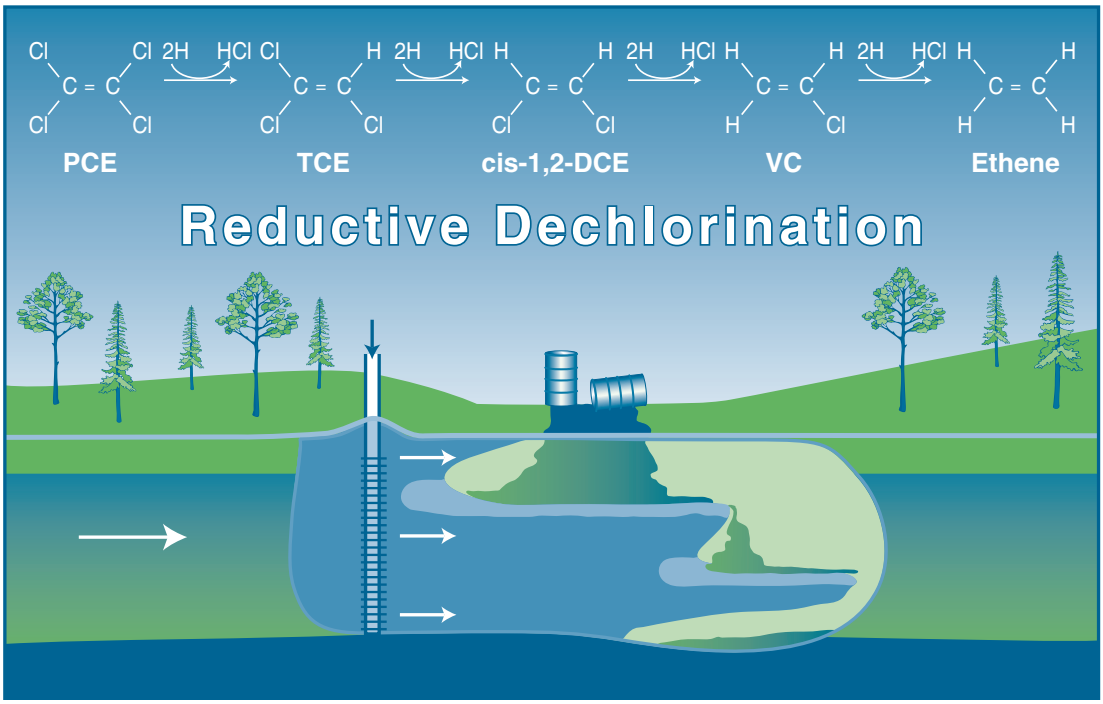
Technical/Regulatory Guidance

In Situ Bioremediation of Chlorinated Ethene: DNAPL Source Zones



June 2008

Prepared by
The Interstate Technology & Regulatory Council
Bioremediation of DNAPLs Team

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- Naji Akladiss, P.E., Maine Department of Environmental Protection, BioDNAPL Team Leader
- Richard Aho, Marquette County Solid Waste
- Jennifer Farrell, Florida Department of Environmental Protection
- Dr. Dibakar (Dib) Goswami, Washington Sate Department of Ecology
- Paul Hadley, California Department of Toxic Substances Control
- Eric Hausamann, P.E., New York State Department of Environmental Conservation
- Bill Morris, Kansas Department of Health and Environment
- Peter Pozzo, North Carolina Department of Environment and Natural Resources
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EXECUTIVE SUMMARY

The Interstate Technology & Regulatory Council's (ITRC) Bioremediation of DNAPLs (BioDNAPL) Team was formed in 2004 with the aim of developing the technical and regulatory guidance needed to support the use of in situ bioremediation (ISB) as a treatment option for subsurface dense, nonaqueous-phase liquids (DNAPLs), particularly those associated with chlorinated ethenes. Chlorinated solvents were once widely used throughout a number of industries, leading to numerous environmental contamination problems. Both the U.S. Department of Defense and the U.S. Department of Energy face DNAPL contamination problems at many of their facilities. DNAPLs, primarily those containing chlorinated ethenes, pose one of the most widespread and prominent types of contamination associated with Superfund sites. Historical and many current DNAPL remediation technologies require the use of energy, fluids, or oxidants to recover or degrade DNAPL. A potential advantage of bioremediation technology is that microorganisms—which can attack the contaminant at or near the DNAPL/water interface, minimizing the need for mobilization—may provide an effective, efficient, and less costly approach to DNAPL source zone remediation.

The objective of this guidance is to provide a systematic understanding of the technical and related regulatory considerations for ISB of chlorinated ethene DNAPL source zones. It is based on scientifically sound and credible evidence supporting the safe and cost-effective application of ISB of DNAPL source areas. The guidance provides the reader with information related to site characterization requirements, application and design criteria, process monitoring, and process optimization.

This guidance focuses on chlorinated ethene DNAPL source zones in the saturated subsurface, where the DNAPL acts as a reservoir that sustains a contaminant plume in groundwater. ISB of such DNAPL source zones relies on microorganisms to convert contaminants to less harmful compounds. ISB involves stimulating the activity of microorganisms already present in the subsurface (biostimulation) or, in some cases, the addition of selected organisms (bioaugmentation). ISB of DNAPL source zones occurs under anaerobic conditions via enhanced reductive dechlorination.

ISB of DNAPL technology has two main components:

- enhanced dissolution and/or desorption of nonaqueous- and/or sorbed-phase contaminant mass
- biological degradation to nonchlorinated, nontoxic end products

The ability of ISB technology to enhance the dissolution and desorption of nonaqueous-phase contaminants to the aqueous phase, where they can be degraded by the microbial population, is what makes the ISB technology applicable to DNAPL source zones. This typically results in faster remediation compared to traditional technologies that are limited by the NAPL dissolution rate (i.e., groundwater extraction). Because it has such a significant impact on the remediation

time frame, enhancement of the NAPL dissolution rate and increasing mass flux are fundamental to the implementation of ISB in a DNAPL source zone.

In a previously published case study document (ITRC 2007a), the BioDNAPL Team established that ISB is a viable, credible, and effective technology for remediation of DNAPL source zones and provides several advantages over traditional source zone remediation technologies. However, as is the case with other remediation technologies, ISB has limitations. Similar to those of other technologies, many of these limitations can be addressed through careful attention to engineering and design and an iterative process of evaluating the system performance followed by optimization.

Some of the most apparent advantages of enhanced ISB include its ability to treat other contaminants present with the chlorinated ethenes, specifically other chlorinated organic compounds, and its ability to be used in combination with a number of other treatment technologies as part of a larger overall site remediation strategy. Also, contaminants are degraded (destroyed) in situ, thereby eliminating secondary waste streams and minimizing potential health and safety concerns. By destroying mass, ISB can shorten the overall remediation time frame at a DNAPL-contaminated site. Finally, capital costs are usually lower than those of other DNAPL source zone treatment technologies.

On the other hand, ISB can be challenged by low aquifer permeability and/or the presence of aquifer heterogeneities and preferential pathways. These natural aquifer characteristics may limit the distribution of amendments throughout the DNAPL source zone, which is a key to the successful implementation of the technology. It should be noted, however, that challenges resulting from the natural aquifer characteristics apply universally to any in situ technology where reagent delivery is required. ISB can also be limited by specific biogeochemical conditions, e.g., where high concentrations of competing electron acceptors or unacceptable aquifer conditions (e.g., low or high pH) exist. This guidance assists the user to understand how to overcome these limiting conditions, if possible.

The length of time required for a microbial system to become fully established and work effectively on a scale relevant to source zone remediation may be months. This allows development of appropriate environmental conditions or the growth of adequate populations of appropriate microbes (i.e., dechlorinating bacteria) to obtain desired rates of treatment able to degrade DNAPL chlorinated ethene compounds. For large source zones, a combination of remedial methods that includes a bioremediation component can be an effective site remedial approach. In 2008 ITRC will begin developing an integrated DNAPL source zone strategy to assist users in the proper selection and application of compatible DNAPL-contaminated site remediation technologies.

The trends that a particular site may follow largely depends on site-specific conditions such as DNAPL architecture, groundwater velocity, lithology, and attenuation parameters. There are models that evaluate the relationship between source depletion and the remedial time frame; however, many researchers, including authors of this guidance, continue to debate the assumptions, variables, and equations in these models. To understand the fundamental approach, we have included in Appendix C of this guidance a preliminary description of the process as it is

understood. We still see this as one of the greatest challenges within the science of ISB of DNAPL source zones and will continue to openly document the results of continued study during ITRC's integrated DNAPL source zone strategy project beginning in 2008.

Additional detailed discussion of the advantages and limitations of enhanced ISB technology is found within the guidance. It is the expectation of the ITRC BioDNAPL Team that this guidance will accelerate technology transfer to and among the states, as well as those charged with site remediation.

GLOSSARY

abiotic. Occurring without the involvement of living microorganisms.

advection. Transport of a solute by the bulk motion of flowing groundwater.

aerobic. Conditions for growth or metabolism in which the organism is sufficiently supplied with molecular oxygen.

aerobic respiration. Process whereby microorganisms use oxygen as an electron acceptor to generate energy.

aliphatic compounds. Acyclic or cyclic, saturated or unsaturated carbon compounds, excluding aromatic compounds.

amendment. Substrate introduced to stimulate the in situ microbial processes (vegetable oils, sugars, alcohols, etc.).

anaerobic. Environmental conditions requiring the absence of molecular oxygen.

anaerobic respiration. Process whereby microorganisms use a chemical other than oxygen as an electron acceptor. Common "substitutes" for oxygen are nitrate, sulfate, iron, carbon dioxide, and other organic compounds.

anisotropy. The property of being directionally dependent, as opposed to "isotropy," which means homogeneity in all directions.

bacteria. Any of a group of prokaryotic unicellular round, spiral, or rod-shaped single-celled microorganisms that are often aggregated into colonies or motile by means of flagella that live in soil, water, organic matter, or the bodies of plants and animals and that are autotrophic, saprophytic, or parasitic in nutrition and important because of their biochemical effects and pathogenicity.

bioaugmentation. (specific to this guidance) The addition of beneficial microorganisms into groundwater to increase the rate and extent of anaerobic reductive dechlorination to ethene.

biodegradation. Breakdown of a contaminant by enzymes produced by bacteria.

biofouling. Biofouling occurs when bacteria attach, grow, and block the well screen, filter pack, or formation surrounding a nutrient delivery well, thereby limiting or preventing the proper function of the well (see ESTCP 2005a, www.estcp.org/Technology/upload/ER-0429-WhtPaper.pdf).

biomass. Material produced by the growth of living material. (Specific to this guidance, the "living material" will be microorganisms.)

bioremediation. Use of microorganisms to biodegrade contaminants in soil and groundwater.

biostimulation. (specific to this guidance) The addition of an organic substrate or nutrients into groundwater to stimulate anaerobic reductive dechlorination.

biotransformation. Microbiologically catalyzed transformation of a chemical to some other product.

chlorinated solvent. Organic compounds with chlorine substituents that commonly are used for industrial degreasing and cleaning, dry cleaning, and other processes.

chlorinated ethene. Organic compounds containing two double-bonded carbons and possessing at least one chlorine substituent.

cometabolism. A reaction in which microorganisms transform a contaminant even though the contaminant cannot serve as an energy source for growth. The microorganisms require the presence of other compounds (primary substrates) to support growth.

compliance monitoring. The collection of data which, when analyzed, can allow for the evaluation of the contaminated media against standards such as soil and or water quality regulatory standards, risk-based standards, or Remedial Action Objectives.

conceptual site model (CSM). A hypothesis about how contaminant releases occurred, the current state of the source zone, and current plume characteristics (plume stability).

control plane. (response boundary) The location of the control plane, or response boundary, is defined as a location within the source area or immediately downgradient of the source area where changes in the plume configuration are anticipated due to the implementation of the ISB DNAPL source zone treatment. The response boundary should not be confused with the term "point of compliance," which the Environmental Protection Agency defines as the point where media-specific standards (e.g., maximum contaminant levels, risk-based cleanup goals) must be achieved (EPA 2002b).

dense, nonaqueous-phase liquid (DNAPL). A water-immiscible organic liquid that is denser than water (e.g., tetrachloroethene).

DNAPL architecture. The spatial distribution of DNAPL mass in the subsurface.

desorption. The converse of "sorption."

diffusion. The process of net transport of solute molecules from a region of high concentration to a region of low concentration caused by their molecular motion in the absence of turbulent mixing.

dilution. A reduction in solute concentration caused by mixing with water at a lower solute concentration.

dispersion. The spreading of a solute from the expected groundwater flow path as a result of mixing of groundwater.

electron. A negatively charged subatomic particle that may be transferred between chemical species in chemical reactions.

electron acceptor. A compound to which an electron may be transferred (and is thereby reduced). Common electron acceptors are oxygen, nitrate, sulfate, ferric iron, carbon dioxide, manganese, and chlorinated solvents, such as tetrachloroethene and its daughter products trichloroethene, *cis*-1,2-dichloroethene, and vinyl chloride.

enhanced bioremediation. An engineered approach to increasing biodegradation rates in the subsurface.

flux. Rate of flow of fluid, particles, or energy through a given surface.

ganglia. DNAPLs that are cut off and disconnected from the main continuous DNAPL body.

growth substrate. An organic compound upon which a bacteria can grow, usually as a sole carbon and energy source.

hydraulic conductivity. The capability of a geologic medium to transmit water. A medium has a hydraulic conductivity of unit length per unit time if it will transmit in unit time a unit volume of groundwater at the prevailing viscosity through a cross section of unit area, measured at right angles to the direction of flow, under a hydraulic gradient of unit change in head through unit length of flow.

hydraulic gradient. The change in hydraulic head (per unit distance) in a given direction, typically in the principal flow direction.

hydrolysis. Decomposition of a chemical compound by reaction with water, such as the dissociation of a dissolved salt or the catalytic conversion of starch to glucose.

inorganic compound. A compound that is not based on covalent carbon bonds, including most minerals, nitrate, phosphate, sulfate, and carbon dioxide.

in situ bioremediation. (specific to this guidance) The use of biostimulation and bioaugmentation to create anaerobic conditions in groundwater and promote contaminant biodegradation for the purposes of minimizing contaminant migration and/or accelerating contaminant mass removal.

mass balance. Quantitative estimation of the mass loading to the dissolved plume from various sources, as well as the mass attenuation capacity for the dissolved plume.

mass loading. Contaminant released to the environment (in this case the aquifer or unsaturated zone) from the source material.

mass transfer. (specific to this guidance) The irreversible transport of solute mass from the nonaqueous phase (i.e., DNAPL) into the aqueous phase, the rate of which is proportional to the difference in concentration.

metabolism. The chemical reactions in living cells that convert food sources to energy and new cell mass.

methanogen. Strictly anaerobic Archaeabacteria able to use only a very limited substrate spectrum (e.g., molecular hydrogen, formate, methanol, carbon monoxide, or acetate) as substrates for the reduction of carbon dioxide to methane.

microcosm. A batch reactor used in a bench-scale experiment designed to replicate the microbial conditions present in the groundwater environment.

microorganism. An organism of microscopic or submicroscopic size, including bacteria.

mineralization. The complete degradation of an organic compound to carbon dioxide.

monitored natural attenuation (MNA). The term "natural attenuation" refers to naturally occurring processes in soil and groundwater environments that act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in those media. These in situ processes include biodegradation, dispersion, dilution, adsorption, volatilization, and chemical or biological stabilization or destruction of contaminants (ITRC 1999a). When scientists monitor or test these conditions to make sure natural attenuation is working, it is called "monitored natural attenuation" (EPA 2001).

process monitoring. The collection of information documenting the operation of a system's engineered components.

performance monitoring. The collection of information which, when analyzed, allows for the evaluation of the performance of a system on environmental contamination.

natural attenuation. Naturally occurring processes in soil and groundwater environments that act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in those media.

oxidation. Loss of electrons from a compound.

plume. A zone of dissolved contaminants. A plume usually originates from a source and extends in the direction of groundwater flow.

pool. An accumulation of DNAPL above a capillary barrier.

rebound. After contaminant concentrations in groundwater have been reduced through in situ treatment and the treatment is terminated or reduced, the return of concentrations to elevated levels due to the continued release of mass from a source zone beyond the natural attenuation capacity of the groundwater system.

reductive dechlorination. The removal of chlorine from an organic compound and its replacement with hydrogen.

response boundary. See "control plane."

saturated zone. Subsurface environments in which the pore spaces are filled with water.

sorption. The uptake of a solute by a solid.

source zone. The subsurface zone containing a contaminant reservoir sustaining a plume in groundwater. The subsurface zone is or was in contact with DNAPL. Source zone mass can include sorbed and aqueous-phase contaminant mass as well as DNAPL.

substrate. A molecule that can transfer an electron to another molecule and/or provide carbon to the microorganism. Organic compounds, such as lactate, ethanol, or glucose, are commonly used as substrates for bioremediation of chlorinated ethenes.

sulfate reducer. A microorganism that exists in anaerobic environments and reduces sulfate to sulfide.

volatilization. The transfer of a chemical from its liquid phase to the gas phase.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDICES

IN SITU BIOREMEDIATION OF CHLORINATED ETHENE: DNAPL SOURCE ZONES

1. INTRODUCTION

Treatment of dissolved-phase chlorinated ethenes in groundwater using in situ bioremediation (ISB) is an established technology; however, its use for dense, nonaqueous-phase liquid (DNAPL) source zones is an emerging application. This guidance is the logical successor to two previous ITRC documents: *Overview of In Situ Bioremediation of Chlorinated Ethene DNAPL Source Zones* (ITRC 2005a) and *In Situ Bioremediation of Chlorinated Ethene DNAPL Source Zones: Case Studies* (ITRC 2007a). The latter includes an overview of how currently available technologies and ISB can be combined to treat DNAPL source zones. After examining both research and case histories, the Interstate Technology & Regulatory Council (ITRC) Bioremediation of DNAPLs (BioDNAPL) Team concluded that ISB of DNAPLs source zones is a viable technology and can be an effective component of a treatment plan for chlorinated ethene source zones. In some sites it may be a sole remedy; in many sites it will be one component of a larger remedial strategy.

NOTE: If you intend to use this guidance to implement in situ bioremediation of DNAPLs, it is recommended you read Section 1 in its entirety.

1.1 Purpose and Objectives

The purpose of this technical and regulatory guidance document (referred to throughout as "this guidance") is to provide the regulatory community, stakeholders, and practitioners (consultants) with the general steps regulators and practitioners can use to objectively assess, design, monitor, and optimize ISB treatment of DNAPL source zones. The objective is to provide adequate technology background for the user to understand the general and key aspects of ISB for treatment of chlorinated ethene DNAPL source zones. It describes technology-specific considerations for application of ISB of source zones but is not intended to be a step-by-step instruction manual for remedial design.

1.2 Definition of a DNAPL Source Zone

The National Research Council (NRC 2004) defines a groundwater contamination source zone as follows:

> …a saturated or unsaturated subsurface zone containing hazardous substances, pollutants or contaminants that acts as a reservoir that sustains a contaminant plume in groundwater, surface water, or air, or acts as a source for direct exposure. This volume is or has been in contact with separate phase contaminant (NAPL or solid). Source zone mass can include sorbed and aqueous-phase contaminants as well as contamination that exists as a solid or NAPL.

For the purpose of this guidance, a DNAPL source zone includes the zone that encompasses the entire subsurface volume in which DNAPL is present either at residual saturation or as "pools" that accumulate above confining units (Mackay and Cherry 1989, Cohen and Mercer 1993, Rao

et al. 2001). In addition, the DNAPL source zone includes regions that have come into contact with DNAPL and may be storing contaminant mass as a result of diffusion of DNAPL into the soil matrix (Chapman and Parker 2005). Figure 1-1 depicts a conceptual model of a DNAPL source zone within the saturated zone.

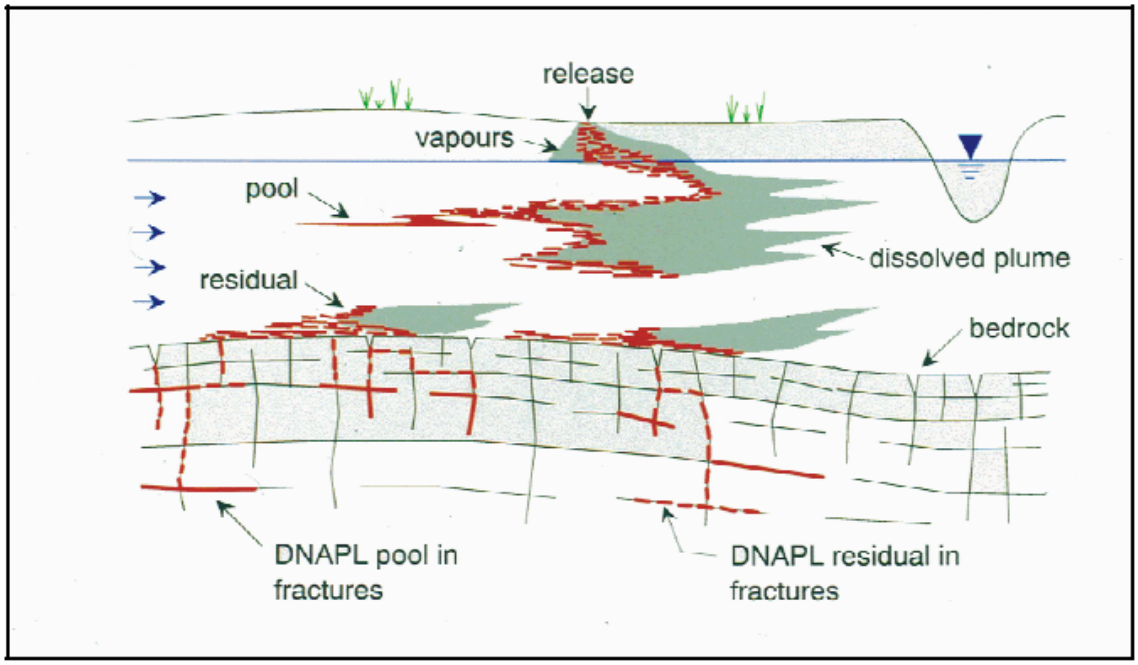


Figure 1-1. Conceptual site model of a DNAPL source zone.
(*Source:* U.K. Environmental Agency 2004)

Although DNAPLs may be present in both the vadose and saturated zones, the discussion of ISB of DNAPL source zones in this guidance is focused on the treatment of DNAPL source zones within the saturated zone only. *Strategies for Monitoring Performance of DNAPL Source Zone Remedies* (ITRC 2004) provides additional information on DNAPL source zones.

1.3 Setting Goals for ISB of DNAPL Source Zones

The two goals of any DNAPL source treatment technology are to reduce the mass of contaminants within the source area and to prevent migration above unacceptable levels. Enhanced ISB (EISB) technology reduces source mass and controls flux through the enhanced dissolution and desorption of DNAPL constituents into the aqueous phase and subsequent microbially mediated degradation processes. Although EISB of DNAPL source zones has been demonstrated in the field at a few chlorinated solvent sites, expectations for source zone depletion rates must be realistic. The following sections describe requirements necessary to support the realistic determination of goals for ISB of a DNAPL source zone.

In many cases remediation of DNAPL will either not achieve or will not sustain maximum contaminant levels (MCLs) in the source zone. Accordingly, realistic goals reflecting the limitations of any DNAPL remediation method to meet MCLs in the source area are necessary.

1.3.1 Conceptual Site Model

Before setting performance goals for a DNAPL source zone remediation project, a conceptual site model (CSM) must be developed. The CSM is a holistic view of the site characteristics on which the remedial design will be based and should be continually updated as new information becomes available. Some of the key elements of a CSM are information on the contaminant release mechanism, geology and hydrogeology, characteristics of contaminant fate and transport, geochemistry, contaminant distribution, and exposure scenarios.

1.3.2 Performance Objectives

An essential component of the planning process prior to implementing ISB of a source area is to clearly identify the remedial objectives and the means by which the achievement of the objectives will be assessed. NRC (2004) presents an extensive discussion of objectives for source remediation. Remedial objectives are either absolute or functional. These may be distinguished in that absolute objectives are important in and of themselves, while functional objectives are a means by which an absolute objective may be achieved (NRC 2004).

Protecting human health is a common absolute objective although others are possible, including preventing further degradation of groundwater resources and protecting ecosystem health. In general, absolute objectives represent judgments of social value that cannot be readily substituted; however, they are also not easily quantifiable. For a given absolute objective, a number of lower-order functional objectives can be defined as the means by which the absolute objective will be achieved. These functional objectives may be specific to a particular technology or approach to achieving the absolute objective and so may substitute for one another (i.e., achievement of either functional objective meets the absolute objective). Each functional objective must be accompanied by a quantifiable performance metric by which the attainment of that functional objective can be measured or, if it cannot be directly quantified, broken down into subsidiary objectives with specific performance metrics.

For example, a common functional objective is the reduction of contaminant concentrations below criteria at a specified point of compliance; however, since the reduction in concentration resulting from source area remediation may not occur quickly (i.e., it is difficult to measure directly), it may be possible to demonstrate that other, more readily quantifiable functional objectives (subsidiary objectives) will eventually result in the required concentration reduction. Examples of these lower-order objectives (and their quantifiable metrics) that could be used to assess performance following the completion of source remediation include the following (see Figure 1-2):

- removal of contaminant mass from the source area (total mass removed, percentage mass reduction)
- reduction of mass flux at a specified plane located near the source (percentage flux reduction)

Following the achievement of these functional objectives at the source area, it may be possible to demonstrate that other intrinsic or enhanced remedial processes are likely to result in the attainment of the higher-order remedial objectives.

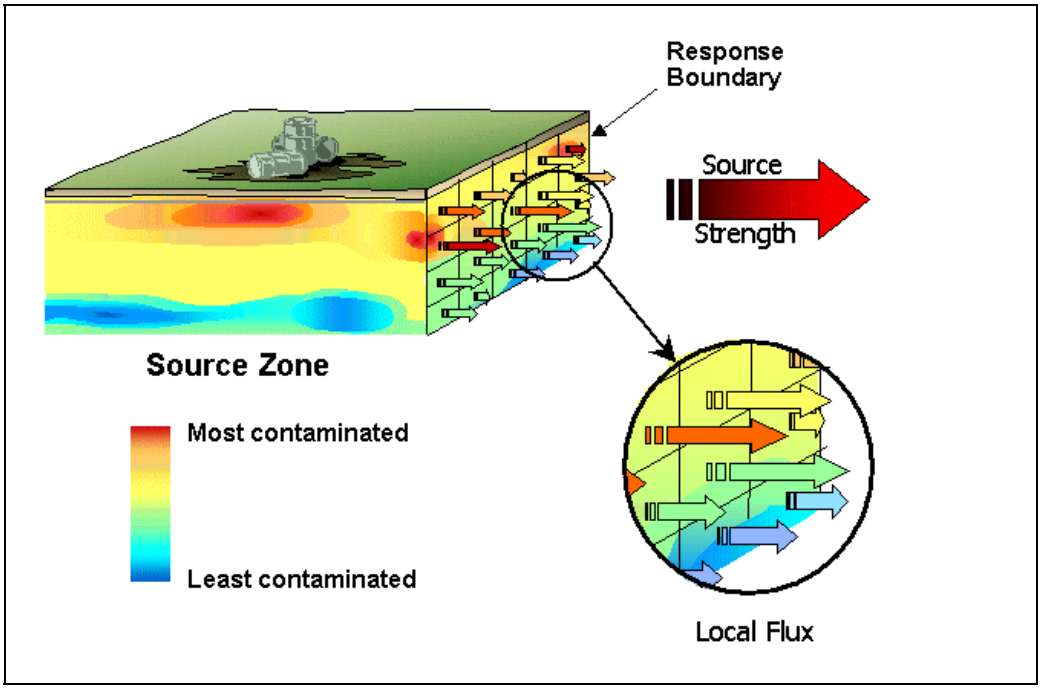


Figure 1-2. Response boundary in relation to the source zone.
(*Source:* Wood et al. 2004)

Other examples of functional objectives that are not readily quantifiable and would require the definition of subsidiary functional objectives include the following:

- preventing the migration of remediation fluids beyond the treatment zone
- reducing the potential for vapor intrusion into buildings
- reducing contaminant plume size
- depleting the source zone sufficiently to allow natural attenuation to sustain the plume stability (ITRC 2008)
- reducing the duration of source treatment

In many cases, long-term monitoring will be required to ensure that attainment of the short-term functional objectives results in the eventual attainment of the absolute objectives.

During source remediation, specific operational objectives may be defined that can be used to optimize the operation of the treatment system. Examples of possible operational objectives include the following:

- preventing the migration of remediation fluids beyond the treatment zone
- minimizing system downtime
- ensuring electron donor distribution throughout the treatment zone

- increasing the rate of reductive dechlorination

The attainment of these operational objectives meets the absolute objective of efficient treatment system operation; however, it does not directly result in the attainment of the principal absolute objective of protecting human health.

1.3.3 Performance Metrics

There are several ways to measure progress of a source zone remediation and many metrics that can be applied. Examples of performance metrics include concentration end points, mass reduction, flux reduction, and remedial system operational parameters. The process of deciding which metrics are appropriate at a given site should involve discussions among the regulators, the public, and the technical team to avoid potential misunderstandings and delays when the performance data become available and decisions are made.

Performance metrics for DNAPL source zone remediation are response-specific parameters defined in the following terms:

- overall site Remedial Action Objectives (RAOs)
- implemented technology(ies)
- location(s) of potentially exposed receptors
- predicted response of the source zone to the implementation of ISB

1.3.4 Baseline Conditions

Establishing a baseline is essential when evaluating the performance of ISB. Some baseline (e.g., regulatory compliance–related) conditions are common to all remedial technologies; others are technology and site specific. Measuring and evaluating preremediation conditions and trends within a DNAPL source zone are typically conducted during site assessment or included in the predesign stage of implementation. Since many performance metrics are based on changes in environmental conditions during treatment (e.g., operational or process criteria may not include environmental media), it is necessary to accurately establish the baseline conditions for a wide variety of parameters prior to treatment.

1.3.5 Technology-Specific Considerations

Assessing the effectiveness of ISB of DNAPL source zone is different from that for most other DNAPL remediation technologies because of the way ISB is implemented. Whereas most traditional DNAPL remediation technologies summarized in Appendix A are deployed as one-time, short-duration remedial actions (weeks to months), ISB at DNAPL source zones is typically applied continuously over a longer time, usually several years.

The traditional metric used to assess ISB of DNAPL source zone performance is groundwater contaminant concentrations. However, groundwater concentrations of the primary constituents being treated should not be the sole criteria for assessing the performance of DNAPL remediation. Redox parameters, degradation products, biological indicator parameters, electron

donors, and fermentation products should also be monitored to document the operation of biodegradation processes. Section 5 provides details on performance monitoring. Another metric that is probably more reliable, but more difficult to demonstrate, is to measure the mass removed from the source itself (i.e., via measurement of DNAPL in soil samples throughout the treatment process). Mass removal is rarely reflected by immediate decreases in aqueous concentration. The dynamics of the ISB process are further discussed in Sections 2 and 5.

The following sections of this guidance describe the mechanisms of ISB of DNAPL source zones (specifically enhanced reductive dechlorination [ERD]) and its advantages and limitations. It further describes factors necessary to assess the applicability of ISB to address specific site conditions and how best to adjust and maintain these conditions to maximize the potential effectiveness of ISB for treatment of DNAPL source zones. The guidance further describes how and why one might select a particular approach and how to design a system for application of ISB in a DNAPL source zone. Finally, it describes how to monitor performance and recognize clues indicating adjustments which would optimize needed system operational performance.

2. ISB TECHNOLOGY DESCRIPTION

This chapter provides an introduction to biodegradation and the fundamental principles underlying ISB of DNAPL source zones. Next, the mechanisms of ISB of chlorinated ethene are described, followed by a discussion of the approaches and principal variations of ISB of DNAPL source zones. Finally, advantages and limitations of ISB are detailed.

2.1 ISB of Chlorinated Ethenes

ISB of chlorinated ethenes involves the stimulation of microorganisms to convert chloroethene contaminants to less harmful compounds. This guidance focuses on active bioremediation of DNAPL source zones under anaerobic conditions. ISB of DNAPL source zones is practiced by many throughout the industry and is sometimes referred to by other names, including ERD, enhanced anaerobic bioremediation, EISB, and anaerobic reductive dechlorination. These terms describe the primary mechanisms responsible for the degradation (reductive dechlorination), whereas ISB of DNAPL source zones refers to the engineered application of the reductive dechlorination mechanism to destroy DNAPL source zones in the subsurface saturated zone. This guidance uses the term “in situ bioremediation” (ISB) to describe both the technology and the mechanism (ERD).

Applying ISB to DNAPL source zones typically involves biostimulation and may involve bioaugmentation. “Biostimulation of ISB” refers to the stimulation of the activity of the microorganisms already present in the subsurface, and “bioaugmentation” is the addition of selected microorganisms to the treatment zone.

ISB of DNAPL source zones generally involves the delivery of an electron donor into the subsurface to stimulate microbial growth and development, creating an anaerobic groundwater treatment zone and generating hydrogen through fermentation reactions. The hydrogen and injected electron donor are referred to as “substrates” (reduced compounds whose oxidation can be linked to reduction of the contaminant compound). The process of aerobic respiration

consumes oxygen and lowers the redox potential of the aquifer to a more anaerobic condition, thereby promoting reductive dechlorination.

ERD relies on a relatively small number of bacterial species able to dehalogenate chlorinated aliphatic hydrocarbons (CAHs) under anaerobic conditions (Vogel and McCarty 1985, Mohn and Tiedje 1992). ERD is a useful approach to treat chlorinated ethenes for several reasons:

- Some compounds, notably perchloroethene (PCE), can be degraded only anaerobically, optimally under deeply reducing conditions (McCarty and Semprini 1994).
- ERD is relatively easy to implement and control under field conditions compared with some approaches, such as cometabolic biodegradation.
- ERD is flexible and inexpensive compared with other source zone treatment technologies.

2.2 Application of ISB to DNAPL Source Zones

ISB has been used for over a decade to treat chlorinated ethenes in the dissolved phase (i.e., in the plume). A contaminant molecule must be in the dissolved phase for effective biodegradation. Recently, it has been demonstrated that dechlorinating organisms can tolerate concentrations of chlorinated ethenes near the solubility limit. This finding has led to testing and development of approaches to extend ERD to DNAPL source zones. Although ERD does not work directly on free-phase DNAPL, it can still be used for source zone remediation because it accelerates the rate of source zone mass removal. The acceleration of source zone mass removal is the result of the following mechanisms:

- increasing the concentration gradient at the DNAPL-water interface, which increases the rate of DNAPL dissolution
- partial biodegradation of parent compounds near the DNAPL-water interface, producing less-chlorinated daughter products (i.e., *cis*-1,2-dichloroethene [DCE] and vinyl chloride [VC]) that are more mobile in groundwater than either trichloroethene (TCE) or PCE
- under some conditions, the electron donor solution and/or its degradation products abiotically enhancing DNAPL mass transfer rates through cosolvency, desorption, and/or dissolved organic matter or surfactant partitioning

Mechanisms that increase the concentration gradient have been documented and accepted in the literature (Seagren, Rittmann, and Valocchi 1993, 1994; Cope and Hughes 2001; Yang and McCarty 2002). The increased mass transfer of degradation products into the dissolved phase can also be significant due to the higher solubility of the daughter products (Carr, Garg, and Hughes 2000). The importance of abiotic dissolution enhancement caused by electron donors appears to be dependent on the specific donors used, as well as on their concentration. For example, Macbeth et al. (2006) showed that high-concentration (5%–10%) whey solutions increased the effective solubility of TCE by a factor of 5–6 in batch and column studies. However, another recent laboratory study concluded that salts of carboxylic acids (e.g., sodium lactate) decrease the solubility of TCE while other donors (lactic acid, acetic acid, and ethanol) increase TCE solubility by up to fourfold at concentrations of 25% (Hood, Major, and Driedger 2007).

