Biodegradation of Tetrachloroethene by Chitin Fermentation Products in a Continuous Flow Column System

Rachel A. Brennan¹; Robert A. Sanford²; and Charles J. Werth³

Abstract: The ability of chitin fermentation products to promote tetrachloroethene (PCE) reduction was evaluated in a continuous-flow column system to identify how different electron donors affect reductive dechlorination. Natural chitin fermentation products were initially used to support PCE reduction. Acetate (3.5 mM) was the dominant fermentation product, followed by propionate (0.1 mM), butyrate (0.1 mM), and hydrogen (100 nM). After chlorinated ethene concentration profiles reached pseudo steady state, the ability of individual fermentation products (acetate, acetate+propionate, propionate, or formate) to support PCE reduction was evaluated. None of the fermentation products tested stimulated dechlorination as well as the suite generated from chitin. After chlorinated ethene concentration profiles reached pseudo steady state, the ability of individual fermentation products (acetate, acetate+propionate, propionate, or formate) to support PCE reduction was evaluated. None of the fermentation products tested stimulated dechlorination as well as the suite generated from chitin. After chlorinated ethene concentration profiles reached pseudo steady state, the ability of individual fermentation products (acetate, acetate+propionate, propionate, or formate) to support PCE reduction was evaluated. None of the fermentation products tested stimulated dechlorination as well as the suite generated from chitin.

DOI: 10.1061/(ASCE)0733-9372(2006)132:6(664)

CE Database subject headings: Biodegradation; Ground-water pollution; PCE; TCE; Kinetics; Columns.

Introduction

The dry-cleaning chemical, tetrachloroethene (PCE), and its daughter products trichloroethene (TCE), cis-1,2-dichloroethene (DCE), and vinyl chloride (VC) are common groundwater contaminants in industrialized nations (Neumann et al. 1994). These chemicals can be remediated under anaerobic conditions by dechlorinating microorganisms if provided with an appropriate electron donor. The electron donor used not only determines the extent and rate of dechlorination for a given microbial community (Gibson and Sewell 1992; Hirl and Irvine 1997), but can also impact the ability to sustain dechlorination activity for the duration of clean up at a contaminated site (Carr and Hughes 1998). There have been numerous studies on the efficiencies of different electron donors to stimulate reductive dechlorination; however, many of the studies have conflicting results. Acetate, butyrate, citrate, ethanol, formate, fumarate, glucose, glycerol, hydrogen, lactate, methanol, propionate, pyruvate, succinate, and sucrose have all been shown to support reductive dechlorination at different rates and to various extents (Krumholz et al. 1996; Schönborn et al. 1997; Lee et al. 1997; Hirl and Irvine 1997; Fennell et al. 1997; Carr and Hughes 1998). Over long-term experiments, some researchers have found equivalent rates of dechlorination regardless of the electron donor fed (Fennell et al. 1997; Carr and Hughes 1998; He et al. 2002).

One reason for the discrepancy between electron donor tests is the diverse physiology encountered among different dechlorinating populations. In particular, some dechlorinators can utilize organic electron donors directly, whereas others are only able to use hydrogen (H₂), a product of fermentation. Although H₂ has long been considered the ultimate electron donor for reductive dechlorination reactions, this is not always the case. He et al. (2002) showed that PCE and TCE dechlorination in the presence of H₂ began only after acetate was formed through H₂/CO₂ acetogenesis, indicating that some key dechlorinators in their system were acetotrophic rather than hydrogenotrophic. Although H₂ is currently the only known electron donor to support the reduction of DCE to VC and ethene (Maymó-Gatell et al. 1997; He et al. 2003), there are several species of PCE and TCE reducing organisms that use acetate, but not H₂, as an electron donor for dechlorination (Sung et al. 2003). Other PCE dechlorinating cultures, such as Sulfitobacterium multiclorans and Desulfotobacterium sp. strain PCE1 can use lactate or H₂ directly as electron donors (Neumann et al. 1994; Gerritse et al. 1996).

Although the aforementioned batch studies provide strong evidence that dechlorination rates depend both on the type of electron donor and on the microbial community, it is not clear how these results apply to the field, where PCE and its daughter products vary spatially. Several continuous-flow column studies (Carr and Hughes 1998; Kao et al. 2001; Yang and McCarty 2002;
Dennis et al. 2003) have been performed to investigate dechlorination kinetics under more realistic conditions. These primarily focused on evaluating the influence of different electron donors on sustaining PCE dechlorination (Carr and Hughes 1998; Kao et al. 2001) and enhancing dense nonaqueous phase liquid (DNAPL) dissolution (Yang and McCarty 2002). Dennis et al. (2003) used a continuous-flow column to investigate the spatial variation of the structure of the microbial consortia relative to the distance from a PCE source zone during treatment with a single electron donor. Although all of these studies are important, none investigated how the type of electron donor affects dechlorination kinetics spatially, or how it potentially changes the structure of the microbial community.

