CHAPTER 12

IN SITU BIOREMEDIATION OF CHLORINATED ETHENE SOURCE ZONES

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12.1 INTRODUCTION

In situ bioremediation (ISB) was quickly adopted during the mid-1990s to treat dissolved chlorinated solvent plumes and to contain source zones by creating permeable reactive barriers (Stroo and Ward, 2010). Initially, ISB was not considered a feasible technology for treating dense nonaqueous phase liquid (DNAPL) source zones, and still faces some skepticism (AFCEE et al., 2004; Borden, 2003; ITRC, 2008). However, the experience of the last decade suggests that it can be a viable technology for treating some source zones. ISB has become one of the most commonly used source remediation technologies because it is relatively inexpensive, sustainable and adaptable to a wide range of site-specific conditions. This chapter summarizes both the promise and the potential pitfalls of using ISB to deplete source zones.

Seagren et al. (1993, 1994) showed that biological activity could enhance the dissolution of nonaqueous phase liquid (NAPL) and thereby shorten the longevity of the source. This work provided an initial theoretical basis for treating chlorinated solvent sources with ISB. However, there was little commercial interest in ISB for source zones for several years, until research demonstrated that dechlorinating bacteria could survive and degrade chlorinated ethenes even at the high concentrations found near trichloroethene (TCE) and perchloroethene (PCE or tetrachloroethene) DNAPL (Nielsen and Keasling, 1999; Harkness et al., 1999; Yang and McCarty, 2000). Following laboratory demonstrations of enhanced dissolution of PCE DNAPL (Carr et al., 2000; Cope and Hughes, 2001), field-scale pilot demonstrations were performed in the early 2000s (Peterson et al., 2000; Battelle, 2004; Hood et al., 2008). Those results have increased the confidence that ISB can be useful for at least some DNAPL source zones (ITRC, 2008).

However, ISB is not appropriate for all chlorinated ethene source zones, particularly those with a significant fraction of DNAPL present as pools (Christ et al., 2005). ISB has been adopted mainly as a primary remediation technology for low-strength DNAPL source zones (Sale et al., 2008) and as a polishing technology for higher-strength sources after more aggressive approaches have been used (ITRC, 2008; Sleep et al., 2006). A recent survey of 118 sites that have used *in situ* source-zone remediation at a field scale (NAVFAC and Geosyntec, 2011) found that ISB had been used at roughly 30% of the sites (Figure 12.1). That survey and others (McDade et al., 2005; McGuire et al., 2006; ITRC, 2008) also have

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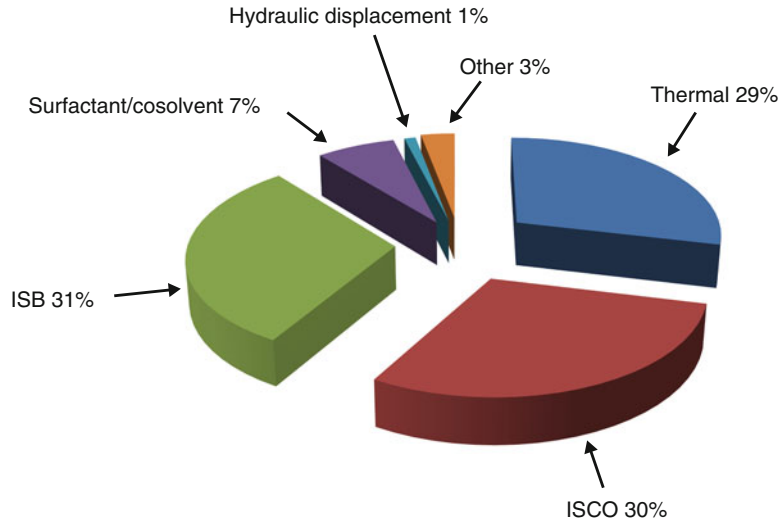

Figure 12.1. Results of survey of field-scale experiences treating chlorinated ethene source zones (from NAVFAC and Geosyntec, 2011). Figure shows the percentages of sites using different *in situ* treatment technologies, from a total of 118 sites. Note: ISCO – *in situ* chemical oxidation.

documented that ISB can achieve reductions in contaminant concentrations that are similar to those measured using other technologies, generally for lower cost.

Despite its rapid adoption it is important to note that, to the authors' knowledge, there are no well-documented case studies of ISB at a heavily contaminated chlorinated solvent site that have demonstrated site-wide reductions in contaminant concentrations to below maximum contaminant levels (MCLs). The technology is slow compared to thermal or chemical treatments, and it is extremely difficult to deliver chemical reagents to all of the contaminated areas in the subsurface; thus, some residual contamination should be expected (ITRC, 2011). Source-zone ISB designs and operations must address several significant challenges that often are not fully appreciated. However, source-zone ISB is a technology that is still developing, and further innovations will occur. Use of ISB for source treatment will continue because it is an economically attractive technology and it also can be a very effective one, given appropriate conditions and remedial objectives.

This chapter summarizes the technical basis for ISB of chlorinated solvent source zones, the advantages and limitations of the technology, the challenges faced when designing and operating an ISB system for a source zone and the options available for addressing site-specific challenges. The chapter also discusses realistic performance expectations and monitoring of these systems and concludes with a summary of the lessons learned to date.

The chapter includes descriptions of four well-documented case studies to provide examples of tank- to field-scale results and the different implementation strategies that have been used. Source-zone ISB is sufficiently innovative that no peer-reviewed report of a full-scale treatment was found in preparing this chapter. However, these case studies provide valuable lessons regarding the appropriate uses of the technology and the performance that can be expected. Finally, the chapter includes a description of a recently developed numerical model of source-zone ISB, with the conclusions drawn from using the model for a range of site conditions.

12.2 TECHNOLOGY DESCRIPTION

12.2.1 Technical Basis

ISB of chlorinated ethenes generally relies on sequential reductive dechlorination, with each step in the process removing one chlorine atom at a time and replacing it with a hydrogen atom (Figure 12.2) (Bradley and Chapelle, 2010). In this sequence PCE is reduced to TCE, which in turn is reduced to dichloroethene (primarily the *cis*-1,2-DCE isomer), which then is reduced to vinyl chloride (VC) and finally to ethene (Vogel and McCarty, 1985; Mohn and Tiedje, 1992). This process has been studied for more than 20 years and has been used to treat chlorinated ethenes in the dissolved phase (i.e., in the plume) for well over a decade (Stroo, 2010). Although several dechlorinating bacteria are capable of the initial steps in dechlorination (PCE to TCE and DCE), to date only bacteria in the genus *Dehalococcoides* (*Dhc*) have been shown to be capable of gaining energy from the complete dechlorination of DCE to VC and eventually to ethene (Maymó-Gatell et al., 2001; He et al., 2003; Löffler et al., 2013b). All known *Dhc* strains are now classified as *Dehalococcoides mccartyi* (Löffler et al., 2013a).

In reductive dechlorination, the chlorinated ethene serves as the electron acceptor, hydrogen serves as the electron donor, and the dechlorinating bacteria generally obtain their carbon from acetate (Bradley and Chapelle, 2010). Stimulating reductive dechlorination typically relies on adding complex organic carbon compounds (electron donor sources such as vegetable oil or lactate) that are fermented, producing acetate and hydrogen. The addition of these electron donor sources into the subsurface stimulates the growth and activity of dechlorinating bacteria by creating a sufficiently anaerobic groundwater treatment zone and generating hydrogen through fermentation reactions (ITRC, 2005). 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 anaerobic treatment zone is created through the consumption of oxygen and other electron acceptors (for example, nitrate and sulfate) during the biodegradation of the electron donor source.

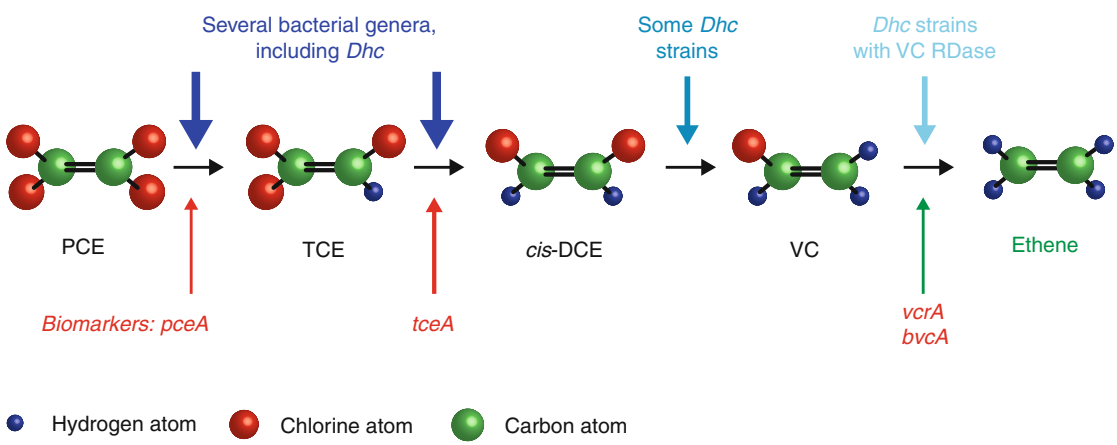

Figure 12.2. Reductive dechlorination of chlorinated ethenes. Bacteria capable of energy-yielding reductive dechlorination of each of the ethenes are noted in *blue text*, and useful biomarkers for particular steps are identified in *red*. *Dhc* = *Dehalococcoides mccartyi*; RDase = reductive dehalogenase; pceA is a marker for PCE RDase; tceA is a marker for TCE RDase; and vcrA and bvcA are markers for two identified VC RDases.

The hydrogen serves as the ultimate electron donor for anaerobic bacteria that reduce the chlorinated ethenes. Thus the electron donor addition both helps create favorable redox conditions (highly reducing methanogenic or sulfate-reducing conditions, with Eh values <-100 milivolts [mV]) and also fuels the reductive dechlorinating bacteria (notably *Dhc*). This form of ISB is often referred to as enhanced reductive dechlorination (ERD), to distinguish it from aerobic bioremediation techniques.

ISB through reductive dechlorination has proven to be a useful approach to treat chlorinated ethenes because it is capable of treating all chloroethenes, and because it has proven to be relatively easy to implement and control under field conditions, compared to other potential biological approaches such as aerobic cometabolism (described below). However ISB is not suitable for all sites. Key limitations include the presence of significant DNAPL accumulations, moderately acidic pH or low alkalinity, low permeability or highly heterogeneous aquifers or very rapid groundwater velocities (Table 12.1).

12.2.2 Enhanced Dissolution and Source Removal During ISB

Under ideal conditions, aqueous phase chlorinated solvents such as PCE and TCE may be reduced all the way to nontoxic end products (ethene and ethane). In some cases, however, substantial amounts of lesser chlorinated compounds (primarily *cis*-DCE) often are produced (Carr et al., 2000; Yang and McCarty, 2000, 2002; Sleep et al., 2006). This incomplete dechlorination has been attributed to insufficient residence time (Amos et al., 2007b), low pH (Adamson et al., 2003; Eaddy, 2008; McCarty et al., 2007; Robinson et al., 2009) and inhibitory parent compound concentrations (Chu et al., 2003; Yu et al., 2005; Sabalowsky and Semprini, 2010a, b).

During ISB in the presence of DNAPL, the parent compound (e.g., PCE) dissolves into the aqueous phase and is then degraded by bacteria. This reduces the PCE concentration in the aqueous phase, allowing more of the DNAPL to dissolve (Seagren et al., 1993, 1994; Cope and Hughes, 2001). However as PCE is degraded, TCE, DCE and VC are produced. Since TCE, DCE and VC are somewhat hydrophobic, these daughter products can partition back into the DNAPL phase. The simultaneous dissolution, biotransformation and back partitioning can lead to temporary sequestering of the daughter products in the DNAPL phase, complicating data interpretation (Ramsburg et al., 2010). Aqueous phase PCE concentrations may be low, even though a substantial amount of this material is still present in the form of DNAPL. Also, the mass flux of TCE, DCE and VC released to the downgradient plume may be lower than the actual production rate because partitioning back into the DNAPL will reduce aqueous phase concentrations of these compounds.

Cleanup of chlorinated solvent source zones is often limited by the low aqueous solubility of the major contaminants (for example, PCE and TCE) and slow mass transfer from DNAPL to the dissolved phase (Kueper et al., 2003). However, ISB can accelerate DNAPL removal through several different mechanisms. Removal of DNAPL during ISB will be accelerated by the increased DNAPL dissolution rate, although repartitioning of the parent or daughter products (e.g., TCE, DCE and VC) back into DNAPL can occur as well, though it may be downgradient of the original DNAPL accumulation. The relative importance of these two competing processes depends on a variety of factors including the transformation rates, effective aqueous solubility of the various chlorinated ethenes, and rate of groundwater flow. If the DCE degradation rate is very high, DCE will not accumulate substantially and the DNAPL removal rate will be controlled primarily by the PCE and TCE degradation rates and dissolution of these compounds by flowing groundwater. However, it is common for the DCE

Table 12.1. Site Conditions that Affect the Applicability of ISB (Modified from AFCEE et al., 2004)

Factor	Favorable Condition	Less Favorable Condition	Impact on ISB	Ease of Managing
Geochemistry				
pH	5.5 to 8	<4.5 or >9	High	Difficult
Temperature	>10°C	<10°C	Low	Difficult
Alkalinity	High (>100 mg/L $CaCO_3$)	Low	Moderate	Difficult
Competing electron acceptors	Low concentrations or influx	High concentrations or influx	Moderate	Moderate
Hydrogeology				
Depth to water	Moderate	Deep or very shallow	Low to Moderate	Difficult
Hydraulic conductivity	Med 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 stagnant	High	Moderate – if slow Difficult – if fast
Aquifer matrix	Granular; unconsolidated media	Rock; consolidated media	High	Difficult
Heterogeneity	Low to moderate	High (>100 variation in local scale K)	Moderate	Difficult
Source-Zone Characteristics				
Contaminant	Single component	Multicomponent mixtures	Moderate to High	Difficult
Depth of source	Shallow	Deep	Low to Moderate	Moderate
Age of source/ plume maturity	Recent (<10 year)	Mature (>10 year)	High	Difficult
Volume	Small (<500 cy)	Large (>500 cy)	Moderate	Moderate
Microbiology				
Native heterotrophic population	Present in pre-ISB screening	Absent in pre-ISB screening	Moderate to High	Difficult
Dehalorespirators	Present in pre-ISB screening	Absent in pre-ISB screening	Low	Easy
Other Factors				
Location of onsite infrastructure	Vapor intrusion risks are acceptable	Vapor intrusion risk is a concern	Moderate	Moderate
Site Access	Site is easy to access	Site is remote or otherwise difficult to access	Moderate	Moderate

Note: cy – cubic yard

degradation rate to be slower than that for TCE and PCE, and for some accumulation to occur, with resulting partitioning of DCE and perhaps VC back into the NAPL phase.

The enhancement of chloroethene dissolution through biotransformation is illustrated in Figure 12.3 (from Yang and McCarty, 2000). Laboratory columns containing droplets of PCE DNAPL were flushed with a feed solution saturated with PCE and containing 1.7 milimolar (mM)

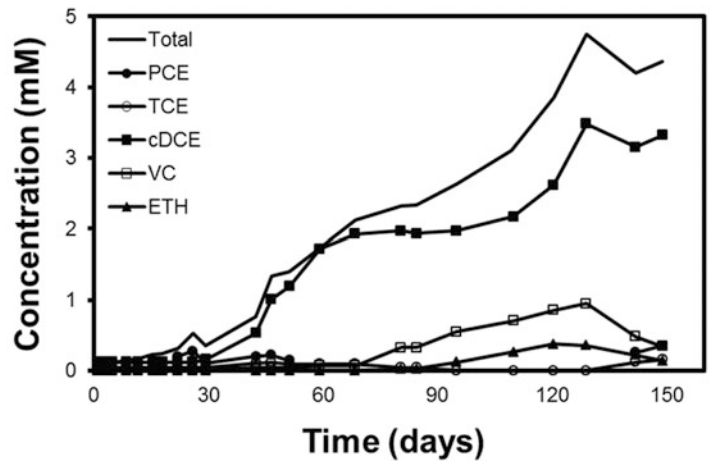

Figure 12.3. Concentration of PCE (*filled circle*), TCE (*open circle*), *cis*-DCE (*filled square*), VC (*open square*), ethene (*filled triangle*) and the total (without marker) in the column effluent with time. Reprinted with permission from Yang and McCarty (2000). Copyright (2000) American Chemical Society.

sodium benzoate and 20 mg/L yeast extract. The columns were then inoculated with an enrichment culture known to degrade PCE to ethene. Throughout the experiment, PCE and TCE concentrations remained low due to rapid transformation of these compounds to DCE, VC, and ethene, even though significant amounts of PCE DNAPL were still present. At the start of the experiment, partitioning of DCE back into the DNAPL droplets could have reduced the flushing rate. However by 45 days, total ethenes (sum of PCE, TCE, DCE, VC and ethene) were greater than the aqueous PCE solubility (~1 mM), demonstrating that ISB was enhancing DNAPL removal. At 130 days, total ethenes were over 4 mM, indicating DNAPL removal was enhanced by more than a factor of 4.

High electron donor concentrations can also impact dissolution rates. Macbeth et al. (2006) reported that high concentrations of dissolved electron donor and/or fermentation products potentially can enhance DNAPL mass transfer rates directly through cosolvency, desorption and/or partitioning from DNAPL to dissolved organic compounds. However, aqueous phase concentrations must be more than 1% (10,000 mg/L) to have a significant impact on TCE solubility (Hood et al., 2007). Low solubility electron donors can also impact chloroethene mobility. Hiortdahl and Borden (2011) reported a four- to five-fold increase in the effective solubility of PCE when flushing columns containing trapped PCE DNAPL with emulsified vegetable oil (EVO). However, partitioning of chloroethenes to oil droplets attached to aquifer material can reduce mobility (ESTCP, 2006).

At some sites, injection and extraction systems are used to recirculate groundwater containing electron donor through the treatment area. The increased groundwater velocity due to recirculation can increase mass transfer rates, potentially accelerating DNAPL removal. In theory, ISB can significantly enhance flushing rates and DNAPL removal. However in practice, the observed enhancement may be lower than expected. Table 12.2 (adapted from Sleep et al., 2006 and Amos et al., 2008) shows the measured enhancements in controlled laboratory experiments. Glover et al. (2007) showed that PCE dissolution could be enhanced by up to a factor of 13 in a 5-centimeter (cm) (2-inch [in]) flow cell. However, the cumulative PCE removal rate was only increased by a factor of 1.7 in 2-dimensional (2-D) aquifer cells containing a nonuniform PCE DNAPL distribution due to a shrinking DNAPL source zone and bioclogging/pore blockage from methane gas generation (Sleep et al., 2006).

Table 12.2. Summary of Selected DNAPL ISB Experiments (Adapted from Sleep et al., 2006 and Amos et al., 2008)

Reference Study	System Description	Major Dechlorination Product(s)	Dissolution Enhancement
Yang and McCarty, 2000	1-D column containing aquifer material and nonuniform residual PCE DNAPL with pentanol as substrate	*cis*-DCE	5 maximum
Carr et al., 2000	Continuous-flow stirred-tank reactor with PCE/tridecane NAPL (0.13 mol/mol PCE/tridecane) with formate as substrate	*cis*-DCE	14 – cumulative based on model comparison
Cope and Hughes, 2001	1-D column containing glass beads with PCE/tridecane NAPL (0.13 mol/mol PCE/tridecane) with pyruvate as substrate	TCE, VC	5.0 to 6.5 cumulative
Yang and McCarty, 2002	1-D column containing aquifer material and PCE DNAPL with different substrates (pentanol, calcium oleate, and olive oil)	*cis*-DCE	Cumulative 1.6 – pentanol 2.3 – oleate 1.9 – olive oil
Adamson et al., 2003	3-D pilot-scale simulated aquifer containing sand and PCE DNAPL with HRC as substrate	*cis*-DCE, VC	Not determined
Sleep et al., 2006	2-D aquifer cell containing aquifer material and PCE DNAPL and methanol–ethanol as substrate	*cis*-DCE, VC	3.3 maximum 1.7 cumulative
Da Silva et al., 2006	3-D pilot-scale simulated aquifer containing sand and PCE DNAPL and HRC and lactate as substrate	*cis*-DCE, VC	1.6
Glover et al., 2007	Custom 5-cm flow cell containing sand mixtures (course- to fine-grained) and PCE DNAPL and methanol as substrate	*cis*-DCE	$<$1.5 to 13
Amos et al., 2008	1-D sand columns with residual PCE DNAPL and pyruvate as substrate	No significant degradation	1
Amos et al., 2008	1-D sand columns with residual NAPL mixture of 25% PCE/75% hexadecane (mol/mol) and pyruvate as substrate	*cis*-DCE	13.6 maximum 4.6 cumulative
Philips et al., 2011	Three layer diffusion cell with TCE DNAPL and formate as substrate	*cis*-DCE	2.4 maximum

Note: mol – mole(s).

