In Situ Bioremediation of DNAPL Source Zones

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FOREWORD

Abstract

EPA's Office of Superfund Remediation and Technology Innovation provided a grant through the National Network for Environmental Management Studies to research *in situ* bioremediation in DNAPL source areas. This report was prepared by an undergraduate student from Tufts University during the summer of 2005. The report is available on the Internet at www.clu-in.org/studentpapers/.

The objective of this report is to provide an overview of *in situ* bioremediation of DNAPL source areas. This report discusses the integral steps when implementing bioremediation, such as site characterization, design considerations, and post-treatment monitoring. In addition, this report also examines the use of bioremediation as a polishing treatment for the source zone. Case studies are included as examples of the use of bioremediation as a stand-alone and a polishing treatment for DNAPL source areas.

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ACRONYMS AND ABBREVIATIONS

CFSTR	continuous-flow stirred tank reactor
DCE	dichloroethene
DNAPL	dense non-aqueous liquid
DO	dissolved oxygen
EPA	Environmental Protection Agency
HRC	hydrogen release compound
INEEL	Idaho National Engineering and Environmental Laboratories
ISB	*in situ* bioremediation
ISCO	*in situ* chemical oxidation
MCL	maximum contaminant level
mV	millivolts
O&M	operation and maintenance
ORP	oxidation reduction potential
PCE	perchloroethene
RAO	remedial action objective
Redox	reduction-oxidation
SEAR	surfactant-enhanced aquifer remediation
TAN	Test Area North
TCE	trichloroethene
TOC	total organic carbon
VC	vinyl chloride
µg/L	micrograms per liter

1.0 INTRODUCTION

1.1 Purpose

The purpose of this paper is to provide an in depth overview of source zone bioremediation for DNAPL source areas. Chlorinated ethenes, which are common dry-cleaning chemicals and degreasers, are the most common source of DNAPL (dense non-aqueous phase liquid) contamination. The presence of DNAPL in the subsurface is a persistent problem for groundwater contamination because of the difficulty to locate and treat DNAPL source areas. Plumes that develop from DNAPL source areas generate large areas of contaminated water, which threatens human and environmental health. Source treatment is advantageous over plume management or containment strategies, as it reduces source longevity, lessens long-term risk, and minimizes down-gradient mass flux (Christ et al. 2005). Because there remains uncertainty whether complete source removal is attainable, containment strategies and plume management methods continue to be employed at many DNAPL sites (EPA 2003). Although no documented DNAPL-contaminated sites have achieved reduction to maximum contaminant levels (MCL), aggressive source zone treatment has proven successful for reducing chlorinated ethene concentrations to levels suitable for natural attenuation and reaching remedial action objectives (RAOs) (EPA 2003, Christ et al. 2005).

Bioremediation of chlorinated ethenes is an emerging technology for treatment of DNAPL source areas. Until the late 1990s, bioremediation only had been used to treat plume contamination, and it was thought that concentrations in the source zone would be toxic to microorganisms. Since then, research has shown that microorganisms degrade contaminants at high concentrations, and degradation may occur at a faster rate in the source area than in the plume (Nielsen and Keasling 1999).

Source zone *in situ* bioremediation (ISB) may reduce the duration for which a site remains impacted by contamination, although complete remediation to MCLs is not likely to be achieved in a short time span. However, by reducing the longevity of a site, the risk of exposure and the cost of treatment often are significantly minimized. Bioremediation offers a potential cost-effective alternative to costly aggressive mass-removal treatments for DNAPL contaminated sites.

1.2 Scope

This document provides background information and design considerations for source zone bioremediation of chlorinated ethenes. Both pilot and full-scale case studies are also included as examples of ISB in the source zone.

Most of the research for bioremediation of DNAPLs has been focused on chlorinated ethenes. In addition, chlorinated ethenes are among the most prevalent DNAPL constituents; hence, the scope of this document will be limited to bioremediation of DNAPL source areas comprised of chlorinated ethene contaminants located in the saturated subsurface environment.

2.0 DENSE NON-AQUEOUS PHASE LIQUID (DNAPL)

Chlorinated ethenes, such as trichloroethene (TCE) and perchloroethene (PCE), are released as organic liquids, which may migrate downwards below the groundwater table when released into the subsurface. As pure-phase product, chlorinated ethenes are dense non-aqueous phase liquids (DNAPLs), which are only sparingly soluble and denser than water (see Table 1). In the saturated zone, chlorinated ethenes may exist in the subsurface environment as a DNAPL in dissolved phase or sorbed phase. Due to the slow dissolution rate of DNAPL, areas in the subsurface containing DNAPL serve as lasting sources of groundwater contamination. Additionally, because of the high toxicity of chlorinated ethenes, even small concentrations in the groundwater pose a health risk (as indicated by the MCL for TCE and PCE for drinking water set by the EPA as 5 ppb).

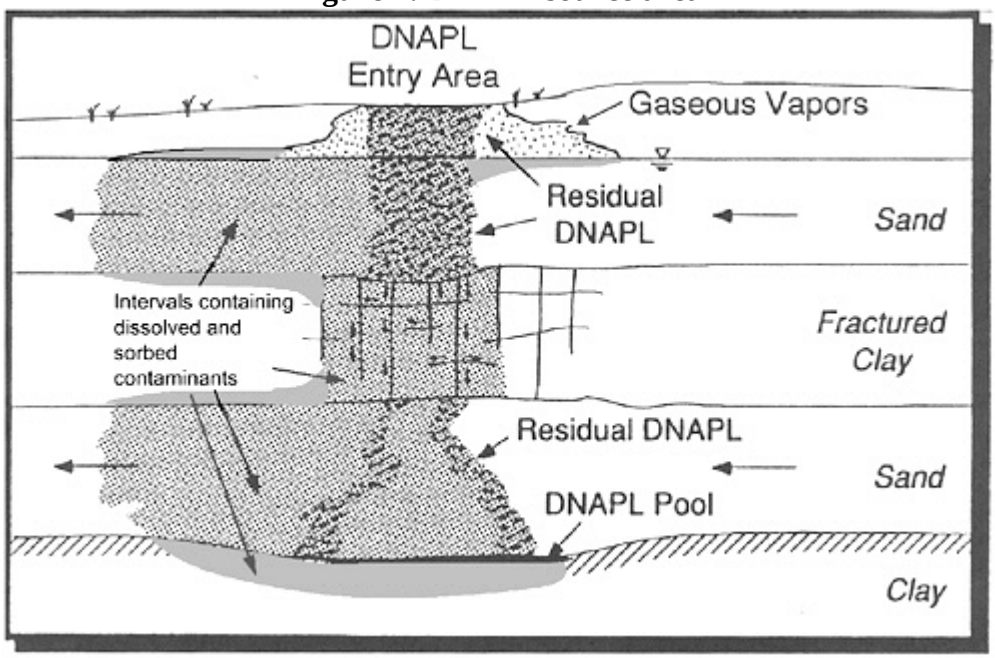
Table 1: Characteristics of Tetrachloroethene and Trichloroethene

Compound	Molecular Formula	Density (g/L @ approx. 20 to 25°C)	Solubility (mg/L @ approx. 20 to 25°C
Perchloroethene (PCE)	C_2Cl_4	1.62	150
Trichloroethene (TCE)	C_2HCl_3	1.46	1,100

Adapted from AFCEE 2004

In the subsurface environment, DNAPL can exist in regions of entrapped ganglia or in higher saturation pools. Immobile, discontinuous ganglia (residual DNAPL) is formed when downward migration occurs, and DNAPL becomes entrapped in the soil pores. Pools form when DNAPL that has migrated downwards encounters a less permeable layer or lens, forcing the DNAPL to spread laterally and accumulate. Pools of DNAPL are areas in which DNAPL is a continuous mass between soil pores (mobile DNAPL). Mobile DNAPL can flow under normal conditions. If the pool accumulates enough to overcome pore entry pressure requirements in the less-permeable lens, downward migration will ensue. The area containing sorbed, residual, and mobile DNAPL is the DNAPL source zone (NRC 2005). Groundwater flowing through the DNAPL source zone becomes contaminated, forming a plume of dissolved-phase contamination downgradient from the source.

