TREATMENT OF TETRACHLOROETHYLENE WITH ANAEROBIC ATTACHED FILM PROCESS

By K. H. Chu and W. J. Jewell

ABSTRACT: A laboratory-scale continuously fed anaerobic attached film expanded bed (AAFEB) reactor is utilized to examine the feasibility of tetrachloroethylene/trichloroethylene (PCE/TCE) dechlorination under methanogenic conditions at 35°C. Sucrose is used as the electron donor. After a short acclimation period (two weeks), the system shows excellent dechlorination capability for treating PCE and TCE. Influent PCE concentrations of 8.2–26 mg/L are reduced to less than 0.2 mg/L (greater than 98% removal) in most cases, even though efforts are made to achieve intermediate removal efficiencies to support kinetic analysis. TCE and cis-1,2-dichloroethylene (cis-1,2-DCE) remain at low concentrations, which indicates that they were not the rate-limiting intermediates throughout much of the test program. Preliminary estimates of the half-velocity coefficient (K) and maximum PCE uptake rate (q max) are 0.25 mg PCE per liter and 22.9 mg PCE per gram VS per day, respectively. Most of the PCE and TCE appear to be converted to vinyl chloride. The cometabolism-like reactions require a minimum of about eight mass units of COD reduction for each mass of PCE dechlorinated with a range of less than five to greater than 10. The results of the study suggest that the AAFEB system with sucrose as carbon and energy source is efficient and rapid enough to appear to be a feasible bioremediation process for PCE and TCE treatment, but further treatment of vinyl chloride is necessary.

INTRODUCTION

Tetrachloroethylene (PCE) and trichloroethylene (TCE), two commonly detected organic pollutants in ground water, were designated as priority pollutants by the U.S. Environmental Protection Agency in 1976 and are strictly regulated under the Safe Drinking Water Act Amendments of 1986. These compounds were widely used in industrial and household cleaning. Their broad usage and inadequate disposal methods have resulted in contamination of soil and ground water.

Two chemical/physical treatments, granular activated carbon and packed-tower aeration, are the most common methods employed because their removal efficiencies can meet the discharge criteria established by the Safe Drinking Water Act. However, these processes do not destroy PCE and TCE, but transfer them from the liquid phase to air or solid phases. Processes are needed that could degrade these compounds to environmentally acceptable materials, such as ethylene, chlorides, carbon dioxide, and hydrogen ions.

The biological transformation of chlorinated compounds is an attractive alternative that has the potential for complete mineralization or biodegradation. Mono- and dihalogenated compounds tend to be easily oxidized in an aerobic environment; conversely, the polyhalogenated chemicals are more easily dechlorinated under anaerobic conditions (Vogel and McCarty 1985, 1987).

TCE was once believed to persist in the aerobic soil environment. How-
ever, recent studies have shown biodegradation by aerobic bacteria containing monooxygenase (methanotrophs) (Wilson and Wilson 1985; Fogel et al. 1986), ammonia-oxidation (ammonia-oxidizing bacteria) (Archer 1989) and dioxygenase (toluene-oxidizing bacteria) (Nelson et al. 1988; Wackett and Gibson 1988). No report has shown that PCE can be mineralized by any of these aerobic bacteria.

Under anaerobic conditions, common microbes including sulfate reducers, nitrate reducers, and methanogens have dechlorination capabilities for PCE and TCE. Previous studies, which were conducted in soil (Klepfer et al. 1985), in organic sediment (Parsons et al. 1984, 1985), with pure cultures (Fathepure et al. 1987; Fathepure and Boyd 1988a, 1988b), and with continuous-flow fixed-film reactors (Bouwer and McCarty 1983a, 1983b; Vogel and McCarty 1985, 1987; Bouwer and Wright 1988), have shown that PCE is sequentially converted to TCE, dichloroethylene (DCE) isomers, vinyl chloride (VC) (Parsons et al. 1984, 1985), and then to carbon dioxide (CO₂) under anaerobic conditions (Vogel and McCarty 1987, 1985). Recently, PCE and TCE were reported to be completely dechlorinated to vinyl chloride and ethylene (Freedman and Gossett 1989).

