

Vinyl Chloride and *cis*-Dichloroethene Dechlorination Kinetics and Microorganism Growth under Substrate Limiting Conditions

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The reductive dechlorination of tetrachloroethene (PCE) and trichloroethene (TCE) at contaminated sites often results in the accumulation of *cis*-1,2-dichloroethene (DCE) and vinyl chloride (VC), rather than the nonhazardous end product ethene. This accumulation may be caused by the absence of appropriate microorganisms, insufficient supply of donor substrate, or reaction kinetic limitations. Here, we address the issue of reaction kinetic limitations by investigating the effect of limiting substrate concentrations (electron donor and acceptor) on DCE and VC dechlorination kinetics and microorganism growth by bacterium VS. For this, a model based on Monod kinetics, but also accounting for competition between electron acceptors and the effect of low electron donor and acceptor concentrations (dual-substrate kinetics), was examined. Competitive coefficients for VC ($7.8 \pm 1.5 \mu\text{M}$) and DCE ($3.6 \pm 1.1 \mu\text{M}$) were obtained and included in the model. The half velocity coefficient for hydrogen, the electron donor, was experimentally determined ($7 \pm 2 \text{ nM}$) through investigating dechlorination over different substrate concentrations. This complete model was then used, along with experimental data, to determine substrate concentrations at which the dechlorinating microorganisms would be in net decay. Notably, the model indicates net decay will result if the total electron acceptor concentration (DCE plus VC) is below $0.7 \mu\text{M}$, regardless of electron donor levels. The ability to achieve sustainable bioremediation to acceptable levels can be greatly influenced by this threshold level.

Introduction

Biological reductive dechlorination is a potential remediation strategy for sites contaminated with the common groundwater pollutants, tetrachloroethene (PCE) and trichloroethene (TCE). One limitation of this approach is the slow

removal of the reductive dechlorination intermediates *cis*-1,2-dichloroethene (DCE) and vinyl chloride (VC), the latter a known human carcinogen. In attempting to obtain complete site remediation, it is therefore crucial to identify and understand the factors resulting in this slow removal. Key factors affecting the degradation of these contaminants are the growth characteristics of the dechlorinating microorganisms and their associated dechlorination kinetics under typical field conditions. To address this, we investigated and subsequently modeled DCE and VC dechlorination kinetics and microorganism growth under the common field scenario of limiting electron acceptor (DCE and VC) and electron donor (hydrogen).

A variety of dechlorination models exist, generally based upon Monod kinetics (1–4). A model developed by Haston (1), consisting of a combination of these models, was considered most suitable for our overall objective and culture characteristics. This model was previously found to describe DCE and VC dechlorination well with electron donor concentrations at nonlimiting levels. However, the model was not evaluated with experimental data for the common field scenario of both electron acceptor and donor being rate limiting, nor was the competition between DCE and VC for electrons from the donor fully investigated (1).

The model developed by Haston (1) has several benefits over other dechlorination models. One advantage is the inclusion of competition between DCE and VC for electrons, important because the dechlorinator under investigation (bacterium VS) uses both VC and DCE as electron acceptors in energy metabolism (5, 6), and cocontamination with them both is common. Another advantage is that the model also predicts growth to be dependent on both VC and DCE concentrations in an additive manner. To our knowledge, this is the first model to consider these important concepts together. Tonnaer et al. (2) used competitive kinetics and additive growth for PCE and TCE dechlorination but not for DCE and VC dechlorination, as it was assumed that different microorganisms reduced these compounds. Fennel and Gossett (3) indicated that the same biomass was responsible for PCE, TCE, and DCE dechlorination but that VC dechlorination was cometabolic, so again competitive kinetics between DCE and VC were not used. Similar to our mixed culture, Bagley (4) suggested that the same group of microorganisms was responsible for DCE and VC dechlorination; however, he only considered competition to be important for VC dechlorination. Another benefit of the model used in the current study is the inclusion of dual-substrate kinetics with a hydrogen threshold (3), which enabled us to study the effect of both electron donor and acceptor concentrations and, furthermore, determine the concentrations for which growth would be limiting.

In the current study, DCE and VC competitive coefficients and the hydrogen half-velocity coefficient were determined. The complete model was then used to evaluate substrate concentrations that would be required for effective and continued DCE and VC removal. This was further investigated experimentally. The model should be useful for predicting dechlorination under substrate-limiting conditions when the microorganisms involved exhibit competitive kinetics between DCE and VC. With this approach the limitations to chlorinated ethene remediation at contaminated sites under such conditions can be evaluated.

