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Diagnostic Tools for Performance Evaluation of Innovative In-Situ Remediation Technologies at Chlorinated Solvent-Contaminated Sites

ESTCP Project ER-200318

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ACRONYMS

ARD	anaerobic reductive dechlorination
B.E.T.™	Bioavailability Enhancement Technology™
bgs	below ground surface
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
CMT	Continuous Multichannel Tubing
COD	chemical oxygen demand
CSIA	compound-specific stable isotope analysis
DCE	dichloroethene
DHC	*Dehalococcoides*
DNAPL	dense non-aqueous phase liquid
DO	dissolved oxygen
DOD	Department of Defense
DOE	Department of Energy
DQO	data quality objective
EGDY	East Gate Disposal Yard
EPA	Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
GC	gas chromotography
gpm	gallons per minute
INL	Idaho National Laboratory
ISB	in situ bioremediation
MLS	multi-level system
MNA	monitored natural attenuation
msl	mean sea level

MW	monitoring well
NAPL	non-aqueous phase liquid
NPL	National Priorities List
ORP	oxidation reduction potential
PCE	tetrachloroethene
PCR	polymerase chain reaction
PID	photoionization detector
ppb	parts per billion
QA	quality assurance
qPCR	quantitative polymerase chain reaction
SPME	solid phase microextraction
TAN	Test Area North
TCA	1,1,1-trichloroethane
TCE	trichloroethene
TDP	Technology Demonstration Plan
T-RFLP	terminal restriction fragment length polymorphism
UIC	underground injection control
USACE	United States Army Corps of Engineers
UWRL	Utah Water Research Laboratory
VC	vinyl chloride
VFA	volatile fatty acid
VOA	volatile organic analysis
VOC	volatile organic compound
WAC	Waste Administrative Code
WDOE	Washington Department of Ecology

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EXECUTIVE SUMMARY

BACKGROUND

In Situ Bioremediation (ISB) has been identified as a promising treatment technology for sites containing chlorinated solvent DNAPL source areas. This technology, however, is considered passive due to the relatively long remedial timeframes associated with the limited rate at which nonaqueous contaminants dissolve or diffuse to where bacteria can degrade them. Recent advances have shown, however, that mass transfer rates from the nonaqueous phase to the aqueous phase (where contaminatns are bioavailable) can be substantially increased during bioremediation (Sorenson, 2002; Macbeth et al., 2006). Therefore, the effective evaluation of key performance metrics, such as the rate of mass transfer, the growth and distribution of microbial populations of interest, contaminant degradation efficiency, and impact to mass flux, are critical to understanding the feasibility of this technology at DNAPL sites.

Bioremediation system performance has historically been evaluated using vertically integrated point measurements of dissolved source contaminant concentrations in aquifers (e.g., to determine changes in maximum concentrations and plume extents). A major drawback to the use of traditional groundwater monitoring with point measurements includes the method's proven inability to determine where a majority of the contaminant mass is located and migrating due to the often spatially complex distribution of dissolved contaminants; variability of hydraulic conductivity, groundwater flow rate and direction; and variation in water level (Einarson et al., 2002; Reinhard et al., 1984; Robertson et al., 1991; Van der Kamp et al., 1994). To improve the evaluation of ISB systems at chlorinated solvent DNAPL sites, a set of innovative diagnostic tools, including 3-D sampling using multi-level systems, passive flux meters, compound-specific stable isotope analysis and molecular microbiological assays, were applied concurrently with conventional techniques at a field site contaminated with DNAPL undergoing a field pilot test to evaluate ISB (i.e., ESTCP ER-0218). ESTCP demonstration project ER-0218 evaluated enhanced mass transfer during ISB operation at a DNAPL source zone at the Ft. Lewis East Gate Disposal Yard (EGDY) using two different whey injection strategies (North Wind, 2008).

OBJECTIVES OF THE DEMONSTRATION

The overall objective of this demonstration project was to evaluate innovative diagnostic tools for the implementation and optimization of bioremediation technology applications. The specific objectives of this demonstration project included: 1) demonstrate the effectiveness of ISB for remediation of chlorinated solvent residual DNAPL contamination using conventional and innovative diagnostic tools, 2) demonstrate efficacy of alternate diagnostic tools for evaluating performance and enhancing implementation of bioremediation in chlorinated solvent source areas, and 3) compare these innovative methods with conventional diagnostic tools that are currently used for assessing bioremediation performance**.**

DEMONSTRATION RESULTS

3-D multi-level systems provided critical information in understanding heterogeneity in the hydraulic system including the presence of vertical gradients, and preferential flow paths within the subsurface at the Ft. Lewis EGDY. In addition, the data provided by the system was key in optimizing the injection design to effectively encompass target horizontal and vertical

contaminant treatment areas resulting in degradation of trichloroethene (TCE) in the areas receiving whey. The use of the 3-D CMT system also allowed for the evaluation of variability in contaminant mass vertically within the treatment areas, and to assess response in contaminant concentrations used to determine mass transfer effects. This provided important information in evaluating mass transfer of residual contaminant mass to the aqueous phase, and evaluating efficiency of reductive dechlorination within the vertical treatment interval. The 3-D CMT system was also used to evaluate mass flux, although there was significant uncertainty in these estimates due to highly variable groundwater flow velocity at the Ft. Lewis site.

The 3-D CMT system was more expensive to install, operate and maintain than the traditional 2-D system, nearly doubling the cost of the monitoring program. However, much of the increased cost was saved in the ability to effectively optimize the ISB system to achieve design objectives in a much shorter timeframe than would be possible with conventional techniques. In addition, monitoring programs can be optimized to include only those parameters necessary for the site (i.e. at Ft. Lewis 3-D monitoring of geochemical parameters was unnecessary), and to reduce analytes and frequency of sampling during different phases of bioremediation operations.

Passive Flux Meters (PFM) provided useful information regarding the variability in groundwater velocity, and contaminant mass flux over the horizontal and vertical extent of the treatment areas. Therefore, PFM was likely a more accurate measure of mass flux compared to estimates assuming constant groundwater flow velocities as this was highly variable at Ft. Lewis. Contaminant response to the different ISB injection strategies, however, was more difficult to assess using PFMs at Ft. Lewis because increases in contaminant mass flux were concomitant to increases in groundwater velocity. Therefore, mass transfer effects due to the ISB injection strategies could not be evaluated using PFMs within the residual source area.

PFM analysis required the installation of additional wells, sampling and analytical costs to the monitoring program, increasing the performance monitoring costs by nearly 50%. Cost savings could potentially be realized, however, by optimizing the ISB operation to target specific zones within the contaminant area that are discharging the greatest mass of contaminants.

Compounds-specific stable isotope analysis (CSIA) was useful in verifying biological degradation of contaminants, although the method detection limits for vinyl chloride (VC) and ethene were higher than standard analytical techniques. Therefore, at Ft. Lewis, CSIA did not provide information regarding the loss in mass balance once cis- dichloroethene (DCE) was converted to VC and ethene. Had the monitoring been sustained until more of the DCE was transformed to VC and ethane, it is likely CSIA would have been able to show the mass balance in spite of the fact that groundwater concentrations would not have shown it.

Molecular tools provided important information on the microbial community, and contaminant-degrading populations, such as *Dehalococcoides*, in particular. Quantitative polymerase chain reaction (qPCR) was determined to be the most cost-effective molecular tool for evaluating the presence, growth and activity of contaminant-degrading populations. Additional analytical costs were offset by providing information as to wither to bioaugment and the ability to optimize the ISB system to achieve optimal environmental conditions for contaminant-degrading populations.

1.0 INTRODUCTION

This report provides the demonstration cost and performance results for the application of innovative diagnostic tools for performance evaluation of in situ bioremediation (ISB) of a chlorinated solvent source area at the Fort Lewis East Gate Disposal Yard (EGDY). This Environmental Security Technology Certification Program (ESTCP) project, ER-0318, was conducted concurrently with ESTCP project ER-0218, which was designed to demonstrate enhanced mass transfer of chlorinated solvent dense non-aqueous phase liquid (DNAPL) using two different biostimulation strategies. The addition of certain electron donors to groundwater has been shown to enhance the mass transfer of DNAPL to the water phase by increasing contaminant bioavailability, and thus increase rates and extent of biological degradation via anaerobic reductive dechlorination (ARD). Improvement of diagnostic tools for quantifying and optimizing bioremediation performance, including ARD and enhanced mass transfer, would greatly augment the application of bioremediation at chlorinated solvent DNAPL sites.

Under ESTCP project ER-0218, two hydraulically isolated treatment cells, each consisting of a network of monitoring wells, an injection well, and an extraction well, were installed at the EGDY non-aqueous phase liquid (NAPL) Area 3. These treatment cells were monitored with various innovative diagnostic tools under ESTCP project ER-0318. The treatment cells were located on the fringe of the DNAPL source area (Treatment cell 1) and within the DNAPL source area (Treatment cell 2). Two injection strategies, or Scenarios, were applied to each treatment cell. Scenario 1 consisted of low concentration (1% w/w) whey powder injections and Scenario 2 consisted of high concentration (10% w/w) whey powder injections. For Treatment cell 1, Scenario 2 was applied within the cell for approximately four months followed by four months of Scenario 1, low concentration (1%) whey powder. For Treatment cell 2, Scenario 1 was applied first for four months followed by Scenario 2, high concentration (10%) whey powder, for an additional four months.

Application of the innovative diagnostic tools during the ER-0218 evaluation of the two injection strategies allowed for evaluation of key parameters critical to the success of enhanced bioremediation in a DNAPL source area. These parameters include distribution of bioremediation amendments and their effects throughout the desired treatment area, enhanced mass transfer and contaminant mass removal measurements, and extent of enrichment of contaminant-degrading microorganisms. In particular, the tools evaluated included:

- 3-D sampling using a multi-level sampling systems – determined the benefits (and costs) of using a 3-D sampling system to evaluate treatment performance using conventional analytical parameters, such as distribution of amendments; redox parameters; biological activity indicators; and distribution, attenuation, and mass flux of chlorinated contaminants. The three-dimensional analysis was also used to determine system hydraulic heterogeneity and vertical groundwater gradients.

- Passive flux meter technology - determined changes in contaminant mass flux in groundwater over time during bioremediation application at discrete locations.

- Compound-specific stable isotope analysis (CSIA) - evaluated degradation mechanisms for contaminants in order to verify biological degradation of contaminants.

- Molecular tools - evaluated presence and time changes of microbial community, including contaminant-degrading organisms, and other key organisms that impact reductive dechlorination during treatment.

The data obtained using these diagnostic tools were compared with the data that would have been obtained using standard practices in order to determine utility and cost-effectiveness of the methods. This cost and performance report details each of the innovative diagnostic tools and describes how they were implemented during the ER-0218 demonstration at Fort Lewis EGDY NAPL Area 3. The background information, objectives of the demonstration, and regulatory drivers are described in the remainder of Section 1. The specific innovative tools are described in Section 2, as well as the advantages and limitations of each technology, while the performance objectives are detailed in Section 3. The demonstration site is described in Section 4 and the overall test design is presented in Section 5. Finally, the performance assessment, cost assessment, implementation issues, and references are presented in Sections 6, 7, 8, and 9, respectively.

1.1 BACKGROUND

Chlorinated solvents comprise two of the top four of the most common groundwater contaminants at hazardous waste sites in the United States (www.atsdr.cdc.gov/cep/07cep). In the Agency for Toxic Substances and Disease Registry report on *Substances Most Frequently Found in Completed Exposure Pathways (CEPs) at Hazardous Waste Sites* 2007, seven of the top 20 contaminants most frequently found in completed exposure pathways at hazardous waste sites on the National Priorities List (NPL) were chlorinated solvents and their intrinsic degradation products, including two of the top four (Pankow and Cherry 1996). Of particular significance is the identification of trichloroethene (TCE) and tetrachloroethene (PCE) as the third and fourth most common contaminants at NPL sites. Not surprisingly, the Department of Defense (DOD) has identified chlorinated solvents at nearly 50% of its 3,212 contaminated waste sites and TCE appears as a major groundwater contaminant at 35% of all DOD sites (EPA 2004), and many of these sites contain chlorinated solvents as dense non-aqueous liquids (DNAPL).

The longevity of chlorinated solvents is thought to be attributable to their widespread use, their hydrophobic nature, and to their relatively oxidized states that prevent them from serving as electron donors for microorganisms. Due to their hydrophobic nature, chlorinated solvents can exist as DNAPLs at many sites. DNAPLs are hydrophobic liquids with a density greater than water. Pertinent to their longevity is the fact that the solubility of the common chlorinated solvents (PCE, TCE, 1,1,1-trichloroethane [TCA], and carbon tetrachloride) ranges from approximately 200 to 1,400 mg/L at 25°C (Sale 1998). These relatively low solubilities play a significant role in limiting mass transfer to the aqueous phase once the solvents contaminate groundwater. Interphase mass transfer (dissolution) of a solvent NAPL into groundwater is governed by the difference between the aqueous solubility of the compound and the actual concentration in groundwater. (Sale [1998] provides an excellent discussion of fundamental interphase mass transfer from DNAPLs.) At typical groundwater velocities, the aqueous concentration of the solvent in the immediate vicinity of the groundwater-NAPL interface approaches the solubility within the first few centimeters of flow along the interface (Bouwer and McCarty 1983) resulting in limited mass transfer into groundwater. For example, if

groundwater flows across a pool of DNAPL (or through an area of residual saturation) several meters long in the direction of flow, mass transfer into the aqueous phase is insignificant along all but the first few centimeters of the flow path. Therefore, groundwater concentrations of chlorinated solvents rarely measure greater than 10% of their solubility despite the presence of large quantities of DNAPL. The result is that chlorinated solvents persist in groundwater for many decades, or perhaps even centuries.

Due to the physical characteristics of DNAPL plumes, the relative recalcitrance of chlorinated solvents, and often complex subsurface heterogeneity, remediation of contaminated groundwater is often considered technically or economically impracticable. Many sites have historically resorted to pump and treat or other containment technologies, which can have significant operations and maintenance costs due to the longevity of the DNAPL sources. In attempting to address the overwhelming costs associated with DNAPL remediation, the Strategic Environmental Research and Development Program has noted (http://www.serdp.com/02SON/CUSON-02-02.html) that:

> *"…the operations and maintenance of engineered containment systems has become a large proportion of DOD environmental budgets, and these costs may continue long into the future. Technologies designed to remove subsurface sources of contaminants, particularly DNAPLs, have received tremendous recent interest. Several approaches have been developed and tested, including thermal treatment technologies, chemical oxidation,* ***bioremediation****, and enhanced physical removal (using cosolvents or surfactants, for example)." [emphasis added]*

Bioremediation has been identified as a promising treatment technology for chlorinated solvent contamination due to relatively low capital costs and minimal generation of secondary waste streams. Bioremediation is non-hazardous to workers and the environment, destroys contaminants in situ, is relatively low maintenance, and minimizes disturbance of the site. Bioremediation, with respect to chlorinated solvent DNAPL source area remediation, however, is limited by the rate at which nonaqueous contaminants dissolve or diffuse to where bacteria can degrade them. Recent advances have shown, however, that mass transfer rates of chlorinated solvents from the nonaqueous phase to the aqueous phase (where they are bioavailable) can be substantially increased during bioremediation (Sorenson, 2002).

Bioremediation system performance has historically been evaluated using point measurements of dissolved source contaminant concentrations in aquifers (e.g., to determine changes in maximum concentrations and plume extents). A major drawback to the use of traditional groundwater monitoring with point measurements includes the method's proven inability to determine where a majority of the contaminant mass is located and migrating due to the often spatially complex distribution of dissolved contaminants, variability of groundwater flow rate and direction, and variation in water level (Einarson et al., 2002; Reinhard et al., 1984; Robertson et al., 1991; Van der Kamp et al., 1994).

To improve the evaluation of ISB systems at chlorinated solvent contaminated sites, a set of diagnostic tools was applied concurrently with conventional techniques at a field site contaminated with TCE DNAPL undergoing a field pilot test to evaluate ISB (i.e., ESTCP ER-0218). The specific diagnostic tools evaluated in this project included:

- 3-D sampling,
- Passive flux meters,
- CSIA, and
- Molecular tools.

1.2 OBJECTIVES OF THE DEMONSTRATION

The overall objective of this demonstration project was to evaluate innovative tools for the implementation and optimization of bioremediation technology applications. The specific objectives of this demonstration project included:

1. Demonstrate the effectiveness of ISB for remediation of chlorinated solvent residual DNAPL contamination using conventional and innovative diagnostic tools,
2. Demonstrate efficacy of alternate diagnostic tools for evaluating performance and enhancing implementation of bioremediation in chlorinated solvent source areas, and
3. Compare these innovative methods with conventional diagnostic tools that are currently used for assessing bioremediation performance.

The scope of the demonstration project included working with University of California Berkeley, University of Florida, Malcolm-Pirnie, and the United States Army Corps of Engineers (USACE) Seattle District to conduct monitoring of bioremediation performance during the ER-0218 demonstration at Fort Lewis EGDY.

1.3 REGULATORY DRIVERS

Solubilities of PCE, TCE, TCA, and carbon tetrachloride range from about 200 to 1,400 mg/L at 25°C (Sale 1998). These solubilities exceed Federal Safe Drinking Water Act maximum contaminant levels (see Table 1-1) by five to six orders of magnitude. The persistence of chlorinated solvents in groundwater, their prevalence, and their solubilities far in excess of health-based levels drive the need for cost-effective remediation technologies.

Table 1-1. Safe Drinking Water Act Maximum Contaminant Levels for Ft. Lewis EGDY Contaminants of Concern.

Compound	Regulatory Limit (μg/L[1])
TCE	5
cis-DCE	70
trans-DCE	100
Vinyl Chloride (VC)	2
1: 40 Code of Federal Regulations (CFR) 141.61	

2.0 TECHNOLOGY

The technologies evaluated under ESTCP project ER-0318 comprise a suite of innovative diagnostic tools applied to ISB of DNAPL source zones. The technologies include 3-D sampling using multi-level sampling wells, CSIA, flux meter analysis, and molecular tools. Detailed descriptions and applications of these tools are described below.

2.1 TECHNOLOGY DESCRIPTION

Enhanced bioremediation for chlorinated solvents has largely focused on anaerobic reductive dechlorination, also termed chlororespiration or halorespiration, a process where anaerobic microorganisms use chlorinated solvents as metabolic electron acceptors for energy generation (Maymó-Gatell et al., 1997; Holliger et al., 1999; Loffler et al., 1999). Bioremediation of chlorinated solvents via reductive dechlorination has been well documented (e.g., Ballapragada et al., 1997; Bouwer and McCarty, 1983; Carr and Hughes, 1998; deBruin et al., 1992; DiStefano et al., 1991 & 1992; Fathepure and Boyd, 1988; Fennell et al., 1997; Freedman and Gossett, 1989; Parsons et al., 1984; Vogel and McCarty, 1985). Reductive dechlorination is a strictly anaerobic process and results in the sequential reduction of highly oxidized contaminants, such as TCE, to daughter products, such as cis-DCE, vinyl chloride (VC), and ethene.

Several microorganisms capable of chlororespiration have been isolated from contaminated and pristine sites. Theses populations are generally strict anaerobes and can be separated into two groups. The first are those capable of reductive dechlorination of TCE to *cis*-DCE which can be classified into a number of phylogenetic groups including *Desulfuromonas* sp. strain BB1, *Desulfuromonas chloroethenica, Sulfurospirillum multivorans, Dehalobacter restrictus* strains PER-K23A and TEA, *Enterobacter* sp. Strain MS1 and *Desulfitobaccterium* sp. strain PCE-S (Holliger 1999). Hydrogen is generally an electron donor for these organisms, except for *Desulfuromonas* sp. strain BB1 and *Desulfuromonas chlorethenica,* which require acetate to support reductive dechlorination of TCE. The second group is capable of complete reductive dechlorination of TCE to ethene, and only includes the obligatory hydrogenotrophic genus *Dehalococcoides* (Maymo-Gatell et al., 1999; Cupples et al., 2003; He et al., 2003; Sung et al., 2006). In addition, the presence of this genus has been linked to the ability to perform complete dechlorination at chloroethene-contaminated field sites (Hendrickson 2002). Therefore, bioremediation strategies increasingly target *Dehalococcoides* for growth and activity through biostimulation or bioaugmentation (Major et al., 2002; Macbeth et al., 2004; Rahm et al., 2006).

Historically, enhanced bioremediation of chlorinated solvents has focused on remediation in dissolved phase plumes. The potential for using bioremediation in chlorinated solvent source zones is now gaining attention due to recent laboratory (Carr et al., 2000; Cope and Hughes, 2001; Yang and McCarty, 2000) and field (Song et al., ,2002; Macbeth et al., 2006) studies. The high potential for cost-effective bioremediation of chlorinated solvent DNAPL source areas has been limited by an incomplete understanding of how best to design, monitor, and predict the performance of bioremediation approaches. Therefore, there is an obvious need for effective diagnostic procedures that allow for rapid and appropriate optimization of field operations leading to more cost-effective cleanup.

This project was conducted concurrently with ESTCP project ER-0218, that demonstrated enhanced mass transfer during bioremediation of NAPL Area 3 at the Fort Lewis EGDY. This project leveraged funds from the ER-0218 project to evaluate technology performance by comparing the use of innovative diagnostic tools to more conventional monitoring techniques during this demonstration. An overview of the innovative diagnostic tools, their application, and the data obtained during the ER-0218 demonstration and used for evaluation in this study are presented in Table 2-1.

Table 2-1. Overview of Innovative Diagnostic Tools.

Diagnostic Tool	Data Obtained
3-Dimensional Sampling using Multiple-Level Sampling Wells	• Differentiate vertical aquifer zones to determine any preferential flowpaths. • Evaluate contaminant mass distribution and flux through discrete vertical zones. • Evaluate distribution of bioremediation amendment both horizontally and vertically within target treatment areas and determine effect on geochemistry, and aqueous contaminant and daughter product concentrations.
Passive Flux Meters	• Measure cumulative water and contaminant mass fluxes in groundwater. • Vertically differentiate zones within the aquifer to determine any preferential flowpaths where significant contaminant mass flux occurs.
CSIA	• Monitor the carbon isotope ratios of TCE and its biodegradation byproducts to differentiate between the effects of groundwater transport, dissolution of DNAPL at the source, and enhanced bioremediation. • Confirm biological reductive dechlorination.
Molecular Tools	• Assess impacts of bioremediation amendment on the biological community. • Determine presence and enrichment of contaminant-degrading microorganisms after treatment. • Monitor microbial community dynamics and correlate population shifts of key organisms with dechlorination performance.

2.1.1 3-D Sampling of Multiple Level Wells

For the ER-0318 demonstration, the Solinst® continuous multichannel tubing (CMT) multilevel system was used for four monitoring wells within each of the two treatment cells (see Figure 2-1 for diagram of CMT well). CMT wells were selected because they are relatively easy to install and are low-cost for shallow targeted depth intervals. The depth interval of interest at the Ft. Lewis EGDY was 10 to 30 ft below ground surface (bgs). The utility of the CMT wells was evaluated for parameters such as: 1) vertical differentiation of preferential flowpaths within the aquifer, 2) delineation of the spatial distribution of contaminants, and byproducts within the vertical intervals, 3) evaluation of contaminant mass flux within the target vertical intervals before and during treatment, and 4) evaluation of the distribution of whey powder throughout the target vertical interval and geochemical changes due to the treatment process (i.e., redox, pH, and fermentation).

2.1.2 Compound-Specific Stable Isotope Analysis

Compound-specific stable isotope analysis (CSIA) is an analytical technique used to generate an isotopic signature or ratio for different compounds. Physical processes, such as dilution, sorption, and volatilization, have very little impact on the isotopic signature of a particular compound (i.e., TCE in groundwater). Other processes, however (i.e., biotic and abiotic degradation), have significant impacts on the isotopic ratios of compounds. During ARD of TCE, mass balance between parent compounds (i.e., TCE) and reductive daughter products (i.e., ethene) is often not observed in groundwater samples, which leads to concerns regarding the actual fate of the contaminants. CSIA represents a powerful tool for monitoring ISB of chlorinated solvents that can specifically evaluate the mechanisms for contaminant concentration reductions (Hunkeler et al., 1999; Sherwood-Lollar et al., 2001; Slater et al., 2001; Song et al., 2002).

Bioremediation is based on the transformation of organic compounds by biological processes, and these processes can cause significant shifts in the ratio of ^{13}C to ^{12}C in both the reactants and products. By comparing the isotopic signature of the parent compound to the degradation byproducts, changes in concentrations can be attributed to physical or degradative processes (Conrad et al., 1997; Landmeyer et al., 1996; Revesz et al., 1995). During bioremediation, microorganisms preferentially utilize molecules with ^{12}C as opposed to ^{13}C, which causes the ratio of $^{13}C/^{12}C$ to increase, or become “heavy”. In addition, the degradative daughter product (i.e., cDCE) is initially predominantly ^{12}C and therefore, the $^{13}C/^{12}C$ is a relatively low value, or “light”. As the parent compound becomes depleted, however, microorganisms begin using ^{13}C, and the daughter product becomes heavier. Once the isotopic signature of the daughter product approaches that of the parent compound, completed degradation (or mass balance) is deduced. Therefore, CSIA of the metabolic byproducts was used to determine the dominant biochemical pathways within specific degradation zones before, during, and after the bioremediation treatment.

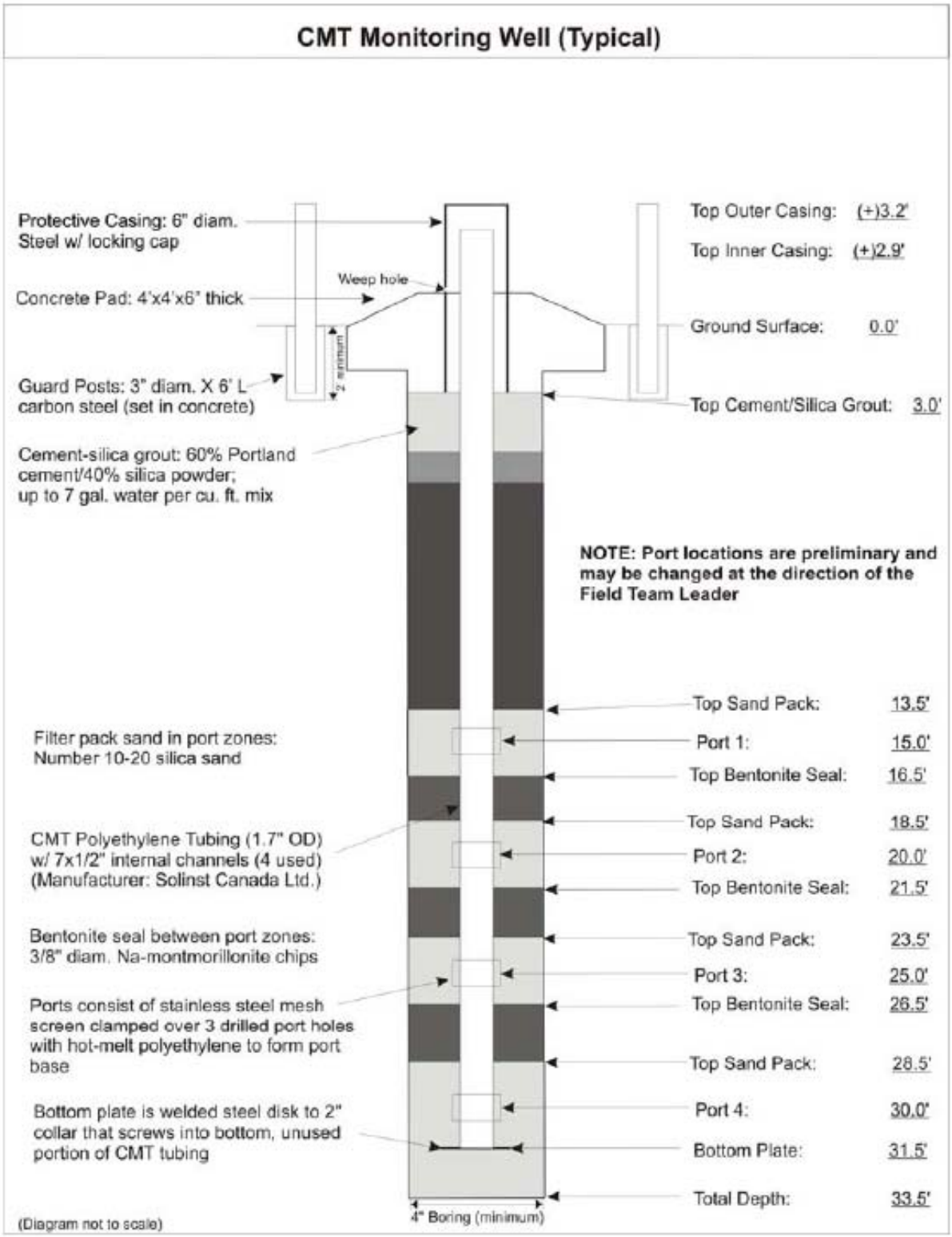


Figure 2-1. Typical CMT Monitoring Well Configuration.

2.1.3 Molecular Tools

Advancements in molecular biology have made it possible to measure the impact of biostimulation on the resident microbial community directly at a contaminated site. Several techniques were evaluated during this demonstration in order to understand their application and utility. The molecular techniques evaluated can be divided into two categories: 1) polymerase-chain reaction (PCR)-based techniques, and 2) florescent microscopy-based techniques. These molecular techniques differ in the way that samples are handled and analyzed and the microorganisms that they target. A summary of the molecular techniques employed as part of this demonstration is presented in Table 2-2.

Table 2-2. Summary of Molecular Tools Evaluated During the ER-0318 Demonstration.

Analysis and Target	Specificity	Data Use
PCR-based techniques		
T-RFLP- *Bacteria*	Kingdom	Community diversity profiling.
T-RFLP- *Archaea*	Kingdom	Community diversity profiling.
qPCR- *Archaea*	Kingdom	Abundance of *Archaea*.
qPCR-*Bacteria*	Kingdom	Abundance of *Bacteria*.
qPCR- 16S rRNA *Dehalococcoides*	Genera	Presence and abundance of the only known organism capable of degrading TCE to ethene.
qPCR- *vcrA*	Species	Presence and abundance of a gene related to the degradation of PCE to ethene in an energy yielding reaction.
qPCR- *tceA*	Species	Presence and abundance of a gene related to the degradation of PCE to vinyl chloride in an energy yielding reaction.
qPCR- *bvcA*	Species	Presence and abundance of a gene related to the degradation of cis-DCE to ethene in an energy yielding reaction.
qPCR- *Methanosarcinales*	Order	Presence and abundance of this order - contains acetoclastic methanogens with optimal temperature ranges from 25 to 60°C.
qPCR- *Methanococcales*	Order	Presence and abundance of this order - contains hydrogenotrophic methanogens with optimal temperature regimes from 35 to 85°C.

Table 2-2. (continued).

Analysis and Target	Specificity	Data Use
qPCR-*Methanobacteriales*	Order	Presence and abundance of this order - contains hydrogenotrophic methanogens with optimal temperature ranges from 37 to 88°C.
qPCR-*Methanomicrobiales*	Order	Presence and abundance of this order - contains hydrogenotrophic methanogens with optimal temperatures from 15 to 40°C.
Fluorescent-microscopy-based tools		
FISH- *Dehalococcoides*	Genus	Presence and activity of the only known organism capable of degrading TCE to ethene.
FISH-*Methanobacteriaceae*	Family	Presence and activity of this family.
FISH-*Methanococcales*	Order	Presence and activity of this order.
FISH-*Methanomicrobiales*	Order	Presence and activity of this family.
FISH- *Methanosarcina*	Genus	Presence and activity of this genus.
FISH-*Methanosarcinaceae*	Family	Presence and activity of this family.
FISH-*Methanosarcinaceae* including *Methanosaeta*	Order	Presence and activity of this order.
FISH- *Methanosaeta*	Genus	Presence and activity of this family.
FISH: Fluorescence in situ hybridization qPCR: Quantitative polymerase chain reactions T-RFLP: Terminal restriction fragment length polymorphism		

Recently, investigators have applied PCR-based molecular techniques to investigate aspects of communities performing reductive dechlorination during bioremediation (Ellis et al., 2000; Flynn et al., 2000; Harkness et al., 1999; Loffler et al., 2000; Macbeth et al., 2004; Rahm et al., 2006; Richardson et al., 2002). By far, the most-wide spread application is the use of quantitative polymerase chain reaction (qPCR) techniques to evaluate populations of *Dehalococcoides* species and the reductase genes *tceA*, *vcrA*, and *bvcA* (Muller et al., 2004; Holmes et al. 2006; Lee et al., 2006; Rahm et al., 2006; Ritalahti et al., 2006; Sung et al., 2006; Lee et al., 2008). In addition, qPCR techniques have also been developed for methanogenic populations of interest (Yu et al., 2005), although these techniques have not been previously applied to a reductively dechlorinating groundwater community.

Two PCR-based techniques were evaluated as part of this demonstration: terminal restriction fragment length polymorphism (T-RFLP) and qPCR. T-RFLP is a technique that generates microbial community chromatographs that illustrate the number of amplified targets (inferred to be species in the application evaluated) and relative abundance of target bacterial and archaeal population(s). The T-RFLP target used to evaluate the populations of interest was the 16S rRNA gene. The 16S rRNA gene codes for the RNA portion of the small subunit of the bacterial ribosome, which is used to make proteins in microbial biosynthesis. A great deal of research has been performed using this gene because it is highly conserved between all microorganisms; however, variability in the DNA sequence of the gene has been shown to be directly related to similarity between different microbial populations, or how closely those populations are related. A great deal of research has been conducted to characterize and catalogue all known *Bacteria* and *Archaea* 16S rDNA sequences into comprehensive databases. 16S rRNA gene-based molecular techniques can be used to tentatively identify individual members of a microbial community, and to assess the relative diversity and abundance of populations within the community. Because of the relatively low cost and ease of application, T-RFLP has become widely used for assessing microbial diversity. When combined with the sequencing of clone libraries (Dunbar et al., 2000; Knight et al., 1999; Lueders and Friedrich, 2000, Richardson et al., 2002; Macbeth et al. 2004), this technique allows the dominant members of a community to be qualitatively tracked over time.

At the Idaho National Laboratory (INL) site, Macbeth et al. (2004) found that the biostimulated methanogenic community was dominated by acetate consumers (acetoclastic) rather than hydrogen consumers (hydrogenotrophic), suggesting that methanogenic competition with the dechlorinators for hydrogen (as suggested by Smatlak et al., 1996) may have been limited at this site. In addition to community-level T-RFLP profiling, methanogenic populations were also evaluated using qPCR techniques (Yu et al., 2005), also targeting the 16S rRNA gene, with specificity to four orders (see Table 2-2) of methanogens. Individual qPCR runs were conducted for each of the specific targets in order to quantify the relative abundance of each within the samples.

For specific analysis of known dechlorinators, most notably those related to *Dehalococcoides*, specific primers can be used during qPCR. A recent study conducted with a wide range of field samples suggests that the presence of these organisms highly corresponds with the ability to stimulate complete dechlorination of PCE and TCE to ethene (Hendrickson et al., 2002). Recent approaches include the use of *Dehalococcoides* - specific primers with quantitative PCR in order to quantify concentrations of these organisms in environmental samples and correlate with observed dehalogenation activity (Chauhan et al., 2002; Holmes et al., 2006; Lee et al., 2006; Ritalahti et al., 2006; Lee et al., 2008).

In addition to PCR - based techniques, whole cell assays using fluorescence in situ hybridization (FISH) molecular probes were also conducted to target a similar suite of organisms as the qPCR assays, based on availability of appropriate FISH probes. FISH probes targeting similar groups of microorganisms (including *Dehalococcoides* and methanogenic populations) to qPCR were evaluated (Table 2-2). FISH was used to evaluate the distribution of active *Dehalococcoides* and methanogenic populations, and to evaluate expression activity, based on RNA, of microbes *in situ* (Amann 1995, Del Nery et al., 2008). Single cells were probed with fluorescently labeled oligonucleotides that hybridize to ribosomal RNA (rRNA). rRNA was the targeted molecule

because of its prevalence in all cells, which leads to a high signal intensity. In addition, different fluorescent dyes were used at the same time, in order to detect several different species (or sub-species) of microbes (i.e., *Bacteria*, *Archaea*, *Dehalococcoides*) at any given time (Raskin et al., 1994).

The application of these molecular tools could be extremely useful for predicting bioremediation performance prior to enhancement and for optimizing injection strategies. For instance, if the dehalogenating organisms are not initially present and are not enriched during initial biostimulation, it would suggest that bioaugmentation should be considered. While these molecular tools are interesting and show promise, the number of DNAPL source sites at which they have been applied to monitor field-scale bioremediation is relatively small. Further work with molecular tools applied in conjunction with other diagnostic tools is required to determine their efficacy for enhancing the cost effectiveness of bioremediation. For the purposes of this study, T-RFLP, qPCR, and FISH were used to track microbial community changes in response to whey powder injections in the two treatment cells within a DNAPL-source area. In addition, methanogens were monitored by qPCR to determine the dominance of acetate or hydrogen consumers. In all cases, the relationship between community structure and overall bioremediation performance was evaluated in order to determine the utility of these methods as predictive and performance assessment tools.

