Kinetics and Modeling of Reductive Dechlorination at High PCE and TCE Concentrations

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Received 26 March 2004; accepted 8 July 2004

Published online 21 September 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/bit.20260

Abstract: Two biokinetic models employing the Michaelis-Menten equation for anaerobic reductive dechlorination of tetrachloroethylene (PCE) and trichloroethylene (TCE) were developed. The models were compared with results from batch kinetic tests conducted over a wide range of PCE and TCE concentrations with two different dechlorinating cultures. One model applies Michaelis-Menten kinetics with competitive inhibition among chlorinated aliphatic hydrocarbons (CAHs), while the other model includes both competitive inhibition and Haldane inhibition at high CAH concentrations. Model simulations with competitive inhibition simulated the experimental results well for PCE concentrations lower than 300 μM. However, simulations deviated from the experimental observations for PCE or TCE concentrations greater than 300–400 μM. The kinetic model that incorporated both competitive and Haldane inhibitions better simulated experimental data for PCE concentrations near the solubility limit (1000 μM), and TCE concentrations at half its solubility limit (4000 μM). Based on the modeling analysis of the experimental results, the PM culture (Point Mugu, CA) had very high Haldane inhibition constants for *cis*-1,2-dichlororethylene (*c*-DCE) and vinyl chloride (VC) (6000 and 7000 μM, respectively), indicating very weak Haldane inhibition, while the EV culture (the Evanite site in Corvallis, OR) had lower Haldane inhibition constants for TCE, *c*-DCE, and VC of 900, 750, and 750 μM, respectively. The BM culture (a binary mixed culture of the PM and EV cultures) had transformation abilities that represented the mixture of the EV and PM cultures. Model simulations of the BM culture transformation abilities were well represented by separate rate equations and model parameters for the two independent cultures that were simultaneously solved. Modeling results indicated that a combination of competitive and Haldane inhibition kinetics is required to simulate dechlorination over a broad range of concentrations up to the solubility limit of PCE and half the solubility limit of TCE.

Keywords: reductive dechlorination; PCE; TCE; competitive inhibition; Haldane inhibition

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Contract grant sponsor: U.S. Environmental Protection Agency-sponsored Western Region Hazardous Substance Research Center
Contract grant number: R-828772

INTRODUCTION

Chlorinated ethylenes are ubiquitous soil and groundwater contaminants (Westrick et al., 1984), for which bioremediation is a promising technology. Anaerobic reductive dechlorination is an important process in the subsurface, especially for the dechlorination of highly chlorinated compounds such as PCE and TCE, which can be present at high concentrations due to the presence of dense non-aqueous phase liquid (DNAPL; Rittmann et al., 1994).

Although intrinsic biotransformation of PCE and TCE occurs, observations at many contaminated sites show transformation often stalls at *c*-DCE and VC (Major et al., 2002). This may be due to an absence of appropriate dechlorinating bacteria. A study of 24 chlorinated ethylene-contaminated sites reported that *Dehalococcoides* organisms were not detected at sites where incomplete dechlorination was observed (Hendrickson et al., 2002). Bioaugmentation with dechlorinating cultures that can completely transform PCE and TCE to ethylene (ETH) can be an appropriate strategy to enhance reductive dechlorination (Ellis et al., 2000; Harkness et al., 1999).

The use of a binary mixed culture consisting of two different aerobic pure cultures has been reported to show interspecies interactions and changes in the rate and extent of biodegradation (Fairlee et al., 1997; Rogers et al., 2000). The studies revealed that the utilization of a binary mixed culture could enhance the biodegradation of mixed pollutants such as toluene, phenol, and benzene. Previously, we reported (Yu and Semprini, 2002a) that a binary anaerobic mixed culture isolated from two different contaminated groundwaters was more effective in reductively dechlorinating PCE to ETH than each individual culture.

Various models have been developed that describe reductive dechlorination of chlorinated ethylenes. For complete reductive dechlorination of PCE to ETH, competitive inhibition of VC dechlorination by the other chlorinated ethylenes was assumed, and model simulations were compared with the experimental data (Tandoi et al., 1994). Fennell and Gossett (1998) simulated both fermentation of electron

donors and competition for the evolved H_2 between hydrogenotrophic PCE dechlorinators and methanogens, but did not consider inhibition between the chlorinated ethylenes. Tonnaer et al. (1997) also incorporated competitive dechlorination between PCE and TCE in a model to predict PCE reductive dechlorination. Garant and Lynd (1998) showed that competitive kinetics for complete reductive dechlorination of PCE achieved better model fits of the experimental data than noncompetitive inhibition. Other modeling studies included competitive inhibition between *c*-DCE and VC with a dechlorinating culture using *c*-DCE and VC as electron acceptors for energy (Cupples et al., 2004; Haston 1999).

Recently, we experimentally determined maximum rates (k_{max}) and half-velocity coefficients (K_S) for each step of the dechlorination of PCE to ETH for two different mixed cultures (Yu, 2003) and evaluated inhibition between chlorinated ethylenes. The study presented here aims to simulate with independently measured kinetic parameters the sequential transformation of PCE and TCE to ETH over a broad range of concentrations up to the solubility limit of PCE and half the solubility limit of TCE in water. The kinetic models developed for each culture were also combined to simulate the transformation ability of a binary mixed culture composed of each dechlorinating culture.

MATERIALS AND METHODS

Chemicals

Chlorinated ethylenes including PCE (99.9%, spectrophotometric grade), TCE (99.9%), and *c*-1,2-dichloroethylene (*c*-DCE) (97%) used for preparing stock feed solutions and analytical standards were obtained from Acros Organics (Pittsburgh, PA). Gaseous VC and ETH (both 99.5%, Aldrich Chemical, Milwaukee, WI) were used to prepare analytical standards. Hydrogen gas (99%, Airco, Inc., Albany, OR) was used as an electron donor for dechlorination and 1-butanol (99.8%, HPLC grade, Aldrich Chemical, Milwaukee, WI) was used as a fermenting substrate.

Analytical Methods

The sample gas concentrations of all chlorinated ethylenes, ETH, and hydrogen were determined using gas chromatography. PCE, TCE, *c*-DCE, VC, and ETH were quantified with an HP-6890 gas chromatograph (GC) equipped with a photoionization detector (PID) and flame ionization detector (FID) connected in series. The GC was fitted with a 30 m × 0.53 mm GS-Q column (J&W Scientific, Folsom, CA), and helium was used as the carrier gas (15 mL/min). Then 100–200 μL batch reactor headspace samples were introduced into the GC. The GC oven was initially held at 80°C for 1.5 min, heated at 65°C/min to 170°C and 40°C/min to 220°C, and kept at 220°C for 2.7 min. Quantification of hydrogen concentrations in headspace gas samples (200 μL) was accomplished using an HP-5890 GC with a thermal conductivity detector (TCD), operated isothermally at 220°C. The gas samples were chromatographically separated with a Carboxen 1000 column (15 ft × 1/8 in; Supelco, Bellefonte, PA), using argon as carrier gas at a flow rate of 15 mL/min. The hydrogen detection limit was 4 n*M* (aqueous concentration).

Enrichment Cultures and Kinetic Batch Reactors

The two anaerobic mixed cultures used in this study were isolated from subsurface samples from Point Mugu Naval Weapon Facility, California (PM) and the Evanite site in Corvallis, Oregon (EV). The third culture used was a binary mixed culture (BM), representing a mixture of the PM and EV cultures (1:1 volume ratio). Details of the enrichment process are provided by Yu (2003). PCR assay targeting genes encoding for 16S ribosomal RNA indicated that the PM and EV cultures had *Dehalococcoides*-like microorganisms present with the ability of complete reductive dechlorination to ETH (Yu, 2003). The two cultures were grown in separate batch mother reactors (total volume 1.2 L with liquid volume of 1 L) as described in Yu and Semprini (2002b) and Yu (2003). The mother reactors were amended with an aqueous TCE or PCE concentration of 100 mg/L for the PM and EV cultures, respectively. 1-Butanol (40–60 mg/L) was added as a fermenting substrate and hydrogen (10% in the headspace) was added as an electron donor. The liquid cultures for kinetic studies were obtained from the batch mother reactor after 50–100% of the PCE or TCE added was converted to ETH.

Batch kinetic reactors [serum bottles—156 mL total volume with 125 mL liquid—fitted with gray chlorobutyl rubber septa (Wheaton Industries, Millville, NJ)] were constructed in an anaerobic glove box. The reactors contained 125 mL enrichment liquid culture and 31 mL anaerobic gas headspace. After construction in the glove box, the reactors were purged with a mixed gas mixture of N_2 (70%), CO_2 (20%), and H_2 (10%) that was treated in a tube furnace to remove trace oxygen. After purging, neat PCE or TCE and butanol were added to each reactor to achieve the desired initial concentration. To evaluate different PCE concentrations (Table I), the kinetic reactors were purged after each experimental run, and higher PCE concentrations were introduced along with butanol and hydrogen. The batch reactors were shaken at 200 rpm and incubated at 20°C. Prior to each experimental run, the total cell concentration, *X*, was determined by a protein analysis, as previously described (Yu, 2003).