It is important to remember that the enhancement factor represents the improvement of dissolution over flushing the source zone with water only. Compared to ambient conditions, active recirculation can significantly increase water movement, especially through the more transmissive portions of the source, accelerating contaminant dissolution and increasing DNAPL removal by both physical and biological processes. If multiple pore volumes of water are flushed through a source zone, even the relatively low enhancement factors

commonly measured (roughly 2–3) could significantly reduce contaminant mass in the source zone, as well as the dissolved concentrations and mass discharge from the source after flushing (ITRC, 2008). Without active flushing, ISB still can reduce source-zone concentrations and mass discharge (McGuire et al., 2006), although mass removal likely will be much slower, and a significant fraction of the contaminant mass may remain after treatment is stopped.

Note also that the enhancement factor generally is calculated as an average enhancement over a relatively large volume, typically the entire source zone. However, the degree of enhancement is likely to differ widely between different regions, based on the spatial differences in the ability to deliver reagents and move water through the subsurface. These variations can be important – for example, lower enhancement in some areas may indicate the need for additional targeted treatment.

12.2.3 Microbiology of Chlorinated Ethene Biodegradation

Several microbiological mechanisms for chlorinated ethene biodegradation exist – direct aerobic oxidation, anaerobic oxidation, aerobic cometabolism, cometabolic reductive dechlorination and direct reductive dechlorination by organohalide respiration. In addition, the microbial activities during ISB can stimulate chemical reduction of chlorinated ethenes, a process termed biogeochemical degradation (Brown et al., 2009). This chapter focuses on anaerobic biodegradation, particularly organohalide respiration, because it is the most important process for source-zone treatment. However, a brief discussion of the other processes is also presented below. More extensive information is available in reviews of the biodegradation of chlorinated ethenes (Bradley and Chapelle, 2010) and of *Dehalococcoides* and reductive dechlorination (Löffler et al., 2013b).

12.2.3.1 Aerobic Oxidation, Anaerobic Oxidation and Cometabolic Biodegradation

Direct aerobic oxidation of chlorinated ethenes is restricted to DCE and VC (Coleman et al., 2002; Hartmans et al., 1985), though VC has the greatest tendency to undergo oxidation. Vinyl chloride oxidation can occur at very low oxygen concentrations that may appear to be anaerobic (Gossett, 2010; Bradley and Chapelle, 2011). Several strains of aerobic bacteria have been found that can grow on VC, and aerobic DCE biodegradation also occurs, although to date only one strain (a *Polaromonas* strain identified as strain JS666) has been isolated and proven to be capable of growth on DCE (Coleman et al., 2002). This process is not effective for the most common parent compounds (PCE and TCE) and is therefore most appropriate for plume treatment and has not been applied to source zones.

Anaerobic oxidation of DCE and VC has been suggested by several studies that have measured mineralization of these daughter products under nominally anoxic conditions (e.g., Bradley and Chapelle, 1996; Bradley et al., 1998). However, it has proven difficult to verify that this mechanism is actually responsible for the observed disappearances, and it has not been used in engineered remediation systems, though it may contribute to losses observed during natural or enhanced biodegradation (Bradley and Chapelle, 2010).

The other important biological process affecting chlorinated solvents is called cometabolism. Cometabolism refers to a situation in which an organism can degrade a contaminant without deriving any benefit, so that for example, it can grow on one compound while fortuitously degrading the contaminant. There are two cometabolic processes affecting chlorinated solvents, termed aerobic and anaerobic cometabolism. These are described briefly in the following paragraphs.

Aerobic cometabolism of TCE has been known for over 20 years (Wilson and Wilson, 1985), and cometabolism of DCE also can occur (McCarty and Semprini, 1994), but no examples of aerobic cometabolism of PCE have been reported. The enzymes responsible for aerobic cometabolism are a variety of oxygenase enzymes that can be expressed by bacteria growing on a range of substrates including alkanes, phenol, toluene, ammonia and VC (Bradley and Chapelle, 2010). This process may be important in mixed-waste plumes where hydrocarbons are present or downgradient of anaerobic bioremediation systems where methane and oxygen mix. However, it has not been applied to treat chlorinated solvent source zones.

Anaerobic cometabolism can occur under reducing conditions, but it is a much less efficient process than the energy-yielding reduction of solvents described in the next section. From a practical perspective, anaerobic cometabolism is largely a side effect of the actions taken to stimulate the *Dehalococcoides* bacteria capable of complete dechlorination to ethene. Cometabolic dechlorinators can be important contributors to the total biodegradation achieved, and they can consume a significant fraction of the total electron donor sources added during biostimulation. Anaerobic cometabolism of PCE and TCE occurs under highly reducing conditions and can be mediated by a wide variety of organisms, including methanogens and other anaerobic bacteria (Bouwer and McCarty, 1983; Fathepure et al., 1987; Vogel and McCarty, 1985). These bacteria contain reduced transition-metal cofactors that fortuitously dechlorinate the solvents (Löffler et al., 2013b). However, the dechlorination rates decrease by an order of magnitude with each chlorine removed, so this process yields very little further reduction of DCE and VC, and little or no ethene is produced (Gantzer and Wackett, 1991). Anaerobic cometabolism can represent simply an inefficient process contributing to the overall removal of the solvents, but it also can pose a problem if it produces a sustained increase in the DCE and especially VC concentrations.

12.2.3.2 Organohalide Respiration and *Dehalococcoides*

The most important microbial process in chlorinated solvent source-zone ISB is direct energy-yielding reductive dechlorination. This process has been referred to by several terms, notably chlororespiration, dechlororespiration, halorespiration and dehalorespiration. The preferred term for the reductive dehalogenation of chlorinated ethenes, as well as similar metabolic processes responsible for the degradation of a wide variety of halogenated compounds, is organohalide respiration (Löffler et al., 2013b). This term refers to the fact that the organisms "breathe" organohalide compounds such as chlorinated ethenes, using them as electron acceptors in the same way that mammals use oxygen (McCarty, 1997).

Organohalide respiration of chlorinated ethenes is restricted to a few genera of bacteria, and respiration of DCE and VC is so far known to be mediated only by strains of *Dehalococcoides mccartyi* (Löffler et al., 2013a). The organisms capable of dechlorinating PCE to *cis*-1,2-DCE include the first such bacterium isolated, *Dehalobacter restrictus* (Holliger et al., 1993; Holliger et al., 1998). Some *Dehalobacter* isolates in fact require PCE or TCE as electron acceptors. Several other PCE-to-*cis*-DCE-dechlorinating bacteria have been identified, including strains of *Desulfuromonas* (Krumholtz et al., 1996; Sung et al., 2003), *Geobacter lovleyi* (Sung et al., 2006), *Sulfurospirillum multivorans* (Luitjen et al., 2003) and *Desulfitobacterium* (Maillard et al., 2005).

The first bacterium known to dechlorinate PCE to VC and ethene was originally named *Dehalococcoides ethenogenes* strain 195 (Maymó-Gatell et al., 1997). Strain 195 grows with PCE, TCE, *cis*-DCE, and 1,1-DCE electron acceptors. However, Strain 195 does not use VC as an electron acceptor and only slowly dechlorinates VC by a cometabolic process (Maymó-Gatell et al., 2001). Later studies have identified other *Dehalococcoides mccartyi* strains that more

rapidly dechlorinate PCE to ethene and can use VC directly as an electron acceptor (He et al., 2003; Sung et al., 2006; Müller et al., 2004). In addition, several mixed cultures have been identified that reduce chlorinated ethenes completely to ethene (Duhamel et al., 2002; Richardson et al., 2002).

Dehalococcoides strains have proven to be very difficult to isolate and grow in pure culture (Löffler et al., 2013b). As a result, typical microbiological methods including plate counts or most-probable-number methods are not reliable methods for estimating *Dhc* numbers. *Dehalococcoides* cells can be detected and counted in an environmental sample, however, based on the quantitative polymerase chain reaction (qPCR) method. The 16S subunit of the ribosomal ribonucleic acid (RNA) of the bacteria in a sample can be extracted from water or soil samples and analyzed by qPCR. Specific 16S rRNA sequences serve as a genetic fingerprint of *Dhc* (Löffler et al., 2000; Fennel et al., 2001), and these sequences have been used to monitor *Dhc* in environmental samples (Hendrickson et al., 2002).

The ability of some *Dhc* strains to respire VC depends on an enzyme, vinyl chloride reductive dehalogenase (VC RDase). Gene probes have been developed for deoxyribonucleic acid (DNA) sequences unique to this enzyme, allowing detection and enumeration of two different VC RDase genes – vcrA (Müller et al., 2004) and bvcA (Krajmalnik-Brown et al., 2004). There are other VC reducing genes that are not detected by these assays (Ritalahti et al., 2006; Scheutz et al., 2008). However, the evidence so far suggests that these markers, and particularly vcrA, are useful for characterization and monitoring at most sites (van der Zaan et al., 2010).

Dhc cells have several interesting features that suggest a highly specialized lifestyle. They require hydrogen as an electron donor and also require a reduced organic compound such as acetate as a carbon source (Löffler et al., 2013b). They do not produce their own vitamin B_{12} and must rely on other bacteria in the community to supply it (He et al., 2007). *Dhc* are not tolerant of even moderate acidity, and activity (particularly VC reduction activity) declines rapidly below a pH of about 6.0 (Vainberg et al., 2006; Fogel et al., 2009). *Dhc* cells are very small, which can be beneficial for bacteria that live on compounds that usually are present at very low concentrations, by maximizing the surface area-to-volume ratio (Duhamel et al., 2004).

The *Dhc* genome is also very small, one of the smallest known for a free-living organism, which is consistent with the high degree of specialization (Giovannoni et al., 2005). The *Dhc* strains share most of their core genes on strongly conserved regions of the genome, but also have so-called high plasticity regions that allow rapid transfer of some genes such as those that code for VC RDases (McMurdie et al., 2009). High plasticity regions may allow the native *Dhc* population to adapt to new substrates without carrying copies of rarely used genes in all of its cells.

Dhc strains may not compete effectively with other bacteria for the early steps in reductive dechlorination, but they flourish when DCE and VC reduction are occurring (Becker, 2006). Therefore, high numbers of *Dhc* in groundwater ($>10^6$ cells/L) are generally found only when complete dechlorination is occurring (Lu et al., 2006, 2009; Van der Zaan et al., 2010), although analyses of the VC RDase gene also should be used to confirm complete biodegradation potential (Ritalahti et al., 2010). Other compounds also can inhibit *Dhc* activity, notably other chlorinated ethenes and cocontaminants such as chloroform (CF) or 1,1,1-trichloroethane (1,1,1-TCA) (Maymó-Gatell et al., 2001; Yu et al., 2005).

The actions taken to stimulate biological reductive dechlorination also may cause chemical reduction of chlorinated ethenes, particularly reductive dechlorination by ferrous iron (Fe[II]) minerals (Cwiertny and Scherer, 2010). This abiotic degradation is sometimes referred to as biogeochemical transformation because it is a result of coupled biological and chemical processes (Becvar et al., 2008). Reduced minerals are formed as a result of the fermentation of organic compounds and the creation of highly anaerobic conditions, and some of these

minerals, such as ferrous sulfides, can directly reduce chlorinated ethenes (Butler and Hayes, 1999; Lee and Batchelor, 2002). This process can be important for natural attenuation (Ferrey et al., 2004), but it also can be responsible for some of the contaminant removal during ISB treatment of a source and may contribute to the sustained treatment observed after source-zone ISB (Adamson et al., 2011).

12.2.4 Practical Implications

The important points for practitioners to understand regarding the microbiology of reductive dechlorination include:

- Several different anaerobic bacteria can gain energy by rapidly reducing PCE and TCE to *cis*-1,2-DCE.
- Only some strains of *Dehalococcoides mccartyi* are known to rapidly dechlorinate all of the chlorinated ethenes to ethene.
- Efficient complete dechlorination depends on the presence of an enzyme (VC RDase). Some forms of this enzyme can be monitored using genetic probes.
- The presence and abundance of *Dhc* and VC RDases can be monitored effectively, and the numbers can be linked to the rate and extent of dechlorination (Lu et al., 2009).
- *Dhc* strains are specialized bacteria that need other organisms, and function in complex consortia, so an entire anaerobic community must be maintained for effective ISB.
- *Dhc* strains require highly anaerobic conditions (preferably methanogenic, with redox potential (Eh) values at least < -100 mV). They are inhibited by even mildly acidic conditions (pH < 6.0) and some cocontaminants (notably TCA and CF).

12.3 TECHNICAL CHALLENGES

ISB has the potential to remediate highly contaminated source zones containing DNAPL. However, several significant technical challenges are involved. The growth and activity of many dechlorinating microorganisms are inhibited by high chlorinated solvent concentrations and low pH. Effectively delivering electron donors to the contaminant can be challenging due to substrate fermentation to methane in areas with low contaminant concentrations, pore blockage with biomass and gas bubbles, and heterogeneity in contaminant distribution and aquifer permeability.

12.3.1 Toxicity

Yang and McCarty (2000) showed that some dechlorinating bacteria can survive at chlorinated solvent concentrations near the aqueous solubility, raising the potential to greatly enhance removal of the more accessible DNAPL (ganglia) and sorbed contaminants (Adamson et al., 2003). However, Amos et al. (2008) reported minimal dechlorination and no enhancement of PCE dissolution in bioaugmented laboratory columns containing PCE DNAPL and concluded that the organism used in this work (*S. multivorans*) did not tolerate saturating PCE concentrations. However when one part PCE was mixed with three parts hexadecane, the effective PCE solubility was reduced to 300 micromolar (μM) (50 mg/L), allowing extensive dechlorination of the mixed PCE DNAPL producing *cis*-DCE.

A variety of investigators have reported toxic inhibition by PCE (Huang and Becker, 2011; Amos et al., 2007a; Yu et al., 2005; Philips et al., 2011) and daughter products (Sabalowsky and

Semprini, 2010a, b; Cupples et al., 2004, Yu et al., 2005). The upper limit for PCE dechlorination appears to vary for different organisms and mixed cultures from approximately 400–800 μM (Yu et al., 2005; Duhamel et al., 2002; Amos et al., 2007a) with higher concentrations tolerated by mixed cultures that can form biofilms and aggregates and thereby provide some biomass protection (Amos et al., 2007a; Sabalowsky and Semprini, 2010b).

12.3.2 pH

Aquifer pH also can have an important impact on dechlorination. During ISB, complex organic substrates (molasses, whey, vegetable oil) and short chain fatty acids are added to the aquifer as an electron donor. The complex substrates are fermented releasing hydrogen (H_2) and acetic acid that can be used by dechlorinators to reduce more highly chlorinated compounds to less chlorinated ones. In the process, H_2 and acetic acid are consumed releasing carbonic acid (H_2CO_3) and hydrochloric acid (HCl), which can cause a drop in pH. A variety of investigators have reported low pH as a contributing factor to reduced dechlorination efficiency (Adamson et al., 2003; Eaddy, 2008; McCarty et al., 2007; Robinson et al., 2009). Inhibition from high chlorinated ethene concentrations also can be compounded by nonneutral pH conditions (Sabalowsky and Semprini, 2010a).

Many biological processes are sensitive to pH and most microorganisms function efficiently in near neutral conditions (Lowe et al., 1993). Zhuang and Pavlostathis (1995) found that neutral pH was optimum for reductive dechlorination by a methanogenic mixed culture capable of dechlorinating PCE to VC. *Desulfitobacterium dichloroeliminans* strain DCA1 has an optimal pH range of 7.2–7.8 but maintained some activity down to pH ~ 5.4 (Fogel et al., 2007). Rowlands (2004) reported that the KB-1™ bioaugmentation culture has an optimal range of 6.0–8.3 and is completely inhibited below pH ~ 5.0. A pH of 6.0–6.8 is optimum for the dechlorination of PCE by the SDC-9™ bioaugmentation culture (Vainberg et al., 2006). Using a bioaugmentation culture enriched from Savannah River Site aquifer material, Eaddy (2008) found that dechlorination of PCE and TCE slowed at pH of 6.0 with increased accumulation of *cis*-DCE and VC. At pH 5.5, reduction of *cis*-DCE to VC and VC to ethene was completely inhibited.

The pH that microorganisms are exposed to during ISB is controlled by a variety of factors including the background aquifer pH and buffering, acidity produced during ISB and alkaline materials added during ISB to control pH decline. In low rainfall areas, the background pH is often neutral to slightly alkaline. However in humid areas, the background pH may be lower than optimal for ISB because of long-term leaching by acidic rainfall. When present, naturally occurring calcium carbonate ($CaCO_3$) can neutralize some acidity, in the process releasing bicarbonate ion (HCO_3^-) and carbonic acid (H_2CO_3). Above the water table, H_2CO_3 will degas as CO_2 essentially stripping acidity from the water. Below the water table, however, CO_2 may not be able to degas causing a buildup of dissolved carbonate (CO_3^{2-}) and HCO_3^-, stopping $CaCO_3$ dissolution. If $CaCO_3$ does not dissolve, it will not be effective in buffering pH (McCarty et al., 2007). Alkalinity can be produced by the reduction of nitrate (NO_3^-), sulfate (SO_4^{2-}) and iron hydroxides ($Fe(OH)_3$). Ion exchange to clays and iron hydroxides can strongly buffer pH. If the pH is near neutral, sorption of H^+ to clays and iron oxides can limit pH declines. However if the pH is already low, large amounts of base may be required to raise the pH because of the large amount of H^+ and other acidic materials sorbed to the aquifer material.

Large amounts of base may be required to maintain neutral pH during ISB. McCarty et al. (2007) calculated that 800 mg/L of bicarbonate alkalinity would be required to reduce the acidity produced during reductive dechlorination of 1.2 mM of TCE using 0.9 mM glucose as the electron donor. According to this calculation, ISB will require approximately six times more

bicarbonate than electron donor. Adding these large amounts of alkalinity can be a logistical challenge and can increase the dissolved solids concentration of the groundwater significantly.

A variety of alkaline materials are available to control pH declines during ISB including hydroxides (NaOH, KOH, $Ca(OH)_2$ and $Mg(OH)_2$) and carbonates (Na_2CO_3, $NaHCO_3$ and $CaCO_3$). Sodium hydroxide and KOH are very soluble, so large amounts of alkalinity can be added to an aquifer. However, concentrated solutions of NaOH and KOH have pH $>$ 13, which is also inhibitory to bacteria. Calcium hydroxide and $Mg(OH)_2$ are much less soluble; thus, water in equilibrium with these materials will have pH values of approximately 12.5 and 10.5, respectively. The lower solubility of these materials, however, makes them more difficult to distribute in the subsurface. As described above, $CaCO_3$ is relatively insoluble, making it a relatively ineffective for controlling pH. Mixtures of Na_2CO_3 and $NaHCO_3$ can be prepared to have a pH between 8.3 and 10, making them useful for ISB. However, carbonates provide relatively little alkalinity per unit mass, so large amounts of these materials are required.

