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Effect of Dechlorinating Bacteria on the Longevity and Composition of PCE-Containing Nonaqueous Phase Liquids under Equilibrium Dissolution Conditions

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The influence of dechlorinating microorganisms on PCE and its reduced end products in the presence of a PCE-containing nonaqueous phase liquid (NAPL) was investigated. Experiments were conducted in continuous-flow stirred-tank reactors (CFSTRs) containing a mixed PCE dechlorinating culture and a model NAPL consisting of PCE and tridecane. Comparisons between biotic and abiotic CFSTRs demonstrated that dechlorination resulted in a factor of 14 increase in PCE removal rates from the NAPL. The formation of dechlorination daughter products trichloroethene and *cis*-dichloroethene were observed, and *cis*-dichloroethene was not dechlorinated further. Partitioning of daughter products between phases caused temporal changes in the chlorinated ethenes distribution within the NAPL. The combined effects of dissolution and dechlorination on the removal of chlorinated ethenes from the NAPL were described using a mathematical model that approximated dechlorination as a pseudo-first-order process. Pseudo-first-order dechlorination rate coefficients for PCE and TCE were determined and were 0.18 and 0.27 h^{-1}, respectively. It was determined that total chlorinated ethenes removal from the NAPL would be achieved in 13 days in biotic CFSTRs, as compared to 77 days in the abiotic CFSTRs—corresponding to an 83% reduction in longevity of the chlorinated ethenes component of the NAPL.

Introduction

Tetrachloroethene (PCE) and trichloroethene (TCE) are among the most frequently detected contaminants in groundwater (*1*, *2*). Because of their limited aqueous solubility and miscibility in other organic solvents, PCE and TCE contamination are often associated with mixed organic nonaqueous phase liquids (NAPLs). Areas in which NAPLs are present, referred to as source zones, represent long term sources of groundwater contamination (*3*), and their presence greatly complicates the ability to restore contaminated aquifers. Pump-and-treat has long been recognized as an ineffective method for source zone restoration (*4*), and the removal of chlorinated ethenes containing-NAPLs, especially those that are denser than water, is considered to be a technical challenge "...unprecedented in the field of groundwater engineering" (*3*). Currently, all accepted remediation technologies focus on source containment and do little, if anything, to reduce the longevity of source zones.

Although microbial reductive dechlorination of PCE and TCE has been well documented (*5*−*15*) and is currently being employed to treat chlorinated ethenes contaminated groundwater (*16*, *17*), dechlorination-based source zone restoration has not been rigorously evaluated. A primary concern for source zone bioremediation is the potential toxicity of high concentrations of contaminants found near the NAPL (*3*). Studies have demonstrated, however, that dechlorination can be sustained at high PCE concentrations including saturation (*15*, *18*−*20*). Furthermore, source zones may represent an ecological niche for certain dehalorespiring microorganisms capable of withstanding high PCE concentrations (*21*−*24*). For these organisms, a PCE-containing NAPL represents a continuous source of terminal electron acceptor.

The extent to which dechlorination can impact source zone longevity (i.e., the time required to exhaust the mass of chlorinated ethenes from the NAPL) could prove to be an important consideration in assessing the duration of natural attenuation scenarios or in the implementation of engineered bioremediation processes in chlorinated ethenes source zones. In the absence of dechlorination, the longevity of the PCE component of the NAPL is controlled strictly by the rate of PCE dissolution. Dechlorination has the potential to increase PCE removal rates by depleting PCE from the aqueous phase in the direct vicinity of the NAPL, thereby increasing the overall mass-transfer of PCE into solution. If dechlorination rates in source zones are sufficiently rapid, it may be possible to substantially reduce the longevity of the PCE component of the NAPL and minimize the time required for site restoration.

A complicating factor in assessing the impact of dechlorination on source zone longevity is the interaction of dechlorination end products with the NAPL itself. PCE dechlorination results in the formation of TCE, *cis*-dichloroethene (*cis*-DCE), and vinyl chloride (VC). These daughter products will partition into the existing NAPL, causing temporal changes in NAPL composition and affecting the overall longevity of the source. Because dechlorination daughter products are contaminants themselves, all chlorinated ethenes must be removed from the source for restoration to be considered complete.

The objectives of this research were to determine whether dechlorinating bacteria can reduce the longevity of PCE in a PCE-containing NAPL and to evaluate the transient effect of dechlorinating bacteria on the chlorinated ethenes distribution between the NAPL and aqueous phase. Experiments were conducted in continuous-flow stirred-tank reactors (CFSTRs) containing a PCE-dechlorinating enrichment culture. Results demonstrated that dechlorination could substantially reduce the longevity of PCE in NAPLs as compared to dissolution alone and that the overall impact of dechlorination within a source zone could be described mathematically using a combined dissolution-dechlorination model.