2.1.4 Mass Flux Analysis

A key element for assessing in-situ remediation of source areas by any technology is developing an understanding of the impact of the remediation on mass flux. For ISB, a key performance criterion is to increase contaminant mass transfer from the non-aqueous phase to the aqueous phase to maximize biodegradation rates while minimizing migration of contaminants out of the treatment area. Therefore, mass flux evaluation is important as a means of assessing both enhanced mass transfer and for evaluating impact of source area treatment on contaminants migrating downgradient of the source area. Mass flux was evaluated in this study using two different tools: 1) groundwater sampling and analysis in 3-D and 2) passive flux meters (PFM). The two methods differed fundamentally in the way that data are collected. The 3-D approach involved the collection of a groundwater samples at discrete points in time using a Solinst CMT multi-level system, and the flux meters involved the collection of cumulative, time-averaged data over longer periods of time in screened monitoring wells with “flux meters.” The PFM is a self-contained permeable unit that is inserted into a well or boring to intercept (but not retain) groundwater flow. Internal to this meter is a matrix of hydrophobic and hydrophilic permeable sorbents that retain dissolved organic and inorganic contaminants, and a conservative tracer. Contaminant masses retained are combined with calculated cumulative fluid flux from residual resident tracer masses to calculate time-averaged contaminant mass fluxes. Mass flux measurements calculated using this cumulative, time-averaged approach were compared to those using the three-dimensional, discrete time approach.

2.2 ADVANTAGES AND LIMITATION OF THE TECHNOLOGY

There are significant advantages of bioremediation as an in situ treatment technology that will be assessed using both conventional and innovative diagnostic techniques. The advantages of bioremediation over other in situ treatment technologies include low risk to human health and the environment during implementation, low secondary waste generation, minimal impacts during operations, and relatively low cost. Additional potential advantages include:

- **Potential for complete source cleanup using one technology, without requirement for separate polishing technologies**—Source removal technologies do not remove all of the NAPL present and often rely on polishing technologies, including ISB and monitored natural attenuation (MNA), to achieve remedial goals. ISB-ARD integrates source removal and polishing, thereby facilitating attainment of cleanup goals by reducing the need for further infrastructure, treatability studies, modification of site conditions, bioaugmentation, etc. that may be required to implement a polishing technology following source removal.

- **Flexibility of implementation**—ISB-ARD is easily scaled to the size of the site, with commensurate cost savings relative to more capital- and energy-intensive technologies. Given the minimal surface infrastructure requirements, the technology is also readily implemented around and under existing structures, and is not disruptive to most commercial or residential property uses. Electron donors can also be selected for enhanced dissolution properties, dechlorination properties, slow versus fast-release properties, etc., for specific applications.

Challenges for this technology include complex lithology, complex residual source mass architecture, unfavorable geochemistry (i.e., low or high pH or high concentrations of competing electron acceptors), insufficient contaminant-degrading biomass, and complex hydraulics (i.e., fracture flow).

The use of innovative diagnostic tools may significantly improve the ability to evaluate, design, and implement a bioremediation system cost-effectively in a residual source area. Technologies currently used to monitor bioremediation systems are generally applied via a network of wells sampled in one vertical horizon each for contaminants and degradation products, including volatile organic compounds (VOCs), dissolved gasses, redox indicators, biological activity indicators, and bioremediation amendments. This approach provides a broad understanding of the system in one dimension. The use of innovative technologies such as CSIA, 3-D sampling strategies, PFM, and molecular microbial tools could provide significant advantages for implementing bioremediation. Table 2-3 lists advantages and limitations of each of the diagnostic tools evaluated.

Table 2-3. Advantages and Limitations of Diagnostic Tools.

Technology	Advantages	Limitations
3-D Multi-level Sampling	• Determine vertical characteristics of aquifer, including gradients • Identifies high permeability zones and areas of predominant contaminant flux • Monitors vertical distribution of electron donor relative to contaminants	• Requires the collection of more samples at any single well, increasing both analytical and data analysis costs
Passive Flux Meter	• Provides simultaneous measurement of both water and contaminant fluxes • Provides long-term monitoring that generates time integrated estimates of both groundwater and contaminant flux	• Requires additional wells • Relatively specialized application
CSIA	• Distinguishes between biological and abiotic degradation of contaminants • Determines mass balance even when concentrations of degradation products in groundwater do not account for all mass	• Relatively specialized and requires detailed knowledge of chemistry • Increases monitoring costs
Molecular Tools	• Evaluates microbial community response to treatment • Evaluates response in growth and activity of key microbial populations, including dehalogenating and methanogenic populations, to treatment • Determines if site is biologically limited and requires bioaugmentation	• Relatively specialized and requires detailed knowledge of microbiology • Increases monitoring costs

3.0 PERFORMANCE OBJECTIVES

Successful bioremediation, as applied to chlorinated solvent source areas in groundwater, realizes many of the benefits of more expensive and hazardous technologies, while retaining its benefits as a low cost, in situ technology. With this in mind, detailed performance objectives have been developed for the implementation, evaluation, and comparison of diagnostic tools for evaluating performance and optimization of ISB in chlorinated solvent source areas. The following represent key bioremediation design and implementation objectives that innovative and conventional diagnostic tools are used to assess:

1. Reduce mass flux emanating from the DNAPL source area.
2. Reduce concentrations of contaminants of concern in groundwater via transformation to innocuous end products within and downgradient from the DNAPL source area.
3. Increase the rate of contaminant mass removal to achieve closure criteria within an acceptable remedial timeframe.
4. Effectively distribute bioremediation amendments within target treatment area.
5. Minimize the frequency of amendment injections.
6. Develop and/or maintain an environment conducive to microbial growth and activity of contaminant-degrading microbial populations.

Table 3-1 illustrates performance objectives, data requirements for conventional and innovative tools, success criteria, and results pertaining to the Ft. Lewis ER-0318 study. The Demonstration was conducted in three phases:

Phase 1: Hydraulic characterization of the treatment cells. This phase of testing established hydrogeologic baseline parameters, including tracer measurements using the multi-level CMT wells to evaluate vertical transport and preferential flowpaths. This phase also evaluated baseline contaminant distribution in groundwater within the two treatment cells.

Phase 2: Baseline testing, during which all diagnostic tools (except FISH) were evaluated to assess the baseline conditions in each treatment cell prior to whey injection. This phase of testing established the contaminant flux baseline parameters using both analytical samples of the CMT wells and PFM. Baseline CSIA values were also determined pre-whey injection, as were the PCR-based molecular tools.

Phase 3: Biostimulation and enhanced mass transfer demonstration, during which all analytical parameters were monitored under two whey injection Scenarios for comparison as well as comparison to Phase 2 conditions. The same analytes described for Phase 2 were analyzed during Phase 3, and FISH analyses were added to evaluate activity of different populations of the microbial community.

Table 3-1 relates the performance objectives to the operational phases of the enhanced bioremediation application.

Table 3-1. Performance Objectives for Evaluation of ISB-ARD Enhanced Mass Transfer Demonstration Using Innovative Diagnostic Tools.

Performance Objective	Data Requirements Conventional Tool	Data Requirements Innovative Tool	Success Criteria	Results
Qualitative				
Demonstrate that new diagnostic tools improve the implementation and optimization of enhanced ISB.	Pre-, during- and post-treatment single point sampling wells with analyses of groundwater parameters such as VOCs, VFAs, COD, sulfate, nitrate, soluble iron, pH, conductivity, and ORP.	Pre-, during- and post-treatment multiple level sampling wells, PFMs, CSIA, and molecular tools.	Demonstrate that innovative tools resulted in efficiencies in the design, operation and/or maintenance of the treatment system to result in more cost-effective treatment.	Innovative tools provided valuable information that improved the design and lead to the successful implementation of ISB in the Ft. Lewis EGDY DNAPL source area.
Quantitative				
Phase 1: Determine hydraulic parameters for injection design including, groundwater gradient, velocity, direction, and residence time as a result of injection.	Pre-treatment, single point sampling wells: conduct pumping and tracer tests.	Pre-treatment multi-level sampling wells: conduct pumping and tracer tests.	Determination of realistic hydraulic parameters that can be used to design an effective injection strategy.	2-D sampling: accurate hydraulic parameters could be measured horizontally using single depth sampling 3-D vertically discrete sampling: necessary to understand predominant vertical flow paths. The high groundwater flow rates of aerobic groundwater into the treatment cells suggested that high carbon loading would be required to maintain reducing conditions. 3-D results, however, suggested that there was significant variability in groundwater velocity within different vertical aquifer zones, and that injection into the low-permeability units could increase retention within the treatment cells.

Table 3-1. (continued).

Performance Objective	Data Requirements Conventional Tool	Data Requirements Innovative Tool	Success Criteria	Results
Phase 1: Determine vertical gradient and preferential flowpaths.	NA	Pre-treatment multi-level sampling wells: conduct pumping and tracer tests.	Assessment of any preferential vertical flow paths, as indicated by high tracer transport in discrete vertical zones.	3-D sampling: was necessary to document significant vertical gradient that resulted in distribution of tracer to depths below the target treatment area. Installation of new injection wells was necessary to inject into desired vertical interval successfully.
Phase 2: Determine contaminant distribution and mass flux pre-treatment.	Pre-treatment, single point sampling wells: collect 3 sampling rounds for contaminants (PCE, TCE, DCE isomers, VC) and degradation daughter products (ethene).	Pre-treatment Multi-level sampling wells: collect 3 sampling rounds for contaminants (PCE, TCE, DCE isomers, VC) and degradation daughter products (ethene). Pre-Treatment flux meter deployment.	Successful determination of contaminant mass distribution to define the target treatment area and determine baseline mass flux within two treatment cells.	2-D sampling: indicated substantially different contaminant mass concentrations within the areal extent of NAPL Area 3, with treatment cell 1 containing substantially less mass that treatment cell 2. 3-D sampling: indicated substantially different contaminant mass distribution both horizontally and vertically. PFM: confirmed that mass flux in treatment cell 2 was substantially greater than treatment cell 1.
Phase 2: Determine geochemistry and carbon concentration within treatment cells pre-treatment.	Pre-treatment, single point sampling wells: collect one round of sampling for carbon, alkalinity and redox and 3 rounds for pH.	Pre-treatment Multi-level sampling wells: collect one round of sampling for carbon, alkalinity and redox and 3 rounds for pH.	Successful determination of geochemical condition and requirements for carbon loading for injection design.	2-D and 3-D sampling: The treatment cells were predominantly aerobic, with the exception of one location in treatment cell 2, which was mildly iron- to sulfate-reducing. Very low levels of carbon were also observed. There was little difference between 2-D and 3-D results.

Table 3-1. (continued).

Performance Objective	Data Requirements Conventional Tool	Data Requirements Innovative Tool	Success Criteria	Results
Phase 2: Determine fate (i.e. attenuation) of contaminants and microbial community contaminant-degrading potential pre-treatment.	Pre-treatment, single point sampling wells: collect 3 sampling rounds for degradation daughter products.	Pre-treatment multi-level sampling wells: collect 3 sampling rounds for degradation daughter products. CSIA: collect one round of samples for CSIA. Molecular Tools: Collect one round of samples for *Dehalococcoides* spp. qPCR and T-RFLP.	Determination of attenuation mechanisms and populations capable of anaerobically degrading TCE.	2-D and 3-D sampling: Attenuation of TCE to cis-DCE was occurring within both treatment areas with 69-80% of the molar mass present as TCE and the remainder as cis-DCE prior to treatment. No VC or ethene was detected at concentrations above the method detection limit in either treatment cell, and results were similar for 2-D and 3-D. CSIA: TCE and cis-DCE were observed prior to treatment. The isotopic ratio of cis-DCE was significantly "lighter" than the TCE, suggesting that it was indeed biologically produced. qPCR analysis indicated the presence of *Dehalococcoides* spp. and reductase genes *tceA*, *bvcA*, and *vcrA* prior to treatment.
Phase 3. Evaluate distribution of whey within target treatment area and impact to geochemical conditions during and post-treatment.	During- and post-treatment single point sampling wells: collect 6 rounds of sampling during- and 2 rounds of samples post-treatment within treatment area monitoring wells.	During- and post-treatment multi-level sampling wells: collect 6 rounds of sampling during- and 2 rounds of samples post-treatment within treatment area monitoring wells.	Distribution of low (<1,000 mg/L) and high (>1,000 mg/L) concentrations of whey to target treatment areas.	2-D and 3-D sampling: Distribution of whey was successfully achieved horizontally (2-D) and vertically (3-D) within target treatment areas during low- (1% w/w) and high- (10% w/w) concentration whey injections.

Table 3-1. (continued).

Performance Objective	Data Requirements Conventional Tool	Data Requirements Innovative Tool	Success Criteria	Results
			Creation of highly reduced redox conditions within treatment are (i.e., methane-producing conditions) and maintenance of pH and alkalinity at levels conducive to contaminant-degrading microbial growth and activity.	In general, higher COD was observed in the deeper CMT depths when injecting 10% whey compared to 1% whey. Where whey was distributed, geochemical conditions changed, notably pH and methane. pH was significantly impacted during both low-and high-concentration whey injections. Low (<5.0) pH was observed in both treatment cells for approximately five months after the start of injections. After this time, the intrinsic buffering capacity increased in both cells and pH recovered to >5.5 between whey injections. Methane-producing conditions were not achieved within the treatment cells for approximately 4-5 months after initiation of Phase 3. By the end of the demonstration, methane was produced. Treatment cell 1 had the highest production rate and maximum methane concentrations. In general, methane concentrations varied at differing depths as measured using the CMT wells.

Table 3-1. (continued).

Performance Objective	Data Requirements Conventional Tool	Data Requirements Innovative Tool	Success Criteria	Results
Phase 3. Impact of treatment on contaminant mass flux.	During- and post-treatment single point sampling wells: collect 6 rounds of sampling during- and 2 rounds of samples post-treatment within treatment area monitoring wells. Collect 5 rounds of samples at downgradient monitoring wells.	During- and post-treatment multi-level sampling wells: collect 6 rounds of sampling during- and 2 rounds of samples post-treatment within treatment area monitoring wells. Flux meter: collect 4 rounds of flux meter data during treatment.	Increase in total VOC and/or ethene mass flux within source area during treatment with high concentration whey powder. Reduction of majority of TCE (>99%) contaminant mass flux within and downgradient from test areas.	3-D sampling: Factor of 2.5 increase in total VOC and ethene mass flux during 10% whey injections compared to pre- and 1% whey injections within source area (treatment cell 2) using flux plane (3-D sampling). Factor of 2.3-3.3 increase in total VOC mass flux during 10% whey injections compared to pre- and 1% whey injections within source area (treatment cell 2) using point measurements at monitoring wells that corresponded to PFM wells. PFM: Factor of 4 increase in total VOC mass flux during 10% whey injections compared to pre- and 1% whey injections within source area (treatment cell 2).
Phase 3. Determine fate (i.e., attenuation) of contaminants during- and post-treatment.	During- and post-treatment single point sampling wells: collect 6 rounds of sampling during- and 2 rounds of samples post-treatment within treatment area monitoring wells. Collect 5 rounds of samples at downgradient monitoring wells.	During- and post-treatment multi-level sampling wells: collect 6 rounds of sampling during- and 2 rounds of samples post-treatment within treatment area monitoring wells.	Degradation of majority (i.e. >90%) of TCE contaminant mass to innocuous end products within treatment area. Complete degradation of TCE to ethene and cessation of VOC flux downgradient of source area.	2-D and 3-D sampling: >99.96 reduction in TCE mass and a 33-52% decrease in total VOC mass. Post- treatment samples illustrate conversion to cis-DCE (52-59% of total mass), VC (33-36% of total mass), and ethene (4-10% of total mass) within treatment cells.

Table 3-1. (continued).

Performance Objective	Data Requirements Conventional Tool	Data Requirements Innovative Tool	Success Criteria	Results
		CSIA: collect 4 rounds of samples during- and one round of samples post-treatment.		CSIA: nearly complete biodegradation of TCE to cis-DCE and VC by the end of the demonstration. Presence of "light" VC indicative of VC generation occurring through biological mechanisms. No ethene, however, was observed with CSIA.
Phase 3. Determine impacts of treatment on microbial community, and populations affecting contaminant-degradation, specifically during- and post-treatment.	NA	Molecular tools: collect 4 rounds of samples during and one round of samples post-treatment.	Evaluate growth and activity of populations either directly or indirectly impacting contaminant-degradation. Verify enrichment of contaminant-degrading populations.	T-RFLP: significant changes in microbial community observed following whey injections. qPCR DHC: 3-4 order of magnitude increase in 16S rDNA DHC, 1-2 order magnitude increase in *tceA*, 2-5 order magnitude increase in *bvcA*, and 3-5 increase n *vcrA* after 6 months of injections. FISH DHC: 4 order magnitude increase in 16S rRNA for DHC after 6 months of injections. qPCR methanogens: 3-4 order magnitude increase in predominantly *Methanosarcinales*. qPCR FISH: 1 order magnitude increase in all methanogenic populations predominated by *Methanosarcinales*, although *Methanomicrobiales* and *Methanococcales* represented a much greater proportion compared to qPCR over 6 months of injections.

4.0 SITE DESCRIPTION

4.1 SITE LOCATION

The EGDY Phase II Remedial Investigation Report (USACE 2002) summarizes the history and characteristics of the test site, and the following discussion is summarized from that report. The Ft. Lewis Logistics Center is located in Pierce County, Washington, approximately 11 miles south of Tacoma and 17 miles northeast of Olympia. The Logistics Center occupies approximately 650 acres of the Ft. Lewis Military Reservation, located at Township 19 North, Range 2 East, Sections 21, 22, 26, and 27. It is bounded on the northwest by Interstate 5 and beyond by the town of Tillicum, on the north by the American Lake Gardens Tract, on the west by the Madigan Army Medical Center, and on the southwest by the Madigan Family Housing Area.

The EGDY is located southeast of the Logistics Center in an otherwise undeveloped portion of Ft. Lewis (Figure 4-1). The EGDY is loosely defined as the area southeast of the intersection of Rainier Avenue and East Lincoln Drive in which landfill trenching and disposal activities historically occurred over an area of approximately 35 acres.

The EGDY is located on an extensive upland glacial drift plain, at an elevation of approximately 290 ft above mean sea level (msl). Trees and shrubs have been cleared from the disposal trench areas.

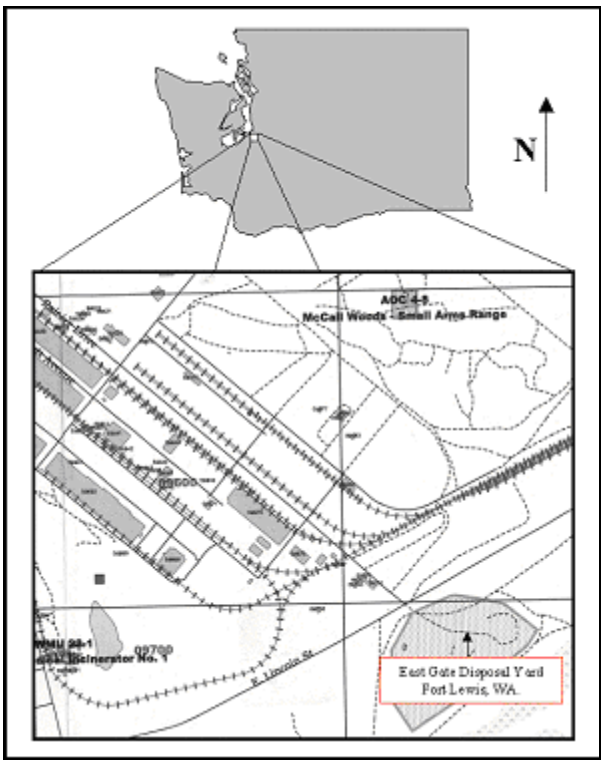


Figure 4-1. Location of the EGDY (USACE, 2008).

4.2 SITE GEOLOGY/HYDROGEOLOGY

At least three glacial and three non-glacial units have been identified in the sediments occurring above sea level at the EGDY, as illustrated in Figure 4-2. These units and a brief description are listed below, sequentially from youngest (shallowest) to oldest (deepest):

- **Holocene-Anthropomorphic Deposits**. These consist of man-made fill in the trench areas and include debris and burned material. These materials typically extend to less than 12 feet below ground surface (ft bgs).

- **Vashon Glacial Drift Deposits**. These consist of glacial deposits including recessional outwash, till and ice contact deposits, advance outwash, and glaciolacustrine silt/clay. Vashon drift deposits typically extend from ground surface to elevations of approximately 210 to 185 ft.

    - **Vashon Recessional Outwash-**Interbedded brown to gray sand gravel and sand with minor silt intervals; also loose, well-graded brown to gray sandy, cobbly gravel.

    - **Vashon Till and Ice Contact Deposits-**Dense, gray silty-sandy gravel and gravelly sandy silt 4 to 35 ft thick were present.

    - **Vashon Advance Outwash-**Interbedded brown to gray sandy gravel and sand, some cobbles, with minor silt interbeds.

    - **Second Non-Glacial Deposits**–Mottled, massive, organic rich, clayey, sandy gravel (mudflows) or lavender silt, peat, sand, and gravelly sand (fluvial overbank deposits).

    - **Third Non-Glacial Deposits**–Lavender silt, peat, sand, and gravelly sand.

The primary aquifers and aquitards are listed below, sequentially from shallowest to deepest:

- **Vashon Aquifer or Upper Aquifer.** The Vashon drift, Olympia beds, and Pre-Olympia drift comprise the Vashon unconfined aquifer. Vashon till and Olympia beds may act locally as discontinuous aquitards within the Vashon aquifer. Vashon outwash and pre-Olympia drift deposits comprise the aquifer materials within the Vashon aquifer. The Vashon aquifer varies in thickness from 100 to 130 ft and is continuous throughout the EGDY.

- **Intermediate Aquitard.** A somewhat laterally continuous till layer may separate the Vashon aquifer locally into an upper and lower permeable unit separated by this relatively low-permeability till or glaciolacustrine silt. This till is notably absent immediately north of NAPL Area 3 where low permeability units do not separate the upper and lower portions of the Vashon aquifer. The demonstration was performed in the upper Vashon aquifer, above the intermediate aquitard.

- **Non-Glacial Aquitard.** A regional aquitard consisting of low permeability second non-glacial deposits separates the Vashon aquifer from the Sea Level (lower) aquifer.

- **Sea Level Aquifer.** Third glacial drift deposits and permeable lower deposits of the second non-glacial unit comprise the Sea Level aquifer. This unit is widely used as a source of groundwater for industrial and municipal use.

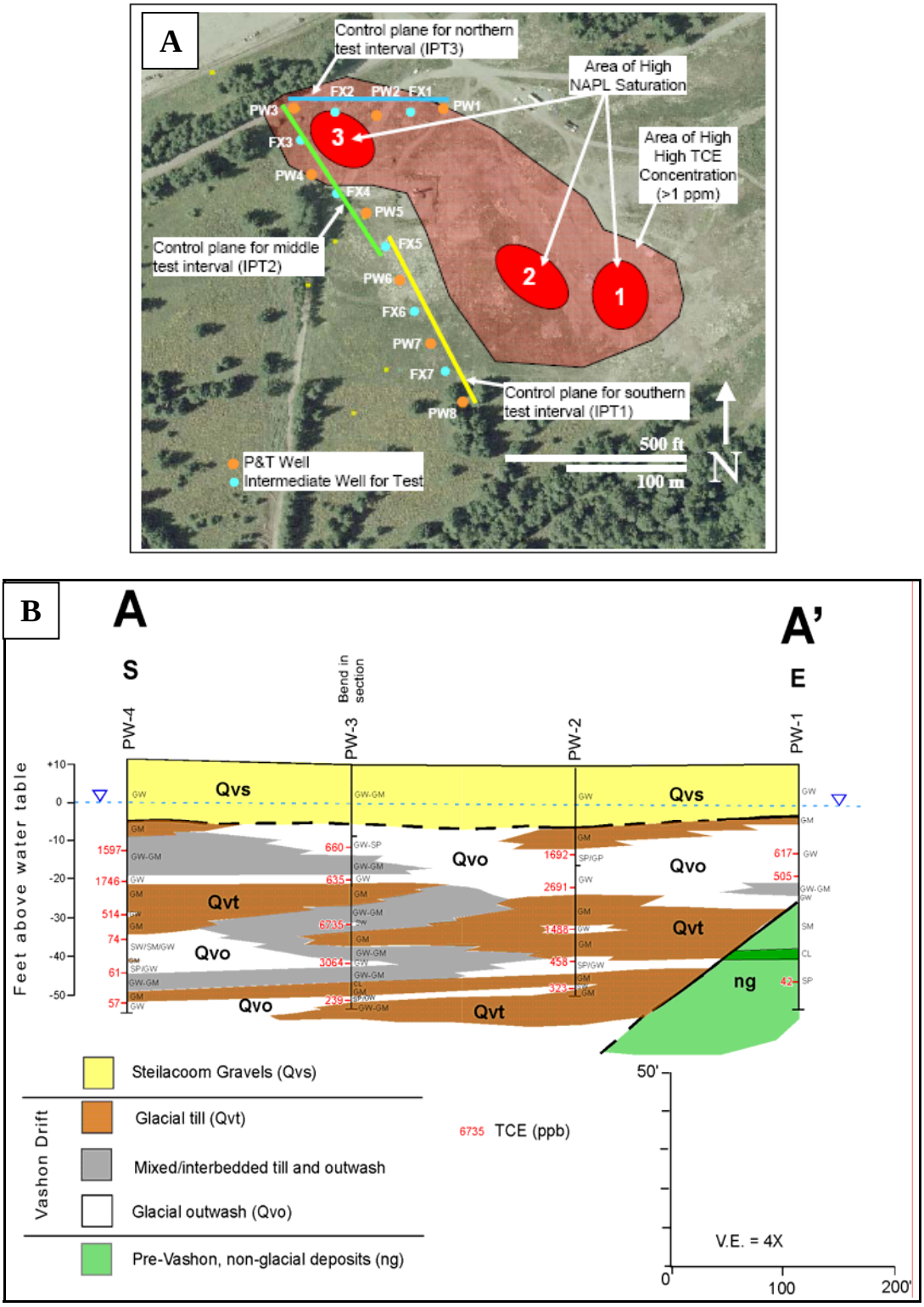


Figure 4-2. Plan View of the EGDY, Including NAPL Area 3 (A) and Cross Section of Lithological Units from a Cross Section Extending South (PW-4) to North through NAPL Area 3 (B) (Truex et al, 2006).

The properties of the Vashon Aquifer are cited in Table 4-1. These properties were used in the initial ISB demonstration design. The hydrologic properties of the demonstration test cells were evaluated as part of Phase 1 of the demonstration.

Table 4-1. Vashon Aquifer Properties Reported in Phase II RI Report.

Property	Value	How determined
Hydraulic conductivity	16 to 114 ft/day	Pumping tests in EGDY
	53 to 1140 ft/day	Pumping tests in I-5 well field
	110 to 3800 ft/day	Tracer tests near EGDY infiltration galleries
	4.4E-03 to 27.2 ft/day	Laboratory permeability tests for EGDY Vashon aquifer materials
Groundwater flow direction	W-SW near NAPL Area 3, as well as NAPL Areas 1 and 2	Contoured water table elevations; NAPL Area 3 elevations recorded in monitoring wells while all extraction wells in the area, except LX-18, were operating.
Horizontal hydraulic gradient	1E-03 to 4E-03	Contoured water table elevations.
Vertical hydraulic gradients	1.1E-02 downward	Multi-level wells in NAPL Area 3
Flow velocities	5E-02 to 15.2 ft/day	
Seasonal water levels	Varies 5 to 6 ft, as much as 14.7 ft over periods of years.	

4.3 CONTAMINANT DISTRIBUTION

NAPL characterization performed as part of the EGDY Phase II Remedial Investigation was used to locate the treatment cells; this characterization is described in the EGDY Phase II Remedial Investigation Report (USACE 2002). During this characterization effort, membrane interface probe (MIP) evaluations completed in NAPL Area 3 indicated high concentrations of dissolved phase TCE (up to 125 mg/L) and DCE (up to 140 mg/L) were observed 3-14 ft bgs. In addition, NAPL contact was observed at one MIP locations with concentrations of 20,000 mg/L observed at approximately 16 ft bgs. Figure 4-3, illustrates the inferred NAPL distribution within NAPL Area 3 based on data gathered during the Phase II RI. Figure 4-3 also illustrates the target areas planned for installation of the two demonstration treatment cells.

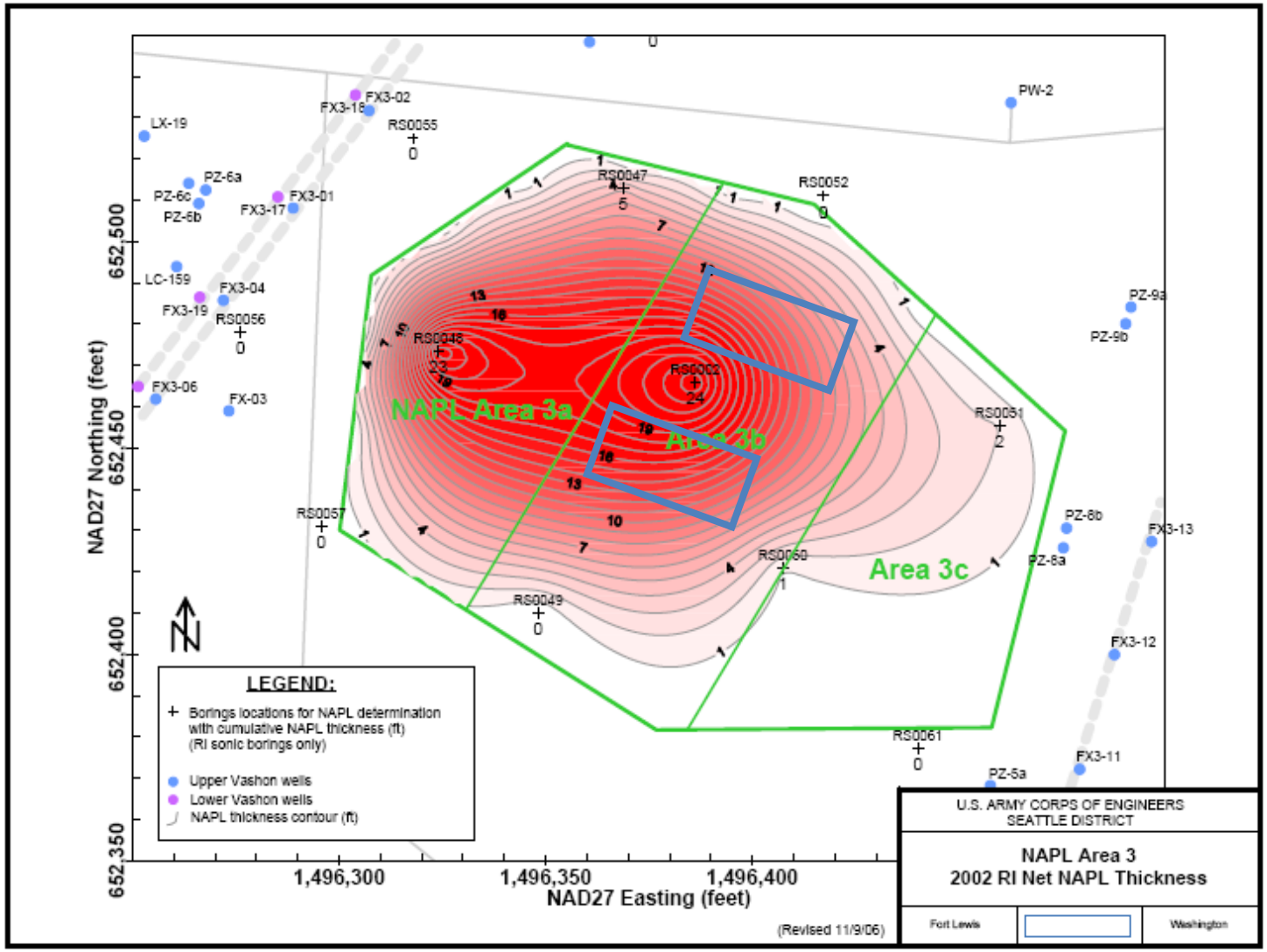


Figure 4-3. Inference of NAPL Distribution Within NAPL Area 3 Based on Data Gathered During the Phase 2 RI (2002) (USACE, 2008). Boxes Highlighted in Blue Indicate Target Treatment Cell Locations.

5.0 TEST DESIGN

5.1 CONCEPTUAL EXPERIMENTAL DESIGN

The experimental design for the ER-0318 demonstration was to identify a sampling and analysis strategy for innovative diagnostic tools that could be implemented during the ER-0218 demonstration in order to determine their utility in evaluating enhanced ISB of a chlorinated solvent source area. All of the diagnostic tools were used to assess the performance of enhanced ISB of the TCE DNAPL residual source at the Fort Lewis EGDY. One aspect of implementing bioremediation in an area containing DNAPL is that degradation of contaminants is limited to the aqueous-phase. Therefore, the rate of contaminant destruction is often limited not by the biological degradation rate but by the dissolution rate of contaminants from the DNAPL to the aqueous phase. Enhanced dissolution of the residual source, therefore, greatly enhances the performance of enhanced bioremediation in residual source areas so long as the biological community is capable of degrading all of the increased aqueous phase contaminants to innocuous products. Therefore, objectives of ER-0218 were to evaluate enhanced mass transfer mechanisms that are significant during application of ISB using whey powder under two operational scenarios conducted in series in two treatment cells. Scenario 1 consisted of low concentration whey powder injections within the treatment area designed to enhance mass transfer from the DNAPL to the aqueous phase by promoting ARD in groundwater surrounding the DNAPL, reducing concentrations in groundwater, and maintaining a high concentration gradient as the driving force for enhanced VOC dissolution. Scenario 2 consisted of relatively high concentration whey powder injections designed to enhance the solubilization of DNAPL in addition to ARD mass transfer mechanisms described for Scenario 1. Comparison of the two scenarios facilitated quantification of the different enhanced mass transfer mechanisms. The innovative diagnostic tools were implemented during the three phases of the ER-0218 demonstration, which consisted of:

- **Phase 1 – Equilibration**. Hydraulic characterization of the treatment cells was conducted. Innovative tools implemented included 3-D monitoring in CMT wells during initial contaminant mass distribution evaluation and tracer studies.

- **Phase 2 – Baseline.** Performance indicators were collected to evaluate electron donor concentrations, geochemistry, and contaminant and degradation daughter product concentrations in each treatment cell prior to whey injection. Innovative tools implemented included 3-D monitoring in CMT wells; flux meters; CSIA; and molecular tools T-RFLP and qPCR for *Dehalococcoides* 16S rDNA, and reductase genes *tceA*, *bvcA*, and *vcrA*.

- **Phase 3 – Biostimulation and enhanced mass transfer demonstration.** Performance indicators were collected in two treatment cells during biostimulation with both low- and high- concentration whey powder injections. Parameters collected during Phase 2 were also collected during Phase 3. In addition, molecular tools targeting qPCR for methanogenic populations, and FISH analysis for *Dehalococcoides* and methanogenic populations, were also conducted.

During each of these three phases, innovative diagnostic tools were applied to augment the data that was already being collected (see Table 5-1 for summary of diagnostic tool application).

Table 5-1. Summary of Implementation of Innovative Diagnostic Tools During Demonstration Phases, Locations Where Samples Were Collected and Analytes.

3-D CMT	**Phase 1 (four tracer tests)**		**Analytes**
	Sample locations	***Treatment cell 1:*** IW-1a, EX-1a, MW1A ports 1-4, MW1B ports 1-4, MW1C ports 1-4, MW1D, ports 1-4 ***Treatment cell 2:*** IW-2a, EX-2a, MW2A ports 1-4, MW2B ports 1-4, MW2C ports 1-4, MW2D ports 1-4	Bromide, Fluorescene/Rhodamine WT, VOCs
	Phase 2 (nine sample rounds for TCE and daughter products and one sample round for carbon and geochemistry) and 3 (23 sample rounds for TCE and daughter products, 16 sample rounds for carbon and 9 sample rounds for geochemistry)		**Analytes**
	Sample locations	***Treatment cell 1:*** MW1A ports 1, 2, 4, MW1B port 4, MW1C port 4, MW1D ports 2, 3, 4 ***Treatment cell 2:*** MW2A ports 1, 2, 4, MW2B port 4, MW2C port 4, MW2D ports 1, 2, 4	VOCs, carbon, and/or geochemistry
PFM	**Phase 2 (one sample round) and 3 (four sample rounds) within treatment cells during Phase 3**		**Analytes**
	Sample locations	***Treatment cell 1:*** FX1A, FX1B ***Treatment cell 2:*** FX2A, FX2B	VOCs, ethene

Table 5-1. (continued).

CSIA	**Phase 2 (one sample round) and 3 (five sample rounds)**		**Analytes**
	Sample locations	***Treatment cell 1:*** MW1A ports 1, 2, 4, MW1B port 4, MW1C port 4, MW1D ports 1, 2, 4 ***Treatment cell 2:*** MW2A ports 1, 2, 4, MW2B port 4, MW2C port 4, MW2D ports 2, 3, 4	Isotopes of TCE, cis-DCE, VC, ethene
PCR-based techniques	**Phase 2 (one sample round) and 3 (five sample rounds)**		**Analytes**
	Sample locations	***Treatment cell 1:*** MW1A port 4, MW1B port 4, MW1C port 4, MW1D port 4 ***Treatment cell 2:*** MW2A port 4, MW2B port 4, MW2C port 4, MW2D port 4	T-RFLP: *Bacteria* and *Archaea*, qPCR: Methanogens
		Treatment cell 1: MW1A ports 1, 2, 4, MW1B port 4, MW1C port 4, MW1D ports 2, 3, 4	*Dehalococcoides 16S rRNA, bvcA, vcrA and tceA genes*
		Treatment cell 2: MW2A ports 1, 2, 4, MW2B port 4, MW2C port 4, MW2D ports 1, 2, 4	
Fluorescent microscopy- based techniques	**Phase 3 (five sample rounds)**		**Analytes**
	Sample locations	***Treatment cell 1:*** MW1A port 4, MW1B port 4, MW1C port 4, MW1D port 4 ***Treatment cell 2:*** MW2A port 4, MW2B port 4, MW2C port 4, MW2D port 4	FISH: *Bacteria* and *Archaea*, *Dehalococcoides* spp., Methanogens

These data were then evaluated for their utility, costs and benefits compared to conventional or other tools that are intended to provide similar information (if applicable). Figure 5-1 illustrates a GANTT chart that shows the field schedule for each of the three Phases and relates the operational activities with key decision points and ESTCP milestones.

