Comparison of Bioaugmentation and Biostimulation for the Enhancement of Dense Nonaqueous Phase Liquid Source Zone Bioremediation

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ABSTRACT: Two 11.7-m^3 experimental controlled release systems (ECRS), packed with sandy model aquifer material and amended with tetrachloroethene (PCE) dense nonaqueous phase liquid (DNAPL) source zone, were operated in parallel with identical flow regimes and electron donor amendments. Hydrogen Releasing Compound (Regenesis Bioremediation Products, Inc., San Clemente, California), and later dissolved lactate, served as electron donors to promote dechlorination. One ECRS was bioaugmented with an anaerobic dechlorinating consortium directly into the source zone, and the other served as a control (biostimulated only) to determine the benefits of bioaugmentation. The presence of halorespiring bacteria in the aquifer matrix before bioaugmentation, shown by nested polymerase chain reaction with phylogenetic primers, suggests that dechlorinating catabolic potential may be somewhat widespread. Results obtained corroborate that source zone reductive dechlorination of PCE is possible at near field scale and that a system bioaugmented with a competent halorespiring consortium can enhance DNAPL dissolution and dechlorination processes at significantly greater rates than in a system that is biostimulated only. *Water Environ. Res.*, **78,** 2456 (2006).

KEYWORDS: dense nonaqueous phase liquid, source zone bioremediation, bioaugmentation, biostimulation.

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Introduction

Tetrachloroethene (PCE) and trichloroethene (TCE) are two of the most prevalent groundwater contaminants in the United States. The focus of research for the remediation of PCE- and TCE-contaminated sites has shifted from technologies such as pump and treat, which only controls the plume, to treatment of the dense nonaqueous phase liquid (DNAPL) source zones, which will reduce the time to reach cleanup standards (Stroo et al., 2003). The DNAPL source zones can be colonized by halorespiring organisms that increase the rate of PCE dissolution (Cope and Hughes, 2001; Yang and McCarty, 2000). The goal of source zone bioremediation technology is to increase the local flux of contaminants into the aqueous phase through reductive dechlorination and the production of more soluble and less hydrophobic metabolites (Adamson et al., 2003) that can be more easily detoxified in situ or removed by alternative technologies (Stroo et al., 2003).

Several types of organisms have been isolated that are capable of dechlorinating PCE to *cis*-dichloroethene (*cis*-DCE), including *Sulfurospirillum multivorans* (Luijten et al., 2003), *Dehalobacter restrictus* (Holliger et al., 1998), *Desulfuromonas michiganensis* (Sung et al., 2003), and *Desulfitobacterium* sp. PCE-1 (Gerritse et al., 1996). *Dehalococcoides* spp. is the only group of organisms known to completely dechlorinate PCE to ethene. In this group, *Dehalococcoides* sp. strains BAV1 and GT are the only known isolates capable of growth on vinyl chloride (VC) (He, Ritalahti, Yang, Koenigsberg, and Löffler, 2003; Sung et al., 2006), but two highly enriched *Dehalococcoides* spp. mixed cultures (VS and KB-1/VC-H_2) also are capable of growth on vinyl chloride (Cupples et al., 2003; Duhamel et al., 2004). All other known *Dehalococcoides* spp. cometabolically dechlorinate vinyl chloride to ethene (He et al., 2005; Maymó-Gatell et al., 1997).

Several field studies have been conducted to evaluate the potential of microbial dechlorination to help remediate sites contaminated with chlorinated solvents. Ellis et al. (2000) biostimulated a TCE-contaminated site, but did not observe dechlorination beyond *cis*-DCE. Following bioaugmentation with a culture capable of complete dechlorination, ethene was detected at this site. Major et al. (2002) also biostimulated a PCE-contaminated site, but had to bioaugment with KB-1, a dechlorinating consortium that contained *Dehalococcoides* spp., before complete dechlorination to ethene was observed. Similar results were observed in a recent bench-scale experiment, where biostimulation alone failed to promote dechlorination, and bioaugmentation with KB-1 enhanced PCE DNAPL dechlorination, with some ethene production (Sleep et al., 2006). In contrast, Lendvay et al. (2003) compared biostimulated bioaugmentation with biostimulation alone to treat a PCE plume at a field site and found that, whereas bioaugmentation was faster and more efficient in controlling the plume, biostimulation alone also enhanced PCE dechlorination to ethene.

The efficacy of bioaugmentation to enhance bioremediation of a wide variety of pollutants remains debatable, as the activity of introduced strains may be difficult to distinguish from indigenous microorganisms. There is also the perception that a similar increase in dechlorination activity could be achieved, in time, simply by conventional biostimulation, which has been demonstrated to be the case at sites contaminated with petroleum hydrocarbons (Alvarez and Illman, 2005; Reinhard et al., 1997; Thomas and Ward, 1989).

In this work, we used experimental controlled release system (ECRS) tanks to compare, in near-field scale, the efficacy of biostimulated bioaugmentation with biostimulation alone. Previous research has demonstrated the advantages of ECRS tanks to assess the efficacy of DNAPL source zone bioremediation, by enhancing mass-balance estimations, while avoiding many of the difficulties inherent in field-scale work (i.e., inadequate estimation of the mass

Table 1—Primers used for characterization of the culture used for bioaugmentation.