Batch kinetic tests of the sequential transformation of PCE and TCE were conducted in duplicate with the PM, EV, and BM cultures (Table I). Neat PCE was added, and the batch reactors were vigorously shaken to achieve target PCE concentrations from 39 μ*M* up to the solubility limit. Tests were conducted at a single TCE concentration of approximately 4000 μ*M*, representing half of its solubility in water. The experimental data are presented

Table I. Experimental conditions of kinetic batch reactors.

	PM		EV		BM		
Run	Initial CAH (μM)	Measured Cell Concentration (mg of protein/L)	Initial CAH (μM)	Measured Cell Concentration (mg of protein/L)	Initial CAH (μM)	Measured Cell Concentration (mg of protein/L)	Relative Proportions of Each Cultures (PM:EV) (mg of protein/L)
1	92[a]	25	39[a]	30	43[a]	28	13:15
2	282[a]	34	282[a]	30	317[a]	31	14:17
3	1128[a]	34	1057[a]	27	1128[a]	30	14:16
4	3875[b]	35	4173[b]	40	4173[b]	37	17:20

[a]PCE.
[b]TCE.

as the average of the duplicate reactors for each kinetic test. Differences between measured values in duplicate reactors were usually smaller than the size of the figure symbols. Butanol was added at the beginning of each experiment to provide more than 3 times the electron donor required for complete dechlorination of PCE and TCE. Hydrogen was also added to the batch reactors' headspace and maintained above 0.03 atm (3% hydrogen in headspace = 24.4 μM aqueous concentration) by adding hydrogen (99%), if necessary.

Microbial Community

A PCR assay targeting 16S ribosomal DNA was performed to identify the microbial populations catalyzing the reductive dechlorination of chlorinated ethylenes. Genomic DNA was extracted from the PM and the EV mixed cultures with a FastDNA spin kit for soil (Qbiogene, Inc., Carlsbad, CA). For each culture, PCR was performed on 5 μL of DNA extracted from the cultures with three different pairs of primers: (1) DSM-205F, *Escherichia coli* 16S rRNA positions 205 to 222 and DSM-1015R, 1033 to 1015, specific for the *Desulfuromonas* (Löffler et al., 2000); (2) DHG-728F, 728 to 750 and DHG-1155R, 1172 to 1155 (Löffler et al., 2000); (3) Fp DHC1, 1 to 17 and Rp DHC-1377, 1385 to 1366 (Hendrickson et al., 2002). The last two primer pairs were specific to the *Dehalococcoids* group (*D. ethenogens* and FL2). Both the PM and the EV enrichment cultures yielded bright positive PCR signals with both DHG and DHC primers, but were negative with the DSM primers. This PCR assay indicated that the PM and EV cultures contain *Dehalococcoides*-like microorganisms (Yu, 2003). Molecular analysis using terminal restriction fragment length polymorphism (T-RFLP) with the restriction endonuclease MnlI was conducted on the extracted DNA. Of the 71 sequences in GenBank identified as *Dehalococcoides* sp., 5 included the recognition sequence for the universal bacterial primer, 27F, used in the T-RFLP analysis. Of these, only three sequences were obtained from TCE contaminated sites undergoing reductive dechlorination and they produced expected terminal fragment lengths (TFLs) of 124 or 125 bp. The culture showed a predominant peak at 123.6 bp, consistent with the expected TFL for *Dehalococcoides* sp., corresponding to 65% of the total sample DNA indicating that the culture is highly enriched in *Dehalococcoides*-like microorganisms. Several other smaller peaks were also present (<7% of total sample DNA each) and most likely represented the fermenting microbial population.

MODEL DEVELOPMENT

A number of models have been developed to describe anaerobic reductive dechlorination for different cultures (Bagley, 1998; Cupples et al., 2004; Fennell and Gossett, 1998; Garant and Lynd, 1998; Haston, 1999; Tandoi et al., 1994; Tonnaer et al., 1997). Competitive Michaelis-Menten kinetics have been commonly used to model reductive dechlorination of PCE and TCE. Tonnaer et al. (1997) used competitive inhibition to model reductive dechlorination of PCE and TCE. Competitive inhibition kinetics were observed and modeled for *c*-DCE and VC dechlorination (Cupples et al., 2004; Haston, 1999). The inhibition constants for *c*-DCE on VC transformation was comparable to its half-velocity coefficients, but the inhibition constant for VC inhibition on *c*-DCE dechlorination was greater than its half-velocity coefficient (Cupples et al., 2004; Haston, 1999). Our previous studies with two anaerobic mixed cultures (PM and EV) indicated inhibition was competitive and the more chlorinated ethylenes inhibited reductive dechlorination of the less chlorinated ethylenes with inhibition constants equal to the K_S values (Yu, 2003). However, the less chlorinated ethylenes very weakly inhibited the more chlorinated ethylenes. PCE inhibited reductive TCE dechlorination, but not *c*-DCE or VC dechlorination, while TCE strongly inhibited *c*-DCE and VC dechlorination. *c*-DCE also inhibited VC transformation to ETH. The experiments and corresponding model simulations were conducted at low CAH concentrations. A recent kinetic study using purified PCE-RDase from *Dehalobacter restrictus* indicated that CAH itself can be inhibitory at high concentrations of PCE and TCE (up to 1000 μM) (Maillard et al., 2003).

Two models were developed here; one with Michaelis-Menten kinetics and competitive inhibition as previously described by Yu (2003), and the other includes both com-

petitive inhibition and Haldane inhibition (Bailey and Ollis, 1986). The models were solved using STELLA (High Performance Systems) using independently measured kinetic parameters and compared with the experimental data. Haldane inhibition constants were fit to experimental results and determined through trial and error analysis. As previously reported by Yu (2003), the PM culture is assumed to grow on PCE, TCE, and *c*-DCE, and the EV culture on PCE, TCE, *c*-DCE, and VC (Pon et al., 2003).

A multiple equilibration method in a single reactor was used to determine k_{max} and K_S values for PCE, TCE, *c*-DCE, and VC as described by Yu (2003). The method involved stepwise increases of the CAH concentration in a single reactor over a short time interval and measuring parent compound disappearance and daughter product production at each concentration. Nonlinear regression of the rate vs. concentration results were used to determine k_{max} and K_S values.

Competitive Inhibition Model

Based on previous studies (Yu, 2003), a competitive inhibition model was used. The kinetic equations for reductive dechlorination by the mixed cultures are presented as Eqs. (1), (2), (3), (4), and (5), with both competitive and Haldane inhibition included. When the Haldane inhibition constant (K_{HI}, μM) is very high (∞), the equation reduces to competitive inhibition only. Electron donor limitations were not included in the model equations by providing H_2 and 1-butanol in excess in the experiments. These equations are simultaneously solved by STELLA using Euler's method.

$$\frac{dC_{PCE}}{dt} = \frac{-k_{max,PCE} X C_{PCE}}{K_{S,PCE} + C_{PCE}} \tag{1}$$

$$\frac{dC_{TCE}}{dt} = \frac{-k_{max,TCE} X C_{TCE}}{K_{S,TCE}\left(1 + \frac{C_{PCE}}{K_{CI,PCE}}\right) + C_{TCE}\left(1 + \frac{C_{TCE}}{K_{HI,TCE}}\right)} + \frac{k_{max,PCE} X C_{PCE}}{K_{S,PCE} + C_{PCE}} \tag{2}$$

$$\frac{dC_{c-DCE}}{dt} = \frac{-k_{max,c-DCE} X C_{c-DCE}}{K_{S,c-DCE}\left(1 + \frac{C_{TCE}}{K_{CI,TCE}}\right) + C_{c-DCE}\left(1 + \frac{C_{c-DCE}}{K_{HI,c-DCE}}\right)} + \frac{k_{max,TCE} X C_{TCE}}{K_{S,TCE}\left(1 + \frac{C_{PCE}}{K_{CI,PCE}}\right) + C_{TCE}\left(1 + \frac{C_{TCE}}{K_{HI,TCE}}\right)} \tag{3}$$

$$\frac{dC_{VC}}{dt} = \frac{-k_{max,VC} X C_{VC}}{K_{S,VC}\left(1 + \frac{C_{TCE}}{K_{CI,TCE}} + \frac{C_{c-DCE}}{K_{CI,c-DCE}}\right) + C_{VC}\left(1 + \frac{C_{VC}}{K_{HI,VC}}\right)} + \frac{k_{max,c-DCE} X C_{c-DCE}}{K_{S,c-DCE}\left(1 + \frac{C_{TCE}}{K_{CI,TCE}}\right) + C_{c-DCE}\left(1 + \frac{C_{c-DCE}}{K_{HI,c-DCE}}\right)} \tag{4}$$

$$\frac{dC_{ETH}}{dt} = \frac{k_{max,VC} X C_{VC}}{K_{S,VC}\left(1 + \frac{C_{TCE}}{K_{CI,TCE}} + \frac{C_{c-DCE}}{K_{CI,c-DCE}}\right) + C_{VC}\left(1 + \frac{C_{VC}}{K_{HI,VC}}\right)} \tag{5}$$

$$\frac{dX}{dt} = Y\frac{dC}{dt} - k_d X \tag{6}$$

In the above equations, C is the CAH aqueous concentration (μM), k_{max} is the maximum specific CAH dechlorination rate (μmol/mg of protein/d), K_S is the half-velocity coefficient (μM), and inhibition constants of each chlorinated ethylene, K_{CI} (μM), were set equal to their respective half-velocity coefficients (K_S) as previously reported (Yu, 2003). X is the total cell concentration (mg of protein/L), and microbial growth was calculated with respect to the transformation rate of each chlorinated ethylene. In the model for biomass growth, values for growth yield, Y (= 0.006 mg of protein/μmol of Cl^- dechlorinated), and decay constant, k_d (= 0.024 d^{-1}), were obtained from the literature (Fennell and Gossett, 1998; Maymo-Gatell et al., 1997).