5.1.1 Treatment Cell Design

Treatment cells were installed within the Ft. Lewis EGDY NAPL Area 3 as described in the ESTCP project ER-0218 Final Report (North Wind, 2008). In order to assess the innovative diagnostic tools described herein, each treatment area was configured with four monitoring wells, which were completed using the Solinst™ CMT system to provide multi-level sampling capability (Figure 2-1). Each CMT well was completed with four sampling ports at discrete depths: Port 1 from 13-14 ft bgs, Port 2 from 17-18 ft bgs, Port 3 from 22-23 ft bgs, and Port 4 from 27-28 ft bgs. Two CMT wells were aligned along the groundwater flow axis between the injection and extraction wells and two CMT wells were aligned cross gradient from the injection and extraction well axis.

A second modification to the treatment cell design was made to facilitate innovative diagnostic tools, which was the installation of two PFM monitoring wells within each treatment cell. The full test cell layout is shown in Figures 5-2 and 5-3, including the new injection (IW), extraction (EW), CMT (MW) and PFM (FW) wells. Figure 5-4 shows an example of the PFM well design. The PFM wells were placed approximately 3 ft downgradient from CMT monitoring wells A and D, which were aligned along the groundwater flow axis for each treatment cell. The configuration was designed to allow for a direct comparison between contaminant flux measurements made from the CMT wells and the PFM wells.

5.1.2 Implementation

The ER-0318 demonstration was implemented in conjunction with ER-0218. The ER-0218 demonstration was implemented in three Phases:

Phase 1: Hydraulic characterization of the treatment cells. This phase of testing established hydrogeologic baseline parameters, including tracer measurements using the multi-level CMT wells to evaluate vertical transport and preferential flowpaths. This phase also evaluated baseline contaminant distribution in groundwater within the two treatment cells.

Phase 2: Baseline testing, during which all diagnostic tools (except FISH) were evaluated to assess the baseline conditions in each treatment cell prior to whey injection. This phase of testing established the contaminant flux baseline parameters using both analytical samples of the CMT wells and flux meters. Baseline CSIA isotope ratios were also determined pre-whey injection, as were the PCR-based molecular tools.

Phase 3: Biostimulation and enhanced mass transfer demonstration, during which all analytical parameters were monitored under two whey injection Scenarios for comparison as well as comparison to Phase 2 conditions. The same analytes described for Phase 2 were analyzed during Phase 3, and FISH analyses were added to evaluate activity of different populations of the microbial community.

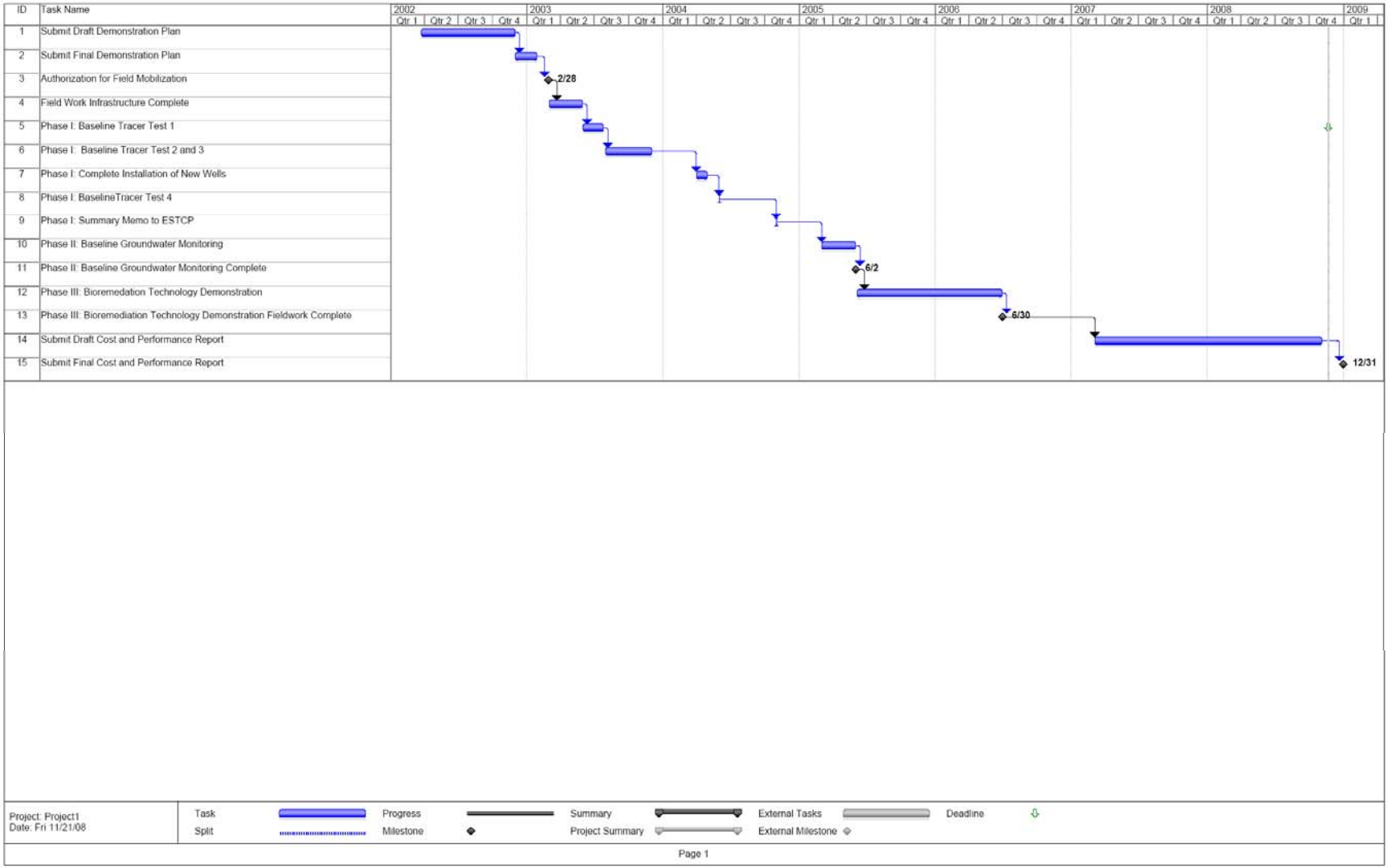


Figure 5-1. Field Schedule for Phases 1, 2, and 3.

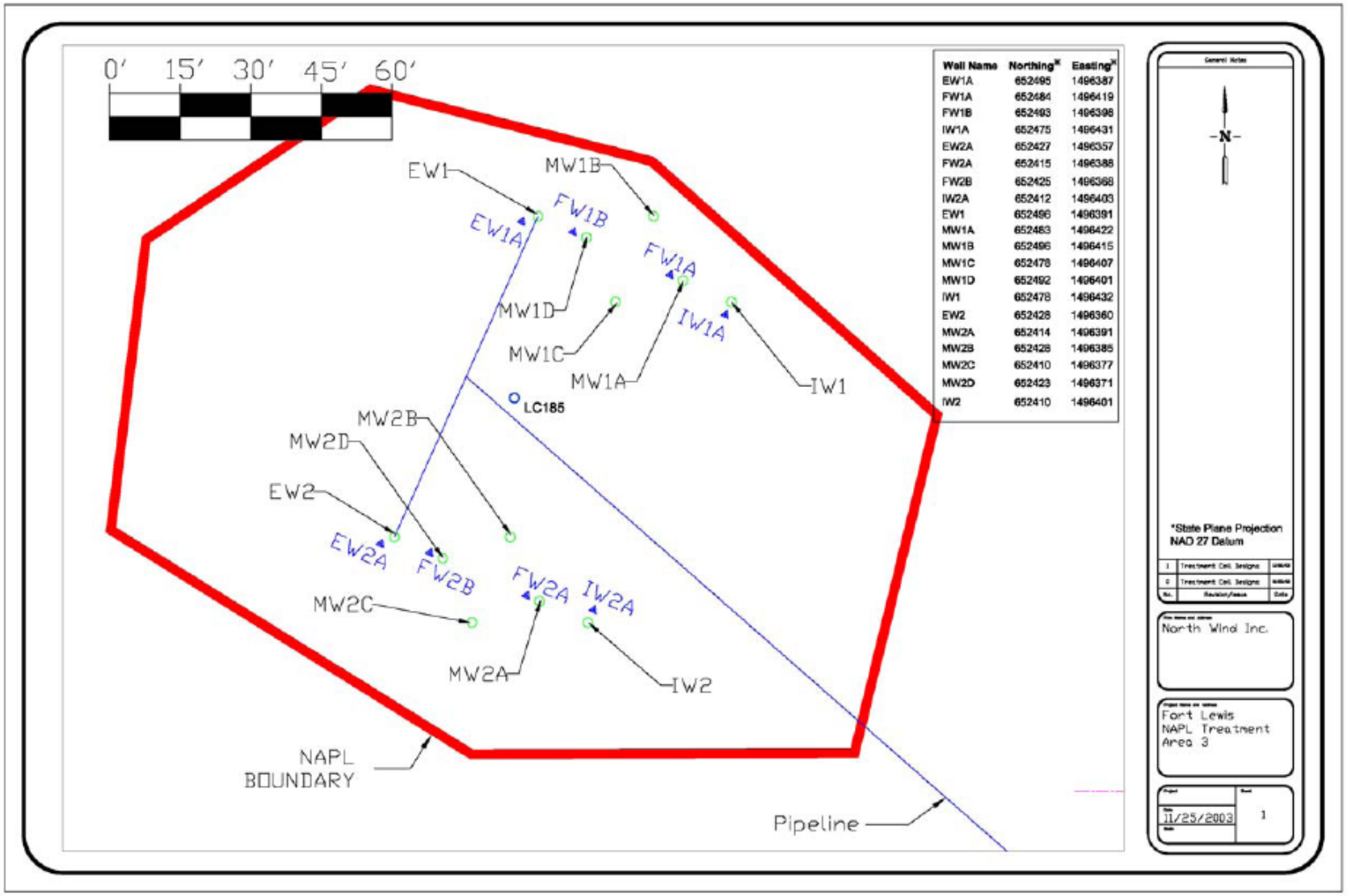


Figure 5-2. Well Locations within Treatment Cells at Ft. Lewis EGDY.

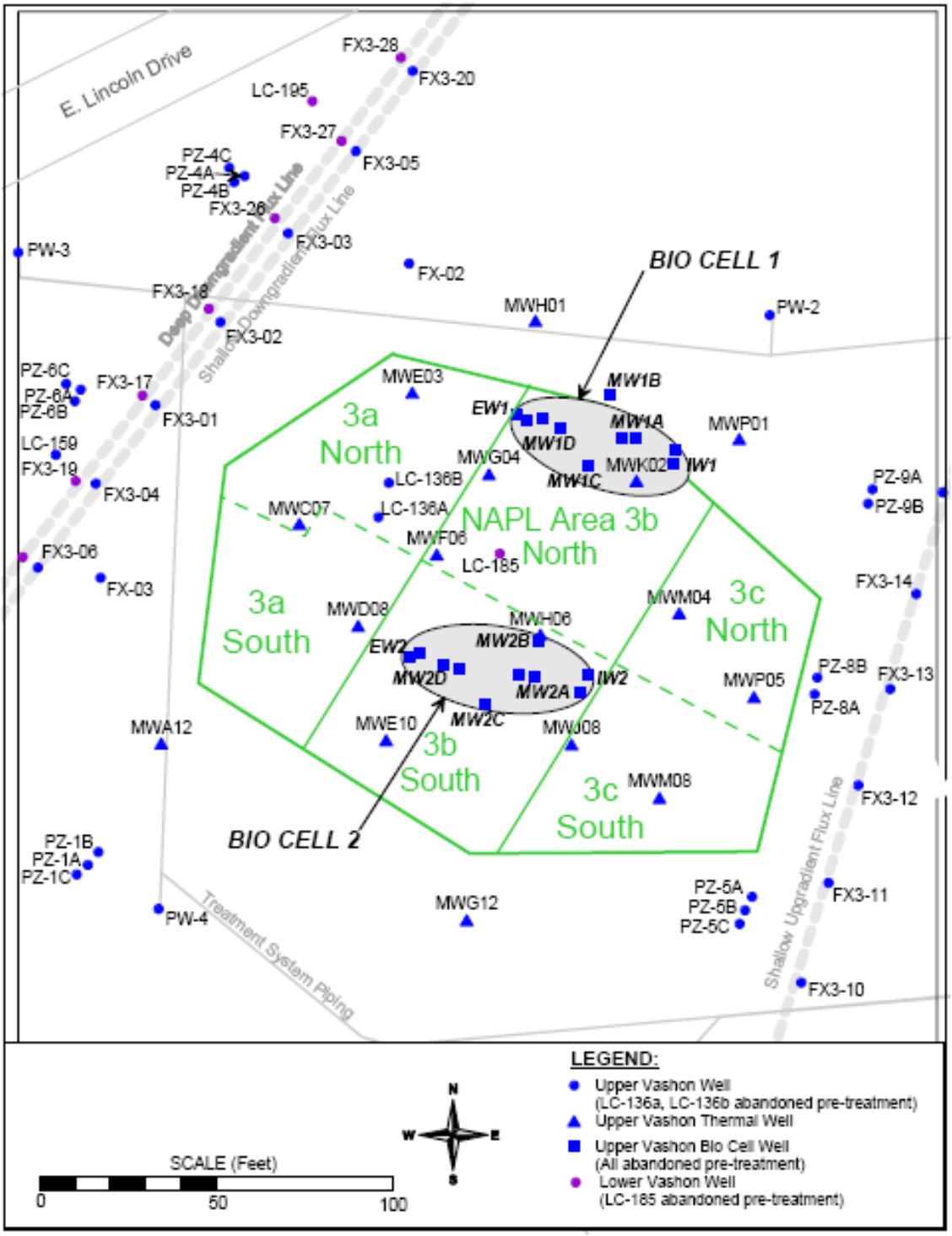


Figure 5-3. Actual Placement of Treatment Cells within NAPL Area 3 (USACE, 2008).

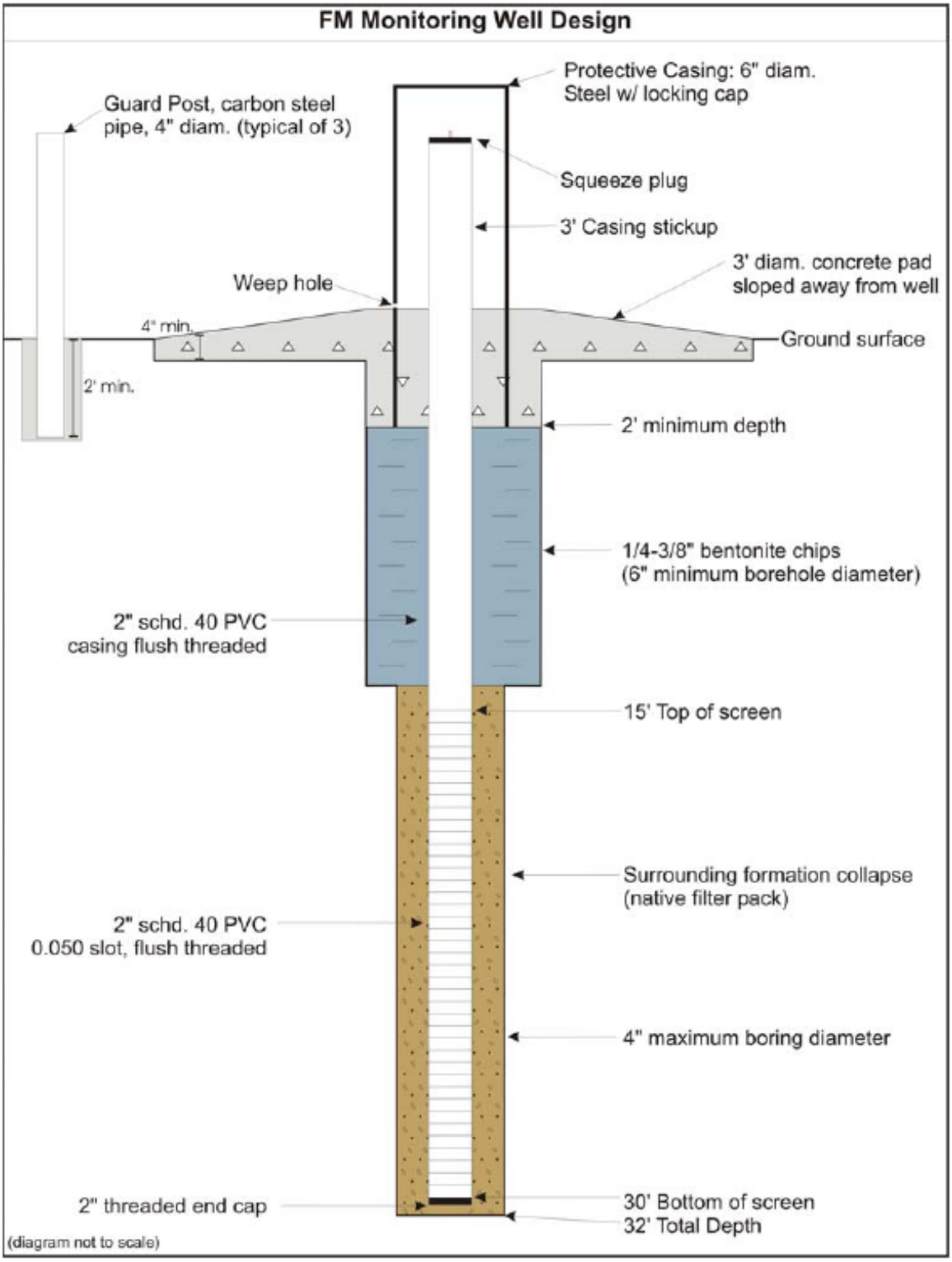


Figure 5-4. Example PFM Well Design.

During Phase 1, 3-D diagnostic tools were used to determine the distribution of contaminants and hydraulic parameters of the aquifer system within the treatment cells. Distribution of contaminants and hydraulic characterization is conventionally determined by evaluating contaminant concentration isopleths in a contaminant plume aerially using discrete monitoring locations in the well field screened over the entire vertical interval of interest. This approach, however, is limited to two dimensions. 3-D CMT wells were used to evaluate contaminant distribution and hydraulics of the system during multiple tracer tests in three dimensions.

During Phases 2 and 3, 3-D CMT monitoring wells were used to evaluate distribution of whey within target treatment areas and the impact to geochemical conditions as a result of low- and high- concentration whey powder injections. One key design parameter for any bioremediation strategy is the efficient and effective distribution of amendments throughout the horizontal and vertical extent of the target treatment area. Therefore, electron donor, or “carbon”, distribution was evaluated by assessing the chemical oxygen demand (COD), and resulting redox conditions in the CMT monitoring wells. Electron donor fate was tracked by evaluating generation and utilization of volatile fatty acids (VFAs), within the system. Samples were collected in three dimensions to ensure that whey was transported both horizontally and vertically throughout the treatment cells. Redox conditions were also monitored to confirm that conditions within the treatment areas were conducive to ARD. Methanogenesis in particular was tracked in order to verify methanogenic redox conditions and to assess the potential extent of competition between dechlorinating bacteria and methanogens.

During Phases 2 and 3, innovative diagnostic tools were used to evaluate contaminant distribution, and transport before, during, and after biostimulation with low- and high-concentration injections of whey powder. 3-D CMT wells were used to evaluate distribution of contaminants and degradation daughter products in three dimensions within the two treatment cells. Contaminant mass flux within the treatment cells was also assessed using both the 3-D CMT wells and PFMs deployed to assess both contaminant and groundwater flux. Total moles of chloroethenes and degradation daughter products were compared between Phases 2 and 3 (Scenarios 1 and 2) to compare contaminant flux rates under low- and high- concentration whey injections.

Contaminant fate was also assessed during Phases 2 and 3 of the ER-0318 demonstration using innovative diagnostic tools. A mass balance was performed using analytical data obtained from the monitoring wells within both treatment cells. Samples were collected periodically from the injection, extraction, and CMT monitoring wells and analyzed for VOCs and the dissolved gasses ethene and ethane. Loss of TCE due to degradation during biostimulation was accounted for by measuring degradation products, including cis- and trans-DCE, VC, ethene, and ethane, in groundwater. In addition, enhanced mass transfer was also evaluated by comparing the molar mass between TCE and degradation products in groundwater.

Using aqueous contaminant and daughter product concentrations to determine contaminant fate has several disadvantages. Often measurement of degradation daughter products, such as VC and ethene, in the aqueous phase results in a loss of the mass balance compared to parent compounds due to volatilization of the daughter products into the vadose zone or mineralization. Therefore, the CSIA tool was evaluated during Phases 2 and 3 to differentiate between the effects of

groundwater transport, dissolution of the residual source, and enhanced bioremediation, and to determine if whey powder injections stimulated complete degradation of contaminants.

The last set of diagnostic tools was used to further elucidate important populations within an indigenous microbial community enriched by whey injections and responsible for degradation of contaminants. Both community level assessment using T-RFLP and techniques to evaluate specific populations (qPCR and FISH) were employed to determine the impact of whey injections on the microbial community. In addition, results from molecular tools were evaluated (relative to the geochemistry and contaminant data) to determine the utility of the tools in evaluating bioremediation performance, and as tools for optimization. In addition, these tools were used to determine if any relationships could be elucidated between methanogenic and dechlorinating populations.

5.2 BASELINE CHARACTERIZATION: PHASES 1 AND 2

Phases 1 and 2 of the demonstration were considered baseline characterization activities. Phase 1 consisted of a series of hydraulic pumping and tracer tests to evaluate the groundwater flow within the two treatment cells. In addition, a round of VOC sampling was conducted to determine contaminant distribution in groundwater.

5.2.1 Phase 1-Hydraulic Characterization

The objective of Phase 1 was to characterize groundwater hydraulic characteristics using pumping and tracer tests within the treatment cells (see ER-0218 Final Report [North Wind 2008] for details). Hydraulic characterization was conducted to obtain parameters necessary to design an effective ISB injection strategy to meet the demonstration objectives. Pumping tests were performed to evaluate sustainable yield of extraction wells and to estimate hydraulic conductivity in the area of the demonstration. Following the pumping tests, tracer studies were conducted to determine the baseline aquifer properties including hydraulic gradient both horizontally and vertically, hydraulic conductivity, residence time, and groundwater velocity and direction. In addition, it was important to establish that the treatment cells were hydraulically isolated from each other.

5.2.2 Pumping and Hydraulic Tests

Following installation of the two treatment cells, the pumping and injection system was tested to determine if it was capable of operating per specifications in the demonstration design. In addition, hydraulic tests, including tracer testing, were conducted to establish the hydraulic properties of the aquifer system. Substantial differences were observed between actual system performance and estimates based on the assumptions stated in the Technology Demonstration Plan (TDP) (North Wind, 2003). The most significant issues with the original treatment system were low water yield from the two extraction wells and a substantial vertical gradient within both treatment cells, resulting in transport of the tracer to the lowest depth of the monitored treatment zone and little to no recovery of tracer in the extraction wells. Therefore, system modification, including the installation of new injection and extraction wells, was conducted such that the treatment system could perform per required specifications.

5.2.3 Tracer Studies

Tracer studies were conducted to determine the baseline aquifer properties including hydraulic gradient both horizontally and vertically, hydraulic conductivity, residence time, groundwater velocity and direction, tracer distribution, and to establish that the treatment cells were hydraulically isolated. Initial tracer studies (conducted June, August, and November 2003) revealed groundwater velocities much higher than originally anticipated, a substantial vertical gradient, and no hydraulic connectivity between the treatment cells. Following installation of new injection and extraction wells, the fourth tracer study (June 2005) revealed that distribution of the tracer throughout the monitored treatment zone was substantially improved, allowing the demonstration to proceed to Phase 2.

5.2.4 Phase 2-Baseline Chemical Characterization

The objectives of Phase 2 were to determine baseline contaminant concentrations and flux under ambient and/or recirculation hydrologic conditions, as well baseline measurements for both conventional and innovative diagnostic tools. Groundwater extraction was only used during injections of whey powder solution. Groundwater was extracted from the extraction wells, pumped through the whey powder injection system, and reinjected into the injection wells. The short-term impacts of injection events on contaminant concentrations were also determined during the baseline phase by conducting an injection without amendment and collecting samples the day of and the day after injection. Groundwater was pumped from extraction wells EW-1A and EW-2A at a rate ranging from 8 to 10 gpm, and was reinjected into injection wells IW-1A and IW-2A without the addition of whey. The injections took place during the weeks March 7, March 21, and April 4, 2005, and the approximate volume of water recirculated is shown on Table 5-2.

Analytical sampling occurring in two and in three dimensions using multiple sampling locations in the CMT monitoring wells (3-D). For the conventional method (2-D), each sample round consisted of collecting analytical samples from one port at each CMT monitoring location and the injection and extraction well locations. Table 5-1 provides a summary of analyses conducted during this phase.

5.3 TREATABILITY OR LABORATORY STUDY RESULTS

No treatability studies or laboratory studies were conducted as part of this demonstration.

Table 5-2. Phase 2 Recirculation Activity Summary.

Month Completed	Volume of Water Recirculated (gal)	
	Treatment Cell 1	Treatment Cell 2
March 2005	1,300	1,300
March 2005	2,000	2,000
April 2005	1,600	1,700

5.4 FIELD TESTING: PHASE 3 BIOSTIMULATION

Actual field conditions observed during the baseline sampling (Phases 1 and 2) indicated that TCE concentrations within treatment cell 1 were much lower than the TCE concentrations in treatment cell 2 making the comparison between the cells difficult to implement as envisioned in the ER-0218 Demonstration Plan (North Wind, 2003). The original plan was to perform one injection scenario in one cell, and one in the other, and then compare the results. However, to collect data that would ultimately be useable to evaluate mass transfer and dissolution in two cells with dramatically different source characteristics, the injection strategy had to be changed. The revised strategy was to perform both injection scenarios in both treatment cells (Table 5-3). This operational change allowed for the direct comparison of the effects of enhanced mass transfer as a result of electron donor concentration-dependent effects within each treatment cell. The composition of whey powder used for injections was comprised of 70 to 75% w/w lactose and 10 to 13% protein. A similar composition of whey was used in previous studies that illustrated enhanced effective solubility with increasing concentration (Macbeth et al. 2006).

Whey Injection: Scenario 1. The Scenario 1 injection strategy entailed high concentration (10% w/w) whey powder injections into well IW-1A of Treatment cell 1 on July 19, 2005, September 13, 2005, and October 4, 2005; and in IW-2A of Treatment cell 2 on November 8, 2005, December 13, 2005, January 15, 2006, and February 22, 2006. Injection flow rates were maintained between 5 and 12 gpm and injections were performed over a period of several hours. The total target volume injected was approximately 1,800 gallons.

Whey Injection: Scenario 2. The Scenario 2 injection strategy entailed low concentration (1% w/w) whey powder injections into well IW-2A of Treatment cell 2 on July 19, 2005, August 16, 2005, September 13, 2005, and October 4, 2005; and in IW-1A of Treatment cell 1 on November 8, 2005, December 13, 2005, January 15, 2006, and February 22, 2006. Injection flow rates were maintained between 5 and 12 gpm and injections were performed over a period of several hours. The total target volume injected varied between approximately 1,800 and 4,000 gallons. The variability in volume was a result of an initial concern that the low concentration whey would be less persistent than the high concentrations whey injections. Once it was demonstrated that whey persisted within the system between injection events with the low concentration whey, the volume was reduced.

Table 5-3. Phase 3 Whey Injection Summary.

	Treatment Cell 1		Treatment Cell 2	
	Volume of Water (gal)	**Concentration of Whey (%)**	**Volume of Water (gal)**	**Concentration of Whey (%)**
June 2005	3,200	4	3,900	3
June 2005	3,200	3	3,200	3
July 2005	1,700	10	4,000	1
August 2005	0[1]	0[1]	1,800	1
September 2005	1,700	10	4,000	1
October 2005	1,900	10	1,800	1
November 2005	1,800	1	1,800	10
December 2005	1,800	1	1,800	10
January 2006	1,800	1	1,800	10
February 2006	1,300	1	1,800	10

[1].No recirculation or injection of whey due to equipment difficulties.
Note: Scenario 2 areas are shaded; Scenario 1 areas are left unshaded.

5.4.1 Bioaugmentation

Biological degradation of TCE to ethene requires the presence and activity of microbial populations capable of complete reductive dechlorination. As mentioned earlier, previous testing of ARD in NAPL Area 1 at EGDY suggested that the indigenous microbial community might be capable of complete dechlorination to ethene. However, the schedule for the field demonstration was very limited due to the impending implementation of a thermal treatment system in NAPL Area 3. Therefore, bioaugmentation was performed in both treatment cells in order to ensure that a dechlorinating microbial community was quickly established. This was accomplished by injecting a laboratory grown culture that was shown to transform TCE completely to ethene under anaerobic conditions. The culture used was a derivative of the Bachman Road culture, and was prepared by the Utah Water Research Laboratory (UWRL) specifically for this purpose. Bioaugmentation entailed injection of approximately 10 liters of culture following the July 2005 whey injection in both test cells (North Wind, 2008).

5.5 SAMPLING METHODS

Table 5-4 summarizes the sample collection strategy. Injection well, extraction well, CMT, and PFM monitoring well locations are illustrated in Figure 5-2. Well locations were selected based on information contained in the EGDY Phase II Remedial Investigation Report (USACE 2002). The test cells were installed in areas where abundant NAPL was suspected to be present. Overall, the monitoring network was designed to provide three-dimensional coverage of the area where NAPL was expected, both along the assumed axis of groundwater flow in the cells and also transverse to the axis.

5.5.1 3-D CMT Analytical

Groundwater sampling was conducted during Phases 1, 2, and 3 of the demonstration to collect a data set that would achieve project objectives. Phase 1 activities included four tracer tests conducted using all ports of the CMT wells in both treatment cells. Phase 2 activities included three rounds of baseline sampling conducted around the three biweekly injection/recirculation events. Each sampling round included collection of samples for VOC and dissolved gas analysis prior to the injection event, immediately following the injection event, and on the day following the injection event for all sample locations. In addition, the groundwater sampling purge parameters of pH, oxidation reduction potential (ORP), specific conductivity, dissolved oxygen (DO), and temperature were measured during each round of sample collection to ensure that representative samples were collected. The field-analyzed parameters, alkalinity and ferrous iron, were analyzed once during the last two baseline sampling events, as were sulfate, nitrate, chloride, chemical oxygen demand (COD). At the same time, specific compounds expected to be introduced with whey powder were analyzed, including acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate. This same sampling strategy was repeated during Phase 3.

Phase 3 sampling activities were conducted around whey powder injections. Three rounds of sampling were conducted before, immediately following, and one day following whey injections conducted in July, August, October, November, and December of 2005 and February 2006. In addition, sampling rounds were conducted 1 (March 2006) and 2 (April 2006) months following the last whey powder injection (February 2006). Analyses for VOC and dissolved gas analysis, groundwater sampling purge parameters pH, oxidation reduction potential (ORP), specific conductivity, dissolved oxygen (DO), and temperature, and samples for COD were conducted for all samples. Analytical parameters alkalinity, ferrous iron, sulfate, nitrate, chloride, and volatile fatty acids acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate were generally analyzed for samples collected prior to whey powder injection, with the exception of the July 2005 sampling event in which samples collected the day following whey injection were analyzed. Volatile fatty acid and anion analyses were not performed on samples collected around the December 2005 sampling event.

Sample Collection. Samples were collected for 1) contaminant concentrations; 2) purge parameters: pH, ORP, specific conductivity, DO, and temperature; 3) field parameters: alkalinity and ferrous iron; 4) anions: sulfate, nitrate, chloride; and 5) electron donor parameters: COD and volatile fatty acids (VFAs). Sample containers, volumes, and holding times are shown in Table 5-4. Details on sample collection and QA are provided in the ER-0218 Final Report (North Wind, 2008). In general, low-flow sampling principles were practiced for all groundwater sampling, and samples were collected using peristaltic pumps and dedicated tubing.

5.5.2 Passive Flux Meters

Two PFM wells FW-1A; FW-1B; FW-2A; FW-2B were installed in each treatment cell along the groundwater flow axis. The wells were screened approximately from 15 to 30 feet below ground surface (note FW-1B was screened 13-28 ft bgs). Approximately one meter upgradient of the PFM CMT multi-level systems (MLS) were installed. The PFMs were deployed just prior to whey injection and remained in place for a duration of approximately three days, which included the whey injection. The first deployment was conducted during Phase 2 in which water recirculation was conducted in the same manner used for whey injection during Phase 3. Four flux meter deployments were conducted during Phase 3.

Table 5-4. 3-D CMT Sample Collection and Analysis Summary.

Analytes	Sample Container Size and Type	Preservative	Analytical Method	Holding Time	Comments
Phase 1					
Field laboratory analyses					
Bromide	One 125-mL HDPE	4ºC	Ion-specific electrode	24 hours	Check for sulfide and/or other anion interference at high concentrations
Iodide	One 125-mL HDPE	4ºC	Ion-specific electrode	24 hours	Same as above
Phases 2 and 3					
Off-site laboratory analyses					
Volatile Fatty Acids (acetate, propionate, butyrate, isobutyrate, valerate, isovalerate)	One glass 40-mL VOA vial	4ºC, filtered with a 0.2 µm	SW-846 8015	7 days	
Anions (chloride, nitrate, sulfate)	One 500-mL HDPE	4ºC	EPA 300.0 SW-846 9056	28 days	
COD	250-mL HDPE	H_2SO_4 to pH<2	EPA 410.1	28 days	
VOCs	Three glass 40-mL VOA vials	4ºC	SW-846 8260B	14 days	No headspace
Ethane/methane	Three glass 40-mL VOA vials	HCl to pH<2 cool to 4ºC	SW-8015M	14 days	No headspace
Field laboratory analyses (priority)					
Alkalinity (2)	250-mL HDPE	4ºC	Hach Method 8203	24 hrs	
Iron (1)	250-ml HDPE	none	Hach Method 8146	30 minutes	Must be analyzed immediately; no headspace

EPA = Environmental Protection Agency
HDPE = high-density polyethylene
VOA = volatile-organic analysis

Table 5-5 provides a summary of the flux meter deployments in relation to the whey injection strategy that was being implemented during the deployment. All five PFM deployments were conducted during periods in which samples were also collected from the corresponding CMT MLS such that a direct comparison of mass flux evaluation using the two techniques could be made.

During each PFM deployment, 12 PFMs were packed at the University of Florida (UF) and shipped overnight to the site. Three 5-foot PFMs were deployed in each well to cover the 15-foot screen interval. Following the three day deployment, the PFMs were retrieved and sampled in 1.25 foot segments thus providing 12 data points for mass flux profiles through the 15-foot screen intervals. The construction, sampling, and analysis followed procedures provided by Annable et al., 2005. Samples were evaluated for TCE and degradation byproducts.

Table 5-5. PFM Deployments and Whey Injection Concentrations.

PFM Deployment	Install	Retrieve	Cell 1 Whey (%)	Cell 2 Whey (%)
1st	03/25/2005	03/28/2005	0	0
2nd	08/15/2005	08/18/2005	0	1
3rd	10/03/2005	10/06/2005	10	1
4th	11/07/2005	11/10/2005	1	10
5th	02/21/2006	02/23/2006	1	10

5.5.3 CSIA

Samples were collected for CSIA during Phase 2 and 3 of the demonstration. Samples were collected in three ports of MWA and MWD and one port from MWB and MWC from each treatment cell (Table 5-1). One round of samples was collected during Phase 2 (March 2005), four rounds were collected during whey injections (July, August and November 2005 and February 2006) and one round was collected approximately two months following cessation of whey injections (April 2006).

Detailed descriptions of the analytical methods can be found elsewhere (Song et al. 2002 and Lee et. al, 2008). 300 μL to 1000 μL of headspace sample were taken from the sample vial and injected into a gas chromatograph-combustion-isotope ratio mass spectrometry system (GC-C-IRMS) as described previously (Song et al., 2002). Briefly, the GC-C-IRMS system consists of a Hewlett-Packard 6890 gas chromatograph that was fitted with a Supelco Supel-Q-Plot capillary column (0.32mm × 60m) for the separation of chloroethenes, a Micromass combustion interface at 850°C, and a Micromass JA Series Isoprime isotope ratio mass spectrometer (Micromass, Manchester, U.K.).

Prior to injection into the GC-C-IRMS, the headspace sample was cryogenically trapped in a stainless steel loop submerged in liquid nitrogen that was connected to a six-port valve in line on the gas chromatograph. Once the valve was activated, the liquid nitrogen was then removed and the loop defrosted with a heat gun, and the cryogenically trapped sample was carried by a helium

gas stream into the GC-C-IRMS. Oven settings for chloroethenes separation varied depending on the compounds being analyzed. For TCE and the DCE isomers, the initial temperature was 80°C for 1 min, ramped to 180°C at 20°C/min, and then held for varying length according to the elution time of the compounds. For VC, the initial temperature was 40°C for 1 min, followed by a ramp of 10°C/min to 80°C then 20°C/min to 180°C. Carbon isotope ratios ($R = {}^{13}C/{}^{12}C$) are expressed in the conventional δ notation and reported in per mil (‰):

$$\delta^{13}C\ (‰) = \left(\left(R_{sample} / R_{standard}\right) - 1\right) \times 1000$$

where

$R_{sample}/R_{standard}$ = carbon isotope ratios for the sample and the Vienna Pee Dee Belemnite (VPDB) standard, respectively.