The highly chlorinated compounds (PCE and TCE) are more easily dechlorinated in an anaerobic environment than under aerobic conditions; however, the dechlorination rate becomes slower and incomplete as the chlorine is removed. The one chlorine ethene, VC, usually accumulates with some being dechlorinated to ethylene.

A general schematic of anaerobic reductive dechlorination is shown in Fig. 1. Stepwise dechlorination may remove one, two, or three chlorine atoms at different rates, leaving mainly VC according to most researchers. Although methanogenic dechlorination appears to be an important alternative remediation process, little quantitative information is presently available and the basis of the process is not well understood. The process has characteristics similar to cometabolism (i.e., it appears to require a readily available reduced carbon energy source, for example); but some attributes do not support this (methanogenic activity does not guarantee that dechlorination will occur since an acclimation phase is often required). In addition, most studies on biotransformation of PCE and TCE have been conducted in batch mode (with serum bottles) or in small, continuous-flow, fixed-film columns. Very few of these studies have analyzed the kinetics of the PCE biotransformation process, especially in an anaerobic attached film system. Thus, it is not known whether the process can be operated under practical conditions and achieve the required exceptionally high effluent quality (less than 5 ppb).

This investigation was undertaken to examine the engineering feasibility of using a methanogenic microbial system in an expanded bed reactor (AABE) to dechlorinate PCE and its by-products (Chu 1991). This was considered the first requirement for the definition of "above-ground" groundwater treatment processes. Eventually, the fundamentals may lead to manipulation of in situ processes for groundwater remediation. The specific objectives were to: (1) Develop a continuously flowing chemostat bioreactor at 35°C with a methanogenic enrichment culture capable of efficiently dechlorinating PCE and TCE; (2) determine the kinetic parameters of PCE dechlorination for continuous flow operation in the AABE system; (3) define minimum organic energy requirements; (4) examine the maximum loading of PCE; and (5) to determine the impact of lower temperatures (20°C) on PCE dechlorination.

**MATERIALS AND METHODS**

**Chemicals**
The chlorinated organic compounds used in the study were: PCE (Eastman Kodak spectrograde, with 0.5% ethanol), TCE (Fisher, Certified ACS, 99.5%), 1,1-dichloroethylene (Aldrich Chemical Corp., Inc., 99%), trans-1,2-dichloroethylene (Aldrich Chemical Corp., Inc., 98%), cis-1,2-dichloroethylene (Aldrich Chemical Corp., Inc., 97%), and vinyl chloride (1.003 ppm volume per volume in nitrogen gas, Matheson Gas Products, Inc.).

**Calibration and Sampling**
PCE/TCE/DCE analyses were performed daily via the procedure described in Jewell et al. (1990). Calibration standards were prepared from stock solutions of chlorinated compounds in 160-mL serum bottles, in which known masses of PCE, TCE, or DCE were added in 100-mL methanol. The liquid concentration of chlorinated species (mg/g methanol) in the stocks were thus well known. To prepare the calibration standards, known masses of stocks solutions were delivered by syringes to 160-mL serum bottles, containing 100 mL of purgable-organic free water (prepared by boiling deionized water for 16 min, then maintaining at 90°C for 1 hour, while bubbling through an inert, contaminant-free gas: helium). The delivered stock masses were determined by weighing the syringes before and after delivery. After preparation, standards were inverted in a shaker water bath (Fisher Scientific, Versa-Bath S, model 224) at 35°C for at least 1 hour of equilibration so that the PCE/TCE partitioned according to Henry's law. Gas samples were withdrawn from bottle headspaces and analyzed by HP5890A GC. With the known total mass of each chlorinated compound in a calibration standard, the headspace concentration (Cg) of each were calculated using Henry's law relationships (Gossett 1987). The lowest stan-
standard concentration used for the calibration curves was 0.025 mg/L for PCE, TCE, and cis-1,2-DCE. Only one VC standard (2.6-mg VC/L) was used. PCE, TCE, and cis-1,2-DCE concentrations were determined using calibration curves established daily. The cis-1,2-DCE isomer represented greater than 90% of the two chlorine ethene molecules under all DCE forming conditions tested. Minimum detectable limits for PCE, TCE, and PCE were approximately 0.002, 0.0025, and 0.001 mg/L, respectively.