The protein concentration was used as the input value for dechlorinating microorganism concentration in the model. The independently measured k_{max} values were also determined on a total protein basis. Thus, protein was the most appropriate measure of dechlorinating biomass, in the absence of the specific biomass of the dechlorinating population. Increase in biomass was also linked only to CAH dechlorination, because the enrichment culture was highly enriched based on the T-RFLP analysis.

The two separate models for the PM and the EV cultures were incorporated to depict PCE dechlorination to ETH and microbial growth of the BM culture. The CAH dechlorination models [Eqs. (1)–(5)] and microbial growth rates [Eq. (6)] were solved simultaneously to simulate the performance of the BM culture with the kinetic values obtained from kinetic studies with the single mixed cultures. The initial protein content of the BM culture was based on the measured protein concentrations at the start of each test, and the relative 1:1 volumetric proportion of the PM and EV cultures was added. Based on their initial protein concentrations of 25 and 30 mg/L for the PM and EV cultures, respectively, the relative starting proportions of each culture were estimated (Table I).

Haldane Inhibition Model

Haldane inhibition (Bailey and Ollis, 1986) was used to model inhibition resulting from high CAH concentrations. The Haldane inhibition for TCE dechlorination is provided in Eq. (2), where TCE concentration appears in the denominator. In contrast to competitive inhibition, where one CAH inhibits the transformation of another CAH, with Haldane inhibition the CAH inhibits its own transformation. Maillard et al. (2003) observed that the transformations of PCE and TCE were inhibited at high concentrations, similar in form

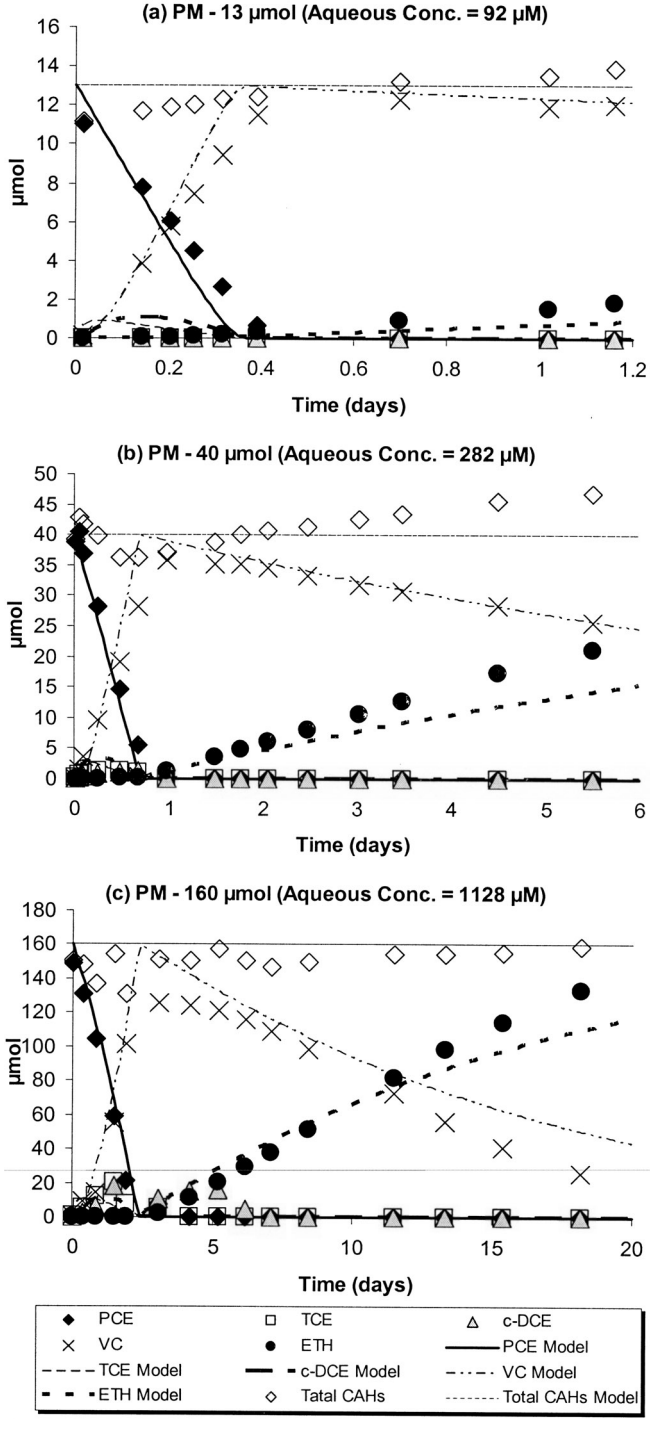

Figure 1. Reductive dechlorination kinetics at different initial PCE concentrations by the PM culture. The lines represent model simulations with competitive inhibition kinetics.

to Haldane inhibition. In model simulations presented here both Haldane and competitive inhibition among chlorinated ethylenes was used and compared with the experimental data [Eqs. (1)–(5)]. Haldane inhibition at high PCE concentrations was not included in this study, since no significant inhibition at PCE concentrations near its solubility limit was indicated in our study. Similar observations were made in the study of Maillard et al. (2003).

RESULTS

Reductive Dechlorination of PCE by the PM Culture

Figures 1 and 2 present results of a PCE transformation test by the PM culture at aqueous concentrations ranging from 92 to 1128 µ*M*, and corresponding model simulations with competitive inhibition. The transformation of VC to ETH

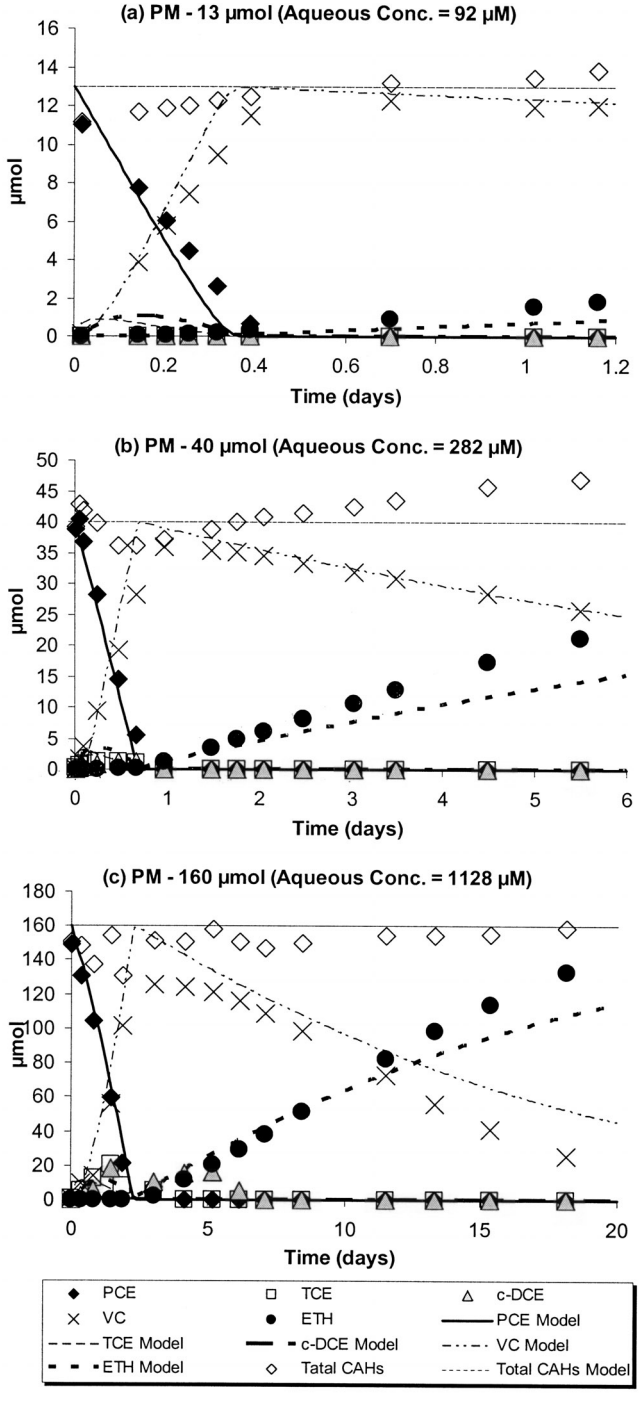

Figure 2. Reductive dechlorination kinetics at different initial PCE concentrations by the PM culture. The lines represent model simulations with both Haldane and competitive inhibition kinetics.

was very slow compared to the other CAHs (Figs. 1 and 2). PCE was transformed rapidly, with little accumulation of TCE or *c*-DCE. At an initial PCE concentration of 92 μ*M*, TCE and *c*-DCE were not detected, and VC was very slowly transformed to ETH. The rate of VC transformation to ETH increased with higher initial PCE concentrations, resulting from a high K_S value of 602 μ*M* reported by Yu (2003). The aqueous concentration of PCE with the PM culture, when fed neat PCE of 160 μmol, exceeded the PCE solubility limit of 900 μ*M* (Yaws, 1999). Simulations were performed using the total mass added to yield a computed aqueous concentration of 1128 μ*M*. The initial PCE concentrations in model simulations were generally higher than the measured PCE aqueous concentrations. The difference between the simulated and measured concentrations possibly resulted from incomplete and slow dissolution of the neat PCE added. However, these differences were insignificant because PCE was dechlorinated very rapidly at the beginning of the tests.

