be successful. ***Further site evaluation using existing data and the use of additional pre-design screening techniques may lower the risk that enhanced bioremediation is improperly applied at marginal or questionable sites.*** Section 4 leads the user through a discussion of these additional pre-design evaluation methods.

SECTION 4

PRE-DESIGN

The preliminary screening criteria presented in Section 3 are only intended to determine whether enhanced anaerobic bioremediation is an appropriate technology for a site. Once a site has been selected for an enhanced *in situ* bioremediation application, a site-specific evaluation is required before a field application can be designed and implemented with confidence. This section describes the methods and tools available to make informed decisions for poorly characterized, marginal, or questionable sites. The following should be considered before proceeding to the design phase:

Site-specific conditions should be reviewed prior to design and implementation of an enhanced anaerobic bioremediation system.

Pre-design techniques (such as microcosm studies) may be used to better assess whether a bioremediation system will stimulate complete anaerobic dechlorination, but at a cost.

An analysis regarding the use of these tools should be conducted before proceeding with field implementation.

- Consider applying the technology if it can be applied in a manner that is economically competitive with other technologies, or if the potential cost-savings are worth taking the risk on unknown performance.

- The risk of failure increases for sites where complete anaerobic dechlorination is not currently occurring, and the site hydrogeology and geochemistry is not well-understood.

- For marginal or questionable sites, conduct an analysis of proceeding with system design and implementation versus collecting additional data to evaluate whether complete anaerobic dechlorination can be stimulated.

Evaluating the potential for stimulating rapid and complete anaerobic dechlorination involves characterizing initial site conditions and using selected tools and analyses to increase the level of confidence that bioremediation can be sufficiently enhanced. Figure 4.1 illustrates the steps used in a site-specific evaluation of engineered anaerobic bioremediation, starting with an analysis of existing biogeochemical data. In some cases, existing data that demonstrate that anaerobic dechlorination occurs naturally at the site may provide confidence in proceeding directly with a field application of enhanced bioremediation. But in many cases, a more extensive evaluation may be required for poorly characterized sites or sites that may be marginal for various reasons (e.g., pH extremes, high salinity). This is beneficial because the cost of modifying or replacing a field-scale bioremediation system can be high relative to the cost of the pre-design techniques described in this section.

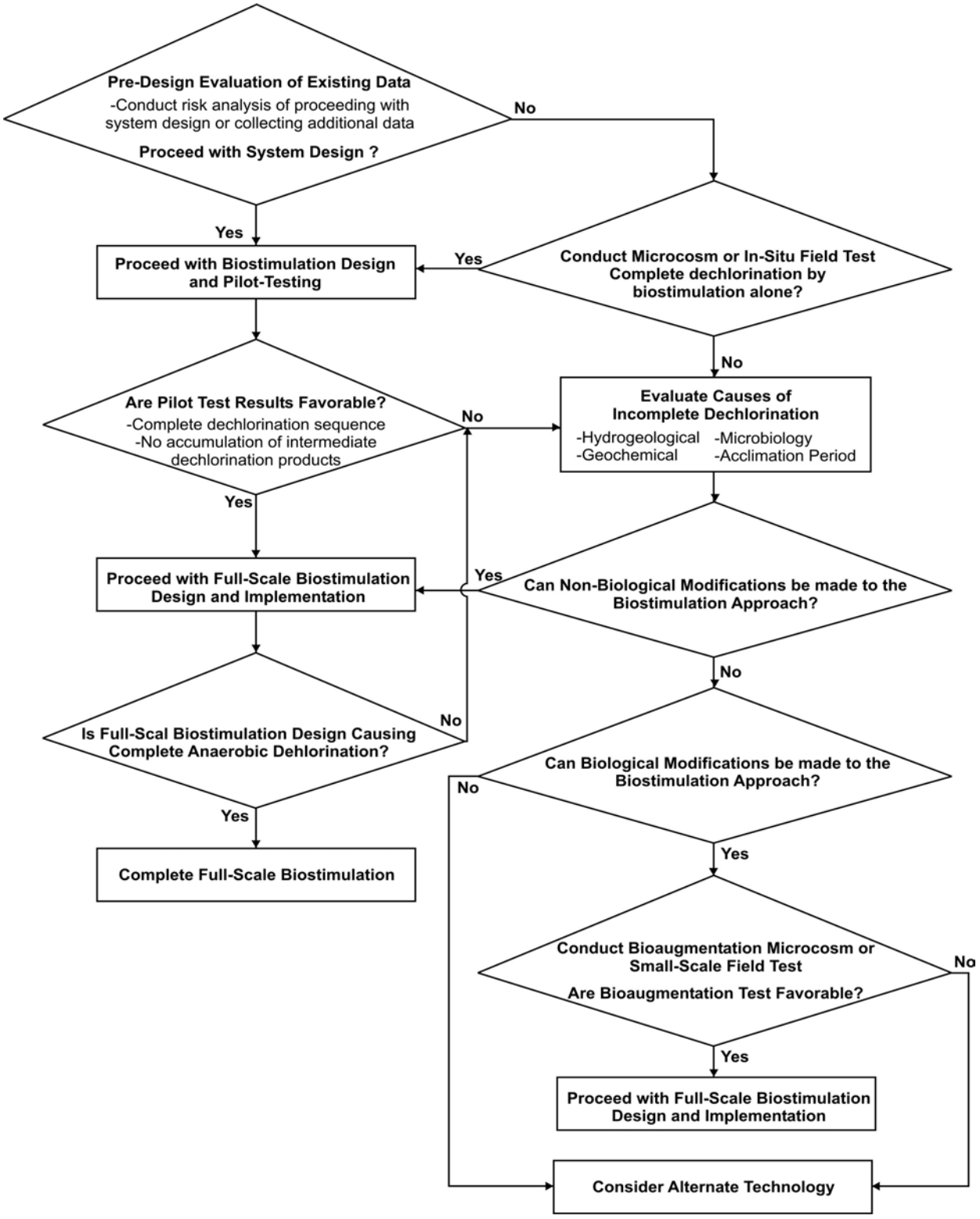


Figure 4.1 Site-Specific Evaluation for Enhanced Anaerobic Bioremediation

4.1 EVALUATING EXISTING DATA

Evaluation of existing site characterization data is the first step in determining whether additional pre-design testing is required, and what additional information may be required prior to design and implementation of an enhanced bioremediation system. The following pre-design considerations should be evaluated using the existing data:

- **Contaminant Plume and Source Delineation.** CAH source zones or contaminant plumes are often difficult to characterize (e.g., DNAPL distribution). A decision is required as to whether additional source zone or plume delineation is beneficial, or whether the system design adequately accounts for the uncertainty associated with the delineation. For example, increasing the size of the treatment zone may eliminate the need for additional source zone delineation. In other circumstances, a cost/risk analysis may indicate that the cost of source area delineation would be offset by the potential savings realized with a smaller treatment system; or that the risk associated with inadequate source zone treatment (i.e., failure to meet remedial objectives) is too high.

- **Hydrogeology.** Inadequate characterization of the site hydrogeology can lead to bioremediation systems that fail to meet remedial objectives. Difficult hydrogeologic conditions may limit the ability to effectively distribute substrate throughout the treatment area (e.g., low permeability, a high degree of aquifer heterogeneity, and/or preferential flow paths). In some cases, the hydrogeologic conditions may dictate the type of substrate and system configuration that can be applied. Additional characterization of hydrogeologic conditions may be warranted if the site is insufficiently characterized.

- **Microbial Sufficiency.** In aerobic or mildly anaerobic aquifer systems, conditions may not be appropriate for anaerobic dechlorination to occur naturally, and an assessment of whether complete anaerobic dechlorination can be stimulated cannot be made with confidence. In this case, a cost/risk analysis may be performed to determine whether the risk of initiating a field application without this assessment is acceptable, or whether microcosm studies or small-scale field tests should be considered to reduce this risk.

- **Carbon Source.** In addition to microbial sufficiency, the selection of a carbon source may influence the fermentation pathways that will predominate and the efficiency with which the substrate is utilized for anaerobic dechlorination. Current literature indicates that a wide variety of organic substrates are capable of supporting anaerobic dechlorination (Parsons, 2002a). Substrate selection should be driven by a site-specific feasibility assessment including ability to implement, cost, and a demonstrated ability to support complete anaerobic dechlorination.

Field testing of multiple substrates is generally not practical, and field pilot testing using the most “feasible” substrate is a common approach. However, for large-scale systems where substrate type may have a significant impact on cost and performance, microcosm studies using site-specific soil and groundwater samples may be a justifiable investment to evaluate the fermentation pathways and dechlorination efficiency of multiple substrate types. Multiple

substrate microcosm trials typically cost on the order of $50,000 to $100,000 to conduct properly and take 8 to 12 months to complete.

Methods for characterizing site hydrogeology and the nature and extent of contaminants are well developed within the environmental industry. Methods to determine microbial sufficiency or whether bioaugmentation is required for an enhanced bioremediation application are less well developed. The following sections describe the methods and techniques available to evaluate whether microbial and biogeochemical conditions are suitable to proceed with design and implementation of a bioremediation system, or whether additional data should be collected.

4.1.1 Reviewing Field Data for Anaerobic Biodegradation Potential

The primary objective of enhanced anaerobic bioremediation applications is to stimulate anaerobic dechlorination of CAHs to levels protective of human health and the environment. Because anaerobic dechlorination occurs sequentially, both the parent CAHs and their dechlorination products must be degraded to protective levels. Therefore, it is important to evaluate the potential for complete dechlorination of CAHs to innocuous end products to occur. It also may be beneficial to evaluate the potential for other degradation processes, such as aerobic oxidation of VC in a downgradient redox recovery zone, to achieve the same end result.

Evaluating the potential to stimulate anaerobic dechlorination at a site has much in common with evaluating natural attenuation processes. Both assessments are based on a review of degradation byproducts, contaminant trends, electron donors, electron acceptors, metabolic byproducts, geochemical indicator parameters, and hydrogeology. However, evaluating the potential for enhanced anaerobic bioremediation requires extrapolating current site conditions to predict the impact of adding large quantities of organic substrate to the aquifer system.

There are site characteristics that indicate the potential for anaerobic dechlorination to occur naturally. As discussed in Section 3.2, these site characteristics may be described as follows:

- **Highly Anaerobic Type 1 Sites** are characterized by relatively high levels of organic carbon, resulting in sulfate-reducing and methanogenic conditions. Evidence of anaerobic dechlorination should be apparent under these conditions. Lack of evidence of complete reductive dechlorination (e.g., to VC and ethene) may be due to substrate limitations. Complete dechlorination should be stimulated by substrate addition at highly anaerobic sites if even low concentrations of dechlorination end products (e.g., VC and ethene) are observed.

- **Mildly Anaerobic Type 2 Sites** are characterized by mildly anaerobic conditions due to the presence of moderate levels of natural or anthropogenic carbon. Limited anaerobic dechlorination may be occurring, such as transformation of PCE and TCE to *cis*-DCE. In many cases, the lack of effective or complete dechlorination is due to a deficiency of carbon substrate. A measurable and sustained conversion of *cis*-DCE to VC and ethene should be achievable via addition of an organic substrate.

- **Aerobic Type 3 Sites** are naturally aerobic aquifers which exhibit little, if any, evidence of anaerobic microbial activity. It is often not possible to determine the potential for complete anaerobic dechlorination from site characterization data. Such sites may require an extended lag time to establish a population of appropriate dechlorinating organisms, given application of appropriate levels of organic substrate.

Experience with MNA of naturally aerobic (Type 3) chlorinated solvent sites that have subsequently been impacted with sufficient substrate (e.g., via a fuel release) to sustain highly anaerobic conditions over periods of several years indicates that anaerobic dechlorination of CAHs will likely occur, given a sufficient acclimation period.

If existing data clearly indicate a Type 1 site with evidence of dechlorination end-products (e.g., VC and ethene), then design and implementation of enhanced bioremediation can be pursued with confidence. In the case of Type 2 or Type 3 sites where the prevailing site conditions are not suitable for complete anaerobic dechlorination, additional site evaluation (Sections 4.2 through 4.4) should be considered. The existence of site-specific factors explaining less than complete anaerobic dechlorination should be incorporated into the CSM and feasibility assessment at each candidate site. Examples of site-specific factors could include high native electron acceptor supply (e.g., frequent infiltration of oxygenated water, high nitrate or sulfate concentrations) or low concentration or poorly degradable carbon sources. These factors should be evaluated, since effective enhanced bioremediation must be designed to overcome or correct these less than optimal conditions.

4.1.2 Geochemical Requirements for Anaerobic Dechlorination

Regardless of whether the appropriate dechlorinating microorganisms are present within the impacted aquifer system, anaerobic dechlorination will only occur under the appropriate geochemical conditions. Because redox conditions are largely a result of the amount of organic carbon and electron acceptor present, an evaluation of the site geochemical conditions provides an indication of the degree to which the system is carbon limited. In most cases, a carbon deficiency can be readily overcome by substrate addition.

Anaerobic reductive dechlorination of CAHs will only occur under appropriate geochemical conditions. Sufficient organic carbon must be present for the growth and development of anaerobic microorganisms capable of degrading the CAHs present.

In most cases, a deficiency of organic carbon can be overcome by addition of an organic substrate.

Many natural geochemical conditions that are not appropriate for anaerobic dechlorination to occur naturally need not be a barrier to implementation of enhanced anaerobic bioremediation, if the implementation will remedy these undesirable conditions. However, caution is warranted for sites where high rates of native electron acceptor flux occurs, due to a combination of elevated levels of DO, nitrate, or sulfate combined with a high rate of groundwater flow. Sites with pH outside of the range of 5 to 9 also may require more thorough biological screening (e.g., microcosm studies). Sites with low alkalinity (less than

100 to 200 mg/L) may require the use of buffers to avoid an excessive drop in pH as a result of substrate addition.

4.1.3 Justification for Proceeding at Sites Lacking Evidence of Anaerobic Dechlorination

A review of the literature indicates that enhanced anaerobic reductive dechlorination can be stimulated at most sites, whether the site is initially anaerobic or aerobic (Parsons 2002a, Appendix E.11, Appendix E.12). However, there are cases where complete dechlorination did not occur within a reasonable timeframe, with stalling at intermediate dechlorination products (e.g., *cis*-DCE). While there may be multiple reasons why complete dechlorination was not achieved, many researchers and practitioners believe that native microbial populations may not always be able to catalyze the complete dechlorination reaction sequence. For sites where aerobic or only mildly anoxic conditions predominate, site characterization data alone may simply not be suitable to determine the potential for complete dechlorination to occur under strongly anaerobic conditions.

Substrate addition has been shown to readily induce anaerobic conditions at many naturally aerobic, Type 3 sites (e.g., see Appendix E.11). However, there may be exceptions where a combination of a high concentration of native electron acceptors and a high rate of groundwater flow may present an electron acceptor demand that is not practical or economical to overcome. The lag time required for the appropriate shift in environmental conditions and microbial succession for development of dechlorinating species will be greater in Type 3 environments, relative to highly anaerobic Type 1 environments. In some cases, degradation of PCE and TCE to *cis*-DCE may proceed fairly rapidly, but development of organisms capable of degrading *cis*-DCE and VC to ethene may take longer due to the need for ecological succession. Contingencies should be included in enhanced anaerobic bioremediation monitoring schedules for Type 2 or Type 3 sites to allow sufficient lag times to occur (12 to 24 months recommended). In addition, schedules should incorporate decision points to assess the need for bioaugmentation to accelerate the process (Figure 4.1).

Given that biostimulation alone has been successful at Type 2 and Type 3 sites, ***there are no compelling reasons not to proceed with enhanced anaerobic bioremediation at sites lacking evidence of naturally occurring anaerobic dechlorination***. However, careful site-specific evaluations, including pilot-scale testing or perhaps microcosm testing, are highly recommended for sites lacking evidence of anaerobic dechlorination prior to full-scale design and implementation. This is primarily because the cost of modifying or replacing field-scale bioremediation systems is typically much higher than the cost of implementing the pre-design techniques described in this section.

4.2 SITE-SPECIFIC EVALUATIONS FOR ENGINEERED ANAEROBIC BIOREMEDIATION

Site screening criteria for potential application of enhanced bioremediation are based on the CSM and qualitative and quantitative evaluations of the potential to stimulate and sustain anaerobic dechlorination over the lifetime of the application. Screening criteria include, but are not limited to:

- **Location of Sensitive Receptors.** The distance to a potential receptor, property boundary, or completed exposure pathway may be an important regulatory consideration. Completion of an exposure pathway may require more aggressive remedial actions.

- **Hydrogeology.** Depth to groundwater and groundwater seepage velocity should be taken into account when designing an enhanced bioremediation system. Aquifer heterogeneity and preferential flow paths will complicate the effective application of enhanced bioremediation.

- **Plume Dynamics.** Is the plume stable, expanding, or receding? How many years of monitoring data are available to make this assessment, and how well understood is the plume? For example, an expanding plume near a potential receptor may not be the ideal site for enhanced bioremediation unless the exposure pathway can be controlled. Alternately, enhanced bioremediation may not be needed at sites undergoing natural attenuation if there is no risk of exposure and the timeframe for remediation is acceptable.

- **Site Infrastructure.** A CAH plume residing beneath or in close proximity to buildings or utilities may not be the ideal candidate site for organic substrate addition due to potential access issues or risk of vapor intrusion (CAHs, methane, or hydrogen sulfide). Mitigation measures may be necessary under these conditions.

- **Organic Substrate Demand.** Consideration must be given to the substrate demand exerted by native inorganic electron acceptors, the demand to drive dechlorination of CAHs, and a substantial safety factor recognizing the inherently inefficient distribution and utilization of the substrate. High substrate demand may result in high costs due to the large quantities of substrate required or the need for frequent substrate addition.

When existing data are too marginal or questionable to support proceeding with a field application for enhanced bioremediation, a number of screening techniques and analytical methods may be used to collect additional information regarding the potential for stimulating complete anaerobic dechlorination.

The following sections describe optional and experimental screening techniques and analytical methods that may be used to evaluate a site for enhanced anaerobic bioremediation; these are above and beyond the data typically collected as part of routine site characterization. These methods focus primarily on whether the native microbial population can be stimulated to completely degrade the CAHs present, and in some cases may be used to evaluate the potential for bioaugmentation to carry sequential dechlorination to completion.

4.2.1 Pre-Design Screening Techniques

Screening techniques that can be used to evaluate sites for enhanced bioremediation include laboratory microcosm studies or small-scale field tests combined with analytical methods to characterize microbial populations and activity. Common (well-established) and emerging (experimental) screening techniques and methods are summarized in Table 4.1. These methods are intended to reduce the uncertainty associated with implementing enhanced anaerobic bioremediation.

Table 4.1 Summary of Microbial Screening Techniques and Supplemental Analytical Methods

Test Method	Description	Information Provided	Usefulness	Limitations	References
Common (Well Established) Techniques					
Evaluation of Existing CAH and Biogeochemical Data	Evaluation of site data for the presence of dechlorination products and appropriate geochemical conditions.	Evidence that reductive dechlorination occurs with native microbial populations, or that reductive dechlorination is limited due to substrate limitation.	Useful for site selection to determine whether complete reductive dechlorination can be enhanced, or whether additional site evaluation is required.	Type 2 or Type 3 sites may not have appropriate conditions for observing reductive dechlorination	See Sections 4.1 and 5
Microcosm Testing	Microcosms tests using site-specific soil and groundwater. Can be used to test either single or multiple substrate types.	Definitive information on the extent of reductive dechlorination that may be achieved. Evidence of predominant fermentation pathways of the substrate selected.	Provides a positive indication that complete dechlorination can be achieved. Useful to evaluate fermentation pathways and efficiency of multiple substrates. May be used to verify the effectiveness of bioaugmentation cultures.	Moderate cost and time. Must be coupled with an engineering assessment or pilot test to evaluate substrate distribution and to define engineering design parameters.	Findlay and Fogel, 2000; Fennel et al., 2001; Loffler et al., 2000; Morse et al., 1998
Single Well Push-Pull Tests	Injection and periodic extraction of a well-characterized groundwater slug in a single well.	Extent and rate of *in situ* reductive dechlorination.	Low-cost field test that provides *in situ* dechlorination rates and field data regarding effectiveness of substrate injection (e.g., injection pressures and flow rates, theoretical radius of influence).	May not observe degradation if groundwater conditions are not sufficiently reducing or insufficient time is allowed for microbial succession and acclimation.	Istok et al., 1997; Haggerty et al., 1997; Hageman et al., 2001; Newell et al., 2000
Field Pilot Tests	Field-scale pilot tests to determine microbial sufficiency.	Extent and rate of *in situ* reductive dechlorination.	Can determine lag times and field degradation rates; used to refine system design parameters.	Time and cost.	Morse at al., 1998; Suthersan et al., 2002
Emerging Techniques					
Isotope Chemistry	Shifts in relative isotope fractions in CAHs over time.	Carbon isotope fractions for chlorinated parent and dechlorination products.	Changes in carbon isotope fractions between chlorinated parent and dechlorination products may provide field evidence for microbial degradation.	Highly experimental. Requires sampling over multiple time periods.	Song et al., 2002; Conrad et al., 1999
Phospholipid Fatty Acid (PLFA)	Profile of the phospholipid content of cell membranes.	Information on biomass concentration, community structure, diversity, and physiological status.	Provides general information on the activity and shifts in the microbial community due to substrate addition.	Does not provide positive identification of dechlorinating species. Excludes methanogens.	White et al., 1997; Stahl, 1997
Molecular Identification of Deoxyribonucleic Acid (DNA) Sequences	Detection of genetic sequences unique to targeted microbial genus and species.	Provides positive identification of a limited number of dechlorinating species.	Positive identification of *Dehalococcoides*-related species, strains of which are known to be capable to complete anaerobic dechlorination of chloroethenes.	Potential for false negative and positive responses, cannot determine the dechlorination potential of the detected species.	See Table 4.2 and Section 4.5

Microcosms (Section 4.3) constructed with site soil and groundwater can be used to assess the presence of dechlorinating microorganisms and whether complete anaerobic dechlorination can be stimulated. Microcosm tests may be a more practical method than field tests to evaluate multiple substrates to compare the extent and rate of dechlorination achieved. In the event complete dechlorination is not observed, microcosms can be amended with bioaugmentation cultures to screen this approach.

Small-scale field pilot tests or in situ substrate utilization ("push-pull") tests (Section 4.4) can often be conducted at a reasonable cost, with the added benefit of determining additional engineering design parameters (e.g., effective substrate distribution, ROI, acclimation periods, and field degradation rates).

One important consideration in using these screening techniques is the amount of time required for the onset of complete dechlorination in both field- and laboratory-scale testing. Pilot tests performed in the field can take a year or more to demonstrate complete dechlorination to innocuous end products. Microcosm studies typically attempt to shorten this lag period by inoculating the microcosms at higher temperatures and agitation, at the expense of using conditions similar to those in the field. As a result, the microcosm results may not be directly applicable to actual field conditions. Frequent sampling for at least the primary contaminants and dechlorination products, including ethene and ethane, is often required if the acclimation time is unknown.

4.2.2 Optional Analytical Methods

Specialized or emerging analytical methods include molecular screening techniques, PLFA analysis, and carbon isotope chemistry. Molecular screening techniques are commonly used in conjunction with laboratory microcosm studies or as a diagnostic tool for field applications where dechlorination appears to be deficient. Molecular screening provides positive identification of a limited number of dechlorinating species. For example, it is possible to detect the presence of *Dehalococcoides*-related species, of which certain strains are known to be capable of complete reductive dechlorination of chlorinated ethenes. Molecular screening methods are described further in Section 4.5.

PLFAs are essential components of the membranes of all cells except Archea (which includes methanogens). Analysis of PLFA profiles provides information on biomass concentration, community structure, diversity, and physiological status. Determination of changes in viable biomass over time is an indicator of microbial growth induced by substrate addition. PLFA profiles may also be used to determine the relative distribution and diversity of brood phylogenic groups of microbes present. However, this method cannot positively identify or distinguish dechlorinating species. Nonetheless, PLFA analysis may be useful as an optional diagnostic tool during a small-scale field test to provide a general indication of the degree to which substrate addition has stimulated microbial growth and how the general microbial community has shifted in response to changing environmental conditions. This type of test provides an indication of whether the current microbial population is relatively diverse or limited to specific classes of microorganisms. However, a decision to change the overall bioremediation approach is typically based on observed changes in contaminants and geochemistry.

Carbon isotope chemistry of parent and dechlorination products can be used as an indicator of microbial degradation of CAHs over time. Microbial degradation of organic compounds favors ^{12}C bonds over ^{13}C bonds, causing the mass of parent compounds to become depleted in ^{12}C and enriched in ^{13}C over time. Therefore, shifts in relative isotope fractions over time indicate microbial degradation (Song et al., 2002; Conrad et al., 1999; Bloom et al., 2000). This type of analysis also may be used to determine whether anaerobic dechlorination is occurring in cases where measurable and substantial decrease in contaminant concentration is not occurring due to desorption effects. Carbon, oxygen, and hydrogen isotope chemistry (tritium for example) can also be used in tracer studies to account for dilution or to determine the ROI of the treatment zone. The laboratory analytical costs may preclude the use of this specialized experimental technique at most environmental sites, but it may be warranted at marginal or poorly performing sites.

4.3 LABORATORY MICROCOSMS

The benefits associated with microcosms may not outweigh the costs of performing them when existing biogeochemical data are favorable. However, when site selection indicators are marginal or questionable, microcosms constructed using site soil and groundwater coupled with molecular identification techniques can be useful in determining whether or not complete dechlorination will likely occur at a site.

Microcosm studies provide information on the potential for native microbial populations to effect complete anaerobic dechlorination of the CAHs of concern to innocuous end products.

However, the artificial conditions under which microcosms are conducted does not mean the results are indicative of what will be accomplished in the field.

4.3.1 Microcosm Design

Microcosms should be carefully designed to answer the questions posed for the study. The ***minimum requirements for a useable microcosm*** study include the following:

- Use of representative site soil and/or groundwater samples collected using reasonably aseptic and anaerobic collection procedures;
- Use of appropriate concentrations of contaminant and substrate;
- Analysis of substrate and contaminant data (including replicate microcosms), including concentrations of chlorinated compounds, ethene and ethane, methane, hydrogen, and volatile fatty acids (VFAs) over time;
- Use of relevant temperatures, media formulations, and controls; and
- Sufficient time for microbial acclimation and growth (6 months minimum).

Microcosm studies should be performed using aquifer matrix material from a number of promising locations. The use of a number of representative field samples (more than two or three) and incubation under field temperatures generally increases the confidence in extrapolating microcosm results to the field. Care must be taken that the samples are not

exposed to air (oxygen), which may be toxic to the dechlorinating microorganisms. Finally, the tests must be carried out by highly qualified technicians to avoid contamination in either the field or the laboratory.

Molecular screening, though not required, is beneficial to determine the dechlorinating species that are facilitating anaerobic dechlorination. In some cases, the dechlorinating species may not be capable of facilitating all of the sequential steps in dechlorination of parent compound to innocuous end products.

4.3.2 Utility of Microcosm Tests

In general, microcosms may be capable of answering the following questions:

- Are native microbial populations capable of the complete anaerobic dechlorination of the chlorinated contaminants of concern given sufficient organic substrate? Note that this is only true to the extent that the microcosm soils are representative of the site as a whole; this can be particularly problematic when the microbial populations are heterogeneously distributed.

- What are the primary fermentation pathways used by native microbial populations for differing substrate types?

- Will an acclimation period occur before complete degradation of dechlorination products is observed?

- Can bioaugmentation enhance the short term rate or extent of dechlorination compared to the native microbial population? Microcosms may also be used to determine whether the introduced culture thrives in the native sediments and to compare bioaugmentation strains.

- Under ideal conditions, mass balance calculations may provide information on the quantity of reducing (electron) equivalents that are channeled toward anaerobic dechlorination as a measure of substrate efficiency. In practice, this is often difficult to achieve.

- For source area applications, microcosms using very high contaminant concentrations (close to solubility) may be used to study concentration or toxicity effects.

In general, if CAHs are not completely degraded after 6 to 9 months of incubation in microcosms amended with an organic substrate, even when appropriate redox conditions and electron donor availability are maintained, then appropriate native dechlorinating organisms may not be present. For chlorinated ethenes, a lack of degradation past *cis*-DCE in the microcosms indicates that 1) it is unlikely that dechlorination in the field will proceed past *cis*-DCE to VC and ethene, and 2) bioaugmentation may be required.

Caution is advised when interpreting microcosm results as being indicative of what can be achieved in the field. Often, microcosm and field results will differ due to the limited number of samples or small sample volumes used to construct the microcosms, and the fact that they

are disturbed. ***Information for field application that microcosms cannot usually provide include the following:***

- Rates of dechlorination that will occur under field conditions,
- Efficiency of substrate utilization that may occur under field conditions,
- Acclimation periods in the field, and
- Any field scale phenomenon such as rates of increased DNAPL dissolution.

The primary disadvantage of microcosms is that the tests may not always accurately reflect subsurface conditions in the field. Microcosm testing must overcome the heterogeneous distribution of dechlorinating populations found in natural aquifer systems. Nonetheless, microcosms are an effective method for determining the potential for complete dechlorination when existing data are insufficient to support proceeding with a field application.

4.3.3 Applying Microcosm Results

There are several examples in the literature that demonstrate the degree to which microcosms were able to predict what could or could not be achieved in the field (e.g., Appendix E.13). For example, a combination of microcosm studies, real-time PCR analysis (described in Section 4.5.1), and site data were used to assess the anaerobic dechlorination potential of indigenous microorganisms in a TCE-contaminated aquifer at Cape Canaveral Air Force Station (CCAFS), Florida (Fennell et al., 2001). The authors concluded that a combination of field data, microcosm studies, and real-time PCR for a specific organism (*Dehalococcoides*) provided complementary information about the potential for the native microbial community to accomplish complete dechlorination via *in situ* substrate addition.

However, sediment and groundwater samples were only collected from two distinct locations, and microcosms from only one of the locations exhibited the presence of *Dehalococcoides* and reduction of TCE to VC and ethene. As a result, the authors acknowledged that the "heterogeneous distribution of dechlorinating activity … points to potential weaknesses in using microcosms to predict responses at a given site." In addition they state, "The time, trouble, and expense involved in running microcosms studies clearly dictate that the locations for testing must be carefully chosen according to the best and most current site data." It should be noted that an extensive VC groundwater plume and elevated levels of ethene occur naturally at the CCAFS site. Given the preliminary screening criteria in Section 3 and the site evaluation discussion in Section 4.1, this site would appear to have highly favorable evidence for natural dechlorination potential and biogeochemical conditions. The observation that only one of the two microcosm results supported complete dechlorination is a further indication that microcosm data should be used with caution.

The Fennell et al. (2001) study further suggests that microcosm studies for candidate sites should be as expansive as possible, including collection of microcosm samples from a number of locations and/or compositing samples from multiple locations. However, performing an expansive microcosm study will be relatively expensive due to the increased number of sample cores needed.

Performing multiple microcosm tests for a representative sample set or for multiple substrate types will increase the upfront costs, and costs for completion of a small field pilot test may be similar. In this case, field-scale pilot testing is recommended, as a pilot test evaluates a much larger, more representative volume of the aquifer, and allows organisms that may be initially present in only a relatively small portion of the aquifer to grow and become more active and widely distributed in the treatment zone after a substrate is introduced.

4.4 SMALL-SCALE PILOT TESTS AND SUBSTRATE UTILIZATION TESTS

In evaluating the potential for applying enhanced anaerobic bioremediation at a site, small-scale pilot tests may be conducted to determine microbial sufficiency, as well as to provide pre-design data on injection well spacing, substrate loading requirements, and injection frequency. Such field tests may preclude the need for laboratory studies. Field tests provide a greater level of confidence in estimating the *in situ* extent and rate of dechlorination, and provide more engineering information for design purposes (e.g., injection pressures and ROI). The cost in time and money is typically similar for laboratory and small pilot-scale efforts. The RABITT protocol (Morse et al., 1998) describes the application of small-scale field pilot tests for soluble substrates. While these pilot tests were linked with microcosm testing, they could also be implemented without the laboratory microcosm effort.

In addition to small-scale pilot tests, "push-pull" tests may also be used to determine 1) transport and mobility of solutes and substrates, 2) biostimulation field degradation rates, and 3) field-scale substrate utilization rates (Kim et al., 2004). Push-pull field tests are described by Istok et al. (1997) for fuel hydrocarbons, for evaluating anaerobic transformation of trichlorofluoroethene (a fluorinated surrogate for TCE) by Hageman et al. (2001), and for assessing aerobic cometabolism of CAHs by Kim et al. (2004). Newell et al. (2000) describe a single well push-pull test using dissolved hydrogen for dechlorination of CAHs at Offutt AFB, Nebraska. Methods to evaluate push-pull test data are also described by Haggerty et al. (1997).

Applying this method to substrate addition for CAHs is complicated by the lag time typically needed to develop microbial populations capable of degrading CAHs. Therefore, "push-pull" substrate utilization tests should be applied only after the aquifer has been conditioned to appropriate anaerobic conditions, usually on the order of 2 to 4 months after injection of a substrate (if necessary).

A substrate utilization test consists of injecting ("pushing") a unit volume of contaminated groundwater with known contaminant and conservative tracer concentrations into the aquifer via a single well. This unit volume is then sampled ("pulled") at discrete time periods and analyzed for the ratio of parent and dechlorination products. The change in parent and daughter product concentrations over time can be used to calculate field degradation rates and the extent of dechlorination. Conservative tracers are used to account for the effects of dilution, and non-conservative tracers are used to account for sorption. The volume of groundwater injected, and the timing and spacing of the sampling periods, should be designed such that the injected groundwater remains within the immediately vicinity of the test well over the duration of the test, generally from 48 hours up to 2 or 3 months (Hageman et al., 2001). Therefore, these tests may not be practical in high flow aquifers.

4.5 MOLECULAR SCREENING TECHNIQUES

Molecular screening methods based on genetics have only recently been developed for application in the environmental field, and the number of microbial species and strains that can be positively identified is limited. The following sections describe the two experimental methods most commonly employed for enhanced bioremediation.

4.5.1 Molecular Identification Methods

Molecular screening methods are based on the detection of gene sequences unique to individual microorganism species. In particular, molecular identification targets the 16S rDNA gene because it contains conserved and highly variable sequences that can be used to identify groups and species of anaerobic microorganisms. The method consists of the following four steps:

DNA Extraction → Amplification → Sequencing (if necessary) → Identification

The most common experimental methods using these steps to assess the presence of dechlorinating species and available on a commercial basis are summarized in Table 4.2. These include PCR and denaturing gradient gel electrophoresis. Other analytical methods may be used by university researchers, but are beyond the scope of this discussion.

Polymerase chain reaction. Once rDNA has been extracted from environmental samples (typically by enzymatic/chemical methods), it is amplified using PCR. PCR targets specific regions within the 16S rDNA by using short pieces of DNA (called PCR primers) that are chemically synthesized and have a known sequence that will bind to the corresponding complementary sequence of the 16S rDNA gene. The PCR process replicates (i.e., amplifies) a specific-sized piece of this gene. The ability to detect specific gene sequences is highly dependent on the availability and quality of appropriate PCR primers.

The PCR products are transferred to a gel that is then subjected to a current of electricity (standard gel electrophoresis). The amplified PCR products have a known size, and will migrate to a location based on their molecular size (as verified by an internal "ladder" of gene fragments of known size). If the PCR product is present, it will form a visible band when exposed to ultraviolet light (Figure 4.2, photo courtesy of Microbial Insights, Inc.). Gel bands of expected size indicate a positive result for bacterial species (e.g., *Dehalococcoides*), whereas the absence of bands indicates that the species is not present. In this case, the sequencing and identification is inherent to the primers used. Note that Sample 7 in Figure 4.2 indicates a negative response for *Dehalococcoides.*

Although typical PCR is not truly quantitative, there may be a correlation between the “band intensity” and the initial amount of gene extracted from the sample. A correlation must first be established for any given location, and band intensity may reflect the bias in the PCR method itself. Quantitative real-time PCR methods are being developed (available now on a limited basis) that can be used to establish the initial number of genes present in a sample. Quantitative PCR is subject to detection limits, and is not an absolute method to determine the presence of a targeted species. Furthermore, the concentration of genes in environmental samples that can be used to determine whether complete anaerobic reductive dechlorination will occur has not been established.

Table 4.2 Molecular Genetic Identification Methods

Test Method	Description	Information Provided	Usefulness	Information Not Provided	Example References
Polymerase Chain Reaction with Gel Electrophoresis	Qualitative amplification method for DNA sequencing and identification.	Qualitative identification of microorganism species based on use of species-specific primers.	High correlation between complete degradation of chlorinated ethenes and presence of *Dehalococcoides.* Can screen multiple areas of site.	Specific only for known dechlorinators, such as *Dehalococcoides*, for which primers have been developed. May exclude other species or consortia known to have similar capabilities.	Fennel et al., 2001; Hendrickson et al., 2002a and 2002b
Real-time Polymerase Chain Reaction with Gel Electrophoresis	Quantitative amplification method for DNA sequencing and identification.	Quantitative identification of microorganism species based on use of species-specific primers.	Same as above. Changes in concentration of known dechlorinating species over time indicate that growth of the targeted species has been stimulated.	Same as above.	Ritalahti et al., 2003
Denaturing Gradient Gel Electrophoresis	Analysis provides a determination of the types of organisms present and their general physiological status.	Qualitative identification of multiple species based on use of "universal primers."	Provides detailed information on microbial community. Can identify dechlorinators whose extracted 16S rDNA genetic sequence is established in a genetic database.	Specific only for known dechlorinators; excludes other species that have not yet been identified.	White et al., 1997; Stahl, 1997

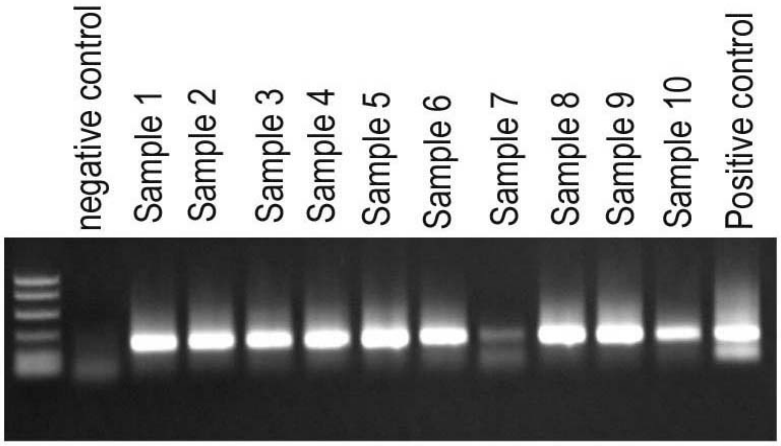


Figure 4.2 Gel Band Responses for *Dehalococcoides* Species for Multiple Samples.

In contrast, **denaturing gradient gel electrophoresis** uses "universal primers" that will similarly attach to the 16S rDNA gene and cause it to be replicated. However, instead of replicating the gene for just one microorganism, it replicates the genes for all the microorganisms in the sample. The base-pair sequence from each microorganism is unique, and will "unwind" at different rates as it migrates through a gel and is exposed to increasing concentrations of urea. When the 16S rDNA gene fully opens, it stops migrating within the gel. As a result, different bands form (versus the single band in PCR), with each band representing one species or strain of bacteria (Figure 4.3).

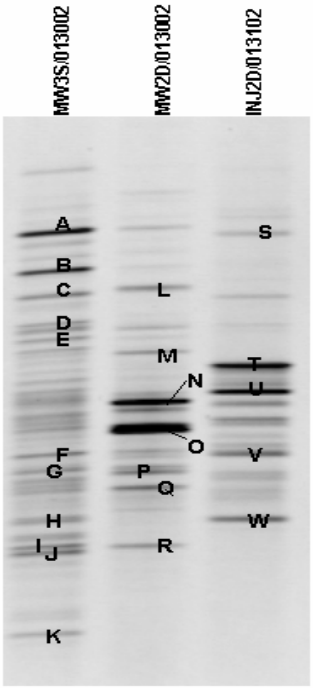


Figure 4.3 Denaturing Gradient Gel Electrophoresis Image of Amplifiers from a Conserved Region of Bacterial 16S rDNA

Banding patterns and relative intensities of the recovered bands provide a measure of differences among the communities. Labeled bands are excised and sequenced.

To sequence and identify the species present, the bands are then excised from the gel and the amplified 16S rDNA gene is sequenced (profiled) to obtain the order of base-pairs of nucleotides. The sequence of nucleotides is compared to a database of known microorganisms (such as the GenBank database) to obtain the identification. In this case, identification of the microbial species present depends on whether they can be matched in available databases to established gene sequences.

4.5.2 Using Molecular Identification Data

Of these two methods, PCR is the most commonly used because of its ease, and because it can be adjusted to target only the species or strains of interest. However, experimental molecular techniques alone should not be used as a site screening tool to determine the potential for complete anaerobic dechlorination to be stimulated by substrate addition. This is mostly due to the following:

1) These methods are subject to minimum detection limits with the potential for false negatives or positives (Section 4.5.3). This is particularly the case for Type 2 and Type 3 sites, where there is insufficient organic substrate or reducing conditions for the growth and development of anaerobic dechlorinating bacteria. Suitable bacteria may be present, but are either inactive or at too low a concentration for detection.

2) These techniques cannot currently identify the particular strains of dechlorinating bacterial species (e.g., *Dehalococcoides*) known to facilitate all of the sequential steps to innocuous end products. Current 16S rDNA gene-based approaches alone are insufficient to distinguish *Dehalococcoides* populations with different dechlorination characteristics.

3) PCR analysis overlooks many other species of bacteria that are capable of anaerobic dechlorination. It remains to be seen if there are other organisms in the environment as yet unidentified that can catalyze the conversion of *cis*-DCE or VC to ethene.

Rather, these tools are better suited as supplemental analyses for evaluation of microcosm or field pilot studies once anaerobic conditions suitable for the growth and development of native dechlorinating bacteria have been induced (i.e., microbial populations are sufficient for positive identification). Molecular screening techniques also may be suitable as a diagnostic tool for optimization of bioaugmentation systems after implementation, where the targeted microbial species can be positively identified by the analytical method.

While direct detection of *Dehalococcoides* species does indicate the potential to achieve complete dechlorination, there are differences in the ability of different strains of *Dehalococcoides* to dechlorinate the chlorinated ethene sequence, and strain identification may be required. Furthermore, a single direct detection does not mean that *Dehalococcoides* is sufficiently active in the subsurface for effective dechlorination, whereas increasing signal PCR "band intensity" or quantitation over time for the same sampling point would suggest the growth and activity of *Dehalococcoides* species.

Research indicates that different strains of dechlorinating bacteria have specific reductase genes that indicate the dechlorination capacity of the microorganism for different chlorinated compounds (Müller, et al., 2004; Krajmalnik-Brown, et al., 2004; and Hölscher, et al., 2004).

Gene probes capable of identifying and sequencing these reductase genes are in development and may one day allow microbiologists to determine the dechlorination capacity of the strains of dechlorinating species detected in field cultures. Further research and development is required before this method is suitable for or commercial use.

4.5.3 Potential for False Negatives and False Positives

The detection of *Dehalococcoides*-related microorganisms (or other dechlorinating species) is subject to false negatives due to practical detection limits and microbial heterogeneity. Analytical results depend on the primer sets used, and commercial and research laboratories use different primer sets. The absence of detectable *Dehalococcoides* in several site samples only suggests (but is not conclusive) that *Dehalococcoides* organisms are absent from the site. Non-detection of *Dehalococcoides* may be a result of the detection limit of the assay, or due to aquifer heterogeneity. Detection limits are a concern, although PCR assays can detect as few as 100 gene copies per liter. Aquifer heterogeneity may result in a particular sample not containing *Dehalococcoides* DNA, even at sites that contain this organism at other locations.

DNA may not be extractable from a sample simply because a particular sample contains no (or very low) biomass and not because *Dehalococcoides* is actually absent from the site. Groundwater samples are typically analyzed, whereas biomass is overwhelmingly associated with the solid aquifer matrix (soil). The proper collection and analyses of soil samples is more specialized and costly relative to groundwater samples. Confirmation of DNA extraction and the use of multiple PCR primers is recommended to limit the potential for false negatives. Confidence in negative results can be increased when a larger number of samples are assessed and when "non-*Dehalococcoides* bacterial DNA" is detected in these samples. This indicates that DNA was successfully extracted from the samples but that *Dehalococcoides* DNA was not detectable.

The potential also exists for false positives due to interferences. For example, Ritalahti et al. (2003) advise caution when using terminal restriction fragment length polymorphism for 16S DNA analysis, as there is potential for false positives using this method. This is another analytical molecular screening technique that is less commonly used.

4.6 WHEN SHOULD BIOAUGMENTATION BE CONSIDERED?

Some practitioners use bioaugmentation in an attempt to overcome the problem of DCE or VC accumulation or stall and to accelerate complete dechlorination. Bioaugmentation can shorten lag times or improve the rate of dechlorination in environments where native dechlorinating species are poorly distributed, present at low population densities, or not an ideal strain.

Bioaugmentation is not a universally accepted practice, and at present there is insufficient field experience to make its benefits and risks clear. Much of the disagreement revolves around adaptation time. It has been observed at numerous locations that dechlorinating species require as long as 12 to 36 months of substrate addition to grow to concentrations that provide timely and complete dechlorination of DCE and VC to ethene. Therefore, most practitioners agree that given sufficient substrate and the right geochemical conditions, populations capable of complete dechlorination will eventually appear.

Microbial sufficiency and life-cycle costs can be assessed to determine when to use bioaugmentation. One approach is simply to bioaugment as soon as anaerobic conditions are induced after system start-up. Another approach is to first add an organic substrate to a site for a period of up to 2 years; if it is clear that sufficient dechlorination is not occurring, then bioaugmentation could be attempted.

The risk associated with bioaugmentation is largely the adaptability and competitiveness of the introduced strain and the additional cost; many practitioners believe that bioaugmentation is unnecessary at most sites. The risks associated with waiting 2 years are largely the delay and cost of operations during the period of ineffective treatment. As more providers of bioaugmentation products enter the market, the cost of bioaugmentation should decrease substantially.

Bioaugmentation is a potential option for any bioremediation project, either from initiation or as a contingency measure should the bioremediation project stall at intermediate dechlorination products or fail to produce significant biodegradation.

Bioaugmentation should be considered when native dechlorinating species capable of complete dechlorination of the CAHs are not present, are poorly distributed, or are present at low population densities.

However, bioaugmentation may not be suitable for many sites, and bioaugmentation cultures are not readily available for all classes of CAHs.

A pragmatic approach many practitioners follow is to do a cost/benefit analysis considering the cost of bioaugmentation, its potential benefits, and the risk of not using bioaugmentation.

Section 5.4 and Appendices E.9 and E.13 present approaches in use by bioaugmentation practitioners; their presentation here is not intended as an endorsement of these approaches. It is possible that bioaugmentation will, in time, become more proven and widely accepted, but at present it is still a developing technology.

The assessment as to whether bioaugmentation is desirable may occur at various stages in implementing enhanced anaerobic bioremediation (Figure 4.1). In questionable cases (i.e., where the feasibility of enhanced bioremediation is not clear up front), assessing the need for bioaugmentation may begin prior to field implementation. If site screening indicates unfavorable biogeochemical conditions, then microcosm studies may be the first step in evaluating microbial sufficiency. If complete dechlorination is not observed in microcosms, then bioaugmentation cultures may be added to determine if this approach can establish complete dechlorination.

More commonly, an assessment of the need for bioaugmentation will be made when pilot- or full-scale field test performance data fail to meet remedial objectives (e.g., accumulation of *cis*-DCE). In such cases, assessing the value of or need for bioaugmentation at a given site is based on the observed extent and rate of dechlorination, as well as whether a sufficient acclimation period has been allowed. Appropriate geochemical conditions and sufficient substrate loading should be ruled out as causes for incomplete dechlorination before bioaugmentation is considered.

A common and reasonable practice is to do a cost/benefit analysis before proceeding with bioaugmentation. The practitioner should consider the cost of bioaugmentation and weigh that against the risks of proceeding without it. At some sites, the cost of bioaugmentation will be less than the cost of conducting tests to determine its necessity. The question of time is also important; if achieving complete dechlorination over a longer period (on the order of a year or so) is acceptable, then it makes sense to start the process without bioaugmentation. If there is more urgency and cost is less of a concern, then it could be reasonable to bioaugment from startup.

In summary, bioaugmentation is an emerging practice that some practitioners believe holds the promise of faster, more effective remediation. It is an option for any bioremediation project, either from initiation or as a contingency measure should the bioremediation project (without bioaugmentation) stall at intermediate dechlorination products or fail to produce significant biodegradation. Laboratory microcosm and pilot-scale field tests are currently the primary mechanisms for conducting these evaluations. As field applications of this new technology increase, that body of information should eventually provide practitioners and RPMs with a basis for more accurate and reliable predictions of the cost and performance of using bioaugmentation.

SECTION 5

SYSTEM DESIGN AND ENGINEERING

There are a number of system and engineering design considerations for applying enhanced anaerobic bioremediation: remedial objectives and suitable technical approaches, system configurations, substrate options, substrate delivery options, mixing and delivery systems, implementation constraints, and implementing bioaugmentation. The primary objective of a system design for enhanced bioremediation is to effectively deliver the substrate throughout the subsurface environment at a rate that creates and maintains environmental conditions optimal for anaerobic dechlorination of CAHs. Hydrogeology, groundwater geochemistry, and microbiology are site-specific conditions that may place constraints on system design, and should be kept in mind throughout the design process.

The different systems described in this section also vary in the amount of capitol construction and O&M needed to implement them. A cost estimating tool in development by the Air Force, Navy, and ESTCP (Project CU-0125) is useful in evaluating the relative costs of implementing varied system designs. The program will be available on the ESTCP (www.estcp.org) and AFCEE (http://www.afcee.brooks.af.mil/products/techtrans/treatmenttechnologies.asp) web pages.

5.1 REMEDIAL OBJECTIVES AND TECHNICAL APPROACH

In general, the remedial objective of enhanced anaerobic bioremediation is restoration of contaminated groundwater to pre-existing levels of beneficial use. As discussed in Section 3.1, typical remedial objectives which may be addressed via engineered anaerobic bioremediation include remediation or containment of CAH source areas or dissolved plumes. Project- or site-specific remedial objectives may vary accordingly.

Objectives for bench- or pilot-scale applications are less comprehensive based on the reduced scale of application. The objective of bench-scale tests may be to demonstrate microbial sufficiency or to select an optimal substrate in terms of utilization and efficiency. The objectives of pilot-scale field tests typically include demonstrating the ability of enhanced *in situ* bioremediation to stimulate complete dechlorination of CAHs to levels that would meet site-specific regulatory goals, and refinement of engineering design criteria for full-scale application.

The appropriate technical approach for implementing enhanced anaerobic bioremediation will be site-specific and based on a strategy that considers final remedial objectives, feasibility of the application, regulatory issues, and cost. System configurations that can be used to meet these remedial objectives are described in the following section.

5.2 SYSTEM CONFIGURATIONS

The following subsections describe the three most common strategies and associated system configurations for applying enhanced bioremediation to source areas, in containment biobarrier configurations, and for plume-wide treatment. Figure 5.1 graphically illustrates common configurations of injection wells for source area grids and linear containment biobarriers.

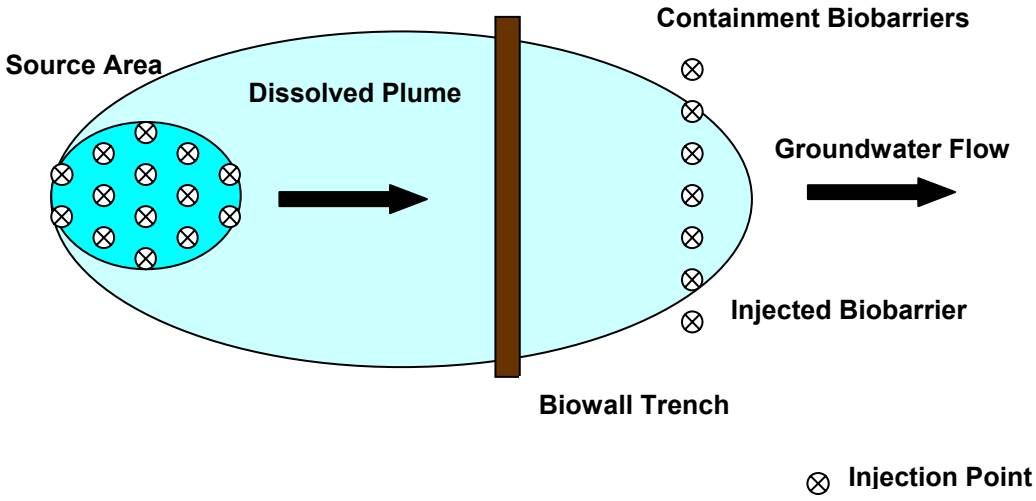


Figure 5.1: Schematic of Source Area and Biobarrier Injection Configurations

5.2.1 Source Area Treatment

All site closure strategies ultimately have to address contaminant sources. ***Without source treatment and accompanying reduction of mass flux from the source area, enhanced anaerobic bioremediation strategies that treat only dissolved contaminants may require operation for an indefinite period of time.*** Historically, other treatment options for source reduction have been employed at sites where DNAPL sources have either been identified or inferred based on dissolved concentrations that approach solubility thresholds. With some special considerations, remediation of DNAPL sources using enhanced anaerobic bioremediation holds promise (e.g., Adamson et al., 2003).

Applications of enhanced anaerobic bioremediation have been successful at reducing contaminant concentrations at several sites with DNAPL (observed or inferred) using a variety of substrates including lactate (e.g., Appendix E.1), molasses (e.g., Appendix E.2), HRC® (e.g., Appendix E.4), and vegetable oil (e.g., Henry et al., 2003a).

Source area treatment is often employed in situations where downgradient migration of the dissolved contaminant plume is being adequately controlled by natural attenuation processes or by another remediation process, such as a biobarrier or hydraulic containment. Substrate addition may cease once contaminant concentrations in the source area have been reduced to target concentrations. However, there has been inadequate study to date to determine the potential for rebound in contaminant concentrations (due to the continued presence of

untreated DNAPL or sorbed contaminant mass) once the source area aquifer becomes less reducing. In the event of contaminant rebound, it should be relatively easy to restore the anaerobic reactive zone with renewed substrate addition.

Another emerging strategy for source area treatment, for which little field data has been published to date, is the injection of a low solubility hydrophobic material such as vegetable oil directly into the source zone for sequestration of contaminant mass. The vegetable oil enables short-term sequestration of contaminant mass due to partitioning into the oil and a reduction in mass flux in groundwater due to a lowering of hydraulic conductivity. Long-term contaminant destruction is achieved by providing a carbon source to stimulate anaerobic dechlorination. Ultimately the oil will degrade and contaminant mass will be released from the oil back into an environment that is optimal for anaerobic dechlorination to occur. This strategy was employed by the Air Force at the Hangar K site at CCAFS, Florida (Parsons, 2002b) and at the Landfill 5 site at Hickam AFB, Hawaii (Parsons, 2003) using direct injection into the DNAPL source area. The Army used a different approach at the Defense Depot Hill Utah BRAC-51 site by spraying vegetable oil into a source zone excavation (Parsons, 2001). The ESTCP is currently funding a project intended to demonstrate and document the sequestration aspects of this process (Sequestration of a DNAPL Source with Vegetable Oil, CU-0319).

Application of enhanced anaerobic bioremediation in a source area may result in temporal fluctuations in contaminant concentrations. This may occur simply by displacement, or potentially by mass transfer of the source constituent and dechlorination products to the aqueous phase due to enhanced dissolution from DNAPL or desorption of contaminant mass from the aquifer matrix (Section 2.3). While this effect is often not observed or is temporal, RPMs and their contractors should be prepared to account for its occurrence. Frequent and early reporting of monitoring results obtained prior to system equilibration and prior to demonstration of effective biodegradation may be counter-productive.

To intercept and treat any mobilized contaminant mass in sensitive situations, it may be desirable to establish a reaction zone downgradient of a source area prior to implementing substrate addition in a DNAPL source (Suthersan et al., 2002). This decision should be based on the relative strength of the source and the nature of the downgradient buffer zone. The greater the source strength and the more sensitive or shorter the downgradient buffer zone is, the greater the need is to control the potential impacts of enhanced dissolution or desorption.

5.2.2 Biobarrier Containment Systems

Biobarrier systems can be used to intercept and treat contaminant plumes as a containment measure. These systems rely on the migration of contaminated groundwater through a permeable reactive zone. Therefore, key design parameters of biobarriers include: 1) a continuous reaction zone, oriented perpendicular to groundwater flow, that is of sufficient cross-sectional area to intercept the entire contaminant plume, 2) sufficient residence time within the reaction zone for the complete dechlorination of contaminants in groundwater, and 3) maintaining permeability to avoid groundwater flow around the barrier system.

Biobarriers are commonly placed along or near a property line or other boundary established for regulatory compliance. The biobarrier itself and the zone immediately downgradient should be viewed as a combined treatment zone such that achievement of site-

specific groundwater quality goals are achieved at the desired downgradient point of compliance. The location of a biobarrier can also be influenced by practical considerations (e.g., located near a road for drilling or trenching access or in available open areas in developed settings).

Passive or semi-passive biobarriers created using soluble substrates typically consist of a series of substrate injection wells established along a line perpendicular to groundwater flow. Continuous or frequent injection is required to maintain the reaction zone. Biobarriers constructed by injection of long-lasting viscous fluid substrates (e.g., HRC® or vegetable oils) may be less expensive to deploy in terms of capital and O&M costs. Eventual replacement of slow-release substrates for biobarrier systems may still be required if the design life for remediation extends longer than the lifespan of the substrate.

Solid substrates placed in trenches may have higher up-front capital costs, but potentially little O&M cost other than performance monitoring. Biowall trenches provide for uniform distribution of substrate because the continuity of the trench eliminates potential problems associated with aquifer heterogeneity. The long-term need for substrate replenishment with solid substrates is not well known, as there has not been extensive experience with these systems. The life-cycle cost for any type of biobarrier containment system will be higher if the source of the CAHs upgradient of the biobarrier is not addressed concurrently.

5.2.3 Plume-wide Treatment Strategies

Plume-wide treatment is an aggressive approach implemented to create an anaerobic reactive zone across large portions of the impacted aquifer, resulting in more rapid remediation. Small plumes may be treated with plume-wide enhanced anaerobic bioremediation systems that address the entire extent of contamination. For this scenario, injection points are typically spaced in a grid pattern or in multiple staggered rows throughout the entire contaminated portion of the aquifer. For larger plumes, it is more likely that forced gradient or recirculation configurations utilizing a smaller number of wells to influence a greater volume of the aquifer will be employed.

Figure 5.2 is an example of a plume-wide treatment configuration used to treat a small plume beneath a former drycleaner site in Wisconsin (Appendix E.2). After an initial injection of molasses during demolition of the existing building, two rows of permanent injection points were constructed and left in place for remedial operations after reconstruction of the facility. In this case, the substrate volume, strength, and injection frequency were sufficient to create a downgradient reaction zone that encompassed the entire footprint of the contaminant plume. Site closure was achieved using this approach (see Appendix E.2).

The higher up-front costs of plume-wide treatment for larger plumes should be weighed against the costs of longer-term O&M and performance monitoring associated with alternate approaches. In addition, the timeframe for remediation can be drastically reduced, reducing long-term liability. However, there is a practical limit to the size of the plume that can be treated due to cost considerations and/or the presence of existing infrastructure. Plume-wide applications may be cost prohibitive for large plumes or cost inefficient for low concentration plumes. In such cases, several approaches can be combined. For example, a source area may be targeted for remediation using a grid configuration, combined with a linear barrier configuration upgradient from a downgradient point of compliance.

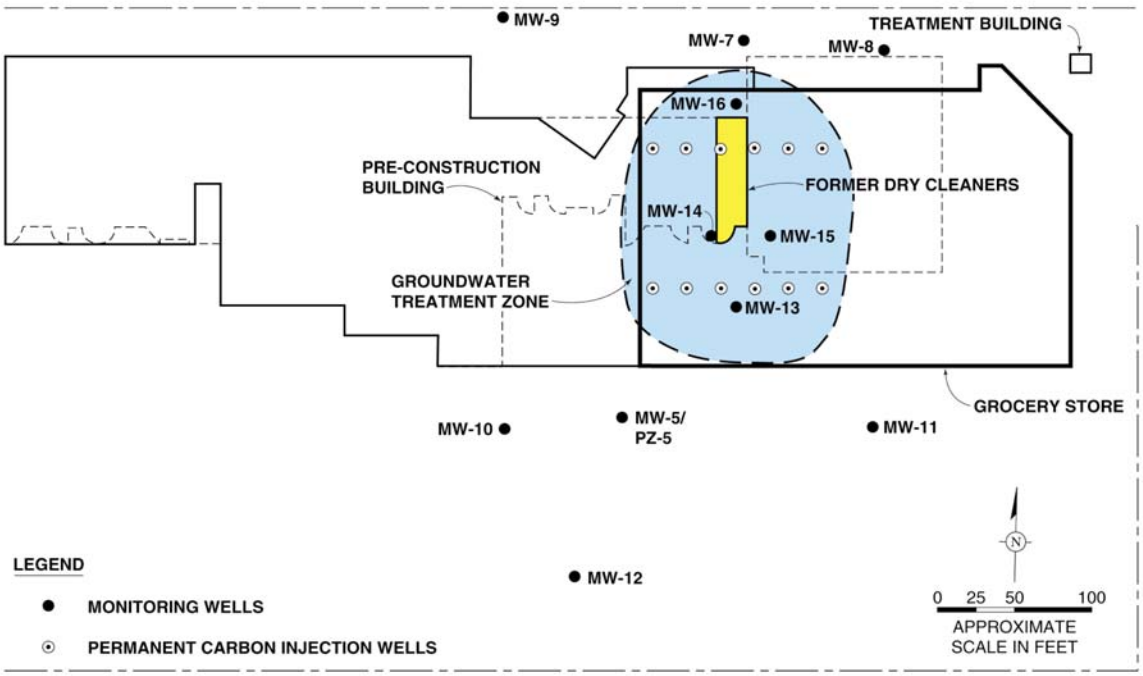


Figure 5.2 Injection Configuration for Plume-wide Treatment of a Small Drycleaner Site

5.3 SUBSTRATE (ELECTRON DONOR) OPTIONS

Selection of a substrate is often based on contractor experience or familiarity, or as a result of commercial marketing. However, there is no reason that the RPM should not consider the full range of substrates and application configurations described in this document, because they all have been shown to stimulate anaerobic dechlorination of CAHs.

There is no reason that the RPM should not consider the full range of substrates and application configurations described in this document.

All substrates described in this section have been shown to stimulate anaerobic reductive dechlorination of CAHs.

As the efficacy of enhanced anaerobic bioremediation is demonstrated in the field for multiple substrate types and under a variety of field conditions, the argument as to what constitutes a "superior" substrate becomes less relevant than matching an appropriate substrate to the site-specific conditions, system configuration, and effective delivery methods. The development of AFCEE protocols for soluble carbohydrates (Suthersan et al., 2002), and for vegetable (edible) oils (in development), are aimed at providing guidance to RPMs and their contractors on implementing the use of these substrates at DoD facilities. In addition, most vendors of bioremediation products provide technical assistance to customers who purchase their product.

Table 5.1 is a summary list of substrates used for enhanced anaerobic bioremediation. The selection of an appropriate substrate should take into account expected performance in developing appropriate anaerobic reactive zones, the rate at which the substrate is used (efficiency of use), substrate availability, and cost to implement (life-cycle cost, including cost of O&M).

Substrates applied for enhanced bioremediation differ in the rate at which the material becomes available for biodegradation and is degraded, in the complexity of their composition, and in their cost. Many substrates are being selected from the wide variety of available low-cost food-grade products such as molasses, HFCS, vegetable oils, and whey. Less complex substrates such as lactate (including HRC®), butyrate, and ethanol may target more specific fermentation reactions. The production of low-molecular-weight acids (e.g., propionate and butyrate) that are further fermented to produce hydrogen is common to degradation reactions that occur with most of these substrate types. Therefore, these substrates are similar with respect as to how hydrogen is generated and how anaerobic dechlorination is stimulated.

Extensive bench-scale work has been performed by numerous researchers to test several potential organic carbon substrates (e.g., Fennel et al., 1997; Gibson et al., 1994; Schollhorn et al., 1997; Gao et al., 1997; Gibson and Sewell, 1992; Lee et al., 2000; Castellanos et al., 2002; Yang and McCarty, 2000a and 2002; Harkness et al., 2003). Many of these microcosm tests have revealed distinctions between substrates in terms of reaction times, degradation rates, and substrate efficiency.

However, caution is advised when transitioning these bench scale results to the field. Many laboratory research studies use highly enriched cultures with basal mediums and nutrients, inoculated at high temperatures and levels of agitation. As a result, they may not be indicative of performance under *in situ* field conditions. When using bench-scale tests to compare substrates for field-scale applications, the tests must use site-specific soil and groundwater, with the tests conducted at realistic temperatures and agitation levels.

A common goal is to minimize overall project cost by minimizing the number of required injection points, the number of injection events, and substrate cost (Harkness, 2000). While attainment of these objectives is typically impacted by the physical and chemical characteristics (i.e. phase and solubility) and cost of the substrates under consideration, none of these objectives will necessarily be satisfied by the selection of any one substrate. For example, the use of large volumes of soluble substrate may minimize the number of injection points; but the substrate itself may have a higher purchase cost, require more frequent injections, and have higher O&M costs (e.g., control of biofouling in wells).

It should be noted that HRC® is the only commercial product listed on Table 5.1 that is formulated and sold specifically for enhanced anaerobic bioremediation. HRC® is sold by Regenesis, a company that specializes in bioremediation products. Although not listed on Table 5.1, other bioremediation products are available, including products for emulsified vegetable oils (EOS Remediation, Inc., and Remediation and Natural Attenuation Services, Inc.) and for lactate and chitin products (JRW Technologies, Inc.). The suppliers of these bioremediation products generally provide technical support; the costs of this support are not charged directly, but are considered overhead and paid out of the product cost. It is therefore possible that the use of these products could reduce other engineering or consulting costs, and this should be considered in the overall cost/benefit analysis.

Table 5.1 Summary List of Substrates (Electron Donors) Used for Enhanced Anaerobic Bioremediation

Substrate	Bulk Price per Pound (dollars)	Level of Experience (number of field applications)	Applications and System Configurations	Key Considerations
Soluble Substrates				
Sodium Lactate	1.00 to 2.00	Moderate (> 20)	Soluble substrates may be used for source area, biobarrier, or plume-wide applications. Applied using direct injection and/or recirculation wells. Direct-push techniques may be used to install small-diameter injection points. Typically installed in rows of injection points. The mobility of soluble substrates allows for greater distance between rows of injection wells relative to slow-release substrates.	Requires periodic injection and process monitoring. Ability to adjust substrate strength, volume, and injection frequency over time is an advantage for optimizing system performance. However, adjusting substrate loading rates and mixing ratios during the initial phase of injection is often necessary to achieve target substrate levels, to avoid adverse impacts to pH, and to maximize radius of influence. Process monitoring and optimization increase the cost of O&M during startup, and the life-cycle cost of O&M for soluble substrate systems is high relative to other substrate options. Maintenance may be required for biofouling.
Propionate, Butyrate	2.00 to 3.00	Low (< 10)		
Methanol, Ethanol	0.10, 0.20 to 0.25	Low (< 10)		
Molasses	0.25 to 0.35	High (> 100)		
Refined Sugars (high fructose corn syrup)	0.25 to 0.30	Moderate (> 20)		
Slow-Release Substrates				
Hydrogen Release Compound (HRC®)	5.00 to 7.00	High (>400)	Slow release substrates may be used for source area or plume-wide treatment in grid configurations, or may be used for biobarriers using rows of injection points perpendicular to groundwater flow. Well spacing on the order of 5- to 15-foot centers is required. Use of direct-push injection in shallow contaminant plumes is the most cost-effective application. May also be injected into deeper injection wells, although HRC® will require special handling (such as heating or pushing the substrate with glycerin).	HRC® may be effective for periods of 9 to 18 months and typically requires reinjection; HRC-X™ may be effective for 3 to 4 years. There may be practical limits to the depths that HRC® can be injected. Vegetable oils are a potential one-time application with effectiveness demonstrated for periods of up to 3 years. Emulsification is typically required for effective distribution. High saturation emulsions may lower hydraulic conductivity. Use of dilute emulsions may require additional injection after 12 to 24 months.
Vegetable Oil / Commercial Emulsion Products	0.20 to 0.40/ 2.00 to 4.00	Moderate (> 40)		

(continued)

Table 5.1 Summary List of Substrates (Electron Donors) Used for Enhanced Anaerobic Bioremediation (concluded)

Substrate	Bulk Price per Pound (dollars)	Level of Experience (number of field applications)	Applications and System Configurations	Key Considerations
Experimental Substrates				
Whey (fresh/powdered)	0.05 (fresh)/ 1.00 to 1.50 (powdered)	Experimental (< 5)	Powdered whey is water soluble and may be applied in a manner similar to soluble substrates. Fresh whey is a viscous fluid and may be used in a manner similar to HRC® and vegetable oil emulsions.	The long-term effectiveness of whey is under study, and may be limited to three to 12 months. Whey will require some special handling (mixing) to prepare for injection.
Mulch and Compost	0.00 to 0.25[a/]	Experimental (< 5)	Typically installed in trenches as biobarriers. Also may be used for as backfill of source excavations, or as surface amendments.	Installation uses established construction methods. Long-term effectiveness under study. Mulch is often a free commodity in most communities. However, costs up to 10 dollars per cubic yard may be incurred for processing and handling.
Chitin	2.00 to 4.00	Experimental (< 5)	Source area or biobarrier applications using slurry injection or conventional trenching techniques	Delivery techniques still in development; effectiveness still under study.
Hydrogen Gas (direct electron donor)	0.05 to 0.11 (per standard cubic foot)	Experimental (< 5)	Source area grids or biobarriers using biosparge wells, permeable membranes, or recirculation with hydrogen amendment.	Delivery techniques still in development; effectiveness still under study.

The use of multiple substrate types in combination also is rapidly growing. In aquifer systems that are naturally aerobic, it may be desirable to use an easily distributed and highly degradable soluble substrate (e.g., ethanol or lactate) to rapidly induce anaerobic, reducing conditions and reduce the microbial lag phase attributed to anaerobic bacteria. Then a longer-lasting, "slow-release" substrate (e.g., vegetable oil, chitin, or whey) to sustain the reaction zone and used to minimize the cost of maintaining the treatment system. An example of this can be found in Appendix E.3, where HFCS and whey were combined as a fast-acting and slow-release substrate combination. Regenesis has used this approach with the production of fast-acting HRC®-primer and longer-lasting HRC-X™ products (Appendix E.5). Thus, using a mixture or combination of fast- and slow-acting products may be desirable in some cases.

Important factors to consider in selecting a substrate include application configuration, delivery and distribution requirements, system O&M requirements, site infrastructure or land use, and overall life-cycle cost. The following subsection describes delivery options, and a more detailed description of the design approach and mixing and delivery systems for each of the substrate types is included in Section 5.5.

5.4 SUBSTRATE DELIVERY OPTIONS

There are a multitude of system configurations and delivery strategies that can be used to distribute organic substrates in the subsurface. Injection of liquid substrates directly through direct-push or permanent injection wells, groundwater recirculation systems, infiltration galleries, and trenches all may be used to deliver substrate to the impacted aquifer. Table 5.2 summarizes these delivery options according to substrate type and system configuration.

Table 5.2 Enhanced Anaerobic Bioremediation Delivery Options

	System Configuration		
Substrate Type	**Source**	**Barrier**	**Plume-Wide**
Soluble (e.g., lactate, molasses)	Periodic injection into source. Recirculation across source.	Periodic injections into linear injection well configurations oriented perpendicular to groundwater flow (substrate drift).	Periodic injections in grid arrays or multiple linear rows of wells. Large-scale recirculation (extraction and injection).
Slow-Release (HRC®, vegetable oils)	Infrequent injection into source (may be one time for vegetable oils).	Infrequent injection into linear rows of injection points oriented perpendicular to groundwater flow.	Infrequent injection in grid arrays (may be one time).
Solid Substrates (mulch, chitin)	One-time or very infrequent addition (e.g., placement in source area excavation).	One-time or very infrequent addition to linear trenches oriented perpendicular to groundwater flow.	May not be practical for large plumes. Potential using combination of source and multiple barrier configurations.
Gaseous Hydrogen (experimental)	Biosparge injection into source (pulsed injection).	Biosparge injection in linear rows perpendicular to groundwater flow (continuous to semi-continuous).	May not be practical for large plumes.

5.4.1 Direct Injection

The most commonly used methods to deliver liquid substrates are via installed injection wells or direct-push well points, or by direct injection through temporary direct-push probes.

Direct-push methods are commonly used for shallow groundwater applications in unconsolidated formations at depths less than approximately 50 feet. This technique is constrained by soil characteristics such as grain size (i.e., gravel and cobbles inhibit use of direct-push technology) or degree of cementation. Direct injection of liquid substrates can be made through direct-push (e.g., Geoprobe®) probes. This technique does not leave well points in place, and is only practical for long-lasting substrates such as HRC®, vegetable oil emulsions, or whey slurries. These substrates release carbon over periods of 6 months to several years, and typically require injection on 7.5- to 15-foot centers to treat the target zone.

In other cases, direct-push methods are used to install semi-permanent well points having design lives of less than 3 or 4 years. This type of well consists of a small-diameter screen and riser pipe (0.5- to 1.0-inch diameter), and is commonly used where injections will be required, but the long-term need for more permanent wells is minimal. Direct-push well points are suitable for both soluble and viscous liquid substrates, but care must be taken to seal and grout the well points in place to withstand the designed injection pressure and to prevent bypass of the substrate to the vadose zone or ground surface.

Permanent injection wells are typically installed for use with soluble substrates where continuous or multiple injections of substrate or recirculation are required. Use of permanent injection wells is also necessary where depth or soil lithology make use of direct-push technology impractical. Existing monitoring or extraction wells from previous investigation or remediation activities may be used when screened in appropriate horizons and located within appropriate portions of the plume. Horizontal wells can also be employed for shallow or thin contaminant plumes, or for plumes beneath buildings or other structures.

Permanent injection wells are installed using conventional drilling techniques such as hollow-stem auger, air rotary, or rotasonic drilling. Typical well construction consists of 2- to 4-inch-diameter Schedule 40 or 80 polyvinyl chloride (PVC) screen and riser with slotted screens sized for the formation. Because most substrates are injected under pressure, these wells must be properly sealed and grouted to prevent bypass of the substrate to the ground surface or vadose zone.

Injection Well Location and Spacing. Injection well configuration includes injection well layout, injection intervals, and spacing. Injection wells are typically located in rows oriented perpendicular to the direction of groundwater flow; multiple rows of wells may be installed in a grid configuration or to construct multi-line biobarriers (e.g., Figure 5.2).

The depth and thickness of the targeted treatment zone will impact selection of a drilling technique and the vertical spacing of well screen interval(s). The injection well screen should intercept the zone of contaminated groundwater that is to be treated. For thick treatment zones (i.e., greater than 15 to 20 feet), multiple injection points installed in a cluster at each location and screened across different intervals are recommended. Alternately, injection over thick intervals can be performed in a single well that has multiple screens separated by packers during injection.

Horizontal well spacing is primarily a function of the degree to which substrate can be distributed laterally in the vicinity of each injection well. An effective ROI should be calculated based on the volume and type of substrate used, taking into account the mixing and dispersion of the substrate that will occur with advective transport or through a recirculation system. Well spacing perpendicular to groundwater flow may range from 5 feet for passive systems in low permeability silts and clays, to 50 feet or more in high permeability formations utilizing recirculation techniques. More typically, horizontal well spacing for passive systems varies from 10 to 15 feet for viscous fluid substrates, to 20 to 30 feet for larger volume soluble substrate systems. In a low permeability aquifer, distribution of the substrate by advection will be limited, and the system may be diffusion dominated.

Spacing of wells parallel to groundwater flow should be based on migration of substrate at concentrations sufficient to maintain the reaction zone as groundwater migrates along the flow path between the points of injection. Suthersan et al. (2002) recommend a 100-day travel time distance as an optimal spacing of injection wells parallel to the direction of groundwater flow for plume-wide treatment. However, they also suggest that this distance could be increased to trade-off capital and initial operating costs for a longer treatment duration.

5.4.2 Recirculation

The most common recirculation systems are well systems consisting of a closed network of extraction and injection wells (Figure 5.3). Recirculation increases the retention time of contaminated groundwater in the treatment zone. The rate at which groundwater passes through the system depends on the rate of recirculation and the natural groundwater flux through the recirculation system. Therefore, design of recirculation systems must consider hydraulic conductivity, aquifer heterogeneity, and hydraulic gradient.

Substrate amendments applied in recirculation systems are more readily controlled and distributed throughout the treatment zone relative to passive systems. Recirculation systems also are capable of capturing a much greater volume of the aquifer, allowing much greater distances between wells. However, most small-scale recirculation pilot systems still use well spacings on the order of 3 to 10 feet, which is not practical for a full-scale system. Highly permeable and uniform lithologies are required to use well spacings on the order of 50 to 100 feet. Groundwater modeling and tracer testing is therefore highly recommended when designing large-scale recirculation systems.

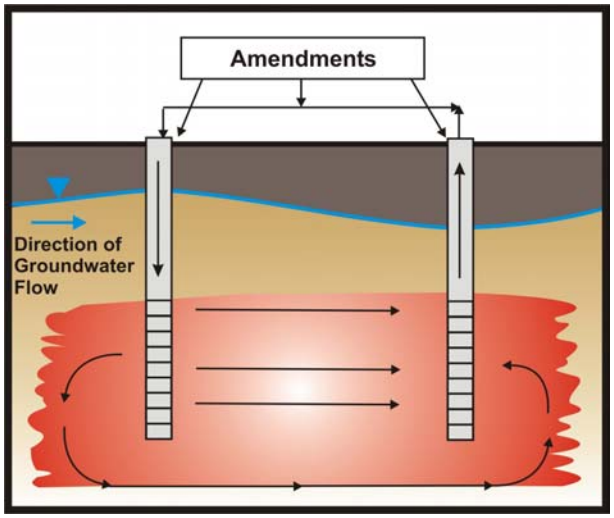


Figure 5.3 Schematic of a Horizontal Recirculation System

Small-scale recirculation systems have most commonly been used in connection with validation of the RABITT protocol (Morse et al., 1998; AFRL et al., 2001) and with bioaugmentation (e.g., Ellis et al., 2000; Major et al., 2002; Lendvay et al., 2003). These pilot

systems were primarily designed to capture the contaminant plume and to achieve an accurate contaminant mass balance to document degradation rates. An example of a larger field-scale recirculation system is described in Appendix E.9 for the Aerojet Facility in California.

A discussion of a number of recirculation configurations also can be found in Section 2 of the ITRC *Technical and Regulatory Requirements for Enhanced In Situ Bioremediation of Chlorinated Solvents in Groundwater* document (1998). Figure 5.4 is a schematic diagram of a vertical circulation system using horizontal injection/ extraction wells. Biofouling of recirculation wells is sometimes an issue (Section 5.6.5), and operating plans may need to include well maintenance schedules.

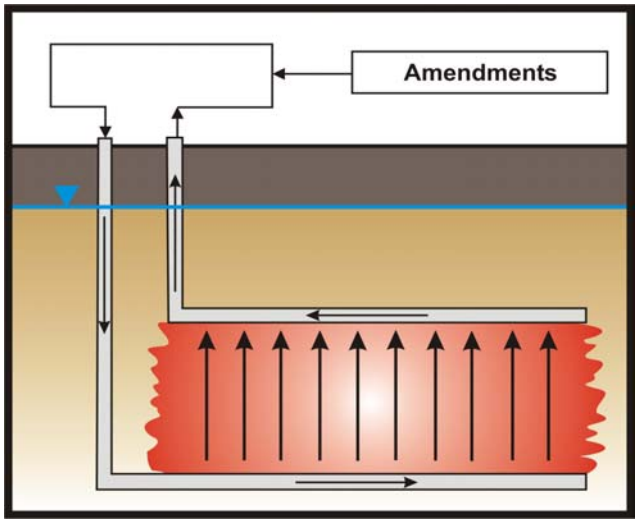


Figure 5.4 Schematic of a Vertical Recirculation System

Recirculation approaches may be the only effective method to achieve more uniform distribution of substrates and amendments at sites with difficult hydrogeological conditions (e.g., lack of a natural hydraulic gradient). Recirculation may also be considered for shorter-term applications that cannot be achieved through less aggressive, more passive methods. For example, recirculation may be useful to circulate groundwater from the greater contaminant plume through an established bioaugmented treatment zone (see Appendix E.4 for an example).

5.4.3 Biowall Trenches

Biowalls using solid substrates are typically constructed in a trench or excavation in a permeable reactive barrier configuration. This treatment method relies on the natural flow of groundwater through the biowall to promote contact with slowly soluble organic matter. This configuration is particularly suitable for low permeability or highly heterogeneous formations, as the formation is physically removed and the biowall trench effectively exposes the contaminant plume to the solid substrate fill material. Perforated pipe can be laid on the top or bottom of the fill material to amend the biowall material with liquid substrates or other amendments in the event the system needs to be modified to deliver more dissolved substrate mass or to alter geochemical conditions. Trenches or infiltration galleries may also be used for gravity flooding of dissolved substrates.

Trenches may be installed using either continuous one-pass trenchers designed for installing subsurface utilities or hydraulic excavators (which are basically backhoes with extended booms). Trench depths are limited by the type of equipment used, the stability of the formation, and the ability of the equipment to excavate the formation. Continuous trenching is not practical in hard, consolidated bedrock. If loose, non-cohesive,

unconsolidated sediments are present, a slurry may be used to keep the trench open during construction.

Other variations of using solid substrates in flow-through configurations include surface amendment infiltration plots (Groundwater Services, Inc. (GSI) 2001; Haas et al., 2000), burial of mulch in excavations, and the recirculation of contaminated groundwater through mulch bioreactors (Parsons, 2003).

5.5 SUBSTRATE MIXING AND DELIVERY

Previous subsections have described options for system configuration, substrate type, and delivery method. This subsection provides further discussion regarding engineering considerations for design of substrate mixing and delivery systems.

5.5.1 Treatment Zone Volume and Radius of Influence

Enhanced bioremediation systems rely on the uniform delivery of substrate throughout the intended treatment zone. As the size of the CAH plume increases, the ability to treat the entire plume volume becomes increasingly difficult and costly. Many large plumes at DoD sites fall into this category (i.e., plumes that are several thousand feet in length and tens of feet thick). Even in barrier configurations that are several hundred feet long, the volume of the treatment zone is enormous. For example, a barrier treatment system 600 feet long by 30 feet wide with an aquifer thickness of 40 feet and a porosity of 40 percent contains approximately 2.15 million gallons of pore water.

Table 5.3 provides an illustrative example of the volume of substrate required to achieve a target ROI for different injection scenarios. This example assumes a uniform and radial distribution pattern that may not be realistic, but provides a useful reference point for the dimensions of potential affected areas. The practitioner should recognize that injection of substrate volumes greater than a few tens of thousands of gallons may become costly and problematic, and cause significant displacement of the contaminant plume. Most practitioners inject lower volumes of higher concentration substrate mixtures and rely on advection and dispersion to mix the substrate with contaminated groundwater. Injection of substrate volumes less than 10 percent of the aquifer treatment zone will limit displacement effects, but this approach requires that relatively high rates of advection and dispersion exist at the site for effective mixing to occur.

Effective mixing of substrate with the contaminant plume is one of the most difficult design challenges for enhanced anaerobic bioremediation.

Injection of large volumes of substrate may cause significant displacement of the contaminant plume.

One approach is to inject a low volume/ high concentration substrate mixture and to rely on advection and dispersion for mixing, but this requires relatively high rates of advection and dispersion to occur.

Recirculation techniques may be required for sites with low rates of groundwater flow to obtain effective mixing of the substrate and contaminated groundwater.

Table 5.3 Examples of Radius of Influence for Varied Injection Scenarios

Radius of Influence (ROI) (feet)	Aquifer Porosity (percent)	Injection Well Screen (feet)	Volume Required to Achieve Target ROI (gallons)
5	30	10	315
10	30	10	1,260
20	30	10	5,040
50	30	10	31,500
50	30	20	126,000

Site-specific information, such as information on more permeable zones or preferential pathways, also should be used to complete similar estimates of substrate distribution potential and configurations. Although heterogeneity and the processes of advection and dispersion can increase substrate distribution, it is important to apply sufficient volumes of substrate to provide reasonable coverage of the target treatment area. This is especially important for the more soluble, readily biodegradable substrates (e.g., lactate or molasses) because these substrate may degrade before the processes of advection and dispersion can affect a wide distribution of dissolved organic carbon.

The amount of substrate mixture required to treat the entire aquifer zone begins to impact the type of delivery system that can be cost-effectively used. The cost to perform an injection or to operate a recirculation system in terms of labor and substrate cost may be high, perhaps approaching the cost of a pump and treat system. As the scale of application increases and the volume of the aquifer to be treated begins to exceed several million gallons, differences in the relative cost of various substrates also become more significant.

Often the approach is to apply a smaller volume of concentrated substrate and depend on advection and dispersion to distribute the substrate throughout the aquifer system. In fact, use of a substrate such as HRC® may rely entirely on this approach. This approach will be less effective in aquifers having a high degree of heterogeneity, with the potential that large quantities of dissolved substrate will migrate along preferential flow paths or that effective substrate distribution will be diffusion limited. The magnitudes of transverse and longitudinal dispersion also will affect the radius of influence achieved. In some cases, closer well spacing may be required to achieve an overlapping radius of influence.

Another issue is the mixing and dilution of substrate with groundwater during transport from the point of injection. This may result in non-uniform substrate concentrations throughout the treatment zone. For soluble substrate systems, a compromise is typically required between frequent injection of high volumes of substrate to maintain a more uniform substrate concentration, and infrequent injection of lower volumes of high-strength substrate as a cost-saving measure. Two factors that influence treatment zone volume and radius of influence are substrate (electron donor) loading and lifespan, as discussed below.

5.5.2 Substrate (Electron Donor) Loading and Lifespan

Substrate demand can be described in terms of the electron acceptor demand exerted by the following three categories:

- **Contaminant Electron Acceptor Demand**. Since the CAH mass serves as an electron acceptor during anaerobic dechlorination, there is a stoichiometric relationship for the electron donor (e.g., hydrogen) required to satisfy the electron acceptor requirements.

- **Native Electron Acceptor Supply.** The flux of groundwater and minerals on the solid aquifer matrix include electron acceptors that in many cases are preferentially used over CAHs. Therefore, their presence exerts a demand on the electron donor required to satisfy the removal of more energetically favorable electron acceptors, which must occur before conditions conducive to anaerobic reductive dechlorination are established.

- **Non-Specific Demand.** In a perfect world, one could conduct a complete mass balance and identify each mechanism of substrate utilization. Although the above two substrate demands could encapsulate the full spectrum of substrate demand, a practitioner of enhanced bioremediation must expect that a large percentage of injected substrate, resultant organic acids, hydrogen, and other byproducts will be used by opportunistic microbes for a myriad of life processes, including cell growth. In addition, numerous transformations of the solid mineral matrix may occur. Thus, there is a non-specific substrate demand that is not practical to calculate.

In addition to substrate demand, it is never possible to achieve a high efficiency for either substrate/contaminant contact or substrate utilization. Therefore, practitioners typically include a substantial safety factor when determining substrate loading rates. The combined substrate demand must be met until a contaminant source is depleted or until remedial goals have been met. The practitioner should attempt to estimate the contaminant and electron acceptor demand using site characterization data. "Non-specific" demands and the necessary safety factor can best be semi-quantitatively estimated using information from field pilot tests.

The substrate should be applied at a rate sufficient to lower redox conditions and induce anaerobic dechlorination, but should not be consumed at such a high rate as to be rapidly depleted before migrating throughout the desired treatment area. Limiting the amount of substrate may result in large portions of the treatment area remaining too oxidizing for complete dechlorination. A limited area of excessive substrate (e.g., in the immediate vicinity of the injection wells or substrate source) may be acceptable to provide sufficient substrate after mixing with groundwater to maintain appropriate levels of organic carbon throughout the entire treatment zone.

Substrates that are rapidly depleted require more frequent injection to develop and sustain sufficiently reducing conditions. Hydrogen gas is the most bioavailable and rapidly utilized substrate, while soluble substrates such as methanol are also considered to be readily bioavailable and are therefore depleted relatively quickly (within days to a couple of weeks). Table 5.4 lists the range of substrate concentrations typically used in field applications, and the injection frequency and life-span that can be anticipated with their use.

Table 5.4 Typical Substrate Loading Rates, Injection Frequencies, and Lifespans of Common Organic Substrates

Substrate	Injected Form and Concentration	Targeted Concentration in the Formation	Typical Injection Frequency	Typical Lifespan	Examples
Sodium Lactate, Potassium Lactate, Lactic Acid	Diluted to 3 to 30 percent by weight	50 to 300 mg/L	Continuous to bi-monthly	7 to 60 days	Appendices E.1 and E.10
Butyrate	Diluted to 3 to 30 percent by weight	50 to 300 mg/L	Continuous to bi-monthly	7 to 60 days	Appendix E.10; Morse et al., 1998
Methanol	Diluted to 3 to 30 percent by weight	50 to 300 mg/L	Continuous to weekly	1 to 7 days	Appendix E.10; Morse et al., 1998
Ethanol	Diluted to 3 to 30 percent by weight	50 to 300 mg/L	Continuous to weekly	1 to 7 days	Appendix E.9; Jawitz et al., 2000
Sodium Benzoate	Diluted to 3 to 60 percent by weight	50 to 300 mg/L	Continuous to weekly	1 to 7 days	Turpie et al., 2000; Beeman et al., 1994
Molasses	Diluted to 1 to 10 percent by weight	50 to 500 mg/L	Daily to quarterly	7 to 90 days	Suthersan et al., 2002; Appendices E.2 and E.11
High Fructose Corn Syrup	Diluted to 1 to 10 percent by weight.	50 to 500 mg/L	Daily to quarterly	7 to 90 days	Suthersan et al., 2002; Appendix E.3
Whey (fresh/powdered)	Powdered form can be dissolved; fresh form can be injected as a slurry.	50 to 500 mg/L	Monthly to annually	1 to 12 months	Suthersan et al., 2002; Appendix E.3

(Continued)

Table 5.4 Typical Substrate Loading Rates, Injection Frequencies, and Lifespans of Common Organic Substrates (continued)

Substrate	Injected Form and Concentration	Targeted Concentration in the Formation	Injection Frequency	Typical Lifespan	Examples
Hydrogen Release Compound (HRC®)	Pure product injected at 4 to 12 pounds per vertical foot of injection.	100 to 500 mg/L	Annually to biennially for HRC® (one-time injection may suffice in some cases). One-time injection for HRC-X™ product.	9 to 18 months for HRC®; 3 to 5 years for HRC- X™	Appendices E.4, E.5, and E.13
Vegetable Oil (food-grade soybean oil)	Oil-in-water emulsions with 5 to 15 percent oil by volume; or neat oil injection (source areas only). Water push typical.	100 to 500 mg/L	One-time emplacement. May require a second injection for very dilute emulsions.	2 to 5 years	Appendix E.6; Lee et al., 2001; Skladany et al., 2001; Henry et al., 2003a
Mulch and Compost (cellulose)	Mixed with sand at 20 to 60 percent mulch or compost by volume.	100 mg/L to 1,000 mg/L TOC within biowall reaction zone	One-time emplacement	Unknown, thought to be 5 years or more	Appendix E.7; Henry et al., 2003b; Cowan, 2000
Chitin	Powdered form injected as a slurry or bulk product in a trench.	100 to 500 mg/L	One-time emplacement	Unknown, thought to be 5 years or more	Martin et al., 2002; Sorenson et al, 2002a; Harkness et al., 2003
Hydrogen Gas	Pure hydrogen gas or less volatile mixtures with nitrogen.		Continuous (permeable membranes) to weekly (pulsed gas sparging)	1 to 7 days	Appendix E.8; Newell et al., 2001 and 2002

The rate at which organic carbon is delivered to the aquifer (i.e., loading rate) depends on: 1) the volume of substrate (or substrate mixture), 2) the concentration of the active ingredients in the substrate mixture, 3) the frequency of injection, and 4) the degree of groundwater flux through the treatment zone and resulting rates of mixing and dilution. Substrate loading rates are typically reported as mass of substrate per unit volume of groundwater treated.

A substrate loading rate is calculated such that native electron acceptors are fully utilized (depleted) in the reactive zone, while at the same time leaving sufficient electron donor to dechlorinate the contaminant mass flux. Generally these calculations are based on stoichiometric reactions using hydrogen equivalents to calculate the substrate mass required to deplete the available electron acceptor flux. These computations require that the substrate composition, stoichiometry, and utilization efficiency of the anticipated degradation reactions be known.

In practice, the exact stoichiometric reactions and electron acceptor flux that will occur in a natural subsurface system is difficult, if not impractical, to determine. In practice, calculations for determining substrate demand are derived in terms of theoretical hydrogen equivalents produced from a known mass of substrate versus estimated electron acceptor demand. A safety factor of 5 to 20 times may be used to account for the presence of DNAPL or sorbed contaminant mass; for uncertainty in estimating substrate utilization for alternate electron accepting processes (e.g., methanogenesis or solid-phase alternate electron acceptors); and to provide for a design contingency. Care should be taken to use a loading rate that is not excessive (i.e., use of excessive safety factors) to avoid creation of low pH conditions or secondary impacts to groundwater quality.

For soluble substrates, this loading rate is factored into the amount of substrate delivered per injection event. For slow-release substrates, the loading rate is multiplied by the design life of the system (typically 1 to 5 years) and all the substrate is injected at once. These calculations should be used only as initial guidelines for calculating substrate loading rates (Appendix C). Field data collected during pilot testing provide a direct indication of the effectiveness of a particular loading rate, and whether it is appropriate for stimulating anaerobic dechlorination or whether modifications are required.

Alternately, the substrate loading rate for soluble substrates is often based on achieving an empirical TOC concentration in the groundwater that passes through the treatment area. The volume and strength of the substrate are estimated to achieve a particular target level in the aquifer after mixing and dilution. For example, Suthersan et al. (2002) suggest that loading rates for soluble substrates of between 0.001 and 0.01 pounds of organic carbon per gallon of groundwater flux per day are sufficient to create and maintain a reducing reactive zone. The loading rate should also be sufficient to maintain between 50 and 100 times as much TOC in the reactive zone as there is CAH in the target area (i.e., 50 to 100 mg/L of TOC for every 1 mg/L of CAH). Soluble organic substrate will be degraded and depleted as it flows with groundwater, causing a TOC gradient between the point of injection and the downgradient treatment zone. This is why higher concentrations of TOC are required at the point of injection to maintain sufficient TOC concentrations throughout the designated downgradient treatment zone.

Typical substrate lifespans are listed in Table 5.3. In theory, the practitioner has the option of using low-volume/high-concentration or high-volume/low-concentration injections. However, the system will operate more effectively if optimal conditions are uniformly maintained. Infrequent injections of high-concentration substrate mixtures tend to disrupt maintenance of uniform conditions. Establishing a balance between frequency of injection, substrate concentration, substrate duration (lifespan), and maintenance of optimal conditions for anaerobic dechlorination is a primary objective of field pilot testing, so that optimum conditions will be maintained in the full-scale system to follow.

For low-permeability or low-flow aquifers, the amount of substrate required is reduced because rates of groundwater and contaminant flux are low. In this case, a single injection of a slow-release substrate is a cost effective way to maintain proper substrate loading. Conversely, if the groundwater velocity is high, the contaminant and electron acceptor flux will require a much higher carbon loading rate. In this situation, more frequent injection and higher loading rates of either soluble or slow-release substrates will be required.

The frequency of injection is also a function of the rate at which the substrate is depleted. As microbial populations are stimulated and the quantity of biomass increases over time, a given quantity of substrate may be depleted more rapidly. Therefore, careful monitoring of the rate at which substrate is depleted is required to determine if loading rates remain sufficient. Typically, higher loading rates are used at the onset of system operation to grow the microbial biomass. After a large quantity of biomass is established, the increased rate of substrate depletion may be offset to some degree due to biomass being used as a secondary substrate. In some systems, the amount of substrate required to maintain the system may decline over time.

In summary, the practitioner must choose what is maintainable over what may be a conceptually optimum injection regime. Analytical data (e.g., DO, ORP, pH, TOC, and VFAs) from the injection and monitoring wells within the treatment zone are used to confirm that an appropriate reactive zone has been established. Field pilot testing is often the most practical way to optimize substrate loading rates.

5.5.3 Soluble Substrate Systems

5.5.3.1 Suitability of Soluble Substrate Systems

The high solubility and low viscosity of soluble substrates make them easy to handle, mix, inject, and distribute by advection in the subsurface. As a result of these properties, the potential exists to increase the ROI and reduce the number of injection points by dispersing a larger volume of substrate from a single injection point. Therefore, these substrates may be better suited for treating very deep or thick contaminated aquifers where drilling costs are high. An example of treating a large portion of a deep and thick aquifer with a single injection well can be found in Appendix E.1.

Because they are rapidly degraded and readily mixed with groundwater, soluble substrates may not be suitable for high-flow aquifers where a high degree of mixing and replenishment of competing electron acceptors occurs. These conditions may make it difficult to maintain sufficiently reducing conditions. In addition to the need for frequent injection of soluble substrates, other operational costs need to be recognized. Optimization of dosing strategies

can take a long time (to adjust the concentrations and frequencies, for example). Frequent high concentration injections can lead to pH changes that require buffer additions. Frequent injections can also increase the problems associated with biofouling, potentially a significant cost item.

Suthersan and Payne (2003) describe how reducing zones are established downgradient of the point of injection as electron acceptors are reduced in the presence of organic substrate (Figures 2.1 and 5.5). The zone most conducive to anaerobic dechlorination, characterized by sulfate-reducing and methanogenic conditions, may occur several to tens of days of travel time from the point of injection. These reducing zones may vary spatially as microbial growth and higher microbial activity cause more rapid depletion of electron acceptors. Under low flow conditions, these reducing zones may be localized very close to the point of injection.

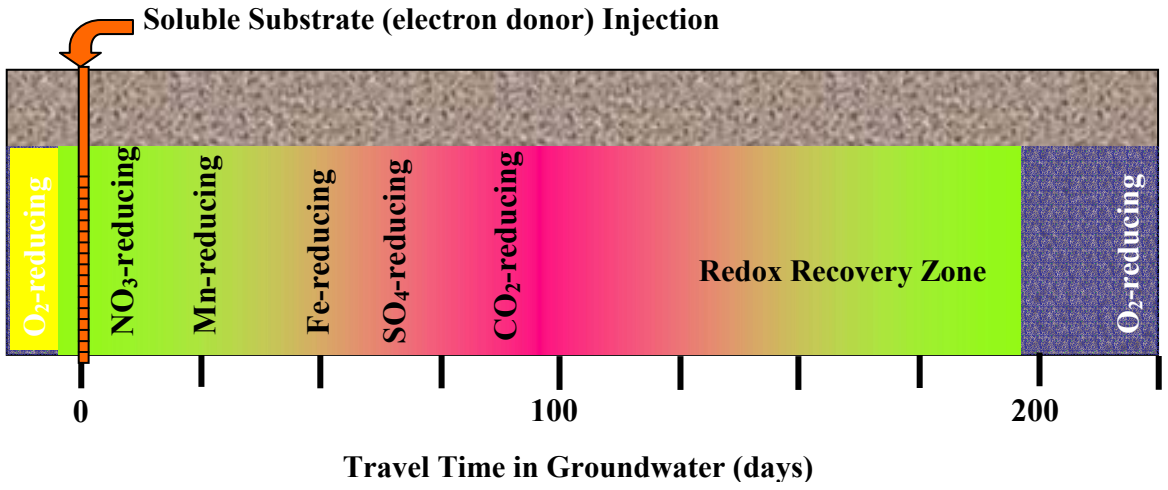


Figure 5.5 Reducing Zones Established Downgradient of Injection in a High-flow Aerobic Aquifer (Suthersan and Payne, 2003)

5.5.3.2 Soluble Substrate Types

Sodium lactate and molasses are the most common substrates applied as a dissolved phase, although other soluble substrates are also used, including ethanol, methanol, butyrate, and sodium benzoate. Lactate is used as a relatively simple substrate (compared to molasses) and is available in the form of lactate salts (sodium or potassium lactate), with lactic acid being the dissociated free form in water. Lactate salts are typically mixed at a concentration of 3 to 30 percent in water, although mixtures as high as 60 percent have been used. The user should be aware of the presence of trace metals in some lactate products, but higher purity commercial products are available.

Molasses is comprised primarily of sugars (sucrose), but may contain other minor constituents such as sulfur, sulfate, and metals that may be of potential concern. Higher grades of molasses or HFCS can be used in situations where the addition of additional sulfur

or other impurities to an aquifer is undesirable. Molasses is typically injected in a water solution of 10 percent molasses or less (Suthersan et al., 2002), although historically molasses has been injected at concentrations as high as 88 percent at the Washington Square Mall site, Wisconsin (Maierle and Cota, 2001).

The choice of low-volume/high-concentration versus high-volume/low-concentration mixtures is affected by how the mixture will disperse and migrate in groundwater to achieve the desired concentration throughout the mixing zone. In general, low-concentration mixtures are more suitable for automated or recirculation systems, and high concentration mixtures are used for more infrequent batch injection. Other factors to be considered include density effects and dilution.

Soluble substrate mixtures will have varying density, depending on the substrate type and strength. Lactate and molasses mixtures will be heavier than water, while ethanol and methanol mixtures will be lighter. In general, vertical migration of soluble substrate due to density differences is not an issue with low-concentration mixtures or with recirculation or forced gradient systems. The higher density of a substrate such as lactate can be advantageous in cases where it is desirable to have a substrate mixture migrate downward from partially penetrating wells, or migrate to deeper zones where DNAPLs may exist (Sorenson, 2003a).

5.5.3.3 Mixing and Delivery of Soluble Substrates

Delivery strategies employ either injection into the aquifer for distribution via advection under natural hydraulic gradients, or recirculation where extracted groundwater is amended with the substrate and reinjected for distribution under an enhanced or forced gradient. Injection of substrate in the first case may be accomplished by either gravity feed or pressure injection. Injection under pressure is preferred because substrate delivery will be more uniform across the entire injection interval and the substrate can be added at a faster rate.

Soluble substrates are typically applied in a continuous or periodic (pulsed) mode to maintain a specified reaction zone. This requires active (either automatic or manual) injection systems that are labor and equipment intensive relative to passive, slow-release substrate systems. However, the cost of system operation may be offset by the ability to modify and optimize the substrate mixture and delivery rates over time and the ability to distribute the substrate more rapidly and uniformly throughout the treatment zone. In practice, it must be recognized that aquifer heterogeneity will exert a substantial limitation on all substrate distribution systems, including soluble substrates. Example descriptions of soluble substrate systems can be found in Appendices E.1 through E.3.

Soluble substrate injection systems can use either a centralized automatic system, or manual injections can be made in batch mode. A typical system configuration for batch mode injection is shown on Figure 5.6. Cost is a primary factor in determining which method to use, but site-specific factors such as facility operations and infrastructure need to be considered. For example, where injection wells are located in high traffic areas, a central distribution system using underground piping for delivery may be desirable, even though it adds to the overall system cost. In other cases, installation of a centralized delivery system may not be feasible due to the presence of utilities and other site infrastructure, and manual batch additions at individual injection wells may be required.

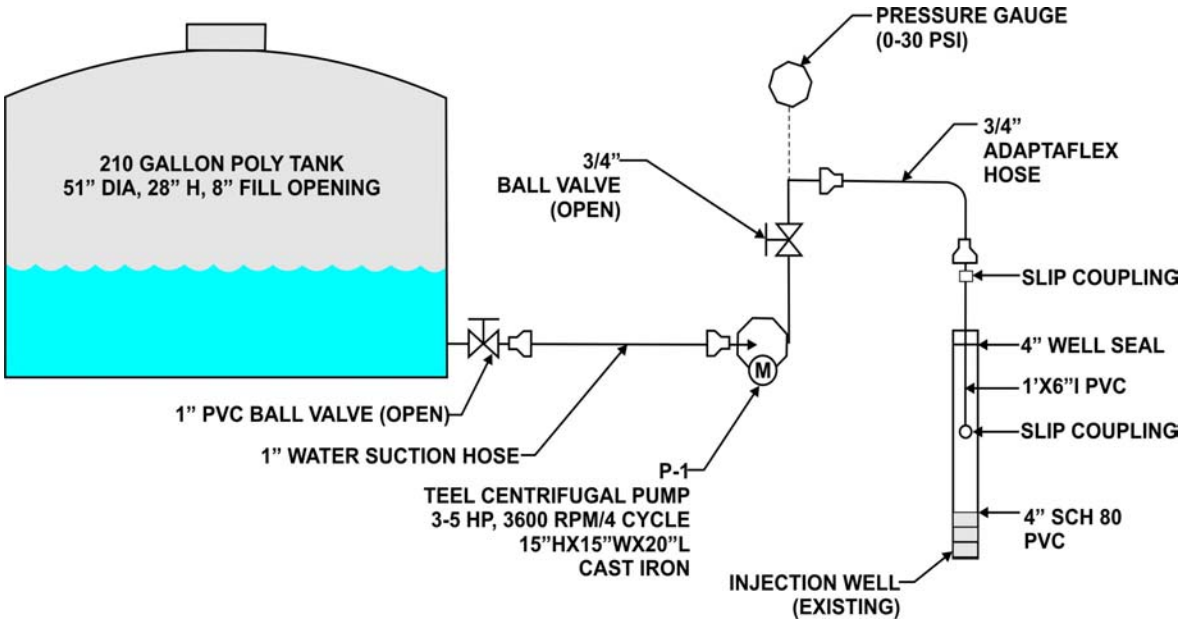


Figure 5.6 Soluble Substrate Injection System (modified from Suthersan et al., 2002)

A uniform substrate mixture can be prepared using a variety of methods; examples can be found in Suthersan et al. (2002). In general, mixing systems use power-operated submersible pumps and/or powered mixers. A series of pumps and mixing tanks are used to meter and mix the appropriate amount of substrate with potable water or extracted groundwater. Power-operated mixers can be used to agitate the solution while the mixing tanks are filled. A programmable logic controller can monitor and adjust the mixing rates automatically. The substrate mixture can be injected directly to a centralized delivery system using a general purpose centrifugal pump, or stored in a batch tank.

The main components of a batch delivery system consist of a mixing vessel, a centrifugal pump, a mixing device, and associated piping, fittings, pressure gauges and flow indicators. A suitable mixing vessel is a polypropylene tank, the size of which can be selected based on the desired volume of injection and/or the availability of transport equipment. A common application is the use of a 250-gallon tank that can be temporarily deployed in a standard pick-up truck bed and is large enough for most individual well batch injections. Mobile systems can also use larger trailer-mounted mixing tanks ranging up to 2,000 gallons in volume.

Dilution is an issue when injecting large volumes of soluble substrate mixed with potable water or uncontaminated groundwater with the objective of achieving widespread distribution of the substrate. While some mixing with contaminated groundwater will occur due to advection and dispersion, large volumes of the contaminated groundwater are displaced. When feasible, higher substrate concentration and lower injection volume is desirable to reduce the amount of displacement and dilution of contaminated groundwater. Recirculation systems avoid this issue, and these systems may be more effective for treating very large aquifer volumes.

In summary, soluble substrates are applicable to most site conditions except aquifers with very high or very low groundwater velocities. They are particularly well-suited for very deep

aquifers, where the number of injection wells that can be practically and cost-effectively installed is more limited. The primary disadvantage to soluble substrates is the requirement for repetitive injection due to their rapid degradation. Permanent injection systems may interfere with facility operations in some cases. Adjusting substrate loading rates and mixing ratios during the initial phase of injection is often necessary to achieve target TOC levels, avoid adverse impacts to pH, and to maximize ROI. This need for optimization increases the costs of O&M during startup, and the life-cycle cost of O&M for soluble substrate systems is high relative to other substrate options.

5.5.4 Viscous Fluid Substrate Systems

5.5.4.1 Suitability of Viscous Fluids as Long-lasting Substrates

The most common viscous fluids used to stimulate anaerobic dechlorination are HRC® and vegetable (edible) oils. Once these substrates are injected into the subsurface, they are intended to be immobile; however, they create mobile plumes of soluble substrate that are dispersed by advection, dispersion, and diffusion. The primary benefit of these substrates is that they require infrequent injection (often only once) with no O&M requirements other than performance monitoring. Ideally, stable reactive zones are created by sustaining dissolved organic carbon concentrations in excess of 100 mg/L for long periods of time. The plumes of dissolved substrate generated from these substrates are readily degraded, and the reaction zone generally does not extend more than a few tens of feet from the point of injection.

While the injection configurations used for these two substrates are similar, their physical and chemical properties vary greatly, requiring distinctly different delivery methods. The high viscosity of these substrates makes it more difficult to inject them into the aquifer matrix. Injection of the viscous HRC® products and vegetable oil require specific types of pumps and pressurized injection systems. Vegetable oils are frequently applied as emulsions, which substantially reduces viscosity. However, the cost of the specialized equipment is minimal (can often be rented or purchased for less than a few thousand dollars) compared to the cost savings that are achieved by eliminating the need for a more permanent system for frequent injection.

5.5.4.2 Hydrogen Release Compound (HRC®) Applications

HRC® is a bioremediation product supplied by Regenesis Bioremediation Products. As per the manufacturer's product literature, various forms of this product are available, but all contain proprietary mixtures of polylactate esters, glycerol, and other materials. These products are designed to provide a slow release of lactic acid and dissolved organic carbon to stimulate *in situ* hydrogen production for the biodegradation of contaminants like chlorinated solvents. The slow release nature of these products is facilitated by the nature of the polymeric materials as well as the viscosity of the preparation.

HRC® has been demonstrated to provide sufficient levels of lactic acid for effective treatment under a variety of aquifer conditions. Because the base product cannot be modified for injection (other than by the manufacturer), the ability to control substrate loading is limited to well spacing and the quantity emplaced per vertical foot of the injection interval. The use of fast-acting HRC®-primer or long-lasting HRC-X™ formulations may be considered based on site-specific conditions.

Application of HRC® is best suited for relatively shallow groundwater plumes where direct-push technology can be used to effectively cover large areas of a plume or create long barriers. For example, upwards of 175 direct-injection points were used in a grid configuration to inject approximately 5,000 pounds of HRC® at the Dixie Cleaners Site in Jacksonville, Florida (Murray et al., 2001; Watts et al., 2002). Shallow barrier configurations also are common, and examples are described in Appendices E.4 and E.5. Finally, HRC® can also be applied within excavations after removal of source area soils.

For deeper applications, HRC® can be delivered by injection in screened wells using glycerin in an effort to chase the product into the formation (Appendix E.4). HRC® and HRC-X™ are highly viscous and are injected using specialized equipment and pumps available from the manufacturer. HRC-X™, and in some cases HRC®, must be heated to reduce viscosity prior to being injected. Depending on the depth of injection and ambient air and groundwater temperatures, it may also be necessary to heat the injection push rods by injecting or circulating steam or hot water through them.

The HRC® products are typically injected at rates of 4 to 12 pounds per vertical foot (lb/ft) of aquifer to be treated. HRC® has a density of approximately 11 pounds per gallon, and the physical distribution of the substrate in a radial direction is only a few inches from the actual point of injection. The rate at which dissolved lactic acid and glycerol released from the substrate product migrates from the point of injection depends on the advective groundwater flow velocity, and will be dominated by the rate of diffusion in low-permeability aquifers. Typical injection point spacing varies from 5-foot centers for low-permeability lithologies to 7.5- to 15-foot centers for more permeable lithologies.

The rate of application (lb/ft) of HRC® or HRC-X™ can be calculated using a spreadsheet-style program provided by the manufacturer, taking into account site-specific conditions including hydrogeology, contaminant levels, and competing electron acceptors. The program also takes into account the size of the treatment area and number of injection points so that the rate of application falls within practical limits.

Once in place, operation of the system is limited to performance monitoring. The typical lifespan for the standard HRC® product ranges from 9 to 18 months, and depends to some extent on the rate of groundwater flow and alternate electron acceptor flux. It is not unusual for additional injections of HRC® to be required, particularly in biobarrier configurations that typically have a design life of several years. When effective contaminant reduction has been achieved, secondary injections of HRC® will typically require less product (perhaps 50 percent) than the initial application to treat a smaller aquifer volume or to maintain the effectiveness of the system. The use of the recently developed HRC-X™ product is gaining in frequency because the vendor claims a lifespan of 3 to 5 years, sufficient to remediate most sites with only a single application. Field demonstration tests are the most accurate way to estimate projections for the amount of product and frequency of injection that would be required for full-scale remedial systems using HRC®.

5.5.4.3 Vegetable (Edible) Oil Applications

Vegetable (edible) oil systems have been used in source area or biobarrier configurations, commonly employing direct-push technology. Applications at Travis AFB, California, and CCAFS, Florida, are examples of grid configurations to treat source areas (Henry et al.,

2003a). An example of a barrier configuration at Altus AFB, Oklahoma, is included in Appendix E.6.

Some early applications used injection of straight oil with a water push or used straight oil to backfill source area excavations, but use of oil-in-water emulsions is currently the most common form of application. Direct injection of straight vegetable oil may still be useful for source areas as a long-term containment or source reduction measure (see Section 5.2.1). The Navy has also sprayed mulch and compost with vegetable oil to enhance substrate loading in biowall trenches at the Naval Weapons Industrial Reserve Plant in McGregor, Texas. Note that contaminants are likely to at least temporarily partition into the oil phase until the oil degrades. This fact may be advantageous in some situations, but can also complicate assessment of reductions in contaminant concentrations.

Refined soybean oil is the most common oil used. Other oils may be used as well, and Borden (2002) has shown that different vegetable oils degrade at different rates, as measured by gas production in microcosms. This may be used to advantage in cases where it is desirable to inject large amounts of substrate while at the same time limiting the rate of oil biodegradation. Otherwise, substrate loading is more readily controlled by modifying the oil saturation in the emulsion (typically 5 to 10 percent). Oil saturations higher than 10 to 15 percent may cause a large reduction in hydraulic conductivity.

Oil-in-water emulsions are readily injected under pressure through direct-push probes. In applications using sealed injection wells or points, it is common to overdevelop the well and use the development water as the makeup water for the emulsion or for a water push to increase the ROI of the substrate. Thus, a large volume of substrate can be reinjected to obtain an ROI (typically 10 to 20 feet) limited only by the quantity of emulsion and water push injected. This practice reduces the potential for displacement and dilution of contaminated groundwater. It also minimizes any disruption of native geochemical conditions in the aquifer resulting from introduction of a foreign water source, although in practice some oxygenation of the extracted groundwater is likely to occur.

It should also be noted that the distribution of buoyant substrates like vegetable oils or oil-in-water emulsions will preferentially flow into more permeable zones in the upper sections of the injection screen interval. A gravity-driven or low pressure injection approach may not effectively distribute oils or emulsions into deeper contaminated intervals.

Injection pressures greater that the overburden pressure (approximately 1 pound per square inch [psi] per vertical foot) may cause hydraulic fracturing of the aquifer formation. This may lead to preferential flow of the substrate mixture along open fractures, resulting in non-uniform distribution. Unless hydraulic fracturing is intentional, injection pressures should be carefully monitored to prevent fracturing of the formation. In low permeability formations (silts and clays), hydraulic fracturing may be used to inject the substrate into the formation. In this case, uniform distribution of the soluble component of the substrate mixture (e.g., metabolic acids) will be diffusion limited, a slow process.

Emulsions can be mixed using static in-line mixers, high-speed shear mixers, or dairy homogenizers. Diaphragm pumps (which are capable of handling back pressure), flow meters, and mixing tanks are used to mix the emulsion to the desired composition. There are numerous emulsifiers (surfactants) used in the food industry for vegetable oils, but the most

common emulsifiers used for enhanced bioremediation applications include food-grade lecithin, polysorbates, mono and diglycerides, glycerol mono-oleate, or some combination of these. It is also common to mix a rapidly degraded soluble substrate such as lactate into the emulsion to condition the aquifer and establish reducing conditions more rapidly.