The reference CO_2 gas standard for the GC-C-IRMS was calibrated using a dual-inlet mass spectrometer (VG Prism series II).

Based on repeated analyses of laboratory standards, the uncertainty associated with the isotope measurement is ± 0.5 ‰ (2σ).

5.5.4 Molecular Tools

Samples were collected for molecular evaluation during Phase 2 and/or 3 of the demonstration. For qPCR analysis of *Dehalococcoides* spp. and reductase genes *tceA*, *bvcA*, and *vcrA*, samples were collected in three ports of MWA and MWD and one port from MWB and MWC from each treatment cell (Table 5-1). One round of samples was collected during Phase 2 (March 2005), four rounds were collected during whey injections (July, August, and November 2005 and February 2006) and one round was collected approximately 2 months following cessation of whey injections (April 2006). For qPCR analysis of methanogenic populations, including *Methanosarcinales*, *Methanococcales*, *Methanobacteriales*, and *Methanomicrobiales*, as well as FISH analysis, samples were collected from Port 4 of all of the CMT monitoring wells within each treatment cell. Samples were analyzed during four sample rounds conducted during Phase 3 (July, August, and November 2005, and February 2006). The methods for these analyses have been published previously and are listed in Table 5-6.

For T-RFLP, community profiling was also conducted on groundwater collected from Port 4 of each monitoring well during the four Phase 3 sampling events (July, August, and November 2005 and February 2006). Details of the analysis are described elsewhere (Macbeth et al. 2004).

Table 5-6. Molecular Targets and References for Methods.

Molecular Target	Purpose	Reference
qPCR		
Archaea	Abundance of Archaea.	Suzuki, 2000, *Appl. Environ. Microbiol*. 6(11):4605-4614.
Bacteria	Abundance of Bacteria.	
16S rRNA DHC	Presence and abundance of the only known organism capable of degrading TCE to ethene.	Lee et al., 2008, *Appl. Environ. Microbiol*. 74: 2728-2739.
vcrA	Presence and abundance of a gene related to the degradation of PCE to ethene in an energy yielding reaction.	
tceA	Presence and abundance of a gene related to the degradation of PCE to vinyl chloride in an energy yielding reaction.	
bvcA	Presence and abundance of a gene related to the degradation of cis-DCE to ethene in an energy yielding reaction.	
Methanosarcinales	Presence and abundance of this order of methanogens-this order contains the only known acetogenic methanogens with optimal temperature ranges from 25 to 60°C.	Yu and Lee et al., 2005, *Biotechnology & Bioengineering*, 89(6):670-678.
Methanococcales	Presence and abundance of this order of methanogens-this order contains hydrogenotrophic methanogens with optimal temperature regimes from 35 to 85°C.	
Methanobacteriales	Presence and abundance of this order of methanogens-this order contains hydrogenotrophic methanogens with optimal temperature ranges from 37 to 88°C.	
Methanomicrobiales	Presence and abundance of this order of methanogens-this order contains hydrogenotrophic methanogens with optimal temperatures from 15 to 40°C.	

Table 5-6. (continued).

Molecular Target	Purpose	Reference
FISH		
Eubacteria	Universal target of almost all prokaryotes	Raskin, L., et al., 1994, *App. Environ. Micro.* **60**:1241-1248.
Archaea	Universal target of almost all *Archaea*	
Methanobacteriaceae	Targets one order of *Methanobacteriales*.	Del Nery, et al., 2008. *Bioresource Tech.* 2018-2024.
Methanococcales	Targets one class of *Methanococci*.	Raskin, L., et al., 1994, *App. Environ. Micro.* **60**:1241-1248.
Methanomicrobiaceae	Targets one order of *Methanomicrobiales*.	
Methanosarcinaceae including *Methanosaeta*	Targets one order of *Methanosarcinales* as well as the genus *Methanosaeta*.	
Methanosaeta	Targets only the genus *Methanosaeta*, comprised of strictly acetoclastic populations.	
Dehalococcoides, some spp. incl. *ethenogenes*	Targets only the genus *Dehalococcoides*, which is known to reduce PCE/TCE to ethene.	Fazi, S., et al. 2007, *Systematic App. Micro.* **31**:62-67.

5.6 SAMPLING RESULTS

5.6.1 3-D CMT sampling

3-D CMT sampling was conducted during Phases 1, 2, and 3 of the demonstration. Details of the sampling results are presented in the sections below. Overall, the 3-D sampling provided valuable information that was key in the design and implementation of enhanced bioremediation at the Ft. Lewis EGDY.

5.6.1.1 Phase 1. 3-D CMT sampling

Initial tracer studies (beginning in June, July, and November 2003) revealed groundwater velocities much higher than originally anticipated (Figure 5-5), a substantial vertical gradient, no hydraulic connectivity between the treatment cells, and an injection system that was ineffective at distributing tracer through the high concentration residual contaminant zone due to vertical heterogeneity in permeability (see North Wind 2008 for details). Following installation of new injection and extraction wells, the fourth tracer study (June 2004) demonstrated effective distribution of the tracer throughout the targeted treatment zone. A summary of results is presented in Table 5-7. The results of these evaluations were used to design the whey injections conducted for Phases 2 and 3.

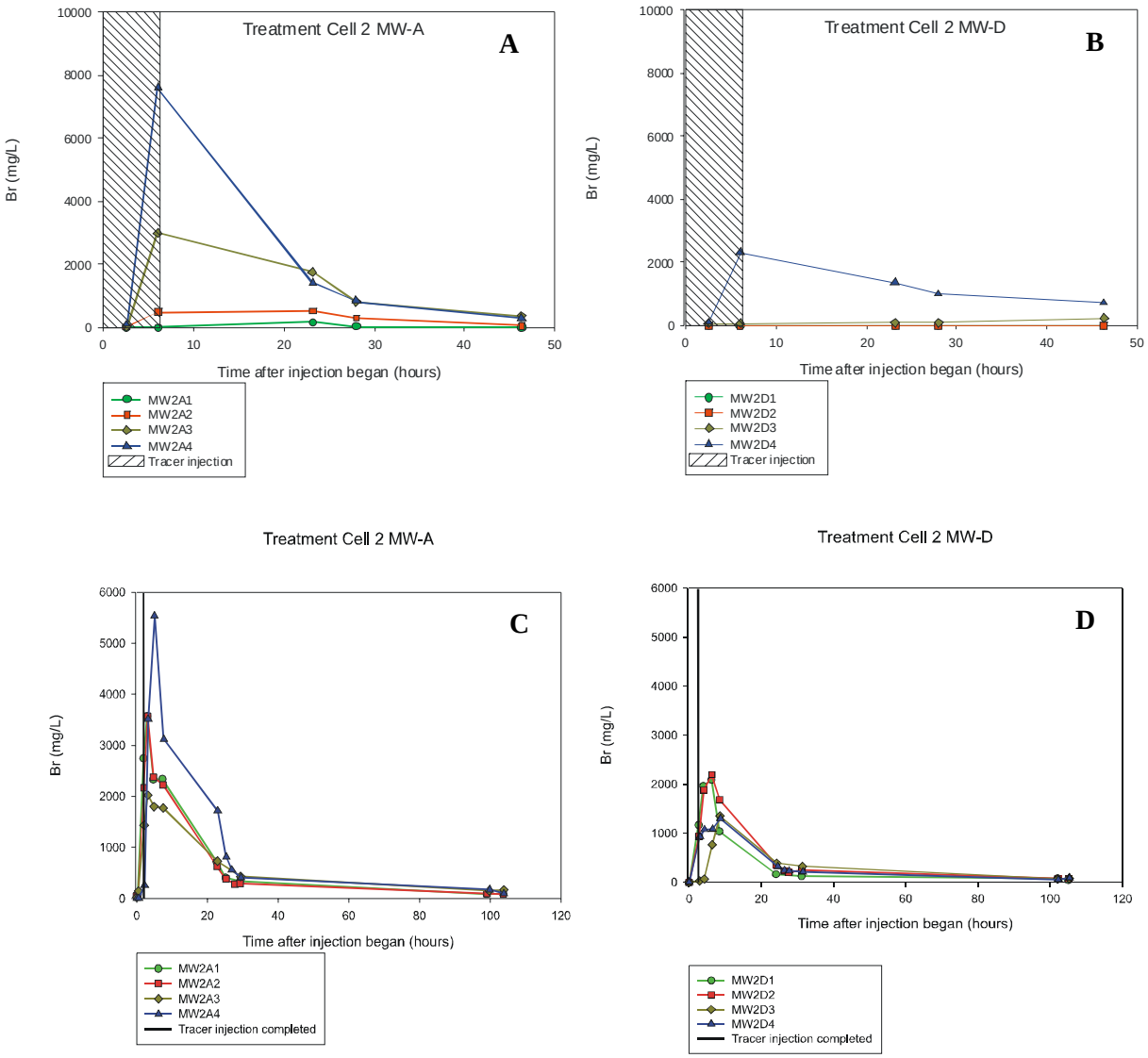


Figure 5-5. Comparison of Vertical Distribution of Tracer in Monitoring Wells A and D in Treatment Cell 2 Following Bromide Injections in the Original (A and B) and New (C and D) Injection Wells.

Table 5-7. Overview of Hydraulic Parameters Determined During Hydraulic Testing of the Two Demonstration Treatment Cells.

Activity Objective	Performance Confirmation Method	Expected Performance	Resultant Outcome
Tracer Test 1 (June 2003)			
Verify gradient	Measure water levels and calculate gradient	Gradient East to West	Gradient East to West
Verify extraction well production rates	Run pumping tests	10-20 gpm	EW1 – 4 gpm EW2 – 2.8 gpm
Estimate groundwater velocity, travel time, and tracer distribution	Measure peak breakthrough of bromide	Peak breakthrough 1 week at extraction well	Travel times for both treatment cells less than 12 hours Significant vertical gradient noted
Verify hydraulic isolation of treatment cells	Inject different fluorescent tracers in the two treatment cells	Non-detect in cross-gradient cells	No significant tracer communication was measured between treatment cells
Tracer Test 2 (July 2003)			
Estimate groundwater velocity, travel time, and tracer distribution	Measure peak breakthrough of bromide Perform inverse analytical modeling	Peak breakthrough 1 day at extraction well Adequate curve matching – hydraulic conductivity expected - 54 ft/d	Peak breakthrough between 6 and 24 hours Significant vertical gradient Hydraulic conductivity calculated: Cell 1 - 2.3 to 9.5 ft/d, Cell 2- -3.2 to 16.6 ft/d

Table 5-7. (continued).

Activity Objective	Performance Confirmation Method	Expected Performance	Resultant Outcome
Tracer Test 3 (November 2003)			
Estimate groundwater velocity, travel time, and tracer distribution without pump and treat wells	Measure peak breakthrough of bromide	Improved tracer distribution	Little effect noted, results similar to previous tests
Tracer Test 4 (June 2004)			
Verify extraction well production rates	Run pumping tests	10-20 gpm	EW1A – 10 gpm EW2A – 18 gpm
Estimate groundwater velocity, travel time, and tracer distribution with new injection and extraction wells	Measure peak breakthrough of bromide Perform inverse analytical modeling	Improved tracer distribution Conductivity based on June 2003: Cell 1 - 2.3 ft/d Cell 2 – 3.2 ft/d	Peak breakthrough between 4 and 31 hours Horizontal and vertical tracer distribution much improved Hydraulic conductivity calculated: Cell 1 - 15.0 ft/d Cell 2 - 24.2 ft/d

One of the key considerations in evaluating CMT sampling for hydraulic parameters is the necessity of understanding vertical heterogeneity. At Ft. Lewis EGDY, the aquifer characteristics included a significant vertical gradient and highly variable seasonal changes in groundwater flow and direction. Therefore, design of an effective injection strategy without an understanding of the vertical heterogeneity in flow would have been exceedingly difficult. Assessment of tracer results indicated that the original injection wells were delivering tracer solution primarily to the deepest zone of the monitoring system (>25 ft bgs). Given that the majority of the DNAPL contaminant mass was much shallower than this interval, effective injections required efficient distribution to shallower depths (~10-20 ft bgs). Therefore, the injection wells were re-drilled and completed and screened across a shallower depth interval (10-20 ft bgs) compared to the original 10-30 ft bgs interval. This resulted in much more effective distribution of tracer throughout the target treatment area. Effective design of the injection system was achieved because of the use of 3-D sampling during the tracer evaluation. In one dimension, it would have been difficult to understand where tracer was going and what hydraulic mechanisms were dominating tracer transport (i.e., a vertical gradient and a deep, high permeability zone). The design of the new injections wells had a high probability of successful delivery of injection substrate due to a detailed understanding of the system.

In addition to evaluating tracer distribution in 3-D, a round of sampling was conducted to evaluate contaminant distribution within the two planned treatment areas during Phase 1 (Figure 5-6). This sampling was conducted to evaluate the relative concentrations of TCE laterally and vertically between the two treatment areas in order to design the PFMs effectively. The analysis of the PFMs was conducted at the University of Florida. These data suggested significant variability in groundwater contaminant mass between the two treatment cells, with Treatment cell 1 containing 2-3 orders of magnitude lower concentrations observed at all sample locations. In addition, TCE contaminant mass appeared to be relatively uniform in Treatment cell 1 between the different depth intervals. Distribution of TCE was also relatively uniform in Treatment cell 2 at MWA, MWC and MWD, but at MWB, concentrations were 5-30 times higher in Ports 2 and 3 compared to 1 and 4.

5.6.1.2 Phase 2-3. 3-D CMT sampling

Whey Delivery. Spatial and temporal trends in COD were used to evaluate distribution of whey powder mixtures following 1% and 10% injections (see Table 5-3 for details) to the CMT monitoring locations within the treatment cells. In addition to COD, VFA analysis was used to evaluate whey powder utilization and fermentation. Concentrations of COD and VFAs were generally low or non-detect prior to whey injection (Phase 2) within the treatment cells (data not shown) and dramatically increased following Phase 3 whey powder injections. COD concentrations were generally highest near the injection locations (MWA approximately 10 ft downgradient from the injection well) and along the axis of the treatment cells (MWD approximately 30 ft downgradient from injection well), and were generally much lower at the cross-gradient locations (MWB and MWC 20 ft downgradient and 10 ft cross gradient from injection well, respectively) immediately following injections (Table 5-8).

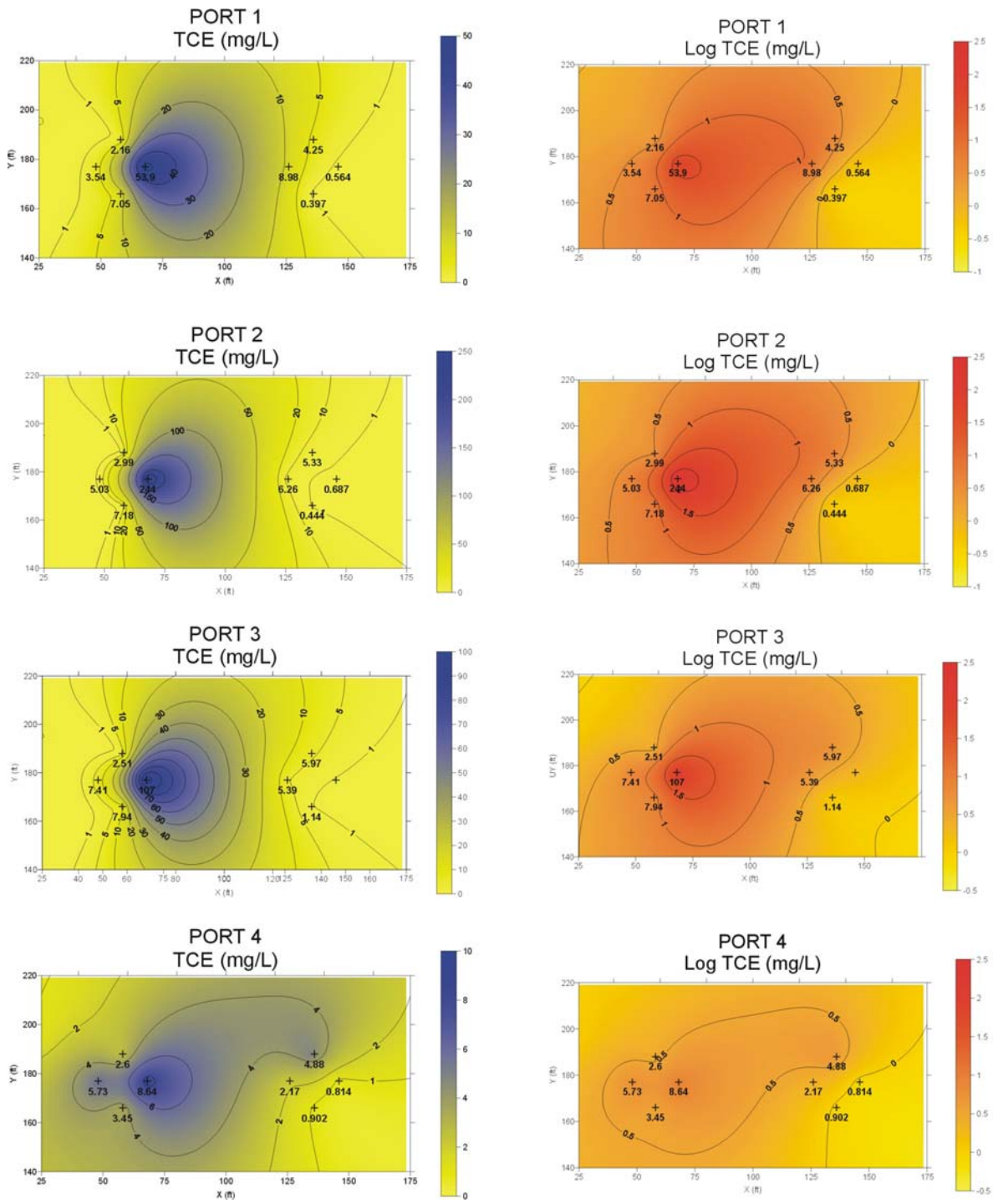


Figure 5-6. Distribution of TCE in Groundwater Within Two Treatment Cells at Four CMT Depth Intervals: 13-14 ft bgs (Port 1), 18-19 ft bgs (Port 2), 23-24 ft bgs (Port 3), and 28-29 ft bgs (Port 4) During Phase 1 Sampling.

Table 5-8. Increase in COD Measured at CMT Locations Before and After Whey Injections.

Sample Locations	Depths (ft)	Treatment Cell 1 - COD (g/L)		Treatment Cell 2 - COD (g/L)	
		July 2005 10% whey	December 2005 1% whey	December 2005 10% whey	July 2005 1% whey
MWA1	13-14	17.40	3.80	16.50	6.9
MWA2	18-19	15.6	3.5	17.1	4.6
MWA4	27-28	69.8	-4.7*	25.3	2.6
MWB4	27-28	8.2	0.3	15.20	0.8
MWC4	27-28	6.50	1.3	5.1	0.4
MWD1	13-14	NS	NS	13.9	7.1
MWD2	18-19	12.50	3.8	21.3	5.9
MWD3	22-23	8.60	2.2	NS	NS
MWD4	27-28	9.5	3.3	16.9	1.9

NS: indicates that the sample port was not sampled.

*: Negative value indicates the carbon concentration prior to injection of 1% whey was greater than the concentration following injection. This is due to accumulation and retention of carbon within the treatment cell between injections.

Vertical distribution of COD following high concentration whey injections resulted in distribution of higher COD at the lower depth interval (27-28 ft bgs) at MWA in both treatment cells. Conversely, low concentration whey injections resulted in higher COD concentrations in the shallow depth intervals (13-14 ft bgs) at MWA. This disparity in COD distribution with depth following the 1% whey injection was observed downgradient at MWD in treatment cell 2, with COD concentrations in port 1 approximately a factor of six higher than observed at port 4 (Table 5-8). A fairly uniform distribution of COD concentrations was observed at MWD during 10% injection in treatment cell 2 and during 1% and 10% injections in treatment cell 1. Despite some minor variability in vertical distribution, overall these data confirmed that whey powder was being distributed within the entire vertical target interval within both treatment cells during 1% and 10% injections.

The fermentation products of whey were evaluated using VFA concentrations in groundwater measured in the CMT monitoring wells. Table 5-9 illustrates results of VFA analysis on samples collected in August 2005 and February 2006, both approximately one month post-injection. The predominant VFAs observed were acetate, propionate and butyrate. In general, these data indicate fairly uniform distribution of total VFAs vertically between sampling depths of a given CMT monitoring well. In addition, concentrations were higher in MWA in treatment cell 1compared to MWD following both 1% and 10% injections. By contrast, the concentrations of VFAs in MWA and MWD of treatment cell 2 were much more uniform. These data indicate that fermentation of whey was occurring at or near all of the sampling depths evaluated following both 1% and 10% whey injections in both treatment cells.

Table 5-9. VFA Profiles Measured at CMT Locations Before and After Whey Injections.

Sample Locations	Depths (ft)	Treatment Cell 1: August 2005 10% whey		Treatment Cell 1: February 2006 1% whey		Treatment Cell 2: August 2005 1% whey		Treatment Cell 2: February 2006 10% whey	
		Total mM	Molar Percentage Acetate: Propionate : Butyrate	Total mM	Molar Percentage Acetate: Propionate : Butyrate	Total mM	Molar Percentage Acetate: Propionate :Butyrate	Total mM	Molar Percentage Acetate: Propionate: Butyrate
MWA1	13-14	14.5	44: 6: 50	12.7	47: 17: 36	4.4	54: 23: 23	9.6	46: 12: 43
MWA2	18-19	15.4	45: 8: 47	9.4	45: 17: 38	5.0	60: 18: 22	8.2	46: 12: 42
MWA4	27-28	19.3	30: 6: 34	10.4	25: 12: 63	5.6	69: 9: 22	3.8	56: 12: 31
MWD1	13-14	NS	NS	NS	NS	3.3	50: 30: 20	1.6	53: 13: 34
MWD2	18-19	6.2	1: 14: 85	1.8	61: 19: 20	3.9	72: 1: 27	3.5	37: 11: 52
MWD3	22-23	5.7	52: 9: 39	1.7	68: 23: 10	NS	NS	NS	NS
MWD4	27-28	4.9	53: 9: 38	1.6	69: 22: 9	7.0	73: 7: 20	16.7	62: 16: 22

NS: indicates that the sample port was not sampled.

Geochemical Impacts. Geochemical impacts were measured within the treatment cells during Phases 2 and 3 to ensure that whey powder injections resulted in conditions conducive to the growth and activity of dehalogenating bacteria, and included parameters to assess both bioactivity and redox. Bioactivity indicators were monitored throughout the demonstration as an indicator of microbial activity within the treatment cells. Bioactivity parameters include pH and alkalinity. Redox parameters were also measured including ORP, DO, nitrate, ferrous iron, sulfate and methane. Anaerobic reductive dechlorination of TCE to ethene is generally most efficient at neutral pH values and when redox conditions are methanogenic, indicated in groundwater by the absence of DO, nitrate, sulfate and the presence of ferrous iron and methane. The changes observed in the bioactivity and redox parameters as a result of whey injections were nearly identical for both treatment cells irrespective of the concentration of whey injected. The focus of the discussion will be on pH and methane.

One of the significant changes in geochemistry observed during whey injections was a reduction in pH. The impact of whey injections on pH is illustrated in Figure 5-7. Each cluster of data and the corresponding average value and one standard deviation from the mean are shown for each period for each of the eight monitoring locations sampled within each treatment cell.

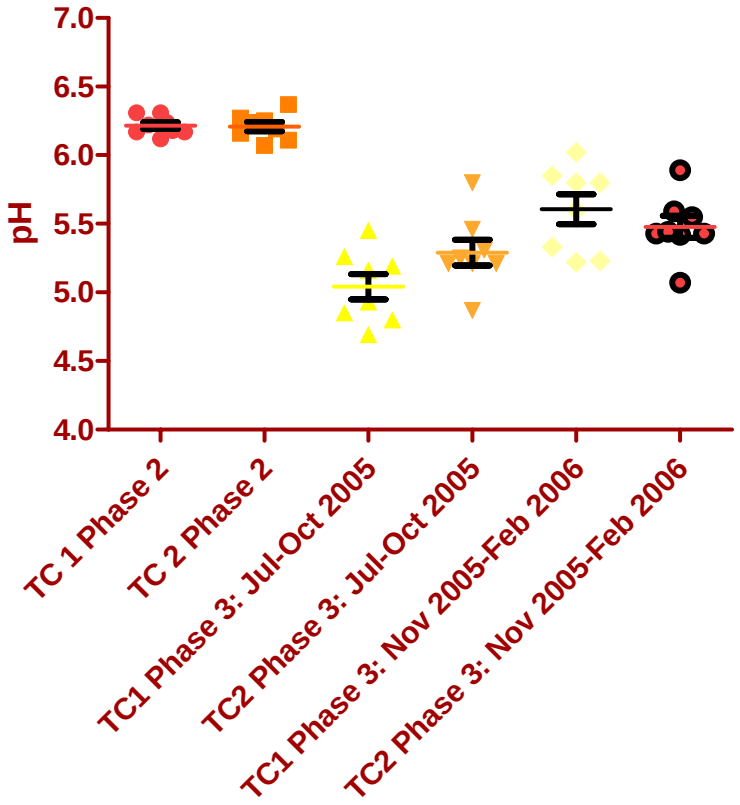


Figure 5-7. Impact of Whey Injection on pH in the Treatment Cells (Lines Represent the Mean and Error Bars Represent One Standard Deviation from the Mean).

In Figure 5-7, the evaluation periods illustrated on the x-axis include:

- Phase 2 includes results from samples collected before and after recirculation for each monitoring location in March and April 2005, the mean value and one standard deviation from the mean.

- Phase 3 (July-October 2005) includes results from samples collected from each monitoring location before and after 1% (Treatment cell 2) and 10% (Treatment cell 1) whey injections in July, August (Treatment cell 2 only) and October 2005, the mean value, and one standard deviation from the mean.

- Phase 3 (November 2005-February 2006) includes results from samples collected from each monitoring location before and after 1% (Treatment cell 1) and 10% (Treatment cell 2) whey injections in November and December 2005, and February 2006, the mean value, and one standard deviation from the mean.

While there was some variability in the observed pH values, the variability did not correlate spatially, (i.e., differences between MWA and MWD or between the vertical intervals) within a given treatment cell. Overall, trends in the data are consistent between the periods evaluated and are summarized:

- **Phase 2:** pH is 6.22 in treatment cell 1 and 6.21 in treatment cell 2. The values are consistent both horizontally and vertically.

- **Phase 3:** July-October 2005. A significant reduction ($p<0.05$) in pH was observed for both treatment cell 1 (1.2 pH unit reduction) and treatment cell 2 (0.9 pH unit reduction) relative to Phase 2. There was not a significant difference in pH between the two treatment cells (i.e., no significant difference between the 1% and 10% whey injection strategy), although the average of treatment cell 2 (receiving 1% injections) was higher (5.6) than Treatment cell 1 (5.3) (receiving 10% injections).

- **Phase 3:** November 2005-February 2006. There was a significant increase ($p<0.05$) in pH in treatment cell 1 (0.6 average pH unit increase from 5.0 to 5.6) relative to the July-October 2005 period, but value was still significantly lower (0.6 average pH units lower) than observed during Phase 2. There was not a significant increase in pH in treatment cell 2, although the average does increase from 5.3 to 5.5, relative to the July-October 2005 period. pH was an average of 0.73 pH units lower in treatment cell 2 than observed during Phase 2.

The second geochemical parameter assessed in detail was methane. Figure 5-8 illustrates methane concentrations observed at discrete vertical depths during Phase 3 in both treatment cell 1 and 2. Overall, a four to five month lag period was observed in both treatment cells before significant methane production was observed. In treatment cell 1, both methane production rate and the maximum concentrations observed were much higher by more than a factor of 2 in treatment cell 1 compared to treatment cell 2. In treatment cell 1, methane concentrations were fairly consistent at a given timepoint between the different vertical sampling intervals. In treatment cell 2, however, the maximum concentration observed was at MWD4 during the February 2006 sampling period. This was a factor of 2 to 5 greater than observed at the shallow depths.

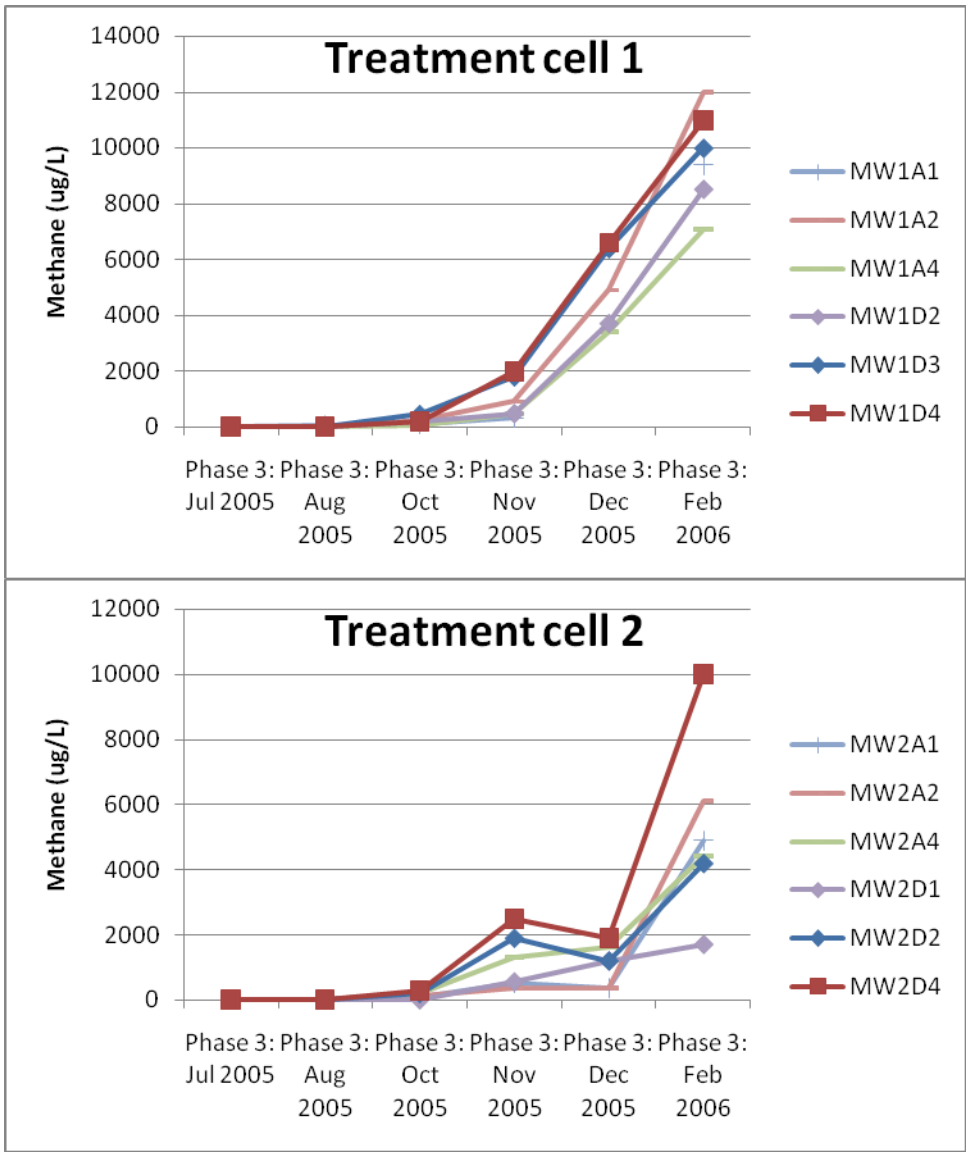


Figure 5-8. Methane Production Observed at Discrete Vertical Depths During Phase 3.

Contaminant Distribution. 3-D CMT sampling was used to determine the distribution of contaminants within the two target treatment cells during Phases 2 and 3 of the demonstration. Figure 5-9 illustrates the change in total molar concentration of TCE and reductive daughter products cis-DCE, VC and ethene during whey powder injections within the MWA and MWD monitoring wells, which were sampled vertically.

Evaluation of the distribution can be summarized as follows:

- Overall, there was a much greater mass of contaminants in treatment cell 2 compared to treatment cell 1 (factor of 3-13 times greater in treatment cell 2).

- In treatment cell 1, MWA contaminant concentrations were generally lower than MWD at all sample depths, and MWD3 (22-23 ft bgs) has consistently the highest contaminant mass observed.

- In treatment cell 1, no significant change ($p>0.05$) in contaminant concentrations (defined as total TCE, cDCE, VC and ethene molar mass concentrations) was observed between any of the Phases 2 and 3 samplings (through February 2006).

- In treatment cell 2, more uniform vertical contaminant distribution was observed in MWA and MWD during Phase 2.

- In treatment cell 2, contaminant concentrations significantly increased ($p>0.05$) during the November 2005 and December 2005 sampling event conducted during 10% whey injections compared to sampling events during both Phase 2 and Phase 3 1% whey injections.

- In treatment cell 2, a significant decline in contaminant concentrations was observed during the February 2006 sampling compared to the November and December 2005 sampling events.

Contaminant Fate. Contaminant fate was also evaluated using the 3-D CMT wells in order to determine the impact of whey injections on contaminants. Biodegradation was evaluated by assessing the molar mass balance between parent compounds (TCE) and reductive daughter products (cDCE, VC and ethene). Figures 5-10 through 5-13 illustrate the total moles of contaminants and reductive daughter projects during Phases 2 and 3.

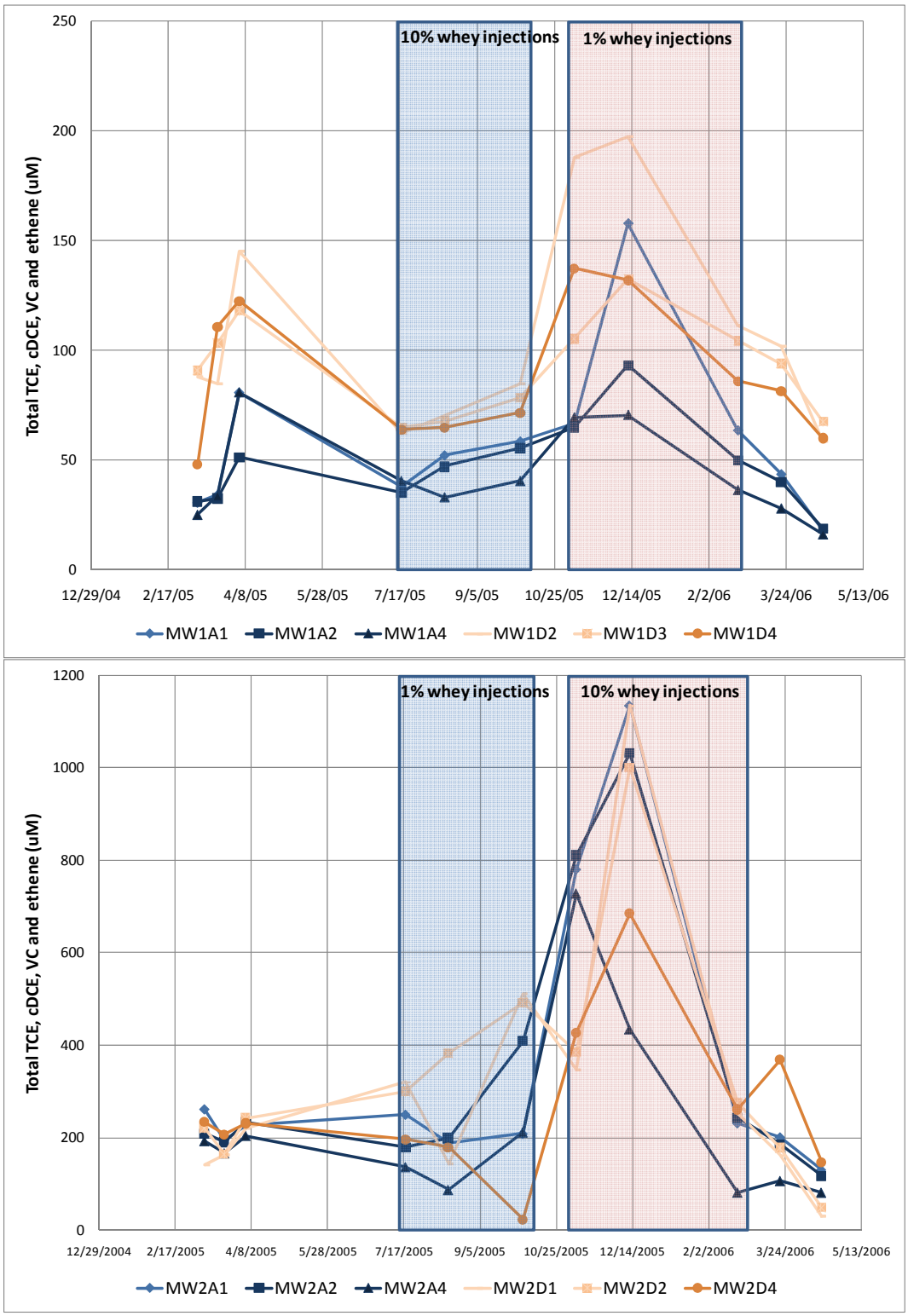


Figure 5-9. Summary of Contaminant Distribution as Total TCE, cDCE, VC, and Ethene in Treatment Cells 1 and 2 Monitoring Wells A and D During Phases 2 and 3.

