Biodegradation of High Concentrations of Tetrachloroethene in a Continuous Flow Column System

MANSOUR ISALOU AND BRENT E. SLEEP*  
Department of Civil Engineering, University of Toronto, Toronto, Ontario, M5S 1A4 Canada

STEVEN N. LISS  
Department of Applied Chemical and Biological Sciences, Ryerson Polytechnic University, Toronto, Ontario, M5B 2K3 Canada

A long-term (2.5 years) study of the anaerobic biodegradation of high concentrations of perchloroethylene (PCE) was carried out in a continuously operated laboratory column filled with sand which was inoculated with biomass from an anaerobic digester. Concentrations of PCE fed to the column were increased from 12 μM to over 600 μM over 21 months, with methanol added as electron donor. Vinyl chloride (VC) was the terminal product of PCE dechlorination for the first 21 months at which point significant conversion of VC to ethylene (ETH) was detected. The onset of ETH production coincided with acetogenesis becoming the primary pathway for methanol metabolism. ETH production occurred in the column in the presence of PCE and TCE. Varying methanol:PCE molar ratios from 1.4 to 7.5 had little effect on the transformation of PCE and TCE to VC. The degradation of VC to ETH was much more sensitive, and VC accumulated when the methanol:PCE molar ratio dropped below 5.0. Withdrawal of PCE from the system for a 5 month period and maintenance of the column on methanol alone did not result in the loss of PCE degradation capability of the consortium.

Introduction

Tetrachloroethene (perchloroethene, PCE), a chlorinated solvent, is a common and persistent contaminant of soil and groundwater (1). It has been demonstrated that, under anaerobic conditions, PCE may be biodegraded to trichloroethene (TCE), dichloroethene isomers (DCEs), vinyl chloride (VC), and ethene (ETH) (2–4). Of those cultures in which dechlorinating organisms have been identified, the end product of dechlorination has usually been either cis-DCE (5–9) or ETH (10). Although organisms responsible for PCE dechlorination have been identified, in situ bioremediation of PCE contamination is likely to be carried out by consortia. The current understanding of the interactions between the various microbial species that comprise the PCE degrading consortia is still very limited, and there is a need for additional studies of these consortia under conditions that simulate as closely as possible, those that would exist in the field during in situ bioremediation.

In applying in situ bioremediation for PCE remediation in the field, it is anticipated that a continuous flow mode of operation would be used. Continuous flow operation results in very different conditions than those existing in microcosms where addition of chlorinated compounds, electron donor, and micronutrients are performed on a periodic batch basis. Conditions tend to vary spatially in a continuous flow or in situ bioremediation system, in contrast to microcosms where conditions are spatially homogeneous, and temporally changing. In addition, in situ bioremediation will be conducted by biomass primarily present as a biofilm. Most microcosm studies have involved suspended growth conditions in a water phase, with no soil present. The rates of dechlorination of PCE and the interactions between the chemical species and the microbial community may be quite different under continuous flow biofilm conditions than under batch suspended growth conditions (11).

A variety of electron donors have been used to support the biodegradation of PCE, including methanol, ethanol, lactate, and butyrate (12). There is strong evidence that many dechlorinating organisms use H₂ produced by metabolism of organic substrates added (13). Competition from methanogens for H₂ can potentially reduce the effectiveness of electron donors added. High concentrations of PCE have been found to inhibit methanogenesis (2), allowing for more efficient utilization of hydrogen produced from the biodegradation of organic substrates. However, Distefano et al. (13) also found that the presence of PCE and TCE inhibited the conversion of VC to ETH in batch studies. The microcosm studies of Distefano et al. (2) are the only studies that have been carried out at PCE concentrations that approach the solubility limit of PCE (900 μM (14)). There have not been any published studies of the biodegradation of high concentrations of PCE in soil columns under continuous flow conditions.

