

Integrated Approach to PCE-Impacted Site Characterization, Site Management, and Enhanced Bioremediation

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Tetrachloroethene (PCE)- and trichloroethene (TCE)-impacted sites pose significant challenges even when site characterization activities indicate that biodegradation has occurred naturally. Although site-specific, regulatory, and economic factors play roles in the remedy-selection process, the application of molecular biological tools to the bioremediation field has streamlined the assessment of remedial alternatives and allowed for detailed evaluation of the chosen remedial technology. The case study described here was performed at a PCE-impacted site at which reductive dechlorination of PCE and TCE had led to accumulation of cis-dichloroethene (cis-DCE) with concentrations ranging from approximately 10 to 100 mg/L. Bio-Trap® samplers and quantitative polymerase chain reaction (qPCR) enumeration of Dehalococcoides spp. were used to evaluate three remedial options: monitored natural attenuation, biostimulation with HRC®, and biostimulation with HRC-S®. Dehalococcoides populations in HRC-S-amended Bio-Traps deployed in impacted wells were on the order of 10³ to 10⁴ cells/bead but were below detection limits in most unamended and HRC-amended Bio-Traps. Thus the in situ Bio-Trap study identified biostimulation with HRC-S as the recommended approach, which was further evaluated with a pilot study. After the pilot HRC-S injection, Dehalococcoides populations increased to 10⁶ to 10⁷ cells/bead, and concentrations of cis-DCE and vinyl chloride decreased with concurrent ethene production. Based on these results, a full-scale HRC-S injection was designed and implemented at the site. As with the pilot study, full-scale HRC-S injection promoted growth of Dehalococcoides spp. and stimulated reductive dechlorination of the daughter products cis-DCE and vinyl chloride. © 2008 Wiley Periodicals, Inc.

INTRODUCTION

Chlorinated ethenes, including tetrachloroethene (PCE) and trichloroethene (TCE), have been used in large volumes as industrial degreasing agents, and PCE is still a common dry cleaning solvent. Extensive use of these compounds combined with improper handling, disposal, and accidental spills has led to TCE and PCE being the most and third-most frequently detected groundwater contaminants at National Priorities List (NPL) sites under the Superfund program (National Research Council, 1994). Corrective action plans at PCE-/TCE-contaminated sites typically employ a tiered approach in which source removal or dense nonaqueous phase liquid (DNAPL) recovery is followed by



monitored natural attenuation (MNA) or enhanced bioremediation to meet groundwater maximum contaminant levels (MCLs). Under anaerobic conditions, PCE can be sequentially dehalogenated through TCE, *cis*-dichloroethene (*cis*-DCE), and vinyl chloride (VC) to ethene via microbially mediated reductive dechlorination (DiStefano et al., 1991; Freedman & Gossett, 1989). Because ethene is an innocuous end product, reductive dechlorination is an attractive treatment mechanism for PCE-impacted sites; however, practical application of the process can be hindered by a few site-specific factors that must be considered during site characterization.

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Site characterization at PCE-contaminated sites involves thorough examination of available chemical, geochemical, and microbiological data to assess the role of reductive dechlorination in an overall site management approach. Analysis of trends in chlorinated ethene concentrations provides the first line of evidence supporting or refuting active reductive dechlorination at a site. Specifically, decreases in parent compound concentrations coupled with production of daughter products (*cis*-DCE, VC, and ethene) indicate active reductive dechlorination. Review of chemical data is not restricted to contaminants of concern, however. Evaluation of site geochemistry and concentrations of competing terminal electron acceptors provides a second, potentially converging line of evidence for reductive dechlorination as a treatment mechanism. Chemical analysis should also include dissolved organic carbon (DOC), volatile fatty acid (VFA), or hydrogen concentrations to evaluate the availability of suitable electron donors. If electron-donor availability is low, microbial activity will be limited by lack of growth-supporting substrates under MNA conditions, and biostimulation by electron-donor injection may be necessary to support reductive dechlorination. Characterization of the site microbial community in terms of biodegradation potential provides a direct method to assess the feasibility of bioremediation as a remedial measure that is particularly important at PCE-impacted sites. While a variety of organisms capable of reductive dechlorination of PCE and TCE to *cis*-DCE have been isolated (Gerritse et al., 1996, 1999; Holliger et al., 1993; Krumholz et al., 1996; Löffler et al., 1996), relatively few organisms have been isolated that are capable of utilizing *cis*-DCE and VC as growth-supporting electron acceptors. To date, the only bacteria that have been isolated that are capable of utilizing *cis*-DCE and VC as growth-supporting electron acceptors belong to the genus *Dehalococcoides*. Thus, the presence of *Dehalococcoides* spp. is considered necessary for the complete reductive dechlorination of PCE and TCE to ethene.

