Kinetics and Inhibition of Reductive Dechlorination of Chlorinated Ethylenes by Two Different Mixed Cultures

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Kinetic studies with two different anaerobic mixed cultures (the PM and the EV cultures) were conducted to evaluate inhibition between chlorinated ethylenes. The more chlorinated ethylenes inhibited the reductive dechlorination of the less chlorinated ethylenes, while the less chlorinated ethylenes weakly inhibited the dechlorination of the more chlorinated ethylenes. Tetrachloroethylene (PCE) inhibited reductive trichloroethylene (TCE) dechlorination but not cis-dichloroethylene (c-DCE) dechlorination, while TCE strongly inhibited c-DCE and VC dechlorination. c-DCE also inhibited vinyl chloride (VC) transformation to ethylene (ETH). When a competitive inhibition model was applied, the inhibition constant (K) for the more chlorinated ethylene was comparable to its respective Michaelis–Menten half-velocity coefficient, Ks. Model simulations using independently derived kinetic parameters matched the experimental results well. Kmax and Ks values required for model simulations of anaerobic dechlorination reactions were obtained using a multiple equilibration method conducted in a single reactor. The method provided precise kinetic values for each step of the dechlorination process. The greatest difference in kinetic parameters was for the VC transformation step. VC was transformed more slowly by the PM culture (kmax and Ks values of 2.4 ± 0.4 μmol/mg of protein/day and 602 ± 7 μM, respectively) compared to the EV culture (8.1 ± 0.9 μmol/mg of protein/day and 62.6 ± 2.4 μM). Experimental results and model simulations both illustrate how low Ks values corresponded to efficient reductive dechlorination for the more highly chlorinated ethylenes but caused strong inhibition of the transformation of the less chlorinated products. Thus, obtaining accurate Ks values is important for modeling both transformation rates of parent compounds and their inhibition on daughter product transformation.

Introduction

Tetrachloroethylene (PCE) and trichloroethylene (TCE) are among the most commonly observed groundwater contaminants (1, 2). Many laboratory studies have shown that PCE acting as an electron acceptor can be reductively dechlorinated to TCE, cis-dichloroethylene (c-DCE), vinyl chloride (VC), and finally to ethylene (ETH) using hydrogen as an electron donor (3, 4). However, the accumulation and persistence of intermediate products such as c-DCE and VC is of concern, since VC is a known carcinogen.

The competition for H2 by dechlorinating microorganisms, nitrate and sulfate reducers, methanogens, and acetogens has previously been reported (5–7). However, few studies have been performed on the inhibition among chlorinated ethylenes during reductive dechlorination. Inhibition of VC dechlorination by other chlorinated ethylenes was indicated by modeling studies of Tandol et al. (8). Haston (9) reported competitive inhibition between c-DCE and VC and showed the inhibition constants were comparable to their respective half-velocity coefficients. Garant and Lynd (10) showed that a competitive inhibition model fit experimental data better than the noncompetitive model for a culture capable of complete dechlorination of PCE to ETH. The kinetic parameters used in their study were obtained from statistical fits of the model to the experimental data, not independently determined. Cupples et al. (11) recently showed competitive inhibition between c-DCE and VC with a dechlorinating culture that grows on c-DCE and VC. However, inhibition studies on each step of the dechlorination process are needed.

Reported here are the results of the kinetic characterization of two different mixed cultures that completely dechlorinate PCE to ETH. A simple multi-equilibration method was developed to determine kmax and Ks values using a single batch reactor. Batch kinetic experiments were also conducted to study chlorinated aliphatic hydrocarbon (CAH) inhibition for each step of reductive dechlorination from PCE to ETH. The inhibition of more chlorinated ethylenes on less chlorinated products was evaluated and vice versa. A model including terms for competitive inhibition was employed and compared with the experimental results using independently determined kinetic parameters.

Materials and Methods

Chemicals. Commercially available PCE, TCE, and VC (no stabilizers) were used in the kinetic experiments. PCE (99.9%), spectrophotometric grade), TCE (99.9%), and c,1,2-DCE (97%) were obtained from Acros Organics (Pittsburgh, PA), and VC and ETH (both 99.5%, Aldrich Chemical, Milwaukee, WI) were used in the preparation of analytical standards and for addition to the batch reactors. 1-Butanol (99.8%, HPLC grade, Aldrich Chemical, Milwaukee, WI) and hydrogen (99%, Airco, Inc., Albany, OR) were used as the electron donors.

