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# Experimental Evaluation and Mathematical Modeling of Microbially Enhanced Tetrachloroethene (PCE) Dissolution

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Experiments to assess metabolic reductive dechlorination (chlororespiration) at high concentration levels consistent with the presence of free-phase tetrachloroethene (PCE) were performed using three PCE-to-*cis*-1,2-dichloroethene (*cis*-DCE) dechlorinating pure cultures (*Sulfurospirillum multivorans*, *Desulfuromonas michiganensis* strain BB1, and *Geobacter lovleyi* strain SZ) and *Desulfitobacterium* sp. strain Viet1, a PCE-to-trichloroethene (TCE) dechlorinating isolate. Despite recent evidence suggesting bacterial PCE-to-*cis*-DCE dechlorination occurs at or near PCE saturation (0.9−1.2 mM), all cultures tested ceased dechlorinating at ∼0.54 mM PCE. In the presence of PCE dense nonaqueous phase liquid (DNAPL), strains BB1 and SZ initially dechlorinated, but TCE and *cis*-DCE production ceased when aqueous PCE concentrations reached inhibitory levels. For *S. multivorans*, dechlorination proceeded at a rate sufficient to maintain PCE concentrations below inhibitory levels, resulting in continuous *cis*-DCE production and complete dissolution of the PCE DNAPL. A novel mathematical model, which accounts for loss of dechlorinating activity at inhibitory PCE concentrations, was developed to simultaneously describe PCE-DNAPL dissolution and reductive dechlorination kinetics. The model predicted that conditions corresponding to a bioavailability number (*Bn*) less than $1.25 \times 10^{-2}$ will lead to dissolution enhancement with the tested cultures, while conditions corresponding to a *Bn* greater than this threshold value can result in accumulation of PCE to inhibitory dissolved-phase levels, limiting PCE transformation and dissolution enhancement. These results suggest that microorganisms incapable of dechlorinating at high PCE concentrations can enhance the dissolution and transformation of PCE from free-phase DNAPL.

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## Introduction

Chlorinated solvents, such as tetrachloroethene (PCE) and trichloroethene (TCE), are widespread contaminants that threaten environmental and human health due to extensive use, accidental release, and improper disposal (*1*). In subsurface environments, chlorinated solvents are often present as dense nonaqueous phase liquids (DNAPLs) (*1*), which can result in persistent elution of contaminant plumes (*2*). Many technologies have been evaluated for containment and treatment of chlorinated ethene plumes, including bioremediation. Anaerobic bioremediation of chlorinated ethenes relies on metabolic reductive dechlorination (i.e., [de]chlororespiration), in which specialized bacteria obtain energy for growth from dechlorination reactions. Several microbial isolates transform PCE to *cis*-1,2-dichloroethene (*cis*-DCE) (e.g., *Desulfuromonas* spp. (*3*, *4*), *Sulfurospirillum* spp. (*5*, *6*), *Geobacter lovleyi* strain SZ (*7*), *Desulfitobacterium* spp. (*8*, *9*), *Dehalobacter* spp. (*10*, *11*), and *Clostridium bifermentans* strain DPH-1 (*12*)). Only members of the *Dehalococcoides* group have been shown to dechlorinate beyond *cis*-DCE to vinyl chloride (VC) and ethene (summarized in ref *13*). None of the *Dehalococcoides* isolates grow with all chlorinated ethenes as electron acceptors (*13*), and efficient and complete dechlorination of PCE to ethene depends on the presence of multiple dechlorinating organisms.

Biostimulation and bioaugmentation have been successfully implemented in the field to promote reductive dechlorination and to contain chlorinated ethene plumes (*13*). Since the rate of contaminant dissolution from the DNAPL source zone (i.e., the DNAPL-containing origin of the plume) remains unchanged, plume containment via bioremediation does not reduce overall remediation time. Physical−chemical remediation strategies (e.g., surfactant flushing, *in situ* chemical oxidation, thermal treatment) have been developed and employed to remove contaminant mass from source zones thereby accelerating remediation times (reviewed in ref *1*). These remediation technologies may not significantly reduce near term environmental risk since they are generally unable to remove all of the contaminant mass (*14*). Microbial reductive dechlorination within source zones provides an alternative method for source zone remediation that may be productive in isolation or as a "polishing" step following physical−chemical remediation (*15*, *16*).

Metabolic reductive dechlorination within PCE-DNAPL source zones was thought to be infeasible due to the toxicity of free-phase PCE and high PCE concentrations to dechlorinating microorganisms. The ability to grow and dechlorinate at high PCE concentrations expected near or in PCE-DNAPL source zones is not a universal characteristic of PCE-dechlorinating isolates (see Table S1, Supporting Information) but has been reported for at least seven PCE-dechlorinating isolates (see Table S1). Additional studies further indicated that mixed dechlorinating consortia can dechlorinate PCE at or near saturated concentrations (e.g., refs *17*−*24*), suggesting that biologically enhanced (bioenhanced) DNAPL dissolution is possible. In bioenhanced DNAPL dissolution, biological activity in proximity of the NAPL−water interface affects interphase mass transfer due to (i) the increase in the driving force for PCE dissolution from the NAPL to the aqueous phase as a result of reduced PCE concentrations in

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the aqueous phase, and (ii) the transformation of PCE to compounds with higher aqueous phase solubilities (e.g., DCEs) (*1*). Bioenhancement of NAPL dissolution has been observed experimentally in sand column and box experiments (*19*, *21*, *25*). Further experiments with batch cultures have demonstrated the ability of some dechlorinating cultures to transform a mixed (PCE/tridecane) NAPL (*17*, *26*) and to achieve complete PCE-DNAPL dissolution (*20*).