In this study, we investigate PCE reduction in a continuous-flow column system using chitin as an electron donor source. We previously showed that the variety of electron donors released during chitin fermentation can support PCE reduction to at least VC in semibatch columns (Brennan 2003). In this work, we initially used chitin hydrolysis and fermentation products to establish PCE reduction under continuous-flow conditions in a series of columns. Then, after chlorinated ethene concentration profiles reached pseudo steady state, we evaluated the ability of individual fatty acids that were part of the chitin fermentation product mixture to support PCE reduction. Individual fatty acids and defined fatty acid mixtures were sequentially tested over short time periods (1 pore volume or 2 days) so that dechlorination kinetics could be evaluated with different electron donors in the presence of a similar microbial community. Previous work by others (Krumholz et al. 1996; Schöllhorn et al. 1997; Lee et al. 1997; Hirl and Irvine 1997; Fennell et al. 1997; Wu et al. 1998; He et al. 2002; Hunter et al. 2002; Kao et al. 2003) evaluated the effects of different electron donors on dechlorination kinetics in batch systems. However, we evaluate how different electron donors affect the spatial distribution of PCE and PCE daughter products in a continuous-flow system for the first time. In a parallel study, we relate these findings to the distribution of the microbial community that developed within the same columns (Brennan 2003).

Materials and Methods

Chemicals

Tetrachloroethene (PCE), trichloroethene (TCE), cis-1,2-dichloroethene (DCE), and vinyl chloride (VC), were obtained from Aldrich Chemical Co. (Milwaukee). Lab grade methane and hydrogen, as well as ultrahigh-purity (UHP) helium and nitrogen gases were obtained from S. J. Smith Welding Supply (Davenport, Iowa). A 100 ppm hydrogen gas standard was obtained from Supelco (Bellefonte, Pa.). A fatty acid standard mix containing C1–C7 acids at 10 mM each was obtained from Supelco (Bellefonte, Pa.). Refined chitin from crab shells (SC-80 grade) in 20–40 mesh size was obtained from Vanson Inc. (Redmond, Wash.).

Groundwater Source

Natural groundwater from the Teays aquifer in Central Illinois was used for this study (Najm et al. 1993). The groundwater was collected under an argon headspace, and was maintained in the dark under anaerobic conditions throughout the experiment by continuously bubbling argon gas through it while stirring.

Supplementary Cultures

The acetotrophic pure culture Desulfitromonas michiganensis strain BB1, which reductively dechlorinates PCE to DCE (Sung et al. 2003), and a mixed culture enriched from Sangamon River sediments (Lodge Park, Piatt County, Ill.), capable of reducing PCE to ethene, were used in this study. Strain BB1 was grown in reduced anaerobic basal salts medium (Löffler et al. 1998) amended with Wolfe’s vitamin solution (Atlas 1997), 1 mM acetate, and ~120 μM PCE. The mixed culture was grown to an approximate population density of 10⁶ cells/mL in a volatile organic chemical interface transfer apparatus (VITA) (Brennan and Sanford 2002) containing reduced anaerobic basal salts medium, Wolfe’s vitamin solution, 4 mM formate, 1–2 mM lactate, and PCE. The two cultures were combined (4% each v/v) in fresh medium, supplemented with vitamins, 4 mM fumarate, 1 mM lactate, and ~120 μM PCE, and allowed to incubate for 2 weeks before being injected into the column experiment. In addition, 125 mL of the original VITA culture was added directly into the Multiport Columns (described in the next section).

Column Setup

To assess the influence of chitin fermentation products on PCE degradation under continuous-flow conditions, column experiments were performed. Heavy-wall glass columns with 3 cm inner diameters and lengths of 31, 63, 31, and 125 cm, were packed with course quartz sand (Global Drilling Suppliers, Inc., Cincinnati), sealed with stainless steel Swagelok fittings and Teflon ferrules, and connected in series with stainless steel three-way valves and 1/16 in. tubing (Fig. 1). The second column was designed with 12 sampling ports spaced evenly along its length so that the fermentation and dechlorination profiles could be accurately measured. The purpose of the third and fourth columns was to provide an extended retention time for any recalcitrant compounds (i.e., vinyl chloride) to degrade. Two identical sets of columns (Systems A and B) were constructed and run under the same conditions for the first 38 days of the experiment.

