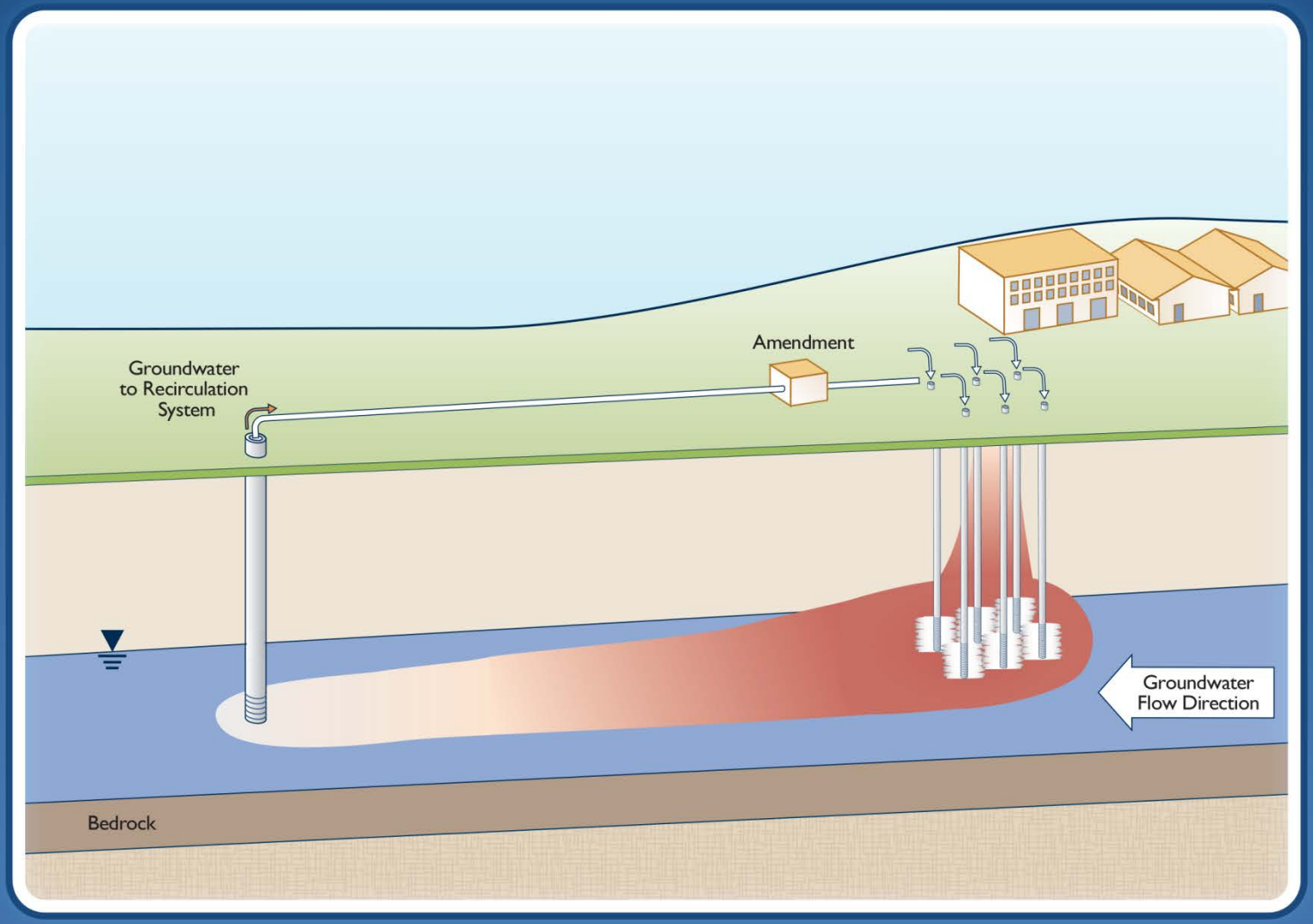
INTRODUCTION TO IN SITU BIOREMEDIATION OF GROUNDWATER



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EXECUTIVE SUMMARY

Bioremediation is an engineered technology that modifies environmental conditions (physical, chemical, biochemical, or microbiological) to encourage microorganisms to destroy or detoxify organic and inorganic contaminants in the environment. The process can be applied above ground in land farms, tanks, biopiles, or other treatment systems (referred to as ex situ) or below ground in the soil or groundwater, referred to as in situ. In situ bioremediation of groundwater has become one of the most widely used technologies for contaminated site treatment because of its relatively low cost, adaptability to site-specific conditions, and efficacy when properly implemented (Stroo 2010).

Introduction to In Situ Bioremediation of Groundwater was prepared by the Office of Superfund Remediation and Technology Innovation (OSRTI) as an introduction to in situ bioremediation of groundwater. This information is intended for U.S. Environmental Protection Agency (EPA) and state agency site managers and may serve as a reference to designers and practitioners. Others may find the EPA's Citizen's Guide to Bioremediation (EPA 2012a) to be a more fundamental and concise reference.

In situ bioremediation (ISB) of groundwater involves the encouragement of indigenous bacterial populations to metabolize target contaminants through the addition of various amendments (biostimulation) to the subsurface environment. In addition to amendments, select strains of bacteria may be added to the subsurface to help treat some sites (bioaugmentation). Bacteria perform coupled oxidation/reduction (redox) reactions to live, and bioremediation exploits these reactions to remove contaminants from contaminated media (soil, air, or groundwater). Bacteria can use different electron acceptors (oxidized compounds) and donors (reduced compounds) in the three major oxidation pathways — aerobic respiration, anaerobic respiration, and fermentation. ISB can use all of these pathways, and contaminant degradation may occur through direct metabolism, cometabolism, or abiotic transformations that may result from biological activities.

Aerobic bioremediation most commonly takes place in the presence of oxygen and relies on the direct microbial metabolic oxidation of a contaminant. The primary concern when an aerobic bioremediation system is designed is delivery of oxygen, which is the electron acceptor. Aerobic bioremediation is most effective in treating non-halogenated organic compounds. Many reduced contaminants can be aerobically degraded by aerobic bacteria already present in the subsurface environment. Oxygen can be added directly to the subsurface, or chemical oxidants can be applied, which release oxygen as they dissolve or decompose. Oxygen and oxygen-releasing compounds can be delivered to the groundwater via several methods, depending on their physical properties, site hydrogeology, and the desired delivery efficiency. The end products of aerobic respiration are usually carbon dioxide and water.

Anaerobic oxidative bioremediation takes place in the absence of oxygen. It relies on other electron acceptors such as nitrate or sulfate for direct microbial metabolic oxidation of a contaminant. This approach is often applied at petroleum-contaminated sites where oxygen has already been depleted. Amendments with soluble sulfate and electron donor are often added to the affected area to stimulate sulfate-reducing conditions to help microbes metabolize the petroleum compounds. A byproduct of this approach is hydrogen sulfide. The hydrogen sulfide can react with the iron at sites where metals such as iron occur naturally to produce iron sulfide or pyrite and reduce the amount of hydrogen sulfide.

Anaerobic reductive bioremediation takes place in the absence of oxygen. It relies on the presence of biologically available organic carbon, which may be naturally present or added to stimulate activity. The organic carbon, also commonly called an organic substrate or an electron donor source, creates and sustains anaerobic conditions by consuming oxygen and other electron acceptors during its biodegradation. It also promotes the bioreduction of oxidized contaminants such as chlorinated solvents (EPA 2001b) by generating hydrogen through fermentation reactions. Because these contaminants exist in an oxidized state, they are generally much less susceptible to aerobic oxidation processes, but they can be reduced by microbes under anaerobic conditions, a process also referred to as enhanced reductive dechlorination (ERD) when applied to chlorinated solvents. In many cases, microorganisms use the oxidized contaminants in a respiratory mechanism and are able to derive metabolically useful energy (EPA 2000, AFCEE 2004). Anaerobic conditions may be used to degrade highly chlorinated contaminants, such as tetrachloroethene (PCE) and trichloroethene (TCE) to ethene, 1,1,1-trichloroethane (1,1,1-TCA) to ethane, carbon tetrachloride (CT) to methane, or perchlorate to chloride and oxygen. Microbially induced reduction of hexavalent chromium to trivalent chromium may be the most common application of bioremediation to metals.

Cometabolism occurs when microorganisms using one compound as an energy source fortuitously produce an enzyme that chemically transforms another compound. Organisms thus can degrade a contaminant without gaining any energy from the reaction. Cometabolic degradation is a process that often happens concurrently in bioremediation systems designed for direct metabolism of contaminants; however, some systems have been designed to specifically take advantage of cometabolic processes. Hazen (2009) indicates that cometabolic bioremediation can occur in environments where contaminant concentrations are well below concentrations that could provide a carbon or energy benefit to the biodegrader. Therefore, this method may be effective at degrading very low concentrations of some contaminants.

Adequate site characterization is critical to designing a successful ISB remedy. The nature and extent of the environmental impacts need to be known, as well as several key characteristics of the affected media. Development of a conceptual site model (CSM) helps guide the characterization and subsequent design, implementation, and operation of the remedy. Application of ISB is highly dependent on site characteristics, such as the aquifer type, baseline geochemistry, and lithology. Bioremediation can change site geochemistry by altering the pH or redox status and, as a result, produce secondary contaminants. Production of secondary contaminants is fairly well understood and is often addressed as part of the design.

The design process commonly includes bench- and pilot-scale treatability studies. These tests may be performed during the feasibility study or remedial design, are used to evaluate whether the proposed bioremediation remedy will be successful, and obtain important design criteria. In general, full-scale implementation is based on the site CSM, remedial objectives, regulatory requirements, and future site use or development. The three primary approaches to full-scale implementation for ISB are classified as active, semi-passive, and passive treatment, distinguished by the need for active groundwater recirculation during operations (Stroo and Ward 2009). Once designed and installed, a bioremediation system requires careful monitoring and possible modifications to optimize performance.

Implementation costs related to almost any technology increase with greater depth and greater treatment volume. Reapplication of amendments, including electron acceptors or donors, will be required at most sites. Some sites may require geochemical adjustment and nutrient amendment. The success of biological technologies is highly dependent on the delivery and longevity of the amendments added to the site and requires a comprehensive performance monitoring program.

This document also highlights several recent trends affecting ISB. These trends include the increasing emphasis on green or sustainable remediation, the use of stable isotopes as diagnostic tools, high-resolution site characterization (HRSC), and three-dimensional visualization and analysis (3DVA) of site data. ISB often results in a smaller on-site environmental footprint than ex situ or non-biological methods because of its relatively low energy use and the minimal equipment and site disruption required to implement it.

Stable isotope analysis can be used to demonstrate that biodegradation is occurring, to discriminate between biological and nonbiological processes and to estimate the rate and extent of contaminant degradation. Stable isotope probing, where compounds enriched in a stable isotope are added to the subsurface, is being used to measure the fraction of degradation directly caused by microbial activity during bioremediation.

Several molecular biological tools (MBTs) are becoming more widely available and cost effective for applications in support of site characterization, remediation, monitoring, and closure, which include but are not limited to microassays, Fluorescence In Situ Hybridization (FISH), and quantitative Polymerase Chain Reaction (qPCR) (ITRC 2013). MBTs can be used to determine if the necessary bacteria with the right genes are present at the site and if they exist at optimal levels.

All remedial technologies, including ISB, require accurate site characterization techniques, such as HRSC. HRSC has become more prominent as sampling and analytical techniques, data evaluation, and visualization methods have improved. HRSC strategies and techniques use scale-appropriate measurement and sample density to define contaminant distributions, and the physical context in which they reside, with greater certainty, supporting faster and more effective site cleanup (CLU-IN 2013). Lithologic, hydrogeologic and contaminant data are often provided by real-time direct sensing and hydraulic profiling technologies such as Laser Induced Fluorescence (LIF), Membrane Interface Probes (MIP), and electrical conductivity (EC) probes. Several software programs are now available to perform 3DVA of site characterization and performance monitoring data. These programs are useful for

designing amendment delivery systems and determining which portions of a plume may require additional amendments. The programs typically use geostatistical kriging procedures to establish the spatial distribution of each parameter in three-dimensional space.

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Table of Contents

Table of Contents, (continued)

Figures

Tables

Appendix

ACRONYMS AND ABBREVIATIONS

3DVA	three-dimensional visualization and analysis
AFCEE	Air Force Center for Engineering and the Environment
AST	Aboveground Storage Tank
BTEX	Benzene, toluene, ethyl benzene, xylene
CAH	Chlorinated aliphatic hydrocarbons
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CLU-IN	Cleanup Information
COC	Contaminant of concern
CSIA	Compound specific isotope analysis
CSM	Conceptual site model
CT	Carbon tetrachloride
CVOC	Chlorinated volatile organic compound
DCA	Dichloroethane: 1,1- and 1,2- isomers
DNA	Deoxyribonucleic acid
DCE	Dichloroethene: cis-1,2-; trans-1,2-; and 1,1- isomers
DNAPL	Dense non-aqueous phase liquid
DPRB	Dissimilatory perchlorate-reducing bacteria
EPA	U.S. Environmental Protection Agency
ERD	Enhanced reductive dechlorination
ESD	Explanation of Significant Differences
ESTCP	Environmental Security Technology Certification Program
EVO	Emulsified vegetable oil
FISH	Fluorescence In Situ Hybridization
gpm	Gallons per minute
HRSC	High-resolution site characterization
ISB	In situ bioremediation
ITRC	Interstate Technology and Regulatory Council
LIF	Laser Induced Fluorescence
LNAPL	Light non-aqueous phase liquid
MBT	Molecular biological tools
mg/L	Milligrams per liter
MIP	Membrane Interface Probe
MTBE	Methyl tert-butyl ether
mV	Millivolts
MVS	Mine Visualization System
NAPL	Non-aqueous phase liquid
NDMA	*N*-Nitrosodimethylamine
NPL	National Priorities List
NRC	Nuclear Regulatory Commission

O&M	Operation & Maintenance
ORP	Oxidation-reduction potential
OSTRTI	Office of Superfund Remediation and Technology Innovation
OSWER	Office of Solid Waste and Emergency Response
PAH	Polycyclic aromatic hydrocarbons
PCB	Polychlorinated biphenyls
PCE	Perchloroethene or tetrachloroethene
PCP	Pentachlorophenol
ppm	Parts per million
PRB	Permeable reactive barriers
psi	Pounds per square inch
qPCR	Quantitative Polymerase Chain Reaction
RDX	Royal Demolition Explosive
Redox	Oxidation/reduction
ROD	Record of Decision
RPM	Remedial project managers
SIP	Stable isotope probing
SVOC	Semi volatile organic compound
TCA	1,1,1-Trichloroethane
TCE	Trichloroethene
TNT	Trinitrotoluene
UST	Underground Storage Tank
VC	Vinyl chloride
vcrA	Vinyl chloride reductase
VOC	Volatile organic compound

1.0 INTRODUCTION

Introduction to In Situ Bioremediation of Groundwater was prepared by the Office of Superfund Remediation and Technology Innovation (OSRTI) as an introduction to in situ bioremediation (ISB) of groundwater. This information is intended for U.S. Environmental Protection Agency (EPA) and state agency site managers and may serve as a reference to designers and practitioners. Others may find the EPA's Citizen's Guide to Bioremediation (EPA 2012a) to be a more fundamental and concise reference.

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This document focuses specifically on in situ groundwater bioremediation. In the context of this document, groundwater remediation is defined as remediation of contaminants that exist below the water table. As a result of phase equilibrium in the subsurface, groundwater remediation must address contaminants dissolved in groundwater as well as those sorbed to the aquifer matrix to be effective. In some cases, even treatment of non-aqueous phase liquid (NAPL) may be needed. Consideration must also be given to the capillary fringe and the smear zone, which can serve as an ongoing source of contaminants to groundwater. This report does not discuss phytoremediation (use of plants to treat groundwater and soil) or monitored natural attenuation (a technology based on monitoring the progress of natural, non-engineered processes that often include biodegradation). Those readers interested in more information on ex situ bioremediation, bioremediation of soil, monitored natural attenuation or phytoremediation may find useful information on EPA's CLU-IN website (www.cluin.org).

The document provides technical information on evaluating and implementing in situ groundwater bioremediation at contaminated sites. Superfund program guidance for selecting and implementing groundwater remedies at Superfund sites can be found in numerous program guidance documents, such as:

- Office of Solid Waste and Emergency Response (OSWER) 9283.1-33, *Summary of Key Existing Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Policies for Groundwater Restoration*, June 2009; and
- OSWER 9283.1-34, *Groundwater Road Map: Recommended Process for Restoring Contaminated Groundwater at Superfund Sites*, July 2011.

For other groundwater guidance, refer to the Superfund groundwater website (www.epa.gov/superfund/health/conmedia/gwdocs).

This document provides (1) a brief technical background on ISB, (2) a summary of the use of ISB for various contaminants, including information on its use for Superfund sites, (3) considerations for implementation of ISB, (4) brief summaries of some important emerging trends affecting ISB, and (5) links to additional sources of information.

1.1 Superfund Project Information

The list of in situ groundwater bioremediation projects accompanying this report in the appendix was derived primarily from the lists in *Treatment Technologies for Site Cleanup: Annual Status Report, Twelfth Edition*, and its successor *Superfund Remedy Report, Thirteenth Edition*, and represents a subset of in situ groundwater bioremediation projects at National Priorities List (NPL) sites. Projects on the list include remedial actions for in situ groundwater bioremediation selected in Superfund Records of Decision (RODs), ROD amendments, and Explanations of Significant Differences (ESDs) for fiscal years 1989 through 2008. These documents are referred as "decision documents." Although decision documents select a general technology such as ISB, the final selection of a specific design is typically deferred to the remedial design phase. Detailed information regarding the remedial design and contaminants treated was compiled for each project on the list based on documents available either on line (for instance, http://cumulis.epa.gov/supercpad/cursites/srchsites.cfm) or in site files. These sources included 5-year reviews, Superfund site summary fact sheets, remedial action reports, and other pertinent documents. Remedial project managers (RPMs) and contractors were contacted for additional clarification as needed. Status information for most projects was last updated in November 2011. More information on project implementation status, design, and performance may be available on the websites related to each site found at the link given above.

1.2 History and Background

Bioremediation is not a new concept. Biological treatment of domestic wastewater has been in use since the mid-1800s, and land treatment has been used for several decades to treat oil and other petroleum wastes by aerobic biodegradation (Loehr 1979). The basic principles and experience from these technologies were adapted to ISB of petroleum (and other contaminants) in the 1980s (Thomas and Ward 1989). ISB has been further developed to treat a wide variety of other contaminants, particularly since the early 1990s, when the potential for enhanced anaerobic treatment became clear (NRC 1993; Alexander 1994).

The first use of ISB was in 1972, when aerobic treatment was used to clean up a Sun Oil pipeline spill in Ambler, Pennsylvania. Treatment consisted of withdrawing groundwater, adding oxygen and nutrients, and recirculating it through the subsurface (Raymond 1977). Aerobic biological treatment or oxidation of petroleum releases gained acceptance throughout the 1970s and 1980s and has been used in several large-scale applications, including the effort to clean up numerous Superfund sites (see for example EPA 1989; Ross 1988).

Anaerobic bioremediation gained popularity when it was recognized as an effective method to remediate chlorinated solvents in groundwater. In 1997 scientists isolated a bacterium originally referred to as *Dehalococcoides ethenogenes* strain 195, the first organism known to completely dechlorinate the common groundwater contaminant perchloroethene (PCE, also known as tetrachloroethene) (Maymo-Gatell 1997). Further studies showed that several related bacteria, all now referred to as strains of *Dehalococcoides mccartyi* (Löffler and others 2012), had the ability to partially or completely dechlorinate PCE and the related chloroethenes. To date, these are the only known organisms with the ability to completely degrade these compounds, which are particularly prevalent groundwater contaminants at Superfund sites. As a result, several demonstration-scale applications of

anaerobic bioremediation were completed in the late 1990s and early 2000s. Several of the demonstration projects went full scale, and today reductive dechlorination, as it is now known, is a widely accepted method for treating halogenated ethenes, ethanes, and methanes (Stroo 2010).

Figure 1 shows the surge in popularity of anaerobic bioremediation for use at NPL sites after the method was successfully demonstrated in the early 2000s, while the use of aerobic bioremediation has remained relatively steady. As indicated in Figures 1 and 2, the selection of anaerobic bioremediation to remediate groundwater at Superfund sites increased dramatically over recent years, and this method is now used at the majority of Superfund sites where ISB technologies have been selected.

As shown in Figure 3, the most common groundwater contaminants addressed by ISB at NPL sites were halogenated volatile organic compounds (VOCs); followed by nonhalogenated VOCs; nonhalogenated semi-volatile organic compounds (SVOCs); and BTEX compounds (benzene, toluene, ethyl benzene and xylenes).

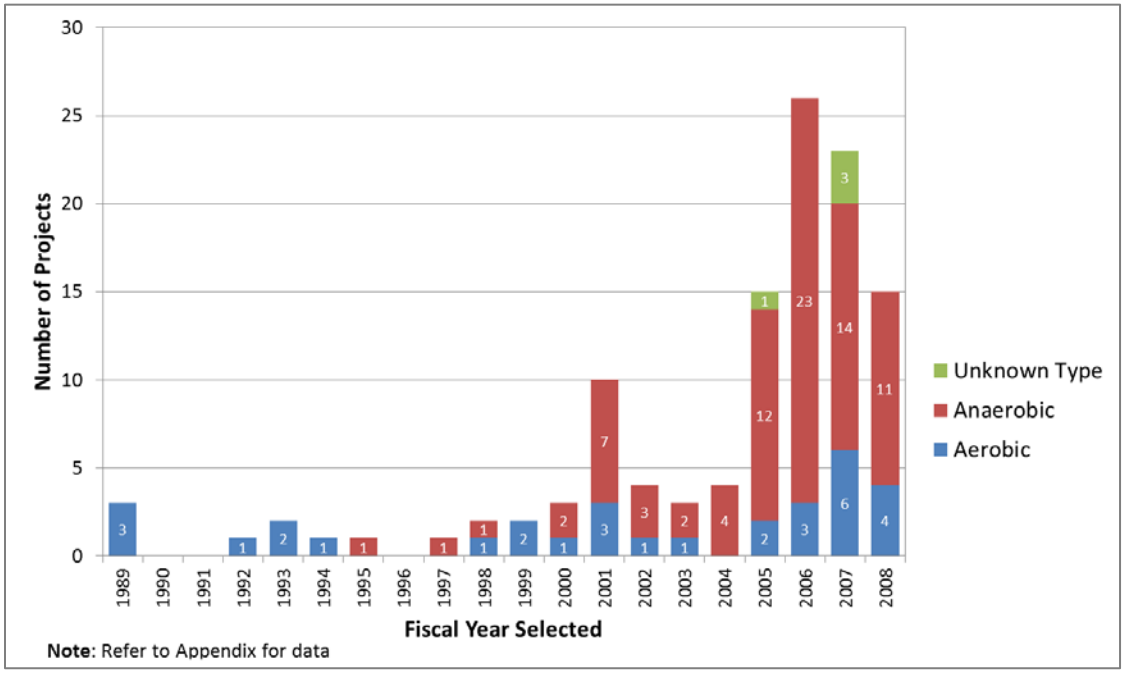

Figure 1. Use of In Situ Groundwater Bioremediation Technologies at Superfund Sites.

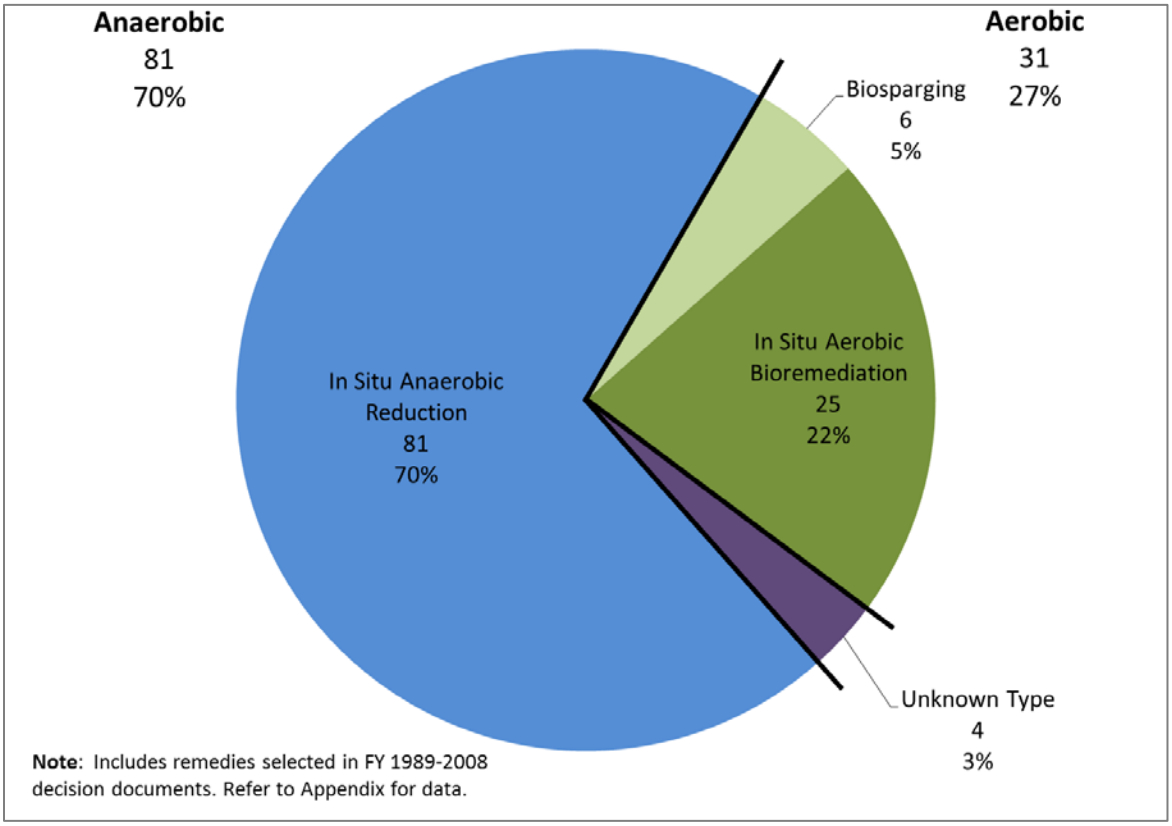

Figure 2. Aerobic and Anaerobic Bioremediation Projects at NPL Sites.

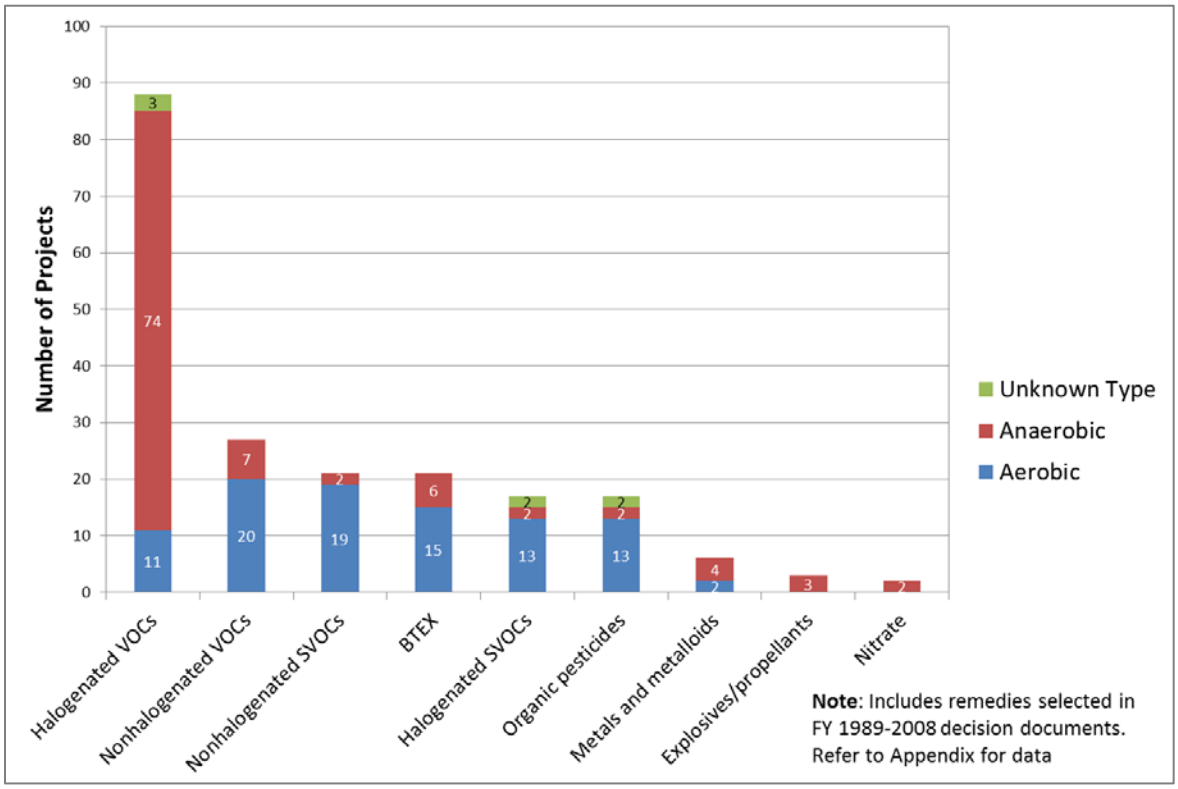

Figure 3. Contaminant Groups Addressed by Bioremediation Technologies at Superfund Sites.