Materials and Methods

Chemicals. The following chemicals were obtained in liquid form: tetrachloroethene (99+%; Acros); trichloroethene (99.5%; Aldrich); *cis*-dichloroethene (97%; Acros); 1,1,1-trichloroethane (99.5%; Aldrich); tridecane (99%; Sigma); pentane (HPLC grade; Acros); methanol (certified ACS; Fisher); and formic acid (88%; Fisher). Vinyl chloride (8%,

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balance N_2), propane (99.95%), and nitrogen (99.999%) were prepared by TriGas. Ethene (99.5%) was procured from Scott Specialty Gases.

Nutrient Medium. Nutrient medium was prepared as outlined previously (*15*), with the following exceptions: $(NH_4)_2HPO_4$ was replaced with 140 mg/L KH_2PO_4; 0.01 M phosphate buffer was substituted for bicarbonate buffer; no reducing agents were added; and vitamins were included (final concentration of 0.04 mg/L 4-aminobenzoic acid, 0.01 mg/L D(+)-biotin, 0.1 mg/L nicotinic acid, 0.05 mg/L Ca-D(+)-pantothenate, 0.15 mg/L pyridoxamine dihydrochloride, 0.1 mg/L thiamine hydrochloride, 0.05 mg/L cyanocobalamin). Formate served as electron donor and was added to nutrient medium (pH 7) to a final concentration of 10 mM.

Analytical. Gas chromatography was used to determine aqueous phase concentrations of all chlorinated ethenes. For PCE and TCE quantification, aqueous samples (150 μL) were extracted in pentane (5 mL) and injected (1 μL) into a gas chromatograph (GC) (Hewlett-Packard) equipped with an electron capture detector (ECD). This method has been described previously, as outlined by Carr and Hughes (*15*). All samples were amended with 1,1,1-trichloroethane (final concentration of 390 μg/L) as an internal standard prior to analysis. Nominal detection limits for PCE and TCE were 12 and 59 μg/L, respectively.

Headspace analysis was used to quantify *cis*-DCE, VC, and ethene. Aqueous samples (5 mL) were added to 70 mL serum bottles capped with a Teflon-lined butyl rubber septum and aluminum crimp cap. Propane (50 μL) was added to each serum bottle as an internal standard. Following equilibration between the aqueous phase and headspace, headspace samples (100 μL) were directly injected onto a GC (Hewlett-Packard) equipped with a flame ionization detector (FID) and a packed column (6 ft. $\times$ 1/8 in OD) containing 60/80 Carbopack B/1% SP-1000 (Supelco). GC operating parameters were identical to those described previously for this analytical method (*15*). Standards were prepared by adding methanol dissolved *cis*-DCE stock solutions and known volumes of VC and ethene to 70 mL serum bottles containing 5 mL of deionized water. Nominal detection limits for *cis*-DCE and VC were 200 and 50 μg/L, respectively.

Determination of Partition Coefficients. Dimensionless partition coefficients (K_i^{o-w}) for PCE, TCE, and *cis*-DCE in tridecane were measured independently. A known mass of each chlorinated ethene was diluted in a known volume of tridecane. The mixture was added to duplicate serum bottles (15 mL) containing deionized water. The final oil-to-water ratio was approximately 0.005 (v/v) to be consistent with oil-to-water ratios used in experimental systems. The serum bottles were capped with Teflon-lined butyl rubber septa and aluminum crimp caps and were stored on a shaker table at room temperature (24 °C $\pm$ 0.57) for a period of no less than 24 h. Bottles were removed from the shaker table and allowed to sit quiescently for phase separation. Duplicate aqueous phase samples (150 μL for PCE and TCE analyses, 5 mL for *cis*-DCE analysis) were removed from each bottle using gastight syringes and analyzed by GC.

Reactor Design. Experiments were conducted in CFSTRs as depicted in Figure 1. The reactors were composed of glass bottles (600 mL) that had been modified on one side to include a glass stopcock with Teflon plug for sampling. The reactors were capped with stainless steel plugs (Rice University Support Shop) kept in place by open-top screw caps and Viton O-rings (American Packing and Gasket Co.). The effluent line extended to the bottom of the reactor to prevent NAPL washout.

Aqueous mobile phases (see protocol section) were continuously pumped into the reactors via a peristaltic pump (Cole Parmer). Reactors were operated with zero headspace.

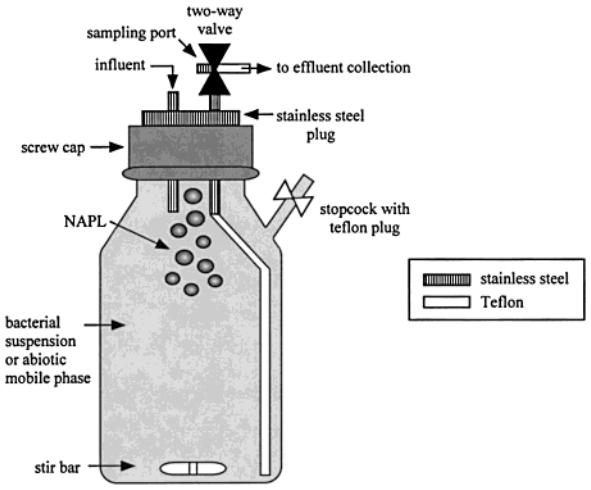

FIGURE 1. Schematic of CFSTRs used in experiments.