Analytical Methods. PCE, TCE, c-DCE, VC, and ETH in the reactors were measured with an HP-6890 gas chromatograph (GC) equipped with a photoionization detector (PID) and flame ionization detector (FID) connected in series. The headspace samples were obtained and transferred using 100 to 250 μL gas tight syringes (Hamilton, Leno, NV). Chromatographic separation of 100–200 μL reactor headspace samples was achieved using a 30 m x 0.53 mm GS-Q column (J&W Scientific, Folsom, CA) with helium as the carrier gas (15 mL/min). The GC oven was initially set 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. Hydrogen concentrations in headspace gas samples (200 μL) were determined using an HP-5890 GC with a thermal conductivity detector (TCD), operated isothermally at 220 °C. The hydrogen samples were chromatographically separated with a Carboxen 1000 column (15 ft x 1/8 in, Supelco, Bellefonte, PA) using argon gas as a carrier gas at 15 mL/min. The hydrogen detection limit was 4 nM (aqueous concentration).

Culture Enrichment and Growth. Two different mixed cultures were enriched and used in this study. Microbial
mixed cultures obtained from Point Mugu Naval Weapon Facility, CA (PM) and the Evanite site in Corvallis, OR (EV), were enriched in two separate batch reactors (total volume 1.2 L with liquid volume of 1 L) fitted with gray chlorobutyl rubber septa (Wheaton Industries, Millville, NJ). The PM culture was originally enriched under anaerobic conditions with aquifer solids and groundwater from the site (12). After 1.5 years, supernatant (300 mL) was transferred into sterile basal medium described by Yang and McCarty (6). The medium was modified to double its buffer capacity (1 g/L of K2HPO4 and 3 g/L of Na2CO3), and sodium chloride and ammonium chloride were replaced with sodium bromide and ammonium bromide, respectively, to make a low-chloride content medium. Initially, 10 mg/L of TCE was added with 5–10 mg/L of butanol. Over a 1 year period, TCE concentrations were increased in steps up to 100 mg/L while maintaining complete reductive dechlorination to ETH. The batch enrichment reactor was operated on a fill-and-draw mode with a hydraulic residence time of about 45 days. After 50 to 100% of the TCE was reductively dechlorinated to ETH, the batch reactor was purged with a furnace-treated gas consisting of N2 (90%) and CO2 (10%). Cells were harvested by replacing 300 mL of the liquid culture with fresh anaerobic medium. Initially, 10 mg/L of TCE was added 20 °C with continuous shaking at 200 rpm. The PM culture was fed TCE as the electron acceptor because the culture was originally unable to transform PCE. After about 3 years of enrichment on TCE, the PM mixed culture started to show PCE dechlorination ability.

The EV culture was enriched from groundwater obtained from the Evanite site in Corvallis, OR (12, 13), using similar enrichment procedures as the PM culture; however, enrichment was on PCE instead of TCE. The batch enrichment reactor was maintained at 20 °C with continuous shaking at 200 rpm. The PM culture was fed TCE as the electron acceptor because the culture was originally unable to transform PCE. After about 3 years of enrichment on TCE, the PM mixed culture started to show PCE dechlorination ability.

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Both of the cultures initially showed very active methanogenesis with butanol added as an electron donor (12). However, no methanogenesis was observed after 2–3 years of enrichment on the high PCE or TCE concentrations.

A PCR assay targeting genes encoding for 16S ribosomal RNA was performed to determine if Dehalococcoides-like microorganisms were present in the mixed cultures. DNA was extracted from the PM and EV cultures using FastDNA spin kits for soil (Qbiogene, Inc., Carlsbad, CA). For each culture, amplification reactions were performed using 5 μL of template DNA and three different primer pairs. One primer pair, developed by Löffler et al. (14), was specific to the hypervariable regions of the 16S rRNA genes of the PCE-dechlorinating Desulfituromonas sp., including D. chloroethenica and D. sp. strain BB1, and consisted of forward primer Dsm-205F, 5′-AACCTTGCGGTCTCAGTC-3′, and the reverse primer Dsm-1051R, 5′-GCCGAACCTGACCCTATGTT-3′. Another primer pair, also developed by Löffler et al. (14), was specific to the hypervariable regions of the 16S rRNA genes of two members of the Dehalococcoides genus, D. ethenogenes and D. sp. strain FL2, and consisted of forward primer Dgh-728F, 5′-AAGGCGGTTTTTATGAGTTGC-3′, and the reverse primer Dgh-1155R, 5′-CGTATTGCGGGGCGAGTCT-3′. The final primer pair, developed by Hendrickson et al. (15), was based on consensus variable and hypervariable regions in the 16S rRNA gene sequences of D. ethenogenes and Dehalococcoides sequences from the Victoria and Pinellas cultures and consisted of forward primer DHC-1F, 5′-GATGAAACGGTGACGCGGC-3′, and the reverse primer DHC-1377R, 5′-GTTGCACATGCCTACCTCA-3′. Both the PM and EV cultures produced bright bands of the expected length when amplified with the Dehalococcoides specific primers sets and produced no bands when amplified with the Desulfuromonas specific primers indicating that Dehalococcoides-like microorganisms were present in both cultures and were likely responsible for the complete reductive dechlorination to ETH. This result was also in accordance with a previous T-RFLP analysis that showed 65% of the universally amplified DNA from the EV culture had a TFL consistent with that predicted from Dehalococcoides sequence information available in GenBank.