Target group	Primer description	Sequence	Reference
Bacteria	Forward	5′-ACGACGGYGGCATTTCTC-3′	(Da Silva and Alvarez, 2004)
	Reverse	5′-GCATGATSGGYACCGACA-3′	
	Probe	FAM-5′-CTTCTGGTTCTTCTGCACCTTGGACACC-3′-TAMRA	
Archaea	Forward	5′-CGGTGAATACGTCCCTGC-3′	(Da Silva and Alvarez, 2004)
	Forward	5′-CGGTGAATATGCCCCTGC-3′	
	Reverse	5′-AAGGAGGTGATCCTGCCGCA-3′	
	Probe	FAM-5′-CTTGTACACACCGCCCGTC-3′-BHQ-1	
Dehalococcoides spp.	Forward	5′-CTGGAGCTAATCCCCAAAGCT-3′	(He, Ritalahti, Aiello, and Löffler, 2003)
	Reverse	5′-CAACTTCATGCAGGCGGG-3′	
Dehalobacter spp.	Forward	5′-GTTAGGGAAGAACGGCATCTGT-3′	(Smits et al., 2004)
	Reverse	5′-CCTCTCCTGTCCTCAAGCCATA-3′	

and composition of DNAPL initially present, inability to operate a parallel independent control study, and the high costs generally associated with experimental work at this scale) (Adamson et al., 2003). This previous work showed that source-zone bioaugmentation could promote dechlorination of PCE to *cis*-DCE, but the enhancement in DNAPL removal and extent of dechlorination relative to biostimulation alone were not addressed. Here, we build on this previous study and report the results of controlled parallel experiments to quantify the benefits of bioaugmentation versus biostimulation for the in situ bioremediation of DNAPL source zones.

Materials and Methods

Chemicals. The following chemicals were obtained in liquid form: PCE (99+%, Sigma-Aldrich, St. Louis, Missouri), TCE (99+%, Sigma-Aldrich), *cis*-DCE (99+%, Sigma-Aldrich), methanol (MeOH) (HPLC grade, Fisher Scientific, Fairlawn, New Jersey), sodium hydroxide (NaOH) (1N, Fisher Scientific), sodium-DL-lactate (60% v/v, Sigma-Aldrich), and Hydrogen Releasing Compound (HRC®) (glycerol tripolylactate, Regenesis Bioremediation Products, Inc., San Clemente, California). Gaseous chemicals obtained from Supelco (Bellefonte, Pennsylvania) included vinyl chloride (8% vinyl chloride, balance nitrogen [N_2]), nitrogen (ultra-high purity), methane (99%), and ethene (99%).

Analytical Methods. Chlorinated compound concentrations in aqueous samples were determined using headspace analysis, as described previously in Zheng et al. (2001). Standards were prepared by adding PCE, TCE, and *cis*-DCE dissolved in methanol, and vinyl chloride, ethene, and methane gases, all at known volumes, to serum bottles (70 mL) containing deionized water (50 mL).

Volatile fatty acids (acetate and propionate) were analyzed as described in Adamson et al. (2003). Chemical oxygen demand (COD) was measured using the closed reflux colorimetric method in *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 1992) using COD vials (Hach Cat. 21259-15, Hach Company, Loveland, Colorado). The input from HRC addition was defined in terms of total COD, which included free aqueous glycerol tripolylactate that had not undergone hydrolysis. Dissolved oxygen and pH (Fisher Scientific) were measured directly in aqueous samples.

Culture Development. A dechlorinating culture was developed from an anaerobic methanogenic consortium that had shown dechlorination activity for over nine years in the laboratory (Zheng et al., 2001). This culture is capable of rapid and complete dechlorination of PCE to ethene (240 μmol/L/d). The culture was maintained in a 20-L high-density polyethylene carboy equipped with ports for injection of nutrients, sodium hydroxide, and PCE. The carboy also had fittings for culture mixing and headspace analysis. The culture was fed 0.25-mM PCE and 3-mM MeOH daily and maintained with an 80-day retention time using a draw-and-fill method. This method allowed for higher cell densities than used in previous bioaugmented ECRS experiments (Adamson et al., 2003), because the culture was fed daily. The total bacterial and archaea concentrations in the consortium, determined by real time quantitative (RTQ) polymerase chain reaction (PCR), as described below, were 3.1×10^9 cell/mL and 2.0×10^8 cell/mL, respectively. Assuming a mass of 1.33×10^{-9} g/cell (Bratbak, 1985), 6390 mg of biomass was added to the tank.

DNA Extraction, Real-Time Polymerase Chain Reaction, and Polymerase Chain Reaction. DNA was extracted using the MO BIO PowerSoil DNA Kit (MO BIO Laboratories, Inc., Carlsbad, California). The manufacturer's protocol was followed, and a bead-beating device (BioSpec Products, Inc., Bartlesville, Oklahhoma) was used for cell lysis. DNA was collected (60 μL) in microfuge tubes (1.5 mL) and stored at −80°C.

The RTQ PCR was used to quantify the number of total bacteria, archaea, *Dehalococcoides* spp., and *Dehalobacter* spp. present in the culture, groundwater, and aquifer material (Table 1). The number of bacteria and archaea were quantified using the methods described by Da Silva et al. (2004). *Dehalobacter* spp. were quantified using the primers developed by Smits et al. (2004). The *Dehalococcoides* spp. were quantified using the primers developed by He, Ritalahti, Aiello, and Löffler (2003), but they were used with a SYBR green approach, as follows. The RTQ-PCR reactions (30 μL) for *Dehalococcoides* spp. and *Dehalobacter* spp. contained 1× SYBR Green PCR Master Mix (Qiagen Inc., Valencia, California), forward and reverse primer (300 nM each), and DNA template (3 μL). The PCR conditions were as follows: 2 minutes at 50°C, 15 minutes at 95°C, followed by 40 cycles of 15 seconds at 94°C and 1 minute at 58°C for *Dehalobacter* spp. or 1 minute at 60°C for *Dehalococcoides* spp. and 30 seconds at 72°C. RTQ-PCR was conducted on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, California).

Standard curves for RTQ-PCR were prepared with a dilution series of genomic DNA using *Dehalococcoides* sp. strain FL2 (obtained from Frank Löffler at the Georgia Institute of Technology, Atlanta), *Dehalobacter restrictus* (DSMZ 9455), *Methanococcus maripaludis* (ATCC 43000), and *Thauera aromatica* strain T1

Table 2—Primers used to characterize the indigenous microorganisms in the tanks and in the culture used for bioaugmentation.