Lecithin and soybean oil emulsions may be suitable for the ability to adhere (sorb) to sandy aquifers with little organic carbon or clay content. Based on manufacturer's recommendations and the author's experience, the lecithin-to-oil ratio should be limited to only that required to create a stable emulsion (typically less than 5 to 10 percent lecithin in oil) to prevent undesired reduction in hydraulic conductivity in the injection zone. The use of emulsifiers such as polysorbate or glycerol monooleate may be appropriate for soils with high clay or organic content (5 percent or greater). Microemulsions may be mobile in sandy soils based on laboratory tank studies conducted by the Colorado School of Mines (Woodward, 2004). Therefore, caution should be used not to apply any singular emulsion product for any given site, due to the highly variable hydrogeological conditions that may be present.

Current commercial food products and food science provide examples of a wide variety of vegetable oil preparations with custom designed characteristics. For example, nondairy creamers like Coffee Mate® are low-viscosity, stable emulsions containing vegetable oil and mono and diglycerides. Similar emulsions with these characteristics are compatible with distribution in aquifer materials with low permeability where the injection of a viscous material would be problematic. An oil emulsion designed for distribution and retention in a more porous soil may have a higher oil content, larger droplet size, and higher viscosity to achieve the desired distribution of substrate and may have a consistency closer to ranch dressing. Physical characteristics like viscosity, emulsion stability and oil droplet size, and biodegradability can be developed using established food preparation techniques. Given the knowledge and practices established in the food industry, the preparation of vegetable oil emulsions suitable to enhanced bioremediation can be designed with a wide range of characteristics.

A critical design parameter is the mean droplet size of the emulsion relative to the mean pore-throat size of the formation. Vegetable oils injected as emulsions can be widely distributed in most aquifers, given that the emulsion droplet size is small relative to the formation pore space. Field preparation using in-line mixers is capable of obtaining average droplet sizes of 5 to 20 microns, while high-speed shear mixers are capable of obtaining droplet sizes of 2 to 15 microns. The droplet sizes produced by these field methods are not as uniform as that achieved by commercial processes such as dairy homogenizers. But in general, these methods and resultant droplet sizes are sufficient for injection into permeable, fine- to coarse-grained sands or fractured formations.

However, to get effective distribution in fine-grained sands and silts, uniform droplet sizes less than 1 micron are needed. Dairy homogenizers are capable of achieving mean droplet sizes in this range, but are not practical for field preparation. Several pre-mixed oil-in-water emulsions are available commercially that meet this requirement (at additional cost) and are highly stable over periods of several months. Caution is advised that these micro-emulsions may be mobile in permeable formations with high flow rates, particularly if the formation contains little organic carbon for adsorption and retardation of the oil fraction. This may be

desirable to treat large aquifer volumes, but without a stable reaction zone, additional injections may be required.

O&M of vegetable oil systems is limited to performance monitoring. Typical lifespans for oil-in-water emulsions are anticipated to be on the order of 2 to 5 years. Life span depends on the emulsion saturation and the rate at which the oil is degraded and, to a lesser extent, on the rate of groundwater flow and alternate electron acceptor flux. Of the vegetable oil applications conducted since 1999, no sites are known to have required reinjection.

5.5.5 Solid Substrates (Mulch and Compost)

Solid substrates are intended to be long-term sources of organic carbon, with anticipated lifespans exceeding 5 to 10 years. Solid substrates that have been used for stimulating anaerobic dechlorination include tree mulch and compost, as well as other agricultural byproducts such as cotton seed hulls. Other investigators have installed trenches and backfilled excavations with a variety of waste cellulose solids (e.g., sawdust and mulch) since the mid-1990s for the treatment of nitrate-contaminated water, and have found little reduction in performance during 7 years of operation (Robertson et al., 2000).

To date, mulch/compost applications have been implemented by the DoD at four installations. Both a pilot- and full-scale shallow groundwater treatment system have been installed by the Air Force at the Building 301 Site at Offutt AFB, Nebraska (Appendix E.7, Haas et al., 2003). Based on encouraging results from Offutt AFB, the Air Force installed a mulch biowall in June 2002 for a shallow groundwater plume at Altus AFB, Oklahoma (Henry et al., 2003b). A third Air Force demonstration biowall has been installed at F.E. Warren AFB, Wyoming. The Navy has installed several compost and mulch biowalls at Naval Weapons Industrial Reserve Plant McGregor (Cowan et al., 2000). The Navy biowalls are intended primarily to remediate perchlorate, but chloroethenes also are being treated.

The general approach used for placing solid substrates includes using established construction techniques to place the bulk materials in a trench, excavation, or surface amendment. Trenching methods should be carefully selected and implemented to avoid potential lowering of the permeability of the trench wall. The development of surface 'skins' that lower the relative permeability of the trench wall may result from infiltration of bioslurries that produces a filter cake on the trench wall, or by smearing of silts and clays across layers of higher permeability (e.g., sands) by the trencher cutting tools.

Biowall trenches are particularly effective for shallow groundwater plumes in aquifers having low to moderate permeability or that are heterogeneous. The continuity of the trench eliminates potential problems of groundwater bypass resulting from preferential flow paths. However, trenching also may interfere with underground utilities or other site infrastructure.

Mulch applications are limited by the depth to which the substrate can be placed and therefore are suitable only for relatively shallow groundwater plumes. Current trenching technologies are limited to depths of approximately 30 to 35 feet in optimal lithologic conditions, although deeper applications are possible by benching down prior to deploying the trenching equipment. Continuous, one-pass trenching machines used to lay utility lines or for installing dewatering trenches are a rapid and effective way to install a biowall trench (Figure 5.7). In general, the greater the saturated thickness, and the sandier and less

consolidated the sediments, the less depth can be achieved without the use of shoring. Highly compacted or cemented lithologies may also limit the ability to trench to required depths.

Figure 5.7 Continuous Trenching for Biowall Installation, Altus AFB, Oklahoma

Biowall trenches can be modified to include wells or perforated pipe for addition of fluid substrates to the system, if necessary. Alternately, wider trenches or multiple parallel trenches may be necessary to increase groundwater residence time within the treatment zone to effectively treat plumes with high groundwater flux or high CAH concentrations.

Another useful application for mulch and compost is to line landfill or source area excavations. Inclusion of a bark-mulch sub-layer in alternative landfill covers also has been proposed. Mulch or compost can be placed in excavations below the water table, but mulch placed above the water table relies on natural or enhanced infiltration (e.g., via recirculation of captured groundwater) to be effective. Surface amendments can be constructed by placing several feet of a mulch or compost on the ground surface or within a shallow excavation (GSI, 2001; Haas et al., 2000). Amendments that rely on precipitation and natural infiltration to leach organic carbon into shallow contaminated groundwater require a favorable water balance between precipitation, evapotranspiration, and infiltration. Climatic conditions will factor strongly into site selection.

The low solubility of solid substrates requires careful consideration of substrate composition, width, and retention time. The degradation characteristics of the wide variety of mulch and compost products that are available are not well documented. However, positive results have been observed at Offutt AFB, Nebraska (GSI, 2001; Aziz et al., 2003) and Altus AFB, Oklahoma (Henry et al., 2003b), which exhibit distinctly different geochemical profiles (Haas et al., 2003). Typically, mulch and compost are mixed with coarse-grained sand or pea

gravel at a ratio of 20 to 60 percent by volume. The percentage of sand or pea gravel added should be sufficient to make the permeability of the biowall material higher than the of the surrounding formation. This maintains a high permeability for groundwater migration or infiltration through the mixture as well as stabilizing the material and preventing compaction. Using a mulch/compost mixture with a high porosity relative to that of the formation increases groundwater retention time in the reaction zone as well. The mulch can be actively composting prior to emplacement, or compost can be added to the mixture, to promote the breakdown and degradation of the organic matter into soluble organics.

Once in place, passive configurations require no maintenance other than routine monitoring. However, the long-term effectiveness of mulch biowalls to sustain anaerobic dechlorination is still being studied.

5.5.6 Experimental Substrates

5.5.6.1 Whey

Cheese whey is perhaps the most chemically complex of the soluble carbohydrates. This complexity potentially makes whey a longer-lasting substrate than simple substrates such as lactate or ethanol. Fresh whey is a byproduct of the dairy industry and can be obtained inexpensively, often for the cost of handling and transportation alone. Powdered whey is more costly, but is easier to obtain, ship, and store. An example of using whey to reduce the frequency of injection is included in Appendix E.3. For that application, whey was mixed with HFCS, and preliminary results indicate that the mixture has a lifespan of approximately 12 months. Therefore, the use of whey has the potential to reduce the O&M requirements of soluble substrate systems if it can be distributed effectively in the subsurface.

5.5.6.2 Chitin

Several grades of bulk chitin are available for application as a solid substrate similar to mulch and compost. Harkness et al. (2003) tested these chitin products in microcosms and found that they were effective at stimulating anaerobic dechlorination. These authors plan on testing bulk chitin in a permeable biowall configuration. Chitin may be more uniform in composition and more predictable in its degradation characteristics than mulch and compost, which can be highly variable. However, bulk chitin has a significantly higher cost than mulch and compost and may not last as long.

Chitin was selected for enhanced anaerobic dechlorination at the Distler Brickyard Site in Louisville, Kentucky (Martin et al., 2002; Sorenson et al., 2002). The chitin was applied by hydraulic fracturing using a chitin, sand, guar gum, and water slurry. This application is unique in that it uses a conventional engineering technique (hydraulic fracturing) to distribute a solid substrate into low-permeability silt and clay sediments. The permeability of the sediments is maintained after injection because the sand serves to prop the fractures open. Preliminary results of the pilot test indicate that anaerobic dechlorination of the primary CAH present, *cis*-DCE, has been stimulated. Elevated ethene concentrations suggest that dechlorination is proceeding to completion.

5.5.6.3 Gaseous Hydrogen

Because microorganisms known to completely degrade PCE to ethene use hydrogen as an electron donor, addition of hydrogen is the most direct approach to stimulating anaerobic dechlorination. Although hydrogen is highly combustible, it is an inexpensive substrate that can be delivered safely with the proper engineering controls. Besides direct addition of hydrogen to groundwater, other methods to deploy hydrogen via hydrogen-releasing compounds, hydrogen-generating electrodes, and permeable membranes also are being developed (Newell et al., 2002; Novak et al., 2002).

The feasibility of distributing uniform concentrations of gaseous hydrogen throughout large portions of a contaminated aquifer is still under research and development. In addition, hydrogen does not provide a carbon source for microbial growth and development. While hydrogen may stimulate activity of dechlorinating species, their growth depends on the availability of a carbon source for cell development. Therefore, the use of gaseous hydrogen may be better suited for aquifers with relatively high quantities of organic carbon (i.e., Type 1 and Type 2 sites).

The Air Force has conducted two pilot-scale treatability tests involving direct addition of hydrogen to groundwater (Newell et al., 2001 and 2002). The first was a pull-push-pull test of groundwater contaminated with DCE at Offutt AFB, Nebraska, in November 1998. Concentrations of DCE decreased from 430 micrograms per liter (µg/L) to non-detectable levels over the 48-hour period of the test, indicating that anaerobic dechlorination of DCE was achieved.

Direct hydrogen injection into the subsurface also was conducted at Launch Complex 15 at CCAFS, Florida (Appendix E.8). The pilot test used low-volume, pulsed biosparging with hydrogen into a sandy aquifer over an 18-month period. Three biosparge points were placed approximately 12 feet apart in a row perpendicular to groundwater flow. Hydrogen gas was sparged into each well at different rates and amounts during the first part of the pilot test. During the final year, most sparge pulses were at 10 to 12 standard cubic feet per minute (scfm) per well for 10 minutes once a week using 100 percent hydrogen gas. To evaluate potential stripping effects of the sparging process, an identically constructed and operated well was sparged with nitrogen. In addition, a side gradient transect of monitoring wells was installed and monitored to evaluate natural attenuation effects.

The treatment zone and the natural attenuation and nitrogen sparge control zones were monitored to determine the effectiveness of the hydrogen addition. Concentrations of TCE and DCE decreased, while an increase in VC, ethene, and methane concentrations was observed. These data suggest that dechlorination proceeded to completion under methanogenic conditions. Based on these results, the Air Force is planning additional testing of hydrogen to stimulate anaerobic dechlorination. A similar system is currently being operated at the Old Jet Engine Test Cell Site at Offutt AFB, Nebraska.

5.6 IMPLEMENTATION CONSTRAINTS

Site conditions such as geochemistry and hydrogeology may impose certain constraints on the design of enhanced bioremediation systems. Operational constraints such as system bypass or biofouling also require consideration.

5.6.1 Geochemical Design Considerations for Substrate Selection

Prevailing geochemical conditions will influence design parameters, particularly substrate strength and the need for amendments or nutrients. Competition for electron donor by native electron acceptors may reduce the efficiency of the treatment system, and greater volumes and/or higher concentrations of substrate may be required to overcome the alternate electron acceptor demand. For example, Morse et al. (1998) and USEPA (1998a) both suggest that excessive levels of sulfate (greater than 20 mg/L) may inhibit anaerobic dechlorination. Even though these authors indicate that anaerobic dechlorination under sulfate-reducing conditions is feasible, they do suggest that sulfate is problematic for the process. Therefore, a higher rate of substrate loading will typically be required at sites with elevated levels of sulfate.

Morse et al. (1998) also suggest that competition from methanogens may never be eliminated, and may be managed by choice and delivery of electron donor. Ballaparaga et al., (1997) however, suggest that dechlorinators have a competitive advantage over methanogens and sulfate reducers at naturally occurring hydrogen concentrations, even under methanogenic conditions. Therefore, strategies that limit the generation of hydrogen to favor dechlorinators may not be necessary (Suthersan et al., 2002, Drzyzga 2002). Conversely, addition of excessive soluble substrate may result in the development of low pH groundwater zones or impacts to secondary water quality. In aquifers where buffering capacity (i.e., alkalinity) is low, buffering additives may be incorporated into the substrate solution.

5.6.2 Hydrogeologic Considerations for Substrate Delivery

Specific hydrogeologic parameters required for the design of an enhanced anaerobic bioremediation system are presented in Table 5.5. These data are required to design a system that effectively delivers substrate at the desired concentration and location within the reaction zone. While a complicated lithology can place constraints on the use of enhanced *in situ* bioremediation, in most cases it will not completely eliminate the technology as a remedial option. Complex lithologies are also likely to be equally problematic for other *in situ* treatment technologies. By properly placing injection wells or using other delivery mechanisms, the technology can be effectively applied in most environments. For example, biowall trenches may be deployed for shallow plumes in tight clay or silt formations. Excavation of the formation and emplacement of a permeable, continuous, and uniform substrate mixture eliminates problems associated with incomplete substrate distribution.

Groundwater velocity, flow direction, and horizontal and vertical hydraulic gradients impact the effectiveness of substrate addition and the extent to which the substrate will spread and mix with the groundwater. The higher the hydraulic conductivity of the formation, the easier it is to deliver the substrate into the subsurface and the greater the ROI for a single injection point. However, higher groundwater flow velocity will reduce the ROI perpendicular to the direction of flow unless the rate of injection is increased proportionately. But in general, injection well spacing can be proportional to the hydraulic conductivity. In high flow environments, recirculation systems can be employed to increase retention time and may be more cost effective than direct injection requiring use of large volumes of substrate. In contrast, low flow environments typically require lower substrate loading rates because the groundwater flux and accompanying dilution of the substrate are reduced.

Table 5.5 Hydrogeologic Parameters Required for Design of Enhanced Anaerobic Bioremediation Systems

Hydrogeologic Parameter	Related Design Criteria
Depth to impacted groundwater	Injection well depth and screen location. Trench depth for solid substrate biowalls.
Width of contaminant plume	Number of injection wells. Length of biowall trenches.
Thickness of contaminant plume	Number of injection points within a well cluster. Trench depth for solid substrate biowalls.
Groundwater velocity	Injection volume and frequency, residence time of the targeted groundwater in the treatment zone. Dilution of substrate. Trench width (residence time) for biowalls.
Hydraulic conductivity (horizontal and vertical)	Efficiency of substrate delivery, extent of reactive zone. Number of injection points within a well cluster.
Heterogeneity, degree of lithologic layering	Identification of preferential flow paths as compared to contaminated intervals. Location of well screens at injection points.
Soil porosity and grain size distribution	Efficiency of substrate delivery. Trench materials for biowalls to maintain higher permeability than the surrounding formation.

Advection and longitudinal dispersion are the main processes by which dissolved organic carbon migrates downgradient from the delivery system; the effects of transverse dispersion, which spread the substrate in directions perpendicular to groundwater flow, are typically insignificant at most sites. Advection is groundwater motion due to bulk fluid flow. Typical groundwater seepage velocities for enhanced anaerobic bioremediation applications range from 30 to 1,000 ft/yr. The horizontal advective groundwater flow rate may be calculated using the following equation:

$$V = KI/n_e$$

where

V = pore water (seepage) velocity (length divided by time [L/T])

K = average hydraulic conductivity (L/T)

n_e = effective porosity of the aquifer matrix

I = the horizontal hydraulic gradient.

It will be difficult to adequately distribute the substrate to contaminated areas in low-permeability areas characterized by a slow rate of groundwater movement. Substrate distribution into these areas will occur via the relatively slow process of diffusion. As a result, these aquifer environments may not be sufficiently reduced in the short term.

5.6.3 Short-Circuiting or Substrate Bypass

Short-circuiting of substrate to the vadose zone or ground surface may occur during pressurized injection of liquid substrates. Care should be taken to properly screen the

injection interval at least several feet below the water table and to seal and grout the borehole annulus. Very long screened intervals (greater than 10 to 15 feet) should be avoided because substrate may preferentially enter the formation at the top of the screened interval due to the increase in vertical hydrostatic pressure with depth.

Another concern is substrate bypass due to aquifer heterogeneity. Any liquid substrate, including aqueous substrate mixtures, will migrate along the pathway of least resistance (highest permeability). In heterogeneous systems, substrate distribution may bypass large volumes of lower permeability aquifer. In practice, this is an unavoidable situation. Higher injection pressures may force more substrate into some finer-grained sediments, but even very high injection pressures dissipate rapidly with distance (within a few feet) from the point of injection due to the exponential increase in surface area. Multiple well points screened in each lithology may be required to avoid short circuiting of substrate to higher permeability zones. Given sufficient time, dissolved organic substrate will migrate into low permeability sediments via diffusion. Furthermore, it should be noted that most contaminant mass also will migrate through zones of higher permeability. Therefore, effective remediation of heterogeneous formations is possible, but will likely require a longer treatment period.

5.6.4 Changes in Hydraulic Conductivity

Impacts to hydraulic conductivity during enhanced bioremediation can be attributed to the following:

- Biological fouling (biofouling) of the aquifer due to biomass growth.
- Gas clogging from excessive amounts of dissolved gases including carbon dioxide, methane, and hydrogen sulfide.
- Physical reduction in relative permeability due to the presence of non-aqueous substrates (e.g., vegetable oils).

Biofouling of the formation due to biomass growth is possible, but was not observed or documented in the case studies reviewed during the preparation of this document. It is not anticipated that biomass growth in the formation will negatively impact the formation hydraulic conductivity for typical enhanced anaerobic bioremediation applications. However, biofouling of injection wells used for injection of soluble substrates has been observed, and may extend a short distance into the formation. Injection well biofouling may impact the ability to effectively inject and distribute substrate, but is not necessarily indicative of a more widespread clogging of the aquifer treatment zone and lowering of the formation hydraulic conductivity.

Gas clogging in the formation may occur when excessive amounts of gases are produced by biological activity, including carbon dioxide, methane, and hydrogen sulfide. The formation of gas bubbles in the aquifer matrix lowers the aquifer permeability to water flow, reducing hydraulic conductivity. A reduction in hydraulic conductivity may cause contaminated groundwater to flow around the treatment zone, impacting the ability to effectively distribute soluble organic substrate. Gas clogging is rarely observed in practice, but practitioners should be aware of the potential for this effect, particularly when levels of methane approach saturation limits (i.e., greater than 20 mg/L).

Hydraulic conductivity reduction due to the physical presence of viscous or non-aqueous phase substrate can be a concern when the substrate occupies a relatively high volume of the aquifer pore space. The typical volume of HRC® used in practice is a very small percentage of the aquifer volume, and impacts on hydraulic conductivity can be considered to be negligible. Vegetable oil emulsions, on the other hand, are distributed through a much greater volume of the aquifer. In this case, a significant reduction in hydraulic conductivity can occur at saturations as low as 10 to 15 percent. Therefore, oil-in-water saturations of 10 percent or less with emulsion droplet sizes smaller than average pore throat sizes are typically used where reduction in hydraulic conductivity is a concern. For solid substrates in trenches or excavations, the permeability of the solid substrate mixture must remain equal to or higher than that of the surrounding formation. In this case, it is typical to mix the substrate with coarse sand or pea gravel to maintain a high permeability.

5.6.5 Biofouling Control Methods

Biofouling of injection or recirculation wells has been observed at several sites due to the growth of biomass or biofilms within the well screen and the surrounding sand pack. Several approaches have been used to mitigate these effects, and biofouling should not be considered a major impediment to enhanced anaerobic bioremediation implementation. Preventative measures typically include pulsed injection, use of a clean water push to remove substrate residue, or the use of non-oxidizing biocides (e.g., Tolcide®) to control growth in the immediate vicinity of the well (Millar et al., 2001). Well rehabilitation may include conventional redevelopment (e.g., surging and pumping, high pressure jetting), chemical methods such as surging and scrubbing with hydrogen peroxide, injection of carbon dioxide under pressure, and application of biocides in conjunction with the preceding measures (e.g., Forman et al., 2001).

5.7 IMPLEMENTING BIOAUGMENTATION

This section presents a summary of approaches used to design and implement a bioaugmentation system for enhancing the *in situ* anaerobic bioremediation of chlorinated solvents in groundwater. This section is included not as an endorsement of bioaugmentation, but for the use of practitioners who have made the decision to bioaugment. Preceding sections of this document address the pros and cons of bioaugmentation. Case studies summarized in Appendices E.9 and E.13 illustrate some bioaugmentation approaches used to date. Although still under development, early indications are that bioaugmentation may provide improved bioremediation performance.

5.7.1 Technical Approaches to Bioaugmentation

Bioaugmentation involves the delivery of selective and enriched microbial cultures into the subsurface to accelerate biodegradation reactions to achieve remediation goals for rapid and complete dechlorination of chlorinated compounds. In the case of the chlorinated ethenes, bioaugmentation applications are performed with anaerobic, dechlorinating microbial cultures that include strains of *Dehalococcoides* bacteria (Ellis et al., 2000; Major et al., 2002; Lendvay et al., 2003).

Direct contact between the contaminant and the remedial reagent(s) is a critical requirement for success with any *in situ* remediation technology. For bioaugmentation to be effective, direct contact between the microorganisms, essential growth factors, and the target contaminant is necessary. The limitations of microbial transport suggest that it may be desirable to inject the culture and bring the contaminant to the cells. In light of the difficulty in locating source areas, effective delivery of the culture to source zones may be difficult. Rather, an *in situ* biological barrier configuration established by injecting the culture into a designated volume of the aquifer may be more favorable in developing an active zone across which the contaminant is either circulated or allowed to pass through with the natural flow of groundwater. Designing a system that provides this mixing and contact requires detailed aquifer testing and evaluation.

Although recirculation systems can make bioaugmentation highly effective, they have associated operational costs and may not be practical at all sites. Most of the field-scale bioaugmentation systems that have been reported in the literature are small-scale pilot tests, which involved extraction of groundwater from downgradient locations, amending with a substrate and bioaugment, and re-injecting the groundwater into an upgradient location (Dybas et al., 2002; Ellis et al., 2000; Appendix E.13).

There is a potential range of bioaugmentation system designs (i.e., passive, semi-passive, active) that may be used to control substrate and bioaugmentation culture delivery, groundwater flow, biogeochemistry, and microbiology to varying degrees. If the site biogeochemistry, microbiology, and flow regime are not ideal, engineering can overcome these conditions to some extent. In general, the most rapid and effective treatment using bioaugmentation is likely to be achieved with fully engineered recirculation systems. Passive bioremediation systems that rely on natural groundwater flow to deliver reagents to the treatment zone are potential bioaugmentation scenarios because of their relatively low capital cost; however, the performance of these systems has yet to be demonstrated and may be different from more active bioremediation systems.

The two primary technical concerns for bioaugmentation are microbial competition from native bacteria and bacterial transport of the bioaugment culture. When adding a non-native bacterial culture to the subsurface, the geochemical and nutritional requirements of the culture must be well understood and provided for in the receiving aquifer. This is accomplished by conditioning the aquifer using biostimulation (substrate alone) prior to injecting the bioaugmentation culture. Therefore, a biostimulation approach is still required, and addition of the bioaugmentation culture is typically not conducted immediately at system startup.

It is not well understood how non-native bioaugmentation cultures will thrive after biostimulation has been conducted for several months or more. It may be more difficult to introduce non-native microbial species after growth of large amounts of native anaerobic biomass that typically occurs over a period of 6 to 24 months of biostimulation. Therefore, it is yet to be determined if the effectiveness of bioaugmentation is a function of when the bioaugmentation culture is added.

Currently, there are no federal and few state prohibitions on the use of mixed, non-genetically engineered cultures for remediation of soil and groundwater. Recent publications by the USEPA on the use of underground injection control permits indicate that bioaugmentation can be considered. However, state regulators may require special permits

for the use of cultures for remediation. For example, the California Regional Water Quality Control Board typically requires site-specific waste discharge permits to use cultures for remediation. Approval from state health departments also may be required in some states. To date, regulatory approval for the injection of commercial bioaugmentation cultures (e.g., KB-1, Bachman Road, Pinellas) has been received from Alaska, California, Delaware, Florida, Massachusetts, Michigan, Nebraska, New Jersey, Pennsylvania, South Carolina, and Texas.

5.7.2 Culture Selection and Estimation of Volume Requirements

Table 5.6 presents a list of documented dechlorinating microbial cultures capable of complete dechlorination of PCE or TCE to ethene, their source and reference, commercial availability, and status of field testing.

Table 5.6 Examples of Mixed Cultures that Dechlorinate PCE or TCE to Ethene

Mixed Culture Name	Source	Reference	Commercially Produced?	Field Tested?
"Cornell" Enrichment	Sewage Treatment Plant, Ithaca, New York	Maymo-Gatell et al., 1997 and 2001	No	No
"Pinellas " Enrichment	Contaminated soil in Florida	Ellis et al., 2000; Harkness et al., 1999	No	Yes
"Victoria " Enrichment	Contaminated soil in Victoria, Texas	Cupples et al., 2003	No	No
"Toronto Main" Enrichment	Toronto Main Sewage Treatment Plant	Dennis et al., 2003	No	No
ANAS Enrichment	Alameda Naval Air Station, California	Richardson et al., 2002	No	No
LEC1 Enrichment	Anaerobic digestor sludge	Adamson et al., 2000	No	No
"Cape Canaveral" Enrichment	Cape Canaveral Air Station, Florida	Fennell et al., 2001	No	No
"Bachman Road" Enrichment	Bachman Road site aquifer in Oscoda, Michigan	Lendvay et al., 2003	No	Yes
KB-1 Dechlorinator	Contaminated soil in Ontario	Duhamel et al., 2002; Major et al., 2002	Yes	Yes
Bio Dechlor INOCULUM	Improved Bachman Road Culture	He et al., 2003a, 2003b	Yes	In Progress

Dehalococcoides typically require the activity of various anaerobic microorganisms to ferment the electron donors to hydrogen for its use, as well to provide various co-factors that *Dehalococcoides* appears to require. As a result, the microbial cultures referenced in Table 5.6, and cultures that may be developed for future bioaugmentation use, are not (nor are they likely to be) composed of single species of *Dehalococcoides*. In contrast, they generally contain other microorganisms. Therefore, it is recommended that cultures are:

1. Demonstrated by the supplier/manufacturer to be consistent and stable over time,
2. Demonstrated to have measurable biodegradation performance,
3. Certified free of known human and animal pathogens,
4. Available in quantities necessary for the desired application, and
5. Easy to handle when delivered at the site.

Various factors affecting the amount of culture required for bioaugmentation, and how they impact the required culture volume, are presented below.

1. **Growth rate of *Dehalococcoides* at given groundwater temperatures**. A general rule is that a 10-degree ^{o}C increase in groundwater temperature will double the growth rate. Temperatures below 4 ^{o}C will likely limit growth, while temperatures exceeding 35 ^{o}C will inhibit *Dehalococcoides*. Doubling times of 10 to 15 days may be realistic for typical aquifer temperatures (e.g., 10 ^{o}C to 20 ^{o}C) when compared to other anaerobic bacteria.

2. **Population density of the culture**. The higher the initial population density of a culture being used for bioaugmentation, the less culture will typically be required to achieve the desired starting cell density in the aquifer. Knowing the initial cell density in the aquifer along with the growth rate of the culture will help assess the anticipated acclimation/lag period.

3. **Volume of treatment area that needs to be inoculated**. This volume is a function of the initial concentration of the target volatile organic compounds (VOCs), estimated degradation rates, desired concentration of VOCs after treatment, and groundwater flow velocity. By establishing the treatment volume and required starting cell density, and knowing the initial cell density of the culture, the total volume of culture required can be calculated.

4. **Minimum desired lag period for production of ethene**. This is a function of the growth rate of the culture and initial cell density achieved in the groundwater after injection. Increasing the culture volume will decrease the time anticipated for observation of complete dechlorination to ethene.

5. **System design**. Passive electron donor systems generally rely on advection, dispersion, and diffusion to distribute electron donors. Distribution of microorganisms will rely on similar mechanisms. Therefore, achieving the desired lag times with a passive system will require injecting the culture at more locations than for a circulation-based design. This may result in a requirement for higher culture volume for more effective distribution, at higher cost.

5.7.3 Selection of Electron Donors for Bioaugmentation

A wide variety of substrates are available to induce anaerobic conditions and provide electron donor to support biological anaerobic dechlorination. The common substrates described in this document have been shown to successfully stimulate anaerobic dechlorination by *Dehalococcoides*, whether present naturally in an aquifer or added via bioaugmentation. Therefore, it would appear that the selection of electron donor is not a critical factor controlling the success of dechlorination by bioaugmentation cultures.

It may be possible to use lower rates of substrate loading and still achieve high rates of degradation under less-reducing conditions when using enriched bioaugmentation cultures. For example, complete degradation was stimulated with bioaugmentation at the California Aerojet facility using ethanol as the substrate (Appendix E.9); the degradation occurred under

iron- to sulfate-reducing conditions that did not promote methanogenesis or solubilization of metals.

5.7.4 Adding Bioaugmentation Cultures to the Subsurface

Cultures should be shipped to the site in vessels that will exclude oxygen. The vessels should be over-packed in a shipping container that is easy to handle, and includes spill containment and absorption. Material Safety Data Sheets (MSDSs), shipping, and other forms should be included in the shipping container or made available by the manufacturer prior to shipment.

Given that *Dehalococcoides* are strict anaerobes, it is not advised that cultures be directly injected into aerobic aquifers. Bioaugmentation should instead be conducted following an aquifer conditioning period, wherein electron donor is added to the target bioaugmentation area to reduce the ORP and create geochemical conditions that will favor the introduction and survival of the introduced microorganisms. In general, bioaugmentation should be conducted when DO concentrations are less than 0.5 mg/L, ORP is less than –100 mV, and ideally when sulfate-reduction and/or methanogenesis are actively occurring.

Prior to bioaugmentation, injection lines and other transfer lines should be purged with argon or another inert gas to displace any oxygen. An argon or inert gas headspace should be maintained above the culture during its injection into wells or transfer lines. The culture should be injected below the water table in the screened interval, and should be allowed to disperse and establish throughout the target treatment zone, in the presence of electron donor, for several days before re-commencing groundwater circulation to the system (for active systems).

5.7.5 Bioaugmentation Performance Monitoring

Performance monitoring for bioaugmented sites is similar to that performed for other enhanced bioremediation applications (Section 6). However, groundwater samples for molecular analysis should be collected before and after bioaugmentation to confirm successful addition of the culture into the aquifer. Molecular analysis can also be used to track the distribution and relative growth of the bioaugmentation microorganisms (e.g., *Dehalococcoides*) within the target treatment area. An increase in the distribution and population density of the bioaugmentation culture over time indicates the establishment and growth of the culture in the subsurface.

Groundwater samples collected at different intervals over time and analyzed for VOCs and dissolved gases can be used to determine trends in the rate and/or production of VC and ethene after bioaugmentation. Correlation of these data with the detection of *Dehalococcoides* indicates successful bioaugmentation.

5.8 SYSTEM DESIGN SUMMARY

There are a number of system and engineering design options for applying enhanced anaerobic bioremediation. Selection of a practical technical approach and system configuration should be based on meeting site-specific remedial objectives. Once remedial

goals and a suitable technical approach (e.g., source reduction or biobarrier containment) are established, the practitioner faces a multitude of substrate and delivery options.

With few exceptions, all of the substrate types described in this section can be used in some form of source area, biobarrier, or plume-wide configuration. Furthermore, all of the substrate types have been demonstrated to stimulate complete anaerobic dechlorination given appropriate geochemical conditions, suitable microbial populations, and sufficient levels of substrate (electron donor). There is no reason not to consider all of the substrate types and system configurations described in this section.

This leads to an obvious question: ***How does the RPM decide which substrate type and delivery system to use?*** It is the opinion of the authors that this decision should be reached by a combination of life-cycle cost analysis and proven effectiveness. In practice, the RPM will be soliciting bids from environmental contractors, and is encouraged to evaluate the life-cycle costs of alternative system designs using the various substrate types. A number of reasonable approaches may be provided, and the relative cost of these systems should be apparent.

In addition to life-cycle costs, the technical merit and effectiveness of the approach also must be evaluated. Comparing case studies of the effectiveness of different approaches for sites similar to that under consideration is one way to evaluate the proposed technical approach. But in many cases, small-scale pilot testing may be the only practical way to prove the effectiveness of a proposed enhanced bioremediation system for a particular site. The cost to modify or change system design or operation after full-scale bioremediation is implemented may prove to be many times the cost of pilot testing. By understanding the principles and practices of enhanced anaerobic bioremediation presented in this document, critically evaluating proposed technical approaches, and exercising sound professional judgment, the RPM will find that enhanced anaerobic bioremediation is an effective remedial option for many sites contaminated with chlorinated solvents.

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SECTION 6

SYSTEM MONITORING

6.1 MONITORING STRATEGIES

Monitoring strategies for enhanced anaerobic bioremediation systems are driven by site-specific hydrogeology and biogeochemistry, and by the configuration and operation of the system used. For example, an active recirculation system using a soluble substrate in a highly permeable sand aquifer may have vastly different monitoring requirements than a passive mulch biowall in low-permeability silts and clays. Regulatory requirements also may dictate certain monitoring protocols and frequency.

System monitoring is generally conducted for three purposes. Baseline contaminant and biogeochemical characterization is conducted prior to substrate addition to refine the CSM, to provide a basis for system design and operation, and to define the baseline for comparison to the performance monitoring data. Process and performance monitoring are conducted after substrate addition for two purposes: 1) to evaluate the need for system modifications that will optimize the performance of the system (process monitoring), and 2) to evaluate the performance of the system with regards to achieving remedial objectives (performance monitoring). Different monitoring strategies and analytical protocols may be warranted for each of these monitoring objectives.

System monitoring is conducted for three purposes:

Baseline Characterization as a basis for design and for performance comparison.

Process Monitoring to optimize system operation and performance.

Performance Monitoring to validate the effectiveness of the system to meet remedial objectives.

Each of these three purposes may have differing analytical protocols, monitoring locations, and monitoring frequencies.

Monitoring protocols and frequency should remain flexible throughout the enhanced anaerobic bioremediation project to incorporate optional diagnostic analyses (e.g., VFAs or dissolved hydrogen), alter the sampling frequency in response to changing conditions, or eliminate parameters that are not providing useful information (i.e., process optimization). This flexibility should be written into monitoring plans to facilitate periodic review and regulatory approval of proposed changes, as well as allowing for optimization of monitoring schedules and budgets. The following subsections briefly describe the key concepts of the different monitoring strategies and sampling protocols.

6.1.1 Baseline Characterization

Baseline contaminant and biogeochemical characterization is used in one of two ways: existing data are insufficient for site selection or design purposes, and additional characterization is required as a "pre-design" step; or data are collected shortly before substrate addition as a basis for comparison of performance results. Baseline characterization typically involves a more extensive analytical protocol than process or performance monitoring. For example, soil characteristics such as fraction of organic carbon (f_{oc}) only need to be characterized once. Note that many of the initial parameters required for adequate baseline characterization may have been analyzed for during previous site characterization activities and need not be repeated.

Recommended, optional, and experimental field and laboratory analyses for soil, soil gas, and groundwater are described below. Most of the sample protocols for contaminant and biogeochemical characterization employ routine methods that are described elsewhere (e.g., AFCEE, 2000; USEPA, 1998a; American Society for Testing and Materials [ASTM], 1997). However, there are certain atypical analyses such as dissolved gases or microbial/molecular analyses that currently can only be performed by a select group of specialty laboratories. The user should consult with the laboratory for special sampling and analytical protocols in these cases. The reader is referred to Section 3 (Preliminary Screening) for further information regarding appropriate baseline sampling protocols.

6.1.1.1 Soil and Soil Gas Analyses

Recommended and optional field and laboratory analyses for soil and soil gas are listed in Table 6.1. Soil parameters recommended for baseline characterization include f_{oc}, primarily to calculate contaminant retardation, and CAHs in suspected source areas. In addition, it is essential that information be collected on the degree of soil layering and the presence and location of preferential flow paths. Data collection methods used to obtain these data must be selected on a site-specific basis, and may include continuous soil cores across the contaminated interval, geotechnical profiling using a cone penetrometer, direct-push permeameters, and borehole flow meters.

Optional soil parameters that may be required for design purposes include grain-size analysis and bioavailable iron. Grain-size analysis gives an indication of the formation permeability, which may limit the types of substrates or system configurations that can be used. Analysis for bioavailable iron may be beneficial for clastic sediments where iron-reduction may be a significant TEAP. Analysis of soil samples for other contaminants (e.g., fuel hydrocarbons or metals) may be beneficial to evaluate existing sources of organic carbon or to address potential concerns that other site contaminants may interfere with or otherwise complicate the bioremediation process (e.g., metals mobilization).

Soil samples are easily obtained during installation of injection or monitoring wells. When in doubt, it is recommended that soil samples be collected and analyzed for optional parameters during baseline characterization to avoid the costs associated with re-mobilization of drilling equipment and personnel.

Table 6.1 Soil and Soil Gas Analytical Protocol for Enhanced Bioremediation

Analysis	Method/Reference (laboratory/field)	Data Use	Data Implications	Frequency of Analysis
Soil Analyses				
Chlorinated Aliphatic Hydrocarbons (CAHs)	SW5035/SW8260B (laboratory)	Data are used to determine the extent and degree of soil contamination, to estimate the sorbed contaminant mass present, and to determine the need for other source removal actions.	A continuing source of contaminant mass from sorbed or free-phase DNAPL must be taken into account in the design and life-expectancy of an enhanced anaerobic bioremediation system.	Recommended. Initial sampling in source area(s) only.
Fraction of organic carbon (f_{oc})	SW9060 modified for soil matrix (laboratory)	The fraction of organic carbon in the aquifer matrix is used to calculate retardation factors for dissolved contaminant transport and to estimate the amount of contaminant mass sorbed to the aquifer matrix.	A large proportion of contaminant mass may be sorbed to the aquifer matrix.	Recommended at initial sampling.
Biologically Available Iron (Fe[III])	Laboratory specialty method (laboratory)	Bioassay with quantification of bioavailable solid-phase ferric iron Fe(III) that is a competing electron acceptor. Optional method that may be used to determine competition from iron reduction. May also affect potential abiotic reactions.	Only recommended for clastic sediments with potential for significant iron concentrations. May also be used as a diagnostic tool if sulfate reduction or methanogenic redox conditions cannot be achieved.	Optional at initial sampling.
Grain Size Analysis	ASTM D-422 (geotechnical laboratory)	Indication of aquifer permeability and pore throat size.	It may be difficult to distribute substrate in fine-grained formations with high silt and clay content.	Optional at initial sampling.
Soil Gas Analyses				
Methane, Oxygen, Carbon Dioxide, and Hydrogen Sulfide	Soil gas analyzer calibrated in the field according to the manufacturer's specifications (field)	Useful for determining biological activity in vadose zone and generation of biogenic methane.	Explosive levels of methane or noxious levels of hydrogen sulfide accumulating in structures or utilities may pose a health risk.	Optional. Recommended when soil vapor exposure pathway exists.
CAHs	USEPA Method TO-3 or TO-4 (laboratory)	Used to determine risk from contaminants in soil vapor and as an alternative to soil sampling to determine the extent of chlorinated contaminants in soil.	CAHs accumulating in structures or utilities may pose a health risk.	Optional. Recommended when soil vapor exposure pathway exists or when preliminary assessment of the extent and magnitude of CAHs in soils is desirable.

NOTES:

Analyses other than those listed in this table may be required for regulatory compliance.

1. "SW" refers to the *Test Methods for Evaluating Solid Waste, Physical, and Chemical Methods*, SW-846, USEPA, 3rd edition, 1986.
2. "ASTM" refers to the *American Society for Testing and Materials*.

Optional soil gas parameters listed in Table 6.1 include field measurements of oxygen, carbon dioxide, methane, and hydrogen sulfide; and laboratory measurements of CAHs. In general, soil gas samples are only analyzed if soil vapor intrusion into nearby utilities or structures could create potentially explosive, toxic, or nuisance conditions. Comparison of methane levels against the lower explosive limit (LEL) is advisable as a safety precaution when elevated concentrations of methane in groundwater are observed. Analysis of select CAHs in soil gas is also warranted in locations proximate to structures or buildings where an inhalation risk may exist. Contingency plans (e.g., SVE) should be considered in locations sensitive to noxious gases or vapor hazards.

6.1.1.2 Groundwater Analyses

Groundwater analyses used for enhanced anaerobic bioremediation are summarized in Table 6.2. Baseline groundwater analyses typically include the following:

- Contaminants and dechlorination products (CAHs, ethene, and ethane)
- Electron donors (dissolved organic carbon [DOC] or TOC),
- Alternate electron acceptors and metabolic byproducts (DO, nitrate, ferrous iron, sulfate, and methane), and
- General water chemistry (ORP, pH, temperature, specific conductivity, chloride, and alkalinity).

These parameters provide basic information on contaminant concentrations and dechlorination breakdown products, redox conditions and prevailing TEAPs, and the buffering capacity of the aquifer (alkalinity and pH). Some of the parameters may have slightly different uses for baseline characterization versus process monitoring. For example, DOC or TOC may be used to identify the natural levels of organic carbon present at a site during baseline characterization to determine whether the site is electron donor limited, and may be used simply as an indicator of substrate strength during process monitoring.

In general, samples should be collected using low-flow purge techniques following appropriate quality assurance / quality control (QA/QC) procedures. DOC (filtered samples) is typically measured for dissolved substrates, while TOC (unfiltered samples) is typically measured where substrate may be present in colloidal or suspended form (e.g., vegetable oils or whey). Selected groundwater parameters such as pH, DO, ORP, ferrous iron, conductivity, and temperature are typically measured in the field due to their instability.

Optional biogeochemical and experimental microbial/molecular analyses used to further the understanding of site conditions within the treatment zone may include, but are not limited to, the following:

- Fuel hydrocarbons,
- Ammonia and nitrite,
- Iron (total) and manganese (II),
- Sulfide or hydrogen sulfide,
- Carbon dioxide,
- Nitrogen and phosphate,

Table 6.2 Groundwater Analytical Protocol for Enhanced Anaerobic Bioremediation

Analysis	Method/Reference (Laboratory/Field)	Data Use	Performance Expectation or Implication	Recommended Frequency of Analysis
Chlorinated Aliphatic Hydrocarbons (CAHs)	SW8260B (laboratory)	Regulatory compliance for contaminants of concern. The values by which success of the remediation system will be measured.	CAHs and dechlorination products are typically expected to decline to less than regulatory compliance levels within the treatment cell after substrate addition.	Recommended for each sampling round.
Total Organic Carbon (TOC) or Dissolved Organic Carbon (DOC)	SW9060, EPA Method 415.1 (laboratory)	Indicator of natural organic carbon present at site during baseline characterization and as an indicator of substrate distribution during performance monitoring. TOC/DOC concentrations greater than 20 to 50 mg/L are desired in the anaerobic treatment zone.	Stable or declining TOC/DOC levels less than 20 mg/L in conjunction with elevated levels of VOCs and alternate electron acceptors indicate additional substrate is required to sustain the treatment zone.	Recommended for each sampling round.
Oxidation-Reduction Potential (ORP)	Direct reading meter, A2580B, or USGS, 1997 (field)	Highly reducing conditions are required for anaerobic dechlorination to occur. The ORP of groundwater provides data on whether anaerobic conditions are present. Used in conjunction with other geochemical parameters, ORP indicates which terminal electron accepting processes (TEAPs) predominate in an anaerobic environment and whether groundwater conditions are optimal for anaerobic biodegradation.	ORP values should remain less than -100 millivolts (mV) within the treatment zone for anaerobic dechlorination to occur. Positive ORP values (greater than 0.0 mV), in conjunction with elevated levels of DO and the absence of TOC/DOC, may indicate that additional substrate is required to promote anaerobic dechlorination.	Recommended for each sampling round. Typically measured at the well head using a flow-through cell to protect samples from exposure to oxygen.
Dissolved Oxygen (DO)	DO meter calibrated in the field according to the manufacturer's specifications (EPA 360.1) (field)	DO should be depleted in an anaerobic bioremediation system. DO less than 0.5 mg/L generally indicates an anaerobic pathway suitable for anaerobic dechlorination to occur.	DO concentrations greater than 1.0 mg/L, in conjunction with elevated levels of CAHs and the absence of TOC/DOC, indicate additional substrate may be required to promote anaerobic dechlorination.	Recommended for each sampling round. Typically measured at the well head using a flow-through cell.
Ferrous Iron (Fe[II])	Colorimetric Hach Method 8146 (field)	Ferric iron is an alternate electron acceptor for microbial respiration in the absence of oxygen and nitrate; reduction of ferric iron produces ferrous iron. Evaluated levels of ferrous iron indicates that the groundwater environment is sufficiently reducing to sustain iron reduction and for anaerobic dechlorination to occur.	Elevated levels of ferrous iron may indicate a competing TEAP to anaerobic dechlorination of CAHs.	Recommended for each sampling round. Typically measured at the well head to protect samples from exposure to oxygen.
Sulfate (SO_4^{2-})	IC method E300.0A (laboratory) or Hach Method 8051 (field)	Sulfate is an alternate electron acceptor for microbial respiration in the absence of oxygen, nitrate, and ferric iron. Depleted concentrations of sulfate relative to background indicate that the groundwater environment is sufficiently reducing to sustain sulfate reduction and for anaerobic dechlorination to occur.	Sulfate levels less than 20 mg/L are desirable, but not required, for anaerobic dechlorination to occur. High levels of sulfate in conjunction with the absence of TOC/DOC indicate additional substrate may be required to promote anaerobic dechlorination.	Recommended each sampling round.

(Continued)

Table 6.2 Groundwater Analytical Protocol for Enhanced Anaerobic Bioremediation (continued)

Analysis	Method/Reference (laboratory/field)	Data Use	Performance Expectation	Recommended Frequency of Analysis
Methane, Ethane, and Ethene	Kampbell *et al.*, 1989 or SW3810 Modified (laboratory)	Elevated levels of methane indicate fermentation is occurring in a highly anaerobic environment and that reducing conditions are appropriate for anaerobic dechlorination of CAHs to occur. Elevated levels of ethene and ethane (at least an order of magnitude greater than background levels) can be used to infer anaerobic dechlorination of CAHs.	Methane levels greater than 1.0 mg/L are desirable, but not required, for dechlorination to occur. Methane levels less than 1.0 mg/L and the accumulation of *cis*-1,2-DCE, VC, or other less-chlorinated CAHs may indicate that additional substrate is required to drive reducing conditions into an environment suitable for reduction of these compounds. If elevated levels of ethene or ethane are not observed, potential accumulation of *cis*-1,2-DCE or vinyl chloride should be monitored.	Recommended each sampling round. May require analysis by a specialty laboratory.
Alkalinity	EPA Method 310.1, or Hach alkalinity test kit model AL AP MG-L, or Hach Method # 8203 (field or laboratory)	Indicator of biodegradation and the buffering capacity of the aquifer (neutralization of weak acids). Used in conjunction with pH, an increase in alkalinity and stable pH indicates the buffering capacity of the aquifer is sufficient to neutralize metabolic acids produced by degradation of substrates.	Concentrations of alkalinity that remain at or below background in conjunction with pH less than 5 indicates that a buffering agent may be required to sustain high rates of anaerobic dechlorination.	Recommended each sampling round.
pH	Field probe with direct reading meter calibrated in the field according to the supplier's specifications (EPA 150.1)	Biological processes are pH sensitive, and the ideal range of pH for dechlorinating bacteria is 5 to 9. Outside this range, biological activity is less likely to occur.	pH levels within a range of 5 to 9 are desirable. pH less than 5 indicates that a buffering agent may be required to sustain high rates of anaerobic dechlorination.	Recommended each sampling round.
Nitrate/Nitrite	IC method E300.1 (laboratory)	Nitrate is an alternate electron acceptor for microbial respiration in the absence of oxygen. Depleted levels of nitrate (relative to background) indicate that the groundwater environment is sufficiently reducing to sustain nitrate reduction.	Indicator parameter only. Nitrate levels less than 1.0 mg/L are desirable for anaerobic dechlorination of CAHs.	Optional. Recommended for each sampling round only if nitrate reduction appears to be a significant TEAP.
Nitrate/Nitrite as Nitrogen (total)	IC Method 353.2 Optional method for Nitrate/Nitrite by E300.1 (laboratory)	Same as above. In most aquifer systems, concentrations of nitrate are naturally much higher than nitrite, and total nitrate/nitrite can be used as an estimate of nitrate.	Same as above.	Optional. Alternative method.

(Continued)

Table 6.2 Groundwater Analytical Protocol for Enhanced Anaerobic Bioremediation (continued)

Analysis	Method/Reference (laboratory/field)	Data Use	Performance Expectation	Recommended Frequency of Analysis
Total Manganese	EPA 6010B (laboratory) or Hach Method 8034 (Field)	Manganese (IV) is an alternate electron acceptor for microbial respiration in the absence of oxygen and nitrate. An increase in dissolved manganese (II) or total manganese indicates that the groundwater environment is sufficiently reducing to sustain manganese reduction and for anaerobic dechlorination to occur.	Elevated levels of dissolved manganese may indicate a competing TEAP to anaerobic dechlorination of CAHs.	Optional. Recommended for each sampling round only if manganese reduction appears to be a significant TEAP.
Sulfide (H_2S)	Hach Method 8131 or similar (field)	Byproduct of sulfate reduction. Sulfide typically precipitates with iron minerals, but elevated levels of sulfide may be toxic to dechlorinating microorganisms.	Elevated levels of sulfide in conjunction with elevated levels of CAHs may indicate that iron-compounds should be added to precipitate sulfides and reduce toxicity effects.	Optional. Recommended when elevated levels of sulfate (> 20 mg/L) are present.
Temperature	Field probe with direct reading meter (EPA 170.1)	General water quality parameter used as a well purging stabilization indicator. Microbial activity is slower at lower temperatures.	Indicator parameter only. Typically used as a well purge stabilization parameter.	Optional.
Specific Conductance	E120.1/SW9050, direct reading meter (laboratory or field)	General water quality parameter used as a well purging stabilization indicator. May correlate with and support interpretations of other geochemical analyses.	Indicator parameter only. Typically used as a well purge stabilization parameter.	Optional.
Major Cations (e.g., iron)	SW6010B (laboratory)	Some metals may be more mobile under highly reducing conditions.	May be required for regulatory compliance of secondary water quality.	Optional.
Nitrogen	E365.1 (laboratory)	Nutrient needed for microbial growth, may be needed as a substrate amendment.	May indicate need for nitrogen amendment.	Optional.
Phosphate	E365.1 (laboratory)	Nutrient needed for microbial growth, may be needed as a substrate amendment.	May indicate need for phosphate amendment.	Optional.
Chloride	IC Method E300.1 or SW9050 (laboratory), or Hach Chloride test kit model 8-P (field)	General water quality parameter. Chloride is produced by anaerobic dechlorination. Elevated levels of chloride may indicate that dechlorination is occurring if observed concentrations are greater than three times background and consistent with CAH molar concentrations.	Indicator parameter only.	Optional.
Carbon Dioxide	Hach Kit Method 8205 (field)	Carbon dioxide is a byproduct of both aerobic and anaerobic degradation. Elevated levels of carbon dioxide indicate microbial activity has been stimulated.	Indicator parameter only.	Optional.
Bromide or Iodide	IC Method EPA 300.1 (laboratory) or field meter (field)	Used as a conservative groundwater tracer.	Indicator parameter for tracer tests only.	Only used with tracer testing.

(Continued)

Table 6.2 Groundwater Analytical Protocol for Enhanced Anaerobic Bioremediation (concluded)

Analysis	Method/Reference (laboratory/field)	Data Use	Performance Expectation	Recommended Frequency of Analysis
Chemical Oxygen Demand (optional)	EPA Method 410.4 or 410.1 (laboratory)	A measure of the oxygen required to oxidize all compounds, both organic and inorganic, in water. Used to determine material load in groundwater subject to oxidation.	Indicator parameter only. May be used as an indication of substrate electron acceptor demand. Redundant with DOC analyses.	Optional.
Biological Oxygen Demand (optional)	EPA Method 415.1 (laboratory)	An indirect measure of the concentration of biologically degradable material present in organic wastes.	Indicator parameter only. May be used as an indication of electron acceptor demand. Redundant with DOC analyses.	Optional.
Volatile Fatty Acids (VFAs) or Metabolic Acids	Laboratory specialty method. EPA Robert S. Kerr Laboratory RSK–SOP 112	VFAs are an indicator of substrate distribution and are also degradation products of more complex substrates (e.g., carbohydrates or vegetable oils). Fermentation of VFAs produces molecular hydrogen for anaerobic dechlorination.	Measurable concentrations of VFAs (greater than 10 to 20 mg/L) are desirable in the treatment zone. The presence of mg/L concentrations of propionate or butyrate is considered favorable. A lack of measurable VFAs in conjunction with elevated levels of VOCs and alternate electron acceptors indicates additional substrate may be required to sustain an anaerobic treatment zone.	Optional. Useful as a diagnostic tool.
Dissolved Hydrogen	USEPA RSK-196, Laboratory specialty method	Specialized analysis used to determine TEAPs. Hydrogen is the primary electron donor used in anaerobic dechlorination. Hydrogen concentrations between 2 and 11 nanomoles per liter (nM/L) are optimal for efficient reductive dechlorination to occur.	Hydrogen levels less than 2 nM/L in conjunction with elevated levels of VOCs and the absence of TOC indicates additional substrate may be required to promote anaerobic dechlorination.	Optional. May be used as a diagnostic tool after substrate addition.
Phospholipid Fatty Acids	Laboratory specialty method	Indicator of biomass and general composition of the microbial population. Can determine relative levels of microbial stress or starvation.	May be useful to evaluate whether significant changes in microbial populations have occurred, but results do not directly support pass/fail determinations or design changes.	Experimental. Only recommended as a diagnostic tool.
DNA sequencing of *Dehalococcoides* species	Laboratory specialty method	Detection of genetic sequences unique to targeted microbial genus and species. See Sections 3 and 6.3.5 for further descriptions of data use.	Positive identification of *Dehalococcoides*-related species indicates potential for complete dechlorination of chlorinated ethenes.	Experimental. Only recommended as a diagnostic tool.

NOTES:

Analyses other than those listed in this table may be required for regulatory compliance.

1. "Hach" refers to the Hach Company catalog, 2003.
2. "A" refers to *Standard Methods for the Examination of Water and Wastewater*, 18th edition, 1992.
3. "E" refers to *Methods for Chemical Analysis of Water and Wastes*, USEPA, 1983.
4. "SW" refers to the *Test Methods for Evaluating Solid Waste, Physical, and Chemical Methods*, SW-846, USEPA, 3rd edition, 1986.
5. "ASTM" refers to the *American Society for Testing and Materials*.

- Dissolved hydrogen,
- COD and BOD,
- VFAs,
- PLFAs, and
- Molecular screening for *Dehalococcoides* species.

Measurement of selected supplemental inorganic parameters (e.g., nutrients such as nitrogen and phosphorus) may be warranted for design purposes if there are no pre-existing data for these parameters. However, no widely accepted criteria exist regarding what are sufficient or insufficient concentrations of these nutrients. Retention of these parameters for subsequent monitoring purposes should be based on the amount of useful information they provide.

Most of the optional and experimental methods are used as diagnostic tools (i.e., VFAs, dissolved hydrogen, PLFAs, and molecular screening for *Dehalococcoides* species), which can be performed later during process or performance monitoring if there is a need for further evaluation of aquifer conditions. For example, dissolved hydrogen may be used as a diagnostic tool for identifying the prevailing biological redox processes. However, it involves the use of a difficult and time-consuming sampling technique that can often be omitted from routine sampling.

6.1.1.3 Hydraulic Characterization

Hydraulic characterization is conducted to evaluate the site hydrogeologic conditions for system design and to determine changes in aquifer characteristics (e.g., loss of permeability or direction of groundwater flow) brought about by substrate addition. The hydraulic conductivity of the aquifer should be measured prior to and after substrate addition to determine any influence of substrate injection. Groundwater elevations should be measured on a routine basis (e.g., quarterly) to determine hydraulic gradients to evaluate changes in groundwater flow direction or velocity. For recirculation systems, or systems delivering a volume of substrate that exceeds 10 percent of the volume of water in the treatment zone, numerical modeling of the hydraulic impact of the system may be warranted.

Tracer Testing. Conservative groundwater tracers can be used to determine aquifer characteristics such as groundwater seepage velocity and aquifer dispersivity to predict the transport of substrate in the subsurface after injection. A tracer such as sodium bromide or sodium iodide is typically injected at a concentration approximately 100 times the respective method detection limit (500 to 1,000 mg/L). This allows for calculation of dispersivity and groundwater seepage velocity under most field conditions. Non-uniform downgradient concentrations also may indicate the presence of preferential flow paths related to aquifer heterogeneity. For recirculation systems, tracer testing is recommended prior to substrate injection to optimize flow rates and plume capture, and to validate system flow models (e.g., see Appendix E.13).

Another application of tracers for passive or semi-passive systems is to mix a conservative tracer with the substrate and track the tracer through the monitoring network under natural or induced gradients to determine ROI. When compared to indicators of substrate strength (e.g.,

TOC or VFAs), and accounting for adsorption, the concentration of the tracer may indicate the rate at which the substrate is used (depleted) with transport in the aquifer.

When using uncontaminated "make-up" water to mix the substrate, the use of a tracer also allows a computation of the magnitude of the effects of dilution caused by injection of a clean substrate mixture. By comparing the concentration of the tracer in the monitoring wells to the injected concentration (assuming an initial groundwater concentration of tracer and true conservative behavior), the observed concentrations of CAHs can then be adjusted for the effects of dilution.

Aquifer Testing. Hydraulic conductivity is a measure of an aquifer's ability to transmit water, and is an important aquifer parameter governing fluid flow in the subsurface. The flow velocity of groundwater and migration of dissolved contaminants are directly related to the hydraulic conductivity of the saturated zone. In addition, subsurface variations in hydraulic conductivity directly influence contaminant fate and transport by providing preferential flow paths for contaminant migration. Estimates of hydraulic conductivity are used to determine residence times for contaminants and tracers, and to determine the seepage velocity of groundwater. By performing these tests in a consistent manner (location and method) before and after substrate addition, impacts on the aquifer (e.g., bioclogging) due to substrate addition can be measured.

The most common methods used to quantify hydraulic conductivity are aquifer pumping tests (Driscoll, 1986), slug (displacement) tests (ASTM, 1997), and single well drawdown tests (Wilson et al., 1997). One drawback to these methods is that they average hydraulic properties over the length of the saturated screened interval. To help alleviate this potential problem, the screened interval of the test wells should be selected after consideration is given to subsurface stratigraphy based on borehole logs. Hydraulic conductivity can be calculated using a variety of methods and corrections for aquifer conditions (e.g., confined versus unconfined), most readily available through commercial software packages. Another method to delineate zones with high hydraulic conductivity using existing wells is the use of an electromagnetic borehole flow meter, as described in USEPA (1998b).

6.1.2 Process Monitoring

System process monitoring is intended to optimize treatment efficiency by ensuring that specified redox conditions (i.e., sulfate reducing to methanogenic), including appropriate ranges of pH, DO, ORP, and TOC, are being maintained in the reaction zone. This type of monitoring is mostly performed in connection with the use of soluble substrate systems using frequent injections or recirculation. In these systems, substrate solution mixtures and flow rates can be readily modified during operation to optimize the reaction zone.

Primary groundwater parameters that are sampled regularly for process monitoring include the following:

- CAHs;
- DOC or TOC;
- pH, ORP, and DO; and
- Nitrate, ferrous iron, sulfate, and methane.

These parameters provide basic information on the efficacy of substrate delivery and the prevailing redox conditions induced in the reactive treatment zone. VFAs are also commonly measured for systems using low-molecular-weight acids such as lactate, propionate, or butyrate. This information can be used to modify the injection regimes for frequent injections of soluble substrates, as appropriate.

The frequency of process monitoring should be a function of system O&M. More frequent monitoring may be required earlier in the process or with more frequent substrate addition, and less frequent process monitoring is required as the system stabilizes at close to optimal conditions. Typical process monitoring frequency may be as often as weekly to biweekly during the first few months of testing, diminishing to monthly to quarterly for the remainder of the system operation. Systems using long-lasting, slow-release substrates also benefit from system modifications, but this is generally limited to determining the need for additional substrate addition over time. This information can be derived from less frequent performance monitoring (e.g., semiannual to annual).

Examples of process monitoring for soluble substrate systems can be found in Appendices E.1 and E.9. Appendix E.1 summarizes the application of a soluble substrate (lactate) by direct injection into a single well in a deep fractured aquifer for Test Area North at the Idaho National Environmental and Engineering Laboratory. The application progressed through several phases, and process monitoring was used to modify the injection and monitoring protocols while the system reactive zone was being expanded. Table 6.3 summarizes some of the results of the process monitoring and how they were used to modify system operation.

Table 6.3 Summary of Process Monitoring at Test Area North at the Idaho National Environmental and Engineering Laboratory, Idaho

Operation Period	Injection Frequency	Monitoring Frequency	Process Monitoring Parameters	Process Monitoring Results
Field Evaluation Months 1-9	Once to twice per week	Eight locations bi-weekly, 11 locations monthly	CAHs, VFAs, COD, carbon dioxide, alkalinity, ammonia, phosphate, DO, nitrate, ferrous iron, sulfate, methane, ORP, pH, temperature, conductivity, and tritium (tracer)	Concentration of substrate was decreased from 60 to 3 percent, injection volume increased from 330 to 6,600 gallons per event. Lactate fermentation predominant.
Pre-Design Phase I Months 9-14	None (substrate depletion test)	Monthly	Same as Field Evaluation	Propionate fermentation predominant, resulted in faster degradation rates.
Pre-Design Phase II Months 15-30	Every 8 weeks (bi-monthly)	Monthly	Same as above, but dropped carbon dioxide and nitrate and reduced phosphate and ammonia to semi-annual	Reduced injection frequency to every 8 weeks to favor propionate fermentation, increased injection volume to 13,000 gallons per event at 3 to 6 percent lactate.
Pre-Design Operations Month 30 to 48 (last data reported)	Approx. every 8 weeks (bi-monthly)	Monthly	Same as Pre-Design Phase II	Increased injection volume to 52,000 gallons per event to expand effective treatment area.

While the process monitoring protocol employed at Test Area North is relatively extensive, and the data are used for performance monitoring as well, Table 6.3 illustrates some of the benefits of conducting process monitoring. In particular, an evaluation of the primary fermentation reactions (determined by analysis of metabolic acids, or VFAs) versus degradation rates indicated that faster rates of anaerobic dechlorination occurred when propionate fermentation (a degradation product of lactate) was predominant, relative to lactate fermentation (Martin et al., 2001). Allowing the lactate substrate to become depleted allowed propionate fermentation to predominate for periods on the order of several weeks. This allowed the injection frequency to be reduced, while using a lower concentration of substrate (primarily to avoid density effects) and increasing injection volumes. In summary, process monitoring at this site allowed for the following:

- Injection frequency was reduced.
- Monitoring frequency and analytical protocol were reduced.
- Effective treatment area was increased.

Each of these actions increased the effectiveness of the system while optimizing injection and monitoring protocols.

6.1.3 Performance Monitoring

Validation of the performance of an enhanced bioremediation system is required to determine the treatment's effectiveness in attaining remedial objectives and operation endpoints. Performance monitoring uses data collected as part of baseline characterization as a basis for comparison. However, the performance monitoring protocol need only incorporate those analytical protocols that provide useful data regarding the degradation reactions and redox conditions that reflect the system performance. Optional or experimental analyses may be incorporated into performance monitoring protocols for diagnostic purposes if they are beneficial to understanding and improving system performance.

The list of parameters measured during performance monitoring should include, at a minimum, the following:

- Parent CAH compounds and their dechlorination products (such as *cis*-DCE, VC, and ethene),
- An indication of substrate strength (TOC or DOC), and
- Indicators of prevalent geochemical conditions (ORP, DO, ferrous iron, sulfate, methane, pH, and alkalinity).

Contaminants of concern and their dechlorination products must be measured to determine treatment effectiveness. Examples of interpreting performance monitoring data are included in Section 6.3.

Monitoring of full-scale systems operating as designed does not need to be as exhaustive as for pilot-scale systems if the groundwater system is well characterized. Certain monitoring

parameters may be dropped from the performance monitoring protocol if they provide little or no useful information. For example, denitrification will not be a significant redox reaction for a site with naturally low levels of nitrate (e.g., less than 1.0 mg/L). Therefore, continued monitoring of this parameter in this case yields little information on the predominant redox reactions that are occurring. The decision to delete a parameter from the sampling protocol should be made on a case-by-case basis. Caution is advised for regulated parameters that may be expected to change with a lowering of ORP. For example, it may take several months for the system to evolve to reducing conditions that may result in elevated levels of dissolved metals. Therefore, caution is advised before dropping such an analysis.

6.2 MONITORING SYSTEM DESIGN

As described previously, system monitoring is generally used for baseline characterization, process monitoring, or performance monitoring. Different enhanced bioremediation systems may have differing monitoring requirements, including monitoring location and frequency.

6.2.1 Monitoring Network Design

Monitoring locations for baseline characterization and performance monitoring should be located both upgradient and at locations within and downgradient of the reaction zone, parallel to the direction of groundwater flow (Figure 6.1). These wells are intended to monitor changing groundwater chemistry over time along the groundwater flowpath through the treatment area. Consideration should be given to the groundwater seepage velocity and the desired frequency of performance monitoring when determining monitoring locations and spacing. Closer well spacing and/or less frequent monitoring may be warranted for sites with low groundwater velocities relative to sites with high groundwater velocities. Rationale for well placement and examples of effective monitoring networks are described in Wiedemeier and Haas (2003).

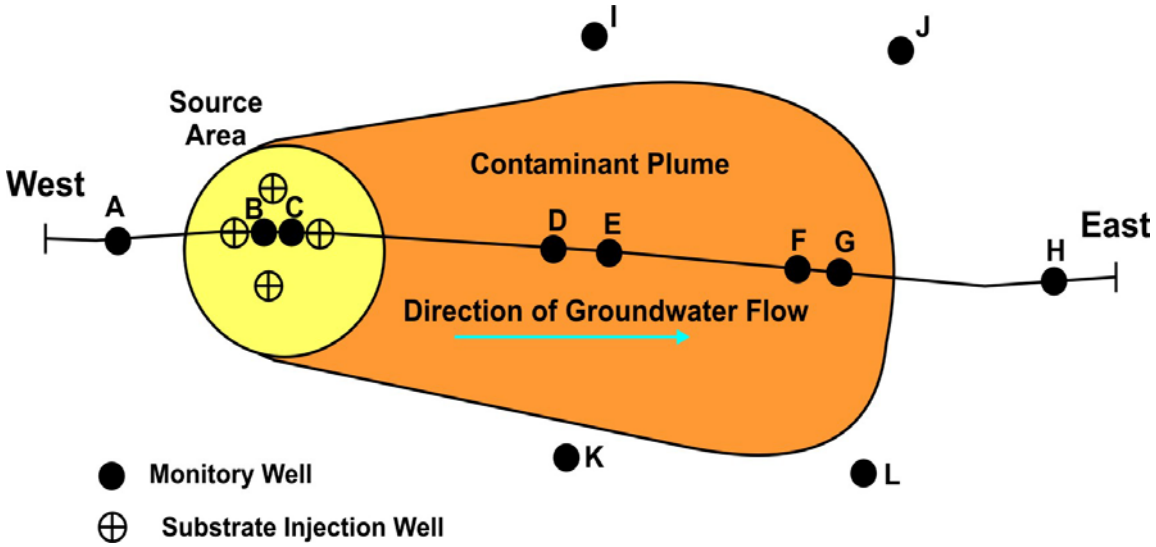

Figure 6.1 Plan View of a Typical Monitoring Well Network for Enhanced Bioremediation

In general, monitoring well screened intervals should be similar to the injection interval(s). It is beneficial to have at least one monitoring location within the injection area screened at multiple depths to determine vertical hydraulic gradients, the potential for vertical migration of substrate, and the vertical extent of the treatment zone. Wells screened in distinct vertical horizons may be required to monitor flowpaths through preferential pathways or plume migration in the presence of vertical hydraulic gradients (Figure 6.2). Cross-gradient well locations are also useful to define the lateral extent of treatment and provide for greater accuracy in mapping hydraulic gradients.

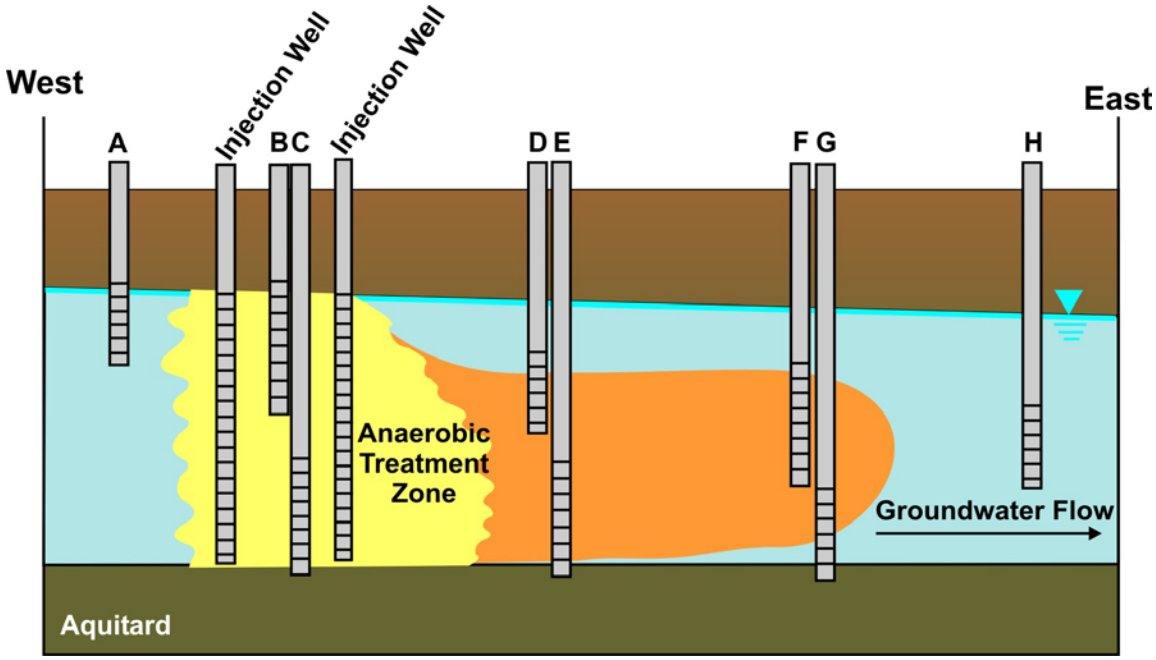

Figure 6.2 Cross-Section View of a Monitoring Well Network for Enhanced Bioremediation

Monitoring locations for process monitoring may be limited to a subset of the existing monitoring network. Process monitoring also may include sampling soluble substrate amendments after mixing and prior to injection, and sampling the injection wells between substrate injections to determine the effects of *in situ* mixing. Downgradient locations within the treatment zone are sampled to determine the area of effective substrate mixing and biostimulation.

6.2.2 Monitoring Frequency

In general, process monitoring for soluble substrate systems should be conducted more frequently (e.g., weekly to monthly) during initial operation of the system and less often (e.g., quarterly to semi-annually) as desired conditions are established. More frequent monitoring is required for active or pulsed systems, in which substrate loading and adjustments to the injection system must be closely monitored. It is often desirable to schedule monitoring to occur between injection events, so that the results of field measurements can be used to refine and adjust substrate strength and injection rate and frequency. The target analyte list for these frequent events may be reduced to basic parameters that provide information on the efficacy of substrate delivery and the redox condition of the treatment zone (Section 6.1.2).

Performance monitoring is typically conducted less frequently than process monitoring, on a quarterly to annual basis for most biostimulation systems. Recirculation systems or systems utilizing bioaugmentation may benefit from more frequent performance monitoring, as substrate distribution and reaction rates are anticipated to be higher. In some instances, longer-term performance monitoring of passive systems may be tied to annual base-wide monitoring programs.

For slow-release substrates where there is no operational component, quarterly to semi-annual monitoring is sufficient to begin with. Typical lag times to stimulate measurable increases in the rate of degradation of chlorinated ethenes (e.g., PCE and TCE to VC and ethene) may be on the order of 6 to 12 months or more. In these cases, frequent sampling on the order of weeks to a month may yield unsatisfactory early results and result in an unjustified lack of confidence in the effectiveness of the system.

6.3 DATA INTERPRETATION

Several methods are available to assess the effectiveness of enhanced anaerobic bioremediation. These include evaluations of changing contaminant concentration/mass over time, changes in groundwater geochemistry, and an evaluation of the efficiency (rate) of biodegradation. The following subsections discuss the contaminant and geochemical changes that occur during enhanced anaerobic bioremediation of CAHs, and some of the common tools and methods used to evaluate and report the effectiveness of an enhanced anaerobic bioremediation system.

6.3.1 Anticipated Changes in CAHs and Biogeochemistry

Groundwater CAH and geochemical data collected during system monitoring can be evaluated to demonstrate whether aquifer redox and geochemical conditions have been modified as planned, and to detect changes in environmental conditions that may optimize or reduce the efficacy of the enhanced bioremediation system. Interpretation of contaminant and geochemical data as it applies to bioremediation of chlorinated solvents is described in further detail in USEPA (1998a), Wiedemeier et al. (1999), and AFCEE (2000).

Monitoring parameters that indicate anaerobic dechlorination of CAHs, or that indicate whether conditions are optimal for the process to occur, include the following:

- Concentrations of parent compounds (e.g., PCE, TCE, 1,1,1-TCA, or CT) are reduced.
- Dechlorination products are being produced (e.g., *cis*-DCE, VC, CA, or CM).
- Ethene and/or ethane are being produced (even low concentrations may indicate anaerobic dechlorination).
- DO concentrations are less than 0.5 mg/L and ORP values are less than 0.0 mV, indicating an anaerobic environment conducive to dechlorination has been achieved.
- Production of Fe(II) and a reduction in sulfate levels further indicate that groundwater conditions are sufficiently reducing for anaerobic dechlorination to occur.

- The production of methane in the groundwater indicates that fermentation is occurring and that the potential for complete anaerobic dechlorination exists.
- Hydrogen concentrations are greater than 1 nmol/L, indicating that sufficient primary electron donor is present to sustain anaerobic dechlorination of CAHs.

Contaminant data is the primary line of evidence used to demonstrate that anaerobic dechlorination is occurring. A reduction in the concentrations of parent compounds coupled with the appearance of dechlorination products can be used to determine the rate and extent to which degradation is occurring.

Assessing biological activity at a field site based on monitoring data can be difficult. Geochemical evaluations are focused on demonstrating that the "footprints" of the expected degradation processes are present. These include indications that alternate electron acceptors have been depleted via utilization of appropriate electron donors, as evidenced by reduced DO, sulfate, and ORP, and increased ferrous iron. The electron donor supply is often measured and tracked by measuring parameters such as TOC or VFAs.

Any practitioner or evaluator of site data should use a multiple converging lines of evidence approach for system performance evaluation and decision-making. A certain percentage of conflicting data will be observed. For this discussion, conflicting data are defined to be individual or multiple results that do not correlate with expected trends or subsurface geochemical conditions. Conflicting data can arise from systematic errors in sampling or analysis. For example, a high DO reading (e.g., 3 to 10 mg/L) in the same well that contains mg/L concentrations of methane typically represents a systematic error, since the production and persistence of methane is inconsistent with the presence of oxygen. The presence of conflicting data should initiate a quality assurance exercise to detect and minimize any systematic errors. But major system modifications should not be initiated due to the presence of conflicting data if multiple lines of evidence support acceptable system performance.

6.3.2 Changes in Contaminant Concentration and Mass

The effectiveness of enhanced anaerobic bioremediation should include an evaluation of contaminant concentration or mass reduction, particularly as reflected in changing molar concentrations of parent and dechlorination products over time. Calculations of both pre- and post-substrate addition contaminant mass can be used to show that the process is working to destroy contaminant mass. In addition, a change in the molar ratio of parent compounds to dechlorination products can be useful in evaluating the extent to which dechlorination is occurring. It is important when evaluating the attenuation of a contaminant plume that the temporal and spatial data demonstrate a clear and meaningful trend in contaminant mass or concentration over time at appropriate monitoring locations.

There are several ways to present data showing changes in contaminant concentrations and plume configuration over time after substrate addition. One method consists of preparing isopleth maps of contaminant concentrations over time. The use of vertical cross-section contour plots oriented along the path of groundwater flow is also recommended to understand the vertical distribution of substrate and contaminant mass.

Evaluating the change in the molar (i.e., molecular) concentration and the molar ratios of parent compounds to dechlorination products can be useful in determining the efficacy of biodegradation brought about by substrate addition. Biodegradation of parent compounds will result in a change in the molar concentrations and ratios of the compounds involved in the reaction.

6.3.2.1 Calculation of Molar Concentrations

Evaluating trends in molar concentrations and ratios can often be more informative than evaluating changes in the parent/dechlorination product concentrations alone (e.g., using concentrations in units of mg/L). The molecular weights of the various parent compounds and dechlorination products vary, with the dechlorination products having progressively lower molecular weights (Table 6.4).

Table 6.4 Molecular Weights for Various Chlorinated Compounds

Compound	Formula	Molecular Weight (grams/mole)
Tetrachloroethene (PCE)	C_2Cl_4	165.83
Trichloroethene (TCE)	C_2HCl_3	131.39
Dichloroethene (DCE)	$C_2H_2Cl_2$	96.95
Vinyl Chloride (VC)	C_2H_3Cl	62.51
Ethene	C_2H_4	28.05
Trichloroethane (TCA)	$C_2H_3Cl_3$	133.41
Dichloroethane (DCA)	$C_2H_4Cl_2$	98.96
Chloroethane (CA)	C_2H_5Cl	64.51
Ethane	C_2H_6	28.05
Tetrachloromethane/ Carbon Tetrachloride (CT)	CCl_4	153.82
Trichloromethane/ Chloroform (CF)	$CHCl_3$	119.38
Dichloromethane (DCM)/ Methylene Chloride (MC)	CH_2Cl_2	84.93
Chloromethane	CH_3Cl_1	50.49
Methane	CH_4	39.49

As a result, the reductive transformation of a given mass of TCE, for example, does not produce the same mass of DCE (e.g., anaerobic dechlorination of 100 µg/L of TCE would produce 73 µg/L of DCE). Conversion of conventional concentrations (e.g., µg/L) to molar concentrations (moles per liter [mol/L]) facilitates assessment of the degree to which reductive transformations occur, because transformation of 1 mole of TCE yields 1 mole of DCE. This conversion is accomplished by dividing the conventional concentration by the molecular weight of the compound. Decreases in the molar concentration of total chlorinated ethenes, for example, indicate that chlorinated ethene mass is being lost and that significant transformation of these compounds to non-toxic end products is occurring. The steps required to calculate molar concentrations and ratios to determine trends over time are as follows:

Step 1 – Molar Concentration: Calculate the concentration of each compound in mol/L for each compound in the reaction sequence using the equation:

$$(1) \qquad \frac{moles_i}{liter} = \frac{C_i}{MW_i}$$

Where: $moles_i$ = moles of compound i
C_i = concentration of compound i (grams per liter)
MW_i = molecular weight of compound i (grams per mole)

Step 2 – Total Molar Concentration: Calculate the total concentration in moles per liter by summing the concentrations of each compound in the reaction sequence.

To illustrate, consider the chlorinated ethenes with PCE as the parent compound:

$$(2) \qquad \sum \frac{moles_{Ethenes}}{liter} = \frac{C_{PCE}}{MW_{PCE}} + \frac{C_{TCE}}{MW_{TCE}} + \frac{C_{DCE}}{MW_{DCE}} + \frac{C_{VC}}{MW_{VC}} + \frac{C_{Ethene}}{MW_{Ethene}}$$

Where: $\sum \frac{moles_{Ethenes}}{liter}$ = total chlorinated ethenes (mol/L)

Step 3 – Molar Fractions: Calculate the molar fraction (ratio) for each compound.

For illustration, consider PCE. This calculation must also be completed for TCE, DCE, VC, and ethene.

$$(3) \qquad MF_{PCE} = \frac{\frac{moles_{PCE}}{liter}}{\sum \frac{moles_{Ethenes}}{liter}}$$

Where: MF_{PCE} = molar fraction of PCE (unitless)

6.3.2.2 Contaminant Molar Concentration Plots

Plots of molar concentrations of parent compounds and dechlorination products can be useful in evaluating the effectiveness of enhanced bioremediation. Figure 6.3 shows conceptually how concentrations of individual compounds change as dechlorination proceeds. Conversely, Figure 6.4 shows a pattern of expected change in contaminant concentrations when large or excessive volumes of substrate are injected (particularly near the injection point) or recirculation causes dilution to be the prominent effect. Without sequential dechlorination, the ratios of the targeted compounds in this case all remain relatively constant, even though all concentrations decline.

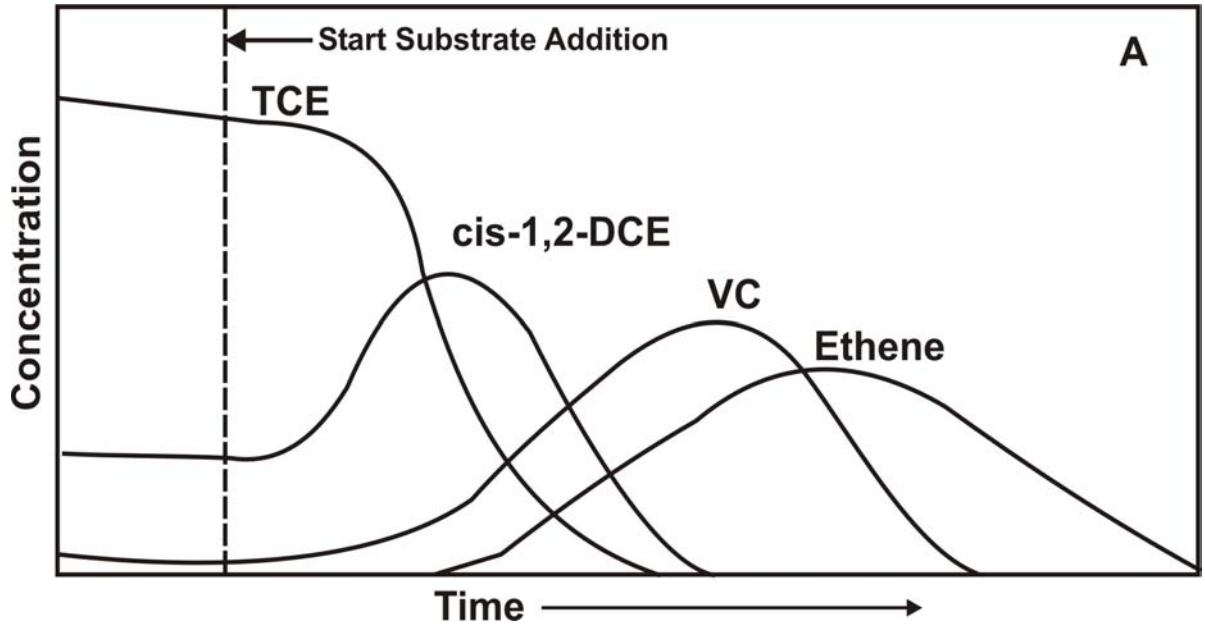

Figure 6.3 Conceptual Changes in Contaminant Molar Concentration over Time with Sequential Anaerobic Dechlorination

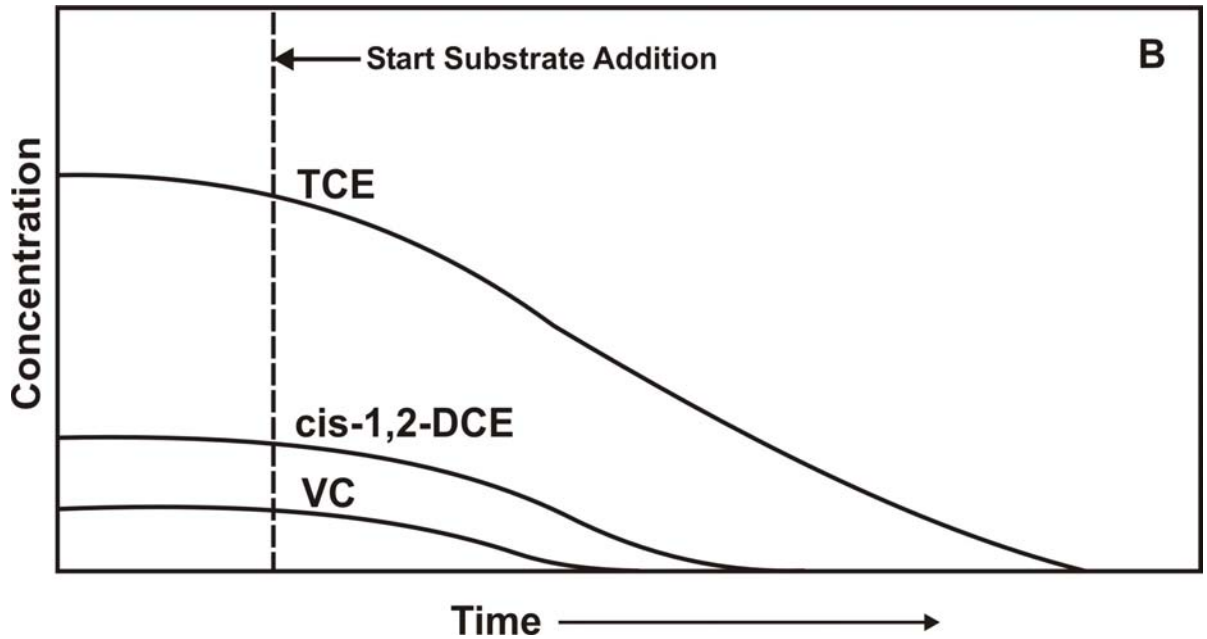

Figure 6.4 Conceptual Changes in Contaminant Molar Concentration over Time with Dilution Only

Figure 6.5 depicts real-world data on how concentrations of individual compounds changed over time at an enhanced bioremediation application at Travis AFB, California. It is clear from this plot that sequential anaerobic dechlorination occurred with a temporal accumulation of the intermediate dechlorination products *cis*-DCE and VC at periods of

approximately 5 and 16 months after substrate addition, respectively. ***The practitioner should exercise care in interpreting early sampling results that indicate a temporal accumulation of intermediate dechlorination products; this trend may be due to kinetic disparity where the intermediate dechlorination product is being generated faster than it is degraded, and not to an absence of appropriate dechlorinating microorganisms.*** Once the more highly chlorinated compounds are depleted (e.g., TCE), then concentrations of the less chlorinated compounds (*cis*-DCE and VC) should decline.

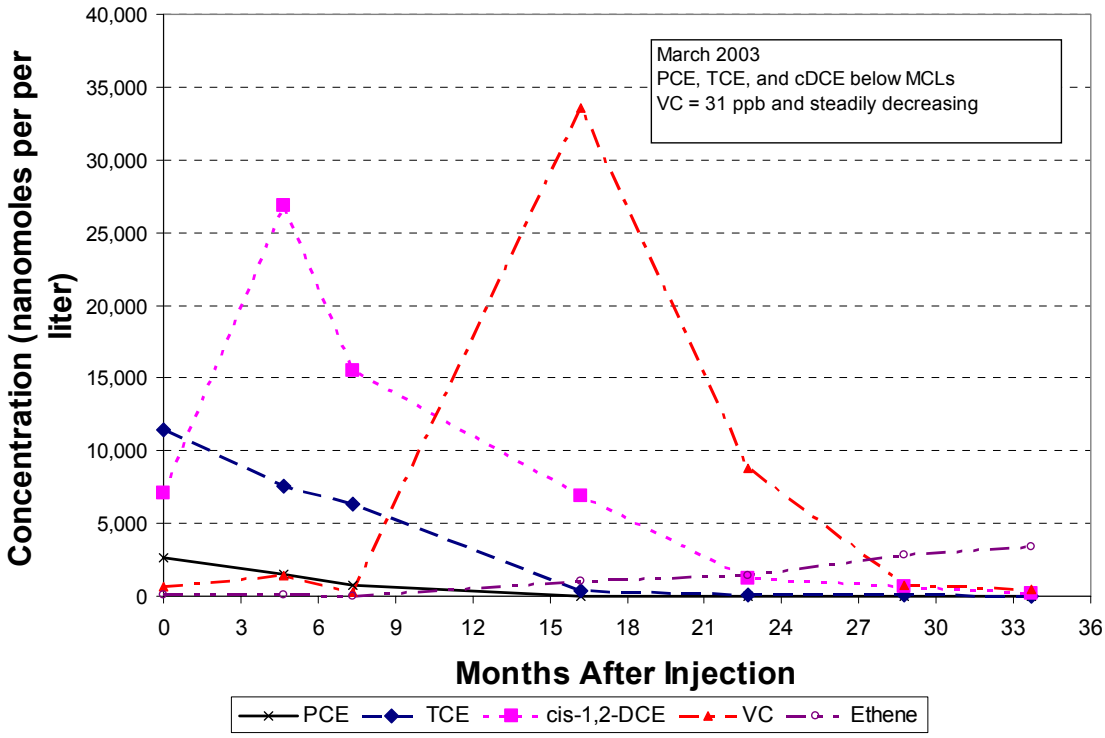

Figure 6.5 Changes in Molar Concentration over Time (Well MW4, Site SS015, Travis AFB, California)

6.3.2.3 Changes in Total Molar Concentration

Figure 6.6 presents a plot of the molar concentration of total chlorinated ethenes (PCE, TCE, DCE, plus VC) versus distance downgradient along the groundwater flow path for several sampling events for a bark mulch biowall at Altus AFB, Oklahoma. Note that ethene and ethane were purposely left out of the calculation because they do not represent contaminant mass (they are innocuous byproducts). The decreasing contaminant concentrations within the biowall shown on Figure 6.6 provides reasonable evidence that contaminant mass is being converted to innocuous end products.

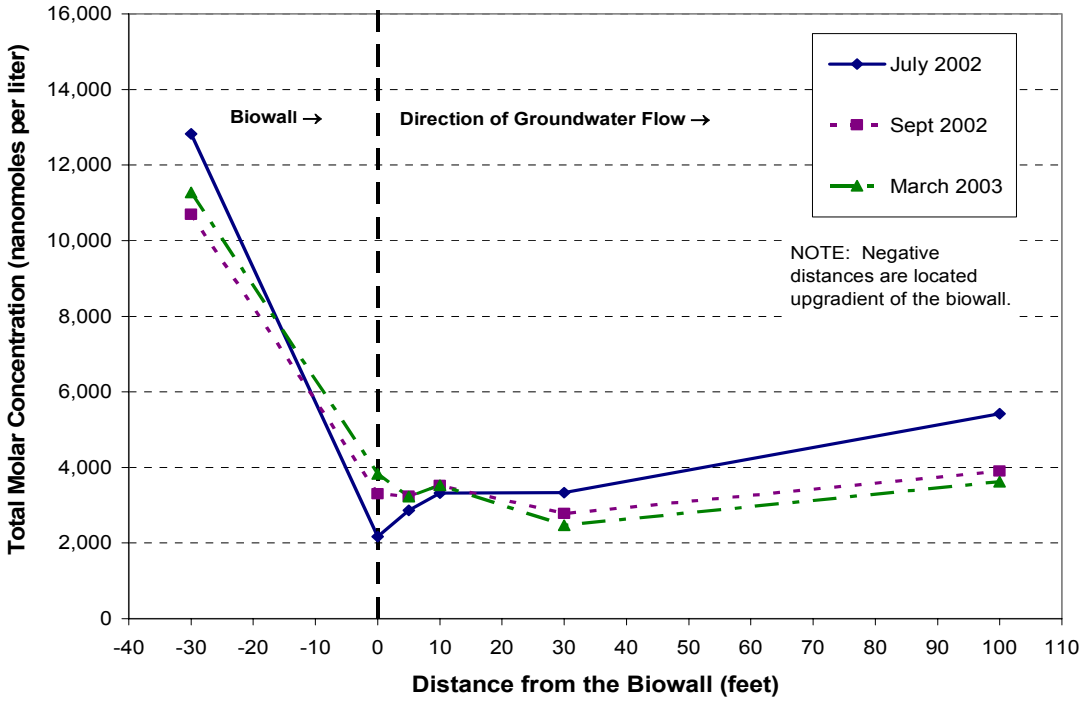

Figure 6.6 Total Molar Concentration over Distance along Groundwater Flowpath through a Mulch Biowall at Altus AFB, Oklahoma

6.3.2.4 Changes in Molar Fractions

A plot of the molar fraction or ratio over time is another method used to determine if biodegradation has been stimulated. In particular, this method is often employed when there is a constant or continuing source of contaminant mass entering a treatment system. ***In this case, the total molar concentration may remain elevated or even increase due to a continuing mass influx, but an increase in the molar ratio of dechlorination products will demonstrate that sequential anaerobic dechlorination is occurring.***

Figure 6.7 is a plot of total molar concentration and molar fractions of individual chlorinated ethenes for the same monitoring location shown in Figure 6.5. In this case, total molar concentration was variable, and actually increased over the first 16 to 18 months of treatment. This increase in total molar concentration could be interpreted as ineffective treatment, but is likely due to enhanced dissolution or desorption of a residual source. However, changes in molar fractions clearly indicate that sequential anaerobic dechlorination was occurring. Once PCE and TCE were depleted, total molar concentration decreased as *cis*-DCE and VC were transformed to innocuous end products.

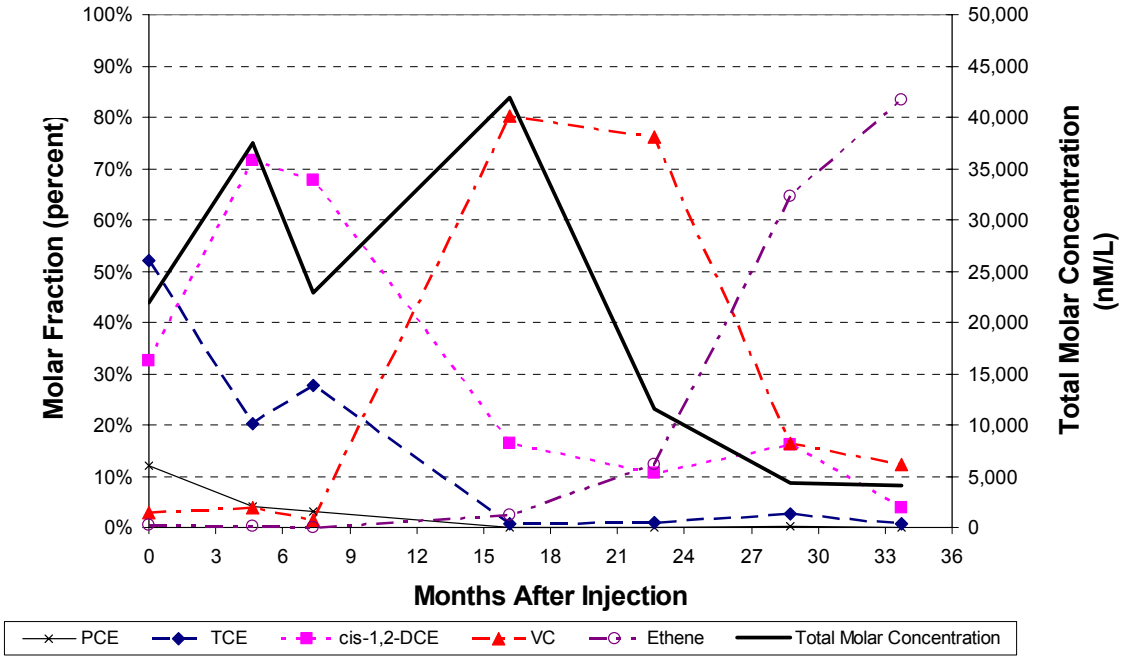

Figure 6.7 Changes in Molar Fraction and Total Molar Concentration over Time (Well MW4, Site SS015, Travis AFB, California)

6.3.3 Statistical Techniques

A number of statistical approaches can be used to evaluate plume stability. The AFCEE *Long Term Monitoring Decision Support Software Package* is an example of computational tools used to evaluate plume stability. This tool uses Mann-Kendall, moving average, and linear regression statistical techniques, as well as a module for center of mass calculations. The program can be accessed on the AFCEE website at http://www.afcee.brooks.af.mil/products/techtrans/treatmenttechnologies.asp. A few of the common statistical techniques available are listed below. Although intended for use in determining the effectiveness of MNA, these techniques may be useful for determining the impact of an enhanced bioremediation application on overall plume dynamics.

- **Regression Analyses.** Contaminant trends can be analyzed by plotting concentration data vs. time, usually in semi-log scale with log concentration being plotted against linear time. Use of a log scale for concentration data facilitates visualization of the relatively large changes in concentration that may have occurred (e.g., a concentration reduction from 1 mg/L to 1 µg/L represents a 1,000-fold reduction). Linear regression calculations can also be used to determine the confidence of any apparent trends in concentration.

- **Mann-Whitney U-Test.** The Mann-Whitney U test (also called the Wilcoxon Rank-Sum Test) is currently being used by the state of New Jersey to determine plume stability (28 N.J.R. 1143). A description of this method can be found in the AFCEE (2000) document *Designing Monitoring Programs to Effectively Evaluate the Performance of Natural Attenuation.* The test is nonparametric (Mann and Whitney, 1947), which means that it does not assume that the data conform to any particular

distribution (e.g., normal, log-normal), and that the outcome of the test is not determined by the overall magnitude of the data points, but depends on the ranking of individual data points.

- **Mann-Kendall Test.** The Mann-Kendall Test is another nonparametric test (Gilbert, 1987) that can be used to define the stability of a solute plume (i.e., stable, diminishing, or expanding) based on concentration trends at individual wells. To evaluate plume stability, four or more independent sampling events are required. The calculation approach is different from the Mann-Whitney test; a description of the method can be found in AFCEE (2000). One can also perform a more sophisticated analysis by comparing the Mann-Kendall S statistic, a calculated confidence level, and the coefficient of variance for the sample data (Gilbert, 1987).

- **Center of Mass Calculations.** A more rigorous evaluation of plume dynamics involves the estimation of the total dissolved mass and the location of the centroid of mass for the plume. Center of mass calculations are described in the AFCEE Monitoring and Remediation Optimization System *(*MAROS) program (GSI, 2003). Under natural conditions, this information may aid in assessing the status of the plume and in interpreting its migration pattern over time. However, enhanced bioremediation systems typically remove mass only from specific portions of the plume, and changes in the centroid of mass over time may not accurately reflect overall plume dynamics. Therefore, results from using this method should be carefully evaluated.

When using these statistical techniques, comparative analyses should be conducted using data from similar hydrogeological conditions (e.g., seasonal variations) and data quality (such as method detection limits). Statistical methods should not be used to analyze apparent trends across data points that are not comparable.

6.3.4 Geochemistry

The variability associated with collecting groundwater samples often makes precise definition of reactions or zones of differing ORP difficult, and the various lines of evidence should be weighed together to determine if substrate addition has stimulated anaerobic dechlorination. The following subsections describe the changes in geochemical conditions that are commonly evaluated for enhanced anaerobic bioremediation.

6.3.4.1 Competing Electron Acceptors

Native electron acceptors potentially compete with anaerobic dechlorination of CAHs. After depletion of DO, anaerobic microbes will use nitrate as an electron acceptor, followed by manganese (IV), ferric iron (Fe[III]), sulfate, and finally carbon dioxide (methanogenesis). These parameters are measured to establish the prevailing redox conditions. In some cases, it is easier to evaluate the byproducts of the reduction (e.g., manganese [II], Fe[II], or methane) of these electron acceptors, which are more readily measured in groundwater samples.

Dissolved Oxygen. DO is the most thermodynamically favored electron acceptor used by microbes for the biodegradation of organic carbon, whether natural or anthropogenic. Anaerobic bacteria generally cannot function at DO concentrations greater than about 0.5 mg/L, and hence anaerobic dechlorination will not occur. Therefore, it is important to

have a source of carbon in the aquifer that can be used by aerobic microorganisms as a primary substrate to deplete DO.

Nitrate. After DO has been depleted in the treatment zone, nitrate may be used as an electron acceptor for anaerobic biodegradation of organic carbon primarily via denitrification. For anaerobic dechlorination to occur, nitrate concentrations in the contaminated portion of the aquifer should be less than approximately 1 mg/L (USEPA, 1998a). Depending on the amount of fermentable substrate and nitrate already present in the aquifer, nitrate may already be low or depleted.

Iron (II) and Manganese (II). In some cases Fe(III) and manganese (IV) are used as electron acceptors during anaerobic biodegradation of organic carbon, but typically they are present in solid mineral form. During this process, Fe(III) is reduced to Fe(II), which is soluble in water. Similarly, manganese (IV) is reduced to soluble manganese (II). Fe(II) and manganese (II) concentrations can thus be used as indicators of anaerobic biodegradation. Care must be taken when interpreting Fe(II) and manganese (II) concentrations because they may be biased low due to co-precipitation with sulfides. Depending on the amount of fermentable substrate and bioavailable iron already present in the aquifer, a site may not exhibit a significant increase in Fe(II) concentrations if Fe(III) is already low or depleted.

Sulfate. After DO, nitrate, manganese, and iron have been depleted in the microbiological treatment zone, sulfate may be used as an electron acceptor for anaerobic biodegradation. Sulfate reduction results in the production of sulfide. Concentrations of sulfate greater than 20 to 50 mg/L may cause competitive exclusion of anaerobic dechlorination. However, anaerobic dechlorination of CAHs may still occur simultaneously with sulfate reduction in many plumes with high concentrations of sulfate.

Methane. During methanogenesis, acetate is split to form carbon dioxide and methane, or carbon dioxide is used as an electron acceptor and is reduced to methane. The fastest rates of anaerobic dechlorination typically occur under sulfate-reducing or methanogenic conditions. However, highly elevated concentrations of methane (greater than 5 to 10 mg/L) also may indicate that the substrate is being consumed by methanogens at the expense of dechlorinating organisms.

6.3.4.2 General Geochemical Indicator Parameters

Geochemical indicator parameters commonly measured during system monitoring include ORP, pH, alkalinity, and chloride.

Oxidation-Reduction Potential. The ORP of groundwater (hydrogen electrode [Eh]) is a measure of electron activity and is an indicator of the relative tendency of a solution to accept or transfer electrons. Redox reactions in groundwater containing organic compounds (natural or anthropogenic) are usually biologically mediated, and therefore the ORP of a groundwater system depends on and influences rates of biodegradation. While the ORP of groundwater generally ranges from -400 mV to +800 mV, most biological processes operate only within a prescribed range of ORP. Therefore, characterizing the range of ORP of the reaction zone provides an indirect indicator of the redox reactions (including anaerobic dechlorination of CAHs) that may be occurring.

pH and Alkalinity. There is a positive correlation between zones of microbial activity and increased alkalinity. Increases in alkalinity result from the dissolution of carbonate minerals driven by the production of carbon dioxide produced by the metabolism of microorganisms. Alkalinity is important in the maintenance of groundwater pH because it buffers the groundwater system against acids generated during both aerobic and anaerobic biodegradation. Biodegradation of organic compounds may generate enough acid to impact the pH of the groundwater. Controlling the range of pH in the reaction zone may be necessary to maintain effective anaerobic dechlorination.

Chloride. During biodegradation of chlorinated hydrocarbons dissolved in groundwater, chlorine atoms are released into the groundwater, resulting in increasing chloride concentrations, which are elevated relative to background concentrations, in groundwater in the contaminant plume. However, high background concentrations of chloride may mask the production of chloride due to anaerobic dechlorination. Therefore, chloride is generally considered as an indicator parameter only.

6.3.5 Microbial Evaluation

Optional analyses used to evaluate microbial activity and the potential for anaerobic dechlorination of CAHs to occur includes dissolved hydrogen, metabolic acids (VFAs), and molecular analysis for specific microbial species.

Dissolved Hydrogen. Molecular hydrogen is the primary electron acceptor used in anaerobic dechlorination and is produced by fermentation reactions. Concentrations of dissolved hydrogen have been used to evaluate redox processes in groundwater systems (Lovley and Goodwin, 1988; Lovley et al., 1994; Chapelle et al., 1995). Significantly, nitrate-, Fe(III)-, sulfate- and carbon dioxide-reducing (methanogenic) microorganisms exhibit different efficiencies in utilizing the hydrogen that is being continually produced. For example, nitrate reducers are highly efficient in utilizing hydrogen and maintain very low steady-state hydrogen concentrations. These characteristic ranges are listed in Table 2.4.

Conversely, sulfate reducers and methanogenic bacteria are progressively less efficient and maintain higher hydrogen concentrations. Because each terminal electron-accepting process has a characteristic hydrogen concentration associated with it, hydrogen concentrations can be an indicator of predominant redox processes. Dechlorinating bacteria also exhibit an efficiency in utilizing hydrogen. If hydrogen concentrations are greater than approximately 1 nmol/L, then rates of anaerobic dechlorination should have environmental significance.

Metabolic Acids. Metabolic acids, or short-chain VFAs, are typically an optional monitoring parameter used for diagnostic purposes. Metabolic acids produced by degradation of the primary substrate indicate microbial activity as well as substrate distribution. A lack of metabolic acids (less than 1 mg/L) usually indicates that additional substrate is required. Furthermore, metabolic acids can be fermented to produce hydrogen for anaerobic dechlorination. Different degradation pathways for the same compound (e.g., lactate) can provide differing dechlorination equivalents. Thus, the distribution of metabolic acids also may indicate the type and efficiency of the degradation pathways that produce hydrogen and the degree to which hydrogen is utilized for anaerobic dechlorination.

Molecular Screening for Dehalococcoides species. Molecular screening techniques are an emerging analyses used for diagnosis of the types and quantities of specific dechlorinating species present or active in the aquifer. Genetic screening 16S rDNA sequences using PCR and/or denaturing gradient gel electrophoresis analyses can be used to identify a few select species of dechlorinating bacteria (Section 4.5). The absence of *Dehalococcoides* species and persistence of intermediate dechlorination products is sometimes used as justification for bioaugmentation. While molecular techniques can yield a positive for the bacterial species *Dehalococcoide*s, other species of bacteria that are capable of anaerobic dechlorination should not be overlooked.

6.3.6 Biodegradation Rate Calculations

If biodegradation has been stimulated by substrate addition, an increase in biodegradation rates should be observed. Calculation of biodegradation rate constants prior to and after substrate addition may help demonstrate the effectiveness of the system. Biodegradation rate constant estimates can be calculated by many methods. The reader is referred to such documents as USEPA (1998a) and Newell et al. (2003) for a detailed discussion of biodegradation rate constant estimation.

In practice, however, biodegradation rates are difficult to determine because enhanced bioremediation systems are seldom in a state of equilibrium. Methods that assume that steady state conditions exist (such as the method of Buscheck and Alcantar, 1995) are generally not appropriate for enhanced bioremediation systems. The addition of an organic substrate causes significant changes in the geochemical conditions and biological activity of the aquifer, which rarely stabilize over the treatment duration. Instead, average degradation rates are typically based on the concentration of the contaminant entering and leaving the treatment system, and the average contaminant residence time. This requires the hydraulics of the system to be well characterized, as well as consideration of the sorptive properties of the contaminant (i.e., retardation) and of dilution effects.

An additional consideration is the transfer of mass from a DNAPL or sorbed phase to the aqueous phase due to enhanced dissolution or desorption. Biodegradation rate calculations that do not take this into account will be conservative in reflecting the actual rate of degradation. Conversely, transfer of mass from the aqueous phase due to partitioning (such as into vegetable oils) or sorption to solid phase substrates (such as mulch) may cause an initial apparent attenuation in aqueous phase concentrations. In these cases, the partitioning or sorptive properties of the contaminants relative to the substrate can be used to estimate mass transfer due to non-destructive mechanisms to calculate a more representative biodegradation rate.

6.3.7 Pilot Test Results and Test Controls

Evaluation of pilot test data should include assessment of whether contaminant mass loss may be due to natural destructive biodegradation or to non-destructive processes such as sorption, dilution, or dispersion. Side-by-side, treated and untreated controls are rarely implemented in most substrate applications. However, substitute “controls” are practical in pilot test design, including upgradient wells and wells with historical data trends in the treatment zone. Upgradient wells can help compensate for the effects of natural attenuation over time. Changes in temporal contaminant concentration trends at the site before and after

implementation of enhanced bioremediation can be used to determine the effectiveness of substrate addition versus natural attenuation processes.

The results from a pilot test can also be used to:

- Demonstrate the efficacy of enhanced bioremediation at a particular site by providing site-specific field data regarding contaminant reduction;
- Determine the substrate loading rate and frequency of injections required to maintain reducing conditions; and
- Define full-scale design parameters (including well spacing and substrate loading) based on substrate distribution, ROI, and the extent to which the reactive zone was established.

Once the pilot test program has defined the critical design criteria, full-scale design can be completed and regulatory approval obtained for full-scale expansion.

6.4 SYSTEM MODIFICATIONS

Table 6.5 lists various system modifications that can be made during pilot-scale operation or full-scale design to deal with undesirable site conditions or developments. Soluble substrate systems that use frequent injections have the most flexibility in modifying injection scenarios. When using infrequent applications of slow-release substrates, potential problems such as the need to add a buffering agent should be evaluated prior to substrate addition, and buffer added during substrate injection as a precautionary measure when in doubt.

Table 6.5 System Modifications for Special Site Conditions

Condition	Modification
Low pH or low buffering capacity	Use of buffer Use of water push for soluble substrates Use of slower-release substrates
Low permeability/groundwater velocity	Closely spaced direct push injections Less frequent injections
High permeability/groundwater velocity	Higher donor loading rates More frequent injections
Inhibitory levels of salinity	Low-sulfate donors (e.g., corn syrup) Higher rates of substrate loading
Buildings above reactive zone	Vapor monitoring systems Vapor control systems (e.g., SVE)
Incomplete dechlorination	Allow for longer lag times Lower the redox environment Bioaugmentation

Modified from Suthersan et al., 2002.

Inadequate or excessive distribution of substrate due to aquifer permeability and/or groundwater flow rates can be adjusted by increasing or decreasing the substrate dose, and/or by modifying injection frequency or well spacing. These modifications are more easily

accomplished for soluble substrates. Substrate application rates may also be increased in the event of inhibitory electron acceptor demand.

Contingencies should be provided when applying the technology near potential preferential vapor migration pathways or accumulation areas such utility corridors and basements. If soil gas monitoring indicates a vapor hazard (e.g., methane), application of remedial measures such as soil vapor extraction may be warranted.

Incomplete or delayed dechlorination is a common limitation of enhanced anaerobic bioremediation. Prior to considering bioaugmentation, the system should be carefully evaluated to ensure that the proper geochemical conditions have been achieved and that a sufficient lag phase has been allowed for ecological succession and development of appropriate microbial consortia. In some cases, bioaugmentation with commercially available cultures or microorganisms from another site can be implemented once it has been determined that indigenous bacterial communities are not suitable for anaerobic dechlorination. The added cost of bioaugmentation also may be justified based on slow rates of degradation or long lag times to meet time-sensitive performance objectives.

6.5 REPORTING OF SYSTEM MONITORING AND PERFORMANCE

A results report should be prepared after the initial operational period (perhaps after 18 to 24 months of operation, with annual updates) that summarizes relevant site data collected during the field test. This report should include a site-specific data review, a description of system installation and substrate addition activities, a detailed chronology, data collection and interpretation, and conclusions and recommendations. In particular, the report should clearly state the objectives and goals of the field application and the extent to which they were achieved, and whether system expansion or a full-scale application is desirable, feasible, and practical. Specific items to discuss in the report include the following:

Remedial Objectives (refer to Sections 3 and 4)**:**

- Overall remedial objectives and required regulatory compliance for the site.
- Specific field test and data quality objectives.

System Installation and Operation (refer to Sections 4 and 5)**:**

- Injection system performance or system construction (e.g., trenching) and any operational or safety issues of concern.
- Delivery system efficiency, including flow rates, injection pressures, volumes, concentrations, and suppliers of injected substrates and amendments.
- Results of process monitoring and modifications made to the system design, including improvements in system performance.
- Extent and uniformity of substrate distribution and ROI.
- As-built drawings, specifications, and catalog cut-sheets.
- Cost summary.

System Performance (refer to Section 6):

- Electron donor loading and utilization rates and the efficiency of electron donor utilization for anaerobic dechlorination as compared to alternate biodegradation processes (e.g., methane production).
- Effective ROI for anaerobic dechlorination of CAHs (including downgradient extent) and apparent electron donor requirements.
- Electron acceptor reduction and prevailing terminal electron accepting processes.
- Extent of anaerobic dechlorination of contaminant mass, including changes in contaminant concentrations and mass considering volatilization, dilution, degradation, and dechlorination product formation and persistence.
- Reaction kinetics and estimated biodegradation rates, including a comparison to natural (background) degradation rates.
- Extent of sequential anaerobic dechlorination, including apparent accumulation of dechlorination products (e.g., *cis*-DCE and VC).
- Evaluation of microbial lag phases and estimated time to meet remedial endpoints.
- System modifications required to optimize performance.
- Contributions or effects of any additional amendments added to the system (e.g., secondary substrates, microbial augmentation, nutrients, or vitamins/cofactors).

Secondary Issues (refer to Section 3.4)

- Secondary impacts to water quality.
- Gas accumulation in the unsaturated zone.
- Impacts on site infrastructure and operations.

Recommendations

- Feasibility and relative cost-effectiveness of enhanced anaerobic bioremediation to meet full-scale remedial objectives.
- Scale-up issues, design considerations, and mitigation or contingency measures.

Based on this information, the report should detail the overall effectiveness of the treatment system and make objective recommendations regarding continued application of enhanced anaerobic bioremediation, and whether continued system operation or system expansion is warranted.

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SECTION 7

ENHANCED ANAEROBIC BIOREMEDIATION REFERENCES

Adamson, D. T., J. M. McDade and J. B. Hughes. 2003. Inoculation of a DNAPL Source Zone to Initiate Reductive Dechlorination of PCE. *Environmental Science & Technology*. Vol. 37:2525-2533. http://pubs.acs.org/journals/esthag.

Adamson, D. T. and G.F. Parkin. 2000. Impact of Mixtures of Chlorinated Aliphatic Hydrocarbons on a High-rate, Tetrachloroethene-dechlorinating Enrichment Culture. *Environmental Science & Technology,* Vol. 34:1959-1965.