To better assess the potential for in situ bioremediation of PCE, studies are needed that incorporate continuous flow conditions and high concentrations of PCE. In this paper, the development and long-term operation of a sand column fed methanol and concentrations of PCE as high as 720 μM, under anaerobic continuous flow conditions, is presented. The progression of biochemical conditions in the column over a period of 2.5 years is described. The effects of varying methanol:PCE ratios and the rates of conversion of PCE to lesser chlorinated compounds are presented.

Materials and Methods

Column System and Inoculation. The studies were conducted in a 2 m tall, 5 cm diameter, stainless steel cylinder, fit with Teflon Mininert valves every 15 cm. All the lines to and from the column were composed of Teflon. The schematic diagram of the setup is illustrated in Figure 1. The column was filled with coarse silica sand and inoculated with biomass obtained from an anaerobic digester at the Metropolitan Toronto Main Treatment Plant. The porosity of the sand was determined to be 0.37 giving a liquid volume of 933 L in the column. Prior to inoculating the column, the biomass was grown on a basal medium (3) containing 3.6 mM of methanol as the primary carbon and energy source for a period of 2 weeks. The column was flushed with N₂ gas prior to inoculation. The microorganisms were then introduced to the column as an aqueous suspension that was fed to the column at a low flow rate (5 mL/h). The column was then continuously fed with basal medium containing methanol, yeast extract (maintained at 50 mg/L over the duration of the study), and PCE. The hydraulic retention time was...
approximately 17 h. Over the 2.5 years of column operation, a variety of PCE and methanol concentrations was fed to the column.

Bromide tracer tests were performed at the end of the 2.5 year study to allow nondestructive determination of the reduction of porosity of the sand in the column due to biofilm growth. The tracer tests were performed by injecting a 0.2 L slug of water containing potassium bromide (0.3 mM) into the column at the same flow rate used for normal column operation. The bromide tracer breakthrough curves were measured at various ports along the length of the column by withdrawing aqueous samples for bromide analysis (Dionex 500 ion chromatograph used for bromide determination). From the breakthrough times for the bromide tracer, the flow rate of water to the column, the cross-sectional area of the column, and the distance to the breakthrough point, the porosity of the various sections of the column was determined.

Analytical Procedures. Metabolites and degradation products were identified by their chromatographic mobility and their mass spectral fragmentation pattern using an HP 5890 Series II GC with a TRIO 1000 Mass Spectrometer (MS) (Fisons Instruments). Concentrations of PCE, TCE, 1,1-DCE, cis-1,2-DCE, and trans-1,2-DCE were routinely determined by withdrawing 2 mL aqueous samples [1 mL liquid sample diluted by 1 mL reagent (Milli-Q) water for concentrated samples] with a Hamilton gastight syringe (Supelco, Inc.). Liquid samples were concentrated in a purge and trap unit and then analyzed using a gas chromatograph (GC) equipped with a 30 m × 0.53 mm × 3 μm film thickness, VOCOL capillary column (Supelco, Inc., Toronto, Ontario). A thermal desorption trap VOCARB 4000 (Supelco, Inc.) was employed. The samples were purged for 11 min with helium at a flow rate of 45 mL/min and desorbed for 4 min at 250 °C. The column temperature program was 35 °C for 4 min ramped to 100 °C at 10 °C/min and held for 2 min. The injector and detector temperatures were 240 and 250 °C, respectively. Helium was used as a carrier gas at a flow rate of 8 mL/min. VC was routinely identified and quantified using the GC/MS. Aqueous samples of 1 mL were added to a 2 mL autosampler vial, and 10 μL headspace samples were taken for injection into the GC/MS. A 30 m × 0.53 mm × 3 μm film thickness HP-624 capillary column (Hewlett-Packard) was used for gas chromatographic separation with an initial hold for 2 min at 4 °C followed by a 15 °C/min ramp to 130 °C.