Target organism or gene	Primer name	Sequence	Reference
Sulfurospirillum spp.	Fp DHSPM 576	5′-GCTCTCGAAACTGGTTACCTA-3′	(Ebersole and Hendrickson, 2005)
	Rp DHSPM 1210	5′-GTATCGCGTCTCTTTGTCCTA-3′	
Desulfuromonas spp.	Desulf For	5′-AACCTTCGGGTCCTACTGTC-3′	(Löffler et al., 2000)
	Desulf Rev	5′-CGGCAACTGACCCCTATGTT-3′	
Desulfitobacterium spp.	Dd1	5′-AATACCGNATAAGCTTATCCC-3′	(El Fantroussi et al., 1997)
	Dd2	5′-TAGCGATTCCGACTTCATGTTC-3′	
Desulfomonile spp.	Dt1	5′-CAAGTCGTACGAGAAACATATC-3′	(El Fantroussi et al., 1997)
	Dt2	5′-GAAGAGGATCGTCTTTCCACGA-3′	
Geobacter lovleyi strain SZ	GeoF	5′-GAATATGCTCCTGATTC-3′	(Sung, 2005)
	GeoR	5′-ACCCTCTACTTTCATAG-3′	
tceA	797F	5′-ACGCCAAAGTGCGAAAAGC-3′	(Magnuson et al., 2000)
	2490R	5′-GAGAAAGGATGGAATAGATTA-3′	
bvcA	bvcAF	5′-TGCCTCAAGTACAGGTGGT-3′	(Krajmalnik-Brown et al., 2004)
	bvcAR	5′-ATTGTGGAGGACCTACCT-3′	
vcrA	vcrAF	5′-CTATGAAGGCCCTCCAGATGC-3′	(Müller et al., 2004)
	vcrAR	5′-GTAACAGCCCCAATATGCAAGTA-3′	

(ATCC 700265D). The linear range for quantification was 10^2 to 10^9 gene copies/mL (r^2 = 0.996) for *Dehalococcoides* sp. strain FL2, 10^2 to 10^9 gene copies/mL (r^2 = 0.991) for *D. restrictus*, 10^2 to 10^8 gene copies/mL (r^2 = 0.990) for *M. maripaludis*, and 10^2 to 10^8 gene copies/mL (r^2 = 0.988) for *T. aromatica* strain T1. The gene copy numbers were calculated as described by Ritalahti et al. (2006) using the following equation:

$$\text{Gene copies} = \left(\text{DNA concentration}\left(\frac{\text{ng}}{\mu\text{L}}\right)\right)\left(\frac{1\,\text{g}}{1,000^3\,\text{ng}}\right)\left(\frac{1}{660\,\text{g DNA}}\right)\left(\frac{6.023 \times 10^{23}\,\text{bp}}{1\,\text{mol}}\right)\left(\frac{\text{copies}}{\text{genome size(bp)}}\right)(\text{volume template}(\mu\text{L})) \quad (1)$$

It was assumed that there was one copy of the 16S rRNA gene for *Dehalococcoides* spp. (Kube et al., 2005; Ritalahti et al., 2006), one copy of the 16S rRNA for the bacteria, one copy for *D. restrictus*, and two copies for *M. maripaludis* (http://rmdb.cme.msu.edu/rmdb/servlet/controller).

Other halorespiring populations and reductive dehalogenase genes were identified using PCR and primers previously developed (Table 2) for *Sulfurospirillum* spp., *Desulfuromonas* spp., *Desulfitobacterium* spp., *Desulfomonile* spp., and *Geobacter lovleyi* strain SZ. If the organisms could not be detected by PCR, a nested PCR approach was used to increase the detection limit. Nested PCR was performed with the universal 8F (5′- AGAGTTTGATCCTGGCTCAG-3′) and 1541 R (5′-AAGGAGGTGATCCAGCCGCA-3′) primers (Ritalahti and Löffler, 2004). The culture used for bioaugmentation was also tested for known reductive dehalogenase genes (Table 2), including *tce*A (TCE to vinyl chloride and cometabolically to ethene), *bvc*A (*cis*-DCE to ethene), and *vcr*A (*cis*-DCE to ethene) (Table 2).

Experimental Controlled Release System. Two 11.7-m^3 near-field-scale ECRS were used to evaluate the relative effects of bioaugmentation and biostimulation on the removal of PCE DNAPL source zones (Adamson et al., 2003; Reeves et al., 2000). One system was bioaugmented with 15 L (3.1×10^9 bacteria/mL) of the anaerobic dechlorinating consortium directly into the source zone and biostimulated by the addition of electron donors upstream of the DNAPL region. This system is referred to as the *bioaugmented tank*. The other system was not bioaugmented, but the indigenous microbial community was biostimulated with the same electron donors. This system is referred to as the *biostimulated tank*.

The experimental system consisted of two metal tanks (5.49 m long, 2.13 m wide, and 1.83 m high) open to the atmosphere (Figure 1). These are the same ECRS systems that were described by Adamson et al. (2003). Fine masonry sand (New Caney, Texas) was emplaced to provide model aquifer material. The physical–chemical properties of the sand used are shown in Table 3. Packing was performed by saturated, continuous fill to a depth of approximately 1 m. This sand–water saturation strategy was designed to enhance distribution of the sand and to minimize mounding, channeling, and other heterogeneities that can occur during packing. The tanks were then drained at a rate of 500 mL/min to induce compaction and then saturated to a depth of 1 m.

Multiple internal sampling or injection points (0.6- and 1.3-cm internal diameter, respectively) were installed using stainless-steel tubing during tank packing. The source water for the ECRS was

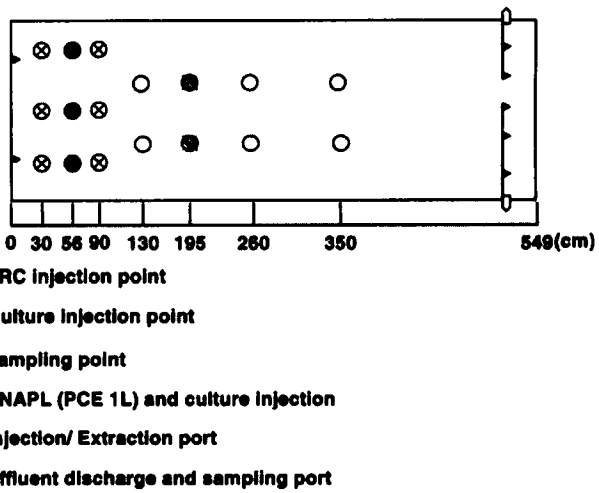

Figure 1—Schematic representation of the ECRS showing sampling wells, DNAPL source, and injection points.