Before the start of the experiment, all columns were positioned vertically on a ring-stand, filled with degassed deionized water (DDW), packed with sand, and then flushed with DDW for 50 pore volumes in the upward direction to remove trace fines and entrapped air. The first column (POM Column) was packed with a mixture of 7.88 g refined chitin and 78.8 g of 20–40 mesh sand (Badger Mining, Berlin, Wis.), giving a ratio of chitin to sand of approximately 1:10 by mass. The chitin and sand used in the first column were chosen to be representative of materials that have been injected into contaminated field sediments to support the in situ bioremediation of chlorinated solvents (Brennan 2003). To buffer the system and prevent detrimental decreases in pH caused by chitin fermentation products, 7 g of 18–45 mesh (0.354–1 mm) limestone chips were added to the end of the POM Column. An in-line pH electrode (Cole-Parmer) was placed in an 800 μL, 3 mm inner diameter, glass flow-through cell (Cole-Parmer) between the first and second columns to continuously monitor the pH of the chitin effluent. The second, third, and fourth columns were packed with 10–18 mesh (1–2 mm) sand only. Larger grained sand was used in these columns to maintain permeability and prevent head loss. After purging with DDW, a full pore volume of combined supplementary cultures was added to Columns 2, 3, and 4, and allowed to incubate for 1 day before the start of the experiment. Throughout the experiment, the columns were kept at room temperature (22±2°C) in the dark.

JOURNAL OF ENVIRONMENTAL ENGINEERING © ASCE / JUNE 2006 / 665
After column preparation and incubation, the experiment was started by pumping groundwater vertically (bottom to top) through the columns at a rate of 8.4 mL/h (linear flow velocity = 0.628 m/day) using a PhD 2000 Programmable Push–Pull syringe pump (Harvard Apparatus, Holliston, Mass.). The retention time of groundwater in the system was 0.5, 1.5, 0.5, and 2 days for the first, second, third, and fourth columns, respectively. A nitrate water tracer test was used to verify these retention times and to calculate the dispersion coefficient, D, for the system. An anaerobic, saturated solution of PCE (~900 µM) was prepared by adding 50 µL neat PCE to 100 mL degassed groundwater in a sealed serum bottle that was continuously shaken on a rotary shaker for at least 7 days. After reaching equilibrium, this PCE solution was delivered to the front of the second column from a 20 mL gas-tight syringe (Hamilton) at a rate of 0.2 mL/h using an infusion syringe pump (Cole-Parmer), giving a final concentration of 22 µM PCE in the system after dilution with the groundwater exiting the POM Column. Aqueous samples were collected from the end of each column and from specific multiports every 3–4 days and analyzed for CH₄, H₂, pH, ammonium, fatty acids, and chlorinated ethenes. The columns were evaluated over a period of 50 days.

**Fatty Acid Step Tests**

To determine how specific chitin fermentation products stimulated dechlorination, fatty acid step tests were run in one column after fermentation had reached pseudo steady state. This was done by removing the Chitin Column from System B after 38 days, and pumping each electron donor through the system with the influent groundwater. For these tests, Multiport Column B was sampled at six evenly spaced ports: 1, 3, 5, 7, 9, and 11. System A was allowed to continue with its Chitin Column in place as a reference for this experiment. The fermentation products that were evaluated were: acetate (5 mM); propionate (0.3 mM); and formate (0.3 mM). Acetate and propionate were selected based on their observed sustained generation during chitin fermentation, and formate was selected as a surrogate for hydrogen. The fatty acid concentrations used in the step test were chosen to be representative or slightly greater than their average observed production during the first 22 days of chitin fermentation, and were in excess of that required for complete dechlorination of the influent PCE. Changes in dechlorination activity were measured for 2 days (approximately 1 pore volume in the Multiport Column) after the introduction of an electron donor before switching to a new one. In this way, the relative contribution of each electron donor to dechlorination activity could be measured with the microbial community that was established using chitin, and the fermentation products of chitin primarily responsible for dechlorination could be determined. At the end of the step tests, the Chitin Column was put back into place after purging it with 3 pore volumes of groundwater to remove any buildup of fatty acids and gases, to evaluate if the original dechlorination activity obtained with chitin could be regained.

**Batch Tests with Column Material**

To test whether the ethene-producing microorganisms had colonized the column sediments, the following batch experiment was performed. Sand samples were extracted from the ends of the third column in System B at the end of the experiment in an anaerobic hood. The samples were aliquoted (~5 g each) to triplicate 160 mL serum bottles containing 100 mL anaerobic basal medium, 1 mL vitamins, 4 mM formate, 2 mM lactate, and 2 µL PCE. After ethene generation was observed, a test was done to determine if these microorganisms could support PCE reduction to ethene in the presence of chitin and groundwater only. The column-derived enrichment from the first batch test was transferred (3% v/v) to triplicate 160 mL serum bottles containing 100 mL degassed groundwater, 1 g limestone chips, 0.05 g chitin, and 1 µL neat PCE. A control bottle was also set up that did not receive any inoculum to test the dechlorination activity of organisms that are naturally present in the groundwater. The bottles were sealed with Teflon-coated rubber stoppers, inverted, incubated in the dark at room temperature, and monitored for 5 months. During this time, additional 1 µL aliquots of neat PCE were added to active bottles as needed.
Chemical Analyses
Aqueous samples from the columns were transferred to headspace vials and allowed to equilibrate for at least 30 min before headspace samples were withdrawn and analyzed for PCE, TCE, DCE, VC, ethene, methane, and hydrogen. Separation of the chlorinated ethenes and methane was conducted using a Perkin-Elmer Autosystem gas chromatograph equipped with a flame ionization detector and a 30 m, 0.53 mm inner diameter GS-Q plot column (J & W Scientific, Folsom, Calif.). The oven was programmed as follows: hold at 35°C for 1.5 min, ramp to 200°C at a rate of 45°C/min, and hold isothermally for 2.25 min. The carrier gas was UHP helium at a flow rate of 26 mL/min. Hydrogen concentrations were determined at room temperature using a reduction gas detector (Trace Analytical, Menlo Park, Calif.). From the headspace concentrations, the aqueous concentrations of the chlorinated ethenes were calculated using Henry’s constants, and methane and hydrogen were assumed to have transferred completely to the gas phase before injection.