The flow rate was maintained at 0.14 mL/min, resulting in a hydraulic retention time (HRT) of 3 days. Reactor effluent was routed via a two-way stainless steel valve to either a sampling port or to a bottle for effluent collection.

Start-up of Biotic Reactors. Effluent from an up-flow column containing a PCE-dechlorinating enrichment culture was used to inoculate the biotic CFSTRs. The PCE-dechlorinating culture, previously described as the methanol/PCE enrichment culture (*15*), had been enriched on methanol and PCE for a period of over 5 years and had consistently dechlorinated PCE (at an influent concentration of 86 mg/L) to VC and ethene. Effluent from the column was collected, purged with N_2, and divided into two CFSTRs. Prior to the onset of experiments, each culture was maintained under continuous flow, completely mixed conditions for a period of 17 days. Nutrient medium was pumped through the systems (HRT = 3 days), and neat PCE (10 μL) was added directly to the contents of both reactors approximately every 72 h to maintain dechlorination activity. The cultures were monitored daily for dechlorination activity, extent of dechlorination, and pH.

NAPL Preparation. A mixture of tridecane and PCE was prepared to simulate a PCE-containing NAPL consisting of an insoluble and recalcitrant organic fraction. PCE was diluted in tridecane to a final weight fraction of 0.12 g PCE/g NAPL (0.13 mol PCE/mol NAPL, based on an average molecular weight of 182 g/mol). The density of the NAPL was 0.81 g/mL.

Protocol for CFSTR Experiments. Abiotic and biotic CFSTRs were run in duplicate. The contents and mobile phase of the abiotic reactors were a 0.005 M $CaCl_2$ solution. Following the initial start-up period, biotic reactors were maintained in the same fashion as the abiotic reactors with the exception of having nutrient medium as the mobile phase. NAPL was added gravimetrically to each reactor (Abiotic 1, 1.63 g; Abiotic 2, 1.67 g; Biotic 1, 1.60 g; Biotic 2, 1.65 g) at the onset of the experiment. Equilibrium dissolution was promoted by vigorous stirring, and flow through the reactors commenced immediately following NAPL addition. Equilibrium dissolution was verified in separate experiments, and it was determined that aqueous PCE concentrations reached equilibrium with the concentration of PCE in the NAPL in less than 10 min. All reactors were operated at 24 °C.

For aqueous PCE and TCE samples, flow and stirring were temporarily halted to allow NAPL to accumulate at the top of the reactor. Samples were taken through the side port using gastight syringes fitted with 6 in. stainless steel needles. Because larger sample volumes were required for *cis*-DCE, VC, and ethene quantification, these samples were collected from the sampling port at the top of the reactor during normal

TABLE 1. Dissolution-Dechlorination Model[a]

$$C_1 = C_{1,0}e^{-K_1 t} \tag{2}$$

$$C_2 = \frac{K'_1}{K_2 - K_1}C_{1,0}(e^{-K_1 t} - e^{-K_2 t}) \tag{3}$$

$$C_3 = \frac{K'_1 K'_2}{(K_3 - K_2)(K_1 - K_3)(K_1 - K_2)}C_{1,0}[(K_3 - K_2)e^{-K_1 t} + (K_1 - K_3)e^{-K_2 t} - (K_1 - K_2)e^{-K_3 t}] \tag{4}$$

$$C_4 = \frac{K'_1 K'_2 K'_3}{(K_2 - K_1)(K_3 - K_1)(K_4 - K_1)}C_{1,0}(e^{-K_1 t} - e^{-K_4 t}) + \frac{K'_1 K'_2 K'_3}{(K_1 - K_2)(K_3 - K_2)(K_4 - K_2)}C_{1,0}(e^{-K_2 t} - e^{-K_4 t}) + \frac{K'_1 K'_2 K'_3}{(K_1 - K_3)(K_2 - K_3)(K_4 - K_3)}C_{1,0}(e^{-K_3 t} - e^{-K_4 t}) \tag{5}$$

$$C_5 = \frac{K'_1 K'_2 K'_3 K'_4}{(K_2 - K_1)(K_3 - K_1)(K_4 - K_1)(K_5 - K_1)}C_{1,0}(e^{-K_1 t} - e^{-K_5 t}) + \frac{K'_1 K'_2 K'_3 K'_4}{(K_1 - K_2)(K_3 - K_2)(K_4 - K_2)(K_5 - K_2)}C_{1,0}(e^{-K_2 t} - e^{-K_5 t}) + \frac{K'_1 K'_2 K'_3 K'_4}{(K_1 - K_3)(K_2 - K_3)(K_4 - K_3)(K_5 - K_3)}C_{1,0}(e^{-K_3 t} - e^{-K_5 t}) + \left[\frac{1}{(K_2 - K_1)(K_3 - K_1)(K_4 - K_1)} + \frac{1}{(K_1 - K_2)(K_3 - K_2)(K_4 - K_2)} + \frac{1}{(K_1 - K_3)(K_2 - K_3)(K_4 - K_3)}\right]\frac{K'_1 K'_2 K'_3 K'_4}{(K_4 - K_5)}C_{1,0}(e^{-K_4 t} - e^{-K_5 t}) \tag{6}$$