Table 3—Properties of sandy material used as matrix in the ECRS.

Parameter*	Units (mg/L [ppm])
Nitrate-nitrogen	3
Phosphorus	2
Calcium	111
Magnesium	11
Sulfur	47
Bioavailable iron	31
Total iron	198
Porosity	0.32
Conductivity	0.97 mmohs cm^{-1}
Organic matter	0.09%

* Soil analysis conducted by Soil, Water and Forage Testing Laboratory, Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas.

from the Rice University (Houston, Texas) tap water supply. The tap water was not dechlorinated before use, because no inhibitory effects were observed previously (Adamson et al., 2003). Each tank was fitted with two influent and two effluent lines. Effluent lines were placed on both sides of each end of the tanks to minimize preferential flow and channeling. Flow was controlled using electronic flow meters (McMillan Co., Georgetown, Texas) in the influent and effluent lines to maintain a near-constant rate (22 to 30 L/h). Activated carbon canisters (liquid-phase activated carbon; total surface area 1050 m^2/g, TIGG Corp., Heber Springs, Arizona) were installed in the effluent lines to remove chlorinated solvents before discharge to the sewer.

The hydraulic characteristics of the tanks were determined using bromide breakthrough curves. Breakthrough data were obtained by continuous injection of a potassium bromide solution (1 kg/L) directly to the influent lines of the tanks using a syringe pump (Harvard Apparatus, Hollistion, Massachusetts), which gave an influent concentration of 1 g/L. Bromide recovery was 100 ± 3%. One pore volume was displaced in 3 to 4 days (Figure 2). The average hydraulic conductivity was 0.17 m/d, corresponding to a seepage velocity of 1.6 m/d. Similar bromide breakthrough curves for both effluent lines in each tank confirmed the absence of preferential flow paths.

To establish DNAPL source zones, neat PCE (1 L total per tank) was added 30 cm from the bottom of the tanks. The PCE was introduced through two sample lines (500 mL each) perpendicular to flow and downgradient (2 m) from the inlet of the tanks (Figure 1). The PCE delivery was accomplished using glass syringes (100 mL) and manual injection under minimal positive pressure. The HRC was added as electron donor directly upstream from the source zones. The HRC was injected to both tanks 7 days after PCE injection, using a direct push geoprobe method developed by the supplier (Regenesis Bioremediation Products, Inc.) (Figure 1). Six locations were chosen for HRC addition. The injection points (Figure 1) were 0.9 and 0.3 m upstream of the PCE addition and were perpendicular to flow. The quantity of HRC injected (22.5 L per tank; 25.3 kg as COD) to the subsurface was based on calculations made by the supplier (Regenesis Bioremediation Products, Inc.), and it was identical (on a source area basis, 1.9 L/m^2) to the quantity of HRC used for the treatment of a PCE-contaminated site (Kean et al., 2000). This amount served to induce

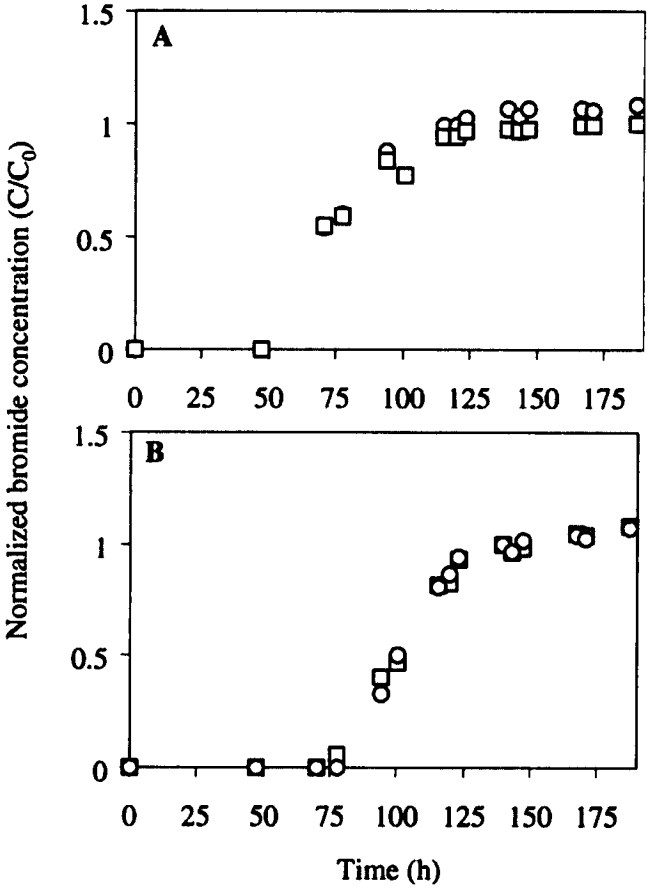

Figure 2—Bromide breakthrough data for the (a) bioaugmented tank and (b) biostimulated tank. Each tank had two effluent ports: □ effluent 1 and ○ effluent 2.

anaerobic conditions by depleting essentially all of the residual oxygen in the soil–water matrix.

The HRC was depleted in the systems after approximately 40 days of operation. Sodium lactate (600 mg/L), a surrogate electron donor, was continuously injected to the influent of both tanks, beginning on day 118. Electron donor injection was performed using two syringe pumps (Harvard Apparatus, Holliston, Massachusetts) connected inline with the influent of both tanks. Injection of lactate continued for 158 days (to day 277) in the bioaugmented tank. The injection of lactate in the biostimulated tank was discontinued at day 232, and the tank was monitored for 45 days to investigate the relationship between electron donor addition and dechlorination potential.