1.3 Microbiology

One component of designing an effective ISB system is to understand the fundamental ecology and physiology of microbes. Microbes have been found everywhere on earth, including environments of extreme heat, cold, and pH, without oxygen, and in the presence of radiation. They are adaptive, resilient, and can thrive in environments impaired by most contaminants. Bioremediation most commonly uses bacteria for treatment, but also includes remediation performed by archaea, protists, and fungi. Microbes used for bioremediation are often referred to collectively as "bacteria," or "bugs" in the bioremediation field.

All microbes have basic requirements for life and growth. The six elements considered essential for life are carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur. The needs of bacteria can be further simplified to three requirements: a carbon source that can be used to build its biomass, an electron donor (such as hydrogen) for the energy it needs to live and reproduce, and a terminal electron acceptor (for example, oxygen) to receive the electrons the bacteria use for energy. Often, the carbon source will serve as the electron donor. Nitrogen, phosphorus, and sulfur will sometimes fulfill the role of electron donor or acceptor, but are more often considered nutrients and are required in smaller proportions than are carbon, hydrogen, and oxygen.

The specific growth rate of bacteria depends on the concentration of a carbon source (substrate) or the nutrient that is most limiting. These growth kinetics are modeled by the Monod equation (Okpokwasili and Nweke 2005), which shows how, at low substrate concentrations, the specific growth rate increases directly with an increase in substrate concentration, while it levels out to approach a constant maximum growth rate when substrate is plentiful. The specific growth rate of bacteria is particularly relevant in bioremediation because bacterial growth rates (and proportionally, breakdown of the contaminant) will likely slow as cleanup progresses, if the contaminant is the substrate.

1.4 Reduction and Oxidation Chemistry and Microbial Metabolism

Bacteria generate the energy they need to live by catalyzing (increasing, initiating, or transforming) chemical reactions that transfer electrons from one molecule, known as the electron donor or reductant, to another molecule, called the electron acceptor or oxidant. When the right electron donor and acceptor are present, bacteria will consume them to grow and divide. The amount of energy generated and available for bacterial growth by each reduction and oxidation or redox pair varies, and each species of bacteria has enzymes to take advantage of only certain redox pairs. The contaminants of concern may act as reductants or oxidants for in situ groundwater remediation.

The various terminal electron acceptors that exist naturally in groundwater are preferentially used and exhausted in a specific order, according to their decreasing redox potential. In the environment, organic matter in the aquifer matrix and groundwater plays the role of electron donor. The vast majority of microbial metabolisms relevant to bioremediation use organic matter as an electron donor, and the bacteria able to generate the most energy from it tend to dominate the microbial population. The amount of energy released during electron transfer is controlled by the redox potential of the terminal electron acceptor. There are a few important groups of bacteria that use inorganic reduced compounds as a substrate. These microbes oxidize many of the same species reduced by anaerobic respiration and

fermentation. Hydrogenotrophic methanogens are examples of bacteria that derive energy from degrading inorganic compounds, since they oxidize hydrogen to water. Nitrifiers include aerobic bacteria and Archaea that oxidize ammonia to nitrate, a process called nitrification that is extremely important to nitrogen cycling. Sulfur-oxidizing bacteria oxidize sulfide to sulfur or sulfate, and iron-oxidizing bacteria convert iron (II) to iron (III).

1.4.1 Aerobic Respiration

Aerobic bacteria use oxygen to oxidize organic molecules by removing electrons and converting the organic molecules to carbon dioxide and water. Because of the high redox potential of oxygen, bacteria able to use oxygen as a terminal electron acceptor will dominate wherever oxygen is present. Above ground, aerobic environments are ubiquitous because they are in contact with the atmosphere, but oxygen below ground surface can quickly be depleted by any aerobic microbial activity in groundwater.

1.4.2 Anaerobic Respiration and Fermentation

When oxygen is not present, bacteria commonly use nitrate, iron (III), manganese (IV), sulfate, carbonate, or other available electron acceptors to oxidize organic matter, producing carbon dioxide and other byproducts (Figure 4). Microbes exist that can use the contaminants for respiration for almost all oxidized contaminants. Bacteria have been identified that use chemicals such as halogenated organic compounds (such as PCE and trichloroethene [TCE]), selenium, arsenic, chromium (VI), technetium (VII), and uranium (VI) as electron acceptors (Palmisano and Hazen 2003). This section discusses the electron acceptors most commonly used by bacteria and most prevalent in the environment.

Nitrate is the first choice for electron acceptor after oxygen is depleted, and many aerobic bacteria possess the enzymes to use nitrate to oxidize contaminants. Reduction of nitrate generates a sequence of byproducts consisting of nitrite ions and the gases nitric oxide, nitrous oxide, and finally nitrogen. Use of nitrate as electron acceptor is termed “denitrification” because it consumes nitrate.

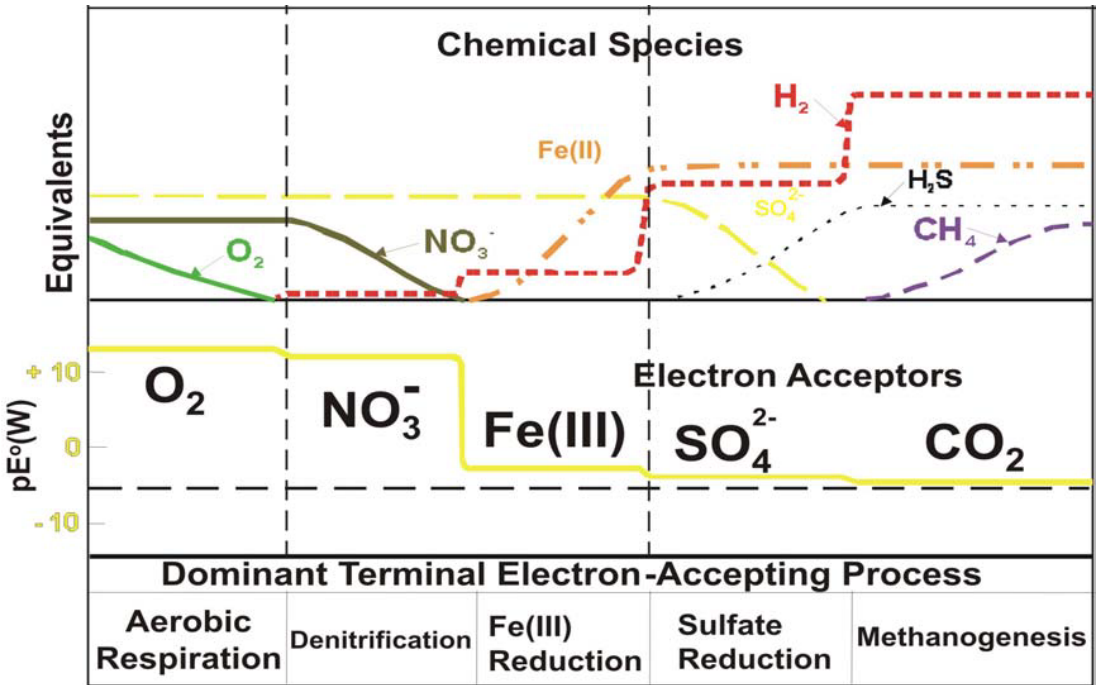

Figure 4. Dominant Terminal Electron-Accepting Process, Electron Acceptors, and Typical Chemical Species Responses (Modified from AFCEE 2004, Bouwer and McCarty 1984)

Manganese and iron are often available for microbial use in the soil or groundwater. Iron-reducing bacteria use iron (III) as an electron acceptor, reducing it to iron (II), or they can use manganese (IV), reducing it to manganese (II). Once iron and manganese have been reduced, sulfate serves as an electron acceptor and is converted by sulfur-reducing bacteria to sulfide, sulfite, or elemental sulfur.

When all external terminal electron acceptors have been exhausted, bacteria can use organic molecules as both electron acceptors and donors in a metabolic pathway called fermentation. Fermentation generates the least amount of energy because only a small fraction of the organic matter available can be readily oxidized by microorganisms and because of the low redox potential of the reactions. Fermentation can be divided into two categories: primary fermentation and secondary fermentation (AFCEE 2004). Primary is the fermentation of substrates and amino acids to various volatile fatty acids, alcohols, carbon dioxide, and hydrogen, while secondary is the fermentation of primary fermentation products.

1.4.3 Direct Metabolism

Most bioremediation systems use a direct metabolic pathway, in which the contaminant of concern is either an electron donor or acceptor, and the remedial system provides the presence of a complementary oxidant or reductant and the right bacteria to take advantage of them. The growth rate of bacteria depends on the concentration of substrate, which is the contaminant. As contaminants are treated by a remedial system, contaminant concentrations may approach the minimum required for bacterial growth, whether it be an acceptor or donor, and cause treatment to slow or stop.

1.4.4 Cometabolism

Cometabolism is a term used to describe biological degradation from which bacteria do not derive any energy. Bacteria secrete metabolic enzymes that break down complex organic matter around them for easier digestion. These enzymes are often nonspecific and can operate on many different substrate molecules, including those that the bacteria itself cannot use for energy. Enzymes such as methane monooxygenase and ammonia monooxygenase are examples of enzymes that can oxidize a wide array of substrates (Hazen 2009). Cometabolic treatment potentially can address even trace levels of the contaminant, as long as the substrate the bacteria require for growth is maintained at acceptable concentrations, because the bacteria do not rely on the contaminant for energy.

Cometabolism was once promoted as a method to treat TCE, but has rarely been used because the intermediate epoxide produced inhibits biological activity. The TCE oxidation byproducts such as TCE epoxide may result in the inactivation of the oxygenase activity caused by damage to the enzymes (Ely, Hyman, and others, 1995). Inhibition and inactivation may be overcome by additional natural substrates (Alvarez-Cohen and McCarty, 1991; Ely and others, 1997). Cometabolism may prove valuable for treating other problematic contaminants such as *N*-Nitrosodimethylamine (NDMA) and 1,4-dioxane (Hatzinger and others 2008; Steffan 2007; Mahendra and Alvarez-Cohen 2006; Fournier and others 2009).

1.4.5 Abiotic Transformation

In some cases, the conditions created to encourage biological breakdown of contaminants will also be conducive to abiotic chemical transformation of the contaminants, which occurs without the help of organisms (Cwiertny and Scherer 2010). Added oxygen will oxidize many compounds without biological catalysis, and hydrolysis of organic contaminants can happen spontaneously. Sulfide produced from anaerobic sulfate reduction will precipitate some dissolved metal contaminants, such as lead, cadmium, zinc, and copper (Lee 2003). Zero-valent iron can be added to support anaerobic bioremediation by producing hydrogen as it oxidizes and to abiotically reduce contaminants.

The biological degradation pathway of 1,1,1-trichloroethane (TCA) generally stalls at chloroethane (ATSDR 2006). Abiotic processes can play a key role in degradation of TCA to non-toxic end products. TCA can be abiotically degraded to 1,1-dichloroethene (1,1-DCE) via a process called abiotic dehydrochlorination (EPA 2009, ATSDR 2006). Figure 5 shows potential biotic and abiotic degradation pathways for common chlorinated VOCs (CVOCs). At sites where TCA and TCE are co-contaminants, TCA can inhibit *Dehalococcoides* from degrading TCE (Duhamel and others 2002). Abiotic dehydrochlorination not only eliminates the inhibitory compound, but also creates a product that can be degraded using the same bacteria and pathway as TCE. Alternatively, commercially available cultures containing *Dehalococcoides* and *Dehalobacter* are capable of biologically degrading mixed plumes of TCA and TCE.

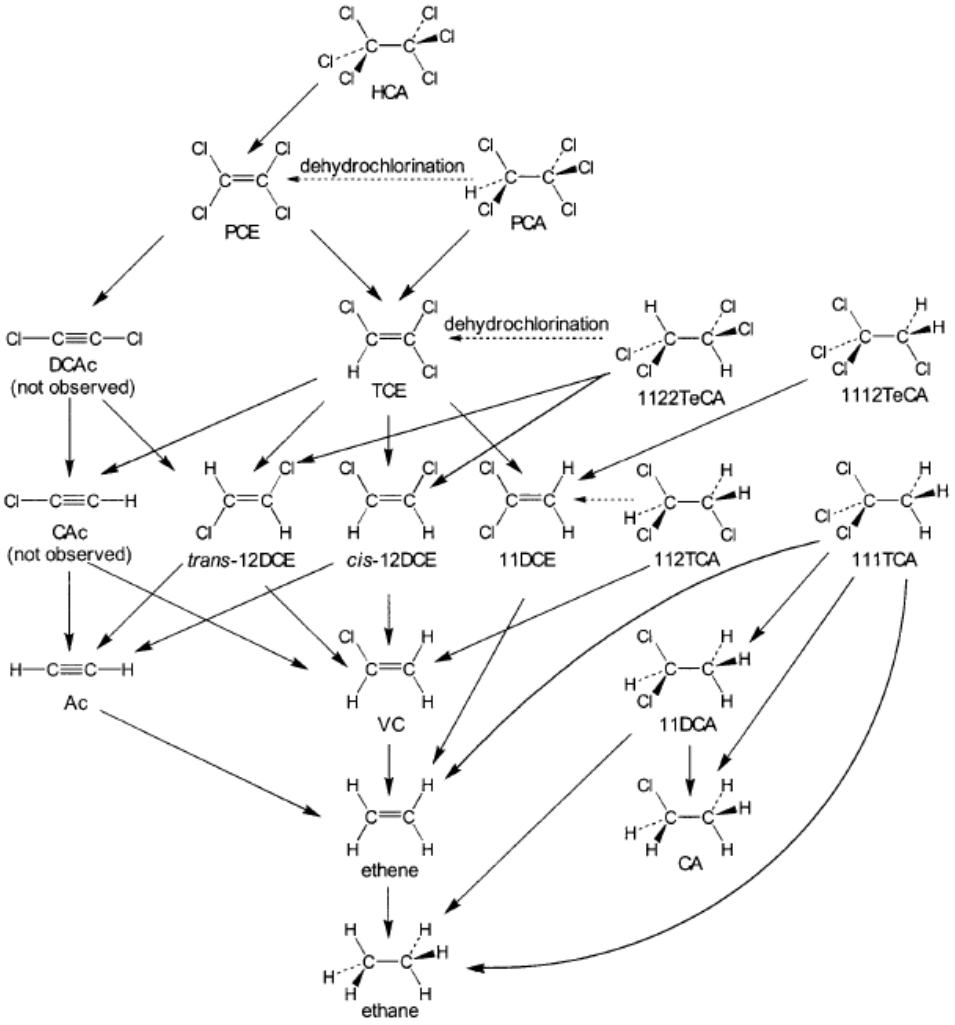

Figure 5. Example Biotic and Abiotic Degradation Pathways of Common CVOCs (EPA 2009, O'Loughlin and Burris 2004)

Magnetite, biogenic iron-sulfide, and other naturally occurring minerals can also contribute to abiotic transformation of chlorinated compounds (EPA 2009).

1.5 Conceptual Site Model

Adequate site characterization is critical to designing a successful remedy. The nature and extent of the environmental impacts and the characteristics and interaction of the affected media need to be known. Development of a conceptual site model (CSM) helps guide the characterization and subsequent design, implementation, and performance of the remedy. The CSM will evolve through the life cycle of a project as additional information is developed and generally includes a visual representation of the site (EPA 2011). At first, the CSM will consist of rough sketches that ideally evolve into a more comprehensive representation of the available data, potentially using three-dimensional visualization and analysis (3DVA), as discussed in Section 4.4. Figure 6 provides an example of a simplified pictorial CSM of an exposure pathway analysis. The CSM is refined throughout the characterization and remediation process at a site (EPA 2011).

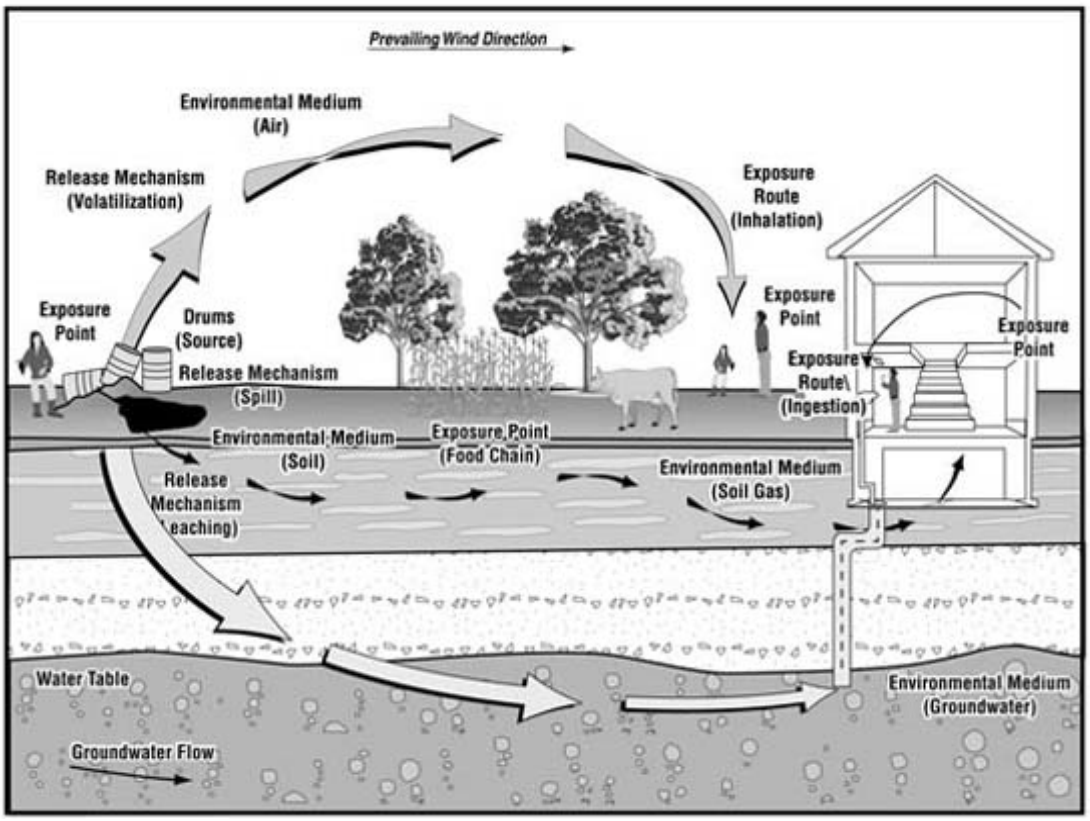

Figure 6. Conceptual Site Model - Exposure Pathway Schematic (ATSDR 2005)

Application of bioremediation is highly dependent on site characteristics, such as the aquifer type and lithology. In particular, the magnitude and distribution of hydraulic conductivity affect the ability to deliver amendments to the subsurface, where they are needed to maintain optimal conditions for the targeted biological processes. Baseline characterization of the microbiology is essential to evaluate whether the right microorganisms are present, if those microorganisms can be stimulated, and to ascertain that no undesirable reactions will occur with the stimulants or daughter products. If bioaugmentation is required or desired, the target treatment area must be properly conditioned to support microbial growth. The following five sections summarize the key components of a CSM, with emphasis on site characterization needed to assess and implement bioremediation.

1.5.1 Land Use and Risk

Important CSM components include past, current, and intended future land use and ecological value. These factors will help to guide the site investigation, evaluate potential human health and ecological risks, establish protective cleanup criteria, and evaluate acceptable control measures. For example, an ISB system for a chlorinated solvent plume typically creates a reduced subsurface environment where methanogenesis occurs naturally. In a residential neighborhood, the design would need to consider vapor intrusion from contaminant vapors and potential methane gas as a critical design factor. Conversely, indoor air quality may not be as important of a design factor in an industrial area dominated by large manufacturing buildings with high air exchange rates.

1.5.2 Geologic Setting

Understanding the geological setting and its heterogeneity is critical to developing a useful CSM. Geological settings have unique characteristics that, if recognized early in the investigation, can help with placement of soil borings and selection of analytical parameters. For example, some geological settings are known for low baseline pH conditions that may adversely affect biological remedies. In other geological settings, bedrock may have a significant influence on the direction of plume migration through fractures, faults, and changes in porosity.

1.5.3 Hydraulic Properties of Contaminated Media

Proper characterization of hydraulic properties of the contaminated media may be one of the most important components of a CSM. The following sections highlight some key hydraulic properties.

1.5.3.1 Hydraulic Conductivity

Hydraulic conductivity (K) is a measure of the ability of an aquifer matrix to transmit groundwater and is important for designing delivery systems. It can be estimated in the field by slug tests, aquifer pumping tests, and vertical hydraulic profiling. Hydraulic conductivities can differ by orders of magnitude within an aquifer that may not be reflected in a single measurement of K. Slug tests are relatively affordable and can be completed at several wells to help understand the distribution of K across a site, but can be difficult to evaluate if significant differences are present between the gravel pack and aquifer materials. Conversely, aquifer pumping tests tend to average K values as a result of their greater radius of influence and may provide less detail regarding a specific location or specific depth interval. Vertical hydraulic profiling provides high-resolution K data but cannot provide specific information regarding the connectivity between higher conductivity zones in various profiles as an aquifer pumping test could. In addition, vertical profiling provides an index of relative K at very small scale, versus measured K at larger scale, which is very useful for understanding site heterogeneity. Often, a combination of aquifer test methods is required to design an ISB system.

1.5.3.2 Porosity and Effective Porosity

Porosity (total porosity) is a measure of the void space in an aquifer. Specifically, total porosity is the volume of the void space divided by the volume of aquifer matrix. Total porosity in bedrock aquifers is described as primary and secondary. Primary porosity is the percentage of the voids in the rock at the time of formation, and secondary porosity refers to the void space from fractures and dissolution (Fetter 2000). Primary and secondary porosity are important for estimating how much contaminant mass may be present and can be useful for estimating amendment quantities. However, effective porosity is often more important because it is a measure of the connected aquifer void space within the aquifer. Effective porosity is lower than total porosity in most geological settings. When effective porosity is low, amendment delivery systems may have difficulty treating the target area because of the poor connections between the aquifer void space and fractures. A value for effective porosity is useful to determine the following:

- The radius of influence of an injection well,
- The total number of injection wells, and
- Whether multiple screened intervals are required for the injection wells.

Effective porosity can be estimated in the field by measuring groundwater flow velocity with a tracer test (USGS 1999).

1.5.3.3 Groundwater Flow Direction and Velocity

Direction of groundwater flow is a key factor driving contaminant transport. Groundwater flows from high to low hydraulic head. The hydraulic heads are often represented on a potentiometric surface map (typically referred to as a water level map). Groundwater flow velocity, or seepage velocity, is a measure of the groundwater flow rate through the aquifer pore space. Groundwater flow velocity is an important parameter for selecting injection well placement and amendment quantities and type. For example, sites with high groundwater flow velocities may require amendment to be added more frequently than sites with low groundwater flow velocities.

High groundwater flow velocities can be incorporated into the amendment delivery design. For example, injection wells can be used upgradient of inaccessible areas (such as under buildings) to deliver amendments, allowing the natural and additional induced flow resulting from injection to transport the amendments to the target treatment areas.

Preferential pathways exist in many geologic settings because of the heterogeneous distribution of more transmissive zones caused by coarser-grained sediments or highly fractured bedrock. Groundwater flow velocity through these zones will be higher than the average groundwater flow at the site. In addition, groundwater flow velocities though preferential pathways can be exaggerated during the application of amendments by the injection pressures. The increased groundwater flow velocities can cause amendments to travel beyond the target application area or reach the ground surface (daylight).

1.5.3.4 Aquifer Matrix Diffusion Potential

Sedimentary aquifers commonly consist of heterogeneous layers or zones of different permeability and transmissivity. Groundwater flows preferentially through more permeable zones as compared with the less permeable zones. Bioremediation is more effective in the more permeable zones because liquid and gas amendments infiltrate much more quickly through high permeability zones (Sale and others 2008).

Contamination often exists in the subsurface for many years before it is detected and remediated. This delay allows the dissolved contaminants the time needed to diffuse from more permeable into less permeable zones within the aquifer system. When remediation begins, the high permeability zones are remediated more quickly and the concentration gradient between high and low permeability zones is reversed. As a result, contaminants in the less permeable matrix will now diffuse back into the more permeable matrix, causing contaminant levels in the more porous matrix to rebound after initial treatment. The general matrix diffusion mechanism is shown in Figure 7. The less permeable matrix of the aquifer becomes a new source area for contamination. Matrix back diffusion may persist for many years after initial treatment.

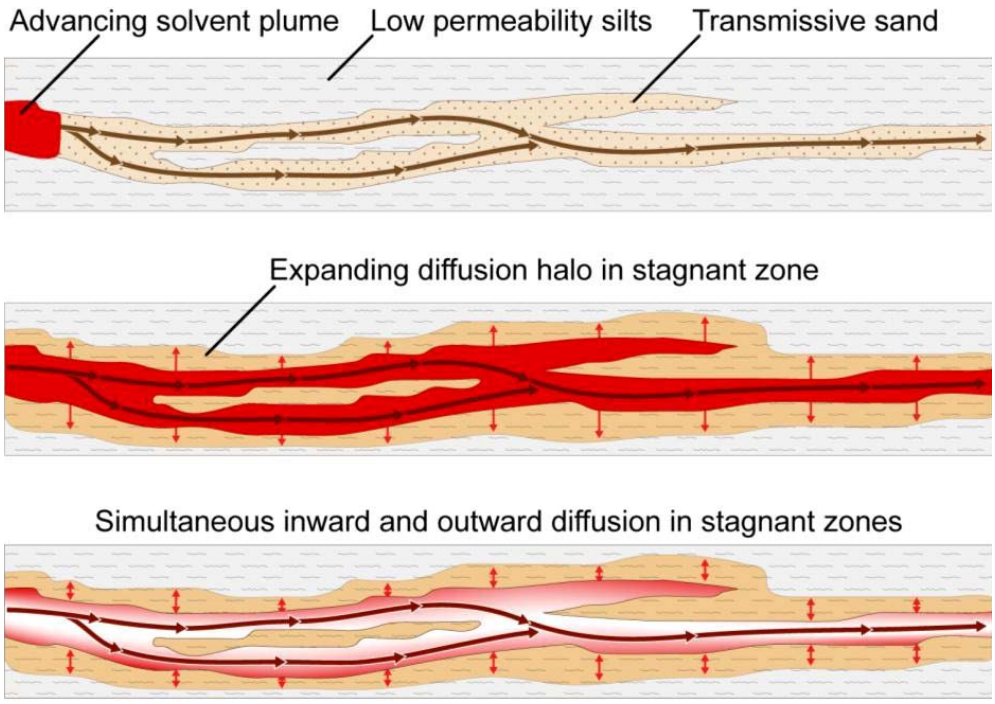

Figure 7. Conceptual Matrix Diffusion Mechanism (Adapted from NRC 2004)

Attainment of cleanup levels can be hindered by the slow release of contaminant mass from matrix back-diffusion. Matrix back-diffusion can be significant where low-permeability zones are present within the unconsolidated aquifer or where a dual porosity system exists as a function of adjacent lithologic units having several orders of magnitude differences in K. Matrix back-diffusion can also play a significant role in bedrock aquifers that exhibit sufficient primary porosity. For example, numerical model simulations have demonstrated that back-diffusion from the matrix pore space (primary porosity) to fractures (secondary porosity) will likely be the time-limiting factor in reaching groundwater cleanup goals in some fractured bedrock environments (Lipson 2005). Matrix back-diffusion is observed much more in sedimentary rocks than in igneous and metamorphic rocks.