**Determination of Cell Concentration.** The total cell concentration, X, in the study was determined by protein analysis using the Pierce Micro BCA Protein Assay Reagent Kit (Pierce, Rockford, IL). All samples were pretreated with the Compat-Able Protein Assay Preparation Reagent Set (Pierce, Rockford, IL) to eliminate potentially interfering substances prior to total protein quantitation. The protein concentration detection limit was 0.5 mg/L.

**Kinetic Study for Determination of kmax and Ks.** An experimental procedure was developed to determine kmax and Ks values for PCE, TCE, c-DCE, and VC dechlorination using sequential equilibrations in batch reactors. The method involved stepwise increases of the CAH concentration over a short time interval and measuring parent compound disappearance and daughter product production at each concentration step (Figure 1). The batch kinetic studies were conducted in 150 mL serum bottles containing 125 mL of liquid media and culture. The culture added to the reactor was harvested from the enrichment batch reactor 10 to 14 days after a batch feeding of PCE or TCE (50 to 100% converted to ETH). The amount of culture used was varied, depending on the test, to yield protein concentrations ranging from 2 to 50 mg/L. The batch reactors were constructed in an anaerobic glovebox and then purged with a furnace-treated mixed gas of N2 (90%) and CO2 (10%), followed by the addition of 10 μL of neat butanol (99.8%) and 0.5–2.0 mL of H2 gas
(99%) as electron donors. The reactors were initially amended with the lowest concentration of a chlorinated ethylene to be tested at a rate of 300 rpm at 20 °C. The rates of parent compound disappearance and daughter product production were generally measured over a period of less than 1 h by repeated headspace sampling. Total CAH mass in the reactor was computed using published Henry’s Law constants (16, 17). Most of the kinetic parameters in the experiments were obtained based on the measurement of dechlorination products due to the greater sensitivity in measuring product production rates. The rates were determined at early time before the product was significantly transformed. After measuring the dechlorination rate at the lowest CAH concentration, the reactor was purged with the mixed gas (90% N₂ and 10% CO₂) for 5–10 min to remove all the dechlorination products from the reactor. H₂ was added, along with a higher concentration of the chlorinated ethylene, and the transformation rates were again measured using the procedures previously described.

The set of kinetic experiments with a particular chlorinated ethylene were usually completed within 10 h to minimize microbial growth. Doubling times for dechlorinating cultures have been reported to be approximately 2 days (18, 19). Thus, the 10 h time period to complete the test was relatively short compared to the doubling time. The kinetic batch experiments were conducted in duplicate or triplicate to ensure that kinetic parameters were statistically reliable. Biogenic c-DCE was used for c-DCE dechlorination rate measurements, since chloroform present in commercial c-DCE has inhibitory effects on CAH utilization (9, 20). Biogenic c-DCE was produced from TCE dechlorination by a mixed anaerobic culture obtained from Site 300 at Lawrence Livermore National Laboratory, CA (LLNL), which dechlorinates TCE to c-DCE (12). An enrichment reactor of the LLNL culture was fed TCE to produce c-DCE, c-DCE dissolved in liquid was obtained from centrifuging the reactor at 8000 rpm and filtering the liquid suspension through a 0.2 μm syringe filter. The filtrate was used as the biogenic source of c-DCE in the kinetic experiments.

**Inhibition Study.** Inhibition of chlorinated ethylenes on transformation of each other was also studied. For example, PCE and TCE were tested for inhibitory effects on each other. The same aqueous concentration of TCE was added to 3 to 4 batch reactors with different concentrations of PCE for the evaluation of PCE inhibition of TCE transformation. Similarly, inhibition of TCE on the reductive PCE dechlorination was evaluated at the same aqueous PCE concentration with different concentrations of TCE. For the experiments of PCE inhibition on TCE dechlorination, the production rates of c-DCE formation were used to determine rates of TCE transformation, since c-DCE transformation to VC was not significantly observed during the test. However, decreases in PCE concentrations were used to quantify TCE inhibition on PCE dechlorination because high concentrations of TCE as an inhibitor were already present. Mass balances showed that at the highest TCE concentration there was less than a 20% difference in rates determined by parent compound disappearance and daughter product production. At lower TCE concentrations the differences were 3 to 7%. For these inhibition studies, several sets of chlorinated ethylenes were chosen including PCE ↔ TCE, PCE ↔ c-DCE, TCE ↔ c-DCE, TCE ↔ VC, and c-DCE ↔ VC. Both inhibitions of the more chlorinated ethylene on the less and the less on the more were studied. PCE inhibition of VC transformation was not studied after it was established that PCE did not inhibit c-DCE transformation and thus would not likely inhibit a further step in the transformation process. The concentrations of CAHs as reactants (not as inhibitors) ranged from 30 to 251 μM. These concentrations were chosen in order to complete the kinetic experiments within 3–4 h. The VC concentration of 251 μM was the highest used because of the high KS values for both cultures.

