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# Natural Attenuation Processes during *In Situ* Capping

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Chlorinated solvents are common groundwater contaminants that threaten surface water quality and benthic health when present in groundwater seeps. Aquatic sediments can act as natural biobarriers to detoxify chlorinated solvent plumes via reductive dechlorination. *In situ* sediment capping, a remedial technique in which clean material is placed at the sediment−water interface, may alter sedimentary natural attenuation processes. This research explores the potential of Anacostia River sediment to naturally attenuate chlorinated solvents under simulated capping conditions. Results of microcosm studies demonstrated that intrinsic dechlorination of dissolved-phase PCE to ethene was possible, with electron donor availability controlling microbial activity. A diverse microbial community was present in the sediment, including multiple *Dehalococcoides* strains indicated by the amplification of the reductive dehalogenases *tceA*, *vcrA*, and *bvcA*. An upflow column simulating a capped sediment bed subject to PCE-contaminated groundwater seepage lost dechlorination activity with time and only achieved complete dechlorination when microorganisms present in the sediment were provided electron donor. Increases in effluent chloroethene concentrations during the period of biostimulation were attributed to biologically enhanced desorption and the formation of less sorptive dechlorination products. These findings suggest that *in situ* caps should be designed to account for reductions in natural biobarrier reactivity and for the potential breakthrough of groundwater contaminants.

## Introduction

It is estimated that 75% of Superfund and RCRA sites are located within one-half mile of surface water bodies, with half of the Superfund sites reporting interaction between contaminated groundwater and surface water (*1*). Contaminants present in groundwater seeps threaten water quality and present dangers to the benthic community as well as to fish and humans. The chlorinated solvents tetrachloroethene (PCE) and trichloroethene (TCE) are common groundwater contaminants (*2*) whose plumes can extend considerable distances from their source to potentially discharge into surface water bodies (*3*−*7*).

Aquatic sediments, because of their inherently anaerobic conditions and abundant carbon and energy sources, tend to have ample microbial mass and diversity to serve as natural biobarriers to detoxify chlorinated solvent plumes. PCE can be biologically transformed under anaerobic conditions through reductive dechlorination to nontoxic ethene and ethane, with corresponding production of hydrogen and chloride ions. During this process, dissolved-phase chloroethenes serve as electron acceptors for the dechlorinating organisms while the source of electrons is typically hydrogen (*8*, *9*), supplied from the oxidation of organic substrates by fermenting microbial communities (*10*, *11*). Multiple dechlorinating organisms have been detected in enrichments originating from river sediments (*12*−*14*), including *Dehalococcoides* strains, which possess the unique ability to dechlorinate dichloroethenes (DCEs) and vinyl chloride (VC) (*15*−*19*). The accumulation of DCEs and VC at contaminated sites is commonly observed, however.

*In situ* capping is a remedial option for contaminated sediments in which clean material is placed at the sediment−water interface to prevent contact between sediment contaminants and the overlying benthic community and surface water. Sand is the traditional material employed for capping, with reactive caps recently designed to sequester or transform sediment contaminants (*20*−*23*). The presence of an *in situ* cap shifts the deposition of labile organic matter to the newly formed cap−water interface, thus removing a source of carbon and organic substrates from the biologically active sediment where biotransformations are most likely to occur. In addition, the employment of some synthetic capping materials have been reported to disrupt groundwater seepage patterns because of low hydraulic conductivities ($\sim 10^{-9}$ cm/s versus for $\sim 10^{-7}$ for clay/silt sediments), creating flow bypassing around the caps and localized zones of discharge with increased flow rates (*24*, *25*). The effect of reduced organic substrate levels, coupled with elevated flow rates, on natural bioattenuation processes in capped sediments is unknown. Such fundamental information is needed for accurate reactive transport modeling and to aid in the design of *in situ* caps subject to advective flows.

Here we present a series of laboratory experiments designed to evaluate the effect of *in situ* capping on the natural bioattenuation of PCE in a natural river sediment. Batch microcosm tests were preformed to evaluate the capacity of the sediment to intrinsically dechlorinate PCE to nontoxic products. Electron donor was added to select microcosms at increasing concentrations to examine its effect on dechlorination. Dechlorination and methane production were monitored within the microcosms, followed by characterization of the dechlorinating microbial community. A sediment column operating with advective flow simulating a groundwater seep was constructed to observe PCE dechlorination within the anaerobic sediment under dynamic conditions. The column influent consisted of only simulated groundwater and dissolved-phase PCE to mimic environmental conditions. Electron donor was again added during the course of the experiment to address limitations on dechlorination. Effluent samples were analyzed for dechlorination products and other geochemical indicators of microbial activity.