Bioaugmentation. The dechlorinating culture was added to the bioaugmented tank after establishment of a residual PCE source zone and depletion of oxygen (dissolved oxygen <0.1 mg/L). Characterization of the culture used is described in Table 4. Bioaugmentation was performed by purging the carboy reactor containing the microbial consortium with nitrogen gas to provide positive pressure in the vessel and to maintain anaerobic conditions. A total of 15 L of culture was added to the bioaugmented tank, which was divided between five injection wells (3 L per well). Two of the lines used for culture injection were the same lines used for PCE addition (bioaugmentation occurred as close to the DNAPL source as possible); the other three lines used were located

Table 4—Characterization of the culture used for bioaugmentation.

Target population or gene	Gene copies/mL
Bacteria	3.1×10^9
Archaea	2.0×10^8
Dehalobacter spp.	3.1×10^9
Dehalococcoides spp.	1.0×10^9
Sulfurospirillum spp.	Present[a]
Desulfuromonas spp.	ND[bc]
Desulfitobacterium spp.	ND[c]
Desulfomonile tiedjei	ND[c]
Geobacter sp. strain SZ	ND[c]
*tce*A	Present
TCE → ETH	
(*Dehalococcoides* spp.)	
*bvc*A	ND[c]
DCEs → ETH	
(*Dehalococcoides* sp. strain BAV1)	
*vcr*A	Present
DCEs → ETH	
(*Dehalococcoides* sp. strain VS or GT)	

[a] Detected with nested PCR.
[b] ND = Not detected.
[c] Not detected with PCR.

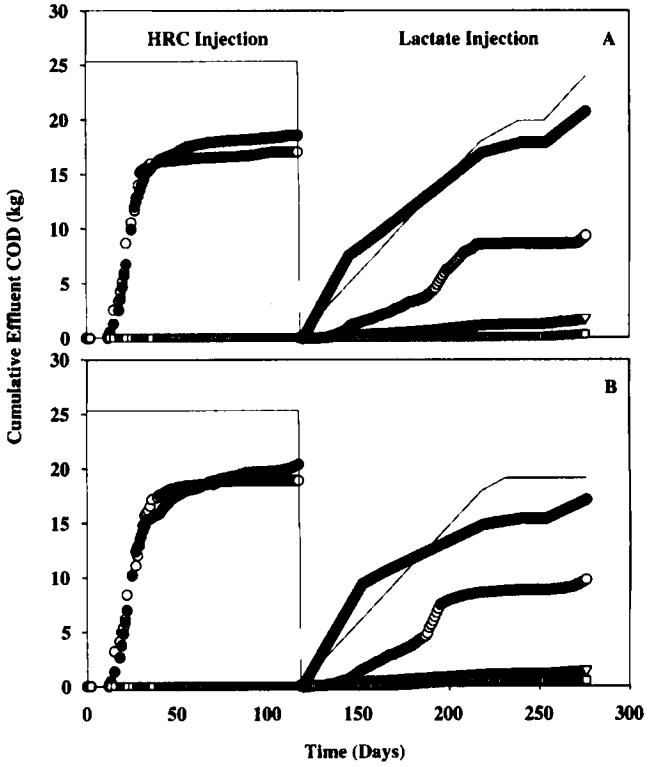

Figure 3—Cumulative effluent COD for the (a) bioaugmented tank and (b) biostimulated tank. Symbols: – influent COD, • effluent COD, ○ effluent propionate and acetate, ▽ effluent lactate, and □ effluent methane.

upgradient (1.5 m) of the PCE injection wells. The amount of culture injected represented 0.4% of the aquifer's pore volume.

Column Studies. Flow-through aquifer columns were used to determine whether the anaerobic culture used for bioaugmentation could enhance dissolution of the DNAPL by biosurfactant production, as a possible mechanism for the high concentration of PCE observed in the effluent of the bioaugmented tank early in the experiment. Three glass columns (15 cm long, 1.5-cm internal diameter) (Da Silva and Alvarez, 2002) were packed with the same sandy material used in the ECRS. All tubing and fittings were Teflon-lined to minimize adsorption losses. Feed solutions were dispensed from gas-tight syringes (100 mL) (SGE, Austin, Texas) at constant flow (1 mL/h) using a syringe pump (Harvard Apparatus). The effluent tubing was adapted for sampling with a 0.64-cm (0.25-in. #28) male luer lock adapter and a thin (30-gauge) disposable syringe needle. A bicarbonate-buffered (1000 mg/L) synthetic groundwater (Vongunten and Zobrist, 1993) was fed continuously (1 mL/h). Synthetic groundwater was used to reproduce similar ionic strength encountered in groundwater. One pore volume was displaced in 7 hours, with a seepage velocity of 5.1 cm/d. The DNAPL source in the columns consisted of neat PCE (0.8 mg) injected with a glass gas-tight syringe (10 μL) below the effluent cap of the column (4 cm). One column was fed continuously with the synthetic groundwater plus 50% v/v ethanol to enhance the dissolution of PCE (positive control). The second column was fed continuously with synthetic groundwater alone (negative control), to define a PCE dissolution baseline. A third column was fed with the same bacterial consortium used to bioaugment the ECRS. Samples (1 mL) were taken over time from each column by attaching the needle from the effluent lines to gas chromatography vials (5 mL), previously sealed with Teflon-lined rubber septa and aluminum crimps. Headspace samples (100 μL) were analyzed for PCE immediately after collection using gas chromatography, as described previously.

Results and Discussion

Experimental Controlled Release Systems Monitoring—Hydrogen Releasing Compound, Lactate, and Effluent Chemical Oxygen Demand. The ECRS tanks were monitored for 276 days. The HRC was largely depleted in the systems after 40 days, as indicated by a decrease in the effluent COD concentration to negligible levels and the resulting stabilization of the cumulative mass of COD exiting the tanks (Figure 3). Possible explanations for the rapid HRC depletion are the enhancement of dissolution rates because of the relatively fast groundwater velocity used (seepage velocity = 1.6 m/d), higher water temperatures (23°C) in the ECRS than would be encountered in the field (typically 10 to 15°C), or possibly the relatively soluble HRC formulation used (glycerol tripolylactate). Most of the added HRC was recovered (73.2 and 80.5% in the bioaugmented and biostimulated tank, respectively) in the effluent as fermentation byproducts, such as acetate and propionate (Figure 3).

