

ADDENDUM TO THE PRINCIPLES AND PRACTICES MANUAL

Loading Rates and Impacts of Substrate Delivery for Enhanced Anaerobic Bioremediation

ESTCP Project ER-200627

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ADDENDUM TO THE PRINCIPLES AND PRACTICES OF ENHANCED ANAEROBIC BIOREMEDIATION OF CHLORINATED SOLVENTS

Project: ER-200627

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ACRONYMS AND ABBREVIATIONS

AFB	Air Force Base
AFCEE	Air Force Center for Engineering and the Environment
BUCHLORAC	buffering of dechlorination acidity (geochemical model)
CA	chloroethane
CAH	chlorinated aliphatic hydrocarbon
CCAFS	Cape Canaveral Air Force Station
CSIA	compound-specific isotope analysis
CSM	conceptual site model
CT	carbon tetrachloride
DCA	dichloroethane
DCE	dichloroethene
DNAPL	dense non-aqueous phase liquid
DO	dissolved oxygen
DOC	dissolved organic carbon
DoD	Department of Defense
DOE	Department of Energy
ESTCP	Environmental Security Technology Certification Program
EOS [®]	Emulsified Oil Substrate [®]
EVO	emulsified vegetable oil
GSI	Groundwater Services, Inc.
HRC [®]	hydrogen release compound
ISCO	in situ chemical oxidation
ITRC	Interstate Technology and Regulatory Council
MBT	molecular biological tool
MCL	maximum contaminant level
mg/L	milligrams per liter
MNA	monitored natural attenuation
MSDS	material safety data sheet
NAVFAC ESC	Naval Facilities Engineering Command/Engineering Services Center
NDMA	N-nitrosodimethylamine
ORP	oxidation reduction potential
PCE	tetrachloroethene
PRG	preliminary remediation goal

ACRONYMS AND ABBREVIATIONS (continued)

RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
SABRE	Source Area BioREmediation Project
SERDP	Strategic Environmental Research and Development Program
TCA	trichloroethane
TCE	trichloroethene
TDS	total dissolved solids
TNT	trinitrotoluene
TOC	total organic carbon
USACE	U.S. Army Corps of Engineers
USEPA	U.S. Environmental Protection Agency
VC	vinyl chloride
VOC	volatile organic compound
ZVI	zero-valent iron

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1.0 INTRODUCTION

A number of improvements and advances for the enhanced in situ bioremediation of chlorinated solvents have been made since the Tri-Services *Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents* guidance document was released in August 2004 (Air Force Center for Engineering and the Environment [AFCEE] et al., 2004). Numerous demonstration and full-scale applications have been completed, yet challenges to successful implementation of the technology remain (Simpkin and Norris, 2010; Suthersan and Payne, 2003). In particular, there remains some uncertainty and a lack of guidance for determining appropriate substrate loading rates and delivery methods based on site-specific conditions.

This addendum to the 2004 *Principles and Practices* document provides a description of a demonstration study conducted to evaluate substrate loading rates, including a summary of limiting factors and challenges to applying enhanced in situ bioremediation. This addendum also summarizes advances made in the field of enhanced in situ bioremediation of chlorinated solvents over the last six years and provides resources and references that may be used to identify and mitigate the limiting factors and challenges that practitioners face when applying the technology.

1.1 OBJECTIVES OF THE DEMONSTRATION

A *Technology Demonstration for Loading Rates and Impacts of Substrate Delivery for Enhanced Anaerobic Bioremediation* was conducted by Parsons for the Environmental Security Technology Certification Program (ESTCP) (Project ER-200627) to supplement existing guidance for enhanced in situ anaerobic bioremediation and to evaluate differing approaches to determining substrate loading rates and the impacts of substrate delivery.

The objectives of this study were to:

- Better understand the effects that substrate amendment loading rates have on substrate distribution and persistence (maintenance of the reaction zone)
- Determine how control of substrate loading rates affects amendment utilization and development of optimal geochemical and redox conditions
- Identify substrate loading rates that have adverse impacts on secondary water quality
- Evaluate the effect that differing substrate types or loading rates may have on hydraulic conductivity
- Develop practical guidelines for designing and optimizing substrate loading rates and injection scenarios for differing substrate types and for differing geochemical and hydrogeologic conditions based on observations from representative case studies.

To achieve these objectives, 15 case studies were evaluated regarding system design, operation, and performance. Quantitative and qualitative performance objectives were developed to

evaluate the case studies and to identify limiting factors for enhanced in situ bioremediation. Supporting information for the case studies may be found in the *Final Technology Demonstration Report* (Parsons, 2010a).

1.2 EVALUATING SUBSTRATE LOADING RATES

Enhanced in situ anaerobic bioremediation involves the delivery of organic substrates into the subsurface to stimulate anaerobic degradation of contaminants in groundwater. Effective application of the technology depends primarily on the delivery of appropriate levels of organic substrate and the development of optimal geochemical and oxidation-reduction (redox) conditions for anaerobic degradation processes to occur.

Substrate loading rates are defined as the volume, concentration, and frequency of injection of organic substrates for in situ anaerobic bioremediation. Insufficient substrate loading rates or nonuniform delivery and mixing may result in areas of the aquifer that are not sufficiently reducing for complete dechlorination to occur, thereby increasing the potential for accumulation of regulated intermediate degradation products. For example, the potential accumulation of dechlorination products cis-1,2-dichloroethene (DCE), vinyl chloride (VC), or chloroethane (CA).

The presence of excessive substrate may result in uncontrolled fermentation reactions (e.g., lowering of pH and formation of undesirable fermentation products such as alcohols and ketones), degradation of secondary water quality (e.g., mobilization of metals), and poor utilization of substrate for anaerobic degradation of the contaminants of concern. The ability for aquifer systems to recover to pre-injection redox conditions and the long-term impacts on secondary water quality after enhanced bioremediation is not well documented.

Given these effects, many enhanced anaerobic bioremediation applications fail to achieve performance expectations or develop unanticipated long-term compliance problems. The cost associated with poor performance (e.g., a need for longer term operation) or with compliance issues such as degradation of secondary water quality (typically requiring additional monitoring or system modifications) may greatly increase the life-cycle costs of full-scale applications. Therefore, determining an appropriate substrate loading rate and an effective distribution method for the various substrate types commonly applied is a critical design and operational objective.

2.0 CASE STUDY EVALUATIONS

The demonstration study consisted of an evaluation of 15 sites based on work plans and results reports and collection of additional field data to fill data gaps necessary to evaluate performance.

2.1 PERFORMANCE OBJECTIVES

The objectives of this study (Section 1.1) were addressed by comparative evaluations of 15 case studies, primarily consisting of Department of Defense (DoD) and Department of Energy (DOE) applications. Additional field sampling and analysis were performed for two sites to support evaluation of the project objectives. Quantitative and qualitative performance objectives developed to evaluate and measure the success of the demonstration sites are listed in Table 1.