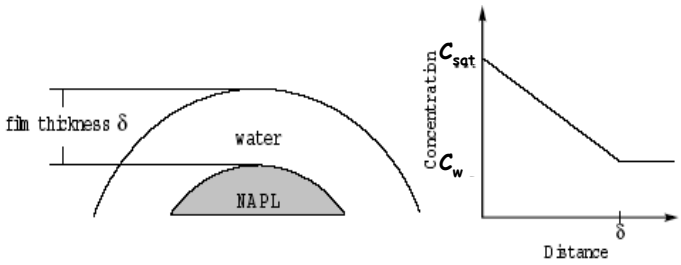
While laboratory studies have been able to quantify DNAPL mass transfer enhancements due to the individual mechanisms described above, observations in the field are more likely to represent the combined effects of all of these mechanisms. These "enhancement factors" have been observed in several sites that were reported in ITRC 2007a, as well as by others (Sorenson 2002, Payne et al. 2001). Macbeth et al. (2006) also documented mass transfer enhancement factors of 2–4 in the field during high-concentration (8%–10%) whey injections relative to lactate injections, inferring that the enhancement was due primarily to the abiotic interaction of the whey and the TCE, as observed in laboratory studies. Similarly, a recent Environmental Security Technology Certification Program (ESTCP) field study demonstrated enhancement factors of 2 to more than 10 during high-concentration whey injections relative to low-concentration injections. These effects were measured both in terms of concentration increases within the treatment areas and in terms of downgradient flux of volatile organic compound (VOC) contaminants (ESTCP 2008).

It is important to note that, because of the enhanced removal of mass from the source area, the contaminant concentrations in the dissolved phase may initially increase at the response boundary. An increase in the dissolution rate by a factor of up to 3 is generally believed to be possible at the field scale, although some sites have seen significantly higher increases than this. Smaller impacts on the dissolution rate are likely with passive source zone treatment configurations in comparison to active circulation systems since active circulation systems generally provide better contact between electron donor, microorganisms, dissolved-phase contaminants, and DNAPL.

In addition to varying goals for different applications, strategies for applying ISB to DNAPL source zones may differ. For some site conditions a passive approach may be used to control migration. In such cases treatment may involve relatively infrequent injections (i.e., yearly or less frequent) of substrate (electron donor), as well as infrequent process monitoring and few modifications to the injection program to optimize performance. In a more active approach, substrates may be injected at intervals of a few weeks to months, with frequent monitoring and modification to maximize source mass depletion. The flexibility of ISB of DNAPL source zones allows for multiple designs and modification of each design to accommodate site-specific conditions. This flexibility and the iterative nature of the optimization process, which is fundamental to ISB of DNAPL source zones, makes it difficult to standardize the application. The basic processes and considerations involved are discussed below to provide a foundation for evaluating various proposed application approaches.

2.2.1 DNAPL Dissolution and Mass Reduction Processes

The time it takes to remove free or sorbed DNAPL phases is a function of how quickly the contaminant mass can be transferred to the aqueous phase. Figure 2-1 conceptually shows the factors affecting how DNAPL dissolves into water.



$$J = \lambda(C_{sat} - C_w)$$

$$\lambda = f(surface\ area, velocity)$$

Figure 2-1. Factors of DNAPL dissolution. (Courtesy of GeoSyntec)

To visualize the concept, imagine a drop of DNAPL on a surface, surrounded by water. There will be a diffusion layer next to the DNAPL drop, which is a film of essentially stagnant water. There will be a concentration gradient across this stagnant film, with the DNAPL compound at its solubility limit (C_{sat}) at the DNAPL surface and decreasing with distance from the DNAPL. The steeper the concentration gradient through the stagnant film layer, the larger the surface area of the DNAPL drop; and the greater the water velocity and mixing of contaminant and water near the stagnant film, the greater the mass flux or transfer (J) of the DNAPL into solution.

Technologies that increase C_{sat} (e.g., a surfactant, cosolvent, or heat) or decrease C_w (concentration in water) (e.g., biodegradation or oxidation) will increase the concentration gradient and enhance J. However, the time required to remove the source also depends on the effective length of the DNAPL pool (an accumulation of DNAPL above a capillary barrier) or source because dissolution first occurs at the point where uncontaminated water first contacts the source zone and where the high concentration gradient promotes the most rapid dissolution. As this dissolved mass migrates downgradient over the remaining DNAPLs, C_w increases locally, which reduces the concentration gradient and lowers J for the remaining DNAPL source zone. This phenomenon is why "effective length" of a source or length of DNAPL pool has a large impact on remediation time frames. Even in the absence of pools of DNAPL, small ganglia (zones of porous media containing DNAPL that are cut off and disconnected from the main continuous DNAPL body) and DNAPL residuals can create equivalent pool lengths in an aquifer.

Figure 2-2 conceptually shows the effects in preceding discussion and demonstrates that even when DNAPL pools are absent, DNAPL residuals can create an effective source or pool length simply because DNAPL mass that is dissolved from upgradient source locations will be close to C_{sat} and inhibit further DNAPL dissolution at downgradient locations. Figure 2-2 conceptually shows that over time, the concentration observed in wells may not decline until most of the source is depleted.

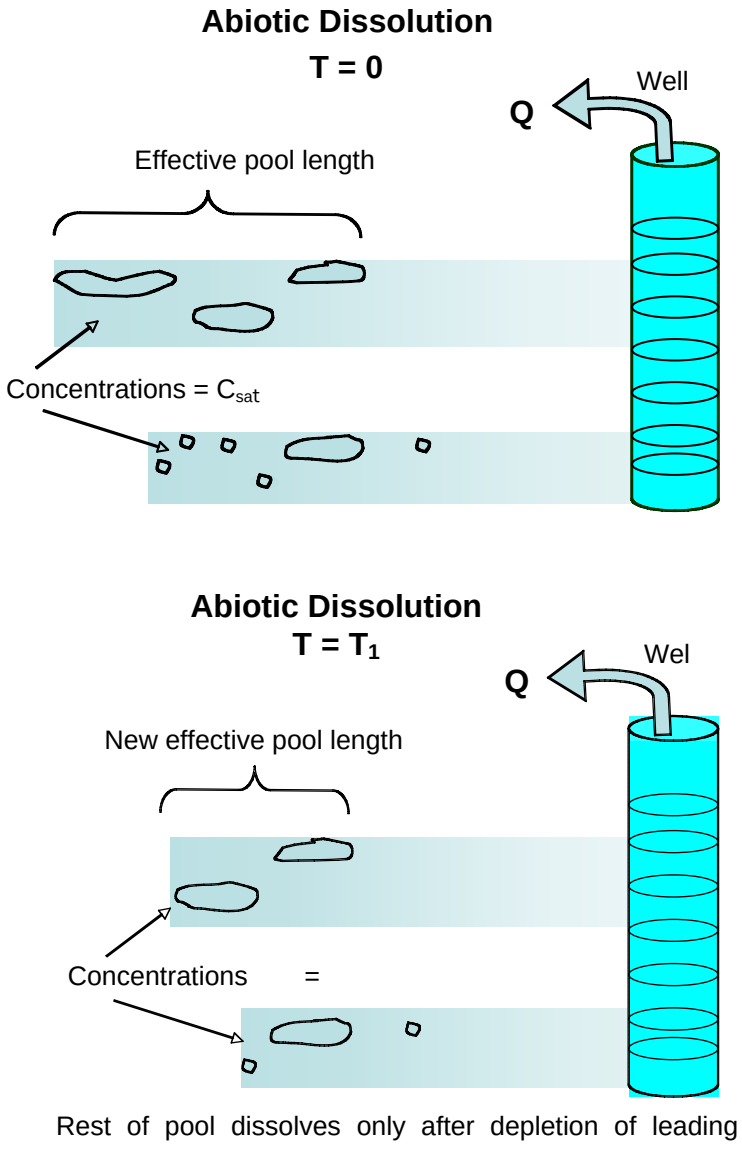


Figure 2-3. Conceptualized impact of biodegradation.
(Courtesy of GeoSyntec)

In contrast to abiotic dissolution, ISB of DNAPL sources works by accelerating dissolution through at least three mechanisms. The first is through decreasing the C_w near the DNAPL or source materials and thereby increasing the concentration gradient. Even if concentrations of the solubilized DNAPL are at inhibitory levels near the DNAPL, bioremediation will also decrease the C_w within the DNAPL source zone and thereby increase the concentration gradient over greater surface areas of DNAPL and sorbed phases. As shown in Figure 2-3, both of these mechanisms create smaller effective pool or source lengths within the source area. This mechanism for enhanced dissolution happens once a robust microbial community has been developed in the DNAPL source area, as the parent contaminants are rapidly degraded after they dissolve into groundwater.

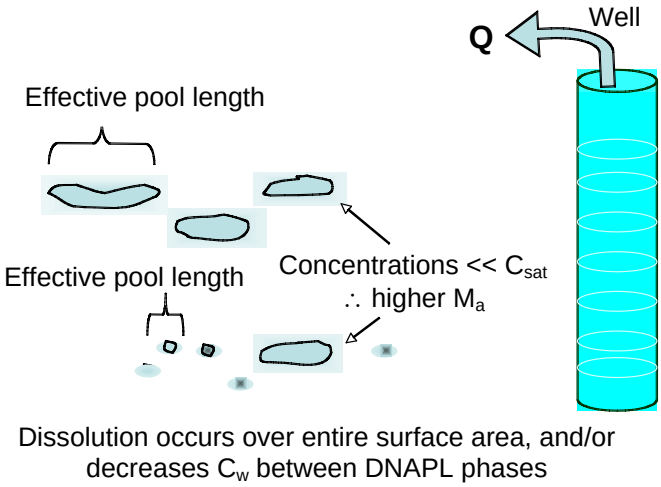


Figure 2-3. Conceptualized impact of biodegradation.
(Courtesy of GeoSyntec)

The second mechanism is related to the fact that the reductive daughter products are more soluble than the parent compounds, allowing for more moles of contaminants to be present in the aqueous phase when degradation is occurring as compared to abiotic dissolution only. The third mechanism for enhanced dissolution is that some electron donors and/or their fermentation products have been shown to abiotically increase the effective solubility of DNAPL contaminants through interfacial tension reductions in increasing C_{sat}, as shown in Figure 2-1.

In practice, it can be difficult to distinguish between the three mechanisms, and in fact, the aggregate effect of the mechanisms is what is important in terms of DNAPL source degradation.

The degree of mass transfer enhancement between abiotic degradation versus bioremediation depends on the DNAPL/source zone architecture (e.g., pool/ganglia ratio) and the ability to deliver amendments throughout this architecture (e.g., close to mass that has diffused into the matrix, penetrated into low-permeability materials, or entered dead-end fractures). There will be little enhancement of mass flux and decrease in cleanup times if the substrate used to stimulate biodegradation is consumed too far from the DNAPL and/or cannot penetrate or be distributed into the DNAPL architecture. Therefore, the selection and application of substrate is a key consideration. In a later section, Table 4-1 presents the characteristics and applicability of various electron donors.

Ideally, the application of ISB to DNAPL source zones leads to an enhanced mass flux of chlorinated ethenes from the source zone and thus to shorter remediation time. However, there is unlikely to be a consistent enhanced mass flux over time because the distribution of accessible DNAPL mass and surface area will change with time. For example, in a hypothetical case, initially 90% of the mass may be associated with ganglia versus 10% as pools. Biological activity may deplete the relatively accessible mass associated with the ganglia quickly, by significantly enhancing the flux over abiotic flux rates. The remaining 10% of DNAPL that might exist as pooled DNAPL will have a lower dissolution rate because of lower surface to mass ratio and less-efficient mixing processes near the DNAPL and will therefore remediate more slowly. Measurements of total dissolved chlorinated ethenes over the treatment period may

show significant mass transfer enhancement early in the process, followed by a decrease in the mass flux.

Biofouling within the DNAPL architecture can also lead to a decrease in mass flux (see ESTCP 2005a). If the biomass/biofilms are established too far from the DNAPL, enhanced concentration gradients will not be established near the DNAPL. As a result, there will be less enhanced flux from the entire DNAPL surface, although the biomass will still reduce the bulk C_w within the source zone. The practitioner may again initially see higher mass flux in the initial phases of bioremediation followed by lower rates. Changes in donor application rates, donor type, or use of different amendment strategies can potentially reestablish previous mass flux rates.

Measuring the mass flux enhancement is not the only method to demonstrate that a source zone is being effectively remediated. For example, with complete dechlorination of PCE to ethene, an increase in flux is shown through the molar sum of the parent plus degradation products, including ethene, during pre- and post-bioremediation monitoring. However, degradation of chlorinated ethenes produces inorganic constituents that are not included in the summation of chlorinated ethenes. For example, the conversion of PCE to *cis*-DCE may be followed by anaerobic oxidation of *cis*-DCE, which produces carbon dioxide, chloride, and water. Similarly, the reductive dechlorination of PCE through *cis*-DCE to VC and ethene may continue further, with production of ethane and/or carbon dioxide, chloride, and water. In some cases, including chloride in the molar summation may improve the overall mass balance, provided that the release of chloride due to degradation of chlorinated ethenes is much greater than chloride background concentrations. Alternatively, stable carbon isotope analyses can be used to demonstrate that these processes are occurring and can also document depletion of parent DNAPL sources as isotope ratios change.

2.2.2 Impact of BioDNAPL Treatment on Source Longevity and Restoration Time Frames

The principal goal of ISB of a DNAPL source zone is to accelerate destruction of the source and its associated plume. Of course, source treatment may also be designed to reduce the flux from the source, to reduce the plume extent, and/or to allow a more passive plume containment approach, such as monitored natural attenuation (MNA). But it is reasonable to expect that source depletion through any technology, including ISB, will reduce the remediation longevity. Source zone bioremediation can be viewed as a method for enhancing the natural depletion of the source and thereby hastening the natural attenuation of the source zone and its plume.

However, the depletion rate of a source is complex and is governed by the hydrogeology within and upgradient of the source area and by the distribution of the various DNAPL phases (free, dissolved, sorbed, and matrix-diffused) within the source area. Current characterization technologies cannot define these characteristics to the degree needed to accurately predict the rate of source mass depletion and the mass flux from a source zone over time. Concurrently, there are little long-term data on the effects of source treatment on source longevity and plume response. Nonetheless, the results from recent laboratory and field studies, along with developments in mathematical models of the effects of treatment on sources and plumes, have led to an improved understanding of the relationships between DNAPL mass, mass flux from source areas, and the responses of plumes over time to partial source depletion. This improved

understanding can enable better evaluations of the benefits of source treatment, including ISB, and improved predictions of the impacts of treatment on the longevity of sources and their downgradient plumes. Although understanding is improved, it is not complete. Many researchers, including authors of this guidance, continue to debate the assumptions, variables, and equations in this prediction. To understand the fundamental approach, we have included a preliminary description of the process, as it is currently understood, in Appendix C of this guidance. We still see full understanding as one of the greatest challenges within the science of ISB of DNAPL source zones.

2.3 Microbiology of Reductive Dechlorination

Various microorganisms have been shown to anaerobically degrade DNAPL compounds. The sequential reductive dechlorination of PCE through TCE, *cis*-DCE and VC to ethene (Figure 2-4) is well documented (Barrio-Lage et al. 1987; Bouwer 1994; De Bruin et al. 1992; DiStefano, Gossett, and Zinder 1991; Freedman and Gossett 1989; Maymó-Gatell et al. 1995; and Vogel and McCarty 1985). However, the efficiency of each step in the dechlorination process can be dramatically different, depending on the environmental conditions and the microbial populations responsible for the reactions. Also, the sequence can appear to stall at an intermediate stage for biological or environmental reasons. This stall may represent a lack of carbon or nutrients in the aquifer, an inability of the microorganisms at the site to completely degrade the chlorinated ethenes, or a kinetic difference under conditions in which the more chlorinated compounds are biodegraded more rapidly than less chlorinated ones. For chlorinated ethenes, this typically results in a buildup of *cis*-1,2-DCE that can be relatively transient or virtually permanent if a microorganism able to degrade *cis*-DCE and conditions suited to that microorganism are not present. Complete reductive dechlorination of chlorinated ethenes requires both microbial populations capable of efficiently completing each step in the dechlorination process and environmental conditions suitable to facilitate each step in the dechlorination process.

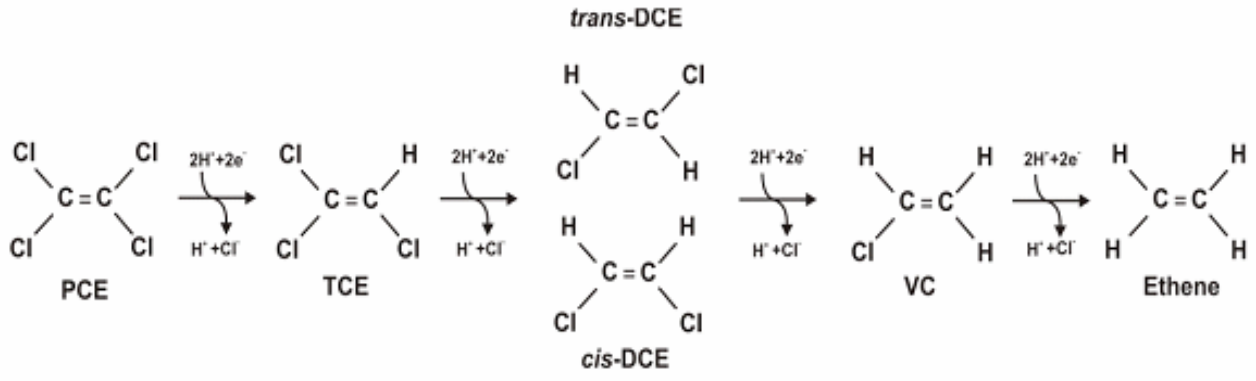


Figure 2-4. Sequential reduction of PCE to ethene by anaerobic reductive dechlorination.

Environmental redox conditions within the target treatment zone must be sufficiently reducing to make the desired reductive dechlorination reactions energetically favorable. During ISB, biodegradation of injected organic substrates depletes the aquifer of dissolved oxygen (DO) and other terminal electron acceptors and lowers the oxidation-reduction potential (ORP) of the groundwater, thereby producing conditions conducive to anaerobic degradation. After DO is

consumed, anaerobic microorganism typically use other electron acceptors in the following order: nitrate, manganese and ferric iron oxyhydroxides, sulfate, and finally, carbon dioxide.

2.3.1 Types of Enhanced Reductive Dechlorination

Three general reaction types of ERD may degrade CAHs under anaerobic conditions:

- *Direct anaerobic reductive dechlorination* is a biological reaction in which bacteria gain energy and grow as one or more chlorine atoms on a CAH molecule are replaced with hydrogen. In the reaction, the chlorinated compound serves as the electron acceptor, and the hydrogen serves directly as the electron donor. Hydrogen used in the reaction is typically supplied by fermentation of organic substrates. Hydrogen can also be introduced by other means, even direct injection. This reaction is sometimes referred to as "halorespiration" or "dehalorespiration."

- *Cometabolic anaerobic reductive dechlorination* is a reaction in which a chlorinated compound is reduced by a nonspecific enzyme or cofactor produced during microbial metabolism of another compound (e.g., the primary substrate) in an anaerobic environment. By definition, cometabolism of the chlorinated compound does not yield any energy or growth benefit for the microbe mediating the reaction (EPA 2000). For the cometabolic process to be sustained, sufficient primary substrate is required to support growth of the transforming microorganisms.

- *Abiotic reductive dechlorination* is a chemical degradation reaction not associated with biological activity in which a chlorinated hydrocarbon is reduced by a reactive compound. Addition of an organic substrate and creation of an anaerobic environment may create reactive compounds, such as metal sulfides, that can degrade CAHs (Butler and Hayes 1999, Lee and Batchelor 2002). In this case, substrate addition indirectly causes and sustains abiotic reductive dechlorination. Abiotic pathways may include hydrolysis, elimination, dehydrodehalogenation, hydrogenolysis, dechloroelimination, and reductive dechlorination by a variety of reactive compounds.

In practice, it is difficult to distinguish among the three reaction types at the field scale, especially when some or all of the reactions may be occurring simultaneously. Enhanced bioremediation applications to date have primarily targeted biotic dechlorination reactions under deeply reducing conditions, but creating anaerobic conditions will likely stimulate all three processes at some subsurface location.

The key reaction that has led to the rapid development of ISB of DNAPL is direct anaerobic reductive dechlorination, whereby microorganisms use chlorinated compounds as electron acceptors to cause reductive dechlorination of the compounds. The microbiology of this process is discussed in the following section.

2.3.2 *Dehalococcoides* and Other Dechlorinating Microorganisms

The ability of some bacteria to completely dechlorinate PCE and TCE to ethene and other innocuous products has made ISB of DNAPL chlorinated ethenes possible. All of the cultures that are capable of dechlorination beyond *cis*-DCE contain organisms in the genus *Dehalococcoides* (Maymó-Gatell et al. 1997; Ellis et al. 2000; Fennell et al. 2001; Richardson et al. 2002; Cupples, Spormann, and McCarty 2003; Dennis et al. 2003). In addition, field sites lacking *Dehalococcoides* spp. have been shown to stall at *cis*-DCE (Hendrickson et al. 2002). Similarly, Lu, Wilson, and Kampbell (2006) showed that the presence of detectable *Dehalococcoides* DNA in groundwater was associated with complete dechlorination, but little attenuation was measured at sites without detectable *Dehalococcoides*. While the presence of *Dehalococcoides* has been linked to the ability to completely degrade chlorinated ethenes to ethene, not all strains of *Dehalococcoides* have the same degradation capabilities. For example the first *Dehalococcoides* strain identified (strain 195) obtains energy from only the first three dechlorination steps (PCE → TCE, TCE → DCE, and DCE → VC) but can transform VC to ethene only through cometabolism (Maymó-Gatell, Anguish, and Zinder 1999; Maymó-Gatell, Nijenhuis, and Zinder 2001). Therefore, the transformation rate of VC to ethene is significantly lower than the other transformation steps, resulting in accumulation of VC. However, other *Dehalococcoides* strains capable of obtaining energy from VC dechlorination have been isolated (He et al. 2003; Cupples, Spormann, and McCarty 2003), and efficient reductive dechlorination of PCE and TCE to ethene has been demonstrated. Therefore, for chlorinated ethenes, biological limitations to achieving efficient reductive dechlorination may significantly impact an ISB treatment if the appropriate strains of *Dehalococcoides* are not native to a site.

One of the greatest technical risks to implementing ISB in a source zone is the potential for inefficient reductive dechlorination and the accumulation of more toxic byproducts (e.g., DCE and VC). This risk can be mitigated, however, using bioaugmentation if it is determined that there is a biological limitation at the site. Several mixed cultures have been successfully developed and are available commercially for bioaugmentation (Ellis et al. 2000, Lendvay et al. 2003, ESTCP 2005b). Bioaugmentation may be considered if there is DCE or VC stall at a site or to hasten the onset of complete degradation and/or increase the overall biodegradation rates (ESTCP 2005b).

There are new diagnostic tools available to help answer the question of whether bioaugmentation may be necessary at a chlorinated ethene DNAPL site. Molecular biological tools (MBTs) are available to identify *Dehalococcoides* and specific reductases that have differing dechlorinating capacities. Several *Dehalococcoides* 16S rRNA gene sequences have been analyzed to date. It is common practice to identify the presence of *Dehalococcoides* at a site by the 16S rRNA gene sequence. While this may suggest the potential for dechlorinating activity, it is insufficient evidence by itself (He et al. 2003) because strains virtually indistinguishable on the basis of 16S rRNA genes have demonstrably different chlorinated-ethene degradation capabilities. A number of new MBTs are now available—assays designed to detect the functional reductive dehalogenase genes (e.g., tceA, vcrA, and bvcA) (Müller et al. 2004) and provide a more complete understanding of dechlorinating capacity at a site. In addition to MBTs, complementary evidence (e.g., microcosms, appropriate field data) can be used to conclusively assess dechlorinating capabilities at a site (ESTCP 2005b).

2.3.3 Substrates (Electron Donors)

Researchers have recognized the role of hydrogen as a direct substrate (electron donor) in anaerobic dechlorination of chlorinated ethenes (Holliger et al. 1993; Gossett and Zinder 1996; Smatlak, Gossett, and Zinder 1996). Laboratory research evaluating *Dehalococcoides*, in particular, has shown that this organism requires symbiotic association with other microbes to obtain required growth factors (i.e., hydrogen, essential nutrients). This requirement has presented particular problems with isolating this genus, and in general, a mixed culture with at least one other distinct strain of bacteria is required to sustain *Dehalococcoides* culture. In one association, the bacterial partner ferments the organic substrate to produce hydrogen, and the *Dehalococcoides* uses the hydrogen as a substrate for anaerobic dechlorination.

The use of hydrogen to sustain reductive dechlorination has several implications regarding the available choices for biostimulating amendments for ISB. Under anaerobic conditions, many microorganisms are capable of fermentation of organic matter to produce hydrogen. Therefore, almost any fermentable substrate can be a potential source of carbon and hydrogen to stimulate reductive dechlorination, including but not limited to naturally occurring dissolved organic carbon (DOC), accidental releases of anthropogenic carbon (e.g., fuel), carbohydrates (sugars), alcohols, oils, solids (e.g., bark mulch, chitin), and complex compounds (e.g., whey and cellulose). Table 2-1 shows examples of fermentation reactions that produce hydrogen. These compounds and their degradation intermediates serve as substrates (carbon sources for growth and energy) for bacteria using a variety of electron acceptors including chlorinated ethenes.

Table 2-1. Examples of fermentation reactions using organic substrates to yield hydrogen

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

(*Source:* AFCEE 2004b)

One consideration for choice of substrates is the consumption requirements that need to be fulfilled to deplete the aquifer system of alternate electron acceptors that compete with chlorinated solvents and inhibit efficient reductive dechlorination. Hydrogen produced by fermentative organisms is rapidly consumed by other microorganisms, including denitrifiers, iron-reducers, sulfate-reducers, methanogens, and dechlorinating microorganisms. For anaerobic reductive dechlorination to occur, dechlorinating organisms must successfully compete with

other microorganisms that also use hydrogen. Table 2-2 shows the relationship between hydrogen and electron-accepting processes. In general, bacteria using oxygen, iron, and sulfate generally outcompete *Dehalococcoides* for available hydrogen, so these alternate electron acceptors must be depleted before efficient reductive dechlorination to ethene will occur.

Table 2-2. Examples of reactions using hydrogen as the substrate

Electron acceptor	Electron-acceptor (reduction) half reaction
Oxygen	$2H_2 + O_2 \Rightarrow 2H_2O$ aerobic respiration
Ferric iron	$H_2 + H^+ + FeOOH \Rightarrow Fe^{2+} + 2H_2O$ "ferric oxyhydroxide" dissolution/reduction
Sulfate	$4H_2 + H^+ + SO^{2-}_4 \Rightarrow HS^- + 4H_2O$ sulfate reduction
Carbon dioxide	$4H_2 + CO_{2,g} \Rightarrow CH_{4,g} + 2H_2O$ methanogenesis
PCE	$H_2 + C_2Cl_4 \Rightarrow C_2HCl_3 + HCl$ PCE reductive dechlorination
TCE	$H_2 + C_2HCl_3 \Rightarrow C_2H_2Cl_2 + HCl$ TCE reductive dechlorination
DCE	$H_2 + C_2H_2Cl_2 \Rightarrow C_2H_3Cl + HCl$ cis-1,2-DCE reductive dechlorination
VC	$H_2 + C_2H_3Cl \Rightarrow C_2H_4 + HCl$ VC reductive dechlorination

(*Source:* AFCEE 2004b)

2.3.4 Stoichiometry

The in situ generation of hydrogen (H_2) does not guarantee that it will be used solely for anaerobic reductive dechlorination. Thus, a direct stoichiometric relationship does not exist between hydrogen produced and chlorinated ethenes degraded in the subsurface or laboratory environment. However, although the efficiency of hydrogen use for reductive dechlorination is often estimated to be low, the stoichiometric relationships for the direct anaerobic dechlorination of CAHs are favorable. For example, on a mass basis, 1 mg of H_2 will dechlorinate PCE (21 mg), TCE (22 mg), DCE (24 mg), and VC (31 mg), assuming 100% use of H_2 by the dechlorinating microorganisms (Gossett and Zinder 1996). These relationships translate to a minimal hydrogen requirement to sustain efficient reductive dechlorination.

Laboratory studies have shown that fermentation that results in a slow, steady, low-level release of hydrogen over time will maximize dechlorination potential while minimizing methanogenic competition for the available hydrogen. However, field experience has shown that sustained delivery of low levels of hydrogen throughout a site can create its own challenges, e.g., biofouling. An alternative strategy is delivery of large quantities of electron donor, which is converted to high concentrations of hydrogen. The abundance of hydrogen reduces competition among dechlorinators, methanogens, and other hydrogen consumers, allowing each to approach or achieve their metabolic maxima.

2.4 Amendments for ISB of Chlorinated Ethene DNAPL

As discussed in Section 2.3.2, microorganisms must exist in sufficient quantity and species to ensure degradation. If they are absent or in low concentrations, then bioaugmentation may be necessary. Effective remediation may require biostimulation if the microorganisms naturally present are insufficient to effectively biodegrade chlorinated ethenes at the appropriate scale for the site.

2.4.1 Biostimulation

Microorganisms that degrade contaminants may be naturally present in the subsurface but in inadequate numbers to facilitate effective biodegradation at a scale that is relevant to remediation. This condition may require biostimulation by injection of a substrate (electron donor), nutrients, and/or other materials (e.g., buffers) into the subsurface to stimulate microbial growth and activity or establish supportive geochemical conditions. For the degradation of chlorinated ethenes, the injected substrate provides a carbon source for cell growth and ferments to produce hydrogen. Hydrogen is the preferred electron donor for reductive dechlorination. Biometabolism of the added substrate, hydrogen, and other simple organics also facilitates consumption of competing electron donors and establishment of methanogenic and/or sulfate-reducing conditions. In some cases addition of other amendments (in addition to electron donor) should be considered to enhance conditions for bacterial growth and metabolism. Other amendments could include nutrients (e.g., nitrogen and phosphorous) and/or buffering agents.

2.4.2 Bioaugmentation

Bioaugmentation involves the injection of microorganisms into the subsurface for the purpose of degrading contaminants. Bioaugmentation intends to increase the overall degradation rate where the indigenous microbial populations cannot completely degrade a contaminant or the degradation rates are too low to meet the remedial goals in an acceptable time period. For example, the bacteria needed to degrade PCE and TCE are present at most sites; however, bacteria able to degrade *cis*-DCE and VC are not always present or may not be present in sufficient numbers.

The microorganisms used in bioaugmentation are available as cultivated, well-characterized, mixed populations (consortia) of bacteria that can be purchased for his purpose. These consortia include fermentative bacteria that produce hydrogen, the substrate (electron donor) for reductive dechlorination. Complete mineralization of chlorinated ethenes has been achieved only with *Dehalococcoides*. Other species capable of reductive dechlorination include *Dehalobacter*, *Sulfurospirillum*, *Clostridium*, *Desulfuromonas*, and *Desulfitobacterium*.

2.5 Advantages and Limitations

Prior sections have discussed various advantages and limitations of ISB at DNAPL source zones. ISB of DNAPL source zones has some advantages over other remediation techniques typically applied in DNAPL source zones. However, like all technologies, EISB also has some potential limitations.

2.5.1 Advantages

The advantages of ISB in the source area include the following:

- increases the rate of desorption or dissolution of chlorinated solvents, thus shortening the overall remediation time frame of the site when applied near the DNAPL/water interfaces
- may treat other contaminants mixed with the chlorinated ethenes
- may be used in combination with several other treatment methods as part of an overall site strategy
- has demonstrated performance through case studies (ITRC 2007a)
- exhibits few health and safety concerns compared to other source zone technologies
- degrades contaminants in situ without creating a secondary waste stream
- requires low maintenance when persistent electron donors are used and natural buffering is adequate
- provides minimal impact to existing site infrastructure
- normally has lower capital cost than other source zone treatment technologies

2.5.2 Limitations or Challenges

ISB of DNAPL source zones is limited under the following conditions:

- Aquifer permeability and preferential pathways inhibit distribution of substrate throughout the DNAPL source zone. This is a universal issue with in situ remedial technologies that rely on injection and distribution of amendments within the subsurface.
- Unacceptable aquifer geochemical conditions (e.g., low or high pH) inhibit biological activity.
- Biofouling occurs in electron donor injection and recirculation wells.
- A long time frame is required (several months or years) to develop appropriate environmental conditions or a microbial community capable of complete degradation.
- Limitations in the microbial populations create the potential for incomplete degradation and the buildup of *cis*-DCE or VC (referred to as *cis*-DCE or VC "stall").
- A decrease in pH and changes in redox enhance the solubilization of metals and the formation of undesirable products (e.g., hydrogen sulfide, methane, and other noxious gases) and an increase in the total dissolved solids (TDS).

The following sections describe the attributes of the site that do and do not lend themselves to ISB of DNAPL source zones. Most are not limitations but characteristics that must be overcome during the application and system design.

2.6 Decision Making

Figure 2-5 is a flow diagram of the investigation, design, and operational monitoring steps that are required for ISB of DNAPL source zones (by ERD). At most sites, comprehensive sampling is conducted to establish a baseline characterization of the site geochemistry and hydrogeology. Additional sampling is conducted during final design and pilot study phases. Sampling programs are then narrowed during full-scale operation, providing only the data needed to support operational decision making. If the ISB system responds as designed, the operational configuration is maintained and optimized as necessary until the project goals (RAOs) are achieved. Conversely, if the system fails to respond as expected during full-scale operations, a diagnostic sampling program is undertaken, with an expanded list of variables. The system is reconfigured and operation is resumed, or in some cases an alternative technology may be applied.

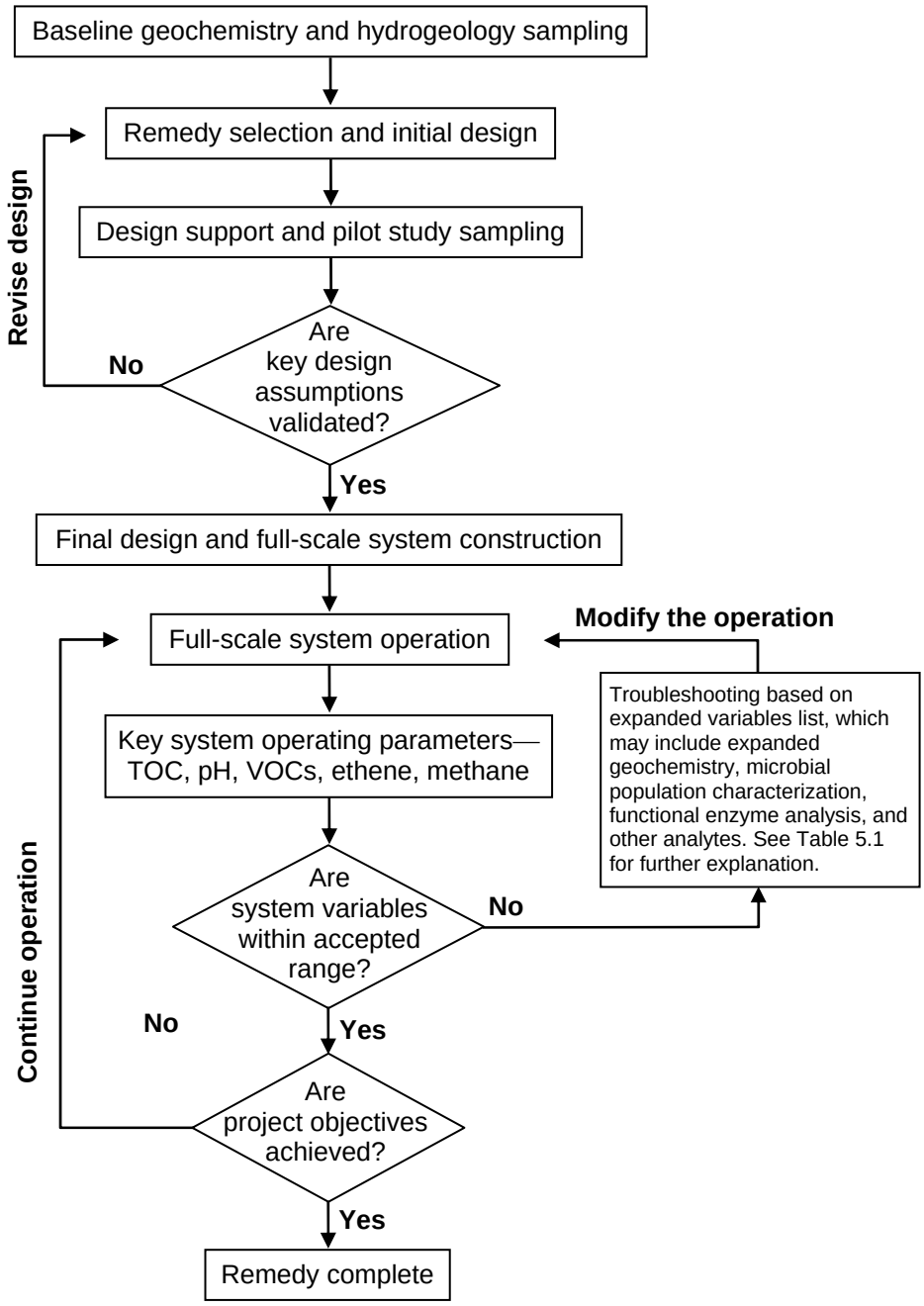


Figure 2-5. Decision making. (Courtesy of Arcadis)

Sections 3–5 describe the details of conducting particular phases of the project. Each section is highlighted within the diagram to keep users oriented within the assessment, design, and operation of an ISB of DNAPL source zone project.