**Model Development**

**Kinetic Model for Reductive Dechlorination.** The dechlorination rate of a given CAH can be described using Michaelis–Menten kinetics (21, 22). Reductive dechlorination of CAHs can be affected by several factors, including inhibition among chlorinated ethylenes, electron donor concentrations, and CAH toxicity (9). However, for these kinetic experiments the chlorinated ethylene concentration was considered to influence only the kinetic parameters, kmax and Ks. This was regarded as a valid assumption because the initial dechlorination rates were measured before concentrations of the product CAHs could accumulate to inhibitory or toxic levels (23). Electron donor limitations were also excluded in the experiments by adding H₂ and butanol in excess. Thus, a Michaelis–Menten model based on electron acceptor concentrations was used including terms for the transformation and production of the CAH of interest

\[
\frac{dC_{LY}}{dt} = -\frac{k_{\text{max,Y}}C_{LY}}{K_{S,Y} + C_{LY}} + \frac{1}{1 + \frac{V_C}{V_L}K_{HCC}}
\]

where Cᵢ is the CAH aqueous concentration (μmol/L), kmax is the maximum specific CAH dechlorination rate (μmol/mg of protein/day), X is the biomass concentration (mg of protein/L), and Kᵢ is the half-velocity coefficient (μmol/L). Since the kinetic experiments were conducted with batch bottles consisting of gas and aqueous phases, the Michaelis–Menten equation was modified (24), where Vᵥ and Vₐ are the volumes of gas and aqueous phases, respectively, and the dimensionless Henry’s constant (Hᵥᵥ) was used for each chlorinated ethylene (16, 17). For measurement of the dechlorination rates, the total CAH mass in the bottle was computed from headspace measurement using published Henry’s constants (16, 17). The aqueous CAH concentration in eq 1 is related to the total mass of each CAH via a mass balance equation (Cᵢ = M(Vᵥ + VₐHᵥᵥ)). For multiple equilibration tests with eq 1, the values of kmax and Kᵢ were estimated without the production term, using a nonlinear least-squares regression fitting program [Solver in Excel (Microsoft, Redmond, WA) and S-PLUS (Insightful, Seattle, WA)].

**Competitive Inhibition among Chlorinated Ethylenes.** Competitive inhibition among chlorinated ethylenes was included in the modeling analysis (9, 10). The reductive dechlorination rate of a CAH was modeled with competitive inhibition kinetics using eq 2

\[
\frac{dC_L}{dt} = -\frac{k_{\text{max,L}}X_C_L}{K_{S,L} + 1 + \frac{C_{1,L}}{K_{1,L}} + \frac{C_{2,L}}{K_{2,L}}} + C_L
\]

where inhibition constants of each chlorinated ethylene are expressed as K₁,i and K₂,i (μmol/L). As will be described in the Results and Discussion, the respective half-velocity coefficients were used for the inhibition constants. The model included TCE, c-DCE, and VC as the major intermediates in PCE dechlorination to ETH, not 1,1-DCE or t-DCE.

Based on the results of the inhibition experiments, a conceptual inhibition model was developed (Figure 2). The equations for reductive dechlorination by the anaerobic cultures including appropriate inhibition terms are presented
in eqs 3–6. The proposed inhibition patterns (Figure 2) and resulting equations assume PCE inhibits TCE transformation, TCE inhibits both c-DCE and VC transformation, and c-DCE inhibits VC transformation. As the inhibition results will show, the less chlorinated ethylene inhibition on more chlorinated ethylenes was very weak and therefore was not included in the model equations.

\[
\frac{dC_{\text{PCE}}}{dt} = -k_{\text{max, PCE}}X_c C_{\text{PCE}} + k_{\text{max, PCE}}X_c C_{\text{TCE}} + k_{s_{\text{PCE}}} + C_{\text{PCE}}
\]

\[
\frac{dC_{\text{TCE}}}{dt} = -k_{\text{max, TCE}}X_c C_{\text{TCE}} + k_{\text{max, PCE}}X_c C_{\text{TCE}} + k_{s_{\text{TCE}}} + C_{\text{TCE}}
\]

\[
\frac{dC_{\text{c-DCE}}}{dt} = -k_{\text{max, c-DCE}}X_c C_{\text{c-DCE}} + \frac{k_{\text{max, TCE}}X_c C_{\text{TCE}}}{k_{s_{\text{TCE}}} + C_{\text{TCE}}}
\]

\[
\frac{dC_{\text{VC}}}{dt} = -k_{\text{max, VC}}X_c C_{\text{VC}} + \frac{k_{\text{max, TCE}}X_c C_{\text{TCE}}}{k_{s_{\text{TCE}}} + C_{\text{TCE}}}
\]

Equations 3–6 were combined to generate the series of rate equations that include transformation, production, and partitioning, as shown in eq 1.