Table 1. Case study performance objectives.

Performance Objective	Data Requirements	Success Criteria
Quantitative Performance Objectives		
Determine ability to uniformly distribute substrate	Post-injection concentrations of soluble organic carbon in groundwater	Achieving the concentration of substrate targeted in the design at all monitoring locations within the reaction zone is considered successful.
Determine if optimal geochemical conditions were achieved	Pre- and post-injection concentrations of geochemical indicator parameters in groundwater	Achieving highly reducing conditions with oxidation reduction potential (ORP) less than -200 millivolts (mV) throughout the reaction zone is considered successful.
Determine remediation effectiveness	Pre- and post-treatment contaminant concentrations in groundwater	A greater than 99% reduction in compound-specific concentrations is considered successful. A greater than 90% reduction in total molar concentration of chlorinated aliphatic hydrocarbons (CAH) is considered successful.
Determine impacts to secondary water quality	Post-treatment concentrations of secondary water quality parameters (e.g., dissolved metals such as iron and manganese)	Maintaining concentrations of secondary water quality parameters below applicable regulatory standards downgradient of the reaction zone is considered successful.
Determine impacts on hydraulic conductivity	Pre- and post-treatment measurements of hydraulic conductivity	A less than 50% decrease in hydraulic conductivity is considered successful.
Determine substrate persistence and long-term effectiveness	Post-treatment concentrations of contaminants and soluble organic carbon at the end of the intended design life of the application	A rebound in concentrations of less than 1.0% of the initial contaminant concentration after the application has been completed is considered successful.
Qualitative Performance Objectives		
Determine need for and cost of additional injections or monitoring	Actual work performed is compared to the application design plan. The cost of additional work is calculated when data are available, or a qualitative assessment is made when cost data are not available.	An application that does not require additional injections or monitoring beyond that in the original design is considered successful.
Application in difficult hydrogeological conditions	Site geology (permeability, heterogeneity) and groundwater hydraulics (hydraulic conductivity, hydraulic gradient, and rate of groundwater flow)	An application where permeability, heterogeneity, or the rate of groundwater does not limit effectiveness is considered successful. Guidelines are developed from examples where they impacted the effectiveness of the application

2.2 EVALUATION METHODS

The distribution of substrate was evaluated using concentrations of soluble organic carbon measured within the intended reaction zone. Concentrations achieved are compared to target concentrations described in the application design (i.e., work plans). These data are used to better understand the effects that substrate amendment loading rates (volume, concentration, and frequency of injection) have on substrate distribution (mixing and radius of influence). Achieving optimal geochemical conditions was evaluated by analyzing indicator parameters of anaerobic conditions such as dissolved oxygen (DO), ORP, nitrate, manganese, ferrous iron, sulfate, and methane.

Pre- and post-treatment concentrations of contaminants were evaluated to determine the effectiveness of the remedy. Success was evaluated by comparing concentrations to site-specific performance criteria, if established. Otherwise, a reduction in contaminant concentration of 99% or greater or a reduction in the total molar concentration of CAHs of greater than 90% was considered successful.

The term “secondary water quality” is used in this document to refer to water-quality issues that result from substrate addition, apart from the primary contaminants being treated. Secondary water quality parameters that were evaluated included pH, chloride, total dissolved solids (TDS), sulfide, and dissolved metals or semi-metals (iron, manganese, arsenic, and selenium).

The hydraulic conductivity of the aquifer may be impacted by physical, chemical, or biological processes. Pre- and post-treatment of hydraulic conductivity (typically from slug tests) were evaluated to determine the degree to which hydraulic conductivity within the reaction zone may have been reduced.

Effective enhanced bioremediation applications must sustain the reaction zone over the design life of the application. Substrate persistence and long-term effectiveness were evaluated using concentrations of soluble substrate and contaminants over the design life of the application. A rebound in contaminant concentrations after the remedy was halted was evaluated when possible. At least one year of post-remediation contaminant data from the treatment zone was considered sufficient to evaluate potential rebound.

The cost associated with poor performance or compliance issues may significantly increase the life-cycle costs of full-scale enhanced in situ bioremediation applications. Actual work performed was compared to the application design or work plan to determine whether additional work was required.

Finally, there are limits to the hydrogeological conditions under which enhanced in situ bioremediation may be applied. A qualitative assessment was made to determine whether performance was related to adverse site conditions such as low permeability sediments, a high degree of heterogeneity, or high rates of groundwater flow.

2.3 SUBSTRATE ESTIMATING TOOL

A substrate estimating tool was developed to assist the practitioner in evaluating a site for an enhanced in situ bioremediation application. A complete description is included in Appendix B of the *Final Technology Demonstration Report* (Parsons, 2010a). The primary objectives of this tool are to:

- Evaluate the site-specific conditions regarding hydrogeology and geochemistry in regard to the demand exerted by both natural and anthropogenic electron acceptors
- Screen for site conditions that require special consideration, such as excursion of pH outside a range optimal for dechlorinating microorganisms
- Evaluate and compare the concentrations of differing substrate types necessary to meet the electron acceptor demand.

This tool was used during the case study evaluations to compare the substrate amendment designs and actual quantities used to the substrate requirements calculated by the tool using site-specific electron acceptor demand.

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3.0 LIMITING FACTORS FOR ENHANCED IN SITU BIOREMEDIATION

A number of limiting factors were identified during the case study evaluations that commonly impact the effectiveness of enhanced in situ bioremediation applications. These limiting factors and the best practices to mitigate them are summarized in the following sections.

3.1 ABILITY TO UNIFORMLY DISTRIBUTE SUBSTRATE

The ability to effectively distribute substrate is often impacted by site-specific lithology (low or high permeability, heterogeneity) and groundwater hydraulics (low or high rates of groundwater flow). In some cases the quantity of substrate that can be injected is limited by a low aquifer buffering capacity and pH excursion. These observations reinforce the need and benefits of conducting adequate site characterization prior to design and implementation of substrate addition. In most cases these conditions can be mitigated by modifying the injection mixture and substrate loading rate (e.g., more frequent and less concentrated substrate solutions, or adding a buffering amendment) or delivery technique (e.g., closer spaced injection points and larger injection volumes).

3.2 ACHIEVING OPTIMAL GEOCHEMICAL CONDITIONS

The most common geochemical problem for the demonstration case studies was an adverse excursion (lowering) of pH, resulting from a combination of low buffering capacity of the aquifer and high concentrations of dissolved organic carbon (DOC). Control of the substrate loading rate is critical when treating aquifers with low buffering capacity. Mitigation measures include careful screening of the site to determine whether a buffering compound should be added to the injection protocol and selecting substrate delivery techniques that provide for more uniform distribution of substrate without excessive “spikes” in DOC. In practice, there will be a range of substrate concentrations that decreases with distance from the point of injection. This will result in a range of redox conditions as illustrated in Figure 1. In some cases, achieving optimal redox conditions over a larger volume of the subsurface may require overstimulation of the aquifer at the point of injection. Therefore, a compromise is often required between overstimulation in the immediate injection area versus creating a larger overall treatment zone.