3. ASSESSING THE APPLICABILITY OF BIOREMEDIATION

This section discusses how to determine whether ISB is appropriate at a site, based on the characteristics of the DNAPL chlorinated ethene source zone and aquifer. It describes site characterization approaches and CSM development, along with an analysis of key factors influencing ISB applicability. Figure 3-1 highlights the assessment phase, during which comprehensive sampling characterizes the site geochemistry and hydrogeology.

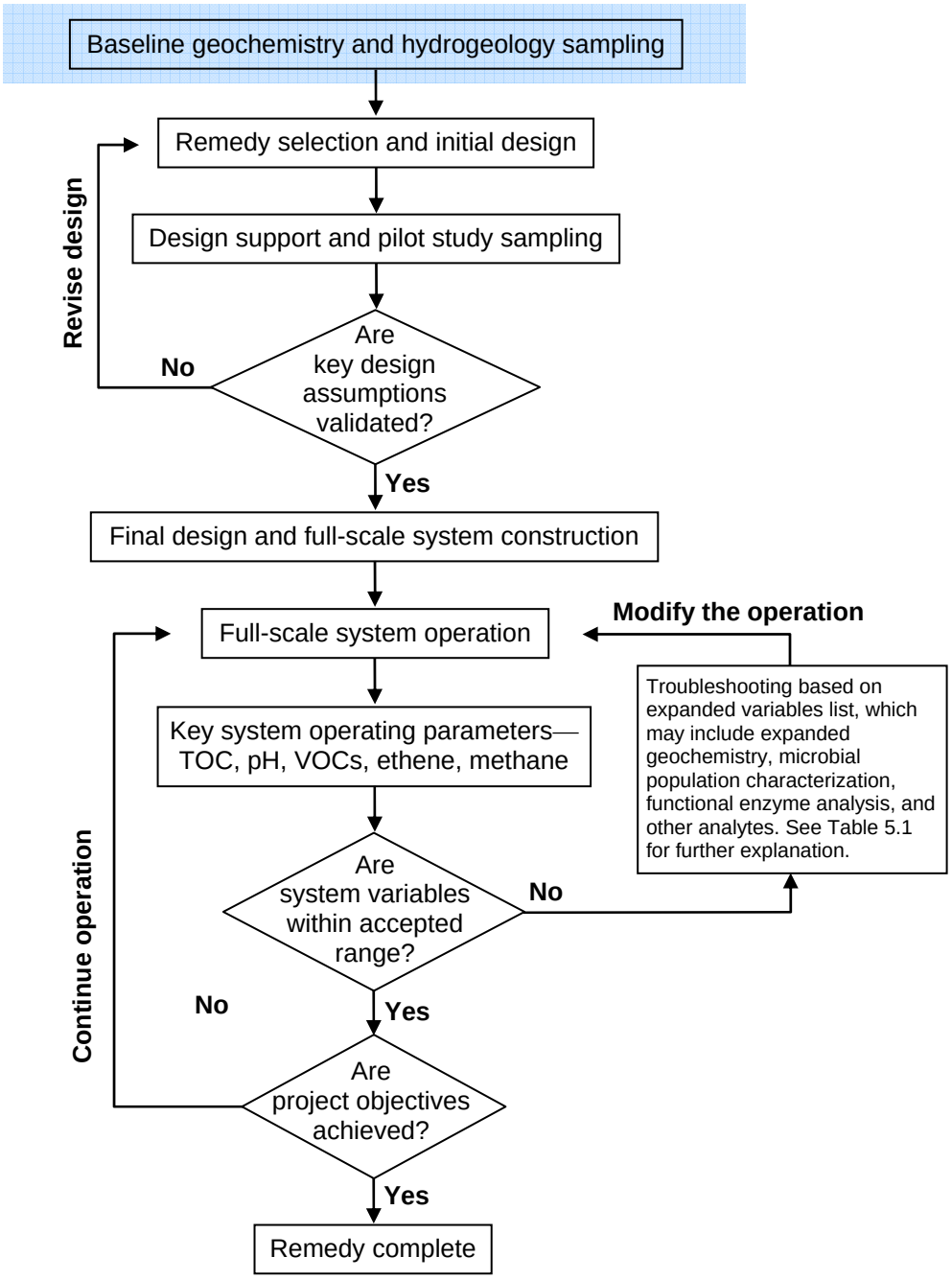


Figure 3-1. Decision making—Assessment. (Courtesy of Arcadis)

3.1 Site Characterization and Conceptual Site Model

Before considering ISB for treatment of a DNAPL source zone, detailed knowledge of the geochemical and hydrogeological characteristics of the source zone, including the DNAPL architecture, contaminant fate and transport mechanisms, and potential exposure pathways, must be obtained. The techniques used to characterize a DNAPL site and develop this knowledge are described in various documents, including, among others, *An Introduction to Characterizing Sites Contaminated with DNAPLs* (ITRC 2003a) and *An Illustrated Handbook of DNAPL Transport and Fate in the Subsurface* (U.K. Environmental Agency 2004)

Development of a comprehensive CSM is the first step in assessing the potential applicability of ISB at a DNAPL site. Contaminant distribution, environmental data (geology, hydrogeology, geochemistry, and microbiology) and other information (e.g., past disposal practices, proximity of the site to occupied structures) collected during site characterization are the CSM's basis. The CSM presents relevant data in a readily shared, concise form, typically a two- or three-dimensional representation of site conditions pertinent to understanding the problem. For example, Figure 3-2 is a schematic of a DNAPL source zone and the adjacent surface and subsurface conditions. As additional data are collected, they are used to refine the CSM, which is used during final design and task planning (e.g., designing a performance monitoring program).

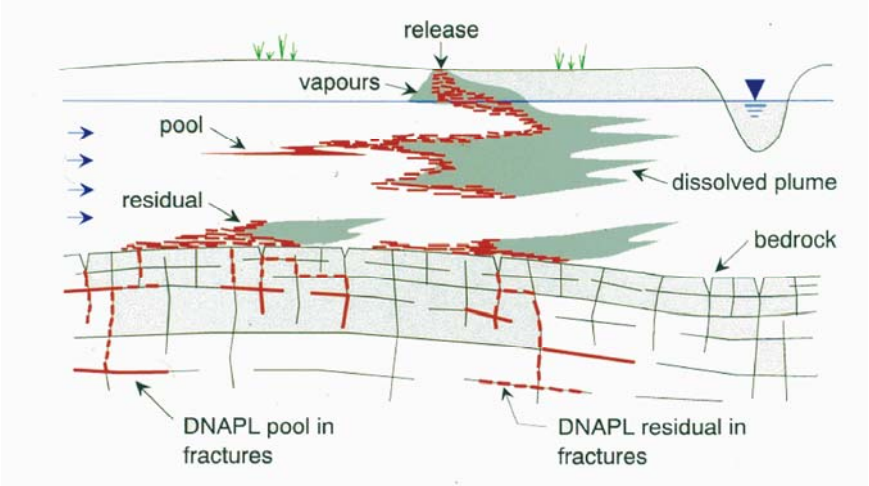


Figure 3-2. Conceptual model of DNAPL source zone. (*Source:* ITRC 2004)

3.2 Assessing the Applicability of ISB

Whether ISB can be applied successfully to a particular site, either as the primary treatment or in combination with other treatment alternatives, depends on a combination of factors specific to the site and the degree to which the favorable factors can be maintained or optimized and the limiting factors can be overcome. Table 3-1 lists general site conditions that should be considered to assess whether or not these characteristics will have favorable or unfavorable impacts on bioremediation or, in the extreme, will prohibit bioaugmentation. This table is not a list of requirements but a list of parameters that should be considered during the evaluation phase. The information presented is a general guide to these factors, not a quantitative scoring system or a feasibility study–type analysis.

Table 3-1. Site conditions that affect applicability of ISB to treat DNAPL source zones
(From AFCEE 2004b with parameters added by members of the ITRC BioDNAPL Team)

Factor	Condition		Effect on ISB	Ease of controlling or overcoming
	Favorable	Less favorable		
DNAPL source zone characteristics				
DNAPL distribution/architecture	Residual phase	DNAPL pools	Moderate to high	Difficult
Pool to ganglia ratio	Low	High	Moderate to high	Difficult
Contaminant	Pure	Mixtures of chlorinated ethenes, ethanes, and/or methanes	Moderate to high	Difficult
Other contaminants and cosolvents or mixed hydrocarbons in DNAPL	Fuel-related mixtures	Mixtures of chlorinated ethenes, ethanes, and/or methanes and oil and grease, which may contain heavy metals	Moderate	Easy to moderate
Depth of source	Shallow	Deep	Low to moderate	Moderate
Age of source/ plume maturity	Recent (<10 years)	Mature (>10 years)	High	Difficult
Volume	Small (<500 yd^3)	Large (>500 yd^3)	Moderate	Moderate
Hydrogeology				
Depth to groundwater	Moderate	Deep or very shallow	Low to moderate	Difficult
Target treatment zone thickness	Thin (10s of feet)	Thick (100s of feet)	Low	Moderate
Hydraulic conductivity	Medium to high (>1 ft/day)	Low (<1 ft/day)	High	Moderate
Groundwater velocity	>0.1 ft/day or <3 ft/day	>3 ft/day or <0.1 ft/day	High	Difficult
Aquifer matrix	Granular, unconsolidated media, primary porosity dominates	Rock, consolidated media, secondary porosity dominates	High	Difficult
Heterogeneity/anisotropy	Low to moderate (e.g., sands)	High (e.g., karst) or multilayered sediments, (i.e., high K layered with low K, glacial alluvium)	Moderate to high	Difficult (tracer study may be required)
Fraction of organic carbon	Low (<1%)	High (>1%)	Low	Difficult

Table 3-1. Site conditions that affect applicability of ISB to treat DNAPL source zones (continued)

Factor	Condition		Effect on ISB	Ease of controlling or overcoming
	Favorable	Less favorable		
Geochemistry				
Total alkalinity	High (>100 mg/L as $CaCO_3$)	Low	Moderate	Difficult
pH	5–8	<4.5 or >9	High	Moderate to difficult
Oxidation reduction potential (mV)	<–50	>0	Low	Moderate
Metals	Iron and Mn minerals absent	Significant presence of minerals containing reduced Fe and Mn	Low	Moderate
Temperature	>10°C (50°F) and <35°C (95°F)	<10°C (50°F) and >35°C (95°F)	Low	Difficult
Competing electron acceptors (nitrate, sulfate, etc.)	Low	High	Low	Easy
Microbiology				
Native heterotrophic population	Present in pre-ISB screening	Absent in pre-ISB screening	Moderate to high	Difficult in extreme sites (e.g., desert aquifers)
Anaerobic oxidizers	Present	Absent/poorly distributed	Low	Difficult
Dehalococcoides ethenogenes	Present	Absent/poorly distributed	Moderate to high	Easy (may be dependent on geology)
Dehalorespirators other than *D. ethenogenes*	Present	Absent/poorly distributed	High	Easy (may be dependent on geology)
Other factors				
Proximity of receptors to groundwater plume	>6 months travel time	<6 months travel time	Moderate	Moderate
Location of on-site infrastructure	Risk from vapor intrusion is acceptable	Target treatment zone is near sensitive infrastructure	Moderate	Moderate
Site access	Easy to access	Site is remote or otherwise difficult to access	Moderate	Moderate

3.3 Evaluation Approach

Evaluation of ISB at a DNAPL source zones is a "weight of evidence" process. However, the number of less favorable characteristics that can be cost-effectively overcome at a given site depends on the remedial goals and potentially applicable alternative remedial technologies. Overcoming a number of less favorable conditions increases costs and possibly remediation time frames. Table 3-1 is not a comprehensive presentation of every factor at a given site that might influence the effectiveness of ISB of DNAPL source zones; it is rather a list of common factors that have been observed to impact ISB at a number of sites. The following subsections discuss the factors presented in Table 3-1 in more detail, as well as potential interactions between or among factors.

3.3.1 DNAPL Source Zone Characteristics

The single set of factors that distinguish an assessment of the applicability of ISB to source zones as opposed to the dissolved phase are the physical characteristics of the source zone itself, including the DNAPL distribution and architecture. In general, the targeted subsurface volume (i.e., its area, thickness, and depth below grade) is critical to treatment system design. Within that volume the DNAPL architecture (i.e., the distribution of DNAPL mass within the source zone) influences ISB performance by controlling the ability of the injection program to achieve contact between the injectate and the DNAPL. Since dissolution is highly dependent on the surface area of the DNAPL, ISB is favored at sites where DNAPL is predominantly distributed as residual, nonaqueous-phase saturation and is less likely to be successful at sites where there is significant DNAPL mass accumulated or pooled on lower-permeability geologic units. This concept may be expressed as a ratio of DNAPL mass in low-saturation residual regions to DNAPL mass in high-saturation pool regions. DNAPL distributed as residual mass has more surface area than DNAPL in pools. Increased surface area increases dissolution rates, which leads to faster mass degradation and allows improved electron donor delivery to and contact with the DNAPL. If applied prior to ISB, other remediation technologies (e.g., water flooding, surfactant flushing) that remove or disperse DNAPL pools and increase DNAPL surface area can improve the performance of ISB.

The contaminants present in the source zone can have significant impact on the applicability of ISB. Currently, ISB is primarily applicable to chlorinated ethenes, which can be biodegraded to ethene, a nontoxic end product. DNAPL consisting of a single contaminant (e.g., TCE) are more favorable for ISB than a mixed contaminant source. At a sufficiently high concentration, some common co-contaminants (e.g., 1,1,1-trichloroethane [TCA], chloroform) can inhibit dechlorinating bacteria. The biodegradation of other chlorinated solvents is less likely to result in the formation of a nontoxic end product. Conversely, some nonchlorinated co-contaminants (e.g., alcohols, petroleum hydrocarbons) can serve as electron donors, providing energy to dechlorinating organisms. In this case, DNAPLs mixed with electron donors may enhance the dissolution rate by promoting the growth of dechlorinating microorganisms close to the DNAPL-water interface, which increases the rate of source mass removal. However, nonchlorinated co-contaminants (e.g., oil, grease, and petroleum hydrocarbon fuels) that initially functioned as

electron donors are eventually consumed, allowing hydrogeologic conditions to return to background and slowing or stopping reductive dechlorination.

Another important source zone characteristic is the age of the source or how long the source zone has been present in the aquifer. In older source zones, much of the readily accessible mass may have already dissolved in the groundwater. The remaining DNAPL may be relatively inaccessible to dechlorinating microorganisms. Further, both residual and dissolved mass may have diffused into low-permeability geologic materials (e.g., clay, shale), again becoming less accessible to dechlorinating microorganisms, though also less likely to dissolve into the groundwater.

3.3.2 Hydrogeology

Site hydrogeology can have a tremendous effect on the applicability of ISB, which is optimized by achieving a uniform distribution of amendments. Many hydrogeologic characteristics are, at best, difficult to control or, at worst, cannot be controlled. For example, hydraulic conductivity, aquifer matrix material, and the degree of heterogeneity cannot be manipulated.

ISB of DNAPL source zones in low-conductivity, highly heterogeneous, and/or fractured rock aquifers is very difficult. However, these sites are also the most significant challenges for other in situ remediation technologies, and ISB can still be appropriate if sufficient resources are available and applied. For example, numerous injection wells may be constructed at sites with less favorable hydrogeologic conditions to ensure distribution of substrate (electron donor) throughout the contaminated area. In addition, the cost per well may be high (e.g., in fractured rock). Sites with low groundwater velocity or a highly heterogeneous aquifer matrix may require forced gradients (i.e., recirculation) to adequately distribute substrate. This approach requires more wells, larger and more complex systems, and more intensive operations than a higher-hydraulic-conductivity, unconsolidated-media site.

Target Treatment Zone Thickness. The thickness of the zone requiring treatment affects the capital investment required for injection and monitoring well installation. Zones thicker than 20–30 feet may require nested injection wells to deliver amendment across the targeted thickness. Also, a thicker treatment zone requires a greater volume of substrate to cover the same radius from the injection well.

Hydraulic Conductivity. Higher hydraulic conductivity allows better distribution of substrates from fewer injection wells or even fewer recirculating wells. However, it should be noted that higher porosity and hydraulic conductivity also require larger injection volumes to achieve the same radius of influence (ROI) compared to a lower-porosity system. Sites with hydraulic conductivity so low that delivery via wells is limited require more injection locations and may benefit from delivery of amendments using fracturing techniques. While these properties cannot be manipulated, certain techniques, such as fracturing, can be implemented to improve the applicability of ISB. Limitations of hydraulic fracturing are not discussed herein but should be considered in low-hydraulic-conductivity zones. Fracturing creates new pathways and may increase the overall conductivity of the fractured aquifer, but it may not improve contact with the source zone mass.

Groundwater Velocity. High-velocity groundwater (e.g., greater than 3 ft/day) reduces lateral spreading of added amendments and may require more closely spaced injection points or use of recirculation and/or pumping strategies to spread amendments perpendicular to groundwater flow. Rapid groundwater velocities rapidly flush electron donor solution from the treatment zone and could result in the need for more frequent injections to maintain adequate electron donor levels. Higher velocities also increase the mass flux of competing electron acceptors entering the treatment zone and the electron donor mass required to consume them. Very low groundwater velocity (e.g., <0.1 ft/day) limits transport and provides less directional control of amendments unless pumping is used to increase groundwater flow rates during application. Low groundwater velocity must also be considered in the placement of monitoring points to recognize system performance in a reasonable period of time. On the positive side, very-low-velocity groundwater systems typically require less frequent electron donor injections to consume competing electron acceptors due to lower influx into and through the treatment area.

Heterogeneity/Anisotropy. Heterogeneity and anisotropy of an aquifer impact the required effort to uniformly distribute amendments and enhance the formation of preferential flow paths, which control the movement of amendments and DNAPL/dissolved phases in the subsurface. Aquifers with high degrees of heterogeneity and anisotropy may require more vertical/lateral injection points or active pumping to overcome mixing and delivery uncertainties.

Fraction of Organic Carbon. Chlorinated ethenes adsorb to natural organic matter, typically quantified in terms of fraction of organic carbon (f_{oc}) in the aquifer. The chlorinated ethene mass adsorbed to the f_{oc} will act as a reservoir, releasing chlorinated ethenes to groundwater as dissolved concentrations decline during remediation. This phenomenon can significantly increase the time required to reduce contaminant concentrations below remedial goals. Introduced organic electron donor can act similarly, though its own degradation and the ability of some of its metabolic products to act as surfactants or solvents increase the release of adsorbed chlorinated ethenes.

3.3.3 Geochemistry

Several key geochemical parameters can affect the applicability of ISB of DNAPL source zones. Near neutral pH, high alkalinity (buffering capacity), high groundwater temperatures, and low to moderate concentrations of competing electron acceptors favor ISB. The most important point regarding geochemistry is that most factors are difficult to control or overcome if they are natural properties of the aquifer (for instance, a naturally low pH) because they require manipulation of the basic aquifer conditions on a very large scale. An unfavorable geochemical factor that can often be overcome is a high concentration of competing electron acceptors. Overcoming this condition can usually be achieved by more time and injection of more substrate.

Total Alkalinity. Total alkalinity buffers against acid produced during the fermentation of organic carbon substrates. Liquid and solid materials (e.g. sodium bicarbonate) can be added to increase the buffering capacity of the groundwater; however, these should be added with care due to the potential risks associated with overdosing of these compounds, including excess gas generation and decreased permeability. Also, the use of amendments to increase the alkalinity

provides only temporary solutions and does not significantly affect the buffering capacity of the system as a whole.

pH. If insufficient buffering capacity is available, pH will decrease due to production of volatile fatty acids (VFAs) during fermentation of electron donors. The target pH range for efficient stimulation of dechlorinating bacteria is 5–8 s.u. Outside this pH range, the efficiency of dechlorination reactions can be negatively impacted. As with alkalinity, additional amendments (e.g., sodium hydroxide) can be added to maintain pH within the target range; however, these should be added with care due to the potential risks associated with overdosing of these compounds, including excess mineral precipitation and decreased aquifer permeability, and negative impacts to the microbial community resulting from wide swings in pH in a short period of time. Also, the use of amendments to increase the pH provides only temporary solutions and does not significantly affect the buffering capacity of the system as a whole.

Oxidation Reduction Potential. ORP can be used as a quantitative measure of natural conditions prior to initiating ISB of DNAPL source zones and after ISB is initiated to indicate the changes in redox due to ISB. Naturally low ORP indicates that a shorter time may likely be needed to achieve strongly reducing (methanogenic) conditions and complete dechlorination to ethene. However, field measurement of ORP can be challenging if one of the many elements which compose the parameter couples (e.g., ferrous/ferric iron) is disproportionately abundant. Due to the potential for interference with this measurement in ISB systems, care should be taken in the use of the ORP parameter to assess redox conditions. Rather, measurement of the individual electron acceptors and/or reduced products is a more reliable approach to assessing redox conditions, both before and during implementation of ISB.

Metals. The solubility of some oxidized metals (e.g., iron, arsenic, and manganese) increases under reducing conditions. In extreme cases, engineering controls may be required to address the solubilization of these metals. In most cases, monitoring will be sufficient to determine whether the concentrations of these metals attenuate downgradient of the source area. Experience has shown most mobilized metals oxidize and precipitate when groundwater conditions return to background.

Temperatures. As a rule of thumb, biological metabolic rates double with each 10°C (50°F) increase in temperature. Most microorganisms have a maximum growth rate at temperatures below 40°C (104°F). At low temperatures reaction rates may be too low for sufficient dechlorinating activity to occur. Most aquifers within the United States exhibit acceptable temperatures for ISB to occur. Adjusting groundwater temperature is impractical for large volumes but may be economical in small DNAPL source zones.

Sulfides. Soluble sulfide measurements are inexpensive and provide an independent check on electrode-based methods for redox measurement. Electrode-based measurement of redox can be variable-based on the preparation of the electrodes and field capability. If there is measurable sulfide in a system, then the redox is typically below –200mV. Soluble sulfide determination is an optional screening-level analysis to assist in establishing baseline geochemistry. Soluble sulfide is reactive and complexes with available metals, predominantly iron. These complexes typically precipitate, thereby removing sulfide and iron from solution. Thus, unless sulfide and

iron are recorded over time, low concentrations of both may be erroneously interpreted to indicate moderate to high redox when, in reality, conditions are methanogenic.

Competing Electron Acceptors. Other electron acceptors (e.g., oxidized metals, oxygen, nitrate, and sulfate) compete with dechlorinating microorganisms for electron donors and hydrogen. Additional electron donor must be injected to deplete these competing electron acceptors before reducing conditions are created and significant dechlorination can occur.

3.3.4 Microbiology

The microbiology of a site before implementation of ISB in a DNAPL source zone may have a surprisingly low impact on assessment of the applicability of ISB. Even if the appropriate bacteria are apparently not present under pre-ISB conditions due to low numbers or actually absent, experience has shown there is a good chance that these bacteria will develop and grow to sufficient numbers once substrate is added to the subsurface. If the appropriate dehalorespiring bacteria are still not present after addition of substrate and establishment of methanogenic conditions, they can be added to the aquifer (bioaugmentation). Therefore, microbial monitoring as a pre-ISB screening tool is not recommended due to the high potential for a false negative indication. The presence of appropriate geochemical conditions and dechlorination products are better indicators of the potential use of ISB. Assessment of the microbiology after the initiation of ISB can be a potentially useful trouble-shooting tool if ISB is not producing the expected results.

One exception is in the case of desert aquifers, which sometime lack heterotrophic bacteria. In this case, bioremediation can be difficult or nearly impossible to implement because these bacteria perform many of the supplemental reactions that are essential to ISB (i.e., fermentation of substrate, depletion of competing electron acceptors, and production of necessary cofactors). Without these synergistic reactions and by-products, dehalogenating bacteria cannot thrive.

Anaerobic Oxidizers. Microorganisms capable of oxidizing organics to CO_2 under anaerobic conditions may be able to degrade partially dechlorinated daughter products in or near the source area (e.g., sulfate- or iron-reducing conditions). Anaerobic oxidizers are not widely distributed, but the presence of these microorganisms is not absolutely required because reductive dechlorination will proceed under proper conditions. However, anaerobic oxidation will increase the overall dechlorination rate.

Dehalococcoides and Other Dechlorinating Microorganisms. The first dechlorination steps, in which relatively insoluble parent compounds are converted to more soluble daughters, are carried out by dechlorinating microorganisms that are ubiquitous in the environment. In the source area, partial dechlorination is sufficient to increase the rate of source mass removal, although ensuring that complete dechlorination occurs can be critical to contaminant plume control. If insufficient dechlorination to ethene occurs naturally, bioaugmentation is a viable and proven approach.

3.3.5 Other Factors

If receptors are located close to the source zone, they may be at risk of exposure to incomplete degradation products (e.g., VC) or to unfavorable secondary water quality issues (e.g., iron, manganese or arsenic). In these cases, ISB can still be implemented, but downgradient hydraulic control may be needed to act as a "safety net" to prevent exposure of downgradient receptors. For example, if the source area is close to an operating municipal well field, the risk of daughter products or anaerobic water possibly containing dissolved metals or organic carbon reaching the production wells must be assessed before considering ISB as a stand-alone remedy.

Another factor is the proximity of on-site infrastructure or personnel that may be affected by vapor-phase contaminants or methane that is produced during source zone ISB. At some sites, methane generation has resulted in vadose zone accumulation of methane under building slabs or in well casings. Also, if the dechlorination process stalls at DCE or VC, the potential exists for vapor-phase VC to migrate upward to and through the vadose zone and possibly cause vapor intrusion issues. These concerns can often be addressed through air sampling or engineering or administrative controls.

One final issue that can impact the applicability of ISB to source zones is site access. Aqueous substrates can have a more dramatic impact on enhancing dissolution from a residual source than slow-release substrates (ITRC 2005a). However, aqueous substrates have a significantly shorter longevity in the subsurface compared to that of slow-release donors. Aqueous substrates may need to be injected more frequently, which in turn requires more frequent site access. Thus, if a site is remote or otherwise difficult to access, one must consider the tradeoff between selecting a slow-release substrate and performing less-frequent injections and thus having less effect on source dissolution, versus selecting an aqueous substrate that will more quickly mobilize and treat the source but will require more-frequent mobilizations to the site.

3.4 Threshold Scenarios/Conditions: Potential Show-Stoppers

While some may say that no site is ideally suited to ISB of DNAPL source zones, many limitations can be overcome with proper engineering, flexible regulatory environments, and/or financial resources. In some cases ISB is more appropriate as a companion technology to more aggressive or direct mass-removal treatments, by conducting ISB either following more-aggressive treatment or in adjacent areas during more-aggressive treatment of other areas. Like any remedial technology, ISB proceeds more readily under optimum conditions and less quickly at less favorable sites. ISB implementability dictates the preferred remedial strategy and may even cause the project team to look at another technology. With this in mind, the following scenarios highlight possible site conditions that are potential ISB "show-stoppers," that is, cases in which ISB would be infeasible. Note that these same conditions would hinder the application and performance of virtually all in situ remediation technologies except the most expensive, which were developed to address one or more of these scenarios. ISB, when practical, is still a flexible, low-cost remedial method.

Scenario 1: Large Volumes of Mobile DNAPL, Inaccessible DNAPL Mass. Source zones with large volumes of mobile DNAPL are generally not suitable for ISB treatment of the DNAPL

source without first implementing some level of physical DNAPL removal. Areas with mobile DNAPL may be controlled using a downgradient reactive biobarrier to control plume concentrations emanating from the source although this approach will not reduce the DNAPL source longevity. Alternately, a more aggressive approach may be undertaken involving physical DNAPL removal (e.g., thermal treatment, source excavation, DNAPL pumping) followed by in situ treatment using ISB or other technologies. Sites may also have a large amount of known chlorinated ethene DNAPL contamination that is rendered inaccessible by other components of the source. For example, oil and grease may effectively surround a smaller chlorinated ethene DNAPL mass. In this case, the DNAPL cannot be remediated by ISB until the oil and grease are naturally weathered away or removed or destroyed using an aggressive remediation technology.

Scenario 2: Geochemical or Other Limiting Condition (e.g., Low or High pH, Temperature). Sites may have a geochemical or water quality condition that could limit DNAPL ISB. For example, groundwater with pH <5 is unfavorable for growth of *Dehalococcoides* bacteria. If the low pH is caused by local co-contamination or by fermentation of the added substrate, then it can be relatively easy to overcome by adding a buffer. However, if the background pH of the aquifer is <5, then ISB may not be appropriate because it can be very difficult to raise and maintain pH on an aquifer scale.

Scenario 3: Low Hydraulic Conductivity and Preferential Flow. Because the method of substrate delivery can limit the success of ISB, subsurface conditions that affect the ability to deliver amendments throughout the source zone, such as low-permeability soils or fractured bedrock (which may have very high groundwater flow rates), should be carefully evaluated. Fortunately, many of these factors can be mitigated so that ISB is workable although site characteristics that foster reductive dechlorination may exist in only a portion of the targeted treatment zone and may vary over time as the biogeochemical conditions evolve during ISB.

Scenario 4: Proximity of Sensitive Receptors (Buildings/Well Fields). Sites where the source area is near sensitive receptors may not be appropriate for ISB. While this factor is discussed in Table 3-1 as one that can be overcome, there may be some situations where it cannot be mitigated. For example, if the source area is too close to an operating municipal well field, the risk of daughter products or anaerobic water containing dissolved metals or organic carbon reaching the production wells may be too great to consider ISB as a stand-alone remedy. In these cases, other remediation technologies should be considered. Some form of containment (biowalls or hydraulic containment) may need to be coupled with DNAPL source zone ISB.

4. APPLICATION DESIGN

The topics addressed in Sections 1–3 of this guidance should be considered prior to undertaking the design of an ISB application for treatment of a DNAPL source zone. It is important to have a CSM of the DNAPL distribution within the source zone and the controlling characteristics of the hydrogeology of the subsurface. Clear goals for the DNAPL source zone treatment should be established prior to undertaking a system design, and the potential site specific applicability of ISB to achieve these goals must be understood. Additional sampling is conducted during final design and pilot study phases to confirm and expand the CSM. Sampling programs are then

narrowed during full-scale operation, providing only the data needed to support operational decision-making (see Figure 4-1).

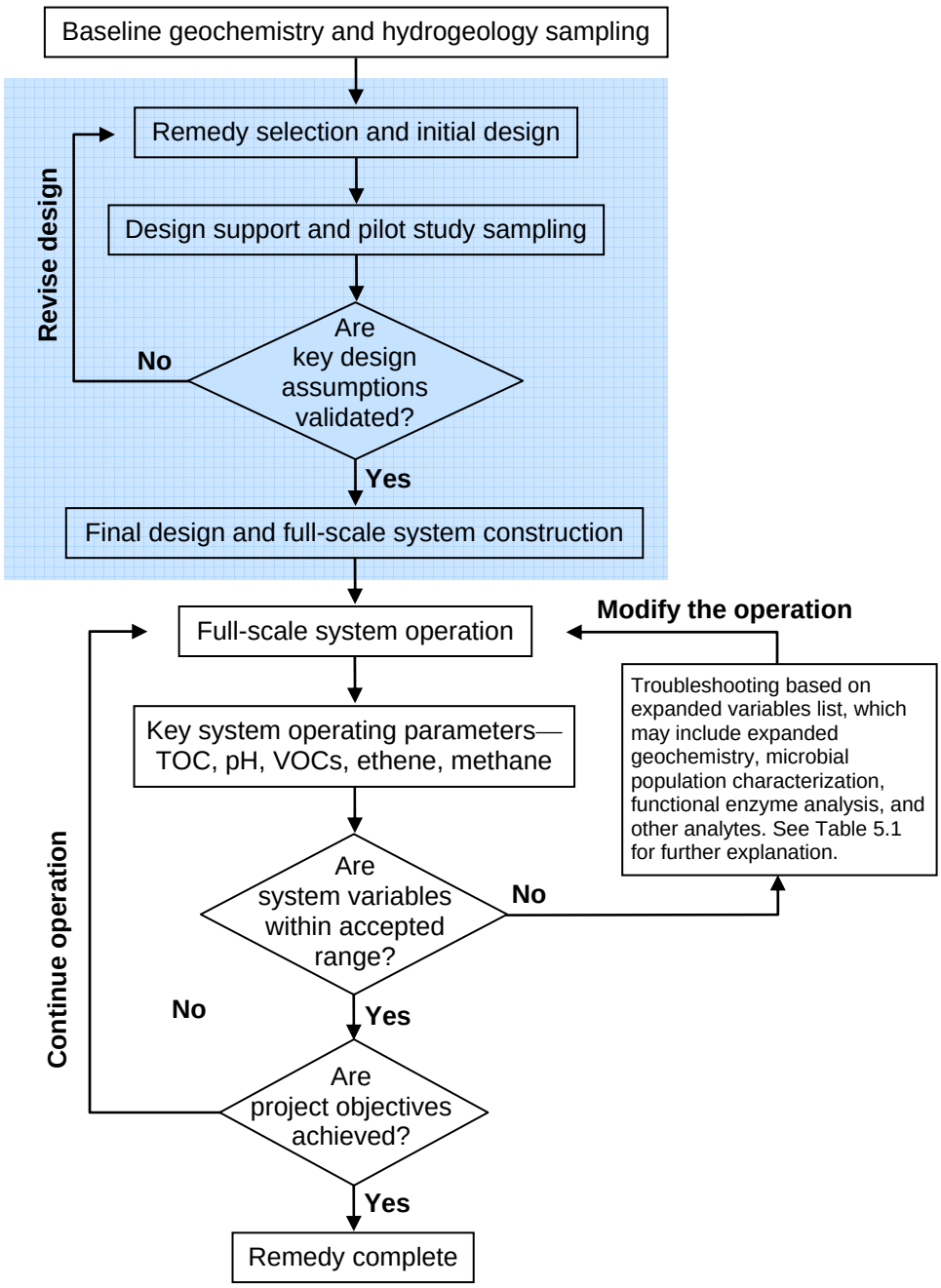


Figure 4-1. Decision making—Application design. (Courtesy of Arcadis)

Ultimately, known site constraints and process components must be accounted for during full-scale system design. The fundamental design elements in the application of ISB include the following five components or process elements:

1. *Determine the Aquifer Redox.* Oxidative bacteria dominate aquifers in which electron acceptors (e.g., O_2, NO_3^-, Fe^{3+}, Mn^{+4}, and SO_4^{2-}) are abundant in the groundwater flowing into the DNAPL source zone and, in the case of iron and manganese, in the aquifer matrix. Determine aquifer oxidation/reduction status; the flux of natural, competing electron acceptors in the groundwater; and the availability of bioavailable solids in the aquifer matrix.
2. *Expand Populations of Fermenting Bacteria.* Late-stage dechlorinating bacteria (those that dechlorinate *cis*-DCE and VC) depend on molecular hydrogen (H_2) for reducing equivalents. As discussed, hydrogen is generated, along with mixed organic acids, during fermentation reactions. When the aquifer microbial community enters fermentative metabolism, many partial decomposition products can be observed (e.g., alcohols, ketones, and VFAs). These compounds are then metabolized during consumption of electron acceptors, including chlorinated solvents, and release hydrogen to the system. Though fermenting bacteria are typically abundant, specific strategies to increase their abundance may be necessary in extreme environments (e.g., desert aquifers).
3. *Dissolve and Desorb Nonaqueous Solvent Mass.* Only a small fraction of the solvent mass in DNAPL source zones is in the aqueous (dissolved) phase at any time. To achieve measurable reductions of DNAPL source mass, it is necessary to maximize dissolution and desorption of chlorinated ethenes into the aqueous phase.
4. *Enhance Early-Stage Dechlorination Metabolism.* Several bacterial genera are known to dechlorinate PCE and TCE to *cis*-DCE. This is referred to as early-stage dechlorination. It is possible to dechlorinate PCE and TCE without achieving significant reductions of the *cis*-DCE that is produced.
5. *Initiate (If Necessary) and Expand Late-Stage Dechlorination.* To date, one bacteria genus (*Dehalococcoides*) that dechlorinates *cis*-DCE and VC to ethene has been identified. Some strains of *Dehalococcoides* produce VC reductase and enzymes that complete the last step in dechlorination of VC to ethene. Conditions that favor this species over sulfate reducers and methanogens, which also function best under deeply reducing conditions, are specific and difficult to produce in the field. It may be preferable to supply additional substrate (electron donor) so that the metabolic activity of all three species is maximized.