Materials and Methods

Chemicals. Liquid *cis*-1,2-dichloroethene (97% Aldrich Chem. Co., Milwaukee, WI) and gaseous VC (99.5%, Fluka, Swit-

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zerland) were used to prepare stock solutions and analytical standards. In addition, ethene (1000 PPM and 100 PPM, Scott Speciality Gases, Alltech Associates) and VC (1000 PPM and 10 PPM, Scott Speciality Gases, Alltech Associates) were used as analytical standards, benzoate (sodium salt, 99%, Aldrich Chem. Co.) as a substrate, and sodium sulfide (Aldrich Chem. Co.) as a reducing agent.

Analytical Methods. Analyses of ethene, VC, and DCE were performed with a temperature program (40–220 °C) using a Hewlett-Packard model 5890 Series II gas chromatograph equipped with a flame ionization detector (Hewlett-Packard) and a GS-Q fused-silica capillary column (length, 30 m; inside diameter, 0.53 mm; J&W Scientific). A reduction gas analyzer (Trace Analytical, Inc., Menlo Park, CA) was used to measure hydrogen. Solution concentrations and total mass were calculated using Henry's Law Constants (7).

Culture. A dehalogenating source culture, initially seeded with a culture derived from aquifer material from a PCE-contaminated site in Victoria, TX, was maintained in a closed continuously stirred tank reactor (CSTR). The reactor was anaerobically maintained, as previously described (5), and was either used with no dilution or provided the seed for batch studies. Based on extensive 16S rDNA analyses made over several years, the culture has contained only a single *Dehalococcoides* strain called bacterium VS (5). Previous research indicated that bacterium VS can use both DCE and VC as electron acceptors for growth (5, 6).

Competition for Electrons. Two experiments were conducted to investigate competition between DCE and VC for electrons from the donor. To ascertain the effect of DCE on VC dechlorination, batch bottles (120 mL) were filled with 40 mL of anaerobic media (as previously described without sodium benzoate or TCE (5)) in an anaerobic chamber (10% H₂, 10% CO₂, 80% N₂). The gas phase of the batch bottles was filled with the anaerobic chamber atmosphere so that hydrogen (10%) could serve as the electron donor. Each bottle was inoculated with 20 mL of a rapidly dechlorinating culture grown from a small inoculum (1 mL of CSTR culture) under optimal conditions (>3% H₂, and >40 μM VC). Gaseous VC (99.5%) was added with a gastight syringe (9–79 μL) to two sets of batch bottles to provide each set with a range of initial VC concentrations. To investigate the effect of DCE on VC dechlorination an aqueous solution of DCE was added by syringe (20 μL) to one set of batch bottles.

To ascertain the influence of VC on DCE dechlorination, the above cultures (following completion of the above experiment) were purged (20% CO₂, 80% N₂) to displace ethenes and mixed together in an anaerobic chamber where they were redistributed to clean batch bottles. An aqueous solution of DCE was added by syringe (3.4–30.5 μL) to two sets of batch bottles to provide each set with a range of initial DCE concentrations. To investigate the effect of VC on DCE dechlorination gaseous VC (99.5%) was added (115 μL) to one set of batch bottles.

In both experiments, hydrogen was nonlimiting (>3%), and dechlorination rates (formation of dechlorination product) were measured over 8 h. Each dechlorination rate was determined using a regressions line ($r^2 > 0.98$) through three data points. In all experiments, an abiotic control with media, VC, and DCE was included to demonstrate the culture was required for dechlorination. All bottles were sealed with a butyl rubber stopper and aluminum seal (Bellco Glass, Inc., Vineland, NJ). For each study, following the addition of the chlorinated ethenes, the cultures were moved to a shaker (60–80 rpm) where they remained throughout the experiment (20 ± 2 °C). In all batch studies, headspace samples were periodically removed (250 μL) for chlorinated ethene and hydrogen analyses.

The Effect of Hydrogen on Dechlorination. The influence of the concentration of hydrogen, the electron donor, was

investigated for both VC dechlorination and DCE dechlorination. These studies were conducted in bottles (120 mL) with 60 mL (DCE batch study) or 30 mL (VC batch study) of the CSTR culture taken anaerobically from the reactor. The cultures were then purged (80% N₂, 20% CO₂) to displace ethene. Different hydrogen feed rates and thus, concentrations, were obtained by adding 1–4 small vials (2 mL) (Part number 98380, Alltech Associates), each containing a gas mixture (10% CO₂, 10% H₂, 80% N₂) to the bottles. Hydrogen diffused through the vial septum (TFE/butyl liners, part number 98380, Alltech Associates) to provide a continuous hydrogen supply to the culture. When needed, an aqueous solution of DCE (70 μL) was added by syringe, and gaseous vinyl chloride (99.5%) was added with a gastight syringe (55 μL).