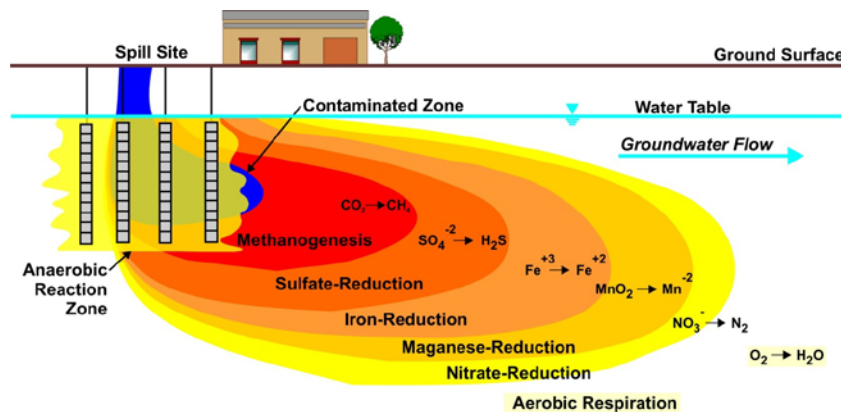


Figure 1. Reducing zones established downgradient of substrate injection.
(from AFCEE et al., 2004)

3.3 DIFFICULT HYDROGEOLOGICAL CONDITIONS

Rates of groundwater flow less than 0.1 ft/day or 37 ft/yr, or greater than 2.7 ft/day or 1000 ft/yr require special design considerations. Low rates of groundwater flow may require closer injection point spacing because migration of soluble organic substrate will be limited. High rates of groundwater flow will require more frequent and higher concentration injections because the substrate may be quickly diluted. In the case of emulsified vegetable oil (EVO) products, the retention of the oil droplets is a critical parameter to sustain adequate substrate concentrations in the reaction zone (Borden et al., 2008). As the degree of aquifer heterogeneity increases, so may the need for closer injection well spacing or for “targeted” injections within lower permeability sediments.

3.4 IMPACTS ON HYDRAULIC CONDUCTIVITY OR BIOFOULING OF INJECTION WELLS

A decrease in hydraulic conductivity (permeability) may result in bypass of contaminated groundwater around the reaction zone or uneven distribution of substrate during subsequent injections. One way to address the potential for loss of hydraulic conductivity is to conservatively design the reaction zone to extend beyond the limits of contaminated groundwater to be treated. For example, a biobarrier or biowall may be installed an additional 20 to 50 ft beyond the edge of the groundwater contaminant plume to avoid potential for bypass around the ends of the reaction zone. It may also be beneficial to provide a degree of overlap (perhaps 20 to 30%) for injection well radius of influence to compensate for reductions in the ability to distribute substrate during subsequent injections.

3.5 SUBSTRATE PERSISTENCE AND LONGEVITY

Concentrations of DOC typically need to be sustained above 50 to 100 mg/L for effective treatment of CAHs over the design life of the application. Buildup of biomass may sustain the reaction zone and limit the amount of rebound that may occur after the initial substrate is depleted. Rebound of concentrations in the treatment zone will depend in large part on whether a residual source of contaminant mass remains upgradient of the treatment zone, or in low permeability sediments within the treatment zone. Adequate characterization and monitoring is needed to ensure that areas of residual contaminants are treated.

3.6 IMPACTS TO SECONDARY WATER QUALITY

Creating an anaerobic groundwater environment may lead to degradation of water quality. The term “secondary water quality” is used to refer to water-quality issues or concerns, apart from the primary contaminants being treated, that result from substrate addition. Production of regulated intermediate degradation products of the primary contaminant (e.g., production of DCE and VC from trichloroethene [TCE]) is not considered a secondary water quality issue for this evaluation. Exceeding secondary water quality standards within the reaction zone may be acceptable if water quality downgradient of the reaction zone is maintained. If concentrations of secondary water quality parameters are maintained below regulatory standards downgradient of the reaction zone, then the application is considered successful in limiting or mitigating any potential adverse impacts.

Table 2 lists common parameters monitored during enhanced in situ bioremediation and associated federal drinking water quality standards. This list is not inclusive, as many U.S. Environmental Protection Agency (USEPA) regions and states enforce additional water quality standards. Several USEPA Region 9 Preliminary Remediation Goals (PRGs) are included in Table 2 as examples. Note that these standards may not be applicable if the impacted groundwater is not a drinking water aquifer, or may not be enforced by all regulatory agencies.

Table 2. Secondary water quality parameters subject to regulatory compliance.
(modified from AFCEE et al., 2004)

Compound or Element	Molecular Formula	USEPA MCL (mg/L) ^{a/}	Secondary Standard ^{b/} (mg/L)	Region 9 PRGs for Tap Water ^{c/} (mg/L)
Volatile Organic Compounds				
Acetone	C ₃ H ₆ O	--	--	5.5
Carbon disulfide	CS ₂	--	--	1.0
Isobutanol	C ₄ H ₁₀ O	--	--	1.8
Methyl ethyl ketone (2-butanone)	C ₄ H ₈ O	--	--	7.0
Total trihalomethanes (includes chloroform)	--	0.080	--	--
General Water Quality Parameters				
Nitrate (as nitrogen)	NO ₃ ⁻	10	--	10
Nitrite (as nitrogen)	NO ₂ ⁻	1.0	--	1.0
Sulfate	SO ₄ ⁻	--	250	--
Chloride	Cl ⁻	--	250	--
pH	--	--	<6.5, >8.5	--
TDS	--	--	500	--
Odor (e.g., sulfide)	--	--	3 threshold odor number	--
Metals/Inorganics				
Arsenic	As	0.01	--	0.045
Selenium	Se	0.05	--	0.18
Iron	Fe	--	0.3	11
Manganese	Mn	--	0.05	0.88

^{a/}USEPA MCL = USEPA maximum contaminant level.

^{b/}USEPA national secondary drinking water regulations are nonenforceable guidelines. However, states may choose to adopt them as enforceable standards.

^{c/}PRGs are USEPA Region 9 preliminary remediation goals for tap water.

Secondary water quality parameters that were evaluated for this study included volatile organic compounds (VOCs) resulting from fermentation reactions (e.g., acetone and methyl ethyl ketone), sulfate and sulfide, chloride, pH, TDS, and dissolved metals or semi-metals (ferrous iron, manganese, arsenic, and selenium). Not all parameters were measured at each site, and often data is available for just a few of these parameters. Nonetheless, the evaluation provided some insight into how much of an issue secondary water quality is and what parameters typically have the greatest potential to create a secondary water quality issue.