At sites impacted by chlorinated ethenes, site managers are faced with a decision point when routine groundwater monitoring suggests that reductive dechlorination is occurring. The first question to be answered is whether MNA is a feasible remedy. If not and biostimulation is deemed necessary to promote reductive dechlorination, site managers must also have a science-based, justifiable method to choose between the wide variety of commercially available electron donors. Furthermore, when characterization of the microbial community indicates prohibitively low concentrations of halo-respiring bacteria, bioaugmentation schemes involving injection of exogenous bacteria may need to be considered if pursuing a bioremediation approach for site management is determined to be appropriate. The case study described here presents a rationale and a cost-effective experimental approach used at a PCE-impacted site to address these issues prior to committing resources to a full-scale course of action.

MATERIALS AND METHODS

Site Description

The study site located in upstate New York (Exhibit 1) is underlain by 10.6 to 12.2 m of fill soil, glacio-lacustrine (glacial lake) deposits, and glacial till overburden. The fill material consists of medium dense, red-brown silty sand containing varying amounts of clay and gravel. The glacial lake deposits that underlie the fill are two separate units consisting of a silty to sandy layer immediately below the fill (upper aquifer) and a clay layer at different depths across the site. The glacial till composition ranges from very dense silty sand and clayey silts to very stiff silty clay with varying amounts of sand and gravel. The till overlies a Vernon shale unit. Depth to water ranges from 0.6 to 1.5 m below ground surface (bgs) with horizontal gradients of 0.001 to 0.023 m/m. The hydraulic conductivity of the impacted unit was estimated at 10^{-4} to 10^{-5} cm/s.

Groundwater contamination is primarily limited to the upper glacio-lacustrine aquifer. Contaminants of concern include PCE, TCE, *cis*-DCE, VC, 1,1,1-trichloroethane (1,1,1-TCA), methylene chloride, and mineral spirits. A groundwater pump-and-treat system was operated at the site for approximately four years as an interim remedial measure and then deactivated. The groundwater system was deactivated and a 2-PHASE™ system (involving multiphase, high vacuum extraction) was installed at the site for contaminant mass recovery. When contaminant mass recovery rates approached asymptotic values, the system was deactivated. In approximately six years of operation, an estimated 9,589 pounds of volatile organic compounds (VOCs) were recovered from the subsurface. When the 2-PHASE system was deactivated, total VOC concentrations in the dissolved plume ranged from 241,000 µg/L (RW-1) in the source area to 288 µg/L (MW-10) at the downgradient edge. To attain site-closure goals, identification of an *in situ* remediation approach to further reduce VOC concentrations was sought. The experimental approach identified in this case study was integral in allowing the site owners to cost-effectively identify, test, and implement an appropriate final remedial approach.

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Groundwater Sampling and Analyses

Groundwater samples were obtained from existing monitoring wells using a peristaltic pump, dedicated Tygon tubing, and low-flow purge techniques. Three 40-mL groundwater samples were sent on ice to a certified laboratory for analysis of volatile organic compounds (U.S. Environmental Protection Agency Method 8260) and VFAs by high-performance liquid chromatography with ultraviolet detection by established US EPA methods. Additional groundwater samples were obtained for laboratory analysis of nitrate (US EPA Method 353.3), sulfate (US EPA Method 300.0), total iron (US EPA Method 200.7), chloride (US EPA Method 9056), dissolved organic carbon (US EPA Method 9060), and dissolved gases (American Society for Testing and Materials Method D1945). Field measurements included dissolved oxygen (Orion DO probe), ferrous iron (phenanthroline AccuVac Ampuls, Hach Company, Loveland, Colorado), and hydrogen sulfide (methylene blue method, Hach Company).