Hydrogen Half-Velocity Coefficient (K_H). Dechlorination over a range of electron donor and acceptor concentrations was investigated to determine K_H. Experiment 1 contained five different, duplicated treatments, consisting of two concentrations of DCE and three of hydrogen. DCE was added in an aqueous solution by syringe (7.6 or 34 μL), and different hydrogen levels were obtained by adding different vial numbers (as above) to the cultures. Experiment 2 consisted of six different, duplicated, treatments with three different concentrations of DCE and two concentrations of hydrogen. DCE was added in an aqueous solution by syringe (3.8, 7.6, or 30 μL), and different hydrogen levels were obtained as above.

The initial active dechlorinating cell concentrations for these experiments were determined by subjecting duplicate cultures to nonlimiting substrate levels and measuring the rate of product formation. From these data, the active cell concentration was obtained by dividing the product formation rate (μmol L⁻¹ d⁻¹) by the maximum utilization coefficient (μmol cell⁻¹ d⁻¹) (ĝ) previously determined for bacterium VS (5). The mean determined cell concentration was then the starting cell concentration for use in the model to estimate K_H.

Dechlorinating Biomass. To confirm the model's prediction of dechlorinating biomass, the active cell concentration of bacterium VS was experimentally determined at the end of the above two experiments (day 8 for experiment 1 or day 8 or 14 for experiment 2). At this time, the cultures were purged (20% CO₂, 80% N₂) and then supplied with nonlimiting concentrations of electron donor and acceptor. Initial product formation rates were determined, and from this active cell concentrations were calculated as above. Resulting cell concentrations were then compared to cell concentrations predicted by the model.

Dechlorination Model. The model equations for DCE, VC, and microorganism growth used in this study are, respectively,

$$-\frac{dDCE}{dt} = \left(\frac{\hat{q}X DCE}{DCE + K_D \left(1 + \frac{VC}{K_{IV}}\right)} \right) \left(\frac{(H - H^*)}{(H - H^*) + K_H} \right) \quad (1)$$

$$-\frac{dVC}{dt} = \left[\left(\frac{\hat{q}X VC}{VC + K_V \left(1 + \frac{DCE}{K_{ID}}\right)} \right) - \left(\frac{\hat{q}X DCE}{DCE + K_D \left(1 + \frac{VC}{K_{IV}}\right)} \right) \right] \left(\frac{(H - H^*)}{(H - H^*) + K_H} \right) \quad (2)$$

$$\frac{dX}{dt} = \mu X \left(\frac{VC}{VC + K_V \left(1 + \frac{DCE}{K_{ID}}\right)} + \frac{DCE}{DCE + K_D \left(1 + \frac{VC}{K_{IV}}\right)} \right) \times \left(\frac{(H - H^*)}{(H - H^*) + K_H} \right) - bX \quad (3)$$

TABLE 1. Growth, Decay, and Substrate Utilization Kinetic Coefficients for the Reductive Dechlorination of DCE and VC by Bacterium VS^a

coefficients	units	DCE	VC	reference
half-velocity (K_D, K_V)	μM	3.3 ± 2.2	2.6 ± 1.9	(10)
max. utilization * bacterium VS concentration ($\hat{q}X$)	$\mu\text{mol Cl}^- (\text{L}\cdot\text{d})^{-1}$	14.3 ± 1.6	10.8 ± 1.1	Figure 1
competition (K_{ID}, K_{IV})	μM	3.6 ± 1.1	7.8 ± 1.5	Figure 1
max. utilization (\hat{q})	$\mu\text{mol Cl}^- (\text{cell}\cdot\text{d})^{-1}$	7.8×10^{-10}	7.8×10^{-10}	(5)
max. growth rate (μ)	d^{-1}	0.4	0.4	(5)
decay rate (b)	d^{-1}	0.09	0.09	(5)
H_2 half-velocity (K_H)	nM	7 ± 2	7 ± 2	Figure 4
initial bacterium VS concentration (X)	cells L^{-1}	5×10^9	5×10^9	Figure 4A–E
		6×10^9	6×10^9	Figure 4F–G

^a 95% confidence intervals for some coefficients are also displayed.