The most common secondary water quality issues include the following, in order of most common occurrence:

- **Dissolved Manganese.** Manganese oxides are common minerals in many aquifer sediments, and reduction of Mn^{4+} to soluble Mn^{2+} is a common occurrence. Manganese does not precipitate or sorb out of solution as readily in an anaerobic environment as ferrous iron (e.g., with sulfide), and dissolved manganese tends to persist farther downgradient.
- **Dissolved Iron.** Dissolved (ferrous) iron is commonly observed at concentrations above its USEPA secondary water quality standard. However, dissolved iron typically precipitates or sorbs out of solution within a short distance of migrating out of the anaerobic reaction zone.
- **pH.** Lowering of pH to below 6.5 is another common occurrence. While low pH by itself may not present a serious health hazard or nuisance issue, it may create other secondary problems. Low pH may enhance the solubility of metals, enhance the potential for adverse fermentation reactions, and inhibit complete dechlorination.
- **Sulfide.** Hydrogen sulfide produced by sulfate reduction has a low odor threshold and is commonly observed during sampling of anaerobic sites with high natural sulfate concentrations (>25 to 50 mg/L). Sulfide attenuates rapidly downgradient of the anaerobic treatment zone and rarely persists as substrate is depleted.

Adverse impacts for dissolved arsenic and selenium appear to be less common, perhaps because minerals containing these elements are present at much lower concentrations in most aquifer sediments. However, it is prudent to evaluate whether arsenic or other heavy metals may be prevalent in the aquifer matrix and what the impact of lowering the pH and ORP state of the aquifer may be on their solubility.

Best practices to mitigate these secondary water quality issues include the following:

- Site screening to identify site-specific potential for secondary water issues. Examples may include characterizing the iron, manganese, and heavy metal content of aquifer sediments and evaluating the buffering capacity of the aquifer (pH and alkalinity).
- Establishing natural concentrations of secondary water quality parameters and determining the beneficial use of the impacted groundwater. Groundwater at many sites is not used for drinking water and secondary water quality criteria may not apply.
- Providing more a more uniform distribution of substrate without “spikes” of highly concentrated substrate solutions, and adding a buffering amendment to control pH.
- Providing for an adequate ORP recovery zone downgradient of the treatment zone.

In many cases providing a downgradient ORP recovery zone is sufficient for impacts on secondary water quality to diminish. This is readily accomplished at many large DoD facilities but may be more difficult to incorporate at small industrial or commercial sites.

3.7 IMPORTANCE OF SITE CHARACTERIZATION

Development of a conceptual site model (CSM) and an understanding of the natural processes that are being stimulated are useful to guide the site selection and design process (AFCEE et al., 2004). This should include an assessment of site-specific data on native electron donors and electron acceptors, metabolic byproducts, geochemical parameters, contaminant trends, and hydrogeology. A CSM also summarizes the fate and transport of contaminants, migration pathways, exposure mechanisms, and potential receptors. Therefore, a CSM provides important information to identify and mitigate the limiting factors described above.

The variety of substrates and configurations that can be used for enhanced in situ bioremediation allows the practitioner to design around these limiting factors. Careful site screening and evaluation of each of these limiting factors will lead to higher rates of success and greater effectiveness of the remedy.

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4.0 DESIGN OF SUBSTRATE LOADING RATES

The substrate products on the market are continually increasing in number and complexity. A common approach with slow release substrates is to calculate a substrate (electron donor) requirement based on estimates of native and contaminant electron acceptor mass, and mass loading through the treatment zone over the design life of the application. Vendors of slow-release substrates (e.g., hydrogen release compound [HRC[®]] and EVO) typically rely on calculated substrate requirements because the product is usually applied in a single injection event (e.g., see Appendix G of AFCEE, 2007). Even so, some designs still focus on achieving a “target” concentration of substrate (DOC) in the treatment zone. More recently, design of EVO applications have focused on the oil retention properties of the aquifer matrix (Borden et al., 2008). EVO products may be modified by the vendor to enhance or reduce retention, e.g., by using ionic versus non-ionic emulsifiers.

Advantages of using soluble substrates include the ability to readily distribute the substrate in the subsurface relative to viscous or solid substrates and the ability to modify the rate at which the substrate is applied over time to achieve the desired biogeochemical conditions. For these reasons soluble substrates are well suited for recirculation systems and for bioaugmentation applications. The primary disadvantage of this approach is the requirement for multiple injections (resulting in higher operations and maintenance costs) and the potential for biofouling of injections wells (GeoSyntec Consultants, 2005a).

4.1 DETERMINING SUBSTRATE REQUIREMENTS

A spreadsheet tool has been developed to assist the practitioner in determining site-specific electron acceptor demand and to estimate the substrate required to meet that demand over the design life of the application (available at [http://serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/\(list\)/1/\(view_all\)/1/](http://serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/(list)/1/(view_all)/1/)). **This tool to evaluate substrate requirements is not intended to be used as a design tool; rather, it is intended only for the purpose of site screening and to evaluate the scientific basis of determining electron acceptor demand and substrate requirements.**

Several providers of organic substrates for enhanced in situ bioremediation provide design tools using similar calculations as the substrate estimating tool. The calculations and assumptions used are not always readily apparent in these design tools. The substrate estimating tool provides information on the reactions, calculations, and assumptions employed in an effort to educate the user on how an estimate of the substrate requirement is determined for a specific site. It is not intended to replace or be used in lieu of a vendor’s proprietary design tool.

The technical basis of the substrate estimating tool is described in Appendix B of the *Final Technology Demonstration Report* (Parsons, 2010a). The substrate estimating tool was used to evaluate the electron acceptor demand for each case study (Appendix C of the *Final Technology Demonstration Report [Parsons, 2010a]*). The electron acceptor demand for individual electron accepting processes (assuming they all go to completion) ranged in percent of the total demand as follows:

- Aerobic Respiration: 0.1 to 12.9%, but typically 2% or less
- Nitrate Reduction: <0.1 to 37.4%, but typically 3% or less
- Manganese Reduction: <0.1 to 16.7%
- Iron Reduction: <0.1 to 26.4%
- Sulfate Reduction: 5.6 to 82.7%
- Methanogenesis: 0.2 to 66.7%, but typically greater than 10%
- Contaminant Reduction (CAHs or perchlorate): <0.1% to 75.8%.

The variability in these percentages reflects the wide range of site conditions that may be encountered. Sulfate reduction and methanogenesis have the greatest potential to dominate electron acceptor demand and to increase substrate requirements. This is due to the magnitude of sulfate concentrations that may occur (up to several thousand mg/L) and to the high utilization rate of hydrogen by methanogenesis (1.99 weight of carbon dioxide produced per weight of hydrogen, e.g., compared to 11.91 weight of sulfate reduced per weight of hydrogen). In source areas, the electron acceptor demand from CAHs may dominate.