On day 118, lactate feeding to both systems was initiated and sustained as an alternative electron donor. Most of the added lactate was recovered (86.5 and 89.6% in the bioaugmented and biostimulated tank, respectively) in the effluent during the following 20 days (up to day 138) (Figure 3). Acetate and propionate (byproducts of lactate fermentation) were detected in the effluent of both tanks, and concentrations increased over time (from day 118 to day 148) during the 30 days after lactate addition. Figure 3 shows a difference in total COD and the effluent acetate plus propionate concentrations, which could be a result of the production of other byproducts of lactate fermentation that were not monitored. The

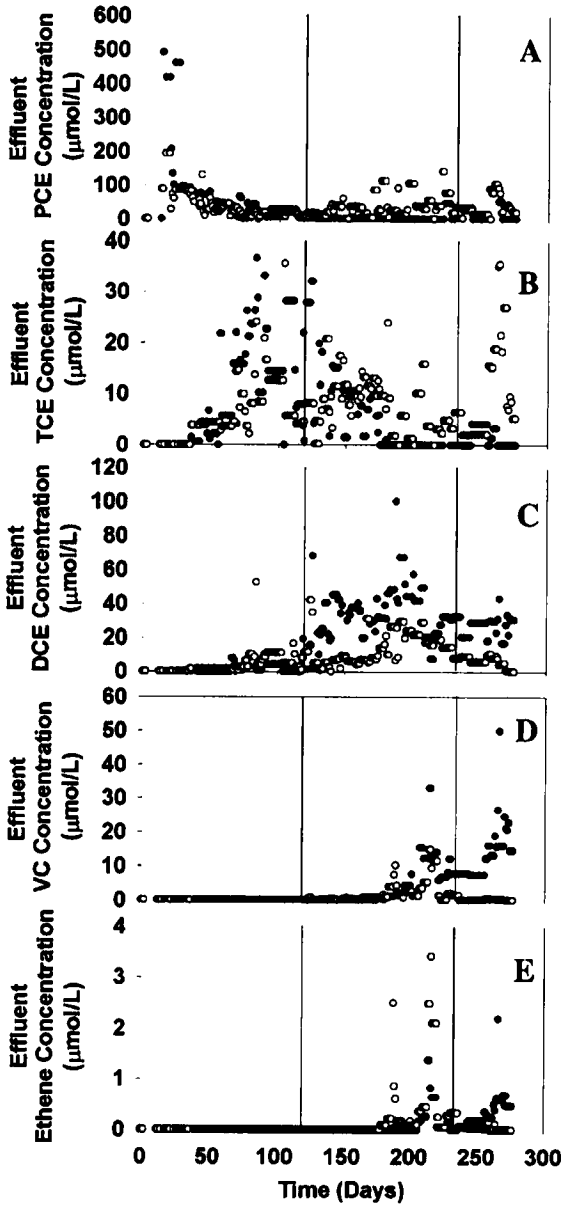

Figure 4—Effluent concentrations of (a) PCE, (b) TCE, (c) *cis*-DCE, (d) vinyl chloride, and (e) ethene. Symbols: • bioaugmented tank and ○ biostimulated tank. The PCE injection was on day 0; HRC was added 6 days before the PCE injection; and bioaugmentation was on day 8. Lactate injection started in both tanks on day 118 (left line) and ceased in the biostimulated tank on day 232 (right line).

missing COD was likely associated with carbon dioxide production and biomass formation during metabolism of the electron donor(s). It is unlikely that other biochemical processes were involved in consuming the missing COD. Based on the bioavailable iron concentration in the sand, iron(III) reduction would have consumed 1 g COD (i.e., <0.01% of the added COD). Cumulative methane production accounted for 300 g as COD (i.e., 1.3%). The influent tap water did contain some sulfate (44 mg/L), but its use as electron acceptor would also account for negligible COD consumption (i.e., <0.01%).

Experimental Controlled Release Systems Monitoring—Chlorinated Ethenes and Ethene. Based on effluent concentrations, both the bioaugmented tank and biostimulated tank demonstrated the stepwise dechlorination of PCE to TCE to *cis*-DCE to vinyl chloride, and then to small amounts of ethene (Figure 4). In the biostimulated tank, PCE was first detected in the effluent on day 13 of the experiment, when an initial spike in the PCE concentration was observed (Figure 4a). After this spike, PCE concentrations remained between 50 and 100 µM for the duration of the experiment. The TCE was detected on day 35, and its concentration increased over time until day 125 (Figure 4b). The *cis*-DCE was first detected in the effluent after day 75 (Figure 4c), but its concentration increased significantly after day 125, when the TCE concentration started to diminish. Vinyl chloride appeared in the effluent after day 124 at a very low concentration, but increased after day 150 (Figure 4d). Ethene was first detected in the effluent on day 152 (Figure 4e). After suspending lactate injection to this tank (on day 232), the effluent concentrations of vinyl chloride and ethene decreased below detection limit. This implies that the dechlorination activity decreased because of the discontinued addition of an electron donor.

In the bioaugmented tank, PCE concentrations in the effluent followed a similar trend to the biostimulated tank; an initial PCE spike almost three times greater than in the biostimulated tank was seen around day 13, and then the concentration of PCE fell sharply (Figure 4a). The TCE was first observed in the effluent near day 35, reached a maximum concentration of 38 µM on day 80, and then started to decrease (Figure 4b). The *cis*-DCE was first detected in the effluent on day 100 (Figure 4c), but the concentrations rapidly increased after day 118, when the TCE concentrations began to decline. Vinyl chloride and ethene were detected on days 159 and 167, respectively (Figures 4d and e). Both vinyl chloride and ethene reached their maximum concentrations in the effluent around day 225, and then stabilized.