Additional applications of amendments may be required to maintain a biologically active zone that will ultimately reduce impacts to below the remedial action objectives. In some cases, several pore volumes of treated groundwater may have to pass through the aquifer before objectives are met. Remediation of aquifers where matrix back-diffusion is a factor may take longer and be more costly. Research is under way to develop ways to estimate the rate of diffusion from the matrix into the groundwater.

1.5.4 Biogeochemistry

The geochemistry of a contaminated aquifer will control whether the necessary bacteria will grow in the subsurface environment and what amendments are needed to help sustain the desired biological processes. The following sections discuss some of the key geochemical parameters required to build a CSM.

1.5.4.1 Contaminant Types

Each contaminant type (for example, hydrocarbons, chlorinated aromatics, and aliphatics) will have an effect on the site geochemistry. For example, excessive organic loading of an aquifer from landfill leachate or a fuel release will result in biological activity that will readily consume oxygen and drive a system to reducing conditions. The resulting reducing environment, in turn, may cause metals to become mobile and create secondary groundwater impacts. Therefore, it is important to delineate the contaminant plume and understand the effects of the contaminants on site geochemistry.

1.5.4.2 pH/Aquifer Buffering Capacity

The pH level in the subsurface is a significant factor for biological activity. The common range of pH for most natural groundwater is between 5 and 8.5 (Kasenow 2010). Optimal ranges for dehalogenating bacteria vary slightly in the literature, from 6 to 8 (AFCEE 2004) and 6.8 to 7.8 (Robinson and others 2009, Middeldorp and others 1999, Cope and Hughes 2001), for example. Sites that are well within the generally recognized optimal pH ranges would pass the initial stages of the screening process for potential biological treatment. However, biological treatment at sites on the margins or just outside the optimal pH ranges should not be rejected until further site biological screening, such as bench testing, is completed (AFCEE 2004). For example, local microbial populations at a site could have adapted to a low pH environment and be able to sustain complete degradation of contaminants, or a low-pH tolerant culture may be commercially available. In addition, pH buffering may be possible and cost effective.

Considerations of pH and buffering capacity for bioremediation are generally less about changing the natural pH conditions — which can be a difficult endeavor for sustained and extended periods — and are much more about establishing or maintaining the optimum microbial conditions after addition of amendments and increased biological activity. Potential limiting pH conditions are common in anaerobic bioremediation as a result of the generation of hydrogen through fermentation reactions and the formation of organic acids that can exceed the buffering capacity of the aquifer. It is important to measure the buffering capacity of the aquifer before a carbon source is added.

There are two laboratory approaches to measure buffering capacity. If an aquifer matrix is rich in limestone and a high natural pH buffering capacity is anticipated, a laboratory acid titration test can be completed on site soil and groundwater samples to determine the level of acid equivalents that will reduce pH to levels outside optimal limits. This approach is referred to alkalinity testing. Alkalinity testing results can be compared with stoichiometric calculations of the amount of electrons anticipated to be liberated (acid to be produced) during the dechlorination process, given the site contaminant and geochemical concentrations and the estimated donor quantities. If calculations indicate that more acid may be produced than the aquifer has the capacity to buffer, a practitioner should consider the addition of a buffering agent. Sodium bicarbonate is a typical pH buffering compound used, but it is a relatively weak buffer and may be most appropriate for bioremediation applications where soluble substrates are injected frequently. Stronger and more persistent buffering compounds such as magnesium hydroxide or sodium phosphates may be used for bioremediation applications where slow-release substrates are used (Henry 2010). The alkalinity testing will also provide an order of magnitude estimate of the amount of buffering agent that will be needed to overcome aquifer acidity and maintain a near-neutral pH.

Acidity testing is appropriate if a site is anticipated to have a low buffering capacity, or is in active remediation with low pH conditions. Acidity testing consists of adding an alkali (such as sodium bicarbonate or sodium hydroxide) to site soil and groundwater in a laboratory setting to determine the equivalents of base needed to overcome aquifer acidity and maintain a near-neutral pH. Alkalinity and acidity tests also provide insight on how the potential buffering requirements of the aquifer may affect the feasibility of the bioremediation being planned.

The pH can also have other effects — besides the direct effect pH has on microorganisms — that could negate the efficacy of bioremediation. For example, a decrease in pH can solubilize toxic metals that were previously insoluble and create secondary environmental impacts. If an aquifer is known or suspected to contain metals that may solubilize if pH is lowered, the practitioner may choose to add a buffering agent to prevent pH from decreasing to a range that may solubilize metals, besides considerations for microorganisms.

1.5.4.3 ORP

Oxidation-reduction potential (ORP) or redox potential describes the tendency of an aqueous solution to either accept or donate electrons when a new species is introduced. Solutions with higher ORP are more likely to oxidize new species, and solutions with lower ORP are more likely to reduce them. Figure 8 provides a summary of ORPs and the associated electron accepting process. A positive ORP is needed for

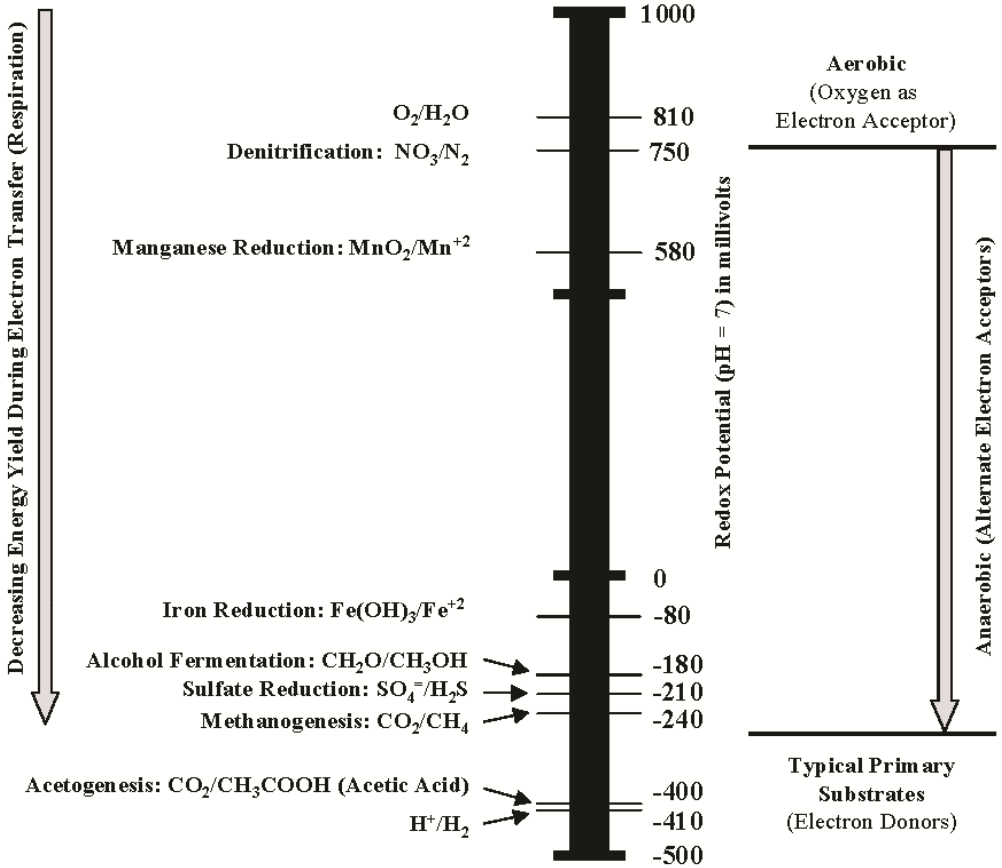

Figure 8. Estimated ORP of Commonly Monitored Species (ITRC 2005)

aerobic oxidation of hydrocarbons and chlorinated solvents, while reductive dechlorination requires a negative ORP, preferably below -200 millivolts (mV). Nitrate reducing conditions occur from 250 to 100 mV; reducing conditions for trivalent iron occur from 100 to 0 mV; reducing conditions for manganese and sulfate occur from 0 to -200 mV; and methanogenesis occurs below -200 mV.

ORP can be a difficult parameter to measure accurately in the field. Down-hole probes or low-flow pumps with flow-through cells are typically the most accurate methods of measuring ORP.

Although additives (substrates) can lower the ORP of a site to allow reductive dechlorination to occur, the more oxidizing the natural conditions, the more substrate is needed, which could lead to other side effects such as low pH and biological fouling (biofouling). Biofouling is attributed to the increase in microbial populations and, perhaps more importantly, to the creation by cells of extracellular polysaccharides. These slimy polysaccharides are important for the accumulation of microorganisms on surfaces or within porous media and can contribute significantly to biofouling of a formation or injection well. A portion of amendment goes to the creation of new bacteria (biomass). Eventually, continued unchecked bacterial growth is likely to reduce circulation and injection of the amendment and may lead to a plugged formation or injection well (ITRC 2002). Biofouling of injection or recirculation wells has been observed at several sites because of the growth of biomass or biofilms with 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 (AFCEE 2004).

1.5.4.4 Temperature

Each species of bacteria has an optimal range of temperature for growth. Growth rates increase with temperature to an optimum near the top of the range and then quickly drop off as temperature increases further. Bacteria are divided into groups based on their preferred temperature ranges: psychrophiles are bacteria that grow best in temperatures below 20˚C, mesophiles thrive between 25 and 35˚C, and thermophiles prefer temperatures between 45 and 65˚C.

Groundwater temperature varies geographically and seasonally and increases with depth. Figure 9 shows the average groundwater temperature across the continental United States. Shallow groundwater can also be locally affected by precipitation as well as subsurface features such as process equipment, utility lines, sewers, and other anthropogenic features. The biodegradation rate will slow as the temperature drops, and many bacteria become inactive at temperatures less than 4˚C. Therefore, bioremediation in northern climates will be slower and may require additional design considerations. For example, the optimal temperature for complete reductive dechlorination of PCE to ethene is between 10 and 30˚C. Below 10˚C, the degradation half-lives of PCE and each of its daughter products are substantially longer than at optimal temperatures (Dennis 2011). In colder environments, simple soluble substrates (sugars or alcohol) may be more effective than more complex non-soluble substrates (vegetable oil) because they are less viscous at lower temperatures and are easier to metabolize. Circulation and subsequent heating of groundwater in a closed circuit could help maintain biodegradation rates as well.

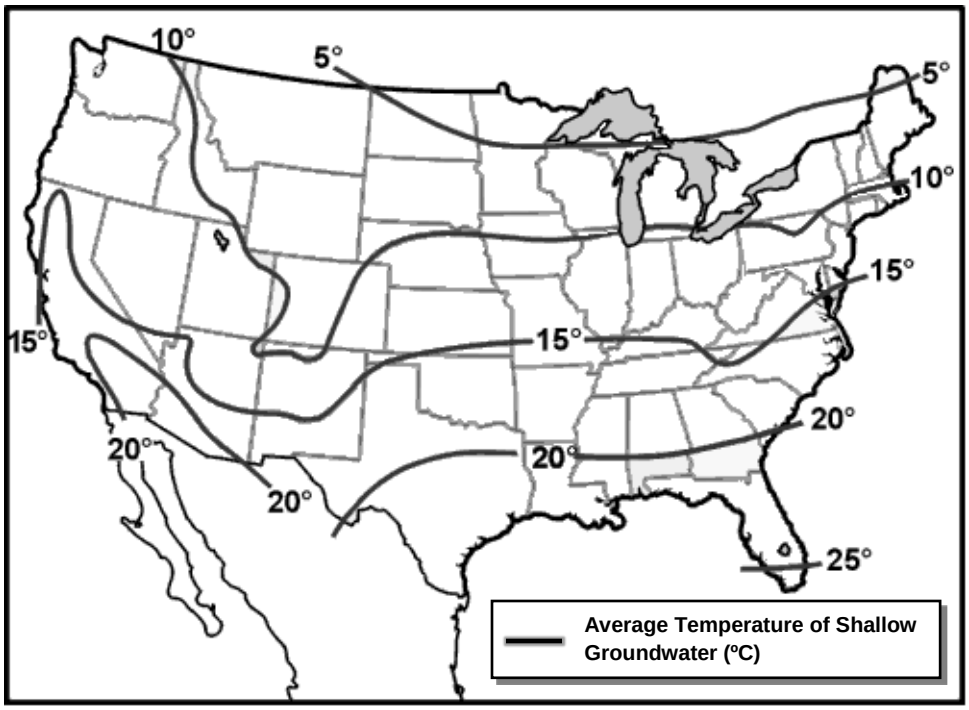

Figure 9. Average Temperature of Shallow Groundwater across the continental U.S. (EPA 2013, Ecosystems Research, Athens, GA)

1.5.4.5 Terminal Electron Acceptor Concentrations

Terminal electron acceptors are the typically native compounds used by organisms for respiration via an electron transfer chain. Aerobes use oxygen as the terminal electron acceptor in the chain, and anaerobes use various terminal electron acceptors. It is important to know the baseline concentrations at the site of potential terminal electron acceptors such as dissolved oxygen, nitrate, manganese, iron, sulfate, and carbon dioxide. These concentrations will describe the current redox state of the groundwater and what quantity of amendments, if any, is necessary to eliminate the native electron acceptors. Native electron acceptors compete with anaerobic dechlorination and must be reduced to a relatively narrow range in the terminal electron accepting process for dechlorination to occur (AFCEE 2004).

If oxidative bioremediation is the targeted process, the presence of potential terminal electron acceptors may mean less electron acceptor needs to be added to achieve complete biodegradation. If reductive bioremediation is the selected remedial technology, competing electron acceptors need to be used by bacteria and become depleted, causing bacteria to sequentially use the next available electron acceptors in the process. Higher concentrations of native electron acceptors would generally indicate that more electron donor will be required than is stoichiometrically demanded to degrade the contaminant itself.

1.5.4.6 Nutrients or Growth Inhibitors

Nutrients are needed to sustain the growth of a bacterial population and include major nutrients nitrogen, phosphorous, potassium, and minor nutrients sulfur, magnesium, calcium, manganese, iron, zinc, copper, and trace elements. Although microbial activity could decrease if nutrients are not available in sufficient amounts, nutrient deficiencies are typically not the growth-limiting factor when poor performance is

observed. In fact, many practitioners do not add any nutrients beyond those that are naturally occurring. Baseline characterization of an aquifer can assist in identifying potential nutrient needs, if any. If necessary, nitrogen and phosphorous are usually added to the bioremediation system in a useable form (such as ammonium for nitrogen and phosphate for phosphorous). However, nutrients can cause soil plugging as a result of their reaction with minerals, such as iron and calcium, to form stable precipitates that fill the pores in the soil and aquifer. Nutrients are required in larger proportions for aerobic systems, compared with anaerobic systems, because of the higher growth rates for aerobic bacteria.

Some nutrients are competing electron acceptors in reductive dechlorination systems; therefore, the amount and forms of nutrients require careful consideration. In some states (Michigan, for example), groundwater antidegradation policies may limit or prohibit nutrient addition to aquifers without a permit or restrict the use of certain compounds or product formulations. Many practitioners have opted to add vitamins, primarily containing B-12, as a supplement at bioremediation sites. Vitamins have been shown to increase rates of bioremediation (Environmental Security Technology Certification Program [ESTCP] 2006). The addition of vitamins, however, is generally not required for bacterial activity to begin and persist, but likely contributes to improved biological performance.

The presence of some compounds may slow or inhibit cell growth. For example, *Dehalococcoides* sp. has been documented to be inhibited by hydrogen sulfide, chloroform, and 1,1,1-trichloroethane (He and others 2005, Duhamel and others 2002). A good understanding of the target bioremediation bacterial population is needed to identify what inhibitors to consider during the feasibility, bench-, or pilot-study phases of a project.

1.5.4.7 Biostimulation and Bioaugmentation

Bioremediation is accomplished through exploitation of microbial metabolism. Biostimulation refers to the addition of an electron donor (substrate) or electron acceptor, and bioaugmentation refers to the addition of the bacteria that can break down the contaminant. It may be a challenge for native bacteria to achieve required contaminant reductions without biostimulation or bioaugmentation for many types of plumes, but it is possible given the right biogeochemical conditions. Naturally occurring biodegradation is more common for contaminants that are degraded by aerobic bacteria (such as gasoline products) in an environment where naturally occurring electron acceptors are common. Significant contaminant reduction via natural biodegradation (without addition of amendments) by anaerobic bacteria (such as chlorinated solvents) is less common. Although reduction of contaminants to intermediate daughter products may occur, complete reduction of contaminants is less likely to occur at sufficient rates to meet remedial objectives. Insufficient organic substrate is the most common limiting factor.

Studies at chlorinated solvents sites with native target microbial populations have shown that bioaugmentation test plots can outperform biostimulation test plots (Lendvay and others 2003). Biostimulation was shown to take three to four times longer to achieve similar contaminant reductions. Bioaugmentation is generally not needed at petroleum sites since the bacteria involved in hydrocarbon bioremediation are ubiquitous in most environments. Conditions in a contaminated aquifer may not be favorable for bacteria to thrive in the subsurface because of a number of potential reasons discussed in

Section 1.6.4, but the presence of a target bacteria in an aquifer is a strong indication that bioremediation is feasible. The absence of a target microbial population, however, does not preclude application of bioremediation at a site. For example, *Dehalococcoides* may be present at a site, but at population densities that are too low to detect and that become detectable only after amendments have been added (biostimulation). The cost of amendments and their delivery to the target treatment zones are often the highest portion of total project costs. Some practitioners consider bioaugmentation in conjunction with amendment addition as a quicker means to obtain the required population densities to reach complete reductive dechlorination. Although bioaugmentation is an additional project cost, its use may reduce remediation time frames, as remedial goals are met more quickly. The net effect is a low total project life cycle cost.

Molecular biological tools (MBTs) are becoming more widely available and cost effective for applications in support of site characterization, remediation, and monitoring to determine microbial populations within aquifers. Quantitative Polymerase Chain Reaction (qPCR) is the mostly commonly used method to determine microbial populations. Other methods include microassays and Fluorescence *In Situ* Hybridization (FISH) (ITRC 2013). Additional discussion regarding MBTs is included in Section 3.3.

1.5.5 Contaminant Distribution

The final component of the CSM described in this document is contaminant distribution. Contaminant distribution is affected by each of the components of a CSM described in Section 1.6. A clear understanding of the contaminant distribution and contaminant phases is critical for the proper design of any remediation system. The contaminant mass distribution is a primary variable for bioremediation sites used to calculate the quantity of amendment and identify the appropriate delivery method. The lack of adequate characterization is one of the main reasons for poor remedial performance. Key characteristics of contaminant distribution are discussed in this section.

1.5.5.1 Source Area

According to Superfund guidance, " 'source material' is defined as material that includes or contains hazardous substances, pollutants or contaminants that act as a reservoir for migration of contamination to ground water, to surface water, to air, or acts as a source for direct exposure" (EPA 1991). The area containing the source is usually where the release has occurred. Typical source areas are attributable to underground and aboveground storage tanks (UST and ASTs), industrial lagoons, landfills, floor drains, septic drainage fields, process equipment, chemical storage, and mine waste rock. The source area may contain significant contaminant mass relative to the whole contaminated area, and impacts to the vadose zone may be significant as well. A site may have multiple source areas. Biological approaches to source area groundwater remediation have become more prevalent in recent years (CL:AIRE SABRE 2010) and include the use of partitioning electron donors to try to attack NAPL source areas. Key design factors are discussed in the field implementation section (Section 3).

1.5.5.2 Non-Aqueous Phase Liquid (NAPL)

Depending on the characteristics and amount of contaminant present, contamination may be completely dissolved in the groundwater or exist as a NAPL, which is typically found within areas considered source areas. NAPL co-exists with water in the pore space of an aquifer. Light non-aqueous

phase liquids (LNAPL) tend to exist in the upper portion of the aquifer, while dense non-aqueous phase liquids (DNAPL) tend to sink through the aquifer until they reach an impermeable formation. However, more often than not, NAPL exists as isolated ganglia between pores in the form of residue rather than as pockets of NAPL that fill all available pores (pooled NAPL) and are difficult to find and recover. The presence of LNAPL is more readily apparent than DNAPL by direct observation of floating product in a well, sheen on water during sampling, and coatings on sampling equipment. Often, the only clue to the presence of DNAPL is if contaminant concentrations are at or near solubility limits or concentrations rebound after some treatment takes place, as more DNAPL dissolves to equilibrate with the newly treated water. However, contaminant rebound can also be attributed to other factors, including but not limited to matrix back-diffusion or inflow of untreated groundwater.

Investigation using high-resolution site characterization (HRSC) strategies and technologies should be considered in areas with potential NAPL, and more specifically DNAPL. The presence of NAPL can make order of magnitude differences in the total aquifer contaminant mass. Conventional investigation methods are more likely to miss DNAPL that may exist over small depth intervals in heterogeneous geology. High resolution site characterization techniques are discussed in Section 4.3.

Over the past several years, the application of ISB to treat DNAPL source areas has become more common. It has been demonstrated that dechlorinating organisms can tolerate concentrations of chlorinated ethenes near the solubility limit (ITRC 2008). Biological degradation occurs only in the dissolved phase, but other mechanisms accelerate source zone mass removal, as stated in ITRC 2008:

- Increasing the concentration gradient at the DNAPL-water interface, which increases the rate of DNAPL dissolution;
- Partially biodegrading parent compounds near the DNAPL-water interface, producing less-chlorinated daughter products (*cis*-1,2-dichloroethene cis-1,2-DCE) and vinyl chloride [VC]) that are more mobile in groundwater than TCE and PCE; and
- Under some conditions, the electron donor solution or its degradation products abiotically enhance DNAPL mass transfer rates through cosolvency, desorption, or dissolved organic matter or surfactant partitioning. Studies are currently under way to demonstrate and validate the application of this approach (ESTCP 2013)

For instance, some bacteria produce natural surfactants that help the bacteria break down the NAPL at its surface interface (Banat and others 2000). Bench and pilot studies have demonstrated that the application of bioremediation in DNAPL source areas is a feasible remediation technology capable of reducing contaminant concentrations in groundwater within the source area and enhancing the removal of non-aqueous and sorbed contaminant mass (Hood and others 2008).

1.5.5.3 Dissolved Plume

The dissolved plume is located in and downgradient of the source area. The shape, concentration, and vertical and horizontal extents are controlled by components of the CSM. The most important factors are the type of contaminant; the initial concentration; and the rates of advection, dispersion, and diffusion. Biological approaches to remediation of dissolved plumes in groundwater have been widely

applied across the United States at aerobic sites since the 1980s and at anaerobic sites since the late 1990s. Important design factors to consider are discussed in the field implementation section (Section 3).

1.5.5.4 Lateral Extent, Thickness, and Depth

The majority of the costs associated with bioremediation are attributable to the quantity of amendments required and the methods needed to deliver them to targeted treatment areas. The lateral extent, the thickness of the affected zone, and the total depth required to reach the contaminated areas strongly influence the selection of a remedial approach. Target treatment areas that are limited in horizontal and vertical extent with low concentrations and limited potential for rebound may be ideal for direct injection and will have relatively low implementation costs. Conversely, target treatment areas that are expansive in horizontal and vertical extent with high concentrations and a high potential for rebound will likely require permanent injection wells with multiple screen intervals, multiple amendment applications, and will have significantly higher implementation costs.

Complex and highly heterogeneous sites often involve several target treatment zones. Each treatment zone may require unique delivery and amendment designs, depending on the differences in hydrogeology, depth, or co-contaminants. As a result, a successful pilot- or bench-scale study directed at one zone does not guarantee success for the other zones. Sites with dissimilar target treatment zones could become more expensive to treat than expected if this level of detail is not addressed at the site characterization and feasibility study stages of a project.

1.5.5.5 Contaminant Mass Flux and Mass Discharge

The final key characteristics of contaminant distribution are mass flux and mass discharge. Mass flux is the flow rate of contaminant mass through a defined area, usually a portion of a plume cross section. Mass flux is expressed as mass per time per area. Mass discharge is the integration of mass flux measured across an entire plume and thus represents the total mass of any contaminant plume conveyed by groundwater through a defined plane. Mass discharge is expressed as mass per time. In addition to defining the source strength and plume attenuation rate, mass flux estimates can identify areas of a plane where most of the contaminant mass is moving. Mass flux and mass discharge can be measured using transect methods, where concentration and flow data are collected from new or existing monitoring points and integrated; well capture and pump tests, where groundwater is extracted from wells while flow and mass discharge are measured; and passive flux meters, which are instruments that estimate mass flux directly within wells (ITRC 2010).

Incorporating mass discharge information into the CSM will help improve remediation efficiency and shorten cleanup time, particularly at sites with multiple source areas or where plumes cross multiple stratigraphic units. Generally, the majority of contaminant mass flows through a small portion of a cross-sectional area of an aquifer. Guilbeault and others (2005) studied three sites in North America using cross-sectional transects that 75% of contaminant mass discharge occurs through 5% to 10% of the plume cross-sectional area. Mass flux and mass discharge are extremely useful parameters to consider in designing an amendment delivery system, though the cost to collect the data needed to calculate mass flux and mass discharge increases with desired accuracy. The added costs may be justified if there

is a possibility that the target treatment area could be reduced or more accurately located and addressed. More targeted treatment can reduce costs and lead to more effective remediation.

2.0 STRATEGIES FOR GROUNDWATER BIOREMEDIATION

There are a wide array of groundwater bioremediation strategies available, each appropriate for specific contaminants and site conditions. The following sections explain the four main categories of bioremediation strategies (aerobic, anaerobic oxidative, anaerobic reductive, and cometabolic), the contaminants these strategies can treat, key microbe summary, summary of electron donors and acceptors, and general implementation approaches.

2.1 Aerobic Bioremediation

Aerobic bioremediation takes place in the presence of oxygen, which is the electron acceptor. With few exceptions, it relies on the direct microbial metabolic oxidation of a contaminant. The primary concern when an aerobic bioremediation system is designed is delivery of oxygen. Aerobic bioremediation technologies have been used at Superfund sites for more than 20 years.

2.1.1 Common Applicable Contaminants

Aerobic bioremediation is most effective in reducing non-halogenated organic compounds to carbon dioxide and water. Typically, aerobic bioremediation is applied to treat BTEX and diesel and jet fuel releases, often from USTS or ASTs (Farhadian and others 2008). Heavier hydrocarbons (those with higher molecular weight), such as lubricating oils, generally take longer to biodegrade than lighter products, but bioremediation can also be feasible for heavier fuels. In addition, although much less common, aerobic bioremediation has also been successfully applied to treat other solvents such as acetone, non-halogenated SVOCs including alkenes and alkanes found in fuels, some polycyclic aromatic hydrocarbons (PAHs), and pesticides and herbicides. For example, 11 Superfund projects in Appendix A selected aerobic treatment to treat the pesticide pentachlorophenol (PCP). Eight of these 11 projects also involve PAHs or naphthalene contamination. Project performance may be available at the relevant websites for these Superfund sites.