Substrate estimates using the substrate estimating tool with a design factor of one times the electron acceptor demand over the design life of each case study were compared to the total amount of substrate applied in practice to calculate an overall design factor. Design factors ranged from approximately one times the electron acceptor demand to 21 times the electron acceptor demand, a considerable range. A more common range from 3 to 10 times the estimated electron acceptor demand was observed for six of 11 case studies.

The highest design factor was applied in an early application of neat vegetable oil in 2000 for a potential dense-non aqueous phase liquid (DNAPL) source area at the Hangar K Site at Cape Canaveral Air Force Station (CCAFS), FL (Parsons, 2007). While the use of neat vegetable became less common once EVO products were available, this case study illustrates that very high substrate loading rates may be considered for DNAPL source area applications. This approach may also be beneficial by enhancing the mass transfer of CAHs from a DNAPL or sorbed phase to the dissolved phase where they may be degraded by microbial processes (Macbeth and Sorenson, 2008).

4.2 USING THE SUBSTRATE ESTIMATING TOOL

The substrate estimating tool is useful to screen site conditions that will impact substrate delivery and utilization. The tool provides an estimate of **total** substrate required over the design life of the application given a user-specified design factor. The tool calculates a time-weighted average concentration of substrate by dividing the total volume of groundwater treated by the total substrate quantity. Figure 2 is an example of the output provided by the substrate estimating tool for a suspected DNAPL source area at the Hangar K Site at CCAFS (Parsons, 2007). In this case, the majority of the substrate requirement was from chlorinated solvents. But for most of the case studies treating dissolved plumes, the demand from sulfate reduction and/or methanogenesis dominates.

Table S.5 Output for Substrate Requirements in Hydrogen Equivalents

Site Name: Hangar K, CCAFS, Florida

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1. Treatment Zone Physical Dimensions

	Values	Units	Values	Units
Width (perpendicular to groundwater flow)	60	feet	18	meters
Length (parallel to groundwater flow)	65	feet	19.8	meters
Saturated Thickness	10	feet	3.0	meters
Design Period of Performance	5	years	5	years

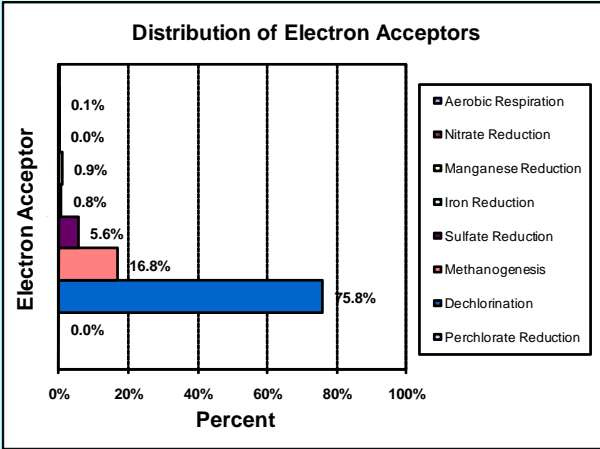
2. Treatment Zone Hydrogeologic Properties

	Values	Units	Values	Units
Total Porosity	0.35	percent	0.35	percent
Effective Porosity	0.25	percent	0.25	percent
Average Aquifer Hydraulic Conductivity	10	ft/day	3.5E-03	cm/sec
Average Hydraulic Gradient	0.001	ft/ft	0.001	m/m
Average Groundwater Seepage Velocity	0.04	ft/day	1.2E+00	cm/day
Average Groundwater Seepage Velocity	15	ft/yr	4.5	m/yr
Effective Treatment Zone Pore Volume	72,950	gallons	276,136	liters
Groundwater Flux (per year)	16,386	gallons/year	62,024	liters/year
Total Groundwater Volume Treated (over entire design period)	154,877	gallons total	586,258	liters total

3. Distribution of Electron Acceptor Demand

	Percent of Total	Hydrogen Demand (lb)
Aerobic Respiration	0.1%	0.081
Nitrate Reduction	0.0%	0.006
Sulfate Reduction	5.6%	4.340
Manganese Reduction	0.9%	0.711
Iron Reduction	0.8%	0.583
Methanogenesis	16.8%	12.989
Dechlorination	75.8%	58.711
Perchlorate Reduction	0.0%	0.000
Totals:	100.00%	77.42

Hydrogen demand in pounds/gallon: 5.00E-04
 Hydrogen demand in grams per liter: 5.99E-02



4. Substrate Equivalents: Design Factor = 1.0

Product	Quantity (lb)	Quantity (gallons)	Effective Concentration (mg/L)	Effective concentration is for total volume of groundwater treated.
1. Sodium Lactate Product	3,589	326	1,338	as lactic acid
2. Molasses Product	2,739	228	1,271	as sucrose
3. Fructose Product	2,163	193	1,339	as fructose
4. Ethanol Product	1,106	160	684	as ethanol
5. Sweet Dry Whey (lactose)	1,706	sold by pound	924	as lactose
6. HRC®	1,311	sold by pound	812	as 40% lactic acid/40% glycerol
7. Linoleic Acid (Soybean Oil)	673	86	521	as soybean oil
8. Emulsified Vegetable Oil	1,122	144	521	as soybean oil

- Notes:**
- Quantity assumes product is 60% sodium lactate by weight.
 - Quantity assumes product is 60% sucrose by weight and weighs 12 pounds per gallon.
 - Quantity assumes product is 80% fructose by weight and weighs 11.2 pounds per gallon.
 - Quantity assumes product is 80% ethanol by weight and weighs 6.9 pounds per gallon.
 - Quantity assumes product is 70% lactose by weight.
 - Quantity assumes HRC® is 40% lactic acid and 40% glycerol by weight.
 - Quantity of neat soybean oil, corn oil, or canola oil.
 - Quantity assumes commercial product is 60% soybean oil by weight.

Figure 2. Example output from the substrate estimating tool.

The substrate estimating tool is also useful to understand how the substrate will be utilized and to screen for potential adverse geochemical conditions. For example, high manganese and iron sites may require monitoring to ensure that secondary water quality is not impacted downgradient of the treatment zone. Alkalinity and pH are included to screen for low buffering capacity.

The quantities and time-weighted average substrate concentrations can be used for comparison to proposed or planned bioremediation applications as a check on the quantities of substrate being proposed or the performance targets for total organic carbon (TOC) or DOC. This should help avoid application of either too little substrate or generating excessive substrate levels. While the substrate estimating tool provides a first approximation of total substrate required, it does not provide for any guidance or indication on how the substrates should be applied. Design tools are often provided by substrate vendors, and the estimated substrate quantity should always be compared to recommendations by the provider or with case studies in the literature.