where \hat{q} is the maximum utilization coefficient ($\mu\text{mol cell}^{-1} \text{d}^{-1}$), X is the concentration (cells L^{-1}) of bacterium VS (therefore, $\hat{q}X$ is the maximum utilization rate on a liquid basis, $\mu\text{mol L}^{-1} \text{d}^{-1}$), b is the decay rate (d^{-1}), and μ is the maximum growth rate (d^{-1}). K_V and K_D are the half-velocity coefficients for VC and DCE (μM), values for which were determined previously for bacterium VS (10) as listed in Table 1. For competition between DCE and VC for electrons from the electron donor, the model was formulated similar to that for enzyme competitive inhibition, although it is recognized here that competition for electrons from the electron donor is mechanistically different from that of competition for a given enzyme. How well this model fits the case in point was investigated. K_{ID} and K_{IV} are the competitive coefficients for DCE and VC (μM), and K_H is the hydrogen half-velocity coefficient (nM) ((5, 10) Table 1). DCE and VC are the solution concentrations of VC and DCE (μM), and H is the solution hydrogen concentration (nM). Mass additions of DCE and VC and hydrogen headspace concentrations were converted to solution concentrations through knowledge of headspace and liquid volumes and use of dimensionless Henry's coefficients, as previously discussed (5). H^* is the hydrogen threshold concentration for dehalogenation (nM). Several researchers have reported H^* values: Fennel and Gossett (3) suggested it was at least as low as 1.5 nM; Yang and McCarty (11) observed a threshold of 2.2 ± 0.9 nM; and Smatlak et al. (12) indicated a value of less than 2 nM. Based on the lowest hydrogen concentration measured during dechlorination in the current study we set H^* equal to 0.9 nM. Hydrogen concentration was measured every 1–4 days and not for every time step (0.5 day) used in the model. Hydrogen concentrations for use in the model at intermediate time steps were taken to be an average of values measured before and after that time step.

Modeling Approach. Kinetic coefficients were determined using nonlinear least-squares analysis with an Excel spreadsheet method previously developed for the evaluation of biochemical reaction rate coefficients (9). The method allows calculation of 95% confidence intervals for the determined coefficients. Using this approach, the VC and DCE dechlorination data (without competitor present) from the competition for electrons experiments were fit to Monod kinetics (eqs 1 and/or 2 without competitive kinetics or dual-substrate kinetics) to determine the maximum utilization coefficient on a liquid volume basis ($\hat{q}X$) for each. Then (using the appropriate $\hat{q}X$ value), the competitive model (eqs 1 and/or 2 without dual-substrate kinetics) was fit to the experimental data for VC and DCE with the competitive acceptor present to obtain the appropriate competitive coefficients (K_{IV} and K_{ID}) along with their 95% confidence intervals. Therefore, for each experiment the nonlinear least-squares analysis contained only one unknown (either $\hat{q}X$ or K_D) reducing the uncertainty associated with the results. Growth (eq 3) was

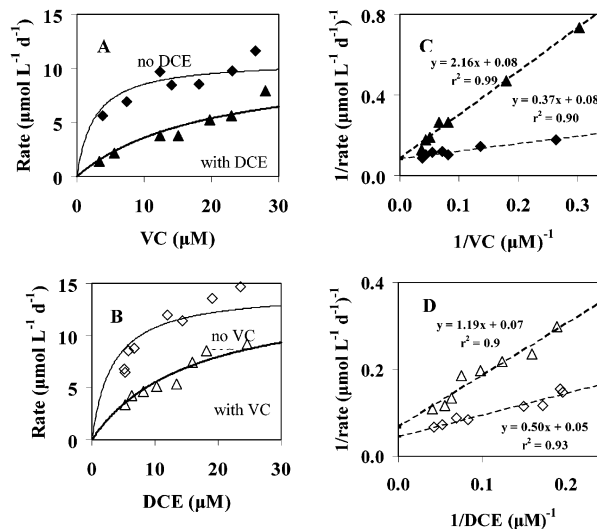


FIGURE 1. (A) VC dechlorination over a range of initial VC concentrations, with DCE ($24 \mu\text{M}$) (solid triangles), and without (solid diamonds). (B) DCE dechlorination over a range of initial DCE concentrations with VC ($30 \mu\text{M}$) (open triangles), and without (open diamonds). For each experiment, the lighter and the heavier lines (A and B) represent the best fit of the model (eqs 1 and 2) to determine the maximum utilization coefficient ($\mu\text{mol Cl}^- (\text{L}\cdot\text{d})^{-1}$) and the competitive coefficient (μM), respectively. Lineweaver–Burk plots for these data; (C) VC dechlorination and (D) DCE dechlorination (D).

not included in these calculations as the experiments lasted less than 8 h during which little growth would have occurred.

Using these competitive coefficients, the hydrogen half-velocity (K_H) coefficient was then determined by fitting the entire model (eqs 1, 2, and 3) to the dechlorination data over a range of electron acceptor and donor concentrations. The initial bacterium VS cell concentration (X) for this modeling phase was determined from dechlorination activity assays (as described above).