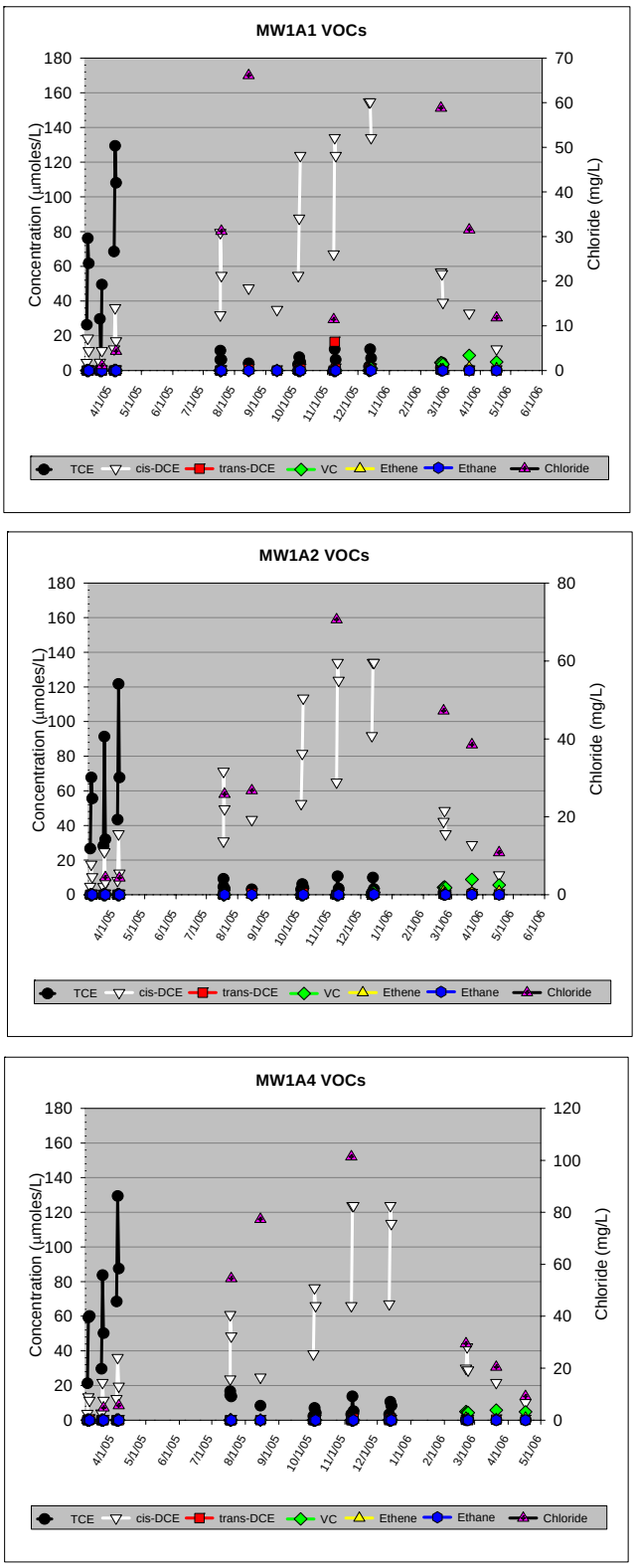


Figure 5-10. Molar Mass Plots of TCE and Reductive Daughter Products During Phases 2 and 3 of the Demonstration at MWA in Treatment Cell 1. Vertical Lines Illustrate Samples Collected Before, During, and After a Recirculation or Injection Event.

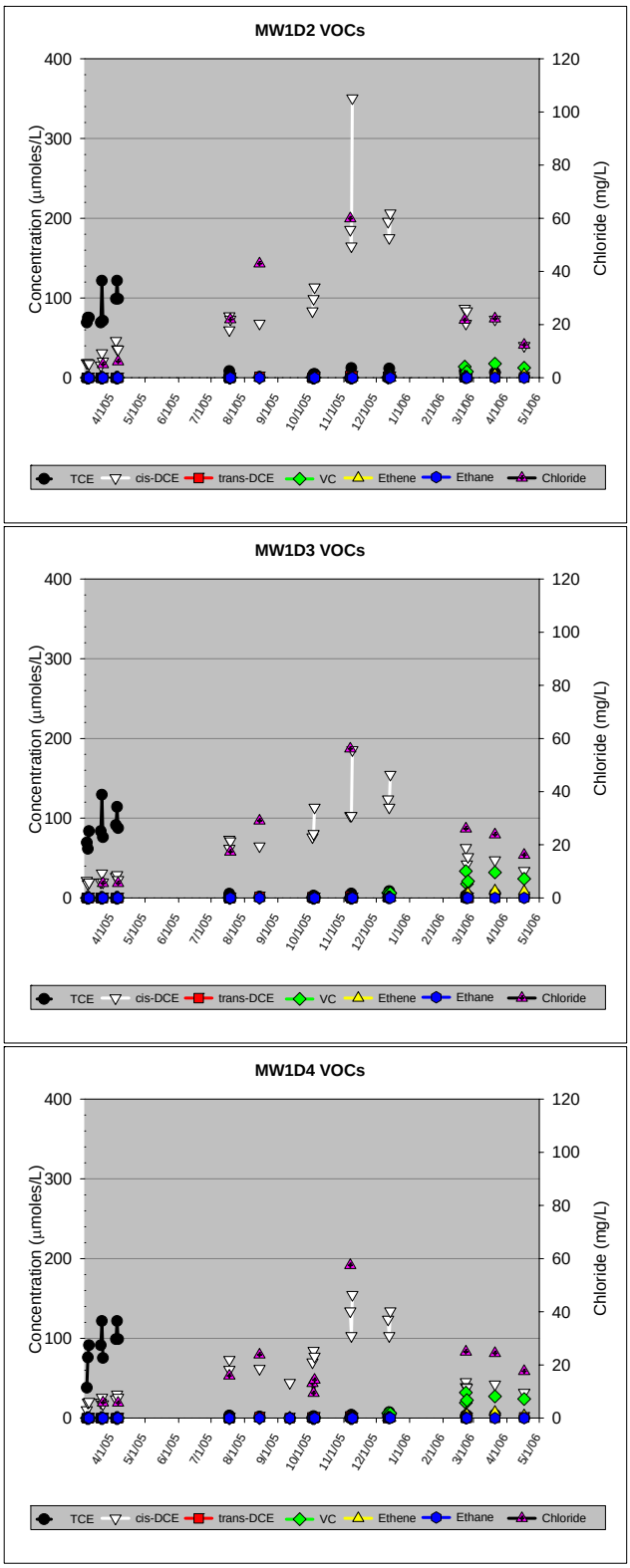


Figure 5-11. Molar Mass Plots of TCE and Reductive Daughter Products During Phases 2 and 3 of the Demonstration at MWD in Treatment Cell 1.

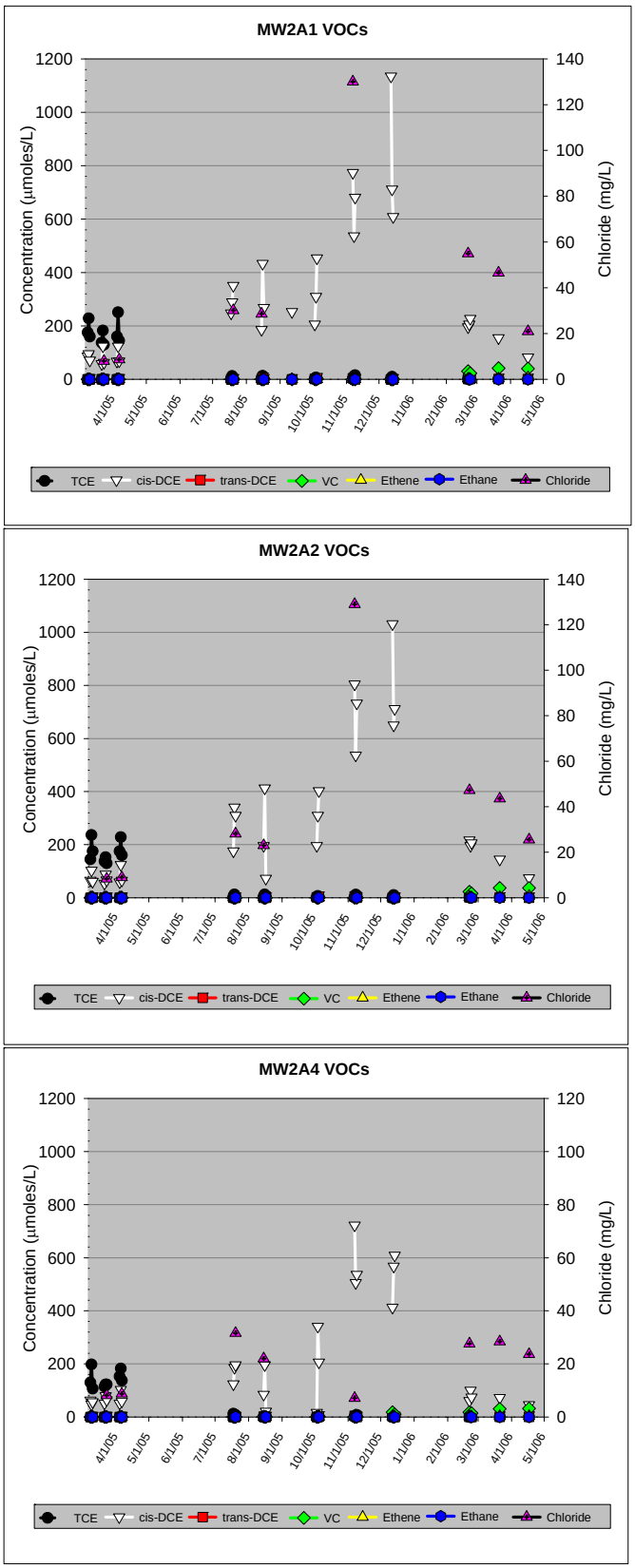


Figure 5-12. Molar Mass Plots of TCE and Reductive Daughter Products During Phases 2 and 3 of the Demonstration at MWA in Treatment Cell 2.

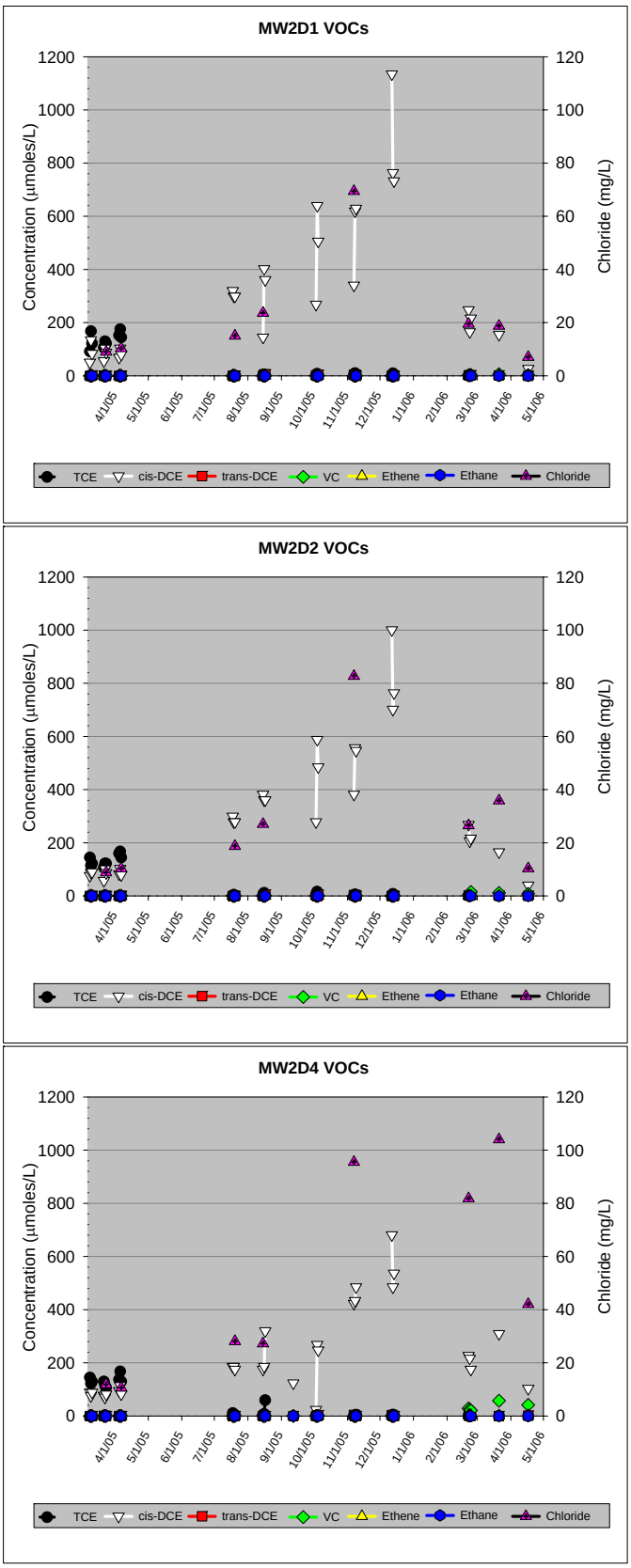


Figure 5-13. Molar Mass Plots of TCE and Reductive Daughter Products During Phases 2 and 3 of the Demonstration at MWD in Treatment Cell 2.

In general, contaminant concentrations during Phase 2 were primarily TCE (70-80% of molar mass at all sampling locations) and cDCE (20-30% of molar mass at all sampling locations) in both treatment cells. Following whey injections nearly all of the TCE was reduced to cDCE, which was the predominant compound observed, representing 88-99% of the total molar mass, until the February 2006 sampling event. For treatment cell 1, Figures 5-9 and 5-10 illustrate the response in molar concentrations of contaminants during Phases 2 and 3. For MWA, contaminant concentrations were similar in magnitude at all depths evaluated for a given sampling point. Therefore, contaminant mass appears to be fairly uniformly distributed vertically in groundwater at this location. After initiation of 10% whey injections in July 2005, a relatively good molar mass balance from TCE to almost exclusively cDCE was observed at all depths. At this location, there was a significant response in contaminant concentrations to injections, resulting in relatively high variability in concentrations observed before, the day of and the day after recirculation (Phase 2) and whey injection (Phase 3) at all depths. This suggests injections significantly influenced contaminant concentrations at this location that was not associated with the whey powder itself, given that similar variability was observed during Phase 2, and was likely the result of injecting groundwater contaminated with higher concentrations of VOCs from the extraction well. This is supported by the trends in concentrations after the February 2006 sampling event.

During the February 2006 sampling event, much lower concentrations of total VOCs were observed at downgradient locations within treatment cell 1 (i.e. MWD) due to biodegradation, which also reduced the concentrations in water that was recirculated during injection. As a result, there was relatively low variability in contaminant concentrations observed before and after the February 2006 injection compared to previous injections. In addition, significantly greater concentrations of VC were observed during the February, March, and April 2006 sampling events. Total contaminant mass during the April 2006 sampling was 35-41% lower than observed during Phase 2 baseline.

MWD in treatment cell 1 showed a similar trend in contaminant concentrations as MWA, but the magnitude of contaminant concentrations observed at this location was initially greater. Again, contaminant concentrations were similar for all depths evaluated at a given sampling point. In addition, the variability in concentrations was also much lower between samples collected before, the day of and the day following injection, which indicates that the recirculation that occurred during injections did not immediately impact contaminant concentrations at this location. In addition, a significant response in VOC concentrations was not observed as a result of transition from 10% whey injections (July-October 2005) to 1% (November 2005-February 2006) whey powder. Overall, degradation of TCE in MWD followed the same trend as MWA, with nearly instant and complete conversion to cDCE following whey injections, with a 6-month lag period before significant production of VC and ethene. Total contaminant mass during the April 2006 sampling was 26-49% lower than the Phase 2 baseline.

Overall, the total contaminant mass in treatment cell 2 was greater than treatment cell 1 (Figures 5-9, 5-12, and 5-13). In treatment cell 1, MWA and MWD contained fairly uniform concentrations of TCE and cDCE vertically within a given sampling point with an average range of total TCE and cDCE of 175-228 μM for all sampling points during Phase 2. Following initiation of whey injections nearly complete conversion to cDCE was observed with a relatively good mass balance at all sampling points with an average range of 185-262 μM during 1% whey

injections. Following initiation of 10% whey injections, however, the total mass of cDCE increased dramatically with concentrations ranging from 303-715 μM for all sample points during the November and December 2005 injections. Significant reductions in cDCE concentrations, and in total contaminant mass, were observed during the February, March, and April 2006 sampling events, concomitant with significant VC and ethene production. Total contaminant mass during the April 2006 sampling was 10-78% lower than the Phase 2 baseline.

5.6.2 Passive Flux Meters

Flux profiles generated using four passive flux meter well are presented for the primary contaminants observed, TCE (Figure 5-14) and DCE (Figure 5-15). In addition Darcy velocity was also evaluated (Figure 5-16). Mass flux and Darcy velocity was compared for five deployment periods:

- Deployment 1: Phase 2, baseline evaluation.
- Deployment 2: Phase 3, 10% w/w injections in treatment cell 1 (although no injection was performed during this period due to mechanical difficulties), and 1% w/w/ injections in treatment cell 2.
- Deployment 3: Phase 3, 10% w/w injections in treatment cell 1 and 1% w/w/ injections in treatment cell 2.
- Deployment 4: Phase 3, 1% w/w injections in treatment cell 1 and 10% w/w/ injections in treatment cell 2.
- Deployment 5: Phase 3, 1% w/w injections in treatment cell 1 and 10% w/w/ injections in treatment cell 2.

The contaminant flux profiles are presented on a log scale due to the dramatic changes in mass flux observed between PFM deployment. The first observation is that treatment cell 2 had higher observed fluxes compared to treatment cell 1 during the baseline sampling (Deployment 1). This observation is in agreement with concentration data collected from groundwater monitoring wells in the treatment cells (North Wind 2008), and with the 3-D CMT data (Figures 5-6 and 5-9).

To compare how mass flux changed during the five deployments, the vertical flux profiles were determined for each well and plotted for the five deployments (Figures 5-14 and 5-15). In addition, the average molar mass flux for each PFM well was determined for each deployment (Figure 5-17). First, it was evident that mass flux of TCE declined and cDCE flux increased 1-2 orders of magnitude between Deployments 1 and 2, following onset of whey injections within the treatment cells. This was consistent with biological reductive dechlorination due to whey injections. In addition, cDCE was the predominant contaminant observed during the rest of the deployments.

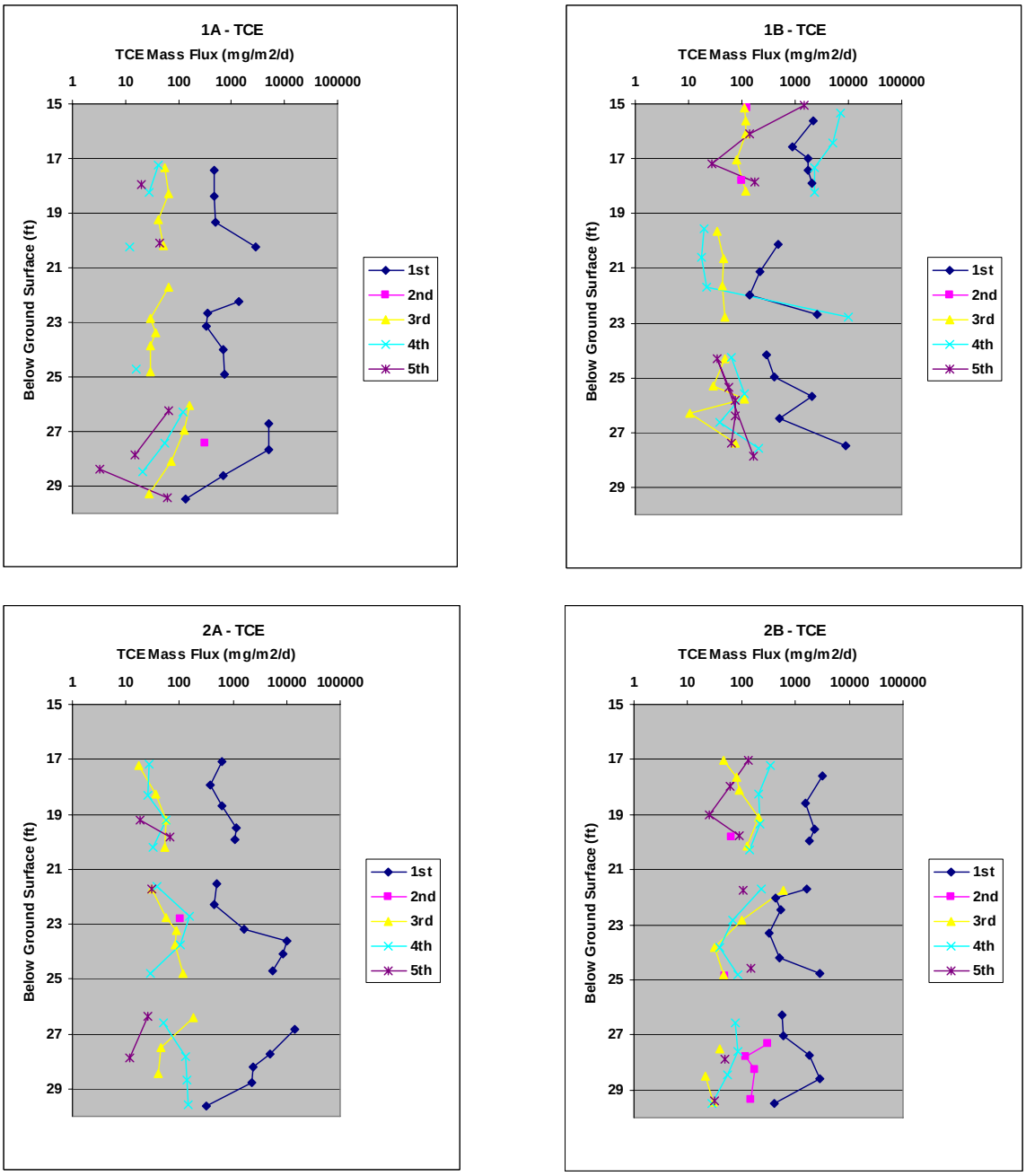


Figure 5-14. PFM TCE Flux Profiles for 5 Deployment Periods.

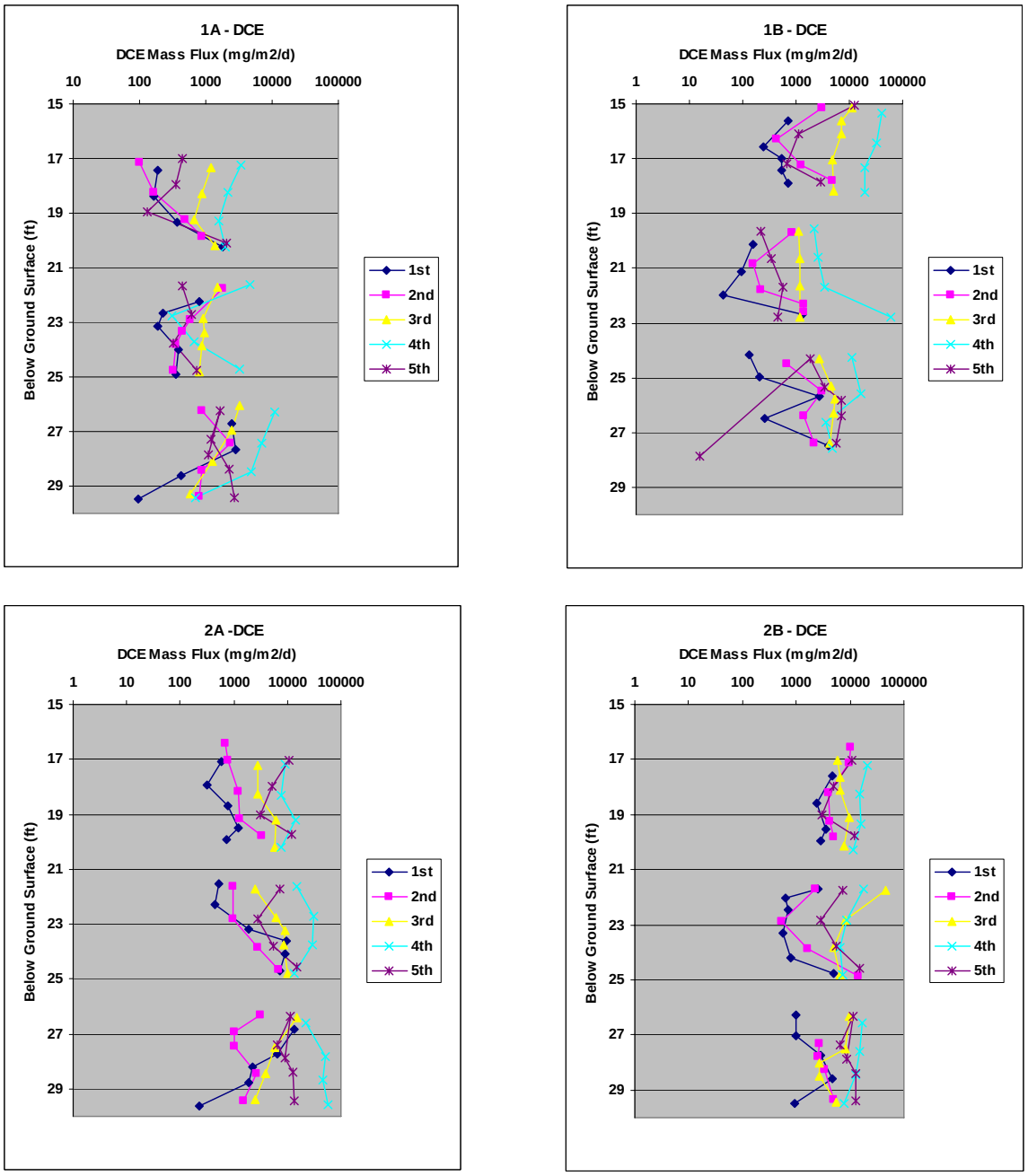


Figure 5-15. PFM DCE Flux Profiles for 5 Deployment Periods.

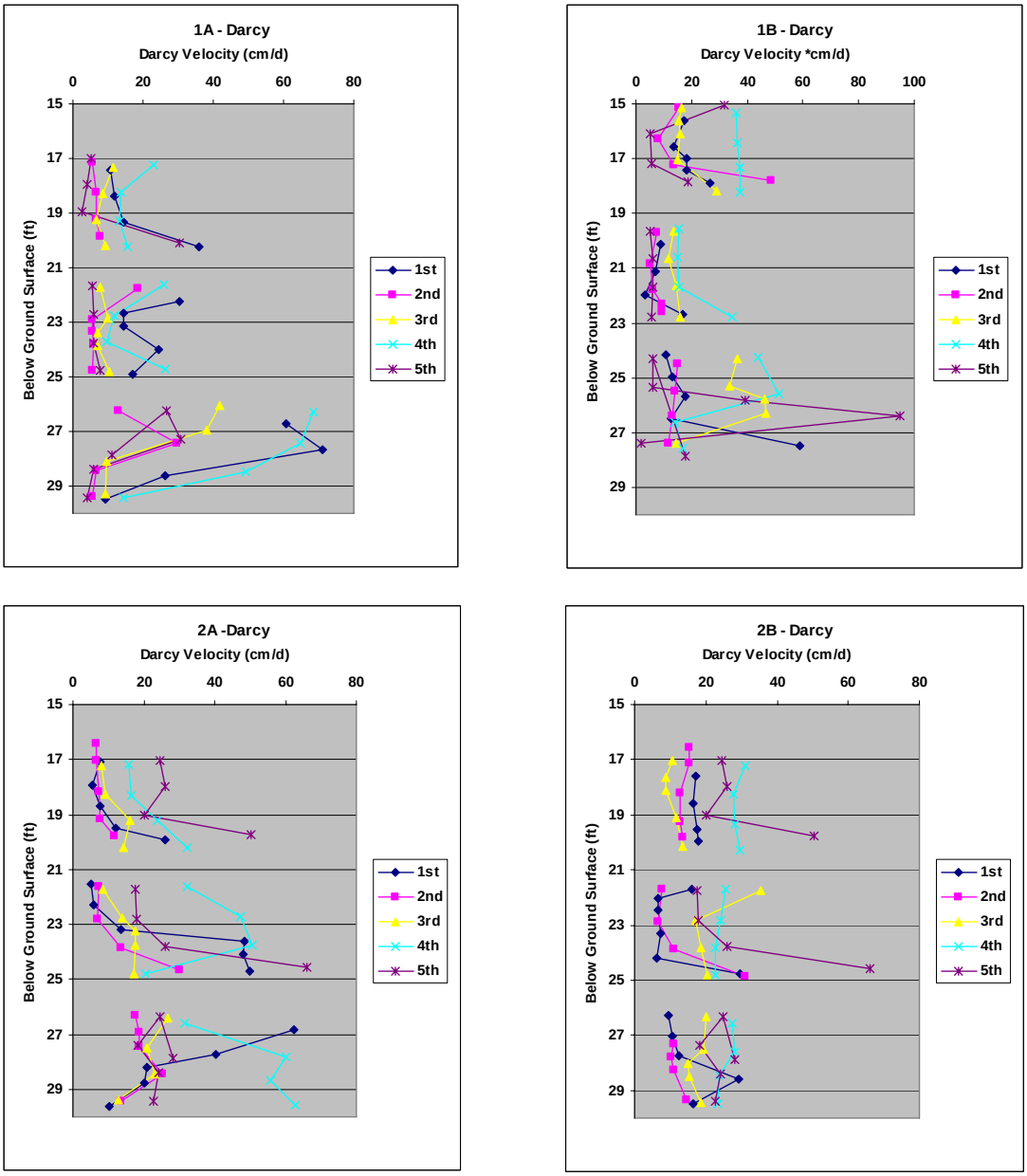
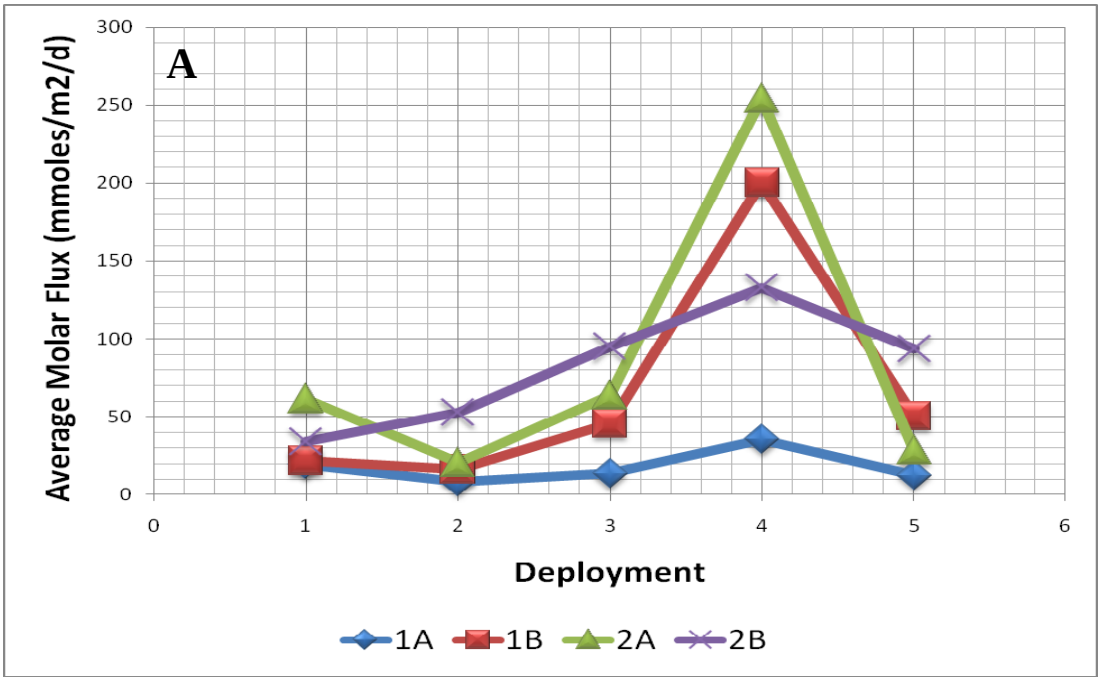


Figure 5-16. PFM Darcy Velocity Profiles for 5 Deployment Periods.



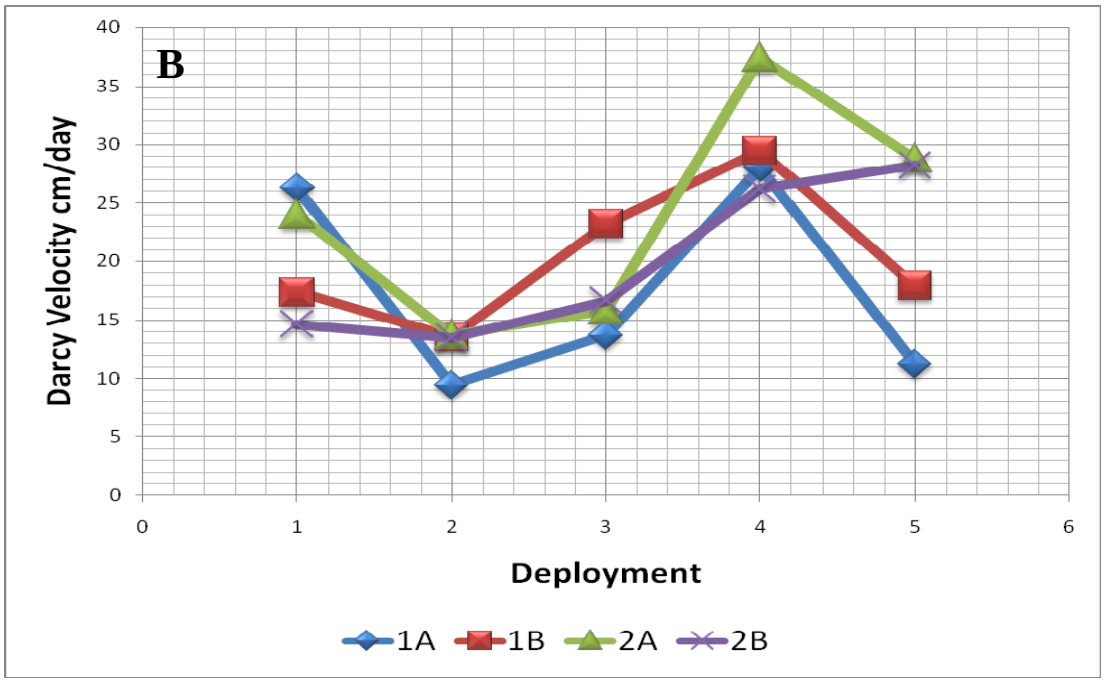


Figure 5-17. Relationship Between Total Average Molar Mass Flux for Each PFM Well for the Five Deployments (A) and Variability in Darcy Velocity (B).

In addition to significant shifts in the composition of VOCs within the treatment cells, substantial increases in the average total molar flux were observed in both treatment cells between Deployments 1, 2 and 3 with the highest flux observed during the 4th deployment. While the composition of contaminants was very different during the first three deployments, the relative magnitude of total mass flux varied from a factor of 0.43-2.13 for the treatment cell 1 and 0.34-2.84 for treatment cell 2 during Deployments 2 and 3 relative to Deployment 1 (Table 5-10). Significant increases in total mass flux were observed during the Deployment 4 in FW-1B (factor 9 increase relative to Deployment 1), and FW-2A and FW-2B (factor 4 increase relative to Deployment 1). During this period, treatment cell 1 had transitioned from receiving 10% whey injections to 1% whey injection and treatment cell 2 had transitioned from receiving 1% whey injections to 10% whey injections. In general, the magnitude of total mass flux, as TCE and cDCE, decreased during Deployment 5 relative to Deployment 4. This is likely due to the production of significant amounts of VC and ethene. Quantities of VC and ethene were only observed during the final PFM deployment and the molar flux of these represented less than 5% to 30% of the observed flux (Table 5-10).

Table 5-10. Total Average Contaminant Mass Flux for Each Flux Meter Location.

	Treatment cell 1: FW-1A mmol/m2/d	**Treatment cell 1: FW-1B mmol/m2/d**	**Treatment cell 2: FW-2A mmol/m2/d**	**Treatment cell 2: FW-2A mmol/m2/d**
Total TCE and DCE mass flux				
Deployment 1	19	22	62	34
Deployment 2	8.2	17	21	54
Deployment 3	14	46	65	97
Deployment 4	36	204	260	136
Deployment 5	11	36	27	91
VC and Ethene mass flux				
Deployment 5 VC	1.3	13	1.5	4
Deployment 5 Ethene	0.05	1.8	0.02	0

The peak average mass flux of DCE in well FW-2A of 25 g/m^2/day can be put in perspective by assuming a vertical interval of approximately 4 m and a horizontal width of 2 m to represent the single well. This gives an integrated mass discharge estimate of about 200 g/day from this single treatment cell. This is a significant mass discharge. For comparison the pre-remedial mass discharge from Area 1 at Ft. Lewis was approximately 640 g/day of TCE and 206 g/day of DCE (Brooks et al., 2008). Thus the enhanced mass discharge generated during the whey injections is substantial from a site wide perspective.