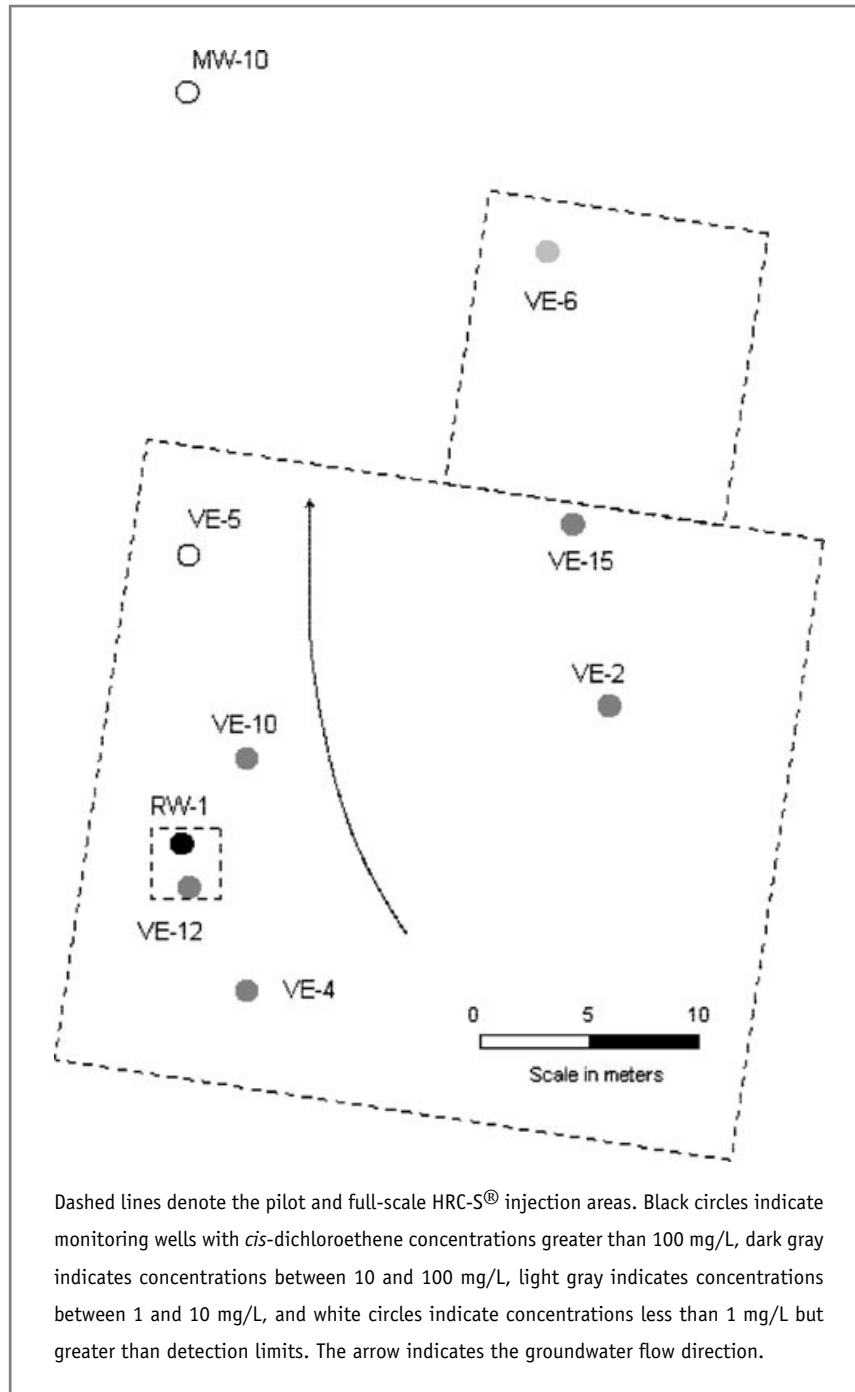


Exhibit 1. Site map

In Situ Bio-Trap[®] Study

Bio-Trap[®] samplers are passive sampling tools containing Bio-Sep[®] beads engineered from a composite of Nomex and powdered activated carbon (PAC) that provides a large surface area (approximately 600 m²/g) that is colonized by indigenous subsurface microorganisms (Sublette et al., 2006). In addition to providing a surface for biofilm formation, the Bio-Sep beads may be “baited” with electron donor compounds during fabrication to

evaluate the impact of biostimulation on the microbial community (Busch-Harris et al., 2006). A total of three different types of Bio-Sep beads were used in the study. During site characterization activities, standard, unamended Bio-Sep beads were used to evaluate the microbial community under MNA conditions. Bio-Traps containing Bio-Sep beads baited with Hydrogen Release Compound (HRC®, Regenesis, San Clemente, California) or HRC-S® (a special HRC formulation designed for sites with high sulfate concentrations, not commercially available) were used to evaluate biostimulation. HRC is a patented, controlled-release, polylactate ester mixture formulated to release lactic acid upon hydration. HRC-S, in addition to the polylactate ester, contains ferrous gluconate, which binds sulfide that can inhibit reductive dechlorination at high concentrations (Hoelen & Reinhard, 2004). All Bio-Traps used in the study were composed of approximately 120 Bio-Sep beads in 11.4-cm polyfluoralkoxy (PFA) tubing with perforations to allow groundwater contact as described previously (Sublette et al., 2006). All Bio-Traps were fastened to a tether and suspended down well for a period of 30 to 60 days. Once recovered from the well, Bio-Traps were placed on ice and shipped overnight to the Microbial Insights laboratory for DNA extraction and quantitative polymerase chain reaction (qPCR) analysis (www.microbe.com, Rockford, Tennessee).