12.3.3 Substrate Delivery to the Contaminant

Effectively delivering the organic substrate/electron donor can be a major challenge and may limit ISB efficiency. For example, Sleep et al. (2006) studied DNAPL ISB in two 2-D boxes packed with homogenized aquifer material with DNAPL source zones containing 10 milliliters (mL) of neat PCE. Each box was flushed with plain groundwater for 112 days followed by electron donor solutions containing methanol, ethanol and/or acetate. One of the boxes was bioaugmented with the KB-1™ culture, while one was not bioaugmented. In the bioaugmented box, the sum of PCE, TCE, *cis*-DCE, VC and ethene reached a maximum of 3.0 mM immediately downgradient from the DNAPL source zone indicating a solubility enhancement of 3.3.

The PCE removal declined after 681 days, even though approximately 35% of the initial PCE DNAPL was still present in the box. The substantial decline in PCE removal corresponded to an increase in methane production, suggesting that electron donor delivery to the DNAPL was being limited by consumption by methanogens or pore blockage by biomass and/or gas bubbles. Numerical simulations by Chu et al. (2003) indicate that pore blockage may divert substrate away from the contaminant, reducing ISB efficiency. In the field, substrate delivery will be further complicated by spatial variations in aquifer permeability. Numerical model simulations presented later in this chapter indicate that spatial variations in permeability, and the resulting potential for much of the subsurface flow to bypass some contaminated regions, may significantly reduce ISB efficiency.

12.4 ADVANTAGES AND LIMITATIONS

Source-zone ISB has several advantages that have made it a popular technology for appropriate sites and remedial objectives. However, it also has important limitations that often are not fully appreciated by practitioners. The advantages and limitations are summarized in the following sections.

12.4.1 Advantages

The advantages of ISB for treating chlorinated solvent source zones include the following:

- *Low cost* relative to certain other technologies, particularly capital costs. ISB has proven to be less costly than other common source treatment technologies, notably ISCO and *in situ* thermal treatment (ISTT) (McDade et al., 2005). Cost is typically site specific, however, and economies of scale can benefit certain technologies.

- *Similar performance to other injection-based technologies*, in terms of reductions in groundwater concentrations and mass discharge. The performance of ISB is typically assessed by monitoring groundwater concentrations of the chlorinated ethenes for some period of time following treatment. By this measure, ISB is expected to yield reductions of roughly one to two orders of magnitude (90–99% reductions) in total concentrations within the source zone at appropriate sites, although the total mass removed by some technologies such as ISTT is generally greater (McGuire et al., 2006). The long-term performance and the reductions in the overall restoration timeframes due to ISB or other source treatment technologies are difficult to evaluate at this time (ITRC, 2008).
- *Flexible design and operations.* ISB is adaptable to a wide variety of site conditions, and the ability to modify or expand the treatment system allows an adaptive management strategy that responds to the inevitable uncertainties while treating source zones. The ISB designs can be adapted to accommodate site infrastructure (buildings and piping) and operations can be adapted to respond to interim results (pulsing or reversing water flow, adjusting electron donor concentrations, adding wells to treat stagnant areas).
- *Ability to treat other cocontaminants.* ISB may treat other contaminants mixed with the chlorinated ethenes, including other solvents (dichloroethane and carbon tetrachloride) as well as other oxidized contaminants of concern that can be found with chlorinated solvents (e.g., hexavalent chromium, perchlorate and some explosives).
- *Ability to combine with other technologies.* As mentioned earlier, ISB may be used in combination with several other treatment technologies as part of a combined remedy. This feature can be particularly valuable for source zones because rarely can any one technology alone result in site closure.
- *Robust treatment.* ISB has proven robust with respect to upsets. Once biomass is built up, the system can handle downtimes and temporary changes in the operating conditions with little effect on the performance, and there is generally ample time to respond to upsets.

12.4.2 Limitations

The key limitations in using ISB to treat chlorinated solvent source zones include the following:

- *Possible increases in VC concentrations.* Temporary or even long-lasting increases in VC concentrations may be a concern at some sites. Such increases raise concerns regarding vapor intrusion into nearby buildings, exposures to workers or the public, and the potential for expansion of plumes. However, temporary increases are expected and generally can be managed successfully.
- *Relatively slow treatment.* Compared to ISTT, which may be completed within a few months of onsite treatment, ISB of a source zone may require many years of active treatment (ITRC, 2007). The time requirement is caused by both the lag time required for acclimation and growth of dechlorinating bacteria before optimal performance can be achieved, and the relatively slow contaminant removal rates possible during ISB.
- *Particularly slow treatment of DNAPL pools.* ISB is probably not appropriate for treating sources with extensive pools of DNAPL because the mass transfer from these pools can be very slow (Glover et al., 2007; ITRC, 2008).
- *Sensitivity to site characteristics.* Site-specific conditions can make ISB difficult or even infeasible. Low permeability or highly heterogeneous sites may be difficult to

treat effectively because of the difficulty in delivering substrates throughout the aquifer. Treatment depths may be limited by the drilling capabilities or by cost. Other site-specific problems might include the presence of competing electron acceptors, inhibitory cocontaminants or geochemical conditions such as unfavorable pH values.

- *Possible microbiological limitations.* Microbial populations capable of complete reductive dechlorination may not be present at a site, or they may be unable to compete with other microbial populations, causing incomplete degradation and accumulations of *cis*-DCE or VC. Bioaugmentation may be needed, or in some cases, aboveground treatment may be required to manage the daughter products.
- *Potential degradation of secondary water quality parameters.* Changes in pH and redox conditions as a result of bioremediation may lead to solubilization of metals such as arsenic or iron or may lead to an increase in the total dissolved solids. Acidification may be a particularly difficult issue that limits biodegradation and requires additions of buffer. The biodegradation process may cause harmful byproducts such as methane or hydrogen sulfide to be produced and remain in the subsurface.
- *Biofouling may cause operational difficulties.* Biofouling of wells may occur, as large concentrations of electron donors cause microbes to proliferate around injection or extraction wells. This biofouling can be controlled in many cases, but often causes increased costs and operational problems (Geosyntec, 2005).
- *Potential inhibition due to acidification.* As discussed earlier, *Dhc* cells are sensitive to pH, especially acidity, and in particular, the final reaction of VC reduction does not seem to occur below a pH of approximately 6.0. The potential for acidification should be considered carefully when selecting an electron donor source and when operating and monitoring the system.
- *Potential for rebound.* Although rebounds in contaminant concentrations within the source zone are not typically observed after ISB treatment, a long post-treatment monitoring record has not been kept, and there is reason to suspect that rebound could eventually occur at some treated sites (Adamson et al., 2011).
- *Potential changes in permeability.* Changes in permeability may occur during ISB for several reasons including (1) biomass clogging, (2) mineral precipitation in pores and (3) mobilization of colloidal particles. Biomass can grow near DNAPL accumulations and divert groundwater around these areas, at least temporarily. Such clogging on a local scale was observed during the ISB demonstration project at Dover Air Force Base (Section 12.10.2) and complicated interpretation of the results (Lebrón et al., 2007). Creation of reducing conditions can cause precipitation of several types of minerals, notably iron sulfides. Colloidal particles could potentially be mobilized by the reduction of iron oxides or changes in interparticle bond strength, but no field evidence indicates that this process represents a significant issue.

12.5 IMPLEMENTATION OPTIONS

ISB is a flexible technology. A wide range of electron donor sources can be used, and they can be delivered in a variety of ways. The technology can be used for different purposes and in different ways, depending on the goals and site characteristics (Figure 12.4 depicts the general design options). For source zones, ISB can be used as the primary technology or as a polishing

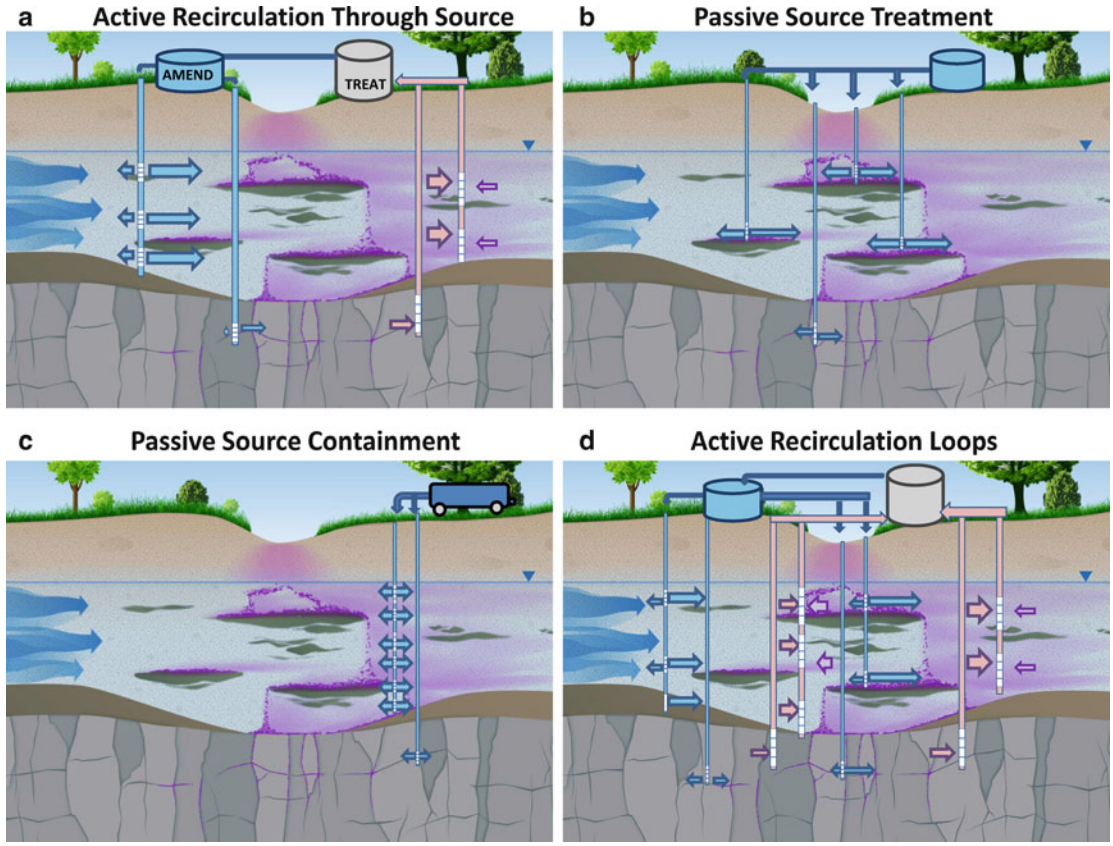

Figure 12.4. Possible designs for ISB systems to treat or contain a source zone. Active systems (a and d) rely on constant or frequent injections of water-soluble rapidly degraded electron donors, generally with some aboveground treatment of extracted water prior to reinjection. Passive systems (b and c) use one-time or infrequent additions of slowly solubilized electron donors.

technology. It can be implemented in a very active manner or in a much more passive approach. Bioaugmentation may or may not be used. Finally, ISB can be combined with other technologies to exploit synergies between biological and non-biological processes. These options are briefly described in the following sections.

12.5.1 Primary or Polishing Technology

ISB may be used as a primary treatment technology for some source zones. It can be very effective for treating chlorinated solvent source zones in which the contamination is present primarily in ganglia or sorbed to the aquifer matrix. However, it may not significantly reduce the cleanup time for sites with a significant fraction of the contamination present as pools (Christ et al., 2005; Table 12.3). Originally, many people assumed that ISB was useful only as a polishing technology following more aggressive technologies such as ISTT. Experience has shown, however, that ISB can be effective even at sites with DNAPL present (ITRC, 2007), and the ability to inject long-lasting electron donors such as vegetable oil can allow the use of ISB to control even high-strength sources and slowly remediate them over several years (ITRC, 2008; Borden et al., 2007).

Table 12.3. Impact of Pool Fraction on Source Longevity Without Treatment, With *In Situ* Bioremediation (ISB) Alone, or With ISB After Surfactant Flushing (Modified from Christ et al., 2005; Reproduced with Permission from *Environmental Health Perspectives*)

Scenario	Source Longevity (years)		
	Natural Gradient Dissolution	Source-Zone Bioremediation	SEAR[a] + Biopolishing
1. No Pools (GTP $= \infty$, PF $= 0$)[b]	36	7	0.01
2. Low PF (GTP > 1, PF < 0.5)	54	11	0.01
3. High PF (GTP < 1, PF > 0.5)	245	50	24
4. Pools Only (GTP $= 0$, PF $= 1$)	817	163	157

[a]Surfactant Enhanced Aquifer Remediation (4% Tween® for 10 days, assumed to remove 99.9% of the PCE mass
[b]Ganglia to Pool Ratio and Pool Fraction (0–1.0)

12.5.2 Active or Passive Treatment

Implementation of ISB can occur in several ways. The most fundamental distinction between different ISB methods is whether the treatment is considered *active* or *passive* (Stroo and Norris, 2009). Active treatment uses recirculation of fluids through the treatment zone on a continuous or near-continuous basis, with constant or frequent injections of a highly soluble electron donor solution such as lactate. In contrast, passive treatment relies on infrequent injections of long-lasting electron donors such as vegetable oils with little or no recirculation of fluids. More information on the types of electron donors available and the treatment options available is provided in AFCEE et al. (2004) and ITRC (2008).

For treating highly concentrated chlorinated solvent source zones, active treatment appears to be the most common approach (ITRC, 2008). It attempts to maximize the delivery of electron donors (and other amendments) throughout the treatment zone, leading to faster remediation. By continuously recirculating fluids through the target treatment zone, contaminant dissolution, and mixing with electron donor is enhanced. Active treatment is also highly flexible. Some or all of the injection and extraction wells can be reversed to improve delivery, additional wells can be added as needed to target stagnant or high-strength areas, and the concentrations and type of electron donor can be changed to respond to changing site conditions. The principal negatives include the relatively high cost for constant operations and maintenance (O&M) and the potential for biofouling of wells.

Passive treatment is a slower process that relies largely on the slow migration of electron donors following injections. Distribution also may be more limited than active treatment, often requiring a larger number of injection points or wells. As a result, passive treatment approaches may be more expensive if drilling costs are high. There is also less opportunity to respond to uncertainties or changing conditions. However, passive treatment largely avoids the problems due to biofouling, and the potential for degradation of secondary water quality parameters may be much lower. Finally, the cost is generally much less than for active treatment, and passive source-zone treatment can be successful under the right conditions and given compatible remedial objectives (Borden et al., 2007).

12.5.3 Mass Removal or Source Containment

The majority of this chapter is concerned with mass removal as the primary purpose of ISB at a source zone. ISB can also be used to contain a source zone, however, by stimulating anaerobic biological activity within and/or immediately downgradient of the DNAPL source

zone, to stop or reduce the flux of contaminants leaving the source zone. Containment is typically achieved by establishing a reactive barrier downgradient of the source, through injection of an electron donor into wells or injection points along a transect perpendicular to the groundwater flow path (see Figure 12.4). Alternatively, physical barriers may be established, with biological treatment in defined areas (so-called funnel-and-gate applications). Finally, hydrophobic electron donors such as vegetable oil may be injected into and near the source zone to sequester the DNAPL and degrade DNAPL constituents over time as they solubilize (Henry et al., 2007). Slow-release electron donors injected directly into source zones can degrade contaminants as they diffuse from lower-permeability zones, potentially enhancing cleanup rates. In contrast to mass removal applications, effective containment typically focuses on achieving and sustaining complete reductive dechlorination of all chlorinated ethenes in the aqueous phase.

12.5.4 Biostimulation or Bioaugmentation

Biostimulation relies on stimulating the native microbial population through creating appropriate conditions for their growth and activity (neutral pH, Eh < -100 mV, available hydrogen). In many cases the native population is fully capable of efficient and complete reductive dechlorination, although it may require a lag time of several months for growth and migration *in situ* (Henry, 2010). Managers may prefer to rely on the native microbial population because of the costs associated with adding bacteria to the subsurface (bioaugmentation), and in some cases because of regulatory concerns about introducing "foreign" organisms or genes.

In some cases, however, bioaugmentation may be needed or helpful, especially for chlorinated solvent contamination (Stroo et al., 2010). Several cultures containing *Dhc* strains capable of compete dechlorination have been developed for bioaugmentation of chlorinated solvent sites (ESTCP, 2005). Bioaugmentation can ensure that microorganisms capable of complete dechlorination are present at a site, and can decrease the lag time before complete dechlorination is observed (Ellis et al., 2000; Geosyntec, 2007; Lebrón et al., 2007). Models useful for designing bioaugmentation systems have recently been published (Schaefer et al., 2009a).

Bioaugmentation may be particularly useful for source-zone remediation for two reasons. First, the O&M costs for active ISB treatment of a source zone can be relatively high, and therefore any time lost due to inefficient treatment can be costly. Second, the fact that the contaminant concentrations are much higher than in the plume means that the potential for VC accumulations and exposure of humans to VC is greater, and bioaugmentation can reduce the magnitude and duration of any VC accumulation. Bioaugmentation also may be useful in treating any residual contamination after aggressive source-zone treatment technologies, such as ISTT, that may kill most or all of the *Dhc* in the target treatment zone (Friis et al., 2006). While deciding whether to bioaugment can be a difficult decision in some cases, the development of molecular biological and stable isotope techniques, and the experience from field-scale use, has improved the decision-making process (Stroo et al., 2013).

12.5.5 Combining ISB with Other Technologies

ISB is well suited to combinations with other technologies, either separated in time or space, or even applied together to exploit useful synergies. Perhaps the first example of combining ISB with another technology was the use of residual surfactants or cosolvents as electron donors (Mravik et al., 2003; Ramsburg et al., 2004). Since it is difficult to remove all of these chemicals from the subsurface after their use to enhance flushing of NAPLs, it makes sense to biodegrade these materials *in situ* and to take advantage of the fact that they can be fermented to produce

hydrogen and to create a reducing environment and thereby stimulate reductive dechlorination (Amos et al., 2007b). However, these materials have proven to be expensive for direct treatment of DNAPL source zones and can have other limitations, such as the potential for enhancing DNAPL migration (NRC, 2005), so this combination has found little commercial use to date.

ISB also is well suited to use after more aggressive DNAPL recovery or destruction technologies, and such treatment trains may have a useful synergy. Significantly, recovery or destruction of DNAPL as an initial step will reduce the subsequent electron donor demand, making ISB more effective and cost-effective. For example, recovery of DNAPL pools using hydraulic displacement (Chapter 8) can be a valuable first step in that the DNAPL-water surface area available for mass transfer is increased, and electron donor demand is reduced. Other treatment technologies also will remove some of the electron donor demand, but may have other potential interactions worth considering.

In situ chemical oxidation may be compatible with ISB because it does not entirely sterilize the treated zone, and the site can be recolonized naturally, or bioaugmented if necessary (Sahl and Munakata-Marr, 2006). In some cases, partial oxidation of native organic compounds may stimulate subsequent biological activity by increasing the dissolved organic carbon (DOC) in the groundwater (Sahl et al., 2007). Most source-zone treatments with ISCO will require additional treatment (Krembs et al., 2010); therefore, ISB after ISCO may be an attractive combination, offering reductions in contaminant mass, followed by sustained biodegradation of the residual contaminants (Hrapovic et al., 2005; Sutton et al., 2010). However, there may be some concerns for subsequent *Dhc* activity, notably the rapid pH changes that can occur, especially after ISCO treatment in poorly buffered systems (Munakatta-Marr et al., 2011). If permanganate is used as the chemical oxidant, substantial amounts of solid phase MnO_2 may be present, requiring additional electron donor to achieve strongly reducing conditions. It often is prudent to wait for some period (months to years) for the geochemical conditions to re-equilibrate after ISCO.

In situ thermal treatment also may be combined with ISB. Although ISTT can leave the subsurface near-sterile, recolonization or bioaugmentation (after electron donor addition) can successfully reestablish reductive dechlorination (Friis et al., 2005). In fact, ISTT may stimulate biological activity after treatment or downgradient from the heated area by increasing the electron donor availability (Costanza et al., 2009; Fletcher et al., 2011). The temperatures typically achieved during ISTT (between 100°C and 300°C) are lethal to *Dhc*, but mild heating (30°C to as much as 40°C) may increase *Dhc* growth and reductive dechlorination rates (Friis et al., 2007). Note that it may take several months after ISTT for the subsurface to cool sufficiently for reductive dechlorination to proceed.