Cumulative mass-balance calculations showed that a significant quantity of the PCE source zone was removed within the first 50 days in the bioaugmented tank (Figure 5a). The total mass removed at the end of the experiment was approximately 90% of the total mass of PCE added to the tank. Of this removal, 59% was removed by dissolution (as measured by the mass of PCE in the effluent) and 31% by dechlorination to lesser chlorinated products, such as TCE and *cis*-DCE. In the biostimulated tank, only 68% of the PCE added to the tank was removed, with 48% being removed by dissolution and 20% by dechlorination (Figure 5b). The lower residual mass of PCE in the bioaugmented tank was partly because of the high concentration of PCE exiting the tank in the beginning of the experiment just after bioaugmentation. This high concentration of PCE measured in the effluent of the bioaugmented tank was initially thought to be caused by biosurfactant properties of the culture that could have enhanced DNAPL dissolution. However, column tests conducted under conditions similar to that in the tanks failed to confirm this hypothesis (see below), contrary to similar phenomena observed in the biodegradation of petroleum hydrocarbons (Francy et al., 1991).

The culture used to bioaugment the tank was capable of complete dechlorination of PCE to ethene, but the concentration of ethene observed in the bioaugmented tank was relatively low (<4 µmol/L). A main cause of the slow ethene production could have been the short contact time resulting from the relatively high groundwater velocity in the tanks (1.6 m/d). Such a fast velocity could have also caused washout of some of the added dechlorinating organisms, which would be conducive to lower ethene production rates.

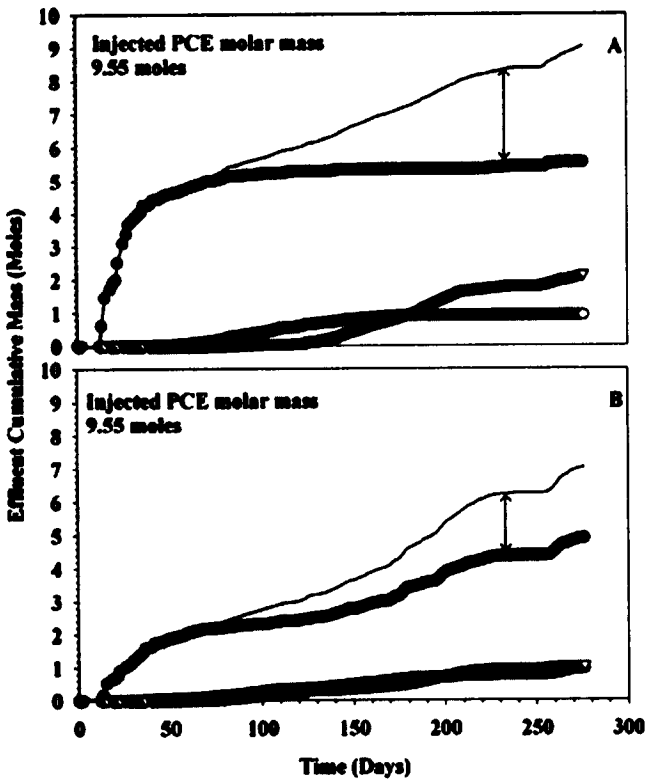

Figure 5—Cumulative effluent concentrations of PCE, TCE, and cis-DCE in the (a) bioaugmented tank and (b) biostimulated tank. Symbols: • PCE, ○ TCE, ▽ *cis*-DCE, and – total chlorinated mass. Arrows show difference in extent of dechlorination between the two tanks.

Insufficient supply of electron donor was also a likely factor that hindered the extent of dechlorination, especially during the time after HRC was depleted (day 40) and before lactate was added (day 118). Although the pH (6.33 ± 0.20 and 6.28 ± 0.14 for the biostimulated tank and bioaugmented tank, respectively) was below the optimum value (6.8 to 7.8) reported for dechlorinating organisms (Middeldorp et al., 1999), it is unlikely that such a small difference hindered ethene production. Interestingly, ethene concentrations observed in this work were much higher than the concentration of ethene observed in a similar ECRS bioaugmentation study conducted by Adamson et al. (2003).

Column Studies. Column studies were performed to test the hypothesis that biosurfactants produced by the culture were capable of displacing DNAPL. The column tests conducted under conditions similar to that in the tanks failed to confirm this hypothesis, contrary to similar phenomena observed in the biodegradation of petroleum hydrocarbons (Francy et al., 1991). Almost all PCE added to the positive control column fed groundwater plus ethanol was recovered (>98%). However, there was not a significant difference between the columns fed groundwater only (negative control) or with the culture used for bioaugmentation. Approximately 50% PCE was recovered in both effluents (Figure 6). Injection of 15 L of culture directly to the DNAPL source zone (conducted under positive pressure) in the bioaugmented tank may have displaced some DNAPL and increased the surface-to-volume ratio of the DNAPL. This could have increased dissolution of PCE and the amount of soluble PCE in the effluent.

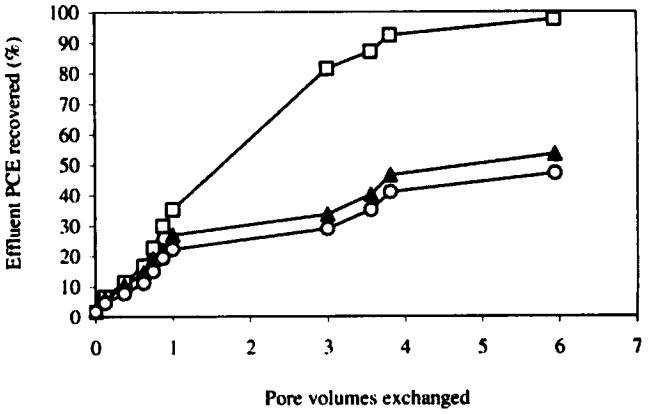

Figure 6—Effluent PCE breakthrough curves for column studies. Symbols: □ 50% (v/v) ethanol, ▲ groundwater, and ○ dechlorinating culture.