Efforts to model metabolic reductive dechlorination have included substrate fermentation, nonlinear dechlorination kinetics (with and without competitor, product, and self-inhibition), and growth of competitor populations (e.g., refs *22*, *27*−*30*). These models are limited to the simulation of dechlorination in single-phase systems, making interpretations of the interplay between dechlorination and dissolution kinetics in the presence of a DNAPL difficult. To date, the only batch model applied to a PCE-NAPL contaminated system assumed first-order dechlorination kinetics and equilibrium dissolution (*26*). While several multidimensional models that include dechlorination and dissolution kinetics have been developed (e.g., refs *31*−*34*), few of these models have been used to specifically investigate bioenhanced DNAPL dissolution.

For the microbial reductive dechlorination process to be widely accepted as a source zone treatment strategy applicable to a wide range of sites and environmental conditions, mechanisms controlling microbial activity in the presence of DNAPL must be elucidated. The objectives of this research were to evaluate the dechlorination performance of four pure cultures at elevated PCE concentrations and to investigate the ability of the cultures to dechlorinate in the presence of PCE DNAPL. A novel dechlorination batch model was developed and utilized to provide insight into the relationship between rate-limited mass transfer from the DNAPL and aqueous phase dechlorination kinetics.

## Materials and Methods

**Chemicals.** PCE (≥99.9%) and TCE (≥99.5%) were purchased from Sigma-Aldrich Co. (St. Louis, MO). *cis*-DCE (99.9%) and *trans*-1,2-dichloroethene (*trans*-DCE, 99.9%) were obtained from Supelco Co. (Bellefonte, PA). Methanol (≥99.9%) was obtained from Fisher Scientific (Hampton, NH). All of the other chemicals used were reagent grade or better unless otherwise specified.

**Cultures and Medium Preparation.** The following pure cultures were used in this study: *Desulfuromonas michiganensis* strain BB1 (DSM 15941, (*4*)), *S. multivorans* (DSM 12446, (*5*, *6*)), *Geobacter lovleyi* strain SZ (DSM 17278, (*7*)), and *Desulfitobacterium* sp. strain Viet1 (*35*, *36*). Reduced anaerobic mineral salts medium was prepared as described (*37*) with the following modifications: $KH_2PO_4$, 0.2 mM; resazurin, 1 $\mu$M; $Na_2S{\cdot}9H_2O$, 0.05 mM (unless otherwise noted); dithiothreitol, 0.5 mM; anhydrous L-cysteine hydrochloride, 0.22 mM; and TES (*N*-tris[hydroxymethyl]methyl-2-aminoethane-sulfonic acid), 10 mM. Vitamins (200-fold concentrated) and $KH_2PO_4$ (200 mM) were added from sterile, anoxic stock solutions after autoclaving. The final vitamin concentrations were as described (*37*), except vitamin $B_{12}$ was at twice the concentration. Serum bottles (160 mL nominal capacity, Wheaton Co., Millville, NJ) were prepared with a $N_2/CO_2$ (80%/20% [vol/vol]) headspace and sealed with Teflon-lined, gray butyl rubber septa (1014-4937, West Pharmaceuticals, Lionville, PA) and aluminum crimp caps (Wheaton Co., Millville, NJ). PCE was added as described below, sterile hydrogen gas was added via syringe using a 25 gauge needle (where indicated), and acetate or pyruvate were added to individual bottles from sterile, anoxic stock solutions with a syringe using a 25 gauge needle. The final aqueous volume following all amendments was 101 ± 1 mL.

**Determination of Dissolved-Phase PCE Tolerance.** In this study, PCE tolerance is defined as the ability to dechlorinate dissolved-phase PCE completely to the strain-specific dechlorination end product (i.e., TCE, *cis*-DCE). In the dissolved-phase PCE tolerance experiments, culture vessels received PCE dissolved in a water miscible carrier phase (i.e., methanol) to facilitate the immediate availability of dissolved-phase PCE to the microorganisms. Preparation of anoxic methanol and the anoxic PCE/methanol stock solution (350 $\mu$L PCE/15 mL methanol) is described in the Supporting Information. Bottles containing medium were amended with the anoxic PCE/methanol stock solution (0.2 mL) and allowed to equilibrate for 2 days, resulting in measured initial aqueous phase PCE concentrations of 240−310 $\mu$M after equilibration (i.e., partitioning between the headspace and aqueous phases). Electron donors and/or carbon sources were added as described above, were in excess throughout the experiment, and varied for each culture: acetate (5 mM) for strain BB1, acetate (5 mM) and hydrogen (10 mL) for *S. multivorans* and strain SZ, and pyruvate (5 mM) and hydrogen (10 mL) for strain Viet1. Each culture was initiated with a 5% (vol/vol) inoculum from a dechlorinating culture grown with PCE (0.33 mM) as the electron acceptor. After significant dechlorination (>80%) of the initial amount of PCE, a series of bottles received different volumes from the PCE/methanol stock solution (aqueous phase PCE concentrations after equilibration are given in parentheses): 0.2 mL (295 ± 10 $\mu$M), 0.4 mL (560 ± 45 $\mu$M), 0.6 mL (830 ± 15 $\mu$M), 0.8 mL (1050 ± 5 $\mu$M), or 1.0 mL (1200 ± 100 $\mu$M). The aqueous methanol concentration was adjusted to 1.2% (vol/vol) in all bottles. Exploratory experiments demonstrated that the final methanol concentration did not affect culture performance (i.e., toxicity), pH, or analyte partitioning. Cultures were immediately shaken by hand and sampled after amendment with the PCE/methanol solution and/or pure methanol. Cultures were incubated upright at 22 °C on a platform shaker at 175 rpm.