In order to compare mass flux data collected using PFMs with the 3-D CMT data, the point concentration data, *C*, was converted to mass flux, *J*. To do this an independently determined Darcy flux, *q*, was used to calculated contaminant mass flux $J=qC$. Two approaches can be used for determining *q*: 1) hydraulic data collected to estimate hydraulic conductivity, *K*, and 2) measured site gradients, *i*, based on triangulation of monitoring well head data, $q=Ki$, and tracer arrival times based on the results of the tracer test. A series of tracer tests were conducted following construction of the test cells in an effort to confirm and improve test cell performance (Table 5-7 and North Wind, 2008). The difficulty in determining the Darcy velocity using the Phase 1 tracer data was that peak breakthough occurred in most of the CMT monitoring ports during the tracer injection. This suggests that the tracer transport time was dominated by the pumping and extraction taking place during tracer injection. This pumping was cut off following the tracer injection period and thus the "back side" or falling limb of the tracer breakthrough curve can be used to estimate natural gradient transport times in the aquifer. Looking at the extraction well breakthrough curve a sharp decline in bromide concentration was observed approximately 26 hours in to the tracer test. Given that tracer injection was terminated at 8 hours this suggests a transport time of 18 hours in the aquifer, which corresponds to a pore water velocity of 5.5 m/day. This number is much higher than typically considered appropriate for the site.

During both tracer tests hydraulic response data were also collected and used to calculate the average test cell hydraulic conductivity. The value for treatment cell 1 was 6 to 15.0 ft/day while that for treatment cell 2 was 24 to 53 ft/day. Using average *K* values (10 and 38 ft/day respectively) and measured gradients that ranged from 0.026 to 0.005 during the deployments, Darcy velocity was calculated as 0.8 to 12 cm/day. In general, these are lower than those measured using PFMs, which had an average of about 20 cm/day. 3-D CMT and PFM based DCE mass fluxes were then compared (Figure 5-18 and 5-19). In general the 3-D CMT based mass flux is lower, likely due to the lower Darcy flux as noted above. The best comparisons are for treatment cell 2 with the higher estimated hydraulic conductivity.

5.6.3 Carbon Stable Isotope Analysis

Both the CMT and the flux meter data suggested a significant reduction in total contaminant concentrations and mass flux between the December 2005 and February 2006 timepoint, which also corresponded to the onset of VC and ethene production. In order to evaluate the mechanisms by which this reduction may be occurring CSIA was conducted. A summary of results is illustrated in Figure 5-20 and is as follows:

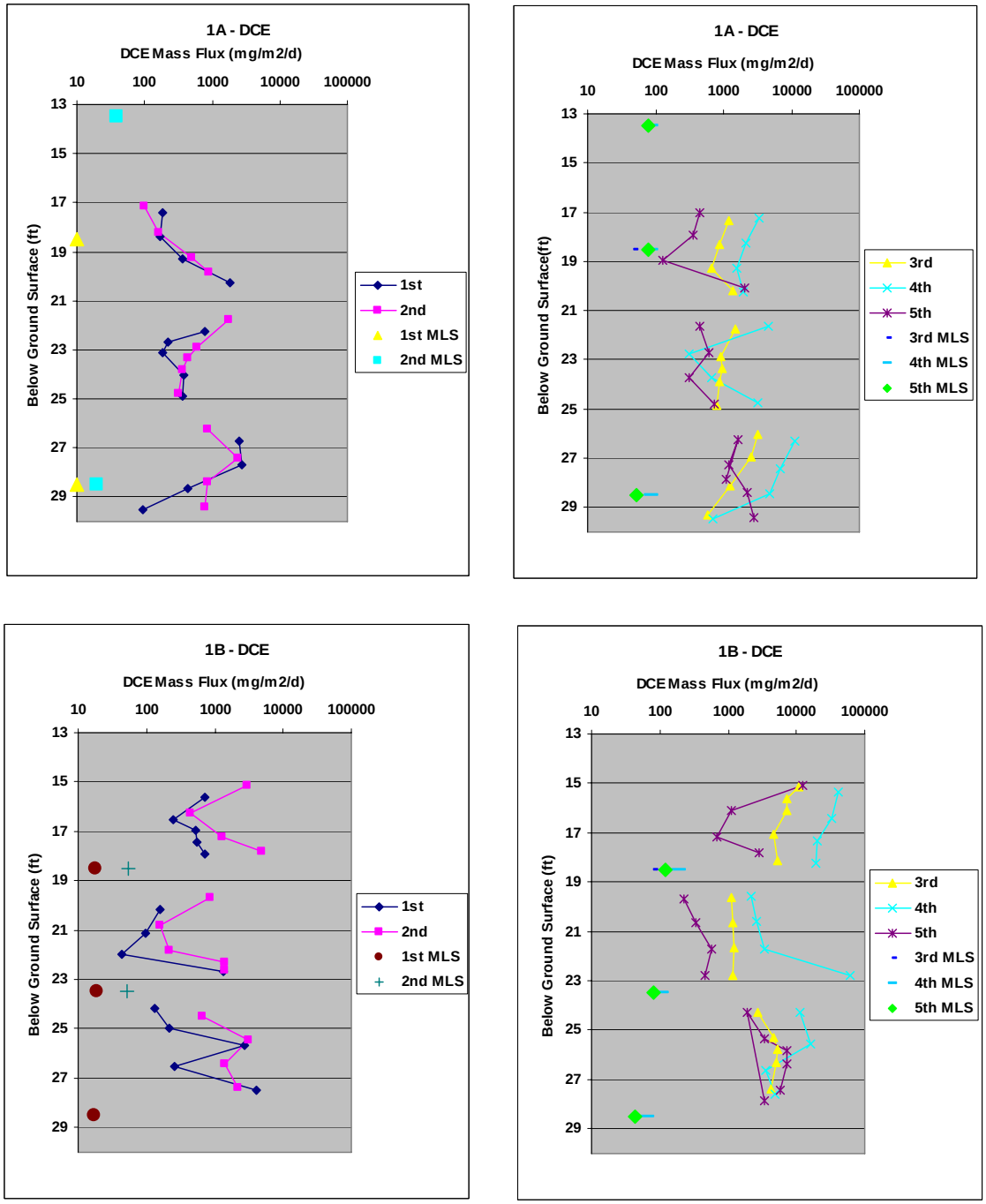


Figure 5-18. PFM DCE Flux Profiles Compared to CMT MLS Based Calculated Mass Flux Using Average Hydraulic Conductivity and Gradient in Treatment Cell 1.

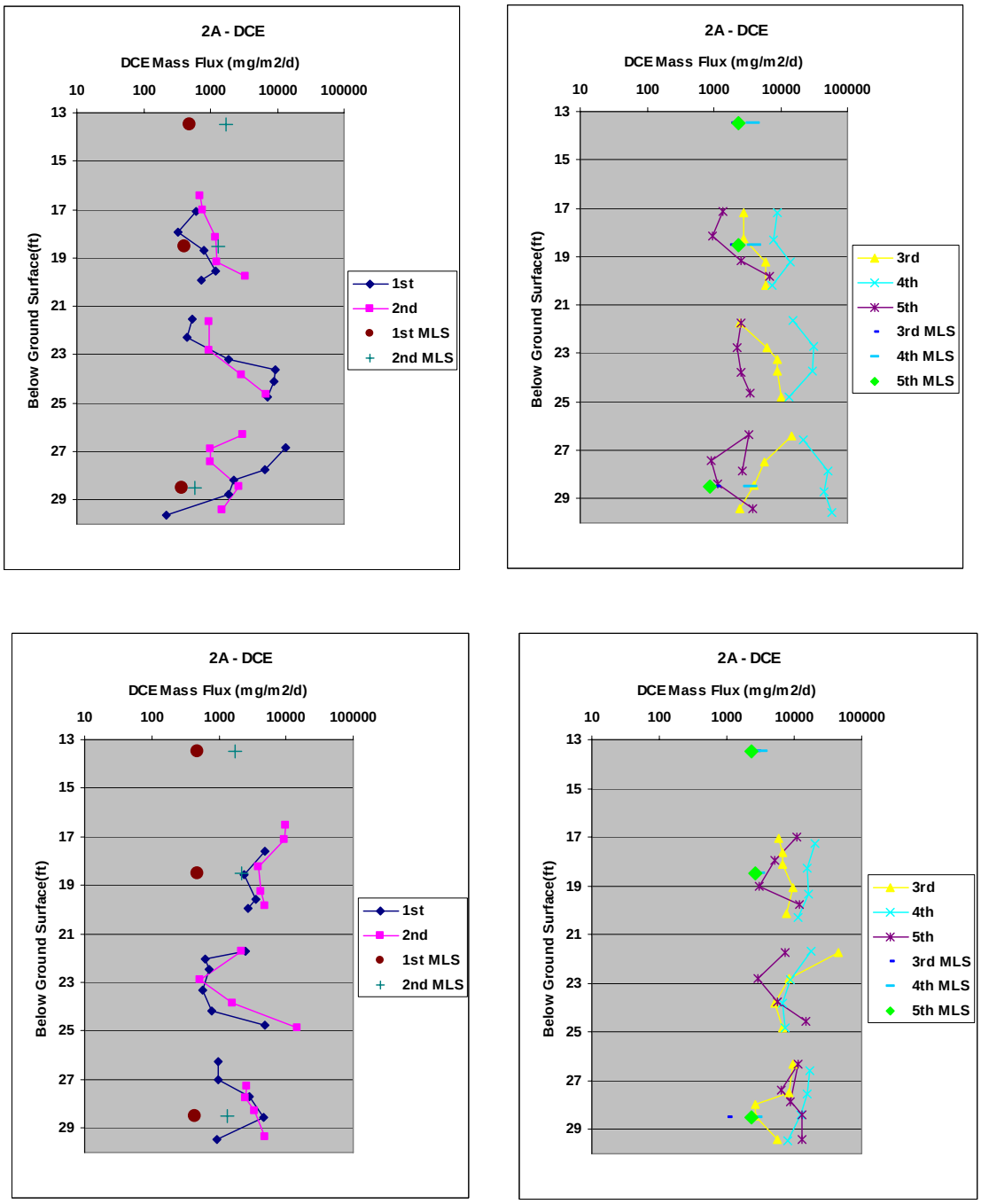


Figure 5-19. PFM DCE Flux Profiles Compared to CMT MLS Based Calculated Mass Flux Using Average Hydraulic Conductivity and Gradient in Treatment Cell 2.

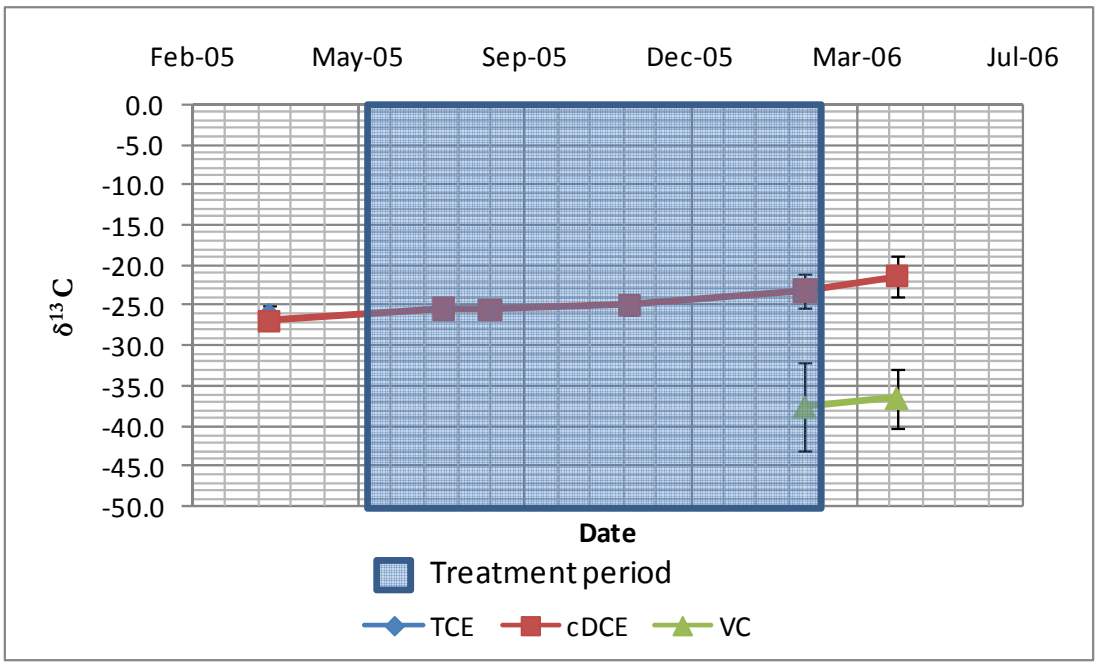


Figure 5-20. Results of CSIA, Values Represent the Mean of All Sampling Points (N=16), Except the April 2006 (N=4), Within Two Treatment Cells and Error Bars Represent One Standard Deviation.

- Phase 2: baseline sampling, the average isotope ratio of TCE was -25.8‰. Across the spatially distributed monitoring wells, the TCE isotope ratio was relatively uniform, ranging from -25.1 to -27.1‰ for both treatment cells. This suggests that prior to Phase 3 biostimulation, the TCE that was at the various locations had been transformed to a similar extent.

- Phase 2: baseline sampling, cDCE was detected by both gas chromatography and isotopic analyses. The cDCE detected was mostly enriched in the light isotope, hence, having an isotope ratio that was more negative than the starting TCE isotope ratio. The average isotope ratio of the cDCE was -27.1‰. This suggests that reductive dechlorination was ongoing prior to biostimulation and bioaugmentation.

- Phase 3: By August 2005, significant dechlorination of TCE to cDCE was measured by gas chromatography, with no detectable VC or ethene. These data were confirmed by the CSIA. While TCE concentrations were below the method detection limit for isotope analysis, cDCE isotope ratio showed an increasing trend and approached the starting TCE isotope ratio, which indicates that the aqueous TCE was completely dechlorinated to form the daughter product cDCE.

- Phase 3: From April to August 2005, the lack of VC production was evident in the cDCE isotope data. The isotope ratio of the cDCE measured during the August sampling was almost identical to the isotope ratio of the starting TCE. Since mass has to be conserved in reductive dechlorination, this indicates that the majority of the TCE was converted to cDCE and the reaction was not going beyond cDCE.

- Phase 3: By November 2005, an increase in isotope ratio of cDCE was measured at some of the monitoring wells. This enrichment suggests that cDCE has started to be converted to VC. The dechlorination beyond cDCE was likely small as little VC was detected by gas chromatography, but the isotope analysis confirmed its occurrence.

- Phase 3: By February 2006, there was solid evidence from the isotope data that dechlorination to VC was occurring. The isotope ratio of VC was much lighter than the cDCE, suggesting that VC was mostly made up of the light isotope from the parent compound cDCE. Correspondingly, an enrichment in cDCE isotope ratio was observed, with some monitoring wells reaching above -20‰.

- Phase 3: April 2006, CSIA was performed in selected monitoring wells and demonstrated continued dechlorination of cDCE to VC as the isotope ratios of the residual cDCE continued to show an increasing trend. Simultaneous with the conversion to VC, dechlorination to ethene was also likely occurring. While no isotope ratio was measured for ethene, an increase in the isotope ratio of VC was observed in three monitoring wells (MW-1A4, -2A4, -2B4), although the increase was not statistically significant. Enrichment in the VC isotope ratio suggests either that its light isotope carbons were being dechlorinated to form the daughter product ethene, or that more of the "heavier" DCE was being transformed to VC, or a combination of both.

5.6.4 Molecular Tools

A suite of molecular tools was applied to evaluate its utility for assessing performance during the ER-0218 ISB demonstration. The tools were broadly categorized as PCR-based methods and whole cell assays that evaluated microbial community structure (T-RFLP) and specific populations of interest (i.e., *Dehalococcoides* genus and methanogenic populations). Results of the molecular evaluation are described below and in the Lee et al. 2008 publication.

5.6.4.1 Evaluation of community Dynamics: T-RFLP

T-RFLP is a technique for measuring the diversity of microbial populations in a community. The procedure involves amplifying the 16S rRNA gene from genomic DNA extracted from microbial cells using a probe that has a fluorescent label. Restriction enzymes are then used which target specific sequences (i.e., every time it sees a "ccgg" in the sequence it cuts the DNA), leaving fragments of different sizes labeled with the fluorescent probe. The size of the cleaved fragment or terminal restriction fragment (T-RF), measured in base pairs (or bp), varies between the microbial populations, or ribotypes. The output of T-RFLP analysis is a chromatogram that represents the number of T-RF ribotypes (used to represent each different population) on the x-axis and the fluorescent intensity of each T-RF on the y axis (used loosely to represent the relative abundance of each population within the community). Therefore, both the number of T-RFs present in a community profile and the relative height of the individual T-RFs provide information about diversity of microbial populations within the microbial community. For instance, if all peaks have similar height, then the relative abundances of various populations are all uniformly distributed within the community T-RFLP profile. In contrast, if some peaks are very large relative to others, then the populations corresponding to those peaks are present in much higher numbers than other populations, and hence dominate the T-RFLP chromatogram. These relationships are used not to

represent the actual diversity within the microbial community but to determine the relative difference in diversity between different samples and between samples collected from the same location over time.

Figure 5-21 illustrates representative T-RFLP profiles of *Bacteria* for the timepoints evaluated for MW2D4. In general, significant shifts in the predominant bacterial populations were observed between each sampling timepoint evaluated. Table 5-11 summarizes the changes in number of T-RFs, diversity and predominant T-RFLP observed for each timepoint for all sampled locations. During the July 2005 sampling event, T-RFs 489 and 490 bp represented >20% of the total community at all sampling points except MW2C4. By August 2005, T-RF 565 bp was predominant at all locations. By the November 2005 and February 2006, the T-RFLP communities had stabilized somewhat and T-RF 95 bp was the predominant T-RF observed at all locations and timepoints except MW1D4 in February 2006. Clone library analysis coupled to T-RFLP analysis of groundwater undergoing whey injections in a chlorinated solvent source area at a site in Idaho identified T-RF 95 as a populations within the genus *Bacteroides* (Macbeth 2008). This populations has been linked primarily to carbohydrate fermentation, including lactose which is the primary component of whey, and production of volatile fatty acids, predominately acetate, propionate, and butyrate in human intestines (McNeil et al., 1978), and in anaerobic digestors (Ueki, Abe et al. 2008). In addition, *Bacteroides* are also associated with protein degradation generating ammonia, carbon dioxide, volatile fatty acids, and branched chain fatty acids (Wrong 1988). Although the identification of this T-RF cannot be confirmed at Ft. Lewis without clone library assessment of the Ft. Lewis samples, it does illustrate the utility of the method.

Table 5-12 illustrates the T-RFLP results targeting *Archaea*, which include methanogens. *Archaea* could not be amplified for the July or August 2005 using PCR to high enough concentrations to run T-RFLP. This suggests that there were relatively low concentration of *Archaea* during these sampling events. T-RFLP analysis could be performed for *Archaea* on the November 2005 and February 2006 sampling events, with the exception of MW2B4 for the November 2005 sampling event. In all of the samples, T-RF 330 predominated the archaeal community comprising 72-100% of the profile. This fragment was associated with the *Methanosarcina* genus, which contains both acetate-utilizing and hydrogen utilizing species, at the INL site undergoing enhanced bioremediation in a source zone (Macbeth et al., 2004). Again, however, clone libraries would need to be conducted on the Ft. Lewis samples to confirm this identification.

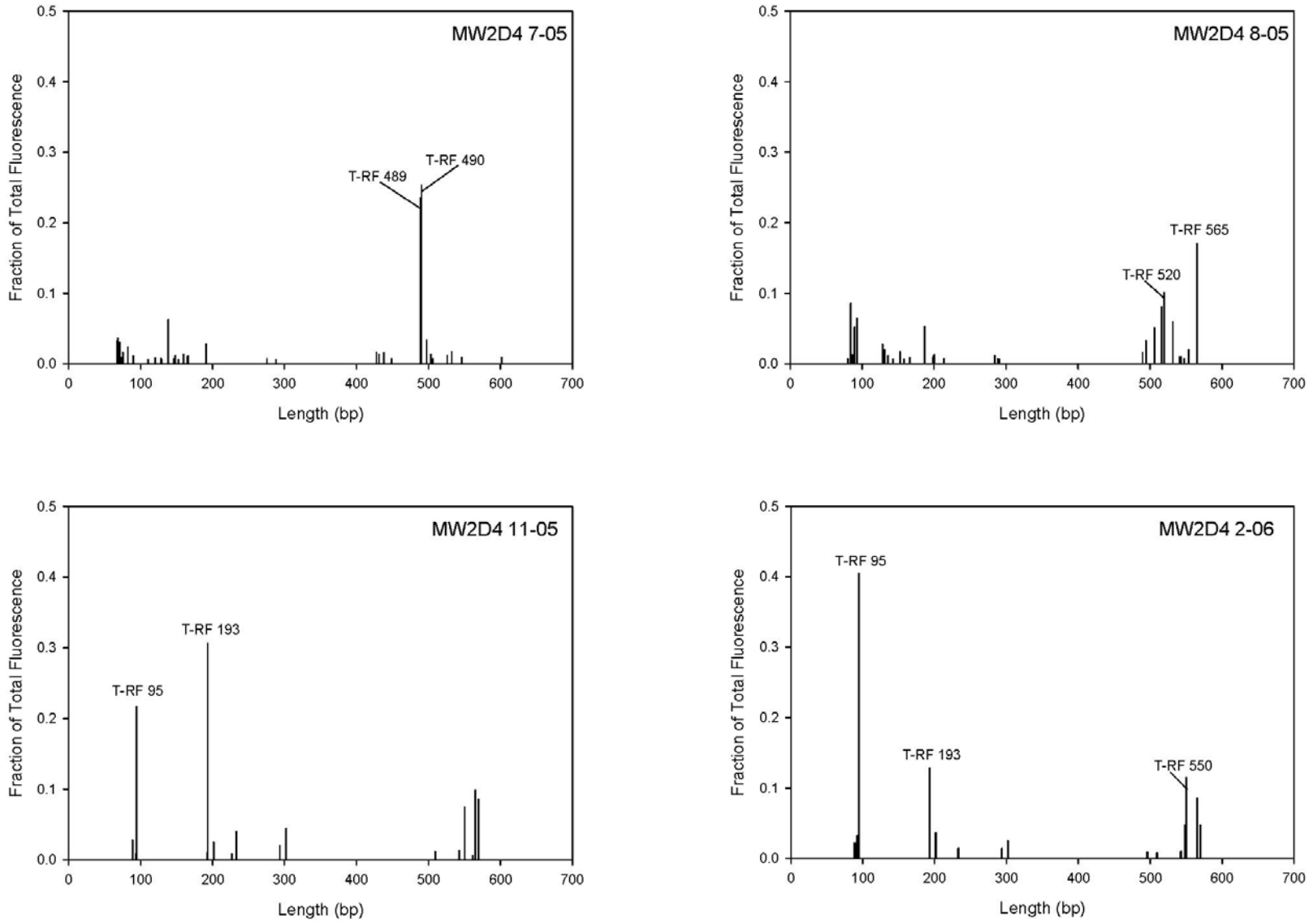


Figure 5-21. T-RFLP Response in *Bacteria* During Phase 3 in MW2D4.

Table 5-11. Summary of T-RFLP Results for *Bacteria*.

Date	Well	S (T-RFs) 2005[a]	Predominant Fragments[d] (bp)
July 2005	MW1A4	17	490 (42) 491 (42)
	MW1B4	39	489 (21), 490 (23)
	MW1C4	45	490 (26)
	MW1D4	11	489 (44), 490 (43)
	MW2A4	45	95 (10, 120 (39), 490 (30)
	MW2B4	36	490 (23)
	MW2C4	34	410 (45)
	MW2D4	34	489 (24), 490 (25)
August 2005	MW2A4	28	84 (17), 532 (15), 565 (15)
	MW2B4	25	84 (14), 92 (37), 565 (12)
	MW2C4	27	532 (12), 565 (24)
	MW2D4	31	520 (10), 565(17)
	MW1A4	20	528 (13), 554(19), 565 (16)
	MW1B4	22	84 (18), 532 (22), 565 (19)
	MW1C4	20	532 (14), 565(25)
	MW1D4	25	84 (12), 532(18), 565(19)
November 2005	MW1A4	18	95 (20), 215 (21), 573 (14)
	MW1B4	23	95 (46), 550 (12)
	MW1C4	18	95 (52), 202 (11)
	MW1D4	15	95 (56), 550(13)
	MW2A4	22	95 (37), 193 (14)
	MW2B4	18	95 (45), 550 (12)
	MW2C4	17	95 (30), 193(18), 550 (11)
	MW2D4	16	95 (22), 193 (31)
February 2006	MW1A4	9	95 (63), 550 (15)
	MW1B4	32	87 (12), 95 (28)
	MW1C4	25	94 (15), 506 (14), 509 (10), 520 (12)
	MW1D4	20	87 (15), 506 (10)
	MW2A4	17	95 (41), 550 (16)
	MW2B4	14	95 (40), 550 (12), 573 (14)
	MW2C4	16	95 (38), 193(20), 550 (11)
	MW2D4	15	95 (41), 193 (13), 550 (11)

a. Number of T-RFs in community profile.
b. Shannon-Wiener diversity index: estimates total diversity as richness and evenness; the higher the number, the more diverse.
c. Shannon-Wiener function: estimates evenness; the closer to 1, the more even the community.
d. Value in parenthesis represents % of total community that the T-RF represented.

Table 5-12. Summary of T-RFLP Results for Archaea.

Date	Well	S (T-RFs)[a]	Predominant Fragments (bp)[b]
November 2005	MW1A4	1	330 (100)
	MW1B4	1	330 (100)
	MW1C4	1	330 (100)
	MW1D4	2	327 (13), 330 (81)
	MW2A4	2	323 (9), 330 (91)
	MW2B4	0	No Data[c]
	MW2C4	1	330 (100)
	MW2D4	2	328 (7), 330 (87)
February 2006	MW1A4	1	330 (100)
	MW1B4	1	330 (100)
	MW1C4	1	330 (100)
	MW1D4	1	330 (100)
	MW2A4	2	325 (28), 330 (72)
	MW2C4	1	330 (100)
	MW2D4	2	325 (8), 330 (89)

a. Number of T-RFs in community profile.
b. Value in parenthesis represents % of total community that the T-RF represented.
c. Sample did not amplify with PCR.

5.6.4.2 Evaluation of Dehalococcoides

Quantitative polymerase chain reaction (QPCR) methods developed by the University of California Berkeley were used to quantify DNA targeting several genes of the genus DHC in environmental samples (Lee et al., 2008). These data were used to determine the indigenous DHC population at Ft. Lewis, the impact of bioaugmentation with a DHC-containing culture, and the growth of DHC coupled to reductive dechlorination performance and geochemistry.

qPCR. QPCR is a semi-quantitative method for estimating the concentration of the target template (i.e., *Dehalococcoides* DNA) within a DNA extraction with high specificity, sensitivity and reproducibility. Using QPCR methods, techniques developed to identify four genes associated with *Dehalococcoides* spp. were targeted. The first qPCR target was the 16S rRNA gene, which is used as the general marker for evaluating all strains of *Dehalococcoides* present in a sample. In addition to the general marker, three functional reductase genes, *tceA*, *vcrA*, and *bvcA*, associated with differing reductive dechlorinating capacities were evaluated. Reductase gene *tceA* was isolated from *Dehalococcoides ethenogenes* strain 195, which reduces PCE or TCE to cis-DCE (cDCE) and VC in energy yielding reactions, but only reduces VC to ethene in

a cometabolic reaction (Magnuson et. al 2000). Reductase gene *vcrA* was isolated from *Dehalococcoides* Strain VS and degrades PCE and TCE energetically all the way to ethene (Muller et al., 2004). Reductase gene *bvcA* was isolated from *Dehalococcoides* Strain BAV1 and degrades PCE or TCE only cometabolically and energetically degrades cis-DCE and VC to ethene (Krajmalnik-Brown et al., 2004). Details of the methods, results and evaluation of the qPCR can be found elsewhere (Lee et al., 2008).

Figure 5-22 illustrates the response of DHC concentrations to the operational phases of the demonstration. In general, relatively low concentrations ($<10^5$ gene copies/L groundwater) of the DHC 16S rRNA and functional reductase genes *tceA*, *bvcA* and *vcrA* were detected during Phase 2 within both treatment cells. Following the onset of whey injections, DHC concentrations increased one to two orders of magnitude in both treatment cells. Bioaugmentation was conducted following the July 2005 whey injection into both treatment cells using a DHC-containing culture. Figure 5-23 illustrates the qPCR results of the bioaugmentation culture. As can be seen, *bvcA* was not detected in the culture, but was present initially in NAPL Area 3. Sampling results from one month post-bioaugmentation (August 2005) generally indicated that average concentrations increased slightly compared to the July 2005 sampling event. Little significant change in DHC concentrations was observed until the February 2006 sampling, when concentrations of all four targets increased by 1-2 orders of magnitude in both treatment cells. In general, the *vcrA* and *bvcA* reductase genes comprised the greatest fraction of the DHC population, with *tceA* genes generally 2-3 orders of magnitude lower in concentration for all timepoints evaluated. In addition, the sum of the functional reductase genes generally equal that of the 16S rRNA gene, which indicates that these three functional genes represent the majority of the *Dehalococcoides* population.

Figures 5-24 through 5-27 illustrate the relationship between TCE, reductive daughter products cDCE, VC and ethene, and DHC 16S rRNA and reductase gene concentrations. Overall, there is no correlation between concentration of DHC and dechlorination rate (data not shown). However, general relationships can be inferred by evaluating results for 16S rRNA, *bvcA* and *vcrA*, which were relatively similar. The 16S rRNA gene is used to evaluate total concentrations of DHC. During the periods of low concentrations of these three genes ($<10^5$ gene copies/L groundwater), TCE and cDCE predominated contaminant mass within the treatment cells. Following the onset of whey injections, nearly complete conversion to cDCE was observed along with an increase in the gene concentrations ($<10^7$ gene copies/L groundwater). This initial increase in DHC is followed by a lag in growth of DHC for nearly four months. Once DHC again increase to high concentrations ($>10^7$ gene copies/L groundwater), VC and ethene are produced. Therefore, a threshold concentration for DHC might exist below which dechlorination of the lower chlorinated ethenes cDCE and VC does not occur.

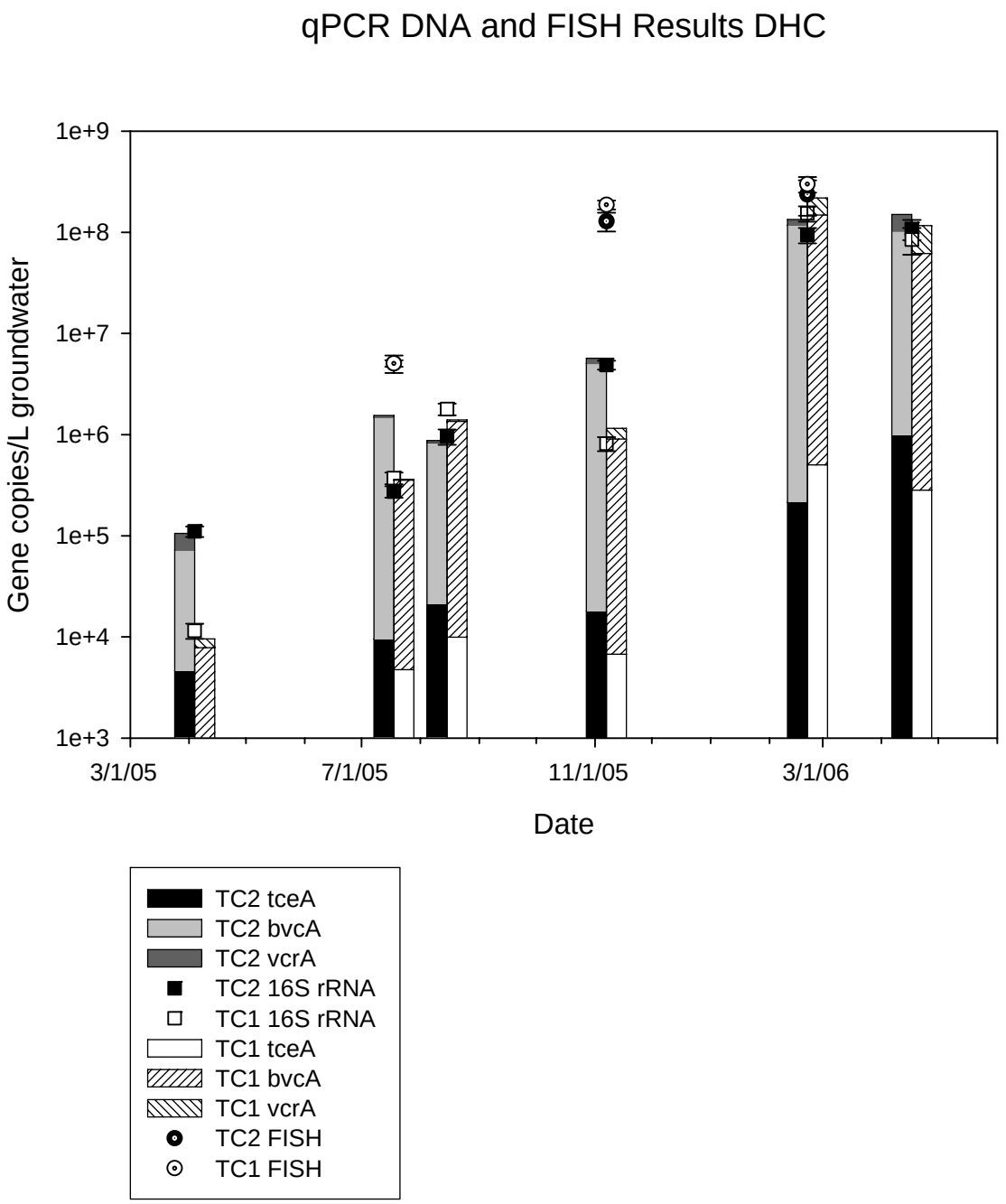


Figure 5-22. Summary of DHC qPCR Results for the 16S rRNA, *bvcA*, *vcrA*, and *tceA* Genes as the Mean (n=4) for Each Treatment Cell and FISH Results Targeting the 16S rRNA Gene.

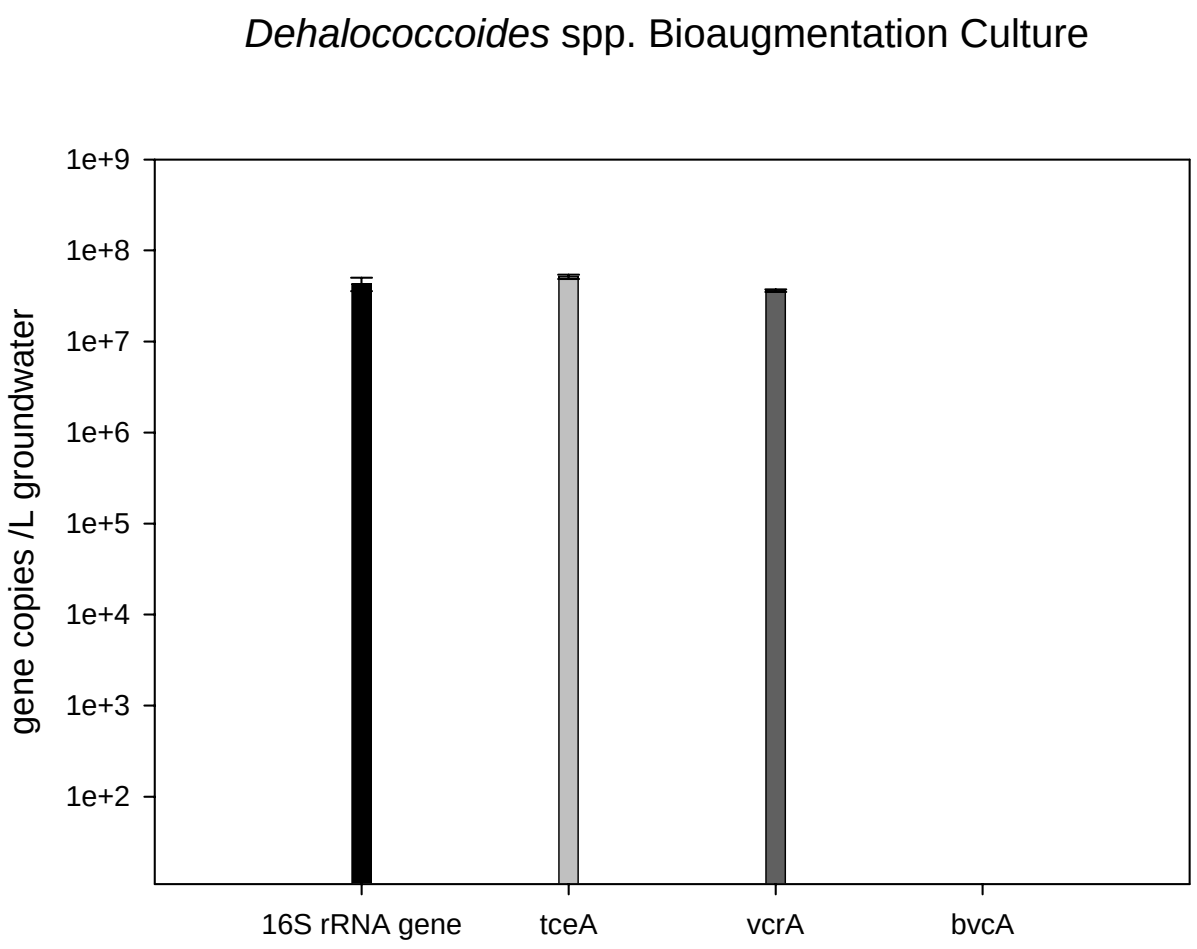


Figure 5-23. qPCR Results of Bioaugmentation Culture used at NAPL Area 3.