Some interest also has been shown in combining ISB with electrokinetics to deliver electron donors into lower-permeability materials (Gent et al., 2001). The concept takes advantage of the ability of electrokinetic remediation systems to preferentially move charged compounds into and through low-permeability materials (Acar et al., 1995). This technique is discussed further in Section 12.12.1.

To date the most commercially successful combination of ISB with another technology has been the combination of *in situ* chemical reduction (ISCR) and ISB. Commercially available materials combine a chemical reductant such as microscale zero-valent iron (ZVI) with electron donors. A material has been developed by the U.S. National Aeronautics and Space Administration (NASA) that combines emulsified edible oil and nanoscale ZVI to target DNAPL source zones (EZVI). In fact the material combines three processes – chemical reduction, biodegradation and partitioning of the DNAPL into the emulsified oil (Quinn et al., 2005). Field testing has indicated EZVI can be effective when delivered successfully. However, effectively delivering the EZVI to the contaminant can be challenging (O'Hara et al., 2006).

12.6 DESIGN CONSIDERATIONS

Designing an ISB system for a source zone involves balancing several interrelated factors (Figure 12.5). Good remediation designs require careful consideration of the site conditions and the available alternatives for implementing any technology. The flexible nature of ISB allows for many options. The design should be based on as complete a conceptual site model (CSM) as possible, one that includes an understanding of the local hydrogeology, the source-zone architecture (an estimate of the amounts of DNAPL in pools and ganglia) and the contaminant distribution between sorbed and DNAPL phases and between regions of differing permeability. Given the importance of delivery for ISB success, the CSM should also include an understanding of the local lithology and the degree of heterogeneity, as well as the adsorptive capacity of the subsurface materials.

An understanding of these subsurface characteristics is important when evaluating the materials that can be used as chemical amendments (particularly the electron donors and pH buffers). The donors and other amendments selected in turn strongly impact the injection and delivery strategy (passive or active treatment) and the design parameters (notably the spatial and vertical distributions of the injection and extraction wells and the well screenings). Finally, the microbiological and biogeochemical conditions should be evaluated before and during startup to determine whether bioaugmentation will be beneficial and/or to modify the design prior to full-scale operation. These issues are discussed briefly in the following sections.

12.6.1 Site-Specific Challenges

Each site poses a unique set of challenges for a source-zone ISB system. These include the site features, as well as regulatory, public, infrastructure and characterization challenges (Simpkin and Norris, 2010). The site features that can present major technical challenges can be classified as results of the subsurface hydraulics, lithology, contaminant distribution, geochemistry, cocontaminants or microbiology of the site. These technical challenges are described briefly in the remainder of this section.

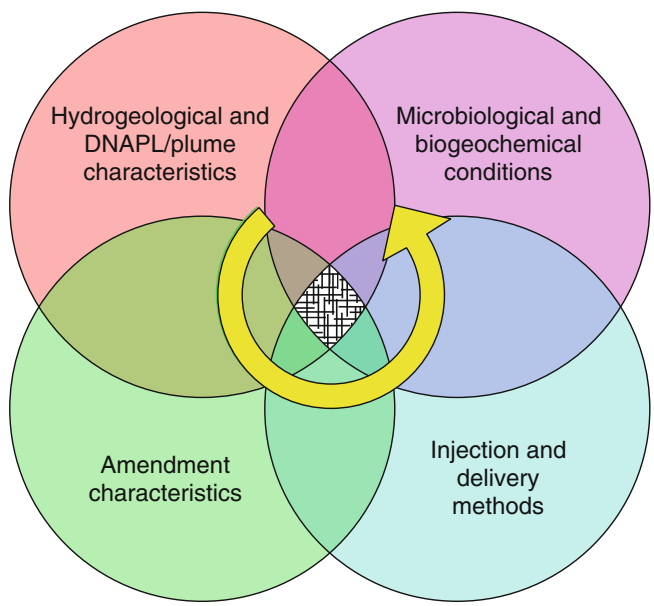

Figure 12.5. Design considerations for implementing ISB of chlorinated solvent source zones.

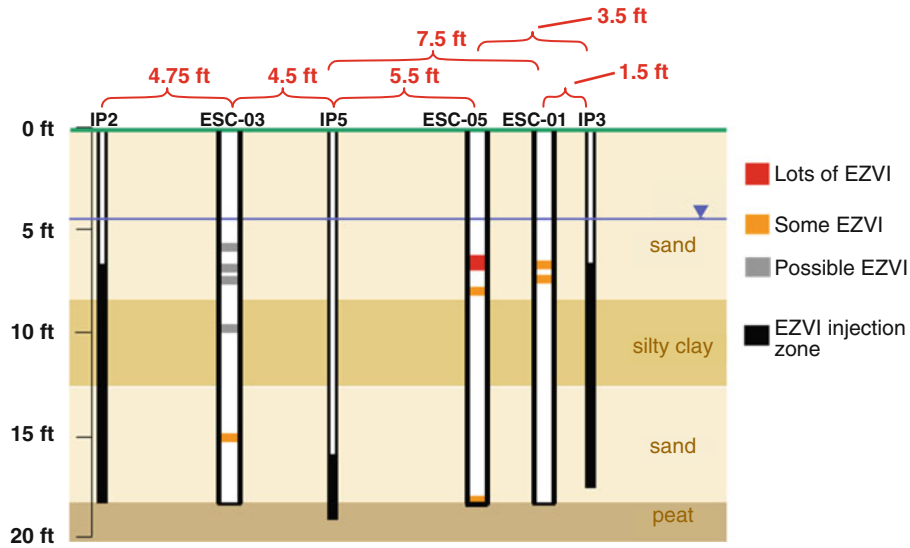

Figure 12.6. Visual observations of the distribution of emulsified oil:ZVI mixture (EZVI) during field demonstration at Cape Canaveral site. Most of the readily apparent black iron particles were found in only a small fraction of the total volume in extracted soil cores (ESC-01, 03, and 05) located near the injection points (IP 1, 2 and 3) within relatively thin sandy layers. Graphic provided courtesy of Geosyntec, Guelph, Ontario, Canada.

Potential hydraulic problems include the presence of preferential pathways and the relatively stagnant zones where little advective flux occurs. Preferential pathways can strongly limit delivery of amendments, even in aquifers in unconsolidated materials lacking clear lenses or fractures that obviously dominate flow. One of the best visual examples of the difficulties faced when injecting electron donors into the subsurface is from the demonstration of EZVI performed at Cape Canaveral in relatively permeable and homogeneous materials (Figure 12.6). In this case, the EZVI material was black, so the injected material could be easily observed in cores taken at varying distances from the injection points. The results from the core observations demonstrate that the vast majority of the material traveled through a very small proportion of the total volume, and any effects are likely to be spatially limited, at least initially (Quinn et al., 2005). The related hydraulic issue is the presence of stagnant zones, where DNAPL treatment is much slower and less effective, because amendments must reach the contaminated areas primarily through diffusion.

Contaminant distribution is almost always a difficult challenge, particularly since most chlorinated solvent sites have been contaminated for more than 20 years, allowing time for slower processes to affect the distribution. Effective treatment is particularly challenging if contaminants have migrated over time into regions with low permeability or poor accessilibity (Feenstra et al., 1996; Chapman and Parker, 2005). In such cases, delivering remedial agents to these zones via injection is difficult, and treatment will be relatively slow and incomplete because it will depend upon the rate of back diffusion. Another aspect of contaminant distribution is the source architecture (Lemke et al., 2004; Fure et al., 2006). Ganglia, particularly those in regions with higher permeability, are much more rapidly depleted than pools, because of the large differences in the surface area:volume relationships. Consequently, even enhanced dissolution by ISB will take a considerable time to remove pooled DNAPL accumulations.

The most common geochemical challenge, beyond overcoming the electron donor demand, is the pH and buffering capacity. Although conditions can be too alkaline for effective

reductive dechlorination, acidity is by far the more common issue. Even slightly acidic conditions (pH < 6.0) can strongly inhibit the complete dechlorination to ethene, and partial dechlorination is inhibited below pH 5.5. Poorly buffered sites easily can become too acidic for dechlorination as fermentation of the electron donors proceeds. Modifying the pH of a significant volume of the subsurface is possible, but it can be costly and difficult. The other common geochemical concern is excessive sulfate, which is inhibitory because sulfate reducers compete with dechlorinators for hydrogen (Heimann et al., 2005). When sulfate concentrations are very high, sulfides can accumulate inhibiting reductive dechlorination by *Dhc* (He et al., 2005).

12.6.2 Amendment Alternatives

Several electron donor formulations have been developed over the last decade, and this rapid development is continuing. The first donors used were lactate and the lighter volatile fatty acids such as butyrate. Lactate is still probably the most commonly used donor for source-zone treatment because it is soluble (so it can be delivered through the source zone via recirculated water), it breaks down to provide both acetate and hydrogen, it is relatively inexpensive and the dosage can be adjusted to respond to site conditions and treatment responses. Other sources have been used including vegetable oils, molasses, and other soluble carbohydrate materials, alcohols (ethanol and methanol), whey, chitin and slow-release forms of lactate, notably Hydrogen Release Compound (HRC®). The properties and characteristics of these materials are summarized in Table 12.4. For source-zone ISB, the soluble materials, especially lactate, are probably the most commonly used, followed by the semisoluble vegetable oils and HRC®.

The soluble electron donor sources (alcohols, fatty acids, sugars) are compatible with either recirculation or direct injection techniques. These materials are inexpensive on a per-volume basis, and the ability to circulate them is a key advantage for source-zone treatment in particular. Contaminated regions distant from injection points can be treated more effectively by constant or near-constant recirculation than by injections, particularly if techniques such as pulsing or reversals in direction are used. Significantly, the active distribution and the extended duration of treatment result in sustained high concentration gradients between the more transmissive and the less permeable regions, promoting the migration of electron donors into the less permeable regions and thereby enhancing treatment of the diffused contaminants.

The less soluble sources (HRC®, vegetable oils) are compatible with passive treatment, involving one-time or infrequent injections to place the materials in the source zone. These materials provide long-term treatment, as they can persist for perhaps 2–5 years, depending on the contaminant levels and in the electron acceptor influx (AFCEE et al., 2004). The materials also may migrate to the less permeable areas following injection, and the DNAPL constituents can partition into the oil phase if these materials are used (ESTCP, 2006). However, the semisoluble electron donors will eventually be depleted, and reinjections may be needed at most sites, especially aerobic sites with rapid groundwater velocities. Slow-release electron donors are also commonly used after more aggressive source depletion or as a temporary barrier downgradient of the source-zone treatment.

It should also be noted that product development is ongoing. Recent developments have included products and procedures that combine materials, for example using lactate for initial treatment and oils for sustained treatment, or products like EZVI that seek to combine biological and chemical reduction. The distinctions between commercial electron donor sources may become less clear, and different products may well be used for different times during overall site remediation (initial treatment or reinjections to address residual contamination) or

Table 12.4. Substrates Used for Enhanced Anaerobic Bioremediation (From AFCEE et al., 2004)

Substrate	Typical Delivery Techniques	Form of Application	Frequency of Injection
Soluble Substrates			
Lactate and Butyrate	Injection wells or circulation systems	Acids or salts diluted in water	Continuous to monthly
Methanol and Ethanol	Injection wells or circulation systems	Diluted in water	Continuous to monthly
Sodium Benzoate	Injection wells or circulation systems	Dissolved in water	Continuous to monthly
Molasses, High Fructose Corn Syrup	Injection wells	Dissolved in water	Continuous to monthly
Whey (soluble)	Direct injection or injection wells	Dissolved in water or slurry	Monthly to annually
Slow-Release Substrates			
HRC® or HRC-X™	Direct injection	Straight injection	Annually to biannually for HRC® (typical); Every 3 to 4 years for HRC-X™; potential for one-time application
Vegetable Oils	Direct injection or injection wells	Straight oil injection with water push or high oil/water content (>20% 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			
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 biannually; potential for one-time application

for different areas or objectives, such as enhanced source removal using lactate vs. injections of edible oils for source containment.

Other chemical amendments also may be needed for optimal treatment. The most common amendment added is an alkaline material to raise the pH. *Dehalococcoides* are pH sensitive, and reductive dechlorination is markedly inhibited below a pH of about 6.0 (Vainberg et al., 2006; Eaddy, 2008). Given that the groundwater at many sites is poorly buffered and mildly acidic before treatment, and fermentation of the large masses of electron donors that can be needed for source-zone bioremediation can be highly acidifying, pH buffering may be needed at many sites (Robinson et al., 2009). Potential water-soluble buffering materials include calcium carbonates ($CaCO_3$), sodium salts ($NaHCO_3$, Na_2CO_3), caustic soda (NaOH), magnesium carbonates ($MgCO_3$) or magnesium hydroxide ($Mg(OH)_2$). Materials also have been formulated specifically for groundwater buffering applications (AquaBupH™, a suspension of buffer particles in emulsified soybean oil). Vitamin B_{12} and yeast extract have been considered for use because *Dhc* relies on external sources of B_{12}. However, it may not be

necessary to add these materials because other bacteria produce sufficient amounts. Similarly, inorganic nutrients may be included in commercial biostimulation products, but research has not demonstrated that they are necessary (AFCEE et al., 2004).

12.6.3 Electron Donor Requirements

The amount of electron donor that needs to be added depends on the amendment type chosen. For many slow-release substrates (EVOs or HRC), the substrate-loading rate is generally calculated on meeting the total demand from both the native electron acceptors (such as oxygen, nitrate and ferric iron) and the contaminant mass, including the continued influx of electron acceptors over the design life (AFCEE, 2007). Soluble substrates can be added frequently, so the loading rate need only achieve and sustain reducing conditions for a few days to weeks.

A spreadsheet tool has been developed to assist practitioners in determining the site-specific electron acceptor demand and the substrate required to meet that demand over the design life of the application (AFCEE et al., 2004). In addition, vendors have developed similar spreadsheets for their own materials. All spreadsheets should be used with some caution, as the uncertainty surrounding the estimates of the masses of contaminants and other electron acceptors within source zones often necessitates using large safety factors to estimate the dosage needed. In fact, it is common to include five- to tenfold increases in the initial electron donor estimates to address this uncertainty (ITRC, 2008).

It is also important to realize that other factors, such as the oil retention capacity of the treatment zone matrix, can determine the amount of electron donor that can be added at any one time (Borden et al., 2008). Lower safety factors may be justified when slow-release substrates are used for long-term control, especially when monitoring during and after a prior technology has yielded accurate estimates of contaminant mass or biodegradation rates. Lower safety factors also may be used for the soluble substrates, because the total demand can be met over numerous injections so the dosage can be adjusted in response to the operational monitoring results.

12.6.4 Injection and Delivery

The options for injection and delivery include direct injection, using permanent wells or direct-push injection points, and recirculation, using either natural gradient flow or forced recirculation. Natural gradient flow involves extracting water from one area, often clean water from upgradient of the contaminated area, amending it with electron donors and other amendments if needed and then injecting it immediately upgradient and/or within the source zone. Forced recirculation involves extraction from downgradient and injection upgradient and/or within the source.

Direct-push injection offers flexibility in the locations and depth intervals treated, but it also offers less control of the treatment area and the ability to deliver amendments. It also is likely to be less costly than establishing permanent wells, though it is important to realize that subsequent reinjections may be needed for adequate treatment. The forced recirculation strategy establishes a closed system that will reduce or eliminate groundwater influx and cause upgradient mounding and flow around the treatment area. Other options worth considering at some sites include the use of horizontal or directional wells to improve delivery, or even use of injection trenches for shallow zones.

When selecting and designing the system, one should ensure that an adequate amendment mass to treat the estimated amount of contaminant and other electron acceptors can be

delivered, that the delivery will be as targeted to the contaminant mass as feasible, and that contingency plans are identified. Source-zone characterization is inherently uncertain, and the field-scale performance of ISB can be difficult to predict accurately, so a well-designed monitoring plan is needed, with clear links to contingency actions. Example contingencies include installing additional injection points to treat possible stagnant or bypassed zones, adding amendments into previously treated areas to target residual contamination, or injecting amendments long after treatment if eventual rebound occurs. The injection point spacing, the amendment concentrations and the injected water volumes should be based on calibrated groundwater models and careful mass estimates, but contingency plans will still be needed given the uncertainties in most site characterizations.

12.7 REMEDIAL OBJECTIVES

Careful definition of the remedial action objectives is always critical, including when using ISB for a chlorinated solvent source zone. Complete mass removal and rapid site closure are not likely using any source-zone remediation technology, and ISB is less aggressive and takes more time than other source-zone technologies such as thermal treatment or *in situ* soil mixing (Kavanaugh et al., 2003). Unfortunately, near-complete removal is needed to meet typical cleanup objectives such as MCLs, and ISB is unlikely to meet such criteria (Wright et al., 2010; ITRC, 2008). Therefore, it often is necessary to develop less stringent *functional* objectives for source treatment, such as mass discharge reduction or removal to the extent that monitored natural attenuation (MNA) can be implemented reliably (NRC, 2005). Some of these functional objectives are relatively easy to achieve using ISB, while others are more difficult, so it is important to develop realistic performance expectations when selecting the technology.

Figure 12.7 provides an initial screening-level evaluation of the ability of ISB to meet common remedial objectives for a source zone. The most common goal is probably to meet concentration criteria at a downgradient location after ISB is completed. This objective is difficult to assess in general, as it is highly dependent on site-specific factors. Based on experience to date, it seems reasonable to expect 90–99% reductions in concentrations immediately downgradient from the source. Similar reductions may be expected eventually at points further downgradient, although it may be less pronounced and take longer to measure if the travel time is significant and if there is significant sorbed contamination between the source and the compliance point.

A very common goal of source-zone ISB is *mass removal* and is based on the total contaminant mass removed or destroyed as a fraction of the estimated original mass. Unfortunately, this objective is often hard to measure accurately, given the uncertainties in most starting source mass estimates. Generally it is possible to measure the direct removal in extracted water, but estimates of mass destroyed are less precise, usually relying on total chloride increases and daughter product concentrations. Estimates of mass removal that have been made indicate more than 90% of the original mass can be removed under favorable conditions (McDade et al., 2005; GeoSyntec, 2004). In many cases, the goal is simply to remove mass to the extent practicable, although many would argue that the goal should be to remove sufficient DNAPL from the source zone to make a significant difference in the future site care requirements (for example, to allow MNA after ISB).

Less commonly, the primary goal of ISB is *flux reduction* (i.e., reductions in the mass discharge from the source and/or the concentrations in groundwater leaving the source zone). In terms of the risks to potential downgradient receptors, flux reduction is a more relevant metric than mass or concentration reductions, and interest in this approach is therefore increasing (ITRC, 2010; Cai et al., 2012). The flux reduction may be more or less than the

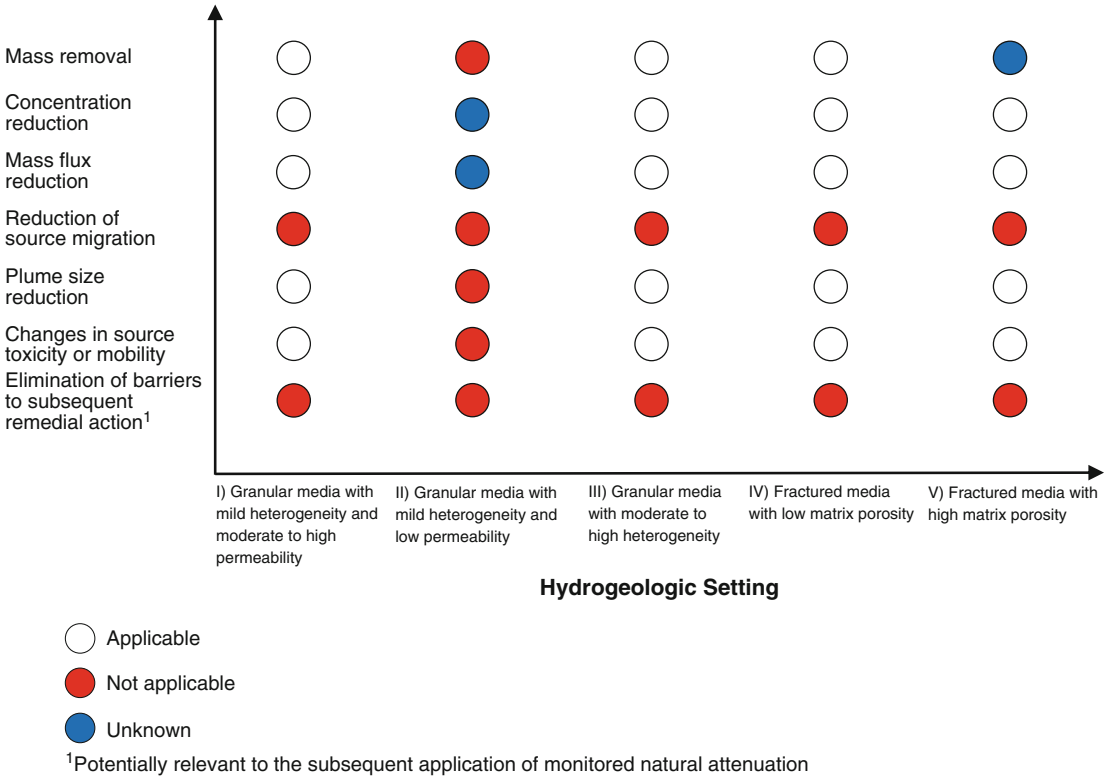

Figure 12.7. Ability of ISB to meet various remedial objectives for source-zone treatment in different hydrogeological settings (modified from ITRC, 2005).

mass removal, depending largely on the DNAPL architecture (Basu et al., 2008). However, if >90% of the mass can be removed, it is likely that a similar reduction in concentrations or discharge should be achieved as well (Sale and McWhorter, 2001; Stroo et al., 2003; Wood et al., 2004).