The reverse procedure was employed for determining the chlorinated concentrations of samples. The liquid PCE, TCE, and cis-1,2-DCE concentrations were determined by small liquid samples taken from the reactor sample ports. To ensure a representative sample, about 10-mL of liquid in the sampling ports was discarded before sampling. A 2-mL sample volume was then transferred into a small vial. The vial with Teflon-faced Neoprene septum was weighed before and after sampling. After weighing, it was inverted and placed in a 35°C water bath for 1 hour before gas analysis. Each sample was done in triplicate.

A chlorinated ethene molar balance was based on the measured liquid and gas concentrations (mole/L) of PCE, TCE, and DCE at certain sample points. These were conducted when VC was thought to be low since minimal measurements were made for this by-product. The recovery percentage of PCE at the beginning of biotransformation and at the time when DCE was at its highest concentration ranged from 99% to 89%. These mass balances also suggested that adsorption of PCE, TCE, and DCE was not significant during continuous flow runs.

**Anaerobic Attached Film Expanded Bed System (AAEF)**

The AAEF system, consisting of an expanded bed reactor, a gas collector, a feed system, and a recycle system, was designed and constructed as a self-contained unit (Fig. 2). A specially fabricated Teflon cone inlet served as the tapered portion of the bed. Copper and stainless steel tubing were used for recycle and feed systems, respectively. All the connections were joined by a small amount of Viton tubing. To prevent copper toxicity to the microbes from the copper tubing, soluble sulfide was maintained at low concentrations by including sulfates in the influent at 10-mg Na₂SO₄/L. Most experiments were performed at 35°C with a few tests conducted at 20°C.

The 4.3-L reactor contained 1.5 L of static bed. The reactor support particles were composed of fused particles of diatomaceous earth and obtained from an ongoing sewage treatment project. The attached biofilm volatile solids were measured in the beginning and end of each continuous flow run. Separate adsorption experiments with clean diatomaceous earth confirmed that they were insignificant.

Throughout the experiments, sucrose was used as the electron donor. The COD/nutrient stock was prepared as shown in Table 1. The feed, consisting of COD and PCE, was mixed by a magnetic stirring system in a 19.4-L glass bottle that was attached to a 50-L Tedlar gas bag to keep a desired liquid PCE concentration.

TCE and PCE integrity tests without media in the reactor were executed in continuous flow modes prior to biological testing. For the TCE integrity test, the reactor was initially filled with 3.5 L of distilled water, and then feed continuously with 5-mg TCE/L in distilled water at a flow rate of 7 L/d. A similar method was used in PCE integrity tests except a feed with 10-mg PCE/L was used.

**FIG. 2. Schematic of Glass/Copper Anaerobic Attached Film Expanded Bed (AAEF) Reactor**

**TABLE 1. Composition of Stock Feed Formula**

<table>
<thead>
<tr>
<th>Component (1)</th>
<th>Source (2)</th>
<th>Stock concentration (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>Sucrose</td>
<td>40 g as COD/L</td>
</tr>
<tr>
<td>N</td>
<td>NH₄Cl</td>
<td>2.9 g as N/L</td>
</tr>
<tr>
<td>P</td>
<td>KH₂PO₄</td>
<td>0.82 g as P/L</td>
</tr>
<tr>
<td></td>
<td>K₂HPO₄</td>
<td>0.26 g as P/L</td>
</tr>
<tr>
<td>Trace nutrients</td>
<td>Yeast extract</td>
<td>0.44 as yeast extract/L</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>NaHCO₃</td>
<td>40,500 mg as CaCO₃/L</td>
</tr>
</tbody>
</table>
Compatibility was acceptable when the gains or losses (through absorption, chemical reaction, or leakage) were minimal (less than 10%) over a four- to five-day period in the absence of biological activity. These integrity tests indicated that the system was compatible with TCE and PCE and that chemical or physical interactions with the chlorinated volatile compounds were insignificant.