Figure 1: DNAPL source area



Source: NRC 2005

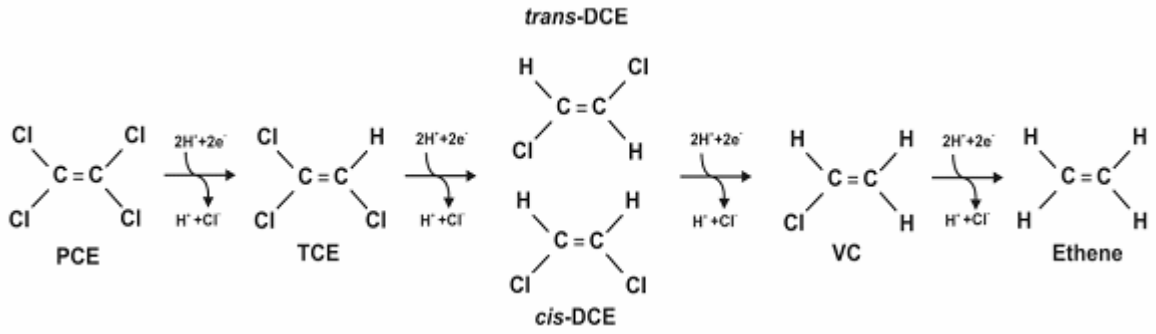
To prevent exposure to contamination or discharge of contaminated groundwater, the DNAPL source zone must either be contained or treated. Enhanced ISB is an innovative technology that can be used to degrade (or transform) contaminants in DNAPL source zones and consequently reduce the mass of DNAPL.

3.0 BIOREMEDIATION

3.1 Introduction to *In Situ* Bioremediation of Chlorinated Ethenes

Bioremediation of chlorinated ethenes is a destruction technology that uses microorganisms to break down contaminants to less toxic end products (e.g., ethene). Chlorinated ethenes can be degraded under anaerobic conditions in the presence of a suitable electron donor through reductive dechlorination (McCarty 1998). Reductive dechlorination is the dechlorination reaction catalyzed by microorganisms in which a chlorine atom is replaced by hydrogen (the electron donor) on an organic compound, such as chlorinated ethene. PCE, which contains four chlorine atoms, is degraded sequentially from PCE to TCE to DCE (dichloroethene) to VC (vinyl chloride) to ethene (as shown in Figure 2). Primarily TCE is degraded to the isomer *cis*-1,2-dichloroethene (*cis*-DCE); infrequently TCE degradation results in the production of *trans*-1,2-dichloroethene (*trans*-DCE). Although DCE and VC are toxic compounds, ethene is a gas that is non-toxic in the environment.

Figure 2: Reductive Dechlorination of Tetrachloroethene to Ethene



Source: http://www.afcee.brooks.af.mil/products/techtrans/bioremediation/default.asp

Reductive dechlorination is the most dominant degradation mechanism for the degradation of chlorinated ethenes by microorganisms (Suthersan and Payne 2005). Reductive dechlorination can occur through either metabolic or cometabolic degradation. In metabolic degradation, microorganisms gain energy from breaking down the contaminant. With cometabolism, the enzymes used for cell metabolism fortuitously act to degrade contaminants, but no energy is gained from the reaction.

Microorganisms can only degrade dissolved contamination (not DNAPL or sorbed contaminant); however, dissolution is enhanced during bioremediation (see section 3.6) resulting in a reduction of DNAPL mass and sorbed contaminant.

Reductive dechlorination can occur naturally to degrade chlorinated ethenes when microorganisms capable of reductive dechlorination (known as reductive dechlorinators) are present and active in the contaminated environments. The remediation of contaminants that occurs through natural processes is called natural attenuation. However, natural attenuation does not occur at all sites or may not be an adequate remedy to reduce contamination to regulatory levels in a suitable timeframe. Alternatively, enhanced ISB is a more active approach for remediation.

Bioremediation can be enhanced by biostimulation or bioaugmentation through engineered systems. Non-ideal conditions usually necessitate additions to the microbial environment to stimulate or improve reductive dechlorination. Through biostimulation, substrates are injected to encourage microbial growth. Bioaugmentation is the process of stimulating bioremediation through the addition of microbial cultures. Bioremediation in the source area is usually implemented through the active approach of enhanced ISB, rather than the passive approach of monitored natural attenuation due to high concentrations of chlorinated ethenes in source areas.

3.2 Biostimulation

The addition of electron donors and nutrients encourage cell growth, increasing the number of microorganisms to degrade contaminants. Substrates provide a carbon source for microbial growth and electron donors for dechlorination. Fermentation of substrates produces fermentation products, such as hydrogen, that supply an electron donor. Hydrogen is the primary electron donor for reductive dechlorination (Yang and McCarty 1998). Acetate also can be utilized as an electron donor by reductive dechlorinators, although a study by He et al. 2002 indicated that hydrogen is a better direct electron donor than acetate. At remediation sites for chlorinated ethenes, hydrogen-producing substrates are frequently used for biostimulation.

Depending on site conditions, slow-release or fast-release hydrogen compounds can be used as substrates. Slow release substrates, such as oils (e.g., olive oil, soybean oil, vegetable oil) and commercially produced Hydrogen Release Compound (HRC®), are relatively insoluble and produce low concentrations of hydrogen. More soluble compounds (e.g., lactic acid, molasses, and lactate) release high concentrations of hydrogen.

Injection of substrates is often used to enhance redox (reduction-oxidation) conditions at sites. Microorganisms preferentially reduce the electron acceptors, which yield the highest free energy from the redox reaction. Oxygen yields the highest free energy; therefore, oxygen is the preferred electron acceptor and has the highest potential for reduction. If conditions are aerobic at the site, aerobic organisms will predominate. The injection of substrate stimulates the microorganisms present, which will result in higher utilization of oxygen in the subsurface. Once the dissolved oxygen content is reduced, anaerobic conditions will develop. Under anaerobic conditions, the order of preferential electron acceptors is nitrate, manganese (IV), iron (III), sulfate, and then carbon dioxide (methanogenesis). Reductive dechlorinators can compete with iron- and sulfate-reducing bacteria, but the best conditions to support reductive dechlorination are at methanogenic conditions. Methanogenic conditions can be achieved through addition of electron donors to drive down the redox potential at the site by stimulating the reduction of electron acceptors with higher redox potentials, such as iron or sulfate. Although PCE and TCE degradation occur within most anaerobic environments, DCE and VC dechlorination occur almost only under sulfate reducing and methanogenic conditions (AFCEE 2004). Therefore, complete reductive dechlorination to ethene without an accumulation of toxic daughter products is most likely to occur under methanogenic conditions.

One concern about injecting electron donors is competition from methanogens. If methanogens out-compete dechlorinators for electron donors, the growth of the dechlorinators will be impeded. However, dechlorinators may thrive in the source zone where methanogens are inhibited by higher concentrations of PCE and TCE (Yang and McCarty 2000). Some research had indicated that hydrogen concentrations should be limited to inhibit methanogens and increase the efficiency of electron donor utilization by dechlorinating bacteria (Yang and McCarty 1998), but more recent research has proven that higher hydrogen concentrations can increase dechlorination even if the efficiency of electron donor utilization is lower (Suthersan and Payne 2005, AFCEE 2004). Also, limiting electron donor supply can lead to reductive dechlorination to stall at DCE, as the electron donor supply may not produce the proper reducing conditions and can prevent complete dechlorination (Suthersan and Payne 2005). An overloading of carbon sources is, however, not cost effective and can result in biofouling due to microbial growth (McCarty 2003). Biofouling is the accumulation of microorganisms, which usually occurs near the injection well and can reduce the permeability of the subsurface, hindering the distribution of the substrate.

3.3 Bioaugmentation

Reductive dechlorination not achieved after biostimulation may indicate a lack of reductive dechlorinators, and the site may require bioaugmentation. Bioaugmentation is used when the population of dechlorinating bacteria is not present at a site, not widely spread, or not populous enough for biodegradation to proceed at an acceptable rate. Dechlorinating bacteria, which degrades PCE and TCE to *cis*-DCE is presumed to be ubiquitous, but dechlorinating bacteria capable of *cis*-DCE and VC degradation are not always found at sites (AFCEE 2004).

Bioaugmentation is not necessary at all sites. Biostimulation alone has been used effectively when reductive dechlorinators have not been detected or are detected in very low numbers through injection of high concentrations of electron donors for an extended period of time to stimulate dechlorination (Suthersan and Payne 2005). However, increasing the population of dechlorinators in bioaugmentation can reduce the lag time before reductive dechlorination occurs and decrease the duration of remediation (Major 2005).

Biostimulation is usually implemented before bioaugmentation to produce an appropriate environment for the bacteria to thrive. For successful bioaugmentation and reduction of time needed for acclimation, the culture must be injected uniformly throughout the source area and suitable conditions for the bacteria must be present (i.e., redox conditions, pH range, supply of electron donors, and presence of nutrients).