After headspace analysis, the aqueous samples were tested for pH, ammonium, and fatty acid concentrations. pH was measured using a Cole-Parmer 5662-52 double junction, combination pH electrode. Ammonium was measured using an Orion 9512 ammonia-selective electrode. Fatty acid concentrations were determined using a Waters 486 (Milford, Mass.) high-performance liquid chromatograph system equipped with a HPX-87H column (Biorad, Hercules, Calif.), as described previously (Brennan 2004).

Model Development
To model the dechlorination activity observed in the Multiport Column at equilibrium when chitin was the electron donor source, the one-dimensional advection-dispersion-reaction equation (ADRE) was used

\[
\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} - u \frac{\partial c}{\partial x} - kc
\]

where \( c \) = concentration of the solute in solution (i.e., [PCE], [TCE], [DCE], or [VC]) (mol L\(^{-1}\)); \( D \) = hydrodynamic dispersion coefficient (m\(^2\) day\(^{-1}\)); \( u \) = linear flow velocity (m day\(^{-1}\)); and \( k \) = first-order reaction (degradation) term (day\(^{-1}\)).

The fermentation of chitin produced electron donors in excess of that required to reduce the chlorinated ethenes in this experiment; therefore, the chlorinated ethenes were assumed to be the rate-limiting substrate and first-order decay of the chlorinated ethenes was assumed. The boundary conditions used for this model corresponded to the experimental conditions: At the start of the experiment the soil column was “clean” and then beginning at time \( t = 0 \), PCE was fed continuously from the column inlet. This can be expressed mathematically as

\[
[PCE](x,0) = 0
\]

\[
\left. u[PCE] - D \frac{\partial[PCE]}{\partial t} \right|_{t=0} = u[PC_{\text{feed}}] \quad [PCE]_{\text{feed}} = 1
\]

\[
[PCE](x \to \infty, t) = 0
\]

Eqs. (2)–(4) were numerically solved using standard finite difference; operator splitting was used to solve the reaction term separate from advection-dispersion terms (Valocchi and Malmstead 1992). The model was evaluated at grid spacings, \( \Delta x \), and time steps, \( \Delta t \), ranging from 0.03 to 0.001 with similar results. With \( \Delta x \) and \( \Delta t \) set to 0.01, the model profiles converged to steady state after approximately one experimental day.

The degradation of PCE with the different electron donors in the fatty acid step tests was approximated by considering only advection and first-order decay under steady-state conditions, as shown in

\[
\ln \left( \frac{[PCE]}{[PCE_0]} \right) = -k \left( \frac{x}{u} \right)
\]

Under the assumption of steady-state conditions, we found that including diffusion negligibly affected first-order rate constants, so this term was not included in Eq. (5).

Model Application
The linear flow velocity was directly calculated from the column flow rate and column dimensions. The dispersion coefficient was obtained from the tritiated water breakthrough profile by using the method of moments (Valocchi 1985). The sequential dechlorination model was fit to the average steady-state spatial profiles obtained at the end of the experiment with chitin (i.e., average of sample time points 43, 46, and 50 days). The dechlorination rate constants of Ballapragada et al. (1997) were used as a starting point for optimizing the model fit to the data. The best values of the rate constants were determined by minimizing the sum of squared errors. The 95% confidence intervals for the rate constants were found by performing a sensitivity analysis on the model (Brennan 2003).

Results
Continuous-Flow Column
The profiles of acetate, butyrate, propionate, and hydrogen concentrations in the effluent of the Chitin Columns in Systems A and B were similar for the first 38 days (Fig. 2). By Day 4, acetate concentrations peaked in the chitin effluent and, although variable, remained high through Day 22, after which they began to decrease steadily. Acetate was the dominant fatty acid produced during chitin fermentation, with an average concentration of 4.6 mM in the first 22 days in Systems A and B. Butyrate reached a maximum value of 1.1 mM on Day 4 (average of Systems A and B), but then was produced sporadically, with an average concentration of 0.3 mM in the first 22 days in both systems. Propionate was consistently produced in both systems, with an average concentration of 0.2 mM in the first 22 days of the experiment. After 25 days, propionate was near the detection limit (0.01 mM) and butyrate was below detection. Formate, isobutyrate, and isovalerate were also intermittently detected, although at lower concentrations (<0.1 mM). Of the fatty acids produced during chitin fermentation, acetate accounted for the majority of the electron donating capacity: an average of 96% in both systems during the first 22 days.