Aerobic bioremediation is more successful for simpler PAH compounds such as naphthalene. Biologically treating more complex cyclic compounds, such as benzo(a)pyrene, is considerably more difficult. Bioremediation of SVOCs and PAHs is far more common in soil remediation applications because these types of contaminants are more likely to be sorbed to soils than dissolved in water. However, bioremediation of groundwater containing select compounds from these groups has been documented (see for example Brubaker and others 1992). Aerobic bioremediation has also been used for a wide range of other contaminants, including vinyl chloride, DCE, methyl tert-butyl ether (MTBE), chlorobenzenes, ketones, some pesticides (such as 2,4-Dichlorophenoxyacetic acid), and some nitroaromatics such as dinitrotoluene.

2.1.2 Key Microbe Summary

Many reduced contaminants can be aerobically degraded by aerobic bacteria already present in the subsurface environment. Many species can metabolize the less recalcitrant organic contaminants because most aerobic heterotrophic bacteria can make use of a range of substrates. Some of the most common aerobic bacteria with the ability to degrade BTEX and PAHs, among other common contaminants discussed in Section 2.1.1 are *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*. These microbes have been well documented to degrade pesticides and

hydrocarbons, both alkanes and polyaromatic compounds (Vidali 2001, Hendrickx and others 2005, Bamforth and Singleton 2005) and are generally considered ubiquitously distributed in the natural environment (Bamforth and Singleton 2005). Fluorescent *Pseudomonas* strains, especially *P. putida* strains, are often isolated as BTEX degraders from BTEX- and gasoline-contaminated sites (Hendrickx and others 2005).

2.1.3 Sources of Electron Acceptor

Oxygen is the electron acceptor required for aerobic bioremediation. Oxygen can be added directly to the subsurface or oxygen-releasing compounds can be applied, which release oxygen as they dissolve or decompose. Common oxygen-releasing chemicals are calcium and magnesium peroxides, hydrogen peroxide, and ozone.

Calcium peroxide and magnesium peroxide break down in water to their hydroxide forms, releasing hydrogen peroxide. Hydrogen peroxide breaks down further in water, completely decomposing into oxygen and water within 4 hours (EPA 2004). Hydrogen peroxide is generally toxic to microorganisms at concentrations above 100 parts per million (ppm), though microbes can tolerate up to 1,000 ppm hydrogen peroxide with proper acclimation. Ozone also decomposes into oxygen in water and is 10 times more soluble in water than oxygen itself (EPA 2004), though it is also toxic to microorganisms at higher concentrations.

Of these chemical amendments, magnesium peroxide and ozone provide the highest relative oxygen delivery efficiency, but magnesium peroxide is a significantly longer-term oxygen-releasing chemical than ozone. Many of these compounds are used in high concentrations as chemical oxidants. Residual oxygen may not have the oxidative power to continue chemical oxidation, but may be sufficient to support biological activity. As a result, it is common to transition to aerobic bioremediation after chemical oxidation remedies are completed.

2.1.4 Delivery Mechanisms

Oxygen and oxygen-releasing compounds can be delivered to the groundwater via several methods, depending on their physical properties, site hydrogeology, and the desired delivery efficiency.

2.1.4.1 Gas-Phase Delivery

Injection of gas into groundwater to stimulate or enhance aerobic biodegradation is called biosparging. The efficacy of biosparging depends primarily on the permeability of the aquifer and the biodegradability of the contaminant (EPA 2004). Intrinsic permeability (related to effective porosity) is a measure of the ability of soil to transmit fluids and is the *single most important characteristic of the soil* in determining the effectiveness of biosparging because it controls how well oxygen can be delivered to the subsurface microorganisms (EPA 2004). Treatment in zones with low permeability will be limited by diffusion. Air, oxygen, and ozone can all be delivered using biosparging.

Biosparging differs from air sparging in that air sparge systems are designed to remove contaminants through volatilization and require a soil vapor extraction system to capture volatilized gases, while biosparging delivers air at lower flow rates as well as nutrients, if needed, to stimulate biodegradation and minimize volatilization. Biosparging has been selected for five projects included in the Appendix A

data set. Some degree of volatilization of contaminants will occur with biosparging (EPA 2004), which has the potential to build up pressure or cause hazardous atmospheres. When biosparging is applied in potentially sensitive areas with basements, sewers, or subsurface confined spaces, biosparging can be combined with soil vapor extraction (EPA 2004) and sub-slab depressurization systems to control vapor-phase contaminants.

An alternative method of dissolving oxygen gas is using gas diffusers. Gas diffusers consist of a cartridge containing a semi-permeable membrane designed to be submerged into a groundwater well and pressurized with oxygen. The semi-permeable membrane allows oxygen molecules to pass through into the liquid, and the increased pressure of oxygen causes the water to become supersaturated compared with oxygen concentrations possible under atmospheric conditions. This method can be used to dissolve air or oxygen in groundwater more efficiently than biosparging, because no gas is lost from bubbling up out of the groundwater. Ozone, however, is too reactive to use with the delicate membranes of the current gas diffusers on the market, and it must be applied to the subsurface in a manner similar to biosparging.

2.1.4.2 Liquid- and Solid-Phase Delivery

Liquid delivery of oxygen to the subsurface can be achieved in several ways to support aerobic bioremediation. The most direct is injection of water supersaturated with oxygen. This method uses a technology similar to that used for gas diffusion discussed in the previous section, but the mechanical infusion of oxygen into the water occurs before it is applied to injection wells. Ozone is also commonly applied as a solution to support aerobic bioremediation. Ozone provides an oxygen delivery efficiency that is higher than other chemical amendments, but lower than biosparging. Hydrogen peroxide can deliver significant oxygen to a saturated zone; however, hydrogen peroxide decomposes and liberates oxygen faster than the oxygen can be biologically used (EPA 1990).

Calcium and magnesium peroxide can be injected into the saturated zone as a solid or in slurry form (EPA 2004). Magnesium peroxide is more commonly used because it dissolves more slowly, prolonging the release of oxygen. In their solid forms, these chemicals can be mixed with water or in slurry for injection. Solids that can be injected are generally fine grained (able to pass through a 0.02-inch opening). Solids in water applications usually require greater and continuous agitation of the mixed product to ensure application of a consistent, homogenized mixture. Lower injection pressures (below 100 pounds per square inch [psi]) are typically adequate for delivery of water-based mixtures. The mixed material can be applied using injection wells with openings of adequate size to allow solids to pass through or applied by direct injection using specialized drilling tooling (for example, Geoprobe Systems Pressure Activated Injection Probe).

Slurries are typically mixed on site and then injected soon after mixing to minimize settling of the product. Larger-volume injections may require intermittent mixing of batches to prevent settling of the oxygen-releasing solids and maintain an even distribution from injection to injection. Slurries are primarily applied via direct injection using the tooling previously mentioned. Slurries are typically injected under higher pressures (100 to 500 psi), depending largely on the receiving material. These

higher injection pressures will fracture soils, in most cases, which need to be considered given specific site conditions.

2.1.5 Common Byproducts

The by-products of aerobic bioremediation are generally carbon dioxide and water. Excessive calcium, magnesium, or iron in groundwater can react with carbon dioxide. The products of these reactions can adversely affect the operation of an ISB system. Crystalline precipitates or "scale" is formed when calcium, magnesium, or iron reacts with phosphate or carbon dioxide. Scale can constrict flow channels and can also damage equipment, such as injection wells and sparge points.

The precipitation of calcium or magnesium phosphates can also tie up phosphorus compounds, making them unavailable to microorganisms for use as nutrients. Precipitation of calcium or magnesium phosphates can be minimized by using tripolyphosphates to act as sequestering agents to keep the magnesium and calcium in solution (prevent the metal ions from precipitating and forming scale) (EPA 2004).

When oxygen is introduced to the subsurface as a terminal electron acceptor, it can react with dissolved iron [Fe(II)] to form an insoluble iron precipitate, ferric oxide. The precipitate can be deposited in aquifer flow channels, reducing permeability. The effects of iron precipitation tend to be most noticeable around injection wells, where the oxygen concentration in groundwater is highest, and can render injection wells inoperable. Lower injection rates and higher pressures are often indicators of a decrease in injection well performance. Routine injection well maintenance may be required.

At least one aerobic pathway is available for all of the BTEX compounds that include degradation to catechol or a substituted catechol. The byproducts of BTEX metabolism are not considered contaminants of concern.

2.2 Anaerobic Oxidative Bioremediation

Anaerobic oxidative bioremediation, like aerobic bioremediation, also relies on the direct microbial metabolic oxidation of a contaminant and is an alternative to aerobic bioremediation in anaerobic aquifers. Generally, aerobic conditions allow for a higher rate of biodegradation of reduced contaminants than anaerobic conditions. As a result, remediation strategies often introduce oxygen to anaerobic environments in an attempt to employ more efficient aerobic microbial processes. However, the overall oxygen demand from dissolved metals such as iron and manganese is often overlooked and underestimated. Even if oxygen demand is accounted for, the result of oxygen delivery may interfere with the injection infrastructure. Oxygen will readily react with dissolved iron(II) to form an insoluble iron(III) precipitate, which decreases the permeability of the aquifer and may foul injection tools and wells. Therefore, it is advantageous to promote anaerobic oxidative bioremediation where oxygen levels are already depleted, an appropriate metabolic pathway exists for the target contaminants, and other conditions are conducive to this approach (as discussed below).

The key concern when an anaerobic oxidative bioremediation system is designed is the availability of a carbon source, nutrients, and an electron acceptor. The rate of degradation is typically limited by the availability of an electron acceptor; however, carbon or nutrient amendments may be necessary as well.

In the absence of oxygen, anaerobes will preferentially use alternative electron acceptors based on the amount of free energy gained from reduction of a given electron acceptor, as described above. The preferential electron acceptor in the absence of oxygen is nitrate, followed by manganese(IV), iron(III), sulfate, and finally carbon dioxide.

2.2.1 Common Applicable Contaminants

Several contaminants can be anaerobically oxidized, including aromatic hydrocarbons, fuels, and some chloroethenes. Aromatic hydrocarbons associated with petroleum and fuel releases such as BTEX will undergo anaerobic oxidative biodegradation. Remediation product suppliers provide sulfate enhanced amendments to promote anaerobic oxidative bioremediation at petroleum-contaminated sites. Toluene and xylenes are more readily oxidized anaerobically than are benzene and ethylbenzene; however, degradation of benzene and ethylbenzene has been documented in manganese and iron reducing environments (Villatoro-Monzón 2003). Naphthalene has also been observed to degrade anaerobically by sulfate-reducing bacteria (Meckenstock 2000). Additional aromatic hydrocarbons that are degraded anaerobically include phenol, cresol, and benzoic acids (Suflita 1991).

Biodegradation of chloroethenes is typically associated with reductive dechlorination processes. However, DCE and vinyl chloride appear to be anaerobically oxidized in iron(III)-reducing and methanogenic conditions (Bradley 2007). Vinyl chloride may also be oxidized in sulfate-reducing and humic acid-reducing environments (Bradley 1997). However, even under nominally anaerobic conditions, very low levels of oxygen (much less than the typical reporting limit of 1 milligrams per liter [mg/L]) may support aerobic biodegradation of chloroethenes, potentially confounding the interpretation of results from laboratory and field tests designed to stimulate anaerobic oxidation (Gossett 2010).

2.2.2 Key Microbe Summary

Several microbes involved in bioremediation can adapt to aerobic and anaerobic conditions. These microbes are called facultative, and while active in both aerobic and anaerobic environments, facultative microbes degrade contaminants at a slower rate in the absence of oxygen. Microbes that use nitrate as an electron acceptor tend to be facultative (Firestone 1982). Facultative microbial action accelerates the depletion of nitrate because it is used as a nutrient as well as an electron acceptor.

Strict anaerobes will be active only in reduced environments and will use electron acceptors such as sulfate or carbon dioxide. Sulfate-reducing bacteria are obligate anaerobes (they require an anaerobic environment to thrive). *Desulfovibrio* is the most well studied sulfate reducer.

2.2.3 Sources of Electron Acceptor

Several inorganic compounds commonly found in aquifers may act as electron acceptors for anaerobic oxidative bioremediation. Various commercial products are available that can supply electron acceptors to drive the anaerobic oxidation process. These products most commonly contain iron(III), nitrate, or sulfate. The selection of an electron acceptor or product will depend on the contaminant and the optimal oxidation-reduction state of the targeted bioremediation process. For example, a product

containing primarily iron(III) should be used if degradation of toluene is observed at a site that is mildly anaerobic and data indicate that the oxidation-reduction state is iron-reducing.

Nitrate is highly soluble in water and, after oxygen, provides the most free energy for microbial action. Nitrate is also mobile in an aquifer. However, nitrate concentrations in groundwater above 10 mg/L have negative toxicological effects on humans and animals. Therefore, care must be taken if groundwater is to be amended with nitrate.

Iron(III) salts are only slightly soluble in water, but when used as an electron acceptor, iron(III) is reduced to iron(II), which is much more soluble in water. Iron(III) has a particularly low electron accepting capacity for its mass, and therefore iron(II) may quickly exceed water quality thresholds in groundwater as it reacts and dissolves.

Sulfate is very soluble in water, will not sorb appreciably, and is generally unreactive. Sulfide, the end product of sulfate reduction, precipitates with iron(II) and is effectively immobilized. However, in acidic environments, sulfide can produce hydrogen sulfide gas, which is toxic to breathe.

2.2.4 Delivery Mechanisms

Since the most common electron acceptors used for anaerobic oxidative bioremediation are soluble in water, the products are typically delivered to the subsurface in a solution via injection wells or direct injection using drilling tooling similar to the delivery methods discussed in Section 2.1.4.2. The key to successful delivery is matching the proper concentration of solution to the remedial objective and avoiding potential negative impacts to the groundwater.

2.2.5 Common Byproducts

Anaerobic oxidative bioremediation may produce metabolic byproducts that can be problematic when oxygen is not the terminal electron acceptor, and if certain compounds exist in an aquifer. The most common of the issues discussed in this section that practitioners have observed is mobilization of metals. Arsenic mobilization is of particular concern in areas with naturally occurring arsenic in soils or bedrock. Mobilized metals will persist until the oxidation-reduction state shifts back to oxidative and metals form oxides, making them generally immobile. The shift back to oxidative conditions occurs naturally downgradient of a biological treatment area; however, an engineered approach such as an air-sparge wall could be installed to induce oxidative conditions if receptors are present.

A few additional examples of problematic byproducts are as follows:

- Nitrate is reduced to nitrite, nitric oxide (reactive intermediate), nitrous oxide (reactive intermediate), nitrogen gas, or a combination of these byproducts, depending on the microbes that are present. The latter three are gaseous byproducts that can dissolve into groundwater to some extent, but will generally escape into the vadose zone; however, the gaseous byproducts may become trapped within pore spaces, displacing water and reducing the hydraulic conductivity of the saturated matrix.

- Manganese (IV) and iron (III) are reduced to soluble manganese (II) and iron (II). These dissolved-phase metals may contribute to secondary groundwater plumes and elevated total dissolved solids.
- Sulfate is reduced to sulfite and sulfide. The end product of sulfate reduction is sulfide. If there are not enough dissolved metals to precipitate the sulfide, hydrogen sulfide gas is generated, which is toxic and flammable and could result in vapor intrusion issues given the depth of the plume and characteristics of any overlying buildings.
- Fermentation generates hydrogen ions, which can lower the pH of the groundwater to levels where the key bacteria cannot survive. In addition, carbon dioxide is reduced to methane, which can support a community of microbes called methanotrophs but could result in vapor intrusion issues.

2.3 Anaerobic Reductive Bioremediation

Anaerobic reductive bioremediation takes place in the absence of oxygen. It relies on the presence of biologically available organic carbon naturally or the application of a reduced carbon source, also commonly called organic substrate, into groundwater to create and sustain anaerobic conditions and the bioreduction of contaminants, such as chlorinated solvents (EPA 2001a), by generating hydrogen through fermentation reactions. Because chlorinated solvents exist in an oxidized state, they are generally much less susceptible to aerobic oxidation processes. However, they are susceptible to microbial reduction under anaerobic conditions. The key concerns when an anaerobic reductive bioremediation system is designed is the competition of native electron acceptors (such as oxygen, nitrate, iron, and iron sulfate) with the contaminant, the presence of bacteria capable of completely reducing contaminants, and the effective delivery of the substrate to all portions of the aquifer that is contaminated.

Injected organic substrates are first fermented to hydrogen and low-molecular weight fatty acids, which in turn provide a source of carbon and energy to the microorganisms. Microorganisms will consume competing native electron acceptors beginning with the most oxidized sequentially to the least oxidized. Once native electron acceptors have been eliminated or depleted, target contaminants will be the most efficient electron acceptors. In many cases, microorganisms use the highly oxidized contaminants in a respiratory mechanism and are able to derive metabolically useful energy (EPA 2000 and AFCEE 2004), requiring either continuous or intermittent substrate replenishment to maintain favorable conditions for the microorganisms. Other processes such as anaerobic cometabolism could also occur in the subsurface as a result of injection of carbon substrates and the creation of reducing conditions.

Anaerobic reductive bioremediation can cost-effectively remediate contaminated sites if the site can be engineered to provide appropriate growth conditions favorable to native contaminant degrading microbes or commercially available microbial cultures, and if contaminants are susceptible to reductive bioremediation (such as chlorinated solvents or perchlorate). Although anaerobic bioremediation has been applied at hundreds of sites to date, many sites have not been closed using anaerobic reductive bioremediation alone. Many factors — including source area mass, the presence of NAPL, aquifer characteristics, and cleanup objectives — will dictate whether other technologies may need to be

implemented before, in concert with, or after implementation of anaerobic reductive bioremediation. ITRC 2011 presents an in-depth discussion of developing an integrated site strategy to account for these factors.

2.3.1 Common Applicable Contaminants

Contaminants that can be degraded using anaerobic reductive bioremediation include halogenated VOCs, munitions, some dissolved metals, perchlorate, and nitrate. Given the increased interest in anaerobic reductive bioremediation, more detail is given in the following sections on these applications. Enhanced reductive dechlorination (ERD) can be used for chlorobenzenes, chlorinated pesticides, and chlorinated SVOCs, but these compounds are more difficult to degrade than chlorinated ethenes (ESTCP 2008).

2.3.1.1 Halogenated VOCs

Anaerobic bioremediation may be used to degrade chlorinated contaminants, such as biodegradation of PCE and TCE to ethene, 1,1,1-TCA to ethane (with further degradation to other non-toxic compounds) and carbon tetrachloride (CT) to methane.

The most common halogenated VOCs include PCE, TCE, 1,2-dichloroethane (DCA), and CT that are called chlorinated aliphatic hydrocarbons (CAH). Generally, the more chlorinated the CAH, the more appropriate it is to use anaerobic versus aerobic degradation processes. However, less chlorinated compounds and dechlorination products such as DCE, VC, and chloroethane can be degraded using either anaerobic or aerobic bioremediation technologies (ESTCP 2008). As seen in Figure 2, halogenated VOCs are the contaminants most commonly treated by in situ groundwater bioremediation projects in the EPA's Superfund program.

At some sites, anaerobic reductive bioremediation of PCE and TCE may undergo incomplete degradation (stalls) to DCE or VC. Many factors can cause stall, such as:

- Reductive dechlorination is most effective and efficient under sulfate-reducing to methanogenic conditions. The inability to achieve these negative redox conditions can cause a stall at DCE.
- Microorganisms generally gain more energy from dechlorination of more highly chlorinated CAHs (such as PCE and TCE). Dechlorination of daughter products (DCE and VC) may not proceed until parent products are sufficiently depleted (AFCEE 2004).
- *Dehalococcoides mccartyi* is the only known bacteria that can achieve complete dechlorination of chlorinated ethenes (Ernst 2009; Löffler and others 2013). Sometimes this bacterium is not present at population densities required to sustain complete dechlorination.
- Co-contaminants or other geochemical conditions such as pH can inhibit the microbial population, such as inadequate electron donor availability or unfavorable geochemistry.

Remediating chlorinated VOCs can be challenging, and the degree of success is subject to hydrogeological and biogeochemical conditions. Chlorinated VOCs can be remediated in situ through anaerobic reductive bioremediation, but it is not appropriate for every site. In addition to limitations from the characteristics of the aquifer that can make it difficult to access the contamination, the timeframe required for complete dechlorination can be months to years, depending on groundwater flow velocity and matrix diffusion. In

addition, native microbial populations can compete with dechlorinating microbes, or other conditions can exist as listed above, that can lead to incomplete degradation pathways. The bioremediation process can change groundwater pH, and redox conditions, such that the solubility of some metals increases and cause secondary water quality impacts. Some of these limitations also apply to other remedial techniques and are not unique to bioremediation (AFCEE 2004).

2.3.1.2 Nitrate and Sulfates

Nitrate is essential for plant growth, but is potentially toxic to human and animal life at moderate concentrations. Sources of nitrate in groundwater include atmospheric deposition from fossil fuel burning, runoff from fertilizer use, leaching from animal wastes from confined animal feedlot operations and dairies, septic tanks and sewage, landfills, and erosion of natural deposits. Impacts to groundwater from sulfate are derived from some sources similar to nitrate sources. Two common sources are agricultural sulfate and geochemistry changes to aquifers that contain sulfide minerals.

Anaerobic reductive bioremediation of nitrate- and sulfate-contaminated groundwater can be achieved by biostimulation of native nitrate-reducing and sulfate-reducing microbial communities. Anaerobic bioremediation was selected to successfully treat nitrates at two projects listed in Appendix A. The conditions that facilitate the process are part of the aquifer reduction and competing electron acceptor elimination step for establishing reductive dechlorination.

2.3.1.3 Perchlorate

Perchlorate is both a naturally occurring and man-made chemical that is used to produce rocket fuel, fireworks, flares, and explosives. Perchlorate-contaminated groundwater can be treated using anaerobic reductive bioremediation or aboveground bioreactors. Perchlorate is degraded by a three-step reduction process where perchlorate is reduced to chlorate, chlorite, and finally to chloride and oxygen. Perchlorate-reducing microorganisms are generally ubiquitous in the environment, but bioaugmentation is sometimes needed to reach population densities required for treatment (ITRC 2005). These microbes produce an enzyme that allows them to lower the perchlorate activation energy for reduction and use the perchlorate as an electron acceptor. Once the aquifer has been conditioned with an electron donor (organic substrate) to eliminate the primary competing electron acceptors — oxygen and nitrate — perchlorate reduction can occur. The presence of molybdenum might be required by the microorganisms (ESTCP 2008; Stroo and Ward 2009). Anaerobic reductive bioremediation has been selected to treat perchlorate or Royal Demolition Explosive (RDX) at three projects listed in Appendix A.

2.3.1.4 Pesticides and Herbicides

Some pesticides and herbicides can be treated using anaerobic reductive bioremediation. Organochlorine pesticides, such as toxaphene and dieldrin, are persistent in the environment and adsorb strongly to soils but do not decompose naturally at a significant rate. Field studies have shown that the application of solid organic carbon and zero-valent iron to groundwater can effectively treat organochlorine pesticides. These amendments create a reduced environment that supports relatively rapid and complete dechlorination of many chlorinated compounds (Seech 2008).

2.3.1.5 SVOCs and PCPs

SVOCs from wood treating wastes include creosote and PCP, which are typically treated using bioremediation in the soil matrix, in sediments, or in mulch. In groundwater, typical treatment includes anaerobic reductive dechlorination followed by anaerobic oxidation. However, there are examples of full-scale in situ implementation that report success using a carbon source supplemented with oxygen (Fields 2010). Since PAHs are the main components in creosote, bioremediation technologies for PAHs can also be considered for creosote contamination (Zhang 2010), although PAHs above three rings are recalcitrant to bioremediation under any scenario because of their typically low solubility.

2.3.1.6 Dissolved Metals

Metals cannot be destroyed through bioremediation technologies. Rather, microbes can remove dissolved metals from solution by reducing them to a more insoluble valence state. Immobilization reduces the mobility of contaminants by altering the physical or chemical characteristics of the contaminant, causing it to precipitate out of solution or to sorb onto the soil. Furthermore, microorganisms can mobilize inorganic compounds through autotrophic and heterotrophic leaching, chelation by microbial metabolites, methylation, and redox transformations (Adeniji 2004).

Microbial alteration of the redox state of either the contaminants or the iron and manganese oxides, which bind most heavy metals, can make metals and metalloids less soluble. Microbially induced reduction of hexavalent chromium to trivalent chromium may be the most common application of bioremediation to metals. Microbial reduction of the highly soluble, oxidized form of selenium to insoluble elemental selenium by microorganisms is a biological mechanism to remove selenium from contaminated surface and groundwater (CLU-IN 2008). The adsorption of metals and metalloids onto microbial biomass can also prevent further migration of these contaminants (Lovley and Coates 1997). Anaerobic reductive bioremediation to promote bacterial sulfate reduction, and consequent precipitation of various insoluble metal sulfides, is a possible remediation technique, as demonstrated at the Stoller Chemical Site in Jericho, South Carolina (CLU-IN 2006).

All of the five anaerobic Superfund projects for metals shown in Figure 3 (and detailed in Appendix A) treat hexavalent chromium. Two of the five also treat cadmium, manganese, and other metals.

2.3.2 Key Microbe Summary

Bacterial species used in anaerobic reductive bioremediation can be highly specific to a particular contaminant. For example, *Dehalococcoides mcartyii* are the only bacteria known to completely convert PCE to ethene, and some strains also break down polychlorinated biphenyls (PCBs). *Dehalobacter* spp. are capable of converting 1,1,1-TCA (known to inhibit the ability of *Dehalococcoides* to dechlorinate TCE*)* to chloroethane. *Pseudomonas stutzeri* KC*, Methanosarcina barkeri, Desulfobacterium autotrophicum, Moorella thermoacetica,* and *Methanobacterium thermoautotrophicum* are each capable of converting carbon tetrachloride to methane. *Geobacter* spp. reduces uranium to a less soluble valence state (Anderson and others 2003). Perchlorate reducers include the species *Dechloromonas aromatic* (Salinero and others 2009), *Moorella perchloratireducens* (Balk and others 2008), and *Sporomusa* sp. (Balk and others 2010). Dissimilatory perchlorate-reducing bacteria (DPRB) are dominated by *Dechloromonas* and *Azospira* spp.

2.3.3 Sources of Electron Donor

The choice of electron donor (substrate) and the selected delivery method are essential components of anaerobic reductive bioremediation. Critical considerations include a substrate's properties (solubility, longevity, cost, and ability to be distributed in the subsurface). Tighter soil matrices can limit the effective distribution of substrate in the target treatment area. Sometimes a single substrate is not sufficient, and combinations of substrates may be required. Effective implementation requires careful design of the mode of delivery and determination of the need for periodic replenishment.

Many materials have been used as electron donors for anaerobic bioremediation. These materials typically fit into one of two categories: quick or slow release compounds. Table 1 lists some common substrates and typical applications methods. Many practitioners and commercially available products use a combination of these types of substrate to capitalize on the advantages of each. In addition, hydrogen and propane gases are used as electron donors, to a much lesser extent than liquid or solid substrates.

Table 1. Substrates used for enhanced anaerobic bioremediation (modified from ITRC 2008, AFCEE 2004)

	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
	Whey (soluble)	Direct injection or injection wells	Dissolved in water or slurry	Monthly to annually
Slow-release substrates	HRC® or HRC-X®	Direct injection	Straight injection	Annually to biennially for HRC (typical), every 3–4 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% oil) emulsions	One-time application (typical)
	Vegetable oil emulsions	Direct injection or injection wells	Low oil content (<10%) microemulsions suspended in water	Every 2 to 3 years (typical)
Solid substrates (barrier wall applications)	Mulch and compost	Trenching or excavation	Trenches, excavations, or surface amendments	One-time application (typical)
	Chitin (solid)	Trenching or injection of a chitin slurry	Solid or slurry	Annually to biennially, potential for one-time application

2.3.3.1 Quick-Release Compounds

Quick-release substrates are typically readily soluble materials and consist of relatively simple molecules (sugars and alcohols). The solubility nature of the substrate allows for wider distribution into the subsurface and general movement with groundwater flow. These substrates are rapidly consumed by

the microbes and require more frequent replenishment. Quick-release compounds include lactate compounds, organic acids, methanol, ethanol, molasses, and high fructose corn syrup (AFCEE 2004).