4.3 RECOMMENDATIONS FOR DESIGN OF SUBSTRATE LOADING RATES

The following recommendations are based on observations from the case studies, including (1) calculation of design factors using the substrate estimating tool, (2) evaluation of overall system performance, and (3) evaluation of limiting factors (Section 3). For slow release substrates injected in a one-time event, a conservative design factor on the order of 3 to 7 times the estimated substrate requirement should be suitable for limiting the potential for insufficient substrate. For soluble substrates, lower design factors on the order of 2 to 3 times the estimated substrate requirement are beneficial to avoid over-stimulating the aquifer and driving down pH. Substrate quantities can be increased if initial loading rates are insufficient to create suitable reducing conditions throughout the treatment zone. The delivery methods for soluble substrates should target uniform substrate concentrations without excessive “spikes” in concentration.

The use of very high substrate concentrations to enhance dissolution of DNAPL into the aqueous phase is an exception to typical substrate loading rates. Solutions with concentrations of lactate as high as 6% by weight, whey as high as 10% by weight, and molasses as high as 1 to 2% by weight have been used for this purpose. These applications require special consideration of the buffering capacity of the aquifer and the system configuration. For example, it may be acceptable to induce adverse geochemical conditions in the source zone to mobilize CAH mass if a suitable downgradient reaction zone for biodegradation and geochemical recovery is established. In most cases, these injections are performed in pulses every 4 to 12 weeks to allow the aquifer geochemistry to stabilize between injections.

4.4 DESIGNING FOR UNCERTAINTY

In practice, the amount of site characterization data that is available or that can be economically obtained is always limited to some extent. Therefore, it is useful to consider practices that mitigate the uncertainty associated with subsurface environments. Examples of system modifications are listed in Table 3.

Soluble substrate systems that use frequent injections have the most flexibility in modifying injection scenarios. When using infrequent applications of slow-release substrates, potential problems such as the need to add a buffering agent should be evaluated prior to substrate

addition, and buffer should be added during substrate injection as a precautionary measure when in doubt.

Table 3. Example enhanced bioremediation system modifications.

Potential Condition	Modification
Low pH or low buffering capacity	<ul style="list-style-type: none"> • Addition of a buffering compound • Use of water push for soluble substrates • Use of slower-release substrates
Low permeability/groundwater velocity	<ul style="list-style-type: none"> • Closely spaced injection points • Targeted injections into low permeability horizons
High permeability/groundwater velocity	<ul style="list-style-type: none"> • Higher substrate loading rates • More frequent injections • Multiple rows of injection wells or biowalls • High retention (coarse droplet) EVO products
Incomplete dechlorination	<ul style="list-style-type: none"> • Microbial characterization • Allow for longer lag times • Lower the ORP environment • Bioaugmentation

Modified from AFCEE et al., 2004 and Suthersan et al., 2002.

Sodium bicarbonate was the most common buffering compound used in the case studies, typically at concentrations in excess of 10,000 mg/L. Sodium bicarbonate is a relatively weak buffering compound (although relatively inexpensive), and may be most suitable for applications using frequent injections of soluble substrates. The use of stronger and more persistent buffering compounds (e.g., sodium carbonates or sodium phosphates) may be necessary for applications using slow release substrates, and further research and product development will be beneficial for sites with low buffering capacity.

Inadequate or excessive distribution of substrate due to aquifer permeability and/or groundwater flow rates can be adjusted by increasing or decreasing the substrate loading rate and/or by modifying injection frequency or well spacing. Substrate loading rates may be increased in the event of inhibitory electron acceptor demand (e.g., sulfate over 50 to 100 mg/L).

Finally, incomplete or delayed dechlorination is a common limitation resulting in accumulation of intermediate dechlorination products. Prior to considering bioaugmentation, the system should be evaluated to ensure that the proper geochemical conditions have been achieved and that a sufficient acclimation period has been allowed for ecological succession and development of appropriate microbial consortia. Bioaugmentation with commercially available cultures can be implemented if it has been determined that indigenous *Dehalococcoides* species are lacking, or do not exhibit the reductase enzymes for complete dechlorination of VC to ethene (e.g., Steffan et al., 2010).

4.5 SUBSTRATE DESIGN TOOLS

The substrate estimating tool is useful to screen site conditions that will impact substrate delivery and reactivity. The tool provides an estimate of **total** substrate required over the design life of the application given a user-specified design factor. The tool also provides a time-weighted average

concentration of substrate for the total volume of groundwater treated. The quantities and time weighted average substrate concentrations can be used for comparison to proposed or planned bioremediation applications as a check on the quantities of substrate being proposed and the performance targets for DOC. This should help to avoid application of either too little substrate or generating excessive substrate levels.

While the substrate estimating tool provides a first approximation of total substrate required, it does not provide any guidance or indication on how the substrates should be applied. Design tools are often provided by substrate vendors, and the estimated substrate quantity should always be compared to recommendations by the provider or with case studies in the literature.

The primary objective when selecting a substrate loading rate is to achieve a uniform distribution of substrate over time and space. Design tools that assist the practitioner with the configuration (well spacing) and injection volumes are being developed and should be incorporated into the design process. An example includes the Edible Oil Substrate Tool being developed under ESTCP Project ER-200626 (Borden et al., 2008), available on the SERDP and ESTCP website at www.serdp-estcp.org.

Two design tools have been recently developed for evaluating buffering requirements to maintain pH at optimal levels for anaerobic dechlorination of chlorinated solvents. The first tool, BUCHLORAC (BUffering of deCHLORination ACidity), was developed by the Source Area BioREmediation (SABRE) project (Robinson and Barry, 2009). The second tool is being developed by Emulsified Oil Substrate[®] (EOS[®]) Remediation under the direction of Dr. Robert Borden. The EOS[®] design tool is based on an Excel spreadsheet and is limited to determining the amount of a substrate/buffering product (AquaBufpHTM) to apply, based on site-specific conditions.

The two tools differ in the input parameters required to determine buffering requirements. In general, the BUCHLORAC model uses speciation of anions and cations in groundwater and the amount of carbonate and iron oxide minerals in the aquifer matrix as input to a geochemical equilibrium model, while the EOS[®] Remediation tool uses direct measurements of soil and groundwater acidity as input to the spreadsheet tool.

5.0 ADVANCES AND RESEARCH FOR ENHANCED IN SITU REMEDIATION

Research over the past 6 years since the 2004 *Principles and Practices* document was published has focused on (1) a greater variety of configurations and better methods of substrate distribution, (2) improved understanding of degradation processes and use of bioaugmentation cultures, (3) improved monitoring methods and tools, and (4) application to an ever expanding list of groundwater contaminants. Future research may lead to exploiting alternative degradation processes such as biogeochemical transformation of chlorinated solvents (Becvar et al., 2008; Lebron et al., 2010) and oxidation of DCE and VC under low DO conditions, based on research by Cox et al. (2010). The following sections highlight several of these advances, with references to some of the research efforts and guidance documents produced or in progress by the ESTCP and AFCEE technology transfer programs.