Hydrogen levels were at their maximum on Day 1, with 11.7 and 82.5 µM measured in the chitin effluent of Systems A and B, respectively. By Day 7, hydrogen concentrations dropped to an average 77 mM in both systems, which were sustained through Day 25. Hydrogen levels increased slightly in System A (that received chitin only) throughout the remainder of the experiment, with an average value of 351 nM from Days 29 to 50. Hydrogen
levels remained relatively constant in System B throughout the fatty acid step test, with an average concentration of 117 nM. The hydrogen levels observed during this experiment are similar to the hydrogen threshold concentrations that have been reported for acetogenesis (336–3,640 nM) and methanogenesis (5–95 nM) (Löffler et al. 1999). Methane concentrations followed the production of hydrogen with a slight lag, peaking at 3.2 mM at 29 days, and then slowly decreasing until reaching 1.3 mM by the end of the experiment (data not shown). As in previous experiments (Brennan 2003), methane accounted for only a small fraction of the chitin carbon added to the system (<5%), indicating that methanogenesis did not significantly affect the electron donating potential available for dechlorination. Low levels of methanogenesis are not unexpected due to the low pH levels observed in the system.

Correlated with fatty acid production, the pH values in the chitin effluent reached a low of 5.96 on Day 4, and then began to steadily increase until returning to a value of 6.7 around Day 40 (data not shown). The average pH throughout the experiment was 6.4. Ammonium concentrations also followed the general trend of the fatty acids, reaching a maximum 1.99 mM NH$_3$-N at Day 4, and then decreasing to a concentration of 0.62 mM NH$_3$-N by Day 36 (data not shown). Based on an average cellular composition of C$_3$H$_6$O$_5$N, a theoretical nitrogen demand of approximately 1.5 with carbon is required on a molar basis to maintain cellular growth (Tchobanoglous and Burton 1991). The NH$_3$-N concentrations in this study, therefore, never reached low enough levels (0.28 mM NH$_3$-N) to be limiting for biological activity, based on the total carbon consumed across the Multiport Column.

As the experiment progressed with time, the gradient of both acetate consumption and PCE dechlorination increased toward the front of the column. The relatively small rise in acetate concentrations observed between Ports 2 and 11 at some of the time points indicates that fermentation of more complex compounds (e.g., N-acetylglucosamine monomers) released from the Chitin Column was still occurring within the Multiport Column. Hydrogen concentrations within the Multiport Column were typically higher than in the Chitin Column effluent, further indicating that fermentation occurred down gradient from the chitin source. After 38 days, fatty acid concentrations exiting the Chitin Column stabilized, and the acetate and chlorinated ethene profiles within the Multiport Column no longer changed, indicating the onset of pseudo steady state. Running mass balance calculations on both oxidation and reduction reactions (reductive dechlorination and methanogenesis) revealed that 99.7±2.9% of the electrons were accounted for from the beginning to the end of the Multiport Column, at the 95% confidence interval.

Dechlorination profiles along the length of Multiport Column A after pseudo-steady-state conditions were reached with chitin as an electron donor source (i.e., after 38 days) were modeled using the one-dimensional ADRE. The resulting first-order rate constants and their 95% confidence intervals are shown in Table 1, as are the fixed parameters used for the model development. Since these rate constants were derived assuming constant biomass, caution should be taken when applying them. The average values of the first-order rate constants were used to develop the concentration profiles that are shown in Fig. 4. These profiles are typical for reductive dechlorination, with PCE degradation showing exponential decay over the length of the column. Although relatively simple and general in approach, the modeled results agree closely with the experimental results for PCE, TCE, and DCE. Model results do not coincide with VC data, however. VC data do not follow a smooth line, possibly because the production of this chlorinated ethene did not reach steady state, or because of the difficulties in sampling this gas in the continuous flow system. The instantaneous PCE dechlorination rate determined with chitin as the electron donor source is within the range of steady-state degradation rates presented in the literature for individual fatty acids. Dechlorination rates from the literature for batch and continuous-culture laboratory experiments, and their corresponding experimental conditions, are listed in Table 2.
Vinyl chloride was observed to accumulate without any reduction to ethene in this experiment, even under the extended retention time of 4.5 days for the whole column system (Columns 3 and 4, data not shown), and despite the fact that an ethene-producing enrichment culture was used as part of the inoculum. Fatty acids and hydrogen were present in sufficient concentration throughout the entire system to support complete dechlorination of PCE to ethene through the 29th day of the experiment; however, no vinyl chloride reduction was observed. To determine if the microbial community in the column system retained the ability to reduce PCE to ethene, batch serum bottle tests with the column material were performed. In the presence of added formate, lactate, and vitamins, ethene generation was observed in 4 fixed-bed column Soil enrichment Chitin This study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column length, L</td>
<td>0.421</td>
<td>m</td>
</tr>
<tr>
<td>Dispersion coefficient, D</td>
<td>$7.8 \times 10^{-8}$</td>
<td>m²·day⁻¹</td>
</tr>
<tr>
<td>Linear flow velocity, u</td>
<td>0.628</td>
<td>m·day⁻¹</td>
</tr>
<tr>
<td>PCE feed concentration</td>
<td>24</td>
<td>µmol L⁻¹</td>
</tr>
<tr>
<td>PCE₀, TCE₀, DCE₀, VC₀</td>
<td>0</td>
<td>µmol L⁻¹</td>
</tr>
<tr>
<td>Experimental time, T</td>
<td>50</td>
<td>day</td>
</tr>
<tr>
<td>Grid spacing, Δx</td>
<td>0.01</td>
<td>m</td>
</tr>
<tr>
<td>Time step, Δt</td>
<td>0.01</td>
<td>day</td>
</tr>
<tr>
<td>$k_{PCE}$ (average±95% C.I.)</td>
<td>6.9±1.8</td>
<td>day⁻¹</td>
</tr>
<tr>
<td>$k_{TCE}$ (average±95% C.I.)</td>
<td>6.6±2.7</td>
<td>day⁻¹</td>
</tr>
<tr>
<td>$k_{DCE}$ (average±95% C.I.)</td>
<td>52.9±106.4</td>
<td>day⁻¹</td>
</tr>
<tr>
<td>$k_{VC}$</td>
<td>0</td>
<td>day⁻¹</td>
</tr>
</tbody>
</table>