These five components or process elements control ISB of DNAPL source zones. Ideally, they can be managed to produce an environment that supports the bacterial species known to drive late-stage dechlorination.

4.1 Screening Potential Bioremediation Approaches

This section provides an overview of the decision logic involved in selecting potential bioremediation approaches for treatment of DNAPL sites. Some of the concepts and criteria also apply to bioremediation in general, while others are specific to DNAPL source zone applications.

Many publications are available describing the application of bioremediation at DNAPL sites and the associated data requirements, including the following:

- *Overview of In Situ Bioremediation of Chlorinated Ethene DNAPL Source Zones* (ITRC 2005a)

- *Strategies for Monitoring the Performance of DNAPL Source Zone Remedies* (ITRC 2004)
- *Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water* (EPA 1998)
- *Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents* (AFCEE 2004b)
- *Protocol for In Situ Bioremediation of Chlorinated Solvents Using Edible Oil* (AFCEE 2007)

4.1.1 Requirements to be Completed Before Selecting an Approach

There is often overlap between the applicability of various bioremediation approaches under consideration as the most appropriate technology for a particular site or location. For example, different amendment formulations may be better suited to certain settings with regard to subsurface permeability, plume size, groundwater flow rates, injection method, and pumping rates.

Technology options are screened relative to the site-specific conditions and remedial objectives early in the design process. A number of factors, such as mix of contaminants, hydrogeologic conditions, regulatory objectives, and short- and long-term goals, are evaluated with respect to their impact on each other and the project. Commonly, the initial process identifies multiple potentially applicable bioremediation approaches, which vary with respect to one or more of the elements below. For each potential ISB approach, the following items must be determined:

- the most appropriate substrate based on site conditions and remedial goals
- the need for additional amendments (e.g., pH buffers, nutrients) required to establish the appropriate biogeochemical environment
- the configuration of the treatment zone (e.g., barrier vs. areal treatment)
- the hydrogeologic constraints on amendment injection and distribution
- the process and performance monitoring required for ISB performance evaluation and optimization

4.1.2 Treatment Configurations

ISB can be implemented to provide source area or dissolved plume treatment or containment or a combination of source area and dissolved plume remediation. Common substrate delivery options include direct injection, recirculation of aqueous substrates, and emplacement of solid substrates via fracturing or in trenches (biowalls). Two treatment configurations used to implement DNAPL source zone bioremediation are areal DNAPL source zone treatment (mass flux reduction) and direct DNAPL source zone treatment.

Areal DNAPL source zone treatment is designed to reduce DNAPL saturations through overall concentration and mass reduction. Mass flux reduction is achieved by stimulating biodegradation of dissolved-phase contaminants immediately adjacent to DNAPL, thereby reducing the contaminant mass available to migrate downgradient.

Direct DNAPL source zone treatment with bioremediation is a more aggressive biological approach compared to approaches designed simply to control flux. During source treatment,

aqueous substrates are injected directly into a source area using injection wells or direct-push technology. This process can chemically and biologically facilitate dissolution or desorption of the DNAPL. An increase in the rate of dissolution of DNAPL to the aqueous phase may be due to the presence of a greater concentration gradient induced by more rapid degradation in the aqueous phase or to reductions in interfacial tension of DNAPL in water caused by the increased abundance of dissolved organics (see Section 2).

Alternatively, one developing approach (see www.estcp.org/Technology/ER-0319-FS.cfm) involves injection of a low-solubility, persistent carbon source, such as free-phase vegetable oil, into a source zone. This step may reduce mass flux by either commingling with or surrounding all or part of the DNAPL mass or through partitioning of the DNAPL into the oil substrate itself. Additionally, injection of a relatively high volume of oil can dramatically reduce available pore space volume and size and, thereby, hydraulic conductivity and groundwater flow, effectively reducing mass flux from the source area (see AFCEE 2005, 2006a, 2006b). Both mechanisms effectively sequester the source zone by reducing its direct contact with the groundwater, thereby reducing the mass transfer rate from the nonaqueous to the dissolved phase.

4.1.3 Amendment Alternatives

Substrates for ISB of DNAPL source zones are designed to stimulate the in situ microbial processes that facilitate electron transfer to the targeted chlorinated organics. Some of the currently available substrates for ISB include petroleum hydrocarbons, vegetable oils, hydrogen-releasing compounds (which can be partially inorganic), sugars, alcohols, organic acids, and other low-molecular-weight organics, as well as food, plant, and animal wastes. Biometabolism of all of these substrates produce hydrogen, which is used during reductive dechlorination and other dechlorination processes.

The key characteristics of each of these substrates are as follows:

- chemical composition
- electron equivalents released per unit mass of amendment
- anticipated microbiological process response
- geochemical impact
- chemical and physical properties
- transport characteristics
- longevity in the subsurface
- purity with respect to inorganic constituents that could present secondary water-quality issues
- cost

These characteristics provide a practical basis for screening the available substrates and selecting the one that is most appropriate for a given site. In practice, however, the substrate's persistence in the environment is most often a key consideration because the impact of other characteristics can be managed during engineering and operations. The following sections describe the amendment screening and selection criteria relevant for ISB of DNAPL source zones.

4.1.3.1 Amendment Screening and Selection

Substrates (electron donor) are available in various forms, including soluble, viscous, solid, and experimental compounds. Combinations of substrates are becoming more common, such as the use of an easily distributed and rapidly degraded soluble substrate combined with a slow-release donor for long-term degradation. Table 4-1 describes specific applications of a range of currently available substrates. AFCEE 2004b also provides a description and discussion of their attributes and limitations.

Table 4-1 Substrates used for enhanced anaerobic bioremediation
(Modified from AFCEE 2004b)

Substrate	Typical delivery techniques	Form of application	Frequency of injection
Soluble substrates			
Lactate and butyrate	Injection wells or circulation systems	Acids or salts diluted in water	Continuous to monthly
Methanol and ethanol	Injection wells or circulation systems	Diluted in water	Continuous to monthly
Sodium benzoate	Injection wells or circulation systems	Dissolved in water	Continuous to monthly
Molasses, high-fructose corn syrup	Injection wells	Dissolved in water	Continuous to monthly
Whey (soluble)	Direct injection or injection wells	Dissolved in water or slurry	Monthly to annually
Slow-release substrates			
HRC® or HRC-X®	Direct injection	Straight injection	Annually to biennially for HRC (typical), every 3–4 years for HRC-X, potential for one-time application
Vegetable oils	Direct injection or injection wells	Straight oil injection with water push or high oil/water content (>20% oil) emulsions	One-time application (typical)
Vegetable oil emulsions	Direct injection or injection wells	Low oil content (<10%) microemulsions suspended in water	Every 2 to 3 years (typical)
Solid substrates (barrier wall applications)			
Mulch and compost	Trenching or excavation	Trenches, excavations, or surface amendments	One-time application (typical)
Chitin (solid)	Trenching or injection of a chitin slurry	Solid or slurry	Annually to biennially, potential for one-time application

Soluble. Soluble substrates may be applied in an aqueous phase with the potential for more uniform distribution throughout the aquifer than slow-release or solid substrates. Soluble

substrates such as ethanol, methanol, benzoate, butyrate, molasses, whey, lactate, and high-fructose corn syrup travel with advective groundwater flow and must be applied continuously or periodically. Some soluble substrates (e.g., lactate) may enhance the solubility of DNAPL. Application of soluble substrates may result in higher operation and monitoring costs because these substrates are rapidly depleted and require frequent injections to maintain adequate hydrogen levels. Frequent or continuous injections of soluble substrates may lead to biofouling.

Slow-Release Substrates. Substrates such as emulsified or pure vegetable oil are relatively mobile compared to solid or highly viscous substrates and distribute more uniformly within the aquifer. Emulsified or pure oils slowly release hydrogen through fermentation of fatty acids. Because of their slow release and uniform distribution, they may require only a single application.

Solid Substrates. Solid substrates are typically hydrogenated vegetable oils. They are heated to facilitate their introduction into the subsurface and release hydrogen as they slowly ferment. Mulch, compost, and chitin are also placed in trenches or other surface impoundments and are typically one-time applications. Chitin can also be injected as a slurry.

Substrate Summary. Fortunately, numerous organic amendments are available, including proprietary formulations containing nutrients, buffers, and other additives used to maximize bioremediation rates. Tables listing various substrates most commonly used in anaerobic reductive dechlorination, including lactate, molasses, vegetable oil, and hydrogen or electron yield compounds, and their consistency, cost, special handling considerations, unique impacts, or other considerations are found in *Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water* (EPA 1998) and *Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents* (AFCEE 2004b).

An important consideration in the selection of a substrate is whether a single injection event or multiple injection events are needed to achieve treatment goals. The substrate injection schedule is based on the treatment configuration and the rates of substrate depletion due to advective flushing and biological utilization. The following section discusses development of an appropriate substrate dose and schedule.

4.1.3.2 Substrate Dose Design

The substrate dose needed to achieve the treatment goals influences project cost and time. The dose should be reflected in the amount of total organic carbon (TOC) desired in the targeted treatment area. Higher concentrations of electron acceptors and higher rates at which they are entering the treatment area will require the dose to be higher to maintain adequate TOC levels.

The substrate dose is commonly expressed in terms of the mass of substrate. However, it is often evaluated in terms of the electron equivalents (EEQs) that are available to support electron transfer to the contaminants and other reducible compounds. The EEQs per kilogram of amendment represents a measure of the amendment strength.

The theoretically required substrate dose is composed of the total EEQ demand imposed by the reducible electron acceptors in the subsurface. This includes not only the target contaminants, but also other organic compounds, oxygen, iron and manganese minerals, nitrate and sulfate, and other minerals. There is uncertainty in accurately determining or estimating the native EEQ demand, and safety factors are commonly applied to the calculated dose. The dose of substrate required to address these electron acceptors, maintain strongly reducing conditions, and support reductive dechlorination may be many times this theoretical dose.

Factors that should be considered when determining the appropriate dose include the following:

- concentration of the target chlorinated ethenes
- concentration of native electron acceptors (e.g., oxygen, nitrate, iron, manganese, and sulfate)
- concentrations of electron acceptors introduced as part of prior remediation efforts (e.g., oxygen, manganese dioxide, or sulfate from in situ chemical oxidation [ISCO])
- the rate of groundwater flow

There is some amount of trial and error, and adjustments in the dose are common.

4.1.4 Supplemental Amendment

In addition to the carbon donor, supplemental subsurface amendments for the application of ISB fall into two classes: those that directly support microbiological growth and those that maintain or create favorable geochemistry. Given the overall complexity of DNAPL source zone bioremediation, the decision to use supplemental amendments is subjective. The following sections provide a brief overview of considerations regarding the potential applicability of supplemental amendments during ISB for bioremediation of DNAPL source zones.

Bioaugmentation. If reductive dechlorination is determined to have stalled at *cis*-DCE or VC (see Section 2.3), then bioaugmentation may be beneficial and should be evaluated. Also, bioaugmentation may be favorable in some cases simply to accelerate the development and growth of an appropriate microbial consortium. The decision to bioaugment can be based on the use of tests to determine the presence of *Dehalococcoides* and/or complementary evidence of dechlorinating activity, including microcosm testing and the collection of appropriate field data (ESTCP 2005c, Stroo et al. 2006).

Nutrients. Optimization of aerobic bioremediation commonly benefits from addition of microbial nutrients such as nitrogen and phosphorus; however, nutrients are not typically rate-limiting for anaerobic bioremediation. To the contrary, under anaerobic bioremediation conditions, nitrate-nitrogen is a competing electron acceptor that must be reduced prior to complete reductive dechlorination. Thus, if the decision is made to provide nitrogen, a reduced form should be used. Phosphorus is rapidly cycled in most bacterial communities and is not often introduced alone.

Geochemistry Amendments. The most common general geochemical amendment for bioremediation of chlorinated solvent sites is a pH buffer such as bicarbonate/carbonate. The production of hydrogen ion (H^+) during reductive dechlorination, as well as production of VFAs

from electron donor fermentation, tends to decrease the pH of the groundwater system. At many sites, the natural buffering capacity of the aquifer matrix is adequate to prevent the development of acidic groundwater pH; however, at some sites, addition of a buffer is needed to maintain near-neutral groundwater pH. The maintenance of near-neutral groundwater pH not only is important for microbial processes, but also has a significant role in secondary groundwater geochemistry.

4.1.5 Conceptual Design Considerations

The purpose of a conceptual design is to identify the main tasks associated with the ISB approach and to develop a cost estimate for decision making. The design is prepared during the 30% design of the remedial approach and is intended to help the engineer and responsible parties evaluate the feasibility of the bioremediation approach to remediate the DNAPL contaminant and achieve the established remedial goals for the DNAPL source zone.

Another consideration in the conceptual design of ISB of DNAPL source zones is to determine how the remedial design will be implemented. This determination is made based on the engineer's understanding of the following:

- completeness of the source area and dissolved-plume delineation
- unsaturated and saturated zone treatment requirements
- physical and chemical properties of the contaminants
- biological processes that affect the distribution of contaminants in the subsurface
- geology and hydrogeology in the treatment zones
- biogeochemical properties of treatment zone
- possible effects of the biological system on aquifer conditions (e.g., changes in mobility of the contaminants, incomplete degradation of daughter products)
- type of delivery methods (e.g., use of injection wells or direct-push injection points)
- type of delivery techniques for the hydrogeology (e.g., low- or high-pressure pumping, low or high amendment volume, bottom-up vs. top-down injection)
- permeability enhancement requirements (e.g., pneumatic, hydraulic, or blast fracturing)
- injection classification of the aquifer
- site access during the implementation and/or operation and monitoring phases
- presence or absence of subsurface utilities in the treatment area
- potential location of the plume relative to site boundaries
- possible impact on potable wells, surface water bodies, or buildings (e.g., vapor intrusion)
- off-site influences on plume migration (e.g., off-site pumping or dewatering associated with construction activities)

Based on an understanding of the factors listed above, the design engineer should consider the following:

- whether additional delineation or site characterization is required
- whether confirmation of existing soil and groundwater data is required (important if using data several years old or generated by others)

- whether treatability testing (bench-scale and/or column studies and/or pilot testing) is needed
- how the regulators regard the technology (i.e., positively or negatively)
- the level of effort required to demonstrate the technology
- whether owners can tolerate the risks associated with the application of the technology
- whether the future land use for the site and surrounding area is compatible with the proposed remedy
- the availability of materials, technology vendors, and experienced subcontractors

In support of the 30% design and because of the assumptions made, contingencies should be included in the design and cost estimate and may include the following:

- increasing the estimated project cost by a percentage (e.g., 20%–30%) based on:
  - level of confidence in the current CSM
  - DNAPL mass, extent, and impacts
  - geology and hydrogeology
  - understanding of site conditions
- probability of an increase in the treatment area and/or depth
- adding an allowance (10%–20%) for additional field time to install the system
- delivery problems (e.g., short-circuiting to surface or utilities conduit)
- increasing the performance monitoring period
- adding an allowance for legal fees, licenses, and permits
- adjusting project cost to reflect net future value

4.2 Design Support Tests

Design support tasks may include bench-scale testing, column studies, field or pilot tests, and injection simulation through modeling. Bench tests usually refer to small-scale studies conducted in the laboratory under controlled conditions. Since oxygen is toxic to *Dehalococcoides* and dechlorination occurs only anaerobically, bench tests must be performed under carefully controlled conditions. These tests can provide significant evidence of dechlorinating activity under either natural conditions or in response to amendment addition.

Field and pilot testing enables the fundamentals of the proposed design to be tested under actual site conditions to confirm the effects of site-specific variables on ISB of the DNAPL source zone. Field tests are often required to collect data necessary to finalize the full-scale design, including the following:

- ability to deliver fluid to the subsurface
- determination of the volume-radius relationship to support determination of injection well spacing
- confirmation of groundwater flow rates to determine the required injection frequency

4.3 Delivery of Substrate and Microorganisms

Substrate delivery approaches range from a one-time injection to frequent or even continuous injection of electron donor. Effective distribution of substrate into the DNAPL source zone is

fundamental to the success of the technology. There are a range of injection scenarios that can deliver bioremediation amendments, including the following:

- direct injection (one injection event or multiple injection events)
  - permanent wells
  - direct-push injection points
- recirculation
  - natural gradient flow
  - forced recirculation

For these amendment injection approaches, an amendment delivery design should demonstrate that the following will be achieved:

- An adequate amendment mass will be delivered.
- A relatively uniform amendment distribution will be achieved throughout the target treatment zone.
- The amendment persistence will be adequate to achieve complete treatment or multiple injections will be used.

All substrate injection plans should include a monitoring plan to verify the injection hydraulics and subsurface distribution of amendment during and after injection. Operational monitoring should determine whether actual injection results are consistent with design objectives. The monitoring and data evaluation criteria should be used to evaluate when substrate reinjection is necessary.

4.3.1 Direct Injection

Direct injection is the process of adding substrates, microorganisms, nutrients, oxidants, or reductants directly into the aquifer at injection points. Direct injection may use direct-push probes or permanent injection wells. Well and injection point locations and spacing depend on site geology and hydrogeology, aquifer and plume characteristics, and the volume of material to be injected. Basic well configurations include wells in the plume and immediately downgradient of the plume source.

A number of different techniques are available to inject substrates into groundwater. The appropriate technique depends not only on the application goal (mass removal or plume containment) but also on the substrate injected.

Direct injection may be used as a semipassive approach with wide injection-point spacing. This technique relies on pulsed injection of large volumes of substrate solution to achieve a large ROI around a single injection point. The approach works best under moderate-to high-conductivity conditions. It can be highly effective for enhancing mass transfer because of the large volumes of high-concentration substrate that are injected into the aquifer. Although the injected substrate may follow preferential pathways in heterogeneous aquifers, the direct injection of large volumes of substrate minimizes bypassing of the DNAPL source zone.

Direct injection may rely on either frequent, single-well injections or less-frequent, multiple-well injections in closely spaced injection points on a grid that covers the DNAPL source zone. These injection approaches are effective at sites with moderate to high groundwater velocities. Sites with groundwater velocities that are very high can be problematic due to low cross-gradient distribution of the substrate within the DNAPL source area.

Direct-injection applications are most cost-effective at shallow groundwater sites where well installation costs are low. Direct injection can enhance mass transfer, but the effective ROI may be limited when low-solubility substrates are used. Also, highly heterogeneous aquifers are problematic for direct-injection approaches because the substrate usually is not distributed evenly around individual injection points.

Direct substrate injection is used for amendments that degrade at a rate that will (a) produce hydrogen at rates and concentrations adequate for ISB despite groundwater movement through the treatment zone and (b) persist for a year or more. The electron donor can also be placed directly in a pit or trench, which is then backfilled. In either case, direct-injected/placed amendments are typically relatively insoluble and immobile and typically release donor over time.

However, in other instances, the direct-injected amendments are soluble and move with the groundwater, particularly when the groundwater flux is slow and the electron donor mass and by-products are not readily depleted by groundwater advection. Occasionally, additional amendment batch injections are needed to complete the treatment process. This approach, while appropriate for small and shallow sites, may not be practical for large and deep sites because the ROI of the direct-injection points is often small, requiring a large number of injection points to distribute the substrate throughout the treatment area.

4.3.2 Recirculation

Recirculation involves groundwater extraction, addition of substrate and other amendments, if needed, and reinjection. The recirculation system is designed to hydraulically control substrate transport through the treatment zone. The distance between injection and extraction wells is dictated by the groundwater flow velocities and the bioremediation process kinetics. Excess amendment (not consumed as it moved from the injection well to the recovery wells) is extracted and recycled in the injected water. This approach uniformly distributes substrate in the subsurface but is typically expensive because of the need to operate a groundwater extraction and reinjection system continuously. Continuous operation requires dedicated equipment and ongoing operations and maintenance and creates a potential for biofouling.

To minimize the latter, groundwater can be recirculated for a limited period (i.e., a few days or weeks to distribute the substrate), after which the recirculation system is shut off for a longer passive phase of several months, during which time the electron donor is consumed. Periodic operation of a groundwater recirculation system may be considerably less expensive than continuous recirculation. Periodic operation of the recirculation system will also result in less biofouling of the injection wells compared to systems that require continuous recirculation of groundwater and injection of substrate.

4.3.3 Practical Considerations

The possible methods of well installation should be considered in the context of site geologic conditions, access constraints, substrate characteristics, etc. as a substrate injection plan is developed. For example, direct-push well installation has been used at DNAPL bioremediation sites for various reasons and with varying results. Consider the following when evaluating the use of direct-push injection for an ISB DNAPL remediation project:

- Direct-push injection offers a great deal of flexibility in injection location, both laterally and vertically.
- Direct push may also offer a cost savings over the installation of dedicated injection wells; however, this cost savings will not be significant if additional injections are necessary.
- Consideration should be given to the site formation, since direct-push injection may not be practical under adverse drilling conditions.

In addition, if the site formation has a low permeability, direct-push injection may result in reagents flowing along the well casing instead of into the formation.

Finally, although direct push may be suitable for sites with incompressible soils, for sites with silt or clay content, the compression associated with direct push may be unacceptable. The compression created near the direct-push location will limit the ability to distribute the reagent away from the injection site. A dual-tube approach, with extraction of excess soil, may be a suitable work-around for direct push in compressible soils.

4.3.4 Injection Challenges

The injection of electron donor solution into an aquifer is one of the difficult technical challenges associated with the ISB process. Several factors affect our ability to inject solutions into the subsurface:

- *Injection Hydraulics.* Aquifers typically cannot accommodate fluid injection at the same rate that fluids can be extracted from a well. In many cases the fluid accommodation rate for a well is only a small fraction of the flow that can be achieved during extraction. Whether injections are conducted through permanent wells or by direct-push methods, injection pressures must remain relatively low to avoid unintentionally fracturing the formation. Payne Quinnan, and Potter (2008) provide more information on well hydraulics and pressure limits.

- *Biofouling.* Biomass buildup can occur in wells receiving electron donor solution, decreasing the fluid injection rate that can be achieved under safe operating pressures. Biofouling can be managed through post-injection rinsing of injection wells (clean water chase) or pulsed biocide injections. ESTCP (2005a) provides information on managing biofouling.

- *Mineral Fouling.* Mineral precipitation can reduce the open area of fluid injection wells and, in some cases, may reduce the effective permeability of aquifer matrix material.

- *Gas Fouling.* Carbon dioxide, methane, and other gases generated during electron donor consumption can accumulate in aquifer pore spaces, reducing the effective permeability of the formation and decreasing the fluid injection rate that can be achieved at safe injection pressures.

Each of these issues presents a manageable engineering challenge for designers and system operators.

4.3.5 Aligning Injection Plan with Treatment Configuration

The first step in developing an injection plan is to select the treatment configuration. Within each basic treatment configuration, discussed in Section 4.1.2, there are many possible scenarios for substrate and/or microorganisms injection. These scenarios combine the injection well layout geometry, well spacing, drilling method, injection volumes, pressures and duration, and flow rates. The selected treatment configuration and site-specific conditions may dictate that one type of amendment or injection approach is more favorable than others. For example, slow-release amendments are generally injected in a batch mode, while soluble donors such as organic acids and alcohols are typically injected on a continuous or periodic recirculation basis, often involving groundwater extraction coupled with reinjection.

Areal DNAPL source zone treatment is intended to reduce DNAPL mass through aggressive treatment and enhanced DNAPL dissolution. This treatment configuration requires amendment delivery throughout the target treatment zone and typically requires an aggressive injection approach with significant overlap between the ROIs of adjacent injection wells. Since substrate consumption rates may be high within a DNAPL source zone, the amendment delivery plan must also ensure adequate substrate over time to support reductive dechlorination of all the DNAPL. Therefore, an injection plan for areal DNAPL source zone treatment must be based on an understanding of the mass of electron acceptors within the zone and the treatment process kinetic rates.

Since areal DNAPL source zone treatment is intended to result in the depletion of DNAPL mass, an important consideration in the injection plan is the degree to which bioremediation will enhance DNAPL dissolution through decreased dissolved-phase concentrations, which drive dissolution, and through surfactant/cosolvent effects of the substrate and its degradation products. The substrate mass must be sufficient to degrade any additional chlorinated ethene mass transferred from the DNAPL phase to the groundwater (ITRC 2005a). Additional amendments can be injected to enhance DNAPL solubilization or control mobilization. In this case, the injection plan may become substantially more complex to accommodate multiple amendments, and groundwater management systems may be needed to control DNAPL mobilization.

Mass flux reduction treats the dissolved contaminant concentrations emanating from the DNAPL source zone. (See ITRC 2008 for a more detailed discussion of mass flux.) A treatment zone is established directly downgradient of the DNAPL source zone. As with other treatment configurations, there are various alternatives for substrate delivery. The considerations for source zone mass flux reduction include groundwater residence times within the treatment zone and the

treatment process kinetic rates. The flux reduction zone needs to be of adequate size so that the combination of groundwater residence time and degradation kinetics is sufficient to meet the established goals. This state can potentially be achieved using a variety of injection approaches, including periodic batch injection of an immobile slow-release substrate or continuous recirculation of a more soluble substrate.

4.3.6 Aligning Injection Plan with Hydrogeologic Conditions

There are two site-specific elements that are the basis for design and that determine the success of ISB in DNAPL source zones:

- *Delineation of a DNAPL Source Zone.* Mapping contaminant mass and distribution in the aquifer is difficult. There are currently no demonstrated methods that accurately and remotely sense DNAPL source mass, although research and development of these technologies are ongoing. The only viable survey methods depend on direct contact with the contaminant. Significant sampling in three dimensions can be expensive.

- *Characterization of the Hydrogeology in DNAPL Source Area.* The injection and effective distribution of substrate (electron donor) solutions into an aquifer to maximize contact with the DNAPL depend on a clear understanding of the controlling hydrogeologic parameters of the site.

The main goal of the injection plan is to deliver adequate amendment with uniform subsurface contact and amendment persistence to degrade the targeted contaminant and achieve treatment goals. Site hydrogeologic conditions influence the distribution of amendments and the uniformity of subsurface contact. Specific hydrogeologic criteria that influence the injection plan include the following:

- heterogeneity and/or low-permeability strata
- preferential pathways (natural and manmade)
- distribution of DNAPL (area, volume, and depths below grade and below the water table)
- location and extent of the saturated treatment zone
- depth to groundwater and other factors that influence injection-well construction costs
- groundwater flow rates through the treatment zone
- geochemical conditions that may either enhance or limit bioremediation and may pose risks related to secondary groundwater quality

Heterogeneity. Heterogeneity includes stratified environments with varying permeabilities or fractured environments. The injection plan must account for the DNAPL architecture and subsurface heterogeneities to ensure that sufficient amendment to degrade all DNAPL is delivered to all parts of the treatment zone. Otherwise, substrate or nutrient concentrations and masses will not be adequate to stimulate ISB and achieve treatment goals.

Distribution of DNAPL. Section 1.2 and Figure 1-1 describe the complexities of a DNAPL source zone. The DNAPL architecture and distribution are influenced by the specific gravity of the DNAPL, groundwater flow velocity, and aquifer matrix. Any aquifer heterogeneities influencing

the distribution of amendments have also influenced the movement and distribution of the DNAPL. Understanding the distribution and condition of the DNAPL is one of the most complex variables of a DNAPL source zone treatment project, and it may be impossible to complete a detailed delineation of DNAPL in the source zone. Even the most detailed characterization efforts can support only estimates of the DNAPL mass present in the source area. ITRC 2003b provides a description of available technologies used to delineate DNAPL source zones.

Saturated Thickness. The saturated thickness targeted for remediation is an important variable in developing the amendment injection plan. First, the saturated thickness is used to develop a contaminant mass balance and substrate dose requirements. Second, the saturated thickness is used to calculate the overall injection volume. And third, in instances of large saturated thicknesses, it may be necessary to inject amendment discretely at multiple intervals, using either direct-push methods or nested permanent wells.

Depth to Groundwater. Depth to water is important in designing an injection plan because it determines drilling methods and influences drilling costs. In general, shallow groundwater depths allow a wider range of drilling methods and result in lower drilling costs. This means that closer injection well spacing may be more cost-effective. Alternately, where the depth to water is large, close well spacing may not be economical, and the injection plan is based on a larger ROI for each injection well. This approach typically requires larger injection volumes and durations or may limit the distribution of substrate.

Groundwater Flow Rates. Native electron acceptors, as well as chlorinated ethenes, must be depleted by the substrate dose. Adequate substrate dose must be delivered not only to accommodate the reduction of target contaminants and native electron acceptors but also to persist at adequate levels as groundwater advection carries electron acceptor into, and electron donor and breakdown organic compounds out of, the treatment zone. In instances of high groundwater velocity through the treatment zone, advective loss of substrate may be significant. The degree of advective loss also depends on the injection configuration and the reinjection frequency. For example, continuous recirculation systems maximize control of groundwater advection within the treatment zone and also recycle electron donor that would otherwise have migrated downgradient beyond the treatment zone.

Geochemical Conditions. Geochemical conditions within the groundwater treatment zone not only influence the selection of an appropriate treatment process but may also influence the selection of an appropriate injection plan. Section 3.3.3 discusses the implications of geochemical site characterization. With respect to the injection plan, geochemical conditions may dictate the need for secondary substrates or a higher degree of hydraulic control over the injection process and/or groundwater flow. For example, low pH may limit the microbiological treatment processes and require injection of a buffer solution. Alternatively, the presence of certain naturally occurring species that are more mobile under reduced conditions (i.e., arsenic, iron, and manganese) may pose a secondary groundwater quality risk. If secondary water quality risks are a site-specific concern, then bench-scale or field pilot-testing can be used to help evaluate the geochemical influence of the treatment process. If secondary water quality geochemistry is a concern and if natural attenuation processes downgradient of the anaerobic

treatment zone are not adequately protective, hydraulic control of groundwater flow may be needed.

4.3.7 Microorganisms

Commercially available cultures for the degradation of chlorinated solvent DNAPL are composed of anaerobic microorganisms. The cultures are typically delivered to the site in airtight containers under an inert gas (e.g., nitrogen or argon) atmosphere. To ensure good activity in the subsurface, it is necessary to avoid exposing the culture to oxygen; therefore, the culture should be delivered (a) below the groundwater surface, (b) under a blanket of inert gas in the well, and (c) through a delivery line that has been purged with inert gas. Finally, the culture should be pushed out of the container and through the delivery line with an inert gas.

The volume of bacteria injected depends on both the desired concentration of the bacteria in situ as well as the amount of time available for the bacteria to reproduce and thereby reach an effective concentration at and within the intended ROI. In situations where the time to remediate DNAPL source zones is not a factor, a relatively small inoculum can be added with the substrate, and the bacteria will grow to an effective concentration over time. Larger volumes of bacteria are added in cases where the onset of degradation must be rapid. Vendors suggest that the volume of the culture be based on the pore volume of the aquifer and the concentration of bacteria in the culture.

4.3.8 Materials Incompatibility

In the design of the remediation infrastructure for a DNAPL source area, consideration should be given to the fact that free-phase chlorinated solvents are incompatible with a number of materials typically used in the construction of monitoring wells, injection wells, sampling equipment, and pumps. There are two aspects to this incompatibility. First, structural integrity can be compromised. For example, TCE can soften or even melt PVC pipe and O-rings and other equipment parts constructed of butyl rubber, and other common materials are also not compatible with chlorinated solvents. Second, contaminants can sorb onto/into and subsequently leach from the well and sampling equipment. Both structural integrity compatibility and water quality measurement accuracy are discussed by McCalou, Jewett, and Huling (1995).

4.4 Integration with Other Technologies

Considerable attention should be paid to the potential economic benefits of coupling one or more biological, chemical, or physical remediation technologies, either in time or location sequence, to facilitate site cleanup. In almost every instance, whether as an engineered effort or as natural attenuation, bioremediation is a component of a sequential treatment scheme targeting source areas. Bioremediation is often incorporated because it is a relatively low-cost treatment alternative that can continue for long periods, even in source areas with high aqueous concentrations of chlorinated solvents. However, the selection and integration of two or more technologies is not without challenges.

Although the principal objectives of a nonbiological source area treatment technology are to reduce VOC concentrations and remove/destroy contaminant mass, these technologies also have

impacts on environmental conditions in the treatment zone. These impacts may be sufficiently harsh that they preclude significant microbial activity, at least temporarily. For example, it is unlikely that significant microbial activity will occur in the presence of a concentrated permanganate solution. However, after the treatment is complete and flowing groundwater has purged the treatment zone, a new set of environmental conditions will develop. These new conditions can include reduced activity of the indigenous microbial community, the increased availability of substrates (e.g., either electron acceptors or substrates) for microbial activity, and changes in pH, ORP, and other geochemical parameters, such as metals or nutrients. Given the ubiquity of microorganisms in the groundwater environment and the resilience of microbial communities to changes in their environmental conditions, it is inevitable that colonizing organisms will establish themselves in the treated zone, resulting in a new microbial community that can exploit the changed environmental conditions. Accordingly, the relevant question for those considering integrating enhanced bioremediation with a more aggressive source technology is, "What impact will this process have on the activity of dechlorinating microorganisms?" Appendix A of this guidance provides summaries of how common remediation technologies for chlorinated ethenes impact environmental conditions along with an overview of their likely impacts on anaerobic reductive dechlorination.

5. OPERATION AND MONITORING REQUIREMENTS

ISB of DNAPL source zones involves injection of degradable substrate (electron donor) into a contaminated aquifer, which modifies the aquifer microbial community to induce reductive dechlorination. The process controls for ISB technology are as follows:

- substrate solution composition (i.e., concentration, volume, and injection frequency)
- aquifer pH that can be adjusted through base or buffer addition
- natural aquifer bacterial consortia that can be augmented with proprietary microorganisms

Process monitoring of the treatment zone is required to determine the following:

- distribution of substrate compared with design objectives
- development of microbial populations relative to baseline microbial populations
- maintenance of optimum geochemical conditions
- maintenance of optimum substrate conditions
- desired extent and rates of biodegradation

During this full-scale operational phase, sampling programs are narrowed, providing only the data needed to support operational decision making (Figure 5-1). If the ISB system responds as designed, the operational configuration is maintained and optimized as necessary until the project goals (RAOs) are achieved. Conversely, if the system fails to respond as expected during full-scale operations, a diagnostic sampling program is undertaken, with an expanded list of parameters. The system is reconfigured, and operation is resumed or, in some cases, an alternative technology may be applied.

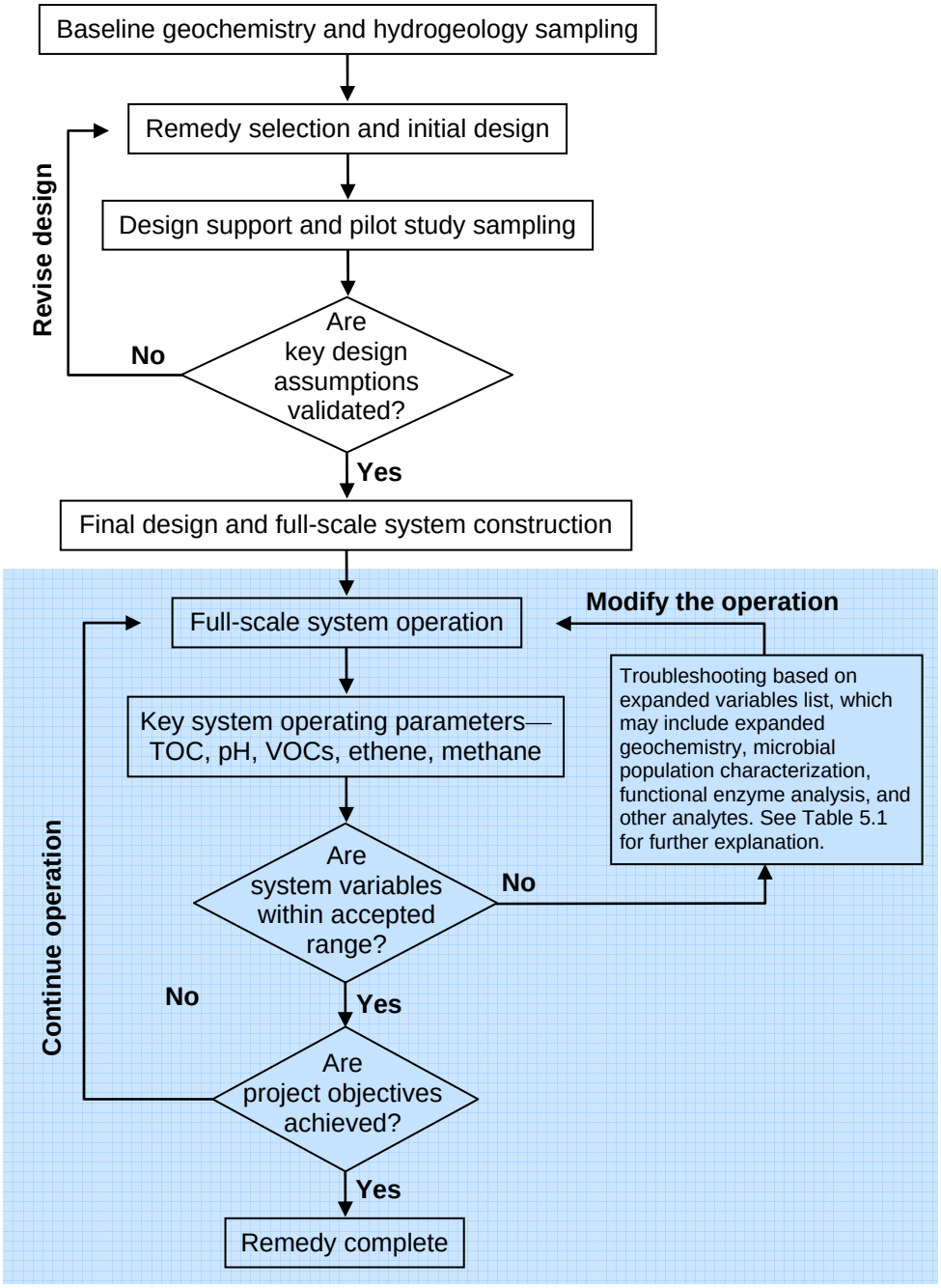


Figure 5-1. Decision making—Operation and monitoring. (Courtesy of Arcadis)

5.1 Operation

The common element of all ISB of DNAPL source zones system operations is the introduction of degradable substrate into the contaminated aquifer matrix in a manner that provides sustained dissolved substrate concentrations within the target treatment zone. The elevated DOC must span a segment of the aquifer matrix large enough to accommodate all of the metabolic processes of ISB of DNAPL, in sequence, along the flow path. Therefore, the groundwater transport time

through the treatment zone must be long enough to achieve the desired contaminant degradation before reaching the point of compliance.