5.1 BIOAUGMENTATION

The science and application of bioaugmentation is now a mature technology with the development of several commercial cultures and a better understanding of distribution techniques (GeoSyntec, 2005a; Stroo et al., 2010; Steffan et al., 2010). Bioaugmentation cultures were initially designed around mixed cultures based on *Dehalococcoides* species to completely degrade tetrachloroethene (PCE) and TCE to ethene. The addition of other dechlorinating species (such as *Dehalobacter*) provides cultures capable of degrading chlorinated ethanes (e.g., 1,1,1-tetrachloroethane [TCA] and 1,2-dichloroethane [DCA]) and chlorinated methanes (e.g., carbon tetrachloride [CT]) (Stroo et al., 2010). Recirculation has traditionally been used to distribute bioaugmentation cultures (e.g., GeoSyntec, 2002). Passive methods of distributing bioaugmentation cultures may lead to even greater use of the technology (ER-200513).

5.2 DNAPL SOURCE ZONE REMEDIATION

The ability to treat DNAPL source areas with in situ bioremediation has been demonstrated as an effective remediation technique (Interstate Technology and Regulatory Council [ITRC], 2008a). The addition of organic substrates has been shown to enhance mass transfer from DNAPL into the aqueous phase where it may be degraded by biological processes (Ward, et al., 2009; Macbeth and Sorenson, 2008; McCarty et al., 2007). Bioaugmentation cultures can survive and grow in the presence of high concentrations of chlorinated solvents (Christ et al., 2005), and may be effective for DNAPL applications (NAVFAC ESC and GeoSyntec, 2007). Research into partitioning electron donors (ESTCP Project ER-200716) may lead to even more effective amendments for DNAPL source area remediation. Partitioning electron donors are electron donors that partition directly into the DNAPL, promoting the growth of dechlorinating biomass close to the DNAPL and enhancing DNAPL dissolution rates. The ESTCP has also developed tools for evaluating the benefits of DNAPL source remediation (Siegrist et al., 2010; Abriola et al., 2008), and for evaluating the impact of treatment on downgradient water quality (Johnson et al., 2008).

5.3 BIOWALLS AND BIOREACTORS

Permeable mulch biowalls (Figure 3) and bioreactors have been demonstrated as effective in situ bioremediation configurations with a protocol document developed by AFCEE (2008). Biowalls are passive, long-term treatment systems that may require infrequent replenishment with EVO (Parsons, 2010b). Biowalls may also be used to treat perchlorate and energetic compounds (Newell, 2008). Solar powered recirculating bioreactors are gaining in popularity as sustainable remedies for source area remediation (e.g., Parsons, 2006). One notable observation of these systems is the potential for stimulating biogeochemical transformation processes, particularly at sites with high sulfate concentrations (Lebron et al., 2010).

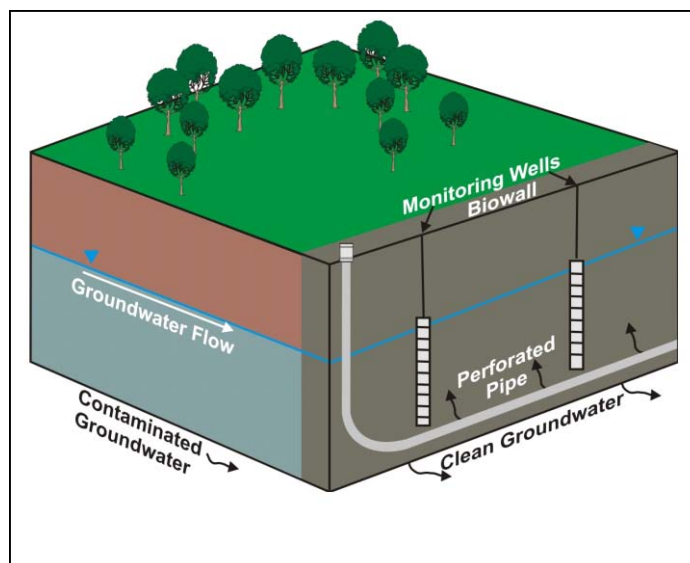


Figure 3. Biowall conceptual design.
(from AFCEE, 2008)

5.4 IMPROVED/ALTERNATIVE DELIVERY TECHNIQUES

Typical delivery techniques include direct injection or re-circulation to distribute organic substrates into an impacted aquifer. The primary limitation to uniform distribution of substrate is aquifer heterogeneity. To optimize substrate delivery, Borden et al. (2008) have developed a design tool for aqueous amendment injection systems using EVO. Other tools are being developed that focus on evaluating and verifying the delivery and distribution of amendments using geophysical techniques (ER-200834 and ER-200717).

Alternate mixing and delivery techniques are being investigated. One potential technique is the use of electrokinetics, where the amendments follow electrical field lines and move through low permeability areas in heterogeneous systems (Gent et al., 2007). Current research includes the use of shear-thinning delivery fluids for enhanced delivery of bioremediation amendments (ESTCP Project ER-200913), and the use of water-soluble polymers in minimizing the effects of geologic heterogeneities to improve remediation amendment delivery and distribution (ESTCP Project ER-1486).

Treatment of CAHs in the vadose (unsaturated) zone is being pursued, particularly using gaseous electron donors. Evans et al. (2009) have explored the use of mixed gases for treating perchlorate and found that hydrogen gas mixed with nitrogen gas was effective. Gaseous hydrogen has previously been demonstrated for treating CAHs in the saturated zone (GSI, 2003), and the use of hydrogen in the vadose zone is currently being demonstrated for CAHs by ESTCP (Project ER-201027).

5.5 ALTERNATE DEGRADATION PROCESSES

Degradation processes other than halorespiration by dechlorinating bacteria have been the subject of intense research over the past few years. Abiotic degradation process by reduced iron minerals is one form of degradation of CAHs that may occur naturally or as a result of substrate addition in high iron/sulfate environments (USEPA, 2009). The Air Force is currently demonstrating the engineered biogeochemical transformation of CAHs by addition of varied iron, sulfate, and substrate amendments to form reactive iron sulfide minerals. These abiotic degradation processes favor dichloroelimination reactions to produce acetylene, instead of a sequential dechlorination reaction to produce cis-DCE and VC (Butler et al., 2009; DeFlaun et al., 2009; Scherer et al., 2007).

The oxidation of DCE and VC under both aerobic and anaerobic conditions is of interest because these compounds are produced and often persist due to the incomplete anaerobic dechlorination of PCE and TCE (Spormann, 2006; Gossett et al., 2004, Bradley and Chapelle, 1998a and 1998b). The ESTCP has initiated research to elucidate the mechanisms and environmental relevance of biodegradation of cis-DCE and VC (ER-1557 and ER-1558). Observations that oxidation of cis-DCE and VC may occur under low DO conditions on the order of 0.5 mg/L (Cox et al., 2010) may lead to greater use of sequential anaerobic/aerobic treatment systems (e.g., ER-201026). Alternately, cultures may be developed that can facilitate the oxidization of VC under anaerobic conditions (ER-1556) or that can oxidize cis-DCE under aerobic conditions (Major et al., 2010).