²least-squares error predictions at the 95% confidence interval.

Table 2. Comparison of the Instantaneous PCE Dechlorination Rate Observed in This Study with Steady-State Degradation Rates Recorded in the Literature and the Corresponding Experimental Conditions

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Reaction rate ($\mu$mol L⁻¹·day⁻¹)</th>
<th>Reactor type</th>
<th>Culture</th>
<th>e-donor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCE → TCE</td>
<td>64²</td>
<td>Fixed-bed column</td>
<td>Soil enrichment</td>
<td>Chitin</td>
<td>This study</td>
</tr>
<tr>
<td>PCE → ethene</td>
<td>16</td>
<td>Batch</td>
<td>Soil enrichment</td>
<td>Methanol or H₂</td>
<td>Aulenta et al. (2002)</td>
</tr>
<tr>
<td>DCE → VC</td>
<td>23</td>
<td>Batch</td>
<td>Dehalococcoides species</td>
<td>Lactate or acetate</td>
<td>He et al. (2003)</td>
</tr>
<tr>
<td>VC → ethene</td>
<td>54</td>
<td>Batch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCE → DCE</td>
<td>63</td>
<td>Biofilm reactor</td>
<td>Enrichment</td>
<td>Lactate or acetate</td>
<td>Hirl and Irvine (1997)</td>
</tr>
<tr>
<td>PCE → ethene</td>
<td>89</td>
<td>Fixed-bed column</td>
<td>Soil/sludge enrichment</td>
<td>Lactate</td>
<td>deBruin et al. (1992)</td>
</tr>
<tr>
<td>PCE → TCE, TCE → DCE, DCE → VC</td>
<td>341, 159, 99</td>
<td>Batch</td>
<td>Enrichment</td>
<td>—</td>
<td>Gerritse et al. (1995)</td>
</tr>
<tr>
<td>PCE → VC</td>
<td>960</td>
<td>Batch</td>
<td>Dehalococcoides ethenogenes strain 195</td>
<td>H₂</td>
<td>Maymó-Gatell et al. (1997)</td>
</tr>
</tbody>
</table>

²This instantaneous rate calculated based on the average PCE concentration observed in the experiment (9.3 µM).

Fig. 4. First-order finite-element model for dechlorination with chitin in the Multiport Column at final times using average rate constants. Model was optimized using least-squares error predictions at the 95% confidence interval. Data points are triplicate windowed averages of the raw data at $t=43$, 46, and 50 days; error bars represent 95% confidence intervals.