3.4 Microorganisms

The species *Dehalococcoides*, *Dehalobacter*, *Sulfurospirillum*, *Desulfuromonas*, *Desulfitobacterium*, and *Clostridium* all have been shown to be capable of reductive dechlorination of chloroethenes (Major et al. 2003, Smidt and de Vos 2004, AFCEE 2004). Yet, *Dehalococcoides* species are the only isolates capable of complete dechlorination (Maymo-Gatell et al. 2001, He et al. 2003). Henderickson et al. (2002)

compiled 21 sites where complete dechlorination was occurring and found the presence of *Dehalococcoides* at all sites. However, at nine sites where only partially reductive dechlorination was occurring, *Dehalococcoides* were not detected. To enhance reductive dechlorination through bioaugmentation, mixed cultures containing strains of *Dehalococcoides* are usually injected (e.g., KB1 and Biodechlor™). However, research is ongoing to determine whether other species are also capable of complete reductive dechlorination.

Although the presence of *Dehalococcoides* is usually necessary for dechlorination of PCE, not all *Dehalococcoides* have the capability for complete dechlorination of highly chlorinated ethenes. One *Dehalococcoides* isolate, *Dehalococcoides ethenogenes* 195, has been identified with the capability of degrading PCE to ethene (Maymo-Gatell et al. 2001). Strain 195 degrades PCE and TCE to VC metabolically, but can only degrade VC to ethene cometabolically (Maymo-Gatell et al. 2001). The cometabolic process is slower than the metabolic degradation of PCE and TCE, causing accumulation of both *cis*-DCE and VC at sites. Accumulation of DCE and VC is detrimental because of DCE and VC's high toxicity and VC's carcinogenetic nature. Maymo-Gatell et al. (2001) also stated that strain 195 requires PCE as a substrate to degrade VC, but high concentrations of PCE and TCE inhibit dechlorination of VC by strain 195.

Another *Dehalococcoides* species strain isolated by He et al. (2003) is BAV1 BAV1 metabolically dechlorinates VC to ethene. Therefore, in the presence of BAV1, reductive dechlorination can proceed without a toxic build-up of VC. Also, BAV1 is the only isolate capable of dechlorinating all DCE isomers. Growth of BAV1 was not supported by PCE and TCE, but co-metabolism of PCE and TCE was detected in the presence of growth-supporting chloroethenes (i.e., VC or DCE). The isolation of BAV1 indicates that DCE stall and VC accumulation may be occurring at sites that lack species capable of degrading DCE and VC and that it may be prevented through bioaugmentation.

For bioaugmentation, a consortium of microorganisms (not only *Dehalococcoides*) is used to promote complete reductive dechlorination. Commercially-available mixed cultures for bioaugmentation are KB-1 and Bio-Dechlor INOCULUM™ (AFCEE 2004).

3.5 Source Zone Bioremediation

One concern about conducting ISB of the source zone is that reductive dechlorinators would not be able to thrive in areas with high concentrations of PCE and TCE, and reductive dechlorination would be limited in DNAPL source zones. Although no study has demonstrated the ability of metabolic reductive dechlorinators to grow on the DNAPL-water interface, reductive dechlorination does occur in the source area. Research has shown that dechlorinators are active at PCE saturation concentrations; however, direct contact with DNAPL may be partially inhibiting (Yang and McCarty 2000). In addition, studies have shown that microorganisms have the capability of facilitating dissolution in the source area (Yang and McCarty 2000, Cope and Hughes 2001, Carr et al. 2000).

3.6 Enhanced Dissolution

Bioremediation may enhance the DNAPL dissolution rates, which would result in a reduction in the longevity of the source zone (Cope and Hughes 2001). Degradation of PCE and TCE to their breakdown products near to the DNAPL interface is envisioned to induce a steep concentration gradient, thereby increasing the rate at which mass is transferred from the NAPL to the aqueous phase. Yang and McCarty (2002, 2003) reported a 3-fold or more enhancement of DNAPL dissolution where reductive dechlorination was occurring in comparison to abiotic systems. Carr et al. (2000) observed a 14-fold increase in PCE removal rates for biotic systems compared to abiotic systems in continuous-flow stirred tank reactors. Enhanced dissolution rates increase the amount of contaminant that is dissolved into the aqueous-phase and is therefore bioavailable to be reduced by microorganisms, unlike the inaccessible DNAPL. The second proposed cause of enhanced dissolution is the production of more soluble daughter products (i.e., *cis*-DCE) compared to the less-soluble parent compounds, PCE and TCE (Carr et al. 2000).

Enhanced dissolution occurs when reductive dechlorination occurs close to the DNAPL interface (McCarty et al. 2003). To encourage growth near the interface and to enhance dissolution, substrates need to be near the interface to supply electron donors to the microorganisms. For the substrate to be close to the interface, the substrate either can be injected at high concentrations, flooding the whole area, or through the addition of a substrate that has an affinity for DNAPL, such as alcohols (Major 2005).

3.7 Enhanced Solubilization

Studies are currently being conducted to determine if bioremediation enhances solubilization. In the DNAPL source zone, most of the chlorinated ethene mass is present as a NAPL or sorbed contaminant. After biostimulation, concentrations of chlorinated ethenes often spike. The spike in chlorinated ethenes is hypothesized to be due to an enhancement in the solubility of NAPL components. Solubility enhancements may be attributed to factors such as the electron donors, fermentation products, and biosurfactants (Payne et al. 2001, Suthersan and Payne 2005).

Some electron donors have shown potential to increase the chlorinated ethene concentration after injection; therefore, more contamination is bioavailable to the microorganisms. Lactic acid and whey are two electron donors that have shown results indicating an enhanced solubility effect (Sorenson 2005, Major 2005). However, not all electron donors enhance solubility. Vegetable oil, for example, may actually reduce the solubility of the contaminants (Borden 2003).

Microorganisms naturally produce biosurfactants, which increase transfer from the NAPL to the aqueous phase, thereby increasing the potential concentration of chlorinated ethenes. Although this mechanism has been suggested for enhanced solubility (Payne et al. 2001), more research is necessary to determine the extent to which biosurfactants increase solubility.

Fermentation products, such as alcohols and ketones, also increase the solubility of chlorinated ethenes. Yet, detectable amounts of fermentation products have not been recorded in bioremediation field studies; therefore, no evidence has been put forth to date to prove that fermentation products have produced an observable affect on solubilization.

4.0 SITE CHARACTERIZATION

4.1 DNAPL Characterization

The source area must be characterized properly for an effective source zone remediation. Due to the difficulty in locating DNAPL, a rule of thumb for assuming the presence of DNAPL is if the concentration of the contaminant in the groundwater is one percent of the saturated aqueous concentration (although 10% aqueous concentrations are used at some sites) (Kram et al. 2001). However, this technique only can be used to indicate whether or not DNAPL is present and not the extent of the contamination. Just one characterization technique cannot accurately assess the extent of the source zone; many tools are needed. The selection of tools for site characterization depends on site conditions, historical site information, and the DNAPL migratory path. Non-geophysical techniques are used for direct and indirect detection of DNAPL. Physical properties are measured by geophysical techniques, which are used to indirectly indicate DNAPL presence (EPA 2004a).

Figure 3: Tools for DNAPL Characterization

Non-Geophysical Tools:	**Geophysical Tools:**
Diffusion Sampler	Electrical Methods
Direct Push Technology	Electromagnetic Methods
In Situ Groundwater Sampling	Radar
Hydrophobic Dye Testing	Magnetic Methods
Tracer Testing	Seismic Methods
Soil Gas Profile	

Adapted from: EPA 2004.

One approach to site characterization, the Triad, incorporates systematic project planning, dynamic work strategies, and real-time measurement technologies to address the heterogeneity encountered at DNAPL sites (EPA 2003). Systematic planning includes laying out clear objectives, optimizing work flow, and focusing on quality control to ensure the data being collected is relevant, accurate, and obtained efficiently. Dynamic work strategies allow flexibility in site characterization; therefore, changes to the location, extent and method of sampling and analysis can be made while still in the field to allow complete characterization in one mobilization. Real-time measurement technologies together with decision support tools allow the conceptual site model to be updated while still in the field, which lets site managers make decisions on site to decide which tools should be used for continued site characterization and where samples should be retrieved in order to obtain an accurate site assessment. The Triad approach usually can result in an accurate site characterization and a reduction in site characterization costs (EPA 2003). More information on the Triad is available at www.triadcentral.org.

4.2 Determining Site Conditions

When evaluating the feasibility of bioremediation and determining design considerations, relevant parameters for determining site conditions include physical, chemical, and biological conditions. Determining site conditions is an integral step for evaluating the effectiveness of ISB.