Methanol and acetate analyses were performed using an HP 5890 Series II GC equipped with flame ionization detector (FID). Initially, a Nukol fused-silica capillary column (Supelco, Inc.) was used for methanol and acetate analysis, but column performance for acetate analysis was poor with aqueous sample injection. In the later stages of the experiment, acetate analyses were performed using a glass column. For these analyses 1 μL aqueous samples (acidified to 0.03 M oxalic acid) were injected into a 2 m × 2 mm ID glass column packed with Carbopack B-DA/4% Carbowax 20M (Supelco, Inc.). The column temperature was held at 175 °C isothermally for 22 min, and the injector and detector temperatures were 200 °C. The carrier gas flow rate was...
adjusted to 25 mL/min and monitored routinely. Methanol and ETH were quantified using an HP 5890 Series II Plus GC and a thermal conductivity detector (TCD). Aqueous samples of 1 mL were put in 2 mL autosampler vials and headspace samples (10 μL) were taken and injected into a 30 m × 0.53 mm Carboxen 1006 Plot fused-silica capillary column (Supelco, Inc.). The initial oven temperature was 35 °C for 3 min followed by a 15 °C/min increase to 170 °C. Both injector and detector temperatures were 230 °C, and the flow rate of helium (carrier gas) was 21 mL/min.

**Chemicals.** Analytical standards for PCE, TCE, 1,1-DCE, cis-1,2-DCE, trans-1,2-DCE, and VC were obtained as mixtures or neat liquids from Supelco Inc. (Toronto, ON). VC (1000 ppm ± 2% in N2) and methanol (99+%) were obtained from Supelco, Inc. (Toronto, ON). ETH was purchased as gas in lecture bottles (99%) from BOC Gases (Toronto, ON). Dimethyl sulfide (DMS, 98%) was obtained from Aldrich Chemical Co. (Toronto, ON). Other chemicals used in this study were reagent grade and were purchased from VWR scientific (Toronto, ON).

**Results**

**Acclimation to High Concentrations of PCE.** The column influent initially contained 7.1 mM methanol and 12 μM PCE. A low concentration of PCE was chosen to allow microbial acclimation to the presence of PCE. After three weeks the inlet PCE concentration was increased to 300 μM. This resulted in a decline in methanol degradation and a 4-fold reduction in methane production rate. Subsequently, the inlet PCE concentration was maintained at about 180 μM and the inlet methanol concentrations was set at 18 mM methanol. The hydraulic retention time was 16.7 h. After achieving stable column operation after 7 months and consistent PCE conversion to TCE, cis-DCE, and VC, the inlet PCE and methanol concentrations were raised to 300 μM and 180 μM, respectively. At these concentrations of PCE and methanol, analysis of the porewater from the sampling ports along the length of the column indicated that PCE was being converted to TCE, DCEs, and VC. The final step in the degradation pathway was VC. All three isomers of DCE were detectable, but cis-DCE was predominant.

After a period of 17 months, the inlet PCE concentration was increased in increments of 60 μM every 2 weeks to 600 μM. The profiles of chlorinated compounds in the column at the end of this period (610 days) are shown in Figure 2a. TCE reached a maximum concentration of 150 μM at 15 cm from the inlet and disappeared by 45 cm from the inlet. VC was the dominant byproduct at the outlet, reaching the peak concentration of 575 μM by 45 cm from the inlet. Concentrations of 1,1-DCE, trans-DCE, and cis-DCE (not shown in Figure 2a) at the outlet of the column were 1.8, 0.6, and 0.4 μM, respectively. ETH production was not detected.

The corresponding methanol, acetate, and methane concentrations at 610 days are shown in Figure 2b. The inlet methanol concentration was 18 mM at this time. A peak acetate concentration of 1.5 mM was measured at 15 cm from the inlet. Acetate was completely consumed by 45 cm from the column inlet, as was methanol. Peak methane concentrations were approximately 2 mM.