### Fatty Acid Step Test

After pseudo steady state had been reached in the Multiport Column, individual and defined mixes of fatty acids were evaluated in step tests to determine which contributed the most toward dechlorination when chitin was used as the electron donor source. The fatty acid step tests were initiated at $t=38$ days in Column System B. Acetate (5 mM) plus propionate (0.3 mM) were the first fatty acids tested, followed by acetate alone (5 mM), propionate alone (0.3 mM), and formate alone (0.3 mM). Although butyrate was produced at somewhat higher concentrations than propionate during the initial stages of chitin fermentation, its production was sporadic and often below detection; therefore, it was not separately tested as an electron donor source. Before the step test was initiated when chitin was the electron donor source, 100% of the influent PCE was converted to VC. During the step...
First-order rate constants for most of the fatty acids increase with distance (i.e., retention time) in the Multiport Column; this may indicate a preferential distribution of microorganisms in the column sand. The slopes of the profiles at the beginning (Ports 1–5, 3.2–16.2 cm) and end (Ports 7–11, 22.7–35.6 cm) of the Multiport Column were separately calculated as \( k_{PCE, minimum} \) and \( k_{PCE, maximum} \), respectively. This was done in an attempt to quantify the variation in dechlorination activity with column length. The slope of the entire profile, the change in \( \ln(PCE/PCE_0) \) between Ports 1 and 11, was also calculated to give an overall rate constant, \( k_{PCE, overall} \) for each electron donor. These estimated rate constants are listed in Table 3. It should be noted that since steady-state conditions were not separately achieved for each electron donor in the step tests, the rate constants presented in Table 3 are approximations only. To further evaluate the effectiveness of the different fatty acids, it is useful to compare their values of \( k_{PCE, maximum} \) with that of chitin. The value of \( k_{PCE, maximum} \) for chitin was only 1.2 times greater than \( k_{PCE, maximum} \) for acetate, but markedly greater than \( k_{PCE, maximum} \) of the other fermentation products tested: 2.5, 3.4, and 5.1 times greater than that of acetate.

Table 3. PCE First-Order Rate Constants Estimated in Different Sections of the Multiport Column for the Electron Donors in the Fatty Acid Step Test

<table>
<thead>
<tr>
<th>Feed (in order of treatment)</th>
<th>( k_{PCE, minimum} ) (day(^{-1}))</th>
<th>( k_{PCE, maximum} ) (day(^{-1}))</th>
<th>( k_{PCE, overall} ) (day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitin</td>
<td>NA</td>
<td>6.1(^a)</td>
<td>6.1(^a)</td>
</tr>
<tr>
<td>Acetate + propionate</td>
<td>0.04</td>
<td>1.8</td>
<td>0.95</td>
</tr>
<tr>
<td>Acetate</td>
<td>1.5</td>
<td>5.3</td>
<td>3.1</td>
</tr>
<tr>
<td>Propionate</td>
<td>0.77</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Formate</td>
<td>0.88</td>
<td>2.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Re-chitin</td>
<td>1.7</td>
<td>5.4(^b)</td>
<td>3.8</td>
</tr>
</tbody>
</table>

\(^{a}\)This rate constant is based on the column performance immediately before the fatty acid step test was initiated.

\(^{b}\)This rate constant was calculated from the slope of the line between Ports 3 and 11.
formate, acetate-propionate, and propionate, respectively. The relative effectiveness of each electron donor source at stimulating PCE dechlorination was found to be dependent upon distance along the flow path.

**Discussion**

In this study, the effect of chitin fermentation products on the kinetics of tetrachloroethene dechlorination was evaluated along the flow path in a column system inoculated with a mixed dechlorinating culture. The complex mixture of chitin fermentation products produced greater dechlorination activity than any of the individual fermentation products tested alone. Acetate gave consistently high rates of PCE degradation along the column length, and appeared to be the chitin fermentation product primarily responsible for the observed dechlorination activity. The electron donor mixture acetate-propionate was expected to give the highest rate of PCE degradation because it most closely resembled the fatty acids released from chitin fermentation; however, it gave one of the lowest rates, second only to propionate. The fact that the acetate-propionate mixture yielded a lower first-order rate constant than acetate alone indicates that propionate may have an inhibitory effect on dechlorination in our system. Propionate typically persists when H₂ concentrations exceed 10⁻⁴ atm (100 ppm, 74 nM) (Lueders et al. 2004). Hydrogen levels ranged from 90 to 440 nM in Multiport Column B during the fatty acid step test, so it is possible that syntrophic microorganisms were inhibited from fermenting propionate to acetate and H₂. Since propionate did not significantly decrease in concentration along the length of the column during the continuous-flow experiment with chitin or during the fatty acid step test, it is unlikely that it was utilized by the existing microbial community. A study by Gibson and Sewell (1992) showed that propionic acid was not degraded in microcosms containing fatty acid mixtures. They suggested that high levels of hydrogen or acetic acid in their experiments may have inhibited the oxidation of propionic acid, and observed that dechlorination was only supported by propionic acid alone after a lag period (Gibson and Sewell 1992). Kennes et al. (1998) found sustained dechlorination activity when acetate, butyrate, methanol, formate, and hydrogen were used as electron donor sources in a mixed PCE dechlorinating culture, but found insignificant dechlorination activity when propionate was used. The reason why propionate should have an inhibitory effect on acetate utilization for dechlorination in our system remains unclear, however.