4.2.1 Physical Parameters

Physical parameters, such as hydraulic gradient and conductivity, will dictate which substrate is best suited and how injections will flow in the subsurface. Under extremely low hydraulic gradients, substrate injection will not have a large radius of influence. Conversely, higher groundwater velocities may also increase the need for substrate or prevent the development of low redox conditions because the substrate will not have a sufficient residence time in the source area. Heterogeneity at a site may lead to preferential pathways for the substrate; therefore, substrate will not be delivered uniformly through the subsurface.

4.2.2 Chemical Parameters

Analysis of the redox potential will indicate whether the aquifer will provide the right conditions for dechlorination. The most rapid dechlorination occurs under highly reducing conditions; methanogenic conditions are the most favorable for dechlorinating bacteria. Less reducing conditions, such as sulfate-reducing ones, often result in biodegradation stalling at *cis*-DCE. Through field evidence, PCE and TCE dechlorinate under sulfate-reducing conditions, but complete dechlorination is more likely to occur under methanogenic conditions (Nelson et al. 2005).

Table 2: Redox Potential and Biodegradation Mechanisms

Bacteria Electron Acceptor Class	Predominant CAH Biodegradation Mechanisms	Redox Potential (mV)
Oxygen-reducing	Aerobic Oxidation	> 600
Nitrate-reducing	Reductive Dechlorination	250-100
Iron (III)-reducing		100-0
Manganese (IV)-reducing		0 to -200
Sulfate-reducing		0 to -200
Methanogenesis		< -200

Biological activity by dechlorinators can be limited if the pH is outside of the neutral range (pH>8 or pH<5). The fermentation of substrates produces hydrogen and organic acids that lower the pH of the aquifer (NRC 1993). High alkalinity levels can buffer an aquifer from changes in pH, thereby keeping the pH in a neutral range.

4.2.3 Biological Parameters

Success when performing anaerobic bioremediation is more likely when dechlorination is already occurring because the dechlorinating population is present and active. A presence of the daughter products, *cis*-DCE and VC, signifies dechlorination. Elevated levels of ethene can indicate complete reductive dechlorination, but background sources of ethene may also cause elevated levels. Accumulation of daughter products and low amounts of ethene suggests that dechlorination is not proceeding through to ethene.

Abiotic degradation pathways for chlorinated ethenes also can be responsible for dechlorination, so biological indicators can be used to affirm the presence of reductive dechlorinators. Microbial assays are one method used to rule out the possibility of abiotic dechlorination. Microbial assays that identify 16S rDNA sequences, which are specific for *Dehalococcoides*-related bacteria, determine if the dechlorinating population is present. It is important to consider that just the presence of *Dehalococcoides* will not predict whether dechlorination can occur, because not all *Dehalococcoides* have the ability to completely dechlorinate PCE and TCE to ethene and other mixed cultures may be capable of complete dechlorination. The benefit of monitoring for *Dehalococcoides* is that if it already has been determined that dechlorination is not occurring, a lack of *Dehalococcoides* may support a decision for implementing for bioaugmentation.

Microcosms are often used to determine the potential for dechlorination. Microcosms are set up and analyzed in the laboratory to determine the efficacy of different substrates and the necessity for nutrient and microbe addition. Dechlorination that does not occur under the controlled conditions of microcosms with sufficient electron donors and nutrients indicates an absence of dechlorinating bacteria.

5.0 SYSTEM DESIGN FOR ENHANCED BIOREMEDIATION

5.1 Design Objectives for Enhanced Source Zone Bioremediation

Determining the priority of remediation goals for a site guides the implementation of source zone bioremediation. Common clean-up goals for bioremediation of DNAPL contaminated sites are:

- Contaminant mass reduction
- Reduction of source longevity and plume life
- Reduction in costs

Contaminant mass reduction can be achieved by enhanced dissolution. It has been suggested that mass removal can be achieved with more efficiency by using electron donors, which can increase the solubility or can partition into the DNAPL. Recirculation wells are often used for increased mass removal to achieve more uniform substrate delivery and substrate/contaminant contact.

If a *reduction in source longevity and plume life* is the main priority for a site, an aggressive approach to ISB is usually implemented. Aggressive ISB treatment often includes bioaugmentation and the use of a soluble substrate. Bioaugmentation will reduce the microbial lag time, and injections of soluble substrates are immediately available as electron donors.

A *reduction in costs* can be achieved by either reducing source longevity or reducing the cost of implementation. For the least expensive design, a cost analysis must be performed and should include costs associated with the site configurations, extent of contamination, substrate selection, injection design, monitoring parameters, and predicted life-span. Often ISB designs that have a higher capital investment (e.g., from costs accrued through bioaugmentation and installation of recirculation), have a reduced lifespan and therefore reduced costs for operation and maintenance (O&M) and monitoring. Conversely, sites that use a more passive approach (e.g., from use of viscous substrates) require less of a capital investment, but have a longer lifespan resulting in more costs associated with O&M and monitoring.

5.2 Substrate Selection for Biostimulation

Substrate selection is dependent on remediation goals and site characteristics. A diverse selection of substrates is available for ISB. Substrates vary in cost, delivery technique, viscosity, and the frequency of injections needed (see table 3).

5.2.1 Soluble Substrates

Soluble substrates have higher mobility in the subsurface than the more viscous substrates, allowing for the most uniform distribution. Soluble substrates are suited for areas of high velocity groundwater flow, as well as areas where contamination reaches

deep levels of the subsurface. The use of soluble substrates can be altered to fit site characteristics by varying concentration, frequency, and volume of injections.

Soluble substrates with a density greater than water, such as lactate and molasses, may reach areas of deeper contamination better than substrates such as ethanol and methanol, which are less dense than water (AFCEE 2004). However, with the use of recirculation wells, substrate density is not an issue of concern. For enhanced mass removal of DNAPL, some soluble substrates may have the potential for enhancing solubility of DNAPL. Lactic acid has shown potential for increasing solubility in preliminary laboratory testing (Sorenson 2005, Major 2005).

Soluble substrates have higher O&M costs because they are utilized quickly, require frequent re-injections, and can cause biofouling. However, soluble substrates are usually the least expensive substrate. Installation of recirculation wells will increase capital costs, but will contain the source zone and usually result in more uniform distribution of substrate, which decreases the life-span of the source area.

5.2.2 Viscous Substrates

Viscous substrates have less mobility in the subsurface than soluble substrates, but release hydrogen slower and last longer. Viscous substrates are usually injected through direct-push technology rather than injection wells because normally only a one-time application is necessary. Viscous substrates release hydrogen slowly over time and remain in the aquifer longer than soluble substrates, reducing the need for reinjection. HRC™ and HRC-X™ release lactic acid, which is fermented into hydrogen for use in reductive dechlorination. Vegetable oil degrades to fatty acids, which are then fermented to hydrogen.

Vegetable oil is most effective when contamination is shallow in the subsurface and when direct-push technology can be used for multiple injections to increase the area of influence. Vegetable oil may reduce mass flux of the contaminant, but may not result in large-scale removal of contaminant mass (Borden 2003). Loss of permeability may also result from oil injections (Lee et al. 2001).

Commercially available HRC™ and HRC-X™ are more expensive substrates, but the cost of the substrates also includes assistance in product application design. HRC™ should last in the subsurface for up to 18 months after injection. HRC-X™ is a more viscous substrate than HRC™ and an injection only should be necessary every 3-4 years. A disadvantage of using HRC™ and HRC-X™ is the inability to alter the configurations of the product, such as concentration and viscosity, as can be done with non-commercial products.

A disadvantage of using viscous substrates is decreased mobility, which may lead to non-uniform distribution of substrate, preventing contaminant/substrate contact. Also, there is a limitation to the depths at which viscous substrates can be applied.

5.2.3 Low-Viscosity Fluids

Low-viscosity fluids, such as emulsified vegetable oil, have more mobility than viscous substrates. This increased mobility allows more uniform distribution in the aquifer and a wider radius of influence. Emulsified oil is not effective for treating contamination in deep layers because of its buoyant nature (AFCEE 2004). Similar to non-emulsified vegetable oil, emulsified oil slowly releases hydrogen through the fermentation of fatty acids and is a long lasting substrate. Depending on site characteristics, application is usually necessary every 2-3 years, but only one application may be needed.

5.2.4 Experimental Substrates

Whey has been used as a substrate for source zone treatment, but only under pilot scale demonstrations. Whey is a longer lasting substrate compared to soluble carbohydrates because of its complex structure. Fresh whey, a waste from the dairy industry, is very inexpensive, but difficult to ship and store. Powdered whey may be preferred because of its ease to ship and store, but is more expensive than fresh whey (AFCEE 2004). Studies have shown benefits of using whey in combination with more soluble substrates to reduce the number injections. Also, preliminary studies by Macbeth et al. (2005a) have shown that injection of whey powder can enhance dissolution of DNAPL.

