Simulated and experimental evaluation of factors affecting the rate and extent of reductive dehalogenation of chloroethenes with glucose

Il-Su Lee, Jae-Ho Bae, Yanru Yang, Perry L. McCarty

Department of Environmental Engineering, Inha Univ., 253 Yonghyundong, Namgu, Inchon, South Korea
Department of Civil and Environmental Engineering, Stanford Univ., Stanford, CA 94305-4020, USA

Received 25 July 2003; received in revised form 9 March 2004; accepted 25 March 2004

Abstract

Carbohydrates such as molasses are being added to aquifers to serve as electron donors for reductive dehalogenation of chloroethenes. Glucose, as a model carbohydrate, was studied to better understand the processes involved and to evaluate the effectiveness for dehalogenation of different approaches for carbohydrate addition. A simulation model was developed and calibrated with experimental data for the reductive dehalogenation of tetrachloroethene to ethene via cis-1,2-dichloroethene. The model included fermentors that convert the primary donor (glucose) into butyrate, acetate and hydrogen, methanogens, and two separate dehalogenator groups. The dehalogenation groups use the hydrogen intermediate as an electron donor and the different haloethenes as electron acceptors through competitive inhibition. Model simulations suggest first that the initial relative population size of dehalogenators and H₂-utilizing methanogens greatly affects the degree of dehalogenation achieved. Second, the growth and decay of biomass from soluble carbohydrate plays a significant role in reductive dehalogenation. Finally, the carbohydrate delivery strategies used (periodic versus batch addition and the time interval between periodic addition) greatly affect the degree of dehalogenation that can be obtained with a given amount of added carbohydrate.

Keywords: Reductive dehalogenation; Tetrachloroethene; Trichloroethene; cis-1,2-Dichloroethene; Glucose; Competitive inhibition model; Vinyl chloride

© 2004 Elsevier B.V. All rights reserved.

E-mail address: jlb@inha.ac.kr (J.-H. Bae).

Present address: Department of Environmental Engineering and Science, Clemson University, Box 340919, Clemson, SC 29634-0919, USA.
1. Introduction

Tetrachloroethene (perchloroethene, PCE) and trichloroethene (TCE) have been widely used as solvents, and uncontrolled disposal in refuse sites and improper management of facilities have made them among the most common groundwater contaminants in the United States (Westrick et al., 1984). Partially dechlorinated ethenes also occur in groundwater including cis-1,2-dichloroethene (cDCE) and vinyl chloride (VC). A potentially economical and environmentally sound process to rectify this problem is biological reductive dehalogenation, in which chlorinated ethenes are used as electron acceptors in energy metabolism under anaerobic conditions (Holliger et al., 1993; McCarty, 1997). In order to accomplish more successful application of reductive dechlorination for in-situ bioremediation, there are areas in need of better understanding. One such area is microbial competition for hydrogen (H2), especially between H2-utilizing methanogens and dehalogenators (McCarty, 1997; Ballapragada et al., 1997). Limiting or threshold H2 concentrations for dehalogenators and other H2-utilizing organisms are quite different. For example, Yang and McCarty (1998) reported that dehalogenators out-compete methanogens and homoacetogens when the H2 level is below 11 nM, the threshold concentration for methanogenesis, while H2 is used primarily for methane production when H2 level exceeds 11 nM. This indicates the importance of maintaining a low H2 concentration in order to obtain efficient utilization of H2 by dehalogenating organisms. The rate of H2 production is affected greatly by the nature of organic substrates or electron donors that may be added to contaminated groundwater to serve as H2 precursors (Yang and McCarty, 1998; Fennell et al., 1997).

Carbohydrates such as molasses are being added to aquifers to serve as electron donors for reductive dehalogenation of chloroethenes as they are inexpensive and non-toxic (Maierle and Cota, 2001; Peeles et al., 2001). However, dehalogenation with carbohydrates as electron donors is not a simple process because of the multiple ways in which H2 is produced and consumed. H2 can be produced in the first step of carbohydrate fermentation to organic acids, next, by organic acid oxidation, and finally through the decay of biomass that is produced (Yang and McCarty, 2000b). H2 is consumed both by H2-utilizing methanogens and dehalogenators, as described earlier. In addition, each group of dehalogenators, one for PCE transformation to cDCE via TCE and the other for cDCE degradation to ethene via VC, competes separately for H2 (Haston, 1999; Cupples et al., 2004). Also, within each group of dehalogenators, there can be multiple dechlorination steps, each of which competitively inhibits the other. Because of the complexity of the many factors that affect the rate and extent of dehalogenation of chlorinated ethenes, a numerical model that effectively integrates the many factors involved can be particularly useful in the design of bioremediation schemes for reductive dehalogenation and for predicting the outcome of various possible remediation strategies.