2.3.3.2 Slow-Release Compounds

Slow-release substrates have low solubility and higher viscosities than quick-release substrates to make them generally immobile and sorb to the aquifer matrix. Low solubility and higher viscosities can pose a challenge for delivery into the subsurface. However, many slow-release substrates are manufactured to allow products mixed with water to behave as a soluble product during the injection process, most commonly in an emulsion with surfactants. These materials return to their insoluble nature after a short period of time (on the order of days) after they are injected. However, breakdown products are soluble and provide some downgradient distribution of substrate. These substrates consist of long-chain molecules intended to limit consumption rates and increase longevity of the material in the subsurface. Longevity is site specific and depends on biological activity, matrix oil retention capacity, and the hydraulic characteristics of the aquifer. Slow-release substrates include soybean oils (neat and proprietary emulsified vegetable oil [EVO] products) and proprietary formulations of polylactate esters and fatty acid esters. Protocol documents are also currently available for design and addition of specific substrate types, such as edible oil (AFCEE 2007).

Solid substrates, such as mulch, compost, and chitin, are generally the longest-lasting substrates, on the order of 5 to 10 years (AFCEE 2004). These substrates are best suited for shallow groundwater plumes, as physical placement of the material is necessary by trenching, excavation, or surface application. However, chitin can be injected in slurry form for particular applications. Solid substrates are often replenished by injecting one or more liquid substrates into the solid substrate material matrix. For example, the mulch biobarriers at the Altus Air Force Base in Oklahoma included piping and other supporting infrastructure to deliver liquid substrate to the biologically active area to maintain electron donor concentrations as the mulch decomposed (AFCEE 2008).

Not all anaerobic bacteria can use slow-release substrates. For example, dissimilatory perchlorate-reducing bacteria (DPRB) cannot break down complex substrates such as edible oils (Coates and Jackson 2008). However, slow-release substrates are gradually consumed by fermenters, which produce simpler organic compounds like those categorized as quick release compounds. The DPRB are able to consume these fermentation products, and thus can still be stimulated with either quick- or slow-release compounds (Borden 2008).

2.3.3.3 Hydrogen and Propane Gas

Direct injection of hydrogen gas is the most direct approach to stimulating anaerobic reductive bioremediation (AFCEE 2004). Some hydrocarbon gases such as propane can also be used. These materials are combustible and pose special health and safety and engineering challenges. However, these gases are generally less expensive than liquid substrates. Delivery methods are similar to those used to deliver oxygen to promote aerobic bioremediation and, include biosparging and permeable-membrane diffusers.

2.3.3.4 Petroleum Hydrocarbons and Other Co-Contaminants

In a mixed contaminant plume, some VOCs such as acetone or petroleum hydrocarbons may be present in the subsurface as a co-contaminant with oxidized target contaminants (such as halogenated compounds). In these cases, an electron donor and acceptor are present, but microbial populations able to use and completely remediate both contaminants may not be present or viable in the aquifer. These microbial populations will need to be bioaugmented with a consortium of other microbes able to achieve complete treatment of contaminants. Commonly, co-contaminants will be exhausted before sufficient remediation of the target compounds has occurred, and additional electron donor may be necessary.

2.3.4 Delivery Mechanisms

Most liquid substrates are delivered to the subsurface in a solution or mixture with water, either groundwater or potable water, via injection wells or direct injection using a drilling tool. Applications anticipated to occur only once may be more cost effective as a direct injection application. However, if there is any possibility that a site would need more than one application, a permanent injection well may likely be more cost effective. The well material costs and possible well rehabilitation over the project life cycle can be less than drilling subcontractor costs, depending on plume depth and time and pressures needed to inject. The longer the remediation timeframe, the more cost-effective permanent injection wells become.

High pressure (100 to 500 psi) liquid injections are worth considering for less permeable zones to induce localized soil fracturing for donor placement. The extent to which soil fracturing will occur is highly dependent on site conditions. Additional information regarding environmental fracturing is located at: www.clu-in.org/techfocus/default.focus/sec/Environmental_Fracturing/cat/Guidance and www.epa.gov/tio/download/citizens/a_citizens_guide_to_fracturing_for_site_cleanup.pdf.

Solid substrates are typically applied by excavating into the saturated zone and placing mulch or compost. These materials are sometimes mixed with a quick- or slow-release substrate and other amendments. The excavation is backfilled with earth back to grade to create a permeable reactive barrier. However, chitin can be ground into a fine powder and mixed with water for application similar to liquid substrates. Figure 10 shows an example of a permeable reactive barrier configuration.

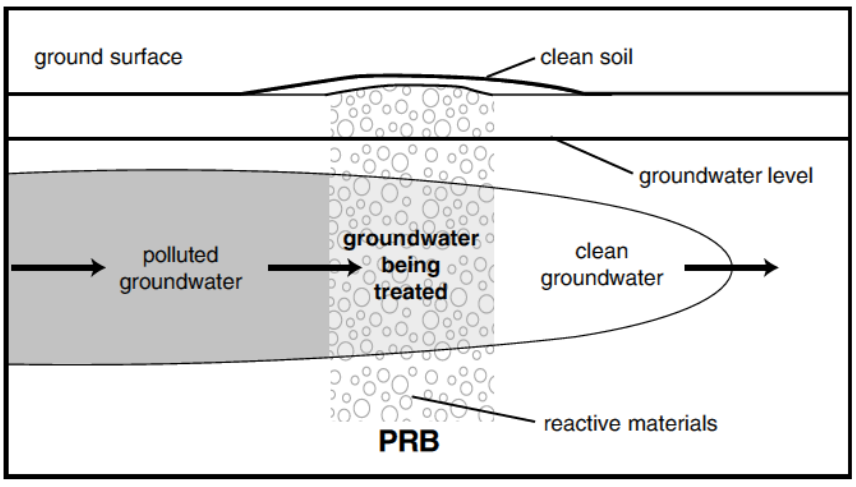

Figure 10. Permeable Reactive Barrier Example (EPA 2001b)

2.3.5 Common Byproducts

The most common problematic byproduct of reductive bioremediation is acidity resulting from fermentation processes. Organic substances are injected to act as electron donors and ensure a highly reducing environment. These substances are fermented in this environment, which generates hydrogen ions and, in the absence of adequate buffering capacity, can lower the pH.

Reductive dechlorination produces chloride ions, the reduced form of the chlorine removed from chlorinated organic compounds. Reductive dechlorination of chlorinated VOCs with multiple substituents produces intermediate daughter products as each halogen atom is sequentially removed. An intermediate dechlorination product for a common contaminant such as PCE is VC, which is more toxic than PCE.

The following lists are some of the most common metabolic byproducts from anaerobic reductive bioremediation and potential issues associated with those byproducts:

- If nitrate is used, byproducts include nitrite, nitric oxide, nitrous oxide, and nitrogen gas. The predominant byproduct depends on the enzymes possessed by the microbes present.
- Iron(II) is far more soluble than iron(III), so iron reduction could exceed iron water quality criteria or create a total dissolved solids issue.
- The end product of sulfate reduction is sulfide. If there are not enough dissolved metals to precipitate the sulfide, hydrogen sulfide gas can be generated, which is toxic.
- Fermentation generates methane, which may necessitate installation of vapor mitigation systems when a building overlies a treatment area. Organic acids are also generated as part of fermentation, which can lower the pH of the groundwater and potentially mobilize metals (notably iron, manganese, and arsenic). The primary concern with mobilization of metals is creating secondary water quality issues at a site. Monitoring the dissolved metals over time may be needed to confirm that any mobilized metals precipitate when the pH and ORP return to the natural state downgradient of the active treatment area. The decrease in pH can also inhibit *Dehalococcoides* and stop the bioremediation process altogether.

2.4 Cometabolic Bioremediation

This section contains less detail than sections for other ISB strategies discussed above because limited full-scale applications of cometabolic bioremediation have been published. Field-scale applications are planned that will provide information on the utility of this bioremediation strategy. Cometabolism occurs when microorganisms using one compound as an energy source fortuitously produce an enzyme that chemically transforms another compound. Organisms thus can degrade a contaminant without gaining any energy from the reaction. Cometabolic degradation is a process that often happens concurrently in bioremediation systems designed for direct metabolism of contaminants; however, some systems have been designed to specifically take advantage of cometabolic processes. Hazen (2009) indicates that cometabolic bioremediation can occur in environments where contaminant concentrations are well below concentrations that could provide a carbon or energy benefit to the biodegrader. Therefore, this method may be effective at degrading very low concentrations of some contaminants.

In particular, aerobic microorganisms that degrade methane (methanotrophic bacteria) have been found to produce enzymes that can initiate oxidation of various carbon compounds. Methanotrophic bacteria can cometabolize many aliphatic compounds and aromatic compounds (Brigmon 2001). Cometabolic bioremediation has been shown to degrade contaminants that are typically recalcitrant or difficult to degrade, such as PCE, TCE, trinitrotoluene (TNT), 1,4-dioxane, and atrazine (Hazen 2009). Monooxygenase enzymes have shown the ability to oxidize PAHs, PCBs, MTBE, pyrene, creosote, TNT, NDMA, and 1,4-dioxane (Hazen 2009, Hatzinger 2011, Steffan 2007), and cometabolic bioremediation has the potential to remediate these groundwater contaminants with further development.

Cometabolic reductive dehalogenation is relevant to large dilute plumes, where contaminant concentrations are too low for direct reductive dechlorination. Cometabolic reductive dehalogenation has been observed for PCE, DCA, and CT, and happens concurrently with direct reductive dechlorination, making it difficult to distinguish the exact contributions of each pathway. Furthermore, there is laboratory evidence of anaerobic cometabolic degradation of hexachlorocyclohexane, BTEX, PAHs, atrazine, and TNT, though these remedies have yet to be used extensively in the field. Common cometabolic bioremediation substrates, enzymes, and contaminants are summarized in Table 2.

Table 2. Common cometabolic bioremediation substrates, enzymes, and contaminants (from Hazen 2009)

Cosubstrates	Methane, Methanol, Propane, Propylene (aerobic)	Ammonia, Nitrate (aerobic)	Toluene, butane, phenol, citral, cumin aldehyde, cumene, and limonene (aerobic)	Methanol (anaerobic)	Glucose, Acetate, Lactate, Sulfate, Pyruvate (anaerobic)
Enzymes (microbes)	Methane Monooxygenase, Methanol Dehydrogenase, Alkene monooxygenase, catechol dioxygenase (Methylosinus)	Ammonia Monooxygenase (*Nitrosomonas, Nitrobacter*)	Toluene Monooxygenase, Toluene Dioxygenase (*Rhodococcus, Pseudomonas, Arthrobacter*)	Alcohol Dehydrogenases (*Pseudomonas, Streptomyces, Corynebacterium*)	Dehalogenase, AtzA, Dichloromethane Dehalogenase (*Dehalococcoides, Methanogens, Desulfovibrio, Clostridium, Geobacter, Clavibacter*)
Contaminants	TCE, DCE, VC, PAHs, PCBs, MTBE, creosote, >300 different compounds	TCE, DCE, VC, TNT	TCE, DCE, VC, 1,1-DCE, 1,1,1-TCA, MTBE	PCE, TCE, DCE, VC, Hexachloro-cyclohexane	BTEX, PCE, PAHs, Pyrene, Atrazine, TNT, etc.

3.0 FIELD IMPLEMENTATION

Treatability studies are commonly performed during or after development of remedial alternatives (feasibility study) where bioremediation is a potential site remedy. Treatability studies generally include bench-scale or pilot-scale studies and are used to further evaluate whether the proposed bioremediation remedy will be successful under site conditions. These studies also provide the design information required for full-scale implementation. Once designed and installed, a bioremediation system will require process and performance monitoring and possible modification to optimize the bioremediation system. The following sections provide design considerations for bench testing, pilot tests, and full-scale implementation. *Delivery and Mixing in the Subsurface: Processes and Design Principles for In Situ Remediation* (Kitanidis and McCarty 2012) provides a more detailed discussion of many topics in this section.

3.1 Treatability Studies

Bench-scale studies or pilot tests are important pre-design steps in determining whether a remedial technology is an implementable, cost-effective, and scalable treatment option. Successful bench-scale studies or pilot tests can justify implementing a full-scale bioremediation treatment system, while failure of bench or pilot tests may indicate that the bioremediation design needs to be reconsidered or possibly abandoned. Treatability studies are not unique to bioremediation, and the general approach to completing these studies is consistent among most in situ remedial technologies. There are several reference documents that present specific treatability study methods for various bioremediation strategies (AFCEE 2004, Stroo 2009).

3.1.1 Bench Test

Bench testing is performed on a sample of the site soil, groundwater, and bedrock, if present, collected for use in laboratory-scale treatment studies. Bench tests are generally used to evaluate:

- The performance of various amendments (biostimulation);
- Substrate demand and loading rates that can subsequently be tested in a pilot test;
- Whether addition of bacterial culture is needed (bioaugmentation);
- What consortium or combination of bacterial cultures is optimal; and
- The treatability of contaminants at different concentrations.

Bench testing is not typically performed for aerobic bioremediation studies and is often referred to as a microcosm study for anaerobic projects. Bench testing allows for easier manipulation and testing of many variables. If site conditions are favorable for a particular bioremediation approach, the cost of a bench test may outweigh the benefits. However, if site conditions are marginal, a bench test could be useful in evaluating whether bioremediation can be applied at a site before additional investment is made in pilot testing (AFCEE 2004). Bench testing may not always accurately reflect subsurface conditions in the field; however, downhole forms of microcosms, called biotraps, can also be used in the field as an alternative to bench studies. A biotrap is a passive sampling tool containing a matrix that encourages colonization by subsurface microbes. Biotraps can be used to test different amendments and microbe consortiums on a microcosm scale and calculate degradation rates.

3.1.2 Pilot Test

Pilot tests are usually small-scale field tests and typically include a set of injection wells or direct injection points and monitoring wells at varying distances within the pilot test treatment area. Monitoring wells may be positioned radially around the injection area when groundwater flow velocities are low, or at various downgradient distances when groundwater flow velocities are naturally higher or a circulation cell will be established. When possible, a tracer test using a conservative tracer, such as an ion salt (such as sodium bromide) or a dye (for example, fluorescein or rhodamine), should be completed as part of the start of the pilot test to help determine groundwater flow paths, dispersion, effective porosity, and velocity.

The results from pilot tests will help identify microbial response to biostimulation and provide design data regarding radii of influence of the injection wells and the performance of the amendment. Pilot test results are used to establish the full-scale injection well spacing and depth interval, as well as the quantity of amendment and the frequency of application. Pilot tests can also be used to evaluate bioaugmentation. A pilot test is generally designed with scalability in mind. For example, the designer will consider whether a given pilot test layout could be scaled up to treat the total target area within the cleanup and cost parameters of the project. In addition, a designer will decide whether other factors need to be considered, such as source water and electricity demands for pumps that may be in more remote locations during full-scale implementation. In some cases, a bench test will not be required if a pilot test is well designed (AFCEE 2004).

If bench testing is not completed, a pilot test may examine the effectiveness of multiple amendments and nutrients (biostimulation) and bacterial cultures (bioaugmentation), rather than simply confirming the results of the bench test. However, pilot testing of multiple amendments or cultures requires some design considerations to differentiate results. One design would be to test various amendments or cultures in different portions of the pilot test area separated by an unamended control area. Another design would be to test amendments sequentially. For example, Kovacich and others (2006) implemented a pilot test to evaluate anaerobic reductive dechlorination of a plume of TCE in groundwater. The pilot test was designed to deliver sodium lactate to a circulation system. Electron donor delivery problems resulted in poor performance and incomplete dechlorination. A second phase of the pilot test was implemented to evaluate direct injection of EVO to the area of the circulation system between the injection and extraction wells. The second phase of the pilot test ultimately showed that the technology could be applied to the site.

Pilot tests should be conducted for a period long enough to determine if complete biological degradation is achieved in addition to obtaining the design data previously mentioned. However, longer pilot tests could provide important information regarding amendment longevity, longer-term microbial and aquifer geochemical responses, potential maintenance issues, and contaminant rebound characteristics.

3.1.2.1 Biostimulation

Amendments can be applied to sites in various ways. Figure 11 shows three common application methods. Biological groundwater amendments have been applied using direct-push injection tooling, permanent injection wells (vertical and horizontal), infiltration trenches, and permeable reactive barriers (PRBs).

Determination of amendment quantities is highly dependent on the specific biological process targeted to achieve remediation. Required quantities can be calculated based on stoichiometry, estimates of biological demand, or rules of thumb found in guidance documents, literature, or provided by product vendors. Another consideration in calculating amendment quantities is the application rate. Application rates can be based on results of bench testing various contaminant concentrations. In the absence of bench testing, application of amendments at a uniform rate across a target treatment area still may not be the best approach. A more cost-effective methodology is often to focus the amendment in the zones (horizontally and vertically) of highest concentrations to address the areas of greatest flux and to reduce the application rate on the margins of the plume. It is important to recognize that the highest concentrations may not be in the most transmissive zones. Full-scale design considerations for source area or dissolved plume treatment are discussed in Section 3.2. Various amendment quantities can be identified for different zones and injection infrastructure can be designed for each zone. High-resolution vertical profiling of the aquifer characteristics and contaminant distribution would result in a more precise and targeted design.

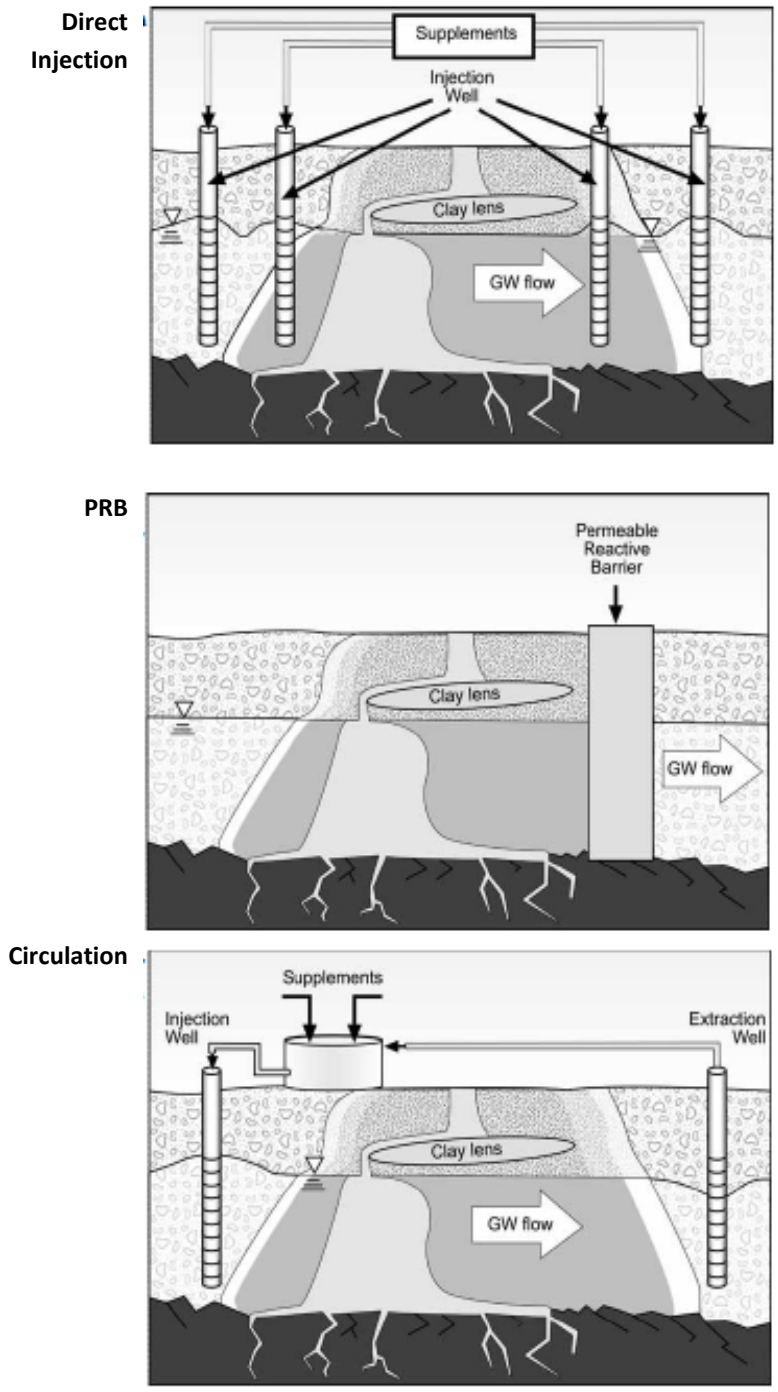

Figure 11. In situ Bioremediation System Configurations (EPA 2000), as adapted

3.1.2.2 Bioaugmentation

A site is typically bioaugmented after an aquifer has been biostimulated and favorable conditions for the target microbial community exist. MBTs can be utilized before, during, and after biostimulation to determine if bioaugmentation is necessary. If a site is selected for bioaugmentation, then bioaugmentation is usually tested as part of a pilot test. During the pilot test, inoculation with multiple cultures (in unique portions of the pilot test area) can be performed to evaluate each culture's performance and confirm bench testing with field data. MBTs can be used throughout the pilot-testing phase of a project to assist in evaluating whether the bioremediation application is performing as designed, how the microbial community changes over time given the electron donor and acceptor use and contaminant degradation, and whether the technology will likely achieve the remedial objectives (ITRC 2013).

3.2 Full-scale Implementation

Results from bench and pilot tests are used to guide full-scale design and implementation. A full-scale implementation approach is selected considering the site CSM, remedial goals, regulatory requirements, and future site use or development to design a remedial system that best meets the needs of the site with the lowest total life cycle cost. Design tools are available to assist with full-scale implementation. For example, several design tools were developed as part of project ESTCP ER-200626.

Figure 12 presents bioremediation treatment approaches applied at NPL sites from 1989 to 2008. For amendment delivery, 41 of the operating or completed projects listed in Appendix A use or used direct

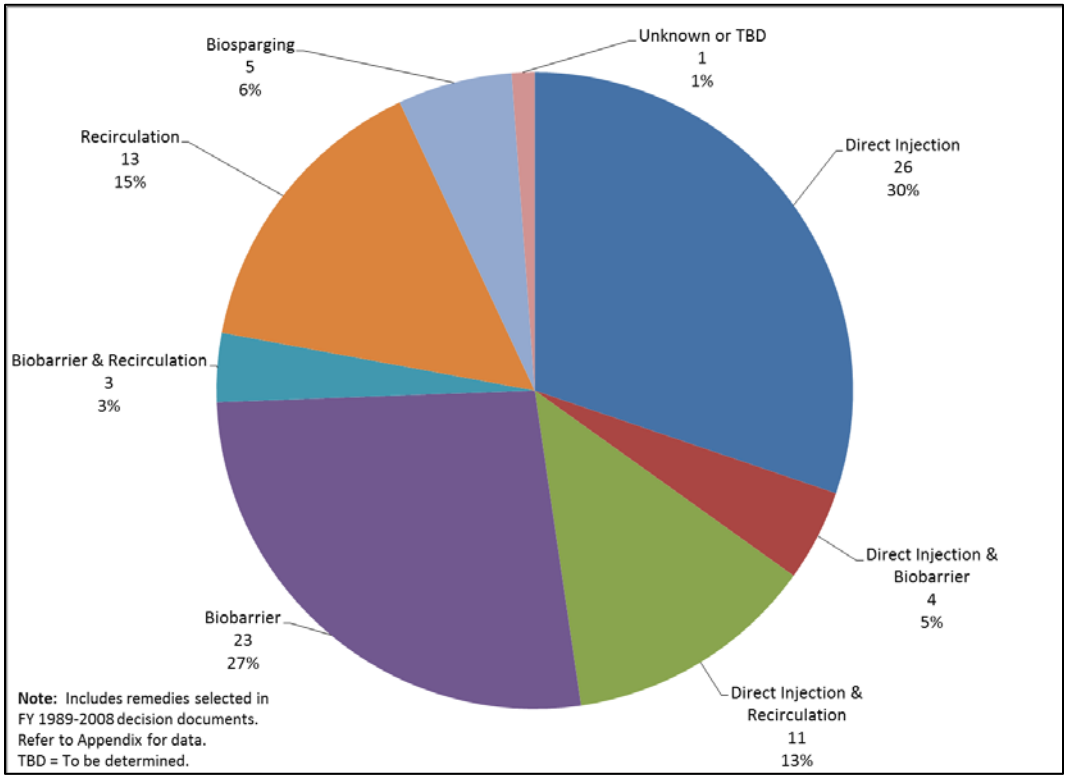

Figure 12. Bioremediation Design Types

injection or biobarriers/PRBs and 27 use or used groundwater recirculation. These categories are not mutually exclusive, and a single project may be counted in more than one category. Bioaugmentation was used at 18 of the projects in Appendix A. This section addresses three primary approaches to full-scale implementation that include active, semi-passive, and passive (Stroo 2009).

3.2.1 Active Treatment Approach

Active treatment approaches to bioremediation include circulation of groundwater in the target treatment area, as shown in Figure 13. Circulation requires significant capital cost to install the extraction wells, injection wells, associated conveyance lines, and the system building or skid where amendments will be stored and metered into the delivery system. Frequent operation and maintenance (O&M) is required to make system checks and adjustments. Circulation also requires a continuous source of power to run the circulation pumps. Water-soluble amendments or amendments that are emulsified are required for effective distribution throughout the target treatment area of a circulation system. Active systems can also distribute bacterial culture, if bioaugmented, much more effectively than semi-passive or passive methods. Circulation approaches can effectively treat target areas in less time and, as a result, may have lower total life cycle costs. Paired injection and extraction typically increases the hydraulic gradient at a site, thus increasing the rate of distribution and delivery of amendments. Active treatment is often applied to source areas and highly concentrated, smaller

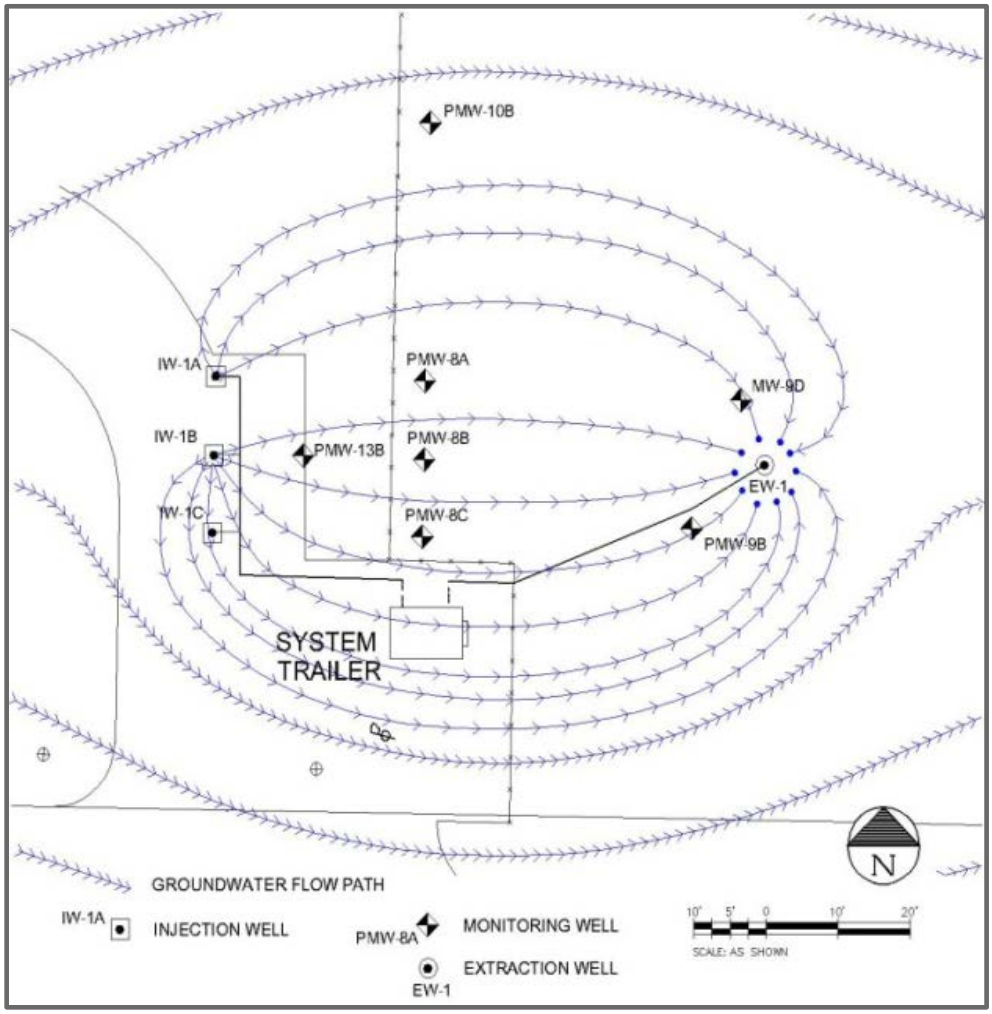

Figure 13. Typical Circulation System Layout (Kovacich and others 2006)

dissolved plumes where elimination of significant mass in a short time can provide the best value to an overall treatment program.