Plume life reduction is often the assumed goal of source depletion. That is, it is assumed that source depletion will reduce the plume longevity to a more reasonable timeframe (e.g., from centuries to <30 years). However, the lifetime of the dissolved phase plume may not be reduced nearly as much as the mass removal might suggest, because plumes tend to exhibit first-order decreases over time, and storage of contaminants in the plume (sorbed phase, diffused mass) can sustain the plume even after the source zone is completely removed or contained (Sale and Newell, 2010).

A related goal that is often the objective of the responsible parties is *life cycle cost reduction* (generally expressed not as total costs but as net present value). This objective can be difficult to define or estimate, given the limited history of any aggressive source depletion efforts. Plumes will continue to require some form of containment for long periods of time following most source depletion efforts, and the long-term costs for these containment technologies can represent a substantial fraction of the life cycle costs, even for MNA. The clearest opportunities to reduce life cycle costs through treatment are at sites where source depletion will allow practitioners to turn off an existing pump-and-treat system and rely on MNA for post-treatment site management. Of course in some cases the rate of spending may be as important, or even more important, than the total life cycle costs, and these economic drivers can affect the decision to treat the source zone as well as the choice of the technology.

12.8 PREDICTING PERFORMANCE

Although the relative contributions of the various mechanisms resulting in mass removal and concentration/flux reduction at a particular site of interest are not always clear, some level of performance prediction is often required as part of ISB remedial designs and remedy evaluations. Past experiences are of value, but mathematical model predictions are often useful. Models provide guidance when selecting ISB initially, when designing the ISB remedy, when deciding whether and how to modify ISB systems during operations, when establishing performance expectations regarding timeframes for mass removal and concentration/flux reduction, and when designing post-remedy monitoring programs. The remainder of this section provides an overview of modeling of source-zone ISB.

Modeling to predict ISB performance generally can be performed using either a simple screening-level model or a more sophisticated model that incorporates several of the important hydraulic, chemical, and biological processes discussed in this chapter. The level of sophistication appropriate for the model chosen will depend on the site conditions, level of existing information, and the modeling objectives. Screening-level models are appropriate for limited resources and information and are often analytical models based on exact solutions to a series of equations. More complex models, requiring more site-specific information and greater resources to develop and use, are generally numerical models (models using numerical time-stepping procedures that allow more realistic models of greater complexity than can be described by analytical models). Modeling is discussed in greater detail in Chapters 5 and 6.

The screening-level models used for simulating natural or enhanced biodegradation are typically based on the concept of first-order decay. First-order decay, which is commonly observed in biodegradation studies, is represented as:

$$C = C_0 e^{-\lambda t} \qquad \text{(Eq. 12.1)}$$

where C is the contaminant concentration of interest at time t, C_0 is the initial concentration, and λ is the decay constant. The decay constant is related to the contaminant concentration half-life ($t_{1/2}$) through:

$$t_{1/2} = \frac{0.693}{\lambda} \qquad \text{(Eq. 12.2)}$$

It is important to point out that using first-order decay to represent biodegradation in a mathematical model is a simplification of what actually occurs. In fact, the assumption of first-order biodegradation kinetics may not apply in all cases, even under controlled laboratory conditions (Simkins and Alexander, 1984), though it often provides a useful approximation of measured degradation rates. Simple first-order decay incorporated into the standard advection–dispersion equation results in a model that can compute decreases in concentration, but cannot explicitly account for processes such as lag, rate of biomass growth, competition, electron donor utilization, and other variables. Nevertheless, the use of analytical and numerical models based on simple first-order decay is prevalent at the present time, and they can provide useful screening-level predictions, even though they are subject to considerable uncertainty.

The most widely used analytical model for chlorinated solvent biodegradation is BIOCHLOR, which has proven useful for many situations. However, BIOCHLOR has significant limitations (West et al., 2008), and recent efforts have focused on improving analytical modeling of chlorinated solvent biodegradation (Burnell et al., 2012). The recent REMChlor model is an analytical model designed to evaluate source treatments, including ISB (Falta et al., 2005).

One source of uncertainty associated with a model based on first-order decay is the adopted contaminant half-life (or decay constant). The half-life is site-specific and can vary

considerably with time and location at any particular site. These issues are often overlooked, and their effects are difficult to quantify. Suarez and Rifai (1999) report half-lives ranging from less than a day to many years for chlorinated ethenes in groundwater. Consequently, relying upon literature sources alone to predict ISB performance is not recommended except at the most basic level of screening. Most remedial designs should rely on site-specific evaluations of half-lives, through model calibration, microcosm studies or column studies (Newell et al., 2002).

An alternative to employing a simple analytical or numerical model based on first-order decay to predict ISB performance is to employ a multiphase/multicomponent reactive transport model. The advantage of employing such a model includes the ability to simulate specific processes of interest such as rate of biomass growth, competition, rate of electron donor utilization, etc. The challenge in employing such sophisticated models stems primarily from the need for site-specific values of a number of coefficients and rate constants, many of which are difficult to obtain in a field setting.

In addition to adopting some level of solute transport modeling to predict ISB performance, it is important to perform groundwater flow modeling to properly assess and design aspects of ISB implementation such as flow from injection wells to extraction wells within closed-loop systems, capture associated with extraction wells, and lateral spreading associated with the delivery of amendments from single-well injections. Groundwater flow modeling is typically performed as part of ISB design, but the model should also be continuously updated and employed as ISB remedy implementation takes place.

One recent approach to numerical modeling (a model called DNAPL3D-RX) is highlighted in the following section, to provide greater detail and insight into the key modeling issues. This modeling effort suggests that ISB may not remove all of the contaminant mass from a source zone and that rebound may occur after ISB at some sites, although it may not occur for months to years following the cessation of active treatment. The rebound is primarily attributable to the dissolution of DNAPL remaining at the end of active treatment. Furthermore, although active ISB treatment for 2–3 years can reduce mass discharge downgradient for several years after treatment is stopped, it may not provide a long-term improvement over natural dissolution alone. These conclusions suggest that practitioners should run such predictive models prior to selecting and designing ISB systems, carefully consider the potential for eventual rebound and include contingency plans to ensure long-term protectiveness.

Practitioners also should be aware that the uncertainty when modeling ISB performance can be considerable. Key sources of uncertainty include the following:

- The assumption of first-order kinetics, which can be a useful approximation but often ignores real-world complexities
- The difficulties in characterizing the spatial variability of hydraulic conductivity that will govern groundwater flow directions and rates of substrate delivery
- The potential for treatment-induced effects such as bioclogging that alter conditions during and after treatment
- The complexities of the subsurface microbial ecology, in which numerous interacting populations change over time and space
- The uncertainties regarding contaminant mass distribution, particularly mass in less permeable regions and mass in DNAPL pools
- Variation in degradation rate over time in response to electron donor addition and changing geochemical conditions
- Difficulty in extrapolating from degradation rates observed in laboratory-scale design studies to those actually achieved during field implementation

Nevertheless predicting ISB performance is critical, and the results can be highly useful in designing the ISB system, developing realistic expectations for ISB performance, and deciding when to stop active treatment or adjust the system to optimize performance. Importantly, the models employed in predicting performance must be updated continuously as the system is operated to reduce uncertainty and reflect the new information from operating and monitoring the system.

12.8.1 Modeling ISB at a Field Scale

To evaluate performance metrics and the potential for rebound following ISB treatment at the field scale, the numerical model DNAPL3D-RX (West et al., 2008; West and Kueper, 2012) was employed. This model simulates the complex biodegradation processes that occur during active treatment of TCE DNAPL source zones in porous media. DNAPL3D-RX is a three-dimensional finite-difference numerical model that integrates two-phase flow simulation (Gerhard and Kueper, 2003; Kueper and Frind, 1991), interphase mass transfer processes (Grant et al., 2007), and the reactive transport model RT3D (Clement, 1997, 2003; Clement et al., 1998).

The example simulations presented here were conducted deterministically for seven idealized template sites impacted with TCE DNAPL. Each template site was varied by one of three integral factors (Table 12.5): (1) TCE DNAPL release volume (small, medium or large); (2) geologic heterogeneity (low, medium or high variability in the hydraulic conductivity); or (3) the mean bulk permeability of the aquifer (low, medium or high). The performance metrics of interest were TCE DNAPL mass removal within the source zone and TCE solute mass flux reduction at a downgradient boundary. In addition, an enhancement factor was computed for both metrics that evaluates the potential for improvement from ISB treatment relative to abiotic processes (no treatment, dissolution only).

Given the complex and dynamic nature of ISB at real sites, models must integrate mechanisms, stoichiometry, and reaction kinetics that capture salient processes at the field scale. For the simulations presented herein, DNAPL3D-RX incorporated the following key biodegradation processes:

- Equilibrium dissolution of TCE DNAPL
- ISB by biostimulation with pulsed (1 day/week) lactate injection
- Lactate is converted *in situ* to H_2 by fermenters
- Competitive consumption of H_2 by both dechlorinators and methanogens

Table 12.5. Summary of Template Sites

Template Site	DNAPL	Initial Mass (kg)	Initial Volume (m^3)	Mean k (m^2)	Variance ln k $(\ln (m^2))^2$
Base case	TCE	3,520	2.41	3.03×10^{-12}	1.74
High mean k	TCE	3,496	2.39	3.02×10^{-11}	1.74
Low mean k	TCE	3,535	2.42	3.04×10^{-13}	1.74
Low heterogeneity	TCE	3,355	2.30	1.87×10^{-12}	0.87
High heterogeneity	TCE	3,186	2.18	7.41×10^{-12}	3.48
Small DNAPL volume	TCE	785	0.54	3.03×10^{-12}	1.74
Large DNAPL volume	TCE	7,343	5.03	3.03×10^{-12}	1.74

- Bacteria are immobile species adhering to soil grains
- The degradation of TCE to *cis*-DCE, where *cis*-DCE is the terminal end product
- TCE degradation to *cis*-DCE via dual-Monod kinetics, including the influence of both biomass concentrations and H_2 concentrations
- No toxic inhibition
- Solute transformation only occurs in the aqueous phase
- Reversible isothermal linear sorption
- First-order decay of biomass
- Bioclogging of soil pores due to biomass growth

The stoichiometric equations and kinetic reactions for each of the above processes are provided below. The governing equations for two-phase flow with sources and sinks, and the reactive transport equations for the mobile and immobile species are omitted here for brevity; the interested reader is referred to the aforementioned citations for those details.

As noted earlier, dechlorination can proceed in a step-wise process reducing PCE to ethene. For this work, only the transformation of TCE to *cis*-DCE is modeled:

$$\mathrm{TCE} + \mathrm{H_2} \rightarrow cis - \mathrm{DCE} + \mathrm{Cl^-} + \mathrm{H^+} + \mathrm{biomass} \qquad \text{(Eq. 12.3)}$$

where TCE is the electron acceptor, the electron donor substrate is H_2, and biomass is explicitly synthesized. Following Fennell and Gossett (1998), H_2 is generated through the *in situ* fermentation of injected lactate given by:

$$\frac{\partial[\mathrm{lactate}]}{\partial t} = -q^{MAX}_{lactate}[X_{lactate}]\left(\frac{[\mathrm{lactate}]}{K_{lactate} + [\mathrm{lactate}]}\right) \qquad \text{(Eq. 12.4)}$$

where q is the maximum utilization rate $\{\mathrm{M\ M^{-1}\ T^{-1}}\}$, K is the Monod half-saturation constant $\{\mathrm{M\ L^{-3}}\}$, and X is the biomass concentration $\{\mathrm{M\ L^{-3}}\}$. The subscript *lactate* denotes a parameter related to the fermentation of lactate to H_2 and the brackets ([ ]) denote molar concentration.

Competition for the consumption of H_2 exists between dechlorinators and methanogens. In the case of the latter species, the utilization of H_2 is given by (e.g., Amos et al., 2007a; Fennell and Gossett, 1998):

$$\frac{\partial\left[\mathrm{H_2^{meth}}\right]}{\partial t} = -q^{MAX}_{meth}[X_{meth}]\left(\frac{\left([\mathrm{H_2}] - H^*_{meth}\right)}{K^{meth}_{H_2} + \left([\mathrm{H_2}] - H^*_{meth}\right)}\right) I_{toxic} \qquad \text{(Eq. 12.5)}$$

where the subscript *meth* denotes the immobile species methanogens, H^* is the threshold H_2 concentration for subsistence, I_{toxic} is the TCE inhibition coefficient accounting for the influence of TCE toxicity on methanogensis and dechlorination (Amos et al., 2007a). The rate of TCE consumption by dechlorinators is given by (Christ and Abriola, 2007; Chu et al., 2003; Fennell and Gossett, 1998):

$$\frac{\partial[\mathrm{TCE}]}{\partial t} = -\frac{q^{MAX}_{TCE}[X_{CE}]}{R_{TCE}}\left(\frac{[\mathrm{TCE}]}{K_{TCE} + [\mathrm{TCE}]}\right)\left(\frac{([\mathrm{H_2}] - H^*)}{K_{H_2} + ([\mathrm{H_2}] - H^*)}\right) I_{toxic} \qquad \text{(Eq. 12.6)}$$

where the subscripts TCE and CE denotes the mobile solute species and the immobile dechlorinator species, respectively, and R is the retardation factor for TCE. For these simulations

$I_{toxic} = 1$ as a conservative bias that promotes greater degradation rates but also increased competition.

Given the above processes, the overall rate of change in H_2 concentration is given by (Christ and Abriola, 2007):

$$\frac{\partial[\mathrm{H_2}]}{\partial t} = F_{lactate}\frac{\partial[\mathrm{lactate}]}{\partial t} - \left(F_{TCE}\frac{\partial[\mathrm{TCE}]}{\partial t} + F_{meth}\frac{\partial\left[\mathrm{H_2^{meth}}\right]}{\partial t}\right) \quad \text{(Eq. 12.7)}$$

where F is the stoichiometric consumption or production coefficient (Bagley, 1998). Lactate serves as the only source of H_2; hence, when lactate injection ceases, no additional sources of H_2 are available for ISB processes, and all H_2 may eventually become depleted.

The biomass synthesis and decay for the fermenters ($X_{lactate}$), methanogens (X_{meth}), and dechlorinators (X_{CE}) can be described by (Fennell and Gossett, 1998):

$$\frac{\partial[X_{lactate}]}{\partial \mathrm{t}} = -Y_{lactate}\frac{\partial[\mathrm{lactate}]}{\partial t} - \lambda_{lactate}[X_{lactate}] \quad \text{(Eq. 12.8)}$$

$$\frac{\partial[X_{meth}]}{\partial \mathrm{t}} = -Y_{meth}\frac{\partial\left[\mathrm{H_2^{meth}}\right]}{\partial t} - \lambda_{meth}[X_{meth}] \quad \text{(Eq. 12.9)}$$

$$\frac{\partial[X_{CE}]}{\partial \mathrm{t}} = -Y_{TCE}\frac{\partial[\mathrm{TCE}]}{\partial t} - \lambda_{CE}[X_{CE}] \quad \text{(Eq. 12.10)}$$

where Y is the biomass yield coefficient $\{-\}$ for each species and λ is a first-order decay rate constant (Cupples et al., 2004).

The production of biomass can lead to the onset of bioclogging of soil pores, and on a larger scale, the biofouling of injection wells and geologic media. The mechanisms and kinetics of bioclogging have been studied by many researches yielding various mathematical representations (Chu et al., 2003; Clement et al., 1996; Thullner et al., 2002; Vandevivere and Baveye, 1992; Vandevivere et al., 1995; Zysset et al., 1994). For this work, the analytical approach represented by Clement et al. (1996) is adopted and modified to simulate reductions in permeability (k) as a function of total biomass (X) growth and decay (see West, 2009). Following Criddle et al. (1991), it is assumed that 20% of the maximum biomass is recalcitrant yielding a permanent reduction in permeability during biomass decay.

The performance metrics of interest for ISB of the TCE DNAPL source zone are total DNAPL mass (M_{DNAPL}) and total boundary mass flux (M_f), where the latter is computed by:

$$M_f = \sum_{i=1}^{N} C_i q_i \quad \text{(Eq. 12.11)}$$

where i denotes an individual node, q is the Darcy flux $\{\mathrm{L\ T^{-1}}\}$, and C is the concentration of TCE at each node $\{\mathrm{M\ L^{-3}}\}$. The metrics M_{DNAPL} and M_f are further utilized to define enhancement factors that directly compare the performance of ISB to abiotic processes only (i.e., no treatment, dissolution only).

The enhancement factor of total DNAPL mass removal (E_m) is given by:

$$E_m = \frac{M^0_{DNAPL} - M^{ISB}_{DNAPL}(t)}{M^0_{DNAPL} - M^{diss}_{DNAPL}(t)} \quad \text{(Eq. 12.12)}$$

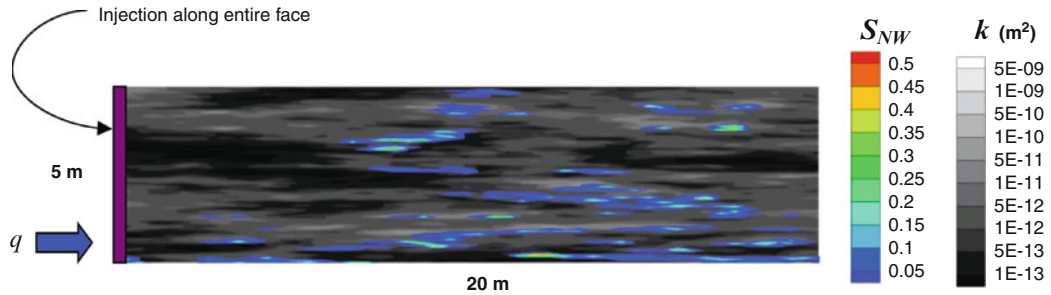

Figure 12.8. Cross-sectional view of model domain utilized for ISB simulations. Groundwater flow is from left to right with amendment injection along the upgradient boundary face. The TCE DNAPL saturations (S_{NW}) and permeability (k) field highlight the influence of geologic heterogeneity on DNAPL distribution in the subsurface.

where M^0_{DNAPL} is the initial DNAPL mass in the domain ($t = 0$), and M^{ISB}_{DNAPL} and M^{diss}_{DNAPL} are the DNAPL mass values for ISB and dissolution only (no treatment), respectively, at time (t). The enhancement factor for total boundary mass flux (E_f) is defined as:

$$E_f = \frac{M_f^{diss}(t)}{M_f^{ISB}(t)} \qquad \text{(Eq. 12.13)}$$

where the superscripts *ISB* and *diss* denote biostimulation and abiotic dissolution (no treatment), respectively. An enhancement factor for either E_m or E_f greater than 1 indicates that ISB performance was greater than dissolution only (no treatment).