**Dechlorination Studies in the Presence of PCE DNAPL.** Dechlorination studies in the presence of PCE DNAPL were performed with strain BB1, strain SZ, and *S. multivorans*. Electron donors and/or carbon sources were added as described above and varied for each culture: acetate (5 mM) for strains BB1 and SZ and pyruvate (5 mM) and hydrogen (5 mL) for *S. multivorans*. In these experiments, the concentration of $NaHCO_3$ was increased to 60 mM to increase the buffering capacity, and the concentrations of $Na_2S$ and L-cysteine were increased to 0.1 mM and 0.27 mM, respectively. Serum bottles containing medium were amended with 0.2 mL of the anoxic PCE/methanol stock solution and allowed to equilibrate for 2 days to yield initial aqueous phase PCE concentrations of approximately 300 $\mu$M. The bottles received a 5% (vol/vol) inoculum of a dechlorinating culture grown with PCE (0.33 mM) as the electron acceptor. Triplicate cultures of strain BB1, strain SZ, and *S. multivorans* were incubated at 22 °C and shaken upright at 150 rpm on a platform shaker. Following complete dechlorination of the initial PCE amendment, the cultures were amended with an additional 0.2 mL of the anoxic PCE/methanol stock solution. After complete dechlorination of dissolved-phase PCE, a 500 $\mu$L gastight syringe (1750, Hamilton Co., Reno, NV) was used to amend the bottles with 50 $\mu$L of neat PCE to form a single PCE droplet (DNAPL). Control cultures were inactivated by the addition of sterile $O_2$ gas (10 mL) after growth of *S. multivorans* on dissolved-phase PCE but before amendment of PCE DNAPL. Additions of hydrogen gas (10 mL at Day 2, 5 mL for all other amendments) to the cultures of *S. multivorans* were performed on Days 2, 4, 5, 6.5, 7, 10, 12, and 15 after DNAPL addition. The experiment with *S. multivorans* was repeated in duplicate vessels shaken at increased agitation (175 rpm), which was assumed to increase

the rate of PCE dissolution from the DNAPL phase. The *cis*-DCE production following DNAPL addition was determined by subtracting the *cis*-DCE aqueous-phase concentration present before DNAPL addition from the *cis*-DCE concentrations measured following DNAPL addition.

**Analytical Methods.** Chlorinated ethenes were quantified via gas chromatography. Aqueous-phase samples (1 mL) were analyzed on a Hewlett-Packard (HP) 7694 headspace autosampler and a HP 6890 gas chromatograph (GC) equipped with a HP-624 column (60 m by 0.32 mm; film thickness, 1.8 $\mu$m nominal) and a flame ionization detector (FID). Standards for chlorinated ethenes were prepared as described (*38*, *39*). Additional information on sample and standard preparation as well as GC and autosampler operational parameters is provided in the Supporting Information.

**Model Development.** Although Michaelis−Menten kinetics have been used successfully to describe the dechlorination process in single-phase batch systems (*27*, *29*, *40*), few models have considered metabolic reductive dechlorination in multiphase systems. Carr et al. (*26*) simulated reductive dechlorination of PCE in a mixed NAPL (PCE/tridecane) system using first-order dechlorination kinetics and assuming equilibrium between the aqueous and nonaqueous phases. To better capture the interplay between experimentally observed contaminant dissolution and dechlorination rates in batch culture vessels, rate-limited dissolution and mixed-order reductive dechlorination kinetics were employed in the present work.

Rate-limited PCE-DNAPL dissolution, $E_{ao}^{PCE}$ (M/T), was modeled using a unidirectional linear driving force approximation (*41*):

$$E_{ao}^{PCE} = -\frac{d(V_o\rho_o)}{dt} = \begin{cases} \kappa^{PCE} a_{ao}(C_{ao}^{PCE-e} - C_a^{PCE}) & \text{if } C_{ao}^{PCE-e} \geq C_a^{PCE} \\ 0 & \text{otherwise} \end{cases} \quad (1)$$

where $V_o$ ($L^3$) is the PCE DNAPL volume, $\rho_o$ ($M/L^3$) is the PCE DNAPL density, $\kappa^{PCE}$ (L/T) is the mass transfer coefficient for PCE through the aqueous-phase boundary layer, $a_{ao}$ ($L^2$) is the interfacial area between the aqueous and organic phases, $C_a^{PCE}$ ($M/L^3$) is the PCE concentration in the bulk aqueous phase, and $C_{ao}^{PCE-e}$ ($M/L^3$) is the PCE concentration in the aqueous phase at equilibrium with the organic phase, which was measured experimentally. Partitioning of reductive dechlorination products (e.g., *cis*-DCE) from the aqueous to the organic phase was assumed negligible due to the relatively small volume of DNAPL (≤50 $\mu$L) used in the batch experiments and the increased aqueous solubility of the dechlorination products. The aqueous−organic interfacial area ($a_{ao}$) was modeled using a diminishing sphere model (*42*), assuming a single DNAPL sphere based on visual observation.

Reductive dechlorination kinetics were based on previously proposed models (*27*, *29*) to account for the growth of PCE-to-*cis*-DCE dechlorinating organisms due to the dechlorination of aqueous-phase PCE to *cis*-DCE with intermediate formation of TCE. Competitive inhibition was modeled following Yu and Semprini (*22*) and product inhibition was neglected (*30*). Self-inhibition at high PCE concentrations was modeled after Luong (*43*). Unlike other self-inhibition models (e.g., Haldane) that have been used successfully to model the gradual decline in transformation rates as substrate concentration increases (*22*), experimental results presented here suggest that dechlorination ceased when PCE concentrations exceeded a maximum tolerable level for the tested cultures. The selected self-inhibition model better represents this terminal inhibition concentration and eliminates the need to quantify another kinetic parameter (i.e., inhibition constant) (*43*). Organic substrate fermentation was not modeled since electron donating substrates (i.e., $H_2$, acetate, or pyruvate) were directly supplied in the medium. Similarly, competitor populations (e.g., methanogens) were ignored since pure cultures were used. Reaction kinetics were described using a modified dual Michaelis−Menten model:

$$R_a^{c'} = k_{max}^c X\left(\frac{C_a^c}{K_S^c \cdot I^c + C_a^c}\right)\left(\frac{C_a^{ED} - C_a^{ED-thresh}}{K_S^{ED} + (C_a^{ED} - C_a^{ED-thresh})}\right) I_T^c \quad (2)$$