Adrian, L., U. Szewzky, J. Wecke, and H. Gorisch. 2000. Bacterial Dehalorespiration with Chlorinated Benzenes. *Nature*, Vol. 408:580-583.

Adriaens, P., M.J. Barcelona, K.F. Hayes, M.L. McCormick, and K.L. Skubal. 2001. Biotic and Abiotic Dechlorination in Iron-Reducing and Sulfidogenic Environments. *Proceedings of the Sixth International Symposium on* InSitu *and On-Site Bioremediation, San Diego, California*, Vol. 6(8):193-199. Columbus, OH: Battelle Press.

Air Force Center for Environmental Excellence (AFCEE). 2003. *Aqueous and Mineral Intrinsic Bioremediation Analysis (AMIBA) of the Pine Bark Mulch Permeable Barrier at Altus Air Force Base SWU-7 (OU-1)*. AFCEE, San Antonio, Texas.

AFCEE. 2000. *Designing Monitoring Programs to Effectively Evaluate the Performance of Natural Attenuation*. AFCEE, San Antonio, Texas. http://www.afcee.brooks.af.mil/products/techtrans/monitorednaturalattenuation/protocols.asp.

AFCEE. 1995. *Technical Protocol for Implementing Intrinsic Remediation with Long-term Monitoring for Natural Attenuation of Fuel Contamination Dissolved in Ground Water.* AFCEE, Brooks Air Force Base, Texas.

Air Force Research Laboratory (AFRL), Battelle Memorial Institute, Cornell University, USEPA, NFESC. 2001. *Reductive Anaerobic Biological In-Situ Treatment Technology (RABITT) Treatability Test Interim Report.* 17 August.

American Society for Testing and Materials (ASTM). 1997. Method D 4044, Test Method (Field Procedure) for Instantaneous Change in Head (Slug Tests) for Determining Hydraulic Properties of Aquifers.

Aziz, C.E., M. Schipper, M.M. Hampton, J. Hansen, and P. Cork. 2003. Full-Scale Mulch Biowall Treatment of a Chlorinated Solvent Plume (abstract). Presented at the Seventh International Symposium of In Situ and On-Site Bioremediation, Orlando, FL. June 2-5, 2003.

Ballapragada, B.S., H.D. Stensel, J.A. Puhakka, and J.F. Ferguson. 1997. Effect of Hydrogen on Reductive Dechlorination of Chlorinated Ethenes. *Environmental Science & Technology*. Vol. 31(6):1728-1734.

Beeman, R.E., J.E. Howell, S.H. Shoemaker, E.A. Salazar, and J.R. Buttram. 1994. A Field Evaluation of *In Situ* Microbial Reductive Dehalogenation by the Biotransformation of Chlorinated Ethenes. In: R.E. Hinchee, A. Leeson, L. Semprini, and S.K. Ong (Eds.), *Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbons*. 14-27. Lewis Publishers, CRC Press.

Bloom, Y., R. Aravena, D. Hunkeler, E. Edwards, and S.K. Frape. 2000. Carbon Isotope Fractionation during Microbial Dechlorination of Trichloroethene, *cis*-1,2-dichloroethene, and Vinyl Chloride: Implications for Assessment of Natural Attenuation. *Environmental Science & Technology*, Vol. 34:2768-2772.

Borden, R.C. 2002. Anaerobic Treatment Using Edible Oil. Presented at AFCEE Clean Up Technology Transfer Workshop, March 4-7, 2002, San Antonio, TX.

Bouwer, E.J. 1994. Bioremediation of Chlorinated Solvents Using Alternate Electron Acceptors. In Norris, R.D., R.E. Hinchee, R. Brown, P.L McCarty, L. Semprini, J.T. Wilson, D.H. Kampbell, M. Reinhard, E.J. Bouwer, R.C. Borden, T.M. Vogel, J.M. Thomas, and C.H.Ward (Eds), *Handbook of Bioremediation*. 149-175. Lewis Publishers.

Bradley, P.M. 2000. Microbial Degradation of Chloroethenes in Groundwater Systems. *Hydrogeology Journal*, Vol. 8:104-111.

Bradley, P.M. and F.H. Chapelle. 2000. Aerobic Microbial Mineralization of Dichloroethene as Sole Carbon Substrate. *Environmental Science & Technology*, Vol. 34: 221-223.

Bradley, P.M., and F.H. Chapelle. 1997. Kinetics of DCE and VC Mineralization under Methanogenic and Fe(III)-reducing Conditions. *Environmental Science & Technology*, Vol. 31:2692-2696.

Bradley, P.M., J.E. Landmeyer, and R.S. Dinicola. 1998a. Anaerobic Oxidation of [1,2-14C]Dichloroethene under Mn(IV)-reducing Conditions. *Applied Environmental Microbiology*, Vol. 64:1560-1562.

Bradley, P.M., F.H. Chapelle, and J.T. Wilson. 1998b. Anaerobic Mineralization of Vinyl Chloride in Fe(III)-reducing, Aquifer Sediments. *Journal of Contaminant Hydrology,* Vol. 31(1-2):111-127.

Bradley, P.M., F.H. Chapelle., and D.R. Lovely. 1998c. Humic Acids as Electron Acceptors for Anaerobic Microbial Oxidation of Vinyl Chloride and Dichloroethene. *Applied Environmental Microbiology*, August, 64:3102-3105.

Buscheck, T. E. and C.M. Alcantar. 1995. Regression Techniques and Analytical Solutions to Demonstrate Intrinsic Bioremediation. In: *Proceedings of the Third International Conference on In Situ and On-Site Bioreclamation Symposium,* Vol. 3(1):109-116. Battelle Press, Columbus, Ohio.

Butler, E.C. and K.F. Hayes. 2000. Kinetics of the Transformation of Halogenated Aliphatic Compounds by Iron Sulfide. *Environmental Science & Technology,* Vol. 34(3):422-429.

Butler, E.C. and K.F. Hayes. 1999. Kinetics of the Transformation of Trichloroethylene and Tetrachloroethylene by Iron Sulfide. *Environmental Science & Technology,* Vol. 33(12):2021-2027.

Carr, C.S., S. Garg, and J.B. Hughes. 2000. Effect of Dechlorinating Bacteria on the Longevity and Composition of PCE-containing NAPL Sources under Equilibrium Dissolution Conditions. *Environmental Science & Technology,* Vol. 34:1088-1094.

Castellanos, M.R., T.A. Peel, M.L. McMaster, J. Adkisson, and S. Dworatzek. 2002. Laboratory Evaluation of Enhanced Bioremediation of Chlorinated Ethenes in Groundwater at the MLP/VAB Area. In: A.R. Gavaskar and A.S.C. Chen (Eds.), *Remediation of Chlorinated and Recalcitrant Compounds – 2002: Proceedings of the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2002)*. Paper 2B-30. Columbus, OH: Battelle Press.

Chamberlain, W.B. 2003. Bionutrient Modeling for Design of *In Situ* Bioremediation. *Pollution Engineering*, April:28-33**.**

Chapelle, F.H. 1993. *Groundwater Microbiology and Geochemistry*. New York: John Wily & Sons.

Chapelle, F.H., P.B. Mahon, N.M Dubrovsky, R.F. Fujii, E.T. Oaksford, and D.S. Vroblesky. 1995. Deducing the Distribution of Terminal Electron-accepting Processes in Hydrologically Diverse Groundwater Systems. *Water Resources Research*, Vol. 31(2):59-371.

Coleman, N.V., T.E. Mattes, J.M. Gossett, and J.C. Spain. 2002. Biodegradation of *cis*-Dichloroethene as the Sole Carbon Source by a β-Proteobacterium. *Applied and Environmental Microbiology*, Vol. 68(6):2726-2730.

Conrad, M.E., D.J. DePaolo, D.L. Song, and E. Neher. 1999. Isotopic evidence for Groundwater Flow and Biodegradation of Organic Solvents at the Test Area North Site, INEEL. In: *Ninth Annual V.M. Goldschmidt Conference*. 58-59. LPI Contribution No. 971. Lunar and Planetary Institute, Houston.

Cope, N. and J.B. Hughes. 2001. Biologically Enhanced Removal of PCE from NAPL Source Zones. *Environmental Science & Technology*, Vol. 35:2014-2021.

Cowan, D. 2000. Innovative Abatement and Remediation of Perchlorate at McGregor, Texas Weapons Plant Site. *Soil Sediment &Groundwater*, Vol. 5:25-26.

Cupples, A.M., A.M. Spormann, and P.L. McCarty. 2003. Growth of a *Dehalococcoides*-like Microorganism on Vinyl Chloride and *cis*-Dichloroethene as Electron Acceptors as Determined by Competitive PCR. *Applied Environmental Microbiology,* Vol. 69:953-959.

Dennis, P. C., B.E. Sleep, R.R. Fulthorpe, and S.N. Liss. 2003. Phylogenetic Analysis of Bacterial Populations in an Anaerobic Microbial Consortium Capable of Degrading

Saturation Concentrations of Tetrachloroethylene. *Canadian Journal of Microbiology,* Vol. 49:15-27.

Devlin, J.F. and D. Muller. 1999. Field and Laboratory Studies of Carbon Tetrachloride Transformation in a Sandy Aquifer under Sulfate Reducing Conditions. *Environmental Science & Technology*, Vol. 33:1021-1027.

Dijk, J.A., J.M. de Bont, X. Lu, P.M. Becker, T. Bosma, H. Rijnaarts, and J. Gerritse. 2000. Anaerobic Oxidation of (Chlorinated) Hydrocarbons. *Proceedings of the Second International In-Situ and On-Site Bioremediation Symposium, Monterey, California*. Vol. 4:63-70.

Driscoll, R.G. 1986. *Groundwater and Wells*. 2nd ed. St. Paul, MN: Johnson Filtration Systems, Inc.

Drzyzga, O. and J.C. Gottschal. 2002. Tetrachloroethene Dehalorespiration and Growth of *Desulfitobacterium frappieri* TCE1 in Strict Dependence on the Activity of *Desulfovibrio fructosivorans*. *Applied Environmental Microbiology*, Vol. 68(2):642-649.

Duhamel, M., S.D. Weher, L. Yu, H. Rizvi, D. Seepersand, S. Dworatzek, E.E. Cox, and E.A. Edwards. 2002. Comparison of Anaerobic Dechlorinating Enrichment Cultures Maintained in Tetrachloroethene, Trichloroethene, *cis*-Dichloroethene, and Vinyl Chloride. *Water Research*, Vol. 36:4193-4202.

Dybas, M. J., D.W. Hyndman, R. Heine, J. Tiedje, K. Linning, D. Wiggert, T. Voice, X. Zhao, L. Dybas, and C.S. Criddle. 2002. Development, Operation, and Long-term Performance of a Full-scale Biocurtain Utilizing Bioaugmentation. *Environmental Science & Technology*, Vol. 36(16):3635-3644.

Ellis, D.E., E.J. Lutz, J.M. Odom, R.J. Buchanan, C.L. Bartlett, M.D. Lee, M.R. Harkness, and K.A. Deweerd. 2000. Bioaugmentation for Accelerated *In Situ* Anaerobic Bioremediation. *Environmental Science & Technology*, Vol. 34(11):2254-2260.

Evans, P.J. and S.S. Koenigsberg. 2001. A Bioavailable Ferric Iron Assay and Relevance to Reductive Dechlorination. *Proceedings of the Sixth International* In Situ *and On-Site Bioremediation Symposium, San Diego, California*, Vol. 6(8):209-215. San Diego, CA.

Fennell, D., A. Carroll, J. Gossett, and S. Zinder. 2001. Assessment of Indigenous Reductive Dechlorination Potential at a TCE-contaminated Site Using Microcosms, Polymerase Chain Reaction Analysis, and Site Data. *Environmental Science & Technology*, Vol. 35(9):1830-1839.

Fennell, D.E. and J.M. Gossett. 1998. Modeling the Production of and Competition for Hydrogen in a Dechlorinating Culture. *Environmental Science & Technology*, Vol. 32(16):2450-2460.

Fennell, D.E., Gossett, J.M., and Zinder, S.H. 1997. Comparison of Butyric Acid, Ethanol, Lactic Acid, and Propionic Acid as Hydrogen Donors for the Reductive Dechlorination of Tetrachloroethene. *Environmental Science & Technology*, Vol. 31(3):918-26.

Ferrey, M.L., R.T. Wilken, R.G. Ford, and J.T. Wilson. 2004. Nonbiological Removal of *cis*-Dichloroethylene and 1,1-Dchloroethylene in Aquifer Sediment Containing Magnetite. *Environmental Science & Technology*, Vol. 38(60):1746-1752.

Findlay, M., and S. Fogel. 2000. Microcosm Test for Natural Attenuation of Chlorinated Solvents. *Soil Sediment & Groundwater,* Vol. 5:13-18.

Flynn, S., F. Löffler, and J. Tiedje. 2000. Microbial Community Changes Associated with a Shift from Reductive Dechlorination of PCE to Reductive Dechlorination of *cis*-DCE and VC. *Environmental Science & Technology*, Vol. 34(6):1056-1061.

Forman, S.R., T. Llewellyn, S. Morgan, C. Mowder, S. Lesage, K. Millar, S. Brown, G. DeLong, D.J. Green, and H. McIntosh. 2001. Rehabilitation of a Biofouled Recirculation Well Using Innovative Techniques. *Proceedings of the Sixth International* In Situ *and On-Site Bioremediation Symposium, San Diego, California*, Vol. 6(7):321-331. San Diego, CA.

Gander, J.W., G.F. Parkin, and M.M. Scherer. 2002. Kinetics of 1,1,1-Trichoroethane Transformation by Iron Sulfide and a Methanogenic Consortia. *Environmental Science & Technology*, Vol. 36(21):4540-4546.

Gao, J., R.S. Skeen, B.S. Hooker, and R.D. Quesenberry. 1997. Effects of Several Electron Donors on Tetrachloroethylene Dechlorination in Anaerobic Soil Microcosms. *Water Research*, Vol. 31(10):2479-2486.

GeoSyntec Consultants. 2004 (in press). *Bioaugmentation for Remediation of Chlorinated Ethenes: Technology Development, Status, and Research Needs*. Prepared for the Environmental Security Technology Certification Program (ESTCP), Arlington, Virginia. www.estcp.org.

Gerritse, J., V. Renard, T.M. Pedro-Gomes, P.A. Lawson, M.D. Collins, and J.C. Gottschal. 1996. *Desulfitobacterium* sp. Strain PCE1, an Anaerobic Bacterium that Can Grow by Reductive Dechlorination of Tetrachloroethene or Ortho-chlorinated Phenols. *Archives of Microbiology,* Vol. 165:132-140.

Gibson, S.A. and G.W. Sewell. 1992. Stimulation of Reductive Dechlorination of Tetrachloroethene in Anaerobic Aquifer Microcosms by Addition of Short-chain Acids or Alcohols. *Applied Environmental Microbiology*, Vol. 58:392-1393.

Gibson, S.A., D.S. Roberson, H.H. Russell, and G.W. Sewell. 1994. Effects of Addition of Different Concentrations of Mixed Fatty Acids on Dechlorination of Tetrachloroethene in Aquifer Microcosms. *Environmental Toxicology and Chemistry*, Vol. 13(3):453-460.

Gilbert, R.O. 1987. *Statistical Methods for Environmental Pollution Monitoring*. New York: Van Nostrand Reinhold.

Gossett, J.M. and S.H. Zinder. 1996. Microbiological Aspects Relevant to Natural Attenuation of Chlorinated Ethenes. In *Proceedings of the Symposium on Natural Attenuation of Chlorinated Organics in Groundwater*. Dallas TX, September 11-13. EPA /540/R-96/509.

Grathwohl, P. 1990. Influence of Organic Matter from Soils and Sediments from Various Origins on the Sorption of Some Chlorinated Aliphatic Hydrocarbons. *Environmental Science & Technology,* Vol. 24:1687-1693.

Groundwater Services, Inc. (GSI). 2003. *Monitoring and Remediation Optimization System (MAROS).* Prepared for the Technology Transfer Division, AFCEE. www.afcee.brooks.af.mil/er/rpo.htm.

GSI. 2001. *Final Report Mulch Biowall and Surface Amendment Pilot Test, Site Building 301, Offutt AFB, Nebraska*. Prepared for the Technology Transfer Division, AFCEE.

Haas, P.E., P. Cork, C.E. Aziz, and M. Hampton. 2000. *In Situ* Biowall Containing Organic Mulch Promotes Chlorinated Solvent Bioremediation. *Proceedings of the Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California, May 2000.* Volume 4:71-76. Columbus, OH: Battelle Press.

Haas, P., J. Gonzales, P. Cork, and B. Henry. 2003. Remedial Performance of Organic Mulch Biowalls at Two Geochemically Distinct Sites. *Proceedings of the 2003 AFCEE Technology Transfer Workshop, San Antonio, Texas, February 2003*. http://www.afcee.brooks.af.mil/ER/techworkshop/postworkshop/wednesday/am/ebcs_part1/7_Haas.pdf)

Hage, J.C. and S. Hartmans. 1999. Monooxygenase-Mediated 1,2-Dichloroethane Degradation by *Pseudomonas* sp. Strain DCA1. *Applied and Environmental Microbiology*, Vol. 65(6):2466-2470.

Hageman, K.J., J.D. Istok, J.A. Field, T.S. Buscheck, and L. Semprini. 2001. *In Situ* Anaerobic Transformation of Trichlorofluoroethene in Trichloroethene-contaminated Groundwater. *Environmental Science & Technology*, Vol. 35:1729-1735.

Haggerty, R., M.H. Schroth, and J.D. Istok. 1997. Simplified Method of "Push-Pull" Test Data Analysis for Determining *In Situ* Reaction Rate Coefficients. *Ground Water*, Vol. 36(2):314-324.

Hansch, C., A. Leo, and D. Hoekman. 1995. *Exploring QSAR – Hydrophobic, Electronic, and Steric Constants*. American Chemical Society, Washington, D.C.

Harkness, M.R. 2000. Economic Considerations in Enhanced Anaerobic Biodegradation. In: G.B. Wickramanayake et al. (Eds.), *Proceedings of the Second International Conference on Remediation of Chlorinated Recalcitrant Compounds, May 22-25, 2000.* Vol. 4:9-14. Columbus, OH: Battelle Press.

Harkness, M.R., A.A. Bracco, M.J. Brennan, K.A. DeWeerd, and J.L. Spivack. 1999. Use of Bioaugmentation to Stimulate Complete Reductive Dechlorination of Trichloroethene in Dover Soil Columns. *Environmental Science & Technology,* Vol. 33(7):1100-1109.

Harkness, M.R, R. Farnum, B. Weesner, D. Foti, W. Wilke, and D. Smith. 2003. The Case for Chitin. *Proceedings of the of the Seventh International Symposium of In Situ and On-Site Bioremediation, Orlando, Florida, June 2003*. Paper A-34. Battelle Press, Columbus, Ohio.

He, J., K.M. Ritalahti, K. Yang, S.S. Koenigsberg, and F.E. Löffler. 2003a. Detoxification of Vinyl Chloride to Ethene Coupled to Growth of an Anaerobic Bacterium. *Nature,* Vol. 424:62-65.

He, J., K.M. Ritalahti, M.R. Aiello, and F.E. Löffler. 2003b. Complete Detoxification of Vinyl Chloride by an Anaerobic Enrichment Culture and Identification of the Reductively Dechlorinating Population as *Dehalococcoides* Species. *Applied Environmental Microbiology*, Vol. 69:996-1003.

He, J., Y. Sung, M.E. Dollhopf, B.Z. Fathepure, J.M. Tiedje, and F.E. Löffler. 2002. Acetate versus Hydrogen as Direct Electron Donors to Stimulate the Microbial Reductive Dechlorination Process at Chloroethene-contaminated Sites. *Environmental Science & Technology*, Vol. 36:3945-3952.

Hendrickson, E.R., J.A. Payne, R.M. Young, M.G. Starr, M.P. Perry, S. Fahnestock. D.E. Ellis, and Ebersole, R.C. 2002a. Molecular Analysis of *Dehalococcoides* 16S Ribosomal DNA from Chloroethene-Contaminated Sites throughout North America and Europe. *Applied Environmental Microbiology*, Vol. 68(2):485-495.

Hendrickson, E.R., L. Buonamici, J. Vidmusky, D.E. Ellis, M.L. McMaster, and D. Major. 2002b. Application and Value of Molecular Techniques to Detect the Members of the Dechlorinating Group, *Dehalococcoides* (abstract). Presented at the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, May 20-23, 2002.

Henry, B.M., D.R. Griffiths, J. Gonzales, and P. Haas. 2003a. Strategies Using Vegetable Oil for Enhanced Bioremediation of Chlorinated Solvents. Presented at the Seventh International Symposium of *In Situ* and On-Site Bioremediation, Orlando, FL, June 2-5, 2003.

Henry, B.M., T. Hartfelder, M. Goodspeed, J.R. Gonzales, P.E. Haas, and D. Oakley. 2003b. Permeable Mulch Biowall for Enhanced Bioremediation of Chlorinated Ethenes. *Proceedings of the Seventh International Symposium of* In Situ *and On-Site Bioremediation, Orlando, Florida, 2-5, June 2003*. Paper K-03. Columbus, OH: Battelle Press.

Henssen, M.J.C., A.W. van der Werf, S. Keuning, C. Hubach, R. Blokzijl, E. van Keulen, B. Albas, C. Haasnoot, H. Boender, and E. Meijerink. 2001. Engineered Full Scale Bioremediation of Chlorinated Ethylenes. *Proceedings of the Sixth International Symposium on* In Situ *and On-Site Bioremediation, San Diego, California*, Vol. 6(8):11-17. Columbus, OH: Battelle Press.

Holliger, C., D. Hahn, H. Harmsen, W. Ludwig, W. Schumacker, B. Tindall, F. Vazquez, N. Weiss, and A.J. Zehnder. 1998. *Dehalobacter restrictus* gen. nov. and sp. nov., a Strictly Anaerobic Bacterium that Reductively Dechlorinates Tetra- and Trichloroethene in an Anaerobic Respiration. *Archives of Microbiology,* Vol. 169(4):313-321.

Holliger, C., G. Schraa, A.J.M. Stams, and A.J.B. Zehnder. 1993. A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth. *Applied Environmental Microbiology*, Vol. 59:2991-2997.

Hölscher, T., R. Krajmalnik-Brown, K.M. Ritalahti, F. von Wintzingerode, H. Görisch, F.E. Löffler, and L. Adrian. 2004 (in press). Multiple Non-identical Reductive Dehalogenase-homologous Genes Are Common in *Dehalococcoides*. Submitted for publication in *Applied and Environmental Microbiology*.

Hopkins, G.D. and P.L. McCarty. 1995. Field Evaluation of In Situ Aerobic Cometabolism of Trichloroethylene and Three Dichloroethylene Isomers Using Phenol and Toluene as Primary Substrates. *Environmental Science & Technology*, Vol. 29(6):1628-1637.

Istok, J.D., M.D. Humphrey, M.H. Schroth, M.R. Hyman, and K.T. O'Reilly. 1997. Single-Well, "Push-Pull" Test for *In Situ* Determination of Microbial Activities. *Ground Water*, Vol. 35(4):619-631.

Interstate Technology and Regulatory Council (ITRC) Work Group. 2002. *DNAPL Source Reduction: Facing the Challenge*. April. http://www.itrcweb.org.

ITRC Work Group. 1999. *Natural Attenuation of Chlorinated Solvents in Groundwater: Principals and Practices*. http://www.itrcweb.org.

ITRC Work Group. 1998. *Technical and Regulatory Requirements for Enhanced* In Situ *Bioremediation of Chlorinated Solvents in Groundwater*. December. http://www.itrcweb.org.

Jawitz, J.W., R.K. Sillan, M.D. Annable, P.S.C. Rao, and K. Warner. 2000. *In Situ* Alcohol Flushing of a DNAPL Source Zone at a Drycleaner Site. *Environmental Science & Technology,* Vol. 34:3722-3729.

Jeffers, P.M., L.M. Ward, L.M. Woytowitch, N.L. Wolfe. 1989. Homogeneous Hydrolysis Rate Constants for Selected Chlorinated Methanes, Ethanes, Ethenes, and Propanes. *Environmental Science & Technology,* Vol. 23:965-969.

Kampbell, D.H., Wilson, J.T., and Vandergrift, S.A. 1989. Dissolved Oxygen and Methane in Water by a GC Headspace Equilibrium Technique. *International Journal of Environmental Analytical Chemistry*, Vol. 36:249-257.

Kim, Y., J.D. Istok, and L. Semprini. 2004. Push-Pull Tests for Assessing In Situ Aerobic Cometabolism. *Ground Water*, Vol. 42(3):329-337.

Koenigsberg, S.S., C.A. Sandefur, K.A. Lapus, and G. Pasrich. 2002. Facilitated Desorption and Incomplete Dechlorination: Observations from 350 Applications of HRC. In: A.R. Gavaskar and A.S.C. Chen (Eds.), *Remediation of Chlorinated and Recalcitrant Compounds – 2002: Proceedings of the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds, May 20-23, 2002, Monterey, CA*. Paper 2B-56. Columbus, OH: Battelle Press.

Krajmalnik-Brown, R., T. Hölscher, I.N. Thomson, F.M. Saunders, K.M. Ritalahti, and F.E. Löffler. 2004 (in press). Genetic Identification of a Putative Vinyl Chloride Reductase in *Dehalococcoides* sp. Strain BAV1. Submitted for publication in *Applied and Environmental Microbiology.*

Kriegman-King, M. R. and M. Reinhard. 1994. Transformation of Carbon Tetrachloride by Pyrite in Aqueous Solution. *Environmental Science & Technology*, Vol. 28(4):692-700.

Krumholz, L.R. 1997. *Desulfuromonas chloroethenica* sp. nov. Uses Tetrachloroethylene and Trichloroethylene as Electron Acceptors. *International Journal of Systematic Bacteriology*, Vol. 47:1262-1263.

Lee, M.D., B. Borden, M.T. Lieberman, W. Beckwith, T. Crotwell, P.E. Haas. 2001. Effective Distribution of Edible Oils – Results from Five Field Applications. *Proceedings of the Sixth International* In Situ *and On-Site Bioremediation Symposium, San Diego, California*, Vol. 6(7):249-256.

Lee, M.D., R.J. Buchanan, and D.E. Ellis. 2000. Laboratory Studies Using Edible Oils to Support Reductive Dechlorination. *Proceedings of the Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California, May 2000*. 77-84. Columbus, OH: Battelle Press.

Lee, W. and B. Batchelor. 2003. Reductive Capacity of Natural Reductants. *Environmental Science & Technology,* Vol. 37:535-541.

Lee, W. and B. Batchelor. 2002. Abiotic Reductive Dechlorination of Chlorinated Ethylenes by Iron-Bearing Soil Minerals. 1. Pyrite and Magnetite. *Environmental Science & Technology*, Vol. 36(23):5147-5154.

Lendvay, J.M., Loffler, M.E. Dollhopf, B. Fathepure, M. Gebhard, R. Heine, R. Hickey, C.L. Major, Jr., E. Petrovskis, J. Shi, J.M. Tiedje, and P. Adriaens. 2003 (in press). Bioreactive Barriers: A Comparison of Bioaugmentation and Biostimulation for Chlorinated Solvent Remediation. Submitted to *Environmental Science & Technology.*

Löffler, F., Q. Sun, J. Li, and J. Tiedje. 2000. 16S rRNA Gene-based Detection of Tetrachloroethene-dechlorinating Desulfuromonas and Dehalococcoides Species. *Applied Environmental Microbiology*, Vol. 66(4):1369-1374.

Lovley, D.R., F.H. Chapelle, and J.C. Woodward. 1994. Use of Dissolved H2 Concentrations to Determine Distribution of Microbially Catalyzed Redox Reactions in Anoxic Groundwater. *Environmental Science & Technology,* Vol. 28:1205-1210.

Lovley, D. R. and S. Goodwin. 1988. Hydrogen Concentrations as an Indicator of the Predominant Terminal Electron-accepting Reactions in Aquatic Sediments. *Geochimica et Cosmochimica Acta,* Vol. 52:2993-3003.

Maierle, M.S. and J.L. Cota. 2001. Complete PCE Degradation and Site Closure Using Enhanced Reductive Dechlorination. *Proceedings of the Sixth International* In Situ *and On-Site Bioremediation Symposium*, Vol. 6(7):149-156.

Maillacheruvu, K.Y. and G.F. Parkin. 1996. Kinetics of Growth, Substrate Utilization, and Sulfide Toxicity for Propionate, Acetate, and Hydrogen Utilizers in Anaerobic Systems. *Water Environmental Research*, Vol. 68:1099-1106.

Major, D.W., M.L. McMaster, E.E. Cox, E.A. Edwards, S.M. Dworatzek, E.R. Hendrickson, M.G. Starr, J.A. Payne, and L.W. Buonamici. 2002. Field Demonstration of

Successful Bioaugmentation to Achieve Dechlorination of Tetrachloroethene to Ethene. *Environmental Science & Technology,* Vol. 36:5106-5116.

Major, D.W., M.L. McMaster, E.E. Cox, B.J. Lee, E.E. Gentry, E. Hendrickson, E. Edwards, and S. Dworatzek. 2001. Successful Field Demonstration of Bioaugmentation to Degrade PCE and TCE to Ethene. *Proceedings of the Sixth International* In Situ *and On-Site Bioremediation Symposium, San Diego, California*, Vol. 6(8):27-34.

Mann, H.B. and D.R. Whitney. 1947. On a Test of Whether One or More Random Variables Is Stochastically Larger than in the Other. *Annals of Mathematical Statistics*, Vol.18:52-54.

Martin, J.P., K.S. Sorenson, L.N. Peterson, R.A. Brennan, C.J. Werth, R.A. Sanford, G.H. Bures, and C.J. Taylor. 2002. Enhanced CAH Dechlorination in a Low Permeability, Variably Saturated Medium. In: A.R. Gavaskar and A.S.C. Chen (Eds.), *Remediation of Chlorinated and Recalcitrant Compounds – 2002: Proceedings of the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2002)*. Paper 2B-54. Columbus, OH: Battelle Press.

Martin, J.P., K.S. Sorenson, and L.N. Peterson. 2001. Favoring Efficient *In Situ* Dechlorination through Amendment Injection Strategy. *Proceedings of the Sixth International* In Situ *and On Site Bioremediation Symposium, San Diego, California*, Vol. 6(7):265-272.

Maymo-Gatell, X., Nijenhuis, I., and S.H. Zinder. 2001. Reductive Dechlorination of *cis*-1,2-dichloroethene and Vinyl Chloride by *Dehalococcoides ethenogenes*. *Environmental Science & Technology,* Vol. 35:516-521.

Maymo-Gatell, X., T. Anguish, and S.H. Zinder. 1999. Reductive Dechlorination of Chlorinated Ethenes and 1,2-dichloroethane by "Dehalococcoides Ethenogenes" 195. *Applied Environmental Microbiology,* Vol. 65(7):3108-3113.

Maymo-Gatell, X., J.M. Gossett, and S.H. Zinder. 1997. *Dehalococcus Ethenogenes* Strain 195: Ethene Production from Halogenated Aliphatics (abstract). In: B.C. Alleman and A. Leeson (Eds.), In Situ *and On-Site Bioremediation,* Vol. 3:23. Columbus, OH: Battelle Press,.

Maymo-Gatell, X., V. Tandoi, J.M. Gossett, and S.H. Zinder. 1995. Characterization of an H_2-utilizing Enrichment Culture that Reductively Dechlorinates Tetrachloroethene to Vinyl Chloride and Ethene in the Absence of Methanogenesis and Acetogenesis. *Applied Environmental Microbiology*, Vol. 61(11):3928-3933.

McCarty, P. 1996. Biotic and Abiotic Transformations of Chlorinated Solvents in Groundwater. *Symposium on Natural Attenuation of Chlorinated Organics in Groundwater*, *September 11-13, 1996*. Dallas, TX: USEPA.

McCarty, P.L., M.N. Goltz, G.D. Hopkins, M.E. Dolan, J.P. Allan, B.T. Kawakami, and T.J. Carrothers. 1998. Full-Scale Evaluation of *In Situ* Cometabolic Degradation of Trichloroethylene in Groundwater through Toluene Injection. *Environmental Science & Technology,* Vol. 32(1):88-100.

McCarty, P.L., and L. Semprini. 1994. Groundwater Treatment for Chlorinated Solvents, Section 5. In: Norris, R.D., Hinchee, R.E., Brown, R., McCarty, P.L, Semprini, L., Wilson, J.T., Kampbell, D.H., Reinhard, M., Bouwer, E.J., Borden, R.C., Vogel, T.M., Thomas, J.M., and C.H. Ward (eds.), *Handbook of Bioremediation*. Lewis Publishers, Boca Raton, Florida.

McMaster, M., M. Bogaart, D. Major, C. Lebron, E. Edwards, P. Morrill, B. Sherwood-Lollar, and T. McHale. 2004. Enhanced Dissolution of a PCE DNAPL Using Bioremediation (abstract). Presented at the *Fourth International Conference on Remediation of Chlorinated and Recalcitrant Compounds,* May 24 – 27, 2004, Monterey, California.

Millar, K., S. Lesage, S. Brown, C.S. Mowder, T. Llewellyn, S. Forman, D. Peters, G. DeLong, D.J. Green, and H. McIntosh. 2001. Biocide Application Prevents Biofouling of a Chemical Injection/Recirculation Well. *Proceedings of the Sixth International* In Situ *and On-Site Bioremediation Symposium, San Diego, California*, Vol. 6(7):333-340.

Morrill, P.L., D.J. Seepersad, G. Lacrampe-Couloume, M. Kaiguo, E.A. Edwards, B.E. Sleep, M.L. McMaster, D.W. Major, and B. Sherwood-Lollar. 2004. Biologically Enhanced Dissolution of Tetrachloroethene: A Stable Carbon Isotope Investigation (abstract). Presented at the *Fourth International Conference on Remediation of Chlorinated and Recalcitrant Compounds,* May 24 – 27, 2004, Monterey, California.

Morse, J.J., B.C. Alleman, J.M. Gossett, S.H. Zinder, D.E. Fennell, G.W. Sewell, and C.M. Vogel. 1998. *Draft Technical Protocol: A Treatability Test for Evaluating the Potential Applicability of the Reductive Anaerobic Biological* In Situ *Treatment Technology (RABITT) to Remediate Chloroethenes.* Prepared for ESTCP. February 23, 1998.

Müller J.A., B.M. Rosner, G. von Abendroth, G. Meshluham-Simon, P. McCarthy, and A.M. Spormann. 2004 (in press). Molecular Identification of the Catabolic Vinyl Chloride Reductase from *Dehalococcoides* sp. Strain VS and its Environmental Distribution. Submitted for publication in *Applied and Environmental Microbiology.*

Murray, W., M. Dooley, and S. Koenigsberg. 2001. Enhanced Bioremediation of Chlorinated Solvents. *Proceedings of the Sixth International* In Situ *and On-Site Bioremediation Symposium*, *San Diego, California*, Vol. 6(7):197-204.

National Academy of Sciences. 2000. *Natural Attenuation for Groundwater Remediation*. Prepublication copy. www.nap.edu/openbook/0309069327/html/r1.html.

Newell, C.J., H.S. Rafai, J.T. Wilson, J.A. Connor, J.A. Aziz, and M.P. Suarez. 2003. Calculation and Use of First-Order Rate Constants for Monitored Natural Attenuation Studies. *Ground Water Issue*. Cincinnati, OH: USEPA.

Newell, C., C. Aziz, J. Hughes, and P.E. Haas. 2002. Two Novel Methods for Enhancing Source Zone Remediation: Direct Hydrogen Addition and Electron Acceptor Diversion. Presentation Materials for the 2002 AFCEE Technology Workshop, San Antonio, TX, March 2002.

Newell, C.J., C.E. Aziz, P.E. Haas, J. Hughes, and T.A. Khan. 2001. Two Novel Methods for Enhancing Source Zone Bioremediation: Direct Hydrogen Addition and Electron Acceptor Diversion. *Proceedings of the Sixth International Symposium on* In Situ *and On-Site Bioremediation, San Diego, California*, Volume 7:19-26.

Newell, C.J., C.E. Aziz, P.E. Haas, J. Hughes, and T.A. Khan. 2000. Results from Two Direct Hydrogen Delivery Field Tests for Enhanced Dechlorination. *Proceedings of the Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California*, Vol. 4:31-37.

Novak, P.J., J.A. Edstrom, L.W. Clapp, R.M. Hozalski, and M.J. Semmens. 2002. Stimulation of Dechlorination by Membrane-Delivered Hydrogen: Small Field Demonstration (abstract and presentation). Presented at the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California, May 20-23, 2002.

Parsons Corporation (Parsons). 2003. *Final Work Plan for a Bioreactor Demonstration at Landfill 3 and Site SS-17, Altus AFB, Oklahoma*. Prepared for ESTCP and Altus AFB, Oklahoma. Revision 1, September.

Parsons Engineering Science, Inc. (Parsons). 2002a. *Final Interim Report: Performance and Cost of Anaerobic Dechlorination, Phase I Site Survey.* Prepared for the Naval Facilities Engineering Service Center (NFESC), Port Hueneme, California, and the Environmental Security Technology Certification Program, Arlington, Virginia. December 11, 2002.

Parsons. 2002b. *Final Phase II Field Feasibility Test for* In Situ *Bioremediation of Chlorinated Solvents Via Vegetable Oil Injection at Hanger K Area, Cape Canaveral Air Force Station, Florida*. Prepared for AFCEE, Brooks City-Base, Texas.

Parsons. 2001. *Final Report, Groundwater Monitoring of Reactive Blanket at BRAC51, June 2000 through July 2001*. Prepared for USACE, Sacramento District, California.

Payne, F.C., S.S. Suthersan, F.Z. Lenzo, and J.S. Burdick. 2001. Mobilization of Sorbed-Phase Chlorinated Alkenes in Enhanced Reductive Dechlorination. In: *Anaerobic Degradation of Chlorinated Solvents: Proceedings of the Sixth International* In Situ *and On-Site Bioremediation Symposium*, Vol. 6(2):53-60. Columbus, OH: Battelle Press.

Pfeiffer, P. 2003. *Abiotic Effects of Vegetable Oil Added to Enhance* In Situ *Bioremediation of Chlorinated Solvents*. Masters Thesis, Colorado School of Mines, Golden, Colorado.

Pfiffner, S.M., A.V. Palumbo, B.L. Kinsall, A.D. Peacock, D. White, and T.J. Phelps. 2000. Microbial Heterogeneity Implications for Bioremediation. *Proceedings of the Sixth International* In Situ *and On-Site Bioremediation Symposium,* Vol. 6(4):73-80. Columbus, OH: Battelle Press.

Rheinhard, M., G.P. Curtis, and M.R. Kreigman. 1990. Abiotic Reductive Dechlorination of Carbon Tetrachloride and Hexachloroethane by Environmental Reductants: Project Summary. EPA/600/S2-90/040. Washington, DC: USEPA.

Richardson, R.E., V.K. Bhupathiraju, D.L. Song, T.A. Goulet, and L. Alvarez-Cohen. 2002. Phylogenetic Characterization of Microbial Communities that Reductively Dechlorinate TCE Based upon a Combination of Molecular Techniques. *Environmental Science & Technology,* Vol. 36:2652-2662.

Ritalahti, K.M., J. He, R. Krajmalnik-Brown, Y. Sung, F.E. Löffler, and S.S. Koenigsberg. 2003. Complete Reductive Dechlorination of Chlorinated Ethenes: Characterization of the Key Players and Implications for Their Specific Detection and Enumeration. Presentation given at the Seventh International Symposium of *In Situ* and On-Site Bioremediation, Orlando, Florida, June 2-5, 2003.

Robertson, W.D., D.W. Blowes, C.J. Ptacek, and J.A. Cherry. 2000. Long-Term Performance of *In Situ* Reactive Barriers for Nitrate Remediation. *Ground Water*, Vol. 38(5):689-695.

Schollhorn, A., C. Savary, G. Stucki, and K.H. Hanselmann. 1997. Comparison of Different Substrates for the Fast Reductive Dechlorination of Trichloroethene under Groundwater Conditions. *Water Research*, Vol. 31(6):1275.

Scholz-Muramatsu, H., A. Neumann, M. Messmer, E. Moore, and G. Diekert. 1995. Isolation and Characterization of *Dehalospirillium multivorans* gen. nov., sp. nov., a Tetrachloroethene-Utilizing, Strictly Anaerobic Bacterium. *Archives of Microbiology,* Vol. 63:48-56.

Sivavec, T.M. and D.P. Horney. 1997. Reduction of Chlorinated Solvents by Fe(II) Minerals. *Proceedings of the 213th American Chemical Society National Meeting*. 115-117. Washington, DC: American Chemical Society.

Skladany, G.J., D. Brown, D.A. Burns, M. Bell, and M.D. Lee. 2001. Biologically Enhanced Reductive Dechlorination. *Proceedings of the Sixth* In Situ *and On-Site Bioremediation Symposium, San Diego, California,* Vol. 6(7):213.

Smatlak, C.R., J.M. Gossett, and S.H. Zinder. 1996. Comparative Kinetics of Hydrogen Utilization for Reductive Dechlorination of Tetrachloroethene and Methanogenesis in an Anaerobic Enrichment Culture. *Environmental Science & Technology*, Vol. 30(9):2850-2858.

Song, D. L., M.E. Conrad, K.S. Sorenson, and L. Alvarez-Cohen. 2002. Stable Carbon Isotope Fractionation during Enhanced *In Situ* Bioremediation of Trichloroethene. *Environmental Science & Technology*, Vol. 36(10):2262-2268.

Sorenson, K.S. 2003a. Aqueous or Slow Release? – Considerations for Substrate Selection. *Proceedings of the 2003 AFCEE Technology Transfer Workshop, San Antonio, Texas*. AFCEE, Brooks City-Base, Texas.

Sorenson, K.S. 2003b. Enhanced Bioremediation for Treatment of Chlorinated Solvent Residual Source Areas. In: S.M. Henry and S.D. Warner (Eds.), *Chlorinated Solvent and DNAPL Remediation: Innovative Strategies for Cleanup.* ACS Symposium Series, Vol. 837:119-131.

Sorenson, K.S., J.P. Martin, R.A. Brennan, C.J. Werth, R.A. Sanford, and G.H. Bures. 2002. *Phase I SBIR Final Report: Development of a Chitin-Fracing Technology for*

Remediation of Chlorinated Solvent Source Areas in Low Permeability Media. North Wind Environmental, NWE-ID-2002-024 Revision 0.

Stahl, D.A. 1997. Molecular approaches for the Measurement of Density, Diversity, and Phylogeny. In: C.J. Hurst, G.R. Knudsen, M.J. McInerney, L.D. Stetzenbach, and M.V. Walter (Eds.), *Management of Environmental Microbiology*. 102-111. Washington DC: ASM Press.

Stroo, H.F., M. Unger, C.H. Ward, M.C. Kavanaugh, C. Vogel, A. Leeson, J.A. Marqusee, and B.P. Smith. 2003. Remediating Chlorinated Solvent Source Zones. *Environmental Science & Technology*, Vol. 37(11):224A-230A.

Suthersan, S. and F. Payne. 2003. Realities of Enhanced Reductive Dechlorination. *Pollution Engineering*. April 1:42-49**.**

Suthersan, S.S., C.C. Lutes, P.L. Palmer, F. Lenzo, F.C. Payne, D.S. Liles, and J. Burdick. 2002. *Final Technical Protocol for Using Soluble Carbohydrates to Enhance Reductive Dechlorination of Chlorinated Aliphatic Hydrocarbons, December 19, 2002.* Submitted to ESTCP and AFCEE under Contract #41624-99-C-8032.

Suthersan, S.S. 2001. *Natural and Enhanced Remediation Systems*. ARCADIS. Lewis Publishers, Boca Raton, Florida.

Tandoi, V., T.D. DiStefano, P.A. Bowser, J.M. Gossett, and S.H. Zinder. 1994. Reductive Dehalogenation of Chlorinated Ethenes and Halogenated Ethanes by a High-rate Anaerobic Enrichment Culture. *Environmental Science & Technology*, Vol. 28(5):973-979.

Turpie, A., C. Lizotte, M.F. DeFlaun, J. Wuinnan, and M. Marley. 2000. Performance of Field-Scale Sequential Anaerobic/Aerobic *In Situ* Bioremediation Demonstration. *Proceedings of the Second International* In Situ *and On-Site Bioremediation Symposium, Monterey, California*.

USEPA. 2003. The DNAPL Remediation Challenge: Is There a Case for Source Depletion? Prepared by the Expert Panel on DNAPL Remediation. EPA/600/R-03/143.

USEPA. Office of Solid Waste and Emergency Response. 2000a. *Engineered Approaches to In Situ Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications.* Division of Solid Waste and Emergency Response. EPA 542-R-00-008. http://www.epa.gov/clu-in.org.

USEPA. 2000b. *Applicability of RCRA Section 3020 to In-Situ Treatment of Groundwater.* Memorandum from Elizabeth Cotsworth, Director, Office of Solid Waste and Emergency Response. December 27.

USEPA. 1998a. *Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Groundwater*. Cincinnati, OH: National Risk Management Research Laboratory, Office of Research and Development, USEPA. EPA/600/R-98/128.

USEPA. 1998b. *Application of the Electromagnetic Borehole Flowmeter*. Office of Research and Development, National Risk Management Research Laboratory, Ada, Oklahoma. EPA/600/SR-98/058.

Vogel, T.M., C.S. Criddle, and P.L. McCarty. 1987. Transformations of Halogenated Aliphatic Compounds. *Environmental Science & Technology*, Vol. 21(8):722-736.

Vogel, T.M. and P.L. McCarty. 1987. Abiotic and Biotic Transformations of 1,1,1-Trichloroethane under Methanogenic Conditions: *Environmental Science & Technology,* Vol. 21(12):1208-1213.

Vogel, T.M. and P.L. McCarty. 1985. Biotransformation of Tetrachloroethylene to Trichloroethylene, Dichloroethylene, Vinyl Chloride, and Carbon Dioxide under Methanogenic Conditions. *Applied Environmental Microbiology*, Vol. 49(5):1080-1083.

Watts, J.J., M.O. Jaynes, J.A. Farrell, and R. Gillespie. 2002. Remedial Action Using HRC under a State Dry Cleaning Program. In: A.R. Gavaskar and A.S.C. Chen (Eds.), *Remediation of Chlorinated and Recalcitrant Compounds – 2002: Proceedings of the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2002)*. Paper 2B-44. Columbus, OH: Battelle Press.

Weaver, J.W., J.T. Wilson, and D.H. Kampbell. 1995. *EPA Project Summary*. EPA/600/SV-95/001. Washington, DC: USEPA.

Weerasooriya, R. and B. Dharmasena. 2001. Pyrite-assisted Degradation of Trichloroethene (TCE). *Chemosphere,* Vol. 42(4):389-396.

White, D.C., H.C. Pinkart, and D.B. Ringelberg. 1997. Biomass Measurements: Biochemical Approaches. In: C.J. Hurst, G.R. Knudsen, M.J. McInerney, L.D. Stetzenbach, and M.V. Walter (Eds.), *Management of Environmental Microbiology*. 91-101. Washington DC: ASM Press.

Wiedemeier, T.H. and P.E. Haas. 2003. Designing Monitoring Programs to Effectively Evaluate the Performance of Natural Attenuation. *Ground Water Monitoring & Remediation*, Vol. 22(3):124-135.

Wiedemeier, T.H., H.S. Rifai, C.J. Newell, and J.T. Wilson. 1999. *Natural Attenuation of Fuels and Chlorinated Solvents in the Subsurface*. New York, New York: John Wiley & Sons.

Wiedemeier, T.H., M.A. Swanson, D.E. Moutoux, J.T. Wilson, D.H. Kambell, J.E. Hansen, and P. Haas. 1996. Overview of the Technical Protocol for Natural Attenuation of Chlorinated Aliphatic Hydrocarbons in Groundwater under Development for the U.S. Air Force Center for Environmental Excellence. *Symposium on Natural Attenuation of Chlorinated Solvents*. 35-59. Washington, DC: USEPA. EPA/540/R-96/509.

Wilson, J.T., H.S. Cho, and F.P. Beck. 1997. Field Estimation of Hydraulic Conductivity for Assessments of Natural Attenuation. In: In Situ *and On-Site Bioremediation, Volume 2*. pp 309-314. Columbus, OH: Battelle Press

Wilson, J.T., C. Lebron, and P. Evans. 2003. Measurement of Bioavailable Iron at Two Hazardous Waste Sites (abstract). Presentation given at the Seventh International Symposium of *In Situ* and On-Site Bioremediation, Orlando, FL, June 2-5, 2003.

Woodward, C. 2004 (pending). *Delivery and Migration of Vegetable Oil and Vegetable Oil Emulsion for Use as a Reductive Dechlorinating Substrate in Saturated Porous Media*. Draft Master's Thesis. Colorado School of Mines.

Yang, Y. and P.L. McCarty. 2002. Comparison between Donor Substrates for Biologically Enhanced Tetrachloroethene DNAPL Dissolution. *Environmental Science & Technology,* Vol. 36(15):3400-3404.

Yang, Y. and P.L. McCarty. 2000a. Biomass, Oleate, and Other Possible Substrates for Chloroethene Reductive Dechlorination. *Bioremediation Journal*, Vol. 4(2):125-133.

Yang, Y. and P.L. McCarty. 2000b. Biologically Enhanced Dissolution of Tetrachloroethene DNAPL. *Environmental Science & Technology*, Vol. 34(14):2979-2984.

Yang, Y. and P.L. McCarty. 1998. Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Anaerobic Mixed Culture. *Environmental Science & Technology*, Vol. 32(22):3591-3597.

Yu, S. and L. Semprini. 2002. Comparison of Trichloroethylene Reductive Dehalogenation by Microbial Communities Stimulated on Silicon-based Organic Compounds as Slow-Release Anaerobic Substrates. *Water Research*, Vol. 36(20):4985-4996.

APPENDIX A

KEY PROJECT PERSONNEL

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APPENDIX A
KEY PROJECT PERSONNEL

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APPENDIX B

SAMPLE STATEMENT OF WORK

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SAMPLE STATEMENT OF WORK

for

ENHANCED ANAEROBIC BIOREMEDIATION OF CHLORINATED SOLVENTS AT DEPARTMENT OF DEFENSE FACILITIES

Contract Number:
Delivery Order:

Date

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SAMPLE STATEMENT OF WORK

for

ENHANCED ANAEROBIC BIOREMEDIATION OF CHLORINATED SOLVENTS AT DEPARTMENT OF DEFENSE FACILITIES

1 INTRODUCTION

This statement of work (SOW) defines requirements for architectural-engineering (A-E) services for environmental restoration using enhanced anaerobic bioremediation, as assigned and in accordance with applicable regulatory guidance. Place of performance shall be at various Government installation(s) in the 50 United States, at various overseas Government locations, and locations of interest to the Government.

The Department of Defense (DoD) is involved in the application of enhanced anaerobic bioremediation for the purposes of groundwater restoration. Previously developed laboratory and field data demonstrates that reductive dechlorination of chlorinated compounds occurs under reducing conditions, where an electron donor is utilized as the main energy source for microbial metabolism. Substrates utilized for enhanced anaerobic bioremediation include both solid and liquid forms and range from readily soluble (e.g., sugars or low-molecular-weight acids) to less soluble (e.g., polymers, oils, or bark mulch) forms. The purpose of this project is to substantially and cost-effectively enhance *in situ* reductive dechlorination. In order to achieve this purpose, the following conditions must be met: 1) The substrate must effectively support reductive dechlorination; 2) A technically-effective and cost-effective distribution mechanism must be deployed; and 3) The application does not create increased hazards (e.g., explosive methane conditions, permanent accumulation of toxic byproducts, or permanent degradation of secondary water quality parameters).

Numerous field-based protocols have been developed by the DoD that allow for the measurement of actual contaminant degradation *in situ*. A similar strategy should be utilized to evaluate enhanced reductive dechlorination. This effort shall involve the application of field methods to measure substrate distribution, electron donor conversion, changes in geochemical profiles, contaminant breakdown, and tracer conservation in chlorinated solvent-impacted unsaturated and saturated zones. In general, the work shall be conducted in accordance with the *Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water* (EPA/600/R-98/128, September 1998; *http://www.epa.gov/ada/reports.html)*.

1.1 SCOPE

In carrying out work assignment(s) issued as a task order (TO) under the contract, the contractor shall: furnish the personnel, services, equipment, materials, facilities and other requirements necessary for, or incidental to, the performance of work set forth herein. Primary technical services shall be performed by individuals that are licensed members of architectural and engineering professions, and individuals in their employ, including

those who are recognized as technical consultants in their respective field. The contractor shall be capable of addressing and interpreting all aspects of environmental law and regulation, including the preparation and presentation of expert testimony if required. Some tasks may require access to or the review of classified material, as identified in individual orders. Some tasks may require planning and logistical support, to include on-site translation and/or interpretation, at various meetings and conferences worldwide. Task Orders will consist of the following types of services:

(1) Development of screening criteria that evaluate site-specific technology applicability to include: safety, target compounds, receptor or discharge locations, current and potential degradation byproducts, site geochemistry, cost-effective distribution systems, mass transfer limitations, and source and remediation lifetimes.

(2) Development of cost-effective field pilot test procedures to determine the efficacy of electron donor addition and enhanced dechlorination at specific DoD sites.

(3) Full-scale implementation of effective and cost-effective electron donor distribution systems for saturated and unsaturated media at DoD designated sites.

(4) Field cost and performance measurement and evaluation of pilot- and full-scale systems.

The Government shall have unlimited rights, in all data, drawings, designs, specifications, notes, and other works developed and utilized in the performance of this contract, including the right to use same on any other Government design or construction without additional compensation to the Contractor. The Contractor hereby grants a paid-up license to the US Government throughout the world to all such works described above to which he may assert or establish any claim under design patent or copyright laws.

1.1.1 Title I Services

The contractor shall conduct all efforts necessary to prepare the design of environmental restoration projects. Title I efforts include all aspects of design such as preparation of contract plans, specifications, scheduling, cost estimates, and preparation of operating and design manuals. Title I efforts also encompass those efforts required to support and develop the design, including: planning and programming; program management; scoping; studies; investigations; evaluations; consultations; conceptual design; value engineering; and operation, monitoring and optimization of environmental treatment or control systems.

1.1.2 Other Environmental A-E Services

The contractor shall provide a full range of environmental management services necessary for the implementation of environmental restoration projects. These efforts include: planning and programming; program management; scoping; studies; services; investigations; evaluations; consultations; conceptual design; value engineering; and operation, monitoring and optimization of environmental treatment or control systems,

as well as other related services for the continuation of an existing environmental program or to establish an initial environmental program.

2 APPLICABLE DOCUMENTS

Comply with all applicable (1) federal, state, and local environmental statutes, instructions, manuals, handbooks, regulations, guidance, policy letters, and rules (including all changes and amendments), and (2) Presidential Executive Orders, in effect on the date of issuance of each TO. For work at overseas locations, contractor shall also comply with all applicable host nation statutes and agreements.

In addition, the contractor shall refer to applicable DoD quality assurance programs such as the Air Force Center for Environmental Excellence (AFCEE) Technical Services Quality Assurance Program and Guidance for Contract Deliverables (GCD), current versions unless previous version is specified in the task order. DoD facility-specific documents shall be identified in each individual TO.

3 ADMINISTRATIVE AND MANAGERIAL REQUIREMENTS

Perform management and planning functions, as well as performance measurement and cost status reporting, during the course of this effort as specified in each TO.

3.1 MEETINGS AND CONFERENCES

3.1.1 Post-Award Meeting/Teleconference

After the issuance of a TO, attend a post-award meeting/teleconference at the location specified by the Contracting Officer's Representative (COR). The purpose of the meeting shall be to become familiar with the work requirements, information, and/or site-specific data addressed under the TO.

3.1.2 Progress Meetings

Attend progress meetings with the installation and/or DoD representative(s), as specified by the COR.

3.1.3 Integration and Planning Meetings

Attend meetings during the project’s execution. The purpose of these meetings shall be to review program integration from the planning, environmental analysis, and design phase through the construction phase. It is through these channels, and oversight meetings detailed in the project action plan(s), that any recommended variations from the project plan(s) and specifications shall be identified.

3.1.4 Attend Public and Regulatory Meetings and Hearings

Attend public and regulatory meetings and hearings as specified by the COR, including meetings to support technical discussions with appropriate regulatory agencies.

3.2 SPECIAL NOTIFICATION

3.2.1 Health Risks

Immediately report to the Contracting Officer (CO) and the COR, via telephone or e-mail, any issues or incidents related to any TOs which may indicate potential imminent risk to contracted, federal, or host nation personnel, the public at large, or the environment. Following the telephone or e-mail notification, a written notice with supporting documentation shall be prepared and delivered within three (3) working days to the CO. Upon request of the CO, or their COR, provide pertinent raw laboratory data within three (3) weeks of the telephone or e-mail notification, documenting the concern and risk.

3.2.2 Identification and Change of Critical Contractor Personnel

Submit an organizational chart displaying key personnel involved in the effort and their respective labor categories as specified in each TO. Notify the COR of all professional personnel to work on specific tasks under the task order. Obtain COR approval of any proposed changes in project personnel along with the steps taken/proposed to ensure there are no impacts to the schedule or costs associated with individual tasks. Identify to the COR all subcontractors to be used under task orders issued pursuant to this SOW, prior to contract and work being initiated. Provide subcontractor qualifications to the COR prior to contract utilization.

3.3 LABORATORIES

The Contractor shall be responsible for data quality assurance/quality control (QA/QC). The Contractor shall perform a laboratory subcontract pre-award audit before the laboratory receives project samples. The contractor shall use only qualified laboratories that have been audited by the Contractor. All audit reports shall be made available to the DoD upon request.

3.3.1 General

Laboratories may be subject to on-site DoD audits of their QA/QC protocols and procedures. All laboratories shall meet Data Quality Objectives (DQOs) specified in task order project-specific Sampling and Analysis Plan(s) (SAP). The labs shall perform QA/QC requirements as specified in the project/site specific SAPs. The analytical capabilities of the laboratory shall be sufficient for the methods specified in the SAP, and the laboratory shall have sufficient through-put capacity to handle the necessary analytical load during all field activities.

3.3.2 On-Site Laboratories

An on-site laboratory may be utilized for the analytical methods required by the approved project- or site-specific SAP. The laboratory shall meet all applicable certification requirements for the necessary analysis methods prior to its implementation. On-site laboratories shall meet the DQO and QA/QC requirements specified in the site-specific SAP. All proposed deviations from the above requirements shall be submitted in writing to the COR for concurrence prior to proceeding with the affected work.

3.4 WORK SITE REQUIREMENTS

3.4.1 Safety Requirements

The Contractor shall be responsible for protecting the lives and health of employees and other persons; preventing damage to property, materials, supplies, and equipment; avoiding work interruptions; and complying with Occupational Safety and Health Administration (OSHA) safety and health regulations and Base safety office requirements. All on-site workers (contractor and subcontractor) performing hazardous operations, including working with hazardous materials, must have completed the OSHA 1910.120 HAZWOPER training and/or other applicable training, plus annual refresher courses. Maintain documentation supporting training records and have a written Health and Safety Plan (HSP) on site available for workers and/or regulatory review. Provide the CO copies of any OSHA report(s) submitted during the duration of the TO.

3.4.2 Work-Site Maintenance

The Contractor shall maintain the work site to: provide for the safety of all individuals in the vicinity of the work site areas, prevent the spread of contamination, provide for the integrity of the samples obtained, and prevent the release of any contamination to the environment. The work site shall be well marked to prevent inadvertent entry into all work areas. Access to work areas shall be monitored and thoroughly controlled. Standard work zones and access points for controlled operations shall be established and maintained as the site conditions warrant. Ensure compliance with any federal, state, host nation, and local regulations and QA/QC protocols and procedures for decontaminating tools, equipment, or other materials, as required. At all times, keep the work area free from accumulation of waste and hazardous materials. Remove non-essential equipment from the work site when not in use. The work-site shall be maintained to present an orderly appearance and to maximize work efficiency. Before completing the work at each sampling site, remove, from the work premises, any rubbish, tools, equipment, and materials that are not property of the Government. Properly dispose of all investigation-derived waste. Upon completing the work, leave the area clean, neat, orderly, and return work site(s) to the original condition.

3.4.3 Minimize Impacts to Existing Operations

The Contractor shall use Global Positioning System (GPS) or standard survey datum at all field locations to mark all points of the field investigation. The installation point of contact (POC) and the COR shall be consulted to properly position sampling locations

(wells, borings, soil gas probes, etc.) with respect to site locations, to minimize the disruption of installation activities, to minimize disruption of natural and cultural resources, and to avoid penetrating underground utilities. The contractor shall coordinate all field survey operations with installation personnel to attain these objectives. Provide for the detection of underground utilities utilizing geophysical or other techniques. All necessary permits, easements, and coordination shall be completed prior to commencement of individual sampling operations.

3.4.4 Storage

The Contractor shall be responsible for security and weatherproofing of stored material and equipment. Equipment or materials used in the work and requiring storage on the installation shall be placed at site(s) designated by the installation POC. At the completion of the work, all temporary fences and structures (used to protect materials and equipment) shall be removed from the installation unless directed otherwise by the COR. Clean the storage area of all debris and material, performing all repairs as required to return the site to its original condition. Maintain an inventory of Government property, a copy of Government property control procedures at the site, and dispose of Government property as directed by the CO.

3.4.5 Site Access Badges

The Contractor shall be responsible for obtaining and monitoring assigned (used by his/her own staff) security badges used during the duration of this contract. All security badges or passes shall be returned to the base POC upon expiration of the badge, upon completion of the project, or when possession of the badge is no longer necessary (e.g., upon removal of contracted personnel from specific projects).

3.4.6 Permits and Site Access Agreements

The Contractor shall be responsible for obtaining all permits and site access agreements required to conduct field exercises.

3.5 PLANNING AND REPORTING REQUIREMENTS

Plan project activities, including the development, implementation, and maintenance of project schedules, events, status of resources, report(s) on the activities, and progress toward accomplishing project objectives, and document for Government review and approval the results of the project efforts for each TO.

3.5.1 WBS Requirements

Prepare and submit for approval a site- and base-specific work breakdown structure (WBS). This WBS shall be used to report the cost and schedule status for each project.

3.5.2 Project Planning Chart

Prepare and submit a project planning chart (PPC) for approval. The PPC shall detail the project schedule and status through the use of Gantt charts, which shall depict percent complete for each task. Schedule activities shall be reported by the approved WBS.

3.5.3 Contractor's Progress, Status, and Management Report

Prepare and submit a Contractor's Progress, Status, and Management Report (CPSMR). The CPSMR shall be site-specific and used to review and evaluate the overall progress of the project, along with any existing or potential problem areas. The CPSMR shall include a summary of the events that occurred during the reporting period, discussion of performance, identification of problems, proposed solutions, corrective actions taken, and outstanding issues.

3.5.4 Funds and Man-Hours Expenditure Report

Implement and maintain a cost accounting system and prepare a Funds and Man-Hours Expenditure Report (FMER) to correlate the status of expensed funds and man-hours against the progress of the work completed. The FMER and associated graphics shall detail the current project status and identify funds and man-hours required to complete the assigned tasks.

4 WORK TASKS

Perform work as specified in each TO. Multi-disciplinary technical capabilities may be required. Services to be performed include support to establish environmental programs, or for continuation of an existing program, including documentation to support funding and execution.

4.1 TASK ORDER SCOPING AND PLAN DEVELOPMENT SERVICES

Perform task order scoping and plan development services to include:

4.1.1 Project Plans

Each TO may require project and/or site-specific planning documents and development requirements. Contractors shall comply with the applicable specifications, procedures and methodologies [such as approved Federal Facilities Agreements (FFAs)] in the site/project specific plan(s). The COR shall approve (in writing) any proposed modification to, or deviation from, any activity described in these documents.

4.1.2 Quality Program Plans (QPPs)

Develop a QPP which will consist of any or all of the following:

4.1.2.1 Health and Safety Plan

The Contractor shall conduct work in accordance with the existing site-specific HSPs. In the event site-specific HSPs are not available, or do not address the proposed work tasks, the Contractor shall prepare a site-specific HSP or HSP addendum. The Contractor shall ensure that all site-specific activities are conducted in accordance with good professional practices.

4.1.2.2 Sampling and Analysis Plan

A SAP shall be developed to ensure use of proper field procedures for collection of representative samples. All field procedures will conform to the TO SAP.

4.1.2.3 Quality Assurance and Quality Control Plan

A QA/QC plan shall be developed to ensure collection of representative and defensible data. All field procedures and laboratory analyses will conform to the TO QA/QC plan.

4.2 STUDIES AND SERVICES

Provide all labor, materials, and services necessary to deliver, for government review and approval, those studies and services that support environmental programs and projects at locations of interest to the Government. These activities are described in the following subsections.

4.2.1 Conceptual Site Model (CSM)

For each site, use validated data supported by acceptable QA/QC results (for example, as measured against AFCEE's Technical Services Quality Assurance Program requirements) and site characterization information to develop or refine, based on newly collected data, the conceptual site model or development profile. The model/project-profile shall define the nature and extent of the projects scope. The AFCEE Technical Services Quality Assurance Program and American Society of Testing and Materials (ASTM) E 1689-95 provides guidance in CSMs/profiles. The complexity and detail of the site model/profile shall be consistent with the nature of the site, its foreseeable problems, and the amount of site/area specific data available. Use the CSM in the field pilot test work plans and field pilot test reports.

4.2.2 Informal Technical Information Reports (ITIRs)

Submit all analytical data, including QC results and cross-reference tables, in ITIRs after intermediate process monitoring events. ITIRs may include development and analysis of alternatives in the event restoration results do not meet DQOs.

4.2.3 Treatability Studies, Bench-Scale Tests, Interim Remedial Actions

The Contractor shall conduct treatability studies, bench-scale tests, and interim remedial measures as directed by the COR to determine the optimum enhanced anaerobic bioremediation approach for each site.

4.2.4 Remedial Action Operations

The Contractor shall operate, maintain, and monitor environmental remedial systems. The Contractor shall shakedown remedial systems that are new or subject to dynamic conditions. The Contractor shall develop operational manuals and standard operating procedures for remedial systems.

4.2.5 Warranty of Installed Equipment and Systems

Assist the Government in resolving warranty issues as requested by the COR. Review documentation of installed equipment/systems and prepare an inventory database detailing their condition, equipment identifiers, equipment/system condition, scheduled maintenance, vendor sources for parts replacement, and warranty expiration dates. Supplement existing operation and maintenance (O&M) documentation to provide a

complete file. Submit inventory database for review and acceptance. Submit Report of Findings outlining the equipment/system condition and selected improvements to optimize operation and associated costs.

4.3 TECHNOLOGY EVALUATIONS AND PILOT-SCALE APPLICATIONS

Evaluate cost, performance, and applicability of methods (field/lab) and technologies for projects and provide a trade-off study of alternative approaches and technologies. Recommendations shall consider cost, schedule, protection of human health and the environment, public acceptance, and technical risk.

4.3.1 Initial Methodologies

Develop initial methodologies and follow-on execution programs for on-site auditing of industry laboratory and field operations, post-installation or post-remediation monitoring, site closure plan(s), and life cycle cost analysis of compliance, pollution prevention and remediation technologies. Analyze experimental designs and provide recommendations concerning adoption of these designs. Audit the performance of new technologies used in environmental and related efforts.

4.3.2 Commercial and Emerging Technologies

Evaluate commercially available and emerging technologies and other project enhancement technologies. Survey and analyze cost and performance data on new and/or innovative project approaches that concern the adoption of these designs. Audit the performance of new technologies used in related efforts.

4.3.3 Site-Specific Field Pilot Test Plans

Phase I sites shall include Operable Units at Multiple DoD Sites. The Contractor shall conduct enhanced *in situ* bioremediation pilot testing at sites to be determined by the COR. The objective shall be to select, implement, and evaluate the best method of substrate distribution, as well as evaluate the cost and performance of enhanced bioremediation via substrate addition. The Contractor shall prepare and complete one revision of a work plan for each site in close coordination with the COR in order to insure that project activities are compatible with site conditions and DoD objectives. Specific emphasis shall be placed upon the selection of the most effective and cost-effective method of substrate distribution.

Field pilot tests shall include all field testing activities necessary to determine the applicability and feasibility of electron donor addition at each site. These data will be used to evaluate whether a longer-term field pilot test is advisable. The site-specific test plans shall utilize existing DoD protocols as a reference and template when applicable. Draft site-specific field pilot test plans shall be submitted to the COR no later than thirty (30) days following the effective date of this task order or modification to this task order.

4.3.4 Site-Specific Field Pilot Test Reports

The Contractor shall compile, analyze, and interpret field test data in site-specific Field Pilot Test Reports. The Contractor shall provide defensible conclusions regarding, but not

limited to: the efficiency of electron donor utilization for reductive dechlorination as compared to metabolic (e.g. methane production) and anabolic (i.e. biomass) processes; contributions or effects of any reagents added to the system (e.g. substrate, microbial consortiums, and vitamins/cofactors); extent and uniformity of reagent distribution (e.g. carbon sources and amendments); loss of electron donor and tracer compounds; effective radii of influence; apparent electron donor requirements; observed changes in site-geochemistry; actual/significant changes in contaminant concentrations and mass (considering volatilization, dilution, degradation, and daughter product formation and persistence); reaction kinetics and contact time; costs; flow rates; injection pressures; volumes, concentrations, suppliers of injected reagents; feasibility and relative cost-effectiveness of expanded-scale implementation. The Contractor shall submit interim sections of Field Pilot Test reports no later than forty-five (45) days following each ground water monitoring event. The draft report shall be submitted no later than following the completion of the second semiannual ground water sampling event. The final reports shall incorporate COR comments and be submitted not later than forty-five (45) days following the completion of the third semiannual ground water sampling event.

4.3.5 Cost and Performance Summaries

Compile a comprehensive evaluation of the applicability and cost and performance data of remedial technologies applied under this effort. Site-specific cost and performance summaries shall be prepared according to the Federal Remediation Technology Roundtable format. The Contractor shall provide electronic copies of all figures, graphs, and text contained in draft and final versions of this report in the Contractor's most up-to-date version of Microsoft Office and in PDF formats. Site-specific draft reports shall be submitted no later than forty-five (45) days following the completion of the final site-specific field sampling event included under this effort.

4.3.6 Field Work

4.3.6.1 Site Characterization and Measurement of Baseline Geochemical and Contaminant Profiles

The Contractor shall characterize initial site-specific geochemical and contaminant conditions in accordance with the *Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water* (EPA/600/R-98/128, September 1998; http://www.epa.gov/ada/reports.html). The Contractor shall evaluate geochemical, metabolic byproduct, and contaminant breakdown product data to evaluate the potential for existing reductive dechlorination, future enhanced reductive dechlorination, and any expected changes in the above profile as a result of substrate addition.

4.3.6.2 *In Situ* System Installation and Monitoring

The Contractor shall conduct a closely controlled validation of *in situ* enhanced bioremediation of halogenated hydrocarbons via substrate addition. *In situ* testing might include the installation of *in situ* test columns, "push/pull" tests, or the installation of an expanded scale substrate emplacement pilot as specified in the site-specific Field Pilot Test Plan. The Contractor shall select those distribution approaches that are closest to proven technologies or have the highest likelihood of success (e.g., multiple direct-push injection points).

4.3.6.3 Pilot Test Initiation

Pilot tests shall be conducted at multiple field sites following the concurrence of the COR. The equipment and site activities to be conducted shall be identified in the site-specific Field Pilot Test Plan and shall be based on site-specific criteria to include at a minimum, hydrogeology, regulatory considerations, COR recommendations, and base-specific parameters (e.g., logistics, site accessibility, amenability to direct push technologies, current evidence in support of natural biodegradation, and compatibility with substrate distribution system). The pilot tests shall be performed according to the Final Field Pilot Test Plan.

4.3.6.4 Mobilization and System Installation

The Contractor shall provide qualified personnel, field equipment, and materials necessary to complete field pilot testing activities. Field pilot testing shall include, but not be limited to the following:

- Installation of an estimated five soil gas/pressure monitoring points (methane monitoring, as appropriate);
- Plumbing of the pilot injection system to the injection well;
- Installation of multiple injection and monitoring wells as specified in site-specific plans; and
- Site-specific geochemical and contaminant sampling and analysis in accordance with the *Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water* (EPA/600/R-98/128, September 1998).

As specified in site-specific plans, soil boreholes shall be advanced for the installation of extraction/injection/monitoring wells. Field logs shall describe site lithology and all field measurements.

4.3.6.5 System Checkout

The Contractor shall verify and document that the all systems are properly configured to operate as designed, efficiently and safely prior to system operation. Initial soil gas concentrations of organic and atmospheric constituents shall be measured in all installed vapor monitoring points. Ground water level measurements shall be made in the system injection well and in any on-site monitoring wells. A brief system test shall be conducted in accordance with site-specific test plans to ensure that the pilot system is operating properly prior to initiating the pilot test.

4.3.6.6 Radius of Influence Testing

The presence and levels of added reagents, tracers, constituents of concern, and critical geochemical parameters shall be measured in all appropriate monitoring points before, during, and after the test in accordance with site-specific test plans. Potentiometric surface measurements shall be conducted in appropriate ground water monitoring wells before, during, and after the test.

4.3.6.7 Process Monitoring

The Contractor shall monitor the system in order to complete a comprehensive mass balance on pilot test systems. Parameters monitored in order to accomplish this mass balance shall include, but not be limited to: a) the mass of contaminants removed from the system via biodegradation versus physical processes in dissolved, vapor, and sorbed

phases; b) electron donor and acceptor supply and utilization; c) metabolic byproduct formation (e.g. vinyl chloride; methane; ethane; ethene; hydrogen sulfide, ferrous iron, manganese, and chloride); and d) hydrogeologic parameters to estimate contaminant flux, electron donor and acceptor flux, and pore volume exchange rates. Aqueous flows shall be quantified, vapor and aqueous concentrations shall be analyzed, injection/extraction rates shall be quantified and recorded, hydrocarbon concentrations shall be determined, and total capital and O&M costs shall be reported. All measurements shall be made using properly calibrated instruments within the linear calibration range of those analytical instruments.

4.3.6.8 Electron Donor Utilization Tests

The Contractor shall conduct electron donor utilization tests in order to estimate total electron donor utilization rates, byproduct production rates (e.g. methane, vinyl chloride, ethene), and biodegradation rates in accordance with site-specific test plans. According to site-specific test plans, and after the area surrounding the injection well(s) has sufficiently equilibrated with the injected fluid, sampling and analysis shall be conducted at all appropriate monitoring points to measure electron donor utilization rates, contaminant reduction rates, and tracer conservation. The Contractor shall incorporate compensations for measured background electron donor utilization rates into all estimates of contaminant degradation and electron acceptor/donor utilization rates.

4.3.6.9 Phase I Extended Testing

The Contractor shall conduct extended testing of pilot systems at sites where contaminant biodegradation rates are significant and transformation product profiles are acceptable. The COR will evaluate biodegradation rates to determine "significant rates", and the Contractor shall not initiate Phase I extended testing without written authorization from the CO. The Contractor shall provide independent equipment consistent with site-specific test plans. Sites to be included in this effort and site-specific hydrogeological parameters are included in an attachment for costing purposes. The Contractor shall submit a site-specific test plan for COR review and approval prior to the conduct of extended field testing activities.

4.3.6.10 Process Waste Handling/Treatment

The Contractor shall provide process and investigative-derived waste handling and treatment/disposal. The Contractor shall exercise all due diligence to minimize any waste streams or allow "uncontaminated" materials to be mixed in with "contaminated" materials. The Contractor shall identify situations where site-specific regulatory compliance requirements do not agree with current costing assumptions/budget levels in the Monthly Progress Report/Cost Summary.

4.3.7 Full-Scale Phase II Applications

The Contractor shall not initiate Phase II extended testing without CO authorization. Such authorization will be in writing, and the Contractor shall not proceed with any further activity identified in Phase II in excess of that specifically identified in the CO's authorization.

At the CO's request, the Contractor shall conduct extended testing of electron donor addition systems. Extended testing shall be conducted at sites where pilot testing is

planned or in-progress, and shall be coordinated with the COR. The Contractor shall submit a site-specific test plan for review to the COR prior to the conduct of extended field testing activities. Extended testing shall include the incorporation of site-specific data into Phase 1 deliverables.

4.4 MISCELLANEOUS DELIVERABLES

4.4.1 Photo Documentation

Prepare photo documentation of site(s) and building(s) under investigation, field activities, and sample locations. Photography of any kind must be coordinated through the installation POC. Include photo documentation in reports as applicable.

4.4.2 Monitoring and Injection Well/Point and Borehole Data

Prepare 1) as-built drawings of each installed well and soil vapor monitoring point installed; and 2) soil boring logs and sieve analysis data collected during investigation and well installation tasks.

4.4.3 Data Management

Collect, prepare, publish, and distribute the data in the quantities and types designated on the Contract Data Requirements List (CDRL). Designate a focal point who shall integrate the total data management effort and manage changes, additions or deletions of data items. Identify items to be added, recommend revisions or deletion of items already listed on the CDRLs as appropriate, and maintain the status of all data deliverables.

4.5 TITLE I SERVICES

Perform all surveys, plans, studies, evaluations, and investigations identified in Section 4 of this SOW as necessary to support design efforts.

4.5.1 Design

The Government shall provide pertinent and available background information concerning the project (e.g., Feasibility Study, Focused Feasibility Study, Record of Decision). Review background data information for completion of an effective design. The major objective of a design project shall be the complete design of a practical and effective system(s) which meets the objectives of the project, maintains regulatory compliance, and incorporates pollution prevention initiatives. The design shall be submitted for review in one to four phases as specified: 30%, draft 100%, and final 100%.

4.5.1.1 Design Plans & Specifications

Develop clear and comprehensive design plan(s) and specifications which shall include the following:

a) Discussion of design strategy and design basis.
b) Discussion of important technical factors.

c) Description of assumptions and their justification.
d) Discussion of possible sources of error and references to possible O&M problems.
e) Engineering shop drawings and catalog cut sheets.
f) Tables listing equipment and specifications.
g) Tables detailing material and energy balances.
h) Appendices including data/results of laboratory or field studies, sample calculations, and derivation of equations.
i) As-built drawings of each installed well and soil vapor monitoring point installed.
j) Soil boring logs and sieve analysis data collected during investigation and well installation tasks.

4.5.1.2 Cost Estimates

As part of the design, develop a detailed cost estimate for construction and implementation of the project. All work items shall detail labor, material, and other costs. Develop life cycle cost estimates for planning and budgeting. These cost estimates shall detail, by fiscal year, the various development costs, construction costs, O&M costs, and long-term monitoring costs. Identify the base year being used for the cost estimates.

4.5.1.3 Operation & Maintenance Plan

As part of the design effort, develop an O&M plan to cover both implementation and long term maintenance. The plan shall include documentation for the comprehensive system, not simply for each component. The O&M plan shall include the following elements:

a) Equipment start-up procedures/specifications.
b) Description of normal O&M.
c) Potential operating problems.
d) Contingency O&M should systems fail.
e) Health & Safety Plan.
f) Description of equipment.
g) Routine monitoring and laboratory testing.

4.5.2 Design Phases

The design shall be submitted for review in one or more phases, as specified. The following lists the various design submittal phases and the approximate percentage of the design which shall be completed at each phase. Submittals shall be reviewed by the Government and written comments shall be provided. Disposition of the comments shall be determined at the respective review meeting. Incorporate the results into the next required design phase submittal.

4.5.2.1 Preliminary Design

Submitted at approximately 30% design completion. Includes all design components as specified in the preliminary design phase.

4.5.2.2 Draft 100% Design

Submitted at approximately 90% design completion. Includes all design components as specified in the preliminary design phase.

4.5.2.3 Final Design

Submitted at 100% design completion. Includes all design components as specified in the draft design phase.

6 GOVERNMENT POINTS OF CONTACT (POCS)

Contracting Agency
Contracting Officer's Representative
Attn: Name
Street Address
DoD Facility, State, and Zip Code
E-mail:
Voice:
FAX:

7 ABBREVIATIONS, ACRONYMS, AND TERMS

A-E	Architect-Engineering
AFCEE	Air Force Center for Environmental Excellence
ASTM	American Society for Testing and Materials
CDRL	Contract Data Requirements List
CO	Contracting Officer
COR	Contracting Officer Representative
CPSMR	Contractor's Progress, Status, and Management Report
CSM	Conceptual Site Model
DoD	Department of Defense
DQOs	Data Quality Objectives
FFAs	Federal Facilities Agreements
FMER	Funds and Man-Hours Expenditure Report
GCD	Guidance for Contract Deliverables
GPS	Global Positioning System
HAZWOPER	Hazardous Waste Operations and Emergency Response
HSP	Health and Safety Plan
ITIR	Informal Technical Information Report
OSHA	Occupational Safety and Health Administration
O&M	Operations and Maintenance
POC	Point of Contact
PPC	Project Planning Chart
QA/QC	Quality assurance and Quality Control
QPP	Quality Program Plan
SAP	Sampling and Analysis Plan
SOW	Statement of Work
TO	Task Order
WBS	Work Breakdown Structure

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APPENDIX C

DETERMINING SUBSTRATE REQUIREMENTS

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APPENDIX C
DETERMINING SUBSTRATE REQUIREMENTS

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APPENDIX C

DETERMINING SUBSTRATE REQUIREMENTS

To stimulate *in situ* anaerobic reductive dechlorination of chlorinated solvents in groundwater, a sufficient mass of organic substrate (electron donor) is required to satisfy both native (inorganic) and chlorinated solvent (organic) electron acceptor demand in the reactive treatment zone (Section 5.5.2). An inadequate substrate loading rate may result in reducing conditions that are insufficient to support complete anaerobic dechlorination of chlorinated solvents, thereby increasing the potential for accumulation of regulated intermediate dechlorination products. Conversely, excessive levels of organic substrate may lead to excessive methanogenesis, inefficient utilization of substrate for anaerobic dechlorination, and an increased potential for long-term adverse impacts to secondary groundwater quality. Therefore, determining an appropriate substrate loading rate is a critical design and operational objective to successful implementation of enhanced anaerobic bioremediation.

Practitioners of enhanced anaerobic bioremediation of chlorinated solvents use a variety of methods to estimate substrate requirements. Determining substrate loading rates is not an exact science, and the degree of uncertainty in the methods currently employed is considerable. To undertake a calculation of this kind may infer an understanding of the complex biological processes that is greater than the current state of the art. To make the process work a substantial design or safety factor is frequently applied, casting some doubt on the value of the calculation. Some practitioners do not perform these calculations and base substrate loading rates on experience, field observations, or practical engineering considerations. Other practitioners always base their design on calculations of this kind. No judgment is intended as to the appropriateness of the calculation or its role in design. The intent of this discussion is only to describe the current state of the practice in estimating substrate requirements. This appendix is not intended as a rigorous guide.

C.1 METHODS USED TO DETERMINE SUBSTRATE DEMAND

Two general approaches have been used to estimate substrate requirements and to derive a substrate loading rate. One approach is to target an empirical concentration of substrate in the reaction zone that is based upon previous experience and experimentation at sites with similar hydrogeology, geochemistry, and contaminant distribution. The other approach is to calculate a substrate (electron donor) requirement based on estimates of native and chlorinated aliphatic hydrocarbon (CAHs, commonly referred to as chlorinated solvents) electron acceptor mass. In practice, the result of applying both methods may be used as a check that the design substrate loading rate is appropriate.

Users of soluble substrates typically use an empirically-based approach because they are able to modify the substrate loading rate on a more frequent basis until the desired geochemical conditions are achieved. Conversely, users of slow-release substrates typically rely on calculated substrate requirements because the product is commonly applied in a single injection event. Spreadsheets to estimate substrate requirements have been developed by vendors of bioremediation products to estimate the quantity (mass or volume) of product that should be applied. These spreadsheets may facilitate selection of substrate type and loading rate during design.

Prior to utilizing either of these approaches, the practitioner should understand the theoretical basis for estimating electron acceptor demand and substrate (electron donor) requirements, and the factors of uncertainty inherent in these methods. ***Caution is urged with any approach to estimating substrate requirements. Given the current state of knowledge, field testing and experimentation may be the only way to optimize substrate loading rates for anaerobic dechlorination of chlorinated solvents.***

C.2 THEORETICAL BASIS FOR DETERMINING SUBSTRATE REQUIREMENTS

In order to determine site-specific substrate loading requirements, the total amount of electron acceptor demand exerted by both native (inorganic) and anthropogenic (i.e., CAHs) electron acceptor mass within and entering the treatment zone over the life-cycle of the application must be estimated. Note that the rate that which the substrate is applied (volume, concentration, and frequency) is equally as important as determining the total substrate demand for the life-cycle of the application (Section 5.5).

C.2.1 Electron and Hydrogen Equivalents

Because anaerobic reductive dechlorination is based on coupled oxidation-reduction (redox) and fermentation reactions, substrate (electron donor) requirements can theoretically be estimated by the amount of ***electron equivalents*** consumed by electron accepting processes utilizing both native and CAH electron acceptors, and the amount of electron equivalents generated by biodegradation of the substrate (electron donor).

For example, consider the following half reactions for reduction of oxygen (O_2) as an electron acceptor:

(1) $2H_2 \Rightarrow 4H^+ + 4e^-$

(2) $O_2 + 4H^+ + 4e^- \Rightarrow 2H_2O$

The reduction of oxygen by these reactions requires the transfer of 4 electrons; provided by hydrogen (H_2) as the electron donor. The net balanced reaction for reduction of oxygen can then be written:

(3) $O_2 + 2H_2 \Rightarrow 2H_2O$

where it takes 2 molecules of molecular hydrogen to reduce 1 molecule of molecular oxygen.

More commonly, practitioners attempt to calculate substrate requirements based on ***hydrogen equivalents*** (i.e., mass of molecular hydrogen). For example, consider the following half reactions for reduction of tetrachloroethene (PCE) as an electron acceptor:

(4) $4H_2 \Rightarrow 8H^+ + 8e^-$

(5) $C_2Cl_4\ (PCE) + 8H^+ + 8e^- \Rightarrow C_2H_4\ (ethene) + 4Cl^- + 4H^+$

The net balanced reaction for reduction of PCE to ethene using hydrogen as the electron donor can then be written:

(6) $C_2Cl_4 + 4H_2 \Rightarrow C_2H_4 + 4Cl^- + 4H^+$

where on a mass basis it takes 4 moles of molecular hydrogen (weight of molecular hydrogen = 4 moles x 2.016 grams per mole [gm/mole] = 8.064 grams) for every mole of PCE (weight of PCE = 1 mole x 165.8 gm/mole = 165.8 grams). More simply stated,

theory predicts that it takes 1.0 gram of molecular hydrogen to degrade 20.6 grams of PCE to ethene based on the molecular weights of the reactants in the above reduction reaction.

Although reduction of PCE to ethene typically occurs sequentially from PCE to trichloroethene (TCE) to dichloroethene (DCE) to vinyl chloride (VC) to ethene, the overall electron and hydrogen equivalents required for complete dechlorination of PCE to ethene remains the same.

C.2.2 Electron Acceptor Demand

The amount of hydrogen required (stoichiometric demand) to reduce other CAHs and native electron acceptors can similarly be calculated given the reaction sequences are known. Table C.1 lists a few examples of some common half reactions that utilize hydrogen as an electron donor for reduction of native electron acceptors and CAHs. Molecular formulas and weights for common compounds involved in anaerobic dechlorination reactions are listed on Table C.2.

Table C.1 Examples of Half Reactions Using Hydrogen as The Electron Donor

Electron Acceptor	Electron-Acceptor (Reduction) Half Reaction
Oxygen	$2H_2 + O_2 \Rightarrow 2H_2O$ *aerobic respiration*
Ferric Iron	$e^- + 3H^+ + FeOOH \Rightarrow Fe^{2+} + 2H_2O$ *"ferric oxyhydroxide" dissolution/reduction*
Sulfate	$4H_2 + H^+ + SO^{2-}_4 \Rightarrow HS^- + 4H_2O$ *sulfate reduction*
Carbon Dioxide	$4H_2 + CO_{2,g} \Rightarrow CH_{4,g} + 2H_2O$ *methanogenesis*
PCE	$H_2 + C_2Cl_4 \Rightarrow C_2HCl_3 + HCl$ *PCE reductive dechlorination*
TCE	$H_2 + C_2HCl_3 \Rightarrow C_2H_2Cl_2 + HCl$ *TCE reductive dechlorination*
DCE	$H_2 + C_2H_2Cl_2 \Rightarrow C_2H_3Cl + HCl$ *cis-1,2-DCE reductive dechlorination*
VC	$H_2 + C_2H_3Cl \Rightarrow C_2H_4 + HCl$ *VC reductive dechlorination*

For example, on a mass basis, 1.0 gram of molecular hydrogen is sufficient to dechlorinate the following mass of chlorinated ethenes, assuming 100 percent utilization of molecular hydrogen by the dechlorinating microorganisms:

- 20.6 grams of PCE to ethene
- 21.7 grams of TCE to ethene
- 24.0 grams of DCE to ethene
- 31.0 grams of VC to ethene

As hydrogen is produced by fermentative organisms, it is rapidly consumed by other bacteria, including denitrifiers, iron-reducers, sulfate-reducers, methanogens, and dechlorinating microorganisms. The production of hydrogen through fermentation does not, by itself, guarantee that hydrogen will be available for anaerobic reductive dechlorination of CAHs. For anaerobic dechlorination to occur, dechlorinators must successfully compete against the other microorganisms that also utilize hydrogen. Thus, a

direct stoichiometric relationship does not exist between hydrogen and CAH degradation in the subsurface or laboratory environment. However, even though the efficiency of utilization of hydrogen for anaerobic dechlorination is often estimated to be relatively low, the stoichiometric relationships for the direct anaerobic dechlorination of CAHs are relatively favorable.

Table C.2 Molecular Weights for Various Compounds Associated with Anaerobic Dechlorination of Chlorinated Solvents

Compound	Formula	Molecular Weight (grams/mole)
Tetrachloroethene (PCE)	C_2Cl_4	165.8
Trichloroethene (TCE)	C_2HCl_3	131.4
Dichloroethene (DCE)	$C_2H_2Cl_2$	96.95
Vinyl Chloride (VC)	C_2H_3Cl	62.51
Ethene	C_2H_4	28.05
Trichloroethane (TCA)	$C_2H_3Cl_3$	133.4
Dichloroethane (DCA)	$C_2H_4Cl_2$	98.96
Chloroethane (CA)	C_2H_5Cl	64.51
Ethane	C_2H_6	30.07
Tetrachloromethane/Carbon Tetrachloride (CT)	CCl_4	153.8
Trichloromethane/Chloroform (CF)	$CHCl_3$	119.4
Dichloromethane (DCM)/ Methylene Chloride (MC)	CH_2Cl_2	84.93
Chloromethane (CM)	CH_3Cl_1	50.49
Methane	CH_4	16.04
Oxygen	O_2	31.98
Nitrate	NO_3^-	61.98
Ferric Iron (Oxyhydroxide)	FeOOH	88.86
Sulfate	SO_4^{2-}	96.02
Carbon Dioxide	CO_2	44.01
Hydrogen	H_2	2.016

C.2.3 Electron Donor Potential

For complete anaerobic reductive dechlorination be effective, sufficient electron equivalents must be provided by electron donors to satisfy both native and CAH electron acceptor demand. Organic substrates may serve as an electron donors to provide the necessary electron equivalents. Hydrogen is thought to be the primary electron donor used in dechlorination reactions. Hydrogen is generated by fermentation of non-chlorinated organic substrates, including naturally occurring organic carbon, accidental releases of anthropogenic carbon (fuel), or introduced substrates such as alcohols, low-molecular-weight fatty acids, carbohydrates (sugars), and vegetable oils.

Theoretically, oxidation of a substrate can produce hydrogen. For example, consider the following half reactions for the oxidation of ethanol:

(7) $3H_2O + C_2H_6O$ *(ethanol)* $\Rightarrow 2CO_2 + 12H^+ + 12e^-$

(8) $12H^+ + 12e^- \Rightarrow 6H_2$

The net balanced reaction for oxidation of ethanol can then be written:

(9) $3H_2O + C_2H_6O \Rightarrow 2CO_2 + 6H_2$

where the oxidation of 1 molecule of ethanol produces 6 molecules of molecular hydrogen. Theoretically, this is the maximum amount of hydrogen that can be produced from ethanol. In the natural environment, the electron equivalents produced by oxidation or fermentation reactions are available to satisfy the electron transfer requirements of coupled reduction reactions.

However, more commonly, ethanol is fermented to acetate. Fermentation of a molecule of ethanol to acetate is shown in the following balanced fermentation reaction:

(10) C_2H_6O *(ethanol)* $+ H_2O \Rightarrow C_2H_3O_2^-$ *(acetate)* $+ H^+ + 2H_2$

In this reaction, the fermentation of 1 molecule of ethanol to acetate produces 2 molecules of molecular hydrogen. This is a more reasonable expectation of hydrogen potential than the coupled oxidation-reduction reactions above. The acetate produced in this reaction may be used directly as a direct electron donor for reduction reactions or may be further fermented to produce hydrogen. Another example of fermentation of sodium lactate (a stable lactate salt solid) to produce hydrogen is provided Section 2.1.4.3. Table C.3 lists a few examples of fermentation reactions where the substrate (electron donor) is fermented to produce hydrogen.

Table C.3 Examples of Fermentation Half Reactions using Organic Substrates as an Electron Donor to Yield Hydrogen

Electron Donor	Electron-Donor (Oxidation) Reaction
Ethanol	$C_2H_6O + H_20 \Rightarrow C_2H_3O_2^- + H^+ + 2H_2$ *ethanol fermentation to acetate*
Methanol	$CH_4O + 2H_20 \Rightarrow CO_2^- + H_2O + 3H_2$ *methanol fermentation*
Acetate	$C_2H_3O_2^- + 4H_20 \Rightarrow 2CO_2^- + 2H_2O + 4H_2$ *acetate fermentation*
Butyrate	$C_4H_7O_2^- + 2H_20 \Rightarrow 2C_2H_3O_2^- + H^+ + 2H_2$ *butyrate fermentation to acetate*
Propionate	$C_3H_5O_2^- + 3H_20 \Rightarrow C_2H_3O_2^- + CO_2^- + H_2O + 3H_2$ *propionate fermentation to acetate*
Lactate	$C_3H_5O_3^- + 2H_2O + \Rightarrow C_2H_3O_2^- + CO2^- + H_2O + 2H_2$ *lactate fermentation to acetate*

Note: Fermentation reactions from Fennel and Gossett (1998) and He et al. (2002).

Given the reactions by which native and CAH electron acceptors are reduced and organic substrates are oxidized or fermented, the theoretical quantity of organic substrate that is required to provide sufficient electron or hydrogen equivalents for complete electron acceptor consumption can be calculated. Therefore, substrate loading rates may be estimated, in terms of these equivalents, based on known stoichiometric reactions for both

the electron acceptor mass present in the treatment zone and the electron donor reactions associated with the substrate(s) applied.

These computations require that the substrate composition and the exact stoichiometry of each anticipated degradation reaction be known. In practice, these calculations only serve as a theoretical guideline for the required substrate loading because of the potential for multiple degradation pathways for some reactants, for variation in natural or CAH electron acceptor flux into the treatment zone, and for electron equivalents to be used for other processes.

For example, nitrate reduction may occur by several processes, including the following:

(11) $2H^+ + 6H_2 + 2NO_3^- \Rightarrow N_2 + 6H_2O$ *(denitrification)*

(12) $H_2 + NO_3^- \Rightarrow NO_2^- + H_2O$ *(nitrate reduction to nitrite)*

(13) $5H_2 + NO_3^- \Rightarrow NH_4^+ + 3H_2O$ *(nitrate reduction to ammonia)*

where 6 molecules of molecular hydrogen are required to degrade 2 molecules of nitrate (NO_3^-) to nitrogen (N_2) by denitrification; 1 molecule of molecular hydrogen is required to degrade 1 molecule of nitrate by nitrate reduction to nitrite (NO_2^-); and 5 molecules of molecular hydrogen are required to degrade 1 molecule of nitrate by nitrate reduction to ammonia (NH_4^+). Therefore, the required number of electron or hydrogen equivalents varies significantly between these three reactions.

For oxidation reactions of complex substrates, such as sucrose ($C_{12}H_{22}O_{11}$), the biodegradation or intermediate reaction sequences that may occur in nature to produce a given quantity of electron equivalents are difficult, if not practical, to predict. Furthermore, these reactions assume that no substrate is converted to biomass by microbial growth (i.e., zero yield). Therefore, the yield of electron equivalents from biodegradation of organic substrates will be less than theoretically possible, and the amount of electron equivalents produced is subject to some uncertainty.

C.2.4 Distribution and Flux of Native and CAH Electron Acceptor Mass

The substrate calculations described above require that the distribution and flux of native electron acceptors be known, including dissolved and solid-phase (e.g., bioavailable iron) electron acceptors. The most abundant dissolved native electron acceptors are dissolved oxygen (DO), nitrate, sulfate, and carbon dioxide (methanogenesis). The most abundant solid-phase native electron acceptors include ferric iron and manganese in the form of hydroxides or oxyhydroxides (See Table C.1 for an example). Similarly, CAH electron acceptor mass may be present in the aqueous phase, sorbed to the aquifer matrix, or present as dense non-aqueous phase liquid (DNAPL). Assuming knowledge of the electron accepting reactions that will occur, estimating total substrate requirements in terms of hydrogen equivalents involves summing the potential hydrogen demand exerted by each individual electron acceptor, and then determining the amount of substrate theoretically required to generate that mass of hydrogen.

The distribution of native electron acceptor mass is typically differentiated as:

1. The amount of dissolved native electron acceptor mass in the treatment zone (one pore volume);
2. The flux of dissolved native electron acceptor mass through the treatment zone over time; and

3. Solid-phase electron acceptor mass within the aquifer matrix of the treatment zone (assumed to be immobile).

Dissolved native electron acceptor mass within the treatment zone is simply the concentration of the dissolved electron acceptor multiplied by the pore volume of the treatment zone (total volume multiplied by total porosity).

Average linear groundwater velocity can be estimated by multiplying a measured or estimated horizontal hydraulic gradient (e.g., foot per foot) times the treatment zone average hydraulic conductivity (e.g., feet per day). Multiplying the average groundwater linear velocity by the area of the treatment zone cross-section through which groundwater will flow (horizontal length x vertical thickness x effective porosity) perpendicular to the direction of groundwater flow yields a groundwater flux in terms of volume per unit time (e.g., cubic feet per day).

Multiplying the groundwater flux by the average upgradient concentration of each dissolved electron acceptor yields an estimated mass flux over time. Concentrations of dissolved native electron acceptors are readily measured by conventional groundwater sampling and analysis techniques.

Calculating native bioavailable solid-phase electron acceptor mass is more difficult. Soil analytical results for iron and manganese mineral concentration and type are typically not available, and are costly to collect due to the need for additional drilling to collect soil samples and specialized procedures for laboratory analysis. Furthermore, it is difficult to determine how much of the iron or manganese minerals are readily available for biological processes. There are also other solid-phase electron acceptors (e.g., arsenic) that may be significant in particular lithologies. Given the current state of practice, a high level of uncertainty is associated with estimating solid-phase electron acceptor mass.

The distribution of CAH electron acceptor mass is similar to that of native electron acceptors, except that the distribution is likely not as uniform due to the nature of the release, and also includes the presence of sorbed CAH mass and possible DNAPL. The average residence time of dissolved contaminant mass in the treatment zone (not accounting for sorption/desorption) can be calculated by dividing the effective treatment zone pore volume (treatment zone volume multiplied by effective porosity) by the groundwater flux. For recirculation systems, the pumping rate and residence time for contaminated groundwater within the system should be assessed; although this is most readily accomplished using numerical flow models.

C.2.5 Designing for Uncertainty in Substrate Loading Estimates

Once the distribution and amount of electron acceptor mass is estimated, the total hydrogen demand exerted by electron accepting processes is typically estimated by summing the electron or hydrogen equivalents required to reduce each electron acceptor species.

The amount of substrate required is then estimated based on the potential electron equivalents or hydrogen mass that is generated by biodegradation of the substrate. In theory, this could be done by balancing half reactions of electron acceptor and electron donor processes. In practice, estimates of the potential amount of electron equivalents or hydrogen mass produced from a given mass of substrate are assumed.

There is a great deal of uncertainty involved in these estimates. The primary factors for uncertainty in substrate demand calculations include (but may not be limited to) the following:

1) ***Microbial Efficiency.*** The stoichiometric reactions described in this section represent only a subset of the possible reactions that may occur in the coupled oxidation-reduction biodegradation reactions that results from the addition of an organic substrate to a natural aquifer. Therefore, there is some uncertainty in estimating the electron equivalents that will be produced per unit mass of substrate, and in how those electron equivalents will be utilized in native and CAH electron accepting processes. Furthermore, substantial amounts of the substrate may be converted to biomass (and not hydrogen) during microbial growth. Therefore, there is an inherent hydrogen production inefficiency that is not accounted for in the theoretical amount of substrate required to completely degrade the estimated native and CAH electron acceptor mass.
2) ***Estimate of Native Electron Acceptor Demand, including the degree of Methanogenesis.*** There is a moderate level of uncertainty in determining the amount of native electron acceptors that are present in the aquifer system. Solid-phase electron acceptors (e.g., bioavailable iron and manganese) are difficult and/or expensive to determine, and many other inorganic species also may exert an electron acceptor demand. Aquifer heterogeneity and seasonal fluctuations in groundwater recharge may further complicate estimates of native electron acceptor demand.

   It is also difficult to determine the amount of substrate that will be utilized for methanogenesis. Biodegradation reactions (including methanogenesis) create large amounts of carbon dioxide, the electron acceptor used in methanogenic reactions. While the supply of carbon dioxide as an electron acceptor is relatively inexhaustible, methanogenesis will be limited until more favorable electron acceptors are depleted. After conditions conducive to methanogenesis are induced, it is difficult to estimate how much substrate will be utilized for methanogenesis relative to anaerobic dechlorination of CAH mass.
3) ***Estimate of CAH Electron Acceptor Demand.*** There is also uncertainty in the amount of CAH mass present in the aqueous, sorbed, or DNAPL phases. The degree of uncertainty in CAH electron acceptor demand is a function of how well the site is characterized. The electron acceptor demand exerted by CAH mass is typically much less than exerted by native electron acceptors, and the uncertainty associated with CAH distribution is therefore considered low relative to native electron acceptors. Nonetheless, the mass of CAHs present in DNAPL or sorbed to the aquifer matrix must be accounted for.

Substrate calculations such as those described in this Appendix should be used only as order-of-magnitude guidelines for calculating substrate loading rates. In practice, design factors on the order of 2 to 10 times the calculated substrate demand are typically used to account for the uncertainty factors described above.

For soluble substrates, substrate requirements are factored into a substrate loading rate, or the amount of substrate delivered per injection event over time. The discussion in this appendix only addresses total substrate demand. ***The rate at which the substrate is applied (amount and frequency) is equally as important as determining a total substrate demand.*** The reader is referred to Section 5.5 for discussion of substrate loading rates.

For slow-release substrates, the loading rate is multiplied by the designed lifespan of the substrate (typically 1 to 5 years) and all the substrate is injected during a single event. The slow-release characteristics of these substrates are intended to release the substrate continuously (controlled loading rate) over the designed lifespan of the application.

Analytical data collected during field sampling provides the best indication of the effectiveness of a particular substrate loading rate, and whether the substrate loading rate is appropriate for stimulating complete anaerobic dechlorination without excessive impacts to secondary water quality. Field analytical data (e.g., DO, oxidation-reduction potential [ORP], pH, total organic carbon [TOC] or dissolved organic carbon [DOC], and metabolic acids) from the injection and monitoring wells within the treatment zone are often used to confirm that the amount of substrate applied has created an appropriate reactive zone. Given the level of uncertainty involved in substrate calculations, many practitioners still utilize an empirical approach as described in the following section. An example of estimating substrate requirements based on calculations of hydrogen demand is presented in Section C.4.

C.3 EMPIRICAL METHODS FOR DETERMINING SUBSTRATE REQUIREMENTS

The exact electron acceptor demand that exists in a natural subsurface system is difficult, if not impractical, to determine. When considering the theoretical basis for substrate requirements, many practitioners base determination of substrate loading rate on achieving an empirical concentration of substrate in groundwater throughout the treatment zone.

Analytical data for TOC (unfiltered samples) or DOC (filtered samples) from injection and monitoring wells is commonly used to measure the effective dilution and mixing of substrate with groundwater and the effective radius of influence of the reaction zone. Analytical data for ORP, pH, and native electron acceptors (e.g., DO, nitrate, iron, sulfate, and methane) are used to establish that the amount of organic substrate added is sufficient to achieve the highly reducing conditions required for effective anaerobic dechlorination of CAHs, and to confirm that an appropriate reactive zone has been established.

For example, Suthersan et al. (2002) suggest that loading rates for soluble substrates of between 0.001 and 0.01 pounds of organic carbon per gallon of groundwater flux per day are sufficient to create and maintain a reducing reactive zone. This equates to a TOC concentration of approximately 15 to 150 milligrams per liter (mg/L). Suthersan et al. (2002) further suggest that the loading rate also should be sufficient to maintain between 50 and 100 times as much TOC in the reactive zone as there is CAH in the target area (i.e., 50 to 100 mg/L of TOC for every 1 mg/L of CAH).

Experience has shown that soluble organic substrates are transported, diluted, and degraded relatively rapidly in groundwater, resulting in a TOC gradient between the point of injection and the downgradient treatment zone. To account for these effects, higher concentrations of TOC are required at the point of injection to maintain sufficient TOC concentrations throughout the designated treatment zone. Therefore, the objective with soluble substrate systems employing direct injection or recirculation is to maintain an effective range of substrate concentration throughout the treatment zone, rather than targeting a singular concentration. Variations in the volume, strength, and/or frequency of substrate addition are used to achieve a particular target concentration range in the aquifer after mixing and dilution.

Controlling and maintaining the "target" substrate concentration over time for slow-release substrates is more dependent on the physical and chemical characteristics of the substrate. Slow-release substrates are designed to release a soluble organic carbon component continuously over a long period of time. Experience has shown that slow-release substrate systems can be effective in maintaining appropriate geochemical conditions for anaerobic dechlorination to occur over periods of months to several years following a single injection or emplacement.

Table C.4 lists some common substrates and the range of substrate concentrations targeted in historical enhanced bioremediation applications. The substrate strength (concentration), volume, and injection frequency may vary widely, even for a single substrate type.

Table C.4 Typical Substrate Loading Rates and Injection Frequencies of Common Organic Substrates

Substrate		Injected Form and Concentration	Targeted Concentration in the Treatment Zone	Typical Injection Frequency
Soluble Substrates	Sodium Lactate, Lactic Acid	Diluted to 3 to 60 percent by weight	50 to 300 mg/L	Continuous to Monthly
	Butyrate	Diluted to 3 to 60 percent by weight	50 to 300 mg/L	Continuous to Monthly
	Methanol	Diluted to 3 to 60 percent by weight	50 to 300 mg/L	Continuous to Weekly
	Ethanol	Diluted to 3 to 60 percent by weight	50 to 300 mg/L	Continuous to Weekly
	Molasses	Diluted to 1 to 10 percent by weight	50 to 500 mg/L	Daily to Quarterly
	High Fructose Corn Syrup	Diluted to 1 to 10 percent by weight	50 to 500 mg/L	Daily to Quarterly
Slow-Release Substrates	Whey (fresh/powdered)	Powdered form can be dissolved, fresh form can be injected as a slurry.	50 to 500 mg/L	Monthly to Annually
	Hydrogen Release Compound (HRC®)	Pure product injected at 4 to 12 pounds per vertical foot of injection.	100 to 500 mg/L	Annually to biennially, less frequently with HRC-X™ product. One-time injection may suffice in some cases.
	Vegetable Oil (e.g., food-grade soybean oil)	Oil-in-water emulsions with 5 to 15 percent oil by volume	100 to 500 mg/L	One-time injection. May require a second injection for very dilute emulsions.
	Mulch and Compost (cellulose)	Mixed with sand at 20 to 60 percent mulch or compost by volume	100 to 1,000 mg/L TOC within biowall reaction zone	One-time emplacement

C.4 EXAMPLE OF ESTIMATING SUBSTRATE DEMAND BASED ON HYDROGEN EQUILAVENTS

As discussed in Section C.2, practitioners have attempted to calculate substrate requirements based on hydrogen equivalents in coupled redox and fermentation reactions. This method calculates the mass of molecular hydrogen required to satisfy native and CAH electron acceptor demands. These computations assume that a limited, known set of stoichiometric degradation reactions occurs, and should be considered order of magnitude estimates only. In practice, large design factors (up to a factor of 10 to 20) have been used to account for uncertainty in hydrogen demand and heterogeneity in aquifer geochemistry.

C.4.1 Hypothetical Site Conditions

Tables C.5, C.6, and C.7 (attached) illustrate a hypothetical example used to illustrate calculation of substrate requirements for design purposes. Table C.5 illustrates calculation of total electron acceptor demand in terms of hydrogen equivalents. Table C.6 lists the molecular formula, molecular weight, and potential hydrogen production for some common substrates based on fermentation reactions. These data are used in Table C.7 to calculate the amount of substrate required to meet the hydrogen demand estimated in Table C.5.

The characteristics of the example site and system design are as follows:

- The treatment zone is 200 feet in length (perpendicular to groundwater flow) and 50 feet in width (parallel to groundwater flow), with a saturated thickness of 20 feet.
- The design period for the substrate calculations is 1 year.
- The groundwater potentiometric surface slopes uniformly in one direction with an average horizontal gradient of 0.005 foot per foot (ft/ft).
- The total porosity, effective porosity, and hydraulic conductivity of the aquifer matrix are assumed to be 30 percent, 20 percent, and 10 feet per day (ft/day), respectively.
- The soil bulk density and fraction organic carbon of the aquifer matrix are assumed to be 1.7 grams per cubic centimeter (gm/cm^3) and 0.5 percent, respectively.
- Contaminant concentrations are uniform throughout the treatment zone. Aqueous phase contaminant concentrations are 2,000 micrograms per liter (µg/L) PCE, 1,000 µg/L TCE, 500 µg/L *cis*-DCE, and 100 µg/L VC.
- The existing groundwater geochemistry is relatively aerobic, with an average DO concentration of 4.0 mg/L, average nitrate concentration of 1.0 mg/L, and average sulfate concentration of 20 mg/L. Anaerobic processes utilizing carbon dioxide as an electron acceptor are expected to generate a concentration of 10 mg/L of methane.
- Anaerobic processes utilizing solid-phase electron acceptors are expected to generate a concentration of 10 mg/L manganese (II) and 20 mg/L ferrous iron (II) for a single pore volume.

The hypothetical site conditions listed above constitute a basic conceptual site model. Application of a substrate for enhanced bioremediation can take many forms in regards to substrate type, injection configuration, and injection frequency. ***For the purposes of this example, the following discussion only describes the calculation of the total hydrogen demand and substrate requirements for a 1-year design life.***

C.4.2 Calculation of Hydrogen Demand

In this example, the total treatment zone volume is 200,000 cubic feet (ft^3) (Table C.5). Given a total porosity of 30 percent, one pore volume is equivalent to approximately 448,900 gallons. The application of Darcy's Law (calculation not shown) yields a groundwater seepage velocity of 0.25 ft/day, or 91.3 feet per year (ft/yr). Based upon an effective porosity of 20 percent (the volume of interconnected porosity through which groundwater will flow), the groundwater flux through the treatment zone is equivalent to approximately 229,300 gallons per year.

The mass of hydrogen required to theoretically reduce the mass of each native electron acceptor species and each CAH species is calculated in Steps 3 and 4 in Table C.5. For example, the hydrogen demand for aqueous native electron acceptor mass in the initial pore volume of the treatment zone is 16.2 pounds (lbs) of molecular hydrogen (Step 3A in Table C.5).

The total hydrogen demand required for the selected 1-year design life is calculated by summing the hydrogen demands for initial aqueous and solid-phase native electron acceptors, initial aqueous and sorbed phase CAH electron acceptors, and the soluble native and CAH electron acceptor mass flux over time. Based upon these calculations, the total electron acceptor demand (in pounds of hydrogen equivalents) for the example site can be summarized as follows:

Initial aqueous native electron acceptor demand in treatment zone:	16.2 lbs
Solid-phase native electron acceptor demand in treatment zone:	2.70 lbs
Initial soluble CAH electron acceptor demand in treatment zone:	0.63 lbs
Sorbed CAH electron acceptor demand in treatment zone:	3.33 lbs
Soluble native electron acceptor flux (per year):	3.95 lbs
Soluble CAH electron acceptor flux (per year):	0.15 lbs

Total Hydrogen Demand for 1-Year Design Life: 27.0 lbs

The design factor typically used by practitioners (to account for microbial efficiency and uncertainty in electron acceptor demand) using this method is between 2 and 10 times the calculated total hydrogen demand of the system. For this example, a design factor of 5 times yields a total hydrogen demand of 135 pounds of molecular hydrogen over 1 year.

C.4.3 Calculation of Substrate Requirements

Each organic substrate is capable of producing a particular mass of hydrogen per unit mass of substrate. This ***hydrogen production potential*** is directly related to the molecular structure of the organic substrate. The hydrogen production potential can be estimated in one of two ways:

1) **Hydrogen potential as the ratio (i.e., percent) of the mass of hydrogen to the sum of the molecular mass of the substrate compound**. For example, the hydrogen production potential of methanol (CH_4O) would be equal to the molecular weight of hydrogen (4 x 1.008 = 4.032 gm/mole) in methanol divided by the molecular weight of methanol (32.04 gm/mole). For methanol, the ratio of molecular weight that is hydrogen is 12.6 percent. The hydrogen production potential (in terms of molecular weight of the substrate that is hydrogen) for several common organic substrates using this method are presented in Table C.8 (attached). This method for calculating the hydrogen production potential of an organic substrate using this method may be oversimplified, but is sometimes used for estimation purposes nonetheless.

2) **Hydrogen potential as the product of fermentation reactions**. For example, the fermentation of methanol illustrated in Table C.3 yields 3 moles of hydrogen for each mole of methanol. Based on molecular weights of the reactants, the ratio of hydrogen produced by weight is 18.9 percent (compared to 12.6 percent calculated in method 1 above). The hydrogen potential for several common

substrates using this method are presented in Table C.7. This is generally a preferred method. Note that the hydrogen potential for complex substrates (sugars, HRC, and vegetable oils) are not well understood and are included primarily for illustrative purposes.

The mass of a particular organic substrate required to meet the total estimated hydrogen demand can be calculated by dividing the total hydrogen demand (including the design factor) of the system by the hydrogen production potential associated with the particular substrate of interest. Table C.7 lists the estimated mass of selected substrates that would be required to meet the hydrogen demand calculated in Table C.5 for design factors of 5 and 10 times the theoretical average demand. For example, the mass of methanol required to meet the example hydrogen demand with a design factor of 5 times (Table C.7) is approximately 715 pounds (135 pounds of hydrogen divided by 0.189). The range of substrate mass required for the example case using a design factor of 5 times ranges from 715 pounds of methanol to approximately 3,015 pounds of refined sugar (fructose) or lactic acid.

The substrate requirements listed in Table C.7 are for 100 percent pure product. When estimating required substrate mass, it is important to account for the fact that some commercially available organic substrate products on the market are less than 100 percent pure product and many are actual mixtures of different organic substrates. For example, HRC® is a mixture of lactate and glycerol, and several commercial vegetable oil emulsion products are mixtures of soybean oil, sodium lactate, emulsifiers, and water. Therefore, when estimating substrate requirements for purchase of substrate products, the composition of a substrate mixture must be known.

The material safety data sheet (MSDS) for HRC® lists the product as ranging from 52.5 to 65.0 percent glycerol tripolylactate and from 35.0 to 47.5 percent glycerol. For practical purposes, one could consider the product 60 percent glycerol tripolylactate and 40 percent glycerol by weight. It is not known by the authors how much of the 60% glycerol tripolylactate yields lactic acid or how much is inactive polymer material. Raymond *et al*. (2003) write a formula for HRC® as $C_{39}H_{56}O_{39}$. If 40% of this compound were lactic acid ($C_3H_6O_3$) and 40% were glycerol ($C_3H_8O_3$), you could conceivably end up with the same amount of hydrogen ions (i.e., 56).