Table 3: Common Substrate Options for Reductive Dechlorination

Substrate	Bulk Price per lb ($)	Delivery Techniques	Frequency of Injection
Soluble Substrate			
Lactate	1.00 to 2.00	injection wells or circulation systems	Continuous to monthly
Methanol	0.10, 0.20, to 0.25	injection wells or circulation systems	Continuous to monthly
Ethanol	0.10, 0.20, to 0.26	injection wells or circulation systems	Continuous to monthly
Molasses	0.25 to 0.35	injection wells	Continuous to monthly
corn syrup	0.25 to 0.30	injection wells	Continuous to monthly
Viscous Fluid Substrates			
HRC	5.00 to 7.00	direct injection	Annually to bi-annually
HRC-X	5.00 to 7.01	direct injection	Every 3 to 4 years
Vegetable Oils	0.20 to 0.40	direct injection or injection wells	One-time application
Low-Viscosity Fluid Substrates			
Vegetable Oil Emulsions	2.00 to 4.00	direct injection or injection wells	Every 2 to 3 years
Experimental Applications			
Whey (soluble)	0.05 (fresh)/ 1.00 to 1.50 (powdered)	direct injection or injection wells	Monthly to annually

Adapted from: AFCEE 2004

5.3 Substrate Delivery for Biostimulation

Source zone depletion occurs when the dechlorinators degrade contaminants near the DNAPL-water interface. Delivering substrate to the DNAPL-water interface will encourage microbial growth as close to the interface as toxic effects will permit. Because of heterogeneity in aquifers, injected substrate often follows preferential pathways and may not reach the contaminants. Different methods of delivery can be used to improve contact between the substrate and the contaminants. By increasing the number of injection points in the source area, there will be more overlap of the substrate, thereby increasing the likelihood of contaminant-substrate contact. Another method is to force the electron donor to reach the contaminant through high pressure injections, so that the substrate can flow through areas of low permeability.

Increased microbial growth around the injection well (biofouling) can reduce permeability and prevent substrate delivery, therefore alternate delivery strategies may be necessary. Biofouling is a common problem when soluble substrates are injected continuously. Reducing the volume of injections and using repeated injections rather than a continuous injection will reduce microbial growth near injection points, thereby preventing biofouling and allowing the substrate to travel freely (Suthersan and Payne 2005).

5.4 Monitoring for Reductive Dechlorination

Monitoring parameters vary depending on site conditions, regulatory needs, and project goals. Systems monitoring includes a baseline analysis, process monitoring, and performance monitoring. Baseline analysis is used for system design and comparison for process and performance monitoring. Conditions commonly monitored for include chlorinated ethenes, total organic carbon (TOC), oxidation-reduction potential (ORP), dissolved oxygen, ferrous iron, sulfate, final degradation products (including methane, ethane, and ethene), alkalinity, pH, and nitrate (see Table 4). Monitoring of chlorinated ethenes (PCE, TCE, *cis*-DCE, VC, and ethene) indicates the extent to which dechlorination occurs. Other parameters indicate why anaerobic degradation would be hindered if complete degradation is not detected.

Table 4: Recommended Groundwater Monitoring Analysis

Analysis	Performance Expectation
Chlorinated Ethenes	Chlorinated ethenes and daughter products are expected to decline
Total Organic Carbon	stable or declining TOC levels less than 20 mg/L in conjugation with elevated levels of VOCs indicate a need for additional substrate
Oxidation Reduction Potential	ORP values should remain less than -100 mV within the treatment zone, greater values indicate a need for additional substrate
Dissolved Oxygen	DO concentrations greater than 1.0 mg/L indicate need for additional substrate to reach anoxic conditions
Ferrous Iron	elevated levels of ferrous iron may indicate a competing terminal electron acceptor to anaerobic dechlorination
Sulfate	sulfate levels less than 20 mg/L are desirable, but not required for anaerobic degradation to occur

Analysis	Performance Expectation
Methane, Ethane, and Ethene	methane levels less than 1.0 mg/L in conjugation with accumulation of *cis*-DCE or VC may require additional substrate to achieve reducing conditions
Alkalinity	concentrations of alkalinity that remain below background with pH of less than 5 may require the addition of a buffering agent
pH	pH levels within a range of 5 to 9 are desirable
Nitrate/Nitrite	nitrate levels less than 1.0 mg/L are desirable for anaerobic dechlorination
DNA Sequencing of *Dehalococcoides* species	positive identification of *dehalococcoides*-related species indicates potential for complete dechlorination of chlorinated ethenes (experimental procedure—recommended only as a diagnostic tool)

Adapted from AFCEE 2004

The usual tool for determining complete dechlorination is monitoring ethene and chloroethene concentrations to determine whether highly chlorinated ethenes are being reduced to ethene. A stoichometric balance should remain for chloroethenes and ethene. Discrepancies in the stoichometric balance could indicate off-site migration. Discrepancies also can be explained by different degradation pathways, such as abiotic degradation. Abiotic degradation of *cis*-DCE has been observed at some sites where ethene is not detected. However, chloroethene concentrations have decreased. Abiotic degradation breaks down *cis*-DCE to carbon dioxide, water, and chloride rather than ethene (Suthersan and Payne 2005).

Through analysis of the monitoring data, the ISB design can be altered to increase the effectiveness of the system either by changing injection strategies, substrate selection, or nutrient addition.

6.0 BIOREMEDIATION AS A SECONDARY TREATMENT

6.1 Introduction to Treatment Trains

In source areas, multiple treatments are often necessary for complete remediation of a site. While successful in removing large quantities of NAPL mass in short time periods, aggressive treatments, such as surfactant-enhancing aquifer remediation (SEAR), co-solvent injections, thermal treatment, and *in situ* chemical oxidation (ISCO), are unlikely to remove all the DNAPL from the source-zone due to the persistence of NAPL pools and the potential for flow bypassing around some areas containing NAPL. Bioremediation is seen as an option for treating post-treatment DNAPL. Multiple treatments have been implemented when one single treatment has not proven successful, but few sites have used treatment trains in which one treatment sequentially follows another within a time span that is best suited to the technologies used. Some technologies have shown additional benefits when paired together, although not all technologies are enhanced when used in a treatment train. This section will look at four technologies: SEAR, co-solvent treatment, thermal treatment, and ISCO. All four have been used in combination with ISB.

6.2 Surfactant-Enhanced Aquifer Remediation (SEAR)

SEAR treatment has been implemented as an aggressive source treatment for DNAPL. Surfactants are injected into the subsurface to increase the solubility of the DNAPL through micellar solubilization. Through increased solubilization of DNAPL, the contaminant can be removed through extraction wells, decreasing the source longevity. Although SEAR has been used successfully to remove greater than 90% of the contamination at some sites, additional treatment or containment is often needed to meet regulatory levels (Christ et al. 2005). The potential benefit for bioremediation would be an immediate reduction in the amount of DNAPL due to SEAR, as areas containing large amounts of DNAPL may limit the effectiveness of bioremediation. Additionally, biodegradable surfactants remaining after SEAR can be fermented, therefore supplying hydrogen for reductive dechlorination. Ramsburg et al. (2004) demonstrated increased reductive dechlorination after SEAR application of a biodegradable, food-grade, ionic surfactant at a PCE-contaminated site. (Refer to Section 8.2 for SEAR case study).

6.3 Co-solvent Flushing

Co-solvent flushing, similar to SEAR, enhances mobilization and solubilization of DNAPL. Ethanol, methanol, and isopropanol are commonly used for co-solvent flushing (ITRC 2003). Co-solvent flushing can remove DNAPL from the site, although post-treatment residual contamination may remain. Since methanol and ethanol have been used as substrates for bioremediation, the co-solvents also can be used to enhance bioremediation to treat the residual contamination. One pilot study analyzed the potential for utilizing excess ethanol from co-solvent flushing to stimulate bioremediation post-treatment at a site contaminated with PCE-DNAPL. The study found that an increase in daughter products, including ethene, were detected after co-solvent flushing treatment (Mravik et al. 2003).

6.4 Thermal Treatment

In situ thermal treatments include steam enhanced extraction, electrical resistive heating, and thermal conductive heating. Bioremediation may be an effective option for remediation of DNAPL remaining after thermal treatment. Because extreme temperatures are not necessary for thermal treatment of chlorinated ethenes (DNAPL-water mixtures boil at temperatures less than 100^{o}C), sterilization is less likely to occur and the elevated temperatures could result in an increase in the biodegradation rate of chlorinated ethenes (EPA 2004b).