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**TABLE 1. Reducing Equivalents Added to PCE Microcosms and Time Required To Achieve Complete Dechlorination of PCE to Ethene for the Initial Three Dechlorination Cycles. Reducing Equivalents Were Added in the Form of Hydrogen and Acetate, Where Indicated**

| microcosm name | reducing equivalents added (mequiv total)[a] | ED to PCE ratio[b] | time required for complete dechlorination (days)[c] | | |
|---|---|---|---|---|---|
| | | | first dechlorination cycle | second dechlorination cycle | third dechlorination cycle |
| no $H_2$ | 0.0 | 0.0 | 82 | 24 | 20 |
| minimal $H_2$ | 0.1 | 1.4 | 80 | 19 | 26 |
| acetate + $H_2$ | 1.3 | 16.6 | 76 | 15 | 10 |
| excess $H_2$ | 2.5 | 31.3 | 56 | 12 | 6 |
| bioaugmented[d] | 0.1 | 1.4 | 33 | 16 | 21 |

[a] Reducing equivalents were calculated based on 2 equiv per 1 mol $H_2$; 4 equiv per 1 mol acetate (*57*). "Minimal $H_2$" and "acetate + $H_2$" microcosms had 3.5% $H_2$ in microcosm headspace and "excess $H_2$" microcosms had 80% $H_2$ in microcosm headspace. [b] Electron donor (ED) to PCE ratio was calculated based on eight reducing equivalents required for PCE to be reductively dechlorinated to ethene, all chloroethene mass being bioavailable, and no loss of reducing equivalents to competing biotic and abiotic processes. [c] Times required for complete dechlorination are averages of duplicates. [d] Bioaugmented microcosms were supplied with a 5% inoculum (volume culture/aqueous volume microcosm) of a PCE to ethene mixed culture (Owls (*27*)) sparged with $N_2$ prior to addition.

## Materials and Methods

**Chemicals**. PCE (99+%, Sigma-Aldrich, St. Louis, MO), TCE (99.5%, Sigma-Aldrich), *cis*-DCE (97%, Acros Organics, Morris Plains, NJ), *trans*-DCE (99.7%, Acros Organics), and 1,1-DCE (99.9%, Acros Organics) were obtained in neat liquid form. Vinyl chloride (8%/$N_2$ balance), ethene (99.5%), ethane (99.5%), and methane (99%) were obtained from Matheson Tri-gas (Parsippany, NJ). Sodium bicarbonate, potassium chloride, magnesium chloride, calcium chloride, sodium lactate syrup (60% vol/vol), ferric chloride, ferrozine (98+%), and potassium bromide were all purchased from Fisher Scientific. Hydrogen gas was purchased from Airgas (Atlanta, GA), and glacial acetic acid (ACS grade) was obtained from Acros Organics.

**Sediment.** Surficial sediment from the Anacostia River, Washington, D.C., was used as the sediment for all experiments. Sediment from the river has previously been characterized and found to contain elevated levels of PCBs, PAHs, metals, and organic pesticides (*24*). The wet sediment was homogenized in the laboratory by mechanical mixing after removal of large debris and shells and transferred to quart-sized mason jars that were stored in the dark at 4 °C.

**Microcosms.** Microcosms were constructed in duplicate by loading serum bottles (70 mL) with wet river sediment and simulated groundwater under anaerobic conditions (5% $CO_2$/5% $H_2$/90% $N_2$) in a glovebox (Coy Laboratory Products, Grass Lake, MI). The ratio of wet sediment to groundwater in the microcosms was 1:1.5 (v/v), following the method of Lorah and Voytek (*7*). The composition of the simulated groundwater was slightly modified from that described by Dries et al. (*26*) and consisted of 3.5 mM $NaHCO_3$, 0.1 mM KCl, 0.25 mM $MgCl_2$, and 0.75 mM $CaCl_2$. The microcosms were sealed with Teflon-lined butyl rubber stoppers and aluminum crimp caps. Neat PCE was introduced into the microcosms with a 10 $\mu$L syringe, and the microcosm mass difference was recorded following injection to determine the amount of PCE loaded. The target aqueous-phase PCE concentration was approximately 0.08 mM after partitioning among gas, solid, and aqueous phases.

Microcosms were supplied with dissolved acetate and hydrogen gas ranging at concentrations from 0 to 2.5 mmol reducing equivalents to test their effect on the times and extent of reductive dechlorination (Table 1). Acetate was added from an anaerobic, sterile stock solution of acetic acid in simulated groundwater. Target hydrogen concentrations were achieved by sparging the microcosm headspace with nitrogen gas and then supplying hydrogen gas directly to the microcosm. When dechlorination was complete (i.e., conversion to ethene/ethane), the microcosms were sparged with $N_2$, recapped under anaerobic conditions, and respiked with the appropriate amount of electron donor and neat PCE. The addition of mineral media, carbon sources (with the exception of the acetate microcosms), and vitamins to the microcoms was avoided to replicate environmental conditions. Active control microcosms were established in duplicate without PCE to ensure that all observed ethenes were experimentally derived. Bioaugmented microcosms were constructed with a 5% inoculum of a PCE to ethene mixed culture (*27*) to test the effect of excess dechlorinating cells on dechlorination times. Sterilized microcosms were prepared as described above but autoclaved at 121 °C for 1 h on two consecutive days prior to PCE addition to ensure that reductive dechlorination observed in the active reactors was not the result of abiotic transformation processes (*28*−*30*). All microcosms were wrapped in foil and incubated at 20 °C on an orbital shaker at 150 rpm. Chlorinated ethenes, ethene, ethane, and methane concentrations were determined from headspace samples of the microcosms.