All the experiments were grouped into four parts: preliminary test of PCE biotransformation (continuous flow test A, CF#A); long-term continuous operation to define longer term kinetic parameters (continuous flow test B1, B2, B3, CF#B1, #B2, #B3); test of lower temperature effect on PCE dechlorination rate (continuous flow test C, CF#C), and higher PCE loading rates to define maximum PCE degradation rate (continuous flow test D1, D2, D3, CF#D1, CF#D2, CF#D3). The operating conditions and overall results are summarized in Tables 2 and 3.

RESULTS

Tests of PCE Biodegradation

In continuous flow test A, the system was continuously fed targeted or calculated concentrations of 10-mg PCE/L and 500-mg COD/L. The resulting PCE and COD loading rates were 29.32-mg PCE/L-d and 1.78 g COD/L-d, respectively. All loading rate data are expressed per liter of operating expanded bed volume unless stated otherwise. Little PCE degradation was observed in the first 230 hours of operation. After this acclimation period, a dramatic change was observed as the PCE concentration decreased from 10 mg/L to 0.5 mg/L, the TCE concentration increased, and DCE appeared. Two types of DCE isomers, 1,1-DCE and cis-1,2-DCE, were observed. Little or no trans-1,2-DCE was detected in the effluent. Because cis-1,2-DCE represented greater than 90% of the DCE present, it was the isomer measured in all runs. VC was observed but unquantified in the gas and the liquid sample when the cis-1,2-DCE concentration began to decrease. At the end of this run, VC and low concentrations of ethylene were detected in the effluent. Thus, the PCE removal efficiency rapidly approached 100%, and the PCE removal rate nearly equaled the loading rate of 29.32-mg PCE/L-d after a relatively brief acclimation period. The rapid onset of anaerobic dechlorination reflected the high rates and efficiencies observed throughout the remainder of the study.

The influent PCE concentrations were increased stepwise from target concentrations of 10–11 mg/L, 15 mg/L and finally to 17.5 mg/L in the first continuous feed run (CF#B1). The average methane content of the biogas was 63.5%, and the pH remained stable at 7.26. When the influent PCE concentration was around 10 mg/L, the effluent PCE concentration decreased to 0.05 mg/L after 10–15 days of operation. At this point, little or no TCE was detected in the effluent. Even though the influent PCE was stepwise increased to 17.5 mg/L, the average effluent PCE concentration remained at 0.04 mg/L or less. Little or no TCE was detected in the effluent. The results of the test indicated that the system could successfully degrade PCE and quickly adapt to high PCE concentrations and loadings.

Substantially increased loading rates were achieved in the next condition (CF#B2). The resulting HRT of the total system was 8.65 hours with 5.4 hours in the expanded bed portion, which was about half that of the first continuously fed run. The average measured influent and effluent PCE concentrations during this run were 20.5 mg/L and 0.15 mg/L, respectively. TCE was detected at a low concentration of 0.04 mg/L and cis-1,2-DCE...
TABLE 3. Results of Anaerobic Expanded Bed PCE Continuous Flow Runs at 35°C