**Degradation of PCE to ETH.** It was hypothesized that the lack of conversion of VC to ETH in the upper portion of the column was due to low numbers of VC degraders, lack of electron donor, or both. The insignificant hydraulic gradients in the upper half of the column, in comparison to the lower half of the column, indicated that the biomass levels were much lower in the upper half of the column than they were in the lower half of the column. To attempt to stimulate biological growth in the upper portion of the column, injection of methanol at the midpoint of the column was started at 777 days. After about 3 months of continuous operation with inlet methanol concentrations fixed at 18 mM and additional midpoint methanol injection (equivalent to a concentration of 2 mM in the column), ETH was detected along the column.

Figure 3a represents a typical profile of PCE, TCE, VC, and ETH after the onset of ETH production. TCE was detected at about 6 μM at the inlet of the column where high concentrations of PCE prevailed. This shows the capability of the microbial community in maintaining dechlorination activities at these inlet concentrations. This also corresponds to the metabolism of some of the inlet methanol to 77 μM of acetate, as shown in Figure 3b. The highest concentration of TCE was about 70 μM, at 15 cm from the inlet. The TCE level decreased to 17.3 μM at 45 cm from the inlet. Significant amounts of VC were still present along the column with the highest concentration of about 150 μM at 90 cm from the inlet. VC decreased by about 25% at 120 cm from the inlet and continued to decrease at a lower rate toward the end of the column. More than 30% of the inlet PCE was degraded to ETH by 15 cm from the inlet. More than 80% of the inlet PCE was completely degraded to ETH by the outlet. Greater than 70% of this degradation occurred before the midpoint methanol injection, the remaining 10% degraded in the upper half of the column.

Figure 3c shows the concentration profile of DCE isomers in the column. All three isomers of DCE were detectable at very low concentrations at the inlet port. cis-DCE was the dominant isomer in the lower half of the column. The highest concentration of cis-DCE appeared at 30 cm from the inlet with a concentration of 4.6 μM. It was further degraded to concentrations of about 0.3 μM by the midpoint and was not detectable (less than 0.1 μM) by 165 cm from the inlet. The other two isomers were trans-DCE and 1,1-DCE, in their order of predominance. trans-DCE did not exceed 0.5 μM, but 1,1-DCE was consistently detected at the outlet of the column. The consistency of this profile over time implies the favorable degradation of cis-DCE over the other two isomers. Levels of cis-DCE and trans-DCE were always below the maximum...
contaminant level of 0.7 and 1.0 μM, respectively, based on United States drinking water standards. On the other hand, 1,1-DCE was usually above the regulated level of 0.07 μM.

In earlier stages of the study (Figure 2b) peak acetate concentrations were less than 1.5 mM and the acetate produced in the lower half of the column was completely removed in the upper half. In contrast, in Figure 3b, acetate concentrations in the lower half of the column exceeded 8.0 mM and increased further above the midpoint methanol injection port. The inlet PCE and methanol concentrations were not changed significantly over the period from 610 to 862 days. Thus, the increased acetate concentration may have been the result of a slowly increasing acetogen population in the column or a decrease in aceticlastic activity. The acetate profile in Figure 3b indicates that acetate concentrations were near the stoichiometric values (half the inlet methanol concentrations) that would be expected from complete conversion of methanol to acetate with no acetate consumption occurring.

Through GC/MS analysis, the presence of dimethyl sulfide (DMS) was confirmed. Typical concentrations were usually around 2.4 μmol/L (150 μg/L) along the column. This compound was most probably biosynthesized in the lower half of the column in the presence of high sulfide concentrations (0.5 g/L added to the basal medium to maintain anaerobic conditions in the column influent), since the DMS concentration in the inlet port was always below the detection limit. There was a slight increasing trend in the DMS concentration across the column with the greatest concentration occurring between inlet port and a distance of 15 cm from the inlet.

**Effect of Methanol:PCE Ratios.** Complete degradation of 600 μM of PCE to ETH requires 4.8 mequiv/L reducing equivalents, assuming that eight reducing equivalents are required for complete reduction of 1 mol of PCE to ETH. This corresponds to a stoichiometric requirement of 0.8 mM of methanol (assuming that methanol is converted to CO₂ producing six reducing equivalents per mole of methanol), which is a small amount compared to the operating methanol concentration of 18 mM. With these operating conditions there was always some methanol detectable along the column (Figure 3b) to support the degradation of remaining VC to ETH. In addition, acetate was being produced in the column (Figure 3b) which could potentially support further VC biodegradation.