[a] Subscript "0" represents the initial condition. Subscripts "1–5" correspond to PCE, TCE, *cis*-DCE, VC, and ethene, respectively. K_i and K'_i are defined as follows: $K_i = (\tau + k_i)/R_i$; $K'_i = k_i/R_{i+1}$.

CFSTR operation. Sampling was initiated 2 h following start-up, and thereafter the abiotic reactors were sampled every 12 to 24 h. The biotic reactors were sampled more frequently (every 8–24 h) to monitor dechlorination activity.

Determination of Microbial Influences on Partitioning Behavior. At the end of the experiment, partition coefficients were measured in the biotic reactors to determine whether changes in partitioning behavior had occurred as a result of microbial activity. Aqueous phase concentrations of chlorinated ethenes were quantified, and then 20 mL of additional tridecane was added to each reactor. The reactor contents were vigorously stirred to allow the chlorinated ethenes to equilibrate between the aqueous and nonaqueous phases. Concentrations of chlorinated compounds remaining in the aqueous phase were determined by GC, and partition coefficients were calculated.

Description of the Dissolution-Dechlorination Model. The reactors were modeled as completely mixed systems where dissolution of NAPL was rapid and could be described by the equilibrium condition, $C_i^o = K_i^{o-w}C_i^w$ (C_i^o and C_i^w (mol/L) represent the concentration of species i in the oil (o) and water (w) phase, respectively). Reductive dechlorination of PCE to the end product ethene was modeled by sequential pseudo-first-order reactions (i.e., constant dechlorination activity was assumed) that accounted for the production and decay of the intermediates TCE, *cis*-DCE, and VC. With this approach, a mass balance for any chlorinated ethene species (i) in the reactor yields the equation

$$R_i\frac{dC_i^w}{dt} = -\tau C_i^w + k_{i-1}C_{i-1}^w - k_i C_i^w \tag{1}$$

where

$$\tau = \frac{Q}{V^w}; \quad R_i = 1 + \frac{V^o}{V^w}K_i^{o-w}$$

t represents time (h); τ is the hydraulic retention time (h^{-1}); Q is the flow through the reactor (L/h); V^o and V^w are the volume (L) of the oil and aqueous phases, respectively; and k_i (h^{-1}) represents the pseudo-first-order dechlorination rate coefficients. R_i (dimensionless) represents the retardation coefficient of a particular chlorinated ethene, and is a function of the partition coefficient and the NAPL to water ratio (V^o/V^w) in the CFSTR. Equation 1 includes removal of species i by dissolution ($-\tau C_i^w$), generation of i due to transformation of the preceding i-1 species ($+ k_{i-1}C_{i-1}^w$), removal of i via dechlorination ($-k_i C_i^w$), and partitioning of the various species between the NAPL and aqueous phase (R_i).

As PCE and daughter products are removed from the NAPL, the volume of the NAPL would be expected to change. Because a maximum NAPL volume change of less than 6% (based on the initial volume percent of PCE in the NAPL mixture) occurred in experiments presented herein, V^o was considered to be constant and temporal changes in the retardation coefficient were neglected. Using eq 1, rate equations for all chlorinated ethenes were solved as coupled differential equations with the initial condition that the only chlorinated ethene present at $t = 0$ was PCE. The resulting analytical solutions to eq 1 for temporal variations in aqueous concentrations of PCE, TCE, *cis*-DCE, VC, and ethene comprise the dissolution-dechlorination model and are presented in Table 1. Equations 2–6 (see Table 1) were used

to fit experimental data, and values for the pseudo-first-order dechlorination rate coefficients were obtained. All model calculations were performed using SigmaPlot (version 4.0).

Results

Determination of Partition Coefficients. Dimensionless partition coefficients for PCE, TCE, and *cis*-DCE in tridecane were determined experimentally at 24 °C. The average partition coefficients from duplicate bottles were as follows: $K_{PCE}^{o-w} = 3060$; $K_{TCE}^{o-w} = 395$; and $K_{DCE}^{o-w} = 94$. Theoretical partition coefficients were calculated using Raoult's Law (NAPL activity coefficients were assumed to be unity in all cases) and aqueous solubilities taken from literature (*25*). Theoretical partition coefficients obtained from these calculations were as follows: $K_{PCE}^{o-w} = 3{,}706$; $K_{TCE}^{o-w} = 517$; and $K_{DCE}^{o-w} = 115$.