<table>
<thead>
<tr>
<th>Run number</th>
<th>pH</th>
<th>Eff. PCE Conc. a (mg/L)</th>
<th>Eff. TCE Conc. a (mg/L)</th>
<th>Eff. DCE Conc. a (mg/L)</th>
<th>PCE conversion rate b (mg/g-VS-d)</th>
<th>PCE removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>(4)</td>
<td>(5)</td>
<td>(6)</td>
<td>(7)</td>
</tr>
<tr>
<td>CF #A</td>
<td>7.15</td>
<td>0.216</td>
<td>0.055</td>
<td>2.388</td>
<td>2.70</td>
<td>97.4</td>
</tr>
<tr>
<td>CF #B1</td>
<td>7.23</td>
<td>0.040</td>
<td>—</td>
<td>—</td>
<td>3.20</td>
<td>99.8</td>
</tr>
<tr>
<td>CF #B2</td>
<td>7.16</td>
<td>0.111</td>
<td>0.040</td>
<td>0.009</td>
<td>7.14</td>
<td>99.5</td>
</tr>
<tr>
<td>CF #B3</td>
<td>7.09</td>
<td>0.659</td>
<td>0.973</td>
<td>1.690</td>
<td>15.6</td>
<td>97.5</td>
</tr>
<tr>
<td>CF #C</td>
<td>7.09</td>
<td>0.111</td>
<td>0.042</td>
<td>0.002</td>
<td>1.60</td>
<td>98.8</td>
</tr>
<tr>
<td>CF #D1</td>
<td>7.05</td>
<td>0.060</td>
<td>0.03</td>
<td>0.115</td>
<td>23.4</td>
<td>99.7</td>
</tr>
<tr>
<td>CF #D2</td>
<td>7.30</td>
<td>1.21</td>
<td>0.629</td>
<td>1.071</td>
<td>22.5</td>
<td>92.5</td>
</tr>
<tr>
<td>CF #D3</td>
<td>7.31</td>
<td>3.61</td>
<td>1.82</td>
<td>2.252</td>
<td>18.2</td>
<td>80.8</td>
</tr>
</tbody>
</table>

Average concentration.

*18.2 g-VSL is used for all runs except that 14.7 g-VSL is used in CF #A.

*At room temperature (18°-23°C).

FIG. 3. Example Dechlorination of PCE at 35°C in AAFEB under Continuous Operation (Test CF#D1) with Influent Flow Rate Varying from 4.4 to 9.1 L/d (HRT bed of 4.3–2.1 Hours)

was measured at 0.01 mg/L. At the 40th hour of the operation, the gas and liquid VC were roughly estimated at 4.5 mg/L and 3.1 mg/L, respectively, accounting for about 50% of the PCE degraded in this test.

Maximum PCE Biodegradation Rates

The data shown in Fig. 3 are representative of the heavy PCE loading rate (209 mg PCE/Lexp/d) when the system was operating efficiently with a high influent concentration of around 20 mg/L. The average PCE removal efficiency was 99.1%, and the average effluent concentration was 0.156 mg/L. TCE was 0.03 mg/L and DCE was 0.115 mg/L. This high loading rate resulted in the highest observed dechlorination rate of 23.4 mg PCE/g-VS-d. A lower COD concentration coupled with a target influent of 20 mg PCE/L were fed at a flow rate of 10.4 L/d (CF#D2). The PCE and COD loading rates were the highest tested (216 mg PCE/L-d and 4 g COD/L-d, respectively). At the conclusion of the runs, the average effluent concentration of PCE was 0.8 mg/L, and TCE was low (0.063 mg/L). DCE was observed to increase in the effluent from 0.22 to 2.5 mg/L, but averaged 1.07 mg/L.

The average PCE removal efficiency was 95%, and 220 mg/L COD was converted. The mean ratio of COD removal to PCE degraded averaged 11.4 mg COD per mg PCE.

In an effort to measure the influence of the primary energy source on dechlorination rates, the COD was decreased to 150 mg COD/L, while the PCE and flow rates were maintained at high levels (test #D3). In this test, the methanogenic system COD removal efficiency decreased significantly. The daily PCE effluent concentration increased from 3.3 mg/L to 12.9 mg/L and TCE concentration in the effluent was approximately 1.9 mg/L. DCE concentrations increased during the first 40 hours of operation from 1.8 mg/L to 2.7 mg/L and then diminished to 0.25 mg/L in the effluent. The average measured COD removal was 30%. This inefficient condition had a mass ratio of ΔCOD/ΔPCE of between six and eight.