where $R_a^{c'}$($M/L^3$-T) is the component $c$ reaction in the aqueous phase, $C_a^c$ ($M/L^3$) is the concentration of chlorinated ethene $c$ in the aqueous phase, $C_a^{ED}$ ($M/L^3$) is the concentration of electron donor in the aqueous phase, $C_a^{ED-thresh}$ ($M/L^3$) is the electron donor utilization threshold concentration (*27*), $k_{max}{}^c$ ($M/M_x$-T) is the maximum utilization rate for chlorinated ethene $c$, $K_S^c$($M/L^3$) is the half-saturation constant for chlorinated ethene $c$, $K_S^{ED}$ ($M/L^3$) is the half-saturation constant for electron donor, $I^c = (1 + (C_a^{c\pm1})/K_S^{c\pm1})$ is the competitive inhibition coefficient for chlorinated ethene $c$, which accounts for competition between PCE and TCE for reducing equivalents (*22*), where $C_a^{c\pm1}$ ($M/L^3$) and $K_S^{c\pm1}$ ($M/L^3$) are the concentration and half-saturation constant for the inhibiting substrate, TCE or PCE, when component $c$ is PCE or TCE, respectively, $I_T^c = (1 - (C_a^c)/C_{a-\max}^c)$ is the substrate inhibition coefficient for chlorinated ethene $c$, which accounts for inhibition at chlorinated ethene (i.e., PCE) concentrations approaching the maximum concentration ($C_{a-max}^c$) at which dechlorination will occur (*43*), and $X$ ($M_x/L^3$) is the cell titer (subsequently referred to as biomass concentration) of the PCE-to-*cis*-DCE dechlorinating bacteria, computed assuming growth due to PCE and TCE dechlorination:

$$\frac{dX}{dt} = Y^{PCE}R_a^{PCE'} + Y^{TCE}R_a^{TCE'} - k_bX \quad (3)$$

where $Y^c$ ($M_x/M$) is the yield of biomass due to the reduction of component $c$, and $k_b$ ($T^{-1}$) is the first-order endogenous decay. Equations 2 and 3 assume that microbial activity (i.e., dechlorination) takes place only in the aqueous phase. Note that for PCE, $R_a^{PCE} = R_a^{PCE'}$, while for TCE and *cis*-DCE, $R_a^{TCE} = R_a^{PCE'} - R_a^{TCE'}$ and $R_a^{DCE} = R_a^{TCE'}$.

Typically, when simulating metabolic reductive dechlorination in batch systems, component mass balance equations are selected to model only the change in mass due to transformation reactions. The aqueous phase volume is assumed to remain constant and the presence of a gas phase is incorporated using Henry's law. While assuming equilibrium between the gas and aqueous phase is appropriate here, temporal changes in the aqueous and gas phase volumes due to sampling must be considered. The component $c$ mass balance equation over all system phases is

$$\frac{d(V_aC_a^c)}{dt} + \frac{d(V_gC_g^c)}{dt} + \frac{d(V_oC_o^c)}{dt} = V_aR_a^c + Q_a^c \quad (4a)$$

Substituting eq 1 for temporal changes in DNAPL mass and incorporating the change in aqueous volume due to sampling then yields

$$\frac{dC_a^c}{dt} = \frac{E_{ao}^c + V_aR_a^c + C_a^cQ_a(1 - K_H^c) + Q_a^c}{(V_a + V_gK_H^c)} \quad (4b)$$

where $C_g^c$ and $C_o^c$ ($m/L^3$) are the gas and organic phase concentrations of chlorinated ethene $c$, respectively, $V_a$, $V_g$, and $V_o$ ($L^3$) are the aqueous, gas, and organic phase volumes, respectively, $K_H^c$ is the non-dimensional Henry's constant,

$Q_a$ ($L^3$/T) is the volumetric rate of aqueous phase sampling, and $Q_a^c$ (M/T) is the mass rate of component $c$ sampling, which were estimated from the experimental sampling frequency and mass balance. To facilitate comparison of model and experimental results, the initial mass of total chlorinated ethene in the model was reduced to equal the total experimental chloroethene mass minus experimental mass balance errors. Equation 4b accounts for changes in the aqueous phase concentration of component $c$ due to (i) interphase mass transfer (first term), (ii) reductive dechlorination (second term), (iii) changes in reactor phase volumes due to aqueous phase sampling (third term), and (iv) component $c$ mass lost due to sampling (fourth term). Substituting eqs 1 and 2 into eq 4b permits the simulation of the aqueous phase concentration of component $c$ in the culture vessel. The model was used to simulate concentrations of the electron donor, PCE, TCE, *cis*-DCE, and the PCE-to-*cis*-DCE dechlorinating population, as well as aqueous, gas, and organic phase volumes.

Equations 1, 2, and 4b represent a system of coupled nonlinear ordinary differential equations. In the simulations, the equations were solved using a high-order explicit finite-difference routine (fourth-order Runge−Kutta) that is part of the high-level technical computing framework, Matlab (version 7.0) (*44*). This solution routine was selected based on results from a recent numerical investigation that compared various solution routines for a similar system of equations (*45*). Best-fit model parameters were obtained by coupling this solution routine with an optimization algorithm that employed derivative-based nonlinear optimization implemented using the FMINCON function in Matlab (version 7.0) to minimize the Nash−Sutcliffe index objective function (*46*). The Nash−Sutcliffe index was selected because of its superior performance over alternative objective functions (e.g., sum of squared errors [SSE]) when multiple components with differing magnitudes are considered (*46*). Trial-and-error and simplex optimization algorithms were also employed with the Nash−Sutcliffe index and the SSE objective functions using multiple initial guesses within the range of values found in the literature to confirm the uniqueness of best-fit model parameters. Calculation of two-parameter correlation coefficients (e.g., $k_{max}^{PCE}/K_s^{PCE}$) following the method of Smith et al. (*47*) confirmed unique parameters were likely obtained ($0.2 < \rho < 0.7$).