The purpose of this study was to provide a better understanding of the reductive dehalogenation process when using carbohydrates and to evaluate the effectiveness for dehalogenation of different approaches for carbohydrate addition. To achieve these goals, a numerical model was developed and calibrated with experimental data. The model
considers competition between methanogens and dehalogenators and also describes the overall reductive dechlorination process for converting PCE or TCE to ethene via cDCE and VC. Then, the model was used to compare different possible carbohydrate delivery strategies for their effectiveness at dehalogenation and their efficiency in use of carbohydrate for dehalogenation.

2. Experimental methods

2.1. Culture and growth medium

For all evaluations, a dehalogenating source culture was developed in a closed continuously stirred tank reactor (CSTR) initially seeded with aquifer material from a PCE-contaminated groundwater site in Victoria, TX. Details of the operating conditions for the reactor and the composition of the media are described elsewhere (Yang and McCarty, 1998). In general, the 3.6-L reactor, maintained at 28 °C, was continuously fed 100 ml/day of a basal salts solution containing 1.7 mM sodium benzoate, 0.98 mM PCE and 20 mg/l of yeast extract.

2.2. Batch experiments

Batch studies were conducted using 120-ml serum bottles, to which 60 ml of the dehalogenating culture was added anaerobically while gas purging with an 80% N2/20% CO2 mixture. The bottles were sealed with rubber stoppers (Bellco Glass, Vineland, NJ), and the adsorption of chlorinated compounds on the rubber stoppers was found to be less than 5% after the first few days (Yang and McCarty, 2000a). Either PCE (20 μmol) or cDCE (30 μmol) was added with syringe to serve as the electron acceptor for the dehalogenating microorganisms. PCE or cDCE, and their metabolic intermediates and products were assumed to be partitioned between headspace and liquid phase according to the following dimensionless Henry’s constants: PCE—0.716, TCE—0.386, cDCE—0.154, VC—1.077 and ethene—8.5 (Yang and McCarty, 2000a). Three different electron donor substrates were evaluated: glucose, soluble glucose fermentation products (SGFP) and biomass resulting from glucose fermentation. The SGFP and biomass substrates were prepared as follows. Basal media containing 10 mM glucose was inoculated with 0.1% of a mixed methanogenic culture (Yang and McCarty, 2000b) that had been grown on a mixture of benzoate, yeast extract, propionate and oleate. After 4 days, when more than 98% of the glucose was depleted (about 4% of electron equivalents was lost in the gas phase according to chemical oxygen demand (COD) measurements), the fermented glucose solution was centrifuged (13,000 × g, 20 min). The resulting supernatant liquid was used as SGFP. The remaining pellet, after washing three times with basal media, was resuspended and used as the biomass substrate. Glucose, the SGFP and the biomass were then added in adjusted amounts that were equal to 1.92 mg of COD to separate 120-ml bottles containing 60 ml of seed taken from the CSTR. In reducing power, the 1.92-mg COD is equivalent to 240 microelectron equivalents (μeq.), and may produce 30 μmol of H2 if 30% of the e− eq. is assumed to be converted into H2 during the fermentation.
process (McCarty, 1982). Duplicates were prepared for each set-up, with the averaged analytical results being reported here. All the bottles were incubated at room temperature on a shaker table (Lab-Line Instruments) at a rate of 100 rpm. Several bottles had no added electron donor and served as controls.

3. Model development

3.1. Kinetics of dehalogenation

A model was developed to simulate the reductive dechlorination of PCE to ethene with glucose. The model builds upon one developed by Fennell and Gossett (1998) for competition between methanogens and dehalogenators and another by Haston (1999) for this culture that includes separate groups of dehalogenators as well as competition between chlorinated ethenes for electrons from electron donors. The model also draws upon reports by Ballapragada et al. (1997), Tonnaer et al. (1997), Haston and McCarty (1999) and Cupples et al. (2003, 2004). Four major assumptions for dehalogenator activity were used in model development:

1. Two separate dehalogenator groups are involved in the complete dehalogenation of PCE: one for PCE transformation to cDCE via TCE and the other for cDCE degradation to ethene via VC.
2. Within each dehalogenator group, competitive inhibition is involved between the chloroethene used as electron acceptors, that is between PCE and TCE for the first dehalogenator group, and between cDCE and VC for the second dehalogenator group.
3. The only electron donor used for reductive dehalogenation by each dehalogenator group is H₂.
4. H₂ is also utilized for methane production, and competition for H₂ exists between dehalogenators and H₂-utilizing methanogens.