As mentioned previously, it is a good practice to compare substrate loading estimates using the hydrogen equivalent method with empirical estimates. As an example, consider the 715 pounds of methanol estimated for the example case. Given an effective pore volume of approximately 448,900 gallons, a groundwater flux of approximately 229,300 gallons per year (Table C.5), and assuming the 715 pounds of methanol substrate is uniformly distributed in space and time, the average dissolved concentration of methanol would be approximately 105 mg/L. This concentration of methanol is within the range typically targeted for methanol of 50 to 300 mg/L (see Table C.4). While there are many uncertainties in estimating substrate loading rates using either empirical or hydrogen demand approaches, the observation that the methanol loading rate estimated by hydrogen demand for this example falls within the typical range used by empirical methods suggests that the hydrogen demand approach provided a reasonable first estimate.

C.5 SUMMARY

Determining substrate requirements is not an exact science, and the degree of uncertainty in the methods currently employed is considerable. While the scientific basis for determining substrate requirements remains an area for further investigation and development, the practitioner of enhanced anaerobic bioremediation must design a substrate loading rate with the methods currently available. The two approaches most commonly employed are to either: 1) target an empirical range of substrate concentration in the reaction zone that is based upon previous experience and experimentation, or 2) calculate a substrate (electron donor) requirement based on estimates of the native and CAH electron acceptor mass and mass flux. In practice, both methods may be performed and used as a check against the other that the substrate loading rate applied is within practical limits used in other successful bioremediation applications.

Practitioners using the methods described in this appendix should recognize the degree of uncertainty involved. One specific concern is that an inadequate substrate loading rate may lead to reducing conditions that are insufficient for complete dechlorination, with the potential for accumulation of intermediate dechlorination products. Conversely, excessive levels of organic substrate may lead to high levels of methanogenesis, low utilization of substrate for anaerobic dechlorination, and potential for adverse impacts to secondary groundwater quality.

Given the state of knowledge and practice, field testing and experimentation may be the only way to optimize substrate loading rates for anaerobic dechlorination of chlorinated solvents. It is anticipated that continued implementation and documentation of enhanced anaerobic bioremediation through substrate addition will lead to an improved understanding and less uncertainty in the design of substrate loading rates.

C.6 REFERENCES

Fennell, D.E. and J.M. Gossett. 1998. Modeling the Production of and Competition for Hydrogen in a Dechlorinating Culture. *Environmental Science & Technology*, Vol. 32(16):2450-2460.

He, J., Y. Sung, M.E. Dollhopf, B.Z. Fathepure, J.M. Tiedje, and F.E. Löffler. 2002. Acetate versus Hydrogen as Direct Electron Donors to Stimulate the Microbial Reductive Dechlorination Process at Chloroethene-contaminated Sites. *Environmental Science & Technology*, Vol. 36:3945-3952.

Raymond, R.L., Jr., M.D. Lee, R.J. Buchanan, and D.E. Ellis. 2003. Cost Implications of Hydrogen Donor Selection for In Situ Bioremediation of Chlorinated Solvents. *Proceedings of the of the Seventh International Symposium of In Situ and On-Site Bioremediation,* Orlando, Florida, June 2003. Paper A-37. Battelle Press, Columbus, Ohio.

Suthersan, S.S, C.C. Lutes, P.L. Palmer, F. Lenzo, F.C. Payne, D.S. Liles, and J. Burdick. 2002. *Final Technical Protocol for Using Soluble Carbohydrates to Enhance Reductive Dechlorination of Chlorinated Aliphatic Hydrocarbons, December 19, 2002.* Submitted to ESTCP and AFCEE under Contract #41624-99-C-8032.

Table C.5 Example Substrate Calculations in Hydrogen Equivalents

NOTE: Shaded boxes are user input.

1. Treatment Zone Physical Dimensions	Values	Range	Units
Length (Perpendicular to predominant groundwater flow direction)	200	1-10,000	feet
Width (Parallel to predominant groundwater flow)	50	1-1,000	feet
Saturated Thickness	20	1-100	feet
Treatment Zone Cross Sectional Area	4000	--	ft^2
Treatment Zone Volume	200,000	--	ft^3
Treatment Zone Total Pore Volume (total volume x total porosity)	448,920	--	gallons
Treatment Zone Effective Groundwater Volume (total volume x effective porosity)	299,280	--	gallons
Design Period of Performance	1	.5 to 5	year

2. Treatment Zone Hydrogeologic Properties			
Total Porosity	0.3	.05-50	
Effective Porosity	0.2	.05-50	
Average Aquifer Hydraulic Conductivity	10	.01-1000	ft/day
Average Hydraulic Gradient	0.005	0.1-0.0001	ft/ft
Average Groundwater Seepage Velocity through the Treatment Zone	0.25	--	ft/day
Average Groundwater Seepage Velocity through the Treatment Zone	91.3	--	ft/yr
Average Groundwater Flux through the Treatment Zone	109,237	--	gallons/year
Soil Bulk Density	1.7	1.4-2.0	gm/cm^3
Soil Fraction Organic Carbon (foc)	0.005	0.0001-0.1	

3. Initial Treatment Cell Electron-Acceptor Demand (one total pore volume)

A. Aqueous-Phase Native Electron Acceptors	Concentration (mg/L)	Mass (lb)	Stoichiometric demand (wt/wt h_2)	Hydrogen Demand (lb)	Electron Equivalents per Mole
Oxygen	4.0	14.98	7.9	1.90	4
Nitrate	1.0	3.75	10.2	0.37	5
Sulfate	20	74.92	10.6	7.09	8
Carbon Dioxide (estimated as the amount of Methane produced)	10	37.46	5.5	6.86	8
Soluble Competing Electron Acceptor Demand (lb.)				**16.2**	

B. Solid-Phase Native Electron Acceptors	Concentration (mg/L)	Mass (lb)	Stoichiometric demand (wt/wt h_2)	Hydrogen Demand (lb)	Electron Equivalents per Mole
Manganese (IV) (estimated as the amount of Mn (II) produced)	10	37.46	27.5	1.36	1
Iron (III) (estimated as the amount of Fe (II) produced)	20	74.92	55.9	1.34	1
Solid-Phase Competing Electron Acceptor Demand (lb.)				**2.70**	

C. Soluble Contaminant Electron Acceptors	Concentration (mg/L)	Mass (lb)	Stoichiometric demand (wt/wt h_2)	Hydrogen Demand (lb)	Electron Equivalents per Mole
Tetrachloroethene	2.000	7.49	20.6	0.36	8
Trichloroethene	1.000	3.75	21.7	0.17	6
cis-1,2-Dichloroethene	0.500	1.87	24.0	0.08	4
Vinyl Chloride	0.100	0.37	31.0	0.01	2
Carbon Tetrachloride	0.000	0.00	25.4	0.00	8
Chloroform	0.000	0.00	12.3	0.00	6
1,1,1-Trichloroethane	0.000	0.00	22.0	0.00	6
1,1-Dichloroethane	0.000	0.00	25.0	0.00	4
1,1-Dichloroethene	0.000	0.00	24.0	0.00	4
Total Soluble Contaminant Electron Acceptor Demand (lb.)				**0.63**	

D. Sorbed Contaminant Electron Acceptors (Soil Concentration = Koc x foc x Cgw)	Koc (mL/g)	Soil Conc. (mg/kg)	Mass (lb)	Stoichiometric demand (wt/wt h_2)	Hydrogen Demand (lb)	Electron Equivalents per Mole
Tetrachloroethene	263	2.63	55.83	20.6	2.71	8
Trichloroethene	107	0.54	11.36	21.7	0.52	6
cis-1,2-Dichloroethene	45	0.11	2.39	24.0	0.10	4
Vinyl Chloride	3.0	0.00	0.03	31.0	0.00	2
Carbon Tetrachloride	224	0.00	0.00	25.4	0.00	8
Chloroform	63	0.00	0.00	12.3	0.00	6
1,1,1-Trichloroethane	105	0.00	0.00	22.0	0.00	6
1,1-Dichloroethane	30	0.00	0.00	25.0	0.00	4
1,1-Dichloroethene	65	0.00	0.00	24.0	0.00	4
Total Sorbed Contaminant Electron Acceptor Demand (lb.)					**3.33**	

(continued)

Table C.5 Example Substrate Calculations in Hydrogen Equivalents (Continued)

4. Treatment Cell Electron-Acceptor Flux (per year)

A. Soluble Native Electron Acceptors	Concentration (mg/L)	Mass (lb)	Stoichiometric demand (wt/wt h_2)	Hydrogen Demand (lb)	Electron Equivalents per Mole
Oxygen	4.0	3.65	7.9	0.46	4
Nitrate	1.0	0.91	10.2	0.09	5
Sulfate	20	18.23	10.6	1.73	8
Carbon Dioxide (estimated as the amount of Methane produced)	10	9.12	5.5	1.67	8
Total Competing Electron Acceptor Demand Flux (lb/yr)				**3.9**	

B. Soluble Contaminant Electron Acceptors	Concentration (mg/L)	Mass (lb)	Stoichiometric demand (wt/wt h_2)	Hydrogen Demand (lb)	Electron Equivalents per Mole
Tetrachloroethene	2.000	1.82	20.6	0.09	8
Trichloroethene	1.000	0.91	21.7	0.04	6
cis -1,2-Dichloroethene	0.500	0.46	24.0	0.02	4
Vinyl Chloride	0.100	0.09	31.0	0.00	2
Carbon Tetrachloride	0.000	0.00	25.4	0.00	8
Chloroform	0.000	0.00	12.3	0.00	6
1,1,1-Trichloroethane	0.000	0.00	22.0	0.00	6
1,1-Dichloroethane	0.000	0.00	25.0	0.00	4
1,1-Dichloroethene	0.000	0.00	24.0	0.00	4
Total Soluble Contaminant Electron Acceptor Demand Flux (lb/yr)				**0.15**	

Total Hydrogen Demand (lb/yr) **27.0**

5. Design Factors and Total Hydrogen Demand

Microbial Efficiency Uncertainty Factor	2X - 5X
Methane and Solid-Phase Electron Acceptor Uncertainty	2X - 5X
Remedial Design Safety Factor (e.g., Substrate Leaving Reaction Zone)	1X - 2X

Total Hydrogen Demand (lb. of H_2) with a 2X Design Factor:	**54**
Total Hydrogen Demand (lb. of H_2) with a 5X Design Factor:	**135**
Total Hydrogen Demand (lb. of H_2) with a 10X Design Factor:	**270**

Table C.6 Hydrogen Produced by Fermentation Reactions of Common Substrates

Substrate	Molecular Formula	Molecular Weight (gm/mole)	Moles of Hydrogen Produced per Mole of Substrate	Ratio of Hydrogen Produced to Substrate (gm/gm)
Lactate (Lactic Acid)	$C_3H_6O_3$	90.1	2	0.045
Acetate (Acetic Acid)	C_2H_4O	44.1	4	0.183
Butyrate (Butyric Acid)	$C_4H_8O_2$	88.1	2	0.046
Ethanol	C_2H_6O	46.1	2	0.088
Methanol	CH_4O	32.0	3	0.189
Refined Sugars (Fructose)	$C_6H_{12}O_6$	180	4	0.045
Complex Sugars (Sucrose)	$C_{12}H_{22}O_{11}$	342	8	0.047
Hydrogen Release Compound[a/]	$C_{39}H_{56}O_{39}$	956	26	0.055
Linoleic Acid (Soybean Oil, Corn Oil, Cotton Oil)	$C_{18}H_{32}O_2$	281	16	0.115

[a/] From Raymond et al.(2003).

Table C.7 Estimated Substrate Requirements for Hydrogen Demand in Table C.5

Substrate (assumes pure product - 100% active ingredient)	Substrate Mass Required to Fulfill Hydrogen Demand Calculated in Table C.5 using Hydrogen Potential in Table C.6 (pounds of substrate)	
	5X Safety Factor	10X Safety Factor
Lactate (Lactic Acid)	3,014	6,028
Acetate (Acetic Acid)	737	1,474
Butyrate (Butyric Acid)	2,948	5,896
Ethanol	1,541	3,083
Methanol	715	1,429
Refined Sugars (Fructose)	3,015	6,029
Complex Sugars (e.g., molasses assuming 100% sucrose)	2,863	5,727
HRC®	2,461	4,921
Linoleic Acid (Soybean Oil, Corn Oil, Cotton Oil)	1,173	2,346

Table C.8 Hydrogen Content for Common Organic Substrates

Substrate	Molecular Formula	Substrate Molecular Weight (gm/mole)	Ratio of Molecular Weight that is Hydrogen
Lactate (Lactic Acid)	$C_3H_6O_3$	90.1	0.067
Acetate (Acetic Acid)	C_2H_4O	44.1	0.092
Butyrate (Butyric Acid)	$C_4H_8O_2$	88.1	0.092
Ethanol	C_2H_6O	46.1	0.131
Methanol	CH_4O	32.0	0.126
Refined Sugars (Fructose)	$C_6H_{12}O_6$	180	0.067
Complex Sugars (Sucrose)	$C_{12}H_{22}O_{11}$	342	0.065
Hydrogen Release Compound[a/]	$C_{39}H_{56}O_{39}$	956	0.088
Linoleic Acid (Soybean Oil, Corn Oil, Cotton Oil)	$C_{18}H_{32}O_2$	281	0.115

[a/] From Raymond et al.(2003).

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APPENDIX D

EVALUATION OF ALTERNATIVE SYSTEMS

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APPENDIX D
EVALUATION OF ALTERNATIVE SYSTEMS

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APPENDIX D

EVALUATION OF ALTERNATIVE SYSTEMS

To prepare this document, multiple case studies were reviewed to assess the relative performance of different enhanced anaerobic bioremediation systems. Site conditions and system design were evaluated to identify factors that contributed to a successful application of enhanced bioremediation, or that limited the effectiveness of the application. Seventeen case studies are reviewed in this appendix to evaluate common attributes of either successful or unsuccessful applications of enhanced bioremediation. Many of the case studies provided by technical contributors in Appendix E were included in this review because they represent typical (if not best case) examples of applying differing substrate types. The performance summaries for various substrate types in Appendices E.10 through E.13 also provided insights obtained from review of multiple field applications. Other selected case studies that illustrate lessons learned from Department of Defense (DoD) applications were provided by restoration or remedial project managers (RPMs) and/or their contractors.

Success of a full-scale enhanced anaerobic bioremediation remedial action can be defined as meeting regulatory compliance standards in a cost-effective and timely manner. While the majority of enhanced anaerobic bioremediation applications to date are pilot-scale systems, their performance can still be evaluated based on whether the results indicate the technology can meet applicable compliance standards, and that expansion to a full-scale system is warranted. Several guidance documents and numerous case studies have been published that describe methods to implement enhanced anaerobic bioremediation. Nonetheless, failures are rarely described in the literature, even though the ratio of sites that have achieved site closure or no-further-action is perhaps less than 10 percent of the number of total applications to date (see Appendices E.12 and E.13 for example).

There are several reasons why the percentage of site closures or no-further-action decisions are low at this time. A majority of applications are pilot-scale evaluations, and many full-scale systems have not been operating for a sufficient length of time to complete their operational life-cycle. In other cases, the enhanced bioremediation application is only part of a larger remedial strategy (e.g., source reduction coupled with monitored natural attenuation [MNA]). However, in many cases technical issues appear to have limited the success of enhanced anaerobic bioremediation applications. Failure to adequately characterize and understand the site hydrogeology, to effectively deliver the substrate throughout the targeted treatment zone, to induce sufficiently reducing conditions, as well as lack of appropriate microbial communities have been cited as limiting factors in the application of enhanced bioremediation. These technical issues are evaluated in the following comparison of alternative systems in order to provide the RPM with a higher level of confidence in implementing and evaluating enhanced anaerobic bioremediation.

D.1 CRITERIA FOR SUCCESS

System performance is often impacted by design and site-specific factors. While success may be difficult to define and is often subjective, some examples of benchmarks for success are listed below:

1. **Regulatory Acceptance:** For example, achieving site closure or designation of no-further-action. Approval to proceed from pilot-scale to full-scale application is also an indication of regulatory acceptance.

2. **Achieving Complete Reductive Dechlorination:** For example, achieving applicable groundwater standards (e.g., federal drinking water maximum contaminant levels [MCLs]) for all targeted contaminant compounds within the treatment zone. Reductions in the mass of chlorinated aliphatic hydrocarbons (CAHs, commonly referred to as chlorinated solvents) without the accumulation of regulated dechlorination products also may be considered an indication of an effective application.

3. **Substrate Distribution:** Achieving uniform distribution of an organic substrate is an indication of a successful engineering design. This includes distribution of substrate at appropriate levels throughout the treatment zone that are conducive to stimulating anaerobic degradation processes.

Achieving both complete anaerobic dechlorination of CAHs and effective substrate distribution are the first steps required to meet regulatory objectives; however, meeting other criteria also may be desirable. For example, elevated levels of secondary water quality parameters (e.g., taste, odor, iron) may occur within the reaction zone. Often these effects are temporal, but in the case of drinking water aquifers enhanced bioremediation may not be successful if secondary water quality standards are adversely impacted. In any event, the true measure of success is the ability to advance the site towards a defined remedial endpoint.

D.2 FACTORS TO CONSIDER IN EVALUATING ENHANCED BIOREMEDIATION

To evaluate selected case studies, several technical questions were considered based on site hydrogeology, substrate distribution, geochemistry, microbial sufficiency, extent of CAH dechlorination, and regulatory considerations. These questions are listed below:

Hydrogeology and Effective Substrate Delivery:

- Was the aquifer hydrogeology adequately characterized, or did inadequate characterization complicate substrate delivery?

- Was the substrate distributed effectively throughout the treatment zone as designed?

Geochemical Conditions:

- Were initial site geochemical conditions conducive to implementation of enhanced anaerobic bioremediation (e.g., relatively low levels of dissolved oxygen [DO], nitrate, sulfate, and bioavailable iron)?

- Did elevated levels of native electron acceptors or other geochemical conditions (e.g., low pH) develop that inhibited complete anaerobic dechlorination?

- Was the level of organic carbon sufficient to induce highly reducing conditions (i.e., as indicated by the occurrence of sulfate reduction and methanogenesis)?

Microbial Sufficiency:

- Was a complete dechlorination pathway observed, or did the system "stall" at an intermediate degradation product (e.g., *cis*-1,2-dichloroethene [*cis*-DCE] or vinyl chloride [VC])?

- Was a sufficient lag time allowed for microbial succession and adaptation (e.g., 12 to 24 months)?

- Was a microbial assessment performed and/or was bioaugmentation required?

Regulatory Considerations:

- Were water quality standards (e.g., drinking water MCLs) achieved?

- Was secondary water quality a factor in the success or failure of the application?

- Was a regulatory decision achieved for full-scale applications, or was full-scale expansion approved for pilot tests?

Each of the case study sites was evaluated using these questions to determine the conditions or developments that may have limited the success of the application. Table D.1 (attached) contains a summary of each site including notable observations pertinent to the questions listed above. The following discussion summarizes these observations and provides lessons learned (advantages and disadvantages) for different substrate types and for bioaugmentation (Sections D.3 through D.6). The references used for these evaluations are listed in Table D.1 and Section D.8. A summary section (Section D.7) is provided that identifies common causes for failure and limitations of applying enhanced anaerobic bioremediation.

D.3 SOLUBLE SUBSTRATE APPLICATIONS

Examples of soluble substrate applications are included from Appendices E.1, E.2, and E.4. Six applications of soluble substrates were reviewed, including applications of lactate and molasses, and a site with an acetate/fructose combination. A discussion of bioaugmentation applications using lactate and ethanol is included in Section D.6.

D.3.1 Lactate Applications

D.3.1.1 Test Area North, Idaho National Engineering and Environmental Laboratory, Idaho

The Test Area North Site is an example of applying sodium lactate in a single injection well to 400 feet below ground surface (bgs) (described in Appendix E.1 and Sorenson, 2003). In this case, injection of lactate stimulated complete anaerobic reductive dechlorination of trichloroethene (TCE) over a treatment cell approximately 500 feet long at depths greater than 200 feet in the plume source area. Sulfate reduction and methanogenesis were observed in the treatment zone, indicating that strongly reducing conditions had been achieved. The system has operated for several years, demonstrating effective treatment of a dense non-aqueous phase liquid (DNAPL) source in a deep fractured-bedrock aquifer. MCLs have been met for TCE and DCE at the base of the aquifer within the immediate injection area. Concentrations of VC remain above the MCL for this compound of 2.0 micrograms per liter (µg/L), but typically do not exceed 15 µg/L. The success of this project is indicated by a Record of Decision (ROD) amendment signed in 2001 that allowed the system to replace pump-and-treat for source area cleanup.

The Test Area North study has shown that soluble electron donor substrates such as sodium lactate can, in some cases, be distributed effectively over relatively large volumes of an aquifer using a relatively small number (only 1 in this case) of injection wells. Pilot test results from this site also indicate that the initial injection of high concentrations of substrate resulted in the rapid formation of strongly anaerobic conditions which are necessary for biologically mediated anaerobic dechlorination. After strongly anaerobic conditions were induced, they were sustained via injection of lower concentrations of substrate. Furthermore, an injection strategy that employs large-volume injections (low substrate concentration) at a lower frequency appears to be more effective and efficient than one that involves relatively small-volume (high substrate concentration) injections at higher frequencies (Martin et al., 2001).

D.3.1.2 IRP Site 24, Naval Base Ventura County, Point Magu, California

Installation Restoration Program (IRP) Site 24 at Naval Base Ventura County, Point Mugu, California, is an example of applying lactate in a shallow recirculation system at depths of 20 to 30 feet bgs (Johnson et al., 1999; Leigh et al., 2000; and Appendix E.10). During the first phase of the pilot test, groundwater was extracted from a single extraction well and reinjected with a lactate amendment into a single injection well approximately 90 feet upgradient for a period of 57 days. Approximately 17 gallons of an 88 percent lactic acid solution was injected per day in a pulsed mode, and groundwater was recirculated at a rate of approximately 10 gallons per minute (gpm). The first phase was successful in reducing concentrations of sulfate from approximately 700 milligrams per liter (mg/L) to less than 25 mg/L. Phase II of the pilot test consisted of a high concentration, pulsed injection of lactate at day 57, recirculation until day 64 to distribute the lactate slug, then monitoring for approximately 3 years.

The pilot test was successful in stimulating complete degradation of TCE and DCE to below applicable MCLs within the treatment zone. Dechlorination of TCE to DCE was observed in the presence of sulfate, but dechlorination of DCE to VC did not appear to occur

until after methanogenic conditions were induced. Concentrations of VC then increased rapidly to a maximum of 2,200 µg/L on day 180 of the pilot test, followed by a slow decline to approximately 441 µg/L at day 930. Ethene was generated from dechlorination of VC, but the rate of VC degradation was deemed too slow to meet remedial objectives (Granade et al., 2003). The observed degradation rate for VC was approximately two orders of magnitude lower than expected based upon laboratory microcosm studies.

It is important to note that available organic acids (acetate and propionate) were consumed by day 181. It is possible that further dechlorination of VC to ethene could have been electron donor (substrate) limited. Additional substrate was not provided over the remaining 3 years of process monitoring. Therefore, it cannot be determined whether degradation of VC could have been accelerated with the addition of more substrate. Laboratory studies suggested that the dechlorination of VC was cometabolic in the presence of TCE. Since TCE had been depleted in the treatment zone, it was thought that further dechlorination of VC would not occur.

The enhanced anaerobic pilot test was replaced with an *in-situ* aerobic cometabolic test using oxygen and methane. VC was effectively oxidized by cometabolism to a non-detectable level at one monitoring location. A full-scale sequential anaerobic/aerobic bioremediation system is currently planned for the site.

D.3.1.3 IRP Site 40, Naval Weapons Station Seal Beach, California

IRP Site 40 at Naval Weapons Station Seal Beach is an example of periodic lactate injection into a single injection well (French et al., 2003; Naval Weapons Station Seal Beach, 2002; and Appendix E.10). Six monitoring locations within a radius of approximately 20 feet were used to evaluate the system performance. The injection strategy included weekly injections of approximately 3,550 gallons of a 3-percent sodium lactate solution, which resulted in the distribution of substrate over an area of approximately 1,300 square feet (ft^2). The weekly injections were continued for 2 months, then temporarily discontinued after significant accumulations of metabolic acids (propionic and acetic acids) occurred. Lower volume injections (approximately 2,250 gallons) were conducted every 3 weeks for the remainder of the 8-month pilot test.

After 4 months of lactate injection, sulfate was depleted to below detection (<50 mg/L), and concentrations of methane increased to as high as 14 mg/L. The onset of anaerobic dechlorination correlated with the occurrence of sulfate reduction and methanogenesis. Once sulfate-reducing and methanogenic conditions were achieved within the treatment area, dechlorination of tetrachloroethene (PCE) to *cis*-DCE was observed. Dechlorination of *cis*-DCE to VC and ethene was not observed, even after achieving methanogenic redox conditions. Molecular analysis for *Dehalococcoides ethenogenes* indicated that this known dechlorinating species was not present within the indigenous microbial community, even after stimulation of microbial activity by addition of a lactate substrate. Thus, it was concluded that the Naval Weapons Station Seal Beach site was limited by a lack of appropriate dechlorinating microorganisms, rather than by a lack of appropriate redox conditions. Dechlorination appears to have "stalled" at *cis*-DCE, and a bioaugmentation pilot test was planned to overcome the microbial deficiency.

D.3.2 Molasses Applications

D.3.2.1 Washington Square Mall, Wisconsin

The Washington Square Mall site in Wisconsin (Appendix E.2; Maierle and Cota, 2001) is an example of applying molasses to the shallow subsurface (approximately 10 to 20 feet bgs) through a closely spaced grid of injection wells. The primary source of contamination in soil at this site was removed by excavation and off-site disposal of approximately 3,123 tons of soil. After dewatering activities associated with the excavation, the remaining contamination in groundwater was largely removed through enhanced anaerobic bioremediation. An initial injection event was implemented in August and September 1998, using 182 temporary direct-push injection points on 10-foot centers. Approximately 3,200 gallons of a dilute molasses solution was injected into the temporary injection points over a period of 11 days. Subsequently, an array of 12 permanent injection points was used to inject an additional 3,000 gallons of molasses solution during four injection periods over a 6-month period from March to September 1999.

Sulfate reducing and methanogenic redox conditions were created, with production of methane levels as high as 6 to 17 mg/L. PCE was dechlorinated to DCE and VC, with concentrations of DCE and VC peaking at 6 and 14 months after the initial injection, respectively. DCE and VC were subsequently degraded to ethene and ethane. PCE was effectively dechlorinated to non-toxic end products within 2 years, without a long-term accumulation of regulated intermediate dechlorination products. Regulatory closure with MNA was granted under Wisconsin Department of Natural Resources (WDNR) flexible closure rules. Low concentrations of DCE and VC persisted after remediation, but concentrations were sufficiently low to allow remediation by natural attenuation under the WDNR Voluntary Party Liability Exemption Insurance program.

D.3.2.2 Hanscom AFB, Massachusetts

Molasses was used to treat TCE in a glacial till aquifer at depths of approximately 35 to 55 feet bgs at Hanscom Air Force Base (AFB), Massachusetts (ARCADIS, 2003). For this pilot-scale application, a dilute molasses solution was injected periodically over 2 years into a single injection well. After the initial injection event in October 2000, 47 injection events were conducted through October 2002. Weekly average substrate loading rates ranged from approximately 50 to 250 pounds of molasses per week, with a final application of approximately 425 pounds in October 2002. The system required frequent injection of substrate (averaging an injection every 2 weeks) during the first 2 years to maintain anaerobic conditions. This was primarily attributed to relatively high rates of groundwater flow (estimated at 290 feet per year) and variable flow direction due to groundwater extraction in other areas of the site. In addition, a water push was required for each injection to avoid an adverse drop in pH at the injection well. Contrary to initial injections, monitoring at 6 months after a last injection of a high dose of substrate indicated that levels of total organic carbon (TOC) and methane continued to remain elevated well after injections were stopped.

Initial groundwater conditions were slightly aerobic, with concentrations of DO ranging up to 1.5 mg/L. Sulfate-reducing conditions were rapidly induced in the immediate vicinity of the injection well, but it took several months for anaerobic conditions to spread throughout the treatment zone. The rate of dechlorination of TCE to *cis*-DCE increased approximately 3

to 5 months after the initial injection of molasses. However, further dechlorination of *cis*-DCE to VC and ethene in the monitoring wells closest to the injection well lagged by a period of 17 to 26 months after the start of molasses injection. Degradation of *cis*-DCE and VC appear to correlate to the onset of methanogenesis. After approximately 2 years of operation, TCE had been reduced to concentrations below the federal drinking water MCL of 5 µg/L. Eventually, concentrations of *cis*-DCE and VC also were reduced. At 7 months after the last injection (May 2003), concentrations of *cis*-DCE and VC had been reduced by as much as 99 percent and 97 percent, respectively, from initial conditions at the most highly treated well. Nonetheless, concentrations of *cis*-DCE and VC persisted above their respective MCLs throughout the remainder of the pilot test area.

In summary, constant adjustments to the rate of substrate loading were required to create anaerobic conditions throughout the treatment zone, and to optimize system performance. The rate of substrate loading at this site required careful monitoring to avoid adverse lowering of pH. However, limiting the rate of substrate loading may also limit the effective radius of influence (ROI) of substrate distribution from the point of injection. Degradation of *cis*-DCE and VC occurred after an acclimation period of 17 to 26 months, which was attributed to either the time required to distribute substrate and deplete native electron acceptors, to the time required for biological acclimation, or both. Given a sufficient acclimation period and effective distribution of substrate, enhanced anaerobic bioremediation can still be an effective remedy for chlorinated ethenes in groundwater at this site.

D.3.3 Other Soluble Substrates (Naval Support Activity Mid-South, Tennessee)

An enhanced bioremediation pilot test using sequential anaerobic/aerobic reaction zones was implemented at Naval Support Activity Mid-South, Tennessee in March of 2000 (EnSafe, Inc, 2002). The aquifer sediments at the site consist of a relatively heterogeneous mixture of fluvial and alluvial sand, silt, and gravel. The aquifer is primarily contaminated with PCE and TCE (up to 2,100 µg/L of TCE detected in baseline sampling), with low levels of *cis*-DCE (less than 20 µg/L). The natural hydraulic gradient is relatively low at approximately 0.006 foot per foot (ft/ft), with an estimated groundwater flow of 31 to 62 feet per year (ft/yr). Therefore, a recirculation approach was employed to deliver the substrate. Substrate addition was used to create an anaerobic reaction zone to degrade PCE and TCE, while a naturally aerobic downgradient reaction zone was intended to degrade residual dechlorination products including *cis*-DCE and VC.

The pilot test was configured such that groundwater was extracted from a single extraction well, amended with substrate and nutrients, and injected into two wells located approximately 125 feet upgradient. The recirculation system was operated for approximately 9 months. Fructose was initially added at a ratio of 1 kilogram (kg) per 1,000 gallons for the first 2 months only. This equates to a substrate loading rate of approximately 260 mg/L. The average flow rate over the 9-month pilot test was approximately 4 gpm. After the 9-month recirculation test, a final high-strength injection of sodium acetate was conducted at a ratio of 11.4 kg per 100 gallons.

During the 9-month recirculation phase, concentrations of TCE within the recirculation cell were observed to decrease by approximately 40 to 60 percent. At the same time, concentrations of *cis*-DCE increased by approximately 2 orders of magnitude. VC was not detected above baseline concentrations during the 9-month recirculation phase.

Concentrations of TOC during the recirculation phase were less than 100 mg/L (averaging less than 20 mg/L), while oxidation-reduction potential (ORP) did not decrease below -200 millivolts (mV) in any portion of the treatment zone. These data suggest that only mildly reducing conditions were achieved. Immediately after injection of the final acetate slug, TOC concentrations in injection wells within the immediate anaerobic treatment zone increased to approximately 1,000 mg/L, and TOC in the nearest monitoring wells increased from approximately 100 to 400 mg/L. At the same time, ORP decreased to approximately -140 mV to -240 mV.

Performance monitoring data, collected approximately 6 months after the recirculation system was shut down, indicate that groundwater remained anaerobic with ORP between approximately -200 to -100 mV, and DO less than 1.0 mg/L. VC was detected approximately 6 months after system shutdown at concentrations as high as 540 µg/L within the injection wells and the closest monitoring locations. To date, VC has not been detected at the furthest downgradient monitoring locations. The pilot test is currently inoperative, pending regulatory approval of a final remedy for the site. MNA with hotspot reduction using direct (passive) injection of sodium acetate is proposed.

This pilot test was partially successful in that TCE was degraded to *cis*-DCE. However, process monitoring data indicate that *cis*-DCE was not being degraded to VC until after a final high-strength dose of substrate was added and the recirculation system was shut down. The generation of VC was attributed to development of a highly reducing stagnant zone in the injection area after recirculation ceased. This observation suggests that the reducing conditions necessary for the dechlorination of DCE to VC were not achieved during recirculation, possibly due to inadequate substrate loading, dilution due to mixing in the reaction zone, or a lengthy microbial acclimation period. *Dehalococcoides ethenogenes* species were detected in groundwater samples from the anaerobic treatment zone after the pilot test was completed, suggesting that the requisite microbial species for complete dechlorination were present when dechlorination of DCE to VC was observed.

D.3.4 Summary of Soluble Substrate Applications

The soluble substrates used in the preceding cases studies were readily injected and distributed in the subsurface, and may be particularly effective in difficult hydrogeologic settings. The applications at Test Area North and Hanscom AFB used only a single well to distribute substrate throughout the treatment zone. Recirculation systems have the potential to impact even larger treatment zones, which may be necessary at sites with complex hydrogeology or low rates of groundwater flow.

The primary disadvantage of using these substrates is the requirement for frequent injection and resulting higher operations and maintenance (O&M) costs relative to slow-release substrates. The cost of O&M alone is a large percentage of the life-cycle costs of soluble substrate systems.

The authors of Appendix E.10 suggest that incomplete dechlorination observed in some cases was either due to 1) an inadequate supply (low concentration) of substrate, resulting in insufficient reducing conditions, or 2) a lack of microorganisms capable of complete anaerobic dechlorination. Limiting the substrate loading rate in order to avoid methanogenesis may result in large areas of the treatment zone not becoming sufficiently

reducing. Although soluble substrates require frequent injection, the substrate concentration and volume can be modified to increase the substrate loading rate, as needed. However, this also may result in extra cost and time to monitor and adjust the substrate volume, concentration, and frequency of injection until an optimal scenario is obtained.

The authors of Appendix E.11 identify difficult hydrogeologic conditions as the cause of failure at three sites, including a high flux of aerobic groundwater, unexpected groundwater flow direction, and inadequate characterization of complex stratigraphy. For example, the demonstration project conducted at Hanscom AFB required modifications to the injection scenario over a period of approximately 1 year to fully deplete native electron acceptors and to induce methanogenic conditions under which degradation of DCE and VC was observed. Temporal fluctuations in the direction of groundwater flow also complicated substrate distribution at this site. The substrate limitation was overcome by adjustments to the system, and system performance objectives were achieved when sufficient substrate loading was accomplished.

In summary, the advantages and disadvantages of soluble substrate systems are listed below.

Advantages of soluble substrate systems include the following:

- Soluble substrates are readily mixed and distributed in the subsurface relative to other substrate types. This may be beneficial for deep treatment zones where direct-push techniques cannot be used. In this case, the high cost of well installation may be offset by the use of soluble substrates that require fewer injection wells for distribution of substrate over larger volumes of the aquifer.

- The ability to vary the concentration, volume, and frequency of injection allows for optimizing substrate delivery and manipulating the geochemical conditions in the reaction zone over time.

- Recirculation systems may be used for hydraulic containment and/or for a greater residence time of contaminants in the reaction zone. Recirculation systems also may treat larger aquifer volumes with fewer wells.

Disadvantages or limitations of soluble substrate systems include the following:

- O&M costs are high relative to long-lasting substrates as a result of the need for frequent injection.

- Biofouling of injection wells may require additional maintenance.

- The use of potable water to make up large volumes of substrate mixture may result in dilution and displacement of the contaminant plume.

- Low-molecular-weight substrates (e.g., lactate or butyrate) can be more expensive than bulk food-grade products, while the presence of impurities may require the use of higher grades of molasses or the use of high fructose corn syrup.

D.4 SLOW-RELEASE VISCOUS FLUID SUBSTRATE SYSTEMS

Eight applications of HRC® and vegetable oil were reviewed to evaluate the performance of slow-release (viscous fluid) substrates.

D.4.1 HRC® Applications

D.4.1.1 Fisherville Mill Site, Massachusetts

Application of HRC® at the Fisherville Mill site in Massachusetts is described in Appendix E.4 and Murray et al. (2001). The primary objectives of the pilot test were to demonstrate that enhanced bioremediation using HRC® can degrade PCE, TCE, and other regulated dechlorination products to ethene, and that the migration of CAHs can be controlled to protect downgradient receptors. HRC® and HRC®-primer were injected in a barrier configuration with three staggered rows of five injection wells spaced approximately 5 to 7 feet apart. Permanent, 2-inch-diameter injection wells were required due to the depth of injection (between 40 and 50 feet bgs) and soil conditions that prevented the use of direct-injection techniques. Glycerin was used to fully chase the HRC® products from the injection well casing.

Initial concentrations of TCE ranged up to 1,600 µg/L, and overall decreases in TCE ranged from 88 to 98 percent in wells located within 25 feet of the injection grid. Elevated levels of organic acids and TOC were reported to persist over a period of 27 months (data not provided). VC and ethene were produced, indicating that appropriate geochemical and microbiological conditions were induced for complete dechlorination of PCE and TCE to ethene. However, concentrations of TCE, *cis*-DCE, and VC at a location within the injection grid (well HLA-1 in Figure 2 of Appendix E.4) remained at 190 µg/L, 260 µg/L, and 56 µg/L, respectively, after 27 months of operation.

The contaminant levels required for compliance at this site were not identified, perhaps due to the fact it was a small demonstration test. Levels of the contaminants substantially exceeded federal MCLs in the downgradient wells at the end of the test, and it would appear that effective use of the technology at this site will require either higher risk-based cleanup standards, a longer life-cycle for the remediation system (i.e., additional injections), or combining the technology with other remedial measures (e.g., MNA for the downgradient portion of the plume).

D.4.1.2 Springdale Cleaners, Portland, Oregon

A combined application using HRC® for a dissolved CAH plume and HRC-X™ for a CAH source area at the Springdale Drycleaner Site is described in Appendix E.5 and in Sandefur et al. (2002). The primary objectives of this pilot test were to determine the effectiveness of using these products for enhanced bioremediation of chloroethenes as measured by the degree to which degradation of PCE could be accelerated; to determine if complete dechlorination of PCE through ethene could be stimulated; and to determine how long the effects of the HRC® and HRC-X™ products would persist. Cleanup levels for both PCE and TCE at this site are 5 µg/L.

For the dissolved phase plume application, 1,900 pounds of HRC® were injected into 22 boreholes advanced using direct-push technology. Within the DNAPL source area, 700 pounds of HRC-X™ were injected into 5 boreholes. Concentrations of organic acids indicated that the substrate was effectively delivered throughout the intended treatment zones. The HRC® product was capable of maintaining elevated concentrations of organic acids within the injection period for a period of approximately 18 to 27 months. The HRC-X™ product was capable of maintaining total organic acid concentrations of between 64 and 1,247 mg/L over the 40-month monitoring period.

Performance monitoring at this site showed significant levels of anaerobic dechlorination of PCE and TCE. Concentrations of *cis*-DCE, *trans*-DCE, and VC were elevated over the duration of the pilot test, but generally declined after peaking 1 to 2 years after injection. Ethene production was limited, but still sufficiently elevated to indicate that anaerobic dechlorination was proceeding to completion. Although significant reduction in the concentrations of all chlorinated ethenes present was observed, concentrations remained above federal MCLs for both the dissolved plume and source area treatment zones throughout the 1,247-day test. This was anticipated in the source area as there is a significant mass flux of PCE and TCE due to the presence of DNAPL and sorbed contaminants. Therefore, repeated applications of HRC® products may be necessary for this site.

Based upon results of the pilot test, a full-scale application was approved by the Oregon Department of Environmental Quality. The site continues to be monitored to determine how long the HRC-X™ product will remain effective. Full-scale application has been postponed due to state funding limitations.

D.4.1.3 Atlas 10 Site, Nebraska

The Atlas 10 Site in York County, Nebraska is an example of the application of HRC® to remediate TCE in groundwater within an unconfined, silty sand aquifer (USACE, 2003). Initial concentrations of TCE at the site were as high as 559 µg/L. Groundwater occurs at depths of approximately 57 to 63 feet, although perched groundwater may occur locally at lesser depths. Baseline groundwater geochemical conditions were highly aerobic, with DO concentrations ranging from 7 to 10 mg/L and ORP ranging as high as +200 mV. In addition, concentrations of total nitrogen (measured as nitrate + nitrite) ranged from 12 to 15 mg/L, and concentrations of sulfate ranged from approximately 30 to 40 mg/L.

In April 2000, approximately 105 pounds of HRC® per point was injected into 15 direct-push points. In December 2000, the injection area was enlarged, and a total of 25 direct-push points were used to inject approximately 232 pounds of HRC® per point into the pilot test area. Lactic acid and a known strain of dechlorinating bacteria (i.e., the Pinellas culture) were also injected into select locations in March 2001. Within the pilot test area, there were two injection point grids located side by side, with three staggered rows of injection points per grid. Injection point spacing was 7.5 feet in one grid, and 15 feet in the other.

Monitoring results for this study indicate that significant reductions in TCE concentrations did not occur, and only limited production of *cis*-DCE (less than 25 µg/L) was observed in one downgradient location near the end of the pilot test. TOC was initially measured at 692 mg/L within the immediate vicinity of the injection array after the first injection. However, within 1 year concentrations of TOC had declined to approximately 22 mg/L. Even though

DO was depleted to less than 1.0 mg/L and ORP to less than -100 mV for a few months within the treatment zone, no degradation of TCE to *cis*-DCE was observed. Nitrate and sulfate concentrations remained above approximately 3.0 mg/L and 20 mg/L, respectively, indicating that there was difficulty in depleting these native electron acceptors. A "low" quantity of *Dehalococcoides* was later measured within the treatment zone, suggesting that anaerobic dechlorination should have been stimulated under appropriate reducing conditions.

It appears that the application of HRC® at the Atlas 10 site was not able to effectively deplete native electron acceptors within the treatment zone. The presence of nitrate due to agricultural activities, and higher than expected rates of groundwater flow due to groundwater pumping for irrigation, are thought to have compounded the problems encountered at this site. In spite of bioaugmentation and the detection of *Dehalococcoides* species, appropriate reducing conditions for effective anaerobic dechlorination were not attained. As a result, the pilot test system is not being considered for full-scale expansion at this site. In general, enhanced anaerobic bioremediation is not thought to be suitable for this site, and groundwater extraction and aeration are being considered as an alternative.

D.4.1.4 SWMU 138, Naval Air Station Dallas, Texas

HRC® was applied at Solid Waste Management Unit (SWMU) 138, Naval Air Station Dallas, Texas in an effort to remediate PCE and TCE in groundwater to concentrations below federal MCLs (CH2M Hill, 2001). PCE and TCE were detected at the site at concentrations up to 110 µg/L and 47 µg/L, respectively. Shallow geologic units to a depth of 30 feet bgs consist of fine-grained fill, alluvial sediments, and weathered shale. Soils are not uniformly saturated, with the depth to water ranging from 8 to 26 feet bgs. It is reported that both unconfined and confined conditions are present within the alluvium at the site. It is believed that groundwater occurs within bedding plane fractures and partings, thin silt lenses parallel to bedding, and expansion joints. Monitoring wells yield low volumes of groundwater, and the rate of groundwater flow is thought to be very low (perhaps less than a few tens of feet per year). Concentrations of DO in groundwater are reported to be less than 1.0 mg/L, and the site has relatively high concentrations of sulfate ranging from approximately 1,600 to 3,400 mg/L.

HRC® was injected in a grid pattern consisting of 32 direct-injection points in July 2000. The product was injected at a rate of approximately 4.5 pounds per vertical foot (45 pounds per point), at a depth of approximately 14 to 24 feet, and with an injection point spacing of approximately 8 feet. In addition, HRC®-primer was injected at a rate of 1.5 to 3 pounds per vertical foot into an additional 10 injection points similarly distributed within the treatment zone grid. The use of HRC®-primer was intended to deplete levels of native sulfate more quickly.

Monitoring results collected over 9 months indicate that limited dechlorination of PCE and TCE to *cis*-DCE occurred in only a few locations within the pilot test area. Overall, consistent trends in the dechlorination of PCE and TCE could not be discerned. Sulfate levels were not depleted, and evidence of methanogenesis was observed at only one location at the end of the pilot test (an increase in methane of approximately 0.5 mg/L). Elevated levels of TOC (greater than 20 mg/L) or organic acids were not observed in any of the pilot test monitoring wells. The lack of substrate distribution was thought to be due to the low permeability of the subsurface soils and a lack of groundwater flow and mixing. As a result,

the pilot test was not considered to be effective for short term (less than 1 year) remediation of chloroethenes at this site. At best, the application might provide for long-term degradation if soluble organic acids released from the HRC® products eventually migrate more widely throughout the treatment zone. Expanded or full-scale application of HRC® was not considered for this site.

D.4.1.5 Summary of HRC® Applications

HRC® has been demonstrated to be an effective substrate for enhanced anaerobic bioremediation, with over 474 field applications since 1999 (Appendix E.12). Complete degradation of PCE and TCE parent compounds to VC and ethene was observed at both the Fisherville Mill Site (Appendix E.4) and the Springdale Cleaners Site (Appendix E.5). Although the ability to reach federal MCLs has yet to be demonstrated at these sites, significant contaminant reductions were achieved. The results obtained for the Springdale Cleaners Site suggests the potential for remediating DNAPL source areas using HRC® products.

Conversely, application of HRC® at the Atlas 10 Site in Nebraska (USACE, 2003) did not stimulate significant reduction of TCE, largely due to a high flux of native electron acceptors (DO, nitrate, and sulfate). Limited substrate distribution and a consequent inability to induce highly reducing conditions was observed at the Naval Air Station Dallas Site (CH2M Hill, 2001) due to low aquifer permeability and lack of groundwater flow. These limitations are not unique to the application of HRC®; rather, they can be problematic with enhanced anaerobic bioremediation applications in general.

D.4.2 Vegetable Oil Applications

D.4.2.1 Altus AFB, Oklahoma

Vegetable oil in the form of an oil-in-water emulsion was applied for a demonstration of the remediation of TCE in shallow groundwater at Site SS-17, Altus AFB, Oklahoma (Appendix E.6; Lee and Lieberman, 2002; and Lee et al., 2003). The soils at Altus AFB consist of silts and clays with little primary permeability. However, there is significant secondary permeability in the form of weathered/fractured bedrock and gypsum dissolution voids. Initial concentrations of TCE at the demonstration site were as high as 1,660 µg/L. The site also exhibits high levels of sulfate, with concentrations ranging from approximately 1,600 to 2,000 mg/L.

A biobarrier configuration was employed by installing six injection wells in a row perpendicular to groundwater flow, spaced 5 feet apart, and screened at a depth of approximately 8 to 18 feet bgs. A total of 760 gallons of emulsion was injected; consisting of approximately 1,270 pounds of soybean oil, 226 pounds of emulsifier (glycerol monooleate and polysorbate 80), 26 pounds of lactate, and 9.8 pounds of yeast nutrient. The emulsion was chased with approximately the same volume of water treated by activated carbon filtration. Monitoring wells were installed at distances up to 40 feet downgradient of the barrier, and process monitoring was conducted over a 13-month period.

Monitoring for TOC and metabolic acids indicates that substrate was distributed as far as 20 feet downgradient of the injection array, but that some downgradient locations closer to the

injection array received little substrate. As was expected, the heterogeneous distribution of TOC is likely due to the presence of preferential flow paths associated with secondary permeability. This was not considered to be detrimental because the mass flux of CAHs through the treatment zone is also anticipated to occur along these preferential flow paths.

Concentrations of TCE and total chloroethenes decreased immediately after injection, although the initial decrease was attributed to dilution and partitioning into the oil. Approximately 7.5 months after injection, the concentration of total chloroethenes returned to more than 90 percent of initial concentrations, indicating the system had reached a quasi-equilibrium, and subsequent changes in concentrations of CAHs were attributed to degradation processes. After 13 months of monitoring, concentrations of TCE at a well located along the centerline of the treatment zone decreased from 1,660 µg/L to less than the limit of detection, while the concentration of *cis*-DCE decreased from 900 to 73 µg/L. During the same monitoring period, concentrations of VC and ethene at this monitoring location increased from 440 to 1,770 µg/L and from 6.9 to 510 µg/L, respectively.

This system was effective in stimulating a complete dechlorination pathway of TCE to ethene, even in the presence of high levels of sulfate. Aquifer heterogeneity and secondary permeability complicated uniform distribution of substrate at this site. Monitoring locations not impacted by substrate addition continue to exhibit elevated levels of CAHs. The system continues to be monitored to determine how long the substrate sustains anaerobic dechlorination, and whether concentrations of VC also decline over time.

D.4.2.2 Site SS015, Travis AFB, California

A field application of vegetable oil was implemented at Site SS015, Travis AFB, California to remediate chlorinated ethenes in a source area hotspot (Parsons, 2004). Initial concentrations of TCE, *cis*-DCE, and VC were as high as 4,000 µg/L, 13,000 µg/L, and 17,000 µg/L, respectively. Lithology at the site consists of low permeability silts and clays, with a groundwater flow rate of less than 30 ft/yr. The site also exhibits high sulfate concentrations ranging from approximately 2,400 to 5,400 mg/L.

The Travis AFB application was designed for source area reduction using a grid configuration. After an initial pilot test in April 2000, the system was expanded in December 2000 and again in April 2002. Four different injection scenarios utilizing straight vegetable oil with a water push and different compositions of oil-in-water emulsions were tested, using approximately 38 direct-push injection points over an area of approximately 2,000 ft^2. The injection points were initially installed as 1-inch-diameter well points in December 2000. However, due to the low permeability of the subsurface formation, these well points could not withstand the injection pressures necessary to inject the total amount of substrate as designed. Well points that failed were successfully replaced by direct injection through the direct-push probe rods in April 2002. Post-injection monitoring occurred from September 2000 to March 2003.

Process monitoring results indicate that elevated levels of TOC (greater than 30 mg/L) were distributed throughout the treatment zone, although the vegetable oil injected as an oil-in-water emulsion appeared to result in a more uniform distribution of substrate. By March 2003, the mass of total CAHs in groundwater had been reduced by over 80 percent. Sequential dechlorination of PCE to TCE to *cis*-DCE to VC to ethene was clearly observed,

with temporal accumulations and subsequent reductions of *cis*-DCE and VC. Concentrations of ethene increased by a factor of 20 to 30. Complete dechlorination was observed even in the presence of sulfate levels that persisted at concentrations of 500 to 1,500 mg/L.

Concentrations of PCE, TCE, *cis*-DCE, and VC were reduced to below federal MCLs at multiple locations. However, due to the low permeability and high sorption potential of the formation, it is likely that uniform reductions in CAHs to MCLs may take several years. Shallow groundwater at the site is not a potable drinking water supply, and the site has been approved for MNA. The Air Force is currently redeveloping the site, and long-term monitoring will continue once construction is completed.

D.4.2.3 Hangar K, Cape Canaveral Air Force Station, Florida

The application of vegetable oil at the Hangar K Site at Cape Canaveral Air Force Station, Florida is an example of injecting neat vegetable oil into shallow groundwater at depths of 20 to 33 feet for remediation of chloroethenes in a suspected DNAPL source area (Parsons, 2002). Baseline concentrations of TCE were measured as high as 300 mg/L. The shallow soils at the site are sandy with high hydraulic conductivity (100 to 500 ft/day). However, the groundwater hydraulic gradient is low (less than 0.0005 ft/ft), resulting in groundwater flow rates of less than a few tens of feet per year. The application used 33 injection points installed at a depth of 22 to 32 feet bgs using direct-push technology; the points were installed in a grid configuration having an area of approximately 3,000 ft^2. Approximately 55 gallons of pure soybean oil was injected into each well point followed by 150 to 200 gallons of native groundwater to immobilize the oil at residual saturations and to increase the substrate ROI.

Process monitoring over a period of 40 months indicates that the substrate was effectively distributed with initial concentrations of TOC as high as 3,200 mg/L in the injection points. Concentrations of TOC after 40 months have declined to less than 50 mg/L at all but one monitoring location, suggesting that the effective lifespan of the substrate at this site is on the order of 4 to 5 years. Due to the viscosity of vegetable oil, it is likely that the distribution of neat oil will only be effective in relatively high permeability, homogeneous formations such as encountered at the Hangar K Site.

Within approximately 18 months of injection, complete dechlorination of TCE to ethene was observed. Within approximately 30 months of injection, concentrations of PCE and TCE were reduced to below federal MCLs at all locations within the treatment zone. Concentrations of *cis*-DCE and VC initially accumulated, but concentrations of *cis*-DCE have declined to less than its MCL at all locations within 40 months of injection. Concentrations of VC also continue to decline, and are below the federal MCL at five locations. Further process monitoring is being considered to evaluate long-term depletion of the vegetable oil substrate.

D.4.2.4 Summary of Vegetable Oil Applications

Vegetable oil is currently being developed as a low-cost alternative substrate, designed to induce or enhance anaerobic dechlorination of CAHs for several years with a single injection. Vegetable oil applications to date have mostly been performed to achieve source reduction using grid configurations, or to construct biobarriers using rows of injection points.

Applications at Altus AFB, Travis AFB, and Cape Canaveral Air Force Station have demonstrated that concentrations of chlorinated ethenes can be reduced by several orders of magnitude to below federal drinking water MCLs. Applications of vegetable oil at Travis AFB and Cape Canaveral Air Station indicate anaerobic dechlorination can be stimulated for periods of at least 3 to 4 years with a single application.

Some difficulty in achieving effective substrate distribution has been encountered, and uniform distribution of the substrate may be complicated by low permeability soils or by a high degree of aquifer heterogeneity. Distribution problems may be expected with injection of neat vegetable oil, and generally this is no longer recommended. Low-viscosity microemulsions are currently the state-of-the-art for distribution of vegetable oils, which allows for more uniform distribution. Stable emulsions with very fine droplet sizes may be difficult to prepare in the field. Alternately, more expensive commercial emulsion products are available.

D.4.3 Summary of Slow-Release Viscous Fluid Substrate Systems

Several applications of HRC® and vegetable oils were evaluated. In summary, the advantages and disadvantages of slow-release viscous fluid substrate systems are listed below.

Advantages of using slow-release viscous fluids include the following:

- Application of HRC® or vegetable oil emulsions can be very cost-effective for treating shallow groundwater plumes using inexpensive direct-push techniques, where close injection point spacing (5- to 15-foot centers) can be utilized.
- The use of fast-acting HRC®-primer and long-lasting HRC-X™ products provides design alternatives for varied site conditions.
- Vegetable oil emulsions are easily modified to fit site-specific conditions. Oil-in-water emulsions can be modified to include fast-acting soluble substrates or to modify the effective oil saturation.
- The effective lifespan of a single application of these substrates may last from 1 to 5 years.
- Slow-release substrates also may be used in trenches and excavations to supplement solid substrates (e.g., spraying vegetable oil onto sand or mulch, or backfilling with a layer of HRC®).

Disadvantages of using slow-release viscous fluids include the following:

- Slow-release substrates typically require more injection points and may not be as cost-effective as soluble substrate applications under very deep or difficult hydrogeologic conditions where injection costs are high.

- High rates of groundwater flow or high rates of native electron acceptor flux may require higher loading rates and additional injections. In some cases (e.g., the Atlas 10 site), the native electron acceptor flux may be too high to overcome.

- HRC® products are relatively expensive compared to other substrate types. However, a cost-benefit analysis should be conducted that considers other cost factors and the overall life-cycle costs.

- Creating stable emulsions in the field with an appropriate droplet size may be difficult in practice. Use of pre-mixed commercial microemulsion products will increase cost.

D.5 SOLID SUBSTRATE APPLICATIONS

The solid substrate case studies reviewed include two applications of bark mulch biowalls for remediation of chlorinated ethenes.

D.5.1 Bark Mulch Applications

D.5.1.1 Building 301, Offutt AFB, Nebraska

A pilot-scale bark mulch biowall was installed by the Air Force at the Building 301 site at Offutt AFB, Nebraska, in January 1999 (Appendix E.7; Groundwater Services Inc., 2001). The biowall trench was designed as a reactive biobarrier to stimulate anaerobic dechlorination of chloroethenes in groundwater. This treatment approach relies on the natural flow of groundwater through the biowall.

A mixture of locally derived bark mulch and sand was emplaced in a trench using a one-pass continuous trencher. The trench measured 100 feet long, 1 foot wide, and 23 feet deep. Subsurface soils at the site consist of silty clay with low hydraulic conductivity (average of 3.5 ft/day). The cohesive nature of these soils facilitated installation of the biowall trench below the groundwater surface. The depth to groundwater is approximately 6 feet bgs, and the advective groundwater flow velocity is reported to be approximately 85 ft/yr. The trench was located to intercept the most contaminated portion of a shallow TCE groundwater plume. Initial concentrations of TCE upgradient of the biowall were as high as 1,900 µg/L, with relatively low concentrations of *cis*-DCE and VC (less than 27 µg/L and 3 µg/L, respectively).

The pilot-scale application was successful in achieving strongly reducing conditions, primarily in the iron-reducing to methanogenic range. Dechlorination of TCE to *cis*-DCE was observed, with limited production of VC, ethene, and ethane. While a complete dechlorination pathway was observed, concentrations of VC did not accumulate and remained less than 3.0 µg/L. Concentrations of *cis*-DCE initially increased by a factor of 45 at 5 months following installation. After 5 months, concentrations of *cis*-DCE measured immediately downgradient of the biowall declined, and TCE also continued to be degraded. Much of the decline in the concentrations of TCE and *cis*-DCE could not be accounted for by conservation of mass of dechlorination products. It is postulated that other degradation processes (e.g., abiotic reductive dechlorination or anaerobic oxidation) may account for degradation of *cis*-DCE and VC without the production of dechlorination products (Appendix E.7; Haas et al., 2003). The mean percent removal of TCE was calculated to be

approximately 75 percent, and the mean percent removal of total chloroethenes was calculated to be approximately 64 percent. Although significant reductions in CAHs were observed, concentrations remained above respective federal drinking water MCLs.

A full-scale biowall was installed at the Building 301 site in July 2001, measuring 500 feet long, 1.5 feet wide, and 25 feet deep (Aziz et al., 2003). For the full-scale system, concentrations of TCE were reduced by up to 92 percent immediately downgradient of the biowall. However, concentrations of *cis*-DCE remain higher than initial concentrations after 1 year of monitoring. The persistence of elevated concentrations of *cis*-DCE suggests that, while the biowall system is effective in degrading TCE, it is less effective in degrading *cis*-DCE. Monitoring where the full-scale biowall and the pilot-scale biowall overlap indicates more promising results; in this area mean concentrations of TCE, *cis*-DCE, and VC measured downgradient of the biowall are all lower than mean concentrations upgradient of the biowall. This observation suggests that the dechlorination of *cis*-DCE and VC is influenced by the residence time in the biowall reaction zone(s). The full-scale system at Offutt AFB continues to be monitored for long-term performance.

D.5.1.2 OU1, Altus AFB, Oklahoma

A mulch and compost biowall was installed at Landfill 3 in Operable Unit 1 (OU1) at Altus AFB, Oklahoma, in June 2002 as a technology demonstration test (Henry et al., 2003 and Haas et al., 2003). The objective of the Altus AFB application was to intercept and contain a shallow TCE/DCE groundwater plume at depths of 6 to 25 feet bgs to prevent surface water discharge and off-base migration, as well as to evaluate the technology for application at other Air Force sites. The biowall measures 455 feet long, 24 feet deep, and 1.5 feet wide. The biowall fill is composed of approximately 50 percent shredded bark mulch, 10 percent cotton burr compost, and 40 percent sand by volume. The sand was added to reduce compaction and maintain permeability. The trench was installed using a continuous chain-driven trencher.

Depth to water at the site ranges from 6 to 8 feet bgs, and the trench was intended to intercept over 80 percent of the groundwater plume contaminant flux, with the remainder of the plume being remediated by natural attenuation. Soils at the site consist of low permeability silt and clay that exhibit secondary permeability in the form of fractures and gypsum dissolution features. Groundwater flow rates average approximately 100 to 120 ft/yr. Similar to conditions for the vegetable oil application described in Section D.4.2.1, groundwater at this site has sulfate concentrations on the order of 1,600 to 2,200 mg/L. Concentrations of TCE immediately upgradient of the biowall have been measured as high as 8,000 µg/L.

The Altus AFB bark mulch wall was successful in stimulating the anaerobic dechlorination of TCE in groundwater. Within 9 months, concentrations of TCE had been reduced to below 5 µg/L (the MCL) within the biowall. Concentrations of *cis*-DCE within the biowall have decreased or remained stable. However, concentrations of *cis*-DCE have increased downgradient of the biowall in the most contaminated portion of the plume, and persist at concentrations in excess of 1,000 µg/L. Concentrations of VC throughout the monitoring network remain less than 5 µg/L after 18 months of monitoring. The overall reduction of in concentrations of total CAHs within the biowall is 86 percent. Much of the reduction in TCE and *cis*-DCE may be attributed to abiotic degradation by reactions with iron monosulfides

(Kennedy and Everett, 2003). However, the production of *cis*-DCE and low levels of VC also suggests that biotic anaerobic dechlorination of TCE is a predominant degradation pathway at this site.

Concentrations of TOC over 30 mg/L (versus a background of less than 5 mg/L) have been observed as far as 30 feet downgradient of the biowall. Concentrations of TOC within the biowall have declined over the first 18 months of monitoring, but remain above 70 mg/L. Sulfate has been depleted, and elevated levels of methane have been observed within the trench. However, immediately downgradient of the trench sulfate concentrations quickly rebound. The rebound in high levels of sulfate may limit effective anaerobic dechlorination in areas downgradient of the biowall.

While the system is effective in overall mass removal, MNA is still required as a polishing process for downgradient portions of the plume. Based on the mass destruction achieved by the demonstration biowall, the Air Force has decided to use this technology as a mass reduction/containment measure for other CAH plumes at Altus AFB. The next generation of biowalls are being installed to depths of 35 bgs, and are being fitted with perforated pipe to allow additional amendment with liquid substrates as a future contingency against depletion of the mulch substrate.

D.5.2 Summary of Bark Mulch Solid Substrate Systems

Two applications of bark mulch in a permeable biowall configuration were reviewed to evaluate the performance of solid substrate systems. The Offutt AFB and Altus AFB biowalls were both capable of effectively reducing concentrations of TCE. Reduction of TCE concentrations to below federal drinking water MCLs occurs in the immediate vicinity of the Altus AFB biowall. Both sites exhibited substantial degradation of *cis*-DCE without an accumulation of VC or ethene. Degradation processes other than biotic anaerobic reductive dechlorination of TCE, *cis*-DCE, and VC are likely occurring, including abiotic degradation by reactive iron-monosulfides. Humic acids in the mulch and compost mixtures may also serve as electron acceptors in energy yielding reactions that result in the oxidation of *cis*-DCE and VC under anaerobic conditions (Bradley et al., 1998). Although exhibiting decreasing trends, *cis*-DCE has persisted at concentrations above initial conditions and above its MCL at both biowall sites. Nonetheless, overall mass destruction rates for total CAHs are impressive, ranging from 64 percent (Offutt AFB pilot-scale biowall) to 86 percent (Altus AFB biowall).

Both of the biowalls appear to be effective at treating shallow groundwater plumes in highly heterogeneous formations having a low to moderate permeability. It is yet to be determined whether retention time and substrate loading using mulch biowalls is sufficient for degrading concentrations of CAHs in excess of 10 to 100 mg/L, or for flow rates greater than 1.0 ft/day. The use of wider trenches (greater than 2 feet in width) or multiple parallel trenches may be necessary to treat higher CAH concentrations at sites with high rates of groundwater flow or high rates of native electron acceptor flux. In summary, the advantages and disadvantages of solid substrate mulch and compost biowalls are listed below.

Advantages related to the use of mulch and compost biowalls include the following:

- Effective for shallow groundwater plumes in low to moderate permeability or highly heterogeneous formations. The continuity of the trench eliminates the potential for

groundwater bypass due to preferential flow paths, or non-uniform distribution of substrate that may occur with delivery of liquid substrate via injection wells.

- Mulch, compost, and sand are relatively inexpensive when purchased in bulk quantities. Tree mulch can often be obtained for the cost of shipping and handling alone.

- Mulch biowalls require no O&M other than periodic performance monitoring. However, it has yet to be determined how many years biowall systems will be able to sustain anaerobic reductive dechlorination of CAHs.

- Trenches can be modified to include wells or perforated pipe for addition of liquid substrates to supplement carbon loading, if necessary. In addition, the relatively small treatment volume of the trench (relative to other substrate configurations) makes biowall systems ideal candidates for relatively low-cost inoculation with bioaugmentation cultures.

Disadvantages or limitations of mulch biowalls include the following:

- The depth that can be trenched in a practical and cost-effective manner is limited to approximately 35 feet bgs. Excavation of a bench for the trenching equipment may provide for an additional 5 to 10 feet of depth.

- Trenching may interfere with site infrastructure and utilities.

- The contaminant retention time in the trench and substrate loading capacity (i.e., rate at which organic carbon is added to the groundwater passing through the trench) may be insufficient to treat concentrations of CAHs in excess of 10 to 100 mg/L. Use of wider trenches or multiple parallel trenches may be necessary to treat higher CAH or to deplete high concentrations of native electron acceptors.

- The effective life-span of mulch biowalls has yet to be determined.

D.6 BIOAUGMENTATION APPLICATIONS

Bioaugmentation applications involve the addition (injection) of a microbial culture known to be capable of complete anaerobic reductive dechlorination of the CAHs present at a site. Two bioaugmentation cases studies are reviewed in the following subsections.

D.6.1 Aerojet General Corporation Facility, Sacramento, California

Bioaugmentation was used to stimulate complete dechlorination of TCE in groundwater at the Aerojet General Corporation Facility in Sacramento, California (Appendices E.9 and E.13; GeoSyntec, 2002). Pilot testing was conducted in two phases. The first phase consisted of a small-scale, closed loop recirculation system, while the second phase involved expansion of the pilot test using a single-pass biobarrier system designed to treat a 600-foot-wide portion of the CAH plume.

The closed loop recirculation system was installed in May 2000, and consisted of a single extraction and single injection well. Following performance of a tracer study to characterize the system hydraulics, groundwater was extracted at a rate of 5 gpm, amended with acetate, and reinjected into the treatment zone. A series of monitoring wells were installed between the extraction and injection wells for process monitoring. Substrate addition alone was sufficient to degrade perchlorate present in groundwater, but was not capable of significant degradation of TCE within a 63-day test period. Following performance of system maintenance from day 64 to day 93, lactate was added until day 157 to evaluate biostimulation using a different substrate. At day 157, only limited dechlorination of TCE to *cis*-DCE had been observed, and a commercial bioaugmentation culture was added to the injection well. After bioaugmentation, production of VC and ethene was observed within 8 days. Concentrations of *cis*-DCE and VC reached maximum values at approximately 33 and 75 days after bioaugmentation, respectively. Concentrations of *cis*-DCE and VC subsequently declined during the remainder of the Phase I pilot test. Furthermore, *Dehalococcoides* species (not detected prior to bioaugmentation) were detected in all monitoring wells within the pilot test area at 75 days after bioaugmentation, indicating that the culture successfully colonized the treatment zone.

Based on the Phase I pilot test results, the system was expanded to extract groundwater from two cross-gradient extraction wells with injection back through the Phase I pilot test area. The substrate amendment was switched to ethanol, and further bioaugmentation was not required because the extracted groundwater passed through the treatment zone inoculated during the Phase I Test. Monitoring results indicated that concentrations of TCE up to 2 mg/L were degraded within 35 to 65 feet of the reinjection well. Concentrations of *cis*-DCE and VC did not accumulate within the bioaugmentation treatment zone because they were rapidly reduced to ethene. In a cross-gradient well outside the zone of bioaugmentation, dechlorination appeared to stall at *cis*-DCE and 1,1-DCE for the duration of the Phase I pilot test.

Another significant observation at the Aerojet Facility is that the rate of ethanol addition could be controlled to reduce the impacts on secondary water quality, without an adverse impact on the rate of CAH dechlorination. Methane concentrations were typically below 0.2 mg/L, suggesting that creation of highly methanogenic conditions was not required to achieve rapid and complete dechlorination when using the bioaugmentation culture.

Lag phases as long as 6 to 24 months are commonly observed for many enhanced bioremediation systems using biostimulation alone before *cis*-DCE and VC are observed to dechlorinate to ethene. For this site, the decision to bioaugment was based on results of biostimulation during the Phase I pilot test and on microcosm studies where dechlorination of *cis*-DCE was not observed after 200 days of testing. The success of the pilot tests at the Aerojet Facility indicates that bioaugmentation is a potential alternative for sites where the ability of biostimulation alone to stimulate complete anaerobic dechlorination is in question.

D.6.2 Bachman Road Residential Wells Site, Michigan

Both biostimulation alone and biostimulation plus addition of a bioaugmentation culture were evaluated in parallel pilot test cells to evaluate the anaerobic dechlorination of PCE in groundwater at the Bachman Road Residential Wells Site in Michigan (Appendices E.10 and E.13; Lendvay et al., 2003, 2001a, and 2001b). Two treatment cells were installed in shallow

groundwater at depths of approximately 10 to 20 feet bgs, with each test cell consisting of a small recirculation system of one extraction well and two injection wells. The injection wells were installed 6 feet apart, with the extraction well located 10 feet downgradient. The test plot treatment areas measured approximately 270 ft^2, and were separated by approximately 20 feet in a cross-gradient direction. Tracer tests were conducted to characterize flow within the test plots and to confirm the test plots were hydraulically separated.

The recirculation system in the biostimulation-only plot was operated without substrate addition for 140 days as a control. Lactate was then added for a period of 121 days to determine the effectiveness of biostimulation alone. Dechlorination of *cis*-DCE occurred approximately 90 days after lactate injection commenced, and the appearance of VC and ethene was observed shortly thereafter. Therefore, an acclimation period of approximately 3 months was required for complete dechlorination of PCE and TCE to VC and ethene to be observed.

Lactate and a bioaugmentation culture were injected into the second treatment cell to determine the relative effectiveness of enhancing the rate of anaerobic dechlorination with the bioaugmentation culture. *Dehalococcoides* species are present naturally at the site, and a locally-derived culture was enriched for the bioaugmentation test. Lactate was injected into the bioaugmentation cell for 29 days to deplete DO and induce reducing conditions prior to adding the bioaugmentation culture. Two hundred liters of the Bachman Road culture was then added to the test cell while lactate addition was continued. The bioaugmentation test was then monitored for 182 days. A considerable portion of the PCE and TCE had been converted to *cis*-DCE during the period when lactate alone was added to condition the test cell. Upon bioaugmentation, the remaining PCE and TCE were converted to *cis*-DCE within 1 week, followed by conversion to VC and ethene. Within 43 days, 92 percent of the total molar concentration of chloroethenes was converted to ethene. The addition of the bioaugmentation culture appears to have reduced the lag time required for complete dechlorination by approximately 3 months.

The results of this pilot test effort clearly show that the addition of the bioaugmentation culture accelerated the onset of complete dechlorination of PCE. In addition, the bioaugmentation culture increased the rates of anaerobic dechlorination. However, the results also indicate that the natural microbial population at this site is capable of complete dechlorination of PCE to ethene. Thus, the decision to bioaugment at this site is likely to be based on economic considerations, versus an issue with microbial sufficiency. In this case, the cost of bioaugmentation should be considered relative to the cost to maintain a biostimulation system over a longer period of time.

D.6.3 Summary of Bioaugmentation Systems

The bioaugmentation applications at the Aerojet Facility in California and the Bachman Road site in Michigan demonstrate that bioaugmentation can be highly effective with small-scale recirculation systems. It has yet to be shown whether bioaugmentation can be used successfully with large-scale recirculation or passive enhanced bioremediation systems. The ability to increase the scale of bioaugmentation systems will be dependent to a large extent on the ability to uniformly distribute the culture over large volumes of the treatment zone.

Most bioaugmentation demonstrations performed to date have used low-molecular-weight soluble substrates (e.g., lactate or ethanol) in recirculation configurations to carefully control aquifer redox conditions (Appendix E.13). The fermentation reactions for low-molecular-weight substrates are generally better understood than for more complex substrates (e.g., molasses or vegetable oils). However, there is no reason to believe that bioaugmentation cannot be effective with all substrate types.

Commercially available bioaugmentation cultures consist predominantly of the microbial strain *Dehalococciodes ethenogenes*. *Dehalococciodes ethenogenes* is the only microbial strain that has been shown to be capable of complete sequential dechlorination of PCE and TCE to *cis*-DCE, VC, and ethene. The ability of these bioaugmentation cultures to completely dechlorinate chloroethanes and chloromethanes is less well understood. The advantages and disadvantages of bioaugmentation are listed below.

Advantages of bioaugmentation include the following:

- Bioaugmentation can be used to reduce acclimation periods and/or to provide populations of known dechlorinating microorganisms where dechlorination is incomplete.

- Bioaugmentation may be used to increase the confidence of using an enhanced anaerobic bioremediation approach. In some cases, bioaugmentation may be cost-effective in that the overall time for remediation can be decreased, thereby reducing costs for O&M and performance monitoring.

- Bioaugmentation in a carefully controlled reaction zone may result in complete anaerobic dechlorination without inducing strongly reducing (i.e., methanogenic) conditions. Avoidance of strongly reducing conditions may not be necessary in most cases, but this capability can be used to advantage where strict adherence to drinking water standards is being enforced (e.g., the Aerojet Facility case study in Appendix E.9).

Limitations of bioaugmentation include the following:

- Enriched cultures may quickly attach to the aquifer matrix and do not migrate as readily as soluble substrates. Therefore, it may be difficult to distribute the cultures throughout large volumes of an aquifer.

- Although the cost of bioaugmentation cultures is decreasing as more commercial vendors enter the market, the cost of the recirculation systems commonly used to carefully control geochemical conditions is high. The ability to inoculate passive treatment systems in a barrier configuration may represent a more cost-effective use of the technology.

D.7 SUMMARY OF LESSONS LEARNED: COMMON CAUSES FOR FAILURE TO ACHIEVE EFFECTIVE ENHANCED ANAEROBIC BIOREMEDIATION

The following subsections describe some common limitations and reasons for failure of enhanced anaerobic bioremediation applications that have been encountered in this survey of cases studies.

D.7.1 Hydrogeology and Substrate Delivery

High rates of groundwater flow, low aquifer permeability, and low hydraulic gradient have all resulted in ineffective applications of enhanced anaerobic bioremediation. In general, a high flow rate results in a high native electron acceptor flux and difficulty in establishing a highly reducing environment. For example, a high flux of DO, nitrate, and sulfate at the Atlas 10 Missile site in Nebraska is thought to have resulted in native electron acceptor demand that could not be overcome by two applications of HRC®. High groundwater flow rates may also limit the effective residence time of CAHs in the reactive zone. This may be a critical design factor for systems such as mulch biowalls, where the reaction zone and residence time may be relatively short.

Low permeability limited the effectiveness of an application of HRC® at the Naval Air Station Dallas site. HRC® and vegetable oil have been applied successfully in low permeability settings at other sites (e.g., the Travis AFB site described in Section D.4.2.2), but it is typical for very long lag times to occur and for distribution of the soluble constituents of these substrates to be diffusion limited (a slow process). A low hydraulic gradient and slow rates of groundwater flow (less than 10 to 30 ft/yr) may similarly inhibit effective distribution of substrate in passive systems. While it is possible that slow migration of soluble substrate via diffusion may occur over a period of several years, for most applications this may not be an acceptable timeframe. The use of recirculation systems that enhance hydraulic gradients and the rate of groundwater flow should be considered for treatment of dissolved plumes in low gradient or low permeability groundwater systems.

D.7.2 Geochemistry, Redox Conditions, and Substrate Loading

Insufficient substrate loading and high native electron acceptor flux can result in redox conditions that are not sufficiently reducing to stimulate effective rates of anaerobic dechlorination. Although anaerobic dechlorination has been demonstrated to occur under sulfate-reducing conditions without methanogenesis (e.g., methane less than 1 mg/L), the number of sites that exhibit complete anaerobic dechlorination under methanogenic conditions would suggest that an attempt to limit substrate loading, in order to prevent methanogenesis and increase substrate utilization, may be counter productive. The cost of additional substrate necessitated by diminished utilization of CAHs for anaerobic dechlorination is small compared to the cost incurred for longer O&M and monitoring due to slower rates of anaerobic dechlorination. Due to aquifer and microbial heterogeneity, controlling subsurface redox conditions with precision is difficult. Such control typically requires frequent substrate injection and/or recirculation, and possibly also temporal variation in loading rates, both of which are labor and cost intensive.

A review of soluble (aqueous) substrate applications in Appendix E.10 notes several sites where anaerobic dechlorination of *cis*-DCE or VC to ethene did not occur until methanogenic

conditions were observed. Methanogenic conditions are often an indication that bioavailable iron and sulfate have been adequately depleted, and that high rates of fermentation are occurring. These conditions favor high rates of anaerobic dechlorination, and should not be considered detrimental. At Naval Support Activity Mid-South (Section D.3.3), production of VC and ethene were only observed after a high concentration slug of acetate was injected at the end of a recirculation test that had utilized a lower substrate loading rate.

The authors of Appendix E.10 suggest that rapid stimulation of strongly reducing (i.e., methanogenic) conditions is one way to expedite an evaluation of microbial sufficiency. If a complete dechlorination pathway is not observed after inducing strongly methanogenic conditions for several months, then it is likely that the requisite microbial species are not present.

In contrast, the Aerojet Facility application illustrated in Appendix E.9 is an example of where bioaugmentation in a carefully controlled reaction zone was successful in stimulating complete anaerobic dechlorination without inducing methanogenic conditions or creating undesirable levels of dissolved metals or other fermentation products. The bioaugmentation culture was capable of complete dechlorination of TCE to ethene without the need to cultivate methanogenic conditions.

D.7.3 Microbial Sufficiency

Microbial sufficiency is likely the most difficult condition to assess during the site selection process. Multiple conditions may cause an accumulation of intermediate dechlorination products (e.g., *cis*-DCE, VC, or 1,2-dichloroethane [DCA]). Examples of incomplete or slow dechlorination of *cis*-DCE or VC include the Point Mugu IRP Site 24 (Section D.3.1.2) and Seal Beach IRP Site 40 (Section D.3.1.3).

No singular analysis, including determining the presence or absence of *Dehalococcoides*, can be used to confirm the lack of a sufficient microbial population. For example, *Dehalococcoides* was detected at the Atlas 10 site in a well that had elevated levels of TOC and metabolic acids. Yet the presence of this species, combined with the presence of reducing conditions and elevated organic carbon levels, did not result in significant degradation of TCE or evidence of complete dechlorination. Quantitative, real-time polymerase chain-reaction (PCR) analysis may provide estimates of the quantity or concentration of *Dehalococcoides* in a sample. However, it is not well known what concentrations are required to effect efficient and complete anaerobic dechlorination. Furthermore, molecular screening methods cannot currently identify the strains of *Dehalococcoides* that may be present. Isolation of different strains in the laboratory indicates they have different dechlorinating capacities.

In addition to the absence of appropriate dechlorinating microorganisms, insufficient substrate loading, failure to achieve sufficient reducing conditions, inhibition due to high levels of native electron acceptors (e.g., sulfate), inhibition due to preferential degradation of more highly-chlorinated compounds in the contaminant mixture, and kinetic disparity all may contribute to accumulation of intermediate dechlorination products. At many sites, a relatively high percentage of the CAH mass may be present in the form of DNAPL or sorbed to the aquifer matrix. Constant dissolution of CAH mass into the groundwater reaction zone may occur. In these cases, *cis*-DCE and VC may be constantly produced by dechlorination of

the parent compounds (PCE and TCE). However, unless the rate of dechlorination of *cis*-DCE and VC is greater than the rate of dechlorination of PCE and TCE, *cis*-DCE and VC will accumulate or persist until the mass of PCE and TCE is depleted. Because *cis*-DCE and VC are typically dechlorinated more slowly than PCE and TCE, this rate disparity should be anticipated.

Given the current state-of-the-practice, it is important to evaluate all potential causes of the accumulation of intermediate dechlorination products and of incomplete degradation pathways before considering the need for bioaugmentation. While bioaugmentation should not be a default remedy for poorly designed or poorly implemented biostimulation applications, there are sites that will benefit from its application. Bioaugmentation has been successfully applied at several sites, and is a potential option for sites with an absence, or insufficient quantities or activity, of appropriate dechlorinating microorganisms.

Application of enhanced anaerobic bioremediation using bioaugmentation at the Aerojet Facility in California and the Bachman Road site in Michigan benefited from bioaugmentation. It should be noted that these projects were specifically designed to demonstrate bioaugmentation using small-scale recirculation systems. Application of a biostimulation-only test typically uses a much different design and allows for a much longer acclimation period. Nonetheless, bioaugmentation was required to meet the project-specific goals of achieving complete dechlorination within a specified time period at these sites.

D.7.4 Regulatory Considerations and Secondary Water Quality

The use of enhanced anaerobic bioremediation was readily accepted by the regulatory agencies associated with the case studies reviewed. Strict adherence to secondary water quality parameters appears to have been enforced only for the Aerojet Facility bioaugmentation application in California (Section D.6.1). There is often a concern that the substrates used should not cause further degradation of water quality, but all of the substrates reviewed do not appear to contain any constituents that would limit their application. Degradation of secondary water quality may occur within the reactive zone as a result of biological activity and creation of highly reducing conditions. In most cases, these effects do not appear to extend a significant distance (perhaps a few tens to a couple hundred feet) from the treatment area. These effects are also thought to be temporal in nature, with groundwater returning to natural conditions after remediation is complete and the substrates are depleted. However, there are insufficient data available to substantiate this claim. The practitioner of enhanced bioremediation should be aware that secondary water quality may be impacted, and be prepared to monitor for appropriate analytes.

D.8 REFERENCES

Appendix E.1 – Case Study of Enhanced Bioremediation of a DNAPL Source Area: Four Years of Data from Test Area North, INEEL

Appendix E.2 – Enhanced Reductive Dechlorination of a PCE Plume using Molasses at a Former Dry Cleaning Site in Wisconsin

Appendix E.4 – Use of Hydrogen Release Compound (HRC®) to Remediate a Chlorinated Solvent Plume in Fisherville, Massachusetts

Appendix E.5 – HRC® Pilot Test at Portland, Oregon Dry Cleaner Site

Appendix E.6 – Enhanced Anaerobic Biodegradation of Trichloroethene Using Edible Oil Substrate (EOS™) in a Permeable Reactive Barrier

Appendix E.7 – Pilot-Scale Mulch Biowall, Building 301, Offutt AFB, Nebraska

Appendix E.9 – Rapid and Complete Treatment of Trichloroethene via Bioaugmentation in an Active Biobarrier

Appendix E.10 – Comparison of Field Sites Undergoing Enhanced In Situ Bioremediation using Aqueous Electron Donors

Appendix E.11 – Enhanced Reductive Dechlorination of CAHs using Soluble Carbohydrates - A Summary of Data from 50 Sites

Appendix E.12 – Hydrogen Release Compound (HRC®): A Review of Published Papers and Case Histories, 1999-2003

Appendix E.13 – Bioaugmentation to Enhance Anaerobic Bioremediation of Chlorinated Solvents in Groundwater: Technology Overview and Design Criteria

ARCADIS Geraghty and Miller, Inc. (ARCADIS). 2003. *Final Report: In-situ Substrate Addition to Create Reactive Zones for Treatment of Chlorinated Aliphatic Hydrocarbons, Hanscom Air Force Base.* Prepared for the Air Force Center for Environmental Excellence and the Environmental Security Technology Certification Program. April 4.

Aziz, C.E., M. Schipper, M.M. Hampton, J. Hansen, and P. Cork. 2003. Full-Scale Mulch Biowall Treatment of a Chlorinated Solvent Plume (abstract). Presented at the *Seventh International Symposium of In Situ and On-Site Bioremediation,* Orlando, Florida, June 2-5, 2003.

Bradley, P.M., F.H. Chapelle., and D.R. Lovely. 1998. Humic Acids as Electron Acceptors for Anaerobic Microbial Oxidation of Vinyl Chloride and Dichloroethene. *Applied Environmental Microbiology*, Vol. 64:3102-3105.

Britto. R., J. Stedman, and M.W. Perlmutter. 2002. Post Remedial Geochemical Activity at a Enhanced Reductive Dechlorination Site (abstract). Poster presented at the *Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds Monterey, California,* May 20-23, 2002.

Casey, C.C., J. Reed, R. Britto, J. Stedman, B. Henry, and T. Wiedemeier. 2002. Field-Scale Evaluation of Soybean Oil and Dissolved Substrates for In-Situ Bioremediation (poster presentation). Poster presented at the *Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds Monterey, California,* May 20-23, 2002.

CH2M Hill Constructors, Inc (CH2M Hill). 2001. *Technical Memorandum, HRC Pilot Study Evaluation at SWMU 138 - Boat Ramp Naval Air Station Dallas, TX.* Revision 01.

EnSafe, Inc. 2002. Personnel communication. Selected data from field testing of a pilot-scale anaerobic-aerobic sequential bioremediation system at Naval Support Activity Mid-South, Tennessee. Response to site survey questionnaire by Michael Perlmutter and John Stedman. October 1.

French, J., A. Rossi, T. Kirk, D. Blackwelder, K. Sorenson, B. Rahm, L. Alvarez-Cohen, S. Le, M. Pound, and P. Tamashiro. 2003. Phased In situ Biostimulation/Bioaugmentation Pilot Testing in a Coastal Aquifer. *Proceedings of the Seventh International Symposium of In Situ and On-Site Bioremediation,* Orlando, Florida, June 2003. Paper A-22. Battelle Press, Columbus, Ohio.

GeoSyntec. 2002. *Pilot Test for In Situ Bioremediation of Perchlorate & Trichloroethene in Groundwater Using an Active Biobarrier.* Prepared for Aerojet, Rancho Cordova, California. Aerojet Document Control No. SR10112086. Final Report, June 2002.

Granade, S., D.P. Leigh, and C.D. Johnson. 2003. Chlorinated Solvent Bioremediation: 3 Case Studies. *Proceedings of the Seventh International Symposium of In Situ and On-Site Bioremediation,* Orlando, Florida, June 2003. Paper A-13. Battelle Press, Columbus, Ohio.

Groundwater Services Incorporated. 2001. *Final Report Mulch Biowall and Surface Amendment Pilot Test, Site Building 301, Offutt AFB, Nebraska*. Prepared for the Technology Transfer Division of the Air Force Center for Environmental Excellence. June 18.

Haas, P., J. Gonzales, P. Cork, and B. Henry. 2003. Remedial Performance of Organic Mulch Biowalls at Two Geochemically Distinct Sites. *Proceedings of the 2003 AFCEE Technology Transfer Workshop, San Antonio, Texas*. Air Force Center for Environmental Excellence, Brooks City-Base, Texas. February.

Henry, B.M., T. Hartfelder, M. Goodspeed, J.R. Gonzales, P.E. Haas, and D. Oakley. 2003. Permeable Mulch Biowall for Bioremediation of Chlorinated Solvents. *Proceedings of the Seventh International Symposium of In Situ and On-Site Bioremediation,* Orlando, Florida, June 2003. Paper K-03. Battelle Press, Columbus, Ohio.

Johnson, C.D., R.S. Skeen, M.G. Butcher, D.P. Leigh, L.A. Bienkowski, S. Granade, B. Harre, and T. Margrave. 1999. Accelerated In Situ Bioremediation of Chlorinated Ethenes in Groundwater with High Sulfate Concentrations. In: *Engineered Approaches for In Situ Bioremediation of Chlorinated Solvent Contamination*. A Leeson and B.C. Alleman (eds.). Battelle Press, Columbus, Ohio. pp. 165-170.

Kennedy, L.G., and J. Everett. 2003. *Aqueous and Mineral Intrinsic Bioremediation Analyses (AMIBA) of the Pine Bark Mulch Permeable Barrier at Altus Air Force Base SMU-7 (OU-1).* Draft Report prepared for the Air Force Center for Environmental Excellence, Brooks City-Base, Texas. November.

Lee, M.D., M.T Lieberman, W. Beckwith, R.C. Borden, J. Everett, L. Kennedy, and J.R. Gonzales. 2003. Pilots to Enhance Trichloroethene Reductive Dechlorination and Ferrous Sulfide Abiotic Transformation. *Proceedings of the Seventh International Symposium of In Situ and On-Site Bioremediation,* Orlando, Florida, June 2003. Paper K-14. Battelle Press, Columbus, Ohio.

Lee, M.D., and M.T. Lieberman. 2002. *Low Cost Emplacement of Insoluble Organic Substrate for Enhanced In Situ Reductive Dechlorination, Altus Air Force Base*. Status report, April 2002 to June 2002. Prepared for the Air Force Center for Environmental Excellence, Contract No. F41624-99-C-8033. May 30, 2002.

Leigh, D.P., C.D. Johnson, R.S. Skeen, M.G. Butcher, L.A. Bienkowski, S. Granade. 2000. Enhanced Anaerobic In Situ Bioremediation of Chloroethenes at NAS Point Mugu. *Proceedings of the Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California*. Vol. C2(4): 229-235.

Lendvay, J.M., F.E. Loffler, M.E. Dollhopf, B. Fathepure, M. Gebhard, R. Heine, R. Hickey, C.L. Major, Jr., E. Petrovskis, J. Shi, J.M. Tiedje, P. Adriaens. 2003 (*in press*). Bioreactive Barriers: A Comparison of Bioaugmentation and Biostimulation for Chlorinated Solvent Remediation. Paper submitted to *Environmental Science & Technology.*

Lendvay, J.M., M.J. Barcelona, G. Daniels, M.E. Dollhopf, B. Fathepure, M. Gebhard, R. Heine, R. Hickey, F.E. Loffler, C.L. Major, Jr., E. Petrovskis, J. Shi, J.M. Tiedje, P. Adriaens. 2001a. Plume control using bioaugmentation with halorespiring microorganisms. *Groundwater Quality 3rd International Conference, Sheffield, UK.*

Lendvay, J.M., P. Adriaens, M. Barcelona, C.L. Major, J. Tiedje, M. Dollhopf, F. Loeffler, B. Fathepur, E. Petrovskis, M. Gebhard, G. Daniels, R. Hickey, R. Heine, and J. Shi. 2001b. Preventing Contaminant Discharge to Surface Waters: Plume Control with Bioaugmentation. *Proceedings of the Sixth International In-Situ and On-Site Bioremediation Symposium, San Diego, California*. Vol. 6(8):19-26.

Maierle, M.S., and J.L. Cota. 2001. Complete PCE Degradation and Site Closure Using Enhanced Reductive Dechlorination. *Proceedings of the Sixth International In-Situ and On-Site Bioremediation Symposium*, Vol. 6(7):149-156.

Martin, J.P., K.S. Sorenson, and L.N. Peterson. 2001. Favoring Efficient In Situ Dechlorination through Amendment Injection Strategy. *Proceedings of the Sixth International In-Situ and On Site Bioremediation Symposium, San Diego, California*, Vol. 6(7):265-272.

Murray, W., M. Dooley, and S. Koenigsberg. 2001. Enhanced Bioremediation of Chlorinated Solvents. *Proceedings of the Sixth International In-Situ and On-Site Bioremediation Symposium, San Diego, California*, Vol. 6(7):197-204.

Naval Weapons Station Seal Beach. 2002. *In Situ Enhanced Bioremediation Pilot Test Report, IR Site 40, NAVWPNSTA Seal Beach*. Storyboard Meeting Presentation. April 25, 2002.

Parsons. 2004. *Final Project Completion Report for a Field Feasibility Test for In Situ Bioremediation of Chlorinated Solvents Via Vegetable Oil Injection at Site SS015, Travis Air Force Base, California*. Prepared for the Air Force Center for Environmental Excellence, Brooks City-Base, Texas. July.

Parsons. 2002. *Final Phase II Field Feasibility Test for In Situ Bioremediation of Chlorinated Solvents Via Vegetable Oil Injection at Hanger K Area, Cape Canaveral Air Force Station, Florida.* Prepared for the Air Force Center for Environmental Excellence, San Antonio, Texas. March.

Sandefur, C.A., K. Parrett, and K.A. Lapus. 2002. Bioremediation of a PCE Plume at a Dry Cleaner Site. In: A.R. Gavaskar and A.S.C. Chen (Eds.), *Proceedings of the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds,* Monterey, California, May 2002. Paper 2B-52. Battelle Press, Columbus, Ohio.

Sorenson, K.S. 2003. Enhanced Bioremediation for Treatment of Chlorinated Solvent Residual Source Areas. In: S.M. Henry and S.D. Warner (Eds.), *Chlorinated Solvent and DNAPL Remediation: Innovative Strategies for Cleanup.* ACS Symposium Series. Vol. 837: 119-131.

United States Army Corps of Engineers (USACE). 2003. *Project Management Plan for Former Lincoln AFB, Atlas Site 10, York, NE.* March 13.

TABLE D.1 SUMMARY OF CASE STUDY RESULTS

Site Name and Location	Observed Dechlorination Pathway	Effectiveness of Substrate Distribution	Site Status	Criteria for Success	Factors Contributing to Success or Failure	References
Soluble Substrates – Lactate						
Test Area North, Idaho National Energy and Environmental Laboratory, Idaho	TCE to *cis*-DCE to VC to ethene.	Substrate distribution was effectively attained with complete dechlorination observed up to 150 feet downgradient of the injection well.	System is being expanded with larger volume injections and a second injection well.	Demonstrate that enhanced bioremediation of a DNAPL source area can reduce CAHs to below MCLs. Demonstrate that enhanced bioremediation can replace groundwater extraction as a final remedy for the source area.	MCLs were achieved for TCE and DCE at the base of the aquifer. Concentrations of VC are generally less than 15 µg/L (October 2003) and VC has not accumulated. The pilot-scale application resulted in a ROD amendment (approved September 2001) to switch from groundwater pump and treat to enhanced *in situ* bioremediation.	Appendix E.1; Sorenson, 2003; Martin et al., 2001
IRP Site 24, Naval Base Ventura County, Point Mugu, California	TCE to *cis*-DCE to VC to ethene (degradation of VC to ethene relatively slow).	Substrate distribution was effective within the recirculation cell. Substrate was allowed to be depleted after last injection at day 57.	Pilot test monitored for 3 years, then converted to aerobic cometabolism. Full-scale sequential anaerobic/aerobic treatment is planned.	Pilot test to demonstrate that lactate addition can remediate TCE and DCE to ethene. Sequential dechlorination of TCE to DCE to VC and ethene were observed over approximately 36 months.	Dechlorination of VC was deemed too slow to meet remedial objectives, and the system converted to aerobic cometabolism. VC was effectively oxidized by cometabolic processes. It is unknown whether injection of additional substrate after 57 days would have stimulated faster dechlorination of VC.	Granade, et al., 2003 Leigh et al., 2000; Johnson et al., 1999; Appendix E.10
IRP Site 40, Naval Weapons Station Seal Beach, California	PCE and TCE to *cis*-DCE. After 8 months of biostimulation, transformation of DCE to VC and ethene was not observed.	Substrate was added by direct injection into a single well, and substrate distribution was effective within the treatment zone.	Biostimulation pilot test conducted for 8 months. A bioaugmentation pilot test is planned.	Pilot test to demonstrate that lactate addition can stimulate complete dechlorination of PCE and TCE to ethene.	Strongly reducing conditions were induced (sulfate reduction and methanogenesis), but after 8 months of lactate injection, dechlorination of PCE and TCE stalled at *cis*-DCE. *Dehalococcoides* species were not detected by molecular screening. A bioaugmentation pilot test is planned.	French, et al., 2003; Naval Weapons Station Seal Beach, 2002; Appendix E.10

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TABLE D.1 SUMMARY OF CASE STUDY RESULTS (CONTINUED)

Site Name and Location	Observed Dechlorination Pathway	Effectiveness of Substrate Distribution	Site Status	Criteria for Success	Factors Contributing to Success or Failure	References
Soluble Substrates – Molasses						
Washington Square Mall, Wisconsin	PCE to TCE to *cis*-DCE to VC to ethene/ethane.	Substrate was effectively distributed using an initial injection into 182 direct-push points and during follow-on injections using a limited array of 12 injection points.	Conditional closure with MNA.	Achieve closure under Wisconsin Department of Natural Resources (WDNR) flexible closure rules (negotiated preventative action limits).	Closure with MNA was granted by WDNR 30 months after the initial application. Closure was based on complete dechlorination of PCE to ethene and ethane. Residual levels of DCE and VC were sufficiently low to allow for closure with MNA.	Appendix E.2; Maierle and Cota, 2001
Hanscom AFB, Massachusetts	TCE to *cis*-DCE to VC to ethene.	Substrate distribution was limited initially; frequent injections were required to extend the zone of influence. Locations without adequate substrate showed only a relatively small decrease in TCE concentration, and incomplete dechlorination.	Pilot test was completed in September 2002. Future remedial actions are under evaluation.	Performance objectives included 1) for concentrations of total CAHs >200 µg/L - reduce concentration by 80% within 1 year.; 2) for concentrations of total CAHs from 50-200 µg/L - reduce concentration by 75% within 1 year; 3) for concentrations of total CAHs <50 ppb - reduce concentration by 50% within 1 year. Show that TCE can be completely dechlorinated to ethene.	Concentrations of total CAHs above 200 µg/L were reduced by over 80% at most locations. Concentrations of total CAHs between 50 and 200 µg/L were reduced by at least 75% at only a few locations. Complete dechlorination to ethene was observed at locations that received a continuous and adequate supply of substrate.	ARCADIS, 2003
Other Soluble Substrates (Fructose/Acetate)						
Anaerobic/Aerobic Pilot Study, Naval Support Activity Mid-South, Tennessee	TCE to DCE during pilot test. Further dechlorination of DCE to VC observed 6 months after a final pulsed injection of acetate.	The system was effective in recirculating groundwater, although distribution of substrate appeared to be limited as levels of TOC averaged less than 20 mg/L throughout the treatment zone.	System is inactive pending regulatory approval of a final remedy for the site. MNA with hotspot reduction using direct injection of sodium acetate is proposed.	Demonstrate complete dechlorination of PCE and TCE to DCE and VC in an anaerobic reactive zone, with complete oxidation of residual DCE and VC in a natural downgradient aerobic treatment zone.	Concentrations of PCE and TCE were reduced by only 40 to 60 percent during active recirculation, with accumulation of *cis*-DCE. The rate and efficiency of substrate loading may not have been adequate to completely degrade TCE, or for dechlorination of DCE to VC and ethene.	EnSafe, 2002; Britto et al., 2002; Casey et al., 2002

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TABLE D.1 SUMMARY OF CASE STUDY RESULTS (CONTINUED)

Site Name and Location	Observed Dechlorination Pathway	Effectiveness of Substrate Distribution	Site Status	Criteria for Success	Factors Contributing to Success or Failure	References
Slow-Release Substrates – HRC®						
Fisherville Mill Site, Massachusetts	TCE to *cis*-DCE to VC to ethene.	Substrate was effectively distributed throughout the treatment zone. Levels of TOC and metabolic acids remained elevated for a period of at least 27 months.	Results of the pilot test are being evaluated for consideration of a full-scale application.	Demonstrate that application of HRC® can remediate PCE, TCE, and dechlorination products to ethene. Demonstrate that application of HRC® can control the migration of CAHs to potential downgradient receptors.	Concentrations of TCE declined by 88 to 98%, coupled with dechlorination of *cis*-DCE and VC to ethene (completed dechlorination pathway). Elevated levels of TOC and metabolic acids persisted for at least 27 months. However, concentrations of TCE, *cis*-DCE, and VC within and downgradient of the treatment zone remain above MCLs.	Appendix E.4; Murray et al., 2001
Springdale Cleaners, Portland, Oregon	TCE to *cis*-DCE to VC to ethene.	Substrate was effectively distributed throughout the treatment zone. Levels of TOC and metabolic acids remained elevated for a period of approximately 18 months (HRC®) to 40 months (HRC-X™).	Pilot testing complete. Full-scale application has been approved and is pending funding for the project.	Demonstrate that application of HRC® can remediate PCE, TCE, and daughter products to ethene. Obtained regulatory approval for full-scale application.	Sequential increases and decreases of TCE, *cis*-DCE, VC, and ethene were observed over approximately 18 months, indicating that PCE was completely dechlorinated to ethene. Concentrations of dechlorination products were observed to rebound after approximately 18 months, indicating that the HRC® substrate had been depleted. Regulatory approval for a full-scale application was granted based upon results of the pilot scale application.	Appendix E.5; Sandefur et al., 2002
Atlas 10 Site, Nebraska	Limited dechlorination of TCE to cis-DCE only.	Elevated levels of TOC and organic acids were only observed in the immediate vicinity of the injection array during the 11-month test.	Site conditions were determined to be unsuitable for enhanced *in situ* bioremediation. Groundwater extraction and *ex situ* treatment using aeration are being considered.	Remediate chloroethenes in groundwater to federal drinking water MCLs.	Significant reductions in TCE concentration did not occur. Only limited production of *cis*-DCE was observed in one location towards the end of the pilot test. Development of a stable and highly reducing reaction zone was not achieved. It is believed that high levels of DO (> 6 mg/L), nitrate (> 10 mg/L), and sulfate (>20 mg/L), combined with a high rate of groundwater flow, inhibited formation of adequate reducing conditions.	USACE, 2003

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TABLE D.1 SUMMARY OF CASE STUDY RESULTS (CONTINUED)

Site Name and Location	Observed Dechlorination Pathway	Effectiveness of Substrate Distribution	Site Status	Criteria for Success	Factors Contributing to Success or Failure	References
Slow-Release Substrates – HRC® (continued)						
SWMU 138, Naval Air Station Dallas, Texas	Limited dechlorination of PCE and TCE to *cis*-DCE. No significant concentration trends could be discerned in 9 months of monitoring.	Elevated levels of substrate were not observed in any monitoring locations during the pilot test. Distribution appears to have been limited by low permeability soils and a lack of advective groundwater flow.	Pilot test completed, full-scale expansion was not recommended.	Reduce concentrations of PCE and TCE to below MCLs within the pilot test treatment zone.	A lack of groundwater flow and mixing is thought to have resulted in insufficient distribution of substrate. Hydrogeologic conditions at this site do not appear to be suitable for use of slow-release substrates. The contractor determined that HRC® did not offer an effective means to provide short term (less than 1 year) degradation of chloroethenes.	CH2M Hill, 2001
Slow-Release Substrates – Vegetable Oil						
Site SS-17, Altus AFB, Oklahoma	TCE to *cis*-DCE to VC to ethene.	Injection of the vegetable oil emulsion was readily accomplished, but uniform distribution was not observed at all locations downgradient of the treatment zone. Distribution appears to be controlled by secondary permeability.	Pilot test results continue to be evaluated.	Determine if a vegetable oil-in-water emulsion can be effectively distributed and a reaction zone maintained in a barrier configuration. Determine whether complete dechlorination of TCE to ethene can be stimulated.	Complete dechlorination of TCE to VC and ethene was observed. Concentrations of TCE were reduced to below detection along the centerline of the treatment zone. Although a complete dechlorination pathway has been observed, concentrations of *cis*-DCE and VC remain elevated above MCLs after 13 months of monitoring. Substrate distribution appears to be controlled by aquifer heterogeneity and secondary permeability.	Appendix E.6; Lee et al., 2003; Lee and Lieberman, 2002
SS-015, Travis AFB, California	TCE to *cis*-DCE to VC to ethene.	Elevated levels of TOC were observed throughout the entire treatment area, indicating that substrate was distributed effectively.	The site is approved for MNA and is being redeveloped by the Air Force. Monitoring will continue after construction.	Demonstration project to determine if vegetable oil is capable of complete dechlorination of TCE to ethene in a high sulfate, low permeability environment, and to evaluate different vegetable oil injection scenarios.	Complete dechlorination of PCE and TCE to ethene was observed, with over 80 percent reduction in total CAH mass site wide. Although MCLs for PCE, TCE, *cis*-DCE and VC were attained at multiple locations, this site may require several years to attain uniform reduction in CAHs to MCLs due to low permeability and slow desorption of CAH mass.	Parsons, 2004

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TABLE D.1 SUMMARY OF CASE STUDY RESULTS (CONTINUED)

Site Name and Location	Observed Dechlorination Pathway	Effectiveness of Substrate Distribution	Site Status	Criteria for Success	Factors Contributing to Success or Failure	References
Slow-Release Substrates – Vegetable Oil (continued)						
Hangar K, Cape Canaveral Air Force Station, Florida	PCE to TCE to *cis*-DCE to VC to ethene.	Based on monitoring data for TOC and metabolic acids, substrate was effectively distributed throughout the source zone area.	Field test complete. Continue to monitor source area for long-term depletion of substrate.	Demonstration project to determine if vegetable oil is capable of complete dechlorination of PCE and TCE to ethene in a source area application. Results were evaluated to determine if federal drinking water MCLs could be achieved.	Sequential increases and decreases of TCE, *cis*-DCE, VC, and ethene were observed over approximately 18 months. Approximately 40 months after injection, MCLs were attained for PCE, TCE, and *cis*-DCE at all locations within the treatment zone. MCLs for VC were also attained at multiple monitoring locations.	Parsons, 2002
Solid Substrates – Mulch Biowalls						
Building 301, Offutt AFB, Nebraska	TCE to *cis*-DCE, with limited evidence of *cis*-DCE to VC and ethene/ethane.	Bark mulch was uniformly distributed within the biowall using a continuous one-pass trencher.	Full-scale biowall system was installed in 2001, with continued long-term monitoring.	Determine effectiveness of a mulch biowall to completely dechlorinate TCE to ethene. Develop operations and cost data to support a full-scale application.	TCE was dechlorinated to *cis*-DCE, with limited evidence of dechlorination to VC to ethene. VC did not accumulate. A full-scale biowall was installed in 2001 based on results of the pilot test. The extent of dechlorination may be limited by the residence time of CAHs in the reactive zone.	Appendix E.7; Groundwater Services Inc., 2001; Aziz et al., 2003; Haas et al., 2003
OU1, Altus AFB, Oklahoma	Primarily TCE to *cis*-DCE. Low levels (< 10 ug/L) of VC were observed. Abiotic degradation of TCE and *cis*-DCE is also occurring.	Bark mulch was uniformly distributed within the biowall using a continuous trencher. Elevated levels of TOC (greater than 20 mg/L) were observed up to 30 feet downgradient of the biowall.	Long-term process monitoring continues. Full-scale biowalls are currently being installed for other CAH plumes at Altus AFB.	Intercept and contain a shallow TCE and DCE groundwater plume in order to prevent surface water discharge and off-base migration. Evaluate the technology for application at other Air Force sites.	Concentrations of TCE within and immediately downgradient of the biowall have been reduced to below the MCL for TCE. TCE was dechlorinated to *cis*-DCE, with no accumulation of VC. The average reduction in concentrations of total CAHs is 86 percent, although elevated concentration of *cis*-DCE persist.	Henry et al., 2003; Haas et al., 2003

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TABLE D.1 SUMMARY OF CASE STUDY RESULTS (CONCLUDED)

Site Name and Location	Observed Dechlorination Pathway	Effectiveness of Substrate Distribution	Site Status	Criteria for Success	Factors Contributing to Success or Failure	References
Bioaugmentation						
Aerojet Facility, California	PCE to TCE to *cis*-DCE to VC to ethene. Also perchlorate to chlorate to chlorite to chloride.	The recirculation system was effective at distributing substrate within the pilot test area. Distribution of the bioaugmentation culture was limited to the immediate area of injection.	Additional pilot testing was recommended. System is being evaluated for full-scale application.	Determine if TCE and perchlorate can be completely degraded through substrate addition. Because microcosm studies indicated that a suitable microbial population may not be present, demonstrate the effectiveness of bioaugmentation to increase dechlorination rates. Assess feasibility of full-scale application.	Substrate addition alone was incapable of completely dechlorinating TCE to ethene within the 90-day test period. PCE was successfully dechlorinated to ethene with the addition of the bioaugmentation culture. The pilot test was also successful in degrading perchlorate to innocuous end products.	Appendix E.9; GeoSyntec, 2002
Bachman Road Residential Wells Site, Michigan	PCE to TCE to *cis*-DCE to VC to ethene	Recirculation systems were effective at distributing substrate and bioaugmentation culture.	Full-scale application under consideration.	Determine relative effectiveness of bioaugmentation (lactate plus bioaugmentation culture) versus biostimulation alone (lactate only) to increase dechlorination rates of PCE and TCE to ethene.	A 92 percent conversion of chloroethenes to ethene was observed in the bioaugmentation test plot within 43 days of inoculation. Dechlorination of PCE and TCE to ethene was observed in the biostimulation test plot after approximately 3 months from the start of lactate injection. The lag time for dechlorination of *cis*-DCE to VC and ethene, and overall dechlorination rates were enhanced by addition of the bioaugmentation culture.	Lendvay et al., 2001a; Lendvay et al., 2001b; Lendvay et al., 2003; Appendices E.10 and E.13

APPENDIX E

ILLUSTRATIVE CASE STUDIES OF SUBSTRATE FIELD APPLICATIONS

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APPENDIX E.1 – CASE STUDY OF ENHANCED BIOREMEDIATION OF A DNAPL SOURCE AREA: FOUR YEARS OF DATA FROM TEST AREA NORTH, INEEL

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CASE STUDY OF ENHANCED BIOREMEDIATION OF A DNAPL SOURCE AREA: FOUR YEARS OF DATA FROM TEST AREA NORTH, INEEL

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Historical waste disposal activities have resulted in a nearly 3-km-long trichloroethene (TCE) plume in groundwater at the Test Area North Facility of the Idaho National Engineering and Environmental Laboratory (INEEL), located in southeast Idaho. Facility process waste consisting of liquid organic, inorganic, and low-level radioactive waste along with sanitary sewage wastewater was injected directly into the Snake River Plain Aquifer via injection well TSF-05 from the mid-1950s to 1972. The plume emanating from the TSF-05 injection well was discovered during routine monitoring operations in the early 1990s. Characterization efforts during the 1990s indicated TCE concentrations in the source area up to 100 mg/L, suggestive of TCE in the dense non-aqueous liquid (DNAPL) phase. In fact, sludge bailed from the former injection well contained as much as 3% TCE by weight. In the formation this sludge acts as a residual source of contamination to passing groundwater.

For purposes of treatment, the 3-km plume was divided into three zones based on TCE concentration: the source area, medial zone, and the distal zone (Figure 1). The source area was defined as an approximately 500-ft long area containing the residual source and associated high aqueous phase concentrations. The 1995 Record of Decision (ROD) selected pump and treat as the default remediation technology for all three plume zones. Continuous pump and treat operations in the source area began in November 1996 and operated for approximately 18 months. However, while the interim pump and treat system was in place, the ROD provided the provision to evaluate innovative technologies for their potential to out-perform pump and treat for source area remediation. The focus of this case study describes the evaluation, official selection, and implementation of enhanced bioremediation for source area remediation at Test Area North.

SITE BACKGROUND

The geology at Test Area North consists of massive basalt flow layers with highly permeable interflow zones. In the area of the TCE plume, the saturated thickness is from 200 to 400 ft below ground surface (bgs). Due to the heterogeneous nature of the geology, the transmissivity values are highly variable. In general the aquifer at Test Area North is highly transmissive, with transmissivity estimates ranging from 1,000 ft^2/day to as high as 500,000 ft^2/day (INEEL, 1996; INEEL, 1998). The groundwater flow direction at Test Area North is eastward near the TSF-05 injection well, and then southward. This explains the general shape of the TCE plume as shown in Figure 1. The groundwater flow velocity in the area of the contaminant plume is approximately 0.5 ft/day (Sorenson, 2000).

TECHNOLOGY EVALUATION PROCESS

An interim pump and treat remedy was installed in the source area and continuous operations began in November 1996. Wells TSF-05 and Test Area North-25 were used interchangeably as extraction wells for these operations (Figure 2, treatment cell plan view). While the interim remedy was operating, an evaluation of five innovative technologies was initiated. The technologies evaluated for their potential to enhance or replace pump and treat included in situ chemical oxidation, abiotic degradation using zero valent iron, hydraulic isolation using grouting, monitored natural attenuation (MNA), and enhanced in situ bioremediation via anaerobic reductive dechlorination. The evaluation process consisted of three steps of increasing effort in which technologies that had encouraging results were retained for further evaluation. The steps in this process included a literature review/paper study, laboratory studies, and a field study. zero valent iron, grouting, and MNA were eliminated from further consideration for source area remediation in the paper study step, and laboratory studies for both in situ chemical oxidation and anaerobic reductive dechlorination were conducted. The lab studies for both technologies yielded positive results; however, a decision was made to proceed with a field test of anaerobic reductive dechlorination prior to in situ chemical oxidation because some intrinsic anaerobic reductive dechlorination was already occurring and it was recognized that the testing of in situ chemical oxidation would be detrimental to the native microbial population and thus impact a subsequent field test of anaerobic reductive dechlorination.

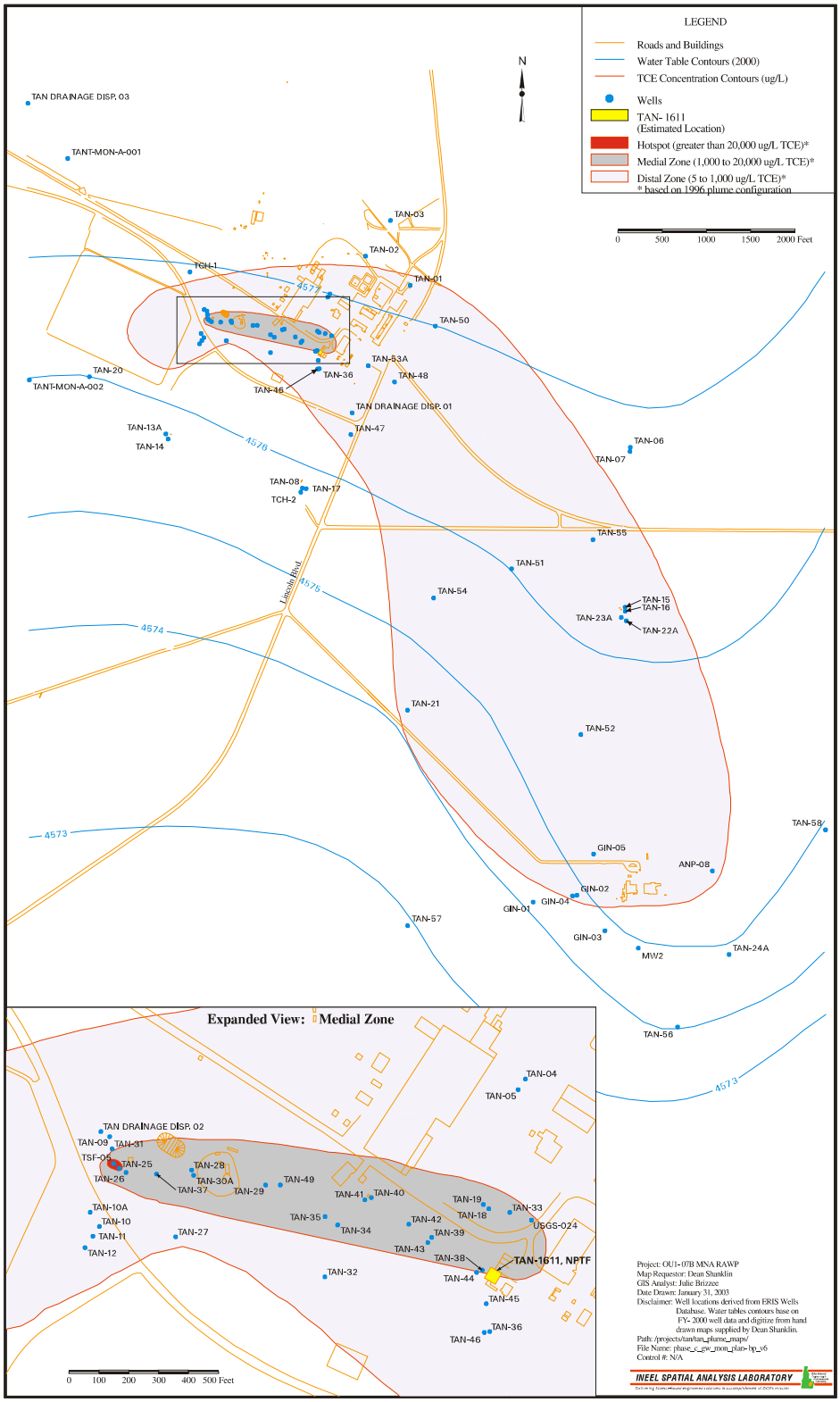

Figure 1. Approximate extent of the Test Area North TCE plume, showing the hot spot immediately adjacent to the TSF-05 injection well (TCE concentrations exceeding 20,000 µg/L), the medial zone (concentrations between 1,000 and 20,000 µg/L), and the distal zone (TCE concentrations between 5 and 1,000 µg/L).

