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Effect of Temperature on Perchloroethylene Dechlorination by a Methanogenic Consortium

Jianwei Gao, Rodney S. Skeen¹, and Brian S. Hooker

ABSTRACT

The effect of temperature on the kinetics of growth, substrate metabolism, and perchloroethylene (PCE) dechlorination by a methanogenic consortium is reported. In all cases, a simple kinetic model accurately reflected experimental data. Values for the substrate and methane yield coefficients, and the maximum specific growth rate are fairly consistent at each temperature. Also, the substrate and methane yield coefficients show little temperature sensitivity. In contrast, both the maximum specific growth rate and the PCE dechlorination yield coefficient ($Y_{PCE}$) are temperature dependent.

INTRODUCTION

Biological treatment has become one of the most appealing technical solutions for contaminated soils and groundwater. In situ bioremediation for chlorinated solvents is one such technology that has gained popularity. Microorganisms rapidly transform chlorinated solvents into nontoxic compounds. In situ biological processes also can circumvent the mass-transport issues that limit the effectiveness of pump-and-treat systems. This advantage results from contaminants being destroyed in place rather than extracted (Skeen et al., 1993).

Most research efforts on bioremediation for chlorinated solvents have focused on exploiting

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aerobic microbial metabolisms to achieve contaminant transformation. As a result, much is known about the biochemistry of these systems (McCarty and Semprini, 1994). In contrast, little is known about the environmental conditions which are necessary to initiate and sustain anaerobic dechlorination (Bouwer, 1994). Hence, laboratory screening tests using microcosms with various additives have served as the basic tool to evaluate in situ bioremediation potential (Petersen, et al., 1994; Gibson and Sewell, 1992; Sewell and Gibson, 1991). The primary goals of these tests are to determine the potential for in situ contaminant destruction and to develop kinetic information to help design an in situ remediation process. This paper reports the effects of temperature on the kinetics of growth, substrate metabolism, and PCE dechlorination by a methanogenic consortium.

MATERIALS AND METHODS

Sediments were obtained from the Yakima River delta in southeastern Washington state and cultivated anaerobically with methanol. A seed culture for these experiments was prepared by combining 20 g of sediment and 40 mL of culture media with 3 g/L of methanol in a 100 mL serum bottle. The culture was continuously maintained by periodically exchanging 20 mL culture solution with fresh media and methanol. The media used both in the seed culture and in subsequent experiments contained (per liter of deionized water): 270 mg KH₂PO₄, 350 mg K₂HPO₄, 530 mg NH₄Cl, 75 mg CaCl₂·2H₂O, 100 mg MgCl₂·6H₂O, 20 mg FeCl₂·4H₂O, 1200 mg NaHCO₃, 250 mg Na₂S·9H₂O, 10 mg C₆H₅NO₃Na₂, 5.0 mg MnCl₂·4H₂O, 0.5 mg H₃BO₃, 0.5 mg ZnCl₂, 0.5 mg CoCl₂·6H₂O, 0.5 mg NiSO₄·6H₂O, 0.3 mg CuCl₂·2H₂O, 0.1 mg NaMoO₄·2H₂O, and 1.0 mg resazurin. One liter of culture medium also contained the following vitamins: 0.02 mg biotin, 0.02 mg folicin, 0.1 mg vitamin B₆, 0.1 mg riboflavin, 0.1 mg thiamine (HCl), 0.05 mg pantothenic acid, 0.05 mg nicotinamide, 0.1 mg vitamin B₁₂, 0.05 mg PAPA, and 0.06 mg lipoic acid.
Growth tests were conducted at 17 and 30 °C to test kinetic expressions for anaerobic metabolism of methanol. In these experiments, 10 mL culture media with approximately 1 g/L methanol was added to 28 mL balsh tubes. Each tube also received 0.5 mL inoculum from a 7-day-old subculture of the original microbial stock. The subculture was prepared by adding 10 mL of the seed culture to a 100 mL serum bottle that contained 50 mL media with 3 g/L methanol. At every sample time, duplicate tubes were sacrificed and analyzed for the concentrations of methane, hydrogen, protein, methanol, and acetate.

Perchloroethylene (PCE) dechlorination tests were conducted at 17 and 30 °C using the fed-batch reactor system reported previously (Petersen et al., 1994). The system was charged with sufficient PCE saturated water and biomass to achieve a nominal aqueous concentration of 1 mg/L and 20 mg dry weight per liter (mg-DW/L), respectively.

Aqueous samples were periodically removed from the reactor and analyzed for the concentration of protein, methanol, acetate, and chlorinated organics (Petersen, et al., 1994). Simultaneously, headspace samples were removed and analyzed for methane and hydrogen. Methane and hydrogen were quantified by gas chromatography (GC) using a thermal conductivity detector and a 4.5-m Carboxen 1000 packed column having a 3.2-mm ID. Chlorinated organics such as PCE, trichloroethylene (TCE), dichloroethylene (DCE), and vinyl chloride (VC) were assayed by a GC using an electron capture detector and a 30-m DB-624 column (J&W Scientific, Fulsom, CA) having a 0.53-mm ID. Methanol concentration was analyzed by GC using a flame ionization detector with a 30-m DB-Wax column (J&W Scientific, Fulsom, CA) having a 0.53-mm ID. Acetate concentrations was determined from a filtered sample using a Dionex 4000i (Dionex, Sunnyvale, CA) ion chromatograph with a Dionex PAX 100 anion exchange column (Dionex, Sunnyvale, CA). Aqueous solutions were analyzed for protein with the Pierce Micro BCA Protein Assay Kit. Cell concentrations were determined in mg-DW/L assuming biomass is composed of 51% protein on a dry weight basis.
RESULTS AND DISCUSSION