A set of experiments was performed to examine the effect of methanol concentration on the kinetics and levels of conversion of PCE to TCE, DCEs, VC, and ETH. Methanol to PCE molar ratios ranging from 7.5 to 1.4 were examined. Table 1 represents typical operating conditions of the column during this phase of study. In terms of electron equivalents supplied, the methanol:PCE molar ratio of 7.5 theoretically provides 5.6 times the required amount of electrons for biodegradation of PCE to ETH, while 1.4 supplies 105% of the electrons required.

The change in methanol:PCE ratio had little effect on the transformation of PCE and TCE over the range of ratios used. However, the conversion of VC to ETH was much more sensitive and VC accumulated when the methanol:PCE ratio dropped to below a molar ratio of 5.0. The molar conversion to ETH dropped from 97 to 35%, with VC outlet concentration increasing from 24 to 470 μM as the methanol:PCE ratio dropped from 7.5 to 1.4 (Table 1).

Figure 4a shows the degradation profile of PCE (at 600 μM) to TCE, VC, and ETH with inlet methanol concentrations of 4.5 mM. This corresponds to a methanol:PCE molar ratio of 7.5. TCE was produced at the inlet port and reached its highest concentration of 21.7 μM by 30 cm from the inlet. It then decreased below detection toward the midpoint of the column. VC was also detected at the inlet port and reached its maximum of 202 μM at 30 cm from the inlet. There was a significant decrease in VC concentration between

<table>
<thead>
<tr>
<th>TABLE 1. Typical Operating Conditions of the Anaerobic Column from 814 to 946 Days after the Initial Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>time (days)</td>
</tr>
<tr>
<td>814</td>
</tr>
<tr>
<td>831</td>
</tr>
<tr>
<td>834</td>
</tr>
<tr>
<td>842</td>
</tr>
<tr>
<td>887</td>
</tr>
<tr>
<td>897</td>
</tr>
<tr>
<td>903</td>
</tr>
<tr>
<td>916</td>
</tr>
<tr>
<td>930</td>
</tr>
<tr>
<td>931</td>
</tr>
<tr>
<td>934</td>
</tr>
<tr>
<td>936</td>
</tr>
<tr>
<td>938</td>
</tr>
<tr>
<td>941</td>
</tr>
<tr>
<td>944</td>
</tr>
<tr>
<td>946</td>
</tr>
</tbody>
</table>

FIGURE 3. Column performance at 862 days. (a) Biotransformation of PCE, (b) methanol, acetate, and methane, (c) dichloroethylene isomers (DCEs).
30 and 75 cm distance from the inlet and the remaining VC persisted along the upper half of the column. VC degradation and subsequent ETH production were not observed above 90 cm from the inlet.

The profiles of methanol, acetate, and methane, with an inlet methanol concentration of 4.5 mM, are shown in Figure 4b. At 60 cm from the inlet, the concentration of acetate was approximately half the inlet methanol concentration (2.3 mM), while the methane concentration was 1.4 mM. If the 2.3 mM acetate was produced from methanol biodegradation, this would have released 9 mequiv/L of reducing equivalents. Production of 1.4 mM of methane would have required 11.4 mequiv/L of reducing equivalents, while 4.8 mequiv/L of reducing equivalents would have been required for the conversion of PCE to ETH and VC (Figure 4a). These results indicate that it is likely that some of the reducing equivalents for dechlorination of PCE and methane formation were produced from the biodegradation of yeast extract (50 mg/L inlet concentration). In previous studies conducted by DiStefano (15), yeast extract was found to provide 42 µequiv of reductant/mg of yeast extract supplied.