There are many permutations of substrate type, injection strategy, and physical plant configuration (e.g., piping, tankage) that can be implemented in an ISB of DNAPL source zone. It is beyond the scope of this guidance to cover every possible operational combination. The tankage, piping, well construction, and system control designs are all within the realm of standard engineering practice and local regulatory controls, and there will be no further discussion of these system elements except for components or issues that are specific to the application of ISB in DNAPL source zones.

There are risks associated with fluid injection in any aquifer. To control and/or minimize these risks during implementation and operation of the ISB system, the following parameters should be evaluated and monitored: injection pressure limits, DNAPL mobilization, response of confined and semiconfined aquifer formations, and groundwater displacement.

5.1.1 Injection Pressure Limits

The injection of fluid into all aquifer formations entails a risk of formation fracturing and loss of fluid into overlying formations. Injection pressures should be designed for each application to minimize unintentional hydraulic fracturing (short-circuiting) and avoidance of drainable DNAPL mobilization through vertical gradient modifications. Direct-push injections or conventional screened injection wells should be tested to ensure that the aquifer can accommodate fluid insertion at the design flow rate. The design documents should clearly express the site-specific operational injection pressure constraints and the basis for their calculation.

5.1.2 DNAPL Mobilization

DNAPL source zones often contain residual NAPL bodies that are not thick enough to generate fluid entry pressures required for vertical movement. The injection of fluid (e.g., substrate) at or above the elevation of a residual NAPL adds to the existing entry pressure and can mobilize DNAPL mass. Although these effects are not common for most ISB applications, it should be considered in all ISB of DNAPL source zone designs.

5.1.3 Confined and Semiconfined Aquifer Formations

In truly confined aquifers, the injection of fluid must be balanced by the extraction of fluid to avoid rupture of the overlying confining layer. In semiconfined aquifers, injection can be accomplished without balancing extraction if the fluid injection rate is held below the aquifer's vertical fluid accommodation rate. If fluid is injected into fully confined aquifers or into semiconfined aquifers at rates that exceed the vertical fluid accommodation rate, the confining layer will be lifted and possibly ruptured. These movements can be monitored at the ground surface with sensitive tilt meters, as are used during engineered fracturing enhancements. Surface heave can impact buildings or utilities in the injection area, and ruptured confinement may spread contaminants beyond the existing distribution. The system design should indicate

whether the target formations are confined or semiconfined and what design and provisions have been undertaken to protect the aquifer and overlying structures.

5.1.4 Groundwater Displacement

Injected fluid volumes typically represent a very small fraction of the aquifer volume, and lateral displacement of groundwater is quite small. In formations with small, mobile pore fractions (especially fractured bedrock systems), there may be displacement of groundwater near the injection wells. In this environment, system designs should indicate how groundwater displacement is to be monitored and what responses will be undertaken if significant displacement is observed. Two issues are associated with displacement:

- Injected fluid can dilute contaminant concentrations in monitoring wells, leading to a false indication of cleanup.
- Contaminated groundwater may be pushed outside of the target treatment zone.

The use of tracers tests will allow confirmation or quantify the distribution of injected fluid and assessment of the extent to which injected fluid has displaced groundwater at the monitored locations. Short-term, cost-effective tracer tests provide valuable information in the design of the remediation program by defining both the flow paths and travel time (Payne, Quinnan, and Potter 2008; Shook, Ansley, and Wyliw 2004). The potential movement can be approximated through calculation of the natural volume of the targeted aquifer and of the distribution distance around a typical injection location. It cannot be assessed through groundwater elevation monitoring during injection events because water is a relatively incompressible fluid and the force of injection, not the distance, will be reflected in such measurements.

5.2 Monitoring Requirements

Three types of monitoring are conducted for ISB of source zones: process, performance, and compliance. Compliance monitoring is not within the scope of the discussion to evaluate the performance of ISB of DNAPL source zones in this guidance. The objectives for process and performance monitoring include the use of different analytical protocols, monitoring locations, and monitoring frequencies.

- Process monitoring is designed to assess whether the system is meeting the design objectives including effective distribution of amendments (electron donor, and bacteria if added), retention of the amendments in the target area, longevity of amendments, potential dilution and displacement of DNAPL, growth of the microorganisms, and potential for biofouling. Process monitoring identifies adjustments to the system for process optimization.

- Performance monitoring is used to assess the effectiveness of the treatment in meeting remedial objectives, including evaluating multiple lines of evidence: concentrations of the contaminants of concern (COCs), mass flux of the COCs (see ITRC 2008, Section 1.5; this is also a subject of the 2008 ITRC integrated DNAPL source strategy project), appearance of the appropriate daughter products and end products of degradation, and changes in the

groundwater geochemistry. An effective performance monitoring plan enables decisions about continued operation or when to shut down a remedial system.

5.2.1 Process Monitoring

Process monitoring evaluates operational objectives established in the design phase and may include ROI of amendment injections, longevity of amendments, expected growth rate of microorganisms (if injected), and avoidance of biofouling. Additional information about mitigation from biofouling can be found in ESTCP 2005a. In terms of biofouling, avoidance and recovery from biofouling affects are identical to those used when using ISB in the dissolved plume of a DNAPL site.

Pressure transducers in wells that have an airtight seal can be used to monitor well head pressure. Well head pressure measurements can be used to monitor the progress of the injection, avoid surface expression of amendments, prevent damage to the injection well or temporary injection point, indicate possible biofouling, and assess influence of the injection to surrounding monitoring wells.

The injection rate is monitored to determine the amount of each amendment injected as well as the configuration of the area of influence. The volume of amendments injected is a key design parameter that is determined from the desired ROI for the injection as well as the amount of each amendment needed for effective degradation. For the electron donor the volume injected is determined by the electron donor demand as well as the desired ROI. Some amendments can be measured directly by laboratory analysis, while other amendments need to be monitored by surrogate analysis such as TOC.

When injecting bacteria, the volume injected as well as the rate of that injection determine the ROI. The volume of bacteria injected depends on both the concentration of the bacteria as well as the amount of time allowed for the bacteria to reach an effective concentration. In situations where the time to remediate is not a factor, a relatively small inoculum can be added along with the electron donor, and the bacteria will grow to an effective concentration over time. Larger volumes of bacteria are added in cases where the onset of degradation needs to be rapid.

The amendment use rate depends on the type of amendment used. The amendment may be monitored by direct detection (e.g., lactate) or may need to be monitored indirectly (e.g., emulsified vegetable oil by TOC or VFA analysis).

5.2.2 Performance Monitoring

Monitoring is required to determine whether ISB is working as designed and to guide adjustments for optimization of system operation. Monitoring wells are typically located along the groundwater flow path, upgradient of, within, and through the DNAPL source zone and downgradient of the ISB treatment zone. The wells are sampled during development of the CSM to understand the baseline site biogeochemistry, chlorinated ethene distribution, and hydrogeology. A wide range of site parameters from Appendix B are often collected to develop the CSM, design the remedy, and evaluate and optimize performance. The duration of

monitoring for ISB in DNAPL source zones occurs over a relatively long time period (several years); therefore, flexible monitoring strategies that allow for the collection of only necessary parameters for a particular operational phase are most often employed. The monitoring requirements and frequency of sampling may be different during the initial characterization and development of the CSM; during remedy selection, system operation, and optimization; and during long-term monitoring.

When system operation commences, a more limited set of key operating parameters is monitored to support operational decision making, as depicted in Figure 5-1. Performance monitoring frequency should be commensurate with the rate of change of the key parameters. If the key operating parameters do not respond as expected, the monitoring parameter list is expanded to support system troubleshooting and possible modification (e.g., substrate addition, bioaugmentation, pH neutralization) during optimization. Contaminant concentrations are a fundamental monitoring data set, and they should be routinely monitored to determine whether the remedy is proceeding at acceptable rates.

The monitoring program should be inclusive enough to gather the required data elements necessary to determine whether the project goals and remedial objectives are being met. In addition, implementation of alternative remedial actions or contingency plans should be included in the design document or monitoring plan and specify triggers when remedial objectives are not achieved that determine when optimization is required. The ongoing use of a specific and established monitoring plan provides important data that can allow for the successful transition into a contingency action and/or delineate the requirements for an exit strategy (site closure).

ISB of DNAPL source zones is monitored by collecting groundwater, gas, and soil samples with analysis of select parameters based on the monitoring objectives. A minimum performance groundwater monitoring program for this technology should include the parent chlorinated ethenes and dechlorination products (e.g., *cis*-DCE, VC), dissolved hydrocarbon gases (methane and ethene), substrate concentration (measured as either TOC or DOC), and typical field parameters (DO, methane, and pH). These parameters may be used to confirm substrate distribution, determine the extent of chlorinated ethene biodegradation, and confirm that the geochemical conditions are minimally suitable for reductive dechlorination.

Geochemical parameters of secondary importance include alternate electron acceptors (including nitrate, manganese, iron and sulfate), substrate (TOC or DOC), and alkalinity. These parameters may be used to further assess the redox environment and the buffering capacity of the aquifer. Other biogeochemical and microbial/molecular analyses used to further understand site conditions within the treatment zone may include VFAs, phospholipid fatty acids, and possibly *Dehalococcoides* enumeration. The suitability of these secondary geochemical, biogeochemical, and molecular analyses, as well as the design of appropriate sampling plans supporting their use, is site specific.

Performance monitoring can also serve as a compliance documentation for reporting requirements contained in approvals or permits from the controlling regulatory agency. Depending on the state requirements, this can include types of amendments and concentration, discharge into the subsurface, hydrogeologic modifications, recirculation, and amendment

distribution. Care should be taken to coordinate with the local regulatory authority for permits and approvals for field testing and operation of an ISB of DNAPL source zones. Table B-1 (Appendix B) provides a monitoring parameters list, the analytical method, use of analytical data, performance expectations, and recommended frequency of analysis.

Modeling. Recently, several modeling tools have been developed to evaluate source depletion and plume response at a DNAPL contaminated site. These data can be used to evaluate effectiveness of remediation in depleting the residual source, reducing contaminant mass flux to the dissolved phase plume, and estimating the remedial time frame. Models can provide valuable guidance in estimating the impact of residual source treatment, either complete or partial; estimating the longevity of a contaminant plume; and assessing the potential of a treatment strategy to cost-effectively achieve remedial objectives (Stroo et al. 2003, Newell and Adamson 2005).

The complexity of chlorinated solvent plumes containing residual sources makes developing, implementing, and appropriately interpreting models challenging. For example, a key modeling parameter is the mass transfer from the DNAPL to the aqueous phase. DNAPL architecture significantly affects the DNAPL dissolution properties and hence the rate that contaminants can be remediated and reliably modeled. DNAPL fingers (i.e., thin, vertically oriented DNAPL zones) dissolve more quickly than DNAPL pools, as the fingers have a higher surface area-to-volume ratio. In general, DNAPL pools with a short length in the direction of flow dissolve faster than larger pools with a longer length in the direction of groundwater flow. DNAPL in zones with no or little groundwater flow persist longer than zones with high groundwater flow. Matrix diffusion, linear desorption, desorption from the fraction with different equilibrium kinetics (Chen et al. 2002), and dispersion all contribute to lower mass flux versus time at DNAPL sites (Newell and Adamson 2005). While several researchers are working on improving the understanding of source zone response and source zone modeling, very few useful predictive tools are currently available for evaluating the benefits of source depletion. In addition, the benefit of source depletion is difficult to predict because of uncertainty in source mass estimates and the distribution of the source mass after treatment (Kavanaugh et al. 2003). Recently, however, several models have been developed to evaluate the multiple parameters necessary for a DNAPL site (Table 5-1). These tools can provide relative estimates of the effect of a certain level of treatment on mass flux and the remedial time frame.

Newell and Adamson (2005) describe the use of four source decay models to evaluate the relationship between source depletion and remedial time frame:

- *Step Function Model*—Mass discharge rate and average source concentration remain constant as long as source mass remains.
- *Linear Decay Model*—Mass discharge rate and average source concentration decrease linearly over time.
- *First-Order Decay Model*—Mass discharge rate and average source concentration follow a first-order decay pattern.
- *Compound Step Function/First-Order Decay Model ("Compound Model")*—Mass discharge rate and average source concentration remain constant until a certain fraction of the source mass is depleted, and then concentrations follow a first-order decay pattern.

Table 5-1. Modeling software used to assess performance of bioremediation of DNAPL source zones

Model	Type of analysis	Significance	Assess source decay?	Assess plume response?	Reference
BIOSCREEN/ BIOCHLOR/ BIOPLUME/ MT3D/RT3D	Solute transport models	Assess source decay and plume longevity by inputting site-specific hydraulic and attenuation parameters.	Yes	Yes	www.epa.gov/ada/csmos/models.html
SourceDK	Decision support system for estimating remediation time frames and assessing the uncertainty associated with those estimates	Remedial time frame decision support tool that evaluates three lines of evidence: empirical data, box model, and process models.	Yes	Yes	www.gsi-net.com/Software/SourceDK.htm
Biobalance Toolkit	Comprehensive evaluation of contaminant plumes including four modules: source, competition, electron donor, and plume	The source module uses simple mass balance models to provide estimates of the reduction in remediation time frame for a given amount of source depletion. It can address the impact of different remediation strategies on the source mass and the mass flux from the source (e.g., reducing flux via a permeable reactive barrier or reducing source mass from a source depletion technology).	Yes	Yes	www.gsi-net.com/Software/biobalancetoolkit.asp#ORDER
Natural Attenuation Software	A combination of analytical and numerical solute transport models	Implemented in three main interactive modules to provide estimates for: • Required source reduction: target source concentration required for a plume extent to contract to regulatory limits (i.e., distance of stabilization • Time of stabilization: time required for a plume extent to contract to regulatory limits after source reduction • Time of remediation: time required for NAPL contaminants in the source area to attenuate to a predetermined target source concentration	Yes	Yes	www.nas.cee.vt.edu/index.php

The trends that a particular site may follow largely depend on site-specific conditions such as DNAPL architecture, groundwater velocity, lithology, and attenuation parameters. Appendix C of this guidance discusses additional modeling variables and assumptions. The team considers models one of the greatest challenges within the science of ISB of DNAPL source zones.

5.3 Data Evaluation

One of the most significant challenges while operating an ISB system in a DNAPL source zone is interpreting the numerous parameters needed to quantify the hydraulic, geochemical, and microbiological conditions of a DNAPL source zone. This section describes the subset of variables, drawn from the wide array of possible analyses shown in Appendix C, that are the main decision-making data set supporting optimal operation of the treatment system. The decision-making data set is restricted to the key system operating parameters (Figure 5-1) to facilitate two objectives: provide direct measurement of the ISB process drivers, whenever possible, and eliminate confusion caused by collection of multiple, sometimes conflicting, parameters to represent a single process element.[1]

During normal ISB operation, the key system operating monitoring parameters in Figure 5.1 provide the information needed to operate the process, and chlorinated ethene, methane, and ethane provide the information needed to evaluate the performance of the full-scale ISB system once the treatment zone is established. Expected patterns resulting from the ISB processes for each of the key operating parameters are shown in the following sections, with a brief explanation of how each variable reflects system performance.

5.3.1 Substrate (Electron Donor or Carbon) Loading

Substrate concentration is one of the most important process control variables for ISB. Figure 5-2 shows substrate concentrations along the groundwater flow path. When the substrate is consumed (returning to background levels), there can be no further molecular hydrogen production and no additional biologically driven dechlorination. If there is insufficient substrate, only partial dechlorination will be observed (e.g., buildup of *cis*-DCE or VC). Substrate concentration data allow the system operator to determine whether sufficient substrate has been added. The volume, concentration, and frequency of injection can be adjusted to change substrate distribution and abundance.

5.3.2 Substrate (Electron Donor or Carbon) Delivery

In addition to substrate loading, another key control variable is the delivery of substrate throughout the target contaminant zone. This can be particularly difficult in highly heterogeneous aquifer matrices. Often, the residual phase contaminants are present within the low-permeability aquifer zones. For example, during direct injection of a substrate through a fully screened well or injection point, the substrate is distributed to the high-permeability zones within the aquifer. Therefore, evaluating substrate concentrations along the vertical extent of the

[1] Data that may be subject to instrument error or interference in an ISB DNAPL application (e.g., DO and ORP) should be used with caution.

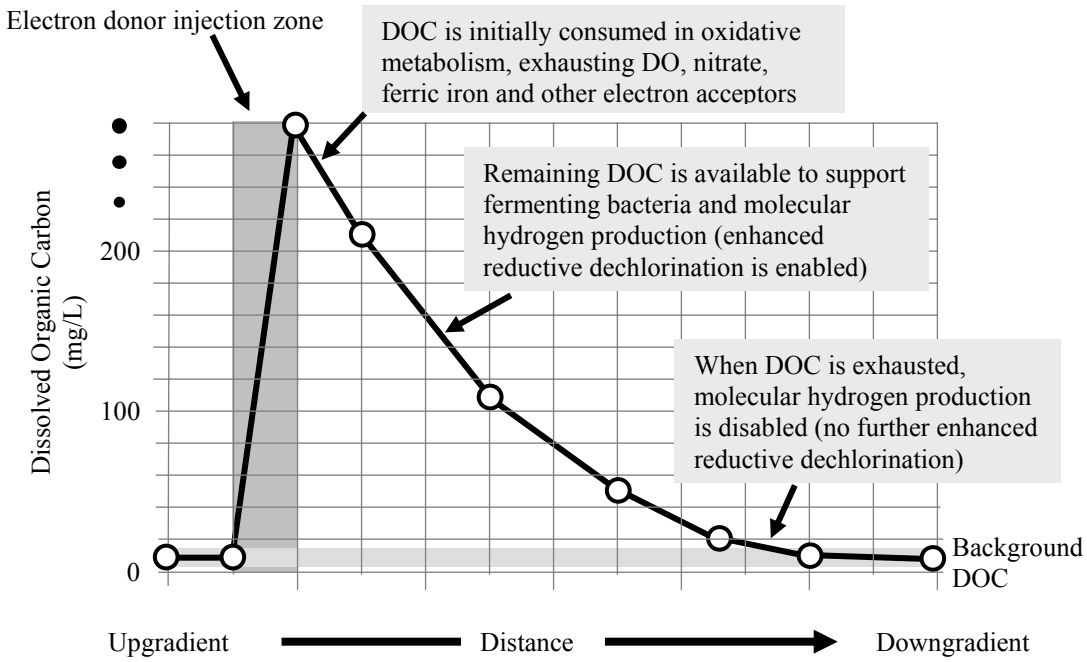


Figure 5-2. Substrate concentrations along the groundwater flow path.

Enhanced reductive dechlorination (ISB of DNAPL) is based on the injection of organic carbon in various forms to achieve DOC concentrations that significantly exceed background levels. Consumption of the carbon exhausts electron acceptors such as DO, nitrate, ferric iron and sulfates. DOC that remains supports fermenting bacteria that form molecular hydrogen, the foodstock of bacteria that dechlorinate solvents such as PCE and TCE.

treatment zone can be useful in evaluating delivery and distribution and can, in turn, be used to determine the most effective injection strategy (see Sections 4.3.2. and 5.1) that may be employed to ensure delivery of substrate throughout the horizontal and vertical extent of the ISB treatment zone. The volume, concentration, and frequency of injection can also be adjusted to change the substrate distribution.

5.3.3 Redox Parameters

The addition of electron donor to an aquifer stimulates bacterial growth that quickly consumes DO. With oxygen depletion, the bacterial community shifts to consume alternative electron acceptors, such as nitrate, manganese, ferric iron, and sulfate. Concentrations of these alternative electron acceptors can be monitored to track the development of bacterial metabolism in response to electron donor loading. The electron acceptor suite provides an indirect indication of microbial community behavior and the adequacy of carbon loading. Measurement of all parameters is an option for routine system operation; the most useful are ferrous iron, methane, and sulfate. Figure 5-3 shows general concentration patterns for electron acceptors along a groundwater flow path through an ISB treatment injection zone. Oxygen and nitrate are rapidly depleted, and reduced iron and manganese increase as bacteria transform the insoluble oxidized forms of these metals. Next, sulfate is transformed as an electron acceptor, generating sulfide that reacts with and precipitates the soluble metals, reducing their dissolved concentration.

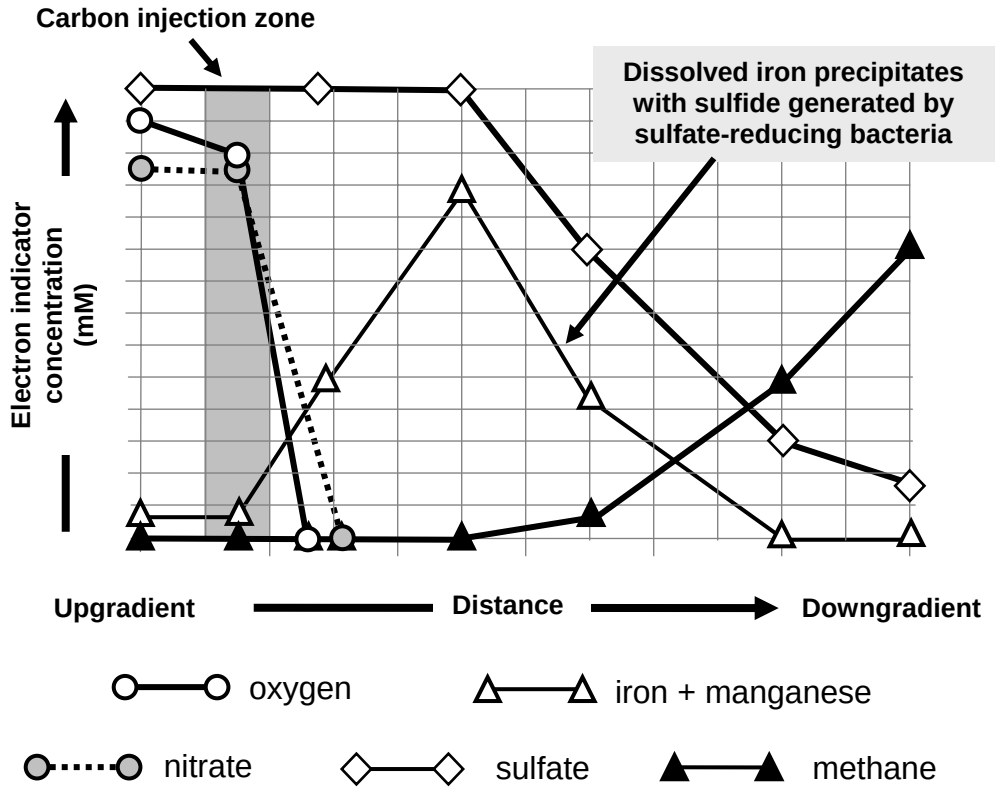


Figure 5-3. Patterns in redox indicator concentrations associated with the enhanced reductive dechlorination process.

Late-stage dechlorination (*cis*-DCE to VC and VC to ethene) reactions typically occur in zones where sulfate reduction is under way or completed and methanogenesis begins. Consequently, methane is one of the key monitoring variables for the completion of the ISB DNAPL process. If strong methane production is observed, late-stage dechlorination products should also be observed. (There is a consensus among BioDNAPL Team members that 5 mg/L methane is an indication of "strong" methane production.) If late-stage dechlorination products are not observed within 90 days after strong methane production is under way and pH and carbon are within accepted ranges, additional sampling should be considered to determine whether dechlorinating bacteria populations are adequate. It is also important to note that dissolved-phase PCE and TCE concentrations associated with DNAPL can be inhibitory to methane-forming bacteria (Payne, Quinnan, and 2008). Consequently, methane formation may be suppressed in systems that are achieving desired dechlorination reactions.

5.3.4 pH

Reductive dechlorination is significantly inhibited when geochemical parameters, such as pH, are not conducive to ERD. For DNAPL applications in particular, success of the treatment depends on maintaining relatively high biodegradation rates to effectively treat high concentrations of contaminants. *Dehalococcoides* spp., the only genera known to completely degrade PCE to ethene, are inhibited at pH <5.5, with complete cessation of biological activity at pH <5.0. Low pH may be an issue in field applications when the ambient aquifer pH is low;

acidic substrates, such as lactic acid, are added to the treatment zone; and substrate fermentation produces VFAs. Therefore, evaluating the change in geochemistry, and in particular looking at the buffering capacity (i.e., alkalinity) of the aquifer, are important to optimizing the bioremediation treatment system. Figure 5-4 shows the general pattern of groundwater pH that may be seen in ISB DNAPL treatment zones under electron donor loading scenarios and fermentation reactions. Near the electron donor injection, bacterial production of VFAs is quite high, and pH is at its lowest point along the flow path. In most formations, carbonate minerals in the aquifer matrix neutralize the acid and increase pH, as shown in Figure 5-4. In some cases, the aquifer buffering capacity is low, and acids produced during fermentation cannot be completely neutralized. In these cases, it may be necessary to limit electron donor loading rates, add buffers, or neutralize the acid with a base (e.g., sodium bicarbonate).

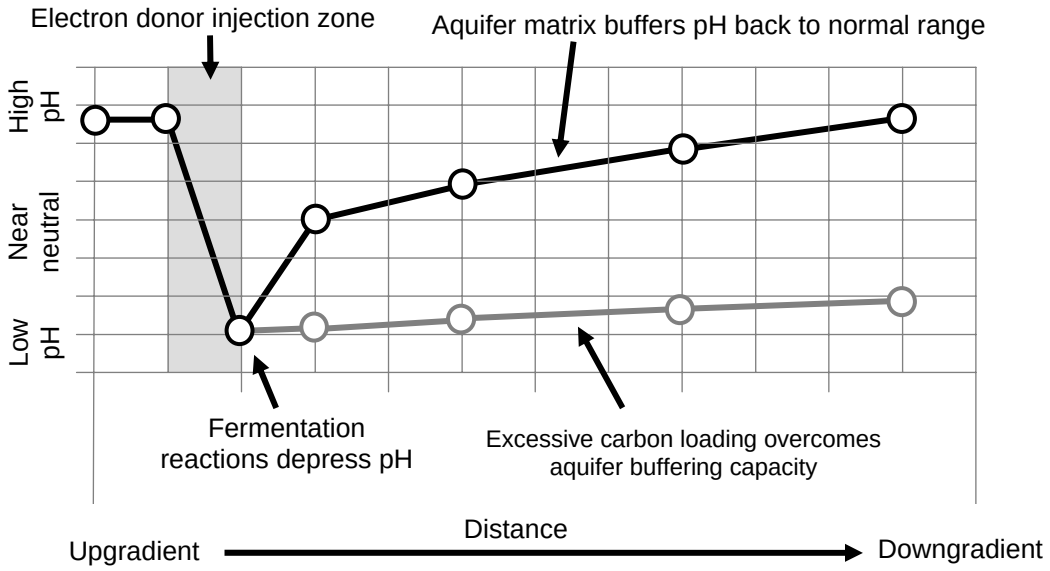


Figure 5-4. Influence of electron donor loading and fermentation reactions on aquifer pH.
At normal electron donor loading rates, reaction with the aquifer matrix minerals buffers the pH back to the normal range. If the electron donor loading is excessive, the aquifer buffering capacity can be exceeded, and the pH depression may extend for a significant distance in the aquifer.

5.3.5 Reductive Dechlorination of Chlorinated Ethene

Decreasing concentrations of chlorinated ethene target compounds (PCE or TCE), appearance and subsequent elimination of dechlorination intermediates (*cis*-DCE and VC), and the appearance of ethene and ethane are the primary lines of evidence used to evaluate the effectiveness of ISB DNAPL source zone treatments. Figure 5.5 shows the pattern that occurs when complete dechlorination is under way and enhanced solubilization of chlorinated ethenes DNAPL mass is occurring. In this example, PCE is the primary contaminant. PCE dechlorination is running faster than *cis*-DCE dechlorination, so *cis*-DCE concentrations increase sharply as the PCE disappears. Because a large portion of the PCE mass is in the nonaqueous phase (sorbed-phase or residual DNAPL), the *cis*-DCE molarity can rise to levels much greater than the original PCE molarity. Increasing molarity of dechlorination products, particularly *cis*-DCE, VC, and ethene, provide strong evidence of DNAPL solubilization. It is important to note that the

pattern of increasing molarities shown in Figure 5-5 is an ideal result and many sites achieve remedial objectives without such a large increase in dechlorination product molarities.

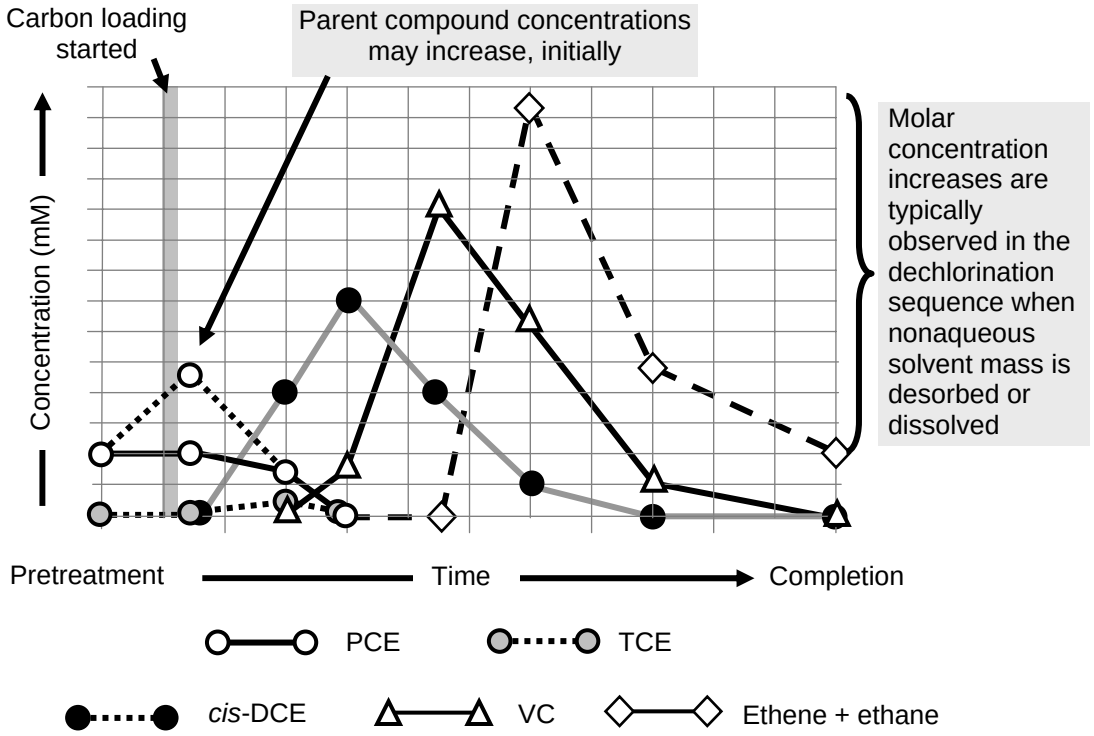


Figure 5-5. Concentration patterns in the chlorinated ethene dechlorination sequence typically observed when DNAPL source mass is dissolved or desorbed during ERD.

5.4 Optimization

From Figure 5-1, if one of the key system operating parameters described in Section 5.3 is not within accepted ranges, then modifications may be required to optimize the treatment system. This may include expanding the analytical parameter list to include a more comprehensive evaluation of the process system such that troubleshooting can be conducted. The following section describe operating variables that may need modification to optimize the system.

5.4.1 Substrate Loading/Delivery

Effective electron donor loading and delivery strategies are key to effective implementation of ISB in DNAPL source zone. As previously discussed, an amendment injection strategy is designed to meet treatment objectives, which include reducing the DNAPL source mass to the extent practicable, reducing the discharge rate or flux from the source zone, preventing the migration of remediation fluids beyond the treatment zone, and reducing contaminant plume dimension. Therefore, the delivery strategy must balance these sometimes different objectives. Table 5-2 presents optimization questions relative to delivery, contaminant fate, and secondary impacts of injections for soluble and viscous amendments.

Table 5-2. Questions to address during optimization

Delivery questions during optimization	Contaminant fate	Secondary impacts
Are you achieving desired distribution over the horizontal and vertical extent within treatment area?	• Are you achieving and maintaining efficient ERD within the treatment area? • Are you achieving desired contaminant mass flux reduction downgradient of the treatment area?	Are there negative geochemical impacts within the treatment area?
Are you achieving desired contact with residual mass?	• Are you achieving desired mass removal rates (i.e., dissolution of residual mass)? • Can removal mechanisms be validated (i.e., biodegradation vs. sequestration of DNAPL)	Are you risking displacement or mobilization of residual mass?

Bioremediation in a DNAPL source zone may entail several electron donor optimization periods during life-cycle operations. For example, early in the life cycle of active treatment, electron donor loading quantities may remain unchanged for some period. Over time, however, loading quantities may be reduced due to changes in the nature and extent of contaminant concentrations within the treatment area, including reduced DNAPL mass, slower rates of DNAPL dissolution, and/or reduced desorption or diffusion of residual phase from the aquifer matrix. The key to determining when a change in the operational strategy may be warranted is to continually evaluate the amendment delivery dose and frequency of injections relative to the change in DNAPL and degradation product concentrations. This parallels the iterative evaluation of the CSM over the duration of the ISB treatment.

5.4.2 Geochemistry

Injection of amendments—in particular electron donor—at high concentrations, such as is often required for bioremediation in a source zone, results in substantial changes in the aquifer geochemistry within the treatment area. These geochemical changes can result in substantial impacts to the reductive dechlorination efficiency. First, sufficient carbon must be delivered within the target treatment zone to induce methanogenic redox conditions (see Figure 5-3). If appropriate redox conditions are not established, the electron donor loading and/or delivery strategy may be modified to drive redox conditions sufficiently low to achieve efficient reductive dechlorination.

In addition, alterations in the electron donor type, loading, and/or delivery strategy may be required if negative effects to pH are observed. If the intrinsic buffering capacity of the aquifer system is not sufficient to maintain pH at acceptable levels, neutralizing and/or buffering agents may be used to adjust the pH in acidic groundwater. Potential neutralizing and/or buffering agents include potassium and sodium hydroxides, ammonium and sodium bicarbonates, calcium hydroxide, and lime. Amendments can be injected at sufficient concentrations to overcome the acidity of both groundwater and the aquifer matrix; however, trying to fully neutralize the aquifer matrix can result in overdosing. Side effects such as precipitation can create significant problems (e.g., reducing permeability within the treatment zone); thus, buffering should be considered only after careful consideration of the potential consequences.