3.2.2 Semi-Passive Treatment Approach

Semi-passive treatment approaches to bioremediation are similar to active approaches. They also include circulation of groundwater and require the infrastructure and water soluble donors listed above, but are typically applied at sites where longer treatment times are acceptable. The primary difference between semi-passive and active systems is that semi-passive systems are not operated continuously. Amendments are circulated throughout the target treatment area in pulses. For example, amendment might be circulated for 3 months, perhaps long enough to circulate one pore volume within the target treatment area. Circulation is halted, and the site is monitored for a time to determine when additional amendments are required. Semi-passive treatment may take more time than active treatment, but may result in less energy consumption, less O&M, and less use of amendment. As a result, semi-passive treatment approaches may have a lower total life cycle cost than active approaches.

3.2.3 Passive Treatment Approach

Passive treatment approaches to bioremediation differ from active and semi-passive approaches in several ways. Groundwater is not circulated for extended periods, slow-release amendments are more often used, relatively little infrastructure is required, and treatment times can be longer. However, amendments and infrastructure can vary widely for passive approaches based on aquifer properties and amendment cost and longevity. Passive treatment approaches rely on natural flow of groundwater to deliver contaminated groundwater to biologically active areas where treatment occurs. The three most common treatment area amendment delivery configurations include a grid of injection points, a line of injection points, or a trench filled with substrate (a PRB), as shown in Figure 14. These treatment configurations and designs have been detailed in recent protocol documents from the Air Force Center for Engineering and the Environment (AFCEE, 2004, 2007, and 2008) and the Interstate Technology and

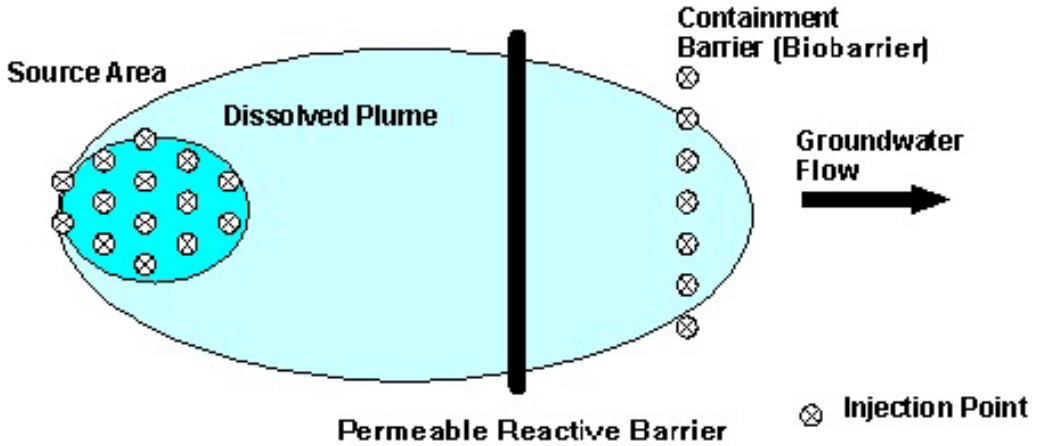

Figure 14. Schematic of Source Area and Barrier Injection Configurations. (Adapted from AFCEE 2004)

Regulatory Council (ITRC 2005). These configurations can also be used in combination with one another

and even in conjunction with semi-passive and active approaches. Additional details regarding each configuration are presented in the following sections.

3.2.3.1 Treatment Area Grid Configuration

Treatment area grids are used to address source areas and smaller dissolved plumes in groundwater, as shown in Figure 14. Treatment areas are often completed by direct-push injections in a grid pattern or by establishing a temporary circulation system to distribute substrate and bacteria, sometimes referred to as biozones. Generally, more closely spaced wells will increase drilling costs but reduce the duration of an injection event. Conversely, wells spaced farther apart will decrease drilling costs but increase the duration of an injection event. For example, the volume of amendment and water required to achieve coverage for each well in a grid spacing of 20 feet is four times the volume required for a grid spacing of 10 feet. Assuming the same injection rate, the time required would increase by a factor of four. (So, for example, an injection into wells with a 10-foot spacing that takes 6 hours would take 24 hours using wells with a 20-foot spacing.) Selecting the most cost-effective grid spacing for each site is highly dependent on drilling and amendment delivery implementation costs and is evaluated on a case-by-case basis. The primary cost drivers can be analyzed to identify the most cost-effective spacing, as illustrated in Figure 15, where the optimal well spacing is 12.5 feet.

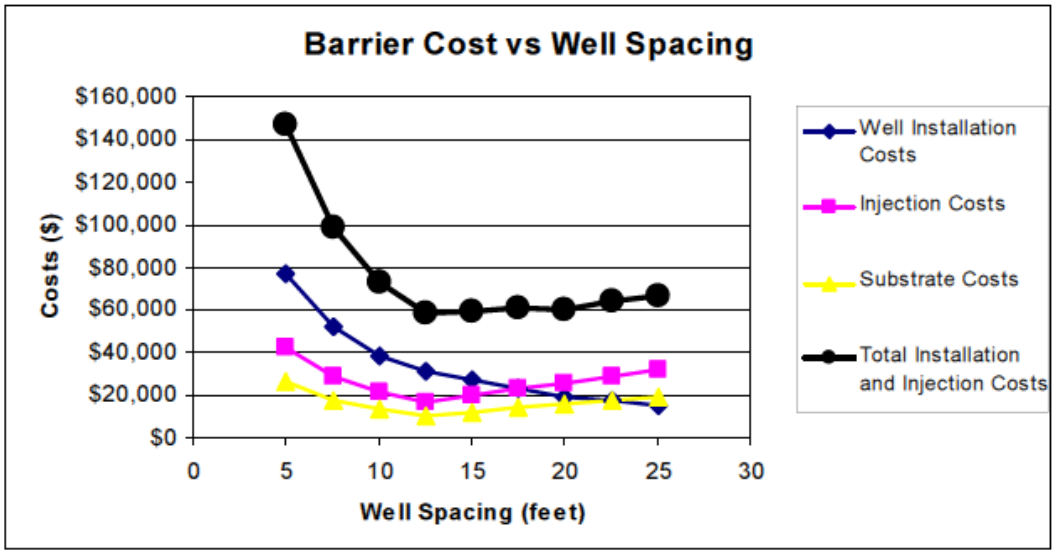

Figure 15. Example Cost Comparison for a PRB with Various Injection Well Spacings (ESTCP 2006)

3.2.3.2 Migrating Plume Barrier Configuration

Passive treatment zones that are created perpendicular to the axis of the plume are often called biobarriers or PRBs. Biobarriers are often applied at sites with large dissolved plumes where active and semi-active approaches are not cost effective. Biobarriers are also used to provide treatment and containment of the plume to prevent off-site migration or discharge to a vulnerable receptor.

3.2.3.3 Circulation Configuration

Circulation systems are one of the most efficient ways to distribute amendments and bacteria and are typically used in active and semi-passive treatment approaches. In a passive treatment approach, circulation systems can be used to distribute emulsified slow-release amendments. Some slow-release amendments remain suspended in emulsion for 5 to 10 days after they are injected. Circulation allows for greater hydraulic control and manipulation (in other words, induces a greater hydraulic gradient) and can be used to create treatment areas beneath buildings, active roads, runways, and other areas with limited site access. However, the total area that can be circulated is limited by the time the amendment remains suspended.

3.2.4 Vertical Application and Distribution

Implementation costs related to almost any technology increase with greater depth and treatment thickness. When injection wells are used, initial injection rates are typically similar to the theoretical transmittance capacity of the screens and are adjusted as needed in the field after startup to ensure distribution across the entire screened interval. Careful consideration for injection well screens is important. Large-diameter wells with high-flow screens may seem to be the best approach to inject fluids, but this approach may not result in effective distribution of amendments. For example, if a selected screen has a theoretical transmittance capacity of 2 gallons per minute (gpm) per foot and its total length is 10 feet, the overall theoretical transmittance capacity is 20 gpm. If the designed injection rate on that screen is only 5 gpm, there is a possibility that the screen will not be fully pressurized and injection materials will enter through the top 2.5 feet of screen or the most permeable interval. Well diameters and screen characteristics (openings size and type [slot versus continuous wrap]) need to be specifically designed with the aquifer material and target injection rate in mind. Once installed, thorough well development is required to maximize injection efficiency.

In a single injection point, multiple screens of shorter lengths (perhaps 5 to 10 feet long) may be required to achieve adequate vertical distribution, rather than one long screen (for example, 20 to 50 feet long). Injection wells intended to provide multiple injection depths are commonly installed as well clusters, sometimes bundeled in the same borehole or as individual wells in separate boreholes but closely spaced together. Figure 16 shows an example of an injection well design with multiple screened intervals within a single borehole that is currently in use at a passive bioremediaton site. The optimal injection well design will be site specific. The well cluster approach may have higher associated drilling costs but result in lower injection costs because downhole packers or other equipment are not needed.

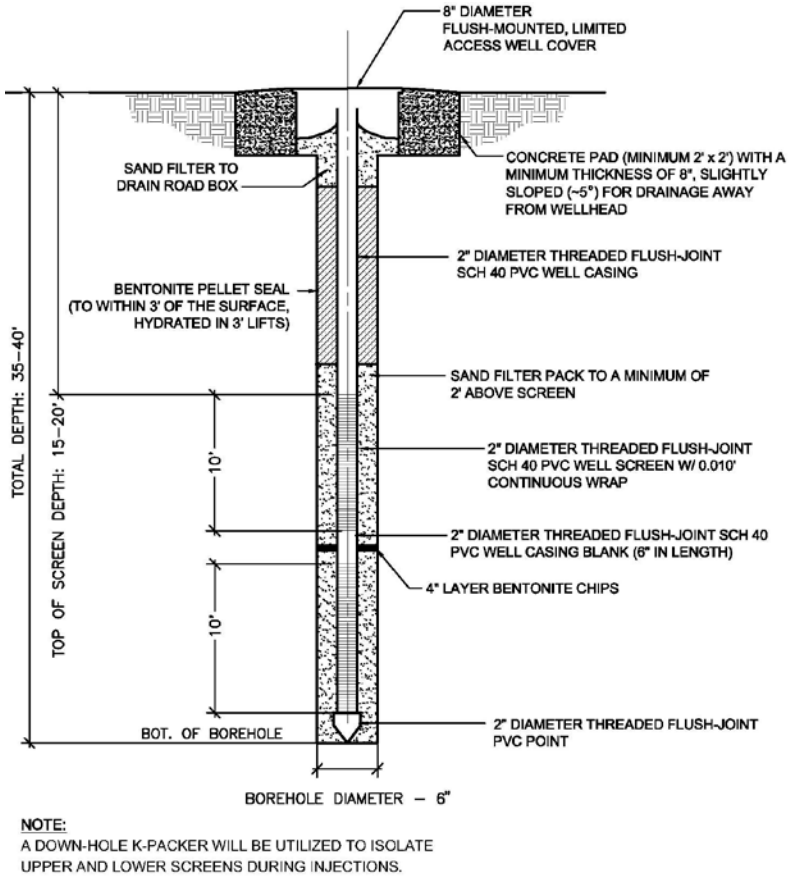

Figure 16. Example Injection Well Design. (Courtesy of Tetra Tech, Inc.)

3.2.5 Maintenance

Re-application of amendments, including electron acceptor or donor, will be required at most sites. Some sites may require geochemical adjustment and nutrient amendments. Maintenance applications are sometimes overlooked during the initial design, and consideration of future drilling costs associated with maintenance applications may require installation of permanent wells. New wells with shorter or focused screens or shorter target injection intervals may be needed in areas where data suggest amendment distribution is inadequate.

The success of biological technologies depends on the presence and persistence of the amendment (electron acceptor or electron donor) and maintaining the geochemical conditions in groundwater that will allow biological populations to flourish. As discussed in Section 1.5.4.2, pH in an aquifer is critical to the performance of bioremediation systems. Adjustment of pH during subsequent maintenance applications, typically with the addition of a base like sodium bicarbonate or sodium hydroxide may be needed; however, pre-design data should inform a practitioner of the potential need for post-installation pH adjustment. An effective performance monitoring program, as discussed in Section 3.3, is required to decide when additional amendments are required to maintain the biologically active zone.

Before amendments are applied to a site, the injection wells must be evaluated to assure they can still operate at their designed injection rates.

Well screen fouling can be a major maintenance issue for aerobic treatment, including calcium and iron precipitation and biological fouling by iron bacteria. Injection well maintenance typically includes well development to remove precipitates and biological films on the screen and gravel pack and usually involves chemical treatment with acids. Biomass buildup in injection wells can also be problematic to reductive dechlorination sites if too much substrate is applied and injection wells are inadequately flushed with water after injection. General maintenance may include use of a downwell video camera to monitor fouling, periodic cleaning with weak organic or inorganic acids, biocides, bleach, or chlorine dioxide for biomass, and well re-development.

3.3 Measuring Performance

As with any site cleanup, it is important to measure progress toward the remedial objectives[1]. Measuring remedial performance is critical to its optimization and long-term applicability to the site. Depending on the remedial objectives, it may take a few years or decades to reach remedial objectives.

Key questions to ask when a monitoring approach is developed include (ITRC 2011):

- What media should be monitored?
- What constituents should be monitored?
    - Beyond the contaminants of concern (COCs), what other parameters should be monitored to establish multiple lines of evidence to evaluate performance?
    - How many lines of evidence are needed for an assessment toward an objective?
- What metrics should be used?
- Where should monitoring points be located?
- When should monitoring occur?

With biologically dependent remedies, the initial monitoring is critical to the overall success of the remedy to ensure aquifer geochemistry has responded as bench and pilot testing suggested; target bacterial communities are established, sustained, and thriving; and to track the general biogeochemical responses to the initial installation. For example, initial performance monitoring could be monthly for a quarter, followed by quarterly for a year, and semi-annually thereafter. It is important to evaluate performance data after each injection event to ensure that expected trends are observed. Even at sites where initial performance is promising, the groundwater monitoring program must consider the possibility of rebound caused by various factors, including matrix diffusion (as described in Section 1.6.3.4).

[1]For Superfund program information on monitoring performance and progress of groundwater remedies, see www.epa.gov/superfund/health/conmedia/gwdocs/pdfs/gwroadmapfinal.pdf

The expected trends vary for each bioremediation strategy, but generally include reductions in contaminant concentrations, stable redox conditions, stable geochemistry, and adequate amendment concentration. However, a short-term increase in contaminant mass may occur initially after installation as a result of changes in the equilibrium between the contaminant phases (adsorbed, dissolved, and NAPL) and liberation of contaminant into the dissolved phase. The following conditions may indicate poor performance of a bioremediation remedy:

- Limited, incomplete, or no reduction, and even increases in contaminant concentrations (although temporary increases in contaminant concentrations can be expected near source zones as a result of increased dissolution);
- Trending toward aerobic conditions in an anaerobic remedy and trending toward anaerobic conditions in an aerobic remedy;
- Increasing concentrations of competing electron acceptors or donors, for anaerobic and aerobic remedies; and
- Sharply decreasing concentrations of amendments or amendment concentrations below those necessary to support bioremediation at a site.

When these conditions occur, modifications in the system may be required to improve performance. Possible modifications may include changes in the method of amendment delivery, changes in the selected amendment, and conditioning the aquifer geochemistry.

Performance monitoring at aerobic bioremediation sites typically tracks three key indicators:

- The concentration of oxygen and compounds being used as a source of oxygen. Dissolved oxygen can be measured in the field;
- The redox conditions within the aquifer and concentrations of primary terminal electron acceptors. ORP measurements in the field can provide the general redox state of an aquifer; and
- The concentration of contaminants and daughter products.

Microbial testing is not commonly conducted with most aerobic bioremediation sites, which are associated with hydrocarbons.

Performance monitoring at anaerobic bioremediation sites typically tracks four key indicators:

- The concentration of competing electron acceptors and resulting reduced states as an indicator of redox conditions. ORP measurements in the field can provide the general redox state of an aquifer;
- The concentration of organic carbon (total and dissolved) and substrate breakdown products, such as volatile fatty acids, to make sure adequate donor is present and in a useable form for the target bacteria populations;
- The concentration of contaminants and dechlorination daughter products. Evaluation of the basis of molar concentration provides insight on the conversion of contaminant mass. Various dissolved hydrocarbon gases, such as ethene and ethane, require specialty analysis other than

the standard VOC scan. Methane is also included in that analysis, which provides further information on the redox conditions; and

- The concentration of the target bacterial populations. Groundwater or filter media through which groundwater has passed can be processed for deoxyribonucleic acid (DNA) sequencing by qPCR or possibly other MBTs (ITRC 2013). qPCR can quantify target bacterial groups (such as *Dehalococcoides*), as well as quantification of a subset of *Dehalococcoides* with genes that can perform vinyl chloride reduction to ethene, vinyl chloride reductase (vcrA).

A performance monitoring program is intended to measure performance at key locations within a plume. Generally, wells would be located within the biologically active area, immediately downgradient of the biologically active area, and farther downgradient at distances based on site seepage velocities, monitoring frequency, and any regulatory requirements.

Overall, densities of targeted bacterial populations should increase with time and reach optimal levels ($>10^7$ cells/L), geochemical conditions must remain favorable, and contaminant levels should decrease in all performance monitoring wells and eventually in downgradient point of compliance wells for successful bioremediation applications. If progress toward remedial objectives is not adequate, reevaluation of a remedy could be warranted (ITRC 2011, EPA 2011).

4.0 EMERGING TRENDS

The field of bioremediation is still a relatively young discipline, and new developments occur each year. The following sections provide a summary of some emerging trends.

4.1 Environmental Remedy Footprint

Increasing attention is being paid to the environmental footprint of implementing a remedy. An environmental footprint includes energy usage, air emissions, water usage, materials usage, and waste generation. ISB treatment options often result in a smaller environmental footprint onsite than ex situ or non-biological methods. For example, ex situ options are energy intensive because of the need to remove and transport large quantities of soil or groundwater. ISB, however, allows treatment without transportation of the contaminated media. The approach to implementing an ISB remedy also can have significant effects on the environmental footprint of the remedy. For example, using extracted groundwater to blend and inject the electron donor has a lower water footprint than using potable water for this purpose. Furthermore, using an electron donor that is a food-grade byproduct or waste product from the food preparation industry can have a lower footprint than using a specially prepared electron donor. Using multiple, long-term direct-push events may have a larger energy and air emission footprint than using permanent injection wells. Thorough consideration of the appropriate design parameters for a successful remedy and consideration of the remedy components that contribute the most to the remedy's environmental footprint can lead to a reduction in the footprint and successful remedies (EPA 2012b).

4.2 Compound Specific Isotope Analysis

The following section references the EPA document, "*A Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants using Compound Specific Isotope Analysis (CSIA).*" CSIA is an environmental forensics technique used to characterize contaminated sites and track the progress of bioremediation and natural attenuation. CSIA measures and compares the ratios of stable isotopes found in compounds of suspected contaminant sources or plumes as well as the feedstock or manufacturing process of materials historically used in the vicinity of the site. Isotopic analysis can help identify various sources of the same compound based on their different isotopic "signatures." It also can be used to evaluate the extent of contaminant degradation caused by microbes. Typical forensic stable isotopes include carbon, hydrogen, chloride, sulfur, and oxygen. However, the majority of the work is done with carbon isotopes (EPA 2008).

There are several techniques to study biodegradation in groundwater that involve the addition of contaminants that are artificially labeled with a carbon isotope (usually 13C-label). Examples include stable isotope probing (SIP) and Bio-Sep beads (media) amended with 13C-labeled substrates. The media can be placed in groundwater wells to conduct in situ SIP studies. The isotopically-enriched contaminant is applied to the medium and the medium is incubated in a well for a given period of time. These techniques work in much the same way as radiocarbon labeling; the 13C-label is used to track the transfer of carbon from the substrate to its metabolites, or to the dissolved inorganic carbon pool, and its subsequent incorporation into the microbial biomass. The disappearance of the label from the substrate pool is convincing evidence that the targeted compound is indeed degrading, and the

identification of 13C-label in microbial biomass is definitive proof that the compound was biologically degraded (EPA 2008). The ITRC *Environmental Molecular Diagnostics Fact Sheets* released in April 2013 (ITRC 2013) also discuss compound-specific isotope analysis and SIP in detail and provide examples of applications and limitations.

4.3 High-Resolution Site Characterization

Effective implementation of remedial technologies, especially in situ methods such as ISB, requires accurate site characterization. In particular, the use of HRSC can vastly improve the CSM. HRSC has become more prominent as sampling techniques, data evaluation, and presentation methods have improved. HRSC strategies and techniques use scale-appropriate measurement and sample density to define contaminant distributions, and the physical context in which they reside, with greater certainty, supporting faster and more effective site cleanup (CLU-IN 2013). The data obtained from HRSC are used to develop an accurate CSM by identifying heterogeneities in the subsurface that significantly influence contaminant distribution, fate, and transport. These heterogeneities can occur at very small scale that conventional investigation strategies and technologies (primarily placing monitoring wells at biased locations to delineate extent of contamination) can miss.

HRSC uses transects of vertical subsurface profiles oriented perpendicular to the direction of groundwater flow. Profiles located along each transect are used to collect high-resolution lithologic, hydrogeologic and contaminant data using real-time direct sensing tools implemented using direct push technology (DPT). Lithologic data are collected using such technologies as cone penetrometer testing (CPT), various electrical conductivity (EC) probes, and hydraulic profiling tools. The hydrogeologic data are best provided by real-time hydraulic profiling tools. Contaminant data are provided using such technologies as Laser Induced Fluorescence (LIF), Membrane Interface Probe (MIP), and Tar-specific Green Optical Screening Tool (TarGOST®).

4.4 3-D Visualization and Analysis of Data and In Situ Sensors

Several software programs are available to perform 3DVA of site characterization and performance monitoring data. These programs are useful for designing amendment delivery systems and identifying which portions of a plume may require additional amendments. Some practitioners are combining in situ sensors (pH, dissolved oxygen, ORP, chloride, and conductivity) and a web-based interface to facilitate continuous monitoring and evaluation additional amendment needs.

The 3DVA programs typically use geostatistical kriging procedures to establish the spatially-accurate distribution of each parameter in three dimensional space. Figure 17 presents an example of three-dimensional kriging using C Tech Corporation's Mine Visualization System (MVS) software. Integrated visualizations can be made when HRSC and performance monitoring data are combined, providing increased understanding of contaminant distribution and behavior. Visualizations can be fully articulated to enable site conditions to be viewed from any vantage point of interest, allowing for more rigorous analysis. However, the visualizations are only as accurate as the data used to prepare them and the software skill and geostatistical knowledge of the modeler. Uncertainty will always exist between data points. Characterizing sites using HRSC will increase data density and reduce uncertainty.

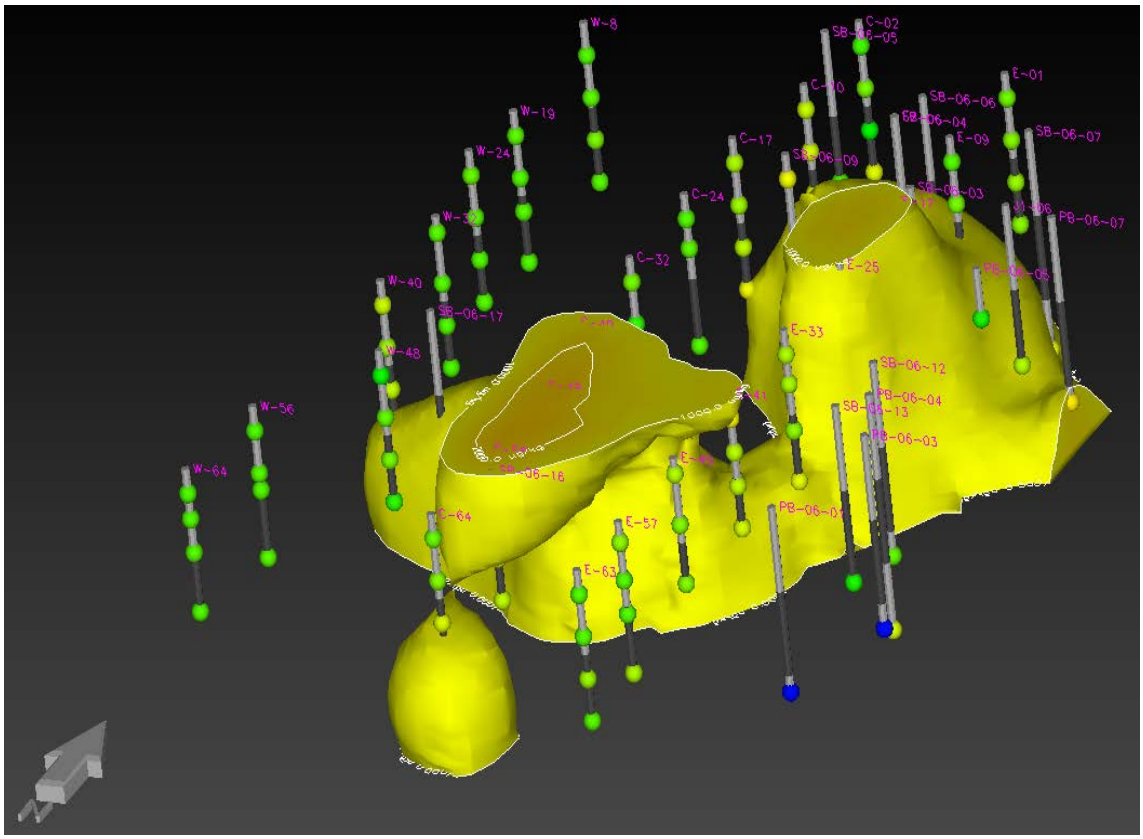

Figure 17. Example of Geostatistical Kriging Analysis of Multi-depth TCE Soil Concentrations. (Courtesy of Tetra Tech, Inc.)

5.0 SUMMARY

ISB can be applied to the treatment of source materials or plumes, and has proven applicable to numerous inorganic and organic contaminants. Several different biochemical pathways have been used, numerous amendments have been developed, and numerous methods have been implemented to deliver those amendments. Implementation of ISB is highly flexible, often using one or a combination of active, semi-passive, or passive delivery systems. Amendments can be liquid, solid, or gaseous to serve as electron donors, acceptors, cometabolites, and nutrients. Table 3 provides a summary of ISB strategies presented in this document. Clear guidance documents on the most widely used forms of ISB have been developed and are widely available. However, site-specific testing is usually appropriate before the final design is completed to help identify the optimal amendment type, amendment quantity and delivery system.

Effective implementation of ISB often requires careful monitoring with the potential for adjustments to the amendments and the delivery system. After treatment, several years may be required before conditions re-equilibrate to pre-treatment and pre-impact conditions. Finally, innovation continues and several emerging trends will affect the selection, design, and operation of ISB systems in the future.