DNA Extraction and Quantitative Polymerase Chain Reaction

DNA was extracted from Bio-Trap samples (approximately 10–20 beads) using a PowerSoil DNA kit (MO BIO Laboratories, Inc., Carlsbad, California) according to the manufacturer's instructions. Quantitative PCR was performed using several PCR primer sets targeting *Dehalococcoides* spp. 16S rRNA genes and functional genes encoding vinyl chloride reductases. PCR primers targeting *Dehalococcoides* spp. 16S rRNA genes (He et al., 2003), TCE reductase genes (Ritalahti et al., 2006), and vinyl chloride reductase genes (Ritalahti et al., 2006) have been described previously. PCR primers and a TaqMan probe specific for the 16S rRNA gene of *δ-proteobacteria* (Stults et al., 2001) were used as an index of potential sulfate-reducing and iron-reducing bacteria. PCR primers targeting the methyl coenzyme M reductase (*mcrA*) gene were used for enumeration of methanogens (Hales et al., 1996). All qPCR was performed by Microbial Insights on an ABI Prism 7300 Real-Time PCR System (Applied Biosystems, Foster City, California).

During site characterization activities, standard, unamended Bio-Sep beads were used to evaluate the microbial community under MNA conditions.

HRC-S Pilot Test

The HRC-S pilot test consisted of eight injection locations in the vicinity of VE-12 and RW-1 (Exhibit 1). All injections were performed using direct-push technologies (Geoprobe) to a depth of 5.2 m bgs. Injection points were spaced at 1.5-m centers in a grid fashion. In total, approximately 380 kg of HRC-S were injected with a loading of approximately 9.2 kg/m. Groundwater samples were collected on a quarterly basis from RW-1, VE-12, and VE-4 for analysis of VOCs, geochemical parameters, and VFAs. Standard, unamended Bio-Traps deployed in study wells were recovered quarterly to assess changes in the microbial community.

Exhibit 2. Initial conditions

Well	VOCs and Ethene ($\mu\text{g/L}$)					Geochemical Parameters (mg/L)					
	PCE	TCE	<i>cis</i> -DCE	VC	Ethene	DO	NO_3^-	Fe^{2+}	SO_4^{2-}	Methane	DOC
RW-1	8,700	2,700	110,000	<500	35	0.26	0	3.6	296	0.24	6.13
VE-4	1,000	2,800	39,000	990	330	0.16	<0.05	6.2	455	0.79	8.48
VE-6	3,300	2,200	4,900	60	2.7	0.34	0.696	<0.1	842	0.029	6.83
VE-10	1,100	1,400	17,000	<250	5	1.01	<0.05	3.8	104	0.06	6.93
VE-12	930	2,100	35,000	2,600	130	0.32	0.06	4.2	186	0.98	4.81
MW-10	27	47	58	7.2	<1	1.5	0.0774	<0.1	425	0.082	2.03

Full-Scale HRC-S Injection

The full-scale HRC-S injection consisted of 100 injection locations encompassing a 715-m² area in the impacted zone. All injections were performed using direct-push technologies (Geoprobe) to a depth of 1.9 m bgs. Injection points were spaced at 3-m centers in a grid fashion. Approximately 2,722 kg of HRC-S were injected with a loading of approximately 6 kg/m. Groundwater samples were collected from RW-1, VE-2, VE-4, VE-5, VE-6, VE-10, VE-12, and VE-15 for chemical and geochemical analysis. Standard, unamended Bio-Traps were deployed in select monitoring wells to monitor changes in the microbial community following HRC-S injection.

RESULTS AND DISCUSSION**Site Characterization**

Groundwater pump-and-treat and 2-PHASE Extraction systems were operated at the site as interim remedial measures to provide hydraulic control and VOC mass recovery. When the 2-PHASE Extraction system was deactivated, site conditions were evaluated to determine whether MNA or enhanced bioremediation would effectively achieve remedial goals. PCE and TCE concentrations were moderate (1 to 10 mg/L) in the study area and decreased appreciably at MW-10, located approximately 61 m downgradient (Exhibit 2). Concentrations of *cis*-DCE, however, were as high as 110 mg/L in the middle of the study area and greater than 10 mg/L within the dissolved plume (Exhibit 1). Except for VE-4 and VE-12, VC and ethene concentrations were low in samples from the remaining wells (Exhibit 2). The detection of daughter products demonstrated that reductive dechlorination of PCE was occurring, but the accumulation of DCE (often referred to as “DCE stall”) suggested MNA alone would not meet overall treatment objectives.