Experimental Controlled Release Systems Monitoring—Methane. Methane concentrations were monitored throughout the experiment, and the quantity of electron donor used for methanogenesis was compared with the amount used for dechlorination. Previous studies demonstrated that methanogens can outcompete dechlorinating organisms in the presence of high hydrogen concentrations (He et al., 2002; Yang and McCarty, 1998). In this study, more electron equivalents (COD) were used for methanogenesis than halorespiration in both tanks (Figure 7). However, in the bioaugmented tank, less methane was produced in relation to the amount of dechlorination compared with the biostimulated tank. Even with the methanogens using more electron equivalents than the dechlorinating organisms in the biostimulated tank, *cis*-DCE, vinyl chloride, and small amounts of ethene were all produced in both tanks. However, the benefit of bioaugmentation was observed with higher (1.6 times) dechlorination activity compared with that in the biostimulated tank (Figure 5). The higher use of equivalents by the halorespiring organisms in the bioaugmented tank was most likely a result of the higher biomass of dechlorinating organisms in this tank.

Microbial Characterization Before Bioaugmentation. Overall, dechlorination activity was observed in both the bioaugmented tank and the biostimulated tank. Microbial analysis (nested PCR) conducted before bioaugmentation showed the presence of specific bacteria capable of dechlorinating PCE to *cis*-DCE (*Dehalobacter* spp., *Sulfurospirillum* spp., and *Desulfuromonas* spp.) and PCE to ethene (*Dehalococcoides* spp.) in both tanks. It is unlikely that the dechlorination activity observed in the biostimulated tank was caused by cross-inoculation from the bioaugmented tank, because all the influent and effluent pipes were run separately. Cross-inoculation caused by spray during bioaugmentation was also unlikely, because the culture was added from a closed container, and all the lines were sealed. The most plausible explanation for the dechlorination activity in the biostimulated tank is that the sand initially contained low concentrations of dechlorinating organisms that eventually proliferated because of selective pressure by PCE and electron-donor amendments.

The sandy material used in this work was obtained from the Brazos River in south Texas, which drains several urban areas, and the possibility that this material had previous exposure to trace levels of chlorinated solvents or naturally occurring chloroorganic compounds cannot be ruled out (Keppler et al., 2002). Our results

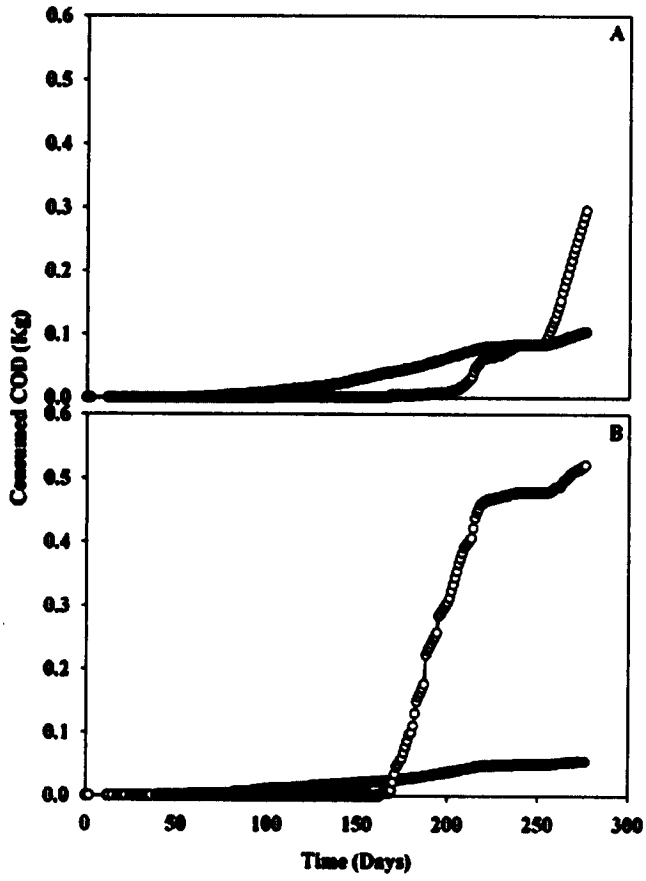

Figure 7—Comparison of electron donor equivalents (as COD) used for methanogenesis versus reductive dechlorination in (a) bioaugmented tank and (b) biostimulated tank. Symbols: ○ methane COD and • dechlorination COD.

support the notion that halorespiring bacteria may be widely distributed in nature. Hendrickson et al. (2002) demonstrated that *Dehalococcoides* organisms are widely distributed in the environment and can survive in a wide range of geographical locations, geological matrices, and climatic zones, possibly consuming naturally produced chloroorganic compounds. Microbial analysis of the groundwater conducted after 160 days of experiment showed that the concentration of *Dehalobacter* spp. was one order of magnitude higher in the bioaugmented tank (10^4 cells/mL) compared with the biostimulated tank (10^3 cells/mL). Because *Dehalobacter* spp. cannot dechlorinate past *cis*-DCE, these results corroborate the higher concentrations of *cis*-DCE observed in the bioaugmented tank.

In this study, biostimulation was performed by adding a solid electron donor (HRC) and later a liquid electron donor (dissolved lactate). Our results were insufficient to distinguish which electron donor delivery approach is more cost-effective for source bioremediation. Whereas liquid-delivery systems (i.e., lactate) can generally achieve good hydraulic control, one potential concern is clogging in areas near the injection well because of excessive microbial growth. In addition, continuous delivery can result in relatively high operation (energy and labor) costs. On the other hand, solid-phase delivery systems (i.e., HRC) provide for a long-term source of electron donor with negligible energy and labor requirements, resulting in lower operational costs. However, depletion of the stimulatory material and the potential for contaminated water to bypass the biostimulated zone, as a result of the lack of hydraulic control, are potential concerns that need to be addressed on a case-by-case basis (Alvarez and Illman, 2005).

Conclusion

This research demonstrated that the dechlorination of a PCE DNAPL can be achieved using both bioaugmentation and biostimulation. Although dechlorination in the biostimulated and bioaugmented tanks followed similar patterns, and some PCE DNAPL may have been displaced during injection of the microbial culture, it was clear, from the overall mass balance of dechlorination products, that bioaugmentation enhanced PCE mass removal (1.6 times) by increasing the local flux of contaminants into the aqueous phase via the production of more soluble and less hydrophobic metabolites (mainly *cis*-DCE). These results suggest that bioaugmentation could significantly aid in the removal of DNAPL source zones in aquifers compared with biostimulation alone.

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