Thermodynamically, formate oxidation coupled to dechlorination provides the greatest free energy, ΔG, of any of the electron donors tested (ΔG_{formate} = −39.7 kJ/mol e⁻; ΔG_{acetate} = −34.1 kJ/mol e⁻; and ΔG_{propionate} = −32.6 kJ/mol e⁻ for oxidation half-reactions under the experimental conditions of the fatty acid step test). However, since formate was apparently completely converted to H₂ before the first sampling port, it is unlikely that it was directly coupled to dechlorination. Rather it would seem that H₂ was the electron donor during the formate-fed step test. The observed H₂ concentration at the first sampling port (440 nM) was significantly less than the formate concentration fed to the system (0.3 mM), indicating that a significant amount of H₂ from formate oxidation was channeled into other processes besides dechlorination, for example, the reduction of bicarbonate to methane. Mass balance calculations revealed that approximately 35% of the formate added to the system was converted to methane by the first sampling port, whereas only 0.15% was converted to measurable H₂. The free energy of H₂ oxidation (ΔG_{H₂} = −26.8 kJ/mol e⁻ for the oxidation half-reaction under the average experimental conditions of the fatty acid step test where H₂ = 120 nM) is considerably less favorable than acetate oxidation, so the moderate dechlorination performance achieved with formate compared to the other electron donors in the fatty acid step test is not unexpected given these observations. The low concentration of H₂ relative to that of other electron donors could have also contributed to kinetic limitations on dechlorination activity.

In general, most electron donors gave higher k_{PCE} values in later sections of the Multiport Column B, indicating a higher density of dechlorinating microorganisms toward the end of the column. For acetate especially, this trend was very strong, with k_{PCE} increasing by 3.5 times from the beginning to the end of the column. Changes in the available electron donor during the fatty acid step test could have caused populations of dechlorinating microorganisms to move (or preferentially grow) from the front toward the back of the column and contribute to higher dechlorination rates in that location. Evidence for this possibility is provided by nested polymerase chain reaction results which indicated that Desulfuromonas strains were indeed found clustered in the sediments at the back of Multiport Column B at the end of the experiment (Brennan 2003).

The results of this study indicate that acetate is important in a chitin-enhanced dechlorination system, whether it is used as an electron donor for acetotrophic chlororespiration, or as a carbon source for hydrogenotrophic dechlorinators. Although acetate can serve as the electron donor for the reduction of PCE to DCE by acetotrophic dechlorinators, the current accepted physiological model indicates that H₂ serves as the electron donor for the subsequent reduction of DCE to VC. Hydrogen also has the ability to serve as the electron donor for the dechlorination of PCE and TCE; however, its low free energy and low concentration relative to that of acetate suggest that the most probable reactions occurring in this experiment are PCE reduction to TCE and DCE coupled with acetate oxidation, and DCE reduction to VC coupled with H₂ oxidation. Although the electron equivalents provided by measurable H₂ are only enough to account for 2.4% of the DCE converted to VC observed in the chitin-fed columns, these measured concentrations do not reflect rates of H₂ consumption (Löffler et al. 1999). The hydrogen flux from fermentation may indeed provide significant electron equivalents for dechlorination without having a high H₂ concentration. In the step test with acetate, H₂ concentrations between 100 and 400 nM were observed along the length of the Multiport Column, indicating that some of the protons liberated from acetate oxidation are likely reduced to H₂ and subsequently used to drive the reduction of DCE to VC. This has been observed previously in acetotrophic PCE-dechlorinating cultures (He et al. 2002).

With the established microbial community that was present in the Multiport Column, the extent of dechlorination activity obtained with the fatty acids examined in the step test is only 25–70% of that originally observed with chitin (Fig. 6). However, when chitin is reapplied nearly all of this activity is regained. This observation suggests that either micronutrients (such as vitamins) released from chitin or other fatty acid-like products not detected may contribute to dechlorination activity. The observed benefit of using chitin over its composite fatty acids is similar to the observation by Fennell et al. (1997), that fermented yeast extract (FYE) supported PCE dechlorination slightly better than surrogate FYE, a synthetic blend of the fatty acids found in FYE.

This experiment is unique in that it enabled spatial and temporal correlations to be made between the nutrient flux from
chitin fermentation and PCE degradation. The rates of chlorinated ethene degradation were shown to be dependent on the electron donor source, which may be due to the distribution of chlororespiring bacteria. The free energies of the individual fatty acids are shown to be useful predictors of dechlorination performance. Chitin fermentation products stimulate the reductive dechlorination of PCE more effectively than any of the fatty acids individually. This fact, combined with the absence of propionate inhibition in the presence of chitin, indicates that other unidentified nutrients must contribute to chitin-enhanced dechlorination.

Acknowledgments

This research was performed with the support of a National Science Foundation (NSF) Small Business Innovative Research (SBIR) Grant (Award No. DMI-0109868). The support of a NSF Graduate Research Fellowship and an Environmental Protection Agency STAR Fellowship for one of the writers (R.A.B.) at the University of Illinois at Urbana-Champaign are also gratefully acknowledged. Frank Löfler of Georgia Institute of Technology is thanked for his donation of Desulfuromonas michiganensis strain BB1. Sean Brennan is thanked for his MATLAB programming assistance.

References