Model simulations without (Fig. 1) and with Haldane inhibition (Fig. 2) yield similar results. The models used the same independently measured kinetic parameters reported in our previous study, as listed in Table II (Yu, 2003). The K_{HI} values for TCE, *c*-DCE, and VC were obtained from heuristic fits of both PCE dechlorination (Figs. 1 and 2) and TCE dechlorination data (Figs. 7a and 8a, as will be discussed). The initial measured cell concentration on a protein basis of 25, 34, 34 mg of protein/L was input as an initial biomass. The model fits for the different initial PCE concentrations captured the overall trends in the experimental data very well. TCE and *c*-DCE did not accumulate, consistent with experimental observations. The results show that both models simulate very well the temporal histories of chlorinated ethylenes, as well as the response to changes in initial PCE concentrations.

Reductive Dechlorination of PCE by the EV Culture

The same procedure of step-wise increase in PCE concentrations was used with the EV culture (Table I). As shown in Figures 3 and 4, almost complete reductive dechlorination to ETH was obtained at all PCE concentrations within 1.2, 3.5, and 15 days, respectively. No lag time to initiate PCE dechlorination was observed even at the highest PCE concentration (1057 μ*M*) (Figs. 3c and 4c). *c*-DCE accumulated at all PCE concentrations tested, and reached a maximum concentration of 700 μ*M* at an initial PCE concentration of 1057 μ*M*. For all PCE concentrations, ETH production occurred after most of *c*-DCE was transformed to VC, indicating *c*-DCE strongly inhibits VC dechlorination to ETH, consistent with the observations of Yu (2003).

Simulations shown in Figures 3 and 4 were performed with initial measured cell concentrations of 30, 30, 27 mg-protein/L, respectively. The simulations with competitive inhibition model fit the experimental data reasonably well for initial PCE concentrations of 39 and 282 μ*M* (Fig. 3a, b). At the highest PCE concentration of 1057 μ*M*, however, the model did not simulate sequential PCE dechlorination as well (Fig. 3c). Simulated *c*-DCE concentrations were much lower than those experimentally observed, and the experimental production and transformation rates of VC and ETH were much slower than predicted by the model simulations (Fig. 3c).

Figure 4 shows model simulations incorporating both competitive and Haldane inhibition kinetics. The K_{HI} values for TCE, *c*-DCE, and VC of 900, 750, and 750 μ*M* were obtained by heuristic fitting of the PCE (Fig. 4) and TCE results (Fig. 8b). The simulation presented in Figure 4a at the lowest PCE concentration provides similar results as those without Haldane kinetics in Figure 3a, indicating little effect of Haldane kinetics at the low initial PCE concentration of 39 μ*M*. At the highest PCE concentration of 1057 μ*M* (Fig. 4c), the simulations including both competitive and Haldane inhibition kinetics fit the experimental data better than those only with competitive inhibition (Fig. 3c). The simulation with both competitive and Haldane inhibition kinetics also better reproduced the experimental results at the intermediate initial PCE concentration of 282 μ*M*.

Table II. Kinetic parameters used in the model simulations.

	PM				EV			
	PCE	TCE	*c*-DCE	VC	PCE	TCE	*c*-DCE	VC
k_{max}[a] (μmol/mg of protein/d)	13.3	124	22.0	2.44	12.4	125	13.8	8.08
K_S[a] (μ*M*)	3.86	2.76	1.90	602	1.63	1.80	1.76	62.6
K_{CI} (μ*M*)	3.86	2.76	1.90	602	1.63	1.80	1.76	62.6
K_{HI}[b] (μ*M*)		900	6000	7000		900	750	750
Y[c] (mg-protein/μmol of Cl^-	0.006	0.006	0.006		0.006	0.006	0.006	0.006
k_d[c](d^{-1})	0.024				0.024			

[a]Yu (2003).

[b]Obtained from the experimental data fitting (Figs. 2, 4, 6, 8).

[c]Maymo-Gatell et al., (1997); Fennell and Gossett (1998). $K_{CI} = K_S$.

Reductive Dechlorination of PCE by the BM Culture

Batch kinetic experiments were also performed with the BM culture representing a 1:1 mixture of the PM and EV cultures (volume basis). At the lowest PCE concentration of 43 μM, 80% of the initial PCE was converted to ETH within 1.2 days (Fig. 5a). The ETH production rate of the BM culture was slightly lower than that of the EV culture for all PCE concentrations tested, but much greater than that of the PM culture. Complete reductive dechlorination at a PCE concentration of 317 μM was achieved on day 4.5, while the EV culture completed PCE dechlorination to ETH at an initial concentration of 282 μM by 3.5 days. However, with the highest PCE concentration of 1128 μM, 100% transformation to ETH was observed

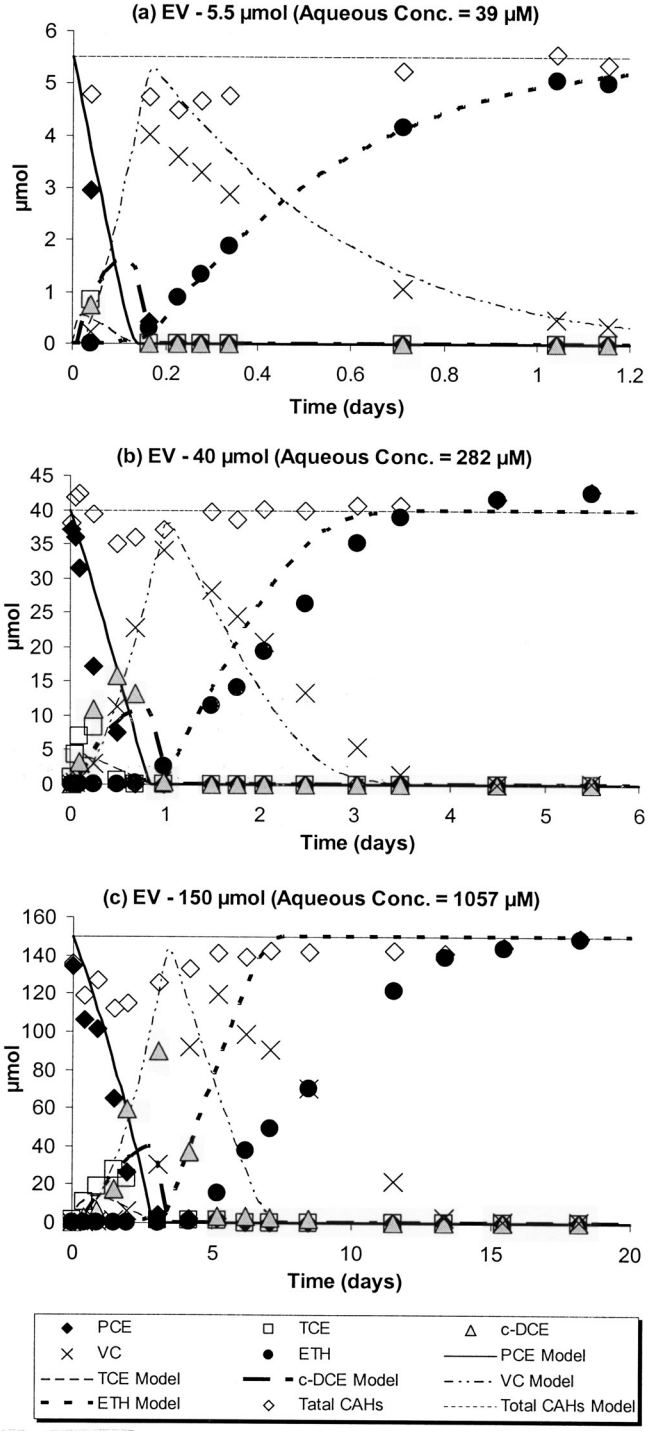

Figure 3. Reductive dechlorination kinetics at different initial PCE concentrations by the EV culture. The lines represent model simulations with competitive inhibition kinetics.