**Kinetic Model for Biomass Growth.** Since VC inhibition experiments lasted several days, the potential growth of culture was included in the model using eq 7. Microbial growth was calculated with respect to the transformation rate of each chlorinated ethylene. The PM culture was assumed to grow on PCE, TCE, and c-DCE. VC transformation to ETH was assumed to be cometabolizable, since the measured $K_S$ value for VC transformation of 602 µmol/L was in the range of that reported for *Dehalococcoides ethenogenes* strain 195, which transforms VC cometabolically. Thus, no biomass growth was coupled with kinetic modeling (25). Unlike the PM culture, microbial growth on each dechlorination step was considered for the EV culture (13).

\[
\frac{dX}{dt} = Y \frac{dC}{dt} - k_{d}X
\]

In eq 7, $Y$ is the growth yield of dechlorinating microorganisms, $dC/dt$ is the rate of concentration change of each chlorinated ethylene over time (µmol/L/day), and $k_{d}$ is the decay constant of the dechlorinating microorganisms (day$^{-1}$). Values for growth yield, $Y = 0.006$ mg of protein/µmol of C1 dechlorinated, and decay constant, $k_{d} = 0.024$ day$^{-1}$, were obtained from the literature (25, 21). For our tests, we do not know the actual biomass, $X$, of the dechlorinating microorganisms, and whether different dechlorinators are active at different steps of the process. Since $k_{\text{max}}$ values were determined on a total protein basis, the values were normalized to the total mixed culture biomass. The simultaneous solution of the model equations was obtained using STELLA Research 5.0 (High Performance Systems, Lebanon, NH).

**Results**

**Determinations of Kinetic Parameters.** The Michaelis-Menten curves for PCE, TCE, c-DCE, and VC transformation by the PM culture determined using the multiequilibrium kinetic method are shown in Figure 3. The triplicate determinations show statistically reliable results were obtained for all the chlorinated ethylenes tested. The Michaelis-Menten curves in Figure 3 show good fits for all chlorinated ethylenes. Table 1 presents $k_{\text{max}}$ and $K_S$ values for the PM and EV cultures for each step of the dechlorination process. The standard deviation of $k_{\text{max}}$ and $K_S$ based on triplicate determinations were approximately 11 and 16% of the parameter value, respectively. For comparison, Haston and McCarty (22) reported standard deviations in $k_{\text{max}}$, and $K_S$ of 17% and 60% of the parameter values, respectively, using single equilibrations in multiple reactors.

Table 1 also presents the $k_{\text{max}}$ and $K_S$ values obtained by Fennell and Gossett (21) for a mixed culture containing *Dehalococcoides ethenogenes* strain 195 and those obtained by Haston and McCarty (22), for comparison purposes. The PM and EV cultures have comparable $k_{\text{max}}$ and $K_S$ values for PCE and TCE (Table 1). For both cultures, the TCE $K_{\text{max}}$ values are a factor of 10 greater than those for PCE. The $K_S$ value for PCE of the PM culture is a factor of 2 greater than the EV culture, while the $K_{\text{max}}$ values are essentially equal, indicating that the EV culture transforms PCE more rapidly than concentrations below the $K_S$ values (1.6 µM). The $k_{\text{max}}$ and $K_S$ values for c-DCE and VC show significant differences between the two cultures. The $k_{\text{max}}$ for c-DCE of the EV culture was 40% lower than that of the PM culture, while the $K_S$ values were almost the same, indicating slower c-DCE biotransformation by the EV culture.

The $K_{\text{max}}$ value for VC by the EV culture was a factor of 3 greater, while the $K_S$ was a factor of 10 lower than the PM culture, indicating much more rapid VC transformation with the EV culture. This result was consistent with the assumption of potential growth of the EV culture using VC as an electron acceptor as discussed by Pon and et al. (19). Recently, VC-grown dechlorinating cultures (*Dehalococcoides* sp. strain BAV1 and *Dehalococcoides* sp. strain VS) were isolated [He et al. (18, 26) and Cupples et al. (19)]. The $K_S$ value (62.6 µM) of the EV culture is closer to those of the VC-grown cultures (5.8 µM and 2.6 µM, respectively) than that of *Dehalococcoides ethenogenes* strain 195 (290 µM) (21), which cometabolically transforms VC. The slower maximum transformation rate and higher $K_S$ value (602 µM) for VC with the PM culture are consistent with cometabolic VC transformation (21, 25). The transformation characteristics of the PM culture are similar to that of *Dehalococcoides ethenogenes* strain 195 that has a high $K_S$ value (21).