Equations 1 through 4 were used to describe growth, substrate consumption, methane production, and PCE dechlorination to TCE by the methanogenic consortium.

\[
\frac{d[X]}{dt} = \frac{\mu_{\text{max}}[X][S]}{([S] + K_s)} \tag{1}
\]

\[
\frac{d[S]}{dt} = -Y_{SX} \frac{\mu_{\text{max}}[X][S]}{([S] + K_s)} \tag{2}
\]

\[
\frac{dM}{dt} = Y_{MX} \frac{\mu_{\text{max}}[X][S]}{([S] + K_s)} V_i \tag{3}
\]

\[
\frac{dTCE}{dt} = Y_{PCE} Y_{MX} \frac{\mu_{\text{max}}[X][S]}{([S] + K_s)} \tag{4}
\]

In these equations \([X]\) represents the aqueous biomass concentration (mg-DWL); \([S]\) the methanol concentration (mole/L); \(M\) the total moles of methane in the reactor; \([PCE]\) and \([TCE]\) the aqueous concentrations of PCE and TCE (mg/L), respectively; and \(V_i\) the liquid volume in the system (L). Only PCE conversion to TCE was described since this was the sole dechlorination step observed during the limited duration of the experiments. A \(K_s\) value of \(1.7 \times 10^4\) mole-methanol/L reported by Gupta et al. (1994) was used in all cases. The form of Equation 4 was chosen based on the observation that PCE dechlorination by methanogenic organisms is strongly linked to methane formation (Rasmussen et al., 1994; Fathepure and Boyd 1988).

In all cases, the model accurately reflected the experimental data. This is demonstrated in Figures 1 and 2 which show the results from growth tests conducted at 17 and 30 °C, respectively. In addition, Figure 3 displays experimental data and model response curves for TCE production at both temperatures. The 30 °C data represents an experiment in which multiple additions of methanol were made up to day 22.
Table 1 summarizes the kinetic coefficients that best described the experimental data for growth tests and dechlorination reactor tests at 17 and 30 °C. These values were determined using the SimuSolv® program (Dow Chemical Company, Midland, Michigan) to calculate optimal model parameter values based on input experimental data sets. Values for $\mu_{\text{max}}$, $Y_{\text{Sx}}$, $Y_{\text{MX}}$ appear to be fairly consistent at each temperature. Also, there is little temperature sensitivity in the substrate and methane yield coefficients. In contrast, the maximum growth rate is temperature dependent with an average of 0.48 day$^{-1}$ at 30 °C and 0.18 day$^{-1}$ at 17 °C. The yield coefficient for PCE dechlorination, $Y_{\text{PCE}}$, is also temperature sensitive. This parameter more than doubles with a temperature reduction from 30 to 17 °C.

The fact that $Y_{\text{PCE}}$ does not remain constant suggests that the dechlorination reaction may be mechanistically different at the two temperatures, as would be encountered if different organisms in the consortium were mediating dechlorination at the two temperatures. The source for this difference is unknown and further work is needed to verify and explain these observations. However, the reported results imply that temperature may have unexpected effects on dechlorination kinetics and care should be taken to conduct microcosm screening tests at aquifer temperatures to assure that activity mimics in situ behavior.

ACKNOWLEDGMENTS

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REFERENCES


Figure and Table Captions

Table 1. Summary of rate data at 17 and 30 °C

Figure 1. Example measured and predicted results of a growth experiment at 17 °C; (a) methanol concentration, (b) biomass concentration, and (c) methane in the culture.

Figure 2. Example measured and predicted results of a growth experiment at 30 °C; (a) methanol concentration, (b) biomass concentration, and (c) methane in the culture.

Figure 3. Measured and predicted trichloroethylene concentration for dechlorination tests at 17 and 30 °C.
Key Word List
Kinetics, Methanogenic, Perchloroethylene, Dechlorination, Anaerobic
Table 1. Summary of rate data at 17 and 30 °C

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Test Type</th>
<th>$\mu_{\text{max}}$ (day$^{-1}$)</th>
<th>$Y_{\text{SX}}$ (mole/g-DW)</th>
<th>$Y_{\text{MX}}$ (mole/g-DW)</th>
<th>$Y_{\text{PCE}}$ (mole/mole)</th>
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<tr>
<td>30</td>
<td>Dechlorination</td>
<td>0.43</td>
<td>0.49</td>
<td>0.27</td>
<td>$3.3 \times 10^{-6}$</td>
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<tr>
<td>30</td>
<td>Dechlorination</td>
<td>0.56</td>
<td>0.39</td>
<td>0.26</td>
<td>$2.0 \times 10^{-6}$</td>
</tr>
<tr>
<td>30</td>
<td>Growth</td>
<td>0.43</td>
<td>0.54</td>
<td>0.41</td>
<td>na</td>
</tr>
<tr>
<td>30 °C Averages:</td>
<td></td>
<td>$0.47 \pm 0.08$</td>
<td>$0.47 \pm 0.08$</td>
<td>$0.31 \pm 0.08$</td>
<td>$(2.7 \pm 0.9) \times 10^{-6}$</td>
</tr>
<tr>
<td>17</td>
<td>Dechlorination</td>
<td>0.19</td>
<td>0.38</td>
<td>0.34</td>
<td>$8.0 \times 10^{-6}$</td>
</tr>
<tr>
<td>17</td>
<td>Growth</td>
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<td>0.37</td>
<td>0.25</td>
<td>na</td>
</tr>
<tr>
<td>17</td>
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<td>0.16</td>
<td>0.37</td>
<td>0.37</td>
<td>na</td>
</tr>
<tr>
<td>17 °C Averages:</td>
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<td>$0.37 \pm 0.00$</td>
<td>$0.32 \pm 0.06$</td>
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</tr>
</tbody>
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na - not applicable or not measured