Numerical simulations of dissolved-phase PCE experimental results (e.g., Figure 1A) were first used to obtain best-fit initial biomass concentrations ($X_0$) and dechlorination kinetic parameters ($k_{max}^{PCE}$, $k_{max}^{TCE}$, $K_S^{PCE}$, $K_S^{TCE}$). While it is often difficult to fit the biomass concentration and maximum utilization rate simultaneously, assumption of a yield value from the literature combined with the simulation of a non-steady biomass enabled the estimation of $X_0$, $k_{max}^{PCE}$, and $k_{max}^{TCE}$ simultaneously. The accuracy of the predicted best-fit initial biomass concentration for each strain was verified by comparison to data from 16S rRNA gene-targeted quantitative real-time PCR analysis on cultures grown under identical conditions (data not shown). Best-fit model parameters were then used, in conjunction with a PCE mass transfer coefficient ($\kappa^{PCE}$) independently derived from an abiotic experiment, to simulate the reductive dechlorination process in the presence of dissolving PCE DNAPL.

## Results

**Dissolved-Phase PCE Tolerance.** Experiments conducted with dissolved-phase PCE indicated that all four pure cultures tested were unable to dechlorinate when aqueous PCE concentrations exceeded ca. 540 $\mu$M. Figure 1 shows representative results for the dechlorination performance of *Desulfuromonas michiganensis* strain BB1 in the presence of dissolved-phase PCE at tolerable and intolerable concentrations. All cultures were established with low PCE concentrations initially (Days 0−2, Figure 1), which was followed by PCE conversion to *cis*-DCE and trace levels (<2.1 $\mu$M) of *trans*-DCE, with the formation of TCE as an intermediate (Figure 1A and B). After amendment of additional PCE to a concentration of 295 ± 10 $\mu$M on Day 2, strain BB1 dechlorinated PCE to *cis*-DCE (Figure 1A). In a parallel experiment (Figure 1B), the concentration of PCE after the Day 2 addition was increased to 690 ± 10 $\mu$M. The aqueous phase concentration of PCE decreased to 560 ± 45 $\mu$M by Day 3 at which point the concentration of PCE remained relatively constant for the duration of the experiment. The initial decrease in PCE between Days 2 and 3, which also occurred when higher PCE concentrations were tested (data not shown), was attributed to partitioning of PCE from the aqueous phase to the headspace. Formation of TCE and accumulation of *cis*-DCE did not occur (Figure 1B, Days 2−6), indicating that strain BB1 did not dechlorinate PCE at the tested concentration. Additional experiments at higher PCE concentrations confirmed that strain BB1 could not dechlorinate PCE at concentrations >560 $\mu$M (data not shown).

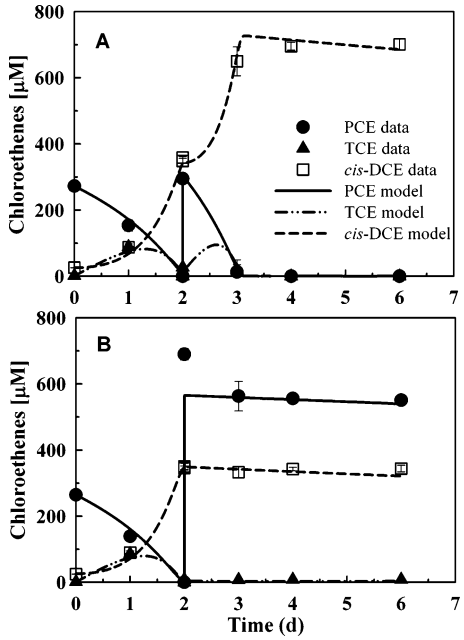


**FIGURE 1. Dechlorination performance of *Desulfuromonas michiganensis* strain BB1 at various PCE concentrations (see Materials and Methods section for details). Low PCE concentrations were used for all initial conditions (Days 0−2), followed by amendment of additional PCE to tolerable (A) or intolerable (B) PCE concentrations. The legend is shared between A and B. All data points represent average values from triplicate cultures, and error bars represent one standard deviation. If no error bars are shown, the standard deviations were too small to be illustrated. The lines represent simulated chlorinated ethene concentrations fitted (Figure 1A) or predicted (Figure 1B) by the developed mathematical model.**

Results obtained for the other three pure cultures tested were similar in that dechlorination ceased or was severely inhibited at PCE concentrations ≥540 $\mu$M (data not shown). *S. multivorans* and *Geobacter lovleyi* strain SZ dechlorinated PCE to *cis*-DCE at PCE concentrations ≤330 $\mu$M but did not dechlorinate PCE to *cis*-DCE at PCE concentrations ≥540 $\mu$M. Limited dechlorination (<25% of the initial PCE mass) of PCE to TCE occurred with strain SZ at PCE concentrations of ca. 540 $\mu$M (13 day incubation, TCE production ceased on Day 8; data not shown). *Desulfitobacterium* sp. strain Viet1 dechlorinated PCE to TCE at PCE concentrations ≤330 $\mu$M, while only limited (<5%) accumulation of TCE occurred at

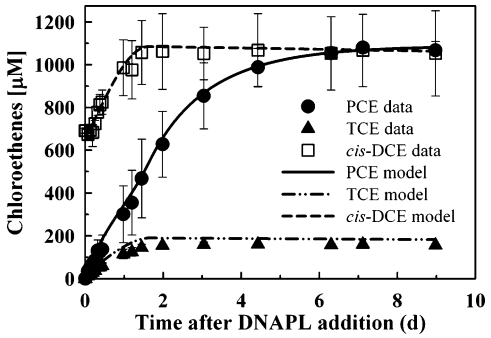


**FIGURE 2. Dechlorination performance of triplicate cultures of *Desulfuromonas michiganensis* strain BB1 in the presence of PCE DNAPL. All data points represent average values from triplicate cultures, and error bars represent one standard deviation. If no error bars are shown, the standard deviations were too small to be illustrated. The lines represent simulated chlorinated ethene concentrations predicted by the developed mathematical model.**