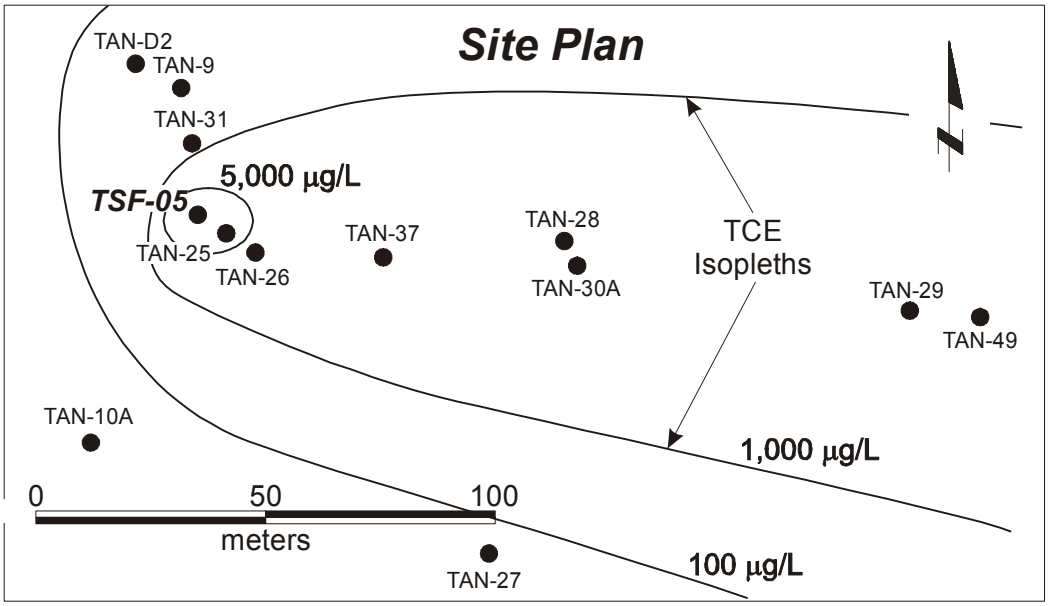

Figure 2. Test Area North TCE source area.

The evidence used to select the anaerobic reductive dechlorination technology for further testing included the results of historical monitoring and laboratory treatability studies. Historical data indicated that the conditions within the source area were favorable for anaerobic reductive dechlorination, and that anaerobic reductive dechlorination was already occurring to some extent. Specifically, the redox conditions were nitrate- to sulfate-reducing as indicated by the low dissolved oxygen and nitrate concentrations and depleted sulfate in some source area wells. Also, direct evidence that anaerobic reductive dechlorination was already occurring included the presence of significant concentrations of cis-DCE in groundwater and vinyl chloride (VC) in the air stripper off-gas. Laboratory microcosm experiments conducted with basalt and groundwater from the Test Area North source area indicated that complete anaerobic reductive dechlorination was stimulated using lactate as an electron donor. Based on these results, a field test of enhanced anaerobic reductive dechlorination via lactate injection was performed.

OVERVIEW OF BIOREMEDIATION ACTIVITIES, OBJECTIVES, AND METHODS

Enhanced bioremediation field activities were initiated at Test Area North in 1998 and have been ongoing since that time. Over the years, the remedy has gone through several phases, each with specific objectives based on the results of the previous phase and the overall progress toward the ultimate goal of achieving the Remedial Action Objectives. Following is a summary of these phases and the objectives and operations for each.

Field activities began in November 1998 with the first phase of activities, the Field Evaluation. The overall objective of the Field Evaluation was to determine whether or not anaerobic reductive dechlorination of TCE could be enhanced through the addition of an electron donor (lactate). The general operations consisted of baseline sampling and a conservative tracer test, followed by lactate injection in TSF-05 and groundwater monitoring. Following this 9-month testing phase, activities shifted toward optimization of the bioremediation operations. This began in October 1999 with Pre-Design Phase I activities, which consisted of no lactate injections and continued groundwater monitoring throughout the treatment cell. The objective of Pre-Design Phase I was to see how the system would respond to the absence of regular lactate injections, utilizing only the residual electron donor (mainly propionate) already present from the Field Evaluation injections. Based on the positive results of Pre-Design Phase I, it was the objective of Pre-Design Phase II to maintain the favorable conditions for efficient anaerobic reductive dechlorination observed during Pre-Design Phase I and to determine the best injection strategy for later

phases. Pre-Design Phase II, beginning in February 2000, consisted of the injection of relatively large volumes of electron donor relatively infrequently (every 8 weeks) compared to the smaller volume, more frequent injections (weekly) used during the Field Evaluation. The implementation of the next phase of activities, Pre-Design Operations, was initiated in May 2001. In general, the objectives of Pre-Design Operations were to continue the optimization of the bioremediation remedy through continued operations (lactate injection and groundwater monitoring) and experimentation with various injection strategies.

For all phases of bioremediation operations, an approximately 500-ft long treatment cell, consisting of an electron donor injection well (Well TSF-05) and 14 monitoring points throughout the treatment cell (including multiple depths in two wells), was used (Figure 2). From November 1998 to December 2000, a continuously operating extraction well (Well TAN-29) was used for hydraulic containment during the initial phases of testing. Monitoring analytes included electron donor parameters (lactate, propionate, acetate, butyrate, and chemical oxygen demand [COD]), biological activity parameters and nutrients (alkalinity, carbon dioxide, pH, ammonia, and phosphate), redox parameters (dissolved oxygen, nitrate, ferrous iron, sulfate, methane, and oxidation-reduction potential), and anaerobic reductive dechlorination parameters (TCE, 1,1-DCE, trans-1,2-DCE, cis-1,2-DCE, VC, ethene, and ethane). In addition, water quality parameters (temperature and specific conductance) and radionuclide contaminants were monitored. A combination of field- and fixed-based laboratories was used in order to obtain real-time data and to maximize the cost-effectiveness of the monitoring program. The parameters carbon dioxide and nitrate were dropped from the monitoring program in October 2000, and phosphate and ammonia analysis was reduced to semi-annual. The monitoring frequency used from November 1998 until October 1999 was biweekly (twice per month), and was subsequently reduced to monthly.

RESULTS

Results are presented chronologically beginning with the Field Evaluation and continuing through the Pre-Design Operations phase. For each phase, three types of data are discussed: electron donors, redox conditions, and anaerobic reductive dechlorination parameters. Three wells are used to represent conditions at different locations within the treatment cell: TAN-25 is located 25 ft from the TSF-05 injection well within the area that contains secondary source sludge material, TAN-26 is located at the base of the aquifer, and TAN-37A is located approximately 140 ft downgradient of well TSF-05, outside the secondary source, in the shallower portion of the aquifer (Figure 2). The results from these wells are summarized in Table 1.

Field Evaluation Phase. As stated above, the goal of the Field Evaluation was to determine if anaerobic reductive dechlorination of TCE could be stimulated through the injection of an electron donor (lactate). Following four weeks of baseline data collection and a conservative tracer test, lactate injection began at Well TSF-05 in January 1999. In general the concentration was reduced over time from 60% to 3% (percent by weight sodium lactate), while the injection frequency during this phase ranged from once to twice per week. Lactate was injected via a single injection well, TSF-05. The general lactate injection strategy for each phase is presented in Table 2.

During the Field Evaluation, significant concentrations of lactate fermentation products propionate (>2,000 mg/L) and acetate (>1,000 mg/L) were observed in source area wells, both shallow (TAN-25) and deep (TAN-26) in the immediate source area. Also, based on an observed molar ratio of propionate:acetate between 1.4 and 2.0 in well TAN-25, it was concluded that the dominant lactate fermentation pathway was via propionate, which produces propionate and acetate in a molar ratio of 2:1. The observed values less than 2:1 were a result of subsequent propionate fermentation to acetate. The significance of the lactate → propionate and acetate pathway here was that propionate has been shown in laboratory studies by other researchers (Smatlak et al., 1996, Fennell and Gossett, 1998) to be a high quality electron donor for anaerobic reductive dechlorination. Lactate can also be oxidized directly to acetate and H_2; however, the observed propionate:acetate ratio suggested that the propionate pathway was the dominant lactate utilization pathway during the Field Evaluation. Electron donor was not observed

after the first month during the Field Evaluation at TAN-37A, 140 ft downgradient of the injection well (Table 1).

Table 1. Summary of results over time for wells TAN-25, TAN-26, and TAN-37A.

Parameters	Units	Baseline	End of the Field Evaluation	End of Pre-Design Phase I	End of Pre-Design Phase II	Pre-Design Operations (Oct 2002)
TAN-25						
Propionate	mg/L	ND	2,442	26	1820	2,543
Acetate	mg/L	ND	1,089	85	1117	2,495
Molar P:A	-	NA	1.8:1	0.3:1	1.3:1	0.8:1
Sulfate	mg/L	37	1	2	0	0
Methane	µg/L	8.2	11,899	8,324	7,060	8,681
TCE	µg/L	386	215	29	<10	<10
Cis-DCE	µg/L	106	651	14	37	227
VC	µg/L	ND	138	22	14	15
Ethene	µg/L	NM?	146	160	55	26
TAN-26						
Propionate	mg/L	ND	1,639	530	<5	49
Acetate	mg/L	ND	1,838	917	15	24
Molar P:A	-	NA	0.7:1	0.5:1	0.1:1	1.7:1
Sulfate	mg/L	29	4	8	0	0
Methane	µg/L	<10	8,189	3,990	22,902	23,038
TCE	µg/L	185	<10	<10	<10	<10
Cis-DCE	µg/L	<10	494	<10	<10	<10
VC	µg/L	ND	290	<10	14	<10
Ethene	µg/L	ND	136	105	70	21
TAN-37A						
Propionate	mg/L	ND	ND	ND	ND	<5
Acetate	mg/L	ND	ND	ND	<5	<5
Molar P:A	-	NA	NA	NA	NA	NA
Sulfate	mg/L	36	34	1	30	26
Methane	µg/L	<10	49	211	19,693	17,496
TCE	µg/L	258	667	210	146	229
Cis-DCE	µg/L	29	350	110	34	34
VC	µg/L	ND	<10	23	14	15
Ethene	µg/L	ND	<10	68	5.2	7.1

Table 2. Summary of Lactate Injections.

Phase	Date	Concentration (wt %)	Volume	Frequency
Field Evaluation	Jan 99 – Sep 99	60%→ 3%	330 → 6,600 gal	Once or twice per week
Pre-Design Phase I	Oct 99 – Jan 00	NA	NA	NA
Pre-Design Phase II	Feb 00 – Apr 01	3-6%	13,000 gal	Every 8 weeks
Pre-Design Operations	May 01 – Oct 02		13,000 →52,000 gal	~ Every 8 weeks

The impact of electron donor injections on in situ redox conditions was significant in the treatment cell. Where electron donor was distributed (TAN-25 and TAN-26), sulfate reduction and methanogenesis were observed (Table 1). Sulfate reduction was seen in both wells TAN-25 and TAN-26 almost immediately following the initiation of lactate injection. Significant methanogenesis was observed within 4 and 5 months in wells TAN-25 and TAN-26, respectively. At the end of the Field Evaluation complete depletion of sulfate and significant methane production was observed in both TAN-25 and TAN-26, indicating methanogenic conditions in these areas. As stated above, electron donor was not distributed to well TAN-37A located 140 ft downgradient. Because of this, redox conditions remained too oxidizing for anaerobic reductive dechlorination at this location. Some evidence of sulfate reduction was observed at TAN-37A; however, this activity was not sustained and sulfate concentrations were similar to baseline levels at the end of the Field Evaluation.

The relative concentrations of TCE, cis-DCE, VC, and ethene are used to assess the extent of anaerobic reductive dechlorination in the treatment area. Anaerobic reductive dechlorination of TCE to cis-DCE was observed in TAN-25 within 5 weeks of the first lactate injection. Further, the concentrations of cis-DCE produced were greater than the original TCE present on a molar basis, indicating that lactate injection was also having the effect of enhancing the partitioning of TCE contaminants from the residual source to the aqueous phase. This was also seen in well TAN-26, which is located at the base of the aquifer. This process is referred to as Bioavailability Enhancement Technology™, patent pending, or B.E.T.™. In well TAN-26, the dramatic increase in TCE immediately following lactate injection was a result of enhanced partitioning of TCE to the lactate followed by density driven flow of the lactate solution to the base of the aquifer (Sorenson, 2002). Once in the aqueous phase, anaerobic reductive dechlorination occurred rapidly, as indicated by the stoichiometric conversion of TCE to cis-DCE at TAN-26 within approximately 9 weeks. Complete anaerobic reductive dechlorination of TCE and cis-DCE to ethene was observed in TAN-25 within about 4 months, and within 5 months in TAN-26. In both wells, the production of ethene corresponded exactly with the onset of methanogenic conditions (Sorenson et al., 2000).

In well TAN-37A, TCE and cis-DCE were present prior to the onset of lactate injection (Table 1). During the Field Evaluation, TCE was observed to fluctuate. At the same time cis-DCE increased, likely as a result of transport from upgradient rather than anaerobic reductive dechlorination occurring at that location. The absence of anaerobic reductive dechlorination at the TAN-37A location was expected during this time because electron donor was not distributed to this portion of the treatment cell, and redox conditions were observed to be unfavorable for anaerobic reductive dechlorination at this location.

The rapid, complete anaerobic reductive dechlorination of TCE and accelerated source degradation observed during the Field Evaluation supported the selection of enhanced bioremediation to replace pump and treat for source area remediation. A ROD Amendment to that effect was signed in September 2001.

Pre-Design Phase I. Following 9 months of lactate injection during the Field Evaluation, concentrations of electron donor up to 4,500 mg/L were present in the aquifer treatment cell. At this time, lactate injections were discontinued for 5 months in order to determine the persistence of electron donor and anaerobic reductive dechlorination reactions within the treatment cell and to evaluate the efficiency of anaerobic reductive dechlorination reactions in the prolonged presence of acetate, propionate, and butyrate (fermentation products) as electron donors. The dominant electron donor at the end of the Field Evaluation was propionate. Following the last lactate injection in September 1999, propionate decreased rapidly as it was utilized by the microbial community. After 5 months, propionate and acetate concentrations were very low, 26 and 85 mg/L, respectively (Table 1). At the TAN-26 location, the electron donors were observed to steadily dissipate following the initial transport of high concentrations of lactate to the base of the aquifer. At the end of Pre-Design Phase I, significant concentrations of propionate and acetate were still present, 530 and 917 mg/L, respectively (Table 1). No electron donor was observed at the TAN-37A location (Table 1).

The redox conditions at the end of the Field Evaluation were methanogenic in both wells TAN-25 and TAN-26 (Table 1). Methane concentrations in both wells had steadily increased since the middle of the Field Evaluation and were observed to stabilize or decrease slightly during Pre-Design Phase I. In well TAN-37A, significant sulfate reduction was observed during Pre-Design Phase I (Table 1), indicating that conditions upgradient of this location were becoming more favorable for anaerobic reductive dechlorination.

In terms of anaerobic reductive dechlorination, the efficiency of the anaerobic reductive dechlorination reactions increased dramatically in well TAN-25 during Pre-Design Phase I as indicated by the decrease in TCE and cis-DCE and the significant increase in ethene (Table 1). At well TAN-26, complete conversion of the cis-DCE to ethene was observed during Pre-Design Phase I (Table 1). At the TAN-37A location, both TCE and cis-DCE decreased while ethene was observed in significant concentrations for the first time. This was direct evidence that anaerobic reductive dechlorination was occurring immediately upgradient of TAN-37A in the downgradient portion of the secondary source zone.

The results from Pre-Design Phase I indicated that conditions in the treatment cell became much more favorable for anaerobic reductive dechlorination in the absence of regular lactate injections when the propionate resulting from lactate fermentation was the dominant electron donor (Martin et al., 2001). As stated above, results from laboratory studies have indicated that propionate is a high-quality electron donor because it produces H_2 for anaerobic reductive dechlorination at a threshold below that required for hydrogenotrophic methanogenesis (Smatlak et al., 1996; Fennell et al., 1997). The absence of lactate and high levels of H_2 for hydrogenotrophic methanogenesis may have minimized competition during this time between dechlorinators and non-dechlorinating organisms such as homoacetogens and hydrogenotrophic methanogens.

Pre-Design Phase II. Based on the results of Pre-Design Phase I, the goal of Pre-Design Phase II was to maintain the favorable conditions for anaerobic reductive dechlorination observed during Pre-Design Phase I by manipulation of the lactate injection strategy. The general strategy for lactate injection in Pre-Design Phase II was to minimize the time of lactate fermentation while maximizing the time of propionate utilization. In order to do this, a strategy that used relatively large volume injections on an infrequent basis was used. Because lactate fermentation happens so rapidly and propionate fermentation is much slower, delivering the lactate in larger volumes on an infrequent basis supports a large but short-lived burst of lactate fermentation activity. The result of this is a large supply of propionate, which is utilized much more slowly and produces a slow and steady source of hydrogen for anaerobic reductive dechlorination that may be less available to hydrogenotrophic methanogens. The injection strategy for Pre-Design Phase II began as 13,200 gal of a 3% (wt% sodium lactate) solution injected every 8 weeks. After two injections, the concentration was increased to 6% (wt% sodium lactate) while the total volume and frequency of injection remained the same.

The lactate injections of Pre-Design Phase II delivered high concentrations of propionate and acetate to the source area (Table 1). In the source area, each injection produced a spike in propionate concentrations similar in magnitude to the concentrations observed during the Field Evaluation. Following each injection, concentrations of propionate, acetate, and lactate (if present) decreased as the electron donor was utilized. The injection strategy of Pre-Design Phase II did not distribute electron donor to the base of the aquifer as indicated by the results from well TAN-26 (Table 1). Similarly, only trace concentrations of electron donor were observed downgradient at well TAN-37A (Figure 5a).

The redox conditions in the source area during Pre-Design Phase II became increasingly reducing as evidenced by the steady methane concentrations observed at well TAN-25 throughout Pre-Design Phase II (Table 1). This was also true of conditions at the base of the aquifer as seen in the results from well TAN-26. Downgradient at well TAN-37A, the enhanced sulfate reduction observed during Pre-Design Phase I was observed to decrease as sulfate concentrations rebounded (Table 1). This indicated that the size of the biologically active zone decreased relative to that of the secondary source and sulfate was again being transported to the TAN-37A location. However, significant levels of methane were observed at TAN-37A during Pre-Design Phase II (Table 1). This was likely a result of transport of methane from the robust methanogenic community upgradient in the TAN-25 area to the TAN-37A location.

While decreased from the levels observed during Pre-Design Phase I, ethene remained the dominant compound at well TAN-25 (on a molar basis), indicating continued anaerobic reductive dechlorination in the source area. At the base of the aquifer, ethene production persisted through Pre-Design Phase II and then decreased as concentrations of primary contaminants were depleted (Table 1). These results indicated that contamination at the base of the aquifer was largely remediated; concentrations of PCE, TCE, cis-DCE, and VC have been near or below the respective MCL values since January 2000 (Table 1). Downgradient at well TAN-37A, the ethene observed during Pre-Design Phase I steadily decreased at the onset of Pre-Design Phase II. At the same time, the decrease in TCE during Pre-Design Phase I reversed itself during Pre-Design Phase II. Concentrations increased, decreased, and finally stabilized at the end of Pre-Design Phase II. These fluctuations were likely a result in fluctuations in mass transfer and anaerobic reductive dechlorination processes in the source area upgradient of TAN-37A.

Figure 3 shows how TCE concentrations changed by the middle of Pre-Design Phase II. Pre-lactate concentrations were measured following 18 months of pump and treat. The lack of TCE near the injection well during Pre-Design Phase II shows the efficiency of TCE degradation in the aqueous phase, but does not indicate that the residual source was completely removed. In general, the results from Pre-Design Phase II indicated that conditions within the source area as indicated by the results from TAN-25 remained conducive to anaerobic reductive dechlorination and ethene was the dominant compound at this location. The base of the aquifer was largely remediated. However, the size of the biologically active zone shrunk relative to the secondary source area as indicated by the results from TAN-37A. This is likely due to the inability of the Pre-Design Phase II injection strategy to distribute electron donor to the downgradient portion of the source area using a single injection well. For this reason, additional modifications to the injection strategy were tested during the subsequent phase, Pre-Design Operations, which began in May 2001. It should also be noted that trans-DCE began to increase during the Field Evaluation and Pre-Design Phase I and persisted throughout Pre-Design Phase II. The source of the trans-DCE might be trace contamination of the original TCE used, or might be biogenic. It was apparent that trans-DCE was more resistant to anaerobic reductive dechlorination, as it persisted in the zone of anaerobic reductive dechlorination.

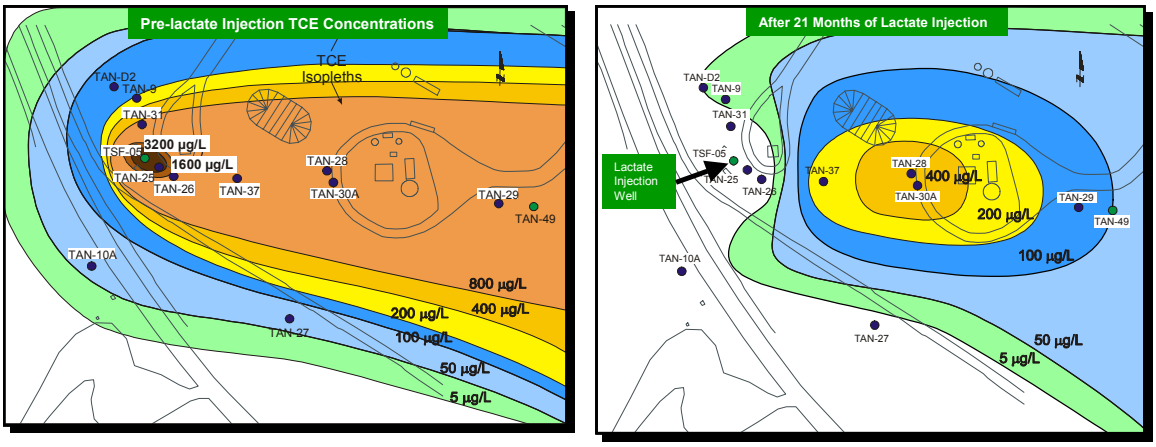

Figure 3. TCE degradation after 21 months of lactate addition as compared to 18 months of pump and treat.

Pre-Design Operations. The goal of the Pre-Design Operations phase was to continue to experiment with the injection strategy to achieve the desired distribution of electron donor within the treatment cell and maximize anaerobic reductive dechlorination efficiency. If the required electron donor distribution could not be achieved with a single injection well, at least one additional injection well would be installed for long-term operations. Variations in the injection strategy during Pre-Design Operations included increasing the volume to as much as 4 times that used during previous injections (Table 2), and varying the concentration in the range of 3 to 6%.

While these changes increased the longevity of electron donor in the system to about 3 months, the change in distribution was marginal. In general, the larger volume injections produced high concentrations of lactate in the source area, as well as propionate and acetate. While relatively high levels were produced, the injection strategies of Pre-Design Operations still did not impact the downgradient portion of the source as evidenced by the lack of electron donor at well TAN-37A (Table 1). The large injection volumes create the potential for dilution of contaminant concentrations, which has been accounted for by analyzing 1) molar contaminant concentrations over time, 2) tritium concentrations, which serve as an internal tracer for the residual source material, and 3) stable carbon isotope ratios of TCE and degradation products (INEEL, 2000; INEEL, 2002; Song et al., 2002).

Redox conditions throughout the treatment cell remained largely stable throughout Pre-Design Operations. Strongly methanogenic conditions were still present in both TAN-25 and TAN-26 (Table 1). It should be noted that complete dechlorination has not been prevented by the methanogenic activity in this area even after several years. Analysis of methanogen DNA suggests that this is probably due to the fact that acetoclastic methanogenesis is far more important than hydrogenotrophic methanogenesis under the field conditions at Test Area North (Wood et al., 2002). The predominance of acetoclastic methanogens may be due in part to the low temperature of groundwater (12^{o}C) in the field (Kotsyurbenko et al., 2001), which is quite different from many of the laboratory studies that have evaluated competition between dechlorinators and methanogens (Fennell and Gossett, 1998). Also, conditions in TAN-37A remained similar to those observed during Pre-Design Operations (Table 1). Sulfate persisted indicating that conditions in the downgradient portion of the source still remained too oxidizing for anaerobic reductive dechlorination. The transport of methane from methanogenic areas in the source area upgradient continued.

In general, anaerobic reductive dechlorination activity remained consistent with those trends observed during Pre-Design Phase II. Ethene in well TAN-25, while lower than that seen in Pre-Design Phase I, was still the dominant compound present at this location up until April 2002. In April 2002, cis-DCE was observed to increase to levels greater than those of ethene on a molar basis. While some fluctuation was

observed, concentrations were between 100 and 200 μg/L. Concentrations of contaminants in the base of the aquifer remained below MCL values (Table 1). Downgradient, TCE remained the dominant compound and was observed to fluctuate in response to each lactate injection (Table 1).

COST INFORMATION

The cost components for this case study were the same as for any bioremediation technology. The cost components include:

- Design and Work Plans
- Electron donor (sodium lactate in this case)
- Delivery system
- Sampling and Analysis
- Reporting

For most of these components, cost is site-specific because it is driven by site requirements and is not a function of the bioremediation technology. For example, the sampling frequency, analyte list, and quality assurance requirements are driven by site requirements and should be the same for all bioremediation technologies for a given site. The delivery system depends on depth to water, aquifer permeability, and other site-specific issues. The sodium lactate cost, however, is not site-specific (except for freight). For large volumes, the cost is generally about $0.75/lb.

Following are some site-specific unit costs to give an idea of the cost to remediate a deep, fractured rock residual source area, with a treatment volume of about 4 million ft^3.

- The average cost to install a monitoring well to 400 ft below ground surface by air rotary drilling at the site is about $100,000. This cost goes up if drilling is in a zone suspected to have significant radiological contamination. In this case, only one new well was installed during the first four years of operation.
- A heated cargo container plumbed with potable water was installed to serve as an injection trailer at a cost of between $60,000 and $100,000.
- The operating costs include lactate injection and sampling and analysis. Lactate is injected approximately once every 2 months. The labor cost per injection is approximately $1800, and the lactate cost at $0.75/lb for the 48 drums (about 29,000 lb) of lactate required to treat 4 million ft^3 is $21,800. Assuming eight injections a year for conservatism, and based on the treatment volume and the lactate injection operating costs (one-time capital costs are not included), this yields a cost of $1.27/$yd^3$/yr for the source zone treatment. The fact that the lactate can treat this volume using a single injection well is a significant advantage because of the cost to install new wells. Ultimately a second injection well will be added to increase the treatment zone to about 6 million ft^3 to completely encompass the residual source area. Some economies of scale will be realized as the lactate volume is not expected to increase proportionally to the treatment volume.
- A complete sampling round at 13 monitoring locations with analysis for a full suite of bioremediation parameters costs approximately $12,000 per round. The analytes include: lactate, volatile fatty acids, chemical oxygen demand, redox-sensitive parameters, tritium, VOCs, dissolved gases, and purge parameters

CONCLUSIONS

This case study presents the results of the large-scale field testing and implementation of enhanced bioremediation for cleanup of a TCE DNAPL source area in a deep fractured rock aquifer at the Test Area North facility of the INEEL. Field activities began with the initial testing phase in November 1998 and have continued with optimization activities since that time. The following provides a summary of the major conclusions drawn from the four years of data presented above.

- Complete anaerobic reductive dechlorination of TCE to ethene was supported through the addition of sodium lactate.
- Complete anaerobic reductive dechlorination was observed to distances of almost 150 ft from the lactate injection well.
- The injection of high concentrations of sodium lactate resulted in enhanced mass removal of TCE from the source material.
- These data supported the decision by the regulators to select enhanced bioremediation to replace pump and treat for source area cleanup. A ROD Amendment to this effect was signed in September 2001.
- Data indicate that enhanced bioremediation has resulted in the restoration of the lower half of the aquifer; all contaminants are currently below MCL values.
- Attempts were made to distribute electron donor throughout the entire treatment cell by manipulating the lactate injection strategy using a single injection well; however, an approach that uses two injection wells and smaller lactate volumes per well to achieve the desired distribution is currently being implemented.

In addition to demonstrating the ability to replace pump and treat with in situ bioremediation via lactate injection for chlorinated solvent source area cleanups, this case study also provides some valuable lessons learned that can be used in developing operating strategies at other sites:

- The use of aqueous electron donors such as sodium lactate allows distribution to be achieved over large areas with a small number of injection wells.
- Competition between dechlorinators and non-dechlorinators can be managed to some extent through the lactate injection strategy. Furthermore competition between dechlorinators and methanogens may not be as significant an issue in the field as in the laboratory, in part due to lower field temperatures.
- Injection strategies that include large volumes at lower frequencies may be superior to smaller volumes at higher frequencies.
- Injecting large volumes of sodium lactate at the beginning of operations allows strongly reducing conditions and complete dechlorination to ethene to be achieved very quickly; optimizing the injection strategy can be performed once complete dechlorination is accomplished.
- The use of high sodium lactate concentrations not only contributes to achieving the necessary reducing conditions quickly, it also contributes to accelerating residual source degradation.

REFERENCES

Fennell, D. E., Gossett, J. M., and Zinder, S. H. 1997. Comparison of butyric acid, ethanol, lactic acid, and propionic acid as hydrogen donors for the reductive dechlorination of tetrachloroethene: *Environ. Sci. Technol.*, Vol. 31(3):918-26.

Fennell, D. E., and J.M. Gossett. 1998. Modeling the Production of and Competition for Hydrogen in a Dechlorinating Culture. *Environ. Sci. Technol*., v. 32, no. 16, pp. 2450-2460.

Kotsyurbenko, O. R., M. V. Glagolev, A. N. Nozhevikova, and R. Conrad. 2001. "Competition between Homoacetogenic Bacteria and Methanogenic Archaea for Hydrogen at Low Temperature." *FEMS Microbiol. Ecol.* 38: 153-159.

Martin, J.P., Sorenson, K.S., and L.N. Peterson. 2001. Favoring Efficient In Situ Dechlorination through Amendment Injection Strategy. *Proceedings of the Sixth International In-Situ and On Site Bioremediation Symposium, San Diego, California*, No. 6(7), p. 265-272. Smatlak, C.R., Gossett, J.M., and Zinder, S.H. 1996. Comparative Kinetics of Hydrogen Utilization for Reductive Dechlorination of Tetrachloroethene and Methanogenesis in an Anaerobic Enrichment Culture. Environ. Sci. Technol., 30(9):2850-2858.

Song, D. L., M. E. Conrad, K. S. Sorenson, and L. Alvarez-Cohen. 2002. "Stable Carbon Isotope Fractionation During Enhanced In-Situ Bioremediation of Trichloroethene." *Environ. Sci. Technol*., 36(10):2262-2268.

Sorenson, K.S. 2000. Biodegradation of TCE Improved with Lactate Injection in Deep, Fractured Rock. *Ground Water Currents*, No. 38, December 2000.

Wood, T., D. Cummings, and K. S. Sorenson. 2002. *Characterizing Microbial Population Shifts of a TCE-Dechlorinating Consortium with the Addition of Alternate Electron Donors.* General Meeting of the American Society for Microbiology, Salt Lake City, UT, May.

APPENDIX E.2 – ENHANCED REDUCTIVE DECHLORINATION OF A PCE PLUME USING MOLASSES AT A FORMER DRY CLEANING SITE IN WISCONSIN

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Enhanced Reductive Dechlorination of a PCE Plume using Molasses at a Former Dry Cleaning Site in Wisconsin

Angie Frizzell, Chris C. Lutes, and Jennine Cota Trask (ARCADIS G&M, Inc.)

Case Study Outline

ARCADIS utilized its patented in-situ enhanced reductive dechlorination process to treat groundwater impacted with tetrachloroethene (PCE) and its daughter products at a former dry cleaning facility in Wisconsin. The soluble, food-grade carbohydrate substrate used to facilitate dechlorination at the site was a dilute molasses solution (Lenzo, 2000; Suthersan, 2000). The use of a full-scale enhanced reductive dechlorination approach at this site resulted in complete PCE degradation and conversion to innocuous end products in less than a 2-year time frame. Regulatory closure was achieved in less than 2.5 years after initiating treatment (Maierle and Cota, 2001).

This case study provides details on the project objectives; site history; hydrogeology and contaminant distribution; site selection criteria; technology design, operation, performance and cost; and a summary of findings and lessons learned from the Wisconsin site.

Remedial/Performance Objectives

The site property was slated for imminent redevelopment at the time that the groundwater plume was discovered. Thus, the remedial objective was to achieve fast closure under Wisconsin Department of Natural Resources flexible closure rules. The multi-part closure criteria include requirements for source control and remediation of groundwater contamination to levels that will not exceed preventive action limits (PALs) beyond property boundaries. PALs for site contaminants are 10 to 100 times lower than Federal Maximum Contaminant Levels (MCLs), at 0.5 µg/L for PCE and trichloroethene (TCE), 7.0 µg/L for cis-1,2-dichloroethene (DCE), and 0.02 µg/L for vinyl chloride (VC). The site was actively remediated to levels above the PALs, after which natural attenuation of residual dissolved contaminants was allowed under the Wisconsin Department of Natural Resources's Voluntary Party Liability Exemption Insurance program.

Site History/Source of Contamination

Prior to 1998, the dry cleaning facility was part of a dilapidated retail center (Figure 1). This property was redeveloped concurrently with the site remediation activities and is currently a successful retail shopping center (Figure 2). Contamination at the site resulted from historic releases of PCE, a common dry cleaning solvent, from a dry cleaning facility that operated within the former retail center. The site location is shown in Figure 3. Initial dissolved PCE concentrations in groundwater were approximately 1,500 to 4,000 µg/L.

A soil remediation program was completed in August 1998 and involved the excavation and off-site disposal of approximately 3,125 tons (3 million kg) of PCE-impacted soils. The excavation extended to the water table, which was even with the top of a saturated sand seam at a depth of

approximately 14 ft (4.3 m) below initial grade. In order to maintain suitable conditions for backfilling and to achieve additional contaminant mass removal, provisions were included for the temporary recovery of groundwater from the base of the excavation. Approximately 88,000

Figure 1: Former dry cleaning facility (pre-redevelopment).

Figure 2: Post-treatment, redeveloped property

gal (335,000 L) of water were pumped from the excavation and discharged to the sanitary sewer in August 1998. It is estimated that approximately 25,000 gal (95,000 L) of this volume was attributable to precipitation or surface water run-in that accumulated in the excavation, and the remainder was groundwater recovered from the sand seam that was penetrated by the deep excavation.

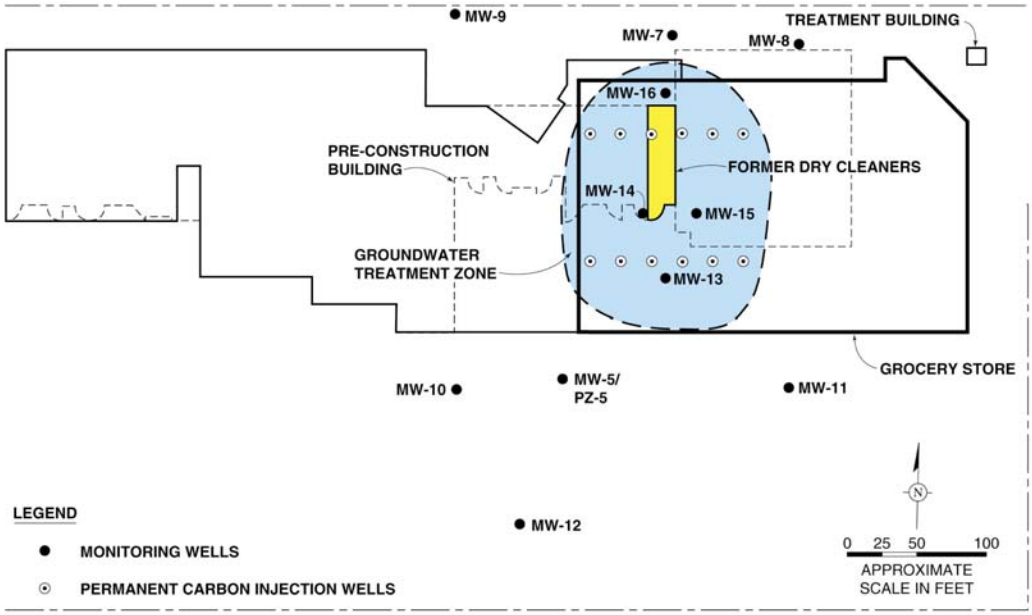

Figure 3: Site Layout

Geology/Hydrogeology/Contaminant Distribution

The lateral extent of affected groundwater was approximately 30,000 ft^2 (3,000 m^2) in plan size, extending to a depth of approximately 20 ft (6 m) below grade. Investigation results suggested that the affected groundwater had spread laterally from the source area primarily through a 2 to 5 ft (0.6 to 1.5 m) thick silt and sand seam that is approximately 13 to 18 ft (4 to 5.5 m) below grade. Within this seam, the extent of impacted groundwater was estimated to be 150 ft (45 m) in width by 200 ft (60 m) in length. A representative geologic cross section is presented in Figure 4.

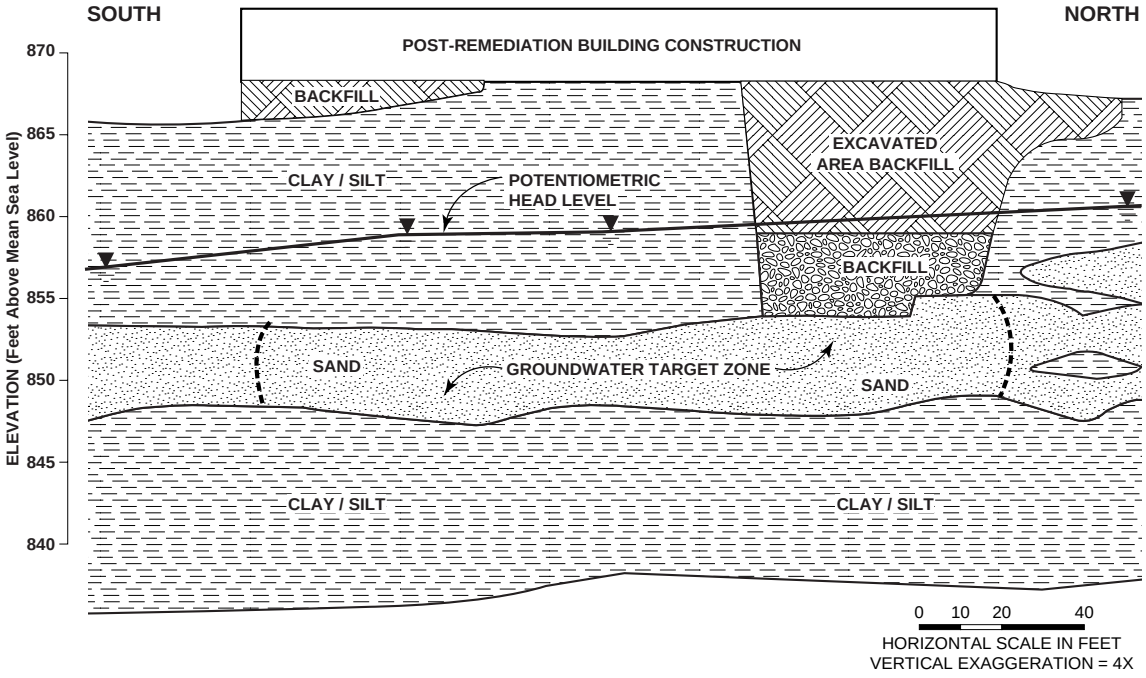

Figure 4: Hydrogeologic cross-section.

Site Selection Criteria

Among the general site selection criteria for enhanced reductive dechlorination technology, as detailed in a recently finalized enhanced reductive dechlorination protocol document (Suthersan, 2002), are the following:

- Site must be at least moderately permeable (K > 0.28 ft/day [10^{-4} cm/s]) and should have a groundwater velocity of 0.08 to 5 ft/day (10^{-5} to 10^{-3} cm/s). Hydraulic conductivity and velocity at the Wisconsin site were approximately 28 ft/day (10^{-2} cm/s) and 0.055 ft/day (1.9 x 10^{-5} cm/s), respectively. Although the velocity was at the low end of the acceptable range, given the small size of the plume and its shallow depth (allowing direct push injections), velocity was judged to be relatively unimportant for reagent distribution.

- Sites should be reasonably well delineated geologically and with regard to contaminant concentration, as the Wisconsin site was.
- The depth of the plume is a factor in determining cost effectiveness, with depths less than 50 ft (15 m) generally being desirable. The depth to the target zone at the Wisconsin site was 13 to 18 ft (4 to 5.5 m).
- Initial pH should be 5-9. The Wisconsin site had a groundwater pH of approximately 7.2 in the target zone before treatment.
- Dense, non-aqueous phase liquid (DNAPL) or sorbed source material is not a barrier to success, but must be carefully considered in locating injection wells and against desired treatment time and goals. Source material was excavated at the Wisconsin site prior to treatment to facilitate rapid closure.
- Aerobic or borderline aerobic/anaerobic starting conditions are preferred. Sites already showing breakdown products are ideal. The Wisconsin site exhibited Type 2 conditions (as defined in U.S. Environmental Protection Agency [U.S. EPA], 1998) prior to treatment, and borderline aerobic/anaerobic geochemistry (dissolved oxygen [DO] <1 mg/L, oxidation-reduction potential [ORP] from –60 to 40 mV). Partial degradation of PCE to TCE was evident before treatment began.

Thus the Wisconsin site met the recommended site selection criteria prior to treatment and was believed to be a good candidate for successful removal of dissolved PCE by enhanced reductive dechlorination.

Technology Description (Design and Operation)

The groundwater remediation process involved the periodic injection of an organic carbon (molasses) solution to enhance the reductive dechlorination of the chlorinated solvents present in site groundwater (i.e., an in-situ bioremediation process). By injecting an organic carbon source, anaerobic and strong reducing conditions were created within the in-situ reaction zone. These conditions created a more suitable environment for the degrading microorganisms to promote both desorption of PCE from the aquifer matrix and enhanced reductive dechlorination (i.e., biodegradation) of the PCE (Payne et al., 2001). Enhanced reductive dechlorination enhances desorption by way of four processes – progressive decreases in organic carbon partitioning coefficient (K_{OC}) values of sequential daughter products, the production of natural biosurfactants by the enhanced microbial population, the production of fermentation products that act as co-solvents, and changes in equilibrium partitioning of contaminants due to the increase in the carbon content of groundwater relative to that of the soil (Suthersan et al., 2002). The dilute molasses solution was injected to create a reactive zone throughout the entire area of impacted groundwater.

The groundwater remediation program was implemented immediately following the source excavation. An initial injection event was conducted in August and September 1998, using 182 temporary Geoprobe® injection points. The Geoprobe® points were advanced in a grid-like pattern across the groundwater target area. The spacing between injection points was approximately 10 ft (3 m). The borings were advanced to intersect the sand seam. Temporary

injection wells installed in the Geoprobe® borings were constructed of 1-in (2.54-cm) diameter polyvinyl chloride (PVC) pipe for the well screen and riser. Bentonite pellets were used to seal the temporary wells.

Edible blackstrap molasses was used for the initial injection. The edible blackstrap molasses is approximately 47% carbohydrates by weight. The other primary constituents of molasses are water, protein and mineral matter (containing chiefly calcium, chloride, magnesium, nitrogen, potassium, sodium and sulfur). Molasses is fully soluble. The organic portion is degradable; analyses of the dilute mixtures used in enhanced reductive dechlorination applications have indicated that metals levels did not exceed MCLs (Lutes, 2003). The molasses solution was mixed in a plastic tank on site using potable water. Approximately 15 to 25 gal (57 to 95 L) of the dilute molasses solution (the dilution ratio was 25 gal [95 L] of water to each gal [3.8 L] of molasses) were injected into each temporary well using a grout pump. Approximately 3,200 gal (12,000 L) of the dilute molasses solution were injected into the temporary injection points over 11 days.

A permanent injection system to be used after the redevelopment of the property was installed concurrently with the initial injection event. Twelve fixed injection wells were installed at the site using conventional hollow-stem auger drilling techniques. The locations of the permanent wells are shown on Figure 3. The fixed injection wells were constructed of 2-in (5-cm) diameter Schedule 40 PVC with 5-ft (1.5 m) screens placed to intersect the sand seam. To facilitate the redevelopment at the site, the injection wells were cut off approximately 6 ft (1.8 m) below ground surface and connected to 1-in (2.54 cm) high-density polyethylene (HDPE) buried conveyance piping.

A network of 1-in (2.54-cm) HDPE conveyance piping was installed below grade between the injection equipment building and the permanent injection wells. Remedial system equipment was housed within a small heated and insulated building. The remedial equipment included a 250-gal (946 L) plastic mix tank, a piping manifold, and a 1/3 horsepower (0.25 kW) rotary gear pump.

After the fixed injection system was installed, four additional injection events were completed at the site. The molasses solution was added to the mix tank and pumped through the manifold to the injection wells at a dilution ratio of 25 gal (95 L) of water to each gal (3.8 L) of molasses. The molasses used for the permanent injection wells was a low-sulfur, cane juice molasses that contained approximately 66% carbohydrates by weight (see previous discussion of other molasses constituents). A total of 3,000 gal (11,300 L) of the molasses solution was injected into the aquifer through the permanent injection wells during four injection events completed over a 6-month period from March 1999 to September 1999.

The quantity of the dilute molasses solution injected into the aquifer and the timing of each event were determined based on changes in biodegradation indicator parameters and the rate of reductive dechlorination determined from groundwater monitoring data. Optimum values for groundwater indicator parameters for the enhanced reductive dechlorination process included

ORP less than –200 mV, total organic carbon (TOC) in the range of 25-100 mg/L, and pH above 5. Due to site redevelopment activities occurring concurrently with the groundwater remediation, post-injection groundwater monitoring did not begin until 6 months after the initial injection event. The site monitoring well network consisted of four monitoring wells within the limits of the plume and eight monitoring wells outside of the plume, as shown in Figure 3.

Technology Performance

The performance of the technology was demonstrated by groundwater monitoring data including initial investigation results, six rounds of monitoring during enhanced reductive dechlorination implementation from February 1999 to April 2000, and four quarterly rounds of rebound monitoring. It is noted that initial sample points installed during the investigation were not retained due to property redevelopment activities, so pre-treatment concentrations indicated in graphics are estimated from the investigation data.

An anaerobic reactive zone produced by the introduction of the carbon source exhibited reduced DO and ORP values (DO of 0.3 mg/L and ORP of -170 to –256 mV), sulfide formation and increasing methane production in treatment-zone wells, indicating that sulfate-reducing or methanogenic conditions had been attained. Typical methane levels during effective treatment were approximately 6,000 to 17,000 μg/L.

Over the 20-month period following completion of soil remediation activities and the initial carbon injection event (August 1998), PCE concentrations within the plume decreased to non-detectable levels (April 2000). As expected, a temporary increase in DCE and VC concentrations occurred in conjunction with the decrease in PCE concentrations. The corresponding build-up of DCE and VC peaked at approximately 6 and 14 months, respectively, after initiating the enhanced reductive dechlorination process. The DCE and VC levels then dropped sharply over the next 6 months. Figures 5 and 6 illustrate the contaminant trends on a mass and molar concentration basis for a monitoring well located within the plume. Within 6 months following the first injection, over 90% of the PCE was degraded to DCE. In addition, within 20 months, over 90% of the PCE in the groundwater plume was degraded to ethene and ethane.

The buildup of the non-toxic, innocuous end products of the reductive dechlorination process (e.g., ethene, ethane, carbon dioxide) indicated that PCE was being completely transformed, and that residual chlorinated aliphatic hydrocarbons (CAHs) were indeed present and required remediation after the excavation/dewatering. The monitoring data collected indicated significant production of ethene and ethane within the groundwater plume. Ethene and ethane concentrations in the four monitoring wells within the plume were approximately one to two orders of magnitude higher than the ethene and ethane levels measured in the monitoring wells located along the fringe of the plume. This was clear evidence that the reductive dechlorination process was going to completion.

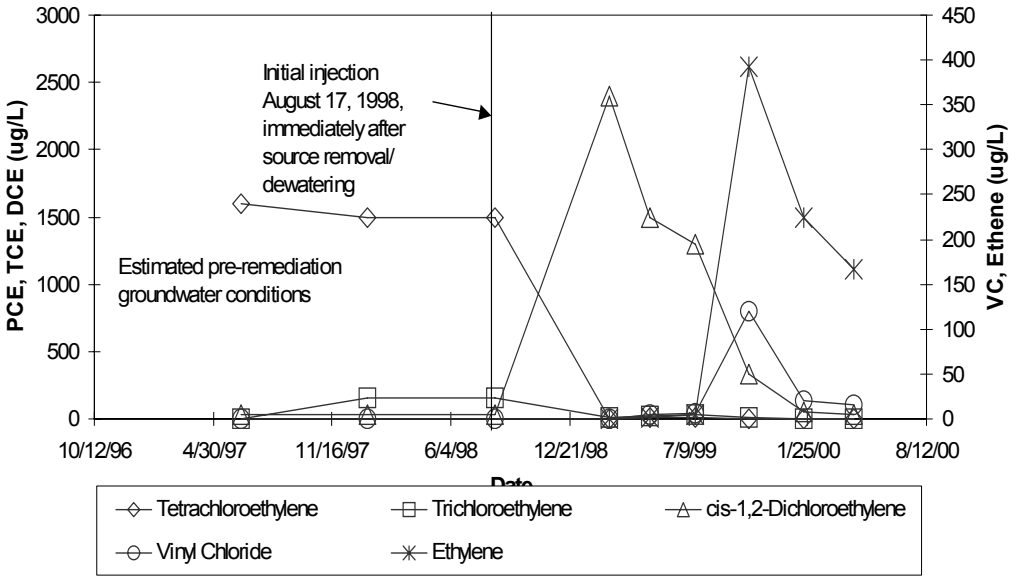

Figure 5: MW-13 contaminant trends.

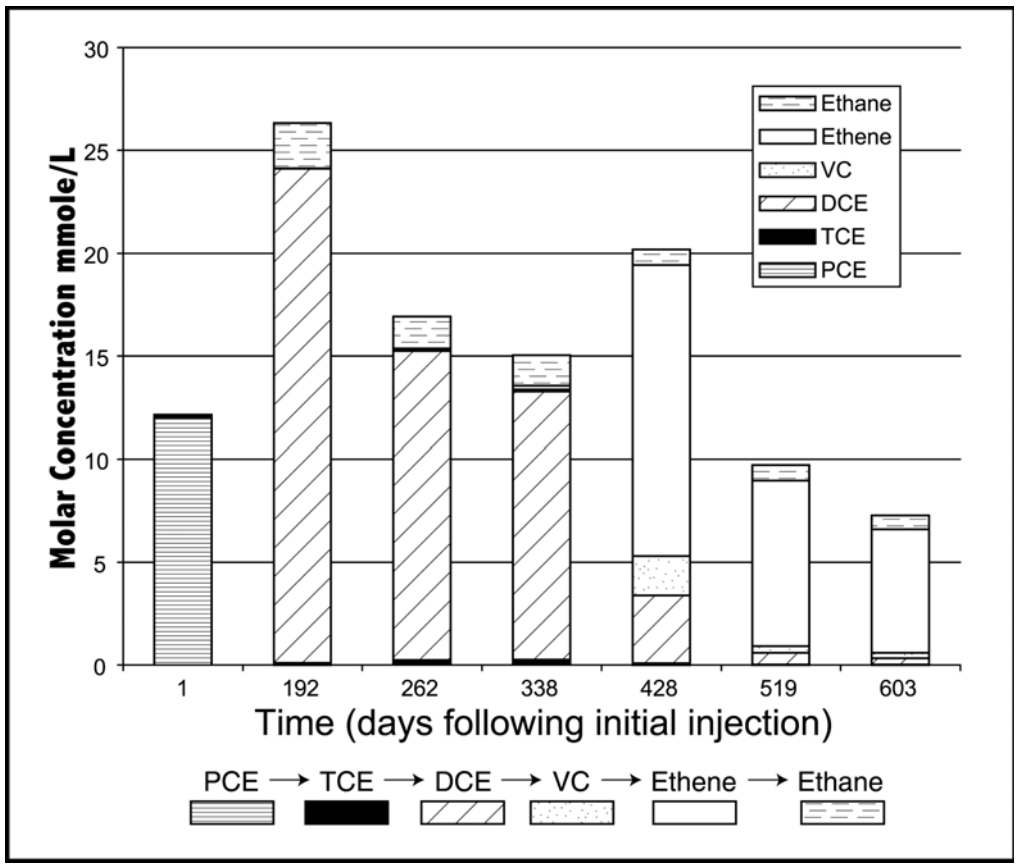

Figure 6: PCE transformation over time at MW-13

Based on the use of first-order degradation kinetics, biodegradation rates for the chlorinated constituents at the site can be determined (U.S. EPA, 1998). Table 1 lists the average site-specific biodegradation rates determined from the collected data for each of the monitoring wells within the groundwater plume. The site-specific biodegradation rates for PCE and TCE are approximately two to eight times higher than average published biodegradation rates under natural (unenhanced) conditions (U.S. EPA, 1998). This demonstrates that the enhanced reductive dechlorination process can greatly accelerate biodegradation rates. Note that the total molasses solution injected was only approximately 2 percent of the total volume of groundwater in the target area, indicating that dilution effects on the observed rates were minimal.

Concentrations of DCE and VC remaining at the completion of the project exceeded PALs, as allowed by the Wisconsin Department of Natural Resources. However, natural attenuation is expected to complete the cleanup at a rate protective of water quality at the downgradient property boundary. Four quarterly rounds of monitoring after April 2000 showed no rebound of CAH levels in the treatment zone.

TABLE 1. Calculated Biodegradation Rates (day^{-1}) for Wisconsin Site

	Monitoring Well			
Compound	**MW-13**	**MW-14**	**MW-15**	**MW-16**
PCE	Not applicable	0.027	Not applicable	0.021
TCE	0.011	0.005	Not applicable	0.023
DCE	0.010	0.004	0.011	0.017
VC	0.015	0.003	0.011	0.018

Technology Cost

Capital costs for application of the enhanced reductive dechlorination technology can vary widely depending on the scale of the project and the injection design (e.g., manual vs. automated, mobile vs. fixed). The delivery system used at Wisconsin site was in the low- to mid-range of relative capital expenditure, making use of both direct push borings and permanent wells for injections. Batch injections were carried out from an on-site mixing and feed system (chosen for ease of use in the cold climate) and subsurface piping. Estimated capital costs for the system were $380,000. Annual operation and maintenance costs for the site, including quarterly groundwater monitoring at 12 wells and minimal upkeep on the delivery system, were estimated at $85,000. The estimated total cost of enhanced reductive dechlorination at the site, independent of source removal, was estimated at $550,000. Itemized project costs are provided in Table 2. The project was contracted under ARCADIS' Guaranteed Remediation Program® (GRiP®).

TABLE 2. ENHANCED REDUCTIVE DECHLORINATION TECHNOLOGY COSTS AT WISCONSIN SITE

Element	Cost ($)
Capital Cost	
Site Characterization, Engineering Design, Planning and Preparation	$100,000
Mobilization/Demobilization/Per Diem	$10,000
Site Labor - Direct Push Points - Injection and Monitoring Wells - Injection System	 $20,000 $15,000 $110,000
Equipment and Appurtenances - Direct Push Points - Injection and Monitoring Wells - Injection System - Substrate - Monitoring Equipment and Supplies	 $25,000 $15,000 $15,000 $2,000 $5,000
Baseline Laboratory Analyses	$8,000
Surveying	$5,000
Reporting	$25,000
Closure Documentation, System and Well Abandonment	$25,000
Total Capital Costs	**$380,000**
Annual Operating and Monitoring Costs	
Mobilization/Demobilization/Per Diem	$10,000
Operation and Maintenance	$20,000
Direct Labor (Process Monitoring)	$20,000
Sampling Equipment and Supplies	$5,000
Laboratory Analysis	$10,000
Reporting	$20,000
Total Annual Operating Costs	**$85,000**

Summary Observations and Lessons Learned

This project demonstrates that source removal and proper implementation of the enhanced reductive dechlorination process can greatly expedite the remediation time frame for PCE contaminated groundwater. Twenty months after implementing the enhanced reductive dechlorination process, PCE concentrations within the plume decreased from pre-remediation levels of approximately 1,500 to 4,000 µg/L to non-detectable levels. Based on stoichiometric relationships, it is estimated that more than 90% of the PCE was degraded to ethene and ethane within the 20-month period. Regulatory approval for site closure was received in January 2001,

less than 2½ years after initiating the enhanced reductive dechlorination process. A year of post-treatment monitoring showed no rebound of CAH levels in the treatment area.

As expected, an increase in DCE and VC concentrations occurred in conjunction with the decrease in PCE concentrations. The corresponding build-up of DCE and VC peaked at approximately 6 and 14 months, respectively, after initiating the enhanced reductive dechlorination process. The DCE and VC levels then dropped sharply over the next 6 months. Ethene and ethane levels increased over two orders of magnitude (exceeding 400 µg/L) in conjunction with the decreasing concentrations of DCE and VC.

References

Lenzo, F. 2000. "*In Situ Treatment Technology* (Chapter 8)," 2nd Ed., Lewis Publishers, CRC Press, Inc., Boca Raton, FL.

Lutes, C.C., V. D'Amato, A. Frizzell, M. Hansen, G. Gordon, P. Palmer, S. Suthersan. 2003. *In-situ Substrate Addition to Create Reactive Zones for Treatment of Chlorinated Aliphatic Hydrocarbons: Hanscom Air Force Base*. Prepared for Air Force Center for Environmental Excellence (AFCEE) and Environmental Security Technology Certification Program (ESTCP). April 4, 2003.

Maierle, M.S. and Cota, J.L. 2001. Complete PCE Degradation and Site Closure Using Enhanced Reductive Dechlorination. *Proceedings of the Sixth International In-Situ and On-Site Bioremediation Symposium*, No.6(7), p.149-156.

Payne, F.C., S.S. Suthersan, F.C. Lenzo and J.S. Burdick. 2001. Mobilization Of Sorbed-Phase Chlorinated Alkenes In Enhanced Reductive Dechlorination. In: *Anaerobic Degradation of Chlorinated Solvents, Proceedings of the International In Situ and On-Site Bioremediation Symposium,* 6(2):53-60.

"Engineered *in situ* Anaerobic Reactive Zones", S.S. Suthersan, US Patent 6,143,177, 2000. http://www.epa.gov/epaoswer/hazwaste/ca/success/r4s_nucl.pdf

Suthersan, S.S, Lutes, C.C., Palmer, P.L., Lenzo, F., Payne, F.C., Liles, D.S., and Burdick, J. 2002. FINAL: *Technical Protocol for Using Soluble Carbohydrates to Enhance Reductive Dechlorination of Chlorinated Aliphatic Hydrocarbons*. ESTCP Contract #41624-99-C-8032, December 19, 2002.

U.S. EPA. 1998. *Technical protocol for evaluating natural attenuation of chlorinated solvents in groundwater*. Cincinnati, OH: National Risk Management Research Laboratory, Office Of Research And Development, U. S. Environmental Protection Agency. EPA/600/R-98/128.

APPENDIX E.3 – ENHANCED REDUCTIVE DECHLORINATION OF A PCE PLUME USING CORN SYRUP AND CHEESE WHEY

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Enhanced Reductive Dechlorination of a PCE Plume using Corn Syrup and Cheese Whey

Angie Frizzell, P.G., Chris C. Lutes, Hoa Voscott and Mike Hansen (ARCADIS G&M, Inc.)

Case Study Outline

ARCADIS used its patented in-situ enhanced reductive dechlorination process to treat groundwater impacted with tetrachloroethene (PCE) and its daughter products at a confidential manufacturing facility in the Western US. The food-grade carbohydrate used to facilitate dechlorination at the site was a dilute solution of high-fructose corn syrup (HFCS) and cheese whey (Suthersan, 2000; Lenzo, 2000). A field pilot test underway at the site is restricted for logistical reasons to infrequent injections of the substrate, necessitating the use of a long-lasting source of carbohydrate. The mixture of HFCS and whey has succeeded in sustaining elevated total organic carbon (TOC) levels in the reactive zone for more than a year following the first injection event, substantially reducing PCE concentrations in groundwater.

This case study provides details on the project objectives; site history; hydrogeology; site selection criteria; technology design, performance and cost; and a summary of findings and lessons learned from the Western US site.

Remedial/Performance Objectives

Several years prior to the enhanced reductive dechlorination pilot test, impacted source-area soils were excavated, and containment and remediation systems were installed by a previous consultant to address downgradient portions of the plume. A funnel-and-gate system with a zero-valent iron treatment gate was installed in 1997 to contain further migration of the plume and to allow for enhanced degradation of chlorinated volatile organic compounds. However, periodic monitoring and additional evaluations indicated that a portion of the plume was migrating around the funnel-and-gate system. In 2001, the funnel-and-gate system was augmented with a pump-and-treat system downgradient of the former source area to further contain impacted groundwater. Elevated concentrations of PCE remained in groundwater in the former source area.

Without source area treatment, remediation at the site could be long-term and costly. ARCADIS evaluated enhanced reductive dechlorination as a means to more aggressively treat the source area in an effort to reduce the life-cycle cost of active remediation. The client ruled out the use of permanent injection wells and frequent injections, so the pilot test was designed for maximum impact from a minimum number of injection events.

Site History/Source of Contamination

Historic site operations resulted in groundwater impacts in a former sump area, primarily in and around groundwater monitoring well W-2R (Figure 1). The downgradient plume extends beyond the pump-and-treat system trench and funnel-and-gate system wall shown in the figure.

The primary source compound was PCE, but as shown in Table 1, daughter products of PCE (trichloroethene [TCE], cis-1,2-dichloroethene [cis-DCE] and trans-1,2-DCE) were also present in 1996-1997. This indicates that dechlorination of PCE was occurring naturally at a slow rate before remediation was attempted.

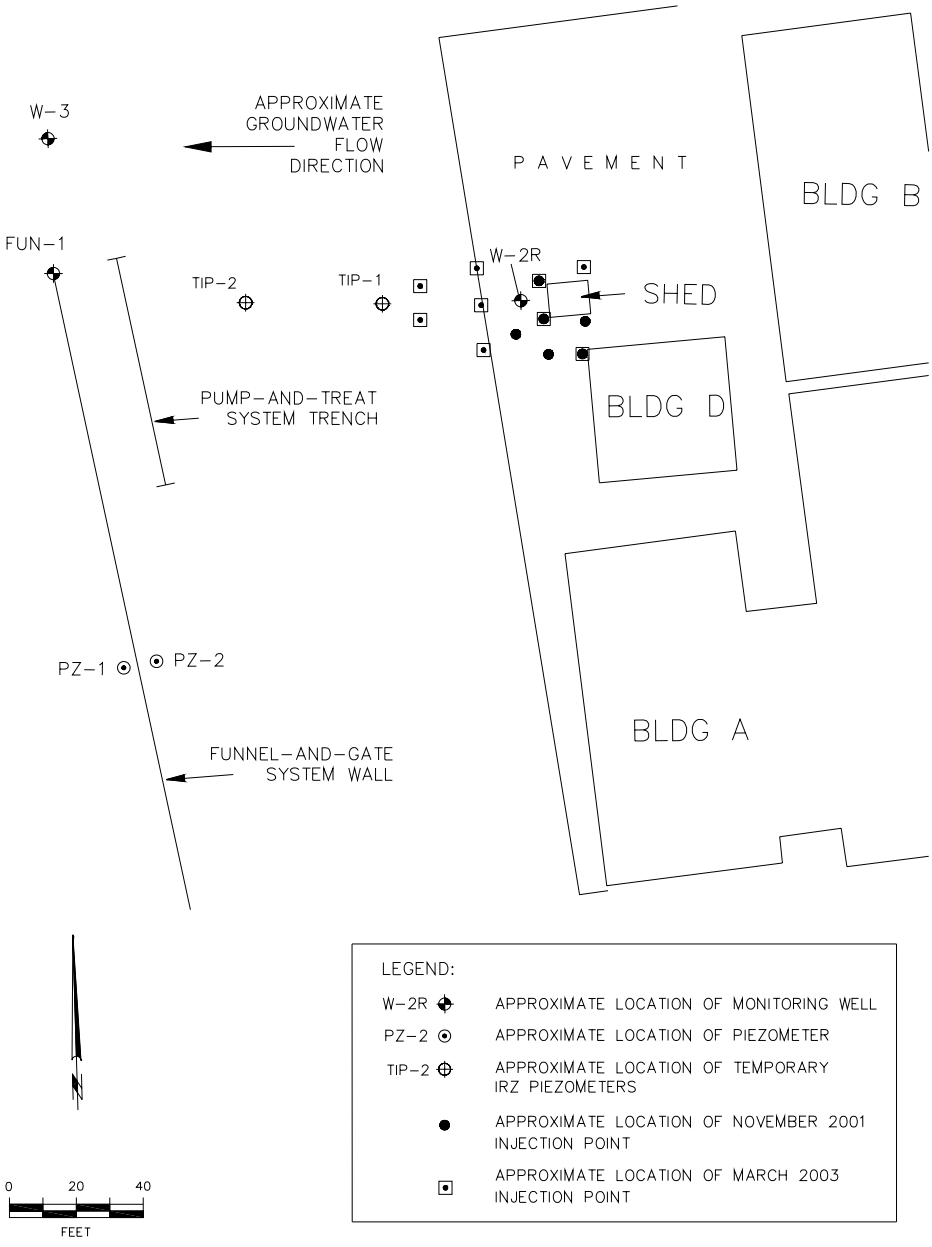

Figure 1: enhanced reductive dechlorination remediation area.

Table 1: Chlorinated VOC and By-Product Data for Western US Site

Date	Sample Location	PCE (µg/L)	TCE (µg/L)	cis-1,2-DCE (µg/L)	trans-1,2-DCE (µg/L)	VC (µg/L)	Ethene (ng/L)	Ethane (ng/L)	Chloride (µg/L)
May-96	W-2R	1,900	54	50	<1	ND	NA	NA	NA
Aug-96		1,700	13	3.9	<1	ND	NA	NA	NA
Feb-97		1,600	270	350	8.3	ND	NA	NA	NA
Aug-97		780	270	460	<10	ND	NA	NA	NA
Feb-98		900	230	410	<25	ND	NA	NA	NA
Aug-98		2,800	78	160	<50	ND	NA	NA	NA
Feb-99		950	120	220	<12	ND	NA	NA	NA
Jul-99		4,500	100	120	<50	ND	NA	NA	NA
Feb-00		2,200	280	370	4.8	ND	NA	NA	NA
Jul-01		4,200	<120	100	<62	<250	<500	<500	30.2
Nov-01	**Injection of cheese whey and corn syrup**								
Dec-01		3,800	560	250	<50	<200	NA	NA	NA
Jan-02		1,600	570	240	<50	<50	NA	NA	NA
Jan-02		2,400	1,500	760	<50	<50	NA	NA	309
Feb-02		1,700	1,600	1,300	<50	<50	1,400	280	286
Mar-02		1,600	1,900	1,800	<50	<50	NA	NA	NA
Apr-02		420	1,400	3,500	<50	<50	2,000	170	225
May-02		720	2,300	8,600	<50	<50	NA	NA	NA
Aug-02		<250	350	9,900	<120	<500	390	<5	239
Sep-02		83	<50	2,400	<50	<50	99	19	188
Nov-02		460	53	12,000	<50	<50	200	60	208
Jan-03		660	77	15,000	<50	<50	340	26	227
Mar-03		660	<200	15,000	<200	<200	NA	NA	NA
Aug-02	TIP-1	1,200	<50	77	<25	<100	1,500	110	46.4
Nov-02		2,000	26	140	<2	<2	220	120	68.1
Jan-03		1,300	18	91	<2	<2	14	7.5	63
Aug-02	TIP-2	570	<20	70	<20	<40	790	37	47.2
Nov-02		530	5	88	<2	<2	190	97	66.4
Jan-03		190	<2	56	<2	<2	<5	<5	58.7

ND: Not detected

Geology/Hydrogeology

The target zone is in a perched, unconfined, heterogeneous sand and gravel aquifer overlying shale bedrock. The perched aquifer is approximately 1 to 5 feet thick depending on seasonal variations. The bedrock surface occurs at approximately 18 to 26 feet below ground surface. Hydraulic conductivity has been estimated at 63 ft/day ($2x10^{-2}$ cm/s) and horizontal velocity under the influence of the pump-and-treat system is approximately 1.5 ft/day ($5x10^{-4}$ cm/s).

Site Selection Criteria

Among the general site selection criteria for enhanced reductive dechlorination technology, as detailed in a recently finalized enhanced reductive dechlorination protocol document (Suthersan, 2002), are the following:

- Site must be at least moderately permeable (K > 0.28 ft/day [10^{-4} cm/s]) and should have a groundwater velocity of 0.08 to 5 ft/day (10^{-5} to 10^{-3} cm/s). Hydraulic conductivity and velocity at the Western US site met these criteria.

- Sites should be reasonably well delineated geologically and with regard to contaminant concentration, as the Western US site was.
- The depth of the plume is a factor in determining cost effectiveness, with depths less than 50 ft (15 m) generally being desirable. The depth to the target zone at the Western US site was approximately 25 ft (7.6 m).
- Initial pH should be 5 to 9. The Western US site had a groundwater pH of approximately 7.1 in the target zone before treatment.
- Dense, non-aqueous phase liquid (DNAPL) or sorbed source material is not a barrier to success, but must be carefully considered in locating injection points and against desired treatment time and goals. Source material was excavated at the Western US site prior to treatment. Based on variable pre-treatment PCE concentrations at W-2R (Table 1), residual DNAPL may have remained in the source area after the soil excavation, but was not judged to be a detriment to success.
- Aerobic or borderline aerobic/anaerobic starting conditions are preferred. Sites already showing breakdown products are ideal. The Western US site exhibited Type 2 conditions (as defined in U.S. Environmental Protection Agency [U.S. EPA], 1998) prior to treatment, and borderline aerobic/anaerobic geochemistry (dissolved oxygen [DO] 0.3 mg/L, oxidation-reduction potential [ORP] 155 mV). Partial degradation of PCE was evident before treatment began, but elevated levels of nitrite-nitrate, sulfate, and ORP, as well as the limited degradable organic carbon sources (electron donors), were likely inhibiting dechlorination under ambient conditions.

Thus the Western US site met the recommended site selection criteria prior to treatment and was believed to be a good candidate for successful removal of dissolved PCE by enhanced reductive dechlorination.

Technology Description (Design and Operation)

The groundwater remediation process involved the injection of an organic carbon (HFCS and cheese whey) solution to enhance the reductive dechlorination of the chlorinated solvents present in site groundwater (i.e., an in-situ bioremediation process). By injecting an organic carbon source, anaerobic and strong reducing conditions were created within the in-situ reaction zone. These conditions created a more suitable environment for the degrading microorganisms to promote both desorption of PCE from the aquifer matrix and enhanced reductive dechlorination, or biodegradation, of the PCE (Payne et al., 2001). Enhanced reductive dechlorination enhances desorption by way of four processes – progressive decreases in organic carbon partitioning coefficient (K_{OC}) values of sequential daughter products, the production of natural biosurfactants by the enhanced microbial population, the production of fermentation products that act as co-solvents, and changes in equilibrium partitioning of contaminants due to the increase in the carbon content of groundwater relative to that of the soil (Suthersan et al., 2002). The dilute carbohydrate solution was injected in an area approximately 20 to 30 feet upgradient of W-2R to create a reactive zone centering on the source area and monitoring well.

Due to site constraints, the carbohydrate mixture was injected using temporary soil borings rather than more permanent, installed injection points. To optimize the duration and effectiveness of the first injection event, both "fast-acting" and "slow-acting" electron donors were used:

- Corn syrup was selected as a "fast-acting" donor to rapidly convert subsurface conditions to a more reducing environment. In addition, corn syrup contains no sulfate, which, if present, acts as a competing electron acceptor (although it can have other benefits, as discussed in Section 4.1.2.2 of Suthersan et al., 2002). Sulfate is naturally elevated at the site.
- Cheese whey was selected as a "slow-acting" donor to maintain the reducing environment over a longer period, after the corn syrup was utilized.

In November 2001, approximately 690 gallons of a diluted corn syrup and powdered cheese whey mixture (Figure 2) were injected in six soil borings in the former source area, upgradient of well W-2R (Figure 1). This volume represents approximately 115 pounds of TOC. The injection was primarily targeted at the impacted water-bearing zone, with injection zones ranging from approximately 5 feet above the water table to the bottom of the boring at bedrock/refusal.

A second injection of 900 gallons was performed in late March 2003. Based on the drop in pH at W-2R following the first injection, a buffer, sodium bicarbonate, was added to the solution to prevent undesirable decreases in pH, which can inhibit enhanced reductive dechlorination. A pH of 5 to 9 in the target zone is optimal. Although substantial dechlorination of PCE was observed at W-2R during several months of low-pH conditions, the process may be enhanced following the second carbon dose by preventing another decrease in pH.

Several indicators were monitored in downgradient well W-2R before and after the carbohydrate injection event to evaluate the progress of the pilot test and to optimize potential future injections. For example, increases in TOC concentration in groundwater are the first indicator that sufficient reagent loadings have been achieved to create a reactive zone large enough to affect well W-2R. Other indicators included general parameters (temperature, specific conductance, pH, ORP), electron donors (TOC, volatile fatty acids), electron acceptors (DO, nitrate-nitrite, sulfate), metabolic by-products (ferrous iron, chloride, methane, ethane, ethene), and chlorinated volatile organic compounds.

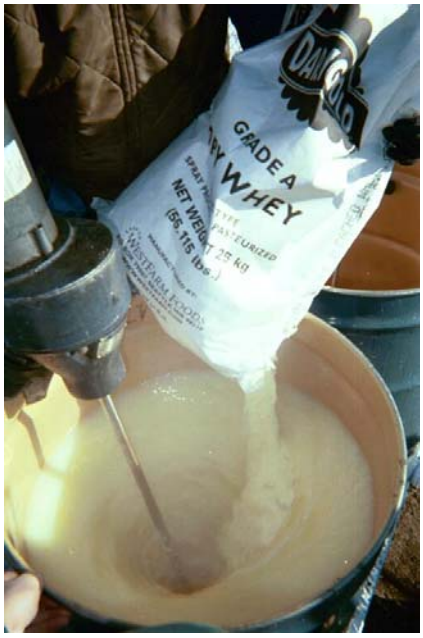

Figure 2: Mixing carbohydrate solution.

Technology Performance

The results of baseline sampling (July 2001) and post-injection sampling (December 2001 through March 2003) are summarized in Tables 1 and 2. Monitoring data following the second injection event are not yet available for publication.

On the basis of 16 months of post-injection data, the pilot test has effectively increased the rate of PCE dechlorination by increasing the electron donors and reducing the electron acceptors. A chart showing chlorinated volatile organic compound and TOC trends is presented in Figure 3.

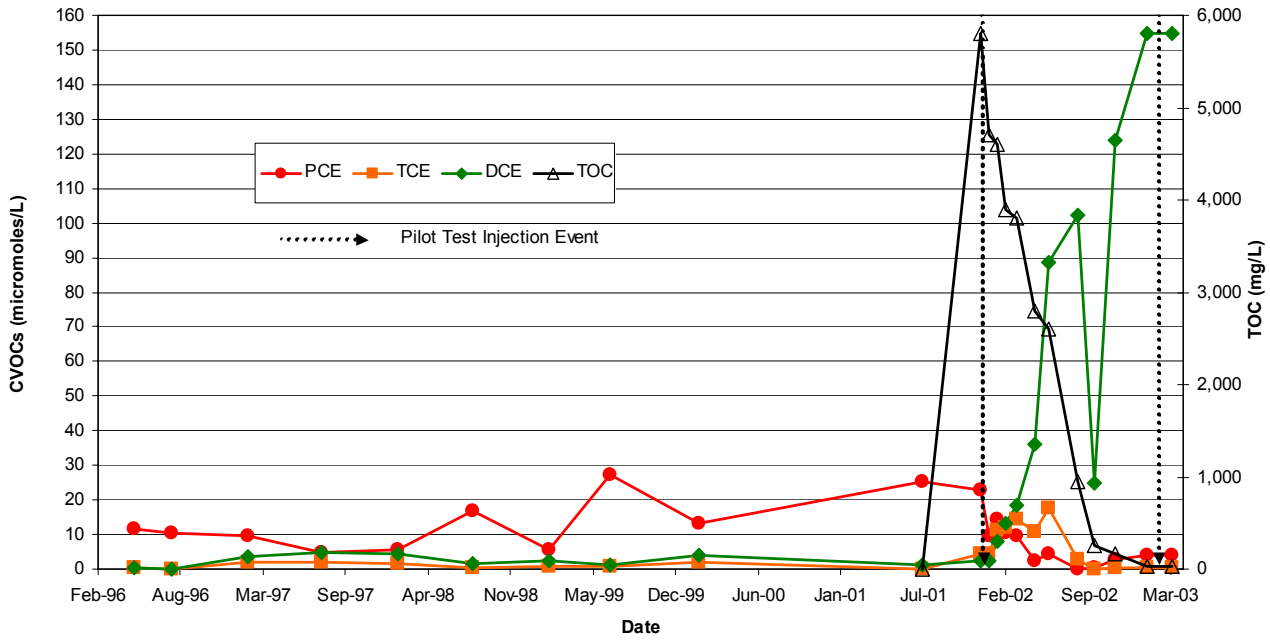

Figure 3: Chlorinated Volatile Organic Compound and TOC Trends for Well W-2R

The main indicator trends observed in well W-2R are presented below and are detailed previously in Table 1 and below in Table 2:

- TOC levels increased from 4 to 5,800 mg/L following the injection, and chlorinated volatile organic compound concentrations were immediately affected, indicating sufficient reagent loading at W-2R. TOC levels gradually declined thereafter, but appeared to be more than adequate to maintain a reductive environment through early 2003.
- The absence of elevated TOC or reductions in chlorinated volatile organic compound levels at wells TIP-1 and TIP-2 (shown in Figure 1) indicates the reactive zone extended less than 50 feet downgradient.
- ORP levels gradually decreased from a baseline level of 155 mV to consistently negative values of –75 to –183 mV, indicating a reducing environment was established.
- pH levels initially decreased due to fermentation of the carbohydrate mixture and creation of volatile fatty acids. Low pH levels (<4) early in the test may have inhibited some microbial activity, but pH soon rebounded to an acceptable level near 5 and above. The initial low pH resulted from the high TOC loading required for the "long residence time" injection event.

Table 2: Process Monitoring Data for Western US Site

Date	Sample Location	pH (s.u.)	DO (mg/L)	ORP (mV)	TOC (mg/L)	Nitrate-Nitrite (mg/L)	Sulfate (mg/L)	Total Sulfide (mg/L)	Ferrous Iron (mg/L)	Methane (µg/L)	Acetic Acid (mg/L)	Butyric Acid (mg/L)	Lactic Acid (mg/L)	Propionic Acid (mg/L)	Pyruvic Acid (mg/L)
Jul-01	W-2R	7.09	0.3	155	4	8.1	660	1.6	NA	0.45 j,b	<1.0	<1.0	<25	<1.0	<10
Nov-01	**Injection of cheese whey and corn syrup**														
Dec-01		4.05	MM	MM	5,800	4.8	629	NA	9	NA	NA	NA	NA	NA	NA
Jan-02		3.67	0.29	88.3	4,700	<0.1	385	NA	NA	NA	NA	NA	NA	NA	NA
Jan-02		4.45	0.13	-130	4,600	<0.1	341	1.1	31.5	NA	NA	NA	NA	NA	NA
Feb-02		MM	MM	MM	3,900	<0.1	281	<1.0	49.1	230	3,800	3,600	<25	380	<10
Mar-02		4.88	MM	-124	3,800	<0.1	189	NA	80.1	NA	NA	NA	NA	NA	NA
Apr-02		4.92	0.85	-183	2,800	<2	105	5.6	97	1,800	3100	2000	<25	300	<10
May-02		4.95	0.74	-164	2,600	<0.1	45.2	NA	102	NA	NA	NA	NA	NA	NA
Aug-02		6.76	0.09	-75.1	940	11	1.6	2.6	0.7	2,800	NA	NA	NA	NA	NA
Sep-02		6.76	0.07	-122.3	250	<0.1	<1	1.6	0.92	8,900	NA	NA	NA	NA	NA
Nov-02		6.79	0.24	-134.1	160	<0.1	<1	2.9	1.5	8,600	NA	NA	NA	NA	NA
Jan-03		6.82	0.75	-175.1	37	<0.1	<1	2.8	1.7	7,300	NA	NA	NA	NA	NA
Mar-03		6.38	0.26	-120.6	29	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Aug-02	TIP-1	6.9	4.08	115.2	12	0.1	953	1	<0.05	1.7	NA	NA	NA	NA	NA
Nov-02		NA	NA	NA	6	9.1	1010	<1	<0.05	7.2	NA	NA	NA	NA	NA
Jan-03		6.8	3.9	62.4	5	8.8	1060	1.1	<0.05	12	NA	NA	NA	NA	NA
Aug-02	TIP-2	7	5.12	85.6	7	11	991	1.8	<0.05	0.96	NA	NA	NA	NA	NA
Nov-02		NA	NA	NA	6	8.8	1010	<1	<0.05	1.6	NA	NA	NA	NA	NA
Jan-03		7.0	3.1	3.1	4	8.8	995	<1	<0.05	3.8	NA	NA	NA	NA	NA

NA: Not analyzed
MM: Machine malfunction

- Of the volatile fatty acids analyzed, acetic, butyric and propionic acids were generated in the reactive zone. Lactic and pyruvic acids have not been detected.
- Competing electron acceptors nitrate-nitrite and sulfate were gradually depleted following the injection to non-detectable levels. As this occurred, methane increased to levels indicative of methanogenesis in the reactive zone.
- PCE concentrations decreased an order of magnitude following the injection, from 4,200 to 460 μg/L. Concentrations of TCE first increased an order of magnitude as the parent compound PCE was dechlorinated, then TCE decreased as the PCE supply dwindled.
- Cis-DCE concentrations rose as the TOC supply fell. The second injection event is intended to boost TOC levels to complete the dechlorination process and reduce cis-DCE concentrations. Vinyl chloride (VC) has not been detected, which may be attributable to a high detection limit (50 μg/L and higher), low production, or rapid consumption. However, ethene has been detected above its baseline level, indicating that a portion of the cis-DCE mass has undergone complete reductive dechlorination.

Technology Cost

The enhanced reductive dechlorination application at the Western US site is at a field pilot scale, and is expected to cost approximately $55,000 for two injection events, process and performance monitoring, and status reporting. For a full-scale project, capital costs for application of the enhanced reductive dechlorination technology can vary widely depending on the project size and the injection design (e.g., manual vs. automated, mobile vs. fixed). Among the variety of electron donors that have been used for enhanced reductive dechlorination, the mixture used at the Western US site falls in the low-to mid-cost range, according to cost information published in Suthersan et al., 2002. Corn syrup and molasses are among the least expensive electron donors at $0.20 to 0.35 per pound of TOC. Whey costs vary between $0.05 (per pound of TOC) for fresh whey to $1.17 for the powdered form used in this project. Typically, costs for the reagent materials used at enhanced reductive dechlorination sites are less than 10% of the project budget.

Summary Observations and Lessons Learned

The enhanced reductive dechlorination pilot test ongoing at the Western US site has demonstrated that enhancement of reductive dechlorination of PCE and its daughter products at the former source area is feasible and effective. The mixture of "fast-acting" HFCS and "slow-acting" cheese whey has proven to provide a long-lasting carbon source for enhanced biodegradation, using materials in the low-to medium cost range. In addition, the pilot study provided valuable interim remediation in terms of actual mass destruction of PCE.

A buildup of cis-DCE has occurred in the reaction zone as the TOC supply from the first injection diminished. Increased ethene levels indicate that complete dechlorination of a portion of the cis-DCE is occurring, possibly at a slow rate in the presence of relatively low TOC levels. Alternately, the microbiological acclimation time required to optimize dechlorination of cis-DCE may not yet have been reached (Flynn et al., 2000). A buildup followed by a decline of cis-DCE over the course of one to two years following system startup is often observed in full-scale enhanced reductive dechlorination systems (Lenzo, 2000). A second injection was performed in

March 2003 to boost and sustain TOC levels in the reactive zone. In addition to reductive dechlorination, several alternate processes of biological cis-DCE transformation have been identified which may ultimately contribute to the cleanup of this compound. Four microbial processes identified by Loeffler (2001) are anaerobic reductive dechlorination (the mechanism exploited by enhanced reductive dechlorination), anaerobic energy-yielding oxidation, aerobic co-oxidation, and aerobic energy-yielding oxidation. Aerobic mechanisms may be capable of transforming cis-DCE at the more aerobic fringes of the reactive zone.

References

Flynn, S., F. Löffler, and J. Tiedje. 2000. Microbial community changes associated with a shift from reductive dechlorination of PCE to reductive dechlorination of *cis*-DCE and VC. *Enivron. Sci. Technol.*, Vol. 34(6):1056-1061.

Lenzo, F. 2000. "*In Situ Treatment Technology* (Chapter 8)," 2nd Ed., Lewis Publishers, CRC Press, Inc., Boca Raton, FL.

Suthersan, S.S, Lutes, C.C., Palmer, P.L., Lenzo, F., Payne, F.C., Liles, D.S., and Burdick, J. 2002. FINAL: *Technical Protocol for Using Soluble Carbohydrates to Enhance Reductive Dechlorination of Chlorinated Aliphatic Hydrocarbons*. ESTCP Contract #41624-99-C-8032, December 19, 2002.

U.S. EPA. 1998. *Technical protocol for evaluating natural attenuation of chlorinated solvents in groundwater*. Cincinnati, OH: National Risk Management Research Laboratory, Office Of Research And Development, U. S. Environmental Protection Agency. EPA/600/R-98/128.

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APPENDIX E.4 – USE OF HYDROGEN RELEASE COMPOUND (HRC®) TO REMEDIATE A CHLORINATED SOLVENT PLUME IN FISHERVILLE, MASSACHUSETTS

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Use of Hydrogen Release Compound (HRC®) to Remediate a Chlorinated Solvent Plume in Fisherville, Massachusetts

Robert D. Norris, Ph.D. (Brown and Caldwell, Golden, CO)

Introduction

Hydrogen Release Compound (HRC®) was used in a pilot demonstration test to enhance reductive dechlorination of perchloroethene (PCE) and trichloroethene (TCE) in groundwater. A substantial amount of data, including concentrations of volatile organic compounds (VOCs), geochemical species, and organic acids were generated because the project was conducted under the United States Environmental Protection Agency (US EPA) Superfund Innovative Technology Evaluation (SITE) Program. Enhanced reductive dechlorination was selected because of its known effectiveness in passively treating PCE and corresponding daughter products down to low regulatory levels.

Remediation and Performance Objectives

The primary objectives of the field test were to:

- Demonstrate that HRC injection can remediate PCE, TCE, and other regulated daughter products down to ethene.
- Demonstrate that HRC injection can control the further migration of VOCs, thus protecting downgradient receptors.

Secondary objectives were to determine:

- The length of time over which HRC metabolites remain within the aquifer.
- How widely the HRC metabolites are distributed and their effect on geochemical parameters.
- The relationship between geochemical parameters and degradation of VOCs.
- The conditions under which cis-DCE reductive dechlorination occurs.

Site Background: History and Contamination Source

The Fisherville Mill site shown in Figure 1 is located on approximately 30 acres of land at 60 to 62 Main Street (Route 122A) in the town of Grafton, Massachusetts. The original Fisherville Mill was constructed in 1832 and was used to produce cotton and woolen items. In the 1950s and 1960s, the mill was used for the manufacture of steel racks, machine tool parts, plastics assembly, aluminum lawn furniture. The mill has stood empty since 1986.

Environmental investigations have been conducted at the site since 1977. A preliminary site assessment was conducted in 1986 in response to a report of oil in the adjacent canal. Subsequent subsurface investigations identified a large light non-aqueous phase liquid (LNAPL) plume (Number 6 fuel oil) and a plume of VOCs, including PCE, TCE, cis-dichloroethene (cis-DCE), and vinyl chloride (VC). The chlorinated ethene groundwater plume is the subject of this pilot study. The source of the solvent contamination is in the area near a loading dock in the northwest corner of the old mill, perhaps from an old dry well located there.

In October 1996, three recovery wells were installed between the mill building and Route 122A to protect the Grafton water supply wells located about 1,200 feet south of the mill building along the western shore of the Blackstone River. Pump and treat operations using these wells continued until a fire in August 1999 completely destroyed the old mill and the groundwater treatment system. The recovery wells have been inactive since the fire.

Geology/Hydrogeology/Contaminant Distribution

The site is located in the Blackstone River Valley, which is approximately 3,000 feet wide in the vicinity of the site. The aquifer is composed of fine to coarse sand and gravel alluvium bounded below by granitic bedrock. A significant silt content is found within the sand and gravel over a 5 to 7 foot thickness at the aquifer base. The unconsolidated alluvium is approximately 50 feet thick at the site.

The water table is located approximately 10 feet below ground surface (bgs) at the location of the pilot test, near MW-1D south of Route 122A. The groundwater flow direction is south to southeast. Annual recharge to the aquifer is estimated to be approximately 15 inches per year. The horizontal gradient is 0.002 in the deep portion of the aquifer (40 to 50 bgs), where the highest concentrations of contamination exist. Hydraulic conductivity has been estimated to range from 200 to 400 ft per day (fpd), and seepage velocities have been estimated to be from 1 to 2 fpd. Retardation coefficients have not been estimated; however, retardation coefficients should be close to 1.0 in this gravel and sand water-bearing zone. Retardation may be somewhat higher in silt materials of the alluvial aquifer, since they typically contain organic matter.

The maximum groundwater concentrations have been discovered beneath the mill building in the deep portion of the aquifer, between 40 and 50 feet bgs. In this area, TCE concentrations exceeded 10,000 μg/L. Wells screened between 5 and 15 ft bgs had low to non-detect measurements of TCE, cis-DCE, and VC. The main plume appears to be migrating in an east-southeast direction toward the river. However, a substantial portion of the plume is migrating in a southerly direction, south of Route 122A in the vicinity of MW-1D located immediately upgradient of the pilot test injection area. This well had concentrations of TCE at 2,740 μg/L before the pump and treat system was installed. After termination of pumping, the concentrations of TCE returned to approximately 2,000 μg/L.

Site Selection Criteria

The site was selected for this intensive study because of extensive documented site history, the presence of only PCE and its degradation products, and its limited site access restrictions allowing the placement of numerous monitoring wells. In order to test the efficacy of enhanced biodegradation at the site, a pilot test was conducted at a location immediately downgradient of MW-1D, south of Route 122A. This location was ideal because it is the location of the highest TCE contamination south of Route 122A, where groundwater flow is headed in the direction of the Grafton water supply wells. Furthermore, it is one of the few locations accessible after the fire and subsequent demolition activities at the old mill building. Following successful pilot demonstration, this location would be ideal for the installation of a larger reactive barrier to

completely cut off plume migration to the south and provide assurances of water supply well protection.

Technology Description

HRC is an ester of glycerol, a three-carbon polyalcohol, and lactic acid. Once injected into the formation, HRC slowly releases lactic acid, which undergoes fermentation, generating molecular hydrogen and a series of carboxylic acids, which act as electron donors for utilization by bacteria that carryout reductive dechlorination. As a result, electron acceptors are consumed, the oxidation/reduction potential (ORP) is reduced, and molecular hydrogen is generated. The result is the creation of conditions favorable for reductive dechlorination of chlorinated ethenes. Because of the slow release nature of HRC, electron donors can be provided and reduced conditions created over an extended period of time. At this site, one injection event of HRC produced conditions favorable for reductive dechlorination for over 2 years. Typically, high seepage velocities and high levels of competing electron acceptors shorten the longevity of HRC. For this study, HRC Primer was also included. This material is miscible with water and releases lactic acid rapidly and may have consumed much of the competing electron acceptors, thus increasing the longevity of the standard HRC product. The combination of HRC Primer and HRC allows for a rapid creation of reducing conditions and long-term effects, without frequent batch additions or a mechanical system.

Technology Performance

Prior to HRC injection, groundwater data obtained from a number of wells in the vicinity of the pilot test area showed that TCE was the predominant VOC present, with some PCE and cis-DCE and low levels of VC also present. Additionally, some 1,1-dichloroethane (1,1-DCA) was present as a byproduct of existing concentrations of 1,1,1-trichloroethane (1,1,1-TCA). These observations are evidence that microorganisms capable of reductive dechlorination, including dechlorination of cis-DCE, are present. The data also indicated that the extent of natural degradation was limited, and thus, HRC injection had the potential to significantly accelerate VOC bioremediation.

Increases in VOC levels were observed following the termination of pump and treat activities after the fire that destroyed remediation equipment and some well heads. Data collected from wells in the vicinity of the test area over the 18 month period prior to injection of HRC showed an increase of VOCs from low levels (some below their detection limits) to levels as high as 2,650 ug/L TCE, stabilizing over the 6 months prior to HRC injection. For example MW-1D, immediately upgradient of the test area had concentrations of approximately 30 ug/L of PCE, 1,300 ug/L of TCE, 66 ug/L of cis-DCE and 2 ug/L of VC on June 1, 2000, as reported in Table 1. The increases in VOCs were also observed in other wells in the immediate vicinity of MW-1D. The short time that was required to stabilize the VOCs is an indication of fairly rapid transport within the sand and gravel water-bearing unit. The relatively stable VOC concentrations make interpretation of the pilot test data somewhat easier; however, one must recognize that while reductive dechlorination is occurring within the test area, additional VOC mass is simultaneously entering the test area from upgradient sources.

The pilot test was initiated by injecting HRC into a barrier perpendicular to the groundwater flow direction, beginning approximately 7 feet downgradient of MW-1D. The barrier, as shown in Figure 2, consists of three staggered rows of five injection points each. Within each row, the points are spaced approximately 7 ft apart, and the rows are separated by approximately 5 ft. Thus, the barrier consists of 15 injection points in an area that is approximately 10 ft long in the direction of groundwater flow, and, due to the staggered positioning of the individual rows, is approximately 35 ft wide perpendicular to the flow. The staggering of the rows allows the approaching groundwater flow to have little chance of migrating through the barrier without contacting HRC or its degradation products. The injection points consist of 2-inch schedule 80 PVC wells screened over the bottom 10 feet of the aquifer, between 40 and 50 ft bgs. HRC Primer and HRC were sequentially injected into each injection point at the rate of approximately 4 pounds per vertical foot of HRC Primer and 6 pounds per vertical foot HRC. The HRC was pushed into the aquifer by injecting sufficient glycerine to fully chase the HRC from within the injection point.

Monitoring wells to assess the performance of HRC were positioned in two arcs downgradient of the HRC injection area and were also screened across the bottom 10 ft of the aquifer. As shown in Figure 2, the first arc consists of three monitoring wells (SP-2B, SP-2C and SP-2D) located approximately 5 feet downgradient of the HRC injection area (row 1 wells). In the second arc, approximately 25 ft downgradient of the HRC injection area, a total of 12 monitoring wells (SP-3C, SP-3F, SP-3G through 3L, SP-3N, SP-3P, SP-3R and SP-3T) were installed (row 2 wells). Two additional monitoring wells were installed: one well (MW-4, not shown in Figure 2) was installed approximately 15 ft downgradient from SP-3P, and the other well (HLA1) was installed within the barrier of HRC injection points. After the first few monitoring episodes, the average downgradient groundwater flow direction was more accurately established, and six monitoring wells in the second arc were identified as "critical wells" for assessing performance under the USEPA SITE program. The critical wells were identified as SP-3K, SP-3C, SP-3L, SP-3N, SP-3P and SP-3R. The other wells in the second arc were deemed to be outside the downgradient area affected by the HRC injection.

Following HRC injection, TCE concentrations decreased after several months with a temporary rebound in a few wells, as shown in Figure 3. Overall decreases in TCE ranged from 88 to 98 percent in all wells except for MW-4 (not shown in Figure 3), which had a 62 percent decrease in TCE. MW-4 is located 15 feet further downgradient from the injection area than are the row 2 wells. The average of wells SP-3 K through SP-3R decreased from 850 ug/L to 52 ug/L.

Concurrent with TCE loss was an increase in cis-DCE concentrations, which eventually decreased as VC and ethene concentrations increased. Thus, over an 18-month period, the complete degradation pathway was evident. Figure 4 shows a representation of the average changes in mass across the test area. The decrease in TCE, as well as the increases and subsequent decreases in cis-DCE and VC mass are shown. Not shown in Figure 4 are the modest increases in ethene (see Table 1 for ethene concentrations). This pattern was observed for:

- Well MW-1D, located 7 feet upgradient from the nearest injection points. Organic acids were also detected at this location, indicating that the HRC may have been distributed to this location during injection.

- Well HLA-1, located within the injection area, located a few feet from some of the injection points.
- The row 1 wells, which are located approximately 5 to 7 feet downgradient of the most downgradient injection points.
- The row 2 wells, which are located 20 to 25 feet downgradient of the nearest injection points.
- Well MW-4 located 15 feet further downgradient than row 2 wells.

The onset of accelerated TCE degradation appeared to occur after 1 month in well SP-2D; after about 4 months in the other row 1 wells and the row 2 wells that are directly downgradient of the injection area; after about 6 months in 1D, HLA-1, and the remaining row 2 wells; and after about 9 months in MW-4. The timing of the onset of accelerated TCE degradation in each location appears to be influenced by their distance from the injection points and the time for organic acids to reach specific wells. Typically, it takes a few to several months for enhanced degradation to be observed following electron donor addition. Thus, the travel times for organic acids, which are quite soluble (little retardation), to the various wells is relatively short compared to typical lag times. Lag times and to a lesser extent, travel times, appear to be limiting when accelerated VOC biodegradation rates are observed. The impact TCE influx from upgradient areas as a result of the rapid groundwater flow is evident from comparing the TCE and cis-DCE data for well MW-1D. In this well, TCE does not decrease until 6 months after injection of HRC, while cis-DCE concentrations increase after 3 months. Thus, TCE conversion to cis-DCE is evident despite the relatively constant TCE concentration over that period.

The response in MW-1D, located upgradient of the injection points, is due largely to the horizontal distribution of HRC during injection. As discussed later, organic acids and changes in geochemistry that reflect impact from electron donors were detected at this location.

As a result of the SITE Program participation, a significant amount of data was collected. The geochemical data collected during the pilot were consistent with the observed changes in VOC concentrations, demonstrating the benefits of HRC addition over extended time periods. Within a few months of HRC injection, the ORP decreased in all wells to negative values) except for MW-1D, which became negative approximately 6 months following HRC injection. ORP levels in some wells were as low as –400 mV. ORP values remained negative in all wells except HLA-1 after 27 months.

Consistent with the ORP and VOC data were the observed increases in dissolved iron (largely ferrous iron /Fe (II) produced from the utilization of ferric iron/Fe(III) as an electron acceptor) that occurred within the first few months, with exception of upgradient well MW-1D where increased dissolved iron was observed after 6 months. Iron (III) is used as an electron acceptor under anaerobic conditions and increases in dissolved iron (iron (II)) indicate the occurrence of biodegradation reactions that contribute to lower reduction potential and conditions which can lead to production of hydrogen.

Alkalinity and dissolved carbon dioxide (CO_2) are indicators of biodegradation, although complex groundwater chemistry, such pH effects and the presence of some cations, can complicate numerical interpretation of the data. Both parameters increased significantly after 3 to 6 months.

Another indication of reducing conditions is an observed decrease in sulfate concentrations. Sulfate concentrations at the beginning of the test were approximately 30 mg/L. Sulfate then decreased after 3 to 6 months, with the concentrations at the time of the last sampling event in the range of 5 to 10 mg/L. Decreased sulfate concentration implies the presence of sulfate reducing bacteria, some of which are also capable of reductive dechlorination.

Lactic acid and its fermentation products (acetic acid, propionic acid, butyric acid, and pyruvic acid) are indicators of HRC's effects. All acids except pyruvic acid were monitored. Total organic acids ranged from 100 mg/L to 1,000 mg/L. The organic acid observations can be summarized as follows:

- Lactic acid was observed in HLA-1 after 3 months and remained at 330 mg/L after 27 months, indicating the longevity of HRC.
- Butyric acid was observed in most wells including the upgradient well, MW-1D (410 mg/L after 13 months before declining). The highest concentration, 8,800 mg/L, was observed in well HLA-1 after 20 months.
- Elevated concentrations of propionic acid appeared after 1 to 3 months and peaked between 6 and 13 months. The highest concentrations were reported for wells HLA-1 and MW-1D. Acetic acid followed a similar pattern to propionic acid.

The organic acid data shows that HRC provided electron donors across the site after a period of one to a few months. The presence of organic acids was demonstrated over 27 months. This shows that HRC can create and support conditions for reductive dechlorination over a period of at least 27 months under site conditions that included a fairly rapid seepage velocity that might be expected to result in the transport of the fairly soluble organic acids out of the study area. The presence of organic acids in the upgradient well MW-1D shows that injected HRC can be distributed several feet away from the injection location.

The organic acid data is consistent with the total organic carbon (TOC) data. TOC was not measured in the baseline sampling event, but was consistently analyzed for in subsequent events. TOC levels showed large increases during the test period in all but a few wells. Increases ranged from a few hundred mg/L to 5,000 mg/L. TOC levels remained substantially elevated after 27 months.

The data set is quite consistent. Where reductive dechlorination is observed, it is supported by the presence of organic acids, increased TOC, low ORP, increased dissolved iron, and reduced sulfate. Dechlorination has occurred from PCE to ethene, demonstrating that the prerequisite microorganisms are present and that HRC can create conditions that are adequate to convert cis-DCE to VC and VC to ethene.

Technology Cost

Costs do not include investigation, design (typically significantly less than for mechanical systems) and planning, or preparation of agency documents prior to implementation. Costs are

for installation and two years of monitoring. Reporting costs are based on typical consulting charges.

Table 2. Project Costs

Installation Costs	
Installation Labor (3 days)[a]	$14,000
Injection wells (3 days)	$20,000
Substrate HRC®, HRC Primer and shipping	$15,000
Base Line Sampling	$5,000
Surveying	$1,000
Completion Report	$5,000
Total Installation Costs[b]	$60,000
Annual Operating Costs[c]	
Mobilization	$2,000
Direct Labor	$6,500
Sampling Equipment and Supplies	$2,000
Laboratory Analysis	$12,800
Project Planning and Reporting	$12,000
Total Annual Operating Costs	**$35,300**
Total Installation and 2 Years Monitoring	**$130,600**

[a]Injection wells were installed.

[b]Does not include additional monitoring wells beyond those installed for delineation. Four additional wells might be considered. This would add installation costs but and sampling/analysis costs for a total of eight wells are included.

[c]Assumes 8 wells sampled quarterly for VOCs, organic acids, gases, and inorganics.

HRC is expected to maintain reducing conditions for 2-3 years. A second application might be necessary, particularly if the upgradient portion of the site is not treated. Follow up treatment, if required, would occur two to three years into the project and would cost significantly less for installation as only addition of product to existing wells would be required (approximately $15,000-$20,000). This project was conducted as a pilot test. As such, the treated area was not as large as would be the case for a full-scale injection. Sufficient information is not available to estimate the cost of full-scale treatment.

Summary Observations and Lessons Learned

The observations from this test can be summarized as follows:

- TCE concentrations were reduced by 88 to 98 percent.
- Cis-DCE was produced and then degraded.
- Vinyl chloride and ethene were produced.
- Geochemical parameters responded as anticipated and were consistent with biodegradation patterns.

- Organic acids and elevated TOC levels were observed across the site and remained elevated through the 27 months of monitoring conducted, as were favorable changes in the geochemistry.

It can be concluded that:

- HRC addition can accelerate reductive dechlorination through ethene.
- That HRC, HRC Primer, and glycerol are an effective combination to initiate and maintain reductive dechlorination.
- The benefits of HRC addition can last as long as 27 months.
- A second addition of HRC would be required to complete remediation or maintain the barrier for an extended time.
- The data supports observations by others that providing sufficient electron donor for a sufficient period of time can carry degradation past cis-DCE.

Appendix of Tables and Figures

Table 1. VOC Concentrations (µg/L)

Well ID	Parameter	6/1/00	7/31/00	12/4/00	7/10/01	12/4/01	5/9/02	9/9/02
1D	PCE	30	35	22	3.32	8.58	7	0.9
	TCE	1300	1600	950	140	693	270	43
	cis-DCE	66	82	660	1400	762	530	850
	vinyl choloride	2.4	3.5	4.1	3.1	10.2	120	78
	Ethene	NS	NS	0.5	1.1	0	67	88
HLA-1	PCE	27	36	30	4.6	5.39	3.8	2.9
	TCE	1600	1500	1500	250	251	150	190
	cis-DCE	68	74	190	980	596	230	260
	vinyl choloride	0.1	3.1	3.4	11	99.5	50	56
	ethene	NS	NS	4.7	NS	81	130	79
SP-2B, SP-2C,	PCE	18.3	22.3	9.2	4	2.5	2.3	1.6
SP-2D[a]	TCE	970	1053	357	183	189	113	80
	cis-DCE	43.0	52.3	643	940	745	400	150
	vinyl choloride	0.9	1.5	2.8	6.5	27.6	40.5	27
	ethene	NS	NS	3.9	5	NS	27	24
SP-3K, SP-3C,	PCE	NS	NS	13.4	1.4	3.5	1.6	2.6
SP-3L, SP-3N[a]	TCE	NS	NS	515	42.5	34.7	17.7	23.5
	cis-DCE	NS	NS	397.5	659.5	642.6	250.5	152
	vinyl choloride	NS	NS	2.2	6.6	11.6	31	62.3
	ethene	NS	NS	2.5	6.1	35.8	49.2	43.4
MW4	PCE	NS	NS	8.8	5.2	9.8	10	12
	TCE	NS	NS	150	43	67.6	50	61
	cis-DCE	NS	NS	130	300	123	96	70
	vinyl choloride	NS	NS	1.1	2.3	20.8	15	8.9
	ethene	NS	NS	NS	NS	NS	NS	18

NS = not sampled
[a]average of group of wells

Figure 1. Fisherville Site Map

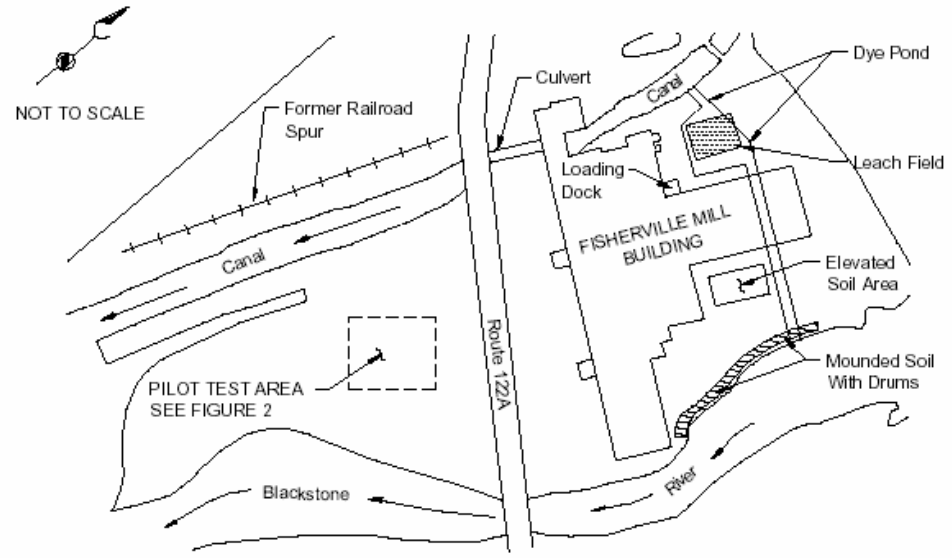


Figure 2. HRC Injection and Monitoring Well Locations

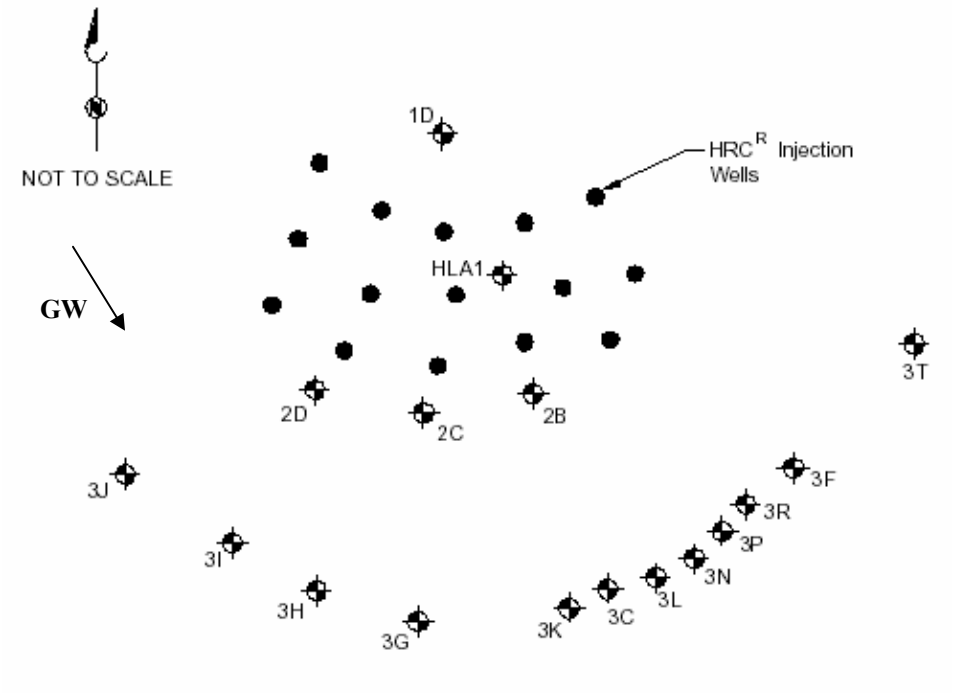


Figure 3. Change in VOC Concentrations Over Time

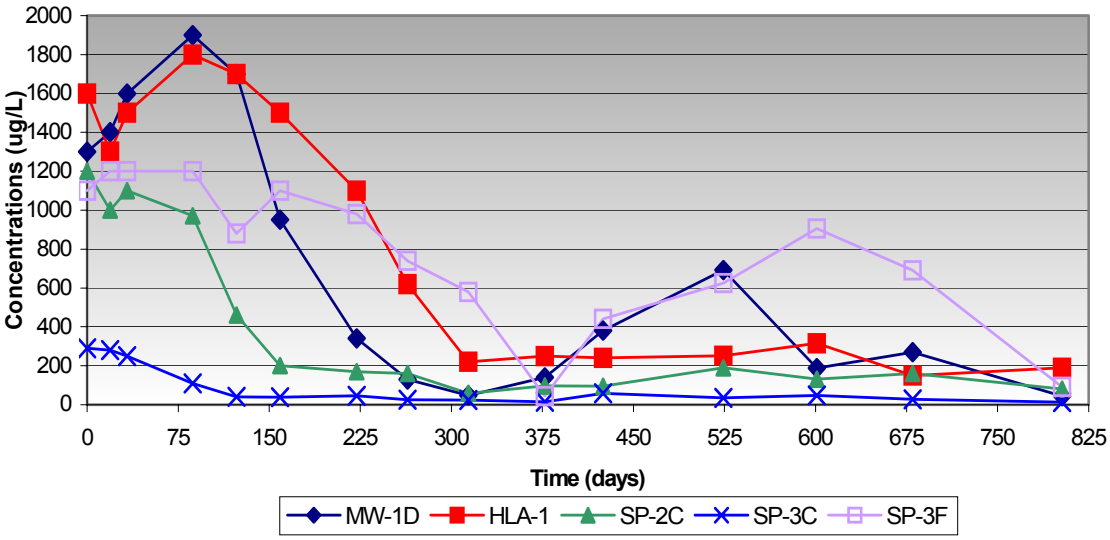
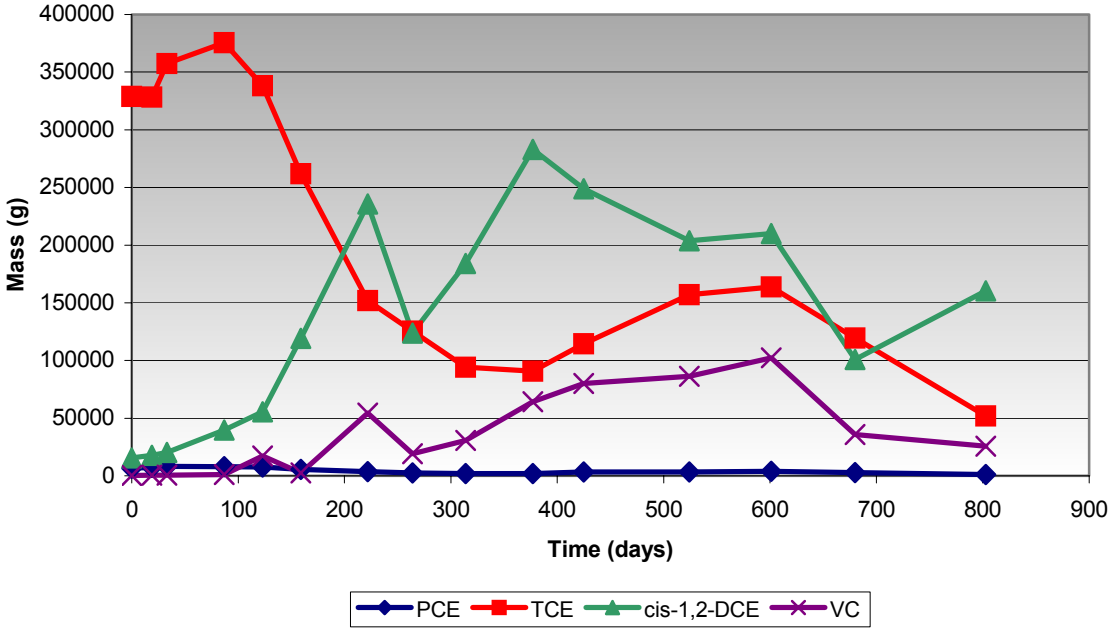


Figure 4. Average Change in VOC Mass Over Time



APPENDIX E.5 – HRC® PILOT TEST AT PORTLAND, OREGON DRY CLEANER SITE

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HRC® and HRC-X™ Pilot Test at Portland, Oregon Dry Cleaner Site

Robert D. Norris, Ph.D. (Brown and Caldwell, Golden, CO)

Introduction

The Oregon Department of Environmental Quality is responsible for addressing groundwater impacts at an active dry cleaner facility located in a strip mall. The Oregon Department of Environmental Quality determined that maintaining current activities at the site required that an unobtrusive, semi-passive remediation technology be used. Accelerated bioremediation using Hydrogen Release Compound (HRC®) within the plume and source area was selected as the remedial approach as it requires modest site access and minimal operation activity. A pilot test was conducted to determine if this option is an appropriate remedy for the reduction of high concentrations of perchloroethene (PCE) and some of its daughter products in site groundwater.