This condition was the least efficient dechlorination experiment. The low efficiency of dechlorination may have been caused by insufficient methanogenic energy substrate. If a ratio of ΔCOD/ΔPCE of around 10 is required, the influent COD of 150 mg/L was not sufficient to dechlorinate 20 mg/L PCE. Since a declining COD removal efficiency occurred, it was not surprising that PCE removal activity also decreased.

In one continuously fed run following a batch test, the system was fed 20 mg/L PCE alkalinity for a period of five days without an organic energy source. A total of 12 reactor volumes were processed without any soluble organic energy source. Within 20 hours, PCE removal efficiency decreased from 99.9% to 43%; the effluent concentration remained at 11 mg/L for most of the run. TCE remained at about 0.1 mg/L and DCE at 0.02 to 0.05 mg/L. Thus, the dechlorination rate averaged 33 mg PCE/L-d or about 1.6 mg PCE/g-VS-d. This rate was less than 10% of the maximum observed rate. This continued low rate of dechlorination may have been supported by endogenous respiration.

Low Temperature Effects

A lower temperature test was conducted at approximately 20° ± 3°C to obtain preliminary estimates of the effects of temperature on PCE dechlorination. The anaerobic system was fed continuously with 500 mg COD/L and 10 mg PCE/L. The influent flow rate was maintained initially at 2.5 L/d for 120 hours to allow the culture time to acclimate to room temperature and then was increased to 4.6 L/d for 96 hours. Under these operating conditions, the pH was 7, the methane content of the biogas was 79%, and biogas was generated at a rate of 0.5 L/d.

Lower temperature had a minimum effect on PCE removal efficiency (Fig. 4). Only small amounts of PCE and TCE, 0.03 mg/L and 0.1 mg/L, respectively, were detected. VC and ethylene were not measured. PCE conversion remained efficient at 20°C, but effluent PCE concentrations equaled DCE values.

Kinetic Parameters

Steady operating conditions to define kinetic parameters (maximum specific removal rates, qmax and half velocity coefficient, K), was the goal of experiments CF#B1, CF#B2, and CF#B3. Loading conditions were varied...
to achieve a range of removal efficiencies by increasing the loading from 39.8 to 202 mg PCE/Lnmph-d.

The \( K_r \) coefficient for PCE degradation in continuous-flow runs was estimated using Michaelis-Menten kinetics and the Lineweaver-Burk linearized form (Grady and Lim 1980). The specific substrate uptake rate, \( q \) (mg/g VS-d), can be expressed as \( q = \frac{q_{\text{max}} \cdot S}{K_s + S} \), where \( q_{\text{max}} \) is the maximum specific rate of substrate utilization (mg/gVS-d), \( S \) is the average bulk solution substrate concentration (mg/L), and \( K_s \) is the half velocity constant (mg/g). The estimated \( q_{\text{max}} = 22.9 \) mg PCE/gVS-d and \( K_s = 0.25 \) mg PCE/L, respectively.

**ANALYSIS**

Mesophilic anaerobic attached films have the potential to treat chlorinated organic compounds. The AAFEB system was stable and operated efficiently in a continuous mode with up to 26 mg PCE/L and 500 mg COD/L. Greater than 99% of the PCE was dechlorinated at hydraulic retention times of less than five hours.

**Biotransformation of PCE in AAFEB System**

The results of this study were consistent with the previous findings of sequential dechlorination of PCE. TCE/PCE was first dechlorinated to DCE, then VC (Barrio-Lage et al. 1986; Parsons et al. 1985, 1984; Vogel and McCarty 1985, 1987). *Cis*-1,2-DCE was shown to predominate over trans-1,2-DCE among three types of DCE isomers. In this study, a microbial acclimation period of 240 hours was observed before anaerobic dechlorination of PCE began in an anaerobic attached film expanded bed reactor. During acclimation, the concentration of PCE decreased while the concentration of TCE increased. Over time, the concentration of TCE decreased while DCE appeared (*cis*-1,2-DCE predominated over 1,1-DCE) and then diminished. After the acclimation period PCE was rapidly depleted.