Concerning the first assumption, the culture used for the experimental study contains the two separate dehalogenator groups (Rosner et al., 1997; Haston, 1999; Cupples et al., 2003, 2004). For this culture, competitive inhibition occurred between PCE and TCE for the first dehalogenator group, and between cDCE and VC for the second dehalogenator group (Haston, 1999; Cupples et al., 2004), and so this aspect was included in the model.

The model takes the following form for the rate of dehalogenation by each dehalogenator group. Here, dehalogenation of cDCE to ethene is illustrated as an example.

\[
- \frac{dS_i}{dt} = \left( \frac{\hat{q}_i X S_i}{S_i + K_i \left( 1 + \frac{S_j}{K_j} \right)} \right) \left[ \frac{S_H - S_{Hi}}{S_H - S_{Hi} + K_H} \right] \times 10^6
\]

(1)

Where subscripts \( i \) and \( j \) represent cDCE and VC, respectively; and \( S_i \) or \( S_j \) = the aqueous concentration of cDCE and VC, respectively (μM); \( S_H \) = the aqueous concentration of H₂.
(nM); \( S^*_H \) = a minimum threshold aqueous concentration for \( H_2 \) (nM), reaction stops when \( S_H \leq S^*_H \); \( q_{\text{max}} \) = the maximum specific dechlorination rate of cDCE per unit dechlorinating biomass (mol/g cell-day); \( X \) = the concentration of dechlorinating biomass (g cell/l); \( K_i \) = the half-velocity coefficient for cDCE dechlorination (\( \mu \text{M} \)); \( K_j \) = the competitive inhibition coefficient for VC on cDCE dechlorination (\( \mu \text{M} \)); \( K_H \) = the half-velocity constant for \( H_2 \) (nM).

A similar equation is used for the dechlorination of VC, but with interchange in the \( i \) and \( j \) subscripts of the above equation.

The specific growth rate of cDCE dehalogenators, \( \mu \), is expressed by the following equation.

\[
\mu = \frac{dX}{dt} = \frac{\mu_{\text{max}} S_i}{S_i + K_i \left( 1 + \frac{S_j}{K_j} \right)} + \frac{\mu_{\text{max}} S_j}{S_j + K_j \left( 1 + \frac{S_i}{K_i} \right)} \left( \frac{S_H - S^*_H}{(S_H - S^*_H) + K_H} \right) - b
\]

(2)

Where \( \mu_{\text{max}} \) = the maximum specific growth rate on cDCE or VC and equals \( Y q_{\text{max}} \) (day\(^{-1}\)); \( Y \) = the yields of biomass per mass of cDCE or VC dechlorinated (g cell/mol); \( b \) = the decay rate (day\(^{-1}\)).

PCE dehalogenation to cDCE can also be expressed with Eqs. (1) and (2), but with the subscripts \( i \) and \( j \) representing PCE and TCE, respectively. Also, the two dehalogenating groups can compete for \( H_2 \) when the appropriate chloroethenes are present.

The third assumption above excludes the possibility that acetate may be used as an electron donor for dehalogenation. While some PCE and TCE dehalogenators are known to use acetate as a donor, this was found not to be the case with this culture. Also, the culture used developed only a very small acetoclastic methanogenic population due to inhibition by the ethene end product (Yang and McCarty, 2000a).

As for the fourth assumption, the culture exhibits strong competition for \( H_2 \) between dehalogenators and \( H_2 \)-utilizing methanogens, and so such competition was included in the model. This competition for \( H_2 \) is related to the relative microbial population sizes and their respective kinetic coefficients—\( K_s \), \( q_{\text{max}} \) and \( Y \). In addition, \( S^*_H \), a minimum threshold value for \( H_2 \) concentration in Eqs. (1) and (2), also plays a significant role in regulating such competition (Yang and McCarty, 1998).

### 3.2. Kinetics of fermentation

This aspect of the model builds closely upon that by Fennell and Gossett (1998). As \( H_2 \) was assumed as the only electron donor for dehalogenation and methane fermentation, the rate and amount of \( H_2 \) production became an important factor affecting both dehalogenation and methane production. Glucose can be fermented by many different pathways yielding hydrogen or formate as well as other organic products such as ethanol, butyrate, propionate and acetate. Hydrogen and formate act as immediately available sources of hydrogen for dehalogenation; ethanol, butyrate and propionate serve as slow releasing substrates for hydrogen production. Acetate does not serve as a source
of hydrogen. The stoichiometric equations related to glucose fermentation used in this study are as follows.