Technology Description

HRC is an ester of glycerol (a 3-carbon polyalcohol) and polylactate (a tetramer of lactic acid). Once injected into an aquifer, it slowly releases lactic acid. This lactic acid undergoes fermentation by indigenous microbes, generating dissolved hydrogen and a series of carboxylic acids (pyruvic, acetic, butyric, and propionic acids). As a result of the introduction of HRC, electron acceptors, like oxygen and nitrate, are consumed, the oxidation/reduction potential (ORP) is reduced, and dissolved hydrogen is generated. These processes create conditions favorable to reductive dechlorination of chlorinated ethenes. Because of the slow lactate release kinetics of HRC, electron donors and reduced conditions can be provided over an extended period of time (typically 12 to 18 months). In addition to the standard HRC, an extended-release, highly-concentrated version of HRC (Hydrogen Release Compound-Extended Release, HRC-X™) has been used at the Oregon site. HRC-X is designed to treat source areas with residual dense non-aqueous phase liquid (DNAPL) and has an anticipated lifetime of 3-5 years.

Remediation and Performance Objectives

The Oregon site consists of a dissolved phase plume and a source area where PCE concentrations in groundwater indicate the presence of DNAPL. Successful remediation requires that both areas be addressed. The Oregon Department of Environmental Quality is both the regulatory agency and the client for this site.

A pilot test approach was selected to determine the efficacy of HRC and HRC-X prior to full-scale application. The performance objective of the pilot test was to push HRC and HRC-X beyond their commonly-accepted end points to determine length of performance, effectiveness over varying conditions, and cost of treatment. Specifically, the objectives of the pilot test were to determine:

- The effectiveness of HRC injection as measured by the degree to which PCE degradation could be accelerated.
- If complete dechlorination (through ethene) of high concentrations of PCE is possible.
- How long the effects of HRC application persist.

- If volatile organic compound (VOC) concentrations would remain low after treatment.

If the pilot test is considered successful, full-scale remediation is expected to be instituted. Remediation goals in Oregon are 10^{-6} risk level for carcinogens and a Hazard Index of 1. For PCE and TCE the practical remediation goal at the site is 5 ug/L.

Site Background: History and Contamination Source

The site is a dry cleaning facility located in a strip mall in Portland, Oregon. The surrounding area is composed mainly of residential properties, with some commercial development. Several utilities (gas, electric, water, and sanitary sewer) run along the west (back side) of the strip mall. An investigation in 1999 revealed that dry cleaning contact water saturated with PCE (150,000 ug/L) and pure phase PCE were probably discharged to a floor-drain, which discharges to a utility trench. Leaks from the floor drain and the utility trench appear to have resulted in impacted soils and groundwater.

Geology/Hydrogeology/Contaminant Distribution

The soils consist of silty clay and silty sand. The depth to groundwater varies from 4 to 7 feet below ground surface (bgs) within the plume and from 2 to 5 feet bgs within the source area. The seepage velocity is estimated at 0.3 ft/day (110 ft/yr). Groundwater generally flows to the west, but flows more to the southwest in the vicinity of the DNAPL pilot test area (Figure 1).

The remediation area, shown in Figure 1, consists of a DNAPL impacted area and an associated plume, which is located down and cross gradient from the DNAPL area. Within the DNAPL area wells (JEMW-4 and JEMW-5), VOC concentrations were as high as 120,000 ug/L of PCE, 8,300 ug/L of trichloroethene (TCE), and 740 ug/L of cis-1,2-dichloroethene (cis-DCE). The dissolved phase plume concentrations (e.g. wells MW-2 and MW-4) were as high as 7,000 ug/L PCE, 480 ug/L TCE, and 130 ug/L cis-DCE. Vinyl chloride (VC) was not detected, indicating a potential "stall" in reductive dechlorination at cis-DCE.

Site Selection Criteria

HRC was selected for a pilot test to determine if the same basic approach could be used to treat both the source area and the plume. The limited accessibility of portions of the site, the documented success of HRC in stimulating the complete conversion of PCE to nonchlorinated end products, and minimal operation and maintenance requirements (sampling only), indicated that HRC was the most favorable technology for the site. Given the active use of this site, multiple injections and repeated site visits were considered too intrusive.

Technology Performance

Application Details. Within the dissolved phase plume, 1,900 pounds of HRC were injected via 22 injection points by means of direct push technology. This method consists of pushing a probe to the desired maximum depth of treatment and injecting the product under pressure as the probe

is withdrawn. The treatment grid covered approximately 1,200 square feet, with an aquifer injection vertical thickness of 22 feet. The application rate was 4 pounds of HRC per vertical foot. Within the DNAPL source area, 700 pounds of HRC-X were added via five injection points. The loading rate was 10 pounds per vertical foot. The location was next to the sewer line, so points were carefully located to avoid puncturing the line.

VOC Data. As shown in Table 1, following addition of HRC to the dissolved phase plume, the observed PCE concentration for HRC injection grid well MW-4 decreased from 340 µg/L to 22 µg/L after about one month. After 287 days, the PCE concentration in MW-4 was less than 5 ug/L and remained low (11 µg/L) after 1,247 days. Following HRC injection, TCE and cis-DCE levels first increased and subsequently decreased over a period of 12 months. The concentration of cis-DCE increased from 230 ug/L to 904 ug/L before reaching 45 ug/L on day 372 and then ranging between 16 ug/L and 654 ug/L through day 1,247. The trans-1,2 dichloroethene (trans-DCE) concentration increased from 160 µg/L pre-baseline to maximum concentrations of 543 µg/L and 420 µg/L after 8 and 372 days, respectively, before decreasing to 20 µg/L on day 1247. VC increased after nine months and peaked at 159 µg/L on day 553, demonstrating reductive dechlorination of cis-DCE and/or trans-DCE as well as the presence of a degradation pathway for VC. Observed ethene production was limited for MW-4.

As shown in Table 1 and Figure 2, the concentration of PCE reported for MW-2, located within the plume area grid, decreased from the base line level of 7,000 ug/L to 4,210 ug/L after 37 days, to less than 50 ug/L after 372 days, and was 101 ug/L after 1,247 days. TCE levels increased from the baseline level of 480 ug/L to 3,550 ug/L before decreasing to less than 50 ug/L on day 372, and were 488 ug/L at day 1,247. The concentration of cis-DCE increased from the base line level of 130 ug/L to 7,900 ug/L before decreasing to 672 ug/L on day 553 and 486 ug/L on day 1,247. VC was initially at non-detect levels, increased to 1,230 ug/L on day 287, declined to 145 ug/L on day 553, and was 110 ug/L on day 1,247. Ethene was produced in MW-2 and ranged from 180 µg/L on day 287 to 43 µg/L on day 1247. The test was conducted longer than the typically longevity of HRC (12-18 months), thus rebound of some of the daughter products (but not the parent compound) is not surprising and suggests that a second addition is justified.

As often is the case, cis-DCE reached concentrations greater than those of the parent compound, reflecting dissolution of the parent compound from the sorbed phase. If only dissolved phase PCE were converted to cis-DCE, the later would be present at approximately half the concentration of the former due to differences in molecular weight. The data from wells MW-4 and MW-2 show that degradation of the more toxic parent products, including sorbed phase contaminants, is proceeding to completion to the non-chlorinated and non-regulated product, ethene.

As shown in Table 1 and Figure 3, a single addition of extended-release HRC-X was effective at achieving substantial treatment of the source (DNAPL) area. PCE concentrations in monitoring well JEMW-4, located immediately downgradient of the injection area, decreased within a short time after injection of HRC-X. A 95% decrease in PCE concentration was observed within 198 days of injection, with a 99.9% reduction achieved after one year. The TCE concentration increased from 8,300 ug/L to 35,900 ug/L at day 198 and then decreased to 298 ug/L at day 553 and to less than 200 ug/L at day 827 (a decrease of greater than 99.4% from the maximum

concentration). PCE and TCE levels remained less than 200 ug/L after 1,247 days indicating that rebound has not occurred.

Here, cis-DCE increased from 740 ug/L to 91,400 ug/L on day 372 and then decreased to 38,400 ug/L on day 827. Cis-DCE then remained relatively constant throughout the remainder of the 1,247-day test period. VC and ethene were not present above their detection limits prior to HRC addition, but were observed at 9,150 ug/L and 318 ug/L, respectively, on day 553 and were reported as 4,900 ug/L and 1,130 ug/L, respectively on day 1,247. There were minimal changes in VOCs following day 553, with parent compound concentrations remaining relatively low. The expected lifetime of HRC-X is 3-5 years. Continued reductive dechlorination may occur after the most recent monitoring event at 1247 days (3.4 years) after HRC-X injection, as suggested by the geochemical and metabolic acid data discussed below. The data clearly show that rebound has not occurred; it thus appears that sufficient electron donor was supplied to address the dissolved and sorbed phases of the parent compound, including that which may have been transported into the treatment area.

Well JEMW-5 is located within the source area and 50 feet crossgradient from the HRC-X treatment area (see Figure 1). Based on starting contaminant concentrations (Table 1) and the groundwater flow direction, JEMW-5 was similarly impacted as JEMW-4, but was not contacted with HRC-X or its breakdown products as indicated from the geochemical and metabolic acid data discussed in the following sections. Thus, it serves as a contaminated reference/control for the contaminant reductions in well JEMW-4. Table 1 shows that, in contrast to nearly 100% reductions in parent products in JEMW-4, there was no overall change in PCE, TCE, or cis-DCE concentrations in well JEMW-5 during the pilot test period. There was no VC or ethene production observed in JEMW-5. A comparison of the unchanged contaminant concentrations in JEMW-5 with the significant contaminant decreases in JEMW-4 yields fairly conclusive evidence that HRC-X stimulated the complete reductive dechlorination of DNAPL/source area-concentrations of dissolved PCE and stimulated degradation past cis DCE.

Metabolic Acids. Upon hydration and contact with aquifer microorganisms, HRC and HRC -X release lactic acid, which is fermented to acetic, butyric, propionic, and pyruvic acids, as well as dissolved hydrogen. These organic acids and dissolved hydrogen serve as electron donors for reductive dechlorination. The total organic acid concentration can be used as a non-conservative tracer to indicate the influence of HRC and HRC-X on the aquifer geochemistry. Most often, lactic and acetic acids are initially observed in high concentrations, with butyric and propionic acids increasing over time. Butyric and propionic acids can be fermented to dissolved hydrogen and serve as “hydrogen storage” compounds. Pyruvic acid is rarely observed in high concentrations, as it is a common metabolic intermediate and is rapidly used by a variety of microorganisms.

Analysis for organic acid concentrations (Table 1) showed that elevated levels of electron donors were present at 553 days (1.5 years) post-injection in the dissolved plume area (MW-2) and 1,247 days (3.4 years) post-injection in the source area (JEMW-4). Except for a few detections at the end of the monitoring period, no organic acids were measured in source area crossgradient well JEMW-5.

In the dissolved plume areas and specifically in well MW-2, lactic (632 mg/L) and acetic acids (129 mg/L) were detected by day 8 post-injection. Later, the total organic acid concentration rose to 1,070 mg/L on day 198 and was maintained at similar concentrations through day 553, before decreasing to 85 mg/L on day 1247. Experience shows that reductive dechlorination is strongly favored when the total organic acids have concentrations greater than 80-100 mg/L; thus, HRC stimulated favorable conditions for reductive dechlorination in MW-2 for at least 18 months. Trends in organic acid concentrations in MW-2 are as follows:

- Lactic acid was observed at 623 mg/L on day 8, varied considerably, and declined from 65 mg/L on day 553 to less than 1 mg/L on day 827.
- Pyruvic acid was observed during the middle of the test at 1-4 mg/L.
- Acetic acid was observed at 129 mg/L on day 8 and at 266 mg/L on day 553, before decreasing to 24 mg/L on day 1,247.
- Butyric acid was first observed at 15 mg/L on day 70, reached a maximum of 297 mg/L on day 553, and then declined to 38 mg/L on day 1,247.
- Propionic acid was first observed at 207 mg/L on day 37, reached 386 mg/L on day 372, and declined to 23 mg/L on day 1,247.

The total organic acid concentrations in MW-4 were much lower than those in MW-2, and they peaked in well MW-4 at 314 mg/L on day 198. This trend may reflect MW-4's location, which is on the edge and slightly downgradient of the HRC injection grid, while MW-2 is located directly in the injection grid. In MW-4, organic acids may be consumed in reductive processes at a similar rate as they are produced from HRC and transported to the well.

In JEMW-4, located in the source area, 25 mg/L of lactic and 12 mg/L of acetic acid were detected in the first 70 days post-injection. Total organic acid concentrations then increased to 269 mg/L (70 mg/L of acetic acid and 199 mg/L of propionic acid) on day 198 before rising steadily to 1,426 mg/L on day 287 and 4230 mg/L on day 1247. These results are indicative of HRC-X's extended release profile and highly concentrated nature. HRC-X was able to maintain total organic acid concentrations of 64 to 4,230 mg/L for 1247 days (3.4 years) and may continue to maintain high concentrations past day 1247, when the most recent monitoring event occurred.

Geochemistry. Geochemical parameters including dissolved iron and manganese, sulfate, and sulfide demonstrated the creation of reducing conditions in wells impacted by HRC or HRC-X (Table 1).

Iron (III) is used as an electron acceptor under anaerobic conditions and increases in dissolved iron (iron (II)) indicate the occurrence of biodegradation of the electron donors and the establishment of reducing conditions. Dissolved iron typically increases as electron donors are consumed and may decrease when electron donor substrates become scarce. Dissolved iron in well MW-2 increased from 23 mg/L on day 8 to 197 mg/L on day 198 and then decreased somewhat to 61 mg/L on day 1247, reflecting the cyclical pattern of HRC-stimulated organic acid production and consumption by biodegradation. Dissolved iron followed a similar pattern in well MW-4 (starting at 11.2 mg/L, peaking at 43 mg/L, and declining to 19.2 mg/L at day 1247), despite the relatively low organic acid concentrations during the monitoring period.)

In well JEMW-4 in the source area, dissolved iron was measured initially at 1.25 mg/L and continually rose to 410 mg/L at day 1247. This pattern indicates that HRC-X has most likely not been depleted after 3.4 years of monitoring. In contrast, dissolved iron in well JEMW-5 remained, for the most part, below 10 mg/L, indicating a lack of electron donor in this well that is outside of the apparent influence of HRC-X.

Manganese responded similarly to iron. In all wells except JEMW-5, dissolved manganese concentrations increased until day 372, when monitoring of manganese was discontinued.

Another indication of reducing conditions is a decrease in sulfate and increase in its reduction product sulfide. Sulfate consumption and sulfide production implies the presence of sulfate reducing bacteria, some of which are also capable of reductive dechlorination. However, reductive dechlorination is energetically favorable at a higher ORP value as compared to sulfate reduction, so the establishment of sulfate reducing conditions is not a prerequisite for reductive dechlorination.

The sulfate concentration in MW-2 at the beginning of the pilot test was 43 mg/L. Sulfate decreased to 1.0 mg/L on day 372 and then increased to 12 mg/L at the end of the pilot study. During this time, sulfide levels increased from non-detect to 1.4 mg/L on day 553. Sulfide is typically rapidly depleted via dispersion, volatilization, or precipitation, so low, non-stoichiometric concentrations from sulfate reduction are expected. Trends in sulfate concentration in MW-4 were not clear and no pattern was established during the pilot study, despite the presence of up to 98 mg/L of sulfate. The lack of sulfate reduction in MW-4 may be due to the moderate concentrations of electron donor, which appears to have created iron reducing, but not sulfate reducing conditions. Similarly, sulfate concentrations in the source area wells, JEMW-4 and JEMW-5, were very low (less than 5 mg/L) and no consistent pattern was observed.

Increased levels of chloride are consistent with the decreases VOC concentrations. Chloride levels in JEMW-4 increased to 120 mg/L on day 287 from less than 20 mg/L at baseline. This corresponds to the conversion of 120 mg/L of PCE to ethene, whereas the initial aqueous concentration of PCE was 98 mg/L. This result provides another indication that desorption and dissolution of residual DNAPL has taken place in the source area that was impacted by HRC-X. In contrast, chloride concentrations in well JEMW-5 remained at less than 25 mg/L for the duration of the pilot study.

Technology Cost

Costs do not include investigation, design (typically significantly less than for mechanical systems and partially offset by no-fee design assistance provided by Regenesis) and planning or preparation of agency documents prior to implementation. Costs are for installation and two years of monitoring. Reporting costs are based on typical consulting charges rather than Oregon Department of Environmental Quality internal costs.

Table 2. Project Costs

Installation Costs	
Installation Labor (3 days)[a]	$4,000
Injection Points (3 days)	$8,000
Substrate HRC®, HRC-X™ and shipping	$21,000
Base Line Sampling	$5,000
Surveying	$1,000
Completion Report	$5,000
Total Installation Costs[b]	$44,000
Annual Operating Costs[c]	
Mobilization	$2,000
Direct Labor	$6,500
Sampling Equipment and Supplies	$2,000
Laboratory Analysis	$12,800
Project Planning and Reporting	$12,000
Total Annual Operating Costs	**$35,300**
Total Installation and two years Monitoring	**$114,600**

[a]Assumes 10 injection points per day (a large system may attain 15-20 injections per day).
[b]Does not include additional monitoring wells beyond those installed for contaminated area delineation. Two to four additional wells might be considered for a full-scale project, adding installation and sampling/analysis costs.
[c]Assumes 8 wells sampled quarterly for VOCs, organic acids, gases, and inorganics.

Cost estimates for HRC and HRC-X should be based on their maintaining reducing conditions from 12-18 months and 3-5 years, respectively. This project was conducted as a pilot test. As such, the treated area was not as large as would be for a full-scale injection. At this site, an initial full-scale injection could be 50% larger for the plume and 8 times larger for the source area. Full-scale costs would thus be approximately $200,000, if no pilot had been conducted. Follow up full-scale treatment, if required, could occur 2-3 years into the project and would cost approximately half to two thirds the original installation costs, as some areas would not require further injections.

Summary Observations and Lessons Learned

In addition to demonstrating that HRC can address PCE plumes by accelerating reductive dechlorination including formation of ethene, the pilot test demonstrated the ability of the extended-release HRC-X to remediate source areas over an extended time. Observations are as follows:

- HRC and HRC-X have been effective for 2.7 and 3.4 years, respectively, based on decreasing contaminant concentrations, the presence of organic acids, and changes in geochemistry.
- Parent compound (PCE and TCE) rebound has not occurred after an extended period.

- HRC addition was successful in overcoming an apparent cis-DCE stall between 200 and 400 days after injection, supporting observations by others that addition of sufficient electron donor for an extended period of time can overcome the stall phenomenon.
- Desorption of parent compounds occurred with subsequent biodegradation.
- A second and full-scale addition of electron donor is required to reach MCLs.
- A full-scale addition is warranted and should occur over a wider area.
- Addition of HRC-X appeared to stimulate a larger mass reduction efficiency than did HRC, but HRC-X took a longer period of time to reach peak efficiency, as measured by organic acid release.

The Oregon Department of Environmental Quality is satisfied with the results of the pilot test and is continuing to monitor the site to determine how long HRC-X will remain effective. Full-scale addition has been postponed due to state funding limitations.

Appendix of Figures and Tables

Figure 1. Springdale Cleaners Site Map

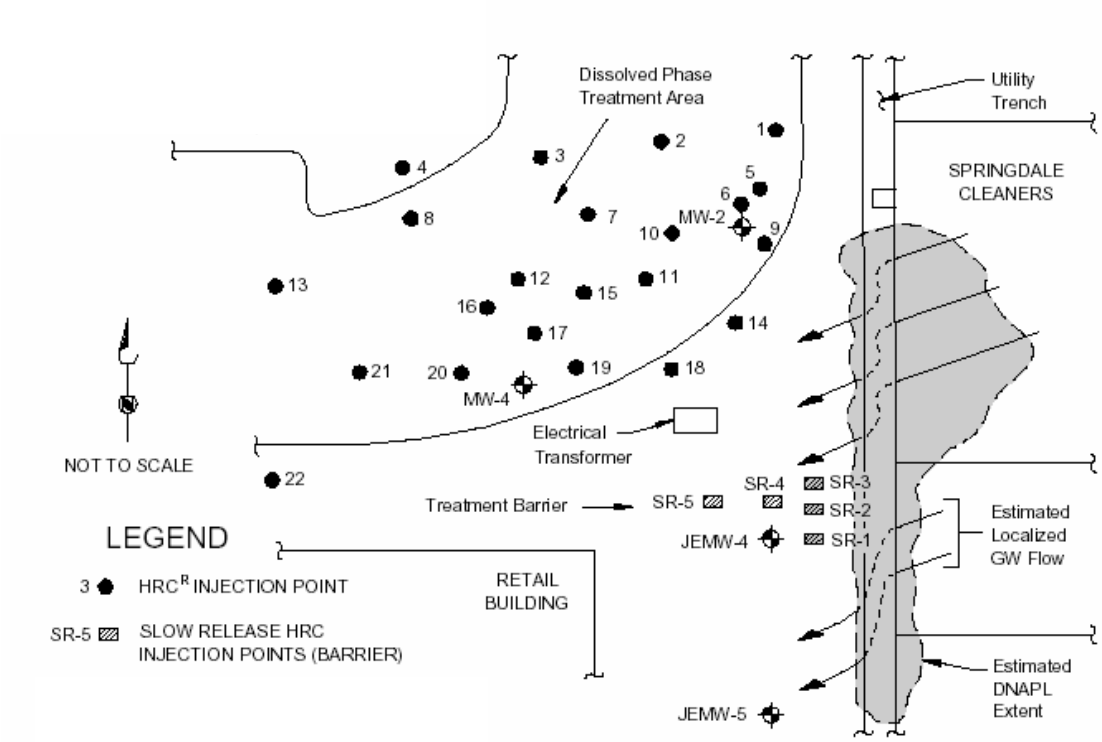

Figure 2. VOC Concentration Changes in the Dissolved Plume Area

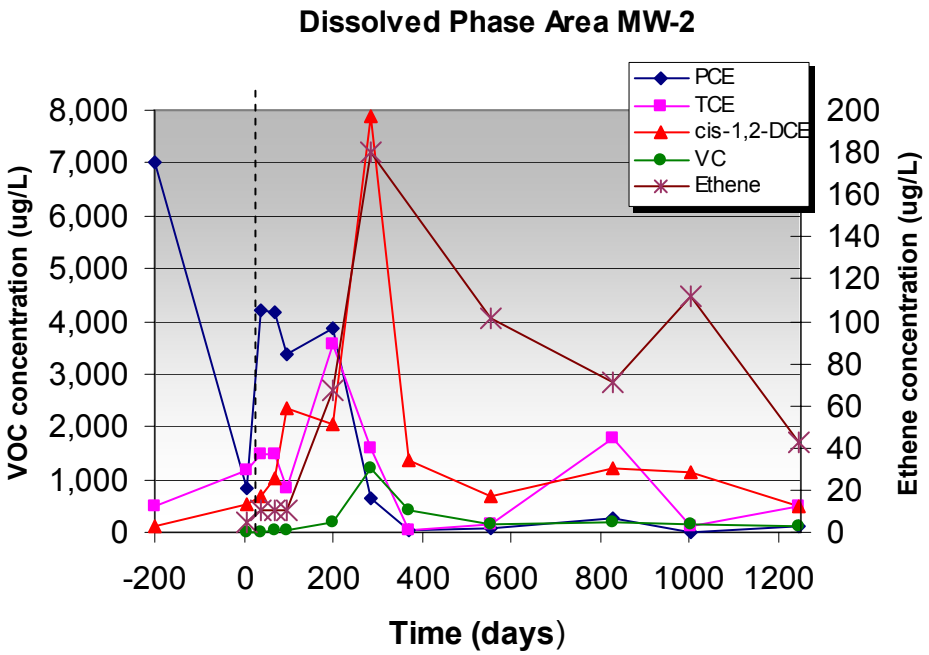

Figure 3. VOC Concentration Changes in the Source Area

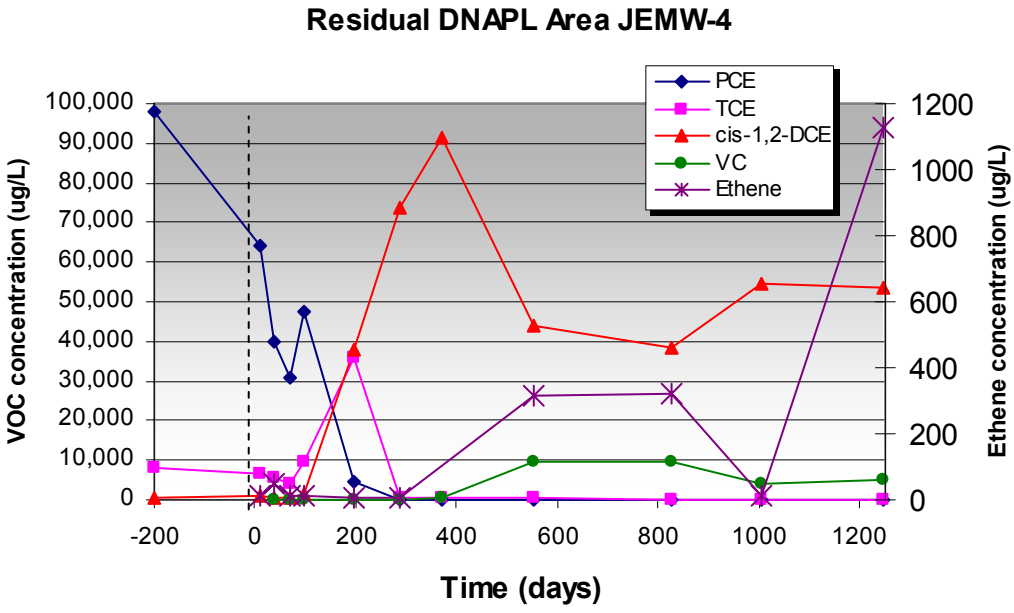

Table 1. VOC Concentrations

Well		Units	5/28/99 -186	12/8/99 day 8	1/6/00 day 37	2/8/00 day 70	3/7/00 day 98	6/15/00 day 198	9/12/00 day 287	12/6/00 day 372	6/5/01 day 553	3/6/02 day 827	8/29/02 day 1003	4/30/03 day 1247
MW-2 (dissolved area grid)	PCE	ug/L	7,000	818	4,210	4,180	3,360	3,870	635	<50	92	274	<10	101
	TCE	ug/L	480	1,190	1,460	1,480	825	3,550	1,580	<50	159	1,790	109	488
	cis-DCE	ug/L	130	542	677	1,010	2,350	2,050	7,900	1,370	672	1,210	1150	486
	trans-DCE	ug/L	93	381	141	86	100	145	323	300	130	135	112	140
	VC	ug/L	na	< 10	na	< 20	< 20	180	1,230	433	145	197	152	110
	Ethene	ug/L	na	< 10	< 20	< 20	< 20	67	180	na	101	71	112	43
	Acetic Acid	mg/L	na	129	87	100	72	223	198	270	266	3.8	113	24.3
	Butyric Acid	mg/L	na	< 1	< 1	15	10	138	149	266	297	< 1	20	37.9
	Lactic Acid	mg/L	na	623	84	42	4	388	299	334	64.6	< 1	< 1	< 1
	Propionic Acid	mg/L	na	< 1	207	195	138	320	292	386	277	< 1	20.1	23.1
	Pyruvic Acid	mg/L	na	< 0.1	< 0.1	< 0.1	< 0.1	1.2	< 0.1	4.2	0.9	< 0.1	< 0.1	< 0.1
	Sulfate	mg/L	43	27	93	98	70	23	21	1	2.5	32.9	8.6	11.9
	Sulfide	mg/L	< 0.2	< 0.1	0.35	0.25	0.1	0.96	0.78	0.37	1.4	0.46	< 0.5	< 0.1
	Iron, diss.	mg/L	na	23	31	41.5	57	138	120	197	135	34.4	17.3	61.1
	Chloride	mg/L	8.9	13	14	< 0.5	12	13	17	28	na	na	na	na
	Mn, diss.	mg/L	na	na	4.22	4.64	11.6	11	10.4	18.6	na	na	na	na
	Redox	mV	na	-84.1	na	120	-6	na	na	na	na	na	na	na
MW-4 (dissolved area grid edge)	PCE	ug/L	340	648	22	26	26.6	4.5	< 5	< 5	17.8	65	1.0	10.6
	TCE	ug/L	180	926	621	534	380	17.5	12	< 5	74.4	306	2.2	122.0
	cis-DCE	ug/L	230	658	904	504	386	489	351	45.2	497	654	16.2	539.0
	trans-DCE	ug/L	160	543	468	232	140	174	302	420	144	41.4	6.2	19.6
	VC	ug/L	na	na	< 5	< 5	< 5	3	62	10.6	159	91.6	5.5	38.4
	Ethene	ug/L	na	< 10	< 20	< 20	< 20	< 10	40	na	< 8	19	11	< 10
	Acetic Acid	mg/L	na	< 1	23	17	22	106	13	24	<1	<1	<1	<1
	Butyric Acid	mg/L	na	< 1	< 1	< 1	< 1	< 1	< 1	< 1	<1	<1	<1	<1
	Lactic Acid	mg/L	na	< 1	< 1	< 1	< 1	< 1	< 1	< 1	<1	<1	<1	<1
	Propionic Acid	mg/L	na	< 1	17	10	11	208	2	12	<1	<1	<1	<1
	Pyruvic Acid	mg/L	na	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	<0.1	<0.1	0.2	<0.1	<0.1
	Sulfate	mg/L	na	5	65	98	82	11	2	< 1	50.5	90.4	2.2	53.4
	Sulfide	mg/L	na	< 0.1	0.3	0.29	0.25	0.15	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	Iron, diss	mg/L	na	11.2	20.3	8.41	11	42.5	32.9	43.4	32.7	4.61	na	19.2
	Chloride	mg/L	na	10	11	< 0.5	11	< 10	10	11	na	na	na	na
	Mn, diss.	mg/L	na	na	2.06	1.18	1.42	6.48	5.87	7.15	na	na	na	na
	Redox	mV	na	-108.4	na	-35	-7	na	na	na	na	na	na	na
JEMW-4 (source area dg)	PCE	ug/L	98,000	63,900	39,800	30,600	47,400	4,420	< 200	79.9	<250	<200	<200	<200
	TCE	ug/L	8,300	6,430	5,450	4,200	9,730	35,900	680	623	298	< 200	<200	<200
	cis-DCE	ug/L	740	871	608	580	1,330	37,900	73,700	91,400	43,900	38,400	54,700	53,500
	trans-DCE	ug/L	170	137	< 1	< 1	< 200	628	588	1,380	808	816	532	558
	VC	ug/L	na	na	< 1	< 1	< 200	< 100	< 200	366	9,510	9,690	4,060	4,900
	Ethene	ug/L	na	< 20	< 100	< 20	< 20	< 10	< 10	na	318	319	14	1,130
	Acetic Acid	mg/L	na	< 1	6	12	32	70	437	247	305	828	883	868
	Butyric Acid	mg/L	na	< 1	< 1	< 1	< 1	< 1	161	170	151	1,280	2060	2680
	Lactic Acid	mg/L	na	< 1	< 1	25	18	< 1	< 1	<1	<1	<5	<1	<1
	Propionic Acid	mg/L	na	< 1	< 1	< 1	14	199	828	560	352	549	597	682
	Pyruvic Acid	mg/L	na	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.5	0.2	<0.5	< 0.1	< 0.1
	Sulfate	mg/L	na	6	2	3	< 1	1	< 1	1	<5	<1	<5	1.3
	Sulfide	mg/L	na	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	<0.1	<0.1	<0.1	<0.1	< 0.5
	Iron, diss	mg/L	na	1.25	0.714	1.94	8.01	8.73	37.1	73	149	192	na	410
	Chloride	mg/L	na	24	20	23	31	80	120	91	na	na	na	na
	Mn, diss	mg/L	na	0.766	0.913	0.94	1.71	3.81	10.4	17.9	na	na	na	na
	Redox	mV	na	43.6	na	7	-43	na	na	na	na	na	na	na
JEMW-5 (source area cg 50 feet)	PCE	ug/L	120,000	60,600	39,000	63,700	51,400	40,600	87,300	108,000	132,000	121,000	66,300	74,000
	TCE	ug/L	4,600	5,630	3,630	5,590	3,860	8,010	7,660	9,850	4,020	3,130	5,340	4,500
	cis-DCE	ug/L	250	355	< 400	406	248	526	775	1,000	<500	<1000	<500	<500
	trans-DCE	ug/L	< 1	< 100	< 400	< 200	< 200	< 100	< 500	< 500	<500	<1000	<500	<500
	VC	ug/L	na	< 100	< 400	< 200	< 200	< 100	< 500	< 500	<500	<1000	<500	<500
	Ethene	ug/L	na	< 20	< 400	< 30	< 10	< 20	< 10	na	< 15	< 13	12	< 10
	Acetic Acid	mg/L	na	10	< 1	< 1	< 1	< 1	< 1	4	<1	<1	< 1	<1
	Butyric Acid	mg/L	na	< 1	< 1	< 1	< 1	< 1	< 1	< 1	<1	<1	< 2	<1
	Lactic Acid	mg/L	na	< 1	< 1	< 1	< 1	< 1	< 1	< 1	<1	<1	< 1	96.9
	Propionic Acid	mg/L	na	< 1	< 1	< 1	< 1	< 1	< 1	< 1	<1	<1	500	<1
	Pyruvic Acid	mg/L	na	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	46.7	< 0.1
	Sulfate	mg/L	na	9	6	6	6	5	5	9	11.4	10	7.9	6.5
	Sulfide	mg/L	na	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	<0.1	<0.1	< 0.1	< 0.1
	Iron, diss	mg/L	na	2.11	2.64	4.5	57	2.09	0.696	0.484	2.15	3.19	0.774	6.36
	Chloride	mg/L	na	24	23	26	25	23	25	22	na	na	na	na
	Mn, diss	mg/L	na	0.684	0.661	0.749	11.6	0.69	0.618	0.565	na	na	na	na
	Redox	mV	na	-22.6	na	-1	-58	na	na	na	na	na	na	na

na = not measured

APPENDIX E.6 – ENHANCED ANAEROBIC BIODEGRADATION OF TRICHLOROETHENE USING EDIBLE OIL SUBSTRATE (EOS™) IN A PERMEABLE REACTIVE BARRIER

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Enhanced Anaerobic Biodegradation of Trichloroethene Using Edible Oil Substrate (EOS™) in a Permeable Reactive Barrier

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Introduction

A novel, low-cost technology has been developed for delivering a low solubility, slowly degradable substrate to the subsurface to enhance the *in situ* biodegradation of a variety of groundwater contaminants including chlorinated solvents, perchlorate, hexavalent chromium, nitrate, and oxidized radionuclides. The EOS™ (Edible Oil Substrate) process blends food-grade vegetable oil and surfactants in a high-speed mixer to generate an oil-in-water emulsion with a small droplet size that can be easily distributed throughout the subsurface (US Patent #6,398,960). The emulsion is injected through permanent wells or temporary direct-push points. Water is subsequently injected to distribute and immobilize the oil. Once in the subsurface, the oil slowly biodegrades over time providing a slow continuous source of dissolved organic carbon (i.e., fermentation products) to support biodegradation of the target contaminants. Degradation of the oil results in removal of oxygen and production of hydrogen (H_2). The hydrogen itself then drives the desired anaerobic biological metabolism. These microbial metabolic transformations are illustrated in the following equations using linoleic acid as a representative fatty acid in soybean oil:

Sequence of Reactions Using Fats or Oils

$C_{18}H_{32}O_2$ (**linoleic acid**)+ 34 H_2O --***Bacteria***--> 18 CO_2 + 50 H_2
C_2HCl_3 (**TCE**) + 3 H_2 --***Bacteria***--> C_2H_4 (**ethene**) + 3 Cl^- + 3 H^+

Implementation of the EOS™ process involves on-site preparation of the emulsion and injection of the emulsion into the treatment zone. The EOS™ can be injected into "hot spots", throughout the plume or as a permeable reactive barrier using conventional wells or direct-push injection points. All materials used in the process are "Generally Recognized as Safe", food-grade materials (21 CFR 184.1400) which typically facilitates obtaining regulatory approval for *in situ* application. The amount of EOS™ injected into the subsurface is determined based on the concentrations of the target compounds, the concentrations of various biodegradation and geochemical parameters, and the geologic and hydrogeologic conditions.

Site Description

The Air Force Center for Environmental Excellence (AFCEE) sponsored a field pilot study at Altus Air Force Base (AFB) in Altus, Oklahoma to evaluate the use of emulsified oil for stimulating *in situ* anaerobic bioremediation of chlorinated solvents. Historical solvent releases of degreasing agents at Altus AFB resulted in a 5,000-ft long chlorinated solvent plume with

TCE concentrations reaching 78,000 µg/L in the source area. Geology at the site consists of reddish-brown, moderately plastic, sandy clay to a depth of roughly 15 feet below ground surface (ft bgs), underlain by fractured clayey shale with occasional gypsum layers. The depth to groundwater is approximately 8 to 10 ft bgs. Most groundwater flow and contaminant transport appears to occur through a series of weathered shale fractures located immediately beneath the surficial clay and within a thick gypsum layer approximately 35 ft bgs. Field observations suggest a groundwater velocity approaching 100 ft/year.

Substrate Preparation and Injection

The area selected for the pilot study was approximately 250 ft downgradient from the source area. A line of six permanent 2-inch polyvinyl chloride (PVC) wells spaced 5 ft apart was installed perpendicular to groundwater flow, and a series of monitoring wells and soil gas monitoring points were installed upgradient and downgradient of the injection wells to allow monitoring of the pilot study. Figure 1 shows the layout of the pilot test area.

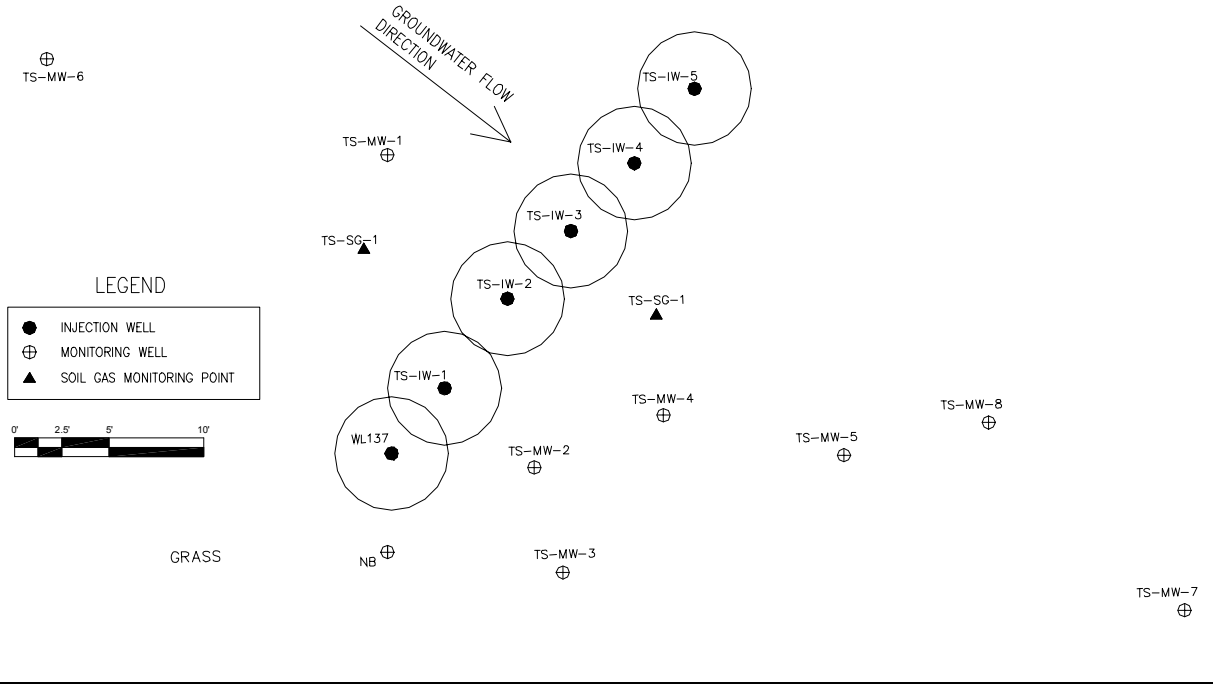


Figure 1. Layout of Injection Wells Forming the Permeable Reactive Barrier and Monitoring Wells

Over a 4-day period in December 2001, a mixture of emulsified soybean oil, lactate and yeast extract was injected through each well to form a 30-ft wide EOS™ permeable reactive barrier that would stimulate reductive dechlorination. Each injection was designed to treat a 6-ft diameter area to provide a small overlap between adjacent injection points. To achieve maximum distribution of the treatment mixture in the upper weathered fracture zone, the wells were screened from 8 to 18 ft bgs. A total of approximately 760 gallons of emulsion was injected consisting of approximately 1,270 lbs of soybean oil, 266 lbs of emulsifier composed of glycerol monooleate and polysorbate 80, 26 lbs of lactate and 9.8 lbs of yeast extract. Significantly more emulsifier was used in the field than required to form a stable emulsion. However, excess surfactant was available and was used to simplify the injection process. All

emulsifiers used were readily biodegradable and, as such, served as additional active substrate for reductive dechlorination. Injection of the emulsion was followed by injection of approximately 800 gallons of water to help distribute the emulsion throughout the treatment zone.

Substrate Distribution

Visual observations and measurements of total organic carbon (TOC) were used to evaluate the distribution of the emulsified oil in the subsurface. During the injection process, emulsified oil was observed more than 20 feet downgradient from the injection points at monitoring well TS-MW-5. Figure 2 shows the distribution of TOC, sulfate and chlorine number, as described below, in the pilot test area on December 18, 2001 (1 day after injection) and January 15, 2003 (13 months after injection).

To aid in evaluating the effects of formation permeability on emulsion distribution, wells were classified as having low, medium, and high conductivities. "Low" represents hydraulic conductivity values of 15 to 40 ft/year; "Medium" is between 80 and 150 ft/year; 'High" is over 500 ft/year. Because slug tests were only performed on selected wells, other field observations were used to provide a qualitative indication of hydraulic conductivity in every well in the pilot test area. Data used in this evaluation included observations from well development activities, flow rates recorded during injection, and visual observations during drilling. Results of this evaluation are shown in Figure 2.

As shown in the figure, immediately after injection, the injection wells had between 7,200 and 33,000 mg/L TOC and elevated TOC levels were observed as far as 20 feet downgradient in well TS-MW-5 (2,200 mg/L). However, some of the monitoring wells closer to the injection points did not show substantial increases in TOC. As expected, the emulsion distribution is highly dependent on the *in situ* permeability distribution. In higher permeability areas, emulsion can be distributed over 20 feet away from the injection points.

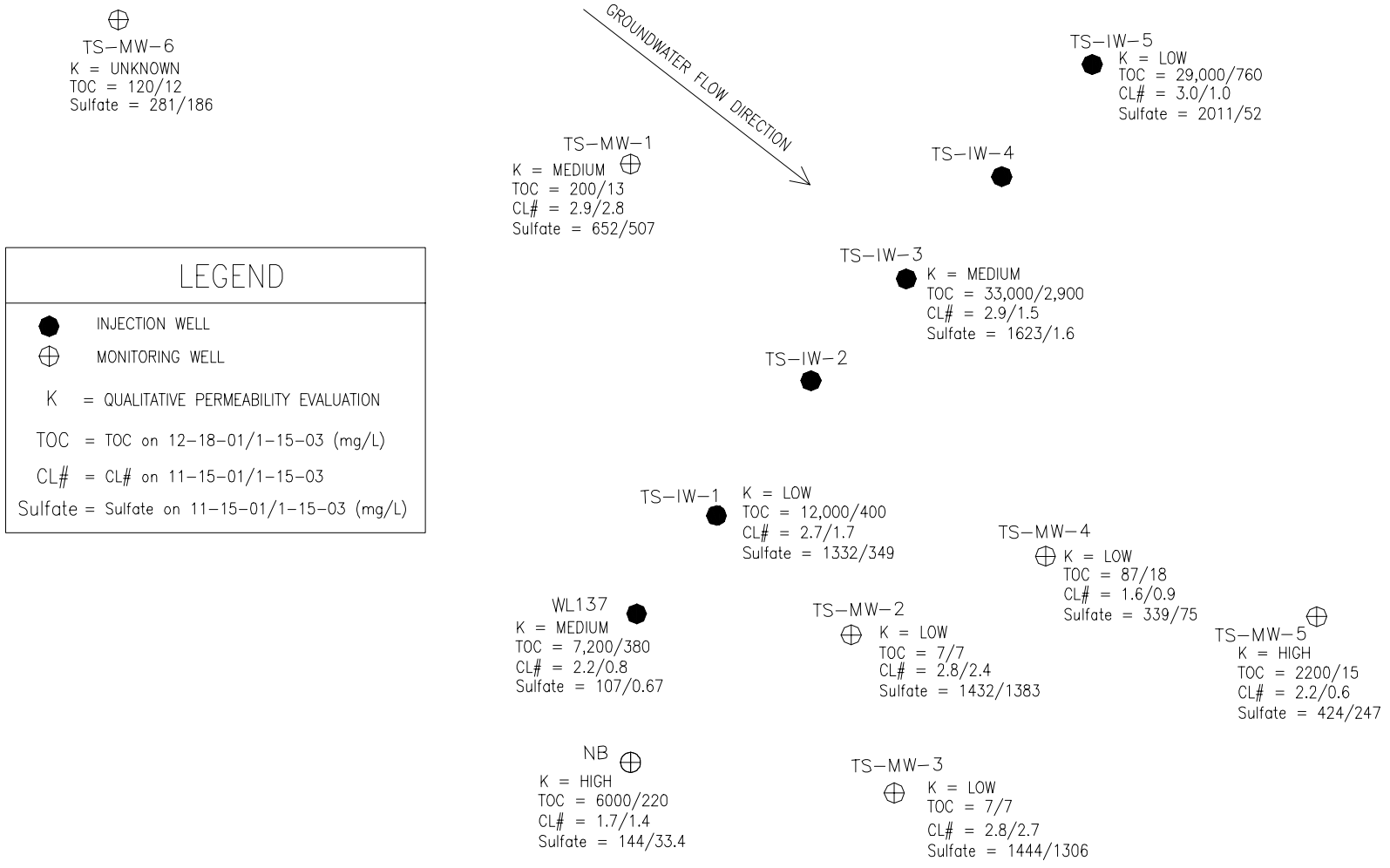
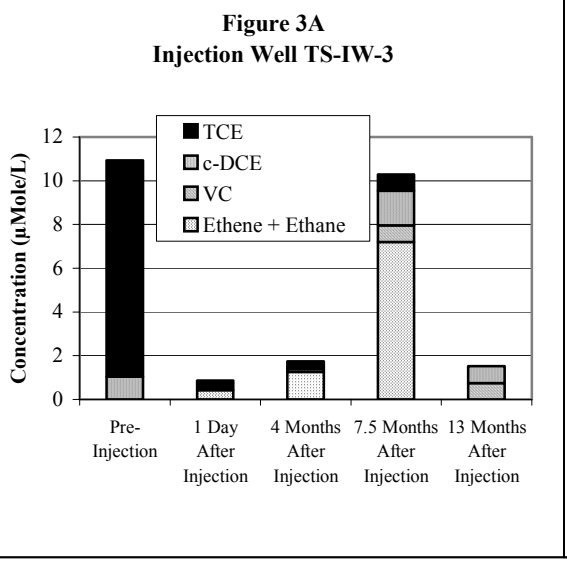


Figure 2. Relative Hydraulic Conductivity, Total Organic Carbon, Sulfate and Chlorine Number throughout Pilot Test Plot

Chlorinated Aliphatic Hydrocarbon Results

The analytical monitoring results from the Altus AFB pilot study show that emulsion injection is effective in stimulating reductive dechlorination processes. TCE concentrations dropped immediately after injection, as illustrated by the data from injection well TS-IW-3 (Figure 3A). Although the concentrations of total ethenes [TCE, *cis*-1,2-dichloroethene (*cis*-DCE), vinyl chloride (VC), ethene and ethane] initially decreased, these temporary reductions were likely due to dilution and/or sorption to the oil. As Figure 3A illustrates, approximately 7.5 months after injection, the concentration of total ethenes (molar concentration) was more than 90 percent of the pre-injection TCE concentration. This demonstrates that dilution/sorption was no longer significant and that the observed reductions in contaminant concentrations were due to biodegradation. Over the 13-month interval since EOS™ injection, TCE has declined from 9.9 µM/L (1,300 µg/L) to below the detection limit (BDL) in the center injection well.



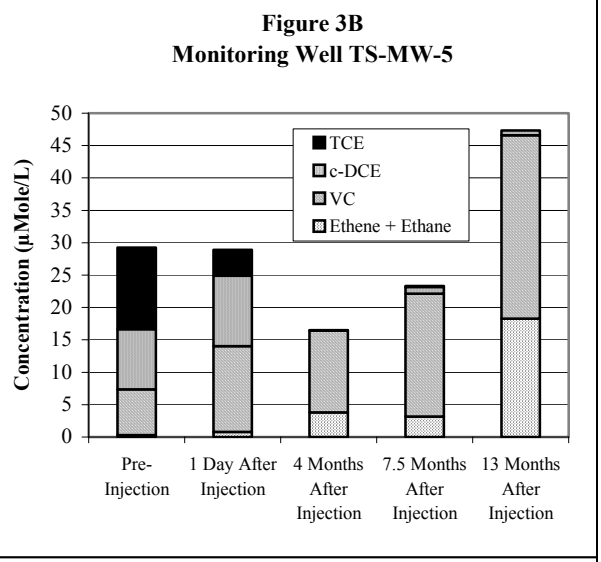


Figure 3. Concentration of TCE and Biodegradation Daughter Products in Injection Well TS-IW-3 (Figure 3A) and Monitoring Well TS-MW-5 (Figure 3B) before and after Injection of Edible Oil Substrate.

Similar results were observed in monitoring well TS-MW-5, 20 feet downgradient of the barrier (Figure 3B). Emulsion reached TS-MW-5 immediately after injection, as evidenced by a rise in TOC to 2200 mg/L one day after application. Post-injection monitoring over 13 months has shown that TCE decreased from 12.6 µM/L (1,660 µg/L) to BDL and *cis*-DCE from 9.3 to 0.75 µM/L (900 to 73 µg/L). There has been a concomitant increase in VC from 7.0 to 28.3 µM/L (440 to 1,770 µg/L) and ethene from 0.25 to 18.2 µM/L (6.9 to 510 µg/L). The increase in total ethenes (molar concentration) in this well may be a result of enhanced desorption/dissolution as dissolved TCE is removed through enhanced reductive dechlorination. Although TOC has substantially decreased from the starting concentration, the continuous downgradient migration of dissolved TOC from areas closer to the injection barrier would be expected to support additional reduction of VC to ethene and ethane.

Chlorine number is another approach for evaluating the effect of anaerobic biotransformation processes, particularly the extent to which sequential degradation of PCE or TCE is occurring. Groundwater containing only TCE would have a chlorine number = 3.0. However, if half of the TCE is reduced to DCE, the chlorine number would decline to 2.5. Chlorine number is calculated as:

$$\text{Chlorine number} = \frac{4\,[\text{PCE}] + 3\,[\text{TCE}] + 2\,[\text{DCE}] + [\text{VC}]}{[\text{PCE}] + [\text{TCE}] + [\text{DCE}] + [\text{VC}] + [\text{ethene}] + [\text{ethane}] + [\text{acetylene}]}$$

where [ ] indicates concentration in moles per liter. When calculating the chlorine number, we have assumed non-detect measurements are equal to zero and that ethene, ethane and acetylene are stable under reducing conditions. The change in chlorine number to <1.0 suggests complete transformation from chlorinated parent molecules to non-chlorinated, non-toxic, end products.

Chlorine number values for the pre-injection monitoring event (November 15, 2001) and the January 2003 monitoring event (13 months after injection) are presented on Figure 2. There was a substantial decline in chlorine numbers in all of the injection wells following emulsion injection. In contrast, there was no significant change in chlorine number in upgradient monitoring well TS-MW-1. In the downgradient monitoring wells, the results were more variable. In TS-MW-5, the chlorine number dropped from 2.17 prior to injection to 0.63 in January 2003 indicating substantial conversion of TCE to lesser-chlorinated compounds. However, in downgradient monitoring wells TS-MW-2 and TS-MW-3 there was no substantial change in chlorine number with time.

The degree of biodegradation is dependent on distribution of emulsion in the aquifer, which is dependent on the aquifer's permeability. In locations of higher permeability where fluids would preferentially flow, a substantial increase in reductive dechlorination processes was observed. In areas with low permeability which would restrict fluid flow, there is no significant enhancement of reductive dechlorination. This effect is illustrated in Figure 2. In wells with TOC > 10 mg/L, the chlorine number is reduced to less than 2.0. However, when TOC is <10 mg/L, chlorine number remains high and there is little evidence for significant reductive dechlorination. This is true whether the well is upgradient, downgradient, or within the barrier.

Bioparameter Results

A variety of bioparameters were monitored over the course of the pilot study to evaluate the effects of emulsion injection to create conditions conducive for reductive dechlorination.

Dissolved oxygen (DO) is used by microbes as an electron acceptor for the biodegradation of organic carbon. The emulsified oil provides a source of carbon for aerobic microbes to metabolize, in turn depleting dissolved oxygen concentrations and creating anaerobic conditions favorable to enhanced reductive dechlorination. Pre-injection DO levels varied widely across the pilot test area. An average DO of 0.82 mg/L was calculated from injection wells WL-137, TS-IW-1 and TS-IW-3 and monitoring wells NB, TS-MW-3 and TS-MW-2 in the south-southwest part of the plot. By contrast, the average DO was 3.9 mg/L in the northern-most injection wells TS-IW-5 and TS-IW-4 and the most eastern monitoring wells, TS-MW-4 and TS-MW-5. The

introduction of organic substrate to the aquifer caused a DO response that was observable 1 day after injections were completed. DO concentrations ranged from 0.08 to 0.36 mg/L in injection and monitoring wells with measurable increases in TOC with DO concentrations generally lower in wells with lower starting concentrations. Since injection, anaerobic conditions have persisted at the site.

The presence of methane above background conditions indicates microbial degradation (methanogenesis) is occurring and conditions are favorable for reductive dechlorination. Methane concentrations have generally increased in the injection and monitoring wells since injection of the oil emulsion. Methane levels above 7,000 µg/L were observed in every injection well sampled in July 2002. Elevated methane levels have also been observed in most of the downgradient monitoring wells, but not in the shallow (5 ft bgs) soil gas monitoring points above the water table.

Substantial amounts of dissolved and solid-phase sulfate are present at Altus AFB. Sulfate can reduce the effectiveness of reductive dechlorination by: (1) competing for available H_2, reducing the rate and extent of reductive dechlorination; (2) producing toxic levels of sulfide that could inhibit reduction dechlorination processes; and (3) accelerating the biodegradation of soybean oil, requiring more frequent emulsion injection. Pre-injection sulfate concentrations as high as 2,011 mg/L were detected in the pilot test area wells. As shown in Figure 4, sulfate levels have dropped dramatically in the wells that were impacted by the emulsion injection (TS-IW-3 and TS-MW-5), and have remained relatively unchanged in wells that were not impacted by the emulsion (upgradient well TS-MW-1 and low permeability well TS-MW-3). Pre- and post-injection sulfate data are also displayed on Figure 2, which illustrates that areas impacted by the emulsion displayed increases in TOC and corresponding decreases in both sulfate and chlorine number.

These data show that competition for available H_2 by sulfate reducers has not inhibited reductive dechlorination processes. Many of the wells have evidence of a black precipitate suggesting that free sulfide is being precipitated with soluble iron as ferrous mono- or di- sulfide, thus preventing accumulation of inhibitory levels of dissolved sulfide. Ferrous mono- sulfide and di- sulfide can abiotically react with TCE, yielding acetylene and other reduced ethenes. Although low levels of acetylene have been detected, the abiotic reaction does not seem to be the dominant TCE removal mechanism at this site.

Figure 4

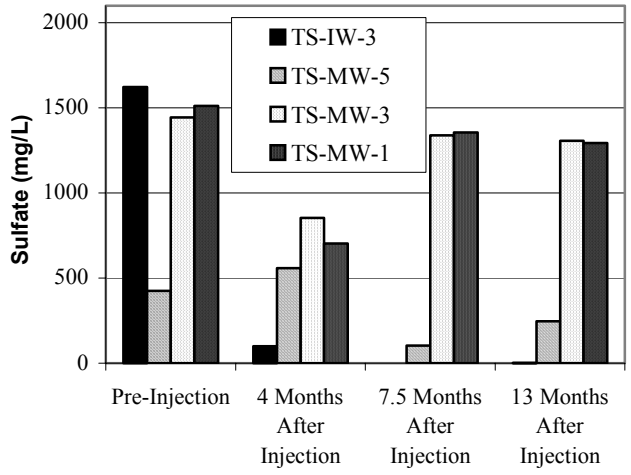


Figure 4. Sulfate concentrations in one injection well and three monitoring wells before and after injection with edible oil substrate. Sulfate was < 2 mg/L in TS-IW-3 at 13 months.

Continued monitoring will be necessary to verify whether the high levels of sulfate in the aquifer accelerate the consumption of the emulsion.

Effect of Emulsion Injection on Permeability

Slug-in and slug-out hydraulic conductivity tests were conducted in the pilot test wells before and after injection of the emulsion to evaluate changes in the aquifer permeability. Pre-emulsion injection hydraulic conductivities varied from 0.02 ft/day to 2.8 ft/day over the approximately 50-ft by 50-ft test area. Emulsion injection did not have a significant impact on the hydraulic conductivity of the injection wells or monitoring wells. In WL-137, which was treated with emulsion, the pre-injection hydraulic conductivity values were 0.34-0.45 ft/day while the post-injection values were 0.20-0.45 ft/day. Similar results were obtained in other injection and monitoring wells that had hydraulic conductivity tests conducted before and after emulsion injection.

Soil Gas Monitoring

Because the pilot test was conducted within the upper unconfined unit, we evaluated the potential for accumulation of methane and other volatile gases in the unsaturated soils overlying the aquifer. Two dedicated soil-gas monitoring points were installed to a depth of 5 ft bgs in the pilot test area to allow monitoring of accumulated volatile organic compounds (VOCs). The headspace of the monitoring points was monitored in the field for percent lower explosive limit (LEL), percent oxygen, hydrogen sulfide, and carbon monoxide using a VRAE monitor. While both soil gas monitoring points had low oxygen readings, neither had detectable LELs. No elevated LEL readings were noted at the surface. This suggests that the methane is being consumed aerobically before it reaches the surface. Neither hydrogen sulfide (H_2S) nor carbon monoxide (CO) was detected in the headspace of the soil gas monitoring points.

Longevity

The longevity of the emulsion in the subsurface is important to achieve continued reductive dechlorination. If the edible oil emulsion biodegraded too rapidly, then the design life of the barrier is reduced and re-injection could be necessary to reduce contaminant concentrations to the desired levels. The barrier at Altus AFB continues to release desirable amounts of organic carbon both within the barrier and to downgradient monitor wells. Approximately 13 months after injection, the TOC in the injection wells was between 850 mg/L and 7,300 mg/L and the TOC at monitoring well TS-MW-5 located 20 feet downgradient of the barrier was over 15 mg/L. Monitoring will continue to evaluate the longevity of the single emulsion injection.

Conclusions

The overall conclusion from the SS-17 pilot test is that addition of slowly biodegradable organic carbon in the form of a soybean oil-in-water emulsion can enhance reductive dechlorination. Although ferrous sulfide and ferrous disulfide have been produced in the vicinity of the barrier at concentrations between six and nine times greater than observed at a background location, there is little evidence for dechlorination via the abiotic pathway leading to acetylene.

Biological enhancement is dependent on the distribution of emulsion in the aquifer. Where contaminated groundwater came immediately in contact with the soybean oil emulsion, we observed a substantial increase in reductive dechlorination processes. This includes both the barrier injection wells and downgradient monitoring wells. In these locations, chlorine numbers generally declined providing strong evidence for significant reductive dechlorination.

Costs

The costs for the tasks involved in the design and implementation of the pilot-scale study are discussed below. Because this test was prepared as a research and development effort, the higher than average costs reflect the expanded effort to collect detailed scientific and engineering data to evaluate the performance of the oil emulsion barrier. On a commercial scale, a significantly reduced pilot test could provide preliminary design information sufficient for a full-scale remedial effort. The cost elements associated with each task at Altus AFB are discussed below:

Work Plan and Barrier Design ($30,000) and Draft Interim Report ($28,700). The work plan and engineering design included evaluation of extensive pre-existing site data provided by others, a preliminary site visit and injection test, preparation and in-house testing of alternate emulsion mixes, and writing a detailed Quality Assurance Project Plan (QAPP) and Health and Safety Plan (HASP). At the end of the performance monitoring period, a thorough and detailed Draft Interim Report was prepared summarizing the data acquired from pre- and post injection sampling activities.

Injection and Monitor Well Installation ($37,000). Six groundwater injection wells were installed 5 ft on center creating a 30-foot long barrier. Each injection well was screened from 8 to 18 ft bgs to intersect contamination in the shallow aquifer above the confining layer. Eight groundwater monitoring wells were also installed within 40 feet of the barrier and one vadose zone soil-gas monitor well was installed on either side of the barrier. Despite the relatively shallow depth of the test (i.e., less than 18 ft bgs), installing wells through the clay and into the weathered shale precluded direct push technologies such as Geoprobe®. Installing permanent injection and monitoring wells using hollow stem auger drilling methods served to provide long-term sampling points for increased data acquisition and evaluation of the pilot-test results. The unit cost per well installed using hollow stem auger drilling methods was $2,300 to $2,500 per well.

Emulsion Preparation and Injection ($24,300). The entire process of preparing the emulsion in the field, injecting it and completing the water chase required 4 days to accomplish. The materials and installation costs are summarized in the following table:

Costs for Installation of Oil Emulsion Barrier at Altus AFB			
	Oil Emulsion Substrate (~1,600 lbs)	**Preparation and Injection of Substrate**	**Total**
Total Cost	$1,300	$23,000	$24,300
Per Injection Well (6 wells)	$215	$3,830	$4,045
Per Linear Ft (30 linear ft)	$43	$767	$810
Per Sq Ft (300 sq ft)	$4.30	$77	$81.30

Performance Monitoring ($52,800). Performance monitoring has included both groundwater and permeability testing with concomitant data evaluation at each of four sampling events performed over the first 13 months of the project. Six injection wells and eight monitor wells were sampled in accordance with the work plan. In addition, slug tests were performed on four wells during each event. Analytical costs represent almost 28 percent of the cost for each sampling event.

Summary and Estimate of Full Scale Costs: The cost for implementing the research and development pilot-project, up to and including 13 months of field evaluation, is $172,800. The information gained is directly applicable for scale up to a full size barrier.

The installation of two staggered 400-ft barriers approximately 20 feet apart (assumed coverage needed at Altus AFB) would incur certain fixed costs including design, work plan and report preparation, that would likely be of similar, or slightly lesser magnitude, than discussed above. Performance monitoring costs would be included in compliance monitoring using pre-existing monitoring wells downgradient of the barriers. Analysis of a few additional parameters in these wells would serve to confirm that the remediation was performing as designed.

Based on the pilot-test information, temporary injection wells could be used and the injection interval could be extended to 10 ft on center. With these changes, unit drilling costs would decrease to approximately $1,100 per well resulting in well installation and abandonment costs of $88,000 for 80 injection wells. Costs for substrate would increase incrementally to $34,300, but costs for injection would be expected to drop to approximately $350 per linear foot as simultaneous injections of multiple wells would decrease time on site. Thus, field costs to install two 400-foot barriers are estimated to be $405,000, or approximately $500 per linear foot of barrier.

APPENDIX E.7 – PILOT-SCALE MULCH BIOWALL, BUILDING 301, OFFUTT AFB, NEBRASKA

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Pilot-Scale Mulch Biowall, Building 301, Offutt AFB, NE

Carol E. Aziz, Ph.D., P.E. and Mark R. Schipper, P.G. (Groundwater Services, Inc.) and Jerry Hansen (Air Force Center for Environmental Excellence)

Case Study

In January of 1999, a pilot-scale in situ mulch biowall was installed at Site Building 301, Offutt Air Force Base (AFB), NE (Groundwater Services (GSI), 2001, Aziz et al., 2001). The 100-ft long, 1-ft wide, and 23-ft deep biowall was constructed using a one-pass trencher and filled with a 50:50 by volume mixture of mulch and sand. The mulch was used as an organic substrate to stimulate reductive dechlorination of the trichloroethylene (TCE) plume.

The main goal of the pilot test was to evaluate the efficacy of mulch to promote the reductive dechlorination of TCE and its daughter products, cis-1,2-dichloroethene (cDCE) and vinyl chloride (VC). The mulch served as an organic substrate to create anaerobic conditions in the aquifer. Once anaerobic conditions were achieved, fermentation of soluble organic substrates derived from the mulch produced hydrogen, which served as the primary electron donor for reductive dechlorination.

Over a 31 month period, the mulch biowall was found to remove 75% of a TCE plume with concentrations ranging up to 1.9 mg/L and 64% of the total chlorinated ethenes passing through the wall. Reductive dechlorination was responsible for some of the removal as evidenced by the production of daughter products, such as cDCE, shortly after installation, but other mechanisms were at play to account for the extent of TCE removal.

Remedial Objectives:

The test was conducted to evaluate the effectiveness of mulch as an organic substrate to stimulate the reductive dechlorination of TCE and its daughter products, cDCE and VC. Other objectives include the development of operation and cost data for a full-scale system.

Site History/Source of Contamination/ Geology/Hydrogeology/Contaminant Distribution/Site Selection Criteria

Site History/Source of Contamination

Offutt AFB is located approximately 5 miles south of Omaha, Nebraska. Building 301 is located in the eastern part of the Base, approximately 4300 ft from Papillion Creek.

The source of TCE contamination is thought to originate from beneath the northwestern corner of Building 301. The TCE stems from manufacturing operations conducted from 1942 to 1965. The plume extends westward approximately 2800 ft from the suspected source area.

Geology/Hydrogeology

The pilot test was conducted 1400 ft downgradient of Building 301. In this area, the subsurface soil material consisted of approximately 1 to 3 feet of fill, overlying either a stiff, black, low plastic, silty clay (topsoil) or a stiff to very stiff, light to reddish brown, low plastic, silty clay (Peoria and Loveland Loess). The depth to groundwater was 6 ft below ground surface (bgs).

The groundwater flow was predominantly westward. The hydraulic conductivity in the alluvial silt and clay near the biowall averaged 3.5 ft/day with a hydraulic gradient of 0.01 ft/ft. Using an assumed effective porosity of 0.15, the computed groundwater seepage velocity was 0.23 ft/day or 85 ft/yr.

Contaminant Distribution

Chlorinated aliphatic hydrocarbon compounds were the primary contaminants of concern in the groundwater. Near the proposed pilot biowall location, TCE ranged from 0.11 to 1.9 mg/L, cDCE ranged from <0.001 to 0.27 mg/L, and VC concentrations ranged from <0.001 mg/L to 0.0025 mg/L. No ethene or ethane was detected in any samples.

Site Selection Criteria

The area 1400 ft downgradient from Building 301 was utilized for the mulch biowall pilot test on the basis of: i) the presence of TCE and degradation products (e.g., cDCE), indicating the presence of dechlorinating bacteria and ii) shallow depth to groundwater (6 ft bgs) to facilitate the installation of the biowall using a one-pass trencher. In addition, Offutt Air Force Base had a ready supply of mulch as a result of a recent storm event.

Technology Description (Design and Operation)

Mulch was used in this pilot test as a source of fermentable organic carbon. Once emplaced in situ, soluble organic matter was leached into the groundwater, where it served as a source of organic carbon for aerobic bacteria. Consumption of the organic material by aerobic bacteria lowered the dissolved oxygen in the aquifer, thereby stimulating anaerobic conditions conducive to fermentation. Fermentation of organic substrates produced hydrogen, which could be used by dechlorinating bacteria for reductive dechlorination. Because mulch is a solid organic substrate, it was best emplaced in situ as a permeable reactive wall.

Using a one-pass trencher, the 100 ft-long by 1-ft wide biowall was installed to a depth of 23 ft to intercept the most contaminated portion of the groundwater plume. The biowall was simultaneously installed and filled to 2 ft below the surface with the mulch-sand mixture. The soil removed from the biowall was deemed non-hazardous and used to cap the biowall.

The fill consisted of a 1:1 by volume mixture of mulch and coarse sand (approximately 850 ft^3 mulch and 850 ft^3sand). The mulch was generated as part of a severe storm cleanup effort. Fallen tree limb and trunk material was passed through a tub grinder and stockpiled. The mulch contained partially composted leaf and twig material as well as some fine wood chips. The

mulch was mixed with sand using a backhoe. The sand was added to minimize settling and to increase the permeability of the wall relative to the surrounding formation. No settling of the biowall has been observed 4 years after biowall installation.

Four 2-inch polyvinyl chloride (PVC) monitoring wells were installed downgradient of the mulch biowall to a depth of 20 ft bgs. All wells were screened between 10 and 20 ft bgs. The downgradient wells were positioned at 10 and 20 ft intervals as shown in Figure 1. Existing wells (located 15 ft upgradient of the biowall) were used as the upgradient wells to monitor untreated ground water.

Two additional monitoring wells were installed, within the contaminated plume area and cross-gradient from the biowall, to act as control wells. Samples taken from these wells were used to compare the rate and extent of chlorinated solvent degradation due to natural attenuation versus mulch addition. A mulch surface amendment was also evaluated, but is not discussed in this case study (GSI, 2001).

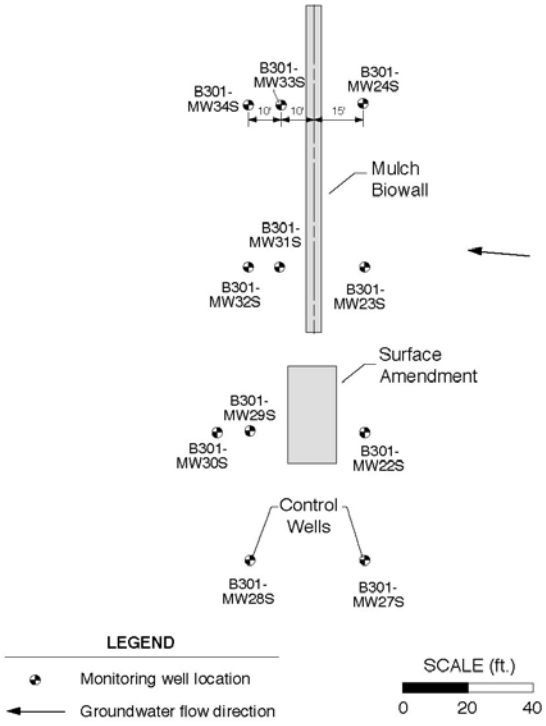

Figure 1. Layout of Monitoring Well Network

Technology Performance

Contaminant Concentration Data vs. Time

Comparison of TCE concentrations upgradient and downgradient of the biowall demonstrate that the mulch biowall effected a significant removal of TCE, as shown in Figure 2. Mean upgradient TCE concentrations ranged from 2 μmole/L (μM) to 16 μM (0.3 to 2.1 mg/L), while mean TCE

concentrations ranged from 0.9 μM to 6 μM (0.1 to 0.5 mg/L) 10 ft downgradient and 1.6 μM to 7.5 μM (0.2 to 0.6 mg/L) 20 ft downgradient of the biowall. Because of the variability of upgradient TCE concentrations, mean concentrations were determined over time. The mean upgradient concentration was 10 μM (1.3 mg/L) and the mean TCE concentration 10 ft downgradient was 2.5 μM (0.33 mg/L), with the biowall removing an average of 7.5 μM (0.97 mg/L). Using a seepage velocity of 85 ft/yr, a width of 100 ft and a depth of 17 ft (amount of the biowall in the saturated zone), approximately 31 moles/yr or 4 kg/yr of TCE were removed by the biowall.

The mulch biowall enhanced reductive dechlorination as demonstrated by the production of daughter products (i.e., cDCE, VC, ethene, and ethane) downgradient of the biowall as shown in Figures 2 through 7.

During the first sampling event at 5 months following biowall installation, the concentration of cDCE increased 45 fold as a result of passing through the biowall. The presence of cDCE downgradient of the biowall was evidence that water was passing through the wall. After 5 months, the amount of cDCE declined, although TCE continued to be removed (Figures 2 and 3). Some of the cDCE was being converted to VC, ethene, and ethane, but much of the decline could not be accounted for by the conservation of mass of the reductive dechlorination end-products.

VC, produced during reductive dechlorination of cDCE, is a carcinogen and generally degrades more slowly than the other chlorinated constituents via reductive dechlorination (Vogel et al., 1987). In this study, small amounts of VC were produced but the concentrations were less than 0.053 μM (3 μg/L). If reductive dechlorination was the only biodegradation mechanism, then VC would be expected to accumulate. Low concentrations of VC may be attributed to rapid degradation of VC by aerobic mineralization (Hartmans et al., 1985) or cometabolism (Vogel, 1994) in aerobic microenvironments. Some VC and cDCE may have been converted to carbon dioxide where methane concentrations were higher, as humic acids found in mulch can act as electron acceptors for the anaerobic microbial oxidation of VC and dichloroethene (Bradley et al., 1998).

The final reduction products of TCE are ethene, and ethane. The production of ethene and ethane increased with time during the first year of operation, suggesting the growth or adaptation of bacteria capable of reductively dechlorinating VC (Figures 5 and 6). Production of ethene and ethane also corresponded to decreasing sulfate levels and increasing methane concentrations in the aquifer.

Because the TCE concentration in the incoming groundwater changed significantly over the course of 31 months, the use of the ratio of cDCE:TCE was informative. This ratio gave an indication of the extent of reductive dechlorination. Upgradient wells showed mean cDCE:TCE ratios of 0.02, while downgradient wells had cDCE:TCE ratios as high as 1.4, as shown in Fig. 7.

Performance Data

The mean percent removals of TCE and total chlorinated ethenes are shown in Table 1 for both the biowall and the control plot. By averaging the upgradient concentrations and concentrations 10 ft downgradient, a mean percent TCE removal of 74.6% was calculated. This compared

favorably with the natural attenuation control plot that showed an average increase of 20% in TCE concentrations. By subtracting the mean total molar concentration of chlorinated constituents downgradient from the mean total molar concentrations of chlorinated constituents upgradient of the biowall, the percent removal of total chlorinated ethenes was calculated. The mean percent removal of chlorinated ethenes was 63.7%, while the control plot had a mean percent increase of 12%. Overall, the mulch biowall achieved significantly greater reductive dechlorination than natural attenuation alone. The amount of complete dechlorination (that is the amount of dechlorination that can be accounted for by ethene and ethane) was only 5%. The percent TCE and total chlorinated ethene removals and dissolved oxygen concentrations 10 ft downgradient of the biowall during the course of the test are shown in Figure 8.

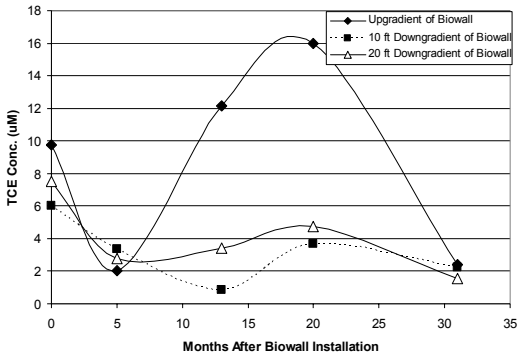

Figure 2. TCE Conc. vs. Time

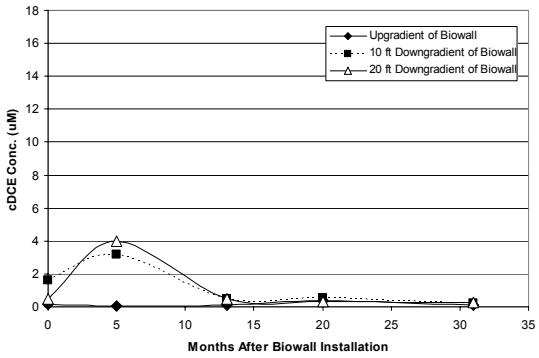

Figure 3. cDCE Conc. vs. Time

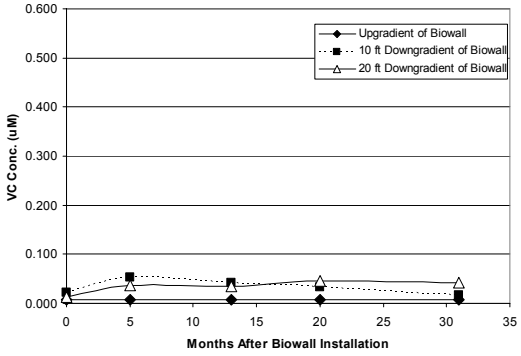

Figure 4. VC Conc. vs. Time

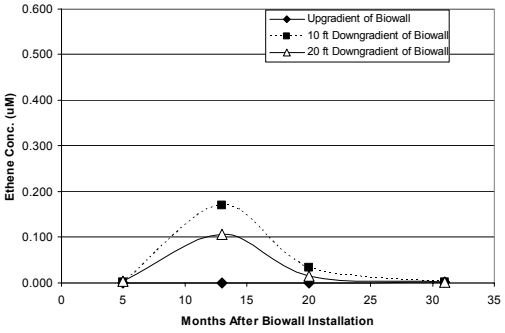

Figure 5. Ethene Conc. vs. Time

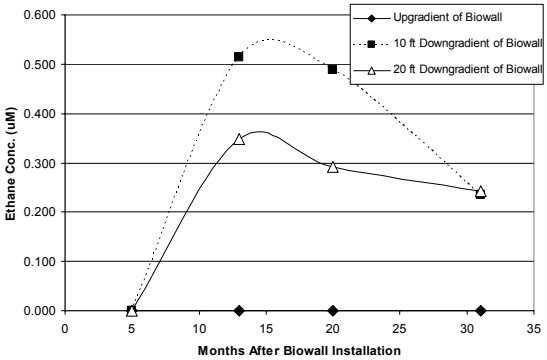

Figure 6. Ethane Conc. vs. Time

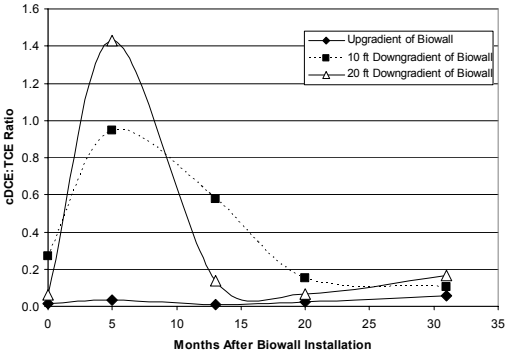

Figure 7. cDCE:TCE Ratio vs. Time

To shed some light on the mechanisms of removal, the change in total constituent concentration upgradient and downgradient of the biowall was examined. Only 40% of the original 10.1 μM of parent and daughter products was found downgradient. Therefore, 60% of the upgradient constituents was unaccounted for. Because of the production of daughter products such as cDCE and VC, it was clear that reductive dechlorination was occurring. The low molar balances indicate that several other mechanisms are at work. cDCE and VC may have been oxidized to carbon dioxide under anaerobic conditions, or aerobic microenvironments may have stimulated the aerobic biodegradation of VC, ethene, and ethane. Lastly, it is possible that sorption of TCE and the daughter products occurred in the mulch biowall.

Table 1: Performance Data for Pilot Scale Biowall Over 31 Months of Operation

Constituent	BIOWALL			MNA CONTROL[1.]		
	Mean Upgradient Conc. (μM)	***Mean Conc. 10 ft Downgradient (μM)***	***% Change***	***Mean Upgradient Conc. (μM)***	***Mean Downgradient Conc. (μM)***	***% Change***
TCE	9.981	2.532	-74.6%	1.293	1.559	+20.6%
cDCE	0.169	1.116	--	0.119	0.045	--
VC	0.008	0.036	--	0.022	0.008	--
Ethene	0.001	0.052	--	0.094	0.029	--
Ethane	0.000	0.310	--	0.001	0.001	--
Total Chlorinated Ethenes	10.158	3.684	-63.7%	1.434	1.612	+12.4%
All Constituents	10.159	4.046	-60.2%	1.530	1.642	+7.3%
TCE to Ethene/Ethane	--	--	+4.8%	--	--	--

Notes:

1. Data were collected over the first 19 months only for the MNA control. Comparison of biowall performance at 19 and 31 months yielded similar results.
2. Mean upgradient biowall concentrations were determined by taking the average concentration in MW 23S and MW 24S over time, while mean downgradient biowall concentrations were determined by taking the mean concentration of MW31S and MW33S over time.

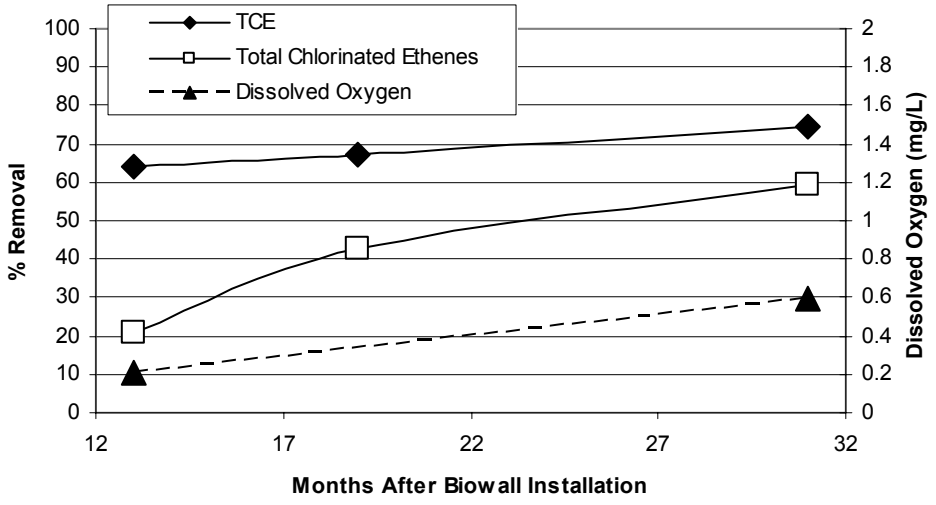

Figure 8. Percent Removals of TCE and Total Chlorinated Ethenes and Downgradient Dissolved Oxygen Concentration Over Time

Natural Attenuation Parameters

The natural attenuation parameter values upgradient and downgradient of the mulch biowall are presented in Table 2. The average parameter values are determined by taking the concentration of upgradient wells MW-23 and MW-24 and the mean concentration of downgradient wells MW 31, 32, 33, and 34.

After installation of the biowall, the aquifer became more anaerobic due to the consumption of oxygen and natural organic matter by aerobic bacteria. Dissolved oxygen concentrations measured 31 months after biowall installation in the downgradient monitoring wells were 42% lower relative to dissolved oxygen concentrations measured in upgradient monitoring wells prior to biowall installation. As oxygen and other alternate electron acceptors were consumed, the redox potential was expected to fall but there was no significant change in redox potential between upgradient and downgradient wells. The optimum redox potential for reductive dechlorination is –100 mV (Wiedemeier *et al*., 1999). Negative redox potentials were not measured in this test, although reductive dechlorination was observed. These higher redox potentials may be the result of mixing of groundwater from different redox zones during sampling or measurement inaccuracies in the redox probe.

Consumption of alternate electron acceptors, such as nitrate, ferric iron, and sulfate acted as additional evidence of reduced conditions as a result of the biowall installation. Nitrate and sulfate levels decreased downgradient of the mulch biowall, by 58% and 21%, respectively. Ferrous iron was present at concentrations below 0.2 mg/L and was not found to increase downgradient of the biowall.

In addition to nitrate and sulfate reduction, carbon dioxide was reduced to form methane through methanogenesis. Methane concentrations increased on average from 0.6 μg/L to 0.6 mg/L. Although methanogens can compete with dechlorinating bacteria for hydrogen, the presence of methanogenic bacteria and active methanogenesis did not preclude reductive dechlorination in this test.

Alkalinity levels, indicative of the production of carbon dioxide from microbial activity, increased 24% downgradient of the biowall. This result is consistent with the decrease in dissolved oxygen through aerobic bacterial activity and the production of carbon dioxide through fermentation processes stimulated by the addition of mulch to the aquifer.

Technology Cost

The attractiveness of this technology is the low cost of the mulch. In this particular case, mulch was obtained free of charge. Mulch can also be purchased for \$5 to 20/yd^3. Handling of the mulch may range up to \$10/yd^3.

A shallow mulch biowall installed using a continuous one-pass trencher will cost approximately \$140-360/linear foot, depending on the length of the biowall and the contractor. Shorter trenches are more expensive on a linear-foot basis. Mobilization and demobilization will add an additional \$20-40K. Biowalls, installed using a continuous one-pass trencher, are generally

Table 2: Natural Attenuation Parameters

Parameter	*Test Method*	*# Samples*	*Upgradient Wells Before Biowall Installation*			*# Samples*	*Downgradient Wells[3] 31 Months After Biowall Installation*			*Diff. of Avg.*	*%Diff.*
			Low	**High**	**Avg.**		**Low**	**High**	**Avg.**		
Dissolved Oxygen, mg/L	Field/Meter	8	0.22	2.24	1.19	4	0.42	1.08	0.69	-0.5	-42%
Redox Potential, mV	Field/Meter	10	127.2	255.3	192.0	4	84.3	313.8	193.8	+1.8	+0.9%
Nitrate as N, mg/L	EPA 300	10	<0.1	5.2	3.2	4	<0.1	2.35	1.35	-1.85	-58%
Ferrous Iron, mg/L	Hach kit	6	<0.2	<0.2	<0.2	4	<0.02[1]	0.448[1]	0.17[1]	--	--
Sulfate, mg/L	Hach kit	10	9	47	31.8	4	20	31	25.1	-6.7	-21%
Methane, µg/L	AM20GAX	8	0.0028	1.6	0.617	4	41	1600	612	+611.4	+99092%
Hydrogen, nM	AM20GAX	8	0.5	2.14	2.14	4	0.41[2]	0.94[2]	0.64[2]	-1.5	-70%
Alkalinity, mg/L	Hach kit	8	90	360	274	4	312	372	340	+66	+24%

Notes:
1. Ferrous iron was measured using EPA Method 6010B 31 months after biowall installation.
2. Hydrogen was not measured 31 months after biowall installation. Hydrogen data are presented for 19 months after installation
3. Downgradient wells include wells located 10 and 20 ft downgradient of the biowall.

limited to a depth of approximately 30 ft. However, DeWind Dewatering of Holland, MI recently reported installation of a 42 ft deep wall using a one-pass trencher and a 17-ft land bench. Deeper biowalls can be constructed using conventional excavation or bioslurry installation, which will increase the cost and installation time.

The benefit of a passive biowall is the low operating and maintenance cost. Once installed the biowall requires no energy or maintenance. Only monitoring of the groundwater is required. One unknown with respect to maintenance costs is the longevity or replacement frequency of the mulch. Over 31 months, no reduction in percent TCE or total chlorinated ethene removal was observed with the mulch biowall as shown in Figure 8. Other investigators have installed walls filled with a variety of waste cellulose solids for the treatment of nitrate-contaminated water and have found little reduction in performance during 7 years of operation (Robertson et al., 2000). Therefore, the mulch biowall can be estimated to last somewhere between 7 and 10 years.

Although mulch appears less effective in treating chlorinated constituents than zero valent iron, it is much cheaper than iron, which costs approximately \$350/ton or \$700/yd^3 (Peerless, Inc., personal communication). Therefore, a potential cost-effective application of the mulch biowall is as a pre- or post- treatment step, in conjunction with zero valent iron walls, to significantly decrease the amount of iron that is required to achieve clean-up objectives.

TABLE 3: COST REPORTING

COST CATEGORY	Sub Category	Costs ($)
FIXED COSTS		
1. CAPITAL COSTS	Mobilization/demobilization	$40 K
	Planning/Preparation	$10K
	Other - Non-Process Equipment - Installation - Engineering - Management Support	 $42K $22K
	Sub-Total ($)	**$114K**
VARIABLE COSTS		
2. OPERATION AND MAINTENANCE	Labor (Reporting)	$12K
	Materials and Consumables	
	Equipment Cost (if rental or lease)	
	Performance Testing/Analysis (5 events)	$55 K
	Sub-Total ($)	**$67K**
	TOTAL TECHNOLOGY COST	**$181K**
	Quantity Treated (kg TCE)	**10.3**
	Unit Cost ($/kg)	**$17.6K**
	Quantity Treated (1000 gallons)	**2795**
	Unit Cost ($/1000 gal))	**$65**

References

Aziz, C.E., Hampton, M.M., Schipper, M., and P. Haas. 2001. Organic Mulch Biowall Treatment of Chlorinated Solvent-Impacted Groundwater. *Proceedings of the Sixth International Symposium on In-Situ and On-Site Bioremediation, San Diego, California*. Volume 6(8), pp. 73-78. Battelle Press, Columbus, Ohio.

Bradley, P.M., F.H. Chapelle, and D.R. Lovley. 1998. Humic Acids as Electron Acceptors for Anaerobic Microbial Oxidation of Vinyl Chloride and Dichloroethene. *Appl. Environ. Microbiol*. 64: 3102-3105.

Groundwater Services, Inc.(GSI) 2001. *Final Report Mulch Biowall and Surface Amendment Pilot Test, Site Building 301, Offutt AFB, Nebraska*. Prepared for the Technology Transfer Division of the Air Force Center for Environmental Excellence. June 18, 2001.

Hartmans, S., J.A.M. de Bont, J. Tramper, and K. Ch.A.M Luben. 1985. Bacterial degradation of vinyl chloride. *Biotechnol. Lett*., 7(6)383-388.

Robertson, W.D., D.W. Blowes, C.J. Ptacek, and J.A. Cherry. 2000. Long-Term Performance of In Situ Reactive Barriers for Nitrate Remediation. *Ground Water. 38*(5):689-695.

Vogel, T.M. 1994. Natural bioremediation of chlorinated solvents. In *Handbook of Bioremediation*. Norris, R.D., R.E. Hinchee, R. Brown, P.L. McCarty, L. Semprini, J.T. Wilson, D.H. Kampbell, M. Reinhard, E.J. Bouwer, R.C. Borden, T.M. Vogel, J.M. Thomas, and C.H. Ward, Eds. Lewis Publishers, Boca Raton, FL, p201-225.

Vogel, T.M., C.S. Criddle, and P.L. McCarty. 1987. Transformation of halogenated aliphatic compounds. *Environ. Sci. Technol*. 21(8): 722-736.

Wiedemeier, T.H., M.A. Swanson, D.E. Moutoux, E.K. Gordon, J.T. Wilson, B.H. Wilson, D.H. Kampbell, P.E. Haas, R.N. Miller, J.E. Hansen, and F.H. Chapelle. 1998. *Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water*. U.S. EPA. EPA/600/R-98/128.

APPENDIX E.8 – PILOT-SCALE LOW-VOLUME HYDROGEN BIOSPARGING PROJECT, CAPE CANAVERAL, FLORIDA

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Pilot-Scale Low-Volume Hydrogen Biosparging Project, Cape Canaveral, Florida

Charles J. Newell and Carol E. Aziz (Groundwater Services, Inc.), Joseph Hughes (Rice University), Patrick Haas (Mitretek Systems, Inc.), and Jerry Hansen (Air Force Center for Environmental Excellence)

Introduction:

To enhance beneficial anaerobic processes for the purpose of bioremediation, numerous research groups have focused on methods to increase the supply of electrons to bacteria capable of dechlorinating chlorinated contaminants. Most researchers and technology developers have concentrated on adding an indirect electron donor (such as lactate, molasses, mulch, edible oil, or other carbon source) that is fermented by subsurface bacteria to produce hydrogen needed to sustain dechlorination reactions.

Low volume pulsed hydrogen biosparging allows for the direct delivery of the primary electron donor, hydrogen, which is introduced directly to the subsurface, eliminating the need for addition of fermentation substrates (Hughes et al., 1997; Newell et al., 1997; Newell et al., 1998; Fisher et al, 1999; Newell et al., 2000; and Newell et al., 2001). Hydrogen gas is added in short pulses during sparging, which improves mixing efficiency in groundwater. Relatively low volumes of gas are added to minimize breakthrough to the surface. This technology is most efficient at sites with dechlorinating bacteria already present at the site (i.e., a Type 1 or Type 2 chlorinated solvent site). In theory, this technology could be applied to Type 3 chlorinated solvent sites, as the hydrogen will be used to remove dissolved oxygen, nitrate, and sulfate from groundwater and make the treatment area anaerobic, but will be less efficient than applications at Type 1 or 2 sites.

Results from an 18-month low-volume pulsed hydrogen biosparging pilot test at Cape Canaveral Air Station, Florida show extensive biological dechlorination of solvents in a 30 x 30 ft (9.1 x 9.1 m) zone located 10 to 25 ft (3.1 to 7.8 m) below the water table in a sandy aquifer (GSI, 2000). The test zone was in or very near a dense non-aqueous phase liquid (DNAPL) source zone, as chlorinated ethene concentrations were very high (~300 mg/L). Hydrogen gas was pulsed into three sparge points at regular intervals (weekly for most of the test) to form residual hydrogen gas bubbles, which then dissolved to deliver donor directly to the test zone.

Remedial Objectives:

The overall objective of the project was to evaluate the efficacy of direct *low-volume pulsed-hydrogen biosparging* as a remedial method for stimulating *in situ* dechlorination of solvents.

Site Description:

Cape Canaveral Air Station is located on a barrier island along the Atlantic coast of Florida, separated from the Florida mainland by the Banana River. Launch Complex 15 is one of a series

of rocket launching facilities located along the easternmost edge of the Base, adjoining the Atlantic Ocean. Shallow groundwater at the site has been impacted by the release of chlorinated solvents, including trichloroethene (TCE), from historic equipment maintenance activity. The site is currently inactive and operations have been partially dismantled.

Geology/Hydrogeology

The near-surface soil/aquifer material at Launch Complex 15 consists of silica sand with some shell, and little clay or organic matter. The sand unit is continuous from the surface to the maximum explored depth of approximately 70 ft below ground surface (bgs), with some silt and clay lenses at depth. Groundwater is typically encountered at 6 - 7 feet bgs.

Groundwater beneath Launch Complex 15 flows north and east toward a man-made drainage canal located a few hundred feet beyond the central facility. The canal collects runoff and shallow groundwater flow from the Launch Complex and flows westward, ultimately discharging to the Banana River. The horizontal groundwater gradient within the uppermost groundwater zone is approximately 0.0007 ft/ft to the east and 0.0011 ft/ft to the north, based on June 1994 potentiometric surface measurements. The average hydraulic conductivity within the shallow groundwater zone has been estimated as 95 ft/day, based on slug test results for 15 shallow monitoring wells. Using this average hydraulic conductivity, a groundwater gradient of 0.0009 ft/ft, and an assumed effective porosity of 0.25, the computed groundwater seepage velocity is 0.34 ft/day (125 ft/yr).

Contaminant Distribution

Groundwater monitoring data for the Launch Complex 15 site indicate chlorinated aliphatic hydrocarbons, including tetrachloroethene (PCE), TCE, *cis*-1,2-dichloroethene (cis-DCE), and vinyl chloride (VC) to be present in the uppermost water-bearing unit. Contaminants found at the highest concentrations include TCE (maximum concentration in the test zone of 87 mg/L), cis-DCE (maximum concentration in the test zone of 370 mg/L), and vinyl chloride (maximum concentration in the test zone of 52 mg/L).

The presence of cis-DCE and vinyl chloride indicates that this is a Type 1 chlorinated solvent site, where indigenous microorganisms are dechlorinating the parent TCE compound to daughter compounds using anthropogenic electron donors at the site. The presence of on-going natural attenuation processes complicated the analysis of the hydrogen delivery system.

Technology Description (Design and Operation):

The hydrogen biosparging pilot test system at Launch Complex 15 utilized a 4-sparge point, 20-monitoring point well network as shown in Figure 1. Three hydrogen sparge points were used to inject hydrogen into the test zone. A total of six multi-level monitoring locations, each with three monitoring points at different depths, were used to evaluate changes in groundwater conditions close to (i.e., within 6 ft) the hydrogen sparge points. A total of 20 single-level monitoring wells were used to evaluate the change in the larger groundwater plume around the test zone. Gas was sparged into each well at different rates and amounts during the first part of the test. During the final year, most sparge pulses were at 10 to 12 standard cubic feet per minute (SCFM) per well for 10 minutes.

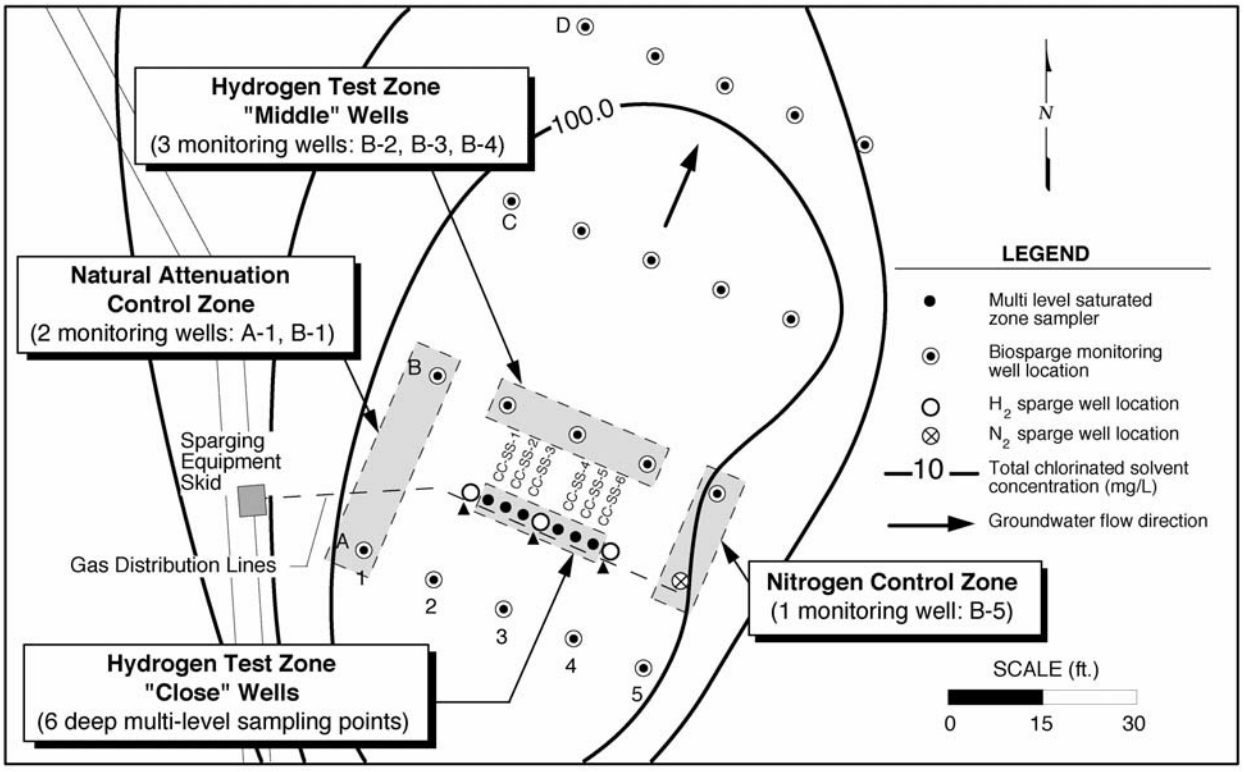

Figure 1. Pilot Test Layout. Monitoring Wells are Shown in Four Rows (A-D) and Five Columns (1 – 5). Six Multi-Levels Samplers are Shown Between H_2 Sparge Points.

Monitoring points were arranged in rows (A-D) perpendicular to the flow direction. The 30 ft spacing between individual rows was based on the 3 month estimated groundwater travel distance (i.e., seepage velocity = 10 ft/month). This was originally designed to allow for the collection of time-series data for a representative groundwater slug as it passed through the sparging zone and moved downgradient.

Two controls were originally designed into the experimental system. One additional sparge point, located on the southeast edge of the test zone, was used to inject molecular nitrogen gas as an inert control to evaluate physical removal processes (i.e., contaminant loss due to volatilization). Second, the wells on the southwest side of the system did not have a corresponding gas injection point, and were originally conceived as a natural attenuation (untreated) control to monitor any changes in baseline conditions. However, the final distribution of hydrogen gas in the subsurface during the course of the test (as indicated by helium tracer) made evaluation of these controls difficult, as is described below.

Technology Performance:

Reduction in Concentration. Contaminant reduction was a key metric of the efficacy of the technology and was analyzed in two ways: 1) evaluation of changes in aerial extent of groundwater constituent concentrations; and 2) changes in the concentrations in different wells and groups of wells.

TCE concentrations near the hydrogen sparge wells decreased significantly over the 18-month pilot test (Figure 2). Wells CC-SS-1 through CC-SS-6 all showed significant reductions in concentration. The maximum reduction in concentration was from 49 mg/L to < 0.5 mg/L in well CC-SS-1. Overall TCE concentrations decreased in the downgradient portion of the plume during the test. TCE removal is significant in that TCE is the parent chlorinated ethene, historically present at highest concentration. Concentrations of cis-DCE concentrations also decreased dramatically near the sparge points (Figure 2). All the monitoring points (CC-SS-1 through CC-SS-6) close to the hydrogen sparge were above 100 mg/L cis-DCE during the initial baseline sampling event. After 18 months only one well (CC-SS-2) exceeded 100 mg/L As in lab studies performed by Carr and Hughes (1998), system performance increased over time, as the dechlorinating bacteria populations grew in the high-hydrogen concentration environment.

VC concentrations decreased in all but one of the near-sparge point monitoring wells (CC-SS-1 through CC-SS-6) (Figure 2). The concentration of VC at well CC-SS-6 increased from 35 to 55 mg/L. The structure of the contours suggests that the system was generating more VC than baseline conditions, most likely as a product of TCE/cis-DCE biotransformation, and that VC was being transported downgradient.

Ethene concentrations decreased in the close monitoring wells, potentially due to volatilization and degassing (Figure 3), but were elevated downgradient of the hydrogen delivery system. The distribution of methane changed, but no dramatic increases were observed (Figure 3). The maximum methane concentration observed in the initial baseline sampling was 3.7 mg/L while the maximum concentration observed after 18 months was 3.1 mg/l.

Originally, one goal of the test was to compare the test zone against the natural attenuation control and the nitrogen control. However, this analysis was complicated by: 1) changes in the plume over time and 2) tracer gas results which suggests that the sparge system delivered hydrogen to monitoring wells in both the untreated natural attenuation and nitrogen control wells. A comparison of the controls vs. the test zone, and the estimated minimum concentration of hydrogen in these locations based on tracer gas concentration, is shown in Figure 4.

The estimated hydrogen concentration shown in Figure 4 was based on the helium concentration. Most of the sparge pulses during the 18-month test were performed using 100% hydrogen gas. However, prior to sampling for the 12 month and 18 month events, a mix of 49% hydrogen, 49% helium, and 1% SF6 was used for the sparge pulse immediately prior to sampling. Helium was observed in B-1 during the 12-month sampling episode, and helium was observed at both B-1 and B-5 during the 18-month episode. If the gases got there via rapid transport of both hydrogen and helium gas in channels during the 10-minute sparge period, then the hydrogen concentration would be similar to the helium concentration immediately after the sparge. The hydrogen would then be consumed in the five days between the mixed-gas sparge event and sampling of the wells, but the helium would not. Using this transport assumption and with helium as a proxy for hydrogen, the potential hydrogen concentration delivered to the control wells was between 0.0004 and 0.002 mg/L. This compares to concentrations of hydrogen under naturally occurring methanogenesis of 10 nM or 0.00002 mg/L. In summary, the control wells may have been exposed to hydrogen concentrations that were 20-100 times higher than those found under natural conditions. By comparison, helium concentrations directly downgradient of the hydrogen sparge wells (at wells B2, B3, and B4) were ranged between 0.05 and 0.15 mg/L.

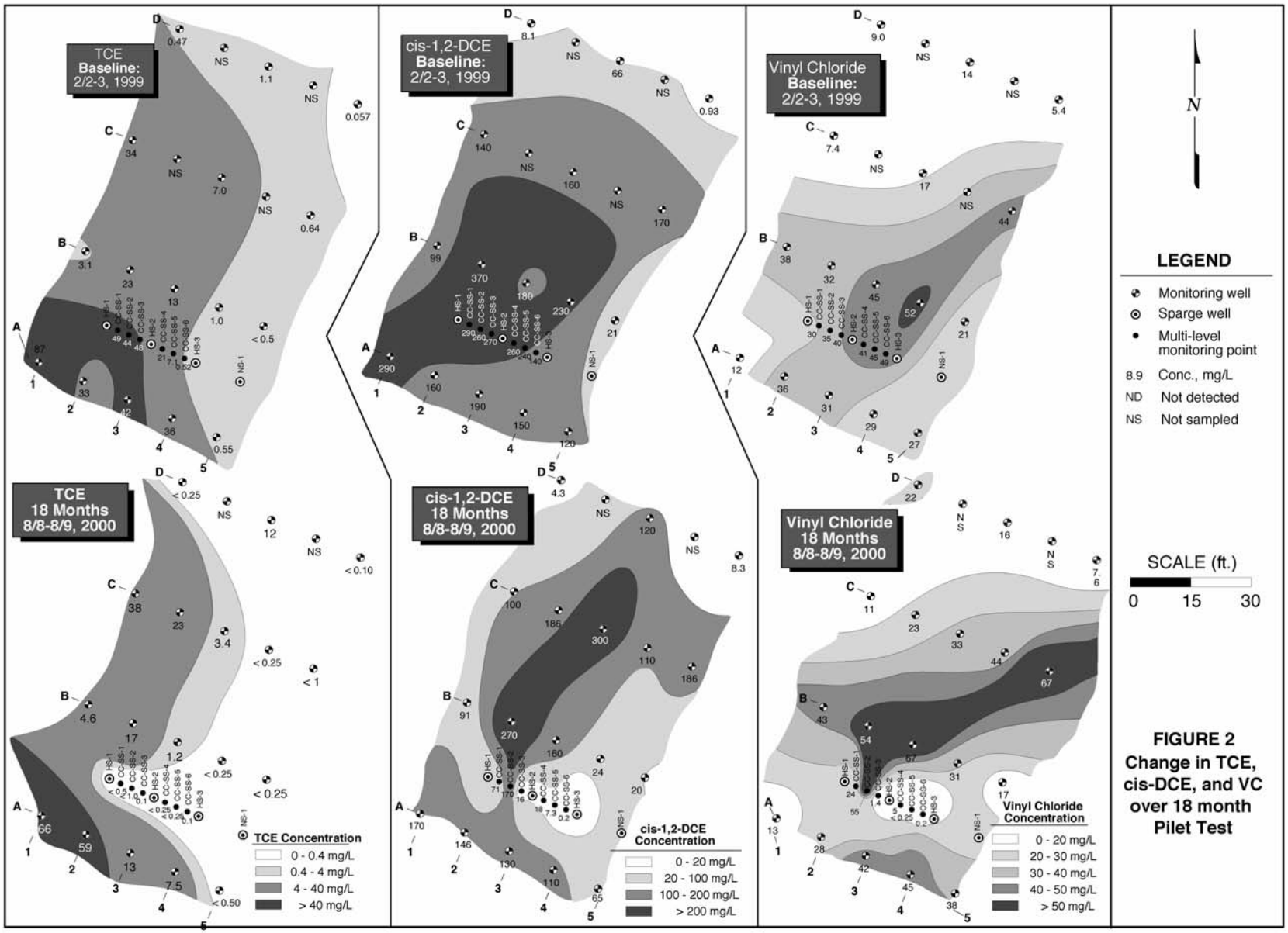

FIGURE 2
Change in TCE, cis-DCE, and VC over 18 month Pilet Test

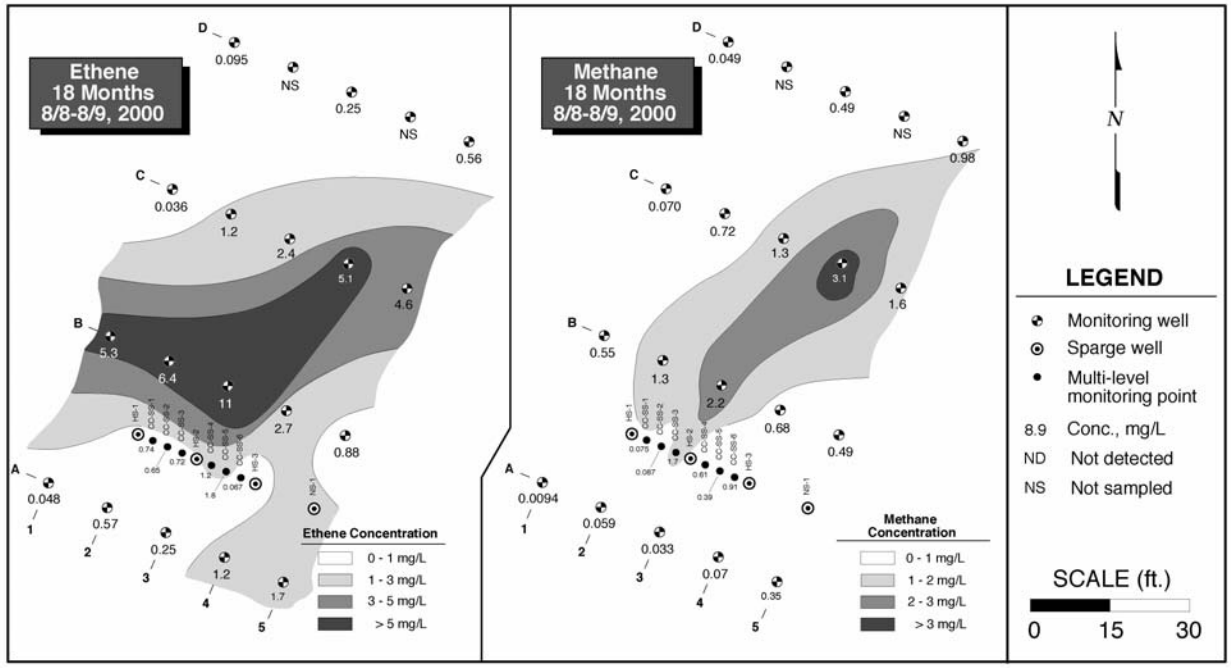

Figure 3. Ethene and Methane Concentrations After 18 Months

An additional line of evidence that the controls were affected by hydrogen occurred after the 18-month intensively monitored pilot test period was over. System operation continued (without helium tracer) and *hydrogen* was observed at well B-5 at 110 nM at 36 months (0.00022 mg/L), or about 11 times what would be expected under natural attenuation conditions (under methanogenesis hydrogen concentrations poise at 10 nM = 0.00002 mg/L).

In summary, significant concentration reductions were observed, both on a percentage basis and on an absolute basis. Removals in wells close to the sparge point were more than 90%, and in downgradient wells there was an approximately 50% reduction in contaminant concentration. More mass reduction was also observed in the test zone than the two controls, although interpretation is difficult as some of this removal may have been due to: 1) unintended hydrogen transport into the groundwater near the control wells; and 2) natural changes in the plume.

Changes Due to Natural Conditions. Two wells unlikely to show any impact from the pilot test system were wells C1 and D1 (Figure 1). These wells were located far from the sparging wells (45 ft and 70 ft, respectively) and were slightly upgradient of other wells in the "C" and "D" rows (i.e., Figure 2 indicates a northern and an eastward flow component along rows C and D based on the vinyl chloride plume). These wells show two different trends, with the closer, higher concentration well decreasing in concentration and the farther, lower concentration well increasing.

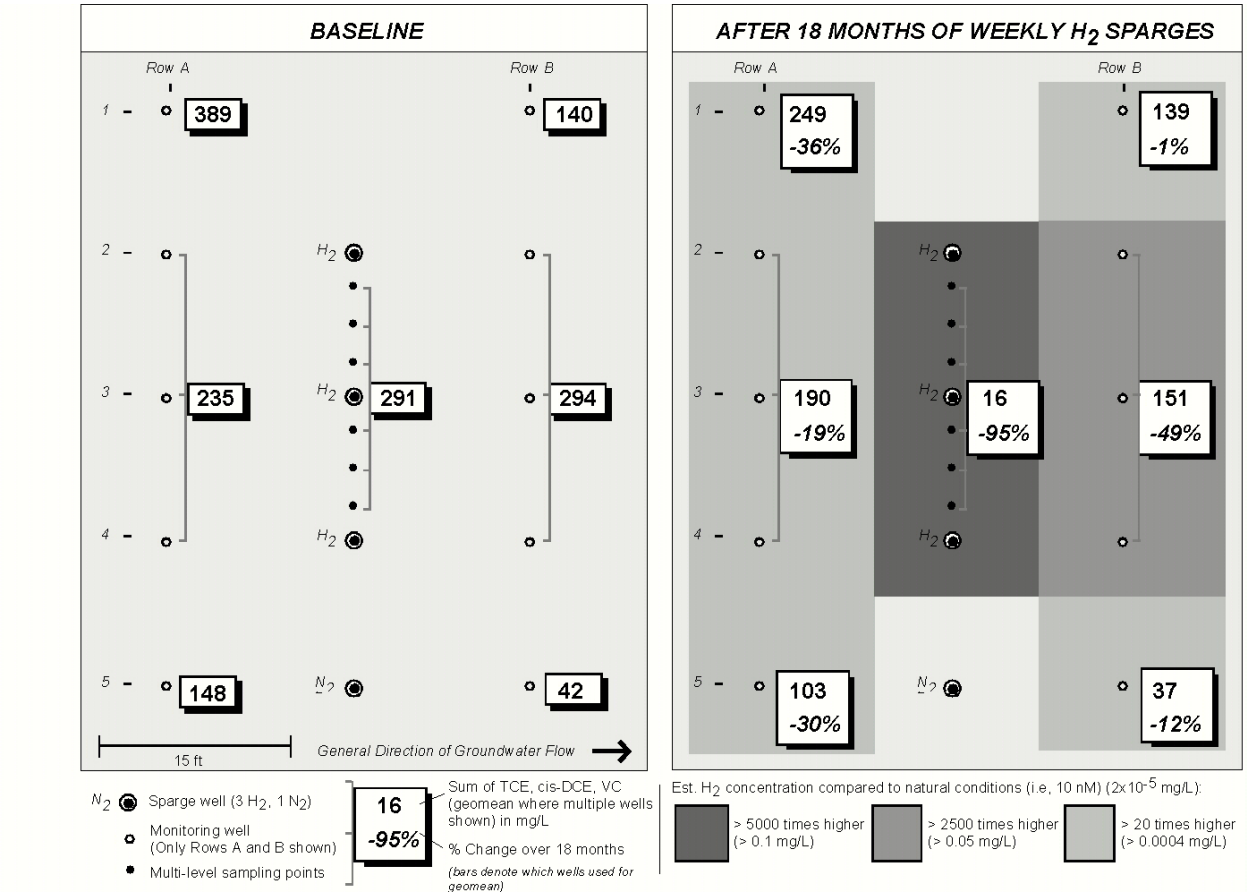

Figure 4. Estimated Hydrogen and Total Chlorinated Ethene Concentration Change Over 18 Months for Rows A and B and for Deep Multi-Level Sampling Points.

TABLE 1. Change in Total Chlorinated Ethenes (CE) in Distant Wells over 18-months. (These wells are less likely to be affected by the sparging)

Well	Initial Total CE Conc. (mg/L)	18-month Total CE Conc. (mg/L)	Percent Reduction Over 18 Months
C1	181	150	-17%
D1	18	27	51%
GEOM. MEAN	56	63	12%

One final analysis was conducted to determine if there was a relationship between dissolved hydrogen delivery (as indicated by the tracer compound, helium) and percent reduction. As shown in Figure 5, higher helium concentrations (which is a 1:1 proxy for expected hydrogen concentration immediately after a sparge event) are related to higher removals. Under natural conditions hydrogen concentrations of less than 0.00002 mg/L (10 nM) would be expected.