**Competitive Inhibition among Chlorinated Ethylenes.** Results of inhibition studies are shown in Figures 4–8. The lines shown in each figure represent model simulations using the series of equations previously described. The model used the independently determined kinetic parameters listed in Table 1 with the initial biomass determined by protein analysis. The measured $K_S$ values were used as putative inhibition constants. Figure 4(a), (b) presents PCE inhibition...
on TCE transformation. As PCE concentrations increased, rates of c-DCE production decreased for both cultures, indicating that TCE dechlorination was inhibited by PCE. The EV culture was more strongly inhibited by PCE than the PM culture. A PCE concentration approximately 2.5 times higher than that used for the EV culture was required to obtain similar inhibition of the PM culture. The effect of TCE on PCE dechlorination was also evaluated (data not shown). PCE dechlorination was not clearly inhibited by TCE, suggesting there is very weak inhibition of TCE on PCE dechlorination.

Inhibition of TCE on c-DCE dechlorination is presented in Figure 4(c),(d). TCE inhibition of c-DCE transformation was observed with both cultures, with the EV culture being more strongly inhibited. The $K_s$ value for TCE of the EV culture was a factor of 1.5 lower than the PM culture, which was consistent with the greater TCE inhibition with the EV culture. Simulations based on the inhibition model given in eq 5 showed good agreement with the inhibition experimental results. TCE inhibition on VC transformation was also examined, as shown in Figure 4(e),(f) for the PM and EV cultures, respectively. TCE strongly inhibited VC transformation to ETH with both cultures. The EV culture more rapidly transformed VC (by a factor of 10) than the PM culture (note the different y-axis scales), even though similar protein concentrations were used. This result is consistent with the EV’s $k_{\text{max}}$ value being a factor of 3 higher and the $K_s$ value being a factor of approximately 10 lower compared to the PM culture (Table 1). The strong inhibition of TCE on VC transformation is consistent with the much lower $K_s$ value for TCE compared to the $K_s$ values of VC for both the PM and EV cultures. The modeling results using eqs 4–6 provide excellent matches to the experimental data using the independently derived kinetic values given in Table 1, and with the $K_s$ values representing the inhibition constants. Neither culture showed clear VC inhibition on TCE dechlorination (data not shown), which was consistent with the findings of Nielsen and Keasling (27).

VC dechlorination rates to ETH in the presence of c-DCE were measured for a longer time compared to other inhibition experiments, due to the slower rates of VC transformation (Figure 5). The initial protein concentration of the EV culture for this test was a factor of 24 greater than the PM culture. This resulted in a much shorter time for VC dechlorination (data not shown), after all c-DCE was essentially transformed to VC (Figure 5(a)–(c)). After complete transformation of c-DCE to VC by the PM culture, ETH production

### TABLE 1. Kinetic Parameters Obtained from the Experiments and Used in Model Simulations and Reported Values from Fennell and Gossett (21) and Haston and McCarty (22)*

<table>
<thead>
<tr>
<th></th>
<th>PM</th>
<th></th>
<th>EV</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{\text{max}}$ (µmol/mg of protein/day)</td>
<td>$K_s$ (µmol/L)</td>
<td>$k_{\text{max}}$ (µmol/mg of protein/day)</td>
<td>$K_s$ (µmol/L)</td>
</tr>
<tr>
<td>PCE</td>
<td>13.3 ± 1.8</td>
<td>3.9 ± 1.4</td>
<td>12.4 ± 0.7</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>TCE</td>
<td>124 ± 17</td>
<td>2.8 ± 0.3</td>
<td>125 ± 14</td>
<td>1.8 ± 0.4</td>
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<tr>
<td>c-DCE</td>
<td>22 ± 2.0</td>
<td>1.9 ± 0.5</td>
<td>13.8 ± 1.1</td>
<td>1.8 ± 0.3</td>
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<tr>
<td>VC</td>
<td>2.4 ± 0.4</td>
<td>602 ± 7</td>
<td>8.1 ± 0.9</td>
<td>62.6 ± 2.4</td>
</tr>
</tbody>
</table>

* All $k_{\text{max}}$ and $K_s$ values for the PM and EV cultures were determined using the multiple equilibration method in a single bottle on triplicate samples. The range of initial CAH concentrations tested with the PM and EV cultures, respectively, were PCE (µM): 0.2–10 and 0.5–20, TCE (µM): 0.4–30 and 0.5–10, c-DCE (µM): 1.0–25 and 0.2–30, and VC (µM): 50–2200 and 30–420.

In Figure 3, Initial reductive dechlorination rates of CAHs by the PM culture. The simple multi equilibration method was used to determine $k_{\text{max}}$ and $K_s$ values. The results of replicate experiments are represented by the different symbols.