Complete reductive dechlorination of PCE to ethene is dependent on three main factors: (1) reducing conditions, (2) the presence of bacteria capable of reductive dechlorination of chlorinated ethenes, particularly *cis*-DCE and vinyl chloride, and (3) available electron donors. To assess the feasibility of MNA as a remedial strategy, each factor was evaluated in turn. Dissolved oxygen (DO) concentrations at the site were

typically less than 1 mg/L, and nitrate concentrations were at or below detection limits (0.05 mg/L). The detection of ferrous iron coupled with high sulfate concentrations and a lack of detectable bisulfide suggested predominantly iron-reducing conditions (Exhibit 2). The efficiency of dechlorination varies under different redox states. Under mildly reducing conditions (iron reduction), PCE dechlorination to TCE and DCE is favorable (Vogel et al., 1987), whereas reductive dechlorination of DCE to VC and VC to ethene appears to require the more reducing conditions of methanogenesis (De Bruin et al., 1992; DiStefano et al., 1991; Freedman & Gossett, 1989) or sulfate reduction. Thus, the available geochemical data suggested that DCE stall was likely under MNA conditions.

To quantitatively evaluate the abundance of bacteria capable of reductive dechlorination of *cis*-DCE and VC, Bio-Trap samplers deployed in impacted monitoring wells were recovered for qPCR enumeration of *Dehalococcoides* spp. *Dehalococcoides* spp. populations were detected in RW-1 but not in VE-6 and MW-10, indicating that, although reductive dechlorination of PCE and TCE was occurring, accumulation of *cis*-DCE was likely under MNA conditions. Groundwater samples were analyzed for volatile fatty acids and dissolved organic carbon to assess electron-donor availability. VFAs were not detected, and low DOC concentrations (approximately 6 mg/L) at the site suggested that reductive dechlorination was limited in part by low electron-donor availability under MNA conditions. Taken as a whole, the combination of chemical, geochemical, and microbiological results showed that while reductive dechlorination was occurring, biostimulation or possibly bioaugmentation was likely to be necessary to achieve remedial goals in an acceptable time frame.

In Situ Bio-Trap Study: Biostimulation Feasibility and Electron-Donor Comparison

An *in situ* Bio-Trap study was designed to determine whether electron-donor addition would stimulate growth of *Dehalococcoides* spp. relative to MNA conditions and to compare two commercial electron-donor formulations (HRC and HRC-S). Hydrolysis of HRC and HRC-S provides lactic acid, which is fermented by indigenous microorganisms to acetic, propionic, and butyric acids with concurrent hydrogen production. The hydrogen produced is generally believed to be the dominant electron donor for halo-respiring bacteria (DiStefano et al., 1992; Fennell et al., 1997). With the high sulfate concentrations at the site, sulfide production following biostimulation was anticipated and led to the inclusion of HRC-S in the study. HRC-S contains ferrous gluconate, which binds sulfide that can inhibit reductive dechlorination at high concentrations (Hoelen & Reinhard, 2004). Individual Bio-Traps with Bio-Sep beads containing no amendments (MNA control), HRC, or HRC-S were deployed for 69 days in RW-1, VE-6, and MW-10 within the dissolved plume and upgradient well MW-24.

Dehalococcoides spp. were not detected in any standard, unamended Bio-Traps deployed in the impacted wells, again indicating that reductive dechlorination of daughter products under MNA conditions would be limited (Exhibit 3). With HRC-baited Bio-Traps, *Dehalococcoides* spp. were only detected in the RW-1 deployed Bio-Trap and only at 10 cells/bead, suggesting HRC amendment would not effectively promote growth of key halo-respiring bacteria. Instead, methanogen populations were approximately 1 to 2.5 orders of magnitude greater in HRC-baited Bio-Traps than in unamended Bio-Traps (Exhibit 3), which was consistent with laboratory sediment microcosm results showing

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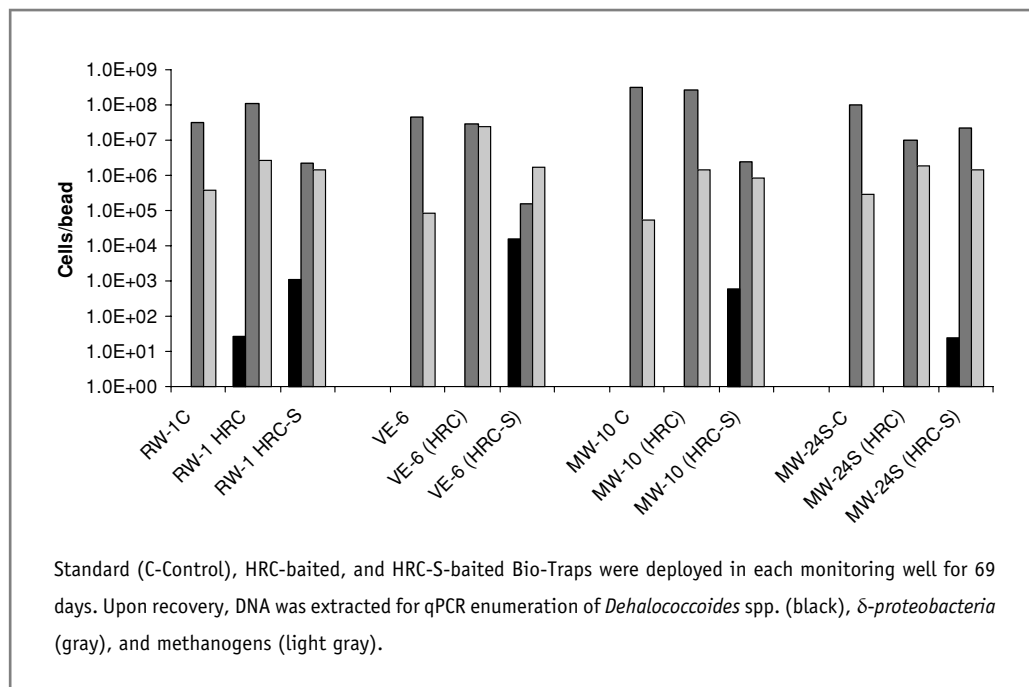