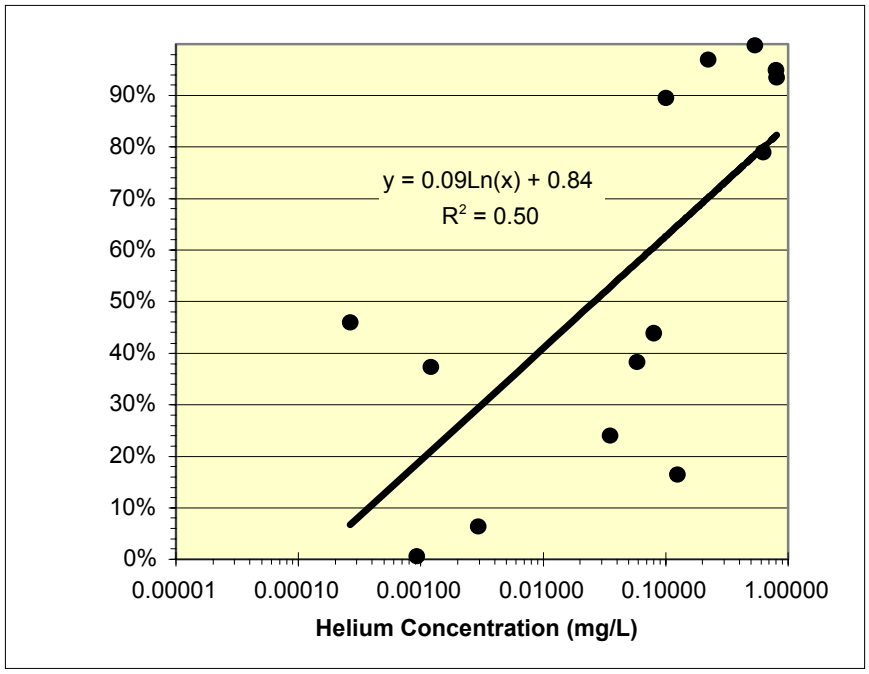

Figure 5. Percent Reduction in TCE + cis-DCE Concentration vs. Log Helium Conc.

In summary, the plume showed significant changes over time. In wells with low concentrations of hydrogen (as indicated by helium concentrations), reductions from 1 to 36 percent were observed. In two wells with no likely impact from hydrogen delivery, one well decreased in concentration by 17% and one well increased in concentration by 51%. Percent reduction correlated well with hydrogen delivery (as indicated by helium tracer concentrations) (Figs. 4-5).

Change Due to Physical Removal. As shown in Figure 4, the nitrogen control well (B5) had a 12% reduction in chlorinated ethenes compared to a 49% reduction in similarly-spaced wells downgradient of the hydrogen sparge wells (middle wells, B2, B3, and B4). However, the B5 well was influenced by the hydrogen sparge system, showing helium tracer at 12 months and 18 months (and hydrogen during the continued operation of the system, at 36 months). To confirm that reductive dechlorination was the primary removal process in the test zone compared to volatilization, the observed changes in concentration at the site were compared against constituent Henry's Law coefficients (Table 2) for the four wells closest to the hydrogen sparge points (CC-SS-1, CC-SS-3, CC-SS-4, CC-SS-6), The results show that the most volatile constituent, VC, had the lowest removal. The parent compound, TCE, had the highest removal.

Table 2. Percent Reduction in the Geometric Mean of Contaminant Concentration Over 18 Months vs. Dimensionless Henry's Law Coefficient

	Henry's Law Coefficient* (dimensionless) (Higher values indicate more volatility)		% Reduction of Geometric Mean Over 18 Months from Close Wells**	
TCE	0.42	↓	96%	↑
cis-DCE	1.33		91%	
VC	3.58		76%	

* From Wiedemeier et al., 1999 ** Wells CC-SS-1, CC-SS-3, CC-SS-4, CC-SS-6

Site Conditions from 18 Months to 36 Months. System operation continued after the 18-month pilot test. The report of this effort (BEM, 2002) concluded that "The August 2001 round of sampling is generally consistent with the previous pilot test data…." Concentrations in all wells in Rows A and B decreased further, except for well B1, which increased. Hydrogen was observed in well B5 at ~ 11 times the concentration that would be expected under intrinsic geochemical conditions, indicating that the nitrogen control may not be an accurate representation of a no-hydrogen condition. As discussed above, helium concentrations in the natural attenuation control wells (A1, B1) indicate that these wells may have been affected by hydrogen delivery.

The site personnel reported that there is a "significant mass" of TCE directly below the pilot study area concentrated at 40 ft bgs. One possible explanation for why only a ~50% reduction in concentration in the downgradient portion of the test zone was achieved is vertical upwelling (possible due to gas addition) that might have continued to deliver contaminant mass to the area sampled by the downgradient wells. More sampling would be required to verify this hypothesis. The operation of the pilot system was terminated after 36 months of operation.

Summary. Several lines of evidence indicate that low-volume pulsed hydrogen sparging reduced the concentrations of TCE and cis-DCE in the test zone. Some VC was produced downgradient of the test zone. Comparative evaluation of planned controls was complicated by the widespread distribution of tracer gas throughout the test zone and spatial and temporal variability in the plume.

Technology Cost

Low-volume pulsed biosparging is best suited for sites where large quantities of donor need to be injected, and where direct-push wells can be used. Simple delivery skids can be constructed for under $20,000, and direct push points can be installed for $500 to $1,000 per well. As indicated by the pilot test results, injection well spacing of 10 to 15 ft should be used for design purposes.

At full operation, the pilot test was using approximately 100 SCF of gas per well per week. The cost for industrial grade hydrogen gas is approximately $0.11 per SCF delivered to a site, or about $0.15 per mole. Therefore the total cost of gas is about $572 per well per year for a typical site.

The entire cost of the pilot test was under $250,000, with 59% going to labor, 11% going to materials, 7% going to travel, and 23% going to drilling contractors and the analytical laboratory. This cost is much greater than would be incurred in a regular field application of the technology due to the large number of monitoring wells (38 monitoring points compared to three hydrogen sparge points), the use of specialty gases with tracers, increased analytical protocols, and higher data analysis/reporting costs.

For comparison purposes, a planning-level budget for a 100 ft by 100 ft treatment zone down to 30 ft was developed and shown on Table 3: Hydrogen injection wells on approximately 15 ft centers installed using a direct-push rig was assumed for this generic design.

Table 3. Cost of Low-Volume Pulsed Biosparging for a 100 ft by 100 ft Treatment Zone

Element	Cost ($)
Capital Cost	
Planning and Preparation	$30,000
Mobilization/Demobilization/Per Diem	$5,000
Site Labor (assume 15 days for well installation @ $75/hr, 5 days for startup)	$15,000
Equipment and Appurtenances - Injection Points (assume $2500/day, 4 wells/day, 49 injection wells) - Process Skid + Shipping - Wellhead Equipment ($100/well) - Manifolds (assume 800 ft 1" PVC @ $5/ft for labor+materials plus $3K fittings)	$31,000 $15,000 $5,000 $7,000
Baseline Laboratory Analyses	$3,000
Surveying	$1,000
Reporting	$20,000
Total Capital Costs	**$134,000**
Annual Operating Costs	
Direct Labor (Process Monitoring) (assume 1 hr per week by on-site technician)	$2,000
Project Management (assume 2 hrs/month @ $80/hr)	$2,000
Hydrogen (assume $30 per 260 ft^3 cylinder) (includes cylinder change out by vendor)	$28,000
Sampling Labor (four events @ 2 days/event) (assume on-site personnel, 2-person team @100/hr combined for both people)	$8,000
Sampling Equipment and Supplies	$4,000
Laboratory Analysis	$10,000
Reporting	$12,000
Annual Operating Costs	**$64,000**

References

BEM. 2002. Space Launch Complex 15 Hydrogen Biosparge Treatability Study. BEM Systems, Inc. Orlando, Florida. September, 2002.

Carr, C. and J. B. Hughes, 1998, High-rate dechlorination of PCE: comparison of lactate, methanol and hydrogen as electron donors: Environmental Science and Technology, 32(12): 1817-1824.

Fisher, R.T., C.J. Newell, P.E. Haas, and J.B. Hughes, 1999. "Treatability Studies of Hydrogen-Enhanced Bioremediation of Chlorinated Solvent-Impacted Media," Engineered Approaches for In-Situ Bioremediation of Chlorinated Solvent Contamination, A. Leeson and B. Alleman, eds., Fifth International In Situ and -Site Bioremediation Conference, April 19-22, 1999, San Diego, California, Battelle Press, pp. 185-190.

GSI. 2001. Low-volume Pulsed-hydrogen Biosparging Pilot Test Final Report. Groundwater Services, Inc. Houston, Texas. June, 2001.

Hughes, J.B., C.J. Newell, and R.T. Fisher, 1997. Process for In-Situ Biodegradation of Chlorinated Aliphatic Hydrocarbons by Subsurface Hydrogen Injection, U.S. Patent No. 5,602,296, February 11, 1997.

Newell, C.J., Rifai, H. S., Wilson, J. T., Connor, J. A., Aziz, J. A. and M. P. Suarez. 2003 . Calculation and Use of First-Order Rate Constants for Monitored Natural Attenuation Studies. Ground Water Issue. U.S. Environmental Protection Agency. February, 2003.

Newell, C.J., C.E. Aziz, P.E. Haas, J. B. Hughes, and T.A. Khan, 2001. Two Novel Methods for Enhancing Source Zone Bioremediation: Direct Hydrogen Addition and Electron Acceptor Diversion, Anaerobic Degradation of Chlorinated Solvents, pg. 19-26, , V. Magar, D. Fennell, J. Morse, B. Alleman, and A. Leeson, eds., In Situ and On-Site Bioremediation: The Sixth International Symposium, Battelle Press, Columbus, Ohio,

Newell, C.J., J.B. Hughes, R.T. Fisher, and P.E. Haas, 1998. "Subsurface Hydrogen Addition for the In-Situ Bioremediation of Chlorinated Solvents," Designing and Applying Treatment Technologies, First International Conference on Remediation of Chlorinated and Recalcitrant Compounds Conference, G. B. Wickramanayake and R. Hinchee, eds., May 21-28, 1998, Battelle Press, Columbus, Ohio, pp. 47-52.

Newell, C.J., P.E. Haas, J. B. Hughes, and T.A. Khan, 2000. "Results From Two Direct Hydrogen Delivery Field Tests For Enhanced Dechlorination," Bioremediation and Phytoremediation of Chlorinated and Recalcitrant Compounds, G. B. Wickramanayake, A. R. Gavaskar, B.C. Alleman, and V.S Magar, eds., The Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds Conference, Monterey, California, May 22-25, 2000, Battelle Press, Columbus, Ohio, pg. 21-38.

Newell, C.J., R.T. Fisher, and J.B. Hughes. 1997. "Direct Hydrogen Addition for the In-Situ Biodegradation of Chlorinated Solvents." *Proceedings of the Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection, and Remediation Conference*. November 12-14, 1997, Houston, TX. pp. 791 - 800.

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APPENDIX E.9 – RAPID AND COMPLETE TREATMENT OF TRICHLOROETHENE VIA BIOAUGMENTATION IN AN ACTIVE BIOBARRIER

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RAPID AND COMPLETE TREATMENT OF TRICHLOROETHENE VIA BIOAUGMENTATION IN AN ACTIVE BIOBARRIER

Evan E. Cox, Neal D. Durant, Michaye L. McMaster, Dave W. Major
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Scott Neville and Larry Bonsack, Aerojet General Corporation, Sacramento, California

1.0 Site History/Source of Contamination

The Aerojet General Corporation facility covers approximately 8,500 acres near Sacramento, California. Since 1953, the facility has been used to manufacture rocket engines for military and commercial applications. Trichloroethene (TCE) and perchlorate (a component of solid rocket propellant) have entered the subsurface at the site as a result of historic operations, and have impacted soil and groundwater quality. Groundwater extraction and treatment systems are operating to contain and remediate subsurface contamination across the facility. Although the groundwater extraction and treatment systems are performing effectively, a variety of supplemental measures are being evaluated to expedite groundwater cleanup.

From 2000 to 2002, Aerojet and the Department of Defense Strategic Environmental Research & Development Program (SERDP) retained GeoSyntec Consultants (GeoSyntec) to conduct a series of field demonstrations to evaluate the performance of in situ bioremediation (both biostimulation and bioaugmentation) to treat TCE and perchlorate in groundwater at the Aerojet facility (McMaster et al. 2001; Cox et al. 2002). The pilot tests were performed in a portion of the site that is located downgradient of a former disposal area (the apparent source of the TCE and perchlorate plume). The pilot test area is located approximately 2,000 feet upgradient of an existing groundwater extraction and treatment system, which extracts groundwater at a rate of 900 to 1000 gpm at the northern site boundary.

2.0 Site Geology, Hydrogeology, and Groundwater Chemistry

The aquifer in the vicinity of the pilot test area consists of a sequence of alluvial sand and gravel deposits. Sampling performed in the pilot test area prior to initiation of the pilot test indicated that the highest concentrations of TCE and perchlorate in groundwater were present at depths ranging between 80 to 100 feet below ground surface. Consequently, the pilot system was designed to treat this depth interval. The natural groundwater flow direction within the pilot test area is approximately west-northwest, with an estimated horizontal hydraulic gradient of 0.008. The horizontal hydraulic conductivity in the pilot test area is estimated to be 30 ft/day. The average groundwater flow velocity (under ambient conditions) in the pilot test area is estimated to be approximately 1 ft/day.

The pilot test area is located within the interior region of the TCE and perchlorate plume. Prior to initiation of the pilot test, the concentration of TCE in the pilot test area ranged from 2.0 to 2.5 mg/L, whereas the concentration of perchlorate ranged from 12 to 15 mg/L. A variety of other constituents were present at low part-per-billion concentrations, including tetrachloroethene (PCE), 1,1-dichloroethene (1,1-DCE), cis-1,2-dichloroethene (cDCE), carbon tetrachloride (CT),

and chloroform (CF). Vinyl chloride (VC), ethene, and ethane were not detected in the pilot test area groundwater. Geochemical data indicated that the aquifer was relatively oxidizing, with dissolved oxygen concentrations ranging from 2 to 5 mg/L and oxidation reduction potential (ORP) ranging from 157 to 263 mV. Consistent with the prevailing aerobic conditions, nitrate and sulfate were present at concentrations of approximately 5 mg/L and 13 mg/L, respectively. Dissolved iron and manganese, and methane were not detected. The combined persistence of TCE, oxidizing redox conditions, and the absence of TCE dechlorination products indicated that conditions were unfavorable for natural biodegradation of TCE. The extent of natural microbial activity in the pilot test area appeared to be limited by a lack of available organic carbon substrates (BOD < 1 mg/L; COD < 20 mg/L).

3.0 Technology Description

The field demonstrations evaluated four principal aspects of in situ bioremediation technology application for TCE treatment: (1) ability of electron donor addition to stimulate TCE (and perchlorate) biodegradation without the addition of exogenous microbes; (2) ability of bioaugmentation to improve the rate and extent of TCE biodegradation; (3) ability to create in situ biologically active zones for plume treatment; and (4) ability to deliver and monitor the distribution and fate of the introduced dehalorespiring bacteria using a DNA-fingerprinting technique. Acetate, lactate and ethanol were individually employed as electron donors for various stages of the pilot tests.

For the bioaugmentation portion of the demonstration, the dehalorespiring microbial culture KB-1™ was delivered to the subsurface in order to seed the test plot and improve the rate and extent of TCE dechlorination to ethene. KB-1™ is a natural (i.e., not genetically-modified), non-pathogenic, dechlorinating bacterial consortia that was enriched from a chlorinated solvent site (Duhamel et al. 2002). Major et al. (2002) demonstrated that KB-1 and soluble electron donors can be injected into the subsurface to achieve complete and rapid dechlorination of TCE, cDCE, and VC to ethene, each with half-lives of a few hours. The KB-1™ culture contains at least three phylogenetic relatives of *Dehalococcoides ethenogenes* strain 195, the only microorganism known to be capable of degrading PCE and TCE completely to ethene (Maymo-Gatell et al. 1997; 2001). Data indicate that *Dehalococcoides* occurs naturally at some sites, but is absent at others (Löffler et al. 2000; Hendrickson et al. 2002). Although a variety of microorganisms are known to dechlorinate PCE and TCE to cDCE, only relatives of *Dehalococcoides* have been shown to achieve complete reductive dechlorination of cDCE and VC to ethene (Fennell et al. 2001). Numerous peer-reviewed studies have found a direct correlation between the presence of *Dehalococcoides* and the extent of cDCE and VC dechlorination (Harkness et al. 1999; Ellis et al. 2000; Löffler et al. 2000; Fennell et al. 2001; Major et al. 2002; Lendvay et al. 2003).

4.0 Pilot Test Approach and System Design

4.1 Overview

The pilot tests were completed in two phases. In Phase I, biostimulation and bioaugmentation were evaluated in a small-scale, closed-loop recirculatory system. Based on the success of Phase I, the pilot system was expanded (Phase II) to create and demonstrate bioremediation

performance using a single-pass biobarrier system designed to intercept and treat a 600-foot wide portion of the TCE/perchlorate plume. Both phases of the test involved extracting contaminated groundwater, amending the groundwater with electron donors, and reinjecting the groundwater to achieve complete reductive transformation of TCE and perchlorate in situ.

4.2 Pre-Design Laboratory Microcosm Studies

Laboratory microcosm studies have previously been conducted for the Aerojet site to evaluate the feasibility of natural and engineered biodegradation of TCE in groundwater. Microcosms were constructed using Aerojet site soil and groundwater, and amended with varying electron donors, including methanol, ethanol, acetate, lactate, molasses, benzoate, and food-waste. In all, 19 different electron donors were tested. The results of the microcosm studies indicated that none of the electron donors tested could stimulate dechlorination of TCE past cDCE within incubations periods of 200 days or more (microcosms were never donor-limited). Based on these results, selected electron donor treatments were bioaugmented with KB-1™ after approximately 200 days of incubation. Figure 1 presents results from an electron donor treatment bioaugmented with KB-1™ (data are averages of triplicate microcosms). Following KB-1™ addition, cDCE dechlorination began immediately, with stoichiometric dechlorination to ethene within weeks.

4.3 Phase I - System Components and Installation

The Phase I pilot system was installed and instrumented in May 2000. Figure 2 presents the layout of the groundwater extraction, electron donor delivery and performance monitoring wells in the pilot test area in plan view. The system was operated by extracting groundwater at a rate approximately 5 gpm from Well 100, amending the groundwater with electron donor (initially acetate, later lactate), and re-injecting the amended groundwater to the aquifer via Well 4385. System operation was controlled via a programmable logic controller and personal computer.

4.4 Phase I - Tracer Test to Characterize System Hydraulics

To characterize the hydraulics of the pilot test area (e.g., pore volume, residence time, etc.), a conservative tracer test was initiated on 19 May 2000. A sodium bromide solution was injected to the aquifer via Well 4385 over an 8 hour period. Breakthrough of the bromide at Wells 3600 and 3601 was monitored via collection of samples on a daily basis (or more frequently during breakthrough) and analysis with field electrodes and ion chromatography. The retention of bromide mass during the tracer test was > 90%, demonstrating a high degree of capture and an excellent system for tracking mass balances on biodegrading chloroethenes. Data from the tracer test indicated that the average travel time for non-retarded particles to reach Wells 3601 and 3600 were estimated at 2.5 and 7 days, respectively. Based on the measured/estimated dimensions of the pilot test area (area of influence of 65 ft long, 56 ft wide and 20 ft thick, with a porosity of 0.3), the pilot test area groundwater pore volume was estimated to be 158,000 gallons, with a residence time of 23 days (for a recirculation rate of 5 gpm).

4.5 Phase I – Pilot Test Execution and Performance Monitoring

The Phase I pilot test consisted of three main operational phases: (1) acetate biostimulation from Day 0 to 63; (2) lactate biostimulation from Day 93 to 157; and (3) bioaugmentation and lactate addition from Day 157 to 280. From Days 64 to 93, the system was shutdown for rehabilitation of delivery/recharge Well 4385. In the first operational phase, acetate was added at a target time weighted average concentration of 50 mg/L. The acetate addition regime consisted of 4 one-hour pulses a day. Although acetate stimulated effective treatment of perchlorate during the first phase, it did not promote significant TCE dechlorination through the first 64 days of operation. Consequently, at Day 93, the electron donor was changed to sodium lactate. As with the acetate addition regime, the lactate addition regime initially consisted of 4 one-hour pulses per day to provide a target time weighted average concentration of 60 mg/L lactate. This addition regime was later modified to provide the same time weighted average concentration of lactate through a single pulse per day, in order to reduce biofouling of the delivery well.

The pilot test area was bioaugmented with approximately 50 L of the KB-1™ culture on 15 December 2000. The KB-1™ culture was delivered to the aquifer by injection via Well 3601. To deliver the KB-1™ culture to the aquifer, stainless steel culture vessels (used for both culture growth and shipping) were pressurized with Argon gas, forcing the culture from the vessel through a delivery line into the screened zone in the well (below the water table). The bioaugmentation process took 4 hours, including setup and demobilization. Following bioaugmentation, the pilot test area was operated as normal. Performance monitoring consisted of semi-weekly measurement of field parameters, and bi-weekly collection of groundwater samples from Wells 3601, 3600 and 100 for analysis of volatile organic compounds (VOCs) and dissolved hydrocarbon gases (ethene, ethane and methane), perchlorate, anions, and volatile fatty acids (acetate, lactate, propionate). Additional groundwater samples were collected prior to and following bioaugmentation to evaluate the presence and transport of *Dehalococcoides* microorganisms in the pilot test area. 16S rRNA-based techniques and PCR analyses were used to detect *Dehalococcoides*.

4.6 Phase II - System Design, Components, and Installation

The objective of the Phase II pilot test was to design, implement, and validate the performance of an active biobarrier capable of controlling plume migration for the core of the TCE/perchlorate plume. Using numerical groundwater flow modeling, a design was developed that consisted of two extraction wells and one injection well. The modeling predicted that two extraction wells, spaced at a distance of 200 feet on either side of injection Well 4385, and pumping at 10 gpm each, would be capable of capturing the core of the TCE/perchlorate plume in the area. This design made use of the Phase I well network, and ensured that the plume width would not be expanded. According to the model, groundwater injected at Well 4385 (20 gpm) was expected to reach Wells 3601, 3600, 100, and 3618 (new) within about 2, 6, 21, and 56 days (a timeframe sufficient to achieve complete dechlorination to ethene).

The Phase II expansion was installed and instrumented from August through October 2001. As illustrated in Figure 5, the Phase II system used the Phase I well network, and added two new extraction Wells 3619 (east) and 3620 (west). While the Phase I system was recirculatory, the

Phase II system was designed as a single-pass, active biobarrier. Existing Well 4385 was used as the electron donor delivery/recharge well. Existing Wells 3601, 3600, and 100 were used as downgradient performance monitoring points. One new downgradient well (3618) and one new transgradient well (3617) were added to improve coverage in the monitoring network.

4.7 Phase II – Tracer Test to Characterize Hydraulics

Conservative tracer testing was initiated on 2 November 2001 to confirm hydraulic connectivity within the pilot test area well network, calibrate the pilot test area numerical model, and refine estimates of residence time and breakthrough at each monitoring well. A bromide solution was injected via Well 4385 as a daily one hour pulse for 14 consecutive days to achieve a time weighted average concentration of 100 mg/L bromide. Breakthrough was measured by collecting samples at the monitoring and extraction wells on a daily to semi-weekly basis. Maximum breakthrough concentrations in Wells 3600, 3617, and 100 (located 35, 50, and 65 feet from the injection well) were 100%, 76%, and 72% of the injected concentrations, confirming that concentration changes attributable to dilution and dispersion along the primary flowpath were minimal. Based on the bromide breakthrough curves, the average travel times for non-retarded particles to reach downgradient performance monitoring Wells 3600, 100, and 3618 were estimated to be 5, 10, and 38 days, respectively. These results compared reasonably well with the travel times predicted by modeling.

4.8 Phase II – Pilot Test Execution and Performance Monitoring

Ethanol was selected as the electron donor for the Phase II pilot test because it was relatively inexpensive and not expected to adversely impact water quality. To minimize biofouling, ethanol was delivered to the pilot test area at a pulse interval of 1 hr/day. The amount of ethanol required to biodegrade the average perchlorate and TCE influent concentrations was estimated based on stoichiometry to be 17 mg/L. A safety factor of 3 was applied to account for uncertainty and biomass production, and the final time weighted average ethanol concentration was approximately 50 mg/L. No additional bioaugmentation of the pilot test area was necessary during Phase II because *Dehalococcoides* that were injected during Phase I appeared to have colonized the aquifer within the pilot test area.

5.0 Technology Performance

5.1 Phase I - Results

The TCE dechlorination results for the Phase I pilot are presented in Figures 3 and 4. During the acetate biostimulation phase (Day 0 to 63), TCE concentrations did not decline significantly, whereas perchlorate was rapidly biodegraded to below detection limits (data not shown). Through Day 63, TCE concentrations declined slightly (about 11%) at Well 3601 but did not decline at Wells 3600 and 100. During the same period, cDCE concentrations remained stable, and VC and ethene were not produced anywhere in the pilot test area. The addition of sodium lactate (Day 93 to 157) slightly enhanced the rate of TCE dechlorination to cDCE (Figure 3). TCE concentrations in the pilot test area wells declined from an average of 1900 to 1700 µg/L over the 62 days, accompanied by an increase in cDCE concentrations from an average of 58

μg/L to an average of 400 μg/L. TCE dechlorination rates over this phase of the pilot test were very slow, with TCE dechlorination half-lives across the pilot test area ranging up to 355 days. More importantly, VC and ethene were not detected in any of the pilot test area wells by Day 157, suggesting that TCE dechlorination beyond cDCE would be extremely slow and, based on the laboratory microcosm results, improbable.

After bioaugmentation at Day 157, the onset of enhanced dechlorination was almost immediate, accelerating the rate of TCE dechlorination and resulting in the initiation of ethene production within 8 days (Figure 4). At Well 3601, TCE and PCE concentrations were consistently below 5 μg/L within 45 days of bioaugmentation (Day 200). The concentration of cDCE reached a maximum value (1,800 μg/L) at Well 3601 at Day 190, and declined rapidly thereafter to less than 70 μg/L by Day 247. VC and ethene production began concurrently at Well 3601 within 8 days of bioaugmentation (Day 163). VC concentrations steadily increased to their maximum of 310 μg/L at Day 232, and then declined rapidly to < 0.5 μg/L by Day 260. Ethene concentrations increased to 460 μg/L by Day 280. On a stoichiometric basis, this concentration of ethene represents the dechlorination of approximately 2,162 μg/L of TCE, which is within 5% of the starting TCE concentration within the pilot test area (2,250 μg/L) at Day 0. Based on these data, the post-bioaugmentation half-lives for TCE, cDCE, and VC were 6, 12, and 3 days, respectively. In addition to the target chlorinated ethenes, several other chlorinated VOCs were also biodegraded to below detection limits, including 1,1-DCE, CT, and CF.

Dehalococcoides was not detected in the pilot test area prior to the initiation of the pilot, nor prior to bioaugmentation at Day 157. Seventy-five days following bioaugmentation, *Dehalococcoides* was detected in all the pilot test area wells. These data, coupled with the persistence of cDCE prior to bioaugmentation, indicate that the KB-1 culture successfully colonized the pilot test area, and catalyzed complete degrade of TCE and cDCE to ethene.

5.2 Phase II - Results

Figure 6 provides a comparison of the relative proportions of TCE, cDCE, VC and ethene at the start of the Phase II test, and at Day 72 following initiation of ethanol delivery. These data show that addition of ethanol to the pilot test area groundwater promoted rapid and complete dechlorination of TCE (2 mg/L) to ethene within 35 to 65 feet from the electron donor delivery well. Of note, the mass balance for the chlorinated VOCs was maintained over the pilot test. At the start of Phase II (Day -1), TCE was the dominant VOC in the biobarrier influent and at all downgradient and transgradient performance monitoring wells (cDCE, VC, and ethene were present in Wells 100 and 3618 as a relic from the Phase I pilot test). By Day 58 (data not shown), ethene was the predominant product at wells located 35 and 65 feet downgradient, within the portion of the pilot test area that was previously bioaugmented with KB-1. By Day 72, TCE and cDCE had reached steady concentrations throughout the pilot test area and were below their respective MCLs at Well 3600 and 100, while VC had declined to below 12 μg/L at well 100, and was continuing to decline. In the non-bioaugmented, transgradient portion of the pilot test area (Well 3617), dechlorination appeared to stall at cDCE and 1,1-DCE for the duration of the pilot test, confirming the importance of bioaugmentation in improving the rate and extent of dechlorination at this site.

The calculated half-life for TCE dechlorination to cDCE under steady state conditions ranged between 1.3 to 3.7 days, while the half-life for complete TCE dechlorination to ethene ranged between 4.1 to 11 days. The TCE dechlorination rates in Phase II were faster than the rates observed in Phase I, suggesting that the efficiency of the dehalorespiring community improved over time, or perhaps that ethanol was a more effective electron donor than lactate. Of note, methane concentrations were much lower than expected during Phase II, typically below 200 μg/L, suggesting that highly methanogenic conditions are not required to achieve rapid and very efficient TCE dechlorination to ethene. In fact, dechlorination efficiency was better in Phase II (compared to Phase I), when methane concentrations were an order of magnitude lower.

6.0 Estimated Cost of Full-Scale Biobarrier Over 30 Year Operation

Creation of a bioaugmentation zone containing KB-1™ typically costs between $1 to $2/yd^3 of saturated aquifer for large sites, and $5 to $10/yd^3 for small sites. At most, bioaugmentation may represent 5% of the capital cost of a bioremediation system. In contrast to electron donor delivery, bioaugmentation is typically completed with a one-time injection, whereas electron donor requires repeated additions.

Based on the results of the pilot tests, a cost estimate was developed for designing, installing and operating a full-scale active biobarrier for a TCE/perchlorate plume having characteristics similar to the Aerojet plume. Key assumptions and system components included: (i) plume width of 3000 ft; depth of 100 ft; (ii) hydraulic conductivity of 30 feet/day; gradient of 0.008; (iii) aquifer discharge rate of 224 gpm; (iv) 4 extraction wells; 3 injection wells; and 6 monitoring wells; and (v) design electron donor demand of 50 mg/L. As shown in Table 1, the estimated net present value (6% discount rate) for installation and operation of the full-scale active biobarrier at Aerojet 30-years is $1.9M (-30/+50%).

7.0 Summary Observations and Lessons Learned

Based on the results of the field demonstrations, the following conclusions can be made:

- Addition of electron donor alone (acetate or lactate) was effective for perchlorate and nitrate treatment, but ineffective for TCE treatment after 157 days of operation.

- While low level TCE dechlorination to cDCE occurred through electron donor addition, dechlorination of cDCE and VC only occurred after the addition of KB-1™, a natural, non-pathogenic culture that contains *Dehalococcoides* microorganisms.

- 16S rDNA-based methods and PCR analysis provided a highly sensitive technique for monitoring the transport and survival of injected dehalorespiring bacteria.

- The Phase II biobarrier provided effective capture/treatment across a significant portion of the TCE plume as a single-pass biobarrier.

- Ethanol was a highly effective and efficient electron donor for anaerobic treatment of TCE, perchlorate, and nitrate. At only a 3:1 donor:acceptor ratio, the level of treatment

success indicates that very little donor was wasted on non-required microbial processes such as methanogenesis. While 50 mg/L lactate generated nearly 5 mg/L methane in Phase I, 50 mg/L ethanol typically generated less than 200 μg/L methane in Phase II.

References

Cox, E.E., R. Borch, T. McAlary, and M. McMaster. 2002b. In Situ Bioremediation of Perchlorate: Comparison of Results from Multiple Field Demonstrations (abstract). *Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds.* Monterey, California.

Duhamel, M., S.D. Weher, L. Yu, H. Rizvi, D. Seepersand, S. Dworatzek, E.E. Cox, and E.A. Edwards. 2002. Comparison of Anaerobic Dechlorinating Enrichment Cultures Maintained in Tetrachloroethene, Trichloroethene, *cis*-Dichloroethene, and Vinyl Chloride. *Water Research.* 36:4193-4202.

Ellis, D.E., E.J. Lutz, J.M. Odom, R.J. Buchanan, C.L. Bartlett, M.D. Lee, M.R. Harkness, and K.A. Deweerd. 2000. Bioaugmentation for Accelerated In Situ Anaerobic Bioremediation. *Environ. Sci. Technol.*, *34*(11), 2254-2260.

Fennell, D., A. Carroll, J. Gossett, and S. Zinder. 2001. Assessment of indigenous reductive dechlorination potential at a TCE-contaminated site using microcosms, polymerase chain reaction analysis, and site data. *Enivron. Sci. Technol.*, Vol. 35(9):1830-1839.

Harkness, M.R., A.A. Bracco, M.J. Brennan, K.A. DeWeerd, and J.L. Spivack. 1999. Use of Bioaugmentation to Stimulate Complete Reductive Dechlorination of Trichloroethene in Dover Soil Columns. *Environ. Sci. Technol.,* 33(7):1100-1109.

Hendrickson, E.R., J.A. Payne, R.M. Young, M.G. Starr, M.P. Perry, S. Fahnestock. D.E. Ellis, and Ebersole, R.C. 2002a. Molecular Analysis of *Dehalococcoides* 16S Ribosomal DNA from Chloroethene-Contaminated Sites throughout North America and Europe. *Appl. Environ. Microbiol.*, February, pp. 485-495.

Lendvay, J.M., F.E. Loffler, M.E. Dollhopf, B. Fathepure, M. Gebhard, R. Heine, R. Hickey, C.L. Major, Jr., E. Petrovskis, J. Shi, J.M. Tiedje, P. Adriaens. 2003 (*in press*). Bioreactive Barriers: A Comparison of Bioaugmentation and Biostimulation for Chlorinated Solvent Remediation. Paper submitted to *Environ. Sci. Technol.*

Löffler, F., Q. Sun, J. Li, and J. Tiedje. 2000. 16S rRNA gene-based detection of tetrachloroethene-dechlorinating desulfuromonas and dehalococcoides species. *Appl. Environ. Microbiol.*, Vol.66(4):1369-1374.

Major, D.W., M.L. McMaster, E.E. Cox, E.A. Edwards, S.M. Dworatzek, E.R. Hendrickson, M.G. Starr, J.A. Payne, and L.W. Buonamici. 2002. Field Demonstration of Successful Bioaugmentation to Achieve Dechlorination of Tetrachloroethene to Ethene. *Environmental Sci. Technol.* Vol. 36:5106-5116.

Maymo-Gatell, X., Nijenhuis, I., and S.H. Zinder. 2001. Reductive dechlorination of cis-1,2-dichloroethene and vinyl chloride by *Dehalococcoides ethenogenes*. *Environ. Sci. Technol.,* Vol.35:516-521.

Maymo-Gatell, X., Gossett, J.M., and Zinder, S.H. 1997. *Dehalococcus Ethenogenes* Strain 195: Ethene Production from Halogenated Aliphatics (abstract). *In Situ and On-Site Bioremediation,* Vol. 3. p. 23. Alleman, B.C. and A. Leeson (Eds.). Battelle Press, Columbus, OH.

McMaster, M., E. Hood, D. Major, C. LeBron, and J. Quinn. 2003. "Evaluation of Bioaugmentation to Enhance DNAPL Dissolution at Multiple Field Sites". *Abstract. Seventh International In Situ and On-Site Bioremediation Symposium.* Orlando, Florida, June 2-5.

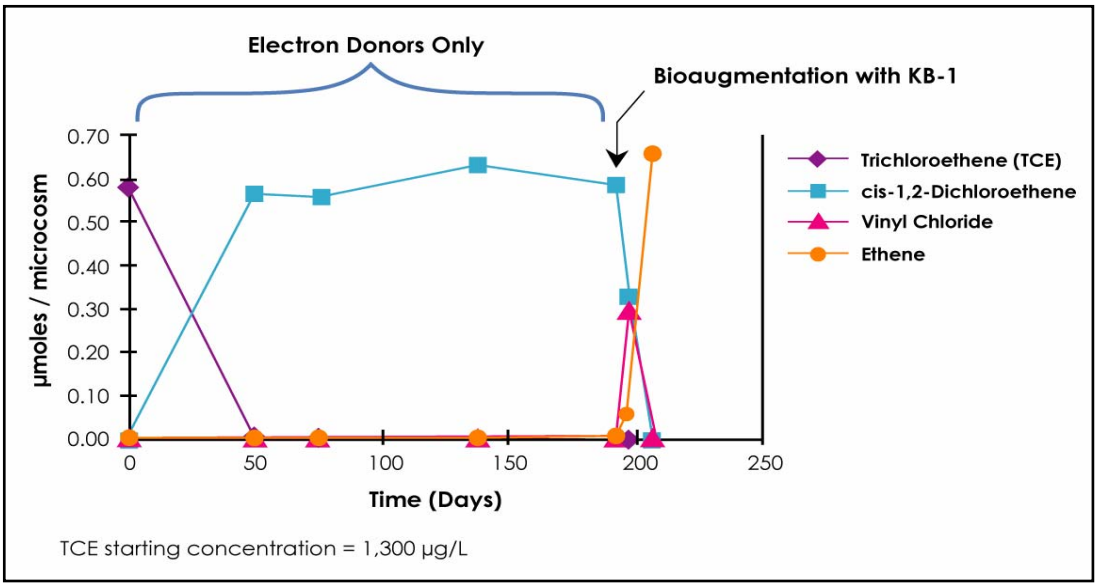

Figure 1. Example results from pre-design microcosm studies. Electron donors tested included methanol, ethanol, acetate, lactate, molasses, benzoate, and food-waste.

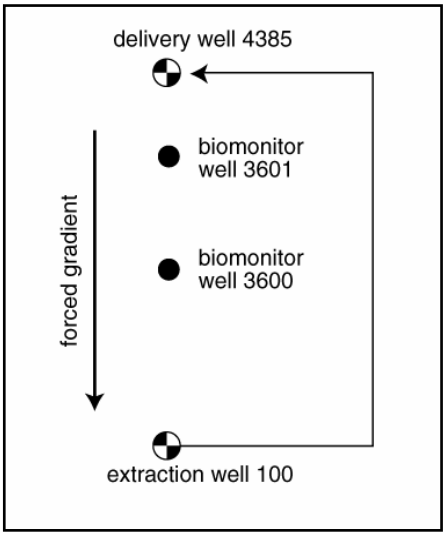

Figure 2. Layout of recirculatory, closed-loop bioremediation system used in Phase I pilot test.

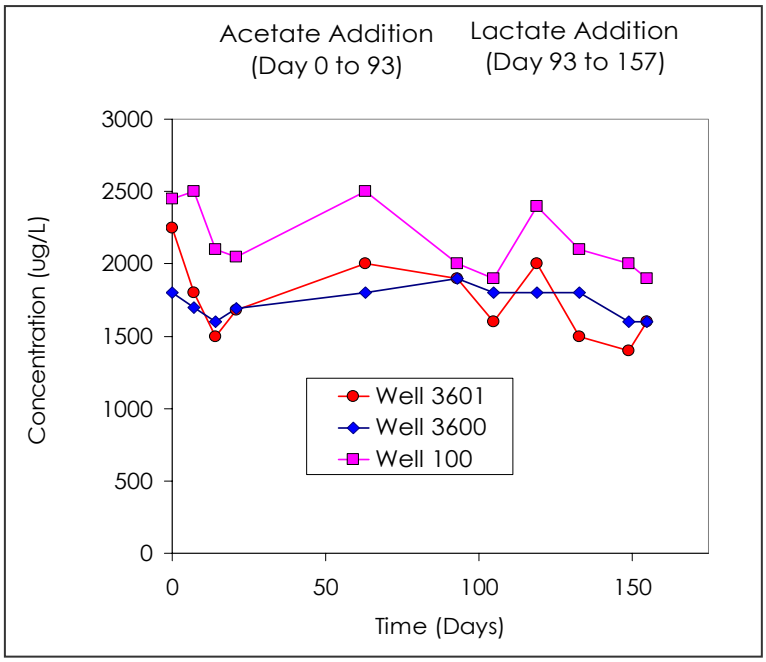

Figure 3. TCE concentrations in groundwater in response to electron donor addition, prior to bioaugmentation, in Phase I pilot test.

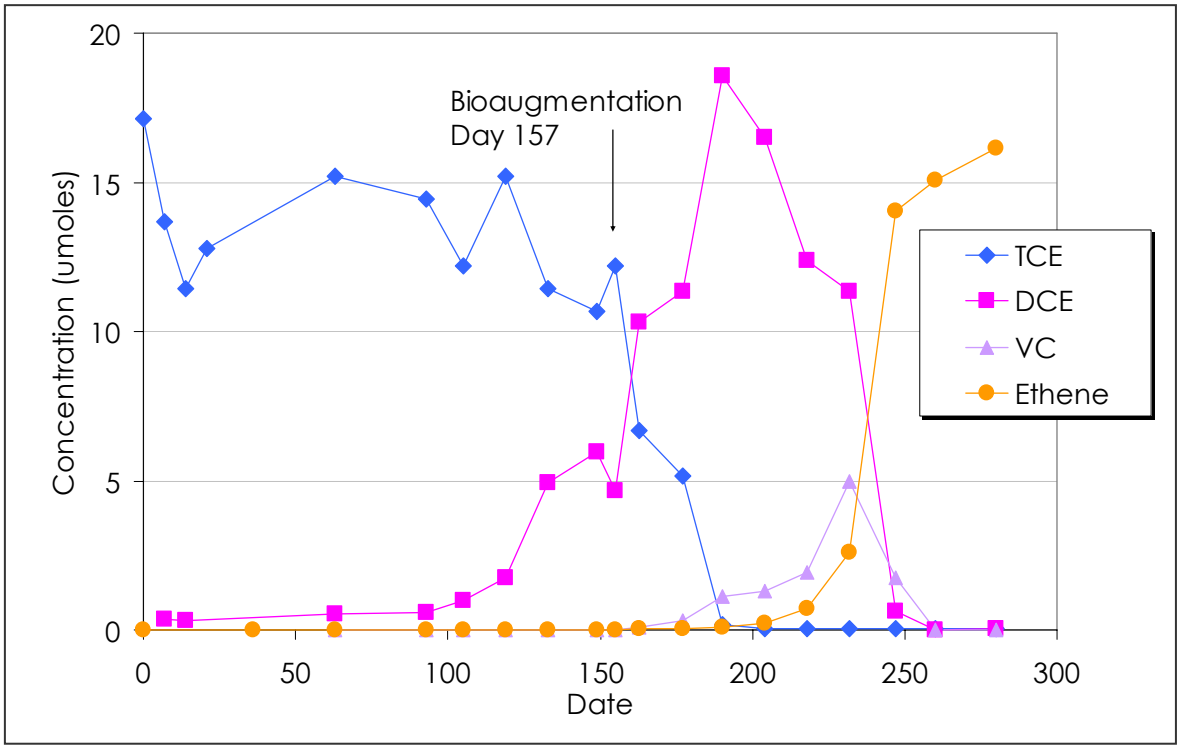

Figure 4. Effect of bioaugmentation with KB-1™ on TCE dechlorination in Well 3601.

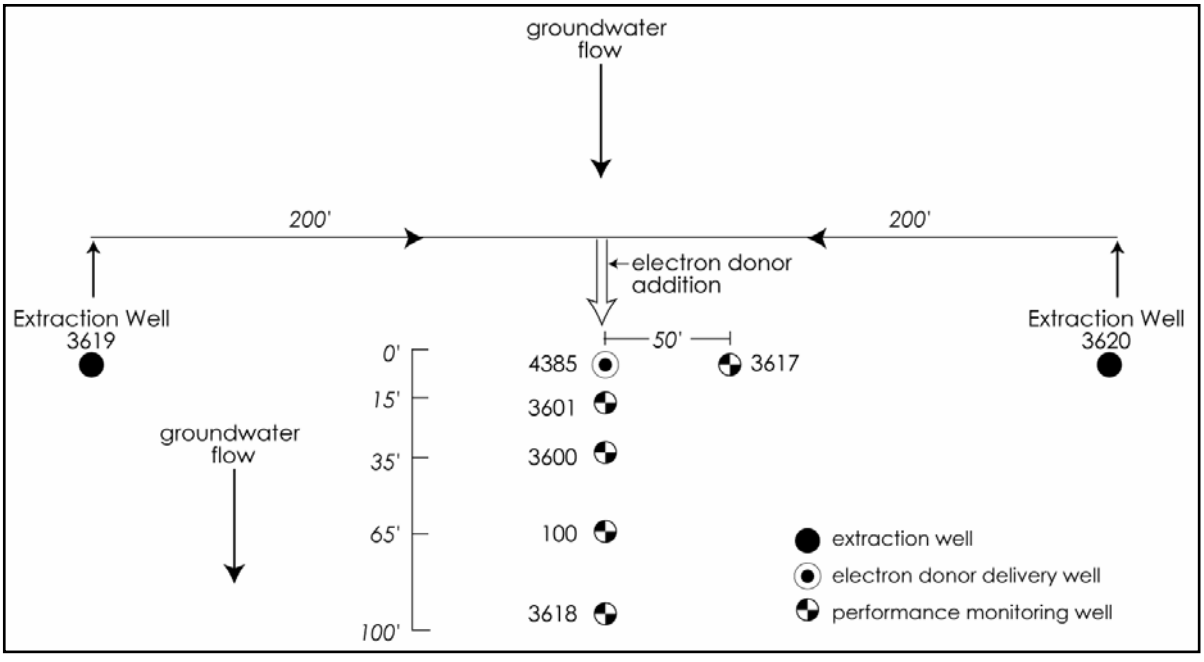

Figure 5. Layout of Phase II pilot system.

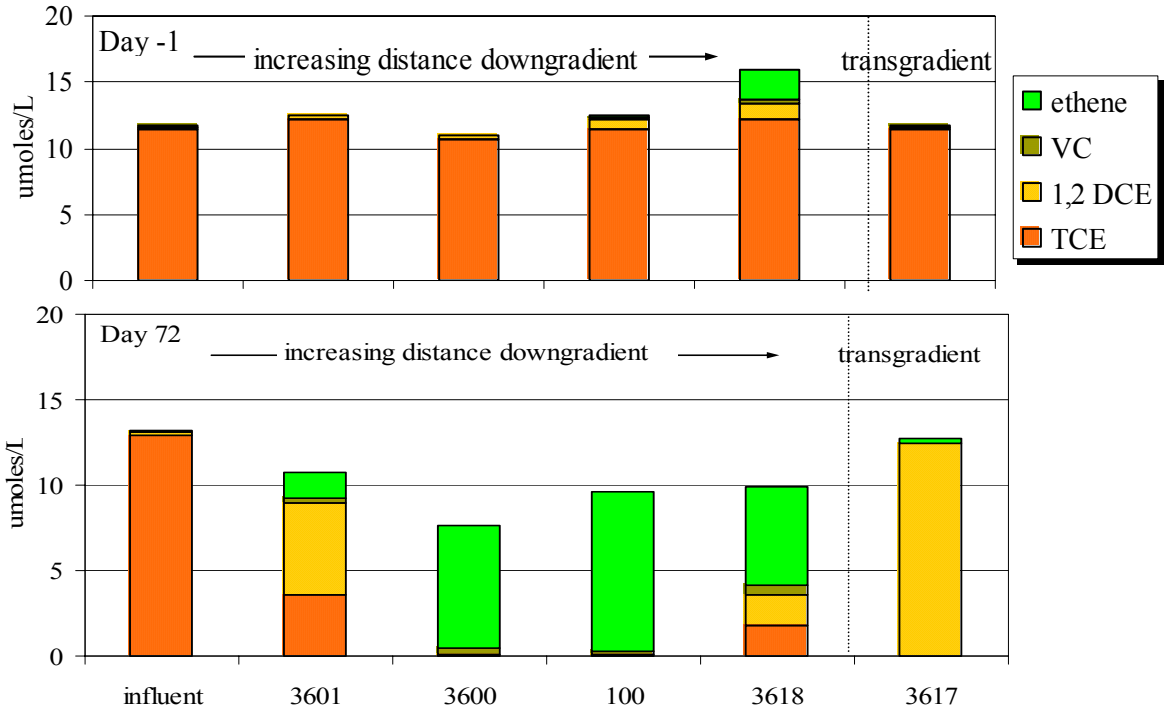

Figure 6. TCE biodegradation results for Phase II.

Table 1: Cost Estimate for an Active Biobarrier for a 3000 Foot-Wide Plume

Item		Total
CAPITAL COSTS		
Well Permitting, Installation, Development, and Waste Handling (100 ft wells)	$	130,000
Trenching, Mechanical, Piping & Electrical	$	136,240
KB-1 Culture	$	29,800
Electron Donor Amendment System	$	110,297
Engineering Design and Start-Up Costs	$	174,800
15% for Contractor Profit (Equipment Only)	**$**	**61,000**
Total Capital Cost	**$**	**642,300**
ANNUAL OPERATION AND MAINTENANCE		
Annual O&M for First 5 Years (quarterly sampling & annual reporting)	**$**	**100,000**
O&M for Remaining Years (assumes semi-annual sampling & bi-annual reporting)	**$**	**86,700**
Sub-Total O&M NPV (6%, 30 Years)	**$**	**1,249,000**
TOTAL ESTIMATED COST (30 YEARS) - FULL-SCALE ACTIVE BIOBARRIER	**$**	**1,891,000**

APPENDIX E.10 – COMPARISON OF FIELD SITES UNDERGOING ENHANCED IN SITU BIOREMEDIATION USING AQUEOUS ELECTRON DONORS

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COMPARISON OF FIELD SITES UNDERGOING ENHANCED IN SITU BIOREMEDIATION USING AQUEOUS ELECTRON DONORS

Tamzen Wood and Kent S. Sorenson Jr. (North Wind Inc., Idaho Falls, Idaho)

INTRODUCTION

One of the key decisions for applying enhanced *in situ* bioremediation of chlorinated aliphatic hydrocarbons is the selection of an electron donor. Aqueous electron donors have been used successfully at many sites because they are easily injected through wells, are easily distributed in the subsurface, are readily available for microbial degradation, and can rapidly reduce local redox conditions to facilitate anaerobic reductive dechlorination. Many aqueous electron donors such as lactate, butyrate, lactose, methanol and acetate, benzoate, and molasses have been used with varying degrees of success for *in-situ* bioremediation. Field application of several of these electron donors is summarized in this case study.

This case study is an evaluation of *in-situ* bioremediation application and performance at several sites using one or more of these aqueous electron donors. An extensive literature search was performed for chlorinated solvent contaminated sites where enhanced *in-situ* bioremediation was applied for six months or greater using aqueous electron donors. A detailed data set that included the hydrogeological characterization, redox conditions, electron donor concentrations, and chlorinated solvent concentrations was preferred so that an informative evaluation could be provided. Unfortunately, very few field sites have collected and published such a data set. Most satisfactory data sets used lactate as an electron donor. Thus, this summary is comprised of six sites using lactate, three sites using butyrate, and one site using a methanol mixture (Tables 1-3). The hydrology, electron donor injection strategy, redox conditions, dechlorination efficiency, and electron donor utilization (where possible) are evaluated and compared for the ten study sites. This evaluation provides many insights into the design, application, and optimization of enhanced *in-situ* bioremediation using aqueous electron donors.

AQUEOUS ELECTRON DONOR INJECTION STRATEGIES

The injection strategy is one of the primary design elements of enhanced *in-situ* bioremediation. The goal is to optimize the distribution of electron donor throughout the contaminated area so that conditions facilitate anaerobic reductive dechlorination. The different injection strategies used at the study sites are listed in Table 2. The first type of injection strategy is continuous injection of the electron donor at relatively low concentrations. The benefits of this design are the continuous input of electron donor allowing for greater concentration control *in situ,* greater hydrologic control of the treatment area, and greater distribution of amended nutrients. Another purported benefit specific to anaerobic reductive dechlorination of chlorinated solvents is that low-concentrations of electron donor may reduce competition between dechlorinating and methanogenic bacteria, thereby increasing dechlorination efficiency. The disadvantages of the design are increased construction and maintenance costs, and greater difficulty achieving reducing conditions, because aerobic water is continually amended to the anaerobic aquifer with low concentrations of electron donor, resulting in decreased overall dechlorination efficiency (unless recirculation of anaerobic groundwater is used).

The second type of injection strategy is the pulsed injection, which is characterized by periodic injections of relatively high concentrations of electron donor. The benefits of this design include less frequent injections, lower maintenance costs, and faster achievement of reducing conditions. A potential disadvantage is a smaller electron donor distribution area because after injection, distribution is dependent upon ambient groundwater velocity.

An extraction component can be included with both continuous and pulsed injection strategies to increase the flow rates achieved within the system. This may be necessary at sites with complex hydrogeology, or low conductivity aquifers, or where regulatory concerns necessitate contaminant control, or hydraulic containment. It may be possible to discontinue extraction once biological containment is demonstrated.

Extracting groundwater downgradient of the injection site provides more hydrologic control, and does not allow the contamination to migrate. The disadvantage of extraction is that the extracted contaminated water must either be disposed of in a waste stream, or recirculated through the electron donor injection well. In the latter system, the waste issue is negated, but the recirculated water may be aerobic, and therefore may introduce oxygen into the anaerobic system and decrease overall efficiency. Also, injection of contaminated water is often a regulatory concern.

RESULTS OF *IN-SITU* BIOREMEDIATION SITES USING LACTATE

Other than molasses, lactate is the most commonly used aqueous electron donor for *in-situ* bioremediation using anaerobic reductive dechlorination. Its anaerobic degradation pathways and behavior have been well defined, and therefore it is easily tracked in the subsurface. Lactate is utilized under anaerobic conditions via two pathways. One pathway results in the production of propionate to acetate in a 2:1 ratio, and the other results in the production of acetate and hydrogen. The predominant lactate utilization pathway(s) at a particular site can be inferred by the propionate: acetate ratio observed. Subsurface microorganisms also use these fermentation products as electron donors. For instance, propionate can be further degraded to acetate and hydrogen. The hydrogen produced in these reactions is used directly for anaerobic reductive dechlorination (Smatlak et al. 1996, Fennell and Gossett 1998) by many dechlorinating bacteria, including *Dehalococcoides ethenogenes* (Maymo-Gatell et al. 1995).

Test Area North. The first lactate case study is Test Area North, located at the Idaho National Engineering and Environmental Laboratory, which contains a residual TCE source area as a result of a disposal injection well, which was used for industrial wastes, including TCE, radionuclides, and sanitary sewage. The contamination source area is approximately 8000 ft^2, and lies within a deep (200-400 ft bgs), fractured basalt matrix underlain by an impermeable sediment interbed (Table 1) (Sorenson, 2000, Martin et al. 2001). The aquifer containing the residual source has sufficiently high hydraulic gradient and groundwater velocity to facilitate the injection of aqueous electron donors (Table 1). Microcosm experiments indicated that lactate facilitated complete anaerobic reductive dechlorination of TCE to ethene using indigenous bacteria from Test Area North soils and groundwater. Therefore, an enhanced *in-situ* bioremediation field evaluation was performed to determine if bioremediation was a viable remedy for the residual source.

The *in-situ* bioremediation injection system at Test Area North consisted of the source injection well, a downgradient (approximately 500 ft) extraction well attached to an air stripper for treatment of extracted groundwater, and ten monitoring wells throughout the treatment area. The injection strategy initially included pulsed weekly injections through the original disposal well of high concentration (3,000-60,000 mg/L), high volume (6,000 gal) sodium lactate (Table 2). These injections resulted in an area of influence of approximately 4000 ft^2 for a total aquifer volume of 800,000 ft^3 using a single injection well. After 3 months of weekly injections, the area impacted by electron donor underwent significant geochemical changes, as sulfate and nitrate were depleted, and significant methane and ferrous iron were generated (Table 3).

The dechlorination progress at Test Area North was dictated by prevailing redox conditions. When sulfate was present, nearly stoichiometric dechlorination of all TCE to *cis*-DCE within the treatment area was observed. When sulfate was depleted and methanogenesis was observed, then dechlorination of *cis*-DCE to VC and VC to ethene was also observed. After nine months of weekly lactate injections, significant propionate and acetate concentrations had accumulated, and so lactate injections were discontinued for five months. During this period, rapid dechlorination of all remaining aqueous TCE and DCE to ethene occurred within the treatment cell. Thus, more efficient anaerobic reductive dechlorination appeared to occur during a period when propionate was the predominant electron donor instead of lactate. After lactate additions resumed, however, efficient anaerobic reductive dechlorination has been maintained for nearly four years. Recent operations have included altering the injection strategy

to increase the volume (12,000-50,000 gal.), and decrease the frequency (every 6 to 8 weeks) to expand the distribution of lactate to encompass the entire residual source area.

Cape Canaveral Building 1381. Cape Canaveral building 1381 was the first "Reductive Anaerobic Biological In Situ Treatment Technology " (RABITT) protocol demonstration site (AFRL, 2001). This protocol specifically outlined required criteria for study sites, including: groundwater contaminated with PCE or TCE greater than 1 ppm concentration, hydraulic conductivities greater than 10^{-4} cm/s, and relatively shallow (<50 ft bgs) and homogeneous aquifers with well-defined stratigraphy so that the injected fluids could be tracked. All of these sites also underwent continuous, low concentration (approximately 260 mg/L) electron donor injections.

As a result of historical waste disposal activities at Cape Canaveral's Facility 1381, an approximately 110 acre TCE, DCE, and VC plume exists, and is believed to discharge to a surface water body adjacent to the site. As part of the RABITT protocol, a site assessment was performed to determine whether the criteria mentioned above were met. Although the field site was naturally anaerobic with concentrations of VC exceeding 1,000 ppb and ethene up to 18 ppb, microcosm studies using site soil and groundwater only achieved partial anaerobic reductive dechlorination to DCE (Parsons, 1999). From these studies lactate was chosen as the electron donor for the field pilot test because it stimulated anaerobic reductive dechlorination in more soil samples from different locations than did the other electron donors evaluated. In this case, however, microcosm studies were not an accurate indicator for completeness of anaerobic reductive dechlorination reactions to ethene in the field.

The subsurface aquifer at the Cape Canaveral field site is unconfined, 35 ft thick, comprised of sands interspersed with silts and clays, and has a groundwater velocity and conductivity conducive for enhanced *in-situ* bioremediation (Table 1). The injection system installed for enhanced *in-situ* bioremediation included two communicating wells, each fitted with 2.5 ft dual screens located at 10 ft and 17.5 ft bgs; one operating in upflow mode and the other operating in downflow mode (Table 2). This system was designed so that continuous injection of electron donor could occur with *in situ* recirculation. This was employed so that mixing of the nutrient amendment could occur, and hydrologic control of the treatment area could be maintained without re-injection of the contaminated water, which was prohibited by Florida law. Thirteen tri-level monitoring probes, and six monitoring wells were also installed up- and downgradient of the injection system.

The continuous injection of 260 mg/L lactic acid at 2 gal/min (Table 2) through the treatment system resulted in a treatment volume of approximately 2400 ft^3. Within the lactate-impacted area significant changes in the ambient geochemistry were observed including the depletion of sulfate (<0.5 mg/L) and redox (<150 mV), and the generation of dissolved iron (>7 mg/L) and methane (3.5 mg/L) (Table 3). In spite of the microcosm results, complete anaerobic reductive dechlorination of TCE to ethene was observed 10 weeks after lactate injection began, and after 25 weeks ethene was the predominant anaerobic reductive dechlorination product detected, with a recovery of ethene approximately 42% that of initial TCE (Table 3).

Naval Air Station Point Mugu. The Naval Air Station Point Mugu has a chlorinated solvent plume covering an approximate area of 5000 ft^2 resulting from leakage from an underground storage tank (Leigh et al. 2000). The subsurface conditions at this site include a confined aquifer comprised of sand overlain by a clay aquitard with sufficiently high hydraulic conductivity and groundwater velocity to allow for aqueous electron donor distribution (Table 1). This site is close to the ocean, and consequently seawater intrusion significantly impacts the geochemistry and very high levels of chloride (5,000 mg/L) and sulfate (700 mg/L) are prevalent. Microcosm studies indicated that complete anaerobic reductive dechlorination of TCE to ethene would not occur in the presence of sulfate. Therefore, sulfate had to be depleted before bioremediation could be successful.

A pilot study for enhanced *in-situ* bioremediation using lactic acid was designed, and included two phases. The objective of the first phase was to isolate the contaminated portion of the aquifer from the prevailing geochemical conditions, and to reduce the high sulfate within the treatment area so that efficient anaerobic reductive dechlorination could occur. The objective of the second phase was to facilitate efficient anaerobic reductive dechlorination by injecting a large slug of lactic acid into the treatment area. To accomplish these goals a dual injection strategy was employed using both continuous injection with recirculation for phase one, and a high concentration/high volume pulsed injection for phase two (Table 2). The injection system was comprised of an injection well and an extraction well fifty feet downgradient with five monitoring wells interspersed between. The continuous recirculation (10 gal/min) of groundwater for six hours followed by a 15 minutes pulse of 17 gallons of lactic acid (88% w/w) resulted in detection of electron donor, as propionate and acetate, throughout the treatment area. These injections resulted in an electron donor area of influence comprising a volume of approximately 50,000 ft^3. Sixty-four days after injection began, the goal of the first phase was achieved and sulfate was depleted (<5 mg/L) throughout the treatment area (Table 3). After this, a high concentration pulse injection was performed, and the recirculation was discontinued. During this phase, significant methane production (to 17 mg/L) was also observed, as was more efficient anaerobic reductive dechlorination (Table 3).

Like the Test Area North site, the prevailing redox conditions at Point Mugu appeared to dictate anaerobic reductive dechlorination progress. Nearly stoichiometric dechlorination of TCE to *cis*-DCE was observed during the period when sulfate was present (Table 3). Not until after sulfate was depleted and methane production began, however, was dechlorination of cis-DCE to ethene observed. The most efficient anaerobic reductive dechlorination to ethene occurred after the large injection, when propionate and acetate were the available electron donors. Thus, like the Test Area North site, anaerobic reductive dechlorination to ethene was not observed until after methanogenic conditions were achieved, and dechlorination efficiency increased when propionate degradation was the predominant hydrogen-producing reaction. One hundred and thirty days after the pulsed injection, however, propionate and acetate were depleted, causing VC and ethene concentrations to level off. Due to a lack of electron donor, no further dechlorination was observed, and the accumulated VC remained. Interestingly, rather than inject more electron donor to the carbon limited system, it was concluded that the degradation rate of VC was too slow, and other aerobic bioremediation treatment options are being considered. This decision was made based on laboratory studies which suggested that VC reduction only occurred in the presence of TCE, and because all TCE within the system had been reduced, further degradation of VC seemed unlikely.

Bachman Road Residential Well Site. This site is located within a highly industrialized area of the Great Lakes region, and as a result of industrial activities, a large groundwater PCE contaminant plume is migrating into Lake Huron (Lendvay et. al. 2001a, 2001b). Table 1 describes the general hydrogeology of the aquifer, which is characterized as a shallow, sandy gravel aquifer perched on a clay aquitard, with relatively high conductivity and groundwater velocity. The injection system (Table 2) was installed to implement a pilot-scale field evaluation, and included two injection wells six feet apart, an extraction well ten feet downgradient, and four monitoring wells for a total treatment area approximately 270 ft^2. Table 2 outlines the injection strategy, which included the continuous injection of low concentration lactate through the entire depth of the aquifer (~8 ft), with extraction only in the lower half of the saturated zone so that dechlorinating microbes located in the deep portion of the aquifer would be circulated and reinjected into the upper portion of the aquifer. The lactate injections influenced an approximately 2,200 ft^3 volume of aquifer.

The injection concentration at the Bachman road site was extremely low (9.0 mg/L), which impacted the overall efficiency of the system. Table 3 outlines the redox conditions and dechlorination achieved for this site. In a monitoring well closest to the injection wells (~3 ft), the redox conditions were approximately –250 mV before injection, and increased to +100 mV after injection began, indicating that

the injection water was not anaerobic. Downgradient monitoring wells (~6 ft) indicated increased ferrous iron concentrations, and decreased dissolved oxygen, suggesting that the lactate was stimulating anaerobic conditions and iron reduction at these locations. Dechlorination of PCE to DCE occurred in the lower aquifer prior to lactate amendment. Indigenous dechlorinating bacteria, including *D. ethenogenes,* were also detected by molecular methods. Despite the presence of a dechlorinating native community, however, twenty days after lactate injections began bioaugmentation was performed using a completely dechlorinating culture. Forty days after this, ethene was the predominant anaerobic reductive dechlorination product detected in the samples taken from the extraction well. The dechlorination ability of the indigenous microbial community cannot be assessed because lactate had only influenced the area for a short time, and the necessary redox conditions were not achieved prior to the bioaugmentation. In any case, lactate successfully stimulated complete dechlorination to ethene throughout the treatment cell.

Dover Air Force Base. Area 6 of the West Management Unit located within Dover Air Force Base is characterized by the presence of chlorinated solvents, including PCE and TCE, in the groundwater as a result of historical disposal practices (U.S. EPA 2000a). The subsurface is comprised of a silt and sand saturated zone approximately 38 ft thick with a relatively high hydraulic conductivity and groundwater velocity (Table 1). A feasibility study of enhanced *in-situ* bioremediation as a treatment option was evaluated using lactate (both sodium salt and acidic forms) as an electron donor. Before the field evaluation, microcosm studies, column studies, and a borehole test were performed and indicated anaerobic reductive dechlorination of TCE only occurred to DCE. Therefore, the pilot study was designed so that after 6 months of lactate injection without ethene production, bioaugmentation would be performed. The field pilot test lactate injection system consisted of three injection wells and three downgradient extraction wells with closed loop-recirculation that were aligned perpendicular to groundwater flow to create a pilot area of approximately 2400 ft^2 (Table 2). The injection and extraction wells were screened over the lower 10 ft of the saturated zone, and six monitoring wells were interspersed within the pilot area and screened at different saturated depths (Table 2).

The injection strategy included the continuous injection of a low concentration sodium lactate (100-200 mg/L) and nutrient solution for 2.75 days followed by an 8 hour flush, then injection for 3.75 days followed by an 8 hour water flush (Table 2). This is the only study that reported biofouling as a problem, reportedly affecting the consistency of injections, the injection concentration, and the consistency of injection rates. Despite these problems, however, significant geochemical changes occurred after lactate injection began as sulfate and nitrate were depleted, ferrous iron increased, and methane production was observed throughout the treatment area (Table 3). Efficient anaerobic reductive dechlorination to ethene, however, was not observed, although nearly stoichiometric conversion of TCE to DCE had occurred throughout the treatment area. Molecular analysis of groundwater samples from the Dover treatment area also suggested that it was biologically limited, as indigenous microbes capable of complete dechlorination, specifically *D. ethenogenes,* were not detected. Therefore, 269 days after lactate injection began bioaugmentation was performed using the Pinellas culture. With continued lactate injection, nearly complete conversion occurred of DCE to ethene throughout the treatment area occurred approximately 7 months after bioaugmentation (Table 3).

Seal Beach. The naval weapons station Seal Beach site 40 was involved in locomotive maintenance activities, which resulted in significant accumulation of chlorinated solvents in underlying soils. Table 1 describes the general hydrogeology of this system, which was characterized as a semi-perched unconfined aquifer 75-200 ft thick, comprised of silty sands intercalated with clays. The injection system installed included one injection well, with six monitoring wells interspersed within a 20 ft radius (Table 2). Initial sulfate concentrations within the contaminated aquifer were relatively high (160-480 mg/L). The *in-situ* bioremediation injection strategy included weekly, pulsed injections of high concentration (3%) sodium lactate to deplete the sulfate within the treatment area. The electron donor injections resulted in distribution of electron donor over an approximately 1300 ft^2 area and 20,000 ft^3 volume (Table 2). After

significant accumulation of propionate and acetate, the weekly injections were discontinued for two months. After this period, half-volume injections were conducted every three weeks for the remainder of the test.

The electron donor injections significantly impacted the geochemistry within the treatment area (Table 3). After four months of injections, sulfate was depleted to below detection limit (<50 mg/L), and ferrous iron (>30 mg/L) and methane (14 mg/L) concentrations increased. Once sulfate-reducing conditions were achieved within the treatment area, complete conversion of PCE to *cis*-DCE was observed. Anaerobic reductive dechlorination of accumulated *cis*-DCE, however, was not observed even after achieving methanogenic redox conditions. Like the Dover AFB site, molecular analysis of the microbial community at Naval Air Station Seal Beach indicated a lack of indigenous bacteria capable of complete anaerobic reductive dechlorination. Thus, it was determined that the Naval Air Station Seal Beach site was biologically limited, rather than limited by redox conditions, and a bioaugmentation pilot test is currently being designed.

RESULTS FOR *IN-SITU* BIOREMEDIATION USING BUTYRATE

Naval Air Station Alameda. All of the field sites reported using butyrate were part of the RABBITT demonstration, as was the Cape Canaveral lactate site (AFRL 2001, 2002). The first of these is the Naval Air Station Alameda site, which was an aircraft engine and repair facility. As a result of cleaning, stripping and plating activities, a chlorinated solvent contaminant plume is located in the subsurface aquifer. Table 1 presents the available hydrogeologic data, which was limited. The aquifer was characterized as unconfined, with relatively low hydraulic gradient and groundwater velocity. Microcosm studies with several aqueous electron donors indicated that sulfate reducers were unable to use butyrate. The butyrate-fed microcosms, however, performed the most efficient anaerobic reductive dechlorination, leading to its selection for the field test. Table 2 outlines the *in-situ* bioremediation injection system, which consisted of three injections wells with nine monitoring wells within a 45 ft^2 area, and an extraction well 93 ft downgradient for recirculation with nutrient amendment. The injection strategy involved the continuous injection of low concentration (270 mg/L) butyrate with yeast extract through a 3-ft section of the contamination zone. These injections resulted in impacts throughout the monitored area, for a total treated volume of approximately 135 ft^3.

The initial redox conditions were generally aerobic, with high sulfate concentrations (>600 mg/L), similar to Point Mugu and Seal Beach. The groundwater extracted and recirculated from the downgradient well was also contaminated with TCE, thereby providing a continuous source of TCE to the treatment area. Eight weeks after injection began, ferrous iron increased (3.0 mg/L), and nitrate, nitrite (<1.0 mg/L), and sulfate decreased (<200 mg/L) throughout the treatment area. After sulfate reducing conditions were achieved, significant TCE reduction to *cis*-DCE occurred. After twenty weeks, however, the average sulfate concentrations were still high (~200 mg/L), yet significant methane production was also observed (0.2 mg/L). Coinciding with the onset of methanogenesis was anaerobic reductive dechlorination of *cis*-DCE and VC, leading to ethene accumulation. Thus, the prevailing sulfate-reducing conditions did not appear to inhibit methanogenesis or complete dechlorination, as was observed at other field sites. This is the only site in this study where this was observed. Ultimately 60% of injected TCE was accounted for as ethene in this system.

Fort Lewis. The second butyrate site was the Fort Lewis East Gate Disposal Yard, Washington, a site of extensive military disposal of liquid and solid wastes (AFRL 2001, 2002). These activities resulted in a contaminant plume of TCE approximately 2 miles long, 3,500 ft wide, and 70 ft thick. Table 1 describes the general hydrogeology of the area, which is characterized as an unconfined aquifer comprised of alluvial sands and gravels intercalated with silt and clay lenses, with high hydraulic conductivity and extremely high groundwater velocity. Microcosm studies indicated that complete, albeit slow, anaerobic

reductive dechlorination of TCE to ethene could be stimulated using sediments and groundwater from the contaminated area.

The injection system was inadvertently installed nearly perpendicular to groundwater flow. This was the result of complex hydrologic conditions, and by pump and treat activities located near the test location. The injection system included three injection wells, with nine monitoring wells downgradient, and comprised a treatment area approximately 90 ft^2 (Table 2). An extraction well was also installed 230 ft cross gradient as a supply of injection water, and also served as an additional source of TCE and DCE. The injection strategy involved the continuous injection of low concentration (270 mg/L) butyric acid with yeast extract and sodium bicarbonate as a buffer to the butyric acid (Table 2). The injections resulted in a butyrate-impacted treatment volume of approximately 270 ft^3.

These injections resulted in significant impacts to the local geochemistry (Table 3). After 20 weeks, dissolved ferrous iron increased (4 mg/L), and sulfate (1 mg/L), nitrate, and nitrite (ND) decreased, and redox conditions ranged from +27 to -100 mV. Methane production was negligible, except after a period when the pump broke at the extraction well and concentrated butyric acid stock solution was injected. Like most of the other sites evaluated, the local redox conditions seemed to dictate the anaerobic reductive dechlorination efficiency within the treatment cell. Dechlorination of TCE to *cis*-DCE occurred after four weeks, and remained efficient throughout the remainder of the pilot test. This occurred even after injections of more concentrated TCE (>10X) (Table 3). Anaerobic reductive dechlorination of *cis*-DCE and VC, however, were spotty, and only trace ethene was ever detected. Microcosms studies indicated that complete dechlorination to ethene was possible at Fort Lewis, which suggests that the area was not biologically limited. Thus, the inability to efficiently dechlorinate *cis*-DCE and VC likely occurred because completely reducing conditions were never achieved due to a lack of sufficient electron donor.

Observations made after the malfunction in the pump from the supply well support the conclusion that the redox conditions were the limiting factor at Fort Lewis. The malfunction resulted in the injection of concentrated butyric acid instead of the low concentration mixture. During this period, the only significant methane ever detected was observed along with the lowest redox potential ever recorded. Likewise, the most efficient dechlorination in the first 28 weeks was observed during this period, as indicated by the highest *cis*-DCE and VC levels seen (other than during the high concentration TCE amendments). Accumulated butyric acid was also degraded faster to acetate (and hydrogen) as indicated by decreased butyrate concentrations and increased acetate concentrations. After the pump was fixed, and low-concentration injection resumed, methane declined, redox rebounded, and butyric acid accumulated to higher concentrations. Concentrations of c*is*-DCE and VC dropped at the next sampling event after the low-concentration injection was resumed. Thus, more efficient anaerobic reductive dechlorination may have been observed if higher electron donor concentrations had been used, allowing redox potential to remain sufficiently negative.

Camp Lejeune. The third site evaluated in the RABBITT demonstration was the Marine Corps Camp Lejeune site 88 (AFRL, 2002). This is the site of a former dry cleaning facility that disposed of spent PCE through a floor sewer drain. The activities resulted in a VOC contaminant plume, including PCE, TCE and DCE. The local hydrology of the system includes an unconfined aquifer comprised of upper and lower zones, with a higher hydraulic gradient in the lower zone (Table 1). Therefore, the *in-situ* bioremediation pilot study was conducted in the lower (45-50 ft bgs) aquifer zone, although microcosm studies performed with sediments from the lower aquifer contamination zone were unable to dechlorinate past DCE. The injection system included the three injection wells, and nine downgradient monitoring wells for a total treatment area of 120 ft^2 (Table 2). Due to problems with the injection wells, however, the injection strategy was modified so that injection occurred in a monitoring well at the center of the original treatment area, and the injection wells were used as monitoring wells. An extraction well was

also installed approximately 100 ft upgradient to the original injection wells to mix the nutrients, and provide an additional source of PCE and trace TCE to the treatment area.

Low concentration, continuous injections of butyric acid (270 mg/L) influenced approximately 360 ft^3 volume of the aquifer (Table 3). This injection strategy had more successful impacts on the geochemistry of the aquifer than the Fort Lewis site (Table 3). After sixteen weeks of injection, nitrate, nitrite (<0.4 mg/L), sulfate (<0.5 mg/L), dissolved oxygen (<0.5 mg/L), and redox (<-250 mV) significantly declined. Methane production (25 mg/L) also began during this period. Unlike the Fort Lewis site, complete anaerobic reductive dechlorination occurred and was also apparently dictated by prevailing redox conditions (Table 3). Dechlorination of PCE and TCE to *cis*-DCE occurred in the presence of significant sulfate, but as with most sites evaluated, reduction of *cis*-DCE to ethene did not occur until methane production was observed. Nearly all injected PCE and TCE were converted to DCE, VC, and ethene. Thus, it was demonstrated that efficient anaerobic reductive dechlorination of PCE and TCE to ethene could be achieved at this site, in spite of microcosm data suggesting anaerobic reductive dechlorination might stop at DCE (like Cape Canaveral).

RESULTS OF *IN-SITU* BIOREMEDIATION USING A METHONOL MIXTURE

Kelly Air Force Base. The Kelly Air Force Base Building 360 site contains several landfill trenches responsible for groundwater VOC contamination, including PCE, TCE, DCE and VC (Major et al. 2001, 2002). Microcosm studies indicated the indigenous microbial community at this site was not capable of complete dechlorination to ethene. Only after bioaugmentation with the KB-1 culture was complete dechlorination of accumulated DCE observed in the laboratory. Initial molecular characterization did not detect *Dehalococcoides ethenogenes* in the contaminated aquifer. The aquifer, however, was aerobic and so likely any indigenous dechlorinators would have been difficult to detect using molecular analysis. Therefore, the pilot study was designed so that bioaugmentation would be performed only after several months of electron donor addition, if the VOCs were not being degraded.

The contaminated unconfined aquifer is comprised of alluvial sands, gravels, and silts, with a very high groundwater velocity (Table 1), similar to Fort Lewis. The injection system consisted of a closed loop recirculation system, including three extraction wells thirty feet downgradient of the one injection well, and five monitoring wells, with a treatment area of approximately 90 ft^2. This site employed a continuous injection strategy of methanol (277 mg/L), and acetate (277 mg/L) with a recirculation system that mixed the nutrient amendments (Table 2).

The electron donor injections resulted in significant geochemical impacts. Seventy days after injection began, the redox conditions went from aerobic to anaerobic as indicated by decreased sulfate (7 mg/L), nitrate (<0.5 mg/L), and redox (-200 mV), and increased methane (6 mg/L), and dissolved iron (3 mg/L) (Table 3). Initially, the anaerobic reductive dechlorination efficiency correlated with the local redox conditions, with nearly stoichiometric dechlorination of TCE to *cis*-DCE. Since ethene was not detected after 176 days, the bioaugmentation culture was anaerobically injected into the aquifer after which all accumulated *cis*-DCE was dechlorinated to ethene (Table 3).

The premise that the Kelly AFB site was biologically limited was tentatively confirmed by subsequent molecular analysis. All of the dechlorinating bacteria detected were identical to those from the bioaugmented culture. Since no new dechlorinators were ever detected, it seems likely that complete dechlorination to ethene may not have occurred without bioaugmentation. *D. ethenogenes*, and ethene production, however, were detected in soils and groundwater from a landfill site 2.4 km away.

DISCUSSION

The case studies evaluated provide useful insight into the design and application of enhanced *in-situ* bioremediation using aqueous electron donors. Aqueous electron donors have many properties that make them ideal for some applications. First, they are by definition highly soluble, which makes radial and

downgradient distribution of electron donor throughout large areas easier. The distribution is also a function of the hydraulic properties of the particular aquifer. For most sites in Table 1, the aqueous electron donors were well distributed throughout the treatment volumes with small numbers of injection wells. In fact, of the 10 cases considered, only two used more than one injection well. A comprehensive understanding of challenging systems can lead to successful injection strategies as occurred for the Test Area North and Point Mugu systems using lactate. This illustrates the importance of developing a sound site conceptual model for use in designing an injection strategy.

Once distributed, the second important property of electron donors is their degradability. Lactate and was a more readily utilized electron donor than was butyrate, as indicated by the degree to which the particular electron donor accumulated in the field (AFRL, 2001). The area impacted by the highly degradable electron donors achieved methanogenic conditions quickly. Therefore, the onset of complete anaerobic reductive dechlorination of TCE to ethene was generally achieved quickly. The first four lactate sites all exhibited methanogenic conditions that were accompanied by complete dechlorination to ethene. At Bachman Road and Dover, bioaugmentation was used and resulted in complete dechlorination, although it was not clearly needed at the former. Seal Beach displayed methanogenic conditions after lactate injection, but dechlorination stopped at DCE, apparently due to a biological limitation at the site.

The three RABITT demonstrations provided insight into the variety of outcomes possible using a low concentration, continuous injection strategy with butyric and lactic acids. Using butyrate at Naval Air Station Alameda all chlorinated ethenes were completely reduced, despite the presence of high sulfate, whereas at Fort Lewis reducing conditions were never achieved, which prevented efficient dechlorination of DCE and VC. The Camp Lejeune site butyric acid injection followed the same general trend as the successful lactate injections, namely that completely reducing conditions were achieved, and dechlorination of *cis*-DCE and VC coincided with methane production. Cape Canaveral was the only RABITT demonstration site that used lactic acid instead of butyric acid as the electron donor. This site achieved methanogenic conditions and anaerobic reductive dechlorination of TCE to ethene.

The two factors in the case studies that resulted in incomplete dechlorination at sites appear to be 1) an inadequate supply of readily degradable electron donor that results in insufficiently reducing conditions, or 2) a lack of organisms capable of complete dechlorination. Given these observations, a logical approach to initiating enhanced *in-situ* bioremediation through anaerobic reductive dechlorination would be to begin with relatively high concentrations of an aqueous electron donor to establish strongly reducing conditions quickly. Once this is accomplished, either complete dechlorination will commence, or any biological limitations will be evident. After complete dechlorination has begun, options for optimizing the longer term bioremediation process such as decreasing injection concentration or frequency, or even switching electron donors could be considered.

The key components for employing enhanced *in-situ* bioremediation of chlorinated solvents are developing an injection strategy that achieves adequate distribution of the electron donor throughout the contaminated area, achieving the appropriate redox conditions within the treatment area, and stimulating capable biology to perform efficient anaerobic reductive dechlorination. A variety of aqueous electron donors have been used successfully at contaminated sites using various injection strategies. A significant objective of any strategy should be to monitor the system with sufficient detail to enable optimization and troubleshooting to be performed when necessary.

REFERENCES

Air Force Research Laboratory (AFRL), Battelle Memorial Institute, Cornell University, USEPA, NFESC. 2001. *Reductive Anaerobic Biological In-Situ Treatment Technology (RABITT) Treatability Test Interim Report.* 17 August.

Fennell, D. E., and J.M. Gossett. 1998. Modeling the Production of and Competition for Hydrogen in a Dechlorinating Culture. *Environ. Sci. Technol.*, v. 32, no. 16, pp. 2450-2460.

Leigh, D.P., C.D. Johnson, R.S. Skeen, M.G. Butcher, L.A. Bienkowski, S. Granade. 2000. Enhanced Anaerobic In Situ Bioremediation of Chloroethenes at NAS Point Mugu. *Proceedings of the Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California*. Vol. C2-4, p. 229-235.

Lendvay, J.M., Barcelona, M.J., Daniels, G., Dollhopf, M.E., Fathepure, B., Gebhard, M., Heine, R., Hickey, R., F.E. Loffler, Major, Jr. C.L., Petrovskis, E., Shi, J., Tiedje, J.M., Adriaens, P. 2001a. Plume control using bioaugmentation with halorespiring microorganisms. *Groundwater Quality 3rd International Conference, Sheffield, UK.*

Lendvay, J.M., Adriaens, P., Barcelona, M., Major, C.L., Tiedje, J., Dollhopf, M., Loeffler, F., Fathepur, B., Petrovskis, E., Gebhard, M., Daniels, G., Hickey, R, Heine, R., and Shi, J. 2001b. Preventing Contaminant Discharge to Surface Waters: Plume Control with Bioaugmentation. *Proceedings of the Sixth International In-Situ and On-Site Bioremediation Symposium, San Diego, California*. No. 6(8), p. 19-26.

Major, D.W., M.L. McMaster, E.E. Cox, E.A. Edwards, S.M. Dworatzek, E.R. Hendrickson, M.G. Starr, J.A. Payne, and L.W. Buonamici. 2002. Field Demonstration of Successful Bioaugmentation to Achieve Dechlorination of Tetrachloroethene to Ethene. *Environmental Sci. Technol.* Vol. 36:5106-5116.

Major, D.W., M.L. McMaster, E.E. Cox, B.J. Lee, E.E. Gentry, E. Hendrickson, E. Edwards, and S. Dworatzek 2001. Successful Field Demonstration of Bioaugmentation to Degrade PCE and TCE to Ethene. *Proceedings of the Sixth International In-Situ and On-Site Bioremediation Symposium, San Diego, California*. No. 6(8):27-34.

Martin, J.P., Sorenson, K.S., and L.N. Peterson. 2001. Favoring Efficient In Situ Dechlorination through Amendment Injection Strategy. *Proceedings of the Sixth International In-Situ and On Site Bioremediation Symposium, San Diego, California*, No. 6(7), p. 265-272.

Maymo-Gatell, X., V. Tandoi, J.M. Gossett, and S.H. Zinder. 1995. Characterization of an H_2-utilizing enrichment culture that reductively dechlorinates tetrachloroethene to vinyl chloride and ethene in the absence of methanogenesis and acetogenesis. *Appl. Environ. Microbiol.*, *61*(11), 3928-3933.

Parsons. 1999. *Final Remediation by Natural Attenuation Treatability Study for Facility 1381 (SWMU 21), Cape Canaveral Air Station, Florida*. Prepared for the Air Force Center for Environmental Excellence. December.

Smatlak, C.R., Gossett, J.M., and Zinder, S.H. 1996. Comparative Kinetics of Hydrogen Utilization for Reductive Dechlorination of Tetrachloroethene and Methanogenesis in an Anaerobic Enrichment Culture. *Environ. Sci. Technol.*, 30(9):2850-2858.

Sorenson, K.S. 2000. Biodegradation of TCE Improved with Lactate Injection in Deep, Fractured Rock. *Ground Water Currents*, No. 38, December.

USEPA. Office of Solid Waste and Emergency Response. 2000a. *Engineered Approaches to In Situ Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications.* Division of Solid Waste and Emergency Response. EPA 542-R-00-008. July. http://www.epa.gov/clu-in.org.

Table 1. Hydrogeology of Study Sites

Site Name (Sorted by Substrate)	Site Hydrogeology	Depth to Groundwater (feet bgs)	Groundwater Velocity (ft/yr)
Lactate			
Test Area North, INEEL	Fractured basalt bedrock impacted from 200 and 475 ft bgs, bounded by thick silty aquitard.	200	88 to 179
Cape Canaveral Air Station- Facility 1381	Unconfined aquifer 35 ft thick comprised of poorly sorted coarse to fine sands and shell material to 35 ft, and small grains, silts and clays from 35 to 48 ft bgs, underlain by a continuous clay unit from 48 to 51 ft bgs.	4 to 7	77
Naval Air Station Point Mugu IRP Site 24	Shallow unconfined aquifer consisting of 10 ft of sand and gravel overlying a 4 ft clay aquitard, and a large confined aquifer.	5	N/A
Bachman Road Residential Well Site	Unconfined sandy gravel aquifer 8 ft thick underlain by a thick, dense clay aquitard.	8	1,800
Area 6, Dover AFB	Unconfined aquifer 38 ft thick comprised of sand with varying amounts of clay, silt and gravel.	10 to 12	140
Installation Restoration Program Site 40 at the Naval Weapons Station, Seal Beach CA	The unconfined, saline aquifer is approximately 75 to 200 ft thick, and is comprised of surficial soils, sands, and silty sands intercalated with low permeability intervals containing clays, silt and silty clay.	8 to 9	N/A
Butyrate			
Naval Air Station Alameda Building 360 (Site #4)	Unconfined shallow aquifer with low groundwater velocity.	4.4 to 6.5	11.4
Fort Lewis East Gate Disposal Yard	Unconfined, shallow aquifer 13 ft thick comprised of brown to black alluvial sands and gravel, with localized lenses of silts and clays to 13 ft bls, underlain with Vashon Till consisting of gray, dense, well-graded gravel in sand, silt, and clay that acts as an aquitard.	10	1,095 to 1,278
Marine Corps Camp Lejeune Site 88	Unconfined, surficial aquifer comprised of two units, the upper is 15 ft thick and a lower unit 45 to 50 ft bgs.	N/A	N/A
Methanol and Acetate			
Building 360, Kelly Air Force Base	Unconfined aquifer comprised of 20 to 40 ft of alluvial gravel, sand, and silt overlying impermeable clay.	5 to 10	1,095

Table 2. Injection Strategy of Study Sites

Site Name (Sorted by Substrate)	Substrate Type, Amount, and Concentration	Injection Frequency	Bioaugmentation or Amendments	Treatment Area		System Configuration
				Surface Area (ft^2)	Thickness (ft)	
Lactate						
Test Area North, INEEL	Sodium Lactate; 60,000 mg/L (V=6,000 gal.)	Weekly	None	4,000	200	Single injection well with 11 monitoring wells in the source area, and multiple downgradient extraction wells with air stripper.
Cape Canaveral Facility 1381	Lactic Acid; 267 mg/L to 2-6 mM	Continuous (2.0 gal/min)	None	240	10	Two communicating (recirculation) wells fitted with dual screen design, with one operating in an upflow mode and one in a downflow mode so that effluent of one well was feeding the influent of the other, 13 tri-level groundwater monitoring probes, and 6 upgradient and downgradient monitoring wells.
Naval Air Station Point Mugu IRP Site 24	Lactic acid; 880,000 mg/L (V=1,020 gal.)	Continuous (10 gal/min); 6 hours inject then 15 minutes of 88% w/w lactic acid.	None	5,000	10	One extraction well, one injection well and five monitoring wells. Groundwater was circulated in a closed loop between the extraction well and the injection well.
Bachman Road Residential Well Site	Lactate; 8.98 mg/L	Continuous (4.0 gal/min)	Bioaugmentation, nitrogen, phosphorous	270	8	Two injection wells; one extraction well; network of monitoring points around the plot.
Area 6, Dover AFB	Sodium lactate; 200 mg/L	Semi-continuous (3.75 gal/min); nutrient for 2.75 days, 8-hour flush; nutrient for 3.75 days, 8-hour flush.	Bioaugmentation with a microbial culture from the DOE Pinellas Site, dibasic ammonium phosphate, and yeast extract	2,400	10	Three injection wells, screen 38 to 48 ft bgs. Closed loop recirculation cell w/three recovery wells. Cyclic injection of nutrients and unamended GW.
Installation Restoration Program Site 40 at the Naval Weapons Station, Seal Beach CA	Sodium lactate; 3,000 mg/L (V=1,775 gal.)	Every 1 to 3 weeks	None	1,300	15	One injection well, with six monitoring wells interspersed within a 20 ft. radius. Weekly injections for 2 months, then none for 2 months, lastly half the volume (1,775 gal.) every 3 weeks.
Butyrate						
Naval Air Station Alameda Building 360 (Site #4)	Butyric acid; 270 mg/L to 3 mM	Continuous (0.17 gal/min)	Yeast extract, to 20 mg/L *in situ*	45	3	Three injection wells, single extraction well with recirculation, and nine monitoring points at depths of 24 to 27 ft bgs.
Fort Lewis East Gate Disposal Yard	Butyric acid; 264 mg/L	Continuous (0.40 gal/min)	Yeast extract, 20 mg/L; Sodium bicarbonate, 279 mg/L; sodium bromide	90	3	Three injection wells spaced 1.5 ft apart; system of six monitoring wells; existing well in zone of contamination used to supply injection water for test plot.
Marine Corps Camp Lejeune Site 88	Butyric acid; 264 mg/L	Continuous (0.16 gal/min)	Yeast extract, 20 mg/L; sodium bromide	120	3	One injection well, nine monitoring wells at depths of 45 to 48 ft bgs.
Methanol and Acetate						
Building 360, Kelly Air Force Base	Methanol; 277 mg/L and Acetate; 277 mg/L	Continuous (1.5 gal/min)	Bromide, KB-1 bioaugmentation	90	25	Closed loop recirculation system consisting of three extraction wells, one injection well and five monitoring wells.

Table 3. Redox Conditions and Dechlorination of Study Sites

Site Name (Sorted by Substrate)	Redox Conditions	Maximum Pre-Treatment Concentrations (mg/L)	Maximum Post-Treatment Concentrations (mg/L)	Comments
Lactate				
Test Area North, INEEL	Nitrate (0 mg/L); Sulfate (0 mg/L); Fe2 (>3.0 mg/L); Methane (>10 mg/L); Redox (<-200 mV)	TCE - 3.2	TCE - < 0.005	Within 6 weeks from the start of lactate injection, nearly 100% of TCE concentrations in groundwater had been dechlorinated to DCE and complete dechlorination was occurring within 4 months.
Cape Canaveral Facility 1381	D.O. (<0.5 mg/L); Fe2+ (7 mg/L); Sulfate (0 mg/L); Redox (<-150 mV)	TCE - 1.58 DCE - 10.95 (dropped to 8.53) VC - 1.25 Ethene - < 0.2	TCE - ND DCE -1.16 VC - 0.5 Ethene - 1.12	RABITT protocol slightly modified because Florida's underground injection control regulations do not allow for reinjection of contaminated groundwater. Reduction of TCE, DCE, and VC by 97, 88, and 66%, respectively. Ethene production accounted for 42% mass.
Naval Air Station Point Mugu IRP Site 24	Sulfate (>0.5 mg/L); Methane (17.0 mg/L)	TCE - 1.7 DCE - 0.75 VC - 0.001 Ethene – N/A	TCE - < 0.005 DCE - < 0.005 VC - 0.015 Ethene - 0.020	Within approximately 180 days TCE and DCE concentrations had been reduced by nearly 100% at one well. However VC concentrations increased by a factor of 15 within the same time period.
Bachman Road Residential Well Site	Redox (100 mV)	TCE - 0.13 DCE - 0.19 VC - 0.25 Ethene - ND	TCE - ND DCE - ND VC - ND Ethene - 0.36	Contaminant concentration reduction, as measured in one monitoring well, was nearly 100% for TCE, DCE, and VC.
Area 6, Dover AFB	Nitrate (0 mg/L); Sulfate (0 mg/L); Methane	PCE - 0.046 TCE - 7.5 DCE - 2 VC - 0.034	PCE - ND TCE - 0.075 DCE - 0.045 VC - 0.020	As of March 1998, 98.5% of TCE and DCE in groundwater were converted to ethene, and 75 to 80% of the TCE and DCE mass had been recovered as ethene.
Installation Restoration Program Site 40 at the Naval Weapons Station, Seal Beach CA	Nitrate (0 mg/L); Fe2 (30 mg/L); Sulfate (0 mg/L); Methane (12 mg/L); Redox (<-200)	PCE - 0.415 TCE - ND DCE - ND VC – ND Ethene-ND	PCE - ND TCE - ND DCE - 0.485 VC – ND Ethene-ND	
Butyrate				
Naval Air Station Alameda Building 360 (Site #4)	Nitrate, Nitrite (>0.5 mg/L); Fe2+ (>2 mg/L); Sulfate (125 mg/L); Redox +27 to 200 mV	TCE - 0.66 to 3.29 TCE (injected)-11.06 DCE–ND DCE (injected)-0.6VC - ND VC (injected) - 0.21 Ethene – ND	TCE - 9.20 DCE - 5.81 VC - 1.56 Ethene - 1.68	Water injected with amendments contained average TCE, cDCE, and VC concentrations of 84.2, 6.5, and 3.4 µM, respectively. Average TCE was reduced by 99% to ethene (60%) by the end of the demonstration.
Fort Lewis East Gate Disposal Yard	Nitrate (0 mg/L); Fe2 (4 mg/L); Sulfate (4 to 6 mg/L); Redox (>+0 mV)	TCE - 1.5 to 6.3 TCE (injected) - 5.2 to 169 DCE - 0.05 to 0.13 VC-ND Ethene-ND	TCE - 78.84 DCE - 77.52 VC - 0.19 Ethene-ND	Injected TCE concentrations spiked as high as 169 mg/L, but this did not prove toxic to the microorganisms. TCE reduced 99.9%, to cDCE and VC. Never got ethene.
Marine Corps Camp Lejeune Site 88	D.O. (>0.5 mg/L); Fe2 (4.4 mg/L); Sulfate (0.5 to 1.5 mg/L); Redox (-270 to -313 mV)	PCE - 3 to 9 PCE (injected)- 3 TCE - 0.2 to 0.7 TCE (injected)- 1.1 to 2.8 DCE - 0.042 to 0.118	PCE - 0.83 TCE - 1.18 DCE - 6.78 VC -4.38 Ethene-0.70	N/A
Methanol and Acetate				
Building 360, Kelly Air Force Base	Nitrate (<0.5 mg/L); Sulfate (7 mg/L); Fe2 (3 mg/L); Methane (6 mg/L); Redox (-200 mV)	PCE - 8.1 TCE - 0.19 DCE - 2.1 VC - ND Ethene - ND	PCE - 0.81 (est.) TCE - 0.019 (est.) DCE - 0.315 (est.) VC –N/A Ethene – N/A	Reported a 90% reduction in PCE. Cis-1,2-DCE degradation was observed only after the addition of the KB-1 microbial consortium amendment.

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APPENDIX E.11 – ENHANCED REDUCTIVE DECHLORINATION OF CAHS USING SOLUBLE CARBOHYDRATES - A SUMMARY OF DATA FROM 50 SITES

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Enhanced Reductive Dechlorination of CAHs using Soluble Carbohydrates – A Summary of Detailed Data from 50 Sites

Christopher C. Lutes, Angie Frizzell, Suthan S. Suthersan (ARCADIS G&M, Inc.)

Introduction

An alternative to conventional groundwater remediation technologies that has been used by ARCADIS at Federal and commercial sites is *In-situ* Reactive Zone technology for the remediation of chlorinated aliphatic hydrocarbons (CAHs) and metals. *In-situ* Reactive Zone involves the addition of a food grade carbohydrate substrate, which serves as a supplemental energy source for microbiological processes in the subsurface. The substrate is typically molasses, but can include high fructose corn syrup, cheese whey, and other carbohydrates (Suthersan et al., 2002). Through the injection of molasses to the subsurface, existing aerobic or mildly anoxic aquifers can be altered to highly anaerobic reactive zones. This creates suitable conditions for the biodegradation of CAHs and/or the precipitation of selected metals in insoluble forms. Thus this technology can be more specifically referred to as enhanced reductive dechlorination or Enhanced Anaerobic Reductive Precipitation.

As of March 2003, ARCADIS has been involved with more than 130 *In-situ* Reactive Zone sites across 5 countries and 26 U.S. states. CAHs are the target compounds at over 110 of the sites. The technology has successfully been applied to the following chlorinated compounds and metals:

- Tetrachloroethene (PCE), trichloroethene (TCE), dichloroethene (DCE), vinyl chloride (VC), 1,1,1-trichloroethane (TCA), carbon tetrachloride (CT), chloroform (CF), chlorinated propanes, pentachlorophenol (PCP), pesticides, trichlorofluoromethane, and perchlorate.
- Hexavalent chromium, nickel, lead, cadmium, mercury, and uranium.

ARCADIS has collected detailed information on the background conditions and performance of *In-situ* Reactive Zone applications company-wide. A survey of that information, including data from 50 enhanced reductive dechlorination sites, is presented in Table 1.

Type and Scale of Implementations

Of the approximately 110 CAH sites, all but one (a bench-scale study) are field applications, and 30 are full-scale implementations. Five enhanced reductive dechlorination sites have achieved closure, and approximately five more have reached regulatory goals and/or are near completion. Other sites are ongoing pilot applications, interim remedial measures, or completed pilot projects that are now in the full-scale design or implementation phase.

Enhanced reductive dechlorination has been implemented in a variety of geometries – in source zones, as barriers in the source areas and in downgradient portions of plumes, and plume-wide. Sizes of treatment areas have ranged from small pilot tests with a single injection point to a full-scale application of 50 acres. Treatment zones range from a few feet to 95 feet in thickness.

Table 1: In-situ Reactive Zone Site Survey

All *In-situ* Reactive Zone Sites		
Category	**Number of Sites**	**Notes**
Total sites	Approx. 130	Active or completed
Bench-scale	2	In progress or complete
Pilot-scale	68	In progress or complete
Interim Remedial Measures	9	In progress or complete
Full-scale in design	7	In progress or complete
Full-scale implemented	33	In progress or complete
Closed	5	
Not yet formally closed but have met MCLs or other remediation goals	Approx. 5	OH site (barrier at BDL); PA CAH/metals site pilot/IRM complete, NFA requested; PA mfg site closure pending; WI drycleaner site post-remed-iation sampling complete; WI industrial site post-remediation sampling complete
Constituents successfully treated	PCE, TCE, DCE, VC, TCA, TCFM, CT, CF, BTEX, U, Cr^{+6}, Ni, Cd, Pb, nitrate	
CAH (Enhanced Reductive Dechlorination) Sites		
Category	**Number of Sites**	**Notes**
Total CAH sites	112	
CAH sites for which biogeochemical data is available	48	
CAH sites for which some indication of success/failure is available	50	
CAH Sites – Successes	45	
Type 1	1	See USEPA, 1998 for Type definition
Type 2	13	See USEPA, 1998 for Type definition
Type 3	11	See USEPA, 1998 for Type definition
No type categorization	20	
Range of starting concentrations	0.018 to 180 mg/L total CAHs	
Range of starting pHs	3.8 to 8.9	
Range of ending pHs	2.9 to 7.9	
Range of starting ORPs	-370 to 420 mV	
Range of ending ORPs	-340 to 440 mV	
Range of ending ethene	0.016 to $>1x10^{7}$ ng/L	
Range of ending methane	0.0002 to 37 mg/L	
CAH Sites – Non-Successes (Inconclusive or Unsuccessful)	4	Liberty Superfund site, Farmingdale, NY (high flux of aerobic water); a pilot test at a northeastern landfill (unexpected groundwater flow direction); a MI dune formation site (inadequate character-ization of complex stratigraphy); Brazil bench-scale (modest HCB removal)
Have Type info	1	Type 2 at Liberty site
Misread geology/hydrogeology	2	Flow direction, complex stratigraphy
Velocity too high	1	High flux of aerobic groundwater
Unproven compound	1	Hexachlorobenzene - modest treatment

Table 1: In-situ Reactive Zone Site Survey (continued)

CAH (Enhanced Reductive Dechlorination) Sites		
Category	**Number of Sites**	**Notes**
CAH Sites - Possible stalling based on buildup of DCE and/or VC	6 (of 15 for which sufficient information is available)	
CAH Sites - Before/after ethene data	26	
No significant increase in ethene	9	
CAH Sites – Before/after methane data	27	
Increase in methane	22	
No change in methane	5	
CAH Sites – Substrate information	54	
Molasses	44	
HFCS	0	
Cheese whey	1	
Molasses/HFCS	1	
Molasses/whey	6	
HFCS/whey	1	
Molasses/CO2	1	
CAH sites – bioaugmentation used	4	Bioaugmentation agents have included anaerobic sludge, POTW supernatant, brewery biosolids, groundwater recirculation from high-performing area to former chemical oxidation test area.

Geology/Hydrogeology/Contaminant Concentrations

Enhanced reductive dechlorination has been implemented in diverse geologic materials, including unconsolidated sediments, partially weathered rock and fractured bedrock. Unconsolidated materials have ranged from highly permeable sands and gravels to low-permeability clays, with depositional environments including dune and beach sands, alluvium, carbonates, glacial till and piedmont saprolite, among many others. Bedrock materials reported at enhanced reductive dechlorination sites include granite, sandstone, shale, limestone, chalk and dolomite.

Reported hydraulic conductivities range from approximately 10^{-6} to 10^{0} cm/s (10^{-3} to 10^{3} ft/day), and horizontal groundwater velocities from approximately 10^{-5} to 10^{-2} cm/s (10^{1} to 10^{4} ft/yr).

Concentrations at successful *In-situ* Reactive Zone applications have ranged from 0.1 to 180 mg/L total CAHs, and up to 140 mg/L of Cr^{+6}. The majority of CAH sites have PCE and/or TCE as the parent compound. Molasses is the substrate used at the majority of the CAH sites.

Site Selection Criteria

Among the general site selection criteria for enhanced reductive dechlorination technology, as detailed in a recently finalized *In-situ* Reactive Zone protocol document (Suthersan et al., 2002), are the following:

- The site should be at least moderately permeable (K >3 x 10^{-4} cm/s [1 ft/day]) but permeabilities of 10^{-6} to 10^{-4} cm/s are marginally suitable. The range of reported hydraulic conductivities at *In-situ* Reactive Zone sites detailed above meets this criterion.
- Groundwater velocity should be between 10^{-5} to 10^{-3} cm/s (0.08 to 5 ft/day). The range of reported velocities at *In-situ* Reactive Zone sites as detailed above slightly exceeds the high end of this criterion. The disadvantage of high velocity is that it generally increases the amount of substrate dosing required to achieve sufficiently reducing conditions. At relatively high delivery rates, the cost of added substrate and more frequent injections may become prohibitive.
- Sites should be reasonably well delineated geologically and with regard to contaminant concentration.
- The depth of the plume is a factor in determining cost effectiveness, with depths less than 50 ft (15 m) generally being desirable.
- A pH close to neutral (5 to 9) is the most conducive to the proliferation of healthy, diverse microbial population. The range of initial pH levels at ARCADIS *In-situ* Reactive Zone sites, listed in Table 1, includes several sites with pH below 5. In aquifers with low pH or low buffering capacity, several methods are used to control pH decreases, such as the use of a buffer solution, the use of a water push to disperse the substrate, and the use of a slower-release substrate.
- Sites with anaerobic or borderline aerobic/anaerobic starting conditions with insufficient TOC can be most rapidly treated. Sites already showing breakdown products are ideal. Approximately two-thirds of ARCADIS' enhanced reductive dechlorination sites have been aerobic before treatment, and about one-third have been borderline aerobic/anaerobic. True anaerobic conditions before treatment have typically occurred only in portions of otherwise more aerobic plumes. In terms of the type of plume behavior as defined in U.S. Environmental Protection Agency (U.S. EPA, 1998), Type 2 and Type 3 conditions predominate and were approximately evenly represented prior to treatment. Type 1 sites are rare in our dataset since they are often more suitable for monitored natural attenuation. The fact that enhanced reductive dechlorination successes are evenly represented by Type 2 and 3 behavior suggests that pre-existing, natural reductive dechlorination is not a pretreatment requirement.
- No large quantities of pooled, dense non-aqueous liquid (DNAPL) are present, or a DNAPL remedy has been selected/implemented but a polishing step needed. Although DNAPL has not been physically observed at any of ARCADIS' enhanced reductive dechlorination sites, several sites with initial CAH concentrations over 100 mg/L have been successfully treated. These high concentrations in groundwater, where no free phase DNAPL is evident, may be indicative of residual or sorbed DNAPL source areas.

Technology Performance

The available data allow us to assess the success or failure of 50 enhanced reductive dechlorination sites, most of which are commercial. Success is evident in 46 of the 50 cases, as defined by quantitative or semi-quantitative evidence of increased rates of contaminant reduction (as compared to pre-treatment data or wells in untreated areas), appearance of daughter products that weren't previously observed, and/or attainment of regulatory goals. A wide disparity in the content of data sets for the many sites precludes establishment of a more specific measure of performance, but details of 19 of the 50 sites can be examined in the literature (a cross-reference

table for specific sites as numbered in the graphics is provided at the end of this document). Reductions in total CAHs for 25 of the "successful" sites (all those for which sufficient data is available) are indicated graphically in Figure 1. The sites shown include pilot tests for which complete CAH degradation was not the objective and several ongoing applications which can be expected to achieve further reductions.

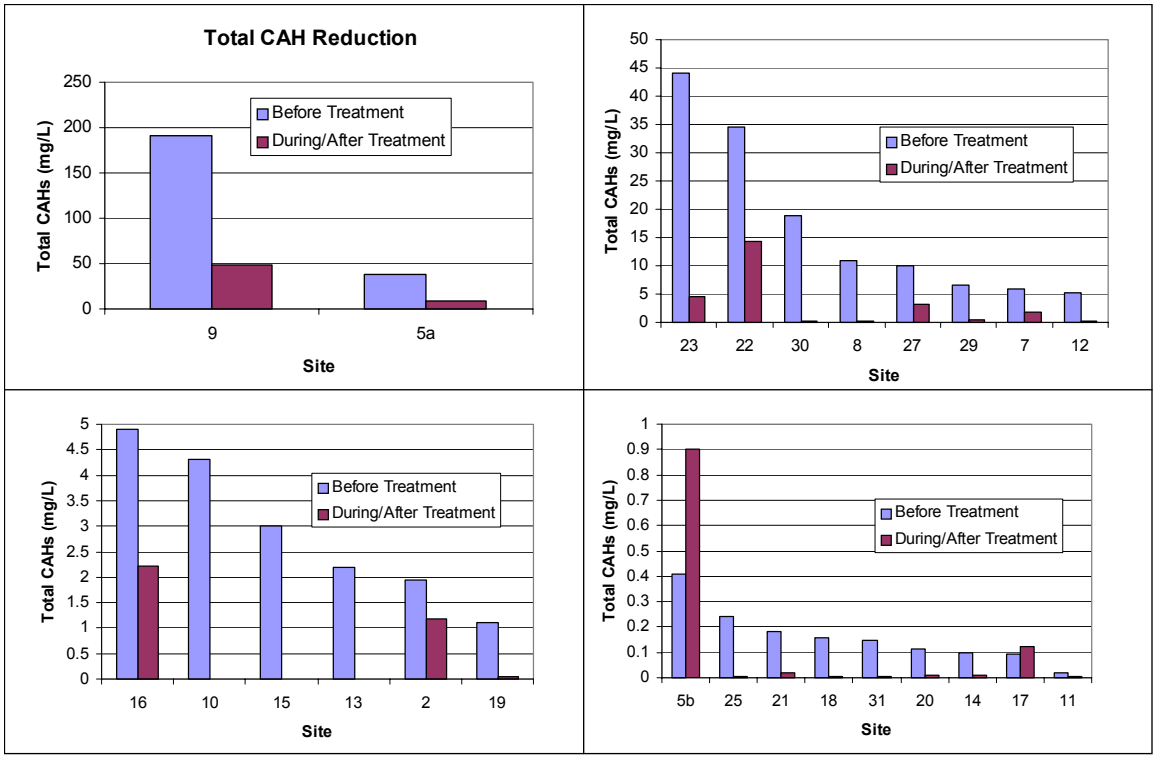

Figure 1. Reduction on Total CAHs at 25 "Successful" Sites (includes pilot tests and ongoing applications)

Starting conditions for enhanced reductive dechlorination sites, as detailed above, are largely aerobic or borderline aerobic/anaerobic, and vary widely in CAH concentrations, geochemistry and groundwater velocity. Before- and after-treatment values of selected parameters at downgradient monitoring points are listed in Table 1:

- pH levels are closely monitored during *In-situ* Reactive Zone implementation to be sure that excessive fermentation is not occurring. A comparison of pH ranges before and after treatment (Figure 2) shows decreases during treatment at more than half of the sites.
- *In-situ* Reactive Zones are designed to create sulfate-reducing or methanogenic conditions, of which ORP values are a gauge. The ranges of starting and ending ORP levels (Figure 2) confirm that decreases in ORP of up to hundreds of millivolts are generally attained.
- Ethene production is one indicator of complete reductive dechlorination of chlorinated ethenes. After-treatment ethene levels are variable, but increases are recorded at 65% of the enhanced reductive dechlorination sites for which data is available. Among the sites not exhibiting increases in ethene, two are at or near closure, indicating either that other routes of contaminant disappearance (for instance, cis-DCE breakdown by anaerobic energy-yielding

respiration, aerobic co-oxidation or aerobic energy-yielding oxidation, or abiotic degradation; or VC mineralization) predominated at those sites or that ethene is being rapidly bio-utilized.

- Methane production is also indicated by the data in Table 1. Increases in methane are recorded at over 80% of the enhanced reductive dechlorination sites for which data is available.

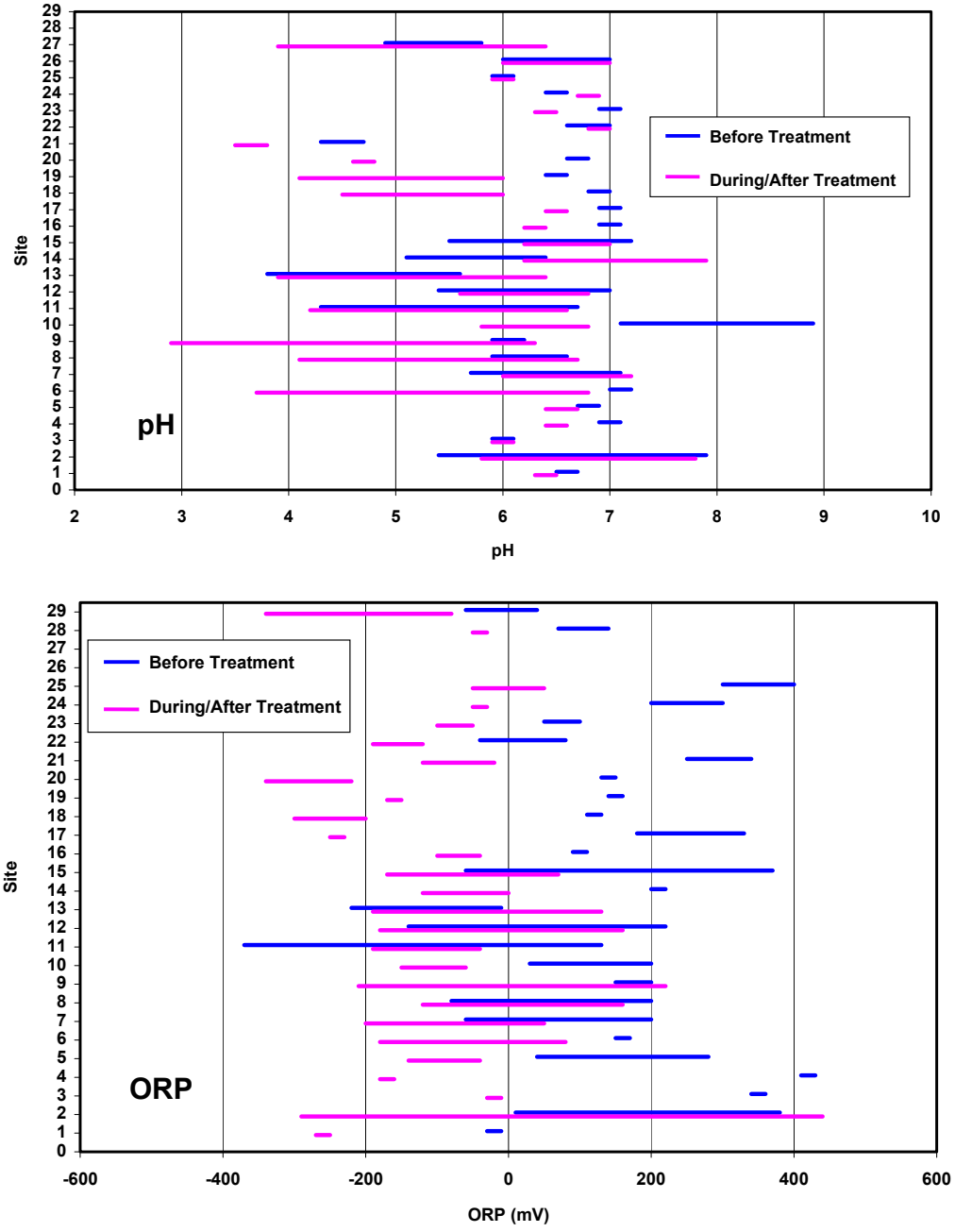

Figure 2. pH and ORP Ranges at "Successful" Sites

Biodegradation Rates. Rates of CAH biodegradation for several ARCADIS enhanced reductive dechlorination sites (Horst et al., 2000; Suthersan et al., 2002; Lutes et al., 2003a), as

calculated using first-order approximation methods (USEPA, 1998), are summarized in Table 2. The sites for which data are listed are TCE sites, one with PCE as a parent compound, with degradation products including cis-DCE and VC. Initial concentrations at the sites ranged from 1.2 to 22 mg/L total CAHs, under a variety of hydrogeologic conditions. The calculation method assumes that a fixed amount of CAH is present and degrades. Where pilot tests are run downgradient of a source area, this assumption is conservative (underestimates degradation rates) because the flux of contaminant from upgradient has not been factored into the rate calculation. Sites 7 and 32 in Table 2 represent enhanced reductive dechlorination applications downgradient from source areas.