Figure 5a presents the degradation profile when inlet PCE and methanol were 720 µM and 2.4 mM, respectively. As can be seen from this figure, PCE and TCE both were present above the midpoint of the column (up to 120 cm from the inlet). Figure 5b shows the metabolism of methanol to acetate and methane with inlet PCE and methanol concentrations of 720 µM and 2.4 mM, respectively. With these inlet conditions, all the acetate produced at 15 cm from the inlet was consumed by 60 cm from the inlet (Figure 5b). The change in the metabolism of acetate relative to the profile in Figure 4b could be due to the lower inlet methanol concentrations and a reduction in hydrogen partial pressure, allowing increased consumption of acetate.

With the inlet methanol concentration of 2.4 mM, significant VC degradation to ETH occurred in the upper half of the column and VC degradation was occurring up to the outlet of the column, indicating the presence of electron donor at the column outlet. However, the rate of VC conversion to ETH in the lower portion of the column was much lower than the rate with an inlet methanol concentration of 4.5 mM.

Rates of PCE Degradation. Tracer tests were conducted to determine the effective porosities and residence times of different sections of the column. The initial sand porosity of 0.37 was reduced to 0.04 for the section from 0 to 15 cm, 0.15 for 30 to 45 cm, 0.21 for 75 to 90 cm, and 0.24 for 150 to 165 cm. Using the information obtained from the tracer tests and degradation profiles of PCE at different periods of the column operation, removal rates of PCE as well as production rates of ETH were calculated. Table 2 shows the removal rates of PCE at different sampling ports over the first 45 cm of the column.

PCE degradation occurred in the first 45 cm of the column and completely degraded to other byproducts. The removal rate significantly increased over time as the microbial community further developed. This was more appreciable in the first 15 cm of the column where the rate increased by an order of magnitude following 1.5 years of the column operation. After stabilizing the inlet conditions, the PCE removal rate was also stabilized with no significant fluctuations. During the adjustment of the methanol:PCE ratio, when the inlet PCE was increased from about 600 to 720 µM with a simultaneous decrease in methanol, the PCE removal rate decreased by more than 20% by 15 cm from the inlet.

An estimate of biofilm volume in the column was made from the porosity reductions estimated from tracer test results. It was assumed that precipitation of minerals from the basal medium was negligible and that the porosity reduction was entirely due to biofilm growth. Published values of 50 mg/mL (dry mass per unit wet volume) biofilm density (16) and 50 wt% of dry biomass as protein (17) were used to estimate the protein content of the biofilm for each section of the column. Using these protein estimates, the volumetric rates were normalized against the protein level of biofilm at different sections of the column (Table 2).

Starvation Study and Maintenance of the Column on Methanol. To determine whether discontinuing the opera-
ETH concentration was 27 \( \text{µM} \) in the inlet, and TCE disappeared by 45 cm from the inlet. The days). PCE was completely degraded by 60 cm from the inlet. Midpoint methanol injection was adjusted to supply around 1.3 \( \text{mM} \) of methanol to the upper half of the column. The column was continuously operated under these conditions (no PCE scenario) for a period of 5 months (947–1097 days). Methanogenesis was the major metabolic activity during this period.

The purpose of the maintenance of the column was to conduct further studies on the microbial community and explore the community structures in different zones of the column. Furthermore, the community in the column could serve as a source of inoculum for future bioaugmentations. However, it was not clear whether the maintenance of the column without PCE would have any detrimental impact on the culture with respect to its dechlorination capability. To elucidate this, a batch of basal medium containing 30 \( \text{µM} \) PCE was introduced to the column. PCE was degraded to below detection by 15 cm from the inlet to TCE and VC. TCE was below detection by 30 cm from the inlet. VC conversion to ETH was very minimal and VC accumulated across the column. Significant methane was also produced at the inlet of the column.

Following this observation, another batch of basal medium containing 60 \( \text{µM} \) of PCE was injected to the column (1099 days). PCE was completely degraded by 60 cm from the inlet, and TCE disappeared by 45 cm from the inlet. The ETH concentration was 27 \( \text{µmol} / \text{L} \) by 120 cm from the inlet, and VC level in the outlet of the column was too small to be quantified. These studies indicate the ability of the dechlorinating organisms to survive considerable lengths of time in the absence of chlorinated compounds.