Results and Discussion

Evaluation of Competitive Kinetics. Research with our culture indicated that DCE and VC were dechlorinated at similar rates (13) and that growth of bacterium VS occurred with either of these compounds (5). Additionally, a partially purified VC-reductase from a culture, highly enriched (>99%) with bacterium VS, reduced DCE and VC at similar rates (6).

DCE and VC were each found to be competitive to the utilization of the other. K_{ID} and K_{IV} were determined to be $3.6 \pm 1.1 \mu\text{M}$ and $7.8 \pm 1.5 \mu\text{M}$, respectively (Figure 1A and B), indicating that DCE (with a lower competitive coefficient) has a more inhibitory effect on VC dechlorination than vice versa.

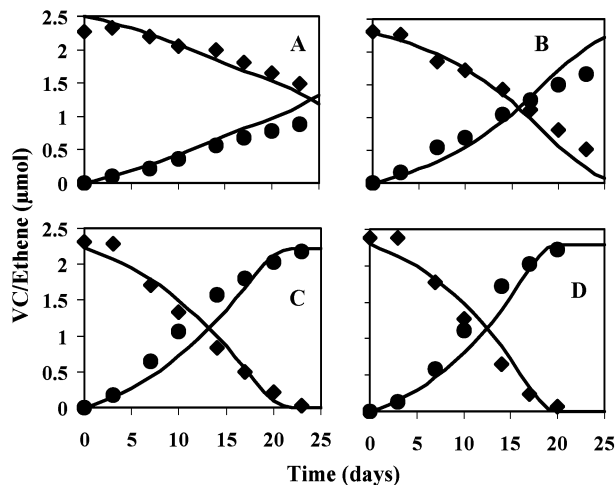


FIGURE 2. Dechlorination of VC over a range of hydrogen concentrations as follows: (A) 5 ± 1 nM, (B) 8 ± 2 nM, (C) 11 ± 2 nM, and (D) 13 ± 2 nM. Points represent measured masses of VC (diamonds) and ethene (circles), and lines represent best fits of the data to the model (see Discussion).

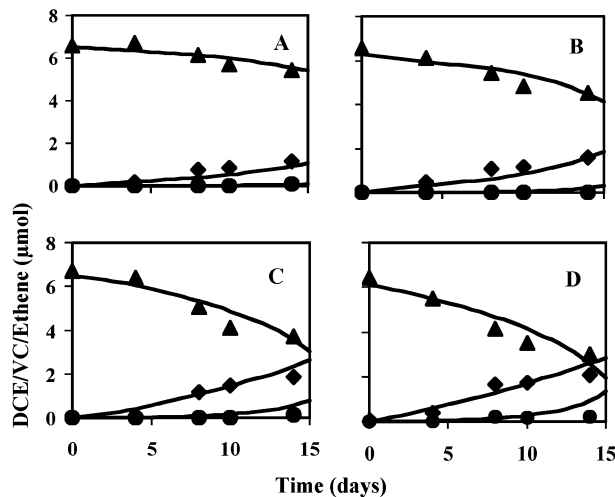


FIGURE 3. Dechlorination of DCE over a range of hydrogen concentrations as follows: (A) 5 ± 2 nM, (B) 8 ± 4 nM, (C) 9 ± 3 nM, and (D) 11 ± 6 nM. Points represent measured masses of DCE (triangles), VC (diamonds), and ethene (circles), and lines represent best fits of the data to model (see discussion).

In comparing the competitive model used here with one similar to the noncompetitive inhibition enzyme model, a lower sum of squares (SOS) value was obtained in a least-squares analysis for the competitive coefficient compared to the noncompetitive coefficient. For the effect of DCE on VC utilization, the SOS values were found to be 3.7 and 16.1 for competitive and noncompetitive kinetics, respectively. Similarly, for the effect of VC on DCE utilization the SOS values were 3.0 and 15.2 for competitive and noncompetitive kinetics, respectively. Thus, the competitive model provided a much better fit of the data. Inverse plots (Figure 1C and D) were also evaluated and show similar near intersections of regression lines on the y-axis, which suggests a good fit with the competitive kinetics model.