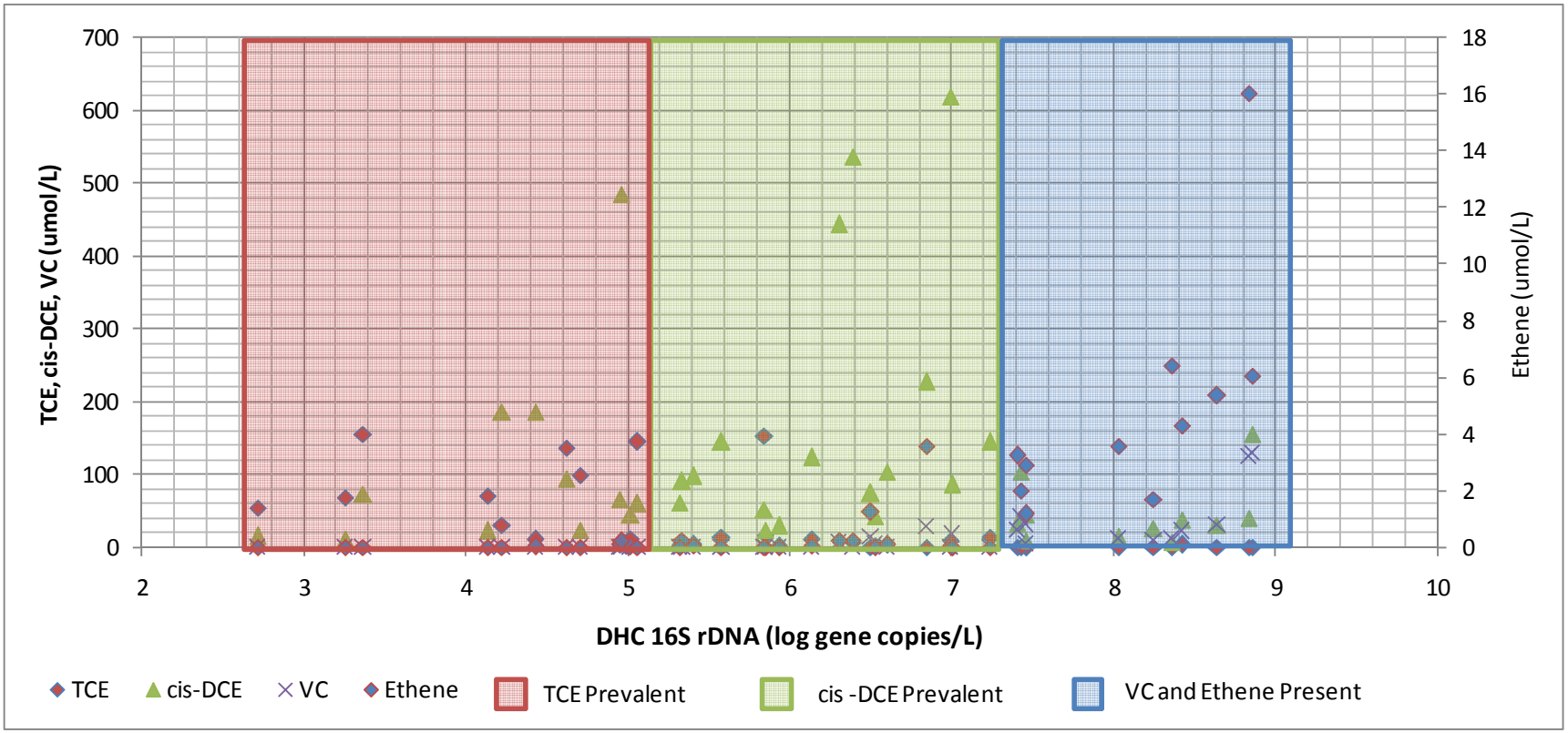


Figure 5-24. Relationship Between TCE and Reductive Daughter Products and Concentrations of DHC 16S rRNA Genes.

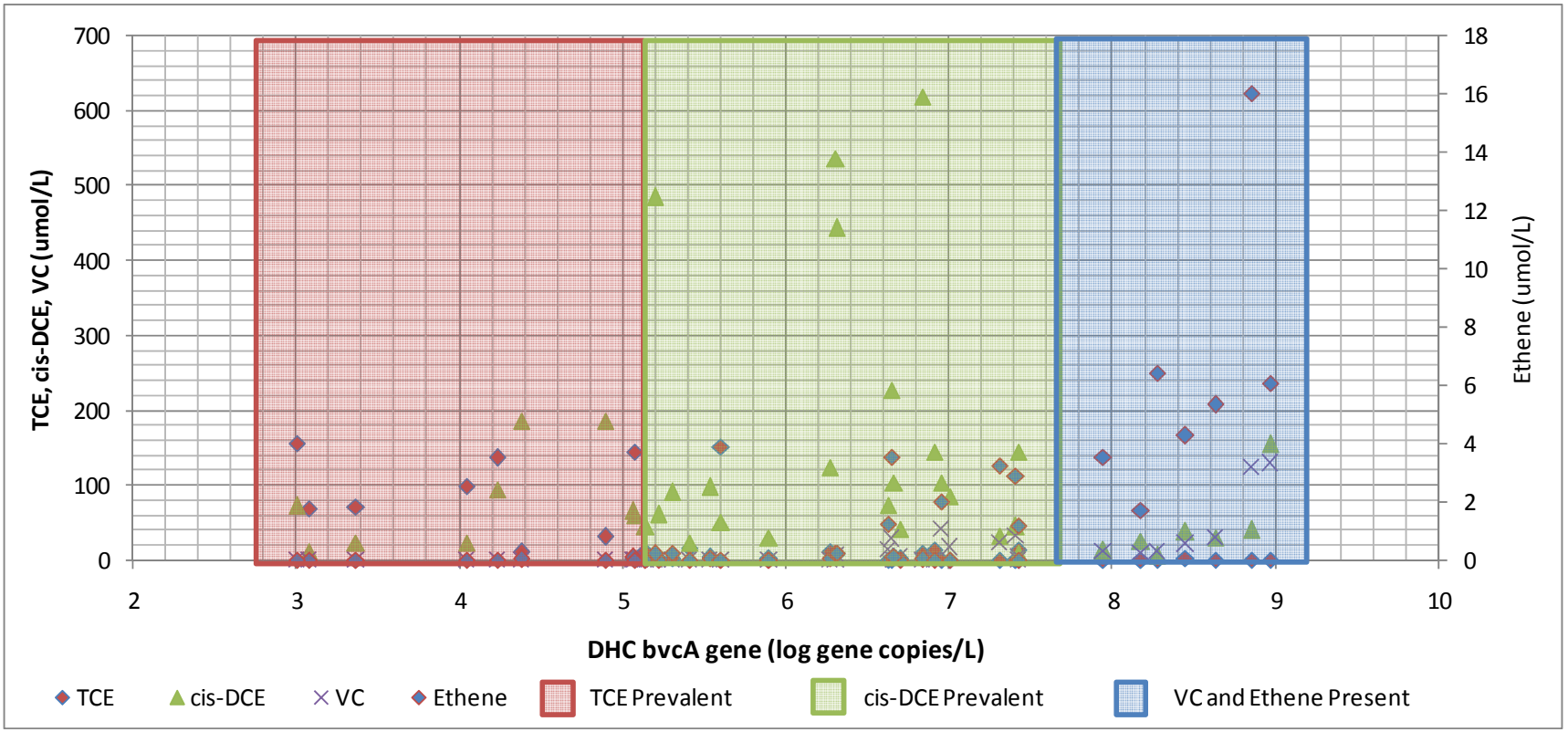


Figure 5-25. Relationship Between TCE and Reductive Daughter Products and Concentrations of DHC *bvcA* Genes.

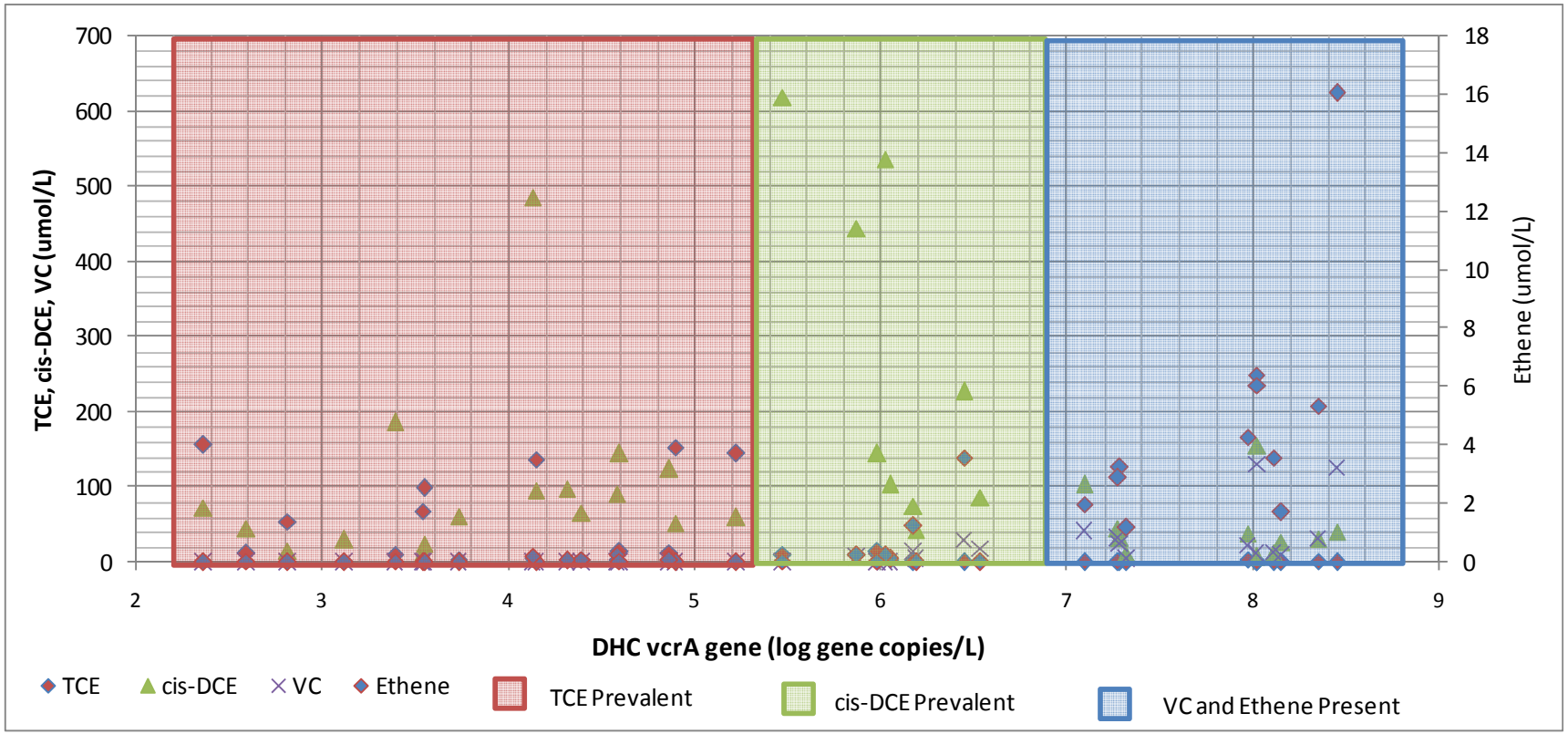


Figure 5-26. Relationship Between TCE and Reductive Daughter Products and Concentrations of DHC *vcrA* Genes.

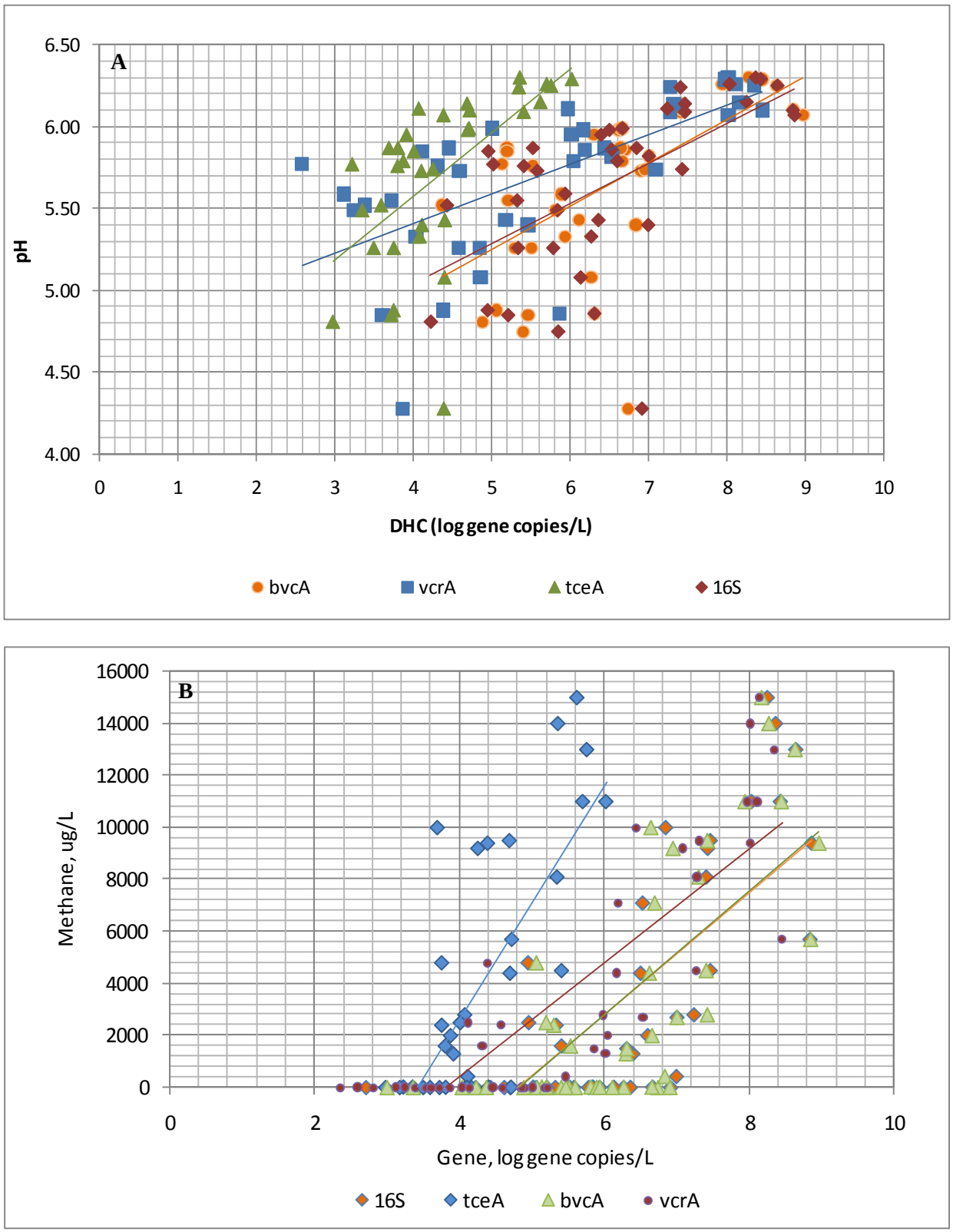


Figure 5-27. Relationship Between DHC qPCR Results and Geochemical Parameters pH (A) and Methane (B).

The relationship between DHC and pH and methane (Figure 5-27) was evaluated in order to determine if these parameters either directly or indirectly influenced DHC growth and activity. For pH, DHC was evaluated during the initial drop in pH to relatively low values immediately following the onset of whey injections through the recovery observed approximately 8 months after injections began (Figure 5-27A). There was a positive correlation between DHC concentrations and pH (R^2 values between 0.36-0.42), with increasing concentrations of DHC with higher pH. In particular, high concentrations of DHC were not observed within both treatment cells until pH values had recovered to >5.5-6.0 (i.e., pH greater than a threshold value). Almost uniformly within both treatment cells, concentrations of DHC 16S rRNA, *vcrA* and *bvcA* genes that exceeded 10^7 gene copies/L of groundwater corresponded to pH values >6.0.

In addition to pH, the relationship between DHC and methane production was also evaluated. DHC is a strict anaerobe and previous studies have indicated that growth and activity are generally most efficient under methane-producing conditions. Consistent with this model, there was a positive correlation between methane production and increasing concentrations of all four of the DHC targets (R^2 values between 0.56-0.63), as illustrated in Figure 5-27B. While this does not necessarily mean that methane is directly affecting DHC growth and activity, it does at least imply that environmental conditions that are conducive to methane production are also conductive to DHC growth and activity.

These results suggest that in order to enrich high concentrations of DHC (>10^7 gene copies/L groundwater) necessary to facilitate efficient reductive dechlorination to ethene, the following conditions were necessary:

1. pH values >6.0

2. Strongly methane-producing conditions.

FISH. FISH was also used to evaluate *Dehalococcoides* spp. FISH is a whole cell assay used to visualize cells by hybridizing RNA with florescent probes that are specific to the desired target. FISH is considered a direct measure of activity since it binds to RNA instead of DNA. In addition, it has the advantage of not requiring DNA or RNA extraction nor does it require amplification, as do PCR-based methods, which can induce inefficiencies and bias into results. The FISH probe targeted 16S rRNA of all known DHC strains for the samples collected in July and November 2005 and February 2006. Figure 5-22 illustrates the results of the DHC FISH results with the qPCR results. During the February and November 2005 samplings, relatively high concentrations of DHC were detected with FISH. In addition, the concentrations measured were higher than measured for qPCR (approximately 1-2 orders of magnitude). By the February 2006 sampling, however, concentrations of DHC as measured for FISH and DHC were similar. Figure 5-28 illustrates the relationship between FISH DHC results and TCE and reductive daughter product concentrations. Similar to qPCR results, no discernable trend between concentrations of DHC and dechlorination rates could be made. However, concentrations of DHC greater than 10^8 were observed to correspond with VC and ethene production at all locations evaluated. Therefore, 10^8 cells/L groundwater appears to be the “threshold” above which production of VC and ethene is observed at Ft. Lewis**.**

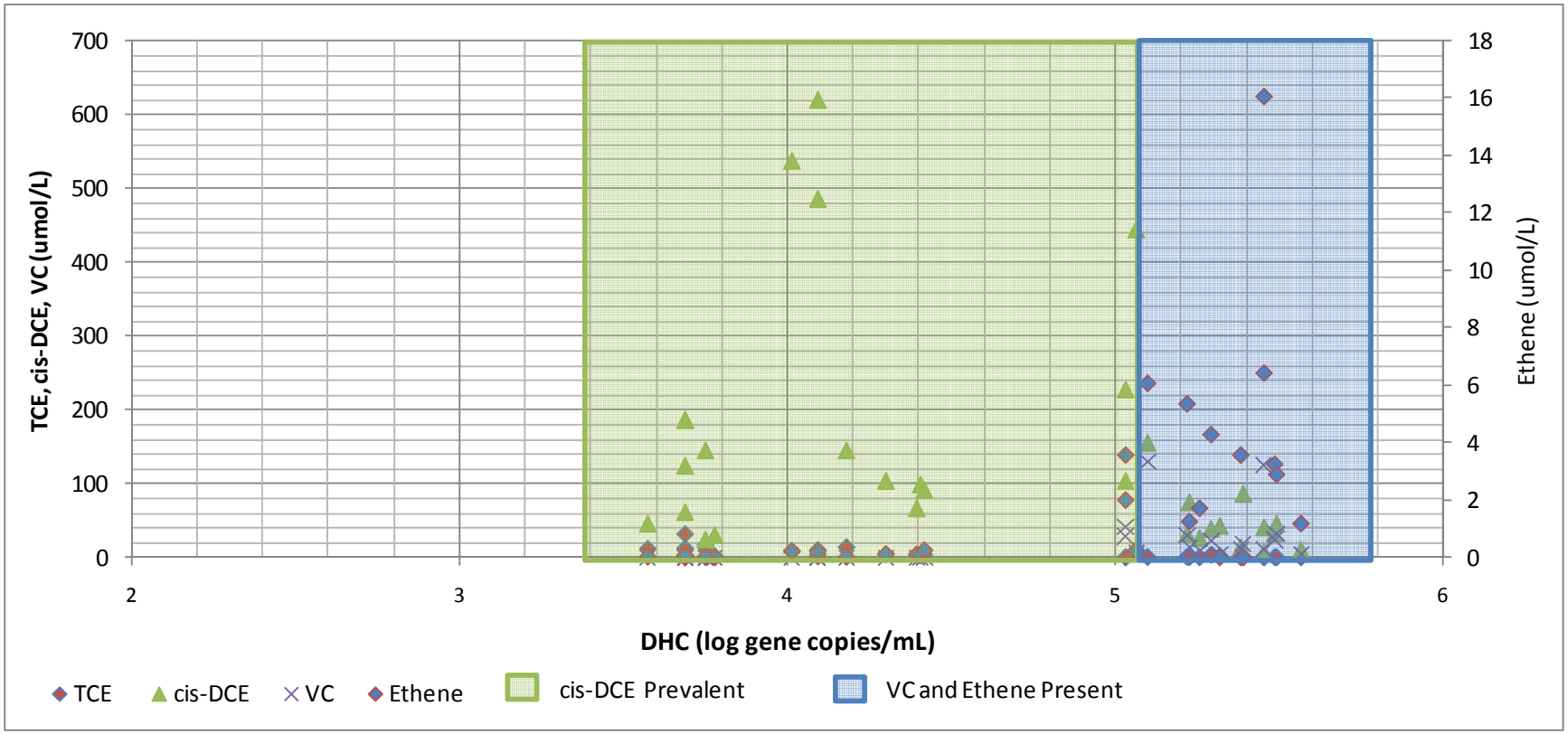


Figure 5-28. Relationship Between TCE and Reductive Daughter Products and Concentrations of Active DHC Cells.

5.6.4.3 Evaluation of Methanogens

Methanogens have long been of interest in reductively dechlorinating communities because they tend to be a significant component of the microbial community in environments conducive to anaerobic reductive dechlorination and have been implicated as potential competitors to DHC for available hydrogen (Fennell 1998) in lab cultures. Subsequent field studies at the INL site where enhanced bioremediation is used to treat chlorinated solvents in groundwater have shown that methane-producing conditions are required for efficient reductive dechlorination, and that at low temperatures, acetate-utilizing and not hydrogen-utilizing methanogens predominate, which do not directly compete with DHC for reducing equivalents (Macbeth et al., 2004). In order to evaluate the relationships between methanogenic populations and DHC, a detailed assessment of specific populations was done using specific qPCR and FISH targets.

qPCR. QPCR was used to evaluate the response of methanogenic populations during the bioremediation treatment at Ft. Lewis. Figure 5-29 illustrates the results of qPCR assessment of four methanogenic orders during Phase 3 operations. One month after whey injections began (July 2005), low concentrations of *Methanosarcinales*, an order containing acetate-, and hydrogen-utilizing methanogens, were observed in both treatment cells, and *Methanococcales*, an order containing hydrogen-utilizing methanogens were observed in treatment cell 1. Five months after whey injections began, concentrations of *Methanosarcinales* increased approximately 1-2 orders of magnitude in both treatment cells, and the *Methanococcales* were non-detect. Eight and nine months after injections began, concentrations of *Methanosarcinales* increased an additional 1-2 orders of magnitude. During these latter sampling events, the total concentrations of methanogens were much closer to measured concentrations of total *Archaea*, which was used as an indicator of total coverage of *Archaea* represented by the target methanogens.

Concentration of methanogens were also compared to DHC (Figure 5-30). The methanogen target with the highest correlation to DHC was total *Methanosarcinales* (R^2= 0.66). This suggests that as this population was enriched during the demonstration, so was DHC.

FISH. Methanogens were also evaluated using FISH analysis. FISH results indicated higher concentrations of methanogenic populations than qPCR (Figure 5-31), especially during the July 2005 event. The predominant populations were also consistent between sampling events with the *Methanomicrobiales*, and *Methanosarcinales*, (also *Methanosaeta* which is included in *Methanosarcinales*) representing 60-70% of the total population. Lower concentrations of *Methanobacteriales* and *Methanococcales* were also consistently detected. Methanogen concentrations increased most dramatically between the Aug. 2005 and Nov. 2005 sampling in both treatment cells.

Figure 5-32 illustrates the correlation between concentrations of DHC and methanogen populations using FISH data. These data illustrate positive correlations (R^2- 0.69-0.81) between increasing concentrations of DHC and methanogens. Similar to the qPCR data, these data suggest that under bioremediation operations conducted at Ft. Lewis, developing an environment that facilitates growth and activity of methanogenic populations also generates conditions conducive to the growth and activity of DHC.

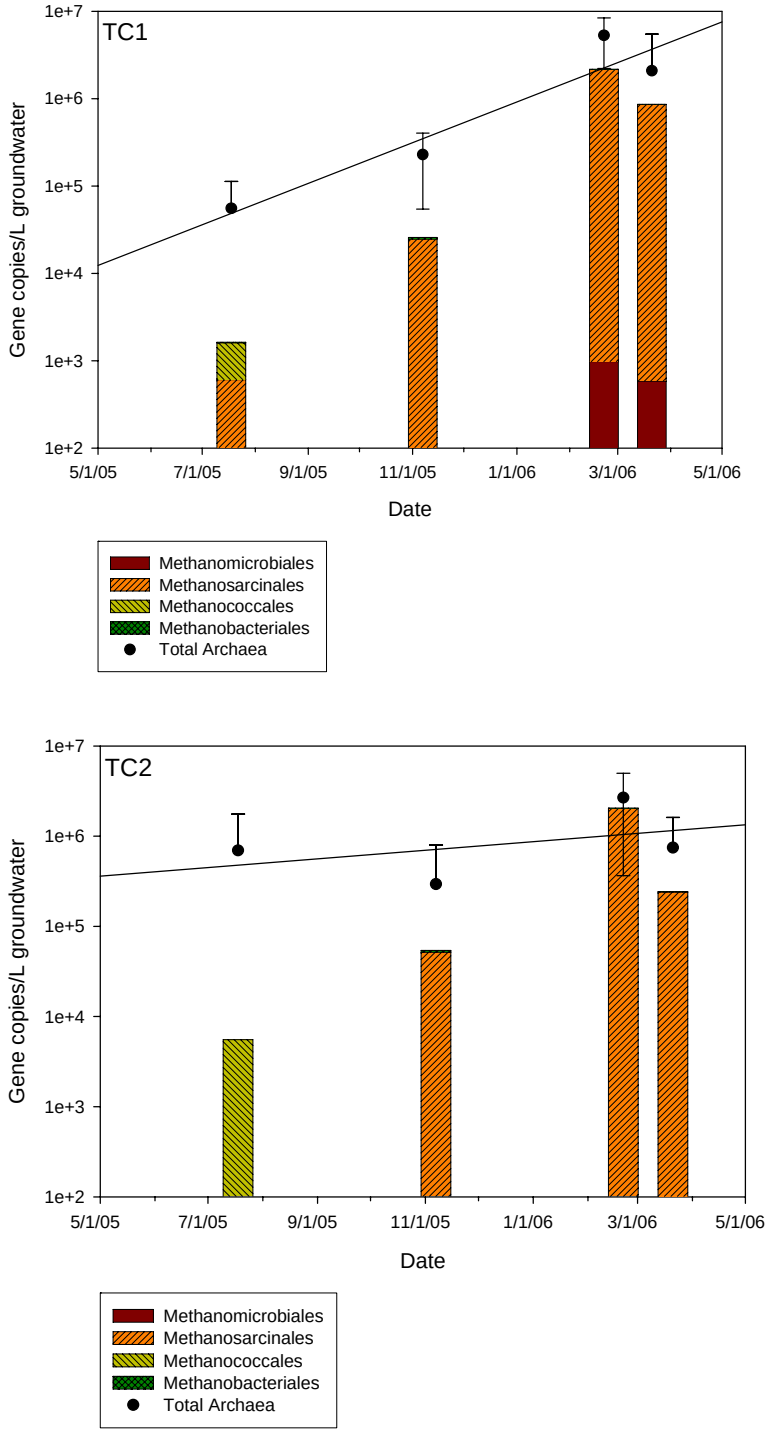


Figure 5-29. Response of Methanogenic Populations (order level) Using qPCR During Phase 3 Operations, Values Represent the Mean of n=4 (Port 4 of each CMT well) Sampling Points for Each Treatment Cell.

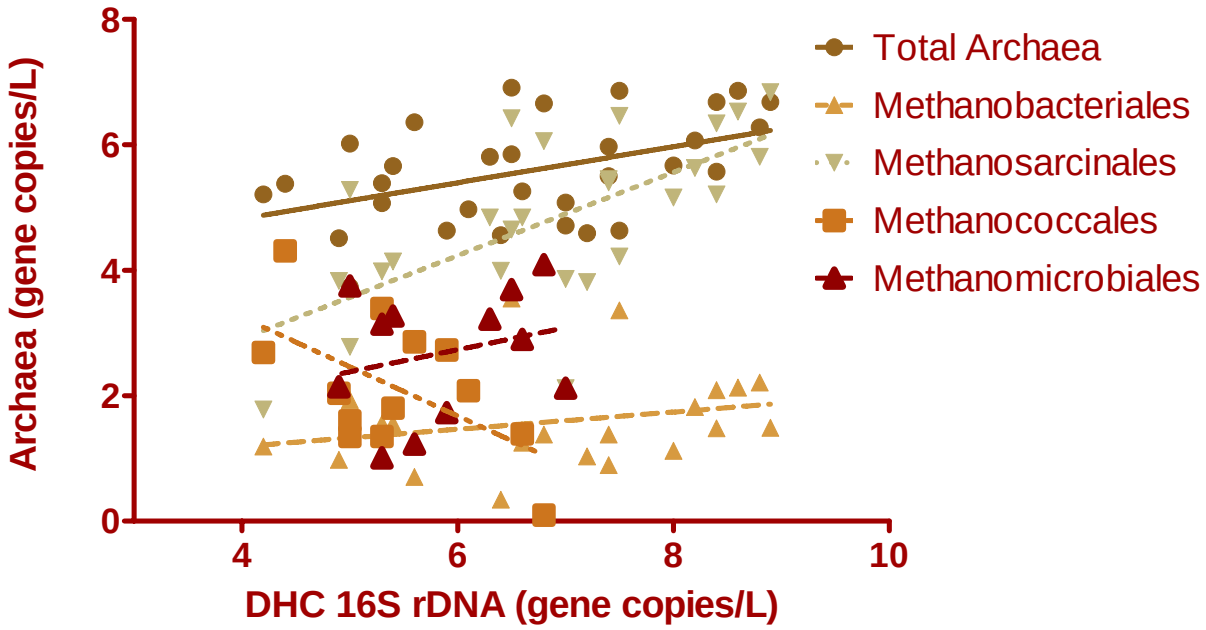


Figure 5-30. Relationship Between Methanogenic Orders and DHC Concentrations Measured using qPCR in Both Treatment Cells.

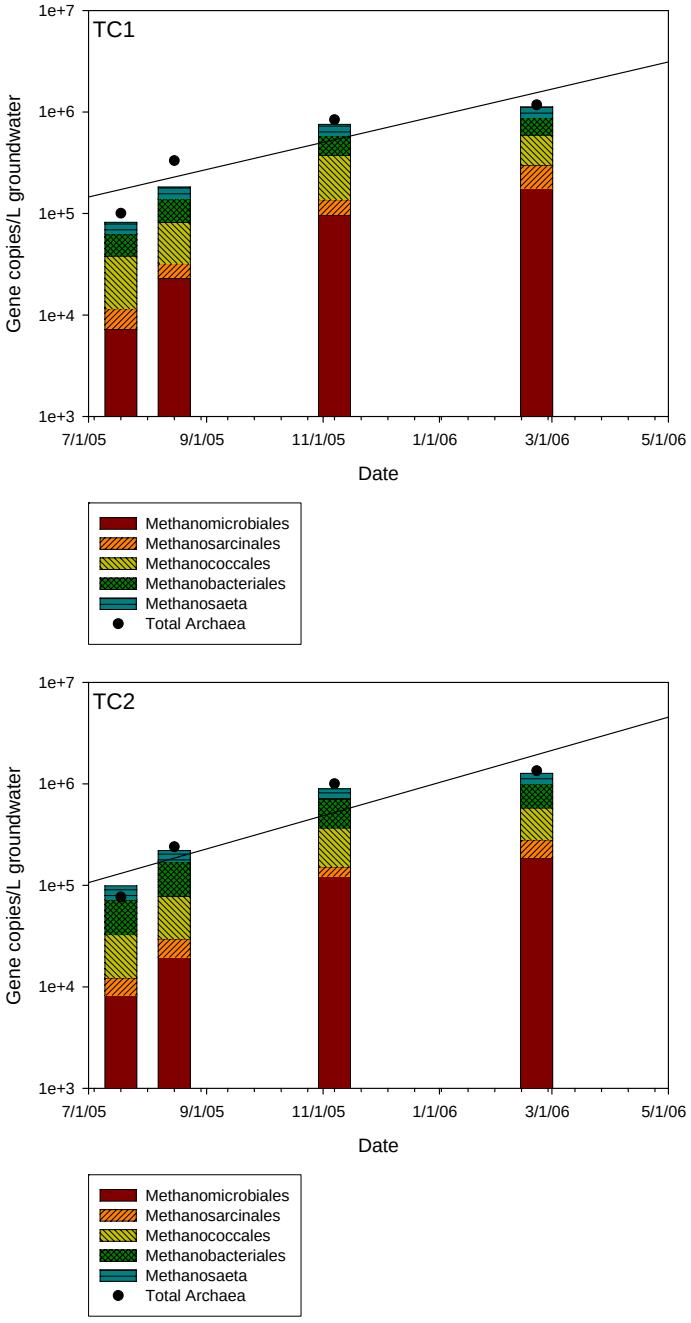


Figure 5-31. Response of Methanogenic Populations Using FISH During Phase 3 Operations, Values Represent the Mean of N=4 Sampling Points for Each Treatment Cell.

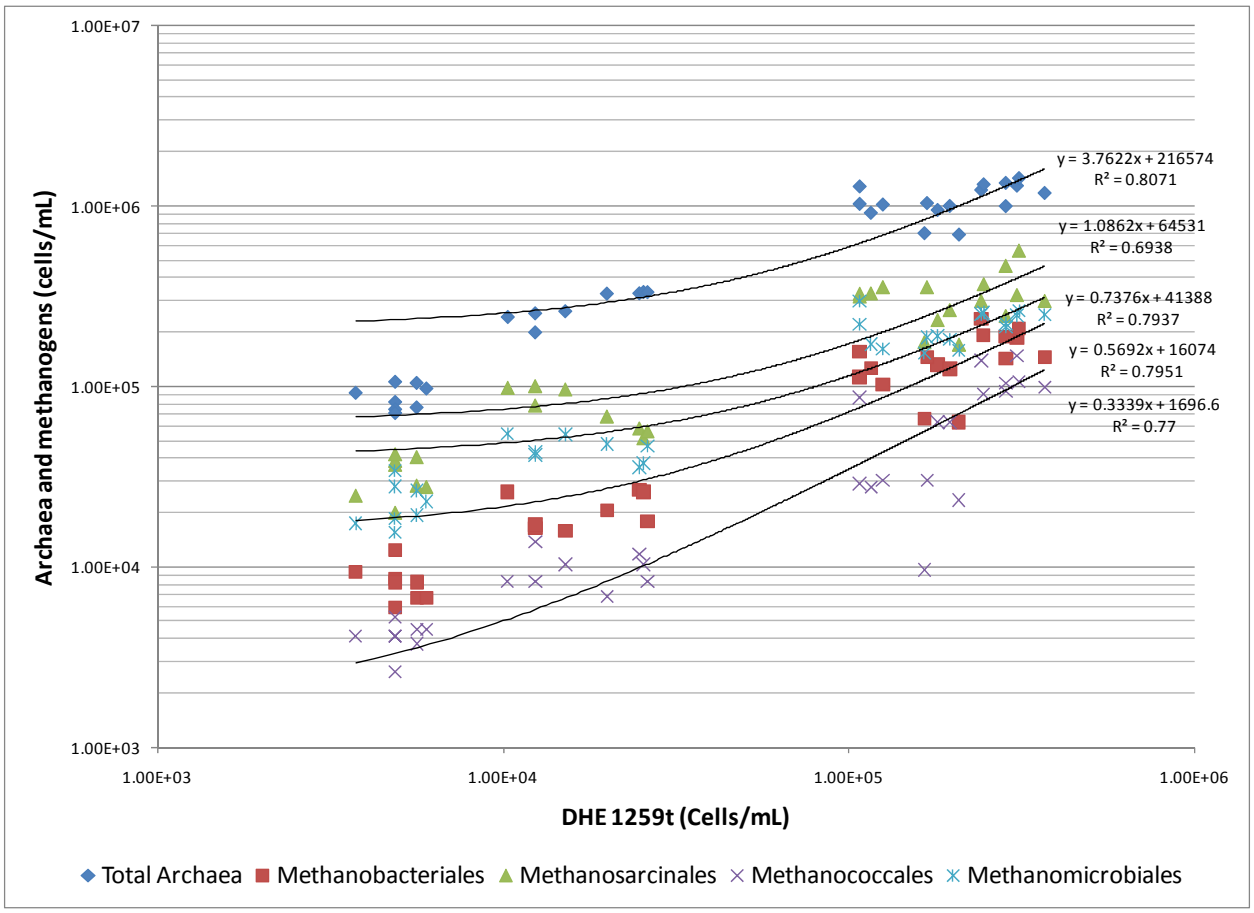


Figure 5-32. Relationship Between Methanogenic Populations and DHC Using FISH During Phase 3 Operations, Values Represent the Mean of N=4 Sampling Points (Port 4 of Each CMT Well) for Each Treatment Cell.