**FIGURE 3.** Initial reductive dechlorination rates of CAHs by the PM culture. The simple multi equilibration method was used to determine $k_{\text{max}}$ and $K_s$ values. The results of replicate experiments are represented by the different symbols.
rates were greater in c-DCE amended reactors. The addition and transformation of c-DCE resulted in higher VC concentrations which led to greater rates of ETH production, as was confirmed in model simulations. VC transformations were very well predicted based on the proposed inhibition model. The VC concentrations used here were well below the $K_S$ value for the PM culture. Thus, an increase in VC concentration would be expected to result in faster VC transformation, as was observed in the experimental results and model simulations. The simulated increase in biomass concentrations over the several day-period of the test are presented in Figure 5(d). A greater relative increase in biomass was predicted for the PM culture, which would result in increased rates of ETH production.

The inhibition of c-DCE on VC dechlorination by the EV culture is shown in Figure 5(e)–(h). Over the time scale of these tests, the simulated increase in cell mass was less than 3% (Figure 5(h)). Although c-DCE was added as an inhibitor, c-DCE was much more rapidly transformed than VC resulting in transient increases of VC concentrations. Figure 5(e)–(h) shows that VC transformation to ETH was increasingly inhibited as the initial aqueous c-DCE concentration was increased. The lags in ETH production are clearly associated with higher concentrations of c-DCE, with ETH production initiated when c-DCE concentrations were reduced to low values. Unlike the PM culture, after complete transformation of c-DCE to VC, the rate of VC dechlorination to ETH was similar in all cases. Due to the lower $K_S$ value for VC with the EV culture, the increase in VC concentrations had little effect on transformation rates, since the initial VC concentration (290 $\mu$M) was significantly higher than the $K_S$ value (62.6 $\mu$M). Also, the higher initial cell mass and short duration of the tests resulted in minimal biomass growth, as predicted by the model simulations. The model simulations did a very good job capturing the temporal changes in c-DCE, VC, and ETH concentrations.

Experiments were performed with both cultures to study PCE inhibition on c-DCE dechlorination (Figure 6). Although the PCE concentration was increased up to 395 $\mu$M and 491 $\mu$M for the PM and EV cultures, respectively, essentially no difference was observed in the rate of c-DCE transformation to VC. Simulations were performed with and without PCE inhibition on c-DCE transformation and compared with the experimental results. Simulations without PCE inhibition more closely matched experimental data than those with PCE inhibition, where inhibition was represented by the
measured $K_s$ value. The experimental results and model simulations indicate that reductive c-DCE dechlorination is unaffected or very weakly inhibited by PCE. c-DCE dechlorination rates were a factor of about 7 higher with the PM culture, due to the higher initial protein content used, and the higher $k_{max}$ value of the culture.

Potential VC inhibition on c-DCE dechlorination was also evaluated. Model simulations were performed with and without VC inhibition and were compared to the experimental results (Figure 7). For both cultures, the experimental observations showed no apparent VC inhibition on c-DCE dechlorination, and there was also no clear difference between the different model simulations. The results show that VC had a very weak inhibitory effect on c-DCE transformation for both cultures, even at VC concentrations as high as 290 $\mu$M, approximately 5 times the $K_s$ value for the EV culture. This result is consistent with the high VC $K_s$ values of the PM and EV cultures of 602 and 62.6 $\mu$M, respectively, and the low $K_s$ values for c-DCE of 1.9 and 1.8 $\mu$M. Thus, VC inhibition on c-DCE transformation was shown to be very weak in our proposed model (Figure 2).

Batch kinetic experiments were performed to study c-DCE inhibition on TCE dechlorination (Figure 8). Interestingly, although the $K_s$ values for c-DCE were similar to those of TCE, rates of TCE dechlorination showed no clear inhibition by c-DCE. The model without c-DCE inhibition simulated the experimental data much more closely than that with inhibition. The differences in the simulations for 0 $\mu$M initial
c-DCE with and without inhibition results from c-DCE formed upon TCE transformation. Some inhibition was observed at the highest c-DCE concentration tested, but it was very weak. These results also indicate that substances inhibitory to TCE transformation were not present in the biologically produced c-DCE. These results show that KS values, when used as inhibition constants, are not representative of less chlorinated ethylene inhibition on more chlorinated ethylene transformation.

Discussion
Several pairs of chlorinated ethylenes (PCE ↔ TCE, PCE ↔ c-DCE, TCE ↔ c-DCE, TCE ↔ VC, and c-DCE ↔ VC) were investigated for transformation inhibition. The experimental and modeling results indicate that more chlorinated ethylenes inhibited reductive dechlorination of the less chlorinated products, except that PCE did not inhibit c-DCE dechlorination. Less chlorinated ethylenes, however, very weakly or did not inhibit the transformation of more chlorinated ethylenes. The KS values for VC for the PM and EV cultures were much higher (602 µM and 62.6 µM, respectively) than the KS values for the parent compounds. Thus, if inhibition were related to the KS values, weak inhibition would be expected, as shown in Figure 7 for VC inhibition on c-DCE transformation. However, very weak inhibition of c-DCE on TCE transformation was also observed, contrary to model simulations using KS values as inhibition constants (Figure 8). Model simulations with inhibition constants much higher than their KS values would be required to simulate the less chlorinated ethylene inhibition on the more chlorinated ethylene. Cupples et al. (11) reported that VC competitively inhibited c-DCE dechlorination and that the VC inhibition constant was three times higher than the KS value for VC. The c-DCE inhibition constant on VC transformation, however, was equal to the KS value for c-DCE. Haston (9) used KS values for c-DCE inhibition on VC transformation when modeling the results of laboratory studies. In a recent study, Cupples et al. (11) determined a competitive inhibition constant (KI) for c-DCE inhibition on VC transformation. The KI value generated for c-DCE was not statistically different than the KS value of c-DCE (KS = 3.3 ± 2.2, KI = 3.6 ± 1.1). Our results were consistent with these studies.