The Effect of Hydrogen on Dechlorination. Hydrogen levels affected both VC (Figure 2) and DCE (Figure 3) dechlorination trends. Even over the narrow hydrogen ranges investigated (5–13 and 5–11 nM) a noticeable difference in dechlorination rate is apparent, indicating that available hydrogen concentration at contaminated sites can have a large impact on dechlorination rates. Model predictions

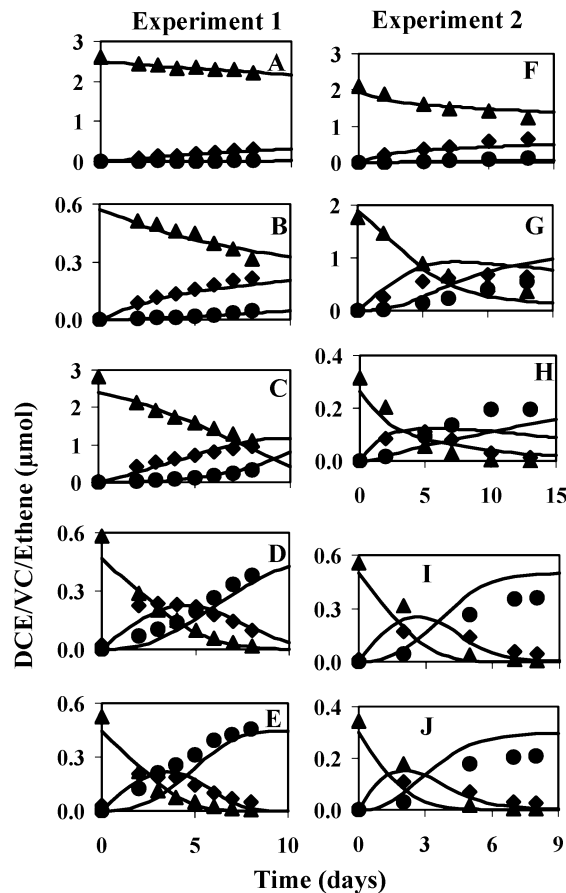


FIGURE 4. Dechlorination over a range of DCE and hydrogen concentrations, respectively, as follows (with DCE masses converted using Henry's law to respective concentration units): Experiment 1, (A) $37 \mu\text{M}$ and 2 ± 0.4 nM; (B) $9 \mu\text{M}$ and 2 ± 0.6 nM; (C) $36 \mu\text{M}$ and 13 ± 7 nM; (D) $7 \mu\text{M}$ and 11 ± 3 nM; (E) $7 \mu\text{M}$ and 25 ± 6 nM; Experiment 2, (F) $30 \mu\text{M}$ and 4 ± 2 nM; (G) $28 \mu\text{M}$ and 18 ± 8 nM; (H) $5 \mu\text{M}$ and 3 ± 2 nM; (I) $7 \mu\text{M}$ and 20 ± 11 nM; (J) $4 \mu\text{M}$ and 10 ± 9 nM. Points represent measured means from duplicates of DCE (triangles), VC (diamonds), and ethene (circles). Lines represent best fits of the data to model to determine K_H . Note differences in axis scales.

illustrated with experimental results in Figures 2 and 3 are discussed below.

Hydrogen Half-Velocity Coefficient (K_H). The ability to compete for an electron donor greatly influences the survival and growth of microorganisms at contaminated sites. An important indicator of a microorganism's ability to compete for hydrogen is the hydrogen half-velocity coefficient (K_H); however, conflicting values have previously been reported for dechlorinating microorganisms. Smatlak et al. (12) reported a relatively high value (100 ± 50 nM), indicating extremely slow growth under conditions of low electron donor. However, Ballagrapada et al. (14) concluded that K_H was lower (9–21 nM), suggesting that dechlorinating microorganisms compete better than previously thought when hydrogen is limiting. To more accurately determine this key coefficient we investigated dechlorination at different hydrogen and DCE concentrations. Using the determined initial active cell concentration for the culture used in these experiments (5×10^9 and 6×10^9 cells L^{-1} for experiments 1 and 2), the models (eqs 1, 2, and 3) were fit to the experimental data from both experiments using nonlinear least squares analyses to determine the hydrogen half-velocity coefficient, resulting in a value of 7 ± 2 nM (Figure 4). This K_H value is similar to that found by Ballagrapada et al. (14) and is significantly lower than that found by Smatlak et al.