**DNA Extraction and PCR.** Partial characterization of the dechlorinating community was performed on an active PCE to ethene dechlorinating microcosm supplied PCE, acetate, and hydrogen. DNA was extracted from five separate 1-g samples of wet sediment (5.04 g total) using the Ultraclean Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA). Procedures of DNA extraction and PCR reactions have been described previously (*27*). Chromosomal DNA from *Dehalobacter restrictus*, *Desulfuromonas michiganensis* strain BB1, *Desulfomonile tiedjei*, and *Geobacter lovleyi* strain SZ were used as positive controls during PCR reactions and gel electrophoresis. The primer sequences for the target 16S rRNA genes have previously been reported and are provided in the Supporting Information (Table S.3). Negative control reactions accompanied all PCR analyses and consisted of nuclease-free water (Fisher Scientific).

**Quantitative Real-Time PCR (qPCR).** Detection of *Dehalococcoides* and reductive dehalogenase genes (RDases) *tceA* (*31*), *vcrA* (*32*), and *bvcA* (*33*) was performed using qPCR. The qPCR reactions for *Dehalococcoides* 16S rRNA genes and the *tceA*, *bvcA*, and *vcrA* genes were performed as described by Ritalahti et al. (*34*). Standard curves for qPCR analysis were prepared with a dilution series of quantified genomic DNA from *Dehalococcoides* sp. strain FL2 or from plasmids carrying a single *Dehalococcoides* RDase gene. The linear quantification range for *Dehalococcoides* sp. strain FL2 was $10^2-10^{10}$ gene copies·mL$^{-1}$ ($r^2 = 0.999$). The linear range for the target RDases was $10^2-10^{10}$ gene copies·mL$^{-1}$ ($r^2 = 0.995$), $10^1-10^{10}$ gene copies·mL$^{-1}$ ($r^2 = 0.997$), and $10^1-10^8$ gene copies·mL$^{-1}$ ($r^2 = 0.990$) for *tceA*, *vcrA*, and *bcvA*, respectively.

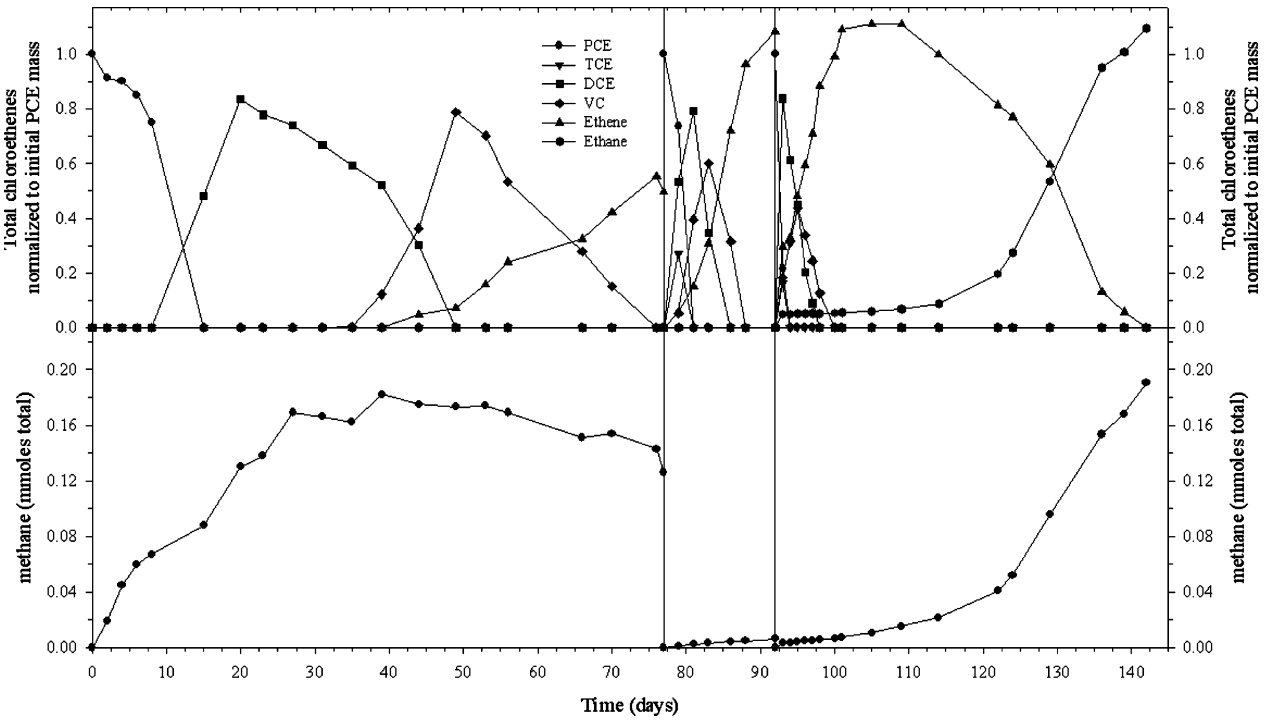


**FIGURE 1. Representative example of the initial three dechlorination cycles and associated methane production for an active PCE microcosm. The example microcosm shown was provided 5 mM acetate and 3.5% $H_2$ in microcosm headspace, resulting in an electron donor to PCE ratio of 16.6. Chloroethene concentrations are plotted as the total mmoles of chloroethene species present in the microcosm (i.e., sum of solid, aqueous, and gas phases) normalized to initial mmoles of PCE introduced into the microcosm. Methane concentrations are plotted as total mmoles within the microcosm (i.e., sum of solid, aqueous, and gas phases). Black vertical lines represent microcosm headspace sparging with nitrogen gas and respike with PCE.**

The gene copy numbers were calculated as described previously (*34*). Genetic and genomic analyses show that these 16S rRNA and the RDase genes exist as single copy genes on *Dehalococcoides* genomes (*34*−*36*).