**Discussion**

In a period of less than 21 months, biomass from an anaerobic digester was able to tolerate and biodegrade concentrations of PCE in excess of 600 \( \text{µM} \) in a continuous flow column filled with sand. This is the first demonstration of complete transformation of PCE to VC at concentrations as high as 600 \( \text{µM} \) in a continuously operated system. DiStefano et al. (2) developed a culture that was able to dechlorinate PCE concentrations up to approximately 330 \( \text{µM} \) at 35 °C in a batch system. They developed this culture from a culture surviving on methanol and low concentrations of PCE.

The endpoint of dechlorination in this study was VC for the first 21 months, with almost complete conversion of PCE to VC. No significant amounts of ETH were detected in the column during this period. Some studies have found that cis-DCE was the end point of dechlorination (5–9). In this study, cis-DCE was the major DCE isomer detected, but the maximum concentrations of cis-DCE observed did not exceed 0.1% of the inlet PCE concentration (Figure 3c). Other researchers (e.g., refs 2 and 3) observed production of VC followed by conversion of VC to ETH under anaerobic batch conditions. Freedman and Gossett (3) found that ETH production occurred 20 days after the start of the experiments. DiStefano et al. (2) reported similar lag times and also noticed a slow trend toward greater ETH production. In our column, very little ETH was produced until after 21 months of operation.

When the column was operated at high methanol inlet concentrations and midpoint methanol injection, there were significant methanol concentrations throughout the column, but VC conversion to ETH was still insignificant. Therefore, the lack of sufficient electron donor was not likely the limitation to further ETH production. PCE and TCE are thought to inhibit conversion of VC to ETH (2, 3). PCE and TCE concentrations were very low in the upper portion of the column, so inhibition of VC degradation in the upper portion of the column was not due to high PCE or TCE concentrations. In contrast, when ETH production started, most of the ETH was produced in the lower portion of the column, where PCE and TCE were present. It is possible that the lack of VC degradation was due to very low numbers of VC degrading organisms initially present in the column.

It is also noted that the onset of ETH production coincided with the establishment of acetogenesis as the primary pathway for methanol metabolism (Figure 3b). This pattern of substantial ETH production and high levels of acetogenesis was also observed toward the end of the study when the methanol:PCE ratios were decreased. When the methanol:PCE ratio was decreased below 5.0, the rate of conversion of VC to ETH declined substantially. At the same time, acetate concentrations also decreased. Acetogenesis was also the primary pathway for methanol metabolism in the culture of Distefano et al. (2). In their study, methanogenesis was completely inhibited after exposure to high concentrations of PCE. About two-thirds of the electron equivalents provided by methanol were used for acetogenesis, with the remaining one-third used for biodegradation of chlorinated compounds.

In contrast to the batch studies of Distefano et al. (2), methanogenesis was still occurring in our column, in the presence of 600 \( \text{µM} \) PCE. Most of the methane was produced in the first 30 cm of the column, where PCE concentrations ranged from 600 to 60 \( \text{µM} \). It is possible that within the biofilm system methanogenesis was occurring in microenvironments not exposed to high concentrations of PCE. Previous studies (2, 13) demonstrated that the electron donor for PCE dechlorination is hydrogen produced in the degradation of methanol. The formation of methane represents a loss of electrons potentially available for biodegradation of chlorinated compounds, but at the high methanol concentrations (and yeast extract concentrations) of the column,