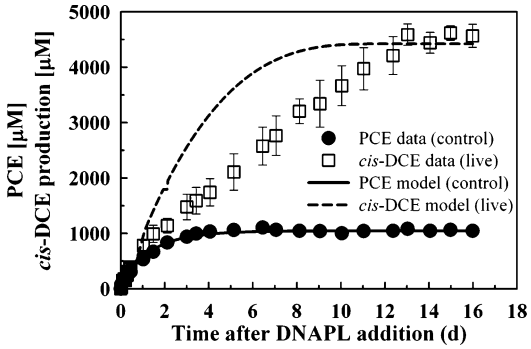


**FIGURE 3. Dechlorination performance of *S. multivorans* cultures in the presence of PCE DNAPL. *cis*-DCE production was calculated by subtracting the *cis*-DCE aqueous-phase concentration present before DNAPL addition from the *cis*-DCE aqueous-phase concentration measured following DNAPL addition and is presented to aid in comparing the initial rate of *cis*-DCE accumulation to the rate of PCE dissolution in the inactivated controls. All data points represent average values from triplicate cultures shaken at 150 rpm, and error bars represent one standard deviation. If no error bars are shown, the standard deviations were too small to be illustrated. The lines represent simulated chlorinated ethene concentrations fitted (inactivated control data) or predicted (live data) by the developed mathematical model.**

PCE concentrations of ca. 540 $\mu$M; PCE dechlorination did not occur with strain Viet1 at PCE concentrations >540 $\mu$M.

**Dechlorination in the Presence of PCE DNAPL.** The dechlorination performance of *Desulfuromonas michiganensis* strain BB1 in the presence of PCE DNAPL is shown in Figure 2. Strain BB1 dechlorinated dissolved-phase PCE (two amendments of 300 $\mu$M each) completely to *cis*-DCE and trace levels of *trans*-DCE (<4 $\mu$M) (data not shown), before the addition of PCE DNAPL. After the addition of PCE DNAPL, PCE dissolution resulted in accumulation of PCE in the aqueous phase (Figure 2). Initially (before Day 2 following DNAPL addition), the cultures accumulated TCE, *cis*-DCE, and trace amounts of *trans*-DCE (~155, ~1050, and <5 $\mu$M, respectively). Dechlorination ceased after 1.4 days when the PCE concentration reached 470 ± 130 $\mu$M (Figure 2). PCE continued to accumulate in the aqueous phase until equilibrium conditions (1070 ± 40 $\mu$M) were reached around Day 6. DNAPL was visible at the conclusion of the experiment. Similar results were observed with strain SZ in the presence of PCE DNAPL, although greater amounts of TCE (310 ± 25 $\mu$M) and *cis*-DCE (1260 ± 180 $\mu$M) accumulated in the aqueous phase after addition of PCE DNAPL (data not shown).

The dechlorination performance of *S. multivorans* in the presence of PCE DNAPL is shown in Figure 3. *S. multivorans* dechlorinated dissolved-phase PCE (two amendments of 300 $\mu$M each) completely to *cis*-DCE and trace levels of *trans*-DCE (<5 $\mu$M) (data not shown), before the addition of PCE DNAPL. For the inactivated controls, PCE dissolution from the DNAPL phase resulted in equilibrium conditions and saturated PCE concentrations (1040 ± 40 $\mu$M) around Day 4 (Figure 3). In the inactivated controls, dechlorination did not occur and the DNAPL droplet remained visible throughout the entire experiment. In live cultures, *S. multivorans* dechlorinated PCE and produced *cis*-DCE (Figure 3) and *trans*-DCE (data not shown). *cis*-DCE and *trans*-DCE accumulated until Day 13, at which time the production of *cis*-DCE and *trans*-DCE leveled off at 4600 ± 200 $\mu$M and 28 ± 2 $\mu$M, respectively. Unlike similar experiments with strain BB1 (Figure 2) and strain SZ (data not shown), PCE did not accumulate in the aqueous phase when *S. multivorans* was grown in the presence of PCE DNAPL. PCE was detected in six of the twelve samples taken before or on Day 3, five of which were at levels <6 $\mu$M, while TCE was only detected in two samples at concentrations <40 $\mu$M. PCE and TCE were not detected in any sample after Day 3. The DNAPL droplet decreased in size (visual observation) throughout the experiment and was completely dissolved after 13 days in the live cultures, corresponding to the time at which *cis*-DCE production ceased. When the experiment with *S. multivorans* was repeated with an increased agitation speed (175 rpm), which presumably increased the rate of PCE dissolution from the DNAPL, transient accumulation of PCE and TCE to maximum aqueous phase concentrations of 124 and 118 $\mu$M was observed at 3 and 6 h after DNAPL addition, respectively (see Figure S1, Supporting Information).

**Model Simulations.** Model simulations of dissolved phase chlorinated ethene concentrations are shown in Figures 1−3. Kinetic coefficients ($k^{PCE}{}_{max}$, $k^{TCE}{}_{max}$, $K_S^{PCE}$, $K_S^{TCE}$, and $X_0$) for reductive dechlorination (summarized in Table 1) were obtained by fitting the model to measured dissolved-phase PCE concentrations for strain BB1 (Figure 1A) and *S. multivorans* (data not shown) using the described optimization method, assuming all other parameters from the literature (see Table 1).

The results shown in Figure 1 demonstrate that the fitted model provided an accurate representation of the concentration data from the dissolved-phase experiments. Incorporation of a substrate inhibition expression correctly predicted the cessation of dechlorinating activity when PCE concentrations approached the experimentally derived inhibitory PCE concentration (i.e., ~540 $\mu$M for strain BB1, Figure 1B). Differences between the simulated and experimental PCE concentrations following amendment of additional PCE on Day 2 (Figure 1B) stem from the model assumption that equilibrium between the gas and aqueous phases occurred instantaneously, while the experimental results indicate a short period (<1 day) of nonequilibrium. The gradual decline of the simulated *cis*-DCE and PCE concentrations after Day 3 (Figure 1A and B, respectively) is due to the model assumption that the $Q_a$ and $Q_a{}^c$ (i.e., losses due to sampling) occur throughout the entire experiment rather than at discreet sampling times.