5.4.3 Reductive Dechlorination

Although complexities associated with the nature and extent of contaminants, geology, hydrology, geochemistry, and biological characteristics of the treatment area are considered in the design process, optimization of many of these parameters to facilitate efficient reductive dechlorination is often required. As discussed in Section 5.3, molar mass balance is used to determine the relative ratio of reductive daughter products and parent compounds to assess the overall efficiency of the dehalogenation reactions. If ERD is not achieving the desired extent of reduction and/or reaction rates to minimize accumulation of undesirable daughter products, optimization may be required. Parameters such as substrate (electron donor) delivery and loading, treatment zone geochemistry, and the microbial populations can be evaluated to determine which parameter(s) need modification to optimize the system.

Bioaugmentation may be considered during optimization activities if it is determined that the rate and extent of contaminant biodegradation are limited not by appropriate availability of amendments or limiting geochemical parameters but by the absence of necessary microbial populations for ERD. Molecular biological tools have been developed to identify the presence of *Dehalococcoides* spp. and the dehalogenase genes and can aid in the determination of whether there is a biological limitation for reductive dechlorination in the ISB DNAPL treatment zone.

5.5 Secondary Impacts and Contingency Planning

Implementation of ISB within a DNAPL source zone can generate several types of secondary water quality impacts that should be monitored and that may require implementation of contingency plans. The three most common secondary water quality impacts are as follows:

- expanding the dissolved plume due to production of partial dechlorination products at higher concentrations than the pretreatment condition
- generating by-products that may create vapor hazards, including methane, hydrogen sulfide, and VC
- solubilizing metals (e.g., arsenic, iron, and manganese) that may migrate outside the original treatment area

5.5.1 Plume Expansion

The treatment of DNAPL source zones requires the removal of contaminant mass. This brings contaminants into solution, thereby increasing dissolved-phase concentrations, at least temporarily. In most systems, it is expected that the solubilization of DNAPL will be accompanied by high rates of dechlorination and that there will be little increase in the size of the dissolved-phase plume. However, if large amounts of nonaqueous mass are solubilized and the dechlorination process is incomplete, it is possible to increase the areal extent of the plume.

5.5.2 Gas-Phase By-Products

Methane and carbon dioxide are generated as metabolic products of degradation of the substrate (electron donor) amendments. Sulfate-reducing bacteria convert dissolved sulfates to sulfides at near-neutral pH; the predominant form of aqueous-phase sulfide is hydrogen sulfide gas. In most

freshwater aquifers the expected concentration of hydrogen sulfide is extremely low. However, in brackish groundwater, sulfate concentrations are often quite high, and hydrogen sulfide concentrations can reach very high levels when the aquifer microbial community reaches the sulfate-reducing stage. Finally, VC, which partitions strongly into the gas phase, may reach high concentrations in ISB of DNAPL source zone treatments if incomplete reductive dechlorination occurs.

Environmental investigations should always evaluate the possibility of vapor generation and vapor intrusion. If vapor intrusion is an immediate risk to human health and the environment, short-term interim mitigation measures should be implemented immediately in suspect buildings, and long-term vapor intrusion strategies added to the site-wide remedial action. Unless the buildings are relatively close to the DNAPL source area, it is unlikely the DNAPL itself will cause a vapor intrusion problem.

Vapor intrusion is one of the most challenging issues facing environmental professionals, regulators, and stakeholders. The dissolved-phase portion of the contaminant plume drives most vapor intrusion investigations. This guidance identifies vapor intrusion as one possible concern that should be considered, but it is not the focus of this guidance. The BioDNAPL Team recommends using the guidance dealing with vapor intrusion site screening and the investigative process found in *Vapor Intrusion Pathway: A Practical Guideline* and *Vapor Intrusion Pathway: Investigative Approaches for Typical Scenarios* (ITRC 2007c, d) and in *Draft Guidance for Evaluating the Vapor Intrusion to Indoor Air Pathway from Groundwater and Soils* (EPA 2002a).

5.5.3 Metals Solubilization

The development of strongly anaerobic conditions results in the solubilization of certain naturally occurring metals as a result of direct reduction and reductive mineral dissolution. The implications of reductive dissolution vary based on the total concentration of labile metals in the aquifer matrix and the specific mineral phases of which they are a part or to which they are bound. Table 5-3 presents several metals that are susceptible to mobilization in an anaerobic environment, most notably iron, manganese, and arsenic.

Table 5-3. Stability of various labile metals

Element	Primary valence states*	Stability
Antimony	III, V	Soluble in both valence states (anionic)
Arsenic	III, V	Soluble in both valence states (anionic)
Chromium	III, VI	III relatively insoluble, VI soluble (anionic)
Iron	II, III	II soluble (cationic), III relatively insoluble
Manganese	II, III, IV	II soluble (cationic), III and IV relatively insoluble
Selenium	II, IV, VI	II insoluble, IV and VI soluble (anionic)
Vanadium	III, IV, V	III and IV relatively insoluble, V soluble (anionic)
Uranium	IV, VI	IV relatively insoluble, VI soluble (cationic)

*Relevant to natural systems

The relevant observations that can be made based on this information are as follows:

- Iron and manganese are susceptible to microbial reduction and are soluble in their reduced valence states. Because these metals are both abundant and ubiquitous, they are significant in terms of their potential for solubilization. Increased levels of dissolved iron and manganese are commonly observed in ISB DNAPL source zone treatments.
- Arsenic is a metalloid that forms oxyanion complexes in the environment, making it soluble in all of its valence states unless it is adsorbed to or incorporated with other minerals. The direct reduction of arsenic from the 5^+ valence state to the 3^+ valence state can increase its solubility by altering the charge of the resulting oxyanion. However, the primary driver for arsenic release is the reductive dissolution of the iron minerals within which it is typically incorporated.

Management of Transient Metals Mobilization. The solubilization and subsequent mobilization of naturally occurring metals is unavoidable in connection with the ISB of chlorinated ethenes. However, the solubilization of these metals is generally a transient phenomenon that can be readily managed as part of the ISB operation. Two key areas must be considered for successful management of metals mobilization in ISB zones: potential migration of mobilized metals beyond the treatment zone and recovery of solubility control within the treatment zone following the end of active treatment.

The primary sequestration mechanisms involved to remove the metals from solution are sorption and precipitation. While additional research is needed to understand (and possibly predict) the degree to which each of these mechanisms contribute, in the absence of oxygen, it is likely that sorption mechanisms are dominant. This is certainly true for arsenic. Transport is severely limited by the strong partitioning between dissolved arsenic, oxyanions (e.g., $H^2AsO_4^-$), and the naturally occurring ferric iron oxide minerals in the aquifer, a process that is further enhanced by the eventual oxidation of arsenite to arsenate. Ferrous iron also sorbs to ferric iron minerals and is susceptible to rapid oxidation in neutral pH environments where oxygen is present. Divalent manganese is more mobile than both arsenic and iron and can travel well beyond the downgradient boundary of an ISB treatment zone. Manganese is less susceptible to sorption and requires more strongly aerobic conditions to reoxidize and precipitate.

Figure 5-6 show concentrations of iron, manganese, and arsenic along the flow path through an ISB zone at a point in time after the reactive zone had been operating for approximately 4.5 years. The site is in an area of the United States where elevated concentrations of arsenic are part of the native aquifer mineralogy. It is clear that the mobility of arsenic and iron is controlled at the boundary of the ISB zone. By comparison, dissolved manganese extends farther downgradient to an area of more strongly aerobic and oxidizing conditions.

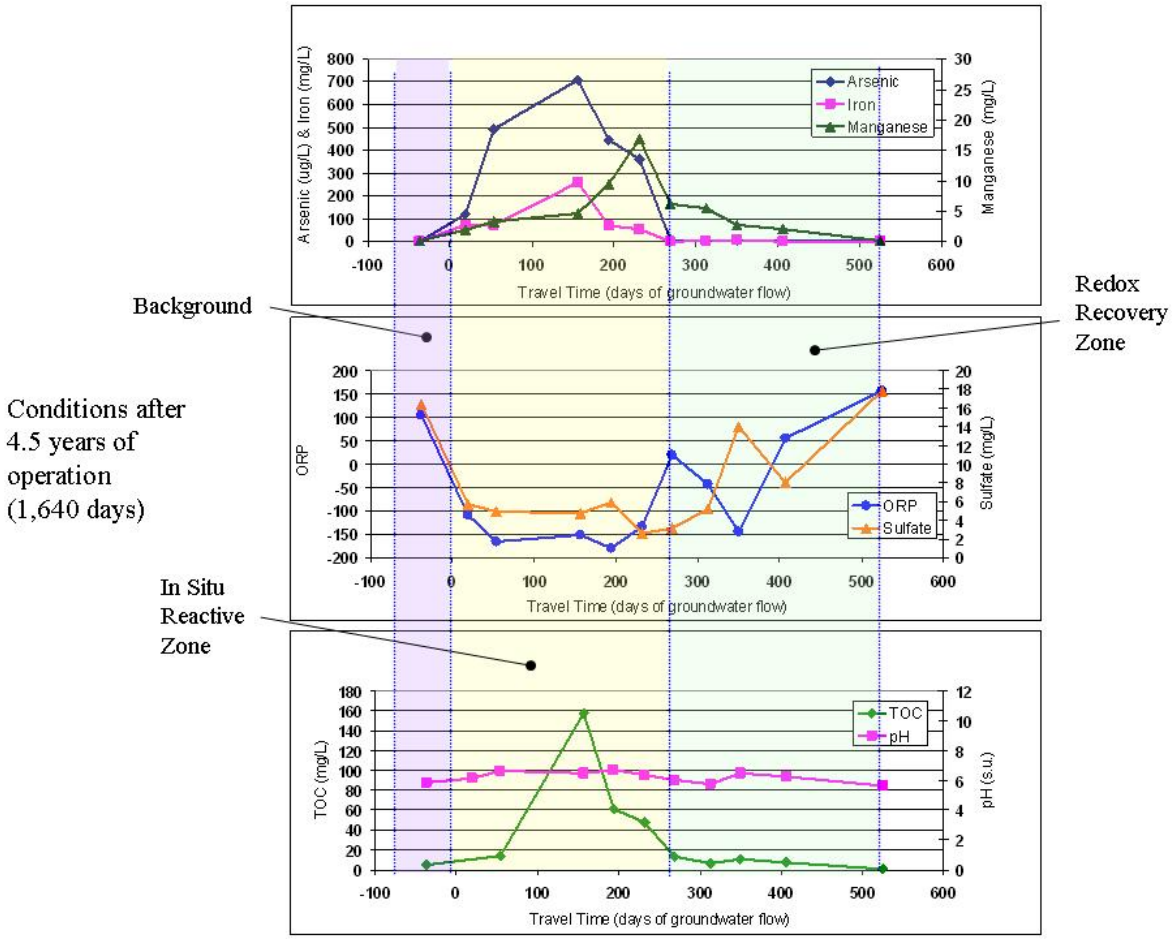


Figure 5-6. Reactive zone profile.

Recovery of pretreatment solubility control typically occurs over an extended time span because reducing capacity, in the form of degradable organic carbon, is inserted into an aquifer matrix as part of the ISB process. A large fraction of the injected carbon mass is converted by aquifer bacteria to gases (carbon dioxide and methane); however, a significant portion can be stored as reduced forms of iron, manganese, and other minerals. ISB is usually operated for an extended period, and the aquifer matrix geochemistry develops a reductive poise in the treatment zone and for some distance downgradient of the dechlorinating zone. The time required for restoration of pretreatment aquifer matrix geochemistry depends on the reducing equivalents embedded in the aquifer matrix during the ISB treatment and the influx of DO and other electron acceptors to the former treatment zone. Depending on the rate of electron acceptor recharge, this has the potential to take a very long time (years). This is typically acceptable in the context of a long-term remediation effort, but the process can be expedited through the injection of oxygen or other electron acceptors into the aquifer.

In summary, the mobilization of metals in ERD zones is a transient concern. Active management of this issue may involve controlling the size of the reactive zone to limit the aquifer volume affected, strategically supplementing the recharge of oxygen (in the redox recovery zone or within a former treatment area), or even enhancing the sequestration process by engineering precipitation

of a sorptive mineral phase like ferric iron oxy-hydroxides. The level of management required varies from site to site, with no active management warranted at many sites.

6. REGULATORY ISSUES

ISB of DNAPL source zone projects faces issues similar to other in situ remediation projects. However, because the treatment zone is a DNAPL source zone, the CSM, sampling methods, and monitoring techniques are varied and often much debated, and because the operative mechanism is biological, there are unique interests and issues that can and should be brought forth by the regulatory agencies. These interests and issues are discussed here with respect to similarities and differences between ISB of DNAPL source zones and other in situ remedial technologies. Also this section discusses the developments in the regulatory arena that clarify the procedures for obtaining the necessary regulatory approvals for injecting remedial fluids into the subsurface.

6.1 Requirements for Underground Injections

In 1998, ITRC's ISB Team published a report documenting confusion regarding the regulatory approval process for the injection of remediation fluids into the subsurface. Much of this confusion was associated with the appropriate application of a section of the Resource Conservation and Recovery Act (RCRA) known as RCRA 3020(b), which suggested that contaminated water needed to be "treated" prior to being injected back into the aquifer. In the case of ISB, using extracted contaminated groundwater in its native state is the best host fluid for introducing remediation fluids and promoting biological growth. Treatment of this extracted groundwater before injection would not only impede the remediation process, which is designed to accomplish destruction in situ, but jeopardize several types of bioremediation treatments. For additional information on the regulatory issues associated with withdrawal and reinjection of contaminated water for bioremediation, refer to Appendix E of *Technical and Regulatory Requirements for Enhanced In Situ Bioremediation of Chlorinated Solvents in Groundwater* (ITRC 1998).

There was also confusion about underground injection control (UIC) regulations. Specifically, the injection or reinjection wells associated with many in situ technologies were often confused with underground injection wells used for disposing of hazardous waste. This confusion arose out of regulations in the Code of Federal Regulations (CFR), Title 40, Section 144.13.

In December 1999, the U.S. Environmental Protection Agency (EPA) issued a brief letter identifying the appropriateness of using ISB, including injection and reinjection of remediation fluids, for site cleanups. In December 2000, EPA provided a more expansive letter (www.cluin.org/products/regs/memo122700.htm), not only reiterating the basic message of the December 1999 correspondence, but clarifying the general path to deployment for most in situ technologies. Both of these letters clarified the interpretation that RCRA 3020(b) does not impede the appropriate use of ISB.

Within the December 2000 letter, EPA also pointed out that injection wells used for remediation of waste sites would be consistent with UIC regulations at 40 CFR 144.13 and does not prohibit

reinjection of contaminated water if ISB treatment is the intended remedial action. Many states have implemented their own UIC programs, while other states have followed the federal UIC requirements. States implementing their own UIC program, which is known as having "primacy," have adopted by reference the UIC passage of 40 CFR 144.13.

- Information on 40 CFR 144.13: www.access.gpo.gov/nara/cfr/waisidx_07/40cfr144_07.html
- Information on UIC programs throughout the United States: www.epa.gov/safewater/uic/primacy2.html
- Information on state programs and contacts: www.epa.gov/safewater/uic/scontact/statescontacts1.html

Since December 2000 there has been an increase of not only ISB projects, but many other in situ technologies. Some states have taken the initiative to recognize the need for a clear approval process for in situ remediation technologies, including the requirements for their UIC programs.

A listing of selected states is provided in Table 6-1, along with some indication as to their regulatory status (implementing the federal program or primacy), adoption (or not) of 40 CFR 144.13, and some discussion as to the status of the approval process for introduction of remediation fluids. For easy reference, 40 CFR 144.26 presents the federal inventory requirements for Class V injection wells, and 40 CFR 144.83 lists states with primacy. As always, individual states should be consulted to clearly obtain the latest changes in their regulatory requirements.

In 1999, the injection of remediation fluids was problematic for many reasons. Skepticism existed on the part of state regulatory agency personnel; relatively few ISB projects had been implemented, even at demonstration scale, from which to build confidence in the technology; and state regulatory agencies were uncertain over how to interpret various regulatory citations, notably RCRA 3020(b) and UIC regulations concerning classification of injection (or reinjection) wells used for remediation. In the ensuing eight years, not only has EPA provided clarity on these matters, but a number of states have taken measures to ensure the path to regulatory approval of in situ technologies.

Exceptions to these issues exist most notably in California, where water basins are regulated independently, and in many jurisdictions discrepancies are apparent between regulatory programs within the same state. For the most part, the acceptability of in situ remedies has increased due largely to an improved regulatory climate facilitated by the type of progress summarized in Table 6-1. Because progress has been made, in many jurisdictions the significant questions still remaining can be addressed by data collection and evaluation and laboratory and pilot studies rather than interpretation of regulations or policy.

Table 6-1. Selected state UIC programs summary

State/ EPA Region	Primacy	Adopted 40 CFR 144.13(c)	Comments
CA/9	Yes	No	EPA-run program. Inventory form can be found at www.epa.gov/safewater/uic/index.html. Needs approval of state managers.
FL/4	Yes	No	Injection wells for remediation projects are classified per 62-528(2)(d) Florida Administrative Code as Class IV Group 4 aquifer remediation wells. www.dep.state.fl.us/water/uic/index.htm
ID/10	Yes	No	A permit is required before using an injection well for groundwater remediation per IDAPA 37.03.03. http://adm.idaho.gov/adminrules/rules/idapa37/0303.pdf
KS/7	Yes, Type II No, Types I and III–V		Adopted under Article 46, 28-46-1, general provisions; developed permit process specifically for remediation wells. www.kdheks.gov/uic/index.html
LA/6	Yes	No	(Adopted similar language in Title 43 Part XVII Subpart 1. State Order No. 29-N-1, Chapter 1, 103 E.1.b.iii) Permitted by rule in most cases. Inventory information for Class V wells is requires to be submitted. www.doa.louisiana.gov/osr/lac/43v17/43v17.pdf
ME/1	Yes	No	(Adopted similar language in Chapter 543 2 D (3) and Chapter 253 5 A) A Class V UIC Well Registration Form is required.
NC/4	Yes	No	(Adopted similar language in Title 15A 2C .0200) Permit program specifically for groundwater remediation. http:/h2o.enr.state.nc.us/admin/rules/documents/2C.doc
NJ/2	Yes	No	(Adopted similar language in N.J.A.C. 7:14A Subchapter 8) Deemed permitted by rule the conditions set forth in N.J.A.C. 7:14A-7.5(b) are satisfied. www.state.nj.us/dep/dwq/7_14a/sub08rul.pdf
PA/3	No	No	EPA-run program. Inventory form can be found at www.epa.gov/safewater/uic/7520s.html. Needs approval of state site manager.
UT/8	Yes	Yes	Program developed for each well class (40 CFR Section 144.13d). Adopted under Administrative code R317-7). www.rules.utah.gov/publicat/code/r317/r317-007.htm
VA/3	No	No	EPA-run program. Inventory form can be found at www.epa.gov/safewater/uic/index.html. Needs approval of state site manager.
WA/10	Yes	Yes	Groundwater remediation under Comprehensive Environmental Resource, Conservation, and Liability Act (CERCLA) and RCRA only for Class IV. Groundwater remediation via Class V wells are permitted by rule for CERCLA, RCRA, and MTCA sites under Chapter 173-218-060 WAC. http://apps.leg.wa.gov/WAC/default.aspx?cite=173-218&full=true
WI/5	Yes	No	(Adopted similar language NR 812.05) An injection well inventory form is required. www.legis.state.wi.us/rsb/code/nr/nr815.pdf

One challenge related to underground injection still remains for in situ remedies that require injection of remedial fluids. Although some states allow variances, which are usually limited to one year, many states do not allow the injection of remedial solutions that contain any element or compound at concentrations above drinking water criteria or comparable limits. While this requirement is reasonable if the injected solution were to be immediately recovered and used for potable purposes, the objective of any remedial injection is to amend all groundwater within the targeted area to enhance bioremediation. Therefore, every effort is made to disperse injected solutions quickly. Most injection procedures effectively dilute each solution an order of magnitude or more during the event. Natural groundwater flow further dilutes the injectate over time. In addition, chemical reactions and biological activity quickly begin to neutralize or consume the injected materials. As federal and state regulators gain experience in the application and performance of ISB and other in situ technologies, they may allow the injection of higher concentrations of remedial agents, such as when needed to optimize ISB.

6.2 State Regulators' Concerns and Considerations

Having established an appropriate regulatory framework for deployment of ISB, the remaining issues of concern to state regulators are primarily related to the predictability and performance of ISB technology. State regulators are often asked for a critical review of a list of technologies to consider for remediation of a source area. Initial concerns and considerations are listed below and are discussed in following sections, with a focus on ISB for DNAPL source zones:

- Technology maturity and success:
  - What is the effectiveness of the technology?
  - What do we know about the technology and on what evidence?
  - What is the cost relative to other technologies?
- What is the time frame for completion of the project?
- Is it expected to meet regulatory goals?
- What are the implementability challenges?
- Is it safe to operate?
- Does it have public acceptance or support? (e.g., Where did it work? Where did it fail?)

All of these considerations are touched upon in the efforts and products of the BioDNAPL Team, including this current guidance. Recognizing that ISB of DNAPL source zones is a relatively new remedial option, some caution should be offered regarding expectations of any such project.

6.2.1 Technical Maturity and Success

One of the challenges facing ISB of DNAPL source zones is the technology's maturity. While there are pilot-scale projects that are completed with some degree of success and optimization, only a few full-scale projects have been implemented with similar results. However, the projects to date have been well documented and show substantial results when compared with other technologies for remediation of DNAPL source zones. The BioDNAPL Team's previous compilation of case studies (see ITRC 2007a) documents several successful applications of ISB of DNAPL source zones.

At this juncture, neither state regulators nor practitioners have enough empirical resources to predict outcomes for ISB of DNAPL source zones. This is in large measure due to the physical constraints associated with achieving a reduction of essentially pure chemical (DNAPL) to a parts per billion dissolved residual. It would be premature to identify what number of successes would have to be achieved in order for anyone—practitioners or regulators—to have great confidence and certainty in ISB's capability to achieve reductions that meet cleanup values traditionally proposed for DNAPL-contaminated waste sites.

Even though, as has been previously stated, the capital cost of ISB of DNAPLs can be less than that of many other DNAPL source zone technologies, the life-cycle costs of DNAPL-contaminated site remediation using ISB of DNAPL source zones are unknown and depend on many factors, including duration of treatment, need for additional injections of substrates and other additives, and duration and extent of monitoring.

6.2.2 Time Frames

ISB is not the only option to address the extended time frame to remediate a DNAPL contaminated site; however, it may shorten the long-term stewardship of the site. This reduction in long-term stewardship depends on the ability to control the mass loading, referred to as the "mass flux" (see Section 6.2.3.1). Case studies have reported that, when implemented properly, ISB for DNAPL source zones can efficiently reduce the mass loading to the dissolved-phase plume, reducing the overall time frame for site remediation.

6.2.3 Achieving Regulatory Goals

The ability to achieve remediation goals at DNAPL sites is an issue that has undergone much deliberation. EPA's *DNAPL Remediation Challenge*: *Is There a Case for Source Depletion?* (EPA 2003) examines the benefits of DNAPL source treatment and the appropriate metrics for quantifying the benefits. The ongoing discussion and evaluation of DNAPL remediation and the benefits of partial source removal affect all types of DNAPL source zone treatment methods. Some regulatory agencies have flexibility in establishing site-specific remedial goals, and this flexibility is particularly important for ISB of DNAPL source zones since it offers alternative mechanisms to achieving site cleanup and in measuring site progress in a phased approach (see Section 5.2.1). Two mechanisms that allow for such flexibility are the use of mass flux and institutional controls.

6.2.3.1 Mass Flux

Mass flux reduction is a potentially valuable performance metric for any DNAPL source zone technology and may be particularly important to the performance monitoring of an innovative technology such as ISB for DNAPL source zones. The goals of removing mass from a source zone include reducing the risks of contaminant migration (via either the dissolved or vapor phase), reducing plume longevity, reducing overall remediation costs, accelerating the natural attenuation of any remaining mass, and speeding the transition to more passive technologies. If properly measured and calculated, mass flux can be a meaningful metric in assessing progress towards these goals. Using source strength as a measure of success, shutdown of the remedial system could be considered when the mass release rate from the source to the groundwater (mass

discharge) falls below the assimilative capacity or "natural attenuation capacity" of the aquifer, defined as a measure of a groundwater system's ability to lower contaminant concentrations along aquifer flow paths. The natural attenuation capacity of groundwater systems depends on hydrologic (dispersion and advection) and biological (biodegradation rates) factors and can be assessed using quantitative models. At present, the quantification of mass flux is an area of active research. More information on mass flux and how it is measured can be found in ITRC 2004 and ITRC 2008.

6.2.3.2 Institutional Controls

ISB at a DNAPL source zone can degrade a significant amount of mass; however, the likelihood of achieving MCLs at any DNAPL site is unpredictable. The remedial action at a DNAPL site will likely require a phased approach to achieve MCLs. In many cases, persons associated with the site, including regulators, recognize that an achievable goal for DNAPL sites would be to reduce risk to the maximum extent possible using available/innovative technologies and adopt a site-use scenario that would protect human health and the environment at an exposure point. This approach would likely require the use of institutional controls (e.g., restricting use or access to the site or resources). Institutional controls are important in the advancement of innovative technologies such as ISB because they offer flexibility in the remedy selection and may be an important component of the exit strategy.

6.2.4 Implementation

The requirements for implementation of ISB DNAPL remediation vary from state to state and with the particular program responsible for the remediation oversight. Approval for implementing ISB of DNAPL source zones may include completing studies to determine the remedial effectiveness. Some implementation concerns, apparent and emerging, are discussed below, as well as how the regulator may choose to address them.

6.2.4.1 Incomplete Dechlorination

Some of the case studies summarized in Section 10, including Dover Test Cell and Tarheel Army Missile Plant, observed incomplete dechlorination. Dover conducted bioaugmentation, which then resulted in complete dechlorination. PCE and TCE underwent dechlorination; however, *cis*-DCE and/or VC did not degrade but instead increased as products of TCE/DCE dechlorination. Because of the higher solubility and mobility of the partially dechlorinated species, compared with PCE and TCE, they may present greater risk to human health and to the environment. Thus, it is important to establish proof of principle that dechlorination is complete before toxic by-products reach some point of compliance and that there will not simply be conversion of PCE and TCE to *cis*-DCE or to VC. It is necessary to establish that ethene is being produced as proof of complete reductive dechlorination. Incomplete reductive dechlorination will be an issue only at some sites, as the organisms that perform the latter dechlorination steps may or may not be present or active (see Section 2.3), given the specific conditions and history of the site in question.

At present there is not sufficient predictive capability to determine *a priori* whether or how well a bioremediation system will work under a particular set of conditions. A pilot study in the field

may be necessary to test that dechlorination is occurring with adequate efficiency and to check for completeness of dechlorination under the field conditions at hand.

In some cases incomplete dechlorination may be addressed with subsequent bioaugmentation to stimulate the microorganisms responsible for dechlorination of the DCE and VC. To ensure that reductive dechlorination is achieved, appropriate performance monitoring should be required (see Section 5.2.2). If a *cis*-DCE or VC plume develops that may reach sensitive receptors, bioaugmentation and perhaps hydraulic containment measures should be part of a contingency plan. This plan will require that some flexibility is built into the remediation program.

6.2.4.2 Mobilization

Historically, regulators have been concerned with the potential for various DNAPL technologies (e.g., surfactant/cosolvent flushing, in situ thermal treatment) to further mobilize DNAPL mass. This potential is discussed in the operation section of this guidance (see Section 5.1.2).

6.2.4.3 Potential for Causing Fractures

Injection of substrates is a common feature of bioremediation schemes. As such, injections will be a common feature of projects to bioremediate DNAPLs. There is a desire to introduce substrates in a manner that disperses them broadly, often through wells. In some cases, the injection pressures will be increased to effect a broader dispersal. If injection pressures are not calculated appropriately and controlled, there is potential for initiating unintended fractures that can create preferential flow paths and limit the effectiveness of the delivery and distribution of the substrate.

6.2.4.4 Vapor Intrusion

Application of bioremediation can also lead to the formation of methane and carbon dioxide gases. Both can affect vapor migration of VOCs in the vadose zone, contributing to vapor intrusion of VOCs or even explosion hazards to nearby buildings, and should be evaluated particularly at sites with shallow water tables and thin vadose zone soils. Vapor intrusion can also be exacerbated as the dissolved phase increases downgradient.

Several guides dealing with vapor intrusion site screening and the investigative process can be found in ITRC 2007c and ITRC 2007d and also in EPA 2002a.

6.2.4.5 Other ISB Concerns

There are emerging issues that deal with the variety of substrates and microorganisms available for ISB. These issues may include regulatory concern over the injection of microorganisms. The regulatory agency may require that the microorganisms under consideration be nonpathogenic and may prefer that the organisms are naturally occurring. Addressing this concern may require additional sampling and studies that should be completed during the design phase. Also, there may be regulatory concerns over impurities, which may have regulatory standards and can be detected in substrates for ISB use. Regulatory agencies may require testing of substrates prior to injection.

Conditions that give rise to reductive dechlorination—strong reducing conditions, absence of oxygen, and abundance of organic substrate—can cause detectable odors in the downgradient groundwater and vadose zone. Such conditions can be considered more than a nuisance, particularly if the substrate daylights or surfaces during the injection event, goes to a spring, or enters a nearby surface water body.

6.2.4.6 Regulatory Concerns over Stakeholder Acceptance

It is a concern of state regulators that a poor reception perhaps even immediate rejection of a proposal to bioremediate a DNAPL source will be the first and perhaps ultimate response of the stakeholders who are active at the site. In addition, the regulator overseeing the project may be the first person in their agency to be asked to evaluate bioremediation of a DNAPL source zone.

Stakeholders typically accept new technologies if there is evidence that they will work on the specific site. This report is based on credible evidence that can help stakeholders understand and accept ISB of DNAPL source zones. Their goals and expectations are similar to those of a regulator. However, where a regulator approaches interested stakeholders with a proposal that has been reviewed, there is a natural apprehension over what their reactions will be.

As discussed in Section 8, stakeholder acceptance of a viable technology is generally possible if effective and honest communication is established. Members of the BioDNAPL Team are available as a resource to regulators tasked with overseeing ISB of DNAPL source zones.

6.3 Lessons Learned

Several issues have been identified that can limit the performance and success of ISB DNAPL applications. These issues were identified during the collection and preparation of *Overview of In Situ Bioremediation of Chlorinated Ethene DNAPL Source Zones* (ITRC 2005a) and *In Situ Bioremediation of Chlorinated Ethene DNAPL Source Zones: Case Studies* (ITRC 2007a) and within the collective experience of the team. Some of the key issues are as follows:

- Successful implementation depends on the expectations and the understanding among the regulators, public, and remediation team.

- Costs to implement additional monitoring parameters depend on the regulatory requirements and may be of concern to the regulator. The ability to implement and evaluate monitoring parameters affects the ability to accurately understand the site remedial progress.

- Inadequate groundwater source zone characterization is often one of the major reasons for problems cited for inadequate remedy performance. Additional site characterization, once remediation has been implemented, is a common response to address inadequate performance. The characterization of a complex DNAPL source area can incur considerable cost; however, costs leading to a thorough understanding of the DNAPL source area will help optimize the remedy and ultimately allow for a more cost-effective remediation through the life cycle of the project. Additionally, during this characterization process, the

unsaturated zone is sometimes not properly characterized, and residual DNAPL remains as an ongoing contaminant source to groundwater. Insufficient soil sampling or characterization due to access limitations, sources beneath buildings, and other obstructions can limit the horizontal and vertical source assessment. Adequate source zone characterization is also affected by the heterogeneity of the soil and the characteristics of DNAPL within the ISB source zone.

6.4 Summary

ISB holds much promise for DNAPL source zones. However, the application of any technology to a source zone is challenging for the persons implementing the remedy as well as for the regulatory community. Consequently, it will be some time in the future before a DNAPL source zones technology is considered fully demonstrated and can be implemented with relative ease and efficiency.

In the past 10 years there have been considerable advances in our understanding and experience in the use of ISB for chlorinated ethene source zones. As more projects are implemented, regulators will be able to gain more confidence in the application of the technology. This guidance can serve as a guide to oversee proposed projects.

7. HEALTH AND SAFETY

The two principal health and safety areas to be considered during implementation of a remedial design are worker protection during site work and safety of the general public from the impacts of investigative and remedial activity. Worker protection is addressed through Occupational Safety and Health Administration regulations; public safety is addressed through permitting, project notification, and/or project planning.

Workers are exposed to two types of risks on remedial projects: exposure to chemicals (contaminants and remediation chemicals) and general construction-related risks. Remediation chemicals can be as innocuous as sugar-based substrates or as potentially harmful as acids, caustics, or peroxides used to maintain wells or equipment. As most contaminants are considered a threat to human health based on exposure levels, it is important to identify potential chemical exposure pathways and minimize worker and public exposure at the design phase through minimization or elimination of chemical use and/or the use of personal protective equipment.

Site work can include probing, drilling, and well installation; excavation and trenching; demolition; mechanical and electrical installation; groundwater sampling; substrate injections; well repair and abandonment; equipment cleaning and repair; site restoration; and other operations on controlled, semicontrolled, and uncontrolled properties. Most of these activities involve tasks that can be inherently dangerous, and as these tasks are usually nonroutine, they present additional challenges to owners, contractors, subcontractors, and the general public. Care must be taken to recognize construction, operations, and monitoring hazards prior to the initiation of site work. This can be accomplished through the development and implementation of site and project specific health and safety plans. Health and safety plans must also include all information as required by the appropriate regulatory authority.

ERD of chlorinated contaminants can result in the formation of potentially more mobile, toxic, and/or higher dissolved concentrations of degradation products that may present a increased risk compared with the original contamination. For example, in some cases, the reductive dechlorination of PCE can result in increased VC concentrations, a compound considered to be a greater health risk than the parent material. The potential volatilization of more toxic degradation products into the vapor phase can cause health and safety concerns in nearby structures. Therefore, it is important to evaluate the potential for vapor migration when designing ISB of DNAPL source zone remedial systems. In sensitive situations, consideration should be given to the performance of vapor monitoring and potential vapor elimination to ensure that site workers and the general public are protected from contaminants and their degradation products.

The use of ERD to treat DNAPL source zones through the addition of a carbon substrate (electron donor) can increase dissolved-phase contaminant mobility, dissolved metals, TDS, and biological and chemical oxygen demands and can change water quality as measured by taste and odor. All of these health and safety considerations should be taken into account when designing an ISB remedial approach. Implementation challenges and how they may be managed are discussed in Section 5.

8. TRIBAL AND STAKEHOLDER CONCERNS

For purposes of this guidance, stakeholders consist of Indian tribes and public stakeholders, including citizens, community groups, advocacy organizations, and local officials. Stakeholders often have valuable information about site characteristics, receptors, history, and future intended use of a site, which can be used to improve the quality of remediation process decisions. This section looks at with the specific concerns of stakeholders.

It is important to note that affected stakeholders are not necessarily limited to those local to the site. For example, those who live downstream of a site may be affected even if they are not in the immediate vicinity. In the identification of affected tribes, it is necessary to consider that tribes may have treaties or other pacts with the federal government that grant them fishing, hunting, or access rights in places that are not necessarily near their present-day reservations. Furthermore, individual states and the Indian community recognize Indian tribes that are not necessarily recognized by the federal government. A list of federally recognized tribes can be found at www.nps.gov/history/nagpra/MANDATES/BIA_List_2007.pdf. A list of tribes that are not federally recognized can be found at www.kstrom.net/isk/maps/tribesnonrec.html. Also, there are some sites where tribes have regulatory oversight; as a result, tribes play an important role that is different from that of stakeholders.

Stakeholders generally show great interest in the contamination problem, remediation process, and effects that these have on human health and the environment. Given the financial, technical, and regulatory complexities inherent in the remediation process, uncertainties in the application of ISB and the poor history of DNAPL source zone remediation, it is highly recommended that effective communication be established with the stakeholders. If the stakeholders have the

opportunity to have meaningful and substantial participation in the decision process, they are more likely to support the difficult policy, budgetary, and technical decisions.

Stakeholders tend to be open-minded about innovative technologies, particularly when these technologies present an increased chance of success and decreased costs compared with more mature technologies. However, stakeholders will raise questions and concerns about the proposed deployment of a novel technology. Two of the questions that stakeholders typically have about an innovative technology are, "Will it work?" and, "Will it do any harm?" Stakeholders should have access to the information that goes into the remediation decision making and, if possible, be included in the decision-making process.

All of the performance issues associated with ISB of DNAPL source zone treatment should be presented with honesty and openness to the stakeholders. These issues include experiences at other sites and the likely scenarios at the site in question. How effective is ISB compared with the alternatives? Will dechlorination be complete? Will the engineered reductive conditions cause mobilization of toxic elements? There are uncertainties associated with all of these questions. The plans to evaluate them and address them at the site should be discussed with the stakeholders, and information should be shared.