**Sediment Column.** An upflow sediment column was assembled to simulate an anaerobic sediment bed modified with an *in situ* cap. The column was constructed using a 2.5 cm i.d. glass chromatography column 30 cm in length (Spectrum Chromatography, Houston, TX) with custom-built stainless steel end plates. A 2.5 cm diameter disc of 80 mesh stainless steel (Small Parts, Inc., Miami Lakes, FL) and a second 2.5 cm diameter disc of finer mesh (5 $\mu$M opening; TWP Inc., Berkeley, CA) were placed in series within the end plates to help distribute flow and prevent the loss of sediment particles. A glass reservoir (15 mL; 0.1 pore volumes) fitted with a stopcock was placed at the column effluent to allow for aqueous sampling. The column was packed with wet, homogenized sediment in a fume hood under a constant stream of $N_2$ and wrapped in foil. Estimates of porosity and water content were obtained from bulk sediment properties (Supporting Information, Table S.1) and column mass difference before and after packing.

Column influent composed of simulated groundwater spiked with dissolved-phase PCE (ranging from 31.4 ± 11.3 $\mu$M to 134.7 ± 47.1 $\mu$M; Supporting Information, Figure S.2) was supplied to the column by a syringe pump at a rate of 5.5 mL·h$^{-1}$ to produce a hydraulic residence time of 1 day and a seepage velocity of 47.0 cm·day$^{-1}$ (Darcy velocity = 25.8 cm·day$^{-1}$). The flow rate was selected to simulate elevated seepage velocities associated with flow bypassing potentially caused by *in situ* capping with relatively low permeability caps. The column seepage velocity of 47.0 cm·day$^{-1}$ is on the upper end of the range of observed field velocities of groundwater seeps (0.0026 to 720 cm·day$^{-1}$ (*37*)). The simulated groundwater was autoclaved at 121 °C for 30 min and sparged with $N_2$ gas for 45 min prior to use. Periodic measurements of the influent indicated that dissolved oxygen levels were routinely below 0.5 mg·L$^{-1}$ and always below 1 mg·L$^{-1}$ (data not shown). The pH of the sparged groundwater was adjusted to circumneutral by the addition of carbon dioxide gas. Dissolved-phase PCE, obtained from a saturated stock solution of neat PCE in anaerobic, sterile simulated groundwater, was added to the influent syringe immediately preceding connection to influent tubing. The column lifetime was divided into three separate phases based on influent conditions: Phase I from 0 to 108 pore volumes; Phase II from 108 to 146 pore volumes; Phase III from 146 pore volumes to the conclusion of the experiment at 180 pore volumes. Phase I was defined by an influent composed of only simulated groundwater and PCE with the goal of obtaining baseline transport and biotransformation data. Phase II was characterized by the addition of 5 mM lactate to the influent from an anaerobic, sterile stock solution intended to stimulate the microbial community. In Phase III, the influent was returned to the initial Phase I conditions, containing only simulated groundwater and aqueous phase PCE.

Influent concentrations of PCE were routinely measured because of variability in the concentration of the aqueous PCE stock and to ensure that no PCE transformations occurred before introduction into the column. Aqueous effluent samples were obtained from the sampling reservoir and transferred to a sealed serum bottle that had an equivalent volume of gas-phase removed immediately prior to injection. Chlorinated ethenes, ethene, ethane, methane, and hydrogen concentrations were determined from headspace samples of these serum bottles. Aqueous samples were obtained from these serum bottles to measure effluent anion concentrations. Colorimetric iron analysis was performed on reservoir samples transferred directly to cuvettes.

**Analytical Methods.** Chloroethenes, ethene, ethane, and methane were separated via gas chromatography (GC) and

**TABLE 2. Partial Characterization and Quantification of Dechlorinating Microorganisms within a Microcosm Supplied Wet Sediment, Simulated Groundwater, PCE, Acetate, and Hydrogen**

| target microorganism or gene | dechlorination activity | result | quantification (gene copies·mL$^{-1}$) |
|---|---|---|---|
| *Dehalobacter spp.* | PCE → DCE | + | not performed |
| *Desulfuromonas spp.* | PCE → DCE | + | |
| *Desulfuromonas spp.* | PCE → DCE | + | |
| *Geobacter* sp. strain SZ | PCE → DCE | N/D[a] | |
| *Dehalococcoides* spp. | PCE → ethene DCEs → ethene | + | $5.2 \times 10^8 \pm 2.3 \times 10^7$ [b] |
| *tceA* enzyme (*Dehalococcoides* sp. strain 195 or strain FL2) | TCE → VC | + | $8.3 \times 10^7 \pm 2.9 \times 10^6$ |
| *vcrA* enzyme (*Dehalococcoides* sp. strain VS or strain GT) | DCEs → ethene | + | $2.1 \times 10^8 \pm 2.1 \times 10^7$ |
| *bvcA* enzyme (*Dehalococcoides* sp. strain BAV 1) | VC → ethene | + | $2.8 \times 107 \pm 1.1 \times 10^5$ |