Exhibit 3. Biostimulation Bio-Trap® study

sulfate reduction and methanogenesis following HRC addition (data not shown). Conversely, *Dehalococcoides* populations in HRC-S-baited Bio-Traps were on the order of 10^3 to 10^4 cells/bead in the source zone (RW-1 and VE-6) and 10^2 cells/bead at the downgradient edge of the plume (MW-10). Also, potential competitors for available hydrogen (δ -proteobacteria and methanogens) were typically lower in HRC-S-baited than in HRC-baited Bio-Traps. In summary, the *in situ* Bio-Trap study results were used to conclude that (1) *Dehalococcoides* populations are low under MNA conditions, (2) HRC injection will not stimulate growth of *Dehalococcoides* and is unlikely to effectively promote reductive dechlorination of *cis*-DCE and VC, (3) biostimulation with HRC-S will stimulate growth of *Dehalococcoides* spp. at the site, and (4) bioaugmentation is not necessary.

Pilot HRC-S Injection

To confirm the results of the *in situ* Bio-Trap study, a pilot HRC-S injection was conducted in the vicinity of VE-12. Groundwater samples were obtained for chemical and geochemical analysis, and standard, unamended Bio-Traps deployed in test wells were sacrificed at discrete time points to monitor changes in the microbial community. Prior to injection, DOC was less than 5 mg/L, VFAs were not present, and *Dehalococcoides* populations were on the order of 10^3 cells/bead. During the first 100 days following injection, *Dehalococcoides* populations decreased to 10^1 cells/bead and *cis*-DCE concentrations increased (Exhibit 4A). At day 99, lactic acid, formed from the hydrolysis of HRC-S, was first detected in groundwater samples. VFA fermentation products were detected, *Dehalococcoides* spp. concentrations returned to 10^3 cells/bead, and the *cis*-DCE concentration decreased from 53 to 30 mg/L (Exhibits 4A and 4B) by day 192. After day 192, *cis*-DCE concentrations continued to decrease and vinyl chloride concentrations temporarily increased due to reductive dechlorination of *cis*-DCE. During this period,