While biological activity can increase at elevated temperatures, microorganisms can be harmed from extremely high temperatures (EPA 2004b). Results from laboratory studies have shown that microbial populations were inhibited after microcosms were heated to 100^{o}C (Friis 2005). However, field studies have shown that reductive dechlorination can occur during the cool-down period after thermal application where temperatures were raised to 100^{o}C (Beyke 2005). Other research has suggested that thermal treatment can stimulate reductive dechlorination (Macbeth 2005).

Future research needs include determining the effects of less aggressive thermal treatment followed by bioremediation. Performing thermal treatment at temperatures lower than the boiling point has the potential to stimulate microbial activity and will be less expensive than the more energy-consuming thermal treatments at higher temperatures.

6.5 *In Situ* Chemical Oxidation (ISCO)

ISCO is implemented by the injection of an oxidant into the source area. Oxidants commonly used for ISCO include permanganate, Fenton's reagent, and hydrogen peroxide (ITRC 2005). Through ISCO, chlorinated ethenes are oxidized to form carbon dioxide, water, and chloride. Oxidizing conditions develop through implementation of ISCO; once oxidizing agents are consumed, the treated area will revert back to pre-treatment conditions (ITRC 2005). At sites containing DNAPL, ISCO can reduce DNAPL mass and lower contaminant concentration, but post-treatment residual DNAPL may cause contaminant concentrations in groundwater to be above MCLs. Enhanced bioremediation can be used as a secondary treatment to reach closure regulations, although the efficacy of enhanced bioremediation after ISCO has not conclusively been determined. One concern with implementing bioremediation after ISCO is that strong reducing conditions necessary for complete reductive dechlorination may be difficult to achieve. Also, when permanganate is used as an oxidant, precipitation of manganese oxides may inhibit TCE dechlorination (Dennis et al. 2005). At a site contaminated with PCE DNAPL, which had been treated with permanganate, the microbial population recovered one year after ISCO treatment. However, microbial populations declined at wells with high permanganate concentrations (MacBeth et al. 2005b). The overall microbial population often increases after ISCO implementation due to an influx of carbon from ISCO. However, it is not known if the specific dechlorinating population also thrives after ISCO.

7.0 FEASIBILITY FOR BIOREMEDIATION OF DNAPLS

7.1 Limitations of Bioremediation

The effectiveness of bioremediation depends on site conditions and DNAPL configuration. Microbial growth thrives only under specific geochemical and hydrological settings. Conditions that limit substrate delivery, such as low hydraulic conductivity and heterogeneous sites, are not conducive to bioremediation. Reducing conditions other than methanogenic conditions will often result in incomplete dechlorination. Although conditions can be engineered to produce microbial growth, an economic analysis must be performed to determine whether an engineered approach will be cost effective compared to alternative treatments.

In addition, bioremediation may be limited in areas containing large amounts of mobile DNAPL (pools). Christ et al. (2005) modeled the relation between source longevity and ganglia-to-pool ratio. High ganglia-to-pool ratios resulted in the greatest reduction in source longevity by source zone bioremediation, but bioremediation was less effective for low ganglia-to-pool ratios where enhanced dissolution is limited by lower specific surface area over which mass can be transferred.

The following are some of the concerns when implementing source area bioremediation:

1) *Toxic Degradation Products*
The toxic degradation products DCE and vinyl chloride may accumulate during bioremediation. However, research has shown that DCE and VC accumulation can be prevented by ensuring that methanogenic conditions, available substrate, and the presence of reductive dechlorinating populations are at the site.

Stalling at DCE can occur because this less chlorinated compound has a lower reduction potential than PCE or TCE. Therefore, when PCE and TCE can be reduced at sulfate-reducing conditions, degradation of DCE will not be as likely to occur. Yu et al. (2005) indicated that highly-chlorinated ethenes can inhibit the dechlorination of less-chlorinated ethenes (except no inhibition was observed of PCE on *cis*-DCE dechlorination).

Accumulation of VC can occur because the degradation rate for VC is slower than for the highly-chlorinated ethenes. Some microorganisms, such as *Dehalococcoides ethenogenes* 195, can only degrade VC through a cometabolic reaction, which is a slower reaction than the metabolic reaction for PCE, TCE, and DCE. Recently, mixed cultures have been used where metabolic degradation of VC has occurred and no VC accumulation was observed.

Aerobic polishing also has been used as a method for degrading DCE and VC after anaerobic bioremediation. DCE and VC can be metabolically degraded aerobically (unlike PCE). Therefore, once PCE has been degraded, aerobic conditions can be stimulated for the degradation of the daughter products. Because aerobic metabolic degradation occurs more rapidly than cometabolic degradation, VC and DCE can be degraded at a faster rate, eliminating the build up of toxic daughter products (EPA 2000).

2*) Impacts on Water Quality and Aquifer Conditions*
Biostimulation and bioaugmentation can change aquifer conditions because of microbial growth. Biofouling can decrease hydraulic conductivity. Microbial growth will also consume nutrients, produce organic acids (which lower pH), increase methane production, and alter redox conditions, all of which result in declined water quality downgradient from the bioremediation site.

3) *Increased Mobility of Contaminants*
Enhancement of dissolution will lead to a reduction in the time required for remediation, but also result in an initial increase in concentration of the contaminants. One method for reducing the risk of mobilization is through the use of recirculation wells to contain the source.

4) *Effectiveness for Bioremediation*
Bioremediation may reduce source longevity, but attaining MCLs for most sites with areas containing high levels of contamination is not always feasible. However, it may be possible that low enough levels can be reached to allow for transition to natural attenuation, allowing for site closure and attainment of RAOs.

5) *Long Term Data Unknown*
Bioremediation of source zone areas is a relatively new technology. Although the immediate effects of bioremediation have been documented, long term effects, such as rebound occurrence and plume reduction, are largely unknown.

7.2 Advantages of Bioremediation

Bioremediation can be an inexpensive and effective process for treating some DNAPL source areas. Field demonstrations have shown that bioremediation can reduce source longevity, reduce DNAPL mass, and reduce concentrations of contaminants. One of the main advantages of bioremediation is that during treatment, exposure to the contaminant is limited because ISB is an *in situ* treatment and no toxic waste needs to be treated. Another advantage is that ISB can offer flexible designs depending on goals and budget by altering substrate choice and injection methods.

8.0 CASE STUDIES

8.1 Biostimulation and Bioaugmentation Case Study: Cape Canaveral Air Force Station, Florida

At Launch Complex 34 in Cape Canaveral Air Force Station, Florida, four remediation technologies had been implemented unsuccessfully to treat a site contaminated with TCE-DNAPL. A pilot study for bioaugmentation and biostimulation was implemented on a portion of the source area to enhance the rate of TCE degradation. Remediation was focused on the upper layer of the aquifer (16 ft – 24 ft below the surface), which contained most of the DNAPL identified in the site characterization. DNAPL was not detected visually, but concentrations of TCE were greater than the solubility level at the test plot indicating DNAPL presence. Contamination at the site predominantly consisted of TCE, although traces of *cis*-DCE and VC were detected, which signified natural attenuation. The redox potential ranged from +54 to + 71 mV.

Recirculation wells were installed to inject the substrate (ethanol) into the source area beginning in October 2002. Ethanol was injected for 14 weeks before bioaugmentation was implemented in February 2003. KB-1 culture, which consists of dechlorinating bacteria including strains of *dehalococcoides ethenogenes,* was then injected to increase the rate of TCE degradation to ethene.

Ethanol was originally injected daily, but biofouling was detected at the injection wells, so the dose was decreased to less than once a day to decrease microbial growth around the injection wells.

Four months after bioaugmentation, soil coring was used to determine if the mass of DNAPL had been reduced. In the treatment zone, pre-treatment concentrations of TCE in the soil reached 8,000 mg TCE/ kg soil, which is greater than the threshold value of 300 mg/kg for TCE DNAPL presence. After treatment, no samples contained TCE concentrations greater than the threshold value, which indicates the absence of DNAPL. The reduction in TCE mass was estimated to be 98.5% total TCE and > 99% of TCE-DNAPL. The degradation products of TCE, *cis*-DCE, and VC increased immediately after biostimulation but reduced post-treatment of bioaugmentation. *Cis*-DCE increased from 31.6 mg/L to 94.7 mg/L after biostimulation, but decreased to 19.4 mg/L after bioaugmentation. The concentration of VC started at less than 1 mg/L. During sampling immediately after bioaugmentation, the concentration of VC increased to 103 mg/L. Post-treatment sampling, one-month after treatment, revealed that VC levels had declined to 8.0 mg/L. The groundwater standard for VC is 1 µg/L. Ethene concentrations, which indicate complete degradation, first decreased after biostimulation but then rose from 573 µg/L to 22,000 µg/L post-demonstration. The decrease in TCE concentrations, along with the presence of VC and *cis*-DCE indicate an enhancement of reductive dechlorination at the site. Also, the increase in ethene signifies that complete dechlorination was occurring due to biostimulation and bioaugmentation.