Recently, Freedman (1990) and Freedman and Gossett (1989) reported that *trans*-1,2-DCE was the predominant DCE isomer and ethylene was detected as the final products, by tracing the labeled [14C] by-products of PCE and TCE biotransformations under methanogenic conditions. In our study, since VC appeared to be the major by-product that accumulated, as measured in a limited number of samples, the transformation of VC to ethylene was considered to be the rate-limiting step. These data suggest a similar tendency of sequential dechlorination of PCE and TCE reported by Freedman and Gossett (1989).

**Implication of Kinetics of PCE Dechlorination**

The low value for \( K_s \) and the relatively high \( q_{\text{max}} = 22.9 \) mg PCE/g VS-d are encouraging because it indicates that there should be little difficulty in treating ground water with a PCE concentration as low as 1 mg/L. Effluent PCE concentrations were often reduced to below 0.01 mg/L, as were DCE and TCE.

Bouwer and McCarty (1985) reported on the degradation of trace halogenated compounds when an easily degradable "primary" substrate (acetate) was added as an energy source for aerobic and anaerobic conditions. A ratio of the maximum specific growth rate of the half velocity constant (\( q_{\text{max}} / K_s \)) for the halogenated material was found to vary from 0.08 for TCE to a maximum of 2.10 for carbon tetrachloride for the best anaerobic biofilm model data fit by these researchers. This ratio for PCE was found to be 0.09 gms/g VS-d in this work, a value similar to that reported for TCE by Bouwer and McCarty (1985).

Microbial biomass in the AAFEB often exceeds 20 g VS/L. Thus, the kinetic analysis indicates that PCE loadings in excess of 400 mgPCE/Lnmph-d can be treated at 35°C. Hydraulic retention times could be short and reaction volume relatively small for these applications. Of course, decreasing temperature will also decrease the removal rate and increase reactor sizes required.

**Temperature Effect on PCE Dechlorination**

Since the temperature of ground water is nearly always lower than the 35°C test temperature, further information on temperature effects is needed. Recently, Freedman (1990) reported that PCE dechlorination rates were greatly affected by temperature decreasing to a small fraction of the 35°C rates when the temperature was 15°C.

Little effect on PCE dechlorination rate was observed when the temperature of the AAFEB unit was decreased from 35°C to 20°C (Fig. 4). Also, low concentrations of intermediates (TCE and DCEs) were maintained at 20°C. These encouraging results were possibly due to large biomass concentrations typical of the AAFEB and low PCE loading rates relative to the PCE dechlorination capabilities of the system.

**Anaerobic Dechlorination Limitations**

Although PCE was efficiently biodegraded, by-products of PCE dechlorination may be more toxic and more volatile than PCE or TCE. This raises questions about the application of the methanogenic system to ground-water remediation. Previous studies have suggested a second stage using a methanotrophic system (Jewell et al. 1990). For this anaerobic-aerobic system,
the higher chlorinated compounds, such as PCE and TCE, are rapidly degraded by the methanogenic system and the lower chlorinated compounds, such as DCE and VC, are mineralized by methanotrophs.

Indirect evidence from this study suggests that the dechlorination rate is suspected to depend on the total mass of the methanogens present, the available electron donors and the efficiency of primary substrate utilization. All of the tests in the study except one showed high dechlorination rates (greater than 96%) under efficient COD removal and methane-production conditions. In the CF#D3, the dechlorination rate decreased rapidly when there was a low COD removal efficiency. In addition, when no organic energy was supplied for five days, the PCE dechlorination removal efficiency decreased to less than half of the original efficiency, also implying the need for a readily available organic energy source. Thus, maintaining a high biomass concentration and sufficient COD removal is important.

A primary energy source was required to support the highest rates of dechlorination. The few instances where incomplete PCE removal occurred due to organic limitations, enabled the organic energy requirements to be estimated. The critical ratio of COD reduction to PCE dechlorination was six to eight when the removal efficiency was primary-substrate limited, i.e., sucrose limited (CF#D3). The ratio was estimated at 11 in one run (CF#D2), where the PCE removal efficiency was less than 100%.