Glucose fermentation:

\[ C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COO^- + 2H_2 + 2CO_2 + H^+ \] (3)

\[ C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COO^- + 4H_2 + 2CO_2 + 2H^+ \] (4)

\[ C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \] (5)

Butyrate and ethanol utilization:

\[ CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2 \] (6)

\[ CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2 \] (7)

Equilibrium between H₂ and formate:

\[ HCOO^- + H^+ \rightarrow H_2 + CO_2 \] (8)

The rates of the above reactions were assumed to follow Monod kinetics. Both butyrate and ethanol oxidations proceed only when they are thermodynamically possible, i.e., when Gibbs free energy of the reactions are negative, which requires low H₂ concentration (Bae, 1991). When not thermodynamically feasible, oxidation of butyrate or ethanol was assumed to stop.

Biomass is formed from the utilization of glucose and its intermediates. Biomass decay was found to be an efficient source of H₂ for dehalogenation (Yang and McCarty, 2000b). Of the 20 electron equivalents associated with 1 mol of biomass (C₅H₇O₂N), 30% or 6 electron equivalents was taken to be converted into H₂, and the remainder into acetate (McCarty, 1982). For biomass decay, it was assumed that 80% of the total organic mass is biodegradable (McCarty, 1975) and that the degradation rate follows first order kinetics.

3.3. Model implementation

The developed model was constructed and implemented in MATLAB 5.3. To solve the systems of ordinary differential equation with initial values, ode23tb, a MATLAB initial value problem solver was used. The time step for calculation was adjusted to yield the relative tolerance smaller than 10⁻⁵. Fennell and Gossett (1998) reported that a small time step was required to avoid numerical instability for the calculation of H₂ concentration. Such instabilities can result when the changes in H₂ concentration are large due to rapid hydrogen production and consumption, especially during early periods of an experiment. The MATLAB software automatically uses time steps that are small enough to yield errors less than that specified.
3.4. Model input

Kinetic coefficients used for the simulations were taken from previous studies, and are summarized in Tables 1 and 2. For the cDCE dehalogenating culture, kinetic coefficients determined previously were used (Cupples et al., 2003). These include a maximum specific growth rate ($\mu_{\text{max}}$) of 0.4/day, a yield value $Y$ of 8.2 g cells/mol consumed for dehalogenation and a $q_{\text{max}}$ of 0.048 mol/g cells/day. The $q_{\text{max}}$ value for PCE and TCE dehalogenators was calculated as 0.366 mol/g cell-day, based upon $\mu_{\text{max}}$ of 3.0/day, which is well within the range reported by Haston (1999). Here, organics such as acetate instead of H$_2$ were assumed to be used for the synthesis of dehalogenators. $K_s$ values for dehalogenation were derived for this culture by Haston and McCarty (1999), except for TCE dehalogenation to cDCE. For TCE dehalogenation, a $K_s$ value of 0.05 A$_M$, instead of the reported value of 1.0 A$_M$ was used to eliminate the accumulation of TCE in the simulations, which was not observed in the experimental studies reported here. Minimum threshold values for H$_2$ concentration, $S_{\text{H*}}$ values for dehalogenators and H$_2$-utilizing methanogens were taken to be 2 and 11 nM, respectively (Yang and McCarty, 1998).

The coefficients for non-dehalogenators are based mainly upon Fennell and Gossett’s (1998) previous report. The decay rate used for all bacterial groups involved including the biomass added from glucose fermentation was 0.05/day, which was developed from the experimental data for this study. This decay rate is the same as that found for the dehalogenating culture used here (Cupples et al., 2003), but twice that used by Fennell and Gossett (1998).

The results of the experimental study were used to calibrate other model coefficients for each of the processes by which H$_2$ is produced. Electron donor conditions for the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Model coefficients for dehalogenators used in simulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron donor</td>
<td>$q_{\text{max}}$ (mol/g cell-day)</td>
</tr>
<tr>
<td>PCE</td>
<td>0.366</td>
</tr>
<tr>
<td>TCE</td>
<td>0.366</td>
</tr>
<tr>
<td>CDCE</td>
<td>0.048</td>
</tr>
<tr>
<td>VC</td>
<td>0.048</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Table 2</th>
<th>Model coefficients for non-dehalogenators used in simulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron donor</td>
<td>$q_{\text{max}}$ (mol donor/g cell-day)</td>
</tr>
<tr>
<td>H$_2$-utilizing methanogens</td>
<td>1.5$^a$</td>
</tr>
<tr>
<td>Ethanol utilizers</td>
<td>0.263$^a$</td>
</tr>
<tr>
<td>Butyrate utilizers</td>
<td>0.588$^a$</td>
</tr>
<tr>
<td>Glucose utilizers</td>
<td>0.225$^a$</td>
</tr>
</tbody>
</table>

$^a$ $q_{\text{max}}$ values were reduced to one half of the reported values to account for temperature differences; temperature was 35 °C for the reported values and room temperature in this experiment.
simulations are summarized in Table 3. The only source of H$_2$ for dehalogenation in the control case was the decay of the seed biomass (10 mg/l). For cases other than the control, the total amount of electron donor added was set to the same value of 240 $\mu$eq per bottle.