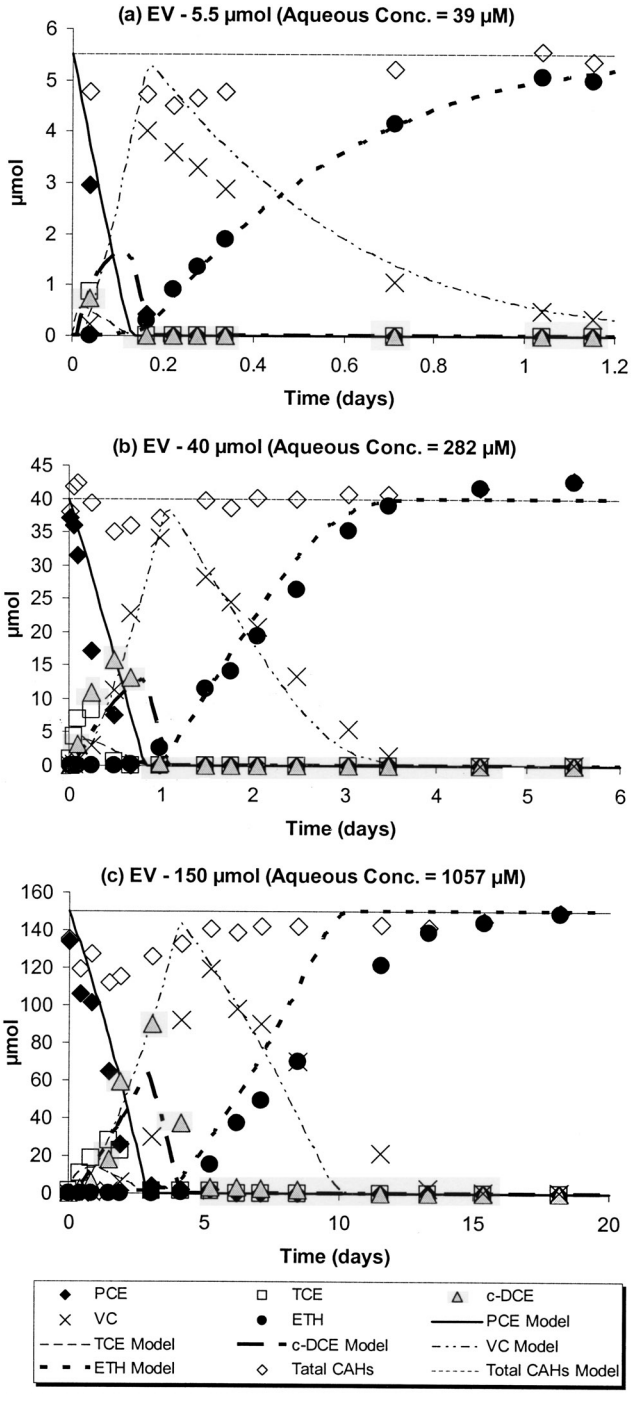

Figure 4. Reductive dechlorination kinetics at different initial PCE concentrations by the EV culture. The lines represent model simulations with both Haldane and competitive inhibition kinetics.

within 15–20 days for both the EV and BM cultures. The maximum *c*-DCE concentration was measured around day 2, yielding an aqueous concentration of 310 μ*M* (about 50% of the maximum concentration observed with the EV culture).

Model simulations of the BM culture were performed by modeling the kinetics for the PM and EV cultures simultaneously, but each had its own biomass and set of kinetic parameters. The initial biomass of each culture is given in Table I. The K_{HI} values obtained from the fitting of the PM and EV experiments were used. Similar to the model fits for the PM and EV cultures, the rate of PCE transformation to TCE was simulated well by models with and without Haldane inhibition at all initial PCE concen-

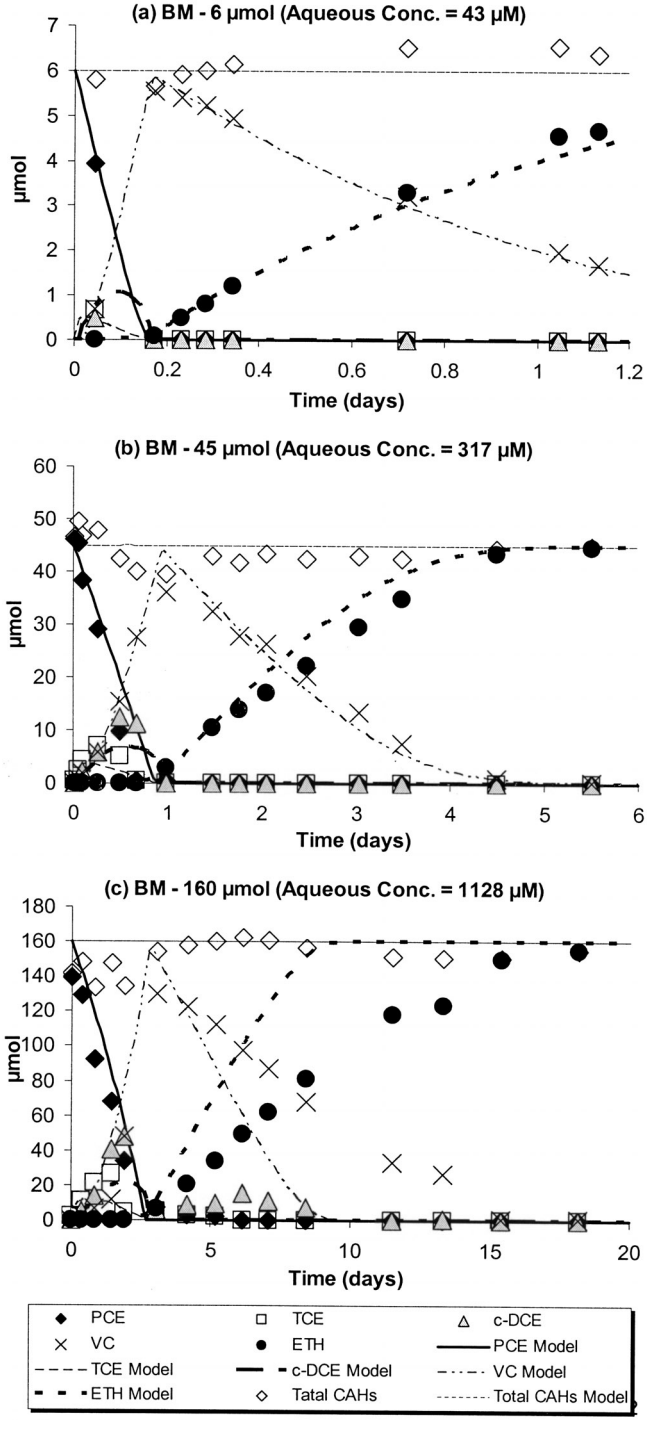

Figure 5. Reductive dechlorination kinetics at different initial PCE concentrations by the BM culture. The lines represent model simulations with competitive inhibition kinetics.

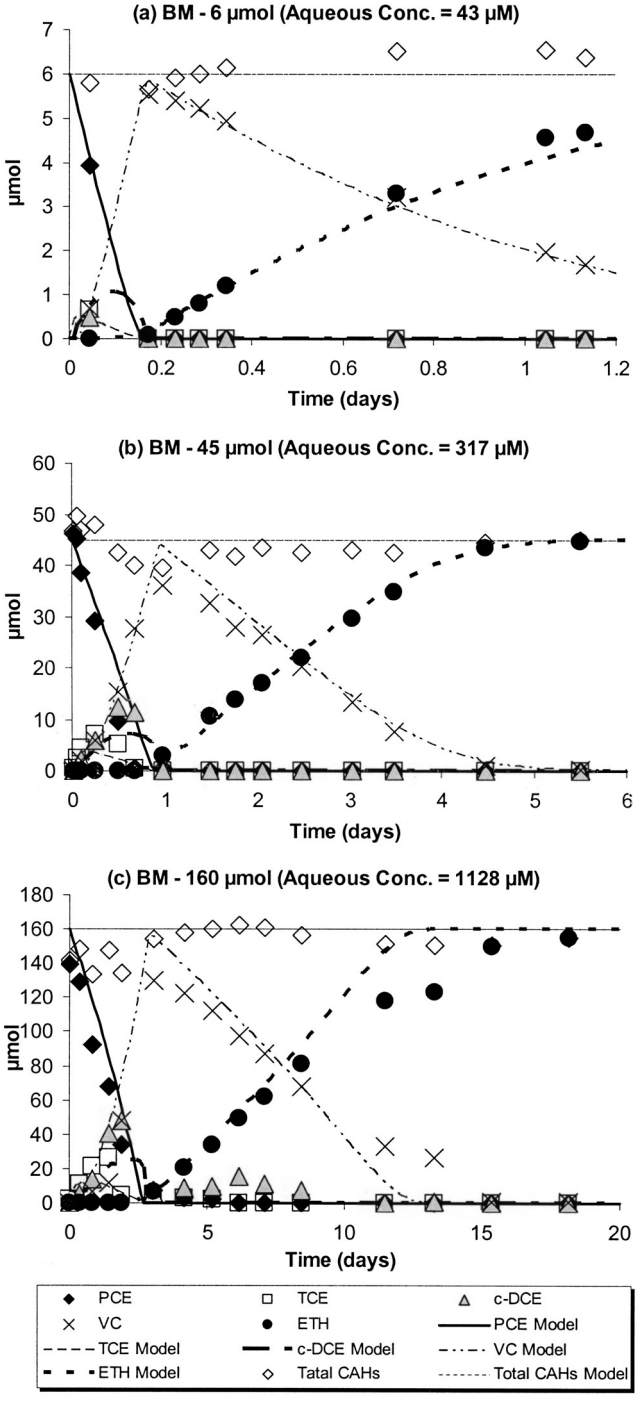

Figure 6. Reductive dechlorination kinetics at different initial PCE concentrations by the BM culture. The lines represent model simulations with both Haldane and competitive inhibition kinetics.

trations, indicating no or very weak Haldane inhibition. Both models showed similar sequential transformation at the lowest PCE concentration (43 μ*M*) as represented in Figures 5a and 6a. At the higher PCE concentrations of 317 and 1128 μ*M*, the model with competitive and Haldane inhibition kinetics provided a better fit to the experimental data than the model with only competitive inhibition kinetics. Like the test results, the model simulations of the BM culture yield results between those obtained by the individual cultures.