Each template site represents a DNAPL source zone measuring 20 m (65.6 ft) long, 10 m (32.8 ft) wide, and 5 m (16.4 ft) thick, comprising both pooled and residual TCE DNAPL. A cross-sectional depiction of the model domain and TCE DNAPL saturations (S_{NW}) at mid-width (5 m) is provided in Figure 12.8. Hydraulic displacement (i.e., water flooding) was conducted on each template site prior to initiating ISB as a preliminary mass removal strategy that reduced the degree of pooled DNAPL. The model was discretized into 0.4 m by 0.4 m by 0.05 m (vertically) blocks for a total of 125,000 nodes. General model input parameters and ISB specific input parameters are provided in West (2009). An average hydraulic gradient of 0.05 was generated across each source zone, which is considered representative of continuous pumping conditions. A constant concentration injection boundary condition was applied to the upgradient face of the domain to simulate an amendment injection trench or line of injection wells positioned further upgradient of the source zone.

Simulations were conducted for each template site for both ISB and dissolution only (no treatment) scenarios. For ISB, lactate was injected at a concentration of 39,130 mg/L at a frequency of 1 day per week for a period of 2.5 years. Subsequently, active injection of lactate was discontinued, while the model was executed for an additional 7.5 years. The total simulation time for all scenarios was 10 years. Both M_{DNAPL} and M_f were computed for each time step, whereas E_m and E_f were selectively computed at 2.5 years (end of injection) and 10 years (end of simulation).

Figure 12.9 presents the DNAPL mass and boundary mass flux for the base case from the initiation of treatment (0 years) to 10 years; similar plots were constructed for all scenarios but are not presented here (see West, 2009). For dissolution only, the initial DNAPL mass of 3,520 kg is reduced to 900 kg over the duration of the simulation. For ISB, the rate of DNAPL mass depletion is less than the dissolution only case for 0–2 years, indicating no enhancement during this initial lag period. Similar occurrences of lag were observed for all template sites

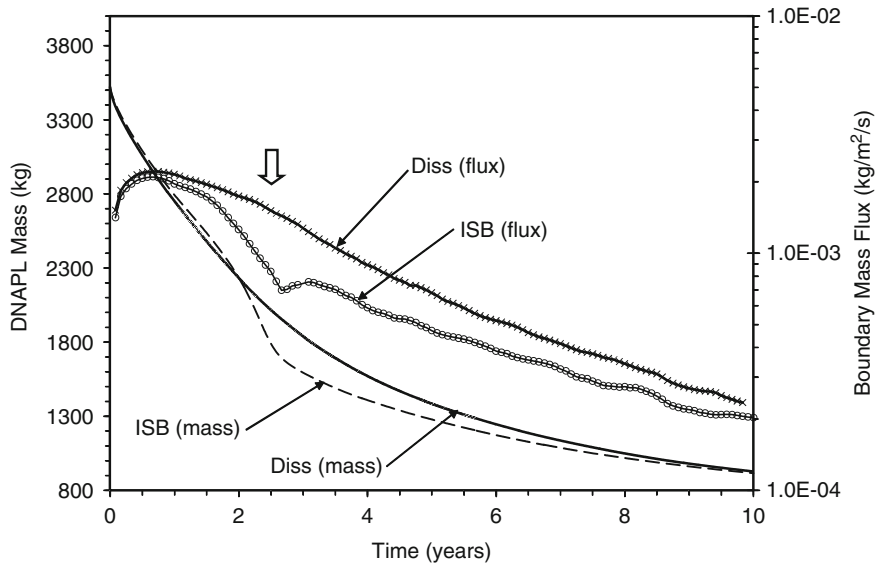

Figure 12.9. Plot of TCE DNAPL mass remaining and TCE boundary mass flux for the base case template site. The *arrow* indicates the time that active injection of lactate for ISB was terminated (2.5 years). Approximately 900 kg of TCE DNAPL mass remained after 10 years for both ISB and dissolution only (Diss).

Table 12.6. Comparison of Enhancement Factors for DNAPL Mass Removal (E_m) and Boundary Mass Flux Reduction (E_f) at the End of Active Injection of ISB and at 10 Years

Template Site	2.5 Years (End of Active Injection for ISB)		10 Years (End of Simulation)	
	E_m	E_f	E_m	E_f
Base case	1.15	1.81	1.00	1.13
High mean *k*	1.12	6.51	1.03	7.48
Low mean *k*	1.53	1.06	1.94	1.34
Low heterogeneity	1.25	1.40	1.01	1.31
High heterogeneity	1.11	7.01	1.01	1.16
Small DNAPL volume	1.15	6.61	1.00	1.75
Large DNAPL volume	1.27	1.58	1.03	1.21

Values greater than 1 indicate that ISB removed more DNAPL mass relative to dissolution or had relatively less boundary mass flux.

with the exception of the high mean k scenario. This reduction in ISB performance relative to dissolution only for M_{DNAPL} is due to bioclogging as biomass preferentially develops at higher saturation nodes that generally coincide with higher permeability pathways. However, from 2 years to the termination of injection (2.5 years) there is an accelerated reduction in DNAPL mass for ISB, leading to an E_m of 1.15 (see Table 12.6).

In contrast, a lag period was not observed with respect to boundary mass flux for the base case, and the $E_f > 1$ for the entire simulation. At 2.5 years the $E_f = 1.81$ with a peak $E_f = 2.07$

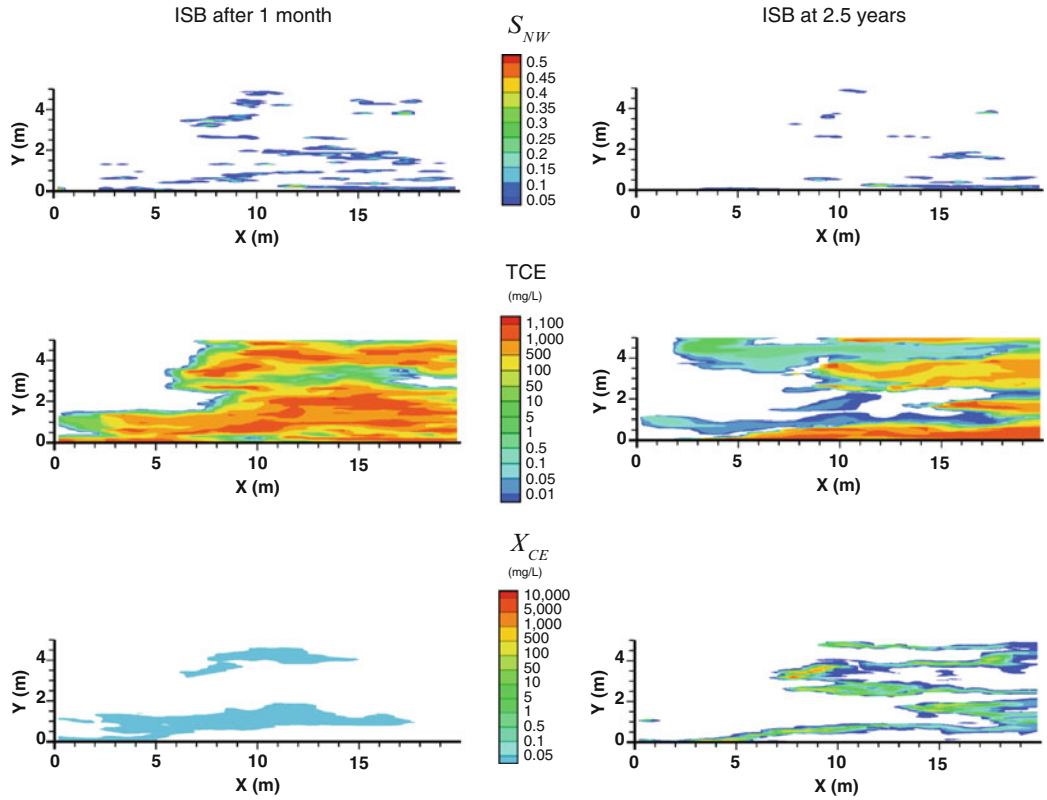

Figure 12.10. Cross-sectional plots of non-wetting saturations (S_{NW}), TCE concentration and dechlorinator biomass (X_{CE}) after 1 month and 2.5 years of ISB. Groundwater flow is from left to right and lactate is injected along the left upgradient boundary face.

occurring shortly after the termination of lactate injection. Following this period, H_2 concentrations cannot be replenished and the rate of dechlorination decreases markedly. In fact, the rebound of mass flux is observed for several months following the termination of injection. Of note is that the numerical model did not allow for accumulated biomass to act as a carbon source and electron donor following the cessation of active treatment. In practice, this biomass may delay the time to which rebound will be observed. We note that further research is required to further understand this process.

For all template sites considered here, the $E_f \geq 1$ throughout the simulation. However, the occurrence of rebound was case dependent: rebound was observed for the base case (medium variance and medium mean k), the large volume scenario, the small volume scenario, the high mean k scenario, and the high heterogeneity scenario. The latter three scenarios exhibited both the greatest E_f at 2.5 years and the most pronounced rebound (see Table 12.6). In these scenarios rebound occurred for 1 year or less following the termination of injection. From Table 12.6 it also can be observed that the high mean k scenario was the only ISB simulation to achieve a greater enhancement in mass flux reduction (M_f) at 10 years than at 2.5 years.

A comparison of DNAPL saturations (S_{NW}), TCE solute concentrations, and biomass growth (X_{CE}) is provided in Figure 12.10 for a longitudinal cross-section through the domain at mid-width (5 m). The first pane of plots is for 1 month after the initiation of ISB, while the alternate pane shows model output at the termination of active lactate injection (2.5 years). The top panes for S_{NW} illustrates mass depletion over the 2.5-year period, where the total

number of nodes with $S_{NW} > 0$ is greatly reduced, as is the overall magnitude of S_{NW} at nodes with DNAPL remaining; similar results occur for dissolution only (no treatment), but the extent and magnitude of depletion is not as pronounced. TCE concentrations are presented in the middle pane, which demonstrates several interesting results.

Initially TCE concentrations are widely distributed throughout the domain with the higher saturation nodes yielding the highest concentrations that are selectively transported through the most transmissive pathways. Some of the solute impacted zones are generated from out-of-plane transport. At 2.5 years, the TCE solute preferential pathways become more pronounced due to geologic heterogeneity, variable DNAPL saturations, spatially selective biomass growth and flow bypassing. The installation of short monitoring well screens or intermittent groundwater grab samples along the downgradient boundary could produce markedly different concentrations depending on vertical placement. Thus a sufficiently dense monitoring/sampling network is required to accurately capture representative concentrations and mass flux signatures from DNAPL source zones.

The bottom pane demonstrates the selective nature of dechlorinator (X_{CE}) biomass growth in these simulations. A low concentration biomass initial condition was assumed at the initiation of ISB to represent background; hence the distribution of low concentration biomass in the plot of ISB after 1 month. However, following an additional 2.4 years of lactate injections (1 day per week), biomass concentrations (expressed in mg/L) increased by up to six orders of magnitude at some nodes. The dechlorinator biomass is highly localized around higher saturation nodes and along higher concentration TCE solute pathways. This marked increase in biomass density may not be as significant when toxicity is incorporated. Despite the absence of toxicity, Table 13.6 summarizes that the maximum simulated enhancement for ISB at 2.5 years for E_m and E_f was 1.53 and 7.01, respectively.

Modeling results demonstrate that enhancements of DNAPL mass removal after 2.5 years of ISB ranged between 1.11 and 1.53 for the template sites and that these enhancement factors generally diminished after 7.5 years of post-treatment monitoring due to dissolution tailing. Conceivably, tailing effects might be less pronounced if a residual H_2 concentration was maintained during post-treatment monitoring. Experimental work with ISB and PCE has found enhancement factors of 2–3 (Sleep et al., 2006; Yang and McCarty, 2002), 5 (Yang and McCarty, 2000) and up to 16 (Cope and Hughes, 2001). In the field, enhanced dissolution may be more limited due to heterogeneity, flow bypassing and many other complicating factors. Only the low mean k scenario demonstrated an increase in E_m during post-treatment monitoring, due primarily the low seepage velocity and slow travel time of amendments relative to the length of the source zone and distance to the downgradient boundary face.

Because ISB is a spatially kinetic process, the positioning of the downgradient monitoring point influences the interpretation of enhancement for a given treatment time: moving the compliance point further up- or downgradient can produce different results. It is important to note that both ISB and dissolution only (no treatment) were unable to completely remediate any of the seven template sites; in all scenarios DNAPL mass and boundary mass flux persisted. In addition, dissolution tailing generally reduced E_m to 1 by 10 years, suggesting that terminating ISB prior to complete DNAPL mass removal is an ineffective long-term strategy.

In general, the enhancement of mass flux reduction (E_f) was greater than the enhancement of DNAPL mass removal (E_m) at both 2.5 and 10 years; the low mean k scenario was the only exception. The magnitude of M_f was variable between template site as was the occurrence, duration and magnitude of mass flux rebound following the termination of injection. It is important to note that E_f is not necessarily correlated to E_m, and significant reductions in boundary mass flux do not necessarily imply significant reductions in DNAPL mass.

12.9 OPERATIONS AND MONITORING ISSUES

12.9.1 Operating and Optimizing ISB Systems

Active remediation of source zones using ISB may require frequent monitoring to optimize treatment. Electron donor and amendment concentrations may need adjustment to respond to process monitoring information. Interim results should be evaluated carefully for evidence of stagnant zones requiring more intense treatment and for evidence of biofouling or other operational problems that need to be addressed before they become serious issues. Pulsing or reversals in extraction/injection wells may improve delivery, and it may be necessary to add wells to target specific areas or depth intervals.

In addition, any technology relying on fluid injection into an aquifer entails several operational risks including contaminant migration, preferential flow and therefore poor distribution, and short-circuiting. To control these risks during operations, the following operational issues should be evaluated carefully: (1) injection pressure limits, (2) DNAPL mobilization, (3) hydraulic responses in confined and semi-confined aquifer formations and (4) groundwater displacement (ITRC, 2008).

Injecting fluids into an aquifer can lead to fracturing and uncontrolled spreading of the fluids, along with any amendments and/or contaminants. Injection pressures should be designed to minimize unintentional hydraulic fracturing and avoid contaminant mobilization. Direct-push injections or conventional screened injection wells should be tested to assure that the aquifer can accommodate fluid insertion at the design flow rate. Conservative tracers such as bromide can be used to identify and quantify any such impacts, and if detected, the injection pressures and volumes should be modified to limit the effects.

Finally, injecting fluids near or above pooled NAPL can alter the capillary pressure and potentially mobilize the DNAPL. This may not be detrimental if the mobilized DNAPL enters already-impacted regions of the subsurface, but is likely undesirable if the mobilization of DNAPL leads to an expansion of the footprint or depth of the source zone. Related to this, the risks associated with drilling through a DNAPL source zone should be evaluated and considered prior to installing the injection, extraction, and monitoring wells needed to support ISB implementation.

Because of the uncertainties inherent in source-zone treatment, especially with an injection-based technology such as ISB, optimization is critical. Optimization is particularly important during the early stages of treatment. The injection volumes, pressures, and amendment concentrations should be estimated based on mathematical modeling and the best available knowledge (see Borden et al., 2008). However, practitioners should be prepared to carefully monitor the actual operations and to adjust the conditions as needed during treatment.

12.9.2 Monitoring ISB Systems

Monitoring an ISB system includes both process and performance monitoring (ITRC, 2008). Process monitoring is used to evaluate whether the system is meeting its design objectives, or if optimization is needed. It includes evaluating amendment distribution and longevity, measuring numbers of specific microbes or gene copies, ensuring that environmental conditions are favorable and that DNAPL is not migrating, and determining the extent and duration of any accumulations of potentially harmful daughter products. Performance monitoring is used to evaluate whether the treatment is meeting the remedial objectives and when it can be shut off. Performance monitoring involves evaluating multiple lines of evidence, including: (1) the concentrations of the parent chlorinated ethenes, and all daughter and end products; (2) any geochemical changes, particularly the impacts to secondary water quality parameters; and possibly (3) the mass discharge and flux of the COCs (ITRC, 2010).

The differences and similarities in these monitoring phases are discussed in detail in ITRC (2008). The following sections briefly describe the key monitoring parameters, the reasons they should be monitored, and issues to consider when measuring these parameters and evaluating the results. The key parameters can be classified into four categories: (1) contaminant concentrations, (2) geochemical characteristics, (3) biological indicators and (4) stable isotopes.

12.9.2.1 Contaminant Concentrations and Mass Discharge

Enhancing the dissolution and degradation of the DNAPL constituents produces a characteristic pattern of sequential increases and subsequent decreases in the daughter product concentrations (Figure 12.11). This pattern should occur over time in a well near DNAPL accumulations, as it reflects the increased rate of formation of daughter products closer to the DNAPL sources. However, the pattern may also reflect the greater aqueous solubilities of the daughter products, so that the concentrations of DCE and VC may temporarily be greater (on an absolute or molar basis) than those of the parent compounds (Carr et al., 2000; ITRC, 2008). Also the parent compound may be degrading faster in solution than some of the daughter products. As a result, the patterns actually observed at many sites where reductive dechlorination is working may differ from the somewhat idealized pattern shown in Figure 12.11.

Ethene, and in some cases ethane, are key breakdown products to monitor because they are innocuous compounds, and their presence at reasonable concentrations (>5–10% of the parent concentration on a molar basis) demonstrates that complete conversion through VC is occurring. However, ethene and ethane generally are not present in stoichiometric amounts, and mass balances are notoriously incomplete at sites undergoing ISB. There are at least three reasons for the difficulty in closing mass balances: (1) some of the ethene and ethane produced is further biodegraded to carbon dioxide and/or methane (Bradley, 2003); (2) non-biological reactions, notably reductive dehalogenation by Fe(II) minerals, can contribute to chlorinated ethene destruction by a different pathway (through acetylene), without production of chlorinated ethene daughter products (Ferrey et al., 2004; Cwiertny and Scherer, 2010); and (3) some aerobic biodegradation of VC (and possibly *cis*-DCE) may be occurring at very low oxygen levels, with conversion to carbon dioxide and chloride and no ethene formation (Coleman et al., 2002; Gossett, 2010).

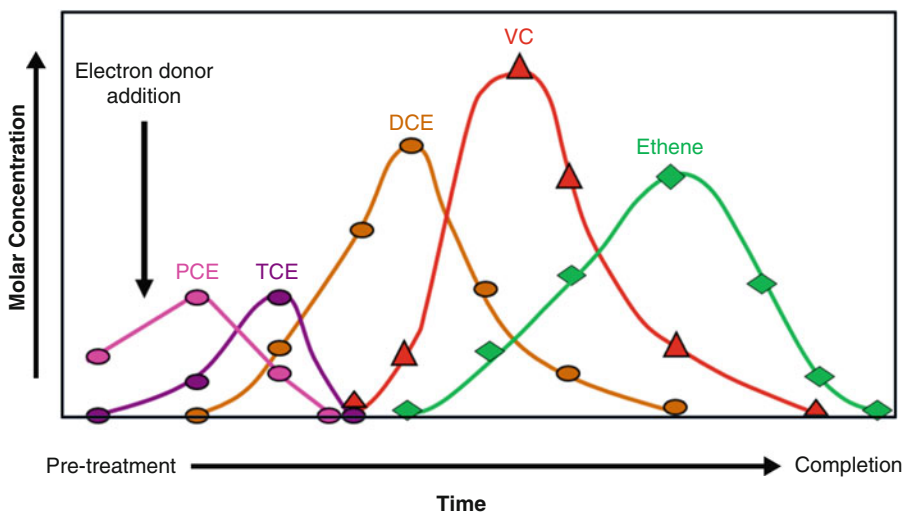

Figure 12.11. Idealized pattern of daughter product appearance and removal during *in situ* bioremediation of a chlorinated solvent source zone (modified from ITRC, 2008).