Source: Battelle 2004

8.2 Biostimulation Case Study: Idaho National Engineering Environmental Laboratory (INEEL)

A field demonstration was conducted at INEEL in Test Area North (TAN) to determine whether ISB was more effective for treating the source area than pump-and-treat. The aquifer at TAN is composed of fractured basalt that was 200 ft deep underlain by an impermeable clay layer. Although conditions in the aquifer were anaerobic, it was not a highly-reducing environment. The contamination consisted predominantly of TCE, although PCE and *cis*-DCE were also detected. The source area treatment was an area 100 ft in diameter where the highest concentrations of TCE were detected. Concentrations of TCE were at 1,000 µg/L, and there was indirect evidence of TCE DNAPL.

Laboratory studies first were conducted to determine the feasibility of ISB at TAN. Microorganisms capable of complete reductive dechlorination were found in the contaminated area. Dechlorination to ethene occurred after the addition of the substrate sodium lactate in microcosm studies.

Biostimulation was implemented for a one-year field demonstration starting in January 1999 prior to full-scale application. The field demonstration was used to determine the effectiveness of applying ISB in the source area for reducing groundwater concentrations and for mass removal of the source.

Sodium lactate was injected biweekly for 9 months through one injection well. The sodium lactate injections were sequentially diluted throughout the 9 months, although the mass of the sodium lactate remained constant. Throughout the treatment, samples were collected from wells to monitor electron donor distribution, chlorinated ethene concentrations, biological activity indicators, redox parameters, and general water quality parameters.

Reductive dechlorination was observed at the site six weeks after biostimulation was implemented. The appearance of *cis*-DCE indicated reductive dechlorination. The increase in the molar concentrations of *cis*-DCE was greater than the decrease in TCE, and concentrations of total chlorinated ethenes also increased after biostimulation. This indicated that the injection of lactate increased the concentration of bioavailable TCE in the aquifer either through enhanced dissolution or mass transport by the electron donor. Because of the increased aqueous concentrations that are bioavailable, enhanced reductive dechlorination will be a faster remediation treatment than pump-and-treat.

The redox conditions were enhanced by the lactate injections. Iron and sulfate-reducing conditions were reached immediately after the lactate injections began. Dechlorination of VC and ethene corresponded with methanogenesis conditions, which occurred after 4 to 5 months of lactate injections. There was no accumulation of VC detected, which was probably due to the highly-reducing conditions obtained in the aquifer. The detection of ethene indicated that the residence time was suitable for complete reductive dechlorination at the site.

Dechlorination was delayed in the wells 40 meters downgradient from the site because of inadequate distribution of the electron donor. Sodium lactate has a higher density than water, which resulted in a downward migration of the lactate in the aquifer; the upper layer downgradient from the injection well had lower concentrations of substrate.

From the field demonstration, it was determined that a full scale application of ISB would be more cost effective and would decrease the source lifespan by 50% than pump and treat. The projected cost for a full scale implementation was $2,091,000 over the span of 15 years. Pump-and-treat would last for 30 years and cost a total of $2,937,000. Bioremediation was also the preferred treatment because the contaminant is destructed *in situ*, which reduces the risk of exposure for workers. For pump-and-treat systems, the contaminated water is extracted from the ground and treated by air stripping, which increases the potential for exposure to workers and releases of contaminants into the air. The field demonstration also supported the use of a high concentration electron donor (lactate) to enhance dissolution and achieve appropriate redox conditions. For the full scale application, the electron donor injection needed to be altered to improve distribution in the aquifer. A second injection well and adjustments in the electron donor concentrations were proposed alternatives.

Source: Peterson et al. 2000

8.3 Bioremediation following Surfactant Treatment: Bachman Road Site Oscoda, Michigan

Monitoring of bioremediation was conducted after surfactant treatment at the Bachman Road Site in Oscoda, Michigan, to determine the potential for applying ISB as a polishing technique. The main contaminant on the site was PCE from a former dry cleaning operation. The estimated groundwater velocity was 0.13 m/day in an unconfined aquifer composed of medium to fine-grained sand. PCE DNAPL was detected at the site through analysis of soil cores.

To treat the source area, a surfactant treatment was implemented. A non-ionic surfactant was flushed through the source area and 19 L of PCE was extracted during the pilot study. Pre-treatment, only trace amounts of PCE daughter products were detected. At 270 days post treatment, an elevated presence of daughter products was detected in the monitoring wells. At 450 days post-treatment, concentrations of *cis*-DCE and other PCE daughter products increased by up to five orders of magnitude in comparison to pretreatment levels. However, VC was only detected at three of the monitoring wells, which were located inside the treatment area, indicating DCE stall. The wells where VC was detected also had elevated levels of the surfactant. Reductive dechlorinating bacteria, including *Dehalococcoides (*BAV-1), had previously been detected at the site.

Fermentation of the surfactant was hypothesized to produce acetate, an electron donor that can be utilized for reductive dechlorination. An increased presence of daughter products after treatment indicated that surfactant flushing stimulated reductive dechlorination at the site. The production of daughter products indicates potential for the application of enhanced ISB post-SEAR to reduce residual DNAPL and chlorinated ethene concentrations to regulatory levels for monitored natural attenuation.

Source: Ramsburg et al. 2004

9.0 CONCLUSION

Enhanced ISB for the source zone is a viable and potentially cost effective method. It has been implemented effectively at sites to reduce contaminant mass, shorten the lifespan of the source area, and reduce groundwater concentrations of chlorinated ethenes. Successful implementation of enhanced ISB requires comprehensive site evaluations and appropriate design considerations. The most important design considerations include achieving proper redox conditions, uniform substrate delivery, and the presence of reductive dechlorinators. An appropriate design will reduce the potential for unsuccessful ISB implementation, which leads to DCE stall and VC accumulation.

Enhanced ISB is not appropriate for all sites. Conditions that prevent microbial growth, areas containing large pools of DNAPL, and low hydraulic conductivity all limit the effectiveness of enhanced ISB. Although conditions can be engineered to overcome the limitations, the cost for altering conditions may be greater than another remediation technology that may be more appropriate for the site conditions. An in-depth cost analysis can be performed to determine whether ISB will be a cost effective option.

To expand the use of enhanced ISB and ensure successful application, more research is needed. A better knowledge of microorganisms capable of dechlorination will lead to better design of engineered ISB systems. Mass reduction can be enhanced if superior substrate delivery technologies were available to achieve substrate/DNAPL contact and uniform distribution. Mass reduction rates could be increased if more research was available on selection of substrates and methods for enhancing dissolution and solubility. Stalling at DCE is still a severe limitation for enhanced ISB, which could be avoided if more research was available on why DCE stall occurs and better methods for achieving complete dechlorination. The use of treatment trains or bioremediation as a polishing technique is a promising application to reach site closures, but needs more research on the most cost-effective and effective implementation techniques.

Enhanced ISB already has been shown to be an effective treatment as indicated by successful application in source areas. More research and better guidance for implementation will lead to more successful implementation.

REFERENCES

AFCEE (Air Force Center for Environmental Excellence). 2004. Principles and Practices of Enhanced Bioremediation of Chlorinated Solvents. Final report.

Battelle. 2004. "Demonstration of Biodegradation of Dense, Nonaqueous-Phase Liquids (DNAPL) through Biostimulation and Bioaugmentation at Launch Complex 34 in Cape Canaveral Air Force Station, Florida." Final Innovative Technology Evaluation Report. Prepared for the EPA.

Beyke, G. and T. Powell. "Heat Enhanced Bioremediation of Chlorinated Solvents Using Electrical Resistance Heating." Presented at the Eighth International In-Situ and On-Site Bioremediation Symposium. June 9, 2005.

Borden, R.C. 2003. "Anaerobic Bioremediation of Chlorinated Solvent Source Zones – What Can be Achieved?" AFCEE Tech Transfer Workshop, San Antonio, TX. http://www.afcee.brooks.af.mil/products/techtrans/workshop/postworkshop03/tuesday/pm/sourcezoneremediation/Borden_abst.pdf

Carr, C.S., Garg, S., and J.B. Hughes. 2000. "Effect of Dechlorinating Bacteria on the Longevity and Composition of PCE-Containing Nonaqueous Phase Liquids Under Equilibrium Dissolution Conditions." *Environmental Science and Technology*, Vol. 34, No. 6, pp. 1088-1094.