[a] N/D = not detected with PCR (nested or direct). [b] Quantification of *Dehalococcoides* and RDase numbers are average of triplicates ± one standard deviation.

analyzed with an FID detector, as described previously (*10*). Hydrogen concentrations were determined from headspace samples using a GC equipped with a reducing gas analyzer (*38*). Iron measurements were conducted using a modified ferrozine method (*39*) with colorimetric determination of ferrous and ferric iron by a Cary 300 UV−vis spectrophotometer. Anions were separated using a Dionex DX-100 ion chromatograph equipped with a Dionex AG4A IonPac guard column and Dionex AS4A IonPac column.

## Results

**Microcosms**. PCE was successfully dechlorinated to the end products of ethene and ethane under methanogenic conditions in all biologically active microcosms. Figure 1 shows a plot of a representative microcosm following the three initial PCE dechlorination cycles. This microcosm was supplied with acetate and hydrogen, resulting in 1.3 mequiv of electrons (electron donor to PCE ratio = 16.6). Temporary stalling of dechlorination at *cis*-DCE occurred during the first dechlorination cycle in most microcosms. The loss of ethene mass balance in the representative microcosm during the first spike was attributed to leakage through the septa after multiple punctures, as evidenced by the loss of methane after approximately 40 days. It is also possible that anaerobic oxidation within the microcosms contributed to the loss of the species (*40*); however, this pathway was not monitored. During the subsequent dechlorination cycles, rapid dechlorination and ethane production occurred. Interestingly, a concurrent increase in methanogenesis was observed during conversion of ethene to ethane, which has been reported previously (*41*, *42*).

The effect of exogenous electron donor supply on the time required for complete dechlorination is presented in Table 1, along with the amount of reducing equivalents provided. It was generally observed that increasing amounts of reducing equivalents (i.e., increasing the electron donor to PCE ratio) decreased the time necessary for complete conversion of PCE to ethene, especially after the second and third PCE additions. Notably, complete dechlorination was observed in microcosms not provided with additional reducing equivalents. These results are in agreement with previous studies reporting that in the absence of nutrient and substrate amendments, sediment can serve as a nutrient source with the system as a whole being electron donor limited (*43*, *44*). The bioaugmented control did not perform substantially better than other microcosms after the initial dechlorination cycle, suggesting dechlorination was not limited by cell numbers. PCE transformation was not observed in the autoclaved control microcosm, with the disappearance of PCE over time attributed to sorption (Supporting Information, Figure S.1). A lumped rate coefficient describing neat PCE dissolution into the aqueous phase, partitioning into the headspace, and sorption by the sediment was obtained in the sterilized controls using a nonequilibrium mass-transfer model, which was used to estimate PCE sorption by the solid phase for mass balance calculations. The organic carbon distribution coefficient ($K_{oc}$) for PCE onto the sediment was measured to be 312 mL·g$^{-1}$, which is similar to a reference value of 265 mL·g$^{-1}$ (*45*).

**Microbial Analysis.** Partial characterization of the dechlorinating microbial community summarized by Table 2 indicated that multiple organisms were responsible for the observed PCE dechlorination in Anacostia River sediment. *Dehalobacter* species, *Desulfuromonas* species, and *Desulfomonile* species were detected in the sediment, all of which are capable of dechlorinating PCE to DCE (*12*−*14*). *Dehalococcoides* and the target reductive dehalogenase genes *tceA*, *vcrA*, and *bvcA* were all amplified from the extracted DNA at concentrations provided in Table 2. Comparison of cell numbers provided by qPCR should be made with caution because of inherent analytical uncertainty (*46*, *47*); however, the technique was successful in identifying which *Dehalococcoides* strains were present, which was the goal of the procedure. Amplification of the *tceA* gene suggests the presence of a *Dehalococcoides* sp. strain 195-type (*31*) and/or FL2-type (*48*) organism, while the presence of the *vcrA* gene suggests either a VS-type (*32*) and/or GT-type (*17*) organism. Detection of the *bvcA* gene suggests the presence of a BAV1-type (*33*) organism. It is interesting that such an active dechlorinating community was detected in the sediment despite the lack of chloroethenes detected in the unamended sediment. This can be attributed to the diverse metabolic capabilities of the organisms, which have been shown to dechlorinate more common sediment contaminants such as chlorobenzenes (*49*), dioxins (*50*), and PCBs (*51*−*54*).