Start-up of Biotic Reactors. Cultures initially dechlorinated PCE to VC, as observed in the original enrichment culture. However, *cis*-DCE became the major reduced end product after several days of operation. By the end of the 17 day start-up period, dechlorination consistently stopped at the level of *cis*-DCE and VC was no longer detected.

CFSTR Experiments. Results from the CFSTR experiments have been summarized in Figure 2 and Table 2. Following NAPL addition in the biotic CFSTRs, a lag phase (1 day) was observed, and limited transformation of PCE occurred. Rapid dechlorination did not commence until after 24 h of operation.

Temporal aqueous phase concentrations of PCE, TCE, and *cis*-DCE are presented for the abiotic and biotic reactors in Figure 2A. The first 6 days of data have been plotted for all four CFSTRs. Aqueous PCE concentrations in the abiotic reactors demonstrated that equilibrium dissolution was maintained throughout the experiment. Decreases in aqueous PCE concentrations in the abiotic systems occurred as the mole fraction of PCE in the NAPL decreased. In the biotic reactors, aqueous PCE concentrations decreased at a faster rate than observed in the abiotic systems. Aqueous TCE concentrations, which started to rise slightly after 18 h, increased steadily until hour 54 and then decreased thereafter. Both PCE and TCE concentrations were at or below detection limits in samples taken after 135 h. Aqueous concentrations of *cis*-DCE peaked at approximately 96 h, remained near constant for a period of 24 h, and then began to decrease as dissolution and washout occurred. VC production was not observed.

The dechlorination rate coefficient in eq 2 was set to zero for calculating the predicted removal of PCE in the abiotic CFSTRs. A value of 0.79 was ascertained for r^2 based on differences between the model predictions and the average observed aqueous concentrations from the two abiotic CFSTRs. Pseudo-first-order dechlorination rate coefficients were calculated by obtaining a least-squares fit of the model to the average concentrations from the two biotic reactors. Because of the initial lag period, the rate coefficients were determined using data from hours 24 to 135 (i.e., 24 h data corresponds to $t = 0$ in eqs 2−6). The pseudo-first-order dechlorination rate coefficients obtained for PCE and TCE were $k_1 = 0.18\ h^{-1}$ ($r^2 = 0.97$) and $k_2 = 0.27\ h^{-1}$ ($r^2 = 0.42$), respectively. The *cis*-DCE pseudo-first-order dechlorination rate coefficient (k_3) was zero in eq 4 since no *cis*-DCE dechlorination was observed. Therefore, the model prediction for removal of *cis*-DCE ($r^2 = 0.89$) only included the production and dissolution of this compound.

Figure 2B presents the cumulative moles of chlorinated ethenes removed from the abiotic and biotic reactors. Data are shown for all four CFSTRs and calculations for cumulative mmoles removed (represented by lines in Figure 2B) were made by integrating the area under the model curves in Figure 2A. For the biotic cumulative model, mass removed over the first 24 h through dissolution and limited dechlorination activity were included to obtain the calculated line in Figure 2B. Despite the initial lag phase, chlorinated ethenes removal was slightly higher in the biotic reactors as compared to the abiotic reactors after 48 h (average cumulative chlorinated ethenes was 0.11 and 0.074 mmol, respectively). PCE and *cis*-DCE were the main constituents of biotic reactor effluent during this time (see Figure 2A). After this period, *cis*-DCE production rapidly increased, and the total cumulative moles removed from the biotic reactors exceeded that of the abiotic reactors by 50% to over 150%. Because *cis*-DCE dechlorination was not observed, the experiments were considered complete once PCE and TCE concentrations in the biotic reactors approached their detection limit (135 h). At this point, the experiments were stopped, and mass balances were performed in all reactors. Abiotic CFSTRs, which had been operating slightly longer than the biotic CFSTRs, were stopped at 144 h.

Temporal changes in NAPL chlorinated ethenes composition are shown in Figure 2C. NAPL composition was calculated using temporal aqueous phase concentrations and measured partition coefficients. In the abiotic CFSTRs, the PCE mole fraction in the NAPL decreased 20−24% over the duration of the experiment. In both biotic reactors, the mole fraction of PCE in the NAPL decreased from 0.13 mol PCE/mol NAPL to approximately 0.01 mol PCE/mol NAPL. Partitioning of *cis*-DCE in these systems resulted in a maximum *cis*-DCE mole fraction of 0.01 mol *cis*-DCE/mol NAPL.

Mass balance data for these experiments are presented in Table 2. In the abiotic reactors, 16−17% of the initial PCE mass was recovered in the effluent after 144 h of operation. The mass of PCE remaining in the reactors was determined for both phases, and 96% of the initial PCE mass was accounted for in Abiotic 1 and 101% in Abiotic 2. Mass balances in the biotic reactors were calculated in the same manner, and the mass recovery was 96% and 104% for Biotic 1 and Biotic 2, respectively.