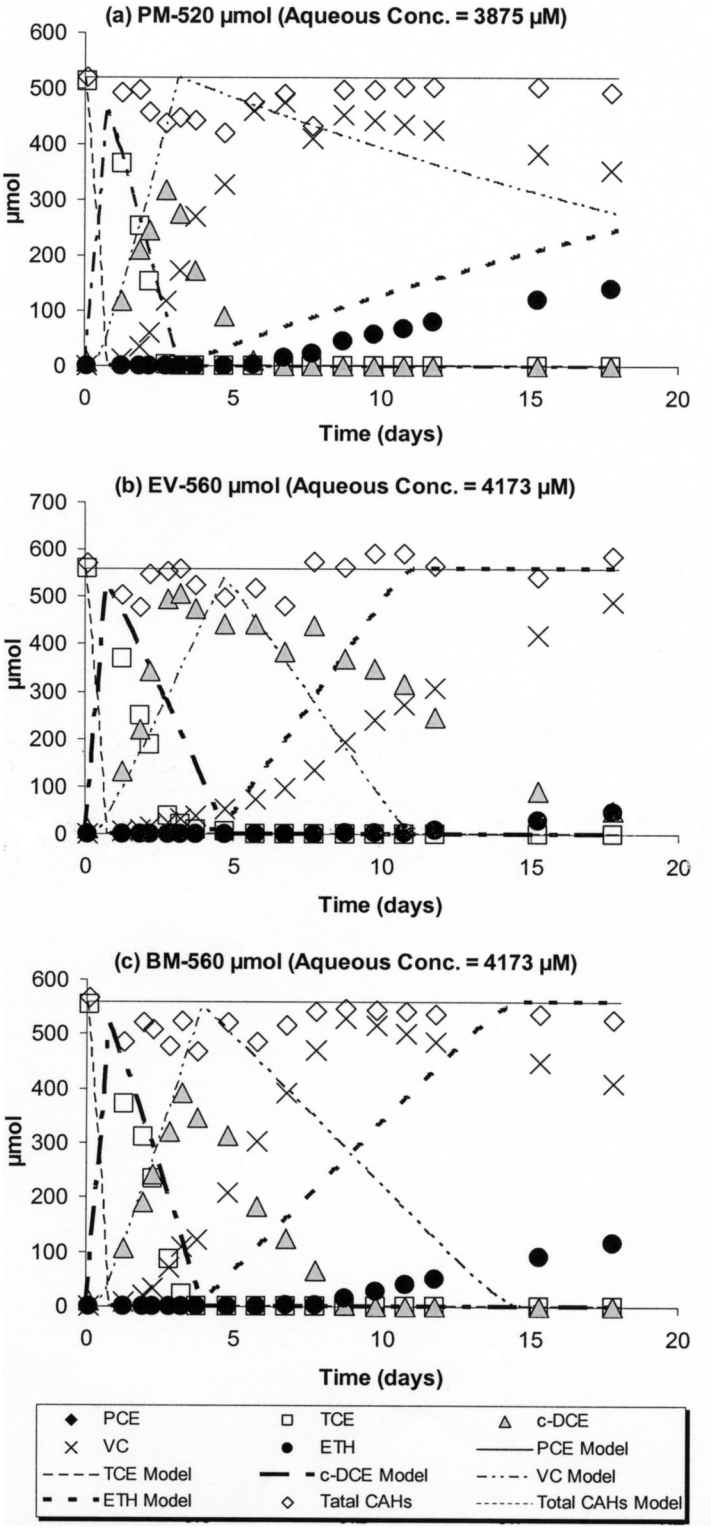

Figure 7. Reductive dechlorination kinetics at high TCE concentrations by the PM, EV, and BM cultures. The lines represent model simulations with competitive inhibition kinetics.

Reductive Dechlorination of a High TCE Concentration

Figures 7 and 8 present the results of batch tests with initial TCE concentrations at half its solubility limit (TCE solubility = 8400 μ*M*; Yaws, 1999). With all the three cultures, TCE was readily dechlorinated within 4 days without any lag time. The highest *c*-DCE aqueous concentration of 3930 μ*M* was observed on day 2–3 with the EV culture, while the PM culture showed the lowest maximum concentration of 2480 μ*M*. Different patterns of ETH production were observed at these high TCE concentrations compared to those observed with PCE. By day 18, the PM culture produced the highest ETH concentration (383 μ*M*), while the lowest occurred with the EV culture. Much slower transformation of *c*-DCE to VC was observed with the EV culture than with the PM and BM cultures. This leads to a slower rate of ETH production, suggesting toxicity or inhibition at high *c*-DCE concentrations.

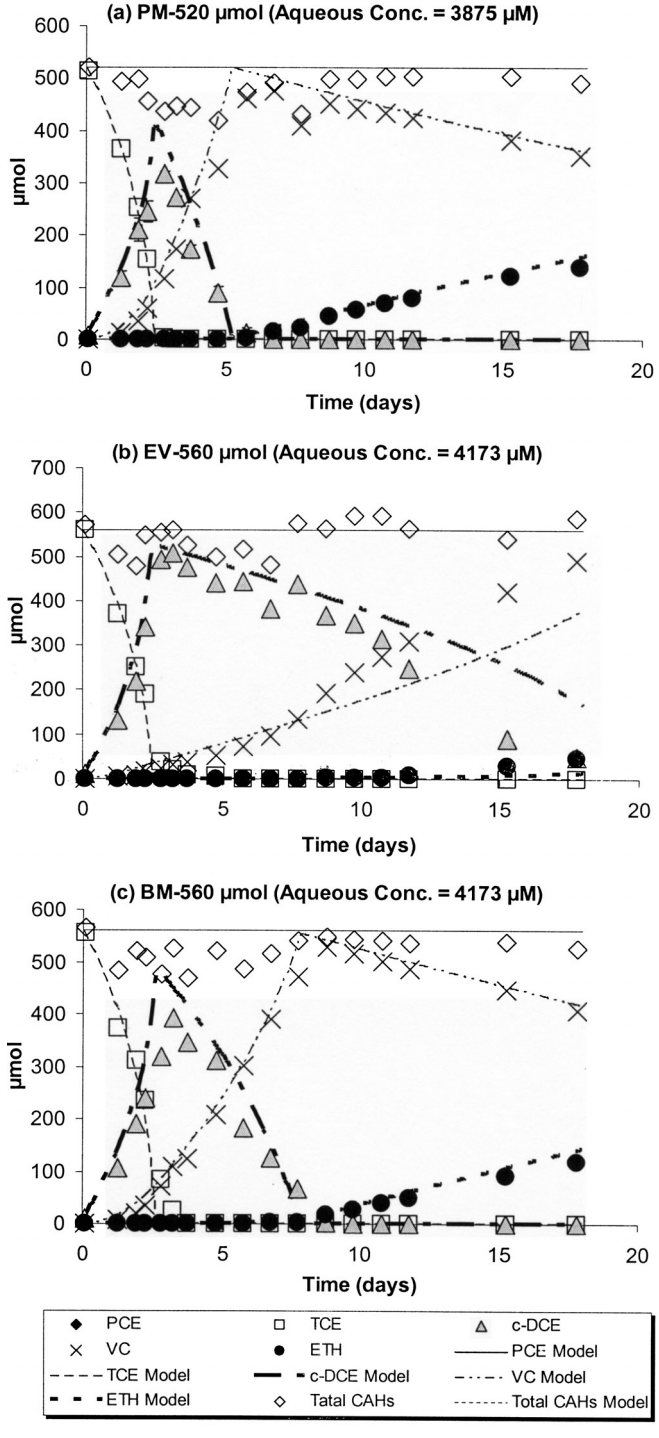

Figure 8. Reductive dechlorination kinetics at high TCE concentrations by the PM, EV, and BM cultures. The lines represent model simulations with Haldane and competitive inhibition kinetics.