**Sediment Column.** Results of the 1-D sediment column are presented in Figures 2A,B and 3A,B. A total of 180 pore volumes of simulated groundwater supplemented with dissolved-phase PCE were flushed through the column at a 1 day retention time. Methanogenesis (Figure 3A), iron sulfide precipitation (Figure 3B), and phosphate elution (Figure 3B) were observed shortly after flow was initiated, all indicators of reducing conditions. Nonideal flow patterns and physical nonequilibrium conditions (e.g., dead end pores) were revealed by nonreactive tracer tests conducted at 5 and 96 pore volumes (Supporting Information, Table S.2). The high degree of dispersion, as indicated by very low fitted Peclet numbers, was attributed to a combination of biogenic gas production and the inherent heterogeneity of natural sediments.

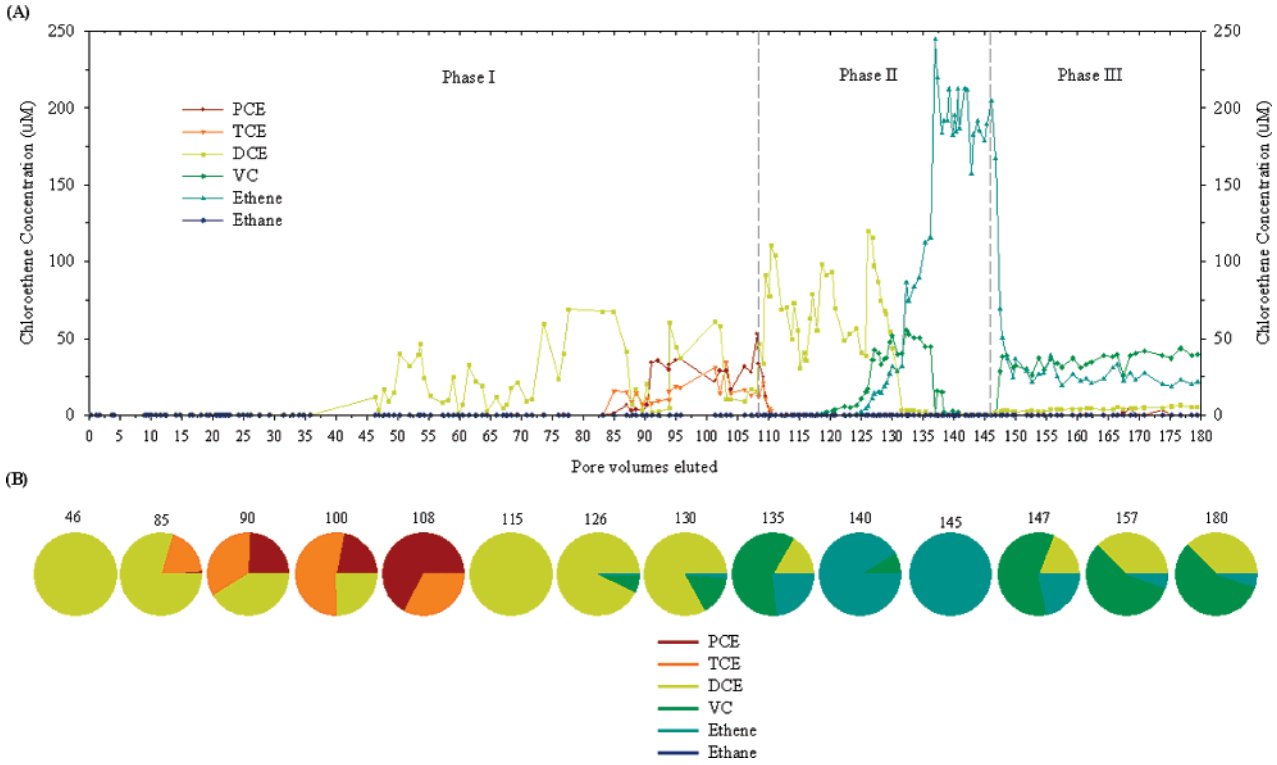


**FIGURE 2. Sediment column aqueous phase effluent concentrations of PCE and its dechlorination products. (A) Effluent concentration of PCE and dechlorination products ($\mu$M). The vertical dashed lines indicate Phase II of the experiment, when lactate was supplied to the influent along with dissolved phase PCE and simulated groundwater. (B) Effluent PCE dechlorination product distribution at selected pore volumes, indicated above each pie chart. Dechlorination products were normalized to the total amount of ethenes and ethane eluted.**