Table 3. Summary of ISB strategies

ISB Strategy	Aerobic	Anaerobic Oxidative	Anaerobic	Aerobic Cometabolism
Key Characteristics	Relies on presence of oxygen	Relies on addition or use of other electron acceptors besides oxygen	Relies on electron donor additions uses contaminants as electron acceptors Anaerobic metabolism includes fermentation, methanogenesis, reductive dechlorination, sulfate- and iron-reducing activities, and denitrification	Relies on addition of cosubstrates for fortuitous degradation of contaminants May be used under aerobic or anaerobic, based on the redox state of the contaminant
Target Contaminants	Petroleum hydrocarbons and some fuel oxygenates Ionic form of metals	Petroleum hydrocarbons present in reducing conditions	Chloroethenes and chloroethanes Perchlorate, Munitions, Chromate, and Nitrate	May be applicable to: PAHs, Explosives, Dioxane, NDMA, PCBs, Pesticides, MTBE, Chloroethenes, Chloroethanes, Chloroform, and methylene chloride
Advantages	Widespread acceptance with documented success for treating target contaminants Aerobic bacteria responsible for degradation are generally ubiquitous in nature	May be applied to highly reduced plumes	Widespread acceptance with documented success for treating target contaminants Documented success in high concentration source material Abiotic degradation often occurs parallel to biological degradation processes	May be able to treat contaminants to low cleanup levels
Limitations	Some petroleum derived plumes are very reduced requiring high doses of oxygen Delivery systems may encounter significant biological fouling	Limited use to date Can be difficult to distinguish from microaerophilic oxidation	Sensitivity to specific range of geochemical conditions May require bioaugmentation with commercially available microbial cultures	Limited use to date in field applications Inhibitory intermediate products can be produced Substrate pulsing may be needed to reduce competitive inhibition between use of substrate and contaminant by the microorganisms

6.0 REFERENCES

Adeniji, Adebowale. 2004. *Bioremediation of Arsenic, Chromium, Lead, and Mercury*. National Network of Environmental Management Studies Fellow Paper Prepared for the U.S. Environmental Protection Agency Office of Solid Waste and Emergency Response. August.

Agency for Toxic Substances and Disease Registry (ATSDR). 2005. Public Health Assessment Guidance Manual (Update). U.S. Department of Health and Human Services. Public Health Service. Atlanta, Georgia.

ATSDR. 2006. Toxicological Profile for 1,1,1-Trichloroethane. July.

Air Force Center for Energy and the Environment (AFCEE). 2004. *Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents*. August. On-line address: www.costperformance.org/remediation/pdf/principles_and_practices_bioremediation.pdf.

AFCEE. 2007. *Final Technical Protocol for In Situ Bioremediation of Chlorinated Solvents Using Edible Oil*. Technical Directorate. Environmental Science Division. October. On-line address: www.clu-in.org/download/remed/Final-Edible-Oil-Protocol-October-2007.pdf

AFCEE. 2008. *Final Technical Protocol for Enhanced Anaerobic Bioremediation Using Permeable Mulch Biowalls and Bioreactors.* Technical Directorate. Environmental Science Division. May. On-line address: www.clu-in.org/download/techdrct/Final-Biowall-Protocol-05-08.pdf.

Alexander M. 1994. *Biodegradation and Bioremediation*. Academic Press, New York, NY. 286 p.

Alvarez-Cohen, L. and P. L. McCarty. 1991. "Product Toxicity and Cometabolc Competitive Inhibition Modeling of Chloroform and Trichloroethylene Transforation by Methanotrophic Resting Cells." *Appl Environ Microbiol.* Vol. 57 No. 4. Pages 1031-37.

Anderson, R.T., H.A. Vrionas, I. Ortiz-Bernad, C.T. Resch, P.E. Long, R. Dayvault, K. Karp, S. Marutsky, D.R. Metzler, A. Peacock, D.C. White, M. Lowe, D.R. Lovley. 2003. "Stimulating the *in situ* activity of Geobacter species to remove uranium from the groundwater of a uranium-contaminated aquifer." *Appl Environ Microbiol.* Vol. No. 69. Pages 5884-91.

Balk, Melike, T. van Gelder, Sander A. Weelink, Alfons J.M. Stams. 2008. "(Per)chlorate Reduction by the Thermophilic Bacterium *Moorella perchloratireducens* sp. nov., Isolated from Underground Gas Storage." *Appl Environ Microbiol*. Vol. No. 74. Pages 403-409.

Balk, Melike, F. Mehboob, A.H. van Gelder, W.I. Riipstra, J.S. Damste, Alfons J.M. Stams. 2010. "(Per)chlorate Reduction by an acetogenic bacterium, *Sporomusa* sp., Isolated from an Underground Gas Storage." *Appl Microbiol Biotechnol*. Vol. No. 88. Pages 595-603.

Bamforth, S.M. and I. Singleton. 2005. *Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions*. *J Chem Technol Biotechnol*. **80**:723–736.

Banat, I. M., Makkar, R. S., Cameotra, S. S. 2000. Potential commercial applications of microbial surfactants. Appl. Microbiol. Biotechnol. 53, 495-508.

Borden, Robert C., M. Tony Lieberman. 2008. *Passive bioremediation of perchlorate using emulsified vegetable oils.* In situ Bioremediation of Perchlorate in Groundwater. Springer Science + Business Media. New York. Pages 155-172.

Bouwer, E.J., and McCarty, P.L. 1984. Modeling of trace organics biotransformation in the subsurface. Ground Water. 22(4):433-440.

Bradley P. M., F.H. Chapelle. 1997. Kinetics of DCE and VC mineralization under methanogenic and Fe(III)-reducing conditions. Environmental Science and Technology. 31:2692-2696.

Bradley, P.M. 2007. Dichloroethene and vinyl chloride degradation potential in wetland sediments at Twin Lakes and Pen Branch, Savannah River National Laboratory, South Carolina. U.S. Geological Survey Open File Report 2007-1028, 21p.

Brigmon, R.L. 2001. *Methanotrophic Bacteria: Use in Bioremediation.* Prepared for the U.S. Department of Energy. Contract No. DE-AC09-96SR18500. On-line address: http://sti.srs.gov/fulltext/ms2001058/ms2001058.html.

Brubaker, G.R. and H.F. Stroo. 1992. In situ bioremediation of aquifers containing polyaromatic hydrocarbons. J. Hazard. Materials 32:163-177.

CL:AIRE SABRE Bulletin SAB 1. 2010. *Project SABRE (Source Area BioRemediation) – an Overview*.

CLU-IN. 2006. Technology News and Trends. February. On-line address: www.clu-in.org/products/newsltrs/tnandt/view.cfm?issue=0206.cfm.

CLU-IN. 2008. Technology News and Trends. January. On-line address: www.clu-in.org/products/newsltrs/tnandt/view.cfm?issue=0108.cfm#4.

CLU-IN. 2013. www.clu-in.org/characterization/technologies/hrsc/index.cfm. Accessed 3/28/2013

Coates, J.D. and W.A. Jackson. 2008. *Principles of Perchlorate Treatment.* In situ Bioremediation of Perchlorate in Groundwater. Springer Science + Business Media. New York. Pages 29-53.

Cope N. and J.B. Hughes. 2001. Biologically-enhanced removal of PCE from NAPL source zones. Environmental Science and Technology. 35:2014-2021.

Cwiertny, D.M. and M.M. Scherer. 2010. Abiotic Processes Affecting the Remediation of Chlorinated Solvents. Pages 69-108 In Stroo HF and CH Ward (eds.), In Situ Remediation of Chlorinated Solvent Plumes. SERDP/ESTCP Remediation Technology Monograph Series, Springer Science & Business Media, LLC. New York, NY. 725 p.

Dennis, P., J. Roberts, and S. Dworatzek. 2011. How Low Can You Go? Bioremediation of Chlorinated Ethenes in Cold Groundwater Abstract and Platform Presentation: REMTEC, Chicago, Illinois. May 16-19.

Duhamel, M., S.D. Wehr, L. Yu, H. Rizvi, D. Seepersad, S. Dworatzek, E.E. Cox, E.A. Edwards. 2002."Comparison of anaerobic dechlorinating enrichment cultures maintained on tetrachloroethene, trichloroethene, cis-dichloroethene and vinyl chloride." *Water Research* 36:4193-4202.

Ely, R.L., Hyman, M.R., Arp, D.J., Guenther, R.B., and Williamson, K.J. 1995. A cometabolic kinetics model incorporating enzyme inhibition, inactivation, and recovery: II. Trichloroethylene degradation experiments: Biotechnology and Bioengineering, v. 46, p. 232-245.

Ely, R.L., K.J. Williamson, M.R. Hyman, and D.J. Arp. 1997. Cometabolism of chlorinated solvents by nitrifying bacteria: kinetics, substrate interactions, toxicity effects and bacterial response: Biotechnology and Bioengineering, v. 54, no. 6, p. 520-534.

Environmental Security Technology Certification Program (ESTCP). 2006. *Protocol for Enhanced In situ Bioremediation Using Emulsified Edible Oil*. ER-0221-Protocol.

ESTCP. 2008. *Protocol Report: Natural Attenuation of Perchlorate in Groundwater: Processes, Tools, and Monitoring Techniques*. ER-0428. August.

ESTCP. 2013. *Fact Sheet: Improving Effectiveness of Bioremediation at DNAPL Source Zone Sites Applying Partitioning Electron Donors*. ER-200716. www.serdp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Persistent-Contamination/ER-200716 (Accessed 3/7/13).

Ernst, T. 2009. *Use of "Dehalococcoides" to bioremediate groundwater contaminated with chlorinated solvents*. MMG 445 Basic Biotechnology. Vol. 5. No. 1; pages 72-77.

Farhadian M and others. 2008. In situ bioremediation of monoaromatic pollutants in groundwater: A review. *Bioresource Technol.* 99:5296-5308.

Fetter, C.W. 2000. Applied Hydrogeology 4th edition

Fields, K.A. and G.B. Wickramanayake (Chairs). 2010. *Remediation of Chlorinated and Recalcitrant Compounds—2010*. Seventh International Conference on Remediation of Chlorinated and Recalcitrant Compounds. Battelle Memorial Institute, Columbus, Ohio. May.

Firestone, M.K. 1982. Biological denitrification. In F.J. Stevenson, ed., *Nitrogen in Agriculture Soils.* American Society of Agronomy, Madison, WI. pp. 289-326.

Fournier, D., J. Hawari, A. Halasz, S.H. Streger, K.R. McClay, H. Masuda, P.B. Hatzinger. 2009. Aerobic biodegradation of *N*-nitrosodimethylamine by the propanotroph *Rhodococcus ruber* ENV425. *Appl. Environ. Microbiol.* 75(15):5088-5093.

Guilbeault, M.A., B.L. Parker, and J.A. Cherry. 2005. Mass and Flux Distributions from DNAPL Zones in Sandy Aquifers. Ground Water 43(1):70-86.

Gossett, J.M. 2010. Sustained aerobic oxidation of vinyl chloride at low oxygen concentrations. *Environ. Sci. Technol.* 44:1405-1411.

Hatzinger P.B., Condee C., McClay K.R., Paul Togna A. 2011, *Aerobic treatment of N-nitrosodimethylamine in a propane-fed membrane bioreactor*. Water Res. 2011 Jan;45(1):254-62.

Hatzinger, P.B. and others. 2008. Bioremediation Approaches for Treating Low Concentrations of N-Nitrosodimethylamine in Groundwater. SERDP Project ER-1456. October. On-line address: www.serdp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Emerging-Issues/ER-1456/ER-1456/(language)/eng-US

Hazen, T.C. 2009. Cometabolic Bioremediation. LBNL-1694E. 16 pages. On-line address: www.clu-in.org/download/techfocus/biochlor/Hazen_cometabolic_bio_2009.pdf

He, J., Y. Sung, R. Krajmalnik-Brown, K. Ritalahti and F. Löffler. 2005. Isolation and characterization of Dehalococcoides sp. strain FL2, a trichloroethene (TCE)- and 1,2-dichloroethene-respiring anaerobe. *Environmental Microbiology*.7 (9), 1442–1450.

Hendrickx, B., W. Dejonghe, W. Boënne, M. Brennerova, M. Cernik, T. Lederer, M. Bucheli-Witschel, L. Bastiaens, W. Verstraete, E.M. Top, L. Diels and D. Springael. 2005. *Dynamics of an Oligotrophic Bacterial Aquifer Community during Contact with a Groundwater Plume Contaminated with Benzene, Toluene, Ethylbenzene, and Xylenes: an In Situ Mesocosm Study*. *Appl. Environ. Microbiol*. vol. 71 no. 7, 3815-3825.

Henry, B. 2010b. Loading Rates and Impacts of Substrate Delivery for Enhanced Anaerobic Bioremediation — Final Report. ESTCP, Project ER-0627, 476 pp. www.clu-in.org/download/contaminantfocus/dnapl/Treatment_Technologies/ER-0627-FR-1.pdf.

Hood E.D. and others. 2008. Groundwater Monitoring & Remediation 28 No. 2, Pages 98-107.

Interstate Technology & Regulatory Council (ITRC). 2002. *A Systematic Approach to In Situ Bioremediation in Groundwater: Decision Trees on In Situ Bioremediation for Nitrates, Carbon Tetrachloride, and Perchlorate*. ISB-8. Washington, D.C.: Interstate Technology & Regulatory Council, In situ Bioremediation Team. On-line address: www.itrcweb.org.

ITRC. 2005. *Overview of In Situ Bioremediation of Chlorinated Ethene DNAPL Source Zones*. BIODNAPL-1. Washington, D.C.: Interstate Technology & Regulatory Council, Bioremediation of Dense Nonaqueous Phase Liquids (Bio DNAPL) Team. On-line address: www.itrcweb.org.

ITRC. 2008. *In Situ Bioremediation of Chlorinated Ethene: DNAPL Source Zones*. BIODNAPL-3. Washington, D.C.: Interstate Technology & Regulatory Council, Bioremediation of Dense Nonaqueous Phase Liquids (Bio DNAPL) Team. On-line address: www.itrcweb.org.

ITRC. 2010. *Use and Measurement of Mass Flux and Mass Discharge*. MASSFLUX-1. Washington, D.C.: Interstate Technology & Regulatory Council, Integrated DNAPL Site Strategy Team. On-line address: www.itrcweb.org.

ITRC. 2011. *Integrated DNAPL Site Strategy*. IDSS-1. Washington, D.C.: Interstate Technology & Regulatory Council, Integrated DNAPL Site Strategy Team. On-line address: www.itrcweb.org.

ITRC. 2013. *Environmental Molecular Diagnostics Fact Sheets.* EMD-2. Washington, D.C.: Interstate Technology & Regulatory Council, Environmental Molecular Diagnostics Team. On-line address: www.itrcweb.org.

Kasenow, M. 2010. *Applied Ground-Water Hydrology and Well Hydraulics (3rd Ed)*. Water Resources Publns. Highlands Ranch, CO.

Kitanidis, P.K. and McCarty, P.L. (eds). 2012. *Delivery and Mixing in the Subsurface: Processes and Design Principles for In Situ Remediation*. SERDP ESTCP Environmental Remediation Technology Vol. 4.

Kovacich, M., D. Beck, P. Rich, and M. Zack. 2006. Direct-Push Injection and Circulation Biobarrier to remediate a TCE Groundwater Plume. Platform Paper – Remediation of Chlorinated and Recalcitrant Compounds, the Fifth International Conference, sponsored by Battelle.

Lee, Ming-Kuo and James A. Saunders. 2003. "Effects of pH on Metals Precipitation and Sorption: Field Bioremediation and Geochemical Modeling Approaches." *Vadose Zone Journal.* Vol. 2; pages 177-185.

Lendvay, J.M., and others. 2003. Bioreactive Barriers: A Comparison of Bioaugmentation and Biostimulation for Chlorinated Solvent Remediation. *Environmental Science and Technology*. Vol. 37; Pages 1422-1431.

Lipson, D.S., and others. 2005. Matrix Diffusion-Derived Plume Attenuation in Fractured Bedrock. *Groundwater.* Vol. 43. No. 1; pages 30-39.

Loehr, R.L., W.L. Jewell, J.D. Novak, W.W. Clarkson, and G.S. Friedman. 1979. Land Application of Wastes. Van Nostrand Reinhold Co., New York, NY.

Löffler F., K.M. Ritalahti, S.H. Zinder. 2013. *Dehalococcoides* and Reductive Dechlorination of Chlorinated Solvents. Pages 39-88 In Stroo H and others (eds), Bioaugmentation for Groundwater Remediation. SERDP/ESTCP Remediation Technology Monograph Series, Springer Science & Business Media, LLC. New York, NY. 361 p.

Lovley, Derek R. and John D. Coates. 1997. "Bioremediation of metal contamination." *Biotechnology*. Vol. 8; pages 285-289. On-line address: http://ijs.sgmjournals.org/content/early/2012/04/23/ijs.0.034926-0.short

Mahendra, S. and L Alvarez-Cohen. 2006. Kinetics of 1,4-dioxane biodegradation by monooxygenase-expressing bacteria. *Environ. Sci. Technol.* 40 (17):5435–5442

Maymo-Gatell, X., Y. Chien, J.M. Gossett, and S.H. Zinder. 1997. "Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene." *Science.* Vol. 276. Pages 1568-71.

Meckenstock, R. U., E. Annweiler, W. Michaelis, H. H. Richnow, and B. Schink. 2000. *Anaerobic Naphthalene Degradation by a Sulfate-Reducing Enrichment Culture*. Appl Environ Microbiol. 66(7): 2743–2747.

Middeldorp PJM, M.L.G.C. Luijten, B.A. van de Pas, M.H.A. van Eekert, S.W.M. Kengen, G. Schraa, A.J.M. Stams. 1999. Anaerobic microbial reductive dehalogenation of chlorinated ethenes. Bioremediation Journal. 3:151-169.

National Research Council (NRC). NRC. 1993. In situ Bioremediation: When Does It Work? National Academy of Sciences. Washington, DC. 184 p.

NRC 2004. Contaminants in the Subsurface: Source Zone Assessment and Remediation, National Academy Press, Washington, D.C. 65p.

Okpokwasili, G.C and C.O. Nweke. 2005. "Microbial Growth and Substrate Utilization Kinetics." *African Journal of Biotechnology*. Vol. 5 (4); pages 305-317.

O'Loughlin, E.J. and D.R. Burris. 2004. "Reduction of halogenated ethanes by green rust." *Environmental Toxicology and Chemistry*. 23: 41–48.

Palmisano, A. and T. Hazen. 2003. *Bioremediation of Metals and Radionuclides: What It Is and How It Works (2nd Edition)*. Lawrence Berkeley National Laboratory.

Raymond, R. L., V. W. Jamison, and J. O. Hudson. 1977. Beneficial stimulation of bacterial activity in groundwater containing petroleum hydrocarbons. American Institute of Chemical Engineers Symposium Series 73(166):390-404.

Robinson C., D.A. Barry, P.L. McCarty, J.I. Gerhard, I. Kouznetsova. 2009. pH control for enhanced reductive bioremediation of chlorinated solvent source zones. *Sci Total Environ*. Aug 1;407(16):4560-73.

Ross, D., H.F. Stroo, and A.W. Bourquin. 1988. "Bioremediation of Hazardous Waste Sites in the USA: Case Histories" in Contaminated Soil. 88. K. Wolf, W.J. van den Brink, F.J. Colon (eds.). Kluwer Academic Publishers, pages 717-725.

Sale, T., C. Newell, H. Stroo, R. Hinchee and P. Johnson. 2008. *Frequently Asked Questions Regarding Management of Chlorinated Solvents in Soil and Groundwater*. ESTCP, Arlington, VA. 33p.

Salinero, K.K., K. Keller, W. S. Feil, H.Feil, S.Trong, G. Di Bartolo, and A. Lapidus. 2009. "Metabolic analysis of the soil microbe Dechloromonas aromatic str. RCB: indications of a surprisingly complex lifestyle and cryptic anaerobic pathways for aromatic degradation." *BMC Genomics*. Vol. No. 10. Page 351.

Seech, A., K. Bolanos-Shaw, D. Hill, and J. Molin. 2008. "*In Situ* Bioremediation of Pesticides in Soil and Groundwater." *Remediation.* Winter Edition. Pages 87-98.

Steffan, Robert J. 2007. *Biodegradation of 1,4-Dioxane*. SERDP/ESTCP ER-1422.

Stroo, H. and C.H. Ward (eds.). 2009. In Situ Bioremediation of Perchlorate in Groundwater. SERDP/ESTCP Remediation Technology Monograph Series, Springer Science & Business Media, LLC. New York, NY. 243 p.

Stroo, H.F. 2010. Bioremediation of Chlorinated Solvent Plumes. Pages 309-324 in Stroo, H.F. and C.H. Ward (eds). In Situ Remediation of Chlorinated Solvent Plumes. SERDP/ESTCP Remediation Technology Monograph Series, Springer Science & Business Media, LLC. New York, NY. 725 p.

Suflita, Joseph M. and Sewell, Guy W. 1991. *Anaerobic Biotransformation of Contaminants in the Subsurface.* Environmental Research Brief. EPA/600/M-90/024. February.

Thomas, J.M. and C.H. Ward. 1989. In-situ biorestoration of organic contaminants in the subsurface. *Environ. Sci. Technol.* 23:760-766.

U.S. Environmental Protection Agency (EPA). 1989. *Bioremediation of Exxon Valdez Oil Spill.* July 31. On-line address: http://www2.epa.gov/aboutepa/bioremediation-exxon-valdez-oil-spill

EPA. 1990. *Enhanced Bioremediation Utilizing Hydrogen Peroxide as a Supplemental Source of Oxygen*. EPA 600-2-90-006.

EPA. 1991. *A Guide to Principal Threat and Low Level Threat Wastes*, OSWER Directive # 9380.3-06FS. November. On-line address: www.epa.gov/superfund/health/conmedia/gwdocs/pdfs/threat.pdf

EPA. 2000. *Engineered Approaches to In situ Bioremediation of Chlorinated Solvents. Fundamentals and Field Applications*. Office of Solid Waste and Energy Response. Division of Solid Waste and Energy Response. EPA 542-R-00-008.

EPA. 2001a. *Use of Bioremediation at Superfund Sites.* EPA-542-R-01-019. September.

EPA. 2001b. *A Citizen's Guide to Permeable Reactive Barriers*. EPA 542-F-01-005. April.

EPA. 2004. *How To Evaluate Alternative Cleanup Technologies for Underground Storage Tank Sites: A Guide for Corrective Action Plan Reviewers*. Prepared by EPA Office of Underground Storage Tanks (OUST). EPA 510-B-94-003, EPA 510-B-95-007, and EPA 510-R-04-002. May.

EPA. 2008. A Guide for Assessing Biodegradation and Source Identification of Organic Groundwater Contaminants using Compound Specific Isotope Analysis (CSIA) Office of Research and Development. National Risk Management Research Laboratory. EPA 600/R-08/148. December.

EPA. 2009. Identification and Characterization Methods for Reactive Minerals Responsible for Natural Attenuation of Chlorinated Organic Compounds in Ground Water. Office of Research and Development. National Risk Management Research Laboratory. EPA 600/R-09/115. December.

EPA 2011. Environmental Cleanup Best Management Practices: Effective Use of the Project Life Cycle Conceptual Site Model. Office of Solid Waste and Emergency Response. EPA 542-F-11-011.

EPA. 2012a. *A Citizen's Guide to Bioremediation.* EPA 542-F-12-003. September.

EPA. 2012b. *Methodology for Understanding and Reducing a Project's Environmental Footprint.* EPA 542-R-12-002. February.

EPA. 2013. Adapted from www.epa.gov/athens/learn2model/part-two/onsite/ex/jne_henrys_map.html. Ecosystems Research, Athens, GA.

U.S. Geological Survey (USGS). 1999. *Design and Analysis of Tracer Tests to Determine Effective Porosity and Dispersivity in Fractured Sedimentary Rocks, Newark Basin, New Jersey*. Water-Resources Investigations Report 98-4126A.

Vidali, M. 2001. *Bioremediation. An overview*. *Pure Appl. Chem.*, Vol. 73, No. 7, 1163–1172.

Villatoro-Monzón, W.R., Mesta-Howard, A.M., Razo-Flores, E. 2003. *Anaerobic biodegradation of BTEX using Mn(IV) and Fe(III) as alternative electron acceptors*. Water Science and Technology. Vol. 48. No. 6. Pp 125-131.