Contaminant monitoring may be required for several years after treatment. Although rebound in contaminant concentrations is typically not observed following ISB application in source zones (McGuire et al., 2006; Adamson and Newell, 2009), few sites have monitoring records extending more than a few years post-treatment, and potential certainly exists for some rebound after the residual effects of treatment (high biomass, back diffusion of electron donors) have subsided, so ongoing monitoring is prudent. In the event that rebound does not occur, such sustained treatment may be attributed to the slow degradation of the microbial biomass produced during active treatment (Sleep et al., 2005; Adamson and Newell, 2009), as well as to the possible storage of reducing power in iron minerals capable of abiotic reductive dechlorination (Elsner et al., 2004) and/or the possible diffusion of electron donors into lower-permeability materials, and their slow back diffusion into the more transmissive regions (Adamson et al., 2011).

Mass discharge is increasingly recognized as a valuable performance metric for partial source-zone remediation, that combines concentration data and the groundwater velocity to measure the mass leaving a source zone (Wood et al., 2004). However, the uncertainty involved in mass flux and discharge measurements can be large and often is not quantified (Li et al., 2007). ISB in particular has important features that need to be recognized by those measuring and interpreting mass discharge results; it involves partial degradation to daughter products as well as possibly the complete reduction to ethene (or ethane) or even abiotic reduction to products such as acetylene that typically are not measured (Brown et al., 2009). Therefore, the mass fluxes of several compounds must be monitored, and the uncertainties involved must be considered (Cai et al., 2012). In addition, ISB often results in a temporary increase in mass discharge, followed by a large (and hopefully permanent) reduction from pre-treatment levels, so it is important to interpret ISB mass discharge data in the context of the time and location of the measurements taken (ITRC, 2010; Cai et al., 2012). Finally, there is some potential for biomass accumulations to change flow paths during ISB, as biomass growth near DNAPL accumulations or in areas exposed to higher levels of organic carbon can cause groundwater at a local scale to be diverted around these areas (Lebrón et al., 2007).

12.9.2.2 Geochemical Characteristics

The main parameters needed to confirm that the redox conditions are appropriate for reductive dechlorination are the dissolved oxygen (DO) concentration and the oxidation–reduction potential (ORP or Eh). DO should be <0.5 mg/L, and the Eh should be <-100 mV (AFCEE, 2007). However, one should keep in mind that DO measurements from the field are notoriously untrustworthy (Wilkin et al., 2001). Other key indicators of the dominant terminal electron accepting processes include dissolved iron (Fe[II]), sulfate and sulfide concentrations, and methane. At a minimum, there should be evidence of ongoing sulfate reduction, and preferably there should be evidence of methanogenesis (detectable CH_4) (Henry, 2010).

Sufficient electron donor should be available to sustain reducing conditions and provide hydrogen for reductive dechlorination. Although fatty acid concentrations are often measured, the DOC concentrations are typically sufficient to ensure that amendments are being distributed as planned and are at levels sufficient to ensure effective treatment. Existing guidance suggests that maintaining DOC $>$ 50 mg/L in monitoring wells within the treatment zone should be sufficient for soluble substrate systems (Suthersan et al., 2002; AFCEE, 2007). However, lower levels of DOC may be acceptable for slow-release substrates such as mulch and vegetable oil.

The pH is a key factor both for characterizing the site's suitability (as discussed earlier) and for ensuring continued effective treatment. The fermentation process can be highly acidifying, especially in poorly buffered aquifers when large amounts of electron donor sources are added,

so ensuring that the pH remains >5.5, or preferably ≥6.0, is important to ensuring that complete dechlorination can continue to occur (Robinson et al., 2009).

Chloride is particularly important to monitor when treating source zones. Chloride is the end product of dechlorination, so the molar volume of chloride produced is a direct measure of the rate of biodegradation occurring (ITRC, 2008). The background chloride concentrations often make it difficult to detect any increase, especially when treating plumes (AFCEE et al., 2004). In source zones, which often have relatively high chlorinated ethene concentrations compared to the background levels of Cl^-, the production of Cl^- often can provide a direct measure of the rate of dechlorination and can be converted back to the original moles of parent compound that were desorbed and degraded during treatment. Estimating the rate of biodegradation on the basis of such data should be carried out using a mathematical solute transport model. Estimating the rate of biodegradation cannot be carried out by simply matching a first-order decay curve to the concentration versus time signature in a monitoring well because that signature is likely influenced by a number of additional processes such as changing rates of upgradient DNAPL dissolution as the DNAPL is depleted, hydrodynamic dispersion and seasonal changes in the direction of groundwater flow.

Other conditions that may need to be monitored include any secondary water quality parameters that may be of concern besides pH, sulfides and DOC (Henry, 2010). Establishing strongly reducing conditions, and lowering the pH, can increase the concentrations of dissolved metals, notably iron, manganese, arsenic and possibly selenium, and generate undesirable fermentation products (ketones and aldehydes, as well as hydrogen sulfide and methane gas).

12.9.2.3 Biological Indicators

Dhc bacteria are very slow-growing and difficult to culture. They are therefore difficult to isolate or enumerate using conventional microbiological methods such as plate counts (Löffler et al., 2013b). Fortunately, molecular biological methods have made it possible to identify and quantify the *Dhc* populations at a site, as well as some of the genes involved in reductive dehalogenation (notably VC RDases). As mentioned previously, the numbers of these biomarkers have been useful for deciding whether bioaugmentation is needed and if ISB systems are operating effectively (Stroo et al., 2012). The technique can also be used to track bioaugmentation cultures or even to discriminate between indigenous and introduced *Dhc* (Holmes et al., 2006).

Quantifying *Dhc* and VC dehalogenase genes (specifically vcrA and bvcA) is done by using the qPCR technique (Cupples, 2008). Although other biomarkers and molecular biology methods may be used, qPCR has proven to be highly sensitive and specific for key gene sequences (SERDP, 2006). Recent work has produced guidance on collecting and analyzing samples by qPCR as well as on interpreting the results of *Dhc* and VC Rdase biomarkers (Lebrón et al., 2008; Ritalahti et al., 2010; ITRC, 2011). One important finding is that onsite biomass collection using filtration techniques is important for accurate analyses, as compared to the more typical practice of shipping the unfiltered groundwater samples to the off-site laboratory (Ritalahti et al., 2010).

12.9.2.4 Stable Isotopes

Compound-specific isotope analysis (CSIA) relies on specialized mass spectrometers to distinguish between stable isotopes of several important atoms, including $^{13}C/^{12}C$ and $^{35}Cl/^{37}Cl$. Biodegradation reactions preferentially deplete the pool of lighter atoms in the parent compound, as these bonds are slightly easier to break. As a result, CSIA of the parent compounds and daughter products can distinguish between biological and non-biological processes affecting the contaminants (Liang et al., 2009) and also can indicate the rate and extent of

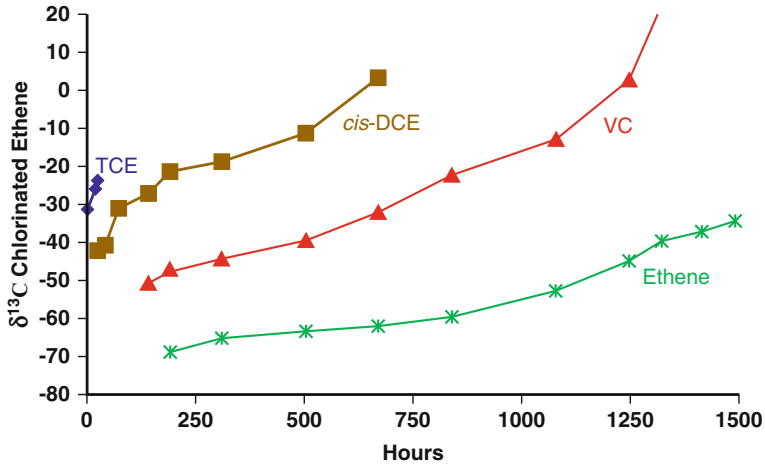

Figure 12.12. Pattern of carbon stable isotope ratios during biodegradation of PCE (modified from Slater et al., 2001). The δ ^{13}C value refers to the normalized ^{13}C/^{12}C ratio, expressed as per mil, or 0/00.

biodegradation (Sherwood-Lollar et al., 2001; Song et al., 2002; Aeppli et al., 2010). CSIA has proven valuable, largely because it provides an unequivocal indication of natural biodegradation and sometimes can discriminate between differing sources (Hunkeler et al., 2008; Wilson, 2010). The typical pattern of sequential enrichment in the heavier isotopes of the parent and daughter products is illustrated in Figure 12.12.

CSIA also can indicate that biodegradation and DNAPL depletion are occurring during source-zone bioremediation. In a well near the DNAPL source material, the expected pattern following electron donor addition (and bioaugmentation, if performed) is a reduction in the concentrations of the parent compounds (PCE and TCE), because these are the most rapidly biodegraded under reducing conditions (Vogel et al., 1987). The ratios of ^{13}C/^{12}C in the PCE in groundwater near a PCE DNAPL, for example, should increase during biodegradation, with an "enrichment factor" that is characteristic of the compound and reaction mechanism (Slater et al., 2001). The TCE, DCE, VC and ethene produced will have relatively low ^{13}C/^{12}C ratios initially, and these ratios will increase as the compounds are further biodegraded. The enrichment factors of parent and daughter compounds can be used to quantify biodegradation rates with greater accuracy than less direct methods (Morrill et al., 2009). However, it is important to realize that, if the well is near DNAPL, a high concentration of PCE in the aqueous phase may represent molecules in equilibrium with the PCE in the DNAPL, and until the DNAPL is depleted extensively the PCE in groundwater will have the same isotope ratio as the DNAPL (Morrill et al., 2009).

12.10 CASE STUDIES

The following case studies present four well-monitored demonstrations of the performance of ISB for source zones. The first two are relatively contained and small-scale. The first is a comparison of biostimulation and bioaugmentation in a controlled reactor (roughly 400 ft^3) containing a known volume of PCE DNAPL. Results indicated roughly a two- to threefold enhancement in flushing due to biological activity. The second is from Dover Air Force Base (AFB), Deleware where a contained test cell was emplaced into the soil, with approximately 1,500 ft^3 of saturated aquifer. Those results indicated a flushing enhancement of between 2 and 4.5, with possibly greater enhancement in the later stages of flushing.

In addition, two field demonstrations are included. In the first, hydraulically isolated test cells were established at Fort Lewis, Washington and mass flux was measured in the cells with or without ISB treatment. The enhancement in mass flux ranged from a factor of 2–8, though some of this enhancement may have been due to the whey used as the electron donor. Finally, the potential for treating an existing TCE source zone by injecting edible oil was tested at Tarheel Army Missile Plant, North Carolina. In this case, the groundwater concentrations of chloroethenes showed a sustained decrease of roughly 90% after the oil injections, with extensive conversion to daughter products.

Together, these case studies suggest that it is reasonable to expect a two- to threefold increase in DNAPL dissolution rate when using active ISB over that achieved by hydraulic flushing alone, during the period of treatment. The results also indicate passive treatment can achieve a 90% reduction in source-zone concentrations, during the period of time that the oil or accumulated biomass persists. None of these case studies address the long-term impacts of ISB, an area where reliable information is still needed.

12.10.1 Rice University Experimental Controlled Release System: Biostimulation and Bioaugmentation

In the first phase of this project (Phase I), two 11.7 m^3 (413 ft^3) experimental controlled release systems (ECRS) were packed with sandy model aquifer material and amended with PCE DNAPL source zones (Figure 12.13). The tanks then were operated in parallel with identical flow regimes and electron donor amendments to measure the impacts of

Figure 12.13. One of the Experimental Controlled Release System tanks being filled prior to the experiment. Photograph provided courtesy of C. Herb Ward, Rice University, Houston, Texas.

bioremediation on the mass discharge of contaminants from the sources. HRC®, and later dissolved lactate, served as electron donors to promote dechlorination. One ECRS was bioaugmented with an anaerobic dechlorinating consortium directly into the source zone, and the other served as a control (biostimulated only) to determine the benefits of bioaugmentation. The presence of halorespiring bacteria in the aquifer matrix prior to bioaugmentation, shown by nested PCR with phylogenetic primers, suggests that dechlorinating catabolic potential may be somewhat widespread. Polymerase chain reaction analyses demonstrated that the bacteria present in the culture used for bioaugmentation in the ECRS prevailed for almost a year. Unfortunately, even with *Dehalococcoides* present, the low concentrations of ethene produced (<1 μM) indicate that complete dechlorination to nontoxic end products was limited.

The results demonstrated that the low concentration of ethene produced in this first phase was not due to washout of the dechlorinating organisms. The experiment also demonstrated that as long as the electron acceptor was not limiting, there was greater energy flow to the dechlorinating populations than to the methanogens. Overall, the results obtained in the Phase I corroborate that:

- Source-zone reductive dechlorination of PCE is possible at near field scale, and
- A system bioaugmented with a competent halorespiring consortium can enhance DNAPL dissolution at significantly greater rates than a system that is biostimulated only.

The Phase II testing compared the fate of a mixed DNAPL source zone under a natural attenuation scenario (no treatment, natural rates of dissolution) with a most probable engineering approach that included biostimulation and bioaugmentation. The same experimental ECRS tanks used in Phase I described above were emptied and repacked with uncontaminated sandy soil. HRC® was continuously added in the influent as a pre-hydrolized (diluted) mixture consisting of 50:50 v/v HRC®: deionized water. The effluent concentration of ethene measured in the biostimulated and bioaugmented tank (~4 μM) was fourfold higher than Phase I. This suggests a more complete dechlorination activity that was most likely the result of the slower groundwater seepage velocity used in this experiment (0.4 m/d) compared to the Phase I experiment (1.6 m/d).

Cumulative mass-balance calculations showed that the total mass removed at the end of the experiment in the biostimulated and bioaugmented tank was nearly 47% of the total mass of PCE added to the tank (Da Silva et al., 2006; see Figure 12.14). Of this removal, 26% was removed by dissolution (as measured by the mass of PCE in the effluent) and 21% by dechlorination to lesser chlorinated products, mainly TCE and *cis*-DCE. In the control tank, 34% of the PCE added to the tank was removed, with 31% being removed by dissolution and 3% by dechlorination. The benefit of biostimulation and bioaugmentation was observed with higher (sevenfold) dechlorination activity compared to the control tank.

These results from a carefully monitored system indicate that biological activity can enhance dissolution from DNAPLs in the subsurface, perhaps by a factor of 2–3. The results also suggest that long periods of time may be required for effective treatment – the 200-day treatment time under these conditions corresponds to roughly 80 pore volumes, but under field conditions it can take several months to years to move a pore volume through a source zone.

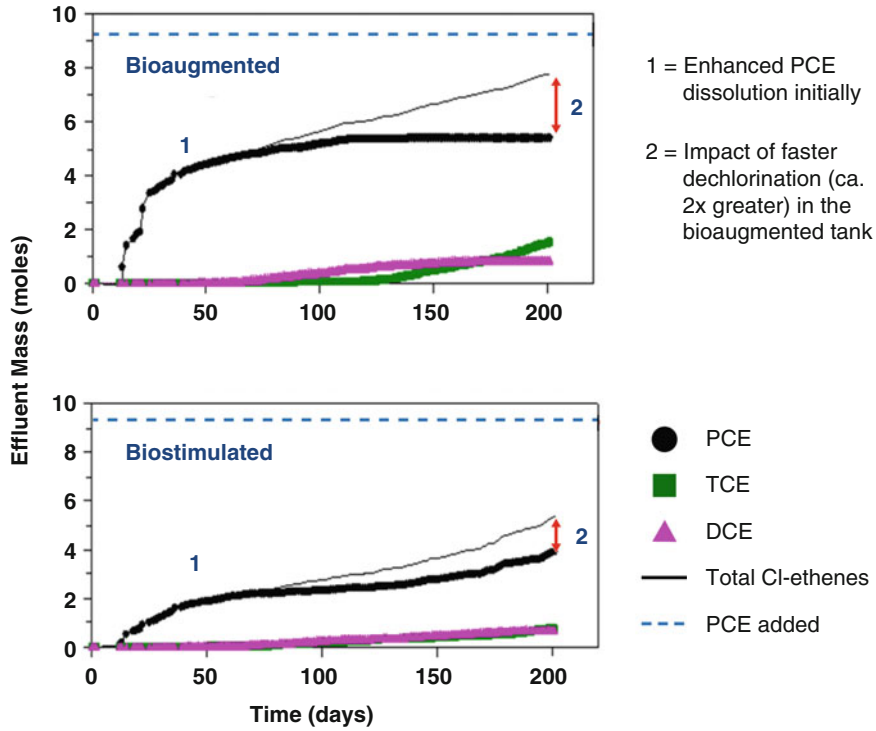

Figure 12.14. Cumulative mass of chloroethenes flushed over time from the bioaugmented and biostimulated only ECRS tanks. Approximately twice as much mass was flushed from the bioaugmented tanks initially than from the biostimulated tank, which showed little evidence of biodegradation until later in the experiment (modified from Da Silva et al., 2006).

12.10.2 Dover AFB: Bioaugmentation and Lactate Recirculation

A known volume of PCE was released in a test cell created by inserting sheet metal walls to a depth of 6 ft (2 m) in place at a shallow site at Dover AFB, Dover, Delaware, USA (Figure 12.15). This demonstration evaluated the use of biological processes to enhance PCE dissolution by establishing a baseline dissolution rate, followed by biostimulation (lactate solution continuously recirculated through the source) and finally by bioaugmentation (addition of KB-1™ with continued recirculation). Initial laboratory tests showed that bioaugmentation was needed for complete dechlorination at this site, and that bioaugmentation could enhance dissolution by a factor of roughly 2–5 compared to the flushing-only baseline (Sleep et al., 2006).

During the field test, effluent from the test cell was analyzed for all chloroethenes as well as chloride. The results confirmed that bioaugmentation was required to promote dechlorination of the PCE to *cis*-1,2-DCE, VC, and ethene and that the dissolution of the DNAPL remaining after the initial water flushing was enhanced by a factor of 2–4.5, depending on the method used to calculate dissolution. The effluent chloroethene concentrations were significantly higher than was predicted based on the kinetics observed prior to bioaugmentation (Figure 12.16).

The field test also demonstrated that bioclogging occurred as a result of electron donor addition, and this clogging eventually caused the flow paths within the test cell to change and prevented delivery of the electron donor to those zones with the greatest amounts of residual PCE. This finding underscores the need for careful performance monitoring during full-scale ISB applications and the need to adequately prevent bioclogging.

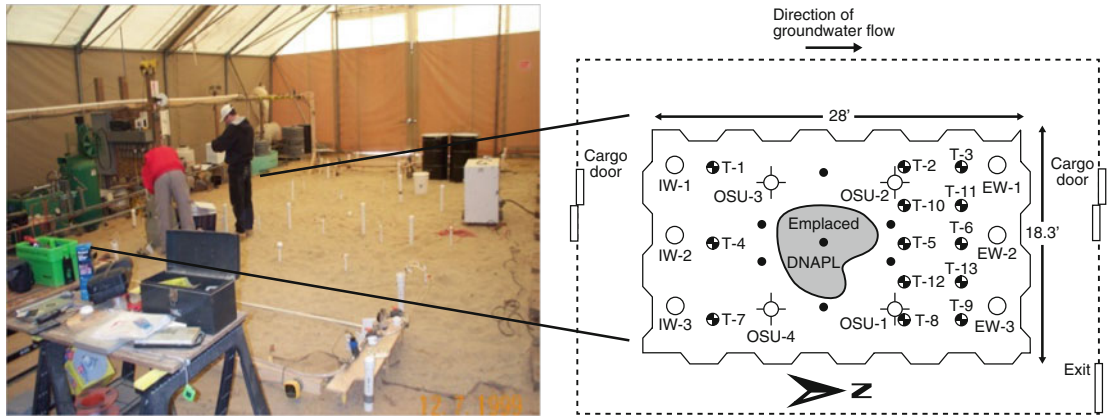

Figure 12.15. Dover AFB test cell during operations, with expanded schematic of the cell showing location of emplaced PCE and injection wells, extraction wells, and all monitoring points.