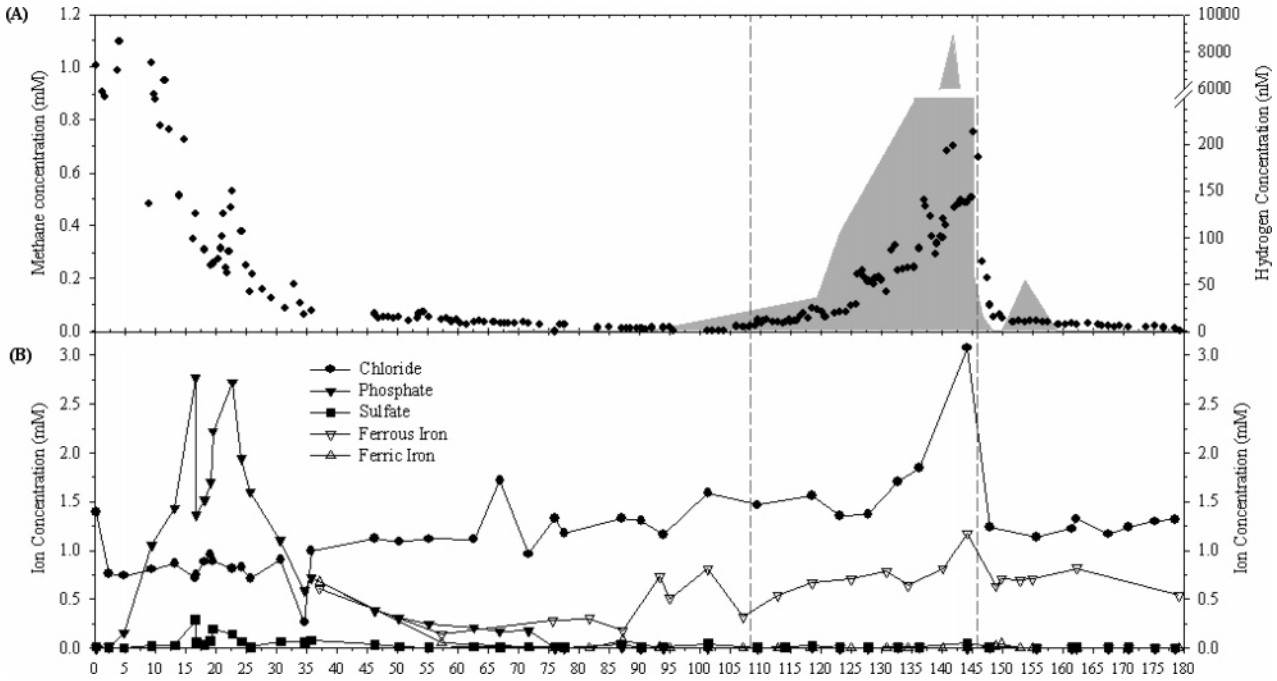


**FIGURE 3. (A) Effluent aqueous methane concentrations (mM, left axis) and aqueous hydrogen concentrations (nM, hatched area). (B) Selected ions in sediment effluent (mM). The vertical dashed lines indicate Phase II of the experiment, when lactate was supplied to the influent along with dissolved phase PCE and simulated groundwater.**

*Phase I.* Figure 2A shows the effluent concentrations of PCE and its dechlorination products, while normalized product distribution at selected pore volumes is presented in Figure 2B. The sorption and partial dechlorination of chloroethenes, along with the loss of methanogenesis, were observed during Phase I of the column experiment. The cause for the scattering of chloroethene effluent concentrations in Figure 2A is unknown, and assessment of column plumbing (e.g., leaks), sampling procedures (e.g., replicate samples, increasing sampling frequency, changing sampling vial), and calibration curves yielded no experimental nor analytical reasons for the scattering. One possible explanation is that the creation of preferential flow patterns within the hydraulically dynamic sediment caused pulsed elution of chloroethene mass. Conant et al. (*3*) characterized a chloroethene plume discharging into a river and reported complex

porewater concentration distributions over relatively small lateral distances due to the presence of preferential flow patterns.

Examination of Figure 2A reveals that effluent *cis*-DCE molar concentrations, beginning at 46 pore volumes, were markedly below the corresponding influent PCE concentrations (Supporting Information, Figure S.2), which was attributed to sorption of chloroethenes by the solid phase. The appearance of PCE and TCE in the column effluent began at approximately 85 pore volumes, and corresponded to an increase of influent PCE concentrations combined with a loss of microbial activity, indicated by methane concentrations approaching nondetect levels beginning at 45 pore volumes (Figure 3A). It was hypothesized that the loss of dechlorination and methanogenic activity was due to a lack of available electron donor, and the addition of lactate to the influent was initiated to test this hypothesis.

*Phase II*. The addition of 5 mM lactate during pore volumes 108 to 146 stimulated the microbial community, evidenced by the revival of dechlorinating and methanogenic activities and a spike in hydrogen concentrations. Effluent chloroethene distribution shifted from a mix of PCE, TCE, and *cis*-DCE prior to lactate addition to solely *cis*-DCE almost immediately following lactate addition (Figure 2B). When *cis*-DCE was the predominant product during Phase II (pore volumes 111 to 124), total chloroethene effluent concentrations averaged 68.6 $\pm$ 22.0 $\mu$M, similar to influent PCE concentrations of 68.9 $\pm$ 16.7 $\mu$M during this period. The appearance of VC and ethene began at approximately 118 and 124 pore volumes, respectively, with ethene eventually becoming the dominant product (Figures 2A,B). Interestingly, between pore volumes 127 to 147, ethene concentrations averaged 172.2 $\pm$ 34.3 $\mu$M, approximately 2.5 times greater than influent PCE concentrations during this period. The increase in dechlorinating activity is accompanied by an increase in chloride at 144 pore volumes (Figure 3B). Additionally, active dechlorinating cells including *Dehalococcoides* eluted from the column during Phase II (Supporting Information, Figure S.3A). Two possible causes for the observed increase in total chloroethene effluent concentrations are the induction of biologically enhanced desorption and the formation of decreasingly sorptive products during the PCE dechlorination pathway. The stimulation of the dechlorinating community removed chloroethenes from the aqueous phase, inducing chloroethene desorption to maintain equilibrium conditions. PCE dechlorination products are also less sorptive (i.e., lower $K_{oc}$ values), allowing for greater aqueous phase concentrations. The combination of these processes may have contributed to the elevated effluent chloroethene concentrations observed during Phase II.