PCE did not inhibit c-DCE transformation. This likely resulted from different microorganisms in the culture or different enzyme systems being responsible for PCE and c-DCE and VC transformation. Reductive dechlorination of c-DCE and VC has been reported to be promoted by different dechlorinators than those capable of reductive dechlorination of PCE and TCE (28). The growth of Dehalococcoides sp. strain VS and Dehalococcoides sp. strain BAV1 are coupled to the dechlorination of c-DCE and VC but not PCE and TCE (18, 19, 26). Dehalococcoides sp. strain FL2 that cometabolically dechlorinates PCE and VC uses only TCE and c-DCE as metabolic electron acceptors (29). Dehalospirillum multivorans, Dehalobacter restrictus strains PER-K23A, and Desulfuromonas sp. can reduce only PCE and TCE in terminal electron accepting processes (14, 30). Another incompletely dechlorinating bacterium (Desulfitobacterium sp. strain PCE1) reductively dechlorinates only PCE to TCE as metabolic electron acceptors (29). The lack of PCE inhibition on c-DCE transformation is consistent with different microorganisms being responsible for PCE and c-DCE transformation. Different enzyme systems responsible for different dechlorination reactions in the same microorganism, as reported by Magnuson et al. (32) for Dehalococcoides ethenogenes, could also explain the lack of PCE inhibition on c-DCE transformation. Inhibition of the more chlorinated ethylenes on the less chlorinated ethylenes was represented well using indepen-
dently determined $K_S$ values as the competitive inhibition constants in model simulations. Experimental results and model simulations both illustrate how low $K_S$ values leading to efficient reductive dechlorination for each chlorinated

FIGURE 7. Comparison of simulations with and without VC inhibition and the experimental data for c-DCE dechlorination by the PM culture (a and b) and the EV culture (c and d). Initial c-DCE concentrations were 96 and 121 µM with cell concentrations of 1.6 and 29 mg/L for the PM and EV cultures, respectively.

FIGURE 8. Comparison of simulations with and without c-DCE inhibition and the experimental data for TCE dechlorination by the PM culture (a and b) and the EV culture (c and d). Initial TCE concentrations were 21.8 and 21.9 µM with cell concentrations of 12 and 10.3 mg/L for the PM and EV cultures, respectively.
ethylene can also cause strong inhibition on daughter product dechlorination. Thus, obtaining $k_{\text{max}}$ values is important for modelling both the rates of transformation of parent compounds and their inhibition on the transformation of daughter products.

A simple multiple equilibration method using a single batch reactor was developed to obtain $k_{\text{max}}$ and $K_i$ values for two dechlorinating mixed cultures. The kinetic parameters measured here are comparable to the values reported by Fennell and Gossett (21) and Haston and McCarty (22). The multiple equilibration method provided precise kinetic values and was simpler to use than the single equilibration method in multiple reactors. Compared with values reported by Haston and McCarty (22), the standard deviations of the kinetic parameters reported here tended to be lower. Studies with direct comparisons of the different methods are needed. The multiple equilibration method might also be adapted to obtain inhibition data required to generate Lineweaver–Burk inhibition plots.

A limitation of the current study is that the actual concentrations of the dechlorinating cultures are not known. The reported $k_{\text{max}}$ values are based on mixed culture protein concentrations. The initial protein concentration when used in model simulations yielded good matches to the experimental data collected over short time scales. The results do indicate that stable communities of dechlorinating microorganisms were maintained in the enrichment reactors over the course of these experiments, since protein based $k_{\text{max}}$ values when used in the simulations fit the results of later inhibition experiments well. As described in Yu and Semprini (33), terminal restriction fragment length polymorphism (T-RFLP) analysis showed a predominant peak at 123.6 bp, consistent with the expected terminal fragment lengths (TFLs) for the Dehalococcoides sp. sequences obtained from GenBank. The peak corresponded to 65% of the amplicon DNA indicating that the culture is highly enriched in Dehalococcoides-like microorganisms. Molecular-based analyses, including clone libraries of the mixed cultures and real time PCR (18, 26), are currently being developed to better enumerate the dechlorinating populations in these cultures.

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