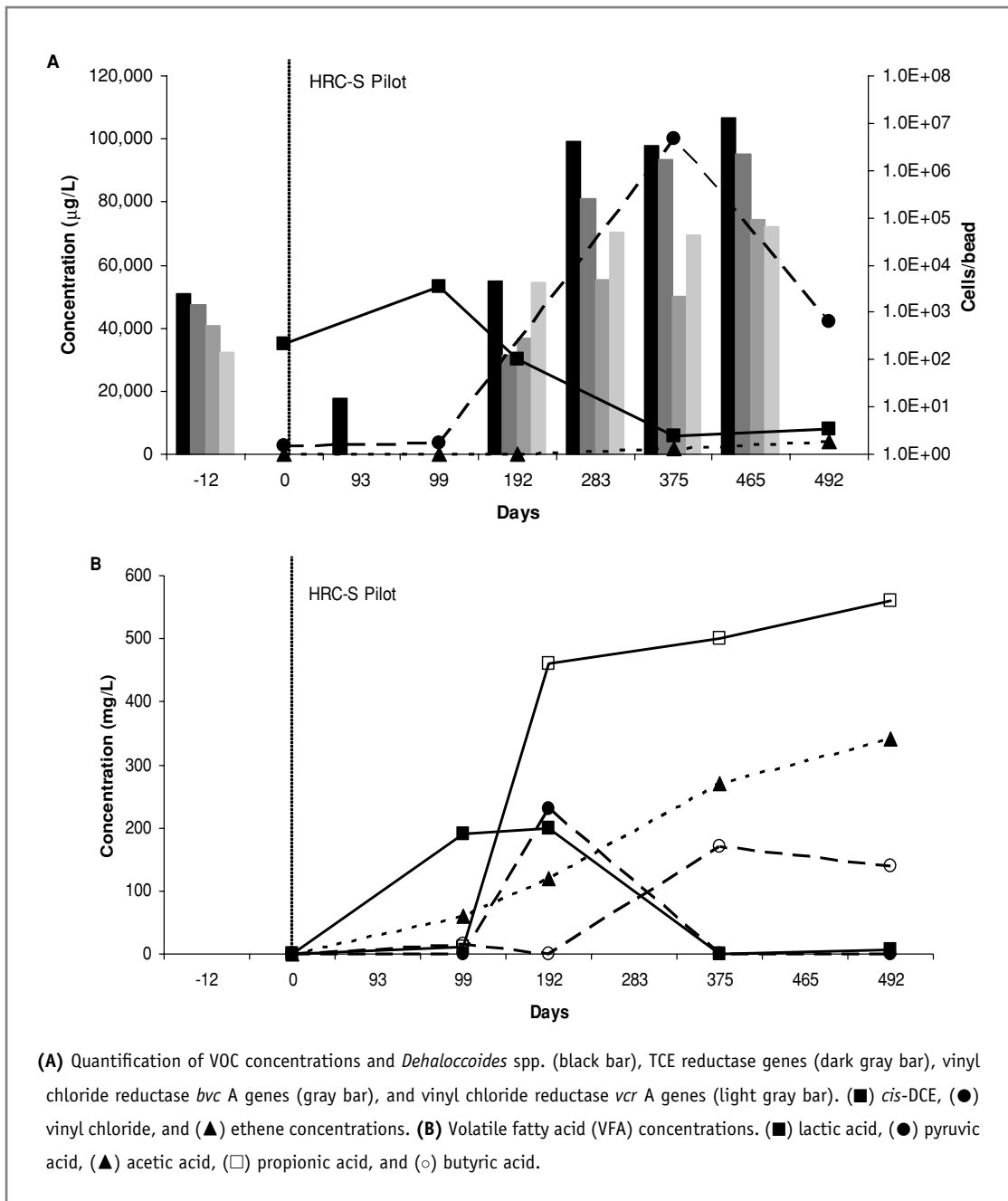


Exhibit 4. HRC-S® pilot study

Dehalococcoides populations increased to 10^6 to 10^7 cells/beam with corresponding increases in vinyl chloride reductase genes. At time points greater than 400 days after injection, high *Dehalococcoides* spp. populations (10^7 cells/beam) were maintained, VFAs were still detected, and vinyl chloride concentrations decreased. Overall, the pilot study at VE-12 demonstrated that HRC-S injection would stimulate growth of *Dehalococcoides* spp. and promote reductive dechlorination of the daughter products *cis*-DCE and VC.

Full-Scale HRC-S Injection

Following the pilot study, a full-scale HRC-S injection encompassing the source zone and downgradient areas of the dissolved plume was conducted (Exhibit 1). In the source area, *Dehalococcoides* spp. populations were on the order of 10^6 to 10^7 cells/bead due to pilot injection activities and remained elevated (greater than 10^6 cells/bead) for over one year (data not shown). Overall, the results of the full-scale injection within the downgradient portion of the dissolved plume (VE-10, VE-2) mirrored those of the pilot study. In VE-10, *Dehalococcoides* populations were moderate (10^3 to 10^4 cells/bead), and copy numbers of TCE and VC reductase genes were relatively low (less than 10^3 copies/bead) prior to full-scale injection (Exhibit 5A). Approximately 200 days after the injection,