Stimulation of the non-dechlorinating microbial community during Phase II is supported by the increase in effluent concentrations of methane, hydrogen, and ferrous iron. Methane concentrations approached nondetect levels prior to lactate addition, but approached initial levels at the conclusion of Phase II (Figure 3A). Figure 3A shows effluent hydrogen concentrations at nondetect levels during Phase I and then significantly elevated levels during Phase II. A substantial increase in hydrogen was observed at 142 pore volumes, almost 3 orders-of-magnitude greater than levels associated with minimum hydrogen thresholds for dechlorinators (*8*, *38*), suggesting that much of the hydrogen being produced was eluting from the sediment column. A modest spike in ferrous iron at approximately 142 pore volumes (Figure 3B) suggests that iron-reducing bacteria were also stimulated within the sediment by lactate addition. Although the addition of electron donor successfully stimulated the microbial community within the sediment, it was unclear whether this activity would be sustained after lactate addition ceased. It was hypothesized that fermentation of organic matter would maintain the observed VC and ethene production during Phase II, and this was tested during Phase III.

*Phase III*. The lack of exogenous electron donor supply during Phase III caused an immediate decrease in microbial activity, demonstrated by the simultaneous reductions of dechlorinating activity, methanogenesis, and hydrogen production. Chloroethene product distributions shifted from complete dechlorination to a mixture of *cis*-DCE, VC, and ethene for the remainder of the experiment (Figures 2A,B). Total chloroethene concentrations immediately decreased from the end of Phase II (172.2 $\pm$ 34.3 $\mu$M) to the beginning of Phase III (67.9 $\pm$ 10.8 $\mu$M) to match influent concentrations (68.9 $\pm$ 16.7 $\mu$M). In addition, active *cis*-DCE and VC dechlorinators were not eluting from the sediment during Phase III, suggesting a change in the active population within the sediment (Supporting Information, Figure S.3B). These reductions, concomitant with the removal of electron donor from the influent, confirm that elevated chloroethene effluent concentrations observed during Phase II were caused by stimulation of the microbial community. This observation may have ramifications for historically contaminated sites with large amounts of sorbed-phase mass that are to be treated using biologically based remediation. Dechlorination of previously sorbed chloroethene mass may occur during the onset of microbial stimulation, leading to a burst of dechlorination products (*55*). Methanogenesis also diminished immediately after the cessation of lactate addition and approached nondetect levels. Hydrogen concentrations declined to nondetect levels, despite an observed peak at 154 pore volumes, while ferrous iron returned to previous concentrations without any notable spikes in concentration.

Reducing equivalents from the oxidation of organic matter apparently were not able to maintain microbial activity in the sediment column experiment, perhaps due to the combined effects of organic matter recalcitrance, electron donor washout, and preferential flow path formation. In natural settings labile organic matter is quickly oxidized at the sediment−water interface, leaving more refractory organic matter subject to burial (*56*). This process may have occurred throughout the sediment column, since the sediment was homogenized and labile organic matter was evenly distributed. In addition, elution of fermentation products from the column removed electron donor from the system prior to utilization. This explanation is supported by the large hydrogen peak observed during Phase II. Finally, the presence of preferential flow paths may have reduced or eliminated contact between dissolved-phase chloroethenes and occluded regions of sediment, effectively decreasing the amount of reducing equivalents available for dechlorinating microbes in the vicinity of the contaminants. Because of the limited availability of electron donor, biostimulation was necessary to achieve complete dechlorination under the operating conditions for the sediment column experiment.

These findings are relevant to *in situ* capping scenarios where contaminated groundwater seeps are present. Our experiments addressed how capped sediment responded to the introduction of new contaminants, but the outcomes should also be applicable in cases when the capped sediment is subject to previous contamination, since reductions in electron donor availability may still occur, leading to subsequent reductions in natural bioattenuation. The observed loss of sedimentary bioattenuation processes suggests that groundwater contaminants can be transported through sediments without substantial biotransformation and discharge into *in situ* caps and surface waters. Therefore, it appears that *in situ* caps should be designed to account for the potential loss of natural biobarrier reactivity and for the breakthrough of groundwater contaminants. The use of reactive caps, whether physicochemical or biologically based, may be a viable solution to changes in natural reductive

dechlorination capacity.


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## Supporting Information Available

Information on sediment properties (Table S.1), tracer results and discussion (Table S.2), primer sequences (Table S.3), PCE sorption results and discussion (Figure S.1), sediment column influent PCE concentrations (Figure S.2), and data demonstrating effluent dechlorination activity (Figures S.3A,B). This material is available free of charge via the Internet at http://pubs.acs.org.

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