The model accurately predicted dechlorination in the presence of PCE DNAPL using the best-fit kinetic coefficients obtained from the dissolved-phase simulations and a best-fit mass transfer coefficient ($\kappa^{PCE} = 1.84 \pm 0.03$ m/d, $C_{ao}{}^{PCE-e} = 1043 \pm 2$ $\mu$M) obtained by fitting the model with $X_0 = 0$ mg/L to PCE dissolution from the PCE DNAPL in the inactivated control (Figure 3). Initial biomass concentrations were assumed to be equal to the initial biomass concentration from the dissolved-phase experiments (see Table 1). Biomass growth due to the supply of dissolved-phase PCE concentra-

**TABLE 1. Summary of Metabolic Reductive Dechlorination (Chlororespiration) Kinetic Parameters[a]**

| Culture | $k_{max}^{PCE}$ ($\mu$mol mg$^{-1}$ d$^{-1}$) | $k_{max}^{TCE}$ ($\mu$mol mg$^{-1}$ d$^{-1}$) | $K_S^{PCE}$ ($\mu$M) | $K_S^{TCE}$ ($\mu$M) | $X_0$ (mg L$^{-1}$) |
|---|---|---|---|---|---|
| *Sulfurospirillum multivorans* | 233.7 $\pm$ 7.9 | 258.8 $\pm$ 24.2 | 13.5 $\pm$ 3.6 | 15.1 $\pm$ 5.4 | 2.5 $\pm$ 1.7 |
| *Desulfuromonas michiganensis* strain BB1 | 128.9 $\pm$ 2.2 | 335.0 $\pm$ 36.8 | 14.2 $\pm$ 2.1 | 23.4 $\pm$ 5.2 | 1.8 $\pm$ 0.8 |

[a] Parameters assumed from the literature (*29*) include: $Y^{PCE} = Y^{TCE} = 4.86 \times 10^{-3}$; $K_S^{ED} = 0.02$ $\mu$M; $C_a^{ED-thesh} = 0.002$ $\mu$M; and $k_b = 0.024$ d$^{-1}$. $C_{a-max}^{PCE}$ was assumed to be 542.8 $\mu$M based on experimental results.

tions below inhibitory values was simulated to estimate the biomass concentration at the time of PCE DNAPL addition (data not shown). For strain BB1, the model predicted PCE dechlorination to TCE and *cis*-DCE before accumulation of PCE in the aqueous phase to inhibitory concentrations (~540 $\mu$M), as was observed experimentally (Figure 2). Furthermore, the model correctly predicted that the dechlorination activity of *S. multivorans* would prevent significant PCE accumulation, resulting in accumulation of *cis*-DCE and ultimately the complete dissolution of the PCE DNAPL, as was observed experimentally (Figure 3). Although the simulated rate of *cis*-DCE accumulation slightly exceeded the observed value, the simulated and experimental time to complete dissolution of the PCE DNAPL was approximately the same (13 days after DNAPL addition). The difference between the measured and predicted rates of *cis*-DCE accumulation could be attributed to (i) assumptions used to model dissolution (e.g., diminishing sphere model), (ii) changes in the dissolution rate due to microbial growth on or near the surface of the DNAPL (*48*), (iii) inhibition of microbial activity at increasing *cis*-DCE concentrations (*4*, *17*), and/or (iv) changes in PCE dissolution rates from the NAPL due to partitioning of *cis*-DCE into the NAPL (*17*, *26*).

## Discussion

Microbially enhanced PCE-DNAPL dissolution has been suggested as a productive PCE and TCE source zone remedy (*17*−*21*, *25*, *26*). Several studies with pure cultures (see Table S1, Supporting Information) and with mixed dechlorinating consortia (e.g., *17*−*24*) have shown that bacterial PCE dechlorination occurs at or near saturated PCE concentrations. For instance, *Desulfuromonas michiganensis* strain BB1 was reported to dechlorinate at saturated PCE concentrations and to grow in the presence of PCE DNAPL (*4*). The results presented here demonstrate that strain BB1 cannot dechlorinate at PCE concentrations above 540 $\mu$M, and similar observations were made for other PCE-to-TCE and PCE-to-*cis*-DCE dechlorinating organisms (i.e., *Sulfurospirillum multivorans*, *Geobacter lovleyi* strain SZ, and *Desulfitobacterium* sp. strain Viet1). The discrepancies in the inhibitory PCE concentrations determined here for strain BB1 and previous results (*4*) are likely due to differences in the experimental methodology including (i) the use of Teflon-lined septa as opposed to butyl rubber stoppers to minimize contaminant loss due to sorption, and (ii) the addition of PCE in a soluble carrier phase (i.e., methanol) to ensure rapid PCE dissolution and availability to the organisms. In the study by Sung et al. (*4*), PCE was added undiluted (i.e., neat) or in a separate-phase insoluble carrier (i.e., hexadecane). The addition of PCE using these techniques is appropriate as long as adequate time is given to ensure equilibrium conditions before initiation of the experiment; otherwise, initial conditions will be at nonequilibrium, yielding PCE concentrations that could be significantly below targeted values. DNAPL dissolution is a slow process, and time is required to establish equilibrium conditions (see Figure 3). The inhibitory PCE concentrations reported in this study for microbial isolates of different phylogenetic lineages accurately reflect dissolved-phase PCE concentrations and demonstrate that the cultures tested cannot dechlorinate PCE at or near the aqueous solubility of PCE. Similar, carefully conducted studies should be performed with other PCE-dechlorinating bacteria (see Table S1) to elucidate culture-specific inhibitory PCE concentrations.

Although the bacterial isolates included in this study failed to dechlorinate at saturated PCE concentrations, these organisms impacted the dissolution and transformation of PCE from free-phase DNAPL. Microbial activity in the presence of PCE DNAPL occurred as long as aqueous PCE concentrations remained below inhibitory concentrations (i.e., the system was not in equilibrium). Such activity might explain one mechanism by which biologically enhanced dissolution occurs with dechlorinating microorganisms incapable of dechlorinating at high or saturated PCE concentrations. The ability to dechlorinate in the presence of DNAPL, resulting in complete DNAPL dissolution and transformation, is likely not restricted to *S. multivorans* but may be observed with the other tested dechlorinating organisms (i.e., strains BB1 and SZ) under the appropriate conditions (e.g., higher biomass concentrations, decreased PCE dissolution rates, attenuation of PCE to non-inhibitory levels due to other processes [e.g., sorption, dispersion]).