Simulations using only competitive inhibition and both competitive and Haldane inhibition are shown in Figures 7 and 8, respectively. The initial measured total cell concentrations for the PM, EV, and BM cultures were 35, 40, and 37 mg-protein/L, respectively. The BM culture consists of 17 mg-protein/L of the PM culture and 20 mg-protein/L of the EV culture, calculated as previously described. Using only competitive inhibition kinetics, the dechlorination of TCE, *c*-DCE, and VC by all three cultures was predicted to occur more rapidly than was actually observed (Fig. 7). Model simulations with both competitive and Haldane inhibitions (Fig. 8) simulated the experimental data much better. For the PM culture, the main differences between the model simulations and the experimental data result from including TCE Haldane inhibition (Fig. 8a). Using a Haldane inhibition constant of 900 μ*M*, the simulations match the temporal response of TCE well, showing an acceleration in rates as concentrations are reduced, consistent with Haldane kinetics. *c*-DCE and VC transformations had weak Haldane inhibitory effects with Haldane inhibition constants of 6000 and 7000 μ*M*. Comparison of the different model simulations for the EV culture showed TCE, *c*-DCE, and VC have significant Haldane inhibition. The model fits the experimental data well with Haldane inhibition constants of 900, 750, and 750 μ*M*, respectively. The transformations of TCE, *c*-DCE and VC were much better predicted by simulations that included Haldane inhibition. Simulations of TCE transformation histories for the EV and BM cultures show the accelerations in rates, observed in the experiments. With Haldane inhibition constants obtained from fitting the data with the PM and EV cultures, the model simulations for the BM culture showed good agreement with the experimental results for both PCE (Fig. 6c) and TCE (Fig. 8c). The simulation of the performance of the BM culture is in between those of the PM and EV cultures, which is consistent with the experimental results.

DISCUSSION

A kinetic model employing Michaelis-Menten equations with a combination of competitive and Haldane inhibition kinetics simulated well the sequential transformation of PCE and TCE over a very wide range of concentrations, for three different anaerobic enrichment cultures. Simulations using the competitive inhibition kinetic model with

inhibition constants set equal to the measured K_S values (Yu, 2003) matched experimental data well for PCE concentrations lower than 300 μM, but deviated from experimental data at PCE and TCE concentrations higher than 300–400 μM. The model that included both competitive and Haldane inhibition kinetics provided much better fits to the experimental results at elevated concentrations.

A number of modeling studies described reductive dechlorination of PCE using competitive inhibition (Cupples et al., 2004; Garant and Lynd, 1998; Haston, 1999; Tandoi et al., 1994; Tonnaer et al., 1997). These studies were all conducted at aqueous PCE concentrations lower than 300 μM. Our modeling and experimental results indicate that a kinetic model that incorporates both competitive and Haldane inhibition kinetics results in a better match to experimental results near the saturation PCE concentration (1000 μM), and for a TCE concentration of 4000 μM, that is 50% of its solubility limit in water. Maillard et al. (2003) reported kinetics for PCE reductive dehalogenase (PCE-RDase) purified from *Dehalobacter restrictus* that exhibited Haldane type kinetics for TCE concentrations higher than approximately 300–400 μM. The results also showed limited inhibition of PCE transformations, consistent with the results of this study.

Additional model simulations, including only competitive inhibition, were run in our study for high concentrations of PCE (1000 μM) and TCE (4000 μM). The cell decay constant, k_d (0.024 d^{-1}) (Fennell and Gossett, 1998) that was used in all the simulations, was increased up to 0.09 d^{-1} as reported by Cupples et al. (2003). This higher decay constant slightly improved the agreement with the experimental data, but the model continued to predict much faster production and transformation rates of CAHs than were observed experimentally.

Changes in total cell concentrations during PCE dechlorination experiments were simulated as shown in Figure 9. PCE kinetic experiments were conducted with the incremental additions of PCE in succession (Table I). Total cell concentrations as measured by protein analysis at the beginning of each run were used as the model simulation inputs. As shown in Figure 9, simulated cell concentrations for the PM, EV, and BM cultures were reasonably consistent with the protein concentrations measured at the beginning of each PCE experiment.

A limitation of this study is that the measured biomass based on protein content does not represent only dechlorinating microorganisms. The cultures, however, were highly enriched with dechlorinating microorganisms, based on T-RFLP analysis. The k_{max} used were based on protein content, resulting in a normalization in rates on a protein concentration basis. The simulated and measured total biomass values appeared to match well at the beginning and end of each PCE experiment. Thus, our assumption that most of the protein was associated with dehlorinating microorganisms seems reasonable.

Haldane kinetic parameters obtained from our model simulation fits to the experimental results were used to construct Haldane rate vs. concentration curves. Figures 10 and 11 present the calculated Michaelis-Menten and Haldane curves for TCE, *c*-DCE, and VC for the two cultures. The PM and EV cultures showed different Haldane inhibition patterns. The PM culture has very high Haldane inhibition constants for *c*-DCE and VC (6000 and 7000 μM, respectively), indicating very weak Haldane inhibition effects. The EV culture, however, showed significant Haldane inhibition for TCE, *c*-DCE, and VC. Figures 10b and c would indicate that little reduction in dechlorination rates should occur at relatively high *c*-DCE and VC concentrations with the PM culture. However, significant decreases in dechlorination rates are expected with increases of TCE concentration for the PM culture (Fig. 10a), and TCE, *c*-DCE, and VC concentrations with the EV culture (Fig. 11a, b, c). One possible explanation for the PM culture having less Haldane inhibition effects is that it was acclimated more to a high TCE concentration of 100 mg/L, while the EV

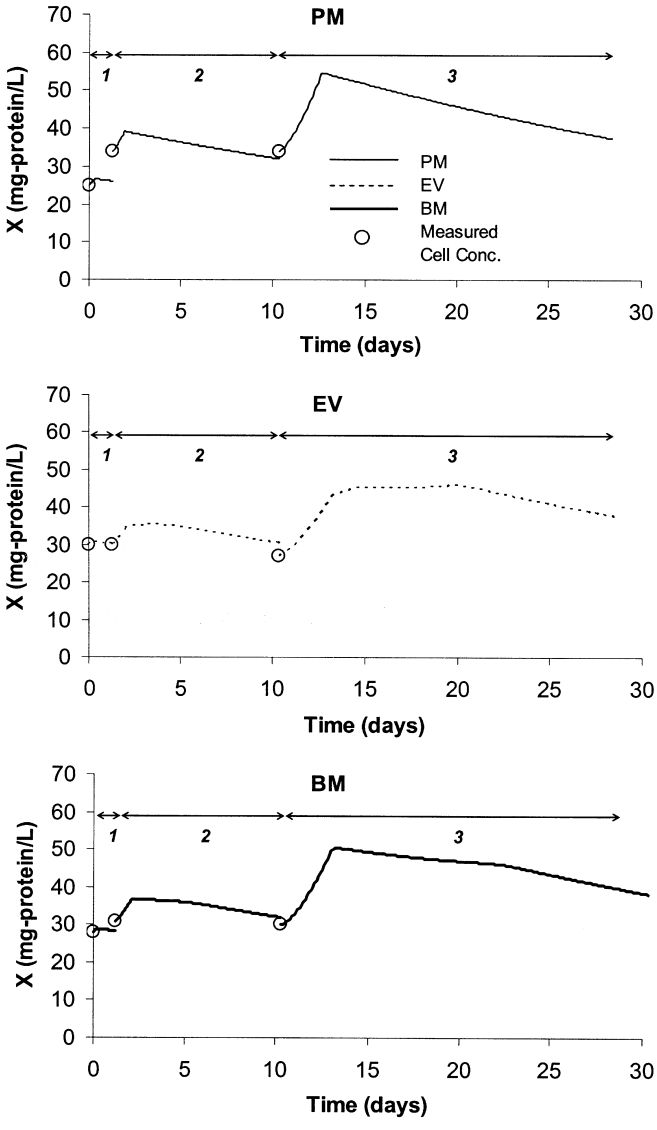

Figure 9. Model simulations of total cell concentrations during PCE reductive dechlorination Runs 1, 2, and 3 as listed in Table I.

culture was enriched with PCE. TCE kinetics using a model with noncompetitive inhibition as reported by Maillard et al. (2003) was compared in Figures 10a and 11a. An inhibition constant of 760 μM (close to the K_{HI} value of 900 μM observed in our study) and a half-velocity coefficient of 23.7 μM were used in their model kinetics. Since Maillard et al. (2003) used purified PCE-RDase, their enzyme-based rates were reduced by a factor of 140 to compare our inhibition patterns with theirs. It is very interesting that our Haldane model used to fit to our experimental data with whole cells has a similar concentration vs. rate