The exact amounts and the fate of electrons remain poorly defined in reductive dechlorination. When 11 mass units of COD were required to dechlorinate one unit of PCE to VC and ethylene, only a small fraction (3.5%) of the total electrons exchanged were used in dechlorination. This calculation is based on the assumption that four moles of electrons are required for one mole of PCE dechlorinated to carbon dioxide. Whenever the primary substrate was limiting, dechlorination rapidly decreased or halted. The PCE dechlorination rates of anaerobic PCE dechlorination remain to be defined—the impact of temperature and the minimum range of temperature required to obtain efficient dechlorination. Clearly, the addition of 50 mg/L of sucrose to remove 1 mg/L PCE, coupled with the possibility of discharging several hundred mg/L of biodegradable organics is an undesirable situation. Operating a methanogenic system at lower temperatures at minimum effluent organic concentrations will be a major challenge for the application of anaerobic technology. Ongoing work in our Cornell University program is attempting to define the minimum requirements for a practical process.

CONCLUSIONS

The following conclusions can be drawn from this study:

1. The AAFEB system constructed with inert materials (glass, copper, Viton tubing, and a small amount of Teflon) efficiently dechlorinated PCE, TCE, and DCE.

2. An acclimation period of 240 hours was required for reductive dechlorination of PCE/TCE. Once it was acclimated to chlorinated compounds, the anaerobic culture dechlorinated PCE and its intermediate by-products (TCE and cis-1,2-DCE) rapidly and efficiently. PCE and TCE were reductively dechlorinated to cis-1,2-DCE. Limited evidence showed that the final products were mainly VC with small amounts of ethylene.

3. Influent concentrations of 8–26 mg/L were reduced to less than 0.2 mg/L in most cases with several hours hydraulic retention time.

4. When the operating temperature was decreased from 35°C to between 18 and 23°C, little adverse effect on PCE dechlorination rate was demonstrated.

5. With a completely mixed model, the q_{max} and K were estimated at 22.9 mg PCE/gVSS·d and 0.25 mg PCE/L, respectively. The maximum PCE uptake rate observed during continuous-flow conditions was 23.4 mg PCE/gVSS·d.

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APPENDIX. REFERENCES


tetachloroethylene under methanogenic conditions,” PhD thesis, Cornell University, Ithaca, N.Y.
tetrachloroethylene and trichloroethylene to ethylene under methanogenic
Jewell, W. J., Fennell, D. E., Nelson, Y. M., Underhill, S. E., Wilson, M. S., and
developing an attached microbial film reactor and kinetics of TCE removal.”
Gas Research Institute Final Report (7/15/87–3/31/89), Gas Res. Inst., Chicago,
Ill.
Kleopfer, R. D., Easlay, D. M., Haas, B. B., Jr., Delhi, T. G., Jackson, D. E.,
ethylene metabolism by microorganisms that degrade aromatic compounds.” Appl.
Parsons, F., and Barrio-Lage, G. (1985). “Chlorinated organics in stimulated ground-
organic solvents in static microcosum.” Envirol. Toxicology and Chemistry, 4, 739–
742.
roethylene and trichloroethene in microcosms and groundwater.” J. Am. Water
to trichloroethylene, dichloroethylene vinyl chloride, and carbon diox-

APPENDIX. NOTATION

The following symbols are used in this paper:

AAFEB = anaerobic attached film expanded bed;
COD = chemical oxygen demand;

K_s = half-rate coefficient; concentration of limiting nutrient that
supports half maximum microbial growth rate expressed
as mg/L;

L_{exp}^d = liter of expanded bed;
PCE = perchloroethene, better known as tetrachloroethene;

q = specific substrate concentration, mg/L;
q_{max} = maximum specific substrate removal rate, mg substrate
per g of microbial VS per day;

S = soluble substrate concentration; mg/L;
TCE = trichloroethene;
trans-1,2-DCE = trans-1,2-dichloroethene;

VC = vinyl chloride;

VS = volatile solids; and

^{14}C = radioactive carbon isotope.