After 135 h, the percent PCE removal in the biotic CFSTRs ranged from 89 to 91%, as compared to abiotic reactors in which percent PCE removal ranged from 16 to 17% (see Table 2). The percent chlorinated ethenes removal (i.e., PCE, TCE, and *cis*-DCE) in the biotic systems was 47−50%. Based on the initial mass of PCE added and the calculated pseudo-first-order dechlorination rate coefficients, the average time required to achieve 90% mass removal of total chlorinated ethenes in the biotic systems was 13 days including the 1 day lag phase. According to model predictions for the abiotic CFSTRs, 90% PCE removal would have been achieved after 77 days of continuous operation.

Determination of Microbial Influences on Partitioning Behavior. At the end of the experiment, the partition coefficient for *cis*-DCE was determined in both biotic reactors. K_{DCE}^{o-w} values were determined to be 102 in Biotic 1 and 110 in Biotic 2, which were not statistically different (95% confidence interval) from the independently measured partition coefficient reported earlier.

Discussion

The objective of this research was to demonstrate the effects of dechlorinating bacteria on the fate of PCE and its reduced end products in the presence of PCE-containing NAPLs. An integral part of this experiment was the determination of tridecane/water partition coefficients for PCE, TCE, and *cis*-DCE, as partition coefficients were critical in understanding and predicting compositional changes of NAPL in biotic systems as well as in obtaining mass balance data. Measured and theoretical partition coefficients were found to compare favorably, although some nonideality was observed for all three compounds. Partition coefficients decreased as the