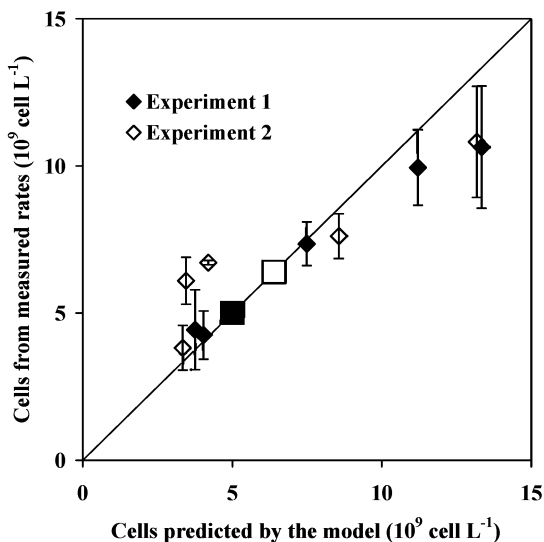


FIGURE 5. Comparison between cell concentrations (filled diamonds for experiment 1 and open diamonds for experiment 2) determined by dechlorination rates and predicted by the model (after 8 days for experiment 1 and after 8 or 14 days for experiment 2), with the line representing perfect correspondence. The filled and open squares represent the initial cell concentration for experiments 1 (5×10^9 cells L^{-1}) and 2 (6×10^9 cells L^{-1}), respectively. Cell concentrations greater than the corresponding square represent a net increase in cells, and concentrations smaller than the corresponding square represent a net decrease over the dechlorination period. Error bars represent the maximum and minimum values ($n=2$).

(12), confirming that growth and dechlorination even under low hydrogen concentrations can be significant.

The determined K_H value was used to predict DCE and VC dechlorination rates in the previously discussed experiments (Figures 2 and 3). However, because the initial cell concentrations were not measured, the initial cell concentrations were the fitting parameters, resulting in values of 4×10^9 and 3×10^9 cells L^{-1} (Figures 2 and 3, respectively).

Dechlorinating Biomass. The dechlorinating cell concentrations at the end of the above two experiments were experimentally investigated using the dechlorination activity assay and compared to those concentrations predicted by the model (Figure 5). For each experiment, the initial cell

concentration is depicted by a square. Final cell concentrations below this value represent cultures that experienced a net decline in population, and those above represent those that experienced a net increase over the study period. Most measured concentrations (perhaps except at the higher concentrations) fall close to the predicted values, providing reasonable confidence in model predictions.

An important parameter for the model's estimation of biomass, thus dechlorination rates, is the decay coefficient. For these investigations the decay coefficient used was based on previous work with this culture (5). A higher decay coefficient would result in a slower overall growth rate, lower dechlorination rate, and hence higher substrate concentrations required for positive growth, whereas a lower decay coefficient would have the opposite effect. Thus, good predictions of substrate limitations require that the decay coefficient be known with reasonable confidence.

Substrate Limitations for Growth. At a field site near a source of PCE or TCE contamination, the concentrations of DCE and VC are often very high such that supplementation with or presence of an electron donor will result in positive growth of dehalogenators and good dehalogenation, provided a seed DCE and VC dehalogenation population is present. However, as the contaminated plume moves away from the source and dehalogenation continues, PCE and TCE concentrations will decrease to zero and the DCE plus VC concentration will drop. The model simulation results in Figure 6 suggest that as the combined concentration of DCE and VC then decreases below $100 \mu M$, organism growth rate will decrease below the maximum rate to an extent that depends on the hydrogen concentration present at that point. At some downgradient location in the plume then, the combined DCE, VC, and hydrogen concentrations will decrease to a level where organism growth rate will become negative. When that occurs, the dehalogenating population either cannot become established or, if bioaugmented, it cannot be sustained. Consequently, unless some other degradation process such as aerobic degradation occurs or an engineered approach that overcomes this limitation is invoked, complete site remediation will fail due to the persistence of DCE and VC. One engineered approach that has proven successful is recycling of the downgradient plume, with below population sustaining concentrations, to a location up gradient where high population-sustaining donor and acceptor concentrations are present (15).

For example, the model indicates that growth will become negative at a DCE + VC concentration of $4 \mu M$ together with

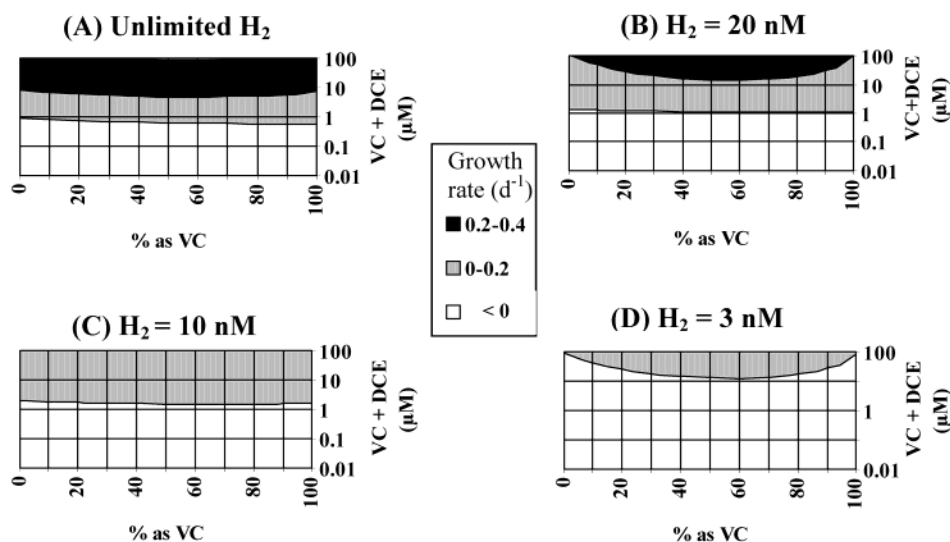


FIGURE 6. Predicted specific growth rates at four different hydrogen concentrations (A–D), with a range of DCE and VC concentrations.