In our experiments, the detection of low, transient PCE concentrations in the presence of PCE DNAPL indicated that the dechlorination rate in cultures of *S. multivorans* was equal to, or possibly limited by, the PCE dissolution rate from the DNAPL. The bioavailability number (*Bn*) has been proposed for quantifying the relationship between the rate of dissolution and the rate of biotransformation (*49*):

$$Bn = \frac{\kappa^{PCE} a'_{ao}}{k_{\max}^{PCE} X_0 / K_S^{PCE}} \quad (5)$$

where $a'_{ao}$ (L$^{-1}$) is the initial specific interfacial area of the DNAPL (interfacial area normalized by aqueous phase volume) and all other values are as described previously. *Bn* is a dimensionless parameter that arises during the derivation of the Best equation, which assumes the mass transfer flux and substrate degradation are approximately equal (quasi-steady-state) (*49*). A *Bn* less than unity represents dissolution-controlled bioavailability, while a *Bn* greater than unity represents conditions where transformation is limited by the catalyst. Using the best-fit dechlorination and dissolution rates determined using the numerical model, *Bn* for the batch experiments was determined to be $1.56 \times 10^{-2}$ and $6.97 \times 10^{-3}$ for strain BB1 and *S. multivorans*, respectively. The results presented here suggest that dechlorinating isolates and mixed cultures incapable of dechlorinating at high or saturated PCE concentrations can continuously dechlorinate in the presence of a DNAPL, leading to dissolution enhancement relative to abiotic conditions, when conditions corresponding to a $Bn < 1.25 \times 10^{-2}$ (e.g., *S. multivorans*) exist. For such dechlorinating cultures, conditions corresponding to a *Bn* greater than this threshold value may not lead to sustained dissolution enhancement and PCE transformation because the catalyst (e.g., strain BB1) will be inhibited as PCE accumulates. Application of the reported *Bn* threshold

to cultures that tolerate saturated PCE concentrations or have different culture-specific inhibitory PCE concentrations should be performed cautiously. A sensitivity analysis found that the *Bn* threshold ranged from 1.0 to 2.0 $\times$ $10^{-2}$ when the culture-specific inhibitory PCE concentration was varied from 25 to 75% of the PCE aqueous-phase solubility, respectively.

DNAPL dissolution in aquifer formations is a complex process that depends on many variables, including the DNAPL saturation distribution, DNAPL interfacial area, porous media heterogeneity, and groundwater flow. The mass transfer coefficient obtained in our experimental system falls within the range of field mass transfer coefficients predicted by commonly employed correlations (e.g., 0.13−4 m/d (*50*)) and is consistent with grid-scale mass transfer coefficients used in multidimensional numerical simulators (e.g., UTCHEM, MISER). Although many of the correlations predict equilibrium aqueous phase contaminant concentrations at the local scale under most environmental conditions (e.g., *42*), dispersive transport and dilution are relevant processes in source zones with heterogeneous NAPL distributions. Dispersive transport and dilution effects may reduce concentrations below levels that are inhibitory to some dechlorinating organisms. Dechlorination activity in zones with tolerable PCE concentrations would further reduce aqueous phase contaminant concentrations and sustain enhanced dissolution of downgradient NAPL ganglia or pools because of the increased mass transfer driving force. Therefore, microbial activity may enhance dissolution in PCE-DNAPL source zones even if the dechlorinating bacteria cannot tolerate high PCE concentrations or are not active in the immediate vicinity of the NAPL-water interface. Future experiments are needed to address the influence of source zone DNAPL architecture and microbial distribution within DNAPL source zones on dissolution processes.

The inability of the tested bacteria to dechlorinate PCE at concentrations >540 $\mu$M does not imply that this is a universal characteristic of PCE dechlorinators, and our results should not be used to discount bioremediation as a viable remedial option at sites where PCE concentrations exceed 540 $\mu$M. Several mixed culture studies suggest that PCE dechlorination occurs at high, even saturated, concentrations of PCE (e.g., *17*−*24*). The ability of these mixed cultures to dechlorinate at elevated PCE concentrations may be due to (i) the presence of dechlorinating strains acclimated to high PCE concentrations, or (ii) dechlorinators that exhibit increased PCE tolerance as members of mixed microbial communities (e.g., biofilms). For example, an increased tolerance to high levels of PCE may be related to the presence of large cell aggregates (i.e., granules), which characterize some dechlorinating consortia (unpublished results). The aqueous-phase concentration that dechlorinating organisms experience within such granules may be below inhibitory levels and lower than bulk (i.e., measured) concentrations due to diffusional limitations or sorption of PCE to cells on the outside of the aggregates. A similar protection mechanism from high concentrations of PCE may also occur within aquifer material biofilms, similar to what has been observed for other stressors (*51*). In many bacteria, environmental stressors (e.g., heat) regulate the production of a large number of proteins that aid in bacterial survival, and such general stress response systems are important for microbial tolerance to other solvents (e.g., toluene) (reviewed in ref *52*). Recently, interspecies communication following stress exposure and the regulation of stress responses have been shown to result in stress-adapted phenotypes (*53*, *54*). At present, the complex interactions among dechlorinators and other microbial populations are poorly understood. Elucidating these interactions, including their potential roles in avoiding PCE inhibition and enhancing dechlorination activity in the presence of PCE DNAPL, is likely to further promote source zone bioremediation.

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## Supporting Information Available

Additional information on laboratory methods, a summary of reported PCE tolerances of dechlorinating isolates (Table S1), and the dechlorination performance of *S. multivorans* in the presence of PCE DNAPL at an increased agitation speed (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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