5.6 IMPROVED MONITORING TOOLS

A number of improvements in monitoring and tools for evaluating enhanced in situ remediation have been made over the past several years. These include test methods and tools using (1) molecular biological tools (MBT) with biomarkers and enzyme probes, (2) the use of compound-specific isotope analysis (CSIA), and (3) techniques and tools for mass flux measurements.

MBTs and functional gene biomarkers may be used to demonstrate that specific microbes capable of performing the desired degradation process are present and active. Functional gene biomarkers have been used for several years to test for the presence of *Dehalococcoides* strains capable of dechlorinating VC to ethene (Krajmalnik-Brown et al., 2004; Müller et al., 2004). Research continues to develop more accurate and predictive MBTs to manage and optimize dechlorination processes at enhanced bioremediation sites (ER-1586, ER-1587, and ER-1588). Efforts are also underway to standardize methods for analysis of nucleic acid-based biomarkers in environmental samples, including sample collection and preservation techniques (ER-1561 and ER-200518). Finally, biomarkers have recently been developed for degradation of

perchlorate (Kirisits et al., 2008) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (Cupples, 2010; ER-1609).

Mass flux measurements are another tool that may be used to evaluate the effectiveness of an enhanced in situ bioremediation application. A mass flux measuring device has been developed by ESTCP (Hatfield et al., 2006), and a mass flux toolkit has been developed to evaluate groundwater attenuation and remediation alternatives (Farhat et al., 2006). ESTCP is also developing a mass flux meter for fractured bedrock (ER-200831).

CSIA is being used in both monitored natural attenuation (MNA) and enhanced in situ bioremediation applications (USEPA, 2008). Research is being conducted on the behavior of compound specific isotopes due to non-destructive processes such the storage of CAHs in low-permeability zones through diffusion and sorption (ER-1739 - in progress). Fractionization of isotopes is used to demonstrate that degradation is occurring. There is potential that the degree of fractionization may indicate which degradation process is occurring, such as biotic versus abiotic dechlorination of CAHs (USEPA, 2008). A tool is also being developed by ESTCP to integrate CSIA into a contaminant transport model for predictive purposes (ER-201029).

5.7 USING ENHANCED IN SITU BIOREMEDIATION IN TREATMENT TRAINS/COMBINED TREATMENT TECHNOLOGIES

Enhanced in situ bioremediation is often used in conjunction with other remediation technologies to achieve site closure. Because enhanced in situ bioremediation promotes naturally occurring degradation processes, it is often used in conjunction with MNA. Used in a more active treatment train, enhanced in situ bioremediation has been applied following source area thermal treatment (e.g., electrical resistivity heating) (Pennell et al., 2009) or following in situ chemical oxidation (ISCO) (Major, 2009). While microbes capable of degrading chlorinated solvents may survive in microclimates within such thermal or chemical treatment zones, bioaugmentation is typically used to reestablish a dechlorinating microbial population following the active treatment. The ESTCP is currently demonstrating the benefits of combining low-energy electrical resistivity heating with either in situ bioremediation or with iron-based reduction using injectable zero-valent iron (ZVI) (Project ER-200719).

5.8 APPLICATION TO OTHER CONTAMINANTS

Perchlorate has been demonstrated to be amendable to enhanced anaerobic bioremediation (Hatzinger and Diebold, 2009; Cox et al., 2009; Stroo and Ward, 2009). Enhanced anaerobic bioremediation has also been demonstrated for treating explosive compounds such as RDX and trinitrotoluene (TNT) (Wade et al., 2010; Newell, 2008). ESTCP continues to fund research for the in situ treatment of perchlorate and energetic compounds (ER-1607, ER-200425, and ER-201028).

N-nitrosodimethylamine (NDMA) is used with propellants and is a carcinogen and emerging groundwater contaminant at a number of DoD and DOE facilities. NDMA may be amendable to enhanced in situ bioremediation (Szecsody et al., 2009; Hatzinger et al., 2008; Finneran et al., 2007). The ESTCP is currently demonstrating an alternative degradation process for NDMA using injection (biosparging) of propane gas and oxygen to stimulate degradation by

propanotrophs, a class of indigenous microorganisms that have been shown to rapidly degrade NDMA to innocuous products (ER-200828). Research is also underway on the degradation processes for the energetic material CL-20 (Hawari, 2006).

Another potential application of enhanced bioremediation using organic substrates is to stimulate anaerobic iron and sulfate reduction to precipitate heavy metal contaminants in iron sulfide systems (Hayes, et al., 2009). Research into degradation of emerging contaminants should continue, while some contaminants such as 1,4-dioxane and NDMA continue to present challenges for in situ remediation. (Steffan, 2007; ESTCP Project ER-1417).

5.9 RESOURCES

Many of the advances and improved science of enhanced in situ bioremediation of chlorinated solvents have been compiled in the Strategic Environmental Research and Development Program (SERDP)/ESTCP monograph series, including the *In Situ Bioremediation of Perchlorate in Groundwater* (Stroo and Ward, 2009), the *In Situ Remediation of Chlorinated Solvent Plumes* (Stroo and Ward, 2010), and a forthcoming monograph on characterization and remediation of chlorinated solvent DNAPL source areas.

Demonstration reports and guidance documents for ESTCP projects related to groundwater remediation (including descriptions of projects in progress) are available online at:

[http://serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/\(list\)/1/](http://serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/(list)/1/)

Similarly, demonstration reports and guidance documents for AFCEE technology transfer initiatives are available online at:

<http://www.afcee.af.mil/resources/technologytransfer/index.asp>

Guidance documents available on the AFCEE Technology Transfer site include the *Principles and Practices of Enhanced Anaerobic Bioremediation for Chlorinated Solvents* (AFCEE et al., 2004), the *Protocol for In Situ Bioremediation of Chlorinated Solvents Using Edible Oil* (AFCEE, 2007), and the *Technical Protocol for Enhanced Anaerobic Bioremediation Using Permeable Mulch Biowalls and Bioreactors* (AFCEE, 2008). Current AFCEE technology transfer initiatives include demonstrations and development of guidance for the in situ biogeochemical transformation of chlorinated solvents.

The ITRC Program also publishes in situ bioremediation guidance documents, at:

<http://www.itrcweb.org/gd.asp>

ITRC technical regulatory guidance documents include the *In Situ Bioremediation of Chlorinated Ethene: DNAPL Source Zones* (ITRC, 2008a) and the *Enhanced Attenuation: Chlorinated Organics* (ITRC, 2008b).

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