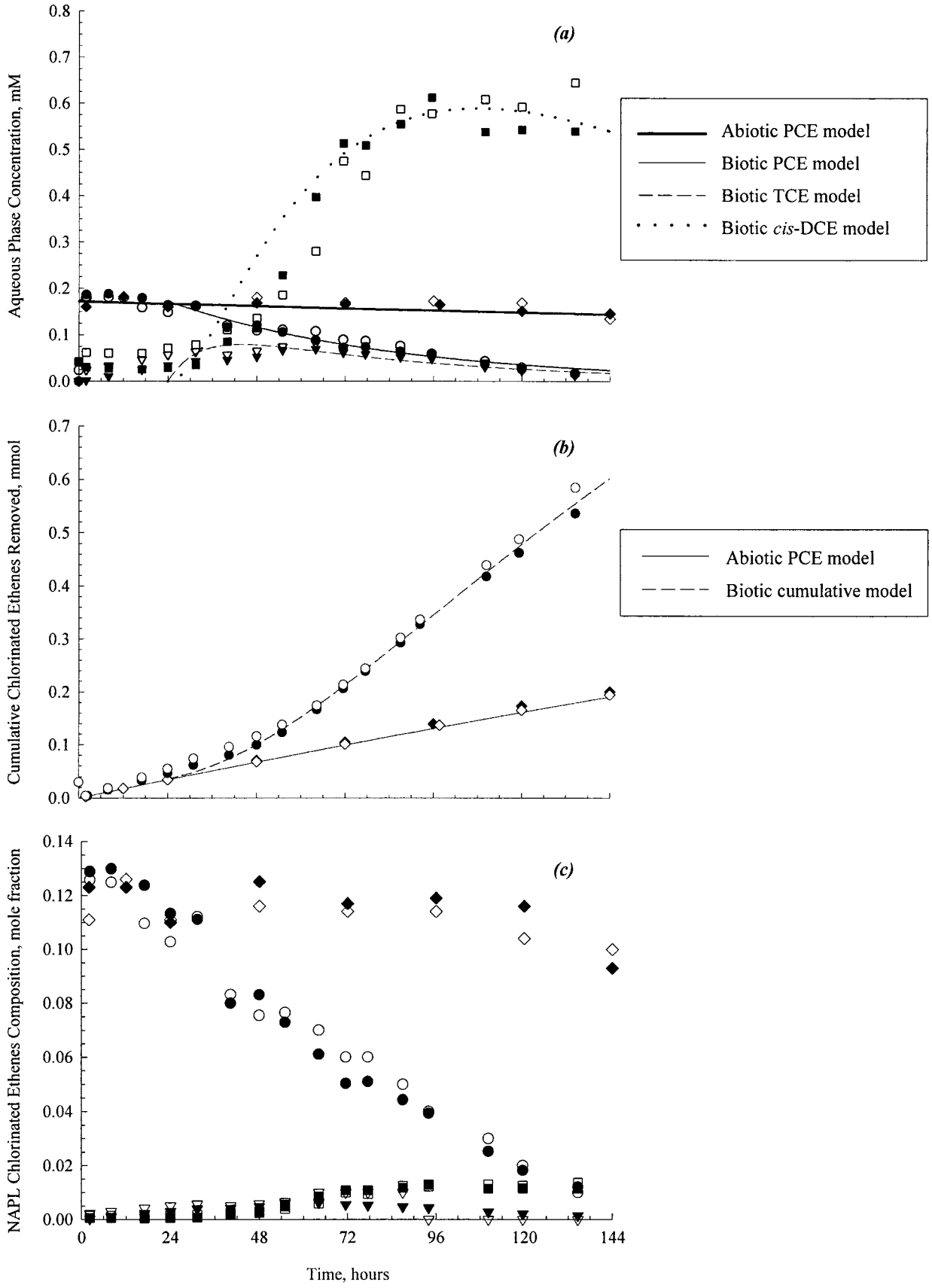

FIGURE 2. Temporal aqueous phase concentrations (a), cumulative moles of chlorinated ethenes collected from abiotic and biotic CFSTRs (b), and changes in NAPL chlorinated ethenes composition (c). In all panels, shaded symbols represent data from Biotic 1 and Abiotic 1, and hollow symbols represent data from Biotic 2 and Abiotic 2. In panels (a) and (c), PCE (●), TCE (▼), and *cis*-DCE (■) values are presented for biotic reactors, and PCE (◆) is shown for abiotic reactors. In panel (b), biotic reactors are represented by (●), and abiotic reactors are represented by (◆). Model predictions are represented by lines.

TABLE 2. Final Chlorinated Ethenes Mass Balance and Percent PCE and Chlorinated Ethenes Removal in Abiotic and Biotic CFSTRs on Day 6[a] of Operation

reactor	PCE added in NAPL (mmol)	chlorinated ethenes recovered in effluent[b] (mmol)	chlorinated ethenes remaining in reactor[c] (mmol)	PCE removal (%)	chlorinated ethenes removal (%)	mass balance (%)
Abiotic 1	1.16	0.20	0.91	17	17	96
Abiotic 2	1.19	0.19	1.01	16	16	101
Biotic 1	1.14	0.54	0.56	89	47	96
Biotic 2	1.18	0.59	0.64	91	50	104

[a] Mass balances were performed in the abiotic reactors after 144 h (6 days) of operation, and in the biotic reactors after 135 h (5.6 days) of operation. [b]The mass of chlorinated ethenes recovered in reactor effluent was determined using aqueous phase concentration data and the volume of effluent produced between samples. [c]The mass of chlorinated ethenes remaining in the reactor includes mass in both the NAPL and aqueous phases and was calculated using final aqueous phase concentration data, measured partition coefficients, and the volumes of both phases.

chlorinated ethene became more reduced (i.e., $K_{PCE}^{o-w} > K_{TCE}^{o-w} > K_{DCE}^{o-w}$), due mainly to their increasing aqueous solubilities. Thus, dechlorination in the presence of a PCE-containing NAPL will yield reduced species that partition more strongly into the aqueous phase, and an increase in the total chlorinated ethenes removal rate from the NAPL is possible.

The prepared NAPL consisted of PCE and a conservative hydrocarbon to imitate a mixed organic waste. This composition was chosen based on the observation that pure phase NAPLs are not commonly encountered and that many chlorinated solvent spills occur in the presence of fuels or other organics such as mineral spirits (*2*, *4*, *26*). BTEX and other constituents that are commonly found in mixed NAPLs and that may serve as an electron donor were omitted from the NAPL as their dissolution would have significantly increased the complexity in describing observed results and transient NAPL composition. However, experiments with NAPLs containing an internally supplied electron donor are of great field relevance and warrant investigation.

The CFSTR experiments were designed to quantitatively determine the effect of dechlorination on the rate of chlorinated ethenes removal from a PCE-containing NAPL as compared to dissolution alone. The experiment was conducted under completely mixed conditions to achieve instantaneous equilibrium dissolution of PCE and to eliminate mass-transfer limitations. Under these conditions, optimal mass depletion of PCE from the NAPL was achieved via dissolution. Dechlorination, which could not exceed the rate of dissolution in this case, represented an additional source of chlorinated ethenes removal (as indicated by the $-k_i C_i^w$ term in the dissolution-dechlorination model). Under mass-transfer limited conditions, as is commonly observed in porous media, dissolution rates may be slower allowing dechlorination to affect the driving force for dissolution by decreasing the bulk phase PCE concentration. Because the experiments and modeling presented herein are for completely mixed systems and do not take into account mass-transfer limitations, they should not be extrapolated to field sites. Experiments taking into account mass-transfer resistances resulting from flow through porous media are currently in progress.

In the biotic CFSTRs, rapid dechlorination commenced after a 24 h lag period, and aqueous PCE concentrations continuously dropped below that which was observed in abiotic controls. The dechlorinating microorganisms were able to achieve 90% PCE removal within 135 h, a removal rate approximately 14 times faster than that determined for the abiotic systems. As dechlorination occurred in the aqueous phase, TCE and *cis*-DCE were formed and equilibrated with the NAPL.