Zhang, Bopeng. 2010. Bioremediation of Creosote-Treated Wood Waste. Submitted in partial fulfillment of the requirements for the degree of Master of Applied Science at Dalhousie University, Halifax, Nova Scotia. October. On-line address: http://dalspace.library.dal.ca/bitstream/handle/10222/13112/Bopeng,Zhang,MASc,BIOE,October 2010.pdf?sequence=1

LINKS TO ADDITIONAL INFORMATION

EPA. Use of Bioremediation of Superfund Sites (542-R-1-019):
www.epa.gov/tio/download/remed/542r01019.pdf

EPA Superfund Remedy Report, 13th edition:
www.clu-in.org/download/remed/asr/13/SRR_13th_MainDocument.pdf
www.clu-in.org/download/remed/asr/13/SRR_13th_Appendices.pdf

EPA Superfund Remedy Report, 12th edition:
www.clu-in.org/download/remed/asr/12/asr12_main_body.pdf
www.clu-in.org/download/remed/asr/12/asr12_print_appendices.pdf
www.clu-in.org/download/remed/asr/12/asr12_online_appendices.pdf

EPA. Monitored Natural Attenuation of Inorganic Contaminants in Ground Water, Volume 2:
http://nepis.epa.gov/Adobe/PDF/60000N76.pdf

Federal Remediation Technologies Roundtable (FRTR). Remediation Technologies Screening Matrix and Reference Guide, Version 4.0:
www.frtr.gov

ITRC Bioremediation of DNAPLs Documents:
www.itrcweb.org/guidancedocument.asp?TID=47

ITRC Enhanced In situ Biodenitrification Documents:
www.itrcweb.org/guidancedocument.asp?TID=74

ITRC In situ Bioremediation Documents:
www.itrcweb.org/guidancedocument.asp?TID=9

EPA Clu-in, Bioremediation
www.clu-in.org/techfocus/default.focus/sec/Bioremediation/cat/Overview/

EPA Clu-in, Bioremediation of Chlorinated Solvents:
www.clu-in.org/techfocus/default.focus/sec/Bioremediation_of_Chlorinated_Solvents/cat/Overview/

EPA Clu-in, Bioventing and Biosparging:
www.clu-in.org/techfocus/default.focus/sec/Bioventing%5Fand%5FBiosparging/cat/Overview/

SERDP/ESTCP, Bioremediation Documents:
www.serdp-estcp.org/Program-Areas/Environmental-Restoration

For additional information on this document, please contact:

Linda Fiedler fiedler.linda@epa.gov	or	Edward Gilbert gilbert.edward@epa.gov

Appendix A: A Selection of Superfund Program *In situ* Groundwater Bioremediation Sites (Remedies Selected FY 1989 to 2008)

Site Name	OU	Document Type	EPA ID	EPA Region	Bioremediation Type	Treatment Approach (Operating & Completed Projects Only)	Contaminants	Status as of March 2012	Start Date	End Date
EASTLAND WOOLEN MILL	01	ROD-A	MED980915474	01	In situ Anaerobic Bioremediation	N/A	Halogenated VOCs	predesign		
FORT DEVENS - OU8 - AOC 50/PCE Spill	08		MA7210025154	01	In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	Sep 2004	present
HANSCOM FIELD/HANSCOM AIR FORCE BASE - Site 1 On-Site Plume	01	ROD	MA8570024424	01	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	2000	present
INDUSTRI-PLEX - Groundwater impacted by West Hide Pile	02	ROD	MAD076580950	01	In situ Aerobic Bioremediation	N/A	BTEX; Nonhalogenated VOCs	predesign		
PARKER SANITARY LANDFILL - OU1	01		VTD981062441	01	In situ Anaerobic Bioremediation	Biobarrier	BTEX; Halogenated VOCs; Nonhalogenated VOCs	operating	2005	present
UNION CHEMICAL CO., INC. - OU 1	01		MED042143883	01	In situ Anaerobic Bioremediation	Unknown or TBD	Halogenated VOCs	completed	2001	2002
BOG CREEK FARM	02	ROD-A	NJD063157150	02	In situ Anaerobic Bioremediation	N/A	Halogenated VOCs	predesign		
BRIDGEPORT RENTAL & OIL SERVICES - Deep Groundwater	02	ROD	NJD053292652	02	In situ Aerobic Bioremediation	N/A	BTEX; Halogenated VOCs; Nonhalogenated VOCs	predesign		
CHEMICAL CONTROL - In situ Bio	00	FYR	NJD000607481	02	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs; Nonhalogenated VOCs	completed	Nov 2002	2004
COLESVILLE MUNICIPAL LANDFILL - In situ Bioremediation	01	ESD	NYD980768691	02	In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	Sep 2002	present
EMMELL'S SEPTIC LANDFILL	02	ROD	NJD980772727	02	Biosparging	N/A	Halogenated VOCs	design		
FEDERAL AVIATION ADMINISTRATION TECHNICAL CENTER (USDOT) - OU 1, Area D - Jet Fuel Farm - Near MW-19S	01		NJ9690510020	02	In situ Aerobic Bioremediation	Recirculation	BTEX; Nonhalogenated VOCs	operating	Jun 2006	present

Appendix A: A Selection of Superfund Program *In situ* Groundwater Bioremediation Sites (Remedies Selected FY 1989 to 2008)

Site Name	OU	Document Type	EPA ID	EPA Region	Bioremediation Type	Treatment Approach (Operating & Completed Projects Only)	Contaminants	Status as of March 2012	Start Date	End Date
HOOKER CHEMICAL & PLASTICS CORP./RUCO POLYMER CORP. - OU 3	03		NYD002920312	02	Biosparging	Biosparging	Halogenated VOCs	operating	Oct 2006	present
ICELAND COIN LAUNDRY AREA GW PLUME - Former Facility Area	01	ROD	NJ0001360882	02	In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	May 2007	present
ICELAND COIN LAUNDRY AREA GW PLUME - Plume Area	01	ROD	NJ0001360882	02	Bioaugmentation, In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	Apr 2007	present
MONITOR DEVICES, INC./INTERCIRCUITS, INC.	01	ROD	NJD980529408	02	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	2010	present
NEPERA CHEMICAL CO., INC.	01	ROD	NYD000511451	02	In situ Aerobic Bioremediation	N/A	BTEX; Halogenated VOCs; Nonhalogenated SVOCs; Nonhalogenated VOCs	being installed	Fall 2011	
ABERDEEN PROVING GROUND (MICHAELSVILLE LANDFILL) - Site 16 DRMO Metal Scrap Yard	06	ROD	MD3210021355	03	Bioaugmentation, In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	2007	present
ABERDEEN PROVING GROUND (MICHAELSVILLE LANDFILL) - Site 23 Building 525 Site	06	ROD	MD3210021355	03	Bioaugmentation, In situ Anaerobic Bioremediation	Direct injection, Recirculation	Halogenated VOCs	operating	2006	present
ABERDEEN PROVING GROUND (MICHAELSVILLE LANDFILL) - Site 28f - Building 3327 UST Site	06	ROD	MD3210021355	03	Bioaugmentation, In situ Anaerobic Bioremediation	Recirculation	Halogenated VOCs	operating	2006	present

Appendix A: A Selection of Superfund Program *In situ* Groundwater Bioremediation Sites (Remedies Selected FY 1989 to 2008)

Site Name	OU	Document Type	EPA ID	EPA Region	Bioremediation Type	Treatment Approach (Operating & Completed Projects Only)	Contaminants	Status as of March 2012	Start Date	End Date
ABERDEEN PROVING GROUND (MICHAELSVILLE LANDFILL) - Site 29 Tower Road Site	06	ROD	MD3210021355	03	Bioaugmentation, In situ Anaerobic Bioremediation	Biobarrier, Recirculation	Halogenated VOCs	operating	2007	present
ABERDEEN PROVING GROUND (MICHAELSVILLE LANDFILL) - Site 32 Building 507 Site	06	ROD	MD3210021355	03	Bioaugmentation, In situ Anaerobic Bioremediation	Biobarrier, Recirculation	Halogenated VOCs	operating	2006	present
ABERDEEN PROVING GROUND (MICHAELSVILLE LANDFILL) - Site 33 Building M600 Site	06	ROD	MD3210021355	03	Bioaugmentation, In situ Anaerobic Bioremediation	Direct injection, Recirculation	Halogenated VOCs	operating	2006	present
ANDREWS AIR FORCE BASE - FT-04	03	ROD	MD0570024000	03	In situ Anaerobic Bioremediation	Direct injection	BTEX; Halogenated VOCs; Nonhalogenated VOCs	operating	Aug 2004	present
ANDREWS AIR FORCE BASE - ST-10 (PD-680 Spill)	07	ROD	MD0570024000	03	In situ Aerobic Bioremediation	Direct injection	BTEX; Nonhalogenated SVOCs; Nonhalogenated VOCs	operating	Sep 2004	present
ANDREWS AIR FORCE BASE - ST-14 (East Side Gas Station) Benzene Plume	11	ROD	MD0570024000	03	In situ Aerobic Bioremediation	Biobarrier	BTEX; Nonhalogenated VOCs	operating	May 2006	present
ANDREWS AIR FORCE BASE - ST-14 (East Side Gas Station) TCE and TCE/CT Plumes	11	ROD	MD0570024000	03	In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	May 2006	present
AVCO LYCOMING (WILLIAMSPORT DIVISION) - Shallow Aquifer	02	ROD	PAD003053709	03	In situ Anaerobic Bioremediation	Direct injection	Metals and metalloids	completed	1997	2000
BRANDYWINE DRMO	01	ROD	MD9570024803	03	Bioaugmentation, In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	Feb 2008	present

Appendix A: A Selection of Superfund Program *In situ* Groundwater Bioremediation Sites (Remedies Selected FY 1989 to 2008)

Site Name	OU	Document Type	EPA ID	EPA Region	Bioremediation Type	Treatment Approach (Operating & Completed Projects Only)	Contaminants	Status as of March 2012	Start Date	End Date
BRESLUBE-PENN, INC.	01	ROD	PAD089667695	03	In situ Anaerobic Bioremediation	N/A	BTEX; Halogenated VOCs; Nonhalogenated VOCs	design		
DEFENSE GENERAL SUPPLY CENTER (DLA)	08	ROD	VA3971520751	03	Unknown Type	N/A	Halogenated VOCs	predesign		
DOVER AIR FORCE BASE - Area 2 Plume	15	ROD	DE8570024010	03	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	2006	
DOVER AIR FORCE BASE - Area 5 Plume	17	ROD	DE8570024010	03	In situ Anaerobic Bioremediation	Direct injection, Recirculation	BTEX; Halogenated VOCs; Nonhalogenated VOCs	operating	2006	
DOVER AIR FORCE BASE - Area 6 Plume	16	ROD	DE8570024010	03	In situ Anaerobic Bioremediation	Direct injection, Recirculation	BTEX; Halogenated SVOCs; Halogenated VOCs; Nonhalogenated VOCs; Organic pesticides	operating	2006	
DOVER AIR FORCE BASE - LF25 Plume	19	ROD	DE8570024010	03	In situ Anaerobic Bioremediation	Direct injection, Recirculation	Halogenated VOCs	operating	2006	
DOVER AIR FORCE BASE - OT41/Building 719 Source Zone - Ongoing Interim Remedy	16	ROD	DE8570024010	03	In situ Anaerobic Bioremediation	Direct injection, Recirculation	Halogenated VOCs	operating	2002	
DOVER AIR FORCE BASE - SS08 Plume	19	ROD	DE8570024010	03	In situ Anaerobic Bioremediation	Direct injection, Recirculation	Halogenated VOCs	operating	2006	
FIKE CHEMICAL, INC.	04	ROD-A	WVD047989207	03	Biosparging	Biosparging	BTEX; Halogenated SVOCs; Halogenated VOCs; Metals and metalloids; Nonhalogenated SVOCs; Nonhalogenated VOCs; Organic pesticides	operating	Jun 2007	present
INDIAN HEAD NAVAL SURFACE WARFARE CENTER - Site 57 Building 292 TCE Contamination Downgradient Plume	01	ROD	MD7170024684	03	In situ Aerobic Bioremediation	N/A	Halogenated VOCs	designed/not installed		

Appendix A: A Selection of Superfund Program *In situ* Groundwater Bioremediation Sites (Remedies Selected FY 1989 to 2008)

Site Name	OU	Document Type	EPA ID	EPA Region	Bioremediation Type	Treatment Approach (Operating & Completed Projects Only)	Contaminants	Status as of March 2012	Start Date	End Date
INDIAN HEAD NAVAL SURFACE WARFARE CENTER - Site 57 Building 292 TCE Contamination Source Zone	01	ROD	MD7170024684	03	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	Dec 2011	present
LETTERKENNY ARMY DEPOT (SE AREA)	10	ROD	PA6213820503	03	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	1999	Jun 2007
MARINE CORPS COMBAT DEVELOPMENT COMMAND	19	ROD	VA1170024722	03	In situ Aerobic Bioremediation	N/A	Halogenated VOCs	predesign		
NAVAL AMPHIBIOUS BASE LITTLE CREEK - Site 11 Plating Shop	05	ROD	VA5170022482	03	In situ Anaerobic Bioremediation	Biobarrier, Direct injection	Halogenated VOCs	operating	Apr 2009	present
NAVAL AMPHIBIOUS BASE LITTLE CREEK - Site 12 Exchange Laundry	06	ROD	VA5170022482	03	In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	Mar 2007	present
NAVAL AMPHIBIOUS BASE LITTLE CREEK - Site 13 PCP Tank	07	ROD	VA5170022482	03	In situ Anaerobic Bioremediation	Biobarrier	Halogenated SVOCs; Halogenated VOCs; Organic pesticides	operating	May 2010	present
NAVAL SURFACE WARFARE CENTER - DAHLGREN - Site 20A Plume	19	ROD	VA7170024684	03	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	2009	
NAVAL SURFACE WARFARE CENTER - DAHLGREN - Site 20B Plume	19	ROD	VA7170024684	03	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	2009	
NAVAL SURFACE WARFARE CENTER - DAHLGREN - Site 23 Plume	19	ROD	VA7170024684	03	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	2009	

Appendix A: A Selection of Superfund Program *In situ* Groundwater Bioremediation Sites (Remedies Selected FY 1989 to 2008)

Site Name	OU	Document Type	EPA ID	EPA Region	Bioremediation Type	Treatment Approach (Operating & Completed Projects Only)	Contaminants	Status as of March 2012	Start Date	End Date
PATUXENT RIVER NAVAL AIR STATION - Site 39 Waste PCE Storage Area (Building 503)	24	ROD	MD7170024536	03	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	Oct 2009	present
SAEGERTOWN INDUSTRIAL AREA	01	ROD-A	PAD980692487	03	In situ Anaerobic Bioremediation	Biobarrier, Direct injection	Halogenated VOCs	operating	2003/2004	present
SAND, GRAVEL AND STONE - OU3 - Shallow GW in Eastern Excavation Area	03	ROD	MDD980705164	03	In situ Anaerobic Bioremediation	N/A	BTEX; Halogenated VOCs; Nonhalogenated VOCs	design		
CAPE FEAR WOOD PRESERVING	01	ROD-A	NCD003188828	04	In situ Aerobic Bioremediation	Direct injection, Recirculation	Nonhalogenated SVOCs	completed	Aug 2001	Sep 2004
DISTLER BRICKYARD - Bioremediation	01	FYR	KYD980602155	04	In situ Anaerobic Bioremediation	Direct injection, Hydraulic fracturing	Halogenated VOCs	operating	Apr 2003	present
ESCAMBIA WOOD - PENSACOLA - High Concentration Plume Areas	02	ROD	FLD008168346	04	In situ Aerobic Bioremediation	N/A	Halogenated SVOCs; Nonhalogenated SVOCs; Nonhalogenated VOCs; Organic pesticides	design		
ESCAMBIA WOOD - PENSACOLA - Source Plume Area	02	ROD	FLD008168346	04	In situ Aerobic Bioremediation	N/A	Halogenated SVOCs; Nonhalogenated SVOCs; Nonhalogenated VOCs; Organic pesticides	design		
FCX, INC. (STATESVILLE PLANT)	03	ESD	NCD095458527	04	In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	May 2007	present
JACKSONVILLE NAVAL AIR STATION - OU3 - Area C Hot Spot	03	ROD	FL6170024412	04	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	Feb 2003	present
JACKSONVILLE NAVAL AIR STATION - OU3 - Area D Hot Spot	03	ROD	FL6170024412	04	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	Dec 2002	present

Appendix A: A Selection of Superfund Program *In situ* Groundwater Bioremediation Sites (Remedies Selected FY 1989 to 2008)

Site Name	OU	Document Type	EPA ID	EPA Region	Bioremediation Type	Treatment Approach (Operating & Completed Projects Only)	Contaminants	Status as of March 2012	Start Date	End Date
JACKSONVILLE NAVAL AIR STATION - OU5	05	ROD	FL6170024412	04	In situ Aerobic Bioremediation	N/A	BTEX; Halogenated VOCs; Nonhalogenated SVOCs; Nonhalogenated VOCs	predesign		
LANDIA CHEMICAL COMPANY - Interim Remedy - Operable Unit 2 Groundwater	02		FLD042110841	04	In situ Anaerobic Bioremediation	N/A	Nitrate	predesign		
MEMPHIS DEFENSE DEPOT (DLA) Main Installation Functional Unit 7 - TTA-1 and 2	2/3/4	ROD	TN4210020570	04	Bioaugmentation, In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	Sep 2006	present
OAK RIDGE RESERVATION (USDOE) - East Bethel Valley VOC Plume (7000-Area)	30		TN1890090003	04	In situ Anaerobic Bioremediation	N/A	Halogenated VOCs	design		
PALMETTO WOOD PRESERVING	01	ROD-A	SCD003362217	04	In situ Anaerobic Bioremediation	Biobarrier, Pneumatic fracturing	Metals and metalloids	operating	Jan 2009	present
PEAK OIL CO./BAY DRUM CO. - Surficial Aquifer	02	ROD-A	FLD004091807	04	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	Jun 2005	present
PICAYUNE WOOD TREATING SITE	00	ROD	MSD065490930	04	Bioaugmentation, In situ Aerobic Bioremediation	N/A	Halogenated SVOCs; Nonhalogenated SVOCs; Nonhalogenated VOCs; Organic pesticides	designed/not installed		
TOWER CHEMICAL CO.	03	ROD	FLD004065546	04	Bioaugmentation, In situ Aerobic Bioremediation	N/A	BTEX; Halogenated SVOCs; Halogenated VOCs; Metals and metalloids; Nonhalogenated SVOCs; Nonhalogenated VOCs; Organic pesticides	predesign		

Appendix A: A Selection of Superfund Program *In situ* Groundwater Bioremediation Sites (Remedies Selected FY 1989 to 2008)

Site Name	OU	Document Type	EPA ID	EPA Region	Bioremediation Type	Treatment Approach (Operating & Completed Projects Only)	Contaminants	Status as of March 2012	Start Date	End Date
USN AIR STATION CECIL FIELD - Site 59 (Hot Spot Nos. 2 and 3)	09	ROD	FL5170022474	04	Bioaugmentation, In situ Anaerobic Bioremediation	Recirculation	Halogenated VOCs	operating	2008	present
AIRCRAFT COMPONENTS (D & L SALES) Chemical Operable Unit OU-2	02		MI0001119106	05	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	Aug 2004	present
GALESBURG/KOPPERS CO. - Deep sand aquifer	01	ESD	ILD990817991	05	In situ Aerobic Bioremediation	Recirculation	Halogenated SVOCs; Nonhalogenated SVOCs; Nonhalogenated VOCs; Organic pesticides	operating		present
GALESBURG/KOPPERS CO. - Shallow till aquifer	01	ESD	ILD990817991	05	In situ Aerobic Bioremediation	Recirculation	Halogenated VOCs; Nonhalogenated SVOCs; Nonhalogenated VOCs	operating		present
KOPPERS COKE - Groundwater OU	01	ROD	MND000819359	05	Biosparging	Biosparging	BTEX; Nonhalogenated SVOCs; Nonhalogenated VOCs	completed	1998	1999
PARSONS CASKET HARDWARE CO. - Alluvial Aquifer	02	ROD	ILD005252432	05	Bioaugmentation, In situ Anaerobic Bioremediation	N/A	Halogenated VOCs	predesign		
PARSONS CASKET HARDWARE CO. - Bedrock Groundwater Aquifer	02	ROD	ILD005252432	05	Bioaugmentation, In situ Anaerobic Bioremediation	N/A	Halogenated VOCs	predesign		
TAR LAKE - OU2	02		MID980794655	05	Biosparging	Biosparging	BTEX; Nonhalogenated VOCs	operating	1998	present
AMERICAN CREOSOTE WORKS, INC. (WINNFIELD PLANT)	01	ROD	LAD000239814	06	Bioaugmentation, In situ Aerobic Bioremediation	Direct injection, Recirculation	BTEX; Halogenated SVOCs; Nonhalogenated SVOCs; Nonhalogenated VOCs; Organic pesticides	operating	Oct 1996	present
GRANTS CHLORINATED SOLVENTS	00	ROD	NM0007271768	06	In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	Dec 2010	present

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Site Name	OU	Document Type	EPA ID	EPA Region	Bioremediation Type	Treatment Approach (Operating & Completed Projects Only)	Contaminants	Status as of March 2012	Start Date	End Date
MCGAFFEY AND MAIN GROUNDWATER PLUME - Hotspot in Groundwater Plume Area	00	ROD	NM0000605386	06	In situ Anaerobic Bioremediation	N/A	Halogenated VOCs	design		
NORTH RAILROAD AVENUE PLUME - Deep Zone	01	ROD	NMD986670156	06	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	Apr 2008	present
NORTH RAILROAD AVENUE PLUME - Downgradient Biocurtain	01	ROD	NMD986670156	06	In situ Anaerobic Bioremediation	Biobarrier, Recirculation	Halogenated VOCs	operating	May 2008	present
NORTH RAILROAD AVENUE PLUME - Source Area and Hotspot	01	ROD	NMD986670156	06	In situ Anaerobic Bioremediation	Recirculation	Halogenated VOCs	operating	May 2008	present
OUACHITA NEVADA WOOD TREATER	01	ROD	ARD042755231	06	Unknown Type	N/A	Halogenated SVOCs; Organic pesticides	predesign		
PANTEX PLANT (USDOE) - Southeast Area ISB System	00	ROD	TX4890110527	06	In situ Anaerobic Bioremediation	Biobarrier	Explosives/propellants; Metals and metalloids; Nonhalogenated SVOCs	operating	Feb/Mar 2008	present
PANTEX PLANT (USDOE) - Zone 11 ISB System	00	ROD	TX4890110527	06	In situ Anaerobic Bioremediation	Biobarrier	Explosives/propellants; Halogenated VOCs	operating	Jun 2009	present
PETRO-CHEMICAL SYSTEMS, INC. (TURTLE BAYOU) - Shallow Groundwater	02	ROD-A	TXD980873350	06	Bioaugmentation, In situ Aerobic Bioremediation	Recirculation	BTEX; Halogenated VOCs; Nonhalogenated SVOCs; Nonhalogenated VOCs	completed	1997	2005
SOL LYNN/INDUSTRIAL TRANSFORMERS - Bioremediation	02	ROD-A	TXD980873327	06	Bioaugmentation, In situ Anaerobic Bioremediation	Biobarrier, Direct injection	Halogenated VOCs	operating	2010	present
HASTINGS GROUND WATER CONTAMINATION - Far-Mar-Co Subsite	06	ROD	NED980862668	07	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	Jul 2010	present

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Site Name	OU	Document Type	EPA ID	EPA Region	Bioremediation Type	Treatment Approach (Operating & Completed Projects Only)	Contaminants	Status as of March 2012	Start Date	End Date
HASTINGS GROUND WATER CONTAMINATION - Second Street OU	20	ROD	NED980862668	07	In situ Aerobic Bioremediation	Biobarrier	BTEX; Nonhalogenated SVOCs; Nonhalogenated VOCs	operating	Nov 2005	present
IOWA ARMY AMMUNITION PLANT - Off-site Groundwater	03	ROD	IA7213820445	07	In situ Anaerobic Bioremediation	Biobarrier	Explosives/propellants; Nonhalogenated SVOCs	operating	Oct 2007	present
LAKE CITY ARMY AMMUNITION PLANT (NORTHWEST LAGOON) - Area 12 Groundwater	01	ROD	MO3213890012	07	In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	Feb 2008	present
LAKE CITY ARMY AMMUNITION PLANT (NORTHWEST LAGOON) - Area 18 Paleochannels	02	ROD	MO3213890012	07	In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	Oct 2007	present
LAKE CITY ARMY AMMUNITION PLANT (NORTHWEST LAGOON) - Area 18 Shallow VOC Source Areas	02	ROD	MO3213890012	07	In situ Anaerobic Bioremediation	N/A	Halogenated VOCs	installed		
LAKE CITY ARMY AMMUNITION PLANT (NORTHWEST LAGOON) - NE Corner Operable Unit Area 16B Plume	03	ROD	MO3213890012	07	In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	Jan 2008	present
LAKE CITY ARMY AMMUNITION PLANT (NORTHWEST LAGOON) - NE Corner Operable Unit Area 17B Downgradient Plume (IRZ Line 5)	03	ROD	MO3213890012	07	In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	Oct 2007	present

Appendix A: A Selection of Superfund Program *In situ* Groundwater Bioremediation Sites (Remedies Selected FY 1989 to 2008)

Site Name	OU	Document Type	EPA ID	EPA Region	Bioremediation Type	Treatment Approach (Operating & Completed Projects Only)	Contaminants	Status as of March 2012	Start Date	End Date
LAKE CITY ARMY AMMUNITION PLANT (NORTHWEST LAGOON) - NE Corner Operable Unit Area 17B Source Area Residual NAPL Zone (IRZ Lines 1-4)	03	ROD	MO3213890012	07	In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	Oct 2007	present
LAKE CITY ARMY AMMUNITION PLANT (NORTHWEST LAGOON) - NE Corner Operable Unit Area 17D Plume	03	ROD	MO3213890012	07	In situ Anaerobic Bioremediation	Biobarrier, Direct injection	Halogenated VOCs	operating	Jan 2008	present
MISSOURI ELECTRIC WORKS - Alluvial Groundwater	02	ROD	MOD980965982	07	In situ Anaerobic Bioremediation	N/A	Halogenated VOCs	predesign		
BOUNTIFUL/WOODS CROSS 5TH S. PCE PLUME	01	ROD	UT0001119296	08	Bioaugmentation, In situ Anaerobic Bioremediation	Biobarrier	Halogenated VOCs	operating	Jul 2011	
BOUNTIFUL/WOODS CROSS 5TH S. PCE PLUME	02	ROD	UT0001119296	08	Bioaugmentation, In situ Anaerobic Bioremediation	Direct injection, Recirculation	Halogenated VOCs	operating	Feb 2011	
F.E. WARREN AIR FORCE BASE - Spill Site 7 (SS-7) Plume	02	ROD	WY5571924179	08	Bioaugmentation, In situ Anaerobic Bioremediation	Direct injection, Hydraulic fracturing	Halogenated VOCs	operating	approx. 2008	present
IDAHO POLE CO.	01	ROD	MTD006232276	08	In situ Aerobic Bioremediation	Recirculation	Halogenated SVOCs; Nonhalogenated SVOCs; Organic pesticides	operating	1997	present
LIBBY GROUND WATER CONTAMINATION - Boundary Injection System	02	ROD	MTD980502736	08	In situ Aerobic Bioremediation	Recirculation	Halogenated SVOCs; Nonhalogenated SVOCs; Organic pesticides	completed	1993	2003

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Site Name	OU	Document Type	EPA ID	EPA Region	Bioremediation Type	Treatment Approach (Operating & Completed Projects Only)	Contaminants	Status as of March 2012	Start Date	End Date
LIBBY GROUND WATER CONTAMINATION - Intermediate Aquifer	02	ROD	MTD980502736	08	In situ Aerobic Bioremediation	Recirculation	Halogenated SVOCs; Nonhalogenated SVOCs; Organic pesticides	completed	1993 (?)	1998
LOCKWOOD SOLVENT GROUND WATER PLUME - Beall Property	01	ROD	MT0007623052	08	In situ Anaerobic Bioremediation	N/A	Halogenated VOCs	predesign		
LOCKWOOD SOLVENT GROUND WATER PLUME - Plume Leading Edges	01	ROD	MT0007623052	08	In situ Anaerobic Bioremediation	N/A	Halogenated VOCs	predesign		
LOCKWOOD SOLVENT GROUND WATER PLUME - SOCO Property	01	ROD	MT0007623052	08	In situ Anaerobic Bioremediation	N/A	Halogenated VOCs	predesign		
MONTANA POLE AND TREATING - Groundwater OU	01	ROD	MTD006230635	08	In situ Aerobic Bioremediation	Recirculation	Halogenated SVOCs; Organic pesticides	completed	1999/2000	2002
ALAMEDA NAVAL AIR STATION - IR Site 16	01	ROD	CA2170023236	09	Unknown Type	N/A	Halogenated SVOCs; Halogenated VOCs; Organic pesticides	predesign		
ALAMEDA NAVAL AIR STATION - IR Site 6	01	ROD	CA2170023236	09	Unknown Type	N/A	Halogenated VOCs	predesign		
ALAMEDA NAVAL AIR STATION - Site 25 Groundwater (Navy OU5/FISCA IR-02)	14	ROD	CA2170023236	09	Bioaugmentation, Biosparging	Biosparging	BTEX; Nonhalogenated SVOCs; Nonhalogenated VOCs	operating	Mar 2009	present
ALAMEDA NAVAL AIR STATION - Site 26 Western Hanger Zone	06	ROD	CA2170023236	09	Bioaugmentation, In situ Anaerobic Bioremediation	Recirculation	Halogenated VOCs	operating	Sep 2010	present
FORT ORD - FT-044 Operable Unit Carbon Tetrachloride Plume, A-Aquifer	12	ROD	CA7210020676	09	In situ Anaerobic Bioremediation	Recirculation	Halogenated VOCs	operating	Sep 2009	present
FRONTIER FERTILIZER	01	ROD	CAD071530380	09	In situ Anaerobic Bioremediation	N/A	Nitrate	predesign		

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Site Name	OU	Document Type	EPA ID	EPA Region	Bioremediation Type	Treatment Approach (Operating & Completed Projects Only)	Contaminants	Status as of March 2012	Start Date	End Date
KOPPERS CO., INC. (OROVILLE PLANT) - Off-Property Plume	01	ROD-A	CAD009112087	09	In situ Aerobic Bioremediation	Direct injection	Halogenated SVOCs; Organic pesticides	operating	Aug 1998	present
KOPPERS CO., INC. (OROVILLE PLANT) - On-Property East Plume	01	ROD-A	CAD009112087	09	In situ Aerobic Bioremediation	Direct injection	Halogenated SVOCs; Organic pesticides	operating	Mar 1998	present
SELMA TREATING CO.	01	ESD	CAD029452141	09	In situ Anaerobic Bioremediation	Direct injection, Recirculation	Metals and metalloids	operating	Mar 2005	present
IDAHO NATIONAL ENGINEERING LABORATORY (USDOE) Test Area North OU 1-07B (OU1) hot spot	01	ROD-A	ID4890008952	10	In situ Anaerobic Bioremediation	Direct injection	Halogenated VOCs	operating	1999	present

Abbreviations/Acronyms:

BTEX = Benzene, Toluene, Ethyl Benzene, and Xylene
ESD = Explanation of Significant Differences
FY = Fiscal Year
FYR = Five-Year Review
ISB = In situ Bioremediation
N/A = Not Applicable
OU = Operable Unit
ROD = Record of Decision
ROD-A = Record of Decision Amendment
TBD = To Be Determined
SVOC = Semivolatile Organic Compound
VOC = Volatile Organic Compound