The glucose fermentation products formed in the experimental study were not measured, but reasonable assumptions based upon commonly observed values were used for model simulations (Bae, 1991; Zoetemeyer et al., 1982). For the glucose case, 1 mol of glucose is fermented into 0.3 mol of butyrate, 1.4 mol of acetate, 0.68 mol of formate and 2.72 mol of H$_2$. The produced butyrate was then further oxidized to acetate as indicated in Eq. (6). Thus, the added 10 $\mu$mol glucose results in the production of 40 $\mu$mol H$_2$.

The glucose fermentation pathway assumed for SGFP production was different from that for the experimental study as there is likely to be a significant difference in H$_2$ levels between the two cases. During the preparation of SGFP substrate with the pulse-added glucose, H$_2$ concentration most probably increased to a level favoring the production of reduced compounds such as ethanol as evidenced by Zoetemeyer et al. (1982). This is different from the experimental studies themselves where H$_2$ was kept much lower because of rapid utilization by both dehalogenators and methanogens. For SGFP formation, 1 mol of glucose was assumed to produce 0.3 mol of butyrate, 1.4 mol of ethanol, 0.24 mol of formate and 0.96 mol of H$_2$. The produced ethanol was then further oxidized to acetate producing H$_2$ as indicated in Eq. (7). As 4% of the glucose electron equivalents was lost as gaseous H$_2$ during SGFP preparation, the sum of butyrate, ethanol and formate was adjusted to give 240 $\mu$eq per bottle.

For simulations, the initial measured biomass, 10 mg/l, was divided among the different trophic groups as summarized in Table 4. The initial concentrations of active PCE and cDCE dehalogenators were taken to be 0.015 mg/l each, which is about the concentration measured by competitive PCR for the cDCE dehalogenators under similar experimental conditions (Cupples et al., 2003). H$_2$-utilizing methanogenic biomass was assumed as 0.5 mg/l. The concentrations of butyrate, glucose and ethanol utilizers were selected to

<table>
<thead>
<tr>
<th>Case</th>
<th>Donor</th>
<th>Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>none</td>
<td>–</td>
</tr>
<tr>
<td>Glucose</td>
<td>glucose</td>
<td>10.0 $\mu$mol</td>
</tr>
<tr>
<td>Biomass</td>
<td>biomass</td>
<td>1.60 mg</td>
</tr>
<tr>
<td>SGFP</td>
<td>ethanol</td>
<td>14.3 $\mu$mol</td>
</tr>
<tr>
<td></td>
<td>butyrate</td>
<td>3.13 $\mu$mol</td>
</tr>
<tr>
<td></td>
<td>formate</td>
<td>1.25 $\mu$mol</td>
</tr>
</tbody>
</table>

* All contained 0.6 mg seed biomass.

* Formate is directly converted to H$_2$ and HCO$_3^-$ before being utilized for methane production or dehalogenation.

<table>
<thead>
<tr>
<th>Case</th>
<th>Donor</th>
<th>Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCE</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>DCE</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>H$_2$-utilizing methanogens</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Butyrate utilizers</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Glucose utilizers</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Ethanol utilizers</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Other biomass</td>
<td>9.41</td>
<td></td>
</tr>
</tbody>
</table>

Table 3
Electron donor conditions assumed for both PCE and cDCE simulations

Table 4
Assumed initial concentrations (mg/l) of each bacterial group in the source culture
yield a best fit for the experimental data. The ‘other biomass’ mostly consists of benzoate utilizers, inert cells and probably fatty acids utilizers.

4. Results and discussion

4.1. Comparison between experimental and simulated results

Comparisons between simulated and experimental results with glucose and its fermentation products are illustrated in Figs. 1 and 2 for the dehalogenation of PCE and cDCE, respectively. Here, the total mass of each chloroethene, instead of the aqueous phase concentration, is presented to give a better overall insight on the mass balance. Also, Table 5 summarizes the electron equivalents recovered by dehalogenation and methane production for both the simulated and experimental cases after 140 days. For the calculation, the production of 1 μmol of chloride by dehalogenation is equal to 2 μe− eq. For example, the production of ethene (ETH) from PCE is equal to 8 μe− eq., while the same ethene from cDCE is equal to 4 μe− eq. One micromole of methane is equivalent to 8 μe− eq.