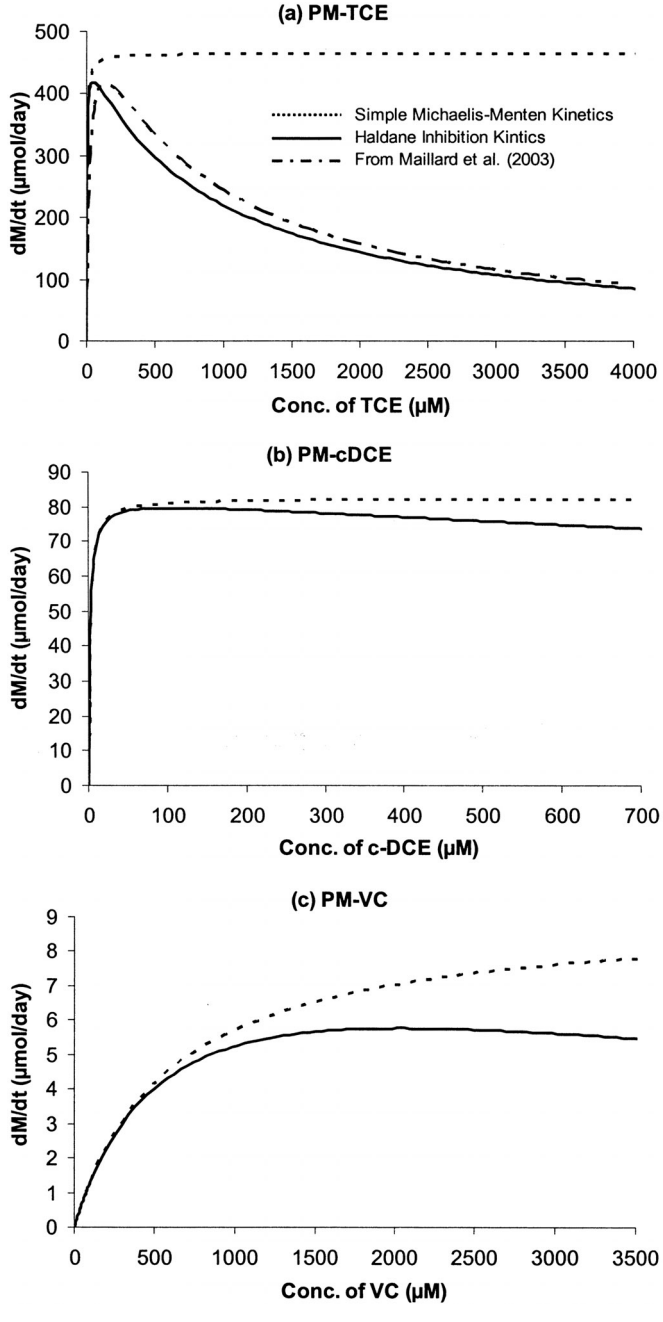

Figure 10. Michaelis-Menten kinetics with and without Haldane inhibition for the PM culture. Rates were determined based on a total dechlorinating cell concentration of 30 mg/L.

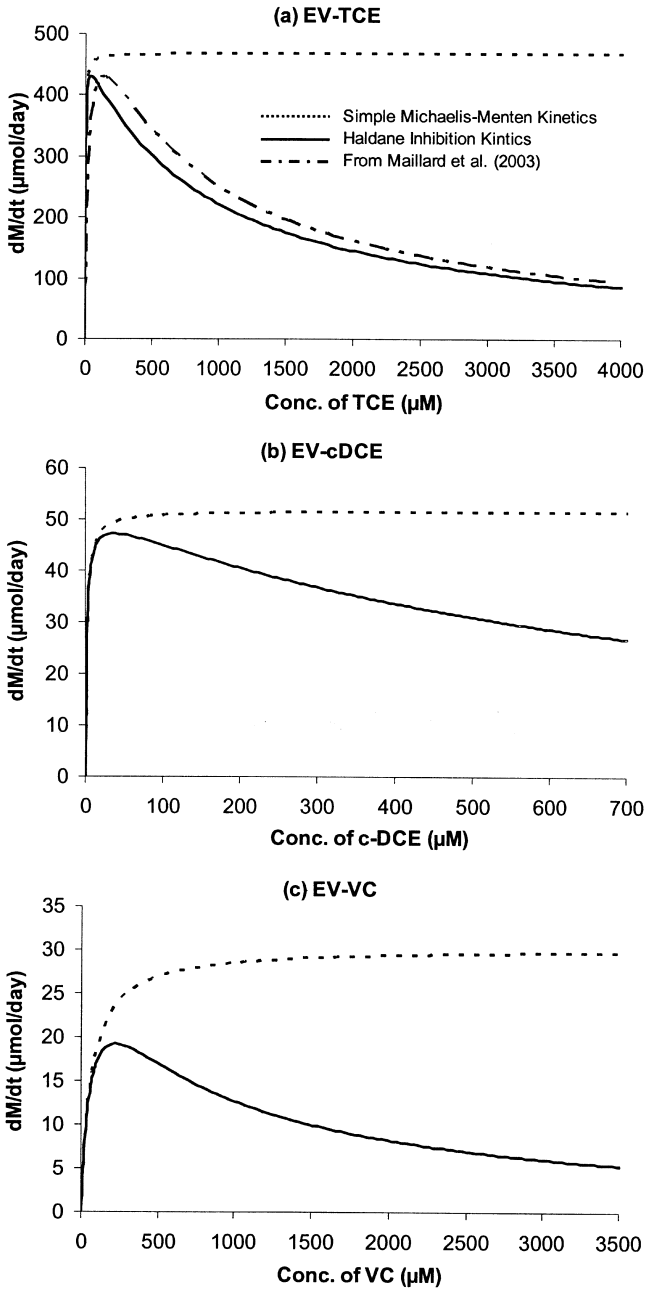

Figure 11. Michaelis-Menten kinetics with and without Haldane inhibition for the EV culture. Rates were determined based on a total dechlorinating cell concentration of 30 mg/L.

dependence as that observed by Maillard et al. (2003). Thus, our observation of Haldane type kinetics at a high TCE concentration is consistent with their observations.

As shown in Figures 1 through 6, the BM and EV cultures more effectively transformed PCE to ETH. However, PCE solubility is much lower than that of the other chlorinated ethylenes. TCE has a solubility of 8400 μM and high concentrations may be found near DNAPL source zones in soil and groundwater. The experimental and modeling results shown in Figures 7 and 8 show sequential dechlorination of TCE at an average initial concentration

of 4073 μ*M*. The pattern of reductive dechlorination between three different mixed cultures differed from that of PCE dechlorination in Figures 1 through 6. The PM and BM dechlorinating cultures showed greater transformations of high TCE concentrations to ETH than the EV culture. The EV and BM cultures, however, show better transformation of PCE to ETH than the PM culture. The results of our modeling analysis indicate factors causing this response. At higher TCE concentrations, the EV culture is experiencing greater Haldane inhibition of *c*-DCE and VC transformation. The experimental results in Figure 8 showed that TCE depletion rates of the PM, EV, and BM cultures are almost the same, but differences for *c*-DCE and VC transformations are observed between the PM and EV cultures. Our modeling studies indicated the EV culture has lower K_{HI} values of 750 μ*M* for both *c*-DCE and VC compared to those of 6000 and 7000 μ*M* of the PM, respectively, indicating higher *c*-DCE and VC concentrations to the EV culture are potential toxic or inhibitory. Strong inhibition of *c*-DCE on VC transformation limits ETH production in all the cultures, as previously observed (Yu, 2003). The modeling presented here indicates that the modeling of *c*-DCE transformation with Haldane kinetics is an important process to predict reductive dechlorination of PCE to ETH at high concentrations.

From the reductive dechlorination results with a broad range of PCE and TCE concentrations, the BM culture obtained transformation abilities of both cultures. This led to overall good performance when both PCE and TCE test results were considered. Bioaugmentation with a binary mixed culture might be more effective at real contaminated sites, where wide ranges in concentrations and contaminant mixture are present. Further investigations utilizing more complex microbial multi-systems of defined cultures with different degradation properties need to be performed to better utilize and understand their potential for application in the polluted environments as suggested by Fairlee et al. (1997) and Rogers et al. (2000).

A variety of reductive CAH dechlorination models have been proposed (Bagley, 1998; Fennell and Gossett, 1998; Garant and Lynd, 1998; Haston, 1999;Tonnaer et al., 1997). Fennell and Gossett (1998) developed a model for complete PCE reductive dechlorination to ETH, in which PCE concentration was a 70 μ*M* PCE aqueous concentration and no inhibition between chlorinated ethylenes was considered. Previously proposed models focused on competitive inhibitions between PCE and TCE (Tonnaer et al., 1997), and *c*-DCE and VC (Cupples et al., 2004; Haston, 1999). To the best of our knowledge, the experimental data and simulations presented here are the first to describe the sequential transformation of PCE and TCE over a wide range of concentrations. Both competitive and Haldane kinetics were required to model the experimental observations for the anaerobic reductive dechlorination of CAHs at high concentrations, possibly produced from DNAPL source zone. Kinetic studies are now needed with these cultures at high concentrations of TCE, *c*-DCE, and VC, to confirm the Haldane kinetics obtained from our heuristic fitting of the high concentration test results.

The research was supported by a research grant from the U.S. Environmental Protection Agency-sponsored Western Region Hazardous Substance Research Center under agreement R-828772. This article has not been reviewed by the agency, and no official endorsement should be inferred. We thank Mark Dolan for his help with the molecular characterization of the cultures.

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