Although the biotic CFSTRs were inoculated with a PCE-dechlorinating culture that routinely dechlorinated PCE to VC and ethene, the cultures lost the ability to dechlorinate beyond the level of *cis*-DCE during the 17 day start-up period. The reason for this loss in dechlorination activity is not known. Slow growth rates have been observed in highly purified enrichment cultures of *cis*-DCE dechlorinators in this laboratory (data not shown), and it is presumed that the short hydraulic retention time in the CFSTRs resulted in the washout of these bacteria from the reactors. Toxicity of high concentrations of *cis*-DCE was not a probable explanation for the loss of *cis*-DCE dechlorination since the initial PCE-dechlorinating culture was continuously fed much higher concentrations of chlorinated ethenes than used in CFSTRs.

For modeling purposes, PCE and TCE dechlorination were modeled as pseudo-first-order processes assuming the activity of dechlorinating microorganisms was constant throughout the experiment. This approach was useful in estimating removal rates of chlorinated ethenes from non-aqueous phase liquids but cannot be used to derive microbial growth kinetics in these systems. Model calculations were found to fit PCE removal ($r^2 = 0.97$) throughout the experiment. Initial TCE and *cis*-DCE concentrations did not coincide with their respective model calculations due to the limited dechlorination that took place during the lag phase. The TCE model did not accurately fit TCE formation during 32−48 h, after which TCE concentrations were consistent with model calculations. Similar observations were made between measured *cis*-DCE concentrations and the *cis*-DCE model, which overpredicted *cis*-DCE formation until approximately 72 h. The reason for the apparent variation in dechlorination activity is not known. One possible explanation is that dechlorination rate coefficients were changing during these periods and eventually became constant. As a whole, however, model calculations were found to be representative of PCE and TCE removal and *cis*-DCE formation and dissolution. In abiotic systems, data demonstrated that effluent aqueous phase concentrations were at equilibrium with PCE concentrations in the NAPL and that measured concentrations were found to closely match ($r^2 = 0.79$) the temporal calculated values.

The cumulative millimoles of chlorinated ethenes removed in the abiotic vs biotic CFSTRs clearly demonstrates the potential impact that dechlorinating bacteria can impart on removal rates of these contaminants from NAPLs. After approximately 6 days of operation, the total chlorinated ethenes removal was approximately three times greater in the presence of a dechlorinating culture as compared to equilibrium dissolution alone. At this time, the NAPL in the biotic reactors contained *cis*-DCE as the primary chlorinated component. Because the *cis*-DCE tridecane/water partition coefficient is 30 times smaller than that for PCE, *cis*-DCE removal is comparatively much more rapid. Thus, the time required to achieve a 90% reduction in total chlorinated ethenes was 77 days in the abiotic CFSTRs and only 13 days in the biotic CFSTRs (including the 1 day lag phase)—corresponding to an 83% reduction in longevity of the chlorinated ethenes component of the source.

It is important to note that the maximum obtainable enhancement of chlorinated ethenes removal is based upon both the rate of dechlorination and the partitioning behavior of the terminal chlorinated ethene. Had TCE been the terminal chlorinated ethene in the CFSTR experiments, the reduction in source longevity would have been diminished. The opposite is true for VC, which has a calculated tridecane/water partition coefficient (K_{VC}^{o-w}) of approximately 33 (using 8400 mg/L as the aqueous solubility). Any production of VC would have decreased the longevity of the source, as VC partitions more strongly into the aqueous phase and is washed out more quickly.

Mass balance data was able to account for the majority of mass in all CFSTRs (96−104%), demonstrating that losses due to sorption or volatilization were minimal. To determine that dechlorination activity was solely responsible for the increase in chlorinated ethenes removal and that microbial activity had not influenced tridecane/water partition coefficients, K_{DCE}^{o-w} values were measured at the end of the experiment. Measured K_{DCE}^{o-w} values in the biotic reactors compared favorably with the value previously determined in the batch experiment ($K_{DCE}^{o-w} = 94$). Thus, dechlorination was assumed to be the sole contributor to the increase in observed removal rates.

In continuously stirred, continuous flow systems, it was demonstrated that dechlorinating microorganisms can impact the longevity of NAPLs as a source of chlorinated ethenes contamination by two distinct mechanisms: first, by depleting PCE from the aqueous phase, causing an increase in the overall mass-transfer of PCE from the NAPL into solution; and last, by reducing PCE to species that partitioned more strongly into the aqueous phase. It was determined that the partitioning behavior of the terminal chlorinated ethene was a key factor in assessing the longevity of the chlorinated ethenes component of the NAPL and that source longevity was diminished as dechlorination daughter products became more reduced. Because dechlorination can substantially impact removal rates of chlorinated ethenes from NAPLs, this process may be an important factor in evaluating the duration of natural attenuation and may lead to bioremediation strategies focused on source zone treatment.

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