Simulated chlorinated ethene concentrations agree reasonably well with the experimental results (Figs. 1 and 2). For all PCE cases, the difference between simulated and experimental e− eq. recovery by dehalogenation was within 10% as indicated in Table 5. It is also interesting to compare the blank and biomass cases as the electron donor for both cases was the same, i.e. biomass. The only difference is that the biomass case had 1.6 mg more biomass. This extra biomass increased the extent of dehalogenation by 64 μe− eq. compared with blank case. A similar increase in dehalogenation, 61 μe− eq., was predicted by the simulation model.

For all the cDCE cases, simulated e− eq. recovery by dehalogenation was greater than the experimental values. However, this difference could be related to the poor experimental mass balances obtained for chlorinated ethenes. In all cDCE cases, the total mass of chlorinated ethenes at the end of the experiments was only about 74–76% of the initial cDCE mass. If the experimental data were corrected for this loss of chlorinated ethenes, more e− eq. would be recovered by dehalogenation and the experimental results would agree better with the simulation results. Both the experimental and simulated increase in dehalogenation from biomass addition (the difference between the blank and biomass cases) were 36 and 44 μe− eq., respectively. This difference is not great considering the experimental loss of chloroethenes. Thus, although there are some limitations due to experimental mass balance difficulties, the simulation results generally described the experimental behavior of chloroethenes and the extent of dehalogenation with a given electron donor.

Experimental results for dehalogenation of PCE with glucose showed similar trends as that with SGFP, suggesting that glucose and its fermentation products produce about the same dehalogenation results. This was also observed with the dehalogenation of cDCE. With biomass addition, both modeled and experimental PCE conversion into cDCE was much slower than with either glucose or SGFP because of the slower production of H2 from cell decay. This slow H2 production does have a positive aspect for dehalogenation as it acts to maintain a low H2 concentration, thus reducing
competition for hydrogen by methanogens (Yang and McCarty, 1998). As a result, the experimental and simulated dehalogenation of cDCE with biomass was more complete than in the other two cases.

Simulated methane productions agree reasonably well with experimental values except in the glucose and biomass cases for the dehalogenation of cDCE (Table 5). For PCE dehalogenation, the difference between experimental and simulated methane production

Fig. 1. Comparisons between simulated and experimental results for reductive dehalogenation of PCE: (a) blank, (b) glucose, (c) SGFP and (d) biomass.
was 4.8–13 μe\(^{-}\) eq. (0.6–1.6 μmol of methane), which equals about 10% of the electrons utilized for dehalogenation and methane production, or less than 5% of the added electron donor. For the glucose and SGFP cases, the initial experimental methane production with cDCE is higher than that with PCE as expected because only one group of dehalogenators then competes with methanogens for H\(_2\) (Figs. 1 and 2). The higher experimental methane production with cDCE can be explained by the simulations. The simulated H\(_2\) concentration with PCE dehalogenation decreased within 1 day to below 11 nM, the threshold
value for H₂-utilizing methanogens (Fig. 1), while it took more than 10 days to reach this level with cDCE (Fig. 2). With a higher simulated H₂ concentration over a longer period, more methane production occurred in the cDCE cases. Second peaks in simulated H₂ concentration resulted with PCE dehalogenation just after the depletion of PCE and TCE occurred, except for the blank case. This is because only one dehalogenating species, rather than two, were then competing with methanogens for H₂.

There is an anomaly in the experimental methane production from glucose with cDCE dehalogenation. Unlike the other cases, methane production continued to increase with time. This higher experimental methane production appears to be related to acetoclastic methanogenesis as a decrease in acetate mass was experimentally found only in this case (decreased by 4 mol between \( t = 30 \) and 140 days).

It is also not clear why there is a difference in methane production between experimental and simulation results in the biomass case for cDCE dehalogenation. Nevertheless, the lower methane production with biomass compared with either glucose or SGFP is reflected in the simulated H₂ level.