6.0 PERFORMANCE ASSESSMENT

6.1 3-D CMT SAMPLING

The 3-D CMT vertical sampling provided valuable information throughout the various demonstration Phases. A summary of the overall performance evaluation of the tool to assess performance of the ER-0218 demonstration for enhanced ISB in a DNAPL source zone includes:

- **Subsurface heterogeneity:** 3-D vertical profiling should be strongly considered at sites with significant subsurface heterogeneity. At Ft. Lewis, it was required to determine specific parameters, such as hydraulic flow and transport, influence of vertical gradients and preferential flow paths. Successful design of an effective injection strategy would have been much more difficult, and costly, without this information.

- **Distribution of whey:** 3-D profiling demonstrated effective horizontal and vertical distribution of whey throughout the target area. Parameters evaluated included COD and VFAs. While COD was useful to verify effective vertical distribution, VFA evaluation was not as useful from a decision-making standpoint. The utility of the VFA data was to verify fermentation of whey, and thus a subset of samples would have been sufficient to meet this objective. In addition, once COD distribution was verified for a given injection strategy, a reduction in number of COD samples would have been sufficient to monitor COD during similar injection events.

- **Evaluation of geochemical impacts:** 3-D profiling was unnecessary for evaluating geochemical impacts at Ft. Lewis. There was little difference in geochemical parameters, such as pH and methane concentrations, measured in groundwater within the different depth intervals. Therefore, 2-D sampling within the treatment area would have been sufficient to evaluate significant changes in geochemistry at this site. This may not be the case, however, for other field sites, especially sites that may have more significant variability in geochemistry with depth (i.e. sites with a much larger vertical contaminant zones).

- **Contaminant distribution and fate:** 3-D profiling was useful for evaluating variability of contaminant and degradation daughter product concentrations spatially within the treatment cells. 3-D profiling was very useful for assessing enhanced mass transfer, assessed using a molar mass balance in contaminant and reductive daughter product concentrations in groundwater, over the various operational phases.

- **Contaminant mass flux:** 3-D vertical sampling was useful for evaluating contaminant mass flux within the treatment cells. However, the high variability in groundwater velocity at the Ft. Lewis site resulted in high uncertainty in the accuracy of the measurements assuming constant groundwater velocity over time.

6.2 FLUX METERS

Flux meter assessment provided useful information for evaluation of enhanced ISB in a DNAPL source zone:

- **Groundwater velocity:** Significant variability in groundwater velocity was observed during the course of the evaluation period at the Ft. Lewis EGDY. Given that this parameter is key in calculating mass flux, understanding variability is important to the overall interpretation of the mass flux data. One significant difference between mass flux calculated using PFMs and the CMT 3-D wells was that for the CMT evaluation a uniform groundwater velocity was assumed for each operational period. Therefore, changes in mass flux were attributed solely to changes in groundwater contaminant concentrations. One important objective in evaluating ISB in a source zone is evaluating mass transfer from the DNAPL to the aqueous phase. Determining enhanced mass transfer factors using PFM data was more difficult because the increases in mass flux were impacted by both changes in concentration and changes in groundwater velocity. Therefore, increases in mass flux could be attributed to higher velocity and not necessarily mass transfer due to enhanced dissolution of residual DNAPL.

- **Mass flux evaluation:** Mass flux profiles collected in the two wells located in each treatment cell showed dynamic changes in flux as a result of the whey injections. Treatment cell 2 indicated higher mass flux and mass discharge during the baseline sampling phase of the study. Increases observed in treatment cell 2 during the 10% whey injection period were the highest observed during the study and represent a significant mass discharge for the entire EDGY area. The PFM- based values incorporated the variability observed in groundwater flow caused by changes in hydrologic conditions. In contrast, mass flux calculated using 3-D CMT sampling data near the flux wells relied on limited knowledge of Darcy flux values for the site. The velocity was assumed to be uniform and thus the variability observed was only based on contaminant concentration differences. The high degree of uncertainty in the Darcy flux magnitude and distribution is the likely reason for differences observed between PFM-based values and 3-D CMT- based values. The higher resolution flux profiles provided by the PFM provide information on both Darcy and contaminant flux spatial variability although it required a greater number of sample analysis similar to the 3-D CMT sampling.

6.3 STABLE CARBON ISOTOPES

CSIA was useful in evaluating contaminant fate within the system at Ft. Lewis, although the monitoring period was not sufficiently long to see the full benefit. Measuring changes in concentration of chlorinated solvents by gas chromatography has been the gold standard in analyzing field samples. However, concentration measurement can be affected by many physical and transport events, making it difficult to attribute concentration changes to contaminant transformation or destruction. At sites where a good mass balance cannot be obtained, this problem is a more serious concern. Stable carbon isotope measurement has the advantage that it is not affect by physical and transport events. By interpreting the changes in isotopic signature of the contaminants, one can determine the nature and extent of the reaction. A summary of the overall performance evaluation of the tool to assess performance of the ER-0218 demonstration for enhanced ISB in a DNAPL source zone includes:

- Mass balance: the mass balance between cDCE was lost once significant VC and ethene were produced within the test cells. CSIA allowed for the interpretation of the isotopic change of the parent compounds (which were at a higher concentration) to infer the transformation patterns of the daughter products.

- Stable carbon isotope data can also be incorporated into predictive groundwater model to simulate transport and rate of transformation of a contaminant. Since concentration measurement might not be a reliable parameter, a predictive model that is built using isotopic data might have advantages over traditional groundwater models.

- Overall, stable carbon isotope data should complement the gas chromatography data and vice versa.

Limitations of the technique include:

- Method Detection Limits. CSIA analysis had a higher detection limit for ethene than did the standard GC analysis. Therefore, while ethene was detected using standard methods, it was not detected using CSIA. Therefore, in order to obtain an accurate isotopic reading, a large volume of groundwater may need to be collected for reductive daughter products that are present in lower concentrations. When analyzing for low concentration, tedious purge-and-trap methods might need to be incorporated prior to analysis to concentrate the sample.

- The chemistry of the groundwater might affect the analysis if there are compounds in the groundwater that co-elute with the target compounds or cause background noise in the mass spectrometry analysis.

6.4 MOLECULAR TOOLS

Molecular tools provided information on the microbial community dynamics as well as growth and activity of specific microbial populations of interest, such as *Dehalococcoides* and methanogenic populations. A summary of the overall performance evaluation of the tool to assess performance of the ER-0218 demonstration for enhanced ISB in a DNAPL source zone includes:

- **Community-level T-RFLP profiling:** These data provided information regarding the shift in predominant bacterial and archaeal populations during enrichment of a microbial community using high-concentration whey powder. While these data can provide interesting scientific information regarding community-level dynamics, they were not necessary to make operational decisions at Ft. Lewis.

- **qPCR for *Dehalococcoides*:** These data were extremely useful in evaluating growth and activity of these contaminant-degrading microbes. First, high initial concentration of indigenous DHC that included all three reductase genes *tceA*, *bvcA* and *vcrA*, followed by growth after whey injection, provided evidence that the bioaugmentation of the site was largely unnecessary. In addition, evaluation of specific strains of DHC that were native to the site, and not present in the bioaugmentation culture (*bvcA*), verified that native DHC were enriched during the biostimulation. Evaluation of qPCR data with contaminant and

geochemical data was very useful in evaluating conditions necessary to enrich and maintain a DHC population capable of efficient degradation to ethene. These data were used to determine key environmental factors that impaired contaminant-degrading efficiency (i.e., pH<6.0). These data can be directly used to define key operational criteria for optimization and maintenance of an efficient bioremediation strategy.

- **FISH for *Dehalococcoides*:** These data were also very useful in evaluating growth and activity of DHC. FISH, however, was relatively redundant to qPCR data. In addition, FISH has not been developed for reductase genes *bvcA*, *vcrA*, and *tceA*, and the technique is more difficult to perform, requires relatively specialized expertise, and is not commercially available.

- **qPCR for Methanogenic populations:** These data were very useful for evaluating methanogenic populations. These data suggested the *Methanosarcinales* population, which contain populations capable of both hydrogen- and acetate- utilizing methanogens, predominated the community. There was a positive correlation between this group and DHC, suggesting that conditions that facilitated the growth and activity of *Methanosarcinales* also facilitate growth and activity of DHC. While these data are useful from a scientific standpoint, they largely weren't used to make operational decisions at Ft. Lewis. However, these results are consistent with Macbeth et al. (2004) in suggesting that competition for hydrogen between dechlorinators and methanogens is not a significant concern for optimizing electron donor injection strategies at field sites with groundwater temperatures of approximately 15 degrees C (or lower). For Ft. Lewis, the use of chemistry data for methane was sufficient to verify that methane-producing conditions necessary for efficient growth and activity of DHC were present (or not). Molecular evaluation methanogenic populations is likely unnecessary unless site-specific conditions require of detailed evaluation of these populations.

- **FISH for Methanogenic populations:** Unlike DHC, FISH probes have been developed to target a wide variety of methanogens and the evaluation was very comprehensive in terms of capturing a more complete representation of total methanogenic populations. In addition, similar to qPCR data, the FISH data suggested that *Methanosarcinales* predominated the population, but went one step further and verified that within the *Methanosarcinales* order the *Methanosaeta* family, containing primarily strict acetoclastic methanogens, predominated. One significant difference between the FISH and qPCR data is that FISH data suggested that *Methanomicrobiales*, a hydrogen-utilizing methanogenic population, were nearly equal in number to the *Methanosarcinales* in both treatment cells. This may be due to inefficiency in the primers used for qPCR.

6.5 SUMMARY OF INNOVATIVE DIAGNOSTIC TOOLS

Table 6-1 provides an overview of the innovative diagnostic tools as they relate to the performance objectives established for evaluating enhanced in situ bioremediation in a DNAPL source zone. In addition, the utility and recommended use of the tools are also provided. Overall, these tools provided important information that allowed for the interpretation, and optimization of ISB performance.

Table 6-1. Summary of Utility of Innovative Diagnostic Tools for Application of ISB for Chlorinated Solvent Source Zones.

Tool	Performance Criteria for ISB in Source Zones	Utility of Innovative Tool	Recommended Use
3-D CMT sampling of wells	Vertically differentiate zones within the aquifer to determine any preferential flowpaths and vertical gradients.	High	Highly recommended for sites with significant heterogeneity.
3-D CMT sampling of wells	Monitor distribution of amendments both horizontally and vertically throughout the treatment area and determine effect on geochemical.	High	Full 3-D suite recommended for evaluation of carbon distribution, while a subset would be sufficient for geochemical evaluation.
3-D CMT sampling of wells	Evaluate contaminant distribution, fate, and transport (i.e. mass flux) within treatment area.	High	Highly recommended for sites remediating source zones.
PFM Analysis	Measure cumulative water and contaminant mass fluxes in ground water.	Moderate	Highly recommended for sites with high heterogeneity and/or with large seasonal variation in groundwater flow velocity if mass flux measurement is desired. Likely most useful downgradient of source zone.
PFM Analysis	Vertically differentiate zones within the aquifer to determine any preferential flowpaths where significant contaminant mass flux occurs.	Moderate	Highly recommend to for sites containing residual source zones in order to determine areas discharging contaminants. This would be most useful downgradient from DNAPL source zone.
CSIA	Monitor the isotope ratios of TCE and its biodegradation byproducts to differentiate between the effects of groundwater transport, dissolution of DNAPL at the source, and enhanced bioremediation. Confirm biological reductive dechlorination.	Moderate	Recommended for sites with complex hydrology or geochemistry where a more detailed understanding of contaminant degradation mechanisms is required.

Table 6-1. (continued).

Tool	Performance Criteria for ISB in Source Zones	Utility of Innovative Tool	Recommended Use
Molecular –T-RFLP	Evaluate microbial community dynamics in response to whey injections.	Low	Not recommended for most applications. May be useful when more detailed understanding of microbial community necessary for particularly complex sites.
Molecular tools-qPCR	Screening tool for presence/absence of desired or indicator organisms; monitoring of growth and distribution of individual organisms	High	Highly recommended for DHC and functional genes *tceA*, *bvcA*, and *vcrA*. Not recommended for methanogens at most sites, unless site-specific conditions necessitate a more detailed understanding of these populations.
Molecular tools-FISH	Provides a count of cells within the groundwater media for a relative quantification. Presence or absence screening of biological markers can be performed.	Low	Redundant with qPCR and generally more labor intensive and expensive. Also, methods not yet developed for evaluating mRNA for *tceA*, *bvcA*, and *vcrA* and/or other strains of DHC. Much better for evaluating methanogenic populations. Not recommended for most applications. May be useful when more detailed understanding of microbial community necessary for particularly complex sites.

7.0 COST ASSESSMENT

An important consideration for evaluating the utility of innovate diagnostic tools is cost. The added cost to implement a monitoring program that includes innovative diagnostic tools was evaluated relative to the added value. In particular, the tools were evaluated in the context of assessing performance of enhanced in situ bioremediation for chlorinated solvent DNAPL source zones. The parameters necessary for the design and implementation of a successful ISB remedial system included remedy performance objectives such as:

1. Reduce mass flux emanating from the DNAPL source area.

2. Reduce concentrations of contaminants of concern in groundwater via transformation to innocuous end products within and downgradient from the DNAPL source area.

3. Increase the rate of contaminant mass removal to achieve closure criteria within an acceptable remedial timeframe.

In addition, specific technology implementation performance criteria were also evaluated including:

1. Effectively distribute bioremediation amendments within target treatment area.

2. Minimize the frequency of amendment injections.

3. Develop and/or maintain an environment conducive to microbial growth and activity of contaminant-degrading microbial populations.

7.1 COST MODEL

Table 7-1 describes the approach and cost assumptions used to determine the cost impact of innovative diagnostic tools to the monitoring program. In order to evaluate the cost impacts of the innovative diagnostic tools, a conventional monitoring program was developed and used as a common basis for comparison. Therefore, a percentage increase in implementing a strategy that includes innovative diagnostic tools could be assessed (and presumably scaled for other applications/sites).

Table 7-1. Approach and Cost Assumptions for Conventional and Innovative Monitoring Programs.

Diagnostic Tool	Approach Assumptions	Cost Assumptions
Conventional Monitoring Program		
2-D sampling	• Collection and analysis of samples from monitoring wells screened over entire vertical interval. • Sample 8 locations within NAPL Area 3 and 8 downgradient locations. • Analyze samples for VOCs, dissolved gasses, carbon, and geochemical parameters. • Sample 8 locations/day	• VOCs $90.00 • Dissolved Gasses $90.00 • VFAs $108.00 • COD $25.00 • Major anions- sulfate, phosphate, nitrate, nitrite $70.00 • Alkalinity $25.00 • Ferrous iron $25.00 • Tracer (bromide and/or iodide)- $70.00 • $150/hour for labor • $200/day for mobilization/per diem • $20/sample materials/shipping • $50/sample for database
Innovative Diagnostic Tools		
3-D sampling	• Collection and analysis of 3 samples at discrete vertical depths at 8 locations within NAPL treatment area • Analyze samples for VOCs and dissolved gasses • Sample 4 locations (12 samples)/day • Assume sample 3depths/well for tracer, VOCs, dissolved gasses and Carbon (COD). • Assume sample 1 depth/well for all other analytical parameters.	• All analytical, labor, mobilization, material/shipping, and database assumptions used for conventional monitoring program assumed here. • $120/foot increased cost for drilling/installation of CMT wells vs. conventional monitoring wells.

Table 7-1. (continued).

Diagnostic Tool	Approach Assumptions	Cost Assumptions
PFM	• Collection and analysis of 8 sampling wells for flux meters • Assume conventional monitoring wells can be used as flux meter wells and so additional wells are not included. • Analyze 3 5-foot PFMs/location for a total of 15 linear feet/ location • Conduct baseline and biannual sampling for 3 years.	• $150/linear foot includes all travel, deployment, retrieval, analysis and reporting.
CSIA	• Collect and analyze samples from 8 locations within treatment area. • Analyze for VOCs and ethene, ethane • Conduct baseline, quarterly sampling for the first year and biannual sampling for 2 years.	• VOC, ethene, ethane $350/sample • $50/sample labor (assuming mobilized during standard round of sampling) • $20/sample materials/shipping • $25/sample database
qPCR	• Collect and analyze samples from 8 locations within treatment area. • Analyze for qPCR for *Dehalococcoides* spp. 16S rRNA, *tceA*, *bvcA* and *vcrA* genes • Conduct baseline, quarterly sampling for the first year and biannual sampling for 2 years.	• qPCR analysis $350/sample • $50/sample labor (assuming mobilized during standard round of sampling) • $20/sample materials/shipping • $25/sample database

7.2 COST DRIVERS

Site- specific factors that affected the ability to implement and evaluate the innovative diagnostic tools included:

1. **Nature of Treatment Zone.** Relatively shallow contaminant treatment zone (approximately 10-30 ft bgs) allowed the use of CMT monitoring wells, which are generally a less expensive option than installing separate vertically discrete monitoring wells at varying depths. At Ft. Lewis the cost to install a conventional monitoring well was $90/ft compared to $210/ft for CMT wells. The shallow treatment depths also allowed for less expensive peristaltic pump (for depths <30 ft bgs) to be used for sampling rather than the more expensive inertial pumps (for depths <150 ft bgs).

2. **Sampling Time.** High well yields resulted in relatively fast sampling with the ability to sample 8-12 locations per day. Low yield aquifer systems may take substantially longer to sample and therefore drive the 3-D sampling costs higher that what were observed at Ft. Lewis.

3. **Hydrogeology.** Relatively high ambient groundwater flow velocities (approximately 5 ft per day) and a significant vertical gradient posed specific challenges that impacted the utility evaluation of the diagnostic tools.

7.3 COST ANALYSIS

NAPL Area 3 of the EGDY was used as the model (described in the Final Report for ER-0218 project) to evaluate implementation of an ISB Remedy with the use of innovative diagnostic tools. Table 7-2 describes the NAPL Area 3 ISB system assumptions used in the evaluation. The cost analysis does not represent the costs accrued during the actual Demonstration, but represent the costs associated with implementation of the innovative diagnostic tools as part of a hypothetical ISB Remedy at NAPL Area 3. Therefore, the assumptions include treatment of the larger area and volume of the entire NAPL Area 3 source area compared to the Demonstration treatment cells. In addition, the analytical sampling plan was modified to reflect, for example, the frequency of sampling that would be typical of a remedy implementation, as opposed to sampling conducted during the Demonstration.

The conventional monitoring program includes the following components:

- Initial tracer study with five sampling events
- Baseline sampling
- 16 performance sampling events over a 3 year treatment operations period.

The monitoring well network includes eight monitoring wells within the NAPL treatment area and eight downgradient monitoring wells. This equated to a total of 80 tracer study samples, and 272 performance monitoring samples.

Table 7-2. Parameters Used as the Basis of the Model Used for ISB for NAPL Area 3 and Costing of Application of Innovative Diagnostic Tools.

Parameter	Value
Site Area	0.5 acre
Contaminated Thickness Treated	20 ft
Treatment Volume	16,000 yd^3
Number of Injection Wells	3
Number of NAPL Area Monitoring Wells	8[a]
Number of Downgradient Monitoring Wells	8
Number of Extraction Wells	2
Duration of Operations	3 years
Frequency of Electron Donor Injection	4/year
Number of Monitoring Events	17[b]

[a] Conventional monitoring assumes fully penetrating wells, and 3-D monitoring assumes multi-level monitoring wells with 3 sampling depths/well

[b] Assumptions included baseline sampling, monthly sampling for six months followed by quarterly sampling for 2.5 years.

For the 3-D CMT sampling, the cost assumptions are the same as the conventional monitoring except that the eight wells within the NAPL treatment area are completed as CMT monitoring wells instead of fully-penetrating monitoring wells, with three sampling depths per well. The analytical parameters for the vertical sampling would be limited to tracer, contaminants of concern and degradation products, carbon, and purge parameters based on demonstration results indicating these were the most relevant parameters for three dimensional sampling. Therefore, the assumptions include 160 tracer samples (32 sampling points and 5 sampling events) and 544 analytical samples for contaminants of concern and degradation products, carbon and purge parameters. For PFM sampling, the deployment is assumed for the eight downgradient monitoring locations. The cost assumptions include additional analytical cost for PFM and databasing results. In addition, nine sampling events are assumed. For CSIA and qPCR sampling, the cost assumptions include the additional analytical cost, and additional costs for sample collection, handling and shipping, and databasing the results. In addition, 8 sample locations and nine sample events (one baseline and 8 performance sampling events) were assumed for a total of 72 samples.

Figure 7-1 provides results of the cost comparison between the conventional monitoring program and the application of innovative diagnostic tools. The total cost for conventional monitoring over the three year operational period is $246,816.

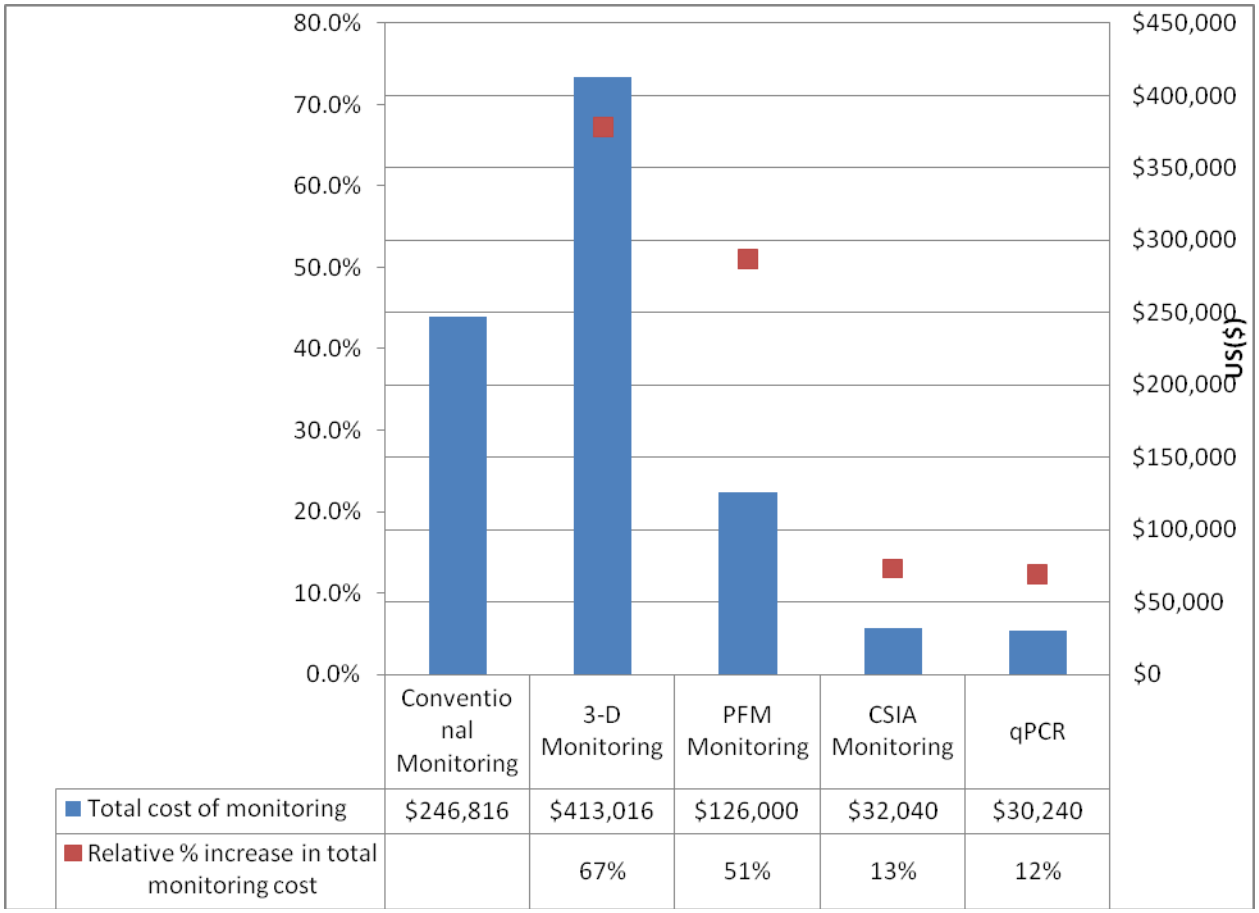


Figure 7-1. Additional Cost for Innovative Diagnostic Tools Included in the Monitoring Strategy Over the Base Case Conventional Monitoring Program.

As shown in Figure 7-1, the incremental cost of implementing the innovative diagnostic tools included:

- An additional $166, 200, or 67%, for the 3-D multilevel monitoring strategy,
- An additional $126, 000 or 51%, for the inclusion of PFM,
- An additional $32,040, or 13%, for inclusion of CSIA,
- An additional, $30,240, or 12%, for inclusion of qPCR.

7.3.1 Cost Benefit of Implementing Innovative Diagnostic Tools

In order to truly understand the utility of the innovative diagnostic tools, a discussion of the benefits of implementing a monitoring program that includes these tools is provided below. The cost-benefit of the innovative diagnostic tools was evaluated relative to the performance objectives identified for ISB of a DNAPL source zone. Tables 7-3 through 7-5 address the three performance objectives including:

1. ***Reduce mass flux emanating from the DNAPL source area (Table 7-3).*** Innovative diagnostic tools that are relevant include the 3-D CMT sampling, and PFM. Table 7-3 illustrates the cost benefit and limitation of the innovative tools relative to the base-case, 2-D sampling.

2. ***Reduce concentrations of contaminants of concern in groundwater via transformation to innocuous end products within and downgradient from the DNAPL source area (Table 7-4).*** Innovative diagnostic tools that are relevant include PFM, 3-D CMT sampling, qPCR, and CSIA.

3. ***Increase the rate of contaminant mass removal to achieve closure criteria within an acceptable remedial timeframe (Table 7-5)***. Innovative diagnostic tools that are relevant include PFM, 3-D CMT sampling, and CSIA.

In addition, specific technology implementation performance criteria were also evaluated and the cost-benefit presented in Tables 7-5 through 7-7 including:

1. ***Effectively distribute bioremediation amendments within target treatment area (Table 7-5).*** The relevant innovative diagnostic tools include the 3-D CMT sampling. Table 7-5 illustrates the cost benefit and limitation of the innovative tool relative to the base-case, 2-D sampling.

2. ***Minimize the frequency of amendment injections (Table 7-6).*** The relevant innovative diagnostic tools include 3-D CMT sampling. Table 7-6 illustrates the cost benefit and limitation of the innovative tool relative to the base-case, 2-D sampling.

3. ***Develop and/or maintain an environment conducive to microbial growth and activity of contaminant-degrading microbial population(s) (Table 7-7).*** The relevant innovative diagnostic tools include the 3-D CMT sampling and qPCR. Table 7-7 illustrates the cost benefit and limitation of the innovative tools relative to the base-case, 2-D sampling.

Table 7-3. Evaluation of Cost-Benefit of Innovative Diagnostic Tools in Evaluating Reduction in Mass Flux from DNAPL Source Area.

Tool	Cost	Benefit	Limitation	Cost Savings for Use of Innovative Tool
2-D sampling (8 sampling locations)	$10,088/sampling event	Cheapest way to evaluate mass flux.	Represents integrated measurement across the entire vertical interval. This can significantly bias data based on placement of sampling pump and contribution of low- and high-flow vertical zones within the screened interval. This results in high uncertainty in both groundwater velocity and mass flux measurements.	NA
3-D CMT Wells (8 sampling locations)	$15,336/sampling event- 3 depths	Ability to evaluate vertical variability in contaminant concentrations resulting in more robust mass flux measurement. Ability to optimize ISB operations (i.e. target injections) to account for variability in contaminant mass within discrete vertical zones.	High uncertainty in groundwater velocity measurement. More expensive than 2-D measurement.	Cost savings due to optimization of injection strategy to target vertical intervals contributing to the greatest mass discharge. Can reduce both volume and mass of injected amendment.
PFM Wells (8 sampling locations)	$18,000/sampling event- 3 PFM/well and 15 sample depths	Greatest vertical resolution of both contaminant mass flux and groundwater velocity. Most robust measurement of mass flux. Allowed for evaluation of variability in groundwater velocity over time and space, which was an important consideration at Ft. Lewis.	Most expensive option, but only minimally more expensive the 3-D sampling per sampling event. However, additional groundwater sampling would still be required to verify results. Only one company, Enviroflux, currently providing the technology	Same as for 3-D CMT wells.

Table 7-4. Evaluation of Cost-Benefit of Innovative Diagnostic Tools for Evaluating Contaminant Degradation Within and Downgradient from DNAPL Source Area.

Tool	Cost	Benefit	Limitation	Cost Savings for Use of Innovative Tool
2-D sampling (16 sampling locations)	$13,248/sampling event	Cheapest way to evaluate contaminant transformation, and effective in evaluating relative concentrations, and generating a molar mass balance.	Cannot evaluate vertical variability in contaminants and degradation daughter products. Does not provide data to support evaluation of vertical zones that are not being addressed by the injection strategy, and hence not treated or treated less efficiently. This can significantly impact treatment timeframe.	NA
3-D CMT Wells (32 sampling locations)	$20,448/sampling event- 3 depths	Ability to evaluate vertical variability in concentrations of parent compounds and reductive daughter products. Ability to identify discrete-vertical zones that may/may not have more efficient degradation occurring.	More expensive than 2-D sampling.	Cost savings due to optimization of injection strategy to target vertical intervals not fully treated with current injection strategy. Can optimize the injection strategy to treat those intervals resulting in greater mass removal rate and shorter remedial timeframe.
PFM Wells (16 sampling locations)	$32,000/sampling event- 3 PFM/well and 15 sample depths	Can evaluate vertical variability in concentrations of parent compounds and reductive daughter products.	Most expensive option, also not as effective at measuring daughter products VC and ethene. Can quantify molar mass flux for mass balance of daughter products but testing in biologically active systems has been limited.	Same as for 3-D CMT wells.

Table 7-4. (continued).

Tool	Cost	Benefit	Limitation	Cost Savings for Use of Innovative Tool
CSIA (8 sampling locations)	$3560/sampling event	Can evaluate mass balance of more labile reductive daughter products, when a concentration-based chemical mass balance is not possible, as long as daughter products are present above the method detection limit.	Adds analytical cost to monitoring program.	Cost savings due to acceptance of biodegradation treatment in the absence of a molar mass balance.
qPCR	$3,360/sampling event	Provides direct evidence regarding the presence of *Dehalococcoides* population, the only known microorganisms capable of complete reduction of chlorinated ethenes to ethene. Allows for the ability to evaluate relative changes in concentration used to evaluate enrichment during ISB.	Adds analytical cost to monitoring program.	Cost savings due to ability to evaluation presence and growth of target contaminant-degrading microorganisms as a direct-line of evidence that biological capability for complete degradation is present.

Table 7-5. Evaluation of Cost-Benefit of Innovative Diagnostic Tools in Evaluating Mass Transfer Within DNAPL Source Area.

Tool	Cost	Benefit	Limitation	Cost Savings for Use of Innovative Tool
2-D CMT Wells	$10,088/sampling event	Cheapest way to evaluate changes in concentration of parent compounds and reductive daughter products to evaluate the molar mass balance and enhanced mass transfer.	Cannot evaluate vertical variability in contaminants and degradation daughter products. Does not provide data to support optimization of injection strategy to maximize delivery to vertical intervals with high contaminant mass. This can significantly affect treatment time.	NA
3-D CMT Wells	$15,336/sampling event- 3 depths	Ability to evaluate vertical variability in concentrations of parent compounds and reductive daughter products that can provide evidence to the architecture of the DNAPL source zone and allow for the targeting of high mass areas in the remedial design. Ability to evaluate magnitude of mass transfer over space both vertically and horizontally to determine whether different vertical zones are achieving efficient mass transfer.	More expensive than 2-D sampling.	Cost savings due to optimization of injection strategy to maximize mass removal of residual source. Can optimize the injection strategy to target specific vertical intervals resulting in greater mass removal rate and shorter remedial timeframe.

Table 7-5. (continued).

Tool	Cost	Benefit	Limitation	Cost Savings for Use of Innovative Tool
PFM Wells	$18,000/sampling event- 5 PFM and 15 depths	Can evaluate vertical variability in concentrations of parent compounds and reductive daughter products.	Most expensive option, also potential limitations for measuring daughter products VC and ethene. Difficult to distinguish mass transfer effects due to influence of changes in groundwater velocity on contaminant mass sorbed to PFM. Based on this, concentration data without groundwater velocity may be a simpler method of evaluating mass transfer effects within DNAPL source area.	Same as 3-D CMT wells.

Table 7-6. Evaluation of Cost-Benefit of Innovative Diagnostic Tools in Distribution and Injection Frequency.

Tool	Cost	Benefit	Limitation	Cost Savings for Use of Innovative Tool
2-D sampling (16 sampling locations)	$13,248/sampling event	Cheapest way to evaluate amendment distribution.	Cannot evaluate vertical variability in amendment distribution. Does not provide data to support evaluation of vertical gradients. This can significantly impact the ability to ensure distribution of amendments across desired vertical profile.	NA
3-D CMT Wells (32 sampling locations)	$20,448/sampling event- 3 depths	Ability to evaluate vertical distribution of amendments and fermentation products.	More expensive than 2-D sampling.	Cost savings due to optimization of injection strategy to target vertical intervals not fully treated with current injection strategy. At Ft. Lewis the injection wells were re-drilled due to inability to distribute tracer to target vertical interval. This allowed for cost effective identification and mitigation of the ineffective injection design, saving significant time and money during the ISB implementation.

Table 7-7. Evaluation of Cost-Benefit of Innovative Diagnostic Tools in Developing and Maintaining Contaminant-Degrading Microbial Populations.

Tool	Cost	Benefit	Limitation	Cost Savings for Use of Innovative Tool
2-D sampling (16 sampling locations)	$13,248/sampling event	Cheapest way to evaluate state of environmental conditions.	Does not provide data to directly evaluate the presence and activity of contaminant-degrading populations. Growth and activity are only inferred though changes in geochemistry and reductive dechlorination response. Using this approach it can take several months to years to determine if the appropriate microbial populations are efficiently degrading contaminants at a site.	NA
qPCR	$3,360/sampling event	Provides direct evidence regarding the presence of *Dehalococcoides* populations. Allows for the ability to evaluate relative changes in concentration used to evaluate enrichment during ISB.	Relatively specialized and no standardized methods.	Given that *Dehalococcoides* is not ubiquitous, qPCR can be used to determine the need for bioaugmentation during the initial design. This can save significant time and money by accelerating the time prior to complete degradation to innocuous end products. Ability to evaluate *Dehalococcoides* populations in response to ISB operations provides data that are key for trouble shooting any conditions that are adversely affecting growth and activity (i.e. pH). This can allow for the optimization of the injection design to facilitate optimal growth and activity and minimize injection frequency.

8.0 IMPLEMENTATION ISSUES

8.1 ENVIRONMENTAL CHECKLIST

North Wind, Inc. did not have to prepare a State of Washington underground injection control (UIC) permit application to inject whey and makeup water extracted from the area of contamination into the aquifer at the Ft. Lewis EGDY due to interpretation of the applicable sections of the Washington Administrative Code Chapter 173-218 WAC Underground Injection Control Program. Specific language in the WAC 173-218-040 UIC well classification including allowed and prohibited wells, allows for Class IV wells to reinject treated ground water . . . "into the same formation from where it was drawn as part of a removal or remedial action if such injection is approved by EPA in accordance with the Resource Conservation and Recovery Act, 40 CFR 144. Such wells must be registered and approved under RCRA and "Class IV wells that are not prohibited are rule authorized, after the UIC well is registered, for the life of the well if such subsurface emplacement of fluids is authorized under the Resource Conservation Recovery Act, 40 CFR 144.23(c)."

RCRA regulations [EPA 1999; specifically 3020(b)] specifically allow for both injection of treatment agents, and reinjection of extracted water amended with bioremediation treatment agents if certain conditions are met: "Specifically, the groundwater must be treated prior to reinjection; the treatment must be intended to substantially reduce hazardous constituents in the ground water – either before or after reinjection; the cleanup must be protective of human health and the environment; and the injection must be part of a response action under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Section 104 or 106, or a RCRA corrective action intended to clean up the contamination." The demonstration met all these conditions and no other permitting requirements were required to implement the demonstration. No emissions were produced by demonstration of the in situ treatment technology.

The State of Washington classifies injection wells into classes based on construction and function. The state requires that all wells be registered and most wells must be rule authorized. The demonstration wells were registered with the WDOE and the injection well was rule authorized for the life of the well because it is authorized under the Resource Conservation Recovery Act, 40 CFR 144.23(c).

8.2 OTHER REGULATORY ISSUES

RCRA provides opportunities for public involvement throughout the remedial action process to expand public access to information about the facility and its activities. Since the small scale ISB demonstration was supplemental to the permitted remedial activities, the actions were not subject to formal public involvement. All activities were performed within the previously disturbed, contaminated area. Generally, ISB is regarded by the public as a safe, effective, low-risk remedial alternative.

8.3 END-USER ISSUES

Monitoring programs using innovative diagnostic tools generally have the same end-user issues as standard analytical monitoring programs. However, there are several technology-specific issues as discussed below.

- The use of passive flux meters has not been established or accepted by the regulatory community. Therefore, use of this tool will require verification sampling using standard analytical techniques. In addition, mass flux as a performance metric is generally a more difficult metric to understand and explain to stakeholders.

- There are currently no standard analytical methods for CSIA, PFM or molecular tools. Therefore, methods, and therefore results, can be highly variable between laboratories conducting this work. Therefore, it is important to at least use the same methods and laboratories on a given project so that results are comparable.

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