Christ, J., Ramsburg, A., Abriola, L., Pennell, K., Loffler, F. 2005. "Coupling Aggressive Mass Removal with Microbial Reductive Chlorination for Remediation of DNAPL Source Zones: A Review and Assessment." *Environmental Health Perspectives.* Vol. 113, No. 4, pp. 465-474.

Cope, N. and J.B. Hughes. 2001. Biologically-enhanced Removal of PCE from NAPL Source Zones. Environ. Science and Technology. 35, 2014-2021.

EPA (Environmental Protection Agency). 2000. Engineered Approaches to *In Situ* Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications. Viewed 2 May 2005. http://cluin.org/download/remed/ engappinsitbio.pdf

EPA. 2003. "The DNAPL Remediation Problem: Is There a Case for Source Zone Depletion?" EPA/600/R-03/143.

EPA. 2004a. Site Characterization Technologies for DNAPL Investigations. EPA 542-R-04-017.

EPA. 2004b. *In Situ* Thermal Treatment of Chlorinated Solvents: Fundamentals and Field Applications. EPA 542-R-04-010.

Friis, Anne Kirketerp, H.J. Albrechtsen, P.L. Bjerg, M. Duhamel, K. Udell, G. Heron. "Anaerobic Dechlorination after Thermal Treatment." Presented at the Eighth International In-Situ and On-Site Bioremediation Symposium. June 9, 2005.

He, J., Y. Sung, M.E. Dollhopf, B.Z. Fathepure, J.M. Tiedje, F.E. Loffler. 2002. "Acetate versus Hydrogen as Direct Electron Donors to Stimulate the Microbial Reductive Dechlorination Process at Chloroethene-Contaminated Sites." *Environmental Science and Technology*. Vol. 36, pp. 3945-3952.

He, J., K.M. Ritalahti, K. Yang, S.S. Koenigsberg, F.E. Löffler. 2003. "Detoxification of Vinyl Chloride to Ethene Coupled to Growth of an Anaerobic Bacterium." *Nature*. Vol 424, pp.62-65.

Hendrickson, E.R., J.A. Payne, R.M. Young, M.G. Starr, M.P. Perry, S. Fahnestock, D.E. Ellis, and R.C. Ebersole. 2002. "Molecular Analysis of Dehalococcoides 16S Ribosomal DNA from Chloroethene-Contaminated Sites Throughout North America and Europe." *Applied Environmental Microbiology*, Vol. 68, pp.485-495.

ITRC (Interstate Technology Regulatory Council). 2003. Technical and Regulatory Guidance for Surfactant/Cosolvent Flushing of DNAPL Source Zones.

ITRC. 2005. Technical and Regulatory Guideline on *In Situ* Chemical Oxidation of Chlorinated Solvent Contaminated Groundwater. Second Edition.

Lee, M.D., B. Borden, M. T. Lieberman, W. Beckwith, T. Crotwell, P. E. Haas. 2001. "Effective Distribution of Edible Oils – Results from Five Field Applications." *Anaerobic Degradation of Chlorinated Solvents*. Eds. V. S. Magar, D. E. Fennell, J.J. Morse, B.C. Alleman, A. Leeson. Columbus, OH: Battelle Press. pp. 249-256.

Kram, M.L., A.A. Keller, J. Rossabi, L.D. Everett. 2001. "DNAPL Characterization Methods and Approaches, Part 1: Performance Comparisons." *Ground Water Monitoring and Remediation*. Vol. 21, pp. 109-123.

Macbeth, T.W., R.A. Wymore, K.S. Sorenson Jr. 2005a. Dissolution and Dechlorination of Chlorinated DNAPLs Stimulated by Whey Powder. Presented at the Eighth International *In Situ* and On-Site Bioremediation Symposium, Baltimore, MD.

Macbeth, T.W., L.N. Peterson, R.C. Starr, K.S. Sorenson Jr., R. Goehlert, K.S. Moor. 2005b. "ISCO Impacts on Indigenous Microbes in a PCE-DNAPL Contaminated Aquifer." Presented at the 8th International *In Situ* and On-Site Bioremediation Conference, Baltimore, MD.

Macbeth, Tamzen. 2005. RE: New Case Study Suggestion from Mark Kluger. Email Communication. 21 June 2005.

Major, D. E. Edwards, P. McCarthy, J. Gossett, E. Hendrickson, F. Loeffler, S. Zinder, D. Ellis, J. Vidumsky, M. Harkness, G. Klecka, and E. Cox. 2003. "Discussion of Environment vs. Bacteria or Let's Play, 'Name that Bacteria.'" *Ground Water Monitoring and Remediation*. Vol. 23, no. 3, pp. 32-48.

Major, Dave (Principal, Geosyntec Consultants, Inc.), telephone conversation, 31 May 2005.

Maymó-Gatell, I. Nijenhuis, and S. Zinder. 2001. "Reductive Dechlorination of *cis*- 1,2-Dichloroethene and Vinyl Chloride by '*Dehalococcoides ethenogenes*.'" *Environmental Science and Technology.* Vol. 35, pp.516-521.

Mvarik, S.C., R.K. Sillan, A.L. Wood, G.W. Sewell. 2003. "Field Evaluation of the solvent Extraction Residual Biotreatment Technology." *Environmental Science and Technology*. Vol. 37, pp. 5040-5049.

NRC (National Research Council). 1993. *In Situ Bioremediation, When does it work?* Washington, D.C.: National Academy Press.

NRC. 2005. Contaminants in the Subsurface Source Zone Assessment and Remediation. Committee on Source Removal of Contaminants in the Subsurface. Washington D.C., The National Academies Press. pp. 256

Nelson, D.K., F.C. Payne, S.S. Suthersan. 2005. "Enhanced Reductive Dechlorination – A Broader Perspective." Contaminated Soils, Sediments and Water: Science in the Real World. Eds. E.J. Calabrese, P.T. Kostecki, J. Dragun. New York: Springer. Vol. 9 pp.69-89.

Nielsen, R.B. and J.D. Keasling. 1999. "Reductive Dechlorination of Chlorinated Ethene DNAPLS by a Culture Enriched from Contaminated Groundwater." Biotechnology and Bioengineering. Vol 62, pp. 160-165.

Payne, F., S. Suthersan, Lenzo, F., Burdick, J. 2001. "Mobilization of Sorbed-Phase Chlorinated Alkenes in Enhanced Reductive Dechlorination." *Anaerobic Degradation of Chlorinated Solvents.* Eds. V. Magar, D. Fennell, B. Alleman, A. Leeson. Columbus, Ohio: Battelle Press. pp.53-60.

Peterson, L.N., Sorenson, K.S., and Starr, R.C. 2000. Field Demonstration Report TAN Final Groundwater Remediation OU 1-07B. Prepared for U.S. Department of Energy. DOE/ID-10718.

Ramsburg, C.A., L.M. Abriola, K.D. Pennell, F.E. Löffler, M. Gamache, B.K. Amos, and E.A. Petrovskis. 2004. Stimulated Microbial Reductive Dechlorination following Surfactant Treatment at the Bachman Road Site. *Environmental Science and Technology,* Vol. 38, no. 22, 5902-5914.

Smidt, H. and W.M. de Vos. 2004. "Anaerobic Microbial Dehalogenation." *Annual Review of Microbiology.* Vol. 58, pp. 43-73.

Sorenson, Kent, (Associate, CDM), telephone conversation, 20 May 2005.

Suthersan, S and F. Payne. 2005. *In Situ* Remediation Engineering. Boca Raton: CRC Press.

Yang, Y. and P.L. McCarty. 1998. "Competition for Hydrogen within a Chlorinated Solven Dehalogenating Anaerobic Mixed Culture." *Environmental Science and Technology*, Vol. 32, No. 14, pp. 3591-3597.

Yang, Y. and P.L. McCarty. 2000. "Biologically Enhanced Dissolution of Tetrachloroethene DNAPL." *Environmental Science and Technology*, Vol. 34, No. 14, pp. 2979-2984.

Yang, Y. and P.L. McCarty. 2002. "Comparison Between Donor Substrates for Biologically Enhanced Tetrachloroethene DNAPL Dissolution." *Environmental Science and Technology*, Vol. 36, No. 15, pp. 3400-3404.

Yang, Y. and P.L. McCarty. 2003. "Response to Comment on "Comparison between Donor Substrates for Biologically Enhanced Tetrachloroethene DNAPL dissolution." *Environmental Science and Technology.* Vol. 37, pp. 2620-2621.

Yu, S. M.E. Dolan, L. Semprini. 2005. "Kinetics and Inhibition of Reductive Dechlorination of Chlorinated Ethylenes by Two Different Mixed Cultures." *Environmental Science and Technology.* Vol. 39, pp. 195-205.