A worthwhile objective when engineering an in-situ reductive dehalogenation system is to use as little electron donor as possible. This is to reduce chemical costs, clogging potential from methane production in-situ and from excess biomass production, the potential fire hazard from methane production, and the organic byproducts that remain in groundwater after treatment. The fraction of donor used for dehalogenation is thus of interest and was determined from the simulations. With both PCE and cDCE, the fraction of donor (including initial biomass) associated with methane production plus dehalogenation was found to be a constant value of about 25–30%. This essentially equals the amount of complex organic material normally found convertible to H₂. The remainder is converted primarily into acetate. For the PCE cases, 24–26% of the donor was utilized for dehalogenation. With cDCE, the respective value was only about 9–16%, with the
remainder being used in methane and acetate production. Competition for H₂ is thus better with PCE dehalogenation than with cDCE dehalogenation, as might be expected because of the better dehalogenation kinetics with PCE and TCE, and also here, two different dehalogenator populations compete for H₂. Also, as previously found, use of H₂ for dehalogenation is more efficient with a slowly decomposing organic compound, such as biomass, than with a rapidly utilized donor such as glucose (Yang and McCarty, 2000b), and this effect is more pronounced in cDCE dehalogenation.

4.2. Importance of initial biomass distribution

The relative initial mass distribution of different trophic groups in the culture used (Table 4) may affect the results of the experimental study. At a given field site where a

---

**Fig. 3.** Simulated effects of the relative ratio of dehalogenator biomass to methanogenic biomass on dehalogenation: (a) PCE and (b) cDCE.
donor may be added to effect reductive dehalogenation, the relative population sizes may be considerably different initially than in the culture used for the experimental studies here. A question then arises as to whether or not this will affect the outcome of donor addition? The simulation model was used to address this question.

Effects of the initial relative ratios of dehalogenators to H₂-utilizing methanogens on the efficiency of dehalogenation were simulated and are illustrated in Fig. 3. Here, the initial concentrations of dehalogenators were varied from 0.1 to 10 times the value used for the simulations of Figs. 1 and 2, while the concentration of methanogens and other simulation conditions were kept the same as before. A small decrease in the initial dehalogenator concentrations over that initially assumed was found to significantly increase methane production and reduce dehalogenation. However, for the PCE case, a 10-fold increase in the dehalogenating biomass from that originally assumed (Table 4) had little additional impact as methane production was then near zero. These results indicate that the initial distribution of biomass can have a large impact on the extent of dehalogenation that results from the addition of a single batch addition of donor. This impact could carry over and significantly affect results of possible subsequent batch additions of glucose, although this possibility was not simulated here.

4.3. Contribution of biomass produced during glucose fermentation for dehalogenation

Biomass was found to be an excellent donor for dehalogenation (Yang and McCarty, 2000b) because of the slow release of H₂ that results. A question arises as to how important is this biomass to the overall dehalogenation that results from glucose addition? To evaluate this question, a comparison was made between the simulated amount of dehalogenation achieved with and without including H₂ production from the decay of glucose-grown biomass.

As indicated in Table 6, without the H₂ produced from the decay of glucose-grown biomass, the simulated total dehalogenation was decreased by 5% and 14% for the PCE and cDCE cases, respectively. The resulting decreases in ethene production were 40% and 25% with PCE and cDCE, respectively. Simulations that do not include biomass decay give very poor fits to the experimental data (results not shown). Thus, growth and decay of biomass from glucose addition can play a significant role in the reductive dehalogenation achieved with this substrate. This may be a surprising conclusion considering the relatively small amount of biomass formed during glucose fermentation. However, the slow decay of biomass makes it a very efficient electron donor for dehalogenation. This conclusion is expected to hold true with any carbohydrate that may be used for in-situ reductive

<table>
<thead>
<tr>
<th>Case</th>
<th>PCE dehalogenation</th>
<th>DCE dehalogenation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (µmol Cl⁻)</td>
<td>Ethene (µmol)</td>
</tr>
<tr>
<td>1. Glucose with biomass decay</td>
<td>51.6</td>
<td>9.83</td>
</tr>
<tr>
<td>2. Glucose without biomass decay</td>
<td>48.9</td>
<td>5.89</td>
</tr>
<tr>
<td>Decrease without biomass decay</td>
<td>5%</td>
<td>40%</td>
</tr>
</tbody>
</table>
dehalogenation, including molasses, starch, and other soluble and readily fermentable carbohydrate forms. Additionally, biomass is likely to remain closer to the point of carbohydrate addition as most of it results from the initial rapid fermentation of carbohydrate, while the SGFP, being soluble, is likely to move further from the injection point. Thus, the biomass and SGFP contributions to dehalogenation would likely be most effective at different locations within the aquifer.

4.4. Optimal donor delivery strategy

The simulation model permitted an evaluation to be made of the effect of different donor delivery strategies on the efficiency of donor usage for dehalogenation. Here, the

![Diagram](image)

Fig. 4. Simulated dehalogenation and methane production with increase in initial glucose dosage: (a) PCE and (b) cDCE.
impact of adding a given amount of donor in increments over time was evaluated and compared with that of a single instantaneous delivery as carried out in the batch experiments. Also, the time between deliveries was varied to determine its effect.