The enhanced reductive dechlorination site data in Table 2 are compared to rates published in Howard et al. (1991; anaerobic aqueous biodegradation) and Aronson and Howard (1997; field studies of anaerobic biodegradation). Calculated half-lives for TCE at enhanced reductive dechlorination sites ranged from 17 to 257 days, improving on the published ranges of 98 to 1653 days (Howard et al., 1991) and 80 to 4080 days (Aronson and Howard, 1997). Half-lives for cis-DCE and VC were also generally lower than the published ranges, as indicated in Table 2.

Site data for TCE and VC are compared graphically to the Aronson and Howard data in Figure 3 (no data for cis-DCE are given in this reference). EPA (1998) notes a likely bias toward high attenuation rates in the Aronson and Howard data. The comparison indicates that in general, faster biodegradation of both TCE and VC was achieved with enhanced reductive dechlorination than under natural attenuation.

Table 2. CAH Biodegradation Rates for Several ARCADIS Enhanced Reductive Dechlorination Sites

Site/Source	TCE		cis-DCE		VC		Reference
	k (1/yr)	HL (days)	k (1/yr)	HL (days)	k (1/yr)	HL (days)	
Site 26	0.98	257	---	---	---	---	a
Site 34	3.95	64	2.45	103	---	---	a
Site 32	3.10	82	3.18	80	2.92	87	b
Site 32	2.33	108	2.15	117	0.95	267	b
Site 22	1.31 - 3.20	79 - 193	1.26	200	0.69	365	b
Site 29	1.83 - 8.40	30 - 139	1.46 - 6.21	41 - 173	1.10 - 6.57	39 - 231	b
Site 33	15.33	17	15.33	17	---	---	b
Site 7	3.16 - 8.98	28 - 80	0.59 - 1.14	223 - 428	2.33	109	c
Range All Sites	0.98 - 15.33	17 - 257	0.59 - 15.33	17 - 428	0.69 - 6.57	39 - 365	a,b,c
Howard et al., 1991	0.15 - 2.58	98 - 1653	0.35 - 2.26	112 - 720	0.35 - 2.26	112 - 720	d
Aronson & Howard, 1997	0.06 - 3.16	80 - 4080	---	---	0.15 - 21.24	12 to 1686	e

HL = half life

a Horst et al., 2000

b Suthersan et al., 2002

c Lutes et al., 2003a

d Howard et al., 1991

e Aronson & Howard, 1997

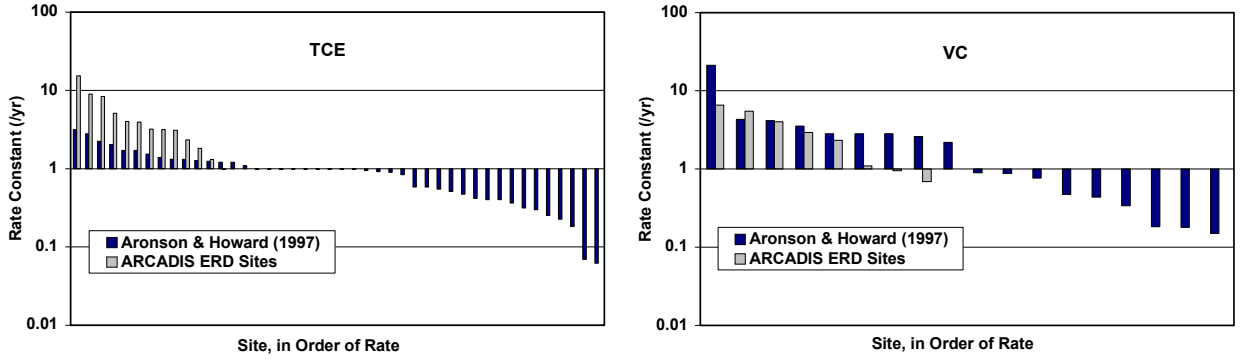

Figure 3. Biodegradation Rate Constants for TCE and VC

Evidence of Stalling. Of 15 CAH sites for which sufficient information is available to evaluate stalling, six show some potential buildup of cis-DCE or VC in the reactive zone. The few which exhibit sustained increases in cis-DCE also show a buildup of ethene, indicating that complete dechlorination is taking place but with DCE degrading more slowly than more halogenated compounds. In addition to reductive dechlorination, several alternate processes of biological cis-DCE transformation have been identified which may ultimately contribute to the cleanup of this compound. Four microbial processes identified by Löffler (2003) are anaerobic reductive dechlorination (the mechanism exploited by enhanced reductive dechlorination), anaerobic energy-yielding oxidation, aerobic co-oxidation, and aerobic energy-yielding oxidation. Aerobic mechanisms may be capable of transforming cis-DCE at the more aerobic fringes of the reactive zone.

"Lag Time" for Complete Dechlorination. The time required to completely dechlorinate a CAH plume by enhanced reductive dechlorination is highly variable, depending on starting conditions. At a given monitoring location, the treatment time includes an initial transport step during which the carbon source is distributed to the monitoring point. Subsequently, a series of electron acceptors must be consumed, in concert with sequential adaptations of the *in-situ* microbiological population. Finally, when the ideal highly reducing conditions are achieved, time is required for degradation of the target constituents (e.g., PCE or TCE), followed by a shift to a population adapted to utilize the breakdown products (e.g., DCE and VC) (Flynn et al., 2000).

The initial carbon supply, redox conditions and the presence of degradation products affect the speed of the microbiological transitions. Under reducing conditions and with partial dechlorination, short treatment times would be expected; at oxidizing sites with no previous degradation, a "lag time" or acclimation time will occur before complete treatment takes place. In ARCADIS' experience, complete dechlorination has occurred in short timeframes at some sites, on the order of months (Appendix A-2.8 in Suthersan et al., 2002 and the Wisconsin dry cleaner site case study in this document discuss site closures within 2 to 2.5 years). Other sites show lag times of 6 months to as long as 2.7 years before the final stages of dechlorination begin to take place.

Unsuccessful Applications. At four of 50 sites, unsatisfactory *In-situ* Reactive Zone performance has been reported (see Table 1). One failed application was attributed to a high flux of aerobic water, which made it impractical to supply enough carbon to drive the system to the required state of reduction. At two sites, incomplete characterization of hydrogeology (groundwater flow direction in one case and stratigraphic complexity in the other) led to failures in directing the carbon source to the target area. These three cases are discussed in detail in Suthersan et al., 2002 (see Appendix A, Sections A.2.11, A.2.14 and A.2.15). Lastly, a bench-scale test was conducted on saturated soil from a site in Brazil containing parts per million levels of hexachlorobenzene (HCB) in soil and groundwater. Enhanced reductive dechlorination achieved only modest removal of HCB, and field-scale pilot test of the technology was not pursued.

Technology Cost

Based on ARCADIS' experience, actual project costs have ranged from approximately $75,000 for a small-scale application and/or pilot study or demonstration-scale project to $1,400,000 for a large plume treatment with a fully automated reagent injection system. Table 3 presents a selection of cost examples with concentration and size information. The full-scale system for the automated site included installation of over 100 reagent injection wells to provide aggressive plume-wide treatment.

Operating costs (including reagent injection, monitoring and reporting) are generally on the order of $50,000 to $100,000 per year. The percentage of the total costs associated with the reagent injections is typically greater than 50%. On the other hand, the actual cost of the reagent itself typically represents less than 10% of the total cost budget.

The cost data presented illustrate the cost-effective nature of enhanced reductive dechlorination technology in addressing CAH contamination in groundwater. For example, two sites have been completed with "no further action" notifications from the regulatory agencies for less than $500,000 each.

Table 3. *In-situ* Reactive Zone Technology Application Costs at a Range of Sites

Site	Estimated Capital Costs	Estimated Annual O&M Costs	Actual or Predicted Costs to Closure	Initial Concentration	Dimensions
Industrial Laundry/Dry Cleaning Facility, Eastern PA	$75,000	$45,000	$250,000	46,000 ug/l PCE	10,000 ft^2 x 20 ft deep
Uranium Processing Facility, Eastern US	$480,000	$65,000	$760,000	5 - 14,000 ug/l PCE (plus U)	19.3 acres or 1200 x 700 ft
Former Metal Pating Site, Western US[1]	$100,000	$150,000	$250,000	24,000 ug/l TCE (plus Cr)	< 2 acres or <87,000 ft^2 x 10 feet deep
Industrial Manufacturing Site, South Carolina	$1,400,000	$75,000	$2,000,000	800 ug/l CT, chloroform, TCE	3.25 acres or 141,600 ft^2 x 10 ft deep
Industrial Site, Northeastern US	$150,000	$80,000	$750,000	120 ug/L PCE	3000 ft long in bedrock - depth varies
Former Dry Cleaner, Wisconsin[2]	$200,000	$100,000	$400,000	1,500-4,000 ug/L PCE	30,000 ft^2 x 5 ft deep
Former Automotive Manufacturing Site, Midwestern, US	$75,000	$60,000	$375,000	800 ug/l TCE	1000 x 400 ft x 20 ft deep
AOC 50, Ft. Devens, Ayer, Massachusetts	$150,000	$150,000	NA[3]	4,000 ug/L PCE	3000 x 400 ft x 40 ft deep

Note:
All costs presented in current dollars.
1 - Site has received regulatory closure.
2 - Site has received regulatory closure.
3 - No Predicted Costs to Closure Available. Pilot study ongoing.

Summary Observations and Lessons Learned

ARCADIS has recently completed a protocol for the *In-situ* Reactive Zone technology, titled "Technical Protocol for Using Soluble Carbohydrates to Enhance Reductive Dechlorination of Chlorinated Aliphatic Hydrocarbons" (Suthersan et al., 2002; soon to be made available on the Air Force Center for Environmental Excellence [AFCEE] web site). Based on ARCADIS' *In-situ* Reactive Zone experience at over 130 CAH sites, several key technical points have emerged regarding CAH bioremediation:

- Bioaugmentation is rarely needed for complete dechlorination to ethene. There is extensive evidence from both the field and the laboratory showing complete dechlorination under conditions that are inhospitable to Dehalococcoides ethenogenes and other species that have been proven to achieve metabolic dechlorination. Injection of bacterial cultures may shorten the lag phase, reducing the time needed to achieve maximum dechlorination rates, but is not required to ensure success.
- ARCADIS believes that co-metabolic and dehalorespiring processes both play a part in reductive dechlorination in real world systems. Abundant evidence exists in the literature to support the existence of co-metabolic, methanogenic processes in reductive dechlorination (Bradley and Chapelle, 1997; Aulenta et al., 2002). Additionally, remedial systems that inherently generate large concentrations of hydrogen *in-situ*, that would be supportive of co-metabolism and would be deemed inhospitable for dehalorespiration, still achieve noteworthy CAH treatment (Newell, 1999). The many different mechanisms of dechlorination (including *Dehalococcoides* and other dehalorespiration, cometabolic, abiotic and anaerobic bio-oxidation processes) are difficult to differentiate or quantify in laboratory or field conditions. Specific microbiological tests at sites that exhibit adequate treatment under methanogenic conditions may provide insight through the process of elimination.
- Suppression of hydrogen levels is unnecessary and may inhibit full dechlorination. Because it is unnecessary to constrain hydrogen levels for the benefit of Dehalococcoides or other dechlorinating organisms, it is also not necessary to limit rates of electron donor consumption in treated aquifers. An in-depth discussion of the role of hydrogen in enhanced reductive dechlorination systems is given in Appendix B of Suthersan et al., 2002.
- Buffers can be used to avoid too much fermentation. Enhanced reductive dechlorination is most effective at a pH range of 5-9, but pH can drop in the presence of substrate. In systems with naturally low pH or low buffering capacity, pH can be controlled by reducing the injection rate or using a buffering agent. At an enhanced reductive dechlorination demonstration at Vandenberg Air Force Base, the groundwater system exhibited a relatively low buffering capacity. In this case, pH was initially controlled by carbon dose control and injection of a clean water push following reagent injection to disperse the dose away from the immediate vicinity of the injection well. These measures failed to sustain the pH at the desired level, so a buffer was added to the reagent. The buffer brought the pH into an acceptable range while allowing a two-fold increase in the initial carbon dose.

- Desorption processes are critical to the performance of these systems. Enhanced reductive dechlorination enhances desorption by way of four processes – progressive decreases in organic carbon partitioning coefficient (K_{OC}) values of sequential daughter products, the production of natural biosurfactants by the enhanced microbial population, the production of fermentation products that act as co-solvents, and changes in equilibrium partitioning of contaminants due to the increase in the carbon content of groundwater relative to that of the soil (Suthersan et al., 2002). This increased desorption allows for greater access to typically "inaccessible" constituent mass, thereby decreasing the time required for source area cleanup and minimizing post-treatment rebound effects.
- Microcosms are rarely needed but "tuning" the field pilot system is vital. If there is a reason in the biogeochemical data to significantly doubt whether the system will be successful, a laboratory microbiological study may be warranted. However, a comparison of various treatability and pilot tests in predicting CAH bioremediation by enhanced reductive dechlorination (Lutes et al., 2002a) concluded that short-term microcosm testing may provide helpful information, but is moderately expensive, time-consuming (four months or more), must be coupled with engineering assessment or field pilot testing to evaluate reagent distribution, and may fail to predict field performance at initially aerobic sites. No reasonable amount of preliminary testing can predict the disparate performance of individual injection wells observed at field scale, thus careful monitoring and tuning of the installed system is essential.

REFERENCES

Aulenta, F., Majone, M., Verbo, P., and V. Tandoi. 2002. Complete dechlorination of tetrachloroethene to ethene in presence of methanogenesis and acetogenesis by an anaerobic sediment microcosm. *Biodegradation*, 13:411-424, 2002**.**

Bradley, P.M., and F.H. Chapelle. 1997, Kinetics of DCE and VC mineralization under methanogenic and Fe(III)-reducing conditions: *Environ. Sci. Technol.*, v. 31, p. 2692 - 2696.

Flynn, S., F. Löffler, and J. Tiedje. 2000. Microbial community changes associated with a shift from reductive dechlorination of PCE to reductive dechlorination of *cis*-DCE and VC. *Enivron. Sci. Technol.*, Vol. 34(6):1056-1061.

Horst, J.F., Beil, K.A., Burdick, J.S., Suthersan, S.S. 2000. Comparison of Natural and Enhanced Rates Through Substrate Amendments. *Proceedings of the Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California*.

Howard, P., Boethling, R., et al. 1991. *Handbook of Environmental Degradation Rates*. Lewis Publishers.

Lutes, C.C., V. D'Amato, A. Frizzell, M. Hansen, G. Gordon, P. Palmer, S. Suthersan. 2003a. *In-situ Substrate Addition to Create Reactive Zones for Treatment of Chlorinated Aliphatic Hydrocarbons: Hanscom Air Force Base*. Prepared for Air Force Center for Environmental Excellence (AFCEE) and Environmental Security Technology Certification Program (ESTCP). April 4, 2003.

Lutes, C., Liles, D., Hansen, M., Burdick, J., Suthersan, S., Hansen, J., Kampbell, D., and D. McInnes. 2002a. Utilization of Treatability and Pilot Tests to Predict CAH Bioremediation. In: A.R. Gavaskar and A.S.C. Chen (Eds.), *Proceedings of the Third*

International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2002). Paper 2B-08. Battelle Press, Columbus, Ohio.

Newell, C, Hass, P.E., Hughes, J.B., and Khan. T.A. 1999. Results from two direct hydrogen delivery field tests for enhanced dechlorination. *Biotechnol. Bioeng.*, *62*, 160-165. (Poster Presented at *Partners in Environmental Technology, Technology Symposium and Workshop* [1999]. Nielsen, R.B., and Keasling, J.D. (Eds.). Washington, DC.)

Suthersan, S.S, Lutes, C.C., Palmer, P.L., Lenzo, F., Payne, F.C., Liles, D.S., and Burdick, J. 2002. *Final Technical Protocol for Using Soluble Carbohydrates to Enhance Reductive Dechlorination of Chlorinated Aliphatic Hydrocarbons, December 19, 2002.* Submitted to ESTCP and AFCEE under Contract #41624-99-C-8032.

USEPA. 1998. *Technical protocol for evaluating natural attenuation of chlorinated solvents in groundwater*. Cincinnati, OH: National Risk Management Research Laboratory, Office of Research and Development, U. S. Environmental Protection Agency. EPA/600/R-98/128.

Available Published Case Studies for Sites Presented in Graphics

Site No.	Reference
2	Suthersan et al., 2002 (App. A-2.10); Lutes et al., 2002a; Lutes et al. 2002b; Lutes et al., 2003a
7	Suthersan et al., 2002 (App. A-2-10); Lutes et al., 2002b; Lutes et al., 2003a
8	Horst et al., 2003
9	Suthersan et al., 2002 (App. A-2-13); Suthersan, 2002 (p.180)
10	Suthersan et al., 2002 (App. A-2-2); Suthersan, 2002 (p.177); Nyer, 2001 (p.378)
11	Suthersan et al., 2002 (App. A-2-5); Beil et al., 2002
12	Suthersan et al., 2002 (App. A-2-6)
13	Suthersan et al., 2002 (App. A-2-9)
15	Panhorst and Page, 2002
22	Suthersan et al., 2002 (App. A-2-3); Burdick et al., 2002b; Lutes et al., 2003b
26	Suthersan et al., 2002 (App. A-2-8); Suthersan 2002 (p.177); Nyer 2001 (p.372)
28	Burdick et al., 2002a
29	Suthersan et al., 2002 (App. A-2-4); Suthersan, 2002 (p.180); Maierle and Cota, 2001; this document
30	Burdick et al., 2003
32	Suthersan et al., 2002 (App. A-2-1); Nyer, 2001 (p.368); Burdick (1998)
33	Suthersan et al., 2002 (App. A-2-12); Suthersan, 2002 (p.180)
---	Also see Suthersan et al., 2002, App. A-2.7, A-2.11, A-2.14 and A-2.15

APPENDIX E.12 – HYDROGEN RELEASE COMPOUND (HRC®): A REVIEW OF PUBLISHED PAPERS AND CASE HISTORIES 1999-2003

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Hydrogen Release Compound (HRC®): A Review of Published Papers and Case Histories 1999-2003

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Introduction

Since 1999, Hydrogen Release Compound (HRC®, U.S. Patent 6,420,594) has been a commercially-available product for engineered bioremediation of anaerobically biodegradable contaminants. The results of some of these applications are chronicled in the 76 publications and case histories listed in Table 1; the majority of these papers were authored by third-party consultants or site owners. Table 1 serves as a site survey of HRC use for bioremediation of many contaminants under many different geologic conditions. It is intended to be used as a reference list to support the information and conclusions presented, which are germane to advancing the "state of the art" in enhanced anaerobic bioremediation. Because the references in Table 1 are published documents, they can be accessed and reviewed, if the reader needs specific information.

In this review, we summarize, using our database of HRC applications and the papers in Table 1, application types, contaminants treated, site types, application locations, injection methods, site lithology and hydrology, and concentration ranges of geochemical species observed. Following this stark recounting of information, we discuss two subtopics that are encompassed by an overarching issue that is highly relevant to the bioremediation community: incomplete dechlorination. These subtopics are: the appropriate geochemical conditions, based on our experiences, for complete dechlorination and the appropriate use of bioaugmentation to achieve site remediation goals.

What is HRC®?

HRC is a polylactate ester that, upon hydration or microbial cleavage of its ester bonds, slowly releases lactate. Lactate serves as an electron donor and carbon source for microbial reductive biodegradation. HRC is a viscous amber-colored liquid that is typically injected into a contaminated aquifer using direct push technology or backfill injection via hollow stem auger. Once in place, HRC creates a plume of lactate and its fermentation products (dissolved hydrogen and other organic acids) downgradient of the injection area and serves to accelerate anaerobic bioremediation processes.

Types of Applications

According to our HRC application database, there have been 474 field-scale applications of HRC worldwide for engineered bioremediation of anaerobically biodegradable contaminants. Of these 474 HRC applications, 20% are pilot scale applications and 80% are full-scale applications, as indicated by the site owner or project manager. The full scale applications range from 2000 square feet to 360,000 square feet; the latter is a full-scale application at the site described in 46.

Based on our application database, the types of HRC applications include grids for source/plume remediation (83%), barriers for plume cutoff or to prevent contaminant migration to sensitive receptors (10%), emplacement in an excavation for residual contamination (6%), and other configurations (1%). For many sites, only one HRC injection (lasting 12-18 months) was completed, with a few notable exceptions for high concentration (e.g. reference numbers 5, 41, 64) and slowly dechlorinating (e.g. 39, 59, 59a) sites. Using engineered bioremediation with HRC, site goals, maximum contaminant levels (MCLs), and site closure or "no further action" status have been reached at many sites (see Table 1 for examples or contact Regenesis for a full list).

Contaminants Treated

From our database, the majority of HRC applications are for bioremediation of chlorinated ethenes (86%) , with chlorinated ethanes (8%) and other contaminants (6%) as the compound classes for the remainder of applications. For the chlorinated ethene HRC applications, the primary contaminant of concern was perchloroethene (PCE) for 29% of applications, trichloroethane (TCE) for 42% of applications, any isomer of dichloroethene (DCE) for 16% of applications, and vinyl chloride (VC) for 13% of applications. Primary contaminants of concern for chlorinated ethane applications consist of 1,1,1 trichloroethane (1,1,1 TCA) (65%) and any isomer of dichloroethane (DCA) (35%). Other applications of HRC have been made for pentachlorophenol (2, 18, 35a), nitrate (8, 32, 33), hexavalent chromium (17, 66), chlorofluorocarbons (15a, 66), perchlorate (66), explosives such as RDX (21, 32, 33), nitrotoluenes, and nitrobenzenes (32, 33), carbon tetrachloride and daughter products (29), neptunium (15), and the pesticide, chlordane (4).

Types of Sites Treated and Studies Performed

As shown in Table 1, HRC has been applied at a wide range of site types, including various manufacturing facilities, dry cleaners, brownfield/redevelopment sites (3, 6), landfills (16, 46), and at a Superfund/CERCLA site (2). Additionally, our database of 474 HRC applications shows that HRC has been part of site remediation activities at 37 US Department of Defense (DOD) sites and several US Department of Energy (DOE) sites. Academic and industrial research on biodegradation has been performed using HRC as a substrate in laboratory studies ranging from biodegradation of pentachlorophenol (35a), reductive dechlorination of perchlorate (56), and DNAPL bioremediation (1) to chemical reduction and precipitation of neptunium (15). At 13 sites in our database, engineered bioremediation with HRC has displaced other treatment methods, such pump and treat systems (e.g. 70, 71). Furthermore, engineered bioremediation with HRC has been bundled with *in situ* chemical oxidation (31), a zero valent iron permeable reactive barrier (38), and soil vapor extraction and pump and treat systems (5) for effective site remediation.

Application Locations

HRC has been used in all but six (Arkansas, Alabama, Maine, Hawaii, North Dakota, and South Dakota) U.S. states, with the greatest number of injections in California-45 applications, New Jersey-37 applications, Florida-36 applications, New York-25 applications, Ohio-23 applications, and North Carolina-21 applications. Worldwide, HRC has been applied to sites in Germany-11 applications, Japan-10 applications, Canada-8 applications, the Netherlands-5 applications, the United Kingdom-2 applications, Belgium-1 applications, and Taiwan-1 applications. Papers describing sites in Japan (12, 24) and Germany (13) have been published.

Novel HRC Injection Methods

The widespread use of HRC as an injectable substrate has stimulated technical professionals and drilling vendors to develop novel HRC-specific injection methods and unique application systems for difficult geologies. For example, a comparison of top-down and bottom-up direct push HRC injections into clay was made in (59) and (59a), who found no difference in the HRC vertical distribution generated by the two methods. Furthermore, to deliver HRC to depths of 38 feet in a dense glacial till (35) and 80 feet in well-sorted sand and gravel (14), combinations of air rotary drilling or solid stem auger and direct push HRC injection were developed and implemented. In (25), HRC was injected at angles of 30 and 15 degrees from vertical, so that areas beneath buildings could be remediated.

Site Lithology
A wide range of site lithologies are amenable to HRC injection, including direct push injection in tight clays (34, 51, 59, 59a) and highly-weathered limestone and shale (6), injection via re-injection wells in saprolite (19), injection via re-injection wells or open boreholes and packers into fractured bedrock (19, 20, 16, 11, 5), and injection into blast fractures in bedrock (34).

Site Hydrogeology
HRC has been injected into and performs well in a broad range of hydrogeological settings (see Table 1). In fact, HRC has routinely been applied at sites exhibiting hydrogeological extremes. HRC has been injected in very low permeability settings, such as at a site with hydraulic conductivities of $1x10^{-5}$ to $2x10^{-5}$ cm/s (6, 28), where reducing conditions (as indicated by negative oxidation reduction potential (ORP) and low dissolved oxygen (DO) concentrations) and organic acids concentrations (lactic, pyruvic, acetic, propionic, and butyric acids) were not detected in wells close to and downgradient of the application area until 4 months after HRC injection. However, at these low permeability sites, reducing conditions were maintained for 14 months after HRC injection. In contrast, HRC has also been injected into aquifers with groundwater flow velocities of 5 to 50 ft/day (8, 27). At these sites, reducing conditions were established downgradient of the HRC injection area in a few weeks, and HRC longevity, as measured by organic acid concentrations and maintenance of reducing conditions, was 10 months.

Contaminant Concentrations
Accelerated reductive dechlorination with HRC has been successfully executed at sites with chlorinated ethene concentrations ranging from a few micrograms per liter to residual DNAPL or source area concentrations. Dechlorination through VC and ethene has been reported at HRC sites with 110 mg/L of PCE and 200 mg/L of TCE (41, 64), 100 mg/L of PCE (26, with HRC-X^{TM}, extended release formula), and 75 mg/L of PCE (5). After HRC injection, 1500 µg/L of 1,1,1 TCA was biodegraded through chloroethane (CA) (22 µg/L) in 68a, and HRC was applied at a site in New York with up to 400 mg/L of 1,1,1 TCA (35).

Organic Acid Concentrations
Lactate is a fermentation substrate for a diverse group of commonly-found soil and aquifer microorganisms. Fermentation of lactate produces organic acids, such as pyruvic, acetic, propionic, and butyric acids, in addition to reduced electron carriers, such as NADH. All of these compounds serve as electron donors for microbial metabolism. During the fermentation process, dissolved molecular hydrogen (H_2), a reduced end-product, is produced to balance the production of oxidized species like carbon dioxide and oxidized organics. Microorganisms also maintain redox balance via conversion of excess NADH to H_2, when H_2 is present in low concentrations in the surrounding environment. Two moles of H_2 (or NADH) are produced when lactate is fermented to pyruvic acid and then to acetic acid. Butyric and propionic acids are produced by specialized microbes when electron donors and H_2 are plentiful. They can later be fermented to H_2 when it is needed.

The majority of HRC sites show elevated organic acid concentrations (>50 mg/L of total organic acids = sum of lactic, pyruvic, acetic, butyric, and propionic acids) for 12 to 18 months (exceptions include the high groundwater velocity sites summarized previously). However, an HRC site in Kinston, NC (4) reports 400 mg/L of total organic acids 2.5 years after injection for chlordane contamination. Additionally, a site where HRC-X was injected for bioremediation of a PCE source area (26) reports 4320 mg/L of total organic acids 3.4 years after the initial injection. At some sites with dense injection grids, HRC creates very high organic acid concentrations. For example, after

significant amounts of HRC, HRC-X, and HRC Primer (a low viscosity version of HRC) were injected into a 3 foot by 10 foot plot at the Alameda Naval Air Station (9), the total organic acid concentration peaked at 96,300 mg/L; however, the total organic acid concentration decreased to 6,300 mg/L within one year after injection.

Hydrogen Concentrations

The lactate that is produced by the breakdown of HRC when it is injected into an aquifer is fermented to dissolved molecular hydrogen (H_2), which is a required substrate for some dechlorinating microorganisms. As with ethene, H_2 is not typically measured at reductive dechlorination sites. The sites that have measured H_2 concentrations report 4 nM of H_2 (62, see www.regenesis.com for H_2) at the low end to 5,300 nM (23) of H_2 at the high end, within one year after HRC injection. Other sites document H_2 concentrations of 240 nM (70), 123 nM (59a), and 40 nM (71), within one year after HRC injection.

Ethene Concentrations

Ethene is a final daughter product in chlorinated ethene reductive dechlorination. The presence of ethene is a qualitative indicator of and one of several lines of evidence for complete reductive dechlorination of chlorinated ethenes. However, ethene is very volatile and readily biodegradable. As a result, stoichiometric amounts of ethene from parent compounds are not usually measured, and a lack of ethene detection at a site does not suggest incomplete dechlorination. Examples of maximum ethene concentrations within a year after HRC injection are 980 µg/L (70), 680 µg/L (41), 500 µg/L (13), and 60 µg/L (59a).

Site Geochemical Parameters

The oxidation reduction potential (ORP) and dissolved oxygen (DO) concentration at most HRC sites are typically decreased to negative ORP values and less than 1-2 mg/L of DO (e.g. 2). The pH within 10 feet of the HRC injection area is rarely outside of the pH 6-8 range (e.g. 8, 23) after injection. Dissolved iron and manganese usually increase in wells impacted by HRC-produced organic acids. The average increases in dissolved iron and manganese concentrations within a year of HRC injection are 10 mg/L of dissolved iron and 5 mg/L of dissolved manganese (e.g. 6). However, at sites with high iron soils, dissolved iron concentrations after HRC injection reaching 200 mg/L (13) and at least 600 mg/L (22, 69) have been reported. Sulfate concentrations at most HRC sites are 50 mg/L or less, with a few notable exceptions, such as 250 mg/L (70, 71) and 273 mg/L (59a). Note that (59a, 70, 71) report complete dechlorination through VC and, for some (59a, 70), significant amounts of ethene were observed over the course of a 1-2 year treatment period, despite the high sulfate concentrations. Recently, HRC has been injected at a site in California with nearly 500 mg/L of sulfate and a site in Montana with 3,000 mg/L of sulfate (data not yet available). We will be closely following the progress of these sites to determine the potential for reductive dechlorination in the presence of high concentrations of sulfate.

Are Methanogenic Conditions Necessary for Complete Dechlorination?

There has been much debate on the optimal *in situ* geochemical conditions for complete reductive dechlorination. This is a complex topic and one better suited for a longer and more rigorous discourse. Instead, we will focus here on the specific question of whether it is necessary for an aquifer to reach fully methanogenic conditions for complete dechlorination to occur. To provide an answer to this question, we present examples of bioremediation sites that support the conclusion that fully methanogenic conditions are not a prerequisite for complete dechlorination. For this exercise, we operatively define methanogenic conditions as present when groundwater

measurements indicate greater than 1.5 mg/L of methane. Complete dechlorination is operatively defined as successive groundwater measurements of >50 μg/L of vinyl chloride or >20 μg/L of ethene.

The first example is a manufacturing site in Cookeville, TN (41, 64). HRC was injected in a grid covering the center and fringes of a PCE and TCE plume. Well PZ-2, located within the injection grid, originally had 10.4 mg/L of PCE and 7.2 mg/L of TCE. Seven months after HRC injection, these contaminants were reduced to non-detect levels, while cis-DCE, VC, and ethene were measured at 83 mg/L, 27.5 mg/L, and 0.6 mg/L, respectively. A baseline methane concentration of 0.13 mg/L was measured, and methane concentrations during the seven months were less than 0.4 mg/L. Within 2.2 years after injection, cis-DCE decreased to 3.8 mg/L, VC decreased to non-detect levels, ethene peaked at 14.5 mg/L, and methane remained below 1 mg/L, with the exception of a single monitoring event with a methane concentration of 1.3 mg/L. No rebound of PCE or TCE was observed in 2.2 years after HRC injection.

The second example is from the Springdale Cleaners site in Portland, OR (26). HRC-X, a concentrated version of HRC with a three year longevity in the subsurface, was injected for residual DNAPL PCE contamination reaching 100 mg/L in well JEMW-4. One and a half years after HRC-X injection, PCE and TCE were each reduced to 0.3 mg/L or less and 43.9 mg/L of cis-DCE, 9.5 mg/L of VC, and 0.3 mg/L of ethene were produced. The maximum methane concentration measured during this period was 0.7 mg/L on day 8 after injection, and methane concentrations steadily decreased to 0.25 mg/L 1.5 years after injection. After 3.4 years of monitoring, no rebound of PCE or TCE was observed, cis-DCE plateaued at 53.5 mg/L, VC decreased to 4.9 mg/L, 1.13 mg/L of ethene was produced, and methane increased only to 0.85 mg/L.

Third and fourth examples are from bioremediation sites at Alameda Naval Air Station in Alameda, CA (7, 9) and near the Duluth International Airport in Duluth, MN (46). At Alameda Naval Air Station, cessation of repeated liquid organic acid and nutrient injections resulted in steady cis-DCE and VC concentrations of 1,310 μg/L and 211 μg/L in well MW-3. After 20 months of cis-DCE and VC plateau, the site was injected with a mixture of HRC, HRC-X, and low-viscosity HRC Primer. In 272 days after injection, the concentration of cis-DCE was reduced by 96% and the concentration of VC was reduced by 89%, with concomitant increases in ethene to a maximum concentration of 78 μg/L. The maximum methane concentration during this period was 600 μg/L. At the site in Duluth, MN, TCE decreased from 354 μg/L to non-detect in 31 weeks, while cis-DCE and VC peaked at 750 μg/L and 23 μg/L, respectively. During the 41 week period, methane did not rise above 1,000 μg/L.

Clearly, methanogenic conditions were not necessary for complete reductive dechlorination at the Cookville, TN, Springdale Cleaners, Alameda Naval Air Station, or Duluth, MN sites. From our experience, rapid and complete dechlorination can occur whether methanogenic conditions are reached or not. Practically, this conclusion is positive as generation of methane is a safety hazard if vadose zone methane fractions reach the lower explosive limit of 5%. This issue of excess methane production is especially relevant at Brownfield and redevelopment sites where excavation for construction may occur. .

Appropriate Conditions for Bioaugmentation

The concept of using bioaugmentation to achieve remediation goals at sites contaminated with chlorinated ethenes has emerged and is at the forefront of innovative engineered bioremediation.

Regenesis offers a commercially-available bioaugmentation microbial culture for reductive dechlorination (Bio-Dechlor INOCULUM™), as well as a phylogenetic dechlorinating organism quantification technology called Bio-Dechlor CENSUS™. Bio-Dechlor CENSUS™ is a Real-Time Polymerase Chain Reaction DNA test for *Dehalococcoides*-type dechlorinating microorganisms and is being co-marketed with Microbial Insights, a commercial microbial laboratory.

Despite these developments, we firmly believe that bioaugmentation should not be the default remedy at a reductive dechlorination site, even if there is an apparent plateau of cis-DCE. Instead, bioaugmentation should be considered as another tool for achieving site remediation goals at certain problem sites that do not respond as desired to substrate additions, due to geochemical or biological reasons. Bioaugmentation should not be employed until an appropriate "differential diagnosis" of the aquifer has been made. A differential diagnosis involves an analysis and assessment of site conditions for the existence of impediments to complete dechlorination, other than a lack dechlorinating microorganisms. These impediments could be potentially alleviated by waiting for the biodegradation process run to completion or by increasing the electron donor concentration, both of which are typically less expensive than bioaugmentation.

A differential diagnosis should only be considered when there is a documented plateau of cis-DCE for 6 months or more and no detection of VC or ethene has been made. Please note that this metric of when incomplete dechlorination is considered problematic is provisional and may change based on our future experience. The first step is to consider unknown sources of cis-DCE. Is there sorbed or vadose zone PCE or TCE that, upon dechlorination, could lead to an increase in dissolved cis-DCE, which may be slower to dechlorinate? Note that for many soil types sorbed PCE and TCE mass is at least 80% of the total PCE and TCE mass (dissolved + sorbed), and a greater fraction of cis-DCE partitions to the dissolved phase. Thus, sorbed mass can "bleed" dissolved cis-DCE, creating the appearance of a plateau. Second, competition from other electron acceptors should be considered. For example, if there is significant dissolved iron production at the site (greater than 20 mg/L), iron may be acting as a competing electron acceptor with cis-DCE. A bioavailable iron test (developed by Pat Evans of CDM and available through New Horizons Diagnostics, see (36)) to determine amount of electron donor necessary to satisfy the iron demand is recommended. These physical and geochemical reasons for incomplete dechlorination can often be corrected with, "more time and more electrons." Finally, if complete absence of the appropriate organisms and/or their presence in suboptimal numbers is suspected, bioaugmentation may be warranted. Using several examples from our site database, we discuss our "differential diagnosis" approach to recommending bioaugmentation.

The first example emphasizes the importance of analyzing for dechlorinating microorganisms after an electron donor substrate has infiltrated the contaminated aquifer. Analyzing for dechlorinating microbes prior to substrate addition can give a false negative because the key microbes need a food source to grow to numbers significant enough for detection. The example site is the Alameda Naval Air Station (7, 9) site discussed previously. Phylogenetic tests for specialized dechlorinating organisms in the genera *Dehalococcoides* (known to be the only microbes capable of dechlorinating cis-DCE) and *Desulfuromonas* (dechloinates PCE to cis-DCE) were performed upgradient of and within the HRC injection grid several months after injection. The phylogenetic tests, which indicate whether a sample is positive or negative for a specific DNA sequence, had positive detections for both genera from samples within the injection area and negative (at current detection limits of approximately 100-1000 cells/ml) for both genera from samples upgradient of the injection area.

This result shows that phylogenetic testing performed prior to substrate addition can lead to incorrect conclusions regarding the microbial status of the aquifer.

A second example is from a dry cleaner site in Florida (39). HRC was injected over a vertical thickness that bracketed lower surficial and intermediate aquifers. Microgram per liter amounts of PCE dechlorinated to 100 to 1,000 µg/L of cis-DCE with no VC detection for 1.5 years after HRC injection. Another injection of HRC was made and, again, no VC was measured in groundwater samples over the course of the year after the second HRC injection. An analysis of site information showed that there was no correlation between cis-DCE concentrations and groundwater levels, indicating recharge from a vadose zone source was unlikely. Additionally, groundwater monitoring data indicated that 30 to 833 mg/L of total organic acids were present throughout the HRC treatment time. Dissolved iron remained at concentrations equal to 5 mg/L on average, sulfate was below the detection limit, and up to 14 mg/L of methane was produced. Thus, lack of substrate and the effects of competing electron acceptors do not seem to be reasons for the cis-DCE plateau. Next, groundwater samples were submitted to two different labs for phylogenetic testing for *Dehalococcoides* species of microorganisms. One lab reported a negative result for all samples, while the other lab reported a moderate positive (result was ++ with a reference standard of +++). These results indicate either that the numbers of *Dehalococcoides* species are close to the detection limit for the phylogenetic test and/or that one lab has a lower detection limit than the other. One explanation for these results is that *Dehalococcoides* species may be present, but were in numbers suboptimal for growth and significant cis-DCE reductive dechlorination. Another possibility is that the phylogenetic tests detected a non-cis-DCE dechlorinating strain of *Dehalococcoides*. This result exemplifies the need for a quantitative test for enumeration of key microorganisms, so that a correlation between the numbers of dechlorinating microbes and cis-DCE dechlorination activity can be made. A further improvement would be a functional gene test that can determine actively-dechlorinating species. In conclusion, bioaugmentation at the site may be an appropriate solution to reach clean up goals, despite a positive test for dechlorinating microorganisms.

The third example is a site at the Kenai River Terrace RV park in Soldotna, AK (22, 69). After HRC was injected in one of two aquifers, PCE in well MW-9, which is located 5 feet downgradient from the HRC injection area, decreased 99% from 2,320 µg/L to 129 µg/L in 451 days. Cis-DCE peaked at 3,800 g/L and then plateaued at concentrations between 1,500 µg/L and 2,000 µg/L for the remainder of the monitoring period. At the same time, dissolved iron increased from 6.5 mg/L to 624 mg/L at day 360. A bioavailable iron test was run on site samples from near well MW-9, with the result that further iron reduction had the potential to produce about 250 mg/L more dissolved iron (meaning the amount of bioavailable iron in the soil was equivalent to a dissolved iron concentration of about 40% the ambient dissolved iron concentration of 624 mg/L). Most likely, ferric iron is acting as a competing electron acceptor with cis-DCE at the Kenai River Terrace RV park site. One option for the site is to inject more HRC and wait for the bioavailable iron to be exhausted. Another option is to convert the aquifer to an aerobic system or otherwise oxidize cis-DCE. Bioaugmentation is also an option, if time is essential for achieving site remediation goals and waiting for iron reduction to cease is not feasible.

Conclusions

With this review, we have presented pertinent facts and conclusions on the results of 474 HRC applications since 1999, 76 of which have been published as shown in Table 1. The information presented here will aid the environmental professional in making intelligent and informed decisions about implementing engineered bioremediation for anaerobically biodegradable contaminants.

Furthermore, we provide insight into and solutions for engineered bioremediation sites where incomplete dechlorination is occurring, including a discussion of bioaugmentation strategies.

Table 1: Hydrogen Release Compound (HRC®) Published Papers and Web Case Histories

Note: Contaminant includes derivatives (e.g. PCE = PCE+TCE+DCE+VC)

Site Name	Site Location	Contaminant	References	Comments
Rice U. Simulated Aquifer Study	Houston, TX	PCE (DNAPL)	(1)Adamson et al., 2003	Complete dechlorination of 90% of added DNAPL after HRC addition and inoculation with dechlorinating culture.
Jennison-Wright Superfund Site	Granite City, IL	Pentachlorophenol (PCP)	(2)Brown et al., 2003	PCP downgradient of the HRC injection decreased 98% in 9 months
The Nipper Building, Former RCA Facility	Camden, NJ	TCE	(3)Daily et al., 2003	In May 2002, the site was granted a conditional "no further action " letter from the New Jersey Department of Environmental Protection (NJDEP).
Pesticide Form. Facility	Kinston, NC	Chlordane	(4)Fennell et al, 2003	At the center of HRC injection area, the concentration of chlordane decreased 94% by day 514.
Dry Cleaners Site	Southeastern MA	PCE	(5)Germano et al., 2003	HRC was used in conjunction with a pump and treat system. Significant decreases in concentrations were observed in groundwater and soil 6 months post-HRC application.
Manufacturing Facility	St. Louis, MI	TCE, TCA	(6)Hippensteel et al., 2003	This site was funded by Missouri Brownfield Redevelopment Program. TCE and TCA were reduced to less than 30 ug/L in 10 months after HRC application.
Alameda Naval Air Station	Alameda, CA	TCE	(7)Koenigsberg et al., 2003	*Dehalococcoides* and *Desulfurmonas* species were active in, but not outside the HRC injection area. DCE and VC were significantly reduced 9 months after injection.
Biosolids Application Field	Columbus, GA	Nitrates	(8)Lathrop et al., 2003	Nitrate concentrations were reduced by 50% in the treatment area after 4 months. However, the high groundwater velocity caused a decreased the HRC longevity.
Alameda Naval Air Station	Alameda, CA	TCE	(9)Lombardi et al., 2003	Total metabolic acids and TOC are reliable tracers for HRC. HRC-X and HRC longevity of at least 15 months.
Manufacturing Facility	Central IL	TCE, TCA	(10)Markley et al., 2003	After two and half years, all constituents of concern within the pilot area showed significant reduction.
Printed Circuits Facility	Northeastern NJ	PCE	(11)Nachlas et al., 2003	One year post-injection of HRC, PCE declined to less than 10% of total VOC mass in the saturated overburden aquifer.
Japan Site	Japan	PCE	(12)Nakashima et al., 2003	Increasing cell size and numbers and decreasing biodiversity were observed in the HRC treatment area.
Germany Site	Northern Germany	PCE, TEA	(13)Oppermann, 2003	After source zone excavation and HRC application, dechlorination to ethene was observed in the source area.
Millville Airport	Millville, NJ	PCE	(14)Pace, 2003	A combination of hollow stem auger drilling with high-pressure injection delivered HRC to 80 feet below grade.
Northwestern U. Lab Study	Evanston, IL	Neptunium, Np(V)	(15)Songkasiri et al., 2003	HRC stimulated the reduction and precipitation of neptunium.
Former Illegal Drug Lab Site	Los Osos, CA	Freon11/113	(15a)Steel et al., 2003	Seven months HRC injection, Freon 11 and Freon 113 concentrations have been reduced by 85% to 90%.
Solid Waste Landfill Site	Northern Georgia	TCE, DCA	(16)Stone et al., 2003	Injection into fractured bedrock.
Multiple Sites	Multiple Locations	PCE, perchlorate	Vigue and Koenigsberg, 2003	Review article by Regenesis
Berkey St. Site	Grand Rapids, MI	Cr(VI)	(17)Wierzbicki et al., 2003	Accelerated Cr(VI) reduction was observed in the treatment area within one year of HRC application.
Multiple Sites	Multiple Locations	PCP	(18)Willett et al., 2003	Review of all HRC applications for pentachlorophenol bioremediation.
West Union	West Union, SC	TCE	(19)Baird et al., 2002; (20)Klutz et al., 2002	HRC injection into saprolite and bedrock aquifers has resulted in the establishment of reducing conditions.
MMR Reactor Study	MA	RDX	(21)Barnes et al., 2002	HRC in 55 gallon soil reactors was very effective in RDX biodegradation, meeting treatment standards in 30 days.

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(Continued)

Site Name	Site Location	Contaminant	References	Comments
Multiple Sites	Multiple Locations	PCE	(22)Koenigsberg et al., 2002	Review paper by Regenesis
Flemington	Elizabeth, NJ	PCE	(23)Kozar et al., 2002	PCE concentrations were decreased by 90%, with contaminant increases in cis-DCE.
Japan site	Japan	PCE	(24)Nakashima et al., 2002	First-order biodegradation constants were calculated.
Arlington Cleaners	Arlington, TX	PCE	(25)Railsback et al., 2002; Koenigsberg and Vigue, 2003	HRC was injected at angles, so that areas under a building could be remediated. The site received conditional closure under TCEQ Volunteer Cleanup Program.
Springdale Cleaners	Portland, OR	TCE	(26)Sandefur et al., 2002; Vigue et al., 2002	PCE in the dissolved-phase plume and the residual DNAPL area decreased by >99% over the course of the pilot study.
Pueblo Chemical Depot	Pueblo, CO	TCE	(27)Schankweiler et al., 2002	HRC stimulated production of anaerobic conditions in a high flow aquifer compared to the aerobic background.
Invensys Control	Old Saybrook, CT	PCE	(28)Skoff et al., 2002	Bioremediation was a viable remedial approach in this low permeability application. 80% of PCE mass was removed.
Rocky Mountain Arsenal	Denver, CO	PCE, Chloroform, DIMP	(29)Vigue and Koenigsberg, 2002	EPA Superfund Innovative Technology Evaluation (SITE) Program site. DIMP (diisopropylmethylphosphonate) is a byproduct from the manufacture of sarin nerve agent.
Dixie Cleaners	Jacksonville, FL	PCE	(30)Watts et al, 2002	Case study by State Coalition for Remediation of Drycleaners
Dry Cleaners Site	Chicago, IL	PCE	(31)Adams et al., 2001	For rapid site closure, HRC was selected to polish an *in situ* chemical oxidation application.
Pueblo Chemical Depot	Pueblo, CO	Nitrates, Explosives	(32)Barnes et al., 2001; (33)Heaston et al., 2001	First site where HRC was used for remediation of nitrates and explosives. Concentrations reduced to MCL's.
Bedrock Site	Princeton, NJ	PCE	(34)Case et al., 2001	Effective delivery of HRC to fractured bedrock environment.
Coopervision Manufacturing	Scottsville, NY	TCA, TCE	(35)Dick et al., 2001	Injection to 38 feet in a dense glacial till required novel injection methods.
PCP lab study	Virginia Beach, VA	PCP	(35a)MacEwen et al., 2001	PCP reduction was >90% in HRC test tubes as compared to 40% in control Daughter products detected.
Multiple Sites	Multiple Locations	cis-DCE	(36)Evans and Koenigsberg, 2001	Evaluation of 13 sites supported bioavailable iron inhibition of cis-DCE dechlorination. Details bioavailable iron assay.
Aquifer Simulation Vessel (ASV)	Anaheim, CA	TCE	(37)Farone and Palmer, 2001	Soil column study comparing the effectiveness of polylactate esters with molasses and vegetable oil.
Mile Hi Cleaners	Aurora, CO	PCE	(38)Fischer et al., 2001	HRC and a zero valent iron wall were used to remediate a dissolved PCE plume.
Contemporary Dry Cleaners	FL	PCE	(39)Kean et al., 2001	Cis-DCE plateau in lower surficial and intermediate aquifers. Dechlorinating organisms detected at the site.
Multiple Sites	Multiple Locations	PCE	(40)Koenigsberg et al., 2001	Review paper by Regenesis
Dixie Cleaners	Jacksonville, FL	PCE	(41)Murray et al., 2001	Groundwater sampling results from the pilot test showed a sharp decrease in the concentrations of PCE and TCE.
Fisherville Mill Site	Grafton, MA	TCE	Murray et al., 2001	EPA Superfund Innovative Technology Evaluation (SITE) Program site
Manufacturing Facility	Cookeville, TN	PCE	Murray et al., 2001	TCE was degraded to below MCLs at the property boundary; TCE source area had mg/L concentrations, successful application in tight clay soils.
Dover Park Dry Cleaning	Yardville, NJ	PCE	(44)North et al., 2001	In the core of the plume, PCE decreased up to 99% within 1 year of HRC injection. Complete PCE biodegradation.
U. of Connecticut Lab Study	Storrs, CT	PCE	(45)Panciera et al., 2001	Dechlorinating microcosms using estuary samples. Compared ethanol, lactate and other organic acids, vegetable oil, and HRC as the electron donors.
Former Landfill Site 7	Duluth, MN	TCE	(46)Semer and Banerjee, 2001	DCE and VC peaked and then decreased at the end of the monitoring period.

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(Continued)

Site Name	Site Location	Contaminant	References	Comments
Santa Clara County	Santa Clara County, CA	PCE	(47)Sharma et al., 2001	Compared performance of commercial HRC with fast-release HRC Primer.
Former Industrial Facility	CO	PCE	(48)South et al., 2001	The groundwater data showed that reductive dechlorination process was accelerated by the injection of HRC.
MMR Microcosm Study	Camp Edwards, MA	RDX, HMX Perchlorate	(49)Weeks et al., 2001	RDX (100ug/L) & HMX (20ug/L) were degraded to less than 0.6 ug/L in 28 days.
Hayden Island Cleaners	Portland, OR	PCE	(50)Anderson et al., 2000	Also as a case study by State Coalition for Remediation of Drycleaners http://www.drycleancoalition.org/
Former Industrial Filter Manufacturer	Rochester, NY	TCE	(51)Boyle et al., 2000; Case et al., 2001	HRC applied to polish source area after removal of extraction system. Site closure based on HRC performance was achieved. Aquifer soil consisted of a tight clay.
Moen Industrial Site	Elyria, OH	DCE	(52)Cornuet et al., 2000	Side-by-side study of aerobic vs. anaerobic biodegradation for DCE & VC.
Hurlburt Field	Tallahassee, FL	TCE	(53)Harms et al., 2000	Goal was to remediate all contaminants in less than 5 years. Complete reductive dechlorination of TCE.
Multiple Sites	Multiple Locations	PCE	(54)Koenigsberg et al., 2000	Review paper by Regenesis
Contemporary Cleaners	Orlando, FL	PCE	(55)Lodato et al., 2000	Also a case study by State Coalition for Remediation of Drycleaners http://www.drycleancoalition.org/
Penn State U. Lab Study	University Park, PA	Perchlorate	(56)Logan et al., 2000	HRC is used as a carbon/energy source by perchlorate-respiring microbes. Removal of perchlorate in 1.5 days.
Unocal Wichita	Wichita, KS	PCE	(56a)Murray et al., 2000; Murray et al., 2001	PCE was degraded from 6 mg/L to 0.2 mg/L within 30 days.
Manufacturing Site	Walled Lake, MI	DCE	Murray et al., 2000; Murray et al., 2001	Results showed large amounts of lactic acid fermentation products.
Vandalia Manufacturing Facility	Vandalia, IL	PCE	(57)Schuhmacher et al., 2000	HRC barrier application.
Dayco Manufacturing Facility	Eldora, IA	TCE	(58)Sheldon and Armstrong, 2000; (58a)2002	Evaluation of HRC in a barrier installed via canisters in wells.
FMC Corporation Site	San Jose, CA	TCE	(59)Zahiraleslamzadeh and Bensch, 2000; (59a) 2001	Compared top-down and bottom-up HRC injection methods and found no difference in vertical distribution of HRC. The bottom-up method was easier to implement.
Watertown Industrial Area	Watertown, MA	PCE	(60)Dooley et al., 1999; Murray et al., 2000	HRC applied to polish source area after removal of extraction system. HRC canisters installed in wells.
Multiple Sites	Multiple Locations	PCE	(61)Koenigsberg et al., 1999	Review paper by Regenesis
Cedarburg Drycleaning Facility	Cedarburg, WI	PCE	(62)Sheldon et al., 1999	First commercial grid application of HRC. *In situ* hydrogen measurements made. Full-scale project was implemented and site now closed.
Decorah Shopping Center Drycleaners	Decorah, WI	PCE	(63)site profile www.drycleancoalition.org\siteprofiles	Case study by State Coalition for Remediation of Drycleaners
Manufacturing Facility	Cookeville, TN	PCE	(64)case study www.regenesis.com Murray et al. (2001)	HRC effectively reduced over 100ug/L of PCE in a tight clay formation.
Manufacturing Facility	Crozet, VA	TCE	(65)case history (H1.7) www.regenesis.com	Fractured bedrock site
Whittaker Ordnance	Hollister, CA	Perchlorate, Cr(VI), Freon	(66)case history (H2.1) www.regenesis.com	First site where HRC was used for remediation of perchlorate, chrome and freon.
Cleaners No. 1,	Kent, WA	PCE	(67)case history (H2.8) www.regenesis.com	Mass reduction of 99.98% after sewer line leak repaired.
Tosco Manufacturing Facility	Burien, WA	PCE	(68)case history (H3.1) www.regenesis.com	This site has mixed commercial and residential property. PCE concentrations in all 6 monitoring wells have shown an average of 70% reduction 159 days after HRC injection.
Hurlburt Field Site	Hurlburt, FL	TCA	(68a)case history (H3.1.4) www.regenesis.com	TCA mass reduction of 75% was observed after HRC application.
Kenai River Terrace	Soldotna, AK	PCE	(68b)case history (H3.2) www.regenesis.com	HRC was effective at degradation of PCE and TCE in upper and lower plume. DCE plateau in lower plume possibly due to

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(Continued)

Site Name	Site Location	Contaminant	References	Comments
			Koenigsberg et al., 2002	high bioavailable iron or lack of appropriate microbes.
TRW Microwave Electronics Facility	Sunnyvale, CA	PCE	(70)consultant prepared case history www.regenesis.com	Pump and treat system replaced by HRC application. Costs were reduced by over 75%. The concentration of PCE reduced to below MCL's. HRC primer application.
Anadite Inc. Facility	Santa Clara, CA	PCE	(71)consultant prepared case history www.regenesis.com	HRC was chosen over pump & treat and chemical oxidation. The HRC application cost $1 million less than the pump & treat system. The concentration of PCE was reduced below MCLs.

References

Adams, R., M. Vigneri, B. Mahaffey and B. Slack. 2001. Rapid Closure Using ISCO and Enhanced MNA to Achieve Site Closure – Case Studies. *Proceedings of the Battelle sponsored 6th International In-situ and On-site Bioremediation Symposium*, San Diego, CA, June 4 – 7, 2001. Battelle Press, ISNB 1-57477-110-8, 2001.

Adamson, D. T., J. M. McDade and J. B. Hughes. 2003. Inoculation of a DNAPL Source Zone to Initiate Reductive Dechlorination of PCE. *Environ. Sci. Technol.* ASAP article, April 17, http://pubs.acs.org/journals/esthag.

Baird, A.M., Maalouf, G., and McDonnell, D., Klutz, T., and S. Sandefur. 2002. Significance of Hydraulic Conductivity in Optimizing HRC® Delivery into a Fractured Bedrock Aquifer. In: A.R. Gavaskar and A.S.C. Chen (Eds.), *Remediation of Chlorinated and Recalcitrant Compounds – 2002*, Proceedings of the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2002). Paper 2H-48. Battelle Press, Columbus, Ohio.

Boyle, S.L., Dick, V.B., Ramsdell, M.N., and Caffoe, T.M. 2000. Enhanced Closure of a TCE Site Using Injectable HRC. *Proceedings of the Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California*. May.

Brown, N. J., N. Fred, S. Mullin and K. Lapus. 2003. The Use of Hydrogen Release Compound (HRC®) for Pentachlorophenol (PCP) Degradation. Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation*, Orlando, FL, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Case, N.L., Boyle, S.L., and Dick, V.B. 2001. Enhanced Bioremediation Under Difficult Geologic Conditions - Case Studies. *Proceedings of the Sixth In-Situ and On-Site Bioremediation Symposium, San Diego, California.* No. 6(7), p. 281-288. (HRC, Former Industrial Filter Manufacturer, Rochester NY Case Study)

Cornuet, T. S., Sandefur, C., Eliason, W. M., Johnson, S. E., Serna, C. 2000. Aerobic and Anaerobic Bioremediation of *Cis*-1,2-DCE and Vinyl Chloride. *Proceedings of the Second International In-Situ and On-Site Bioremediation Symposium, Monterey, California*.

Daily, C. and D. Shattuck. 2003. The Use of Hydrogen Release Compound (HRC®) for Pentachlorophenol (PCP) Degradation. Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation*, Orlando, FL, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Dooley, M.A., Murray, W.A., and S. Koenigsberg. 1999. Passively Enhanced In Situ Biodegradation of Chlorinated Solvents. *Engineered Approaches for In Situ Bioremediation of Chlorinated Solvent Contamination.* In: A. Leeson and B.C. Alleman (Eds.), Proceedings

of the Fifth International In Situ and On-Site Remediation Symposium (San Diego, CA; April 1999). Vol. 5(2):121-127. Battelle Press, Columbus, Ohio.

Evans, P.J., and S.S. Koenigsberg. 2001. A Bioavailable Ferric Iron Assay and Relevance to Reductive Dechlorination. *Proceedings of the Sixth International In-Situ and On Site Bioremediation Symposium,* San Diego, California, No. 6(8), p. 209-215.

Fennell, S., K. Lapus, A Willett and S. Koenigsberg. 2003. Field Pilot Test of Anaerobic Biodegradation of Chlordane Using HRC®. Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation*, Orlando, FL, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Farone, W.A., and T. Palmer. 2001. Comparison of Reducing Agents for Dechlorination in a Simulated Aquifer. *Proceedings of the Sixth International In-Situ and On Site Bioremediation Symposium,* San Diego, California, No. 6(8):103-108.

Germano, M and J. Pecevich. 2003. "Multiple Remedial Techniques Including Bioacceleration Used at Dry Cleaners Site." Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation, Orlando, FL*, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Harms, W.D., Jr., Taylor, K.A., and Taylor, B.S. 2000. HRC-Enhanced Reductive Dechlorination of Source Trichloroethene in an Unconfined Aquifer. *Proceedings of the Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California*.

Hippensteel, T., M. J. McGuire and M. R. Sieczkowi. 2003. Reductive Dechlorination of TCE and TCA during a Brownfield Redevelopment. Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation, Orlando, FL*, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Klutz, T., A.M. Baird, G. Maalouf, and D. McDonnell, and S. Sandefur. 2002. Accelerated Bioremediation of Trichloroethylene: A Comparison between Saprolite an Crystalline Bedrock Aquifers. In: A.R. Gavaskar and A.S.C. Chen (Eds.), *Remediation of Chlorinated and Recalcitrant Compounds – 2002*, Proceedings of the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2002). Paper 2C-46. Battelle Press, Columbus, Ohio.

Koenigsberg, S. S., J. C. Bensch and C. E. Lombardi. 2003." Implications for Bioaugmentation at a RABITT Protocol Site Revisited." Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation, Orlando, FL, June 2-5, 2003*. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Koenigsberg, S.S., C.A. Sandefur, K.A. Lapus, and G. Pasrich. 2002. Facilitated Desorption and Incomplete Dechlorination: Observations from 350 Applications of HRC. In: A.R. Gavaskar and A.S.C. Chen (Eds.), *Remediation of Chlorinated and Recalcitrant Compounds – 2002*, Proceedings of the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2002). Paper 2B-56. Battelle Press, Columbus, Ohio.

Koenigsberg, S.S., Farone, W.A., Sandefur, C.A. 2000. Time-Release Electron Donor Technology for Accelerated Biological Reductive Dechlorination. *Proceedings of the Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California*.

Koenigsberg, S.S., and Farone, W.A.,. 1999. The Use of Hydrogen Release Compound (HRC™) for CAH Bioremediation. In: *Engineered Approaches for In Situ Bioremediation of Chlorinated Solvent Contamination*. A Leeson and B.C. Alleman (Eds.). Vol. 5(2), pp. 67-72. Battelle Press, Columbus, Ohio.

Kozar, M.S., McIlvaine, C.L., Duffy, B.E., and Street, W.M. 2002. Enhanced Degradation of Chlorinated Solvents in Fractured Rock Ground Water Using Subsurface Injection of HRC. In: A.R. Gavaskar and A.S.C. Chen (Eds.), *Remediation of Chlorinated and Recalcitrant Compounds – 2002*, Proceedings of the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2002). Paper 2C-42. Battelle Press, Columbus, Ohio.

Lathrop, S. B., W. T. Tharpe, H. E. Nuttall, and B. G. Turner. 2003. "Pilot-Scale Field Test Results of Enhanced In Situ Denitrification." Presentation given at the 7th International Symposium of In Situ and On-Site Bioremediation, Orlando, FL, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Lodato, M., Graves, D., Kean, J. 2000. Enhanced Biological Reductive Dechlorination at a Dry Cleaning Facility. *Proceedings of the Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California*.

Logan, B. E., H. Zhang, J. Wu, R. Unz and S. S. Koenigsberg. 2000. "The Potential for In Situ Perchlorate Degradation" in *Proceedings of the 2nd International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA*, May 22-25, 2000. Battelle Press, Columbus, OH.

Lombardi, C. E., J. C. Bensch and S. S. Koenigsberg. 2003. "Tracing Rate of Dispersion, Distribution, and Longevity of Electron Donor." Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation, Orlando, FL*, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

MacEwen, S. J., F. Fadullon and D. Hayes. 2001. "Evaluation of Aerobic and Anaerobic Degradation of Pentachlorophenol in Groundwater." *Proceedings of the Battelle sponsored 6th International In-situ and On-site Bioremediation Symposium, San Diego, CA*, June 4 – 7, 2001. Battelle Press, ISNB 1-57477-110-8.

Markley, D. and M. R. Sieczkowski. 2003. In-Situ Biodegradation of Chlorinated Ethanes and Ethenes Using HRC®. Presentation given at the 7th International Symposium of In Situ and On-Site Bioremediation, Orlando, FL, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Murray, W., Dooley, M., Koenigsberg, S. 2001. Enhanced Bioremediation of Chlorinated Solvents. *Proceedings of the Sixth International In-Situ and On-Site Bioremediation Symposium*, No. 6(7), p. 197-204.

Nachlas, P. E. and T. Tesler. 2003. HRC® Remediation of PCE at a New Jersey, Printed Circuit Site. Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation,* Orlando, FL, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Nakashima, M. X. Wu, M. Nishigaki, T. Shigeno, H. Uchiyama and T. Someya. 2003. Remediation Effect on HRC® Injection and Microorganism Environment Change. Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation,* Orlando, FL, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Nakashima, M., Wu, X., Okada, R., and Nishigaki, M. 2002. Enhanced Bioremediation of a Site in Japan Contaminated with Chlorinated Solvents using HRC Injection. In: A.R. Gavaskar and A.S.C. Chen (Eds.), *Remediation of Chlorinated and Recalcitrant Compounds – 2002*, Proceedings of the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2002). Paper 2B-42. Battelle Press, Columbus, Ohio.

North, R.W., Burkett, S.E., Sincock, M.J. 2001. Effective Enhancement of Biological Degradation of Tetrachloroethene (PCE) in Ground Water. *Proceedings of the Sixth International In-Situ and On-Site Bioremediation Symposium*. Vol. 6(7), pp. 189-196.

Oppermann, A. 2003. "Remediation of CHC by Reductive Dechlorination in Germany: a Full-Scale Approach." Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation,* Orlando, FL, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Pace, F. J.. 2003. A Novel Hydrogen Release Compound (HRC®) Injection Method for Complicated Site Geology. Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation,* Orlando, FL, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Panciera, M. A., O. Zelennikova, B. F. Smets and G. M. Dobbs. 2001. "Differential Stimulation of Haloreduction by Carbon Addition to Subsurface Soils" *Proceedings of the Sixth International Symposium on In-Situ and On-Site Bioremediation,* San Diego, California, June 4 – 7, 2001. Battelle Press, ISNB 1-57477-117-5, p69-76, 2001.

Schumacher, T., Bow, W.A., Chitwood, J.P. 2000. A Field Demonstration Showing Enhanced Reductive Dechlorination Using Polymer Injection. *Proceedings of the Second International In-Situ and On-Site Bioremediation Symposium, Monterey, California.* pp. 15-23.

Semer, R., Banerjee, P. 2001. Anaerobic Bioremediation of Trichloroethene Near Duluth International Airport. *Proceedings of the Sixth International In-Situ and On-Site Bioremediation Symposium*, No. 6(7), p. 157-164.

Sharma, P. K., Voscott, H. T., Swann, B. M. 2001. Enhanced CAH Dechlorination Using Slow and Fast Releasing Polylactate Esters. *Proceedings of the Sixth In-Situ and On-Site Bioremediation Symposium, No. 6(7), p. 305-312.*

Sheldon, J. K., and Armstrong, K. G. 2000. Barrier Implants for the Accelerated Bio-Attenuation of TCE. *Proceedings of the Second International In-Situ and On-Site Bioremediation Symposium, Monterey, California*.

Sheldon, J. K., and Armstrong, K. G. 2002. Assessing Ongoing Full-Scale HRC Performance From Source Area to Property Line (abstract). Presented at the *Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds Monterey, California*. May 20-23, 2002.

Sheldon, J.K., Koenigsberg, S.S., Quinn, K.J., Sandefur, C.A. 1999. Field Application of a Lactic Acid Ester for PCE Bioremediation. In: *Engineered Approaches for In Situ Bioremediation of Chlorinated Solvent Contamination*. A Leeson and B.C. Alleman (Eds.). Vol. 5(2), pp. 61-66. Battelle Press, Columbus, Ohio www.environmental-center.com/articles/article1047/article1047.htm.

Skoff, D. E., Holmes, J. S., and Peterson, D. 2002. Time-Release Electron Donor Application in Low-Permeability PCE-Contaminated Aquifer. In: A.R. Gavaskar and A.S.C. Chen (Eds.), *Remediation of Chlorinated and Recalcitrant Compounds – 2002*, Proceedings of the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2002). Paper 2B-51. Battelle Press, Columbus, Ohio.

Songkasiri, W., B. E. Rittmann, D. T. Reed, A. Willett and S. Koenigsberg. 2003. Bioremediation of Neptunium(V) Using Lactate, Hydrogen, or Hydrogen Release Compound (HRC). Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation*, Orlando, FL, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

South, D., Seracuse, J., Garrett, K., and Li, D. 2001. Accelerating the Reductive Dechlorination Process in Groundwater. *Proceedings of the Sixth International In-Situ and On-Site Bioremediation Symposium*, No. 6(7), p. 205-212.

Steele, J. R., R. W. Griffith and P. C. Randall. 2003. "In Situ Enhanced Bioremediation of Freon 11/Freon 113 Groundwater ContaminationUsing HRC®." Presentation given at the *7th*

International Symposium of In Situ and On-Site Bioremediation, Orlando, FL, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Stone, B. M., E. Hood, E. Victor and T. Hughes. 2003. Accelerated Bioremediation of Chlorinated Solvents in a Fractured Rock Aquifer. Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation*, Orlando, FL, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Vigue, B. W. and S. S. Koenigsberg. 2003. Bioremediation on the Fast Track: An Expanding Groundwater Treatment Is Moving Accelerated Natural Attenuation into the Mainstream. Preprint from *Water & Wastewater Products* July/August 2003.

Vigue, B. W. and S. S. Koenigsberg. 2002. Cost Effective Cleanup of DOD Sites Using Slow Release Compounds. *Pollution Engineering*, March, pp 14-17.

Watts, J.J., Jaynes, M.O., Farrell, J.A., and Gillespie, R. 2002. Remedial Action Using HRC Under a State Dry Cleaning Program. In: A.R. Gavaskar and A.S.C. Chen (Eds.), *Remediation of Chlorinated and Recalcitrant Compounds – 2002*, Proceedings of the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2002). Paper 2B-44. Battelle Press, Columbus, Ohio.

Weeks, K. R., S. C. Veenstra, D. Taege, D. L. Hill and B. P. Gregson. 2001. Evaluation of Innovative Groundwater Remediation Technologies at Camp Edwards, Massachusetts. *Proceedings of the Battelle sponsored 6th International In-situ and On-site Bioremediation Symposium*, San Diego, CA, June 4 – 7, 2001. Battelle Press, ISNB 1-57477-110-8, 2001.

Wierzbicki, D., D. Bohan, S. Mullin, J. Peery and A. Willett. 2003. "Hexavalent Chromium Reduction and Immobilization Using Hydrogen Release Compound." Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation*, Orlando, FL, June 2-5, 2003. (Proceedings in press, available Jan. 2004, Battelle Press, Columbus, OH).

Willett, A. and S. S. Koenigsberg. 2003. The Efficacy of ORC® and HRC® for the Accelerated Bioremediation of Pentachlorophenol (PCP) (abstract). Presentation given at the *7th International Symposium of In Situ and On-Site Bioremediation*, Orlando, FL, June 2-5, 2003.

Zahiraleslamzadeh, Z.M., Bensch, J. C. 2000. Enhanced Bioremediation using Hydrogen Release Compound (HRC) in Clay Soils. *Proceedings of the 2nd International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California.*

Zahiraleslamzadeh, Z.M., Bensch, J.C. 2001. Enhanced Bioremediation in Clay Soils. *Proceedings of the Sixth International In-Situ and On-Site Bioremediation Symposium*, No. 6(7), p. 221-228.

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APPENDIX E.13 – BIOAUGMENTATION TO ENHANCE ANAEROBIC BIOREMEDIATION OF CHLORINATED SOLVENTS IN GROUNDWATER: TECHNOLOGY OVERVIEW AND DESIGN CRITERIA

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BIOAUGMENTATION TO ENHANCE ANAEROBIC BIOREMEDIATION OF CHLORINATED SOLVENTS IN GROUNDWATER: SIX CASE STUDIES

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1. INTRODUCTION

Bioaugmentation involves the delivery of specialized microbial cultures into the subsurface to accelerate biodegradation reactions and to achieve specific remediation objectives. In the case of chlorinated ethenes (PCE, TCE, cDCE, and VC), bioaugmentation applications are performed with anaerobic, dehalorespiring microbial cultures that include strains of *Dehalococcoides* bacteria (Ellis et al. 2000; Major et al. 2002; Lendvay et al. 2003). Although bioaugmentation in chlorinated ethene plumes has also been performed with aerobic, toluene-oxidizing cultures (Steffan et al. 1999), research indicates that biodegradation of chlorinated ethenes proceeds most efficiently via anaerobic dehalorespiration reactions (Bradley 2000). In addition, the substantially higher aqueous solubility of organic electron donors, relative to oxygen, favors anaerobic over aerobic in situ bioremediation systems. A defining characteristic of dehalorespiring bacteria is that they are capable of using certain chlorinated compounds as sole substrates for energy and growth. A variety of dehalorespiring microorganisms have been shown to dechlorinate PCE and TCE to cDCE; however, *Dehalococcoides ethenogenes* strain 195 and *Dehalococcoides* sp. strain FL2 are the only pure cultures known to dechlorinate PCE, TCE and cDCE completely to ethene (Maymo-Gatell et al. 1997; Löffler et al. 2000).

Dehalococcoides microorganisms are not ubiquitous in subsurface environments, and a growing body of academic research has observed a clear link between the persistence of cDCE and the presence/absence of *Dehalococcoides* (Löffler et al. 2000; Fennell et al. 2001; Hendrickson et al. 2002). At sites where indigenous *Dehalococcoides* populations are present, properly designed biostimulation approaches (e.g., electron donor addition) may be effective at achieving complete dechlorination of PCE and TCE to ethene. At sites where *Dehalococcoides* are absent, biostimulation approaches commonly result in an accumulation and persistence of cDCE (cDCE "stall"), regardless of how much electron donor is added (Ellis et al. 2000; Major et al. 2002). In this case, bioaugmentation with cultures that contain *Dehalococcoides* can be an effective supplement to a variety of biostimulation designs, including those that involve both passive (e.g., vegoil biobarriers; HRC) or active electron donor delivery systems (e.g., groundwater recirculation cells).

Bioaugmentation with cultures containing *Dehalococcoides* has been implemented at a variety of chlorinated solvent sites. Because the technology is relatively young (the first comprehensive technology demonstration was published by Ellis et al. in 2000), many bioaugmentation projects are not yet complete. Table 1 presents a summary of some bioaugmentation projects that have been performed to date. As illustrated in Table 1 and the case study descriptions that follow, bioaugmentation has been demonstrated to achieve rapid dechlorination of dissolved PCE and TCE in unconsolidated sand and gravel aquifers (e.g., Ellis et al. 2000; Major et al. 2002; Lendvay et al. 2003). Through these successful demonstrations, design of bioaugmentation applications for sand and gravel aquifer applications is becoming relatively standardized. The performance of the technology in fractured bedrock aquifers has not been demonstrated to the same extent as for porous media; however, several ongoing projects have achieved substantial chloroethene reductions and conversion to ethene (e.g., industrial sites in Massachusetts and South Carolina, and the Caldwell Trucking NPL site in New Jersey).

The Department of Defense Environmental Security Technology Certification Program (ESTCP) and National Aeronautic Space Administration (NASA) are sponsoring comprehensive field tests to demonstrate the performance of bioaugmentation for remediating dense non-aqueous phase liquids (DNAPLs). Both the ESTCP and NASA projects, which are using the commercially-available KB-1™ culture, involve quantifying the enhanced dissolution of DNAPL that results from biostimulation and bioaugmentation. A key objective of those projects is to verify observations of laboratory studies that have found that biostimulation and bioaugmentation can accelerate the rate DNAPL dissolution by a factor of 5 to 14 times (Yang and McCarty 2000; Carr et al. 2000; Cope and Hughes 2001). Results from laboratory studies indicate that bioaugmentation may be able to substantially reduce the timeframe for DNAPL site cleanup by accelerating biodegradation and the rate of DNAPL dissolution.

This chapter reviews six case studies involving bioaugmentation applications to achieve anaerobic bioremediation of chlorinated solvents in groundwater. The selected projects represent a range of bioaugmentation applications, and vary in terms of electron donor delivery systems (recirculation and batch injection), operational modes (continuous and pulsed), geologic terrains (fractured rock and sand and gravel aquifers), contaminant conditions (plume, source, and complex mixture), and bioaugmentation cultures. Five of the six case studies represent projects where bioremediation systems at chloroethene-contaminated sites were augmented with cultures that contain *Dehalococcoides* microorganisms. A sixth case study is presented that involves augmentation with the denitrifying microorganism *Pseudomonas stutzeri* strain KC to achieve in situ biodegradation of carbon tetrachloride (CT). Although the electron donor and transformation requirements for strain KC are significantly different than those for *Dehalococcoides* cultures, the strain KC case study is included here because it involves many features and processes with direct relevance to bioaugmentation at chloroethene sites. All of the case studies illustrate state-of-the-science approaches for determining the need for bioaugmentation, designing effective electron donor delivery and bioaugmentation systems, and monitoring bioaugmentation performance.

2. BIOAUGMENTATION CASE STUDIES

Dover Air Force Base, Dover Delaware

Ellis et al. (2000) presented a field-scale demonstration of in situ bioaugmentation for treating dissolved phase TCE at Dover Air Force Base. Comprehensive microcosm studies were performed prior to system design to assess the need for bioaugmentation. The microcosm studies described by Lee et al. (2000). used site soil and groundwater amended with various electron donors including: organic acids such as acetate, benzoate, butyrate, formate, lactate, and propionate; alcohols such as ethanol and methanol; sugars, including sucrose, glucose, and fructose; complex organics such as molasses or yeast extract; other compounds including vitamin B_{12}; ethylene glycol and glutamic acid. Sulfate and bicarbonate were added as electron acceptors in addition to the nitrate, sulfate, iron, and bicarbonate already present in the groundwater and soil. Many treatments were amended with nutrients, vitamins, or trace elements and/or yeast extract. In all, over 1000 individual microcosms were tested during this phase of the study. TCE was reduced to vinyl chloride in only a few microcosm bottles after incubation of up to 500 days. Ethene was not produced in any bottles, even under methanogenic conditions. If *Dehalococcoides* spp. were more widely distributed at this site, then a greater percentage of these microcosms should have converted TCE beyond cDCE. Additional treatability studies were performed using Dover soil and groundwater to assess biostimulation performance in aquifer columns (Harkness et al. 1999). TCE was not reduced beyond cDCE in columns that had been fed electron donors for up to 371 days. However, injection into one column of a small volume of

the Pinellas culture, which contains close relatives of *Dehalococcoides ethenogenes*, stimulated complete dechlorination of cDCE to ethene within 20 days. This effect was repeated in a second column injected with the same culture. Furthermore, VC production was transient in both bioaugmented columns, with rapid conversion to ethene.

The bioaugmentation field pilot test at Dover AFB involved a recirculation design and the addition of lactate (Ellis et al. 2000). The well layout of the pilot test area is illustrated in Figure 1. The layout of the pilot test area and the pumping rates were designed with the aid of groundwater flow modeling and subsequent tracer tests with bromide. The treatment well network consisted of one row of three extraction wells and another row of three injection wells, with each row oriented perpendicular to the prevailing hydraulic gradient. The two rows were 6 m apart. Based on the results of modeling and tracer testing, a combined extraction rate of 11.6 L/min was chosen as the design flow that would achieve sufficient capture. This pumping rate resulted in an estimated residence time of 60 days.

The system was operated in biostimulation mode for the first 269 days, with TCE being stoichiometrically dechlorinated to cDCE, but not to VC or ethene. Lactate was delivered on a 7-day, pulsed feeding schedule to minimize biofouling at the injection wells. The pilot test area was augmented with the Pinellas culture on days 260 and 284 (180L, and 171 L, respectively), and VC and ethene was detected within 90 days of bioaugmentation. A complete mass balance conversion of TCE and cDCE to ethene was achieved within 8 months after bioaugmentation. After completion of the pilot test, Hendrickson et al. (2001) used 16S rDNA-based PCR methods to screen for the presence of *Dehalococcoides ethenogenes*-like bacteria within the pilot test area. One year after the completion of the pilot, close relatives of *Dehalococcoides ethenogenes* were detected throughout the test plot, but were not detected outside the pilot test area, which provided additional evidence that the attainment of complete dechlorination within the test plot was linked to the presence of *Dehalococcoides*. Additional sampling performed 2 and 3 years after the completion of the pilot test detected the continued presence of *Dehalococcoides ethenogenes*-like bacteria within the pilot test area, but again not in the upgradient background wells. These data indicate that the *Dehalococcoides* strains injected into the subsurface can survive for long periods, and continue to dechlorinate as long as an anaerobic environment is maintained.

Kelly Air Force Base, San Antonio, Texas

Major et al (2002) conducted a demonstration of bioaugmentation for treating dissolved-phase PCE, TCE and cDCE at Kelly AFB in San Antonio, Texas. Prior to the demonstration, the site groundwater contained about 1 mg/L of PCE and lower amounts of TCE and cDCE, without any detectable VC or ethene. Analysis with 16S rDNA-based PCR methods did not detect *Dehalococcoides* in any groundwater or sediment samples from the pilot test area. Laboratory microcosm studies showed that non-bioaugmented treatments containing lactate or methanol resulted in stoichiometric conversion of TCE and cDCE, without further dechlorination of cDCE to VC or ethene. Microcosms bioaugmented with KB-1, a halo-respiring culture that contains various strains of *Dehalococcoides*, and methanol stoichiometrically converted all of the TCE to ethene. The field test consisted of three recirculation plots, two that served as control plots, and one that was bioaugmented with KB-1.

Figure 2 illustrates the example performance monitoring results for the bioaugmentation test plot. The test plot was recirculated for 89 days to equilibrate the system and to conduct the bromide tracer test. From day 90 to day 175, methanol and acetate were added as electron donors to establish reduced conditions and to stimulate reductive dechlorination by the indigenous

bacteria. Bioaugmentation with 13L of KB-1 occurred on day 176. Performance monitoring of the control and test plots showed that in the presence of methanol and acetate, the indigenous bacteria could be stimulated to dechlorinate PCE to cDCE. However, no dechlorination past cDCE was observed in the control plots for the remainder of the test. In contrast, VC was detected 52 days after bioaugmentation with KB-1 in the test plot, and by day 318 ethene was the dominant product. Calculated half-lives for degradation were on the order of minutes to hours. 16S rDNA-based PCR methods were used to monitor the migration and growth of KB-1 culture after injection. Molecular monitoring showed that the culture had completely colonized the 9.1 meter-long aquifer test plot within 115 days after the one-time injection of KB-1. The two control plots were installed and operated in the same manner as the test plot, but were never amended with KB-1. In these control plots dechlorination stalled at cDCE, with no VC observed during 216 days of operation. Molecular analysis confirmed that *Dehalococcoides* was not present in the control plots.

Bachman Road Residential Wells Site, Oscoda Michigan

Lendvay et al. (2003) conducted a field demonstration of the relative performance of bioaugmentation and biostimulation through side-by-side closed-loop, recirculatory remediation test plots at the Bachman Road Residential Wells Site in Michigan. Molecular analysis indicated that indigenous *Dehalococcoides* populations existed at the site, and this population was enriched (the Bachman Road Culture) and used for the bioaugmentation plot. Two test plots (4.6 x 5.5 m) were constructed perpendicular to groundwater flow, separated by one plot of the same size. Each plot consisted of an extraction well, two injection wells, and a series of performance monitoring points. A bromide tracer study was performed to quantify the hydraulics of each test plot, and a design recirculation flow rate of 7 gpm was selected for each plot. Both the biostimulation and bioaugmentation plots received lactate as an electron donor. The bioaugmentation test plot was preconditioned with a lactate (0.5 to 1.0 mM) and nutrient feed prior to bioaugmentation. On day 29, 200 L (10^8 cell/mL) of the Bachman Road Culture was introduced into the bioaugmentation plot.

Relative to the control (biostimulation) plot, bioaugmentation resulted in a significant reduction in the time to achieve complete dechlorination to ethene. Complete dechlorination of PCE to ethene was achieved within 6 weeks after inoculation in the bioaugmentation plot, whereas, after 4 months of operation nearly 76% of the PCE was converted to ethene in the biostimulation plot. Important findings of this work include: (1) dechlorination in the bioaugmentation plot was demonstratively linked to the presence of *Dehalococcoides*; (2) *Dehalococcoides* populations grew (measurable numbers increased) as system operation proceeded; (3) addition of *Dehalococcoides* can significantly shorten lag times to the onset of dechlorination; and (4) biostimulation approaches can achieve complete dechlorination to ethene at sites where certain *Dehalococcoides* populations occur naturally.

Industrial Site, Boston, Massachusetts

GeoSyntec and ERM are performing a bioaugmentation pilot test at an industrial facility in Boston (Chang et al. 2002; 2003). Spent organic solvents, primarily TCE, were released to unconsolidated soils through a dry well located interior to the main manufacturing building. The TCE is suspected to have traveled along building pilings downward to the basal unit of fractured bedrock. The pilot test area (pilot test area) is located directly downgradient from the dry well. Concentrations of TCE in the pilot test area range from 30 to 120 mg/L. Due to the proximity to salt water, sulfate and chloride concentrations in shallow bedrock were approximately 400 and 5,500 mg/L, respectively. Pre-design laboratory studies using PCR and 16S rDNA-based

methods detected the presence of an indigenous *Dehalococcoides* population. Microcosms studies confirmed that when supplied with an exogenous electron donor, the indigenous microorganisms could be stimulated to convert TCE to ethene. However, compared to microcosms amended with the KB-1™ culture, the rate of ethene production achieved by the native bacteria was much slower, even after a six month incubation period. Based on the results of the laboratory trials, bioaugmentation was selected for the field pilot test.

A recirculatory, forced-gradient pilot test system was designed based on the demonstrated success of achieving effective reagent delivery and maximum mass balances with these types of pilot-scale systems for bioremediation applications at other sites (e.g., Hopkins and McCarty 1995; McCarty et al. 1998; Ellis et al. 2000). The pilot test area is comprised of an injection well, extraction well, and three monitoring wells. The pilot test area layout was oriented such that the induced gradient was parallel with the prevailing ambient flow direction and hydraulic gradient to minimize leakage from the pilot test area. Prior to performing any biological treatments, the hydraulics of the pilot test area (i.e., flow rates, residence time, capture, mass recovery) were quantified via tracer testing with iodide. The tracer test demonstrated hydraulic connectivity across the pilot test area, but only 15% of the iodide delivered to the injection well was recovered at the extraction well. These results indicated a high degree of mixing between the recirculation cell and ambient groundwater. Subsequent observation in the vicinity of the Site revealed that dewatering activities at a neighboring property caused periodic 90-degree changes in the hydraulic gradient in the pilot test area.

Example results for the pilot system are illustrated in Figure 3. The pilot test area was fed acetate for the first 3 months of operation for the purpose of establishing reducing conditions in the test zone, prior to bioaugmentation. During this preconditioning period, sulfate concentrations and oxidation/ reduction potential (ORP) decreased linearly, and TCE was dechlorinated to cDCE. Dechlorination did not proceed beyond cDCE prior to bioaugmentation. During the fourth month of operation (June 2002), the pilot test area was augmented with KB-1™ and methanol was added as a supplemental electron donor. Acetate addition was discontinued in October 2002 due to site-specific reasons. Bioaugmentation was achieved by transferring 40L of KB-1™ culture from the stainless steel culture containers into the injection well. Argon gas was used to displace the culture from the containers and push it into the well. The bioaugmentation culture volume was calculated based upon a design target of 0.01% of the pore volume in the pilot test area.

As shown in Figure 3, TCE and cDCE have been degraded to below their respective State remediation standards throughout the pilot test area. Transient accumulations of cDCE and VC appeared at peak concentrations that were approximately equivalent to the initial micromolar concentration of TCE. Detectable conversion of VC to ethene began in the latter half of 2002, a few months after bioaugmentation. In 2003, production of ethene has continued to increase; however, VC losses have not been balanced by ethene increases. The cause for this gap is not known, but is likely related, in large part, to dilution of the plume in the pilot test area as a result of the shifting hydraulic gradients at the Site. Molecular assays (PCR and genetic analyses) of groundwater samples collected from the pilot test area after bioaugmentation indicate that the density of *Dehalococcoides* populations in the pilot test area has increased significantly since bioaugmentation.

Caldwell Trucking NPL Site, New Jersey

Golder Associates and GeoSyntec are operating a bioaugmentation/biostimulation system to treat a PCE/TCE DNAPL source area in fractured bedrock groundwater at the Caldwell Trucking

Superfund Site in New Jersey. The system is treating a source area in fractured basaltic bedrock in a test area measuring approximately 120 feet wide, and 40 feet long. The source area was bioaugmented (February 2001) with the KB-1™ culture, and electron donors (methanol, lactate and acetate) are added periodically in a batch mode via multiple injection wells. Groundwater circulation is not a component of this design. Initially, electron donor was added on a weekly basis. After monitoring results showed relatively slow treatment performance, the frequency of donor addition was increased to a daily basis. Example performance monitoring results are provided in Figure 4. As of Fall 2002, results indicated an order of magnitude decline in PCE/TCE concentrations, with an accompanied increase in the concentration of cDCE and VC. There is evidence that cDCE production has peaked and concentrations are starting to decline. TCE concentrations in the well containing the highest TCE concentration (680 mg/L) have declined by 90 percent. Furthermore, the use of molecular probes has demonstrated that the *Dehalococcoides* microorganisms that were injected in the KB-1™ culture have become distributed throughout the test area.

Compared to the other bioaugmentation projects described above, the rate of treatment at Caldwell has been somewhat slower. One primary reason for this observation is that the Caldwell system is treating a DNAPL source area, while the other projects (except for the Boston site) are treating dissolved phase plumes. Other key factors affecting the rate of treatment performance at Caldwell include electron donor delivery design and a presence of chloromethanes and chloroethane co-contaminants. While the Dover AFB, Kelly AFB, Bachman Road, and Aerojet bioaugmentation systems used forced gradient, groundwater circulation to deliver electron donor, the Caldwell system uses batch injection. High concentrations of chloroform (CF) and 1,1,1-trichloroethane at Caldwell also likely compete for electron donor, and slow the relative rate of cDCE and VC conversion to ethene.

Carbon Tetrachloride Site, Schoolcraft, Michigan

Dybas et al. (2002) conducted a full-scale field demonstration of bioaugmentation in an aquifer contaminated with carbon tetrachloride (CT) and nitrate. The demonstration evaluated the performance of bioaugmentation in a biocurtain system designed to intercept and treat the downgradient edge of a CT plume (~ 30 ppb) in a sandy water table aquifer. *Pseudomonas stutzeri* KC was selected for the test because of its known ability to degrade CT without producing chloroform (CF). The requirements for CT transformation by strain KC are (1) adequate concentrations of nitrate and electron donor, (2) anoxic denitrifying conditions, (3) iron-limited conditions, and (4) trace levels of copper. In addition, CT transformation by strain KC is optimal at pH ~8. A pilot study performed at the site previously demonstrated that CT transformation (60 to 65% removal efficiency) could be achieved in situ through inoculation with strain KC, addition of acetate and phosphate, and pH adjustment (Dybas et al. 1998). The pilot study also found that CF generation occurred in regions where strain KC activity was low, and uniform CT transformation was not achieved because of inadequate hydraulic control.

The full-scale system was designed using data and design parameters collected from the pilot test, aquifer characterization, laboratory studies, and solute transport modeling. The full-scale bioaugmentation/biocurtain system was installed in a linear array of 15 adjacent injection/extraction wells aligned perpendicular to the natural groundwater flow gradient. Each well alternatively served as either an injection or extraction well during different operational phases. The full-scale biocurtain was approximately 15 m long. The primary bioremediation additives used were acetate (electron donor), sodium hydroxide (pH adjustment), phosphate (nutrient), and strain KC. An above ground chemical addition system was designed to deliver bioremediation amendments on a weekly basis. System performance was assessed in a series of

monitoring wells installed upgradient and downgradient of the biocurtain. PCR techniques were applied to track the extent of migration of strain KC downgradient of the biocurtain.

The demonstration was performed in seven primary phases: (1) aquifer characterization and system installation (days 1 - 52); (2) tracer testing with bromide and fluorescein to assess solute transport between delivery wells and downgradient monitoring points (days 53 – 72) ; (3) pre-inoculation mixing and adjustment to pH 8.2 (days 73-116); (4) inoculation and feeding (days 117 – 199); (5) re-inoculation and feeding (days 200-313); (6) feeding with reduced acetate concentrations (days 314 to present); and (7) solid-phase evaluation of contaminants and microbes (days 336-342 and 1006-1013). During a typical delivery event, a combined flow rate of 150 L/min groundwater was extracted from alternating delivery wells, circulated through the chemical addition/mixing system, and then injected into adjacent delivery wells. On day 117, the biocurtain was inoculated with 18,900 L of strain KC culture (~10^7 cfu/mL). Thereafter, the delivery system was operated weekly for a 5 hour period to deliver bioremediation additives, followed by a 1 hour reversed flow operation to reduce biofouling at the well screen. On days 200 and 201, half of the delivery well gallery was re-inoculated with 37,000 L of strain KC culture (~10^7 cfu/mL) to increase the cell density downgradient of the biocurtain.

Performance monitoring results are illustrated in Figure 5. Sustained and efficient (98%) removal of CT has been observed in the biocurtain system for over 4 years. Transient levels of CF and H_2S were observed, but both disappeared when the concentration of acetate in the feed was reduced from 100 to 50 mg/L. Denitrification was stimulated by addition of acetate and strain KC, and nitrate levels were reduced to below drinking water standards at both acetate doses. Cell migration after the first inoculation appeared limited, suggesting that much of the strain KC culture might have been attached to sediments close to the point of injection. Nine days after inoculation, strain KC and tracer were detected 1 m downgradient of the delivery well gallery, indicating that some cells had traveled at least as fast as the advective groundwater velocity. The culture was also detected at a few locations 2 m downgradient of the biocurtain. Subsequent monitoring confirmed that the initial inoculation achieved adequate colonization of the biocurtain area. Fifty-three days after the northern half of the biocurtain was re-inoculated, strain KC was detected at all locations sampled along the entire length of the biocurtain network.

The Schoolcraft project represents the longest-sustained successful bioaugmentation application to date. Based on the absence of CF over most of the demonstration, and the apparent colonization and growth of strain KC, it has been concluded that augmentation with strain KC was the principal mechanism for treating CT. However, Dybas et al. (2002) acknowledged that indigenous microorganisms may have also contributed significantly to the degradation of CT. Since no control plot was operated during the demonstration, the influence of the indigenous microflora cannot be known for certain. In any case, the project demonstrated the feasibility of pulsed-pumping operation for achieving effective treatment with low volumes and short durations. Except for the weekly 6 hour period of reagent delivery and groundwater recirculation, the biocurtain was operated as a passive treatment system.

3. SUMMARY

The successful application of bioaugmentation for in situ remediation of chlorinated solvents in groundwater has been demonstrated by multiple research teams at multiple sites. These technology demonstrations have been validated through critical evaluations, expert peer-review, and publication in leading scientific journals. As shown by these demonstrations, bioaugmentation with certain *Dehalococcoides* cultures can achieve in situ dechlorination half lives on the order of hours for chloroethenes, and thereby dramatically shorten the timeframe for

attaining compliance with cleanup criteria. In addition, each of the case studies demonstrated the benefits of applying molecular assays (i.e., 16S rDNA-based PCR) for tracking the growth and transport of bioaugmentation cultures. From these works, it is evident that exogenous cultures can survive, proliferate, and potentially migrate significant distances in the subsurface.

The projects reviewed here all have several recommended design elements in common, including (1) pre-design studies to identify appropriate electron donors and identify the need for bioaugmentation; (2) simple groundwater flow modeling to quantify system hydraulics of treatment areas; (3) tracer testing to calibrate system flow models and confirm connectivity between injection and monitoring points within a treatment area; (4) execution of pilot test to verify feasibility and quantify performance prior to design of a full-scale system; and (5) confirmation of treatment performance through mass balance analysis, geochemical monitoring, and molecular monitoring. It should be recognized that the intensive monitoring programs used in most of these cases studies (i.e., extensive sampling requirements) was only appropriate because these projects were technology demonstrations. The resource requirements for routine pilot- and full-scale bioaugmentation applications are typically less than the examples described here.

REFERENCES

Bradley, P.M. 2000. Microbial Degradation of Chloroethenes in Groundwater Systems. *Hydrogeology Journal*. Vol. 8, p. 104-111.

Carr, C.S., S. Garg, and J.B. Hughes. 2000. Effect of Dechlorinating Bacteria on the Longevity and Composition of PCE-Containing NAPL Sources Under Equilibrium Dissolution Conditions. *Environ. Sci. Technol.,* Vol. 34: 1088-1094.

Chang, P., C. Elder, K. Finneran, D. Major, P. Zeeb, and D. Wanty. 2003. "Geochemical and Microbiological Characterization of in situ Reductive Dechlorination in Fractured Bedrock." *Abstract. Seventh International In Situ and On-Site Bioremediation Symposium.* Orlando, Florida, June 2-5.

Chang, P. R., P.J. Zeeb, C.R. Elder, R.B. Leary, G.A. Demers, D. Major, and D.A. Wanty. 2002. The Evolving Microbiology of an Enhanced Bioremediation Pilot Test Performed in Fractured Bedrock (abstract). Presented at the *Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds Monterey, California*. May 20-23.

Cope, N., and J.B. Hughes. 2001. Biologically-Enhanced Removal of PCE from NAPL Source Zones. *Environ. Sci. Technol.*, (35):2014-2021.

Dybas, M. J., D.W. Hyndman, R. Heine, J. Tiedje, K. Linning, D. Wiggert, T. Voice, X. Zhao, L. Dybas, and C.S. Criddle. 2002. Development, operation, and long-term performance of a full-scale biocurtain utilizing bioaugmentation. *Environ. Sci. Technol.*, Vol. 36(16):3635-3644.

Dybas, M.J., M. Barcelona, S. Bezborodnikov, S. Davies, L. Forney, H. Heuer, O Kawka, T. Mayotte, L. Sepulveda-Torres, K. Smalla, M. Sneathen, J. Tiedje, T. Voice, D.C. Wiggert, M. Witt, and C. Criddle. 1998. Pilot-Scale Evaluation of Bioaugmentation for In-Situ Remediation of a Carbon Tetrachloride-Contaminated Aquifer. *Environ. Sci. Technol.* Vol. 32(22):3598-3611.

Ellis, D.E., E.J. Lutz, J.M. Odom, R.J. Buchanan, C.L. Bartlett, M.D. Lee, M.R. Harkness, and K.A. Deweerd. 2000. Bioaugmentation for Accelerated In Situ Anaerobic Bioremediation. *Environ. Sci. Technol.*, *34*(11), 2254-2260.

Fennell, D., A. Carroll, J. Gossett, and S. Zinder. 2001. Assessment of indigenous reductive dechlorination potential at a TCE-contaminated site using microcosms, polymerase chain reaction analysis, and site data. *Enivron. Sci. Technol.*, Vol. 35(9):1830-1839.

Harkness, M.R., A.A. Bracco, M.J. Brennan, K.A. DeWeerd, and J.L. Spivack. 1999. Use of Bioaugmentation to Stimulate Complete Reductive Dechlorination of Trichloroethene in Dover Soil Columns. *Environ. Sci. Technol.,* 33(7):1100-1109.

Hendrickson, E.R., J.A. Payne, R.M. Young, M.G. Starr, M.P. Perry, S. Fahnestock. D.E. Ellis, and Ebersole, R.C. 2002a. Molecular Analysis of *Dehalococcoides* 16S Ribosomal DNA from Chloroethene-Contaminated Sites throughout North America and Europe. *Appl. Environ. Microbiol.*, February, pp. 485-495.

Hendrickson, E.R., M.G. Starr, M.A. Elberson, J.A. Payne, E. Mack, H.-B. Huang, M. McMaster, and D.E. Ellis. 2001. "Using a Molecular Monitoring Approach to Monitor a Bioaugmentation Plot." In: Leeson, A., B.C. Alleman, P.J. Alvarez, and V.S. Magar (Eds.) *Bioaugmentation, Biobarriers, and Biogeochemistry*. Battelle Press, Columbus, OH. pp. 43-51.

Hopkins, G.D., and P.L. McCarty. 1995. "Field Evaluation of In Situ Aerobic Cometabolism of Trichloroethylene and Three Dichloroethylene Isomers Using Phenol and Toluene as Primary Substrates." *Environ. Sci. Technol.*, 29(6):1628-1637.

Lee, M.D., R.J. Buchanan, D.E. Ellis. 2000. Laboratory Studies Using Edible Oils to Support Reductive Dechlorination. *Proceedings of the Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California*. p. 77-84.

Lendvay, J.M., F.E. Loffler, M.E. Dollhopf, B. Fathepure, M. Gebhard, R. Heine, R. Hickey, C.L. Major, Jr., E. Petrovskis, J. Shi, J.M. Tiedje, P. Adriaens. 2003 (*in press*). Bioreactive Barriers: A Comparison of Bioaugmentation and Biostimulation for Chlorinated Solvent Remediation. Paper submitted to *Environ. Sci. Technol.*

Löffler, F., Q. Sun, J. Li, and J. Tiedje. 2000. 16S rRNA gene-based detection of tetrachloroethene-dechlorinating desulfuromonas and dehalococcoides species. *Appl. Environ. Microbiol.*, Vol.66(4):1369-1374.

Major, D.W., M.L. McMaster, E.E. Cox, E.A. Edwards, S.M. Dworatzek, E.R. Hendrickson, M.G. Starr, J.A. Payne, and L.W. Buonamici. 2002. Field Demonstration of Successful Bioaugmentation to Achieve Dechlorination of Tetrachloroethene to Ethene. *Environmental Sci. Technol.* Vol. 36:5106-5116.

Maymo-Gatell, X., Gossett, J.M., and Zinder, S.H. 1997. *Dehalococcus Ethenogenes* Strain 195: Ethene Production from Halogenated Aliphatics (abstract). *In Situ and On-Site Bioremediation,* Vol. 3. p. 23. Alleman, B.C. and A. Leeson (Eds.). Battelle Press, Columbus, OH.

McCarty, P.L., M.N. Goltz, G.D. Hopkins, M.E. Dolan, J.P. Allan, B.T. Kawakami, and T.J. Carrothers. 1998. Full-Scale Evaluation of In Situ Cometabolic Degradation of Trichloroethylene in Groundwater Through Toluene Injection. *Environ. Sci. Technol.* Vol. 32(1):88-100.

Steffan, R.J., K.L. Sperry, M.T. Walsh, S. Vainberg, and C.W. Condee. 1999. "Field-Scale Evaluation of In Situ Bioaugmentation of Chlorinated Solvents in Groundwater." *Environ. Sci. Technol.* 33(16):2771-2781.

Yang, Y., and McCarty, P.L. 2000b. Biologically enhanced dissolution of tetrachloroethene DNAPL. *Environ. Sci. Technol.*, Vol. *34*(14), 2979-2984.

Table 1. Bioaugmentation Field Demonstrations.

Site Name, Location	VOC	Contaminant Concentration	Geology	Culture	Culture Volume Used (L)	System Scale	Reference
Dover Air Force Base, Dover, DE	TCE	5 mg/L	Silty sands	Pinellas	350	Pilot	Ellis et al. 2000
Kelly AFB, San Antonio, TX	PCE	2 mg/L	Shallow silty gravel	KB-1	13	Pilot	Major et al. 2002
Bachman Road, Lake Huron, MI	PCE		Fine to medium grained sand	Bachman Road	200	Pilot	Lendvay et al. 2003
Aerojet, Sacramento, CA	TCE	2 mg/L	Deep alluvium	KB-1	50	Pilot + Full	Cox et al. 2000; 2002
Caldwell Trucking NPL, NJ	TCE	200 mg/L	Fractured Basalt	KB-1		Full	Finn et al. 2003
Industrial Site, MA	TCE	80 mg/L	Fractured Bedrock	KB-1	40	Pilot	Chang et al. 2002; 2003
Schoolcraft, MI	CT	30 μg/L	Shallow sand	Strain KC	56,000	Pilot + Full	Dybas et al. 1998; 2002
Dover AFB National Test Site, DE	PCE	DNAPL	Shallow sand	KB-1		Pilot	McMaster et al. 2003
NASA LC-34, FL	TCE	DNAPL	Shallow sand	KB-1		Pilot	McMaster et al. 2003

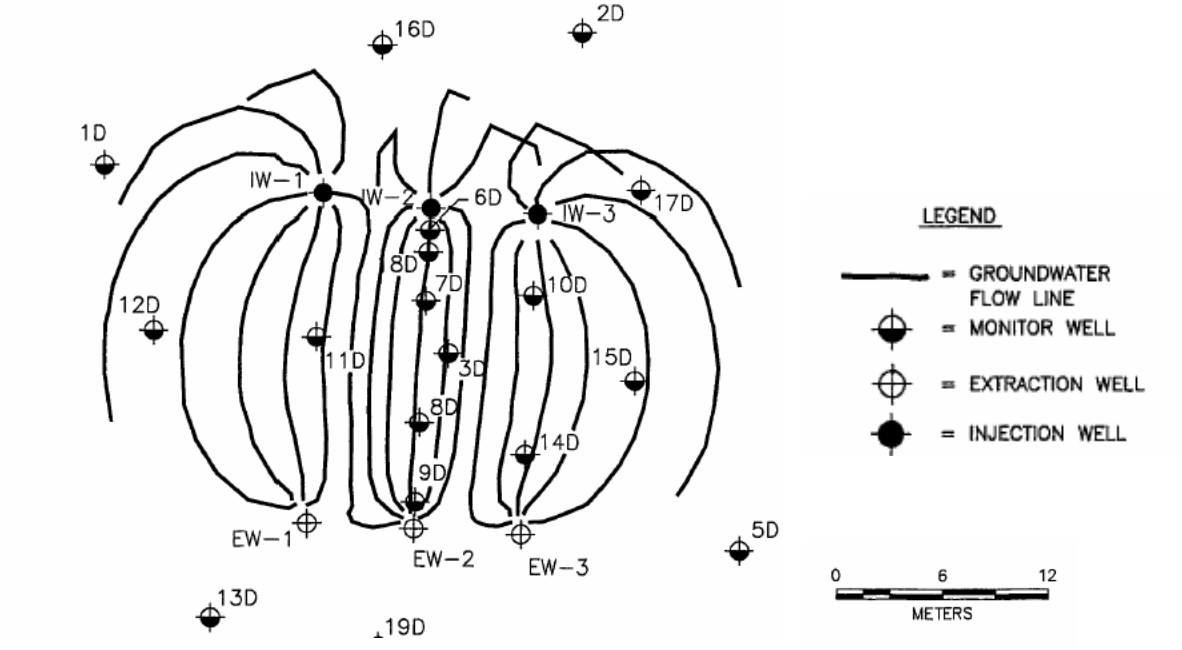

Figure 1. Pilot test well layout and inferred groundwater flow lines for Dover AFB bioaugmentation demonstration (from Ellis et al. 2000). (EW = extraction well; IW = injection well).

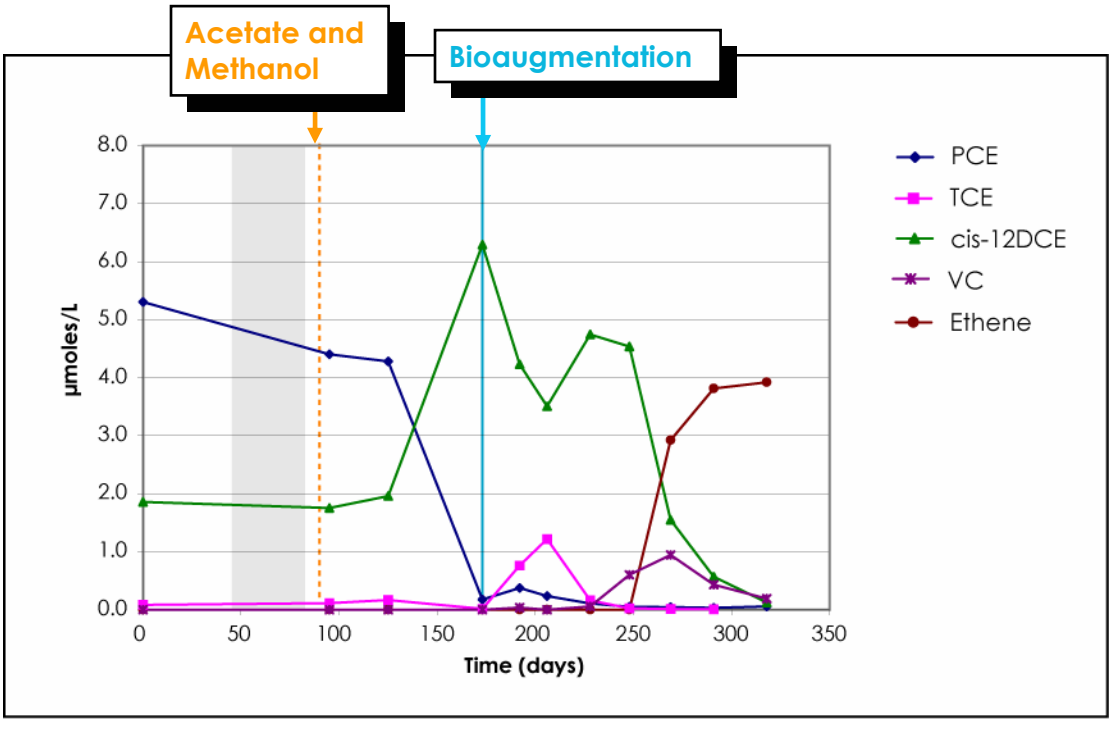

Figure 2. Performance monitoring results for bioaugmentation test plot at Kelly AFB (Major et al. 2002)

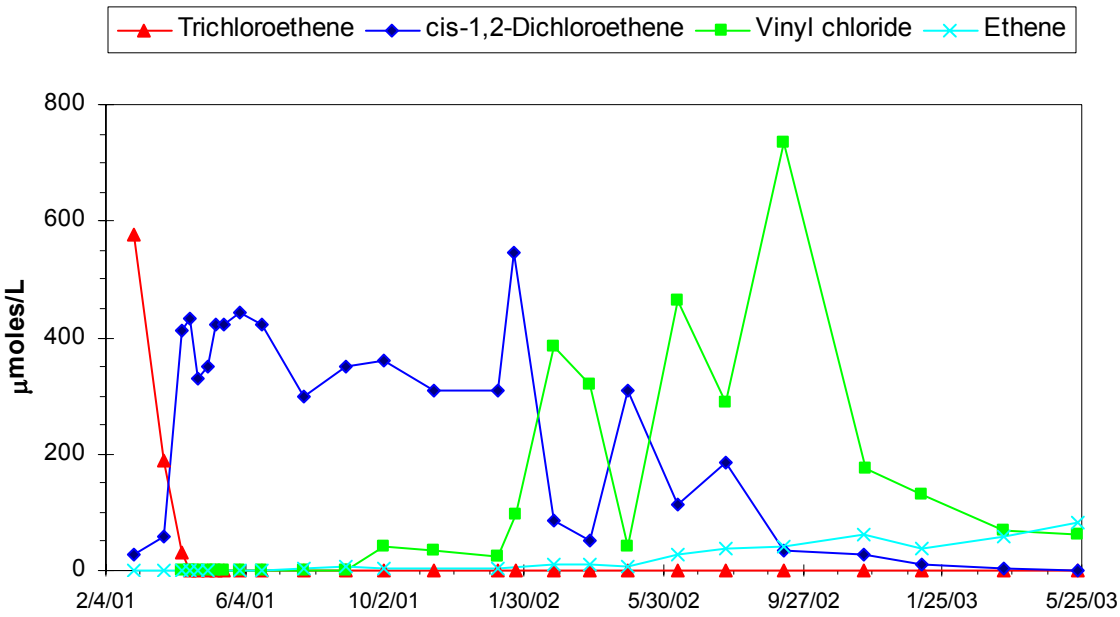

Figure 3. Performance monitoring results (MW-1004B) for bioaugmentation demonstration in fractured bedrock, Boston, Massachusetts.

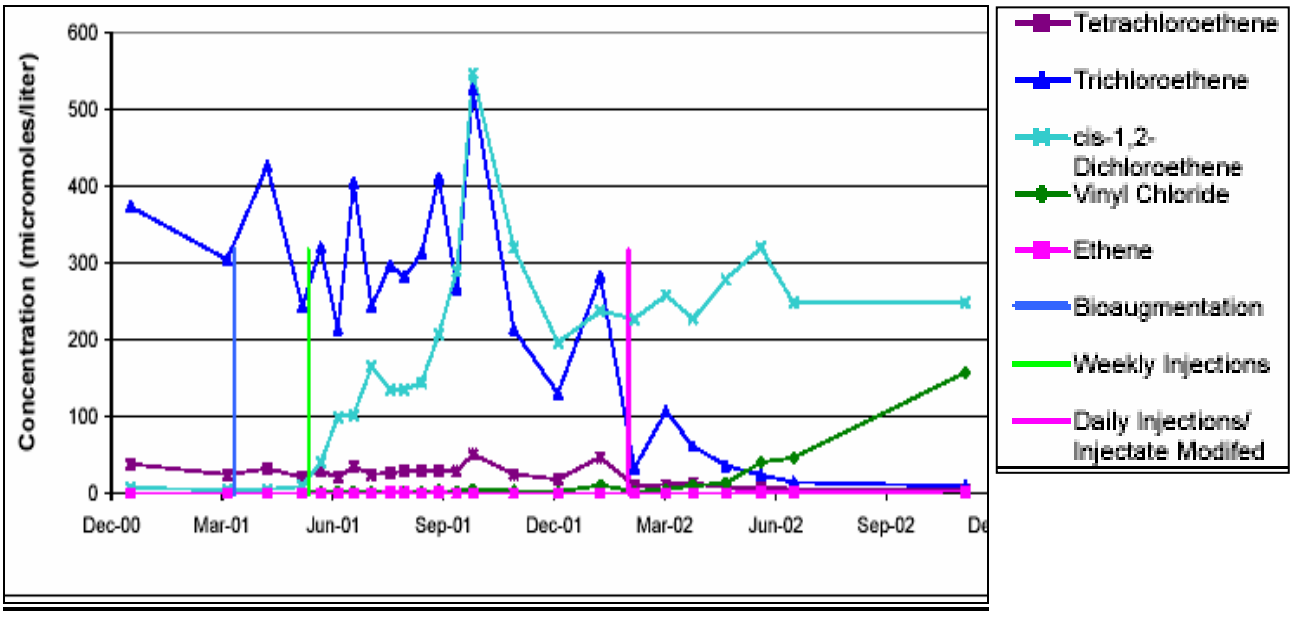

Figure 4. Example performance monitoring results (MW-C14) for bioremediation system at Caldwell Trucking NPL site.

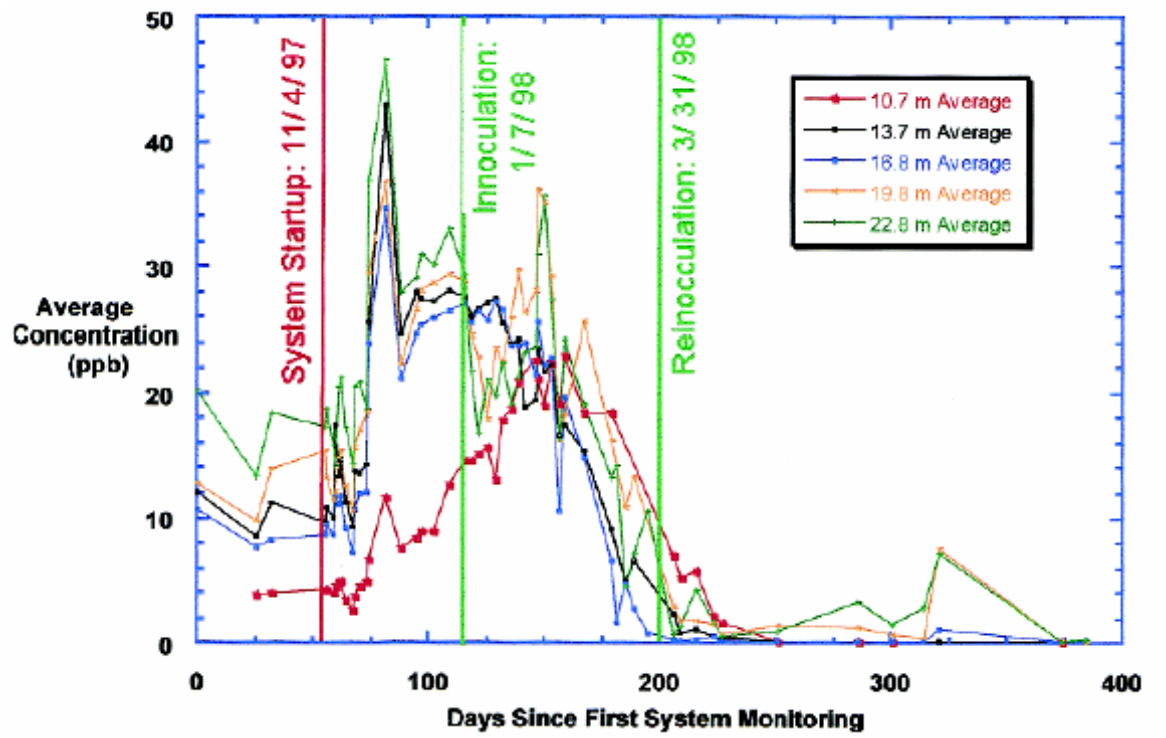

Figure 5. Average CT concentrations for multi-level monitoring wells 9, 10, and 11, located 1 m downgradient of the biocurtain at the Schoolcraft Site (from Dybas et al. 2002).