In the deployment of a technologies such as ISB, situations in the field cannot always be anticipated. Thus, some flexibility is necessary in the remediation management process. This flexibility itself may be a cause for stakeholder concern as it might be perceived as a loss of regulatory control over the remediation process. It is the responsibility of the regulator to address these concerns directly. Such concern is most effectively addressed by the inclusion of public and tribal stakeholders in the problem definition, strategic planning, and process and performance monitoring progress reports and by early, frequent, and ongoing communication with the stakeholders.

Stakeholders can make substantial, positive contributions to a successful remediation process. The key is to involve them early and often.

9. ISSUES UNDER CONSIDERATION

Issue: Currently, there is a substantial body of peer-reviewed literature for ISCO, thermal, zero-valent iron, and surfactant flushing; however, there are relatively few studies that examine any of these technologies from the broader perspective of integrating them with bioremediation as part of a treatment strategy. Accordingly, there is a strong need for both laboratory and field studies examining these approaches and the impact of the primary technology on dechlorination by both indigenous and bioaugmented microorganisms.

> **Resolution:** ITRC has approved a new integrated DNAPL source strategy project, which will investigate the impacts and potential integration of a variety of other remediation technologies with ISB of DNAPL source zones.

Issue: Impact of bioDNAPL treatment on source longevity and restoration time frames. The depletion rate of a source is complex and governed by the hydrogeology in and upgradient of the source area and the distribution of the various DNAPL phases (free, dissolved, sorbed, and matrix diffused) within the source area. Current characterization technologies cannot define these characteristics to the degree needed to accurately predict the rate of source mass depletion and the mass flux from a source zone over time. In addition, there are little long-term data on the effects of source treatment on source longevity and plume responses. Nonetheless, the results from recent laboratory and field studies, along with developments in mathematical models of the effects of treatment on sources and plumes, have led to an improved understanding of the relationships between DNAPL mass, mass flux from source areas, and the responses of plumes over time to partial source depletion. This improved understanding can enable better evaluations of the benefits of source treatment, including ISB, and improved predictions of the impacts of treatment on the longevity of sources and their downgradient plumes. However, though we understand it better, we do not understand it completely. Many researchers, including authors of this guidance, continue to investigate the assumptions, variables, and equations used in modeling the relationship of source treatment to source and plume longevity. To understand the fundamental approach, we have included a preliminary description of the process, as it is currently understood, in Appendix C of this guidance. We still see this as one of the greatest challenges within the science of ISB of DNAPL source zones.

> **Resolution:** This topic will be further evaluated during the integrated DNAPL source strategy project beginning in fall 2008. Because of the complexity of the problem, we expect to see progress but may not reach any predictive conclusions.

Issue: Bioremediation of DNAPL source zones challenges conventional thinking and regulatory agency preferences. Bioremediation of DNAPL source zones causes at least a temporary increase in (dissolved-phase) mass flux away from the source area and also causes at least a temporary expansion of the plumes of daughter products (notably VC when TCE is present). Recent research has suggested that in addition to dissolved-phase expansion that occurs as a result of biodegradation, it may be beneficial to increase the surface area of sorbed-phase material—essentially smearing the material throughout a greater volume of aquifer material—to increase the rate of biodegradation of that material. While these are (or may become) desirable attributes and practices for bioremediation, they contradict conventional thinking and regulatory agency preferences for approaches that collect, concentrate, and limit any form of spreading of contaminants. Consequently, bioremediation of DNAPL source zones challenges conventional views of remediation.

> **Solution:** Clearly understanding the impact of reductive dechlorination on DNAPL (described in this guidance) and effectively monitoring the degradation products and appropriate geochemical parameters will enable the regulatory agency site manager to determine that the system is working regardless of the concentration values and plume geometry. In most cases mobilization and other effects of treatment can be controlled with the proper engineering. The physical mobilization due to drilling or injection is an engineering challenge that will be further addressed by the ITRC integrated DNAPL source strategy project beginning in fall 2008.

Issue: There can be resistance to the use of ISB at DNAPL source zones as a remedial alternative by the regulatory community. This is due, in part, to past experiences with bioremediation being incorrectly applied.

> **Resolution:** The team's opinion is that the science of bioremediation is rapidly growing and improving and the available bioremediation empirical data clearly demonstrate that this technology can be a valid remedial alternative for DNAPL source zones. While not a "silver bullet," ISB of a DNAPL source zone can be an important tool in the remedial toolbox.

Issue: Hydraulic fracturing can be used by ISB applicators to increase the area of substrate delivery into the treatment zone; however, if the mechanism of fractures and the hydraulics of the formation are not understood, uncontrolled fractures can be created in the subsurface which will serve as conduits for electron donor solution transport outside the DNAPL treatment zone and/or limit the distribution within the treatment zone (e.g., preferential channels are created in and outside of the treatment zone).

> **Solution or Caution:** In ISB applications, the engineer should include an evaluation of advantages and disadvantages of controlled fractures in an ISB design. In all cases, performance monitoring should be conducted to confirm that substrate is being delivered throughout the DNAPL zone and that preferential flow paths are not created with or without intentional fracturing.

Issue: An operational challenge encountered during ISB is biofouling, when the amendment injection wells and surrounding zone become plugged with biomass. Biofouling is sensitive to the process design, the site characteristics, and operational design. It is perhaps most challenging for recirculation systems. Treatment of the wells requires cleaning with chemicals and often well redevelopment.

> **Solution:** Biofouling is not unique to ISB of DNAPL source zones but is an operational consideration in all ISB systems. This guidance does not go into detail on measures to avoid or mitigate biofouling since ESTCP (2005a) provides an excellent discussion of the topic.

Issue: Reliance by regulatory agencies on the MCL or other state-specific numeric standards can lead to difficulties, impediments, and even contradictions in remediation of DNAPL source zones. While standards can sometimes be met, for the most part the attainment of low numerical criteria is not feasible for a DNAPL source zone of any significant size. The point of application of an MCL (at the source, at a receptor well, at a property boundary, etc.) can vary among jurisdictions, introducing variations that are difficult to justify in terms of protection of the public health.

> **Resolution:** Reliance on an MCL as a performance metric for a source zone remediation technology may not be appropriate. A thorough discussion of the use of MCLs and other approaches during DNAPL site remediation is intended for the ITRC integrated DNAPL source strategy project.

Issue: Mass flux is difficult to estimate using current methods, and there are uncertainties in relating mass flux to regulatory goals. There will be a near-term bias to continue to monitor performance based only on contaminant concentration.

Resolution: The BioDNAPL Team agrees that the actual use of mass flux measurements is still in the early stages and there are uncertainties. ITRC's integrated DNAPL source strategy project will research the topic of mass flux in 2008. The BioDNAPL Team believes that the future use of mass flux will provide a greater understanding of actual conditions and therefore improved remedial alternative evaluations. There is a large portfolio of currently active research related to the measurement and interpretation of mass flux that will help implement more effective strategies in the future.

10. CASE STUDIES

The abstracts contained in this section are referenced in the text of this guidance. The case studies are contained in a previously completed project report, *In Situ Bioremediation of Chlorinated Ethene DNAPL Source Zones: Case Studies* (ITRC 2007a). Understanding that studies of bioremediation of DNAPLS are becoming more prevalent, the ITRC BioDNAPL Team collected additional summaries of case studies and incorporated them into Table 10-1, immediately following these abstracts.

10.1 Enhanced Anaerobic Bioremediation in a DNAPL Residual Source Zone: Test Area North Case Study

Authors: **R. A. Wymore,** T. W. Macbeth, J. S. Rothermel, L. N. Peterson, L. O. Nelson, K. S. Sorenson, N. Akladiss, and I. Tasker

Abstract: This case study, the Test Area North (TAN) site of the Idaho National Engineering and Environmental Laboratory, involves a TCE residual source area in a deep, fractured basalt aquifer that has been undergoing enhanced bioremediation since January 1999. Complete dechlorination from TCE to ethene was documented within nine months of operation, and sodium lactate injections were shown to enhance TCE mass transfer from the residual source. Since that time, optimization of injection strategies has maintained efficient dechlorination while demonstrating accelerated cleanup at a lower cost by changing to a whey powder amendment that solubilizes DNAPL.

10.2 Enhanced Anaerobic Bioremediation of a TCE Source at the Tarheel Army Missile Plant Using EOS®

Authors: **R. C. Borden,** W. J. Beckwith, M. T. Lieberman, N. Akladiss

Abstract: Emulsified Oil Substrate (EOS®) was used to stimulate anaerobic biodegradation of TCE and PCE at a former Army-owned manufacturing facility located in piedmont North Carolina. Previous use of chlorinated solvents at the facility resulted in soil and groundwater impacts. Ten years of active remediation using soil vacuum extraction and air sparging were largely ineffective in reducing the TCE/PCE plume. In 2002, the Army authorized preparation of

an amended Remedial Action Plan to evaluate ISB methods to remediate TCE in groundwater. The plan evaluated eight groundwater remediation technologies and recommended EOS as the preferred bioremediation alternative for the site.

Eight wells were drilled within the 100 × 100 ft area believed to be the primary source area for the TCE plume. In a first injection phase, dilute EOS emulsion was injected into half of the wells. Distribution of the carbon substrate through the treatment zone was enhanced by pumping the four wells that were not injected and recirculating the extracted water through the injection wells. The process was repeated in a second phase that reversed the injection/extraction well pairs. Overall, 18,480 pounds of EOS was injected and 163,000 gallons of water was recirculated through the source area. Anaerobic groundwater conditions were observed shortly after injection, with a corresponding decrease in both PCE and TCE concentrations. DO, ORP, and sulfate concentrations also decreased post injection, while TCE degradation products, ferrous iron, and methane concentrations increased. The reduction in TCE allowed the Army to meet the groundwater remediation goals for the site.

10.3 Pilot-Scale Evaluation Using Bioaugmentation to Enhance PCE Dissolution at Dover AFB National Test Site

Authors: **C. Lebron,** T. McHale, D. Major, M. McMaster

Abstract: This case study involved a pilot-scale demonstration of the effects of biological activity on enhancing dissolution of an emplaced PCE DNAPL source. It used a controlled-release test cell with PCE as the primary DNAPL in a porous media groundwater system and consisted of laboratory tests and a field-scale pilot test to demonstrate that bioaugmentation can stimulate complete dechlorination to a nontoxic end product and that the mass flux from a source zone increases when biological dehalorespiration activity is enhanced through nutrient (substrate) addition and bioaugmentation. All project goals were met. Important achievements include demonstrating the ability to degrade a PCE DNAPL source to ethene and obtaining significant information on the impacts to the microbial populations and corresponding isotope enrichments during biodegradation of a source area.

10.4 Enhanced Reductive Dechlorination of PCE in Unconsolidated Soils

Authors: **F. C. Payne,** S. S. Suthersan, D. K. Nelson, G. Suarez, I Tasker, N. Akladiss

Abstract: This case study involved ERD of PCE in unconsolidated soils, primarily silts and clays with very low permeabilities. The project results indicated that complete reductive dechlorination had been achieved and provided encouragement that large amounts of nonaqueous solvent can be brought into the reductive dechlorination treatment process by dissolution and desorption, giving support to the contention that the capacity to attack nonaqueous mass is a prerequisite for any effective treatment of DNAPL source zones. The site geology for this project was relatively unfavorable, and further work is needed to confirm that the ERD technology can economically reach a natural attenuation end point for this type of setting.

10.5 Source Area Remediation at a Portland, Oregon Dry Cleaner Site

Author: **R. Gillespie**

Abstract: The Oregon Department of Environmental Quality is responsible for addressing groundwater impacts at an active dry cleaner facility located at a strip mall in Portland. The department determined that maintaining current activities at the site required that an unobtrusive, semipassive remediation technology be used. Accelerated bioremediation using HRC within the source area and dissolved plume, was selected as the remedial approach as it required modest site access and minimal operation activity.

In addition to demonstrating that HRC-X can degrade PCE to ethene by accelerating reductive dechlorination, the pilot test demonstrated the ability of the slow-release HRC-X to remediate source areas over an extended time.

10.6 Demonstration of Enhanced Bioremediation in a TCE Source Area Case

Authors: **Eric Hood,** D. Majors, J Quinn, S. Yoon, A. Gavaskar, and E. Edwards

Abstract: Launch Complex 34 (LC34), a launch facility at the Kennedy Space Center, is the site of historic releases of TCE, which is present in the subsurface as DNAPL. TCE was used in launch operations, which continued up until 1969. The large source zone is partially located below the Engineering Support Building (ESB) at LC34. Up to 40,000 kg of TCE is present in the aquifer below LC34, suggesting that centuries will be required to restore groundwater using natural remediation processes.

A demonstration of EISB of TCE was initiated May 2002 in a test plot located within the ESB. The small test plot was contained entirely within the much larger source area at LC34. The biodegradation mechanism of interest was reductive dechlorination. Reductive dechlorination is the most common biodegradation mechanism for TCE and other chlorinated alkenes (i.e., *cis*-1,2-DCE and VC) in groundwater and can result in complete dechlorination to ethene, a non-toxic degradation product.

In contrast to the prevailing consensus that EISB is not relevant to source zone remediation, the results of this study demonstrated that rapid and complete dechlorination occurred in the presence of very high initial chloroethene concentration (TCE 1,220 mg/L). The study resulted in the removal of >98% of total TCE mass from the test plot. The continued decrease in chloroethene concentrations for two years following the completion of the study suggests that the activity was sustained in the absence of continuous electron donor addition.

10.7 Survey of BioDNAPL Applications

Table 10.1 presents the results of an informal survey, based on the knowledge of ITRC team members and their respective organizations, to gather information on sites where the application of biological treatment of chlorinated solvents was, or is being, used. The objective of Table 10.1 is to demonstrate that ISB of DNAPL source zones is being applied and indicate the general conditions of those applications.

Table 10.1 results show that ISB of DNAPL source areas has or is being applied at over 16 sites, ranging from fractured bedrock to silty clays and sands. Bioremediation of DNAPL sources is being applied predominantly at sites that have concentrations of chlorinated solvents at 1%–10% of their effective solubility. Sixty-eight percent of the projects began as a pilot study or remained solely as a pilot study. The shortest study was only five months and the longest was seven years. Four studies are ongoing. It should be noted that at all sites ISB was performed, with the primary amendments being bacteria, vegetable oil, and whey.

Table 10-1. Bioremediation sites nationwide

Site	Location	Geological setting/ stratigraphy	Source area (DNAPL?)	Size	Contaminant and concentration	Remediation method/ substrate	Scale	Duration	Dates	Reference/ contributor
Tarheel Army Missile Plant	Burling-ton, NC	Clay, sand and gravel	NA	100 × 100 ft	TCE, PCE, *cis*-DCE	Emulsified Oil Substrate (EOS)	Pilot	5 months	6/04–10/04	Borden, Zawtocki, Beckwith
Test Area North	ID	Fractured basalt	Yes	360 × 640 ft	TCE	EISB (whey, lactate)	Full	Ongoing	11/98–present	Wymore, Macbeth, Sorenson
Dover AFB National Test Site	DE	Medium to fine sands	NA	18 × 28 ft	PCE	ISB and EISB (lactate and ethanol)	Pilot	3 years	3/02–5/05	Lebron, McHale, Major, McMaster
Launch Pad 34, Cape Canaveral	FL	Sand and crushed shells	NA	22 × 22 ft	TCE	EISB (ethanol and KB-1)	Pilot	<2 years	4/02–8/03	Hood, Major, Quinn, Yoon, Gavaskar
Demo of Enhanced Bio, PCE Source Area	Undis-closed	NA	NA	400 × 1000 ft	PCE	EISB (molasses)	Demo	3 years	NA	Payne
Source Zone Remediation at Dry Cleaner	Portland, OR	Silty clay and silty sand	NA	1200 ft^2	PCE, TCE, *cis*-DCE	EISB (HRC and HRC-X)	Pilot	5 years	Began 12/99	Willett, Koenigsberg, Parrett, Gillespie
Former Hunter AFB	GA	NA	NA	NA	TCE	Direct injection (lactate/oil, anaerobic bioremediation compound (ABC)	Pilot/ full	NA	NA	Stroo, HGL
Forbes Missile Site	KS	NA	NA	NA	TCE	ABC	Pilot/ full	NA	NA	Stroo, HGL
Arnold AFB (SWMU 10)	TN	NA	NA	50 ×100 ft	PCE, TCE, 1-DCA	EOS and virgin vegetable oil	Pilot	2 years	12/03–3/06	Lee, Terrasystems

Site	Location	Geological setting/ stratigraphy	Source area (DNAPL?)	Size	Contaminant and concentration	Remediation method/ substrate	Scale	Duration	Dates	Reference/ contributor
Hangar K Site, Cape Canaveral AFB	FL	Sand and silty sand	NA	60 × 100 ft	TCE (30 mg/L)	EISB (vegetable oil)	Pilot/ phase II	7 years	6/99–4/06	Ficklen
Site LF05, Hickam AFB	HI	Silty sands and gravel	NA	60 × 60 ft	TCE, DCE, VC	EISB (vegetable oil)	Pilot	2 years	4/03–8/05	Ficklen
Aerospace, Pinellas Park 1	FL	Fine to silty sand	Yes	70 × 170 ft	TCE (470 mg/L)	EISB (Fenton's, EOS, bacteria)	Full	Ongoing	Since 2004	Lisiecki
Aerospace, Pinellas Park 2	FL	Fine to silty sand	Yes	130 × 110 ft	TCE (110 mg/L)	EISB (Fenton's, EOS, bacteria)	Full	Ongoing	Since 2004	Lisiecki
Active Manufacturing Site	MI	Fine to silty sand	Yes	300 × 200 ft	TCE (440 mg/L)	EISB (Fenton's, EOS, whey, bacteria)	Full	Ongoing	Since 2003	Lisiecki
Ft. Lewis EGDY NAPL Area 3	WA	Glacial till	Yes	200 × 150 ft	TCE (up to 250 mg/L	ISB (whey)	Pilot	1 year	06/05–07/06	Macbeth, Sorenson
Portland Oregon Manufacturing Facility	OR	Sand and gravel	Yes	4000 ft^2	TCE up to 1170 mg/L	Reductive dechlorination (vegetable oil)	Full	2 years	8/05–5/07	Larsen, Fiedler

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Appendix A
Other Technologies Used with ISB of DNAPL

OTHER TECHNOLOGIES USED WITH ISB OF DNAPL

A.1 Zero-Valent Iron

Metallic iron, known as zero-valent iron (ZVI), is applied in the subsurface to promote abiotic reductive dechlorination, typically in permeable reactive barrier (or "wall") configuration. ZVI particles are available in a range of sizes, from coarse granules to nanometer-sized particles. Although there is a wide range of installation methods, barriers are often constructed in a wall orientation using conventional trenching techniques. Since the trench extends below the water table, the excavation may be stabilized by filling it with a high-density slurry of a biodegradable material such as guar gum. Following ZVI placement, some of the slurry remains in the subsurface and is readily available as organic substrate for indigenous microorganisms.

Through reaction with water, iron corrosion results in the production of Fe^{+2}, such that

$$Fe^0 + 2\ H_2O \rightarrow Fe^{+2} + 2\ OH^- + H_2\ (g)$$

The production of hydroxide ions results in an increase in pH (typically $9 < pH < 10$), which is inhibitory to reductive dechlorination. Dissolved ferrous iron rapidly precipitates as iron minerals including siderite ($FeCO_3$), pyrite (FeS_2), iron hydroxides [e.g., $Fe(OH)_2$], and magnetite (Fe_3O_4) (Odziemkowski et al. 1998). As a consequence of the production of hydrogen gas, the corrosion reaction results in strongly reducing conditions that favor reductive dechlorination and the reduction of other electron acceptors in groundwater, including oxygen and sulfate.

Downgradient of a ZVI barrier, geochemical conditions quickly return to ambient. At least one field study reports the occurrence of anaerobic biodegradation immediately downgradient of a ZVI barrier (VanStone et al. 2005), indicating the absence of any inhibitory impacts of ZVI on bioremediation.

A.2 In Situ Chemical Oxidation

A.2.1 Permanganate

Remediation applications involve the injection of either sodium or potassium permanganate (MnO_4^-) to degrade chlorinated ethenes. Short-term impacts of permanganate in groundwater include increases in the concentration of the relevant cations and microbial disinfection. However, permanganate is readily decomposed by the natural reduction capacity present in many groundwater systems, resulting in the precipitation of insoluble, brown-black Mn(IV) manganese oxides (MnO_2). These impacts are generally relatively minor.

However, permanganate can cause impacts to groundwater quality through a wide range of geochemical reactions (Table A-1). In addition to reactions with the target contaminant, permanganate oxidizes constituents of the uncontaminated porous media, including natural organic carbon, sulfides, and minerals containing reduced forms of either iron or manganese (Barcelona and Holm 1991). Oxidation of sulfide minerals can produce sulfate (Nelson et al.

2001), while fractions of the insoluble organic carbon content are likely to be only partially oxidized (e.g., Hayes et al. 1989), possibly accounting for increased DOC concentrations at some sites. While the increase in DOC could promote transient increases in dechlorinating activity, the addition of permanganate for the purpose of enhancing anaerobic reductive dechlorination through the release of DOC seems a poor substitute for direct substrate addition.

Table A-1. Potential geochemical impacts of permanganate on groundwater geochemistry

Reaction	Impact	Reference
Oxidation	Oxidation of humic matter, producing DO and CO_2 Oxidation of reduced S (e.g., pyrite, FeS_2) to SO_4^{-2}	
Reduction	Redox dissolution of $MnO^{-2} > Mn^{+2}$ (MnO_2 insoluble) under reducing conditions	Stone 1984
pH buffering/ dissolution	Dissolution of carbonate minerals (Mg^{+2}, Ca^{+2}) Increase in alkalinity (H_2CO_3, CO_3^{-2})	Nelson et al. 2001
Ionic strength	Increased ionic strength (particularly K^+ or Na^+)	

The most significant groundwater impacts are likely to be associated with the presence of MnO_2. Under oxic conditions, manganese is essentially insoluble; however, anaerobic conditions favor Mn reduction and the mobilization of soluble Mn(II) through reductive dissolution (Stone 1984).

Only a limited number of laboratory investigations have evaluated the impacts of ISCO using permanganate on microbial populations and dechlorinating activity. Two field studies have established that diverse microbial communities became established following a large-scale permanganate demonstration (Klens et al. 2001, Azadpour-Keeley et al. 2004), although neither of these studies directly examined dechlorination. While it seems apparent that an active microbial community becomes rapidly reestablished following ISCO, these studies provide only limited insight into the effects on dechlorinating microorganisms. There is at least limited laboratory evidence to suggest that dechlorinating activity can also rebound following ISCO (Rowland et al. 2001; Hrapovic et al. 2005).

Given the disinfection properties of permanganate, in the short term it seems likely that in situ addition of concentrated permanganate solutions will significantly reduce the indigenous microbial population and inhibit further microbial activity as long as residual permanganate is present. However, once that residual is depleted, groundwater will flow into the oxidized zone carrying microorganisms that will reestablish an active microbial population. Alternatively, bacteria that were physically isolated from the oxidizing, aerobic conditions in blind pockets or low-permeability zones may serve to reinoculate the treated area. The new microbial community will consist primarily of those microorganisms with rapid growth rates and/or unique metabolic characteristics that enable them to effectively exploit the environmental conditions (e.g., manganese-reducing bacteria). The presence of manganese dioxide exerts a substantial substrate demand, slowing a transition to a microbial population dominated by degradative microorganisms (e.g., *Dehalococcoides)*, which require a significant shift in redox conditions. Since Mn reduction is thermodynamically favorable relative to reductive dechlorination, it may be the case that the establishment of dechlorinating populations may be possible only in anaerobic niches where MnO_2 has been completely removed.

A.2.2 Modified Fenton's Reagent

The Fenton reaction involves the reaction of hydrogen peroxide (H_2O_2) with a ferrous iron (often in the form of $FeSO_4$) catalyst in acid solution, resulting in the production of ferric iron, hydroxyl ions, and hydroxyl radicals (Walling 1975). The hydrogen peroxide is typically injected at low concentrations (5%–10%). In addition to the hydroxyl radicals, transient reactive oxygen species such as hydroperoxide and superoxide anion may play significant roles (Watts, Bottenberg, and Teel 1999), although the field-scale significance of these species is not well understood. Modified Fenton's reagent, which does not require acid addition, includes a chelating agent to enhance iron solubility.

In the short term, modified Fenton's reagent, like permanganate, is biocidal, although the aggressive reactivity of Fenton's reagent with the aquifer matrix probably limits the extent of the biocidal effects. Table A-2 summarizes the longer-term geochemical impacts of Fenton's reagent. The principal impact of Fenton's reagent is the release of oxygen in the subsurface via hydrogen peroxide decomposition. Application of the Fenton's reagent resulted in elevated concentrations of DO concentrations (up to 24 mg/L) 17 months after oxidant injection (Kastner et al. 2000). Like the deposition of manganese dioxide, it is likely that considerable quantities of oxygen gas can be trapped in the subsurface.

Table A-2. Geochemical impacts of modified Fenton's reagent on groundwater geochemistry

Reaction	Example	Reference
Oxidation	Oxidation of humic matter, producing DO and CO_2 Oxidation of reduced S (e.g., pyrite, FeS_2) to SO_4^{-2}	Miller et al. 1996, Shiple et al. 2002a
Precipitation	Precipitation of Fe^{+2} as $Fe(OH)_3$ and Mn^{+2} as $MnCO_3$, MnO_2, or MnS	Shiple et al. 2002b, Stumm and Morgan 1970
pH buffering	Dissolution of carbonate minerals (Mg^{+2}, Ca^{+2}) Increase in alkalinity (H_2CO_3, CO_3^{-2})	Stumm and Morgan 1970
H_2O_2 decomposition	Increase in DOC Generation of heat	Jones 1999

Similar to permanganate, increases in DOC attributed to dissolution and/or partial oxidation of the organic carbon content of the aquifer matrix have been observed (Shiple et al. 2002b, Miller et al. 1996, Griffith and Schnitzer 1977). The simultaneous addition of oxygen creates conditions that favor aerobic microorganisms.

Recolonizing microorganisms may be significantly impacted by long-term geochemical changes within the Fenton's treatment zone following oxidant injection, particularly by DO that inhibits anaerobic reductive dechlorination. However, only limited data characterizing the impact of Fenton's reagent on resumption of reductive dechlorination are available. Rebounding of aerobic heterotrophic microorganisms capable of hydrocarbon degradation has been observed in nutrient-amended microcosms pretreated with conventional Fenton's reagent (Stokely et al.

1997). In the field, adverse impacts appear to be caused by geochemical impact rather than biocidal effects on the indigenous population (Kastner et al. 2000).

Although to date the phenomenon has not been evaluated in the literature, the reports of supersaturated DO concentrations in groundwater months after application suggest that the application of Fenton's reagent induces aerobic conditions by trapping oxygen gas in the aquifer. Given that the principal biodegradation pathway for chlorinated ethenes is anaerobic reductive dechlorination, DO exerts an additional substrate demand similar to that exerted by manganese dioxide, increasing the amount of substrate required to reestablish and promote anaerobic biodegradation of the chlorinated ethenes.

A.3 Thermal

Several thermal treatment options exist for DNAPL source areas; however, the use of electrical resistance heating (both three and six phase), appears to be gaining favor due to the ability to better control the heating in the subsurface.

As with the use of oxidants, the high temperatures associated with thermal remediation likely inhibit dechlorinating activity. In addition, decreases in biomass concentration, microbial diversity, and catabolic potential (based on carbon use) also have been observed (Friis 2006). Dechlorinating organisms were killed, not merely temporarily deactivated, by the high temperatures used, although dechlorinating activity can be reestablished once the in situ temperature drops below 35°–40°C (Friis 2006). During the post-treatment cooling phase, increases in DOC at concentrations capable of supporting dechlorinating organisms can occur (Friis, Albrechtsen, and Bjerg 2005). Below this temperature range, dechlorination rates become progressively slower (Friis et al. 2007).

The principal impact of thermal treatment on enhanced bioremediation appears to be the sensitivity of dechlorinating organisms, including *Dehalococcoides*, to high temperatures. During the cooling phase, dechlorinating activity may be reestablished by the influx of dechlorinators carried with groundwater flow or bioaugmentation of the target treatment area. For at least the short term following thermal treatment, geochemical conditions in a post-thermal site favor bioremediation in terms of increased availability of substrates, reduced microbial competition for these substrates, and temperatures conducive to high dechlorination rates.

A.4 Surfactant-Enhanced Aquifer Remediation

Surfactant-enhanced aquifer remediation (SEAR) using compounds such as Tween 80 has been used in source areas to remove significant DNAPL mass. An interesting aspect of this technology is that the surfactant also can serve as a substrate for reductive dechlorination. For example, Tween 80 can be fermented to organic acids, ethanol, and hydrogen. However, surfactants also can inhibit the activity of dechlorinating bacteria, though the process appears reversible as the post-treatment surfactant concentration attenuates below inhibitory levels (Amos et al. 2007). As an added benefit, some surfactants adsorb on to the soil; accordingly, they act as a substrate in the treated zone for a prolonged duration. ERD after a SEAR application was observed at the Bachman Road site (Ramsburg et al. 2005).

The likely design strategy for a sequential SEAR/enhanced bioremediation includes initial surfactant flooding to recover as much DNAPL mass as possible using active recirculation of the concentrated surfactant solution followed by a passive bioremediation phase. Following the completion of the DNAPL recovery phase, recirculation could be stopped to allow the recovery of dechlorinating organisms, and/or the zone could be bioaugmented. As the residual surfactant is depleted over time, pulses of surfactant solution (or another substrate) could be periodically injected to ensure that the substrate supply in the treatment zone is adequate.

A variety of factors need further investigation to determine how to optimize the coupling of SEAR with bioremediation:

- How does the design of the active treatment (particularly surfactant selection and injection concentration) affect dechlorinating microorganisms?
- Do the indigenous dechlorinating microorganisms rebound following the active phase, and how is that activity distributed in and downgradient of the source area?
- Do dechlorinating microorganisms rapidly reestablish themselves, or is it relatively advantageous to bioaugment?

Other than the potential inhibition of dechlorinating organisms by high surfactant concentrations, post-treatment impacts of SEAR appear generally beneficial to bioremediation.

A.5 Density-Modified Displacement Methods

Low-interfacial-tension mobilization/displacement of DNAPLs offers potential as an efficient remediation technology for contaminated aquifer source zones. However, displacement of dense DNAPLs is problematic due to the tendency for downward migration and redistribution of the mobilized DNAPL. To overcome this limitation, a density-modified displacement method was developed, which couples in situ density conversion of DNAPLs via alcohol partitioning with low-interfacial-tension NAPL displacement and recovery (Ramsburg and Pennell 2002).

A.6 Issues/Observations/Research Directions

The chemical and physical technologies presented offer a unique opportunity in that they may facilitate the establishment of new, post-treatment microbial community that may be better suited to dechlorination than the indigenous microbial community by eliminating more competitive and abundant bacteria, which can survive under a wider range of subsurface conditions.

Currently, there is a substantial body of peer-reviewed literature for ZVI, ISCO, thermal, and surfactant flushing; however there, are relatively few studies that examine any of these technologies from the broader perspective of integrating them with bioremediation as part of a sequential treatment strategy. Accordingly, there is a strong need for both laboratory and field studies examining these approaches and the impact of the primary technology on dechlorination by both indigenous and bioaugmented microorganisms.

Further, primary technologies may result in changes to the DNAPL architecture that affect the bioremediation rate. For example, primary technologies may change the surface area available for mass transfer or destroy/recover mass not in relatively inaccessible locations, reducing contact between substrates and the DNAPL. Primary technologies can also change the chemical composition of a source area by preferentially depleting some contaminants. For example, if permanganate flushing is used to remove a mixed TCE/TCA source, the more oxidizable TCE will be preferentially removed, leaving the nonreactive TCA in the source. Accordingly, the post-treatment abundance of TCA relative to TCE will present a potential concern if bioremediation is to be used to destroy residual TCE since high concentrations of TCA can inhibit reductive dechlorination of chlorinated ethenes.

Appendix B

Monitoring Metrics for Soil and Groundwater

MONITORING METRICS FOR SOIL AND GROUNDWATER

Table B-1. Monitoring metrics for soil and groundwater

Performance parameter	Method	Data use	Performance expectation	Recommended frequency of analysis
Chlorinated aliphatic hydrocarbons	SW8260B (laboratory)	Regulatory compliance for COCs, the values by which success of the remediation system will be measured.	CAHs and dechlorination products are typically expected to decline to less than regulatory compliance levels within the treatment zone after substrate addition.	Baseline and recommended for each sampling round.
Methane, ethane, ethene	SW3810 Modified (laboratory), Robert S. Kerr Laboratory RSK-175	Elevated levels of methane indicate fermentation is occurring in a highly anaerobic environment and that reducing conditions are appropriate for anaerobic dechlorination of CAHs. Elevated levels of ethene and ethane (at least an order of magnitude greater than background levels) can be used to infer anaerobic dechlorination of CAHs.	Methane levels >1.0 mg/L are desirable but not required for dechlorination to occur. Methane levels <1.0 mg/L and the accumulation of *cis*-1,2-DCE, VC, or other less CAHs may indicate that additional substrate is required to shift reducing conditions into an environment suitable for reduction of these compounds. If elevated levels of ethene or ethane are not observed, potential accumulation of *cis*-1,2-DCE or VC should be monitored.	Recommended for each sampling round. May require analysis by a specialty laboratory.
Total organic carbon (TOC, DOC)	SW9060, EPA Method 415.1 (laboratory)	Indicator of natural organic carbon present at site during baseline characterization and as an indicator of substrate distribution during performance monitoring. TOC/DOC concentrations >20–50 mg/L are desired in the anaerobic treatment zone.	Stable or declining TOC/DOC levels <20 mg/L in conjunction with elevated levels of VOCs and alternate electron acceptors indicate additional substrate is required to sustain the anaerobic treatment zone.	Baseline and recommended for each sampling event.
Dehalococcoides ethogenes (DHE)	Quantified by quantitative polymerase chain reaction	Determine presence of DHE at baseline periods after bioaugmentation.	DHE will be detected and increase as a consequence of adding electron donor to create anaerobic conditions or increase after inoculation with DHE-containing culture.	Baseline prior to injection and quarterly based on the numbers achieved. Once a high titer is measured and growth is ensured, the test may be continued but is not critical.
Ammonia	Distillation/ Titration Method E350.2	Ammonia can represent a form of biologically available nitrogen.	Indicator parameter only.	Baseline.

Performance parameter	Method	Data use	Performance expectation	Recommended frequency of analysis
Nitrate/nitrite	IC Method E300.1 (laboratory)	Nitrate is an alternate electron acceptor for microbial respiration in the absence of oxygen. Depleted levels of nitrate (relative to background) indicate that the groundwater environment is sufficiently reducing nitrate.	Indicator parameter. Nitrate level <1.0 mg/L is desirable for anaerobic ISB.	Optional and troubleshooting. Recommended for each sampling event if nitrate reduction appears to be a significant terminal electron accepting process (TEAP).
Nitrate/nitrite (as nitrogen (total)	IC Method 353.2 optional method for nitrate/nitrite by E300.1 (laboratory)	In most aquifers the concentration of nitrate is naturally much higher than nitrite, and total nitrate/nitrite can be used as an estimate of nitrate.	Indicator parameter. Nitrate level <1.0 mg/L is desirable for anaerobic ISB.	Optional and troubleshooting. Alternative method.
Manganese	EPA 6010B (laboratory) or Hach Method 8034 (field)	Manganese(IV) is an alternate electron acceptor for microbial respiration in the absence of or manganese oxygen and nitrate. An increase in dissolved manganese(II) or total manganese indicates that the groundwater environment is sufficiently reducing to sustain manganese reduction and for anaerobic dechlorination to occur.	Elevated levels of dissolved manganese may indicate a competing TEAP to anaerobic dechlorination of CAHs.	Optional. Recommended for each sampling event only if manganese reduction appears to be a significant TEAP.
Major cations (Ca, Mg, Na, K)		Major cations along with major anions are good general groundwater chemistry parameters and are inexpensive to analyze.	Only as a check if the system is not working as planned.	Baseline and as needed in subsequent sampling events.
Ferrous iron (Fe[II])	Preferred method is to field filter (0.45 µm filter) and ICP 200.7; alternate method: Colorimetric Hach Method 8146 (field)	Ferric iron is an alternate electron acceptor for microbial respiration in the absence of oxygen and nitrate. Reduction of ferric iron produces ferrous iron. Evaluated levels of ferrous iron indicates that the groundwater environment is sufficiently reducing to sustain iron reduction and for anaerobic dechlorination to occur.	Elevated levels of ferrous iron may indicate a competing TEAP to anaerobic dechlorination of CAHs.	Recommended for each sampling round. Typically measured at the well head to protect samples from exposure to oxygen.
Biologically available iron (Fe[III])	Laboratory specialty method (laboratory)	Bioassay with quantification of bioavailable solid-phase ferric iron Fe(III) that is a competing electron acceptor. Optional method that may be used to determine competition from iron reduction. May also affect potential abiotic reactions.	Recommended only for clastic sediments with potential for significant iron concentrations. May also be used as a diagnostic tool if sulfate reduction or methanogenic redox conditions cannot be achieved.	Optional at initial sampling.