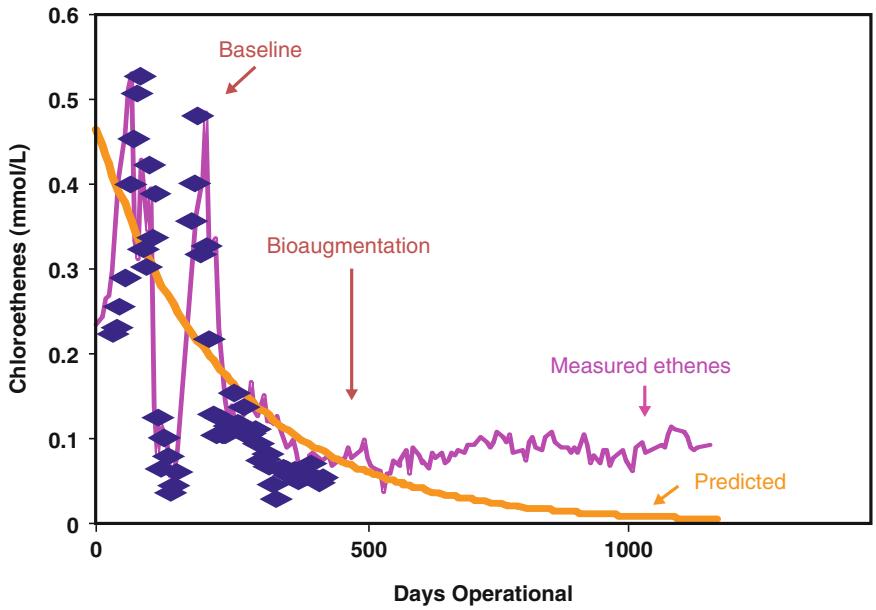

Figure 12.16. Concentrations (molar) of chloroethenes during Dover AFB demonstration. After bioaugmentation, the PCE was largely converted to daughter products, and the total concentration of daughter products increased significantly over the concentrations that were predicted based on the prior kinetics, and over the concentrations measured prior to bioaugmentation. Modified from Lebrón et al. (2007) and Geosyntec (2008).

12.10.3 Fort Lewis East Gate Disposal Yard: Whey Injections

Two hydraulically isolated treatment cells, each consisting of an injection well, an extraction well, and a network of monitoring wells were installed at a DNAPL source zone in Fort Lewis, WA, USA (Figure 12.17; details in Macbeth and Sorenson, 2008). The site had a TCE DNAPL source within a shallow, gravelly aquifer, with rapid groundwater flow (1 ft/day or 0.3 m/day). In Treatment Cell 1, high concentration (10%) whey powder was injected initially, followed by low concentration (1%) whey powder injections. In Treatment Cell 2, the reverse was done: the low concentration whey was injected first, followed by the 10% whey powder solution.

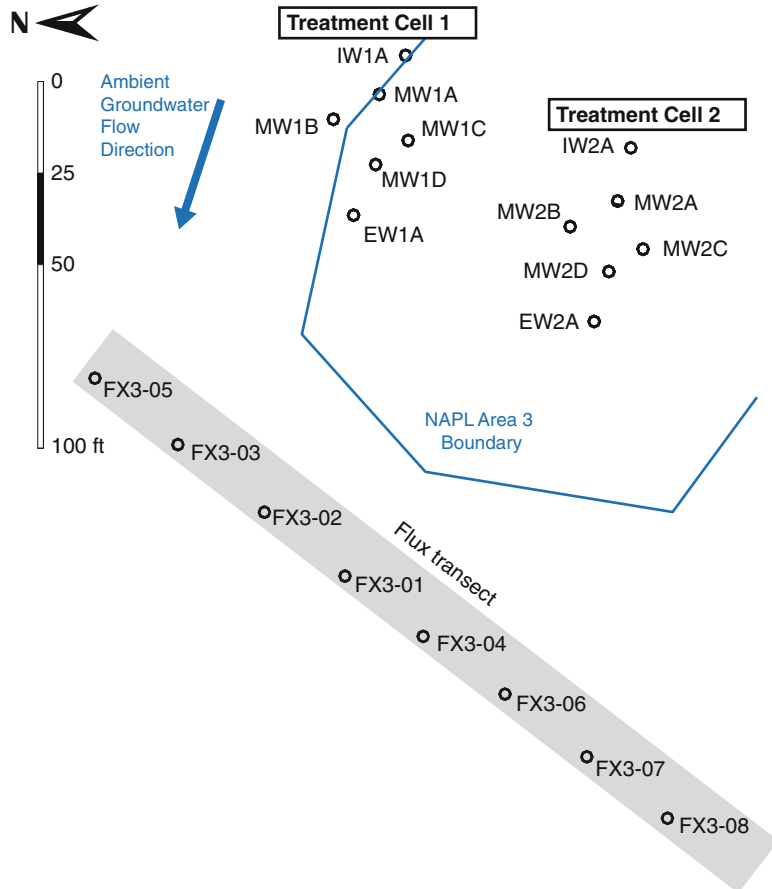

Figure 12.17. Layout of Fort Lewis treatment cells within the TCE source zone and the mass flux monitoring wells located immediately downgradient (from Macbeth and Sorenson, 2008).

Treatment Cell 2, in the core of the source zone, had significantly higher concentrations of TCE and DCE than Treatment Cell 1, located on the fringe of the source. In both cells, whey injection quickly stimulated iron- and sulfate-reducing conditions, and reductive dechlorination of TCE to DCE was rapid and complete. An initial drop in pH due to the whey injections delayed the onset of methanogenic conditions and further dechlorination of the DCE to VC and ethene. Statistical comparisons demonstrated that VOC molar concentrations were significantly increased during treatment (Figure 12.18). The calculated dissolution enhancement factors ranged from approximately 3–8 under optimal conditions (that is, when 10% whey powder was injected). This enhancement apparently resulted from both the increased biodegradation to daughter products and enhanced solubilization of TCE by the organic matter in the whey.

ISB not only increased DNAPL dissolution during treatment, but also the treatment effects persisted after the whey injection stopped. The total VOC concentrations remained well below baseline for at least 4 months after treatment (concentrations were 94–99% lower at the last sampling in May 2006 than in the baseline sampling of July 2005).

The costs of the demonstration were carefully tracked, and a realistic estimate of the full-scale cleanup cost was developed. Based on an assumed 3-year operations period, the total cost for cleanup of the 0.5-acre site was estimated to be \$0.9M (a unit cost of \$56 per cubic yard [yd^3] or \$74/m^3). The actual cost of cleaning up this same source zone using electrical resistance heating (ERH) was \$5M or \$313/yd^3 (\$412/m^3).

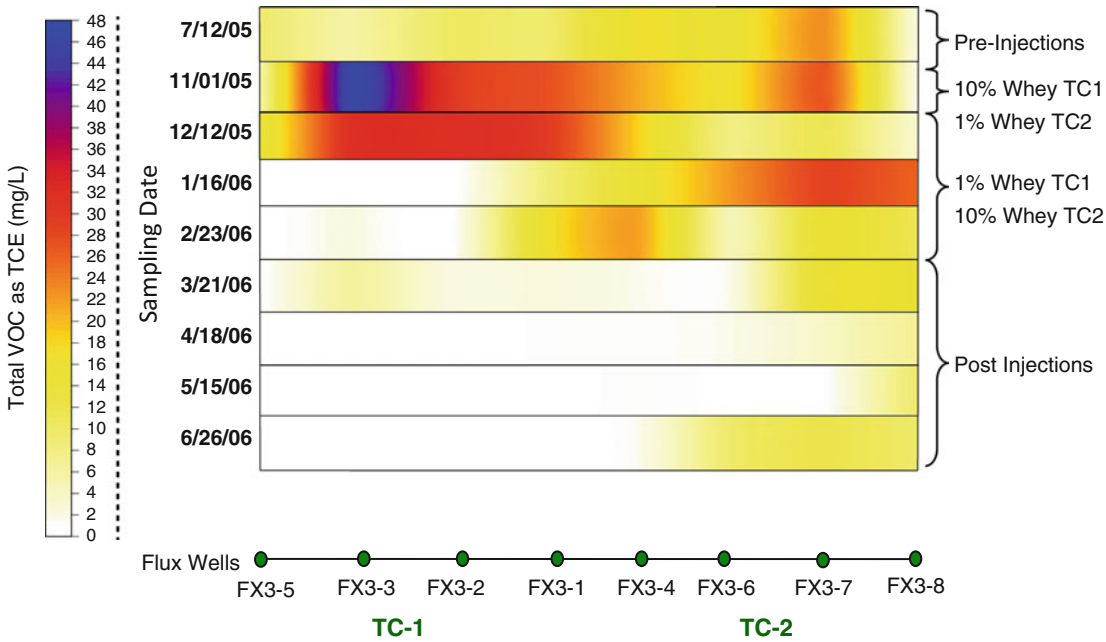

Figure 12.18. Site layout and total VOC concentrations (as mg/L of TCE) in monitoring wells downgradient of the source zone during the Fort Lewis ISB demonstration (from Macbeth and Sorenson, 2008).

12.10.4 Tarheel Army Missile Plant, North Carolina: Edible Oil Injection

In preparation for transfer of ownership of the property, the Army evaluated ISB to treat TCE in groundwater, using commercially available edible oil (EOS®). The pilot test was designed to treat a 100 × 100 ft (approximately 30 m × 30 m) zone that included the primary source zone (Borden et al., 2007). The source and plume were within a more transmissive zone present at the transition from bedrock to weather material where the rock fractures remain open allowing water flow.

The EOS was injected in two separate events, as a dilute emulsion into eight wells (6 in [2 cm]-diameter), spaced approximately 30–35 ft [9–11 m] apart. After each injection, groundwater was recirculated to distribute the emulsion throughout the targeted treatment zone, by extracting and injecting one pore volume from the four well pairs. No bioaugmentation was done. Over the two phases of injections, approximately 18,500 pounds (lb) (8,400 kg) of EOS concentrate were added, and 163,000 gallons (314,000 L) of groundwater were recirculated. The active treatment was completed in two separate injection events over 18 and 32 days.

After injection, the ORP changed from slightly positive to strongly reducing (<-100 mV), DO was no longer detectable, and methane was consistently detected. Volatile organic compounds in groundwater within the source zone were monitored for over a year, and concentrations declined by roughly 90% on a molar basis. Moreover, the contaminants that remained showed evidence of biodegradation. TCE was completely depleted with the production of *cis*-DCE and VC (Figure 12.19).

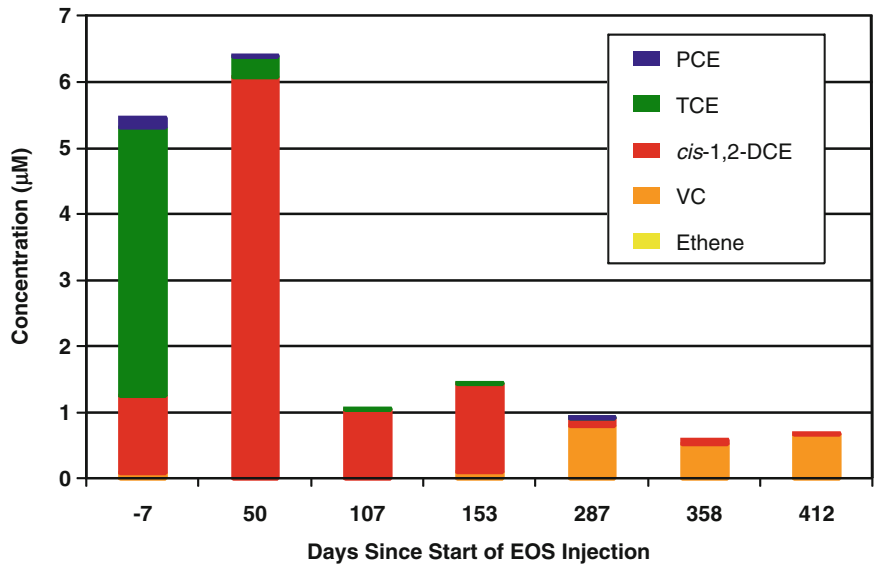

Figure 12.19. Average molar concentrations of chlorinated ethenes in groundwater after source-zone treatment with EOS™ (emulsified edible oil) at Tarheel Army Missile Plant, NC, USA (graphic provided courtesy of Robert C. Borden, North Carolina State University, Raleigh, North Carolina, 2013; updated from ITRC, 2007).

12.11 LESSONS LEARNED

Case studies and performance reviews provide some lessons regarding the use of ISB for remediation of source zones:

- *Set realistic objectives.* Complete cleanup over a few years is unlikely. Reducing mass discharge from a source by 90–99% and achieving similar reductions in contaminant concentrations in the permeable portions of the source zone are reasonable expectations, although lower reductions have been observed in some field-scale treatments (McGuire et al., 2006). However, undiscovered and/or untreated source material, especially in less transmissive zones, generally will require some long-term management.
- *Use adaptive management when possible.* Typically, uncertainty exists in the source characterization and site-specific issues limit performance (stagnant areas, unknown contaminated areas). ISB, like any injection-based technology, is best applied in stages, learning along the way, and optimizing the design and operations over time.
- *The long-term treatment impacts are not fully known.* Some rebound in contaminant concentrations may occur after treatment is completed and the aquifer returns to pre-treatment conditions. Although ISB may remove much of the source zone mass and reduce the short-term mass discharge, model simulations indicate that long-term monitoring in combination with natural attenuation may be required at many sites. Recent research suggests that buildup of bacterial biomass and reduced minerals during ISB may sustain treatment after electron donor addition ends. As a result, contaminant concentrations may not rebound for several years after treatment, providing site managers with time to detect rebound and implement contingency actions, if required.

- *Monitor and address biofouling aggressively.* Adding large amounts of readily degradable materials stimulates more than the dechlorinators. Fouling of wells due to microbial growth or activities is a common problem, and it can be particularly critical for source zones if large amounts of electron donor are needed and water is continuously extracted and injected. Several remedies are available (Geosyntec, 2005), but it is critical to monitor the wells carefully and respond quickly to indications of fouling.
- *Carefully evaluate the concentrations of parent and daughter products.* The distribution and relative concentrations of the different chloroethenes, along with the geochemical parameters that exist prior to treatment, can be useful in designing the eventual ISB system. For example, substantial preexisting dechlorination suggests that less electron donor will be needed to achieve reducing conditions and may demonstrate that a complete dechlorination pathway already exists. Similarly, a lack of VC or ethene given otherwise favorable conditions can suggest a need for bioaugmentation.
- *Effective treatment must overcome significant challenges.* Practitioners considering ISB for a source zone should be aware of several potential difficulties. The electron donor demand can be so great that it is not feasible to supply enough donor and/or pH buffer, or it may cause other problems such as methane production or clogging of the subsurface. Long-term performance of ISB is not clear, and treated sources may still sustain a plume that requires ongoing monitoring or even treatment. These challenges require careful design, monitoring and continuous optimization throughout treatment.

12.12 FUTURE DEVELOPMENTS

ISB for chlorinated ethenes is a rapidly evolving technology, especially when applied to source zones. Source-zone treatment in general has gained acceptance and developed a record of experience only over the last decade. The next decade is likely to see refinement of the approaches taken, applications of the basic principles to more complex sites and more cost-effective monitoring and operation (Suthersan et al., 2011). Moreover, it will probably include deliberate combinations with other technologies, sequentially or simultaneously (ITRC, 2011). This section examines some of the potential future developments in this area.

12.12.1 Improved Understanding and Treatment of Low-Permeability Regions

Understanding if targeted treatment of lower permeability zones is needed, and then designing cost-effective ways to treat these zones, are important issues for ISB. Like any injection-based strategy, most of the amendments move through and remain in the most transmissive regions. In a recent spill, or at the leading edge of a plume, most of the contaminants are in the transmissive regions, and injection-based strategies may be relatively successful. But all too often, source zones already have been exposed for decades, and a large fraction of the remaining mass may be in the lower permeability regions. Targeting amendment delivery to these regions could lead to lower cost and more effective treatment.

One potential approach to improving delivery is to co-inject the amendments with shear-thinning agents (such as gum agar), to temporarily clog the transmissive regions and force amendments into the less permeable areas (Zhong et al., 2008; Newell, 2009). Another possibility is to combine ISB or ISCO or ISCR with electrokinetics (EK) to improve amendment

delivery to the low-permeability zones (Norris et al., 1995, Reynolds et al., 2008; Jones et al., 2011; Wu et al., 2012 a, b). The use of EK as the driving force for amendment migration overcomes the traditional limitations of hydraulic injection approaches and is relatively insensitive to the soil type and the hydraulic permeability.

Ongoing research is examining the fundamental aspects of chlorinated solvent storage in, and release from, lower permeability materials, including sorption to clays and organic materials, and clay-DNAPL interactions. The goal of this work is to improve predictions of the magnitude of contaminant storage in low permeability zones, and help managers decide if treatment is needed to control any back diffusion.

12.12.2 Improving Delivery to DNAPL Accumulations

The difficulty of delivering water soluble electron donors to the DNAPL phase is obvious, and makes effective treatment of sites with DNAPL accumulations very challenging. One solution currently being tested is the formation of mixed DNAPLs containing both the contaminant and electron donor. Partitioning electron donors (PEDs) are organic compounds such as *n*-butyl acetate and *n*-hexanol that can be delivered in water but can partition into the DNAPL phase, enhancing biodegradation at and near the DNAPL:water interfaces (Lebrón, 2007). Laboratory-scale testing has indicated that both these PEDs will partition to DNAPLs and persist for several years, reducing the need for subsequent electron donor reinjections to sustain continued reductive dechlorination (Cápiro et al., 2011). Alternatively, vegetable oils can be injected where the chlorinated solvent partitions into the oil droplets, forming a mixed NAPL that will provide both electron donor and electron acceptor for bacterial growth. This strategy has been tested in the laboratory with EVO (Hiortdahl and Borden, 2011) and in the field with neat vegetable oil injections (Henry et al., 2007). Heating the oil prior to injection may increase the distribution of the oil in the subsurface (Williams, 2003).

12.12.3 ISB at Fractured Rock Sites

DNAPL sources located in bedrock are particularly difficult to treat and manage (Steimle, 2002). It is difficult to characterize the DNAPL nature and extent (Mercer et al., 2008), matrix storage can sustain plumes for decades (Mutch et al., 1993; Parker et al., 1994; West and Kueper, 2010), and delivering amendments can be very challenging (Goldstein et al., 2004). Thermal treatment may be effective, but can be difficult and costly (Johnson et al., 2009; Baston et al., 2010; Chen et al., 2010), so there is considerable interest in less costly technologies, including bioremediation.

Laboratory research has indicated that bioaugmentation can be highly effective in enhancing the removal of DNAPL from fractures within bedrock (Schaefer et al., 2009b, 2010). Limited field testing also has suggested that bioaugmentation can be effective in bedrock (Kane et al., 2005; De Flaun et al., 2006). There is a need for well-monitored field-scale demonstrations to understand the costs and performance, and for improvements in the technology to increase the use of ISB at fractured bedrock sites.

12.12.4 Long-Term Performance Predictions and Improvements

As mentioned earlier, ISB treatment in DNAPL source zones must be sustained over several years in order to achieve significant mass reductions. However, because long-term monitoring data sets are not yet available, there is still some uncertainty regarding the long-term performance. For example, what will be the post-treatment equilibrium state, and how

long will it take before re-equilibration occurs? The modeling results provided in the text box suggest that ISB may have relatively little effect on the total source mass and the mass discharge from the source several years after treatment is stopped, as compared to natural dissolution of the source zone, and that the greatest long-term enhancement will be in heterogeneous and low-permeability aquifers.

There will almost certainly be some remaining mass within the source zone after ISB is terminated. Untreated DNAPL pools and zones of residual DNAPL may still be present, especially in inaccessible areas such as the less permeable regions in highly heterogeneous porous media and in dead-end fractures. These remaining source materials can sustain plumes for decades above regulatory criteria, but they will be attenuated so long as reducing conditions persist, especially if elevated levels of organic carbon and/or reactive reduced iron minerals are still present. Validated models will help determine the need for monitoring and its frequency, better long-term monitoring methods will reduce the costs and materials may be developed specifically to ensure long-term treatment.

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