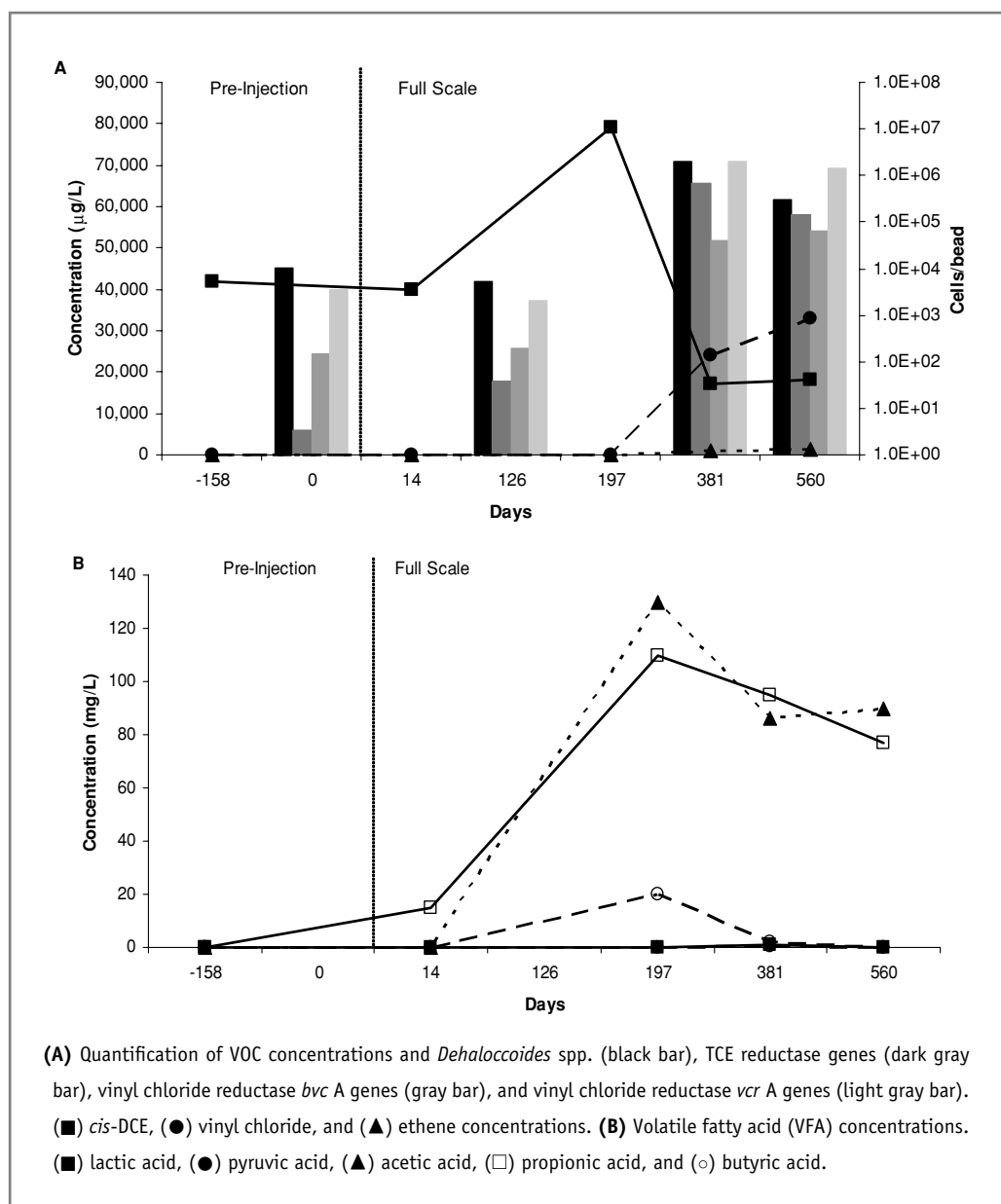


Exhibit 5. Full-scale injection VE-10

lactate fermentation products (acetic and propionic acids) were detected in excess of 100 mg/L (Exhibit 5B). Following production of VFAs, the *Dehalococcoides* population and reductase gene copy numbers increased by two to four orders of magnitude. DCE concentrations decreased substantially, with a concurrent increase in VC and ethene. Similar results were obtained for VE-2, although the *Dehalococcoides* population had decreased by two orders of magnitude by day 560.

CONCLUSIONS

The challenges present at the case study site are typical of many sites impacted by chlorinated solvents. When the efficiency of contaminant mass recovery efforts diminishes, the feasibility of a transition to a bioremediation approach whether via MNA (intrinsic bioremediation), biostimulation, or bioaugmentation is frequently evaluated for meeting site objectives. While site-specific, regulatory, and even economic factors will impact the decision-making process, the fundamental evaluation of remedial alternatives should follow a science-based progression from site characterization through remedy selection based on converging lines of evidence. Ideally, the progression of site activities should be tiered from small-scale (less expensive) test to screen remedial options to larger (more expensive) studies as options narrow and full-scale design parameters are considered. During the characterization phase of the case study, available chemical, geochemical, and microbial data combined to indicate DCE stall. The results of the *in situ* Bio-Trap study not only demonstrated that biostimulation would promote growth of *Dehalococcoides* spp. and overcome the DCE stall but were also used to choose an electron donor (HRC-S) for full-scale application and eliminate alternate remedial alternatives (MNA and bioaugmentation). Essentially, site characterization and the *in situ* Bio-Trap study cost-effectively identified the candidate corrective action, allowing a focused pilot study that confirmed stimulation of halo-respiring bacteria and linked changes in the microbial community to changes in contaminant concentrations. Based on these results, the full-scale corrective action plan was successfully implemented. A reclassification of the site to “No Further Action Required” status is imminent.

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