First, the effect of varying the glucose dosage with instantaneous delivery was simulated, and the results are illustrated in Fig. 4. Here, a glucose dosage of 1 equals the amount used in the experimental studies. Methane production increases linearly as glucose dosage increases for both the PCE and cDCE cases. Total dehalogenation also increases with an increase in glucose dosage up to 5 times (PCE case) and 10 times (cDCE case). Further increases in glucose dosage above these levels results in more methane production and so is wasteful of glucose. However, the time required for complete dehalogenation is shortened with higher glucose additions as higher H_{2} concentrations result, causing faster dehalogenation as well as faster methanogenesis.

To evaluate the effect of periodic donor addition rather than a single batch addition, glucose was assumed to be added 2–10 times at equal time intervals over a 140-day period, which is how long the experimental study was conducted. Here, the total mass of glucose added was kept constant at twice that used in the experimental study. As illustrated

![Graph](image1)

a) PCE dehalogenation  
b)
in Fig. 5, periodic addition of glucose yields more efficient dehalogenation and less methane production for cDCE dehalogenation. For example, the 120 μeq. (30 μmol) of cDCE assumed present was about completely converted into ethene with 10 periodic additions of glucose (0.2 times the experimental dosage at each period) at 14-day intervals. However, with a single addition, the required glucose dosage for complete dehalogenation of cDCE was 10 times the experimental value. Therefore, with periodic addition, the glucose required to achieve complete dehalogenation of cDCE can be reduced by 80%. In contrast, the dehalogenation of the 160 μeq. (20 μmol) of PCE assumed present was not enhanced by periodic donor additions, as competition for H₂ was better with two different dehalogenators acting, as discussed before.

In order to evaluate the desirable interval between periodic glucose additions, 0.2 times the experimental glucose dosage was added 10 separate times, while the interval between each addition was varied between 2 and 14 days. The best result with cDCE was an interval of 10 days, with complete cDCE conversion into ethene being achieved within about 130 days, as illustrated in Fig. 6. An interval of 14 days yielded similar dehalogenation and methane production, but intervals shorter than 10 days produced more methane and less dehalogenation. On the other hand, because of better competition for H₂, dehalogenation of PCE was not affected by the changes in time intervals.

Fig. 5 shows the simulated dehalogenation with 10 periodic additions of glucose with various time intervals between additions: (a) PCE and (b) cDCE.
5. Conclusions

1. Simulation results that include competitive inhibition between different chloroethene electron acceptors for reductive dehalogenation of PCE to cDCE and cDCE to ethene agree well with experimental results using glucose, soluble glucose fermentation products or biomass as electron donors.

2. Simulation results indicate that 24–26% of the electron donor (glucose, biomass and soluble glucose fermentation product) was utilized for dehalogenation for the PCE cases, while the respective value was 9–16% with cDCE. The better efficiency with PCE is the result of better dehalogenator kinetics and the use of hydrogen by two different dehalogenator groups.

3. When the relative initial population of dehalogenators was smaller, dehalogenation was less complete and more methane was produced. This indicates that the relative starting population sizes of dehalogenators and H$_2$-utilizing methanogens at a given field site can greatly affect the degree of dehalogenation obtained for a given amount of donor added.

4. The growth and decay of biomass from soluble carbohydrate fermentation plays a significant role in reductive dehalogenation.

5. The donor delivery strategy used, such as the amount of donor added in each increment and the time between incremental deliveries, can greatly affect the degree of dehalogenation achieved, and thus should be an important consideration in remediation design, not only because it impacts on the efficiency of dehalogenation achieved with a given amount of donor added, but also it affects possible clogging and other problem associated with methane production.

6. The model of reductive dehalogenation developed here can be a useful tool for evaluating different strategies for addition of glucose and other organic compounds to groundwater contaminated with chlorinated ethenes, and may be useful as well for predicting the outcome of bioaugmentation used for enhancing dehalogenation.

Acknowledgements

This research was supported by a cooperative U.S. National Science Foundation/Korea Science and Engineering Foundation research grant (20015-309-01-2), by the U.S. Department of Energy through grant DE-FG07-96ER62300 and by E.I. duPont de Nemours, through the U.S. Environmental Protection Agency sponsored Western Region Hazardous Substance Research Center. Since these organizations have not reviewed this manuscript, no official endorsement should be inferred.

References


vinyl chloride and \textit{cis}-dichloroethene as electron acceptors as determined by competitive PCR. Appl. Environ. Microbiol. 69 (2), 953–959.