Performance parameter	Method	Data use	Performance expectation	Recommended frequency of analysis
Sulfate (SO_4^{2-})	IC Method E300.0A (laboratory) or Hach Method 8051 (field)	Sulfate is an alternate electron acceptor for microbial respiration in the absence of oxygen, nitrate, manganese, and ferric iron. Depleted concentrations of sulfate relative to background indicate that the groundwater environment is sufficiently reducing to sustain sulfate reduction and for anaerobic dechlorination to occur.	Sulfate levels <20 mg/L are desirable but not required for anaerobic dechlorination to occur. High levels of sulfate in conjunction with the absence of TOC/DOC indicate additional substrate may be required to promote anaerobic dechlorination.	Recommend for baseline and each sampling round.
Sulfide	Hach Method 8131 or similar (field)	By-product of sulfate reduction. Sulfide typically precipitates with iron minerals, but elevated levels of sulfide may be toxic to dechlorinating microorganisms.	Elevated levels of sulfide in conjunction with elevated levels of CAHs may indicate that iron compounds should be added to precipitate sulfides and reduce toxicity effects.	Optional. Recommended when elevated levels of sulfate (>20 mg/L) are present.
Hydrogen sulfide	Soil gas analyzer calibrated in the field according to the manufacturer's specifications (field)	Useful for determining biological activity in vadose zone and generation of biogenic methane.	Explosive levels of noxious levels of hydrogen sulfide accumulating in structures or utilities may pose a health risk.	Optional. Recommended when soil vapor exposure pathway exists.
Bromide or iodide	IC Method EPA 300.1 (laboratory) or field meter (field)	Used as a conservative groundwater tracer.	Indicator parameter for tracer tests.	Used only with tracer testing.
Carbon dioxide (CO_2)	Care should be exercised when membrane meters are used in highly reducing environments, Hach Kit Method 8205 (field), alternative method (laboratory)	Carbon dioxide is a by-product of both aerobic and anaerobic degradation. Elevated levels of carbon dioxide indicate microbial activity has been stimulated.	Indicator parameter.	Optional.
pH	Field probe with direct-reading meter calibrated in the field according to the manufacturer's specifications (EPA 150.1)	Biological processes are pH sensitive, and the ideal range of pH for dechlorinating bacteria is 5–9. Outside this range, biological activity is less likely to occur.	pH levels within a range of 5–9 are desirable. pH <5 indicates that a buffering agent may be required to sustain high rates of anaerobic dechlorination. Desorption toward phase equilibrium is the basis of dissolved CAH "rebound," which extends treatment duration.	Baseline and recommended for each sampling event.

Performance parameter	Method	Data use	Performance expectation	Recommended frequency of analysis
Oxidation-reduction potential	Direct-reading meter, A2580B, or U.S. Geological Survey 1997 (field)	ORP of groundwater provides data on whether or not anaerobic conditions are present. Reducing conditions are required for anaerobic dechlorination of CAHs. Used in conjunction with other geochemical parameters and whether or not groundwater conditions are optimal for anaerobic biodegradation.	Positive ORP values (>0.0 mV) in conjunction with elevated levels of DO and the absence of TOC/DOC may indicate that additional substrate is required to promote anaerobic dechlorination.	Baseline and typically measured at the well head using a flow-through cell to protect samples from exposure to oxygen.
Dissolved oxygen	DO meter calibrated in the field according to the manufacturer's specifications (EPA 360.1) (field)	DO should be depleted in an anaerobic bioremediation system. DO <0.5 mg/L generally indicates an anaerobic pathway suitable for anaerobic dechlorination to occur.	DO concentrations >1.0 mg/L in conjunction with elevated levels of CAHs and the absence of TOC/DOC indicate additional substrate may be required to promote anaerobic dechlorination.	Baseline and recommended for each sampling event. Typically measured at the well head using a flow-through cell.
Temperature	Field probe with direct-reading meter (EPA 170.1)	General water quality parameter used as a well purging stabilization indicator. Microbial activity is slower at lower temperatures.	Indicator parameter. Typically used as a well purge stabilization parameter.	Baseline and every subsequent sampling event.
Specific conductance	E120.1/SW9050, direct-reading meter (laboratory or field)	General water quality parameter used as a well purging stabilization indicator. May correlate with and support interpretations of other geochemical analyses.	Indicator parameter. Typically used as a well purge stabilization parameter.	Baseline and every subsequent sampling event.
Fraction of organic carbon (f_{oc})	SW9060 modified for soil matrix (laboratory)	Fraction of organic carbon in the aquifer matrix is used to calculate retardation factors for dissolved contaminant transport and to estimate the amount of contaminant mass sorbed to the aquifer matrix.	A large portion of contaminant mass may be sorbed to the aquifer matrix.	Recommended at baseline sampling.
Natural carbon	SW9060 modified for soil matrix (laboratory)	The fraction of organic carbon in the aquifer matrix is used to calculate retardation factors for dissolved contaminant transport and to estimate the amount of CAH mass sorbed to the aquifer matrix.	A large proportion of contaminant mass may be sorbed to the aquifer matrix.	Recommended at baseline sampling.

Performance parameter	Method	Data use	Performance expectation	Recommended frequency of analysis
Volatile fatty acids	Laboratory specialty method, EPA Robert S. Kerr Laboratory (RSK)–SOP 112	VFAs are an indicator of substrate distribution and are also degradation products of more complex substrates (e.g., carbohydrates or vegetable oils). Fermentation of VFAs produces molecular hydrogen for anaerobic dechlorination.	Measurable concentrations of VFAs (>10–20 mg/L) are desirable in the treatment zone. The presence of mg/L concentrations of propionate or butyrate is considered favorable. Absence of measurable VFAs in conjunction with elevated levels of CAHs and alternate electron acceptors indicates additional substrate may be required to sustain the anaerobic treatment zone.	Optional. Useful as a trouble-shooting parameter.
Alkalinity	EPA Method 310.1 or Hach alkalinity test kit model AL AP MG-L or Hach Method #8203 (field or laboratory)	Indicator of biodegradation and the buffering capacity of the aquifer (neutralization of weak acids). Used in conjunction with pH. An increase in alkalinity and stable pH indicate the buffering capacity of the aquifer is sufficient to neutralize metabolic acids produced by degradation of substrates. Can also be used as measurement of salinity.	Concentrations of alkalinity that remain at or below background in conjunction with pH <5 indicates that a buffering agent may be required to sustain high rates of anaerobic dechlorination. High salinity conditions can inhibit microbiological activity.	Baseline and recommended for each sampling event. Typically measured at the well head using a flow-through cell.
Phosphate	E365.1 (laboratory)	Nutrient needed for microbial growth. May be needed as a substrate amendment	May indicate need for phosphate amendment.	Optional.
Chloride	IC Method E300.1 or SW9050 (laboratory), or Hach chloride test kit Model 8-P (field)	General water quality parameter. Chloride is produced by anaerobic dechlorination of CAHs. Elevated levels of chloride may indicate that dechlorination is occurring if observed concentrations are greater than three times background and consistent with CAH molar concentrations.	Indicator parameter only.	Baseline and every subsequent sampling event.

Appendix C

Impact of BioDNAPL Treatment on Source Longevity and Restoration Time Frames

IMPACT OF BIODNAPL TREATMENT ON SOURCE LONGEVITY AND RESTORATION TIME FRAMES

One of the principal goals of DNAPL source area bioremediation is to accelerate destruction of the source and its associated plume. Of course, source treatment may also be designed to reduce the flux from the source, to reduce the plume extent and/or to allow a more passive plume containment approach, such as MNA. But it is reasonable to expect that source depletion through any technology, including bioremediation, will reduce the source longevity. Source zone bioremediation can be viewed as a method for enhancing the natural depletion of the source and thereby hastening the natural attenuation of the source zone and its plume.

However, the depletion rate of a source is complex and is governed by the hydrogeology in and upgradient of the source area and the distribution of the various DNAPL phases (free, dissolved, sorbed, and matrix diffused) within the source area. Current characterization technologies cannot define these characteristics to the degree needed to accurately predict the rate of source mass depletion and the mass flux from a source zone over time. In addition, long-term data on the effects of source treatment on source longevity and plume responses are inadequate. Nonetheless, results from recent laboratory and field studies, along with developments in mathematical models of the effects of treatment on sources and plumes, have led to an improved understanding of the relationships between DNAPL mass, mass flux from source areas, and the responses of plumes over time to partial source depletion. This improved understanding can allow better evaluations of the benefits of source treatment, including ISB, and improved predictions of the impacts of treatment on the longevity of sources and their downgradient plumes.

Generally, the average concentrations of VOCs emanating from a source are less than the aqueous solubility of the VOCs that compose the DNAPL because of the impact of the geological variability and source zone architecture on mass transfer from sorbed, diffused, and free phases of VOCs and because of the by-passing and mixing of groundwater. Rao et al. (2001) first proposed that the mass flux from a source over time could be approximated by a power function of the DNAPL mass, as shown below:

$$\frac{C_s(t)}{C_o} = \left(\frac{M(t)}{M_o}\right)^{\Gamma} \quad ,$$

where C_o and $C_s(t)$ are, respectively, the initial contaminant concentration and the average concentration at time t of the dissolved VOC leaving the source zone, M_o and $M(t)$ are, respectively, the source zone mass initially and at time t, and Γ is an empirical fitting parameter, which is a function of the heterogeneity of the subsurface. When Γ is unity, then the fraction decrease in the source zone mass will lead to an equal fractional reduction in the average VOC concentration leaving the source zone (i.e., a 50% reduction in source zone mass will lead to a 50% reduction from the initial VOC concentration leaving the source zone).

Falta, Basu, and Rao (2005a, b) described how the DNAPL architecture and effect of homogeneity and heterogeneity would change the relationship between mass discharge and source reductions, and the relationship with Γ values, as depicted in Figure C-1 and summarized below.

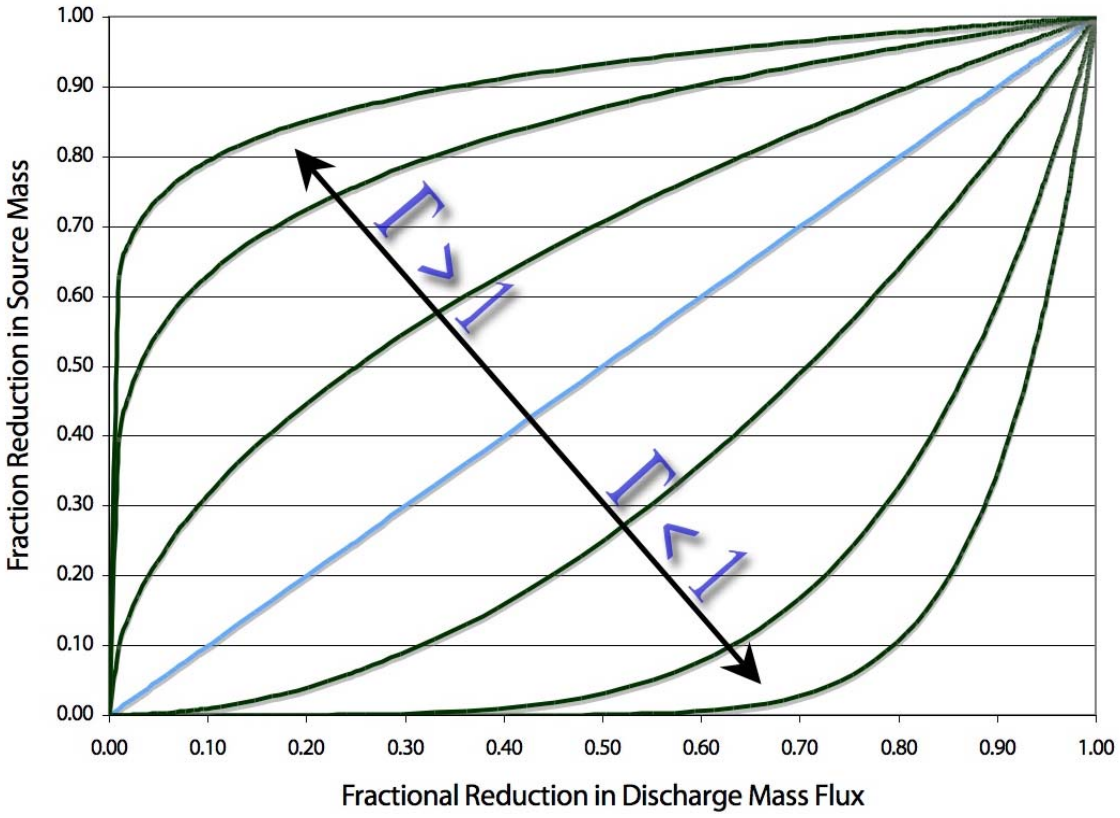

Figure C-1. Fractional reduction in source mass/fractional reduction in discharge mass flux.

- **Γ > 1:** Increasing values are associated with a negative correlation between permeability and DNAPL distribution, such as having most of the DNAPL mass in low-permeability zones. The case with Γ > 1 has also been described as the condition where *heterogeneous DNAPL* is present in a *homogeneous media* (Rao et al. 2001). Concentrations in the dissolved phase immediately downgradient of the source will decrease rapidly as the relatively small fraction of the total DNAPL mass in the higher-permeability areas is removed, followed by a slow decrease in the dissolved concentrations as mass is slowly removed from low-permeability zones (e.g., via diffusion).

- **Γ < 1:** Decreasing values associated with a more positive correlation between permeability and DNAPL distribution, such as DNAPL pools present in a high-permeability zones. The case with Γ < 1 has also described as *homogeneous DNAPL* is present in a *heterogeneous media* (Rao et al. 2001). The dissolved-phase concentrations will therefore decrease slowly until most of the mass is removed, after which the concentrations decrease more rapidly. Note the case Γ = 0.5 is a *linear* decline in concentration until all the mass is depleted, and Γ = 0 is a step function pattern in concentration over the lifetime of the source.

- **Γ = 1:** The case where a *first-order* decline in source concentration will be observed over time and where source concentration is linearly related to source mass. This model is included in both the BIOSCREEN and BIOCHLOR models distributed by EPA.

Falta, Rao, and Basu (2005b) further provided analytical models that considered removal of DNAPL mass by biotic and abiotic processes in addition to dissolution and advective processes to remove DNAPL, as indicated in the following equations:

$$M(t) = \left[\frac{-V_d A C_o}{\lambda_s M_o^{\Gamma}} + \left(M_o^{1-\Gamma} + \frac{V_d A C_o}{\lambda_s M_o^{\Gamma}} \right) e^{(\Gamma-1)\lambda_s t} \right]^{\frac{1}{1-\Gamma}} ,$$

and

$$C_s(t) = \frac{C_o}{M_o^{\Gamma}} \left[\frac{-V_d A C_o}{\lambda_s M_o^{\Gamma}} + \left(M_o^{1-\Gamma} + \frac{V_d A C_o}{\lambda_s M_o^{\Gamma}} \right) e^{(\Gamma-1)\lambda_s t} \right]^{\frac{\Gamma}{1-\Gamma}} ,$$

where V_d, A and λ_s are the Darcy velocity, cross-sectional area of the source perpendicular to groundwater flow, and source decay term over the lifetime of the source (i.e., not the decay term in the plume). Falta, Basu, and Rao (2005a, b) suggested that λ_s would be expected to be small relative to the dissolution of DNAPL mass, "but it could be significant over large time periods, especially when the rate of DNAPL dissolution becomes very small." However, they also noted that the decay term could be large when reductive dechlorination was enhanced in the source area.

Even small λ_s values can dramatically impact treatment times to remove DNAPL mass and reduce the flux (mass discharge rate) over time from source areas. Figure C-2 shows the change in the source zone mass over time for a hypothetical source zone using the above equations. The source zone values for M_o, C_o, V_d, and A were set at 2000 kg, 100 mg/L, 20 m/yr, and 30 m^2, respectively. Falta, Basu, and Rao (2005a, b) reviewed various studies and found that Γ typically ranged 0.5–2, which were used as end points. λ_s was set at 1×10^{-9}, 0.04 (natural rate), and 0.12 (enhanced rate) per year, respectively. These values correspond to source half-lives of approximately 7 million (i.e., no degradation), 17, and 6 years, respectively. These half-lives are in the range reported by Falta, Basu, and Rao (2005a, b). The λ_s value of 0.12 was based on the assumption that enhancing biological activity would increase rates by a factor of three over the natural rate. BioDNAPL field and laboratory studies (referenced in this document) have shown that enhanced biological activity in source areas can increase the rate of DNAPL depletion 2–15 times.

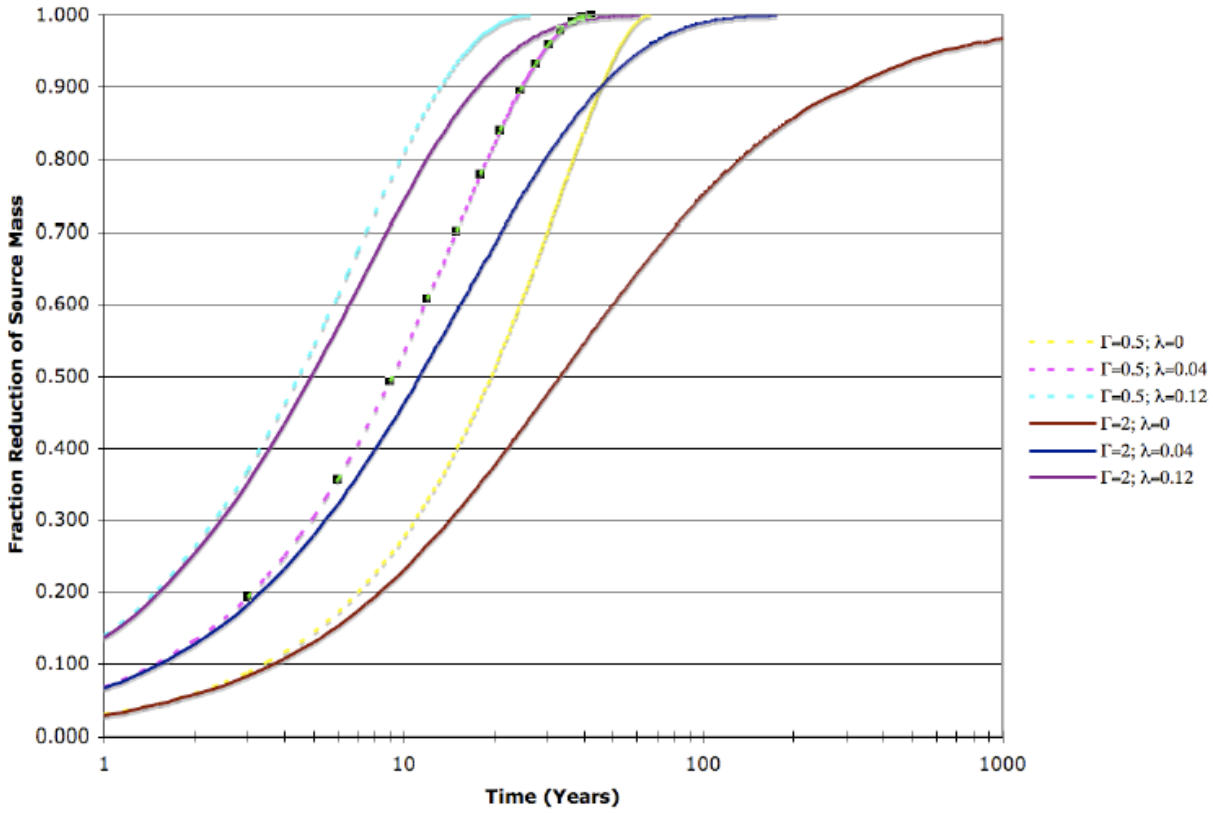

Figure C-2. Fraction reduction of source mass/time.

Figure C-3 indicates that significant concentration reductions can be achieved considerably sooner with a permanent threefold increase in the source zone biodegradation rate, λ_s. For example, the 10 µg/L concentration is achieved in approximately 45 years versus 110 years when Γ is 2.0, and λ_s is increased by a factor of three over the entire 45-year time period. Tables C-1 and C-2 show the predicted impact of achieving different concentration goals (MCL, and 90% and 50% concentration reduction at the downgradient edge of the source area) by similarly increasing the long-term degradation rate in the source zone. However, one important assumption in Table C-1 is that the enhanced biodegradation process is sustained until the target concentration is met. For example, for the case where Γ = 1.0 (Table C-2), concentration is 30 mg/L, λ_s = 0.04, and the target is MCLs, the enhanced biodegradation process would need to be sustained for 175 years (for example, adding electron donor every year for 175 years).

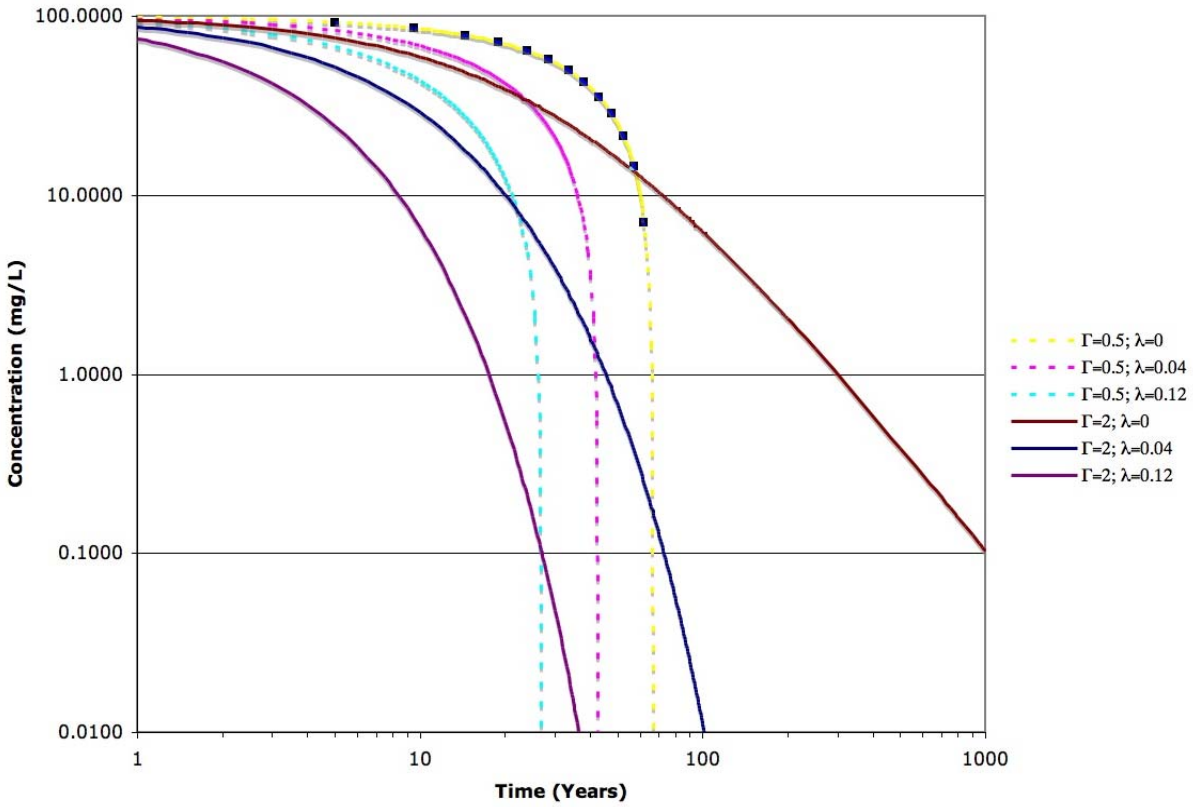

Figure C-3. Reduction in concentration vs. time.

Table C-1. Modeled impacts of source depletion on the time frames required to reduce the concentrations discharging from a chlorinated solvent source area to various target levels assuming source treatment occurs over a one year period

		Target concentration (mg/L)		
		MCL	90% Red.	50% Red.
		0.005	10	50
% Mass removed	**Co (mg/L)**	**Time to achieve target (years)**		
0%	100	143	33	10
80%	100	121	23	0
90%	100	111	0	0

Assumes source type: Γ = 1 and naturally occurring source biodegradation rate of λ_s = 0.04 per year. For cases with "0," the planning level model indicates that partial source depletion project will achieve this target level.

Table C-2. Modeled impacts of source depletion on the time frames required to reduce the concentrations discharging from a chlorinated solvent source area to various target levels assuming continual source treatment throughout lifetime of source

		Target (Concentration)								
		MCL	90% reduction	50% reduction	MCL	90% reduction	50% reduction	MCL	90% reduction	50% reduction
		Γ								
		0.5			1			2		
λ	C_o(mg/L)	Time to Achieve Target (Years)								
0	30	222	200	111	947	256	77	7939	240	46
	100	67	60	33	325	77	23	4404	72	14
0.04	30	85	66	26	175	47	14	104	25	18
	100	42	36	17	144	33	10	109	20	5
0.12	30	44	30	10	66	18	5	35	9	9
	100	27	21	9	65	15	5	39	8	2

Other source decay models are more applicable when enhanced biodegradation is not sustained over the lifetime of the source (i.e., electron donor addition is performed for only a few months or a few years to reduce the source mass). The REMCHLOR model (Falta et al. 2007) allows for the simulation of relatively short-term source depletion projects followed by natural attenuation. This is by far the most common approach used for enhanced biodegradation field projects. For the case of a one-year source depletion project, $\Gamma = 1.0$, and $\lambda_s = 0.04$, the following source longevities were calculated for three different percentages for source mass removal during the first year of a plume management project: 0% (the MNA or untreated case); 80% removal; and 95% removal (Table C-1). Note that the median percent reduction in parent concentration for 21 enhanced biodegradation projects analyzed by McGuire, McDade, Newell (2006) was 96%.

With $\Gamma = 1.0$, the REMCHLOR model assumes that there is slow "tailing" of the remaining mass as residual contaminants in inaccessible and untreated compartments are slowly released. While originally developed for tailing caused by DNAPL dissolution, this model can (in theory) also capture tailing caused by processes such as slow diffusion from low-permeability zones ("matrix diffusion") and equilibrium desorption. In the case with an initial source depletion followed by natural attenuation, there is not "equal benefit for equal work" with regard to the percentage mass removal and percentage reduction in source longevity for cases where the starting concentrations are several orders of magnitude higher than the target concentration (see MCL column, above). Newell and Adamson (2005) present planning-level source decay equations that can be used with a calculator that give the same results as the ones shown above.

The source decay model described above is very simplistic, and assumptions must be taken with caution, as it is important to realize that although these predictive models are valuable, there are still little long-term data available to verify such predictions. Further, the plume response remains difficult to predict—even complete removal of the source may have much less impact on the overall restoration time frame than existing models (such as REMCHLOR) currently predict because contaminant mass released from less-permeable zones within the plume may occur even slower than predicted by REMCHLOR. For more information on the impacts of matrix diffusion, see Newell and Adamson (2005) and Chapman and Parker (2005).

Appendix D

BioDNAPL Team Contacts

BioDNAPL TEAM CONTACTS

Naji Akladiss, Team Leader
ME Dept. of Environmental Protection
17 State House Station
Augusta, ME 04333
207-287-7709
naji.n.akladiss@maine.gov

Rick Ahlers
LFR Inc.
3150 Bristol St., Ste. 250
Costa Mesa, CA 92626
714-444-0111
rick.ahlers@lfr.com

Wilson Clayton, Ph.D.
Aquifer Solutions
29025A Upper Bear Creek Rd.
Evergreen, CO 80439
303-679-3143
wclayton@aquifersolutions.com

Geoff Compeau, Ph.D.
Geosyntec Consultants
1370 Stewart St.
Seattle, WA 98109
208-915-4144
Gcompeau@Geosyntec.com

Mary DeFlaun
Geosyntec Consultants
3131 Princeton Pike
Lawrenceville, NJ 08648
609-895-1400
mdeflaun@geosyntec.com

Robert Downer
Burns and McDonald Engineering Co., Inc
425 South Woods Mill Rd.
Chesterfield, MO 63017
314-683-1536
rdowner@burnsmcd.com

Jennifer Farrell
Florida DEP
2600 Blair Stone Rd., MS#4520
Tallahassee, FL 32399
850-245-8937
jennifer.a.farrell@dep.state.fl.us

Holmes (Donald) Ficklen
HQ AFCEE/TDE
3300 Sidney Brooks
Brooks City-Base, TX 78235-5112
210-536-5290
holmes.ficklen@brooks.af.mil

Linda Fiedler
USEPA
1200 Pennsylvania Ave., NW (5203P)
Washington, DC 20460
703-603-7194
fiedler.linda@epa.gov

Rick Gillespie
Regenesis
9308 Warm Springs Cir.
Plano, TX 75024
972-377-7288
rgillespie@regenesis.com

Dibakar (Dib) Goswami, Ph.D.
WA State Dept. of Ecology
3100 Port of Benton Blvd.
Richland, WA 99354
509-372-7902
dgos461@ecy.wa.gov

Paul Hadley
CA Dept. of Toxic Substances Control
P. O. Box 806
Sacramento, CA 95812-0806
916-324-3823
phadley@dtsc.ca.gov

Eric Hausamann
NY State Dept. of Environmental Control
518-402-9819
eghausam@gw.dec.state.ny.us

Song Jin
University of Wyoming
WRI Building
365 N. 9th St.
Laramie, WY 82072
307-721-2404
sjin@uwyo.edu

Trevor King
Langan Engineering & Environ. Services
2700 Kelly Dr., Ste. 200
Warrington, PA 18976
215-491-6519
tking@langan.com

Carmen Lebron
Naval Facilities Engineering Service Center
1100 23rd Ave., ESC411
Port Hueneme, CA 93043
805-982-1616
carmen.lebron@navy.mil

Jerry Lisiecki
Fishbeck, Thompson, Carr & Huber, Inc.
1515 Arboretum Dr., SE
Grand Rapids, MI 49546
616-464-3751
jblisiecki@ftch.com

Tamzen Macbeth
North Wind Inc.
1425 Higham
Idaho Falls, ID 83402
208-528-8718
tmacbeth@northwind-inc.com

David Major
Geosyntec Consultants, Inc.
130 Research Ln., Suite 2
Guelph, Ontario N1G5G3
519-823-2037
dmajor@geosyntec.com

Jennifer Martin
ARCADIS
375 West Santee
Charlotte, MI 48812
210-215-7078
jennifer.martin@Arcadis-us.com

Beth Moore
U.S. Dept. of Energy
EM-22, FORS 3E066
1000 Independence Ave., SW
Washington, DC 20585
202-586-6334
beth.moore@em.doe.gov

Bill Morris
KS Dept. of Health and Environment
785-296-8425
bmorris@kdhe.state.ks.us

Alec Naugle
California Regional Water Board
1515 Clay St., Ste. 1400
Oakland, CA 94612
510-622-2510
anaugle@waterboards.ca.gov

Eric Nuttall
University of New Mexico–Emeritus
1445 Honeysuckle Dr., NE
Albuquerque, NM 87122
505-269-7840
nuttall@unm.edu

Dr. Mary Jo Ondrechen
Professor of Chemistry/Chemical Biology
Northeastern University
617-373-2856
mjo@neu.edu

Ian T. Osgerby
USACE
696 Virginia Rd.
Concord, MA 01742
978-318-8631
ian.t.osgerby@usace.army.mil

Fred Payne
ARCADIS
375 West Santee
Charlotte, MI 48812
248-376-5129
fpayne@arcadis-us.com

Greg Rapp
NJ Dept. of Environmental Protection
401 E. State St.
Trenton, NJ 08625-0409
609-292-9969
Gregory.Rapp@dep.state.nj.us

Julia Sechen
MA Dept. of Environmental Protection
20 Riverside Dr.
Lakeville, MA 02347
508-946-2791
Julia.Sechen@state.ma.us

G. A. (Jim) Shirazi, Ph. D., P.G.
OK Dept. of Agriculture, Food and Forestry
2800 North Lincoln Blvd.
Oklahoma City, OK 73105
405-522-6144
gashirazi@aol.com

Michael Sieczkowski
JRW Bioremediation, LLC
14321 W. 96th Ter.
Lenexa, KS 66215
913-438-5544
msieczkowski@jrwbiorem.com

Donovan Smith
JRW Bioremediation, LLC
14321 W. 96th Ter.
Lenexa, KS 66215
913-438-5544
dsmith@jrwbiorem.com

Jennifer Smith
Conestoga Rovers & Associates
2055 Niagara Falls Blvd., Ste. 3
Niagara Falls, NY 14304
716-297-6150
jjsmith@craworld.com

Michael B. Smith
VT DEC Waste Mgmt. Division
103 South Main St./West Building
Waterbury, VT 05671-0404
802-241-3879
michael.b.smith@state.vt.us

Hans Stroo
HGL
300 Skycrest Dr.
Ashland, OR 97520
541-482-1404
hstroo@mind.net

Larry Syverson
VA Dept. of Environmental Quality
Box 1105
Richmond, VA 23218
804-698-4271
lwsyverson@deq.virginia.gov

Kimberly A. Wilson
SCDHEC
2600 Bull St.
Columbia, SC 29201
803-896-4087
wilsonka@dhec.sc.gov

Ryan Wymore
CDM
1331 17th St., Ste. 1100
Denver, CO 80202
303-298-1311
wymorera@cdm.com

Fuxing Zhou
VA Dept. of Environmental Quality
629 E. Main St.
Richmond, Virginia 23219
804-698-4126
fzhou@deq.virginia.gov

Appendix E

Abbreviations, Acronyms, and Symbols

ABBREVIATIONS, ACRONYMS, AND SYMBOLS

AFB	Air Force Base
AFCEE	Air Force Center for Engineering and the Environment
bioDNAPL	bioremediation of DNAPLs
°C	degrees Centigrade, Celsius
CAH	chlorinated aliphatic hydrocarbon
CFR	Code of Federal Regulations
cm	centimeter
COC	contaminant of concern
CERCLA	Comprehensive Environmental Resource, Conservation, and Liability Act
CSM	conceptual site model
d	day
DCE	dichloroethene
DHE	*Dehalococcoides ethogenes*
DNAPL	dense, nonaqueous-phase liquid
DO	dissolved oxygen
DOC	dissolved organic carbon
DOD	U.S. Department of Defense
ECOS	Environmental Council of the States
EEQ	electron equivalent
EISB	enhanced in situ bioremediation
EOS®	Emulsified Oil Substrate
EPA	U.S. Environmental Protection Agency
ERD	enhanced reductive dechlorination
ERIS	Environmental Research Institute of the States
ESB	Engineering Support Building
ESTCP	Environmental Security Technology Certification Program
°F	degrees Fahrenheit
ft	foot, feet
gpm	gallons per minute
HRC®	Hydrogen-Release Compound
ISB	in situ bioremediation
ISCO	in situ chemical oxidation
ITRC	Interstate Technology & Regulatory Council
kg	kilogram
L	liter
LC34	Launch Complex 34
m	meter
M	molar
MBT	molecular biological tool
MCL	maximum contaminant level
mg	milligram
MNA	monitored natural attenuation

mV	millivolt
ORP	oxidation-reduction potential
PCE	perchloroethene
PVC	polyvinyl chloride
RAO	Remedial Action Objective
RCRA	Resource Conservation and Recovery Act
ROI	radius of influence
SEAR	surfactant-enhanced aquifer remediation
TAN	Test Area North
TCA	trichloroethane
TCE	trichloroethene
TDS	total dissolved solids
TEAP	terminal electron-accepting process
TOC	total organic carbon
UIC	underground injection control
VC	vinyl chloride
VFA	volatile fatty acid
VOC	volatile organic compound
yr	year
ZVI	zero-valent iron

Symbols

C_{sat}	solubility limit
C_w	bulk liquid
f_{oc}	fraction of organic carbon
J	mass transfer rate
K_{ow}	octanol-water partition coefficient
K_{sp}	mineral solubility product
K	hydraulic conductivity
λ	decay term
μg	microgram
δ	thickness