a hydrogen concentration of 4 nM (a typical concentration found in the field). Regardless of how high the hydrogen concentration, when the DCE plus VC concentration decreases below 0.7 μ M, organism growth rate will be negative, and dehalogenation will not be sustainable (dehalogenation past this threshold is observed in batch experiments only because of a large added dechlorinating population, a scenario not typical at field sites). It is significant that 0.7 μ M VC represents a concentration of 44 μ g/L, well above the U.S. Environmental Protection Agency Maximum Contaminant Level for drinking water of 2 μ g/L. Thus, while microbial reductive dehalogenation can have significant impact in reducing the concentrations of chlorinated ethenes in an aquifer, kinetic limitations of the microorganisms involved make it unlikely with natural attenuation alone that concentrations can be reduced sufficiently by reductive dehalogenation to meet drinking water standards. Other processes such as aerobic degradation or dilution may be required. The extent to which the VC concentration can be reduced is dependent significantly upon the hydrogen concentration that can be maintained. Thus, kinetic limitations need to be understood when considering biological dehalogenation through natural attenuation or engineered bioremediation at a field site. The simulation model presented and evaluated here should be a useful tool in helping to assess both the rate and extent of microbial dehalogenation of PCE and TCE and the chlorinated daughter products in a contaminated aquifer as well as the important boundary conditions that may limit the extent of their removal.

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Literature Cited

- (1) Haston, Z. C. Ph.D. Dissertation, Stanford University, 1999.
- (2) Tonnaer, H.; Alphenaar, A.; de Wit, H.; Grutters, M.; Spuij, F.; Gerritse, J.; Gottschal, J. C. *In Situ and On-Site Bioremediation*; Alleman, B. C., Leeson, A., Eds.; Battelle Press: Columbus, OH, 1997; pp 591–596.
- (3) Fennell, D. E.; Gossett J. M. *Environ. Sci. Technol.* **1998**, *32*, 2450–2460.
- (4) Bagley, D. M. *J. Environ. Eng.* **1998**, *124*, 1076–1086.
- (5) Cupples, A. M.; Spormann, A. M.; McCarty, P. L. *Appl. Environ. Microbiol.* **2003**, *69*, 953–959.
- (6) Mueller, J. A.; Rosner, B. M.; Mesulam-Simon, G.; van Abendroth, G.; McCarty, P. L.; Spormann, A. M. In *103rd General Meeting of the American Society for Microbiology*, Abstract Q-003, Washington, DC, 2003.
- (7) Gossett, J. M. *Environ. Sci. Technol.* **1987**, *21*, 202–208.
- (8) Maymo-Gatell, X.; Chien, Y.; Gossett, J. M.; Zinder S. H. *Science* **1997**, *276*, 1568–1571.
- (9) Smith, L. H.; McCarty, P. L.; Kitanidis, P. K. *Appl. Environ. Microbiol.* **1998**, *64*, 2044–2050.
- (10) Haston, Z. C.; McCarty P. L. *Environ. Sci. Technol.* **1999**, *33*, 223–226.
- (11) Yang, Y.; McCarty P, L. *Environ. Sci. Technol.* **1998**, *32*, 3591–3597.
- (12) Smatlak, C. R.; Gossett J. M.; Zinder, S. H. *Environ. Sci. Technol.* **1996**, *30*, 2850–2858.
- (13) Rosner, B. M.; McCarty, P. L.; Spormann, A. M. *Appl. Environ. Microbiol.* **1997**, *63*, 4139–4144.
- (14) Ballapragada, B. S.; Stensel, H. D.; Puhakka, J. A.; Ferguson, J. F. *Environ. Sci. Technol.* **1997**, *31*, 1728–1734.
- (15) Ellis, D. E.; Lutz, E. J.; Odom, J. M.; Buchanan, R. J.; Bartlett, C. L.; Lee, M. D.; Harkness, M. R.; Deweerd, K. A. *Environ. Sci. Technol.* **2000**, *34*, 2254–2260.

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