---

**TABLE 2. Removal Rate of PCE in Different Zones of the Column**

<table>
<thead>
<tr>
<th>time (days)</th>
<th>PCE concn (µM)</th>
<th>MeOH concn (mM)</th>
<th>column zone</th>
<th>table</th>
<th>0–15 cm</th>
<th>0–30 cm</th>
<th>0–45 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[µmol/(L h)]</td>
<td>[µmol/(L mg of protein)]</td>
<td>[µmol/(L h)]</td>
</tr>
<tr>
<td>119</td>
<td>185</td>
<td>9.4</td>
<td></td>
<td></td>
<td>60</td>
<td>31</td>
<td>24</td>
</tr>
<tr>
<td>177</td>
<td>189</td>
<td>15.6</td>
<td></td>
<td></td>
<td>113</td>
<td>55</td>
<td>37</td>
</tr>
<tr>
<td>578</td>
<td>612</td>
<td>62.3</td>
<td></td>
<td></td>
<td>2481</td>
<td>625</td>
<td>400</td>
</tr>
<tr>
<td>610</td>
<td>599</td>
<td>15.6</td>
<td></td>
<td></td>
<td>2386</td>
<td>594</td>
<td>399</td>
</tr>
<tr>
<td>862</td>
<td>596</td>
<td>13.2</td>
<td></td>
<td></td>
<td>2263</td>
<td>492</td>
<td>388</td>
</tr>
<tr>
<td>931</td>
<td>604</td>
<td>4.5</td>
<td></td>
<td></td>
<td>2410</td>
<td>594</td>
<td>385</td>
</tr>
<tr>
<td>938</td>
<td>725</td>
<td>2.4</td>
<td></td>
<td></td>
<td>1896</td>
<td>595</td>
<td>438</td>
</tr>
</tbody>
</table>

---

Ether concentrations up to approximately 330 \( \text{µM} \) at 35 °C in a batch system.
there appeared to be an adequate supply of electrons for methanogenesis and biodegradation of chlorinated compounds.

The rates of dechlorination estimated in this study are much higher than those estimated in other studies. Skeen et al. (17) measured PCE dechlorination rates of 4 × 10^{-3} \text{mol L}^{-1} \text{h}^{-1} \text{mg}^{-1} protein in a methanol-enriched methanogenic sediment consortium. PCE dechlorination rates reported for Acetobacterium woodii and Methanosarcina sp. are as low as 3.6 × 10^{-3} and 3.5 × 10^{-5} \text{mol h}^{-1} \text{mg}^{-1} protein, respectively (18, 19). The rates estimated for this study were 3–5 orders higher than those of Skeen et al. (17), Egli et al. (18), and Fathepure et al. (19), suggesting that methanogens and acetogens were not the dechlorinating organisms in our system.

de Bruin et al. (20) achieved PCE dechlorination rates of 3.7 \text{mol L}^{-1} \text{h}^{-1} in a lactate-fed fixed-bed column, while Chu and Jewell (21) observed PCE dechlorination rates of 0.01 \text{mol h}^{-1} \text{mg}^{-1} protein in a sucrose-fed anaerobic attached film expanded bed reactor. The highest PCE dechlorination rates measured in our study are 670 times higher than those of Skeen et al. (17), Freedman, D. L.; Gossett, J. M. Appl. Environ. Microbiol. 1989, 55, 2144–2151.

For methanol-fed batch conditions, PCE concentrations of 300 \text{mol L}^{-1} and cultures enriched on PCE for several years, Tandoi et al. (22) reported PCE dechlorination rates of 0.2–0.4 \text{mol h}^{-1} \text{mg}^{-1} volatile solids. The rates of dechlorination observed by Tandoi et al. (22) are within a factor of 4 of those estimated for this study. The similarity in the rates between our continuous flow study and the batch study of Tandoi et al. (22) may be due to the similarity of conditions with respect to the long-term enrichment of the mixed consortia on PCE, the use of high concentrations of PCE, and the use of the same electron donor.

The large buildup of biofilm in our continuous flow column (porosity reduced from 0.37 to 0.04 in the first 15 cm of the column for example), resulting from operation of the column for 2.5 years with high concentrations of PCE and methanol, created significant pressure drops. The pressure drops in the column approached 150 kPa/m toward the end of the study, and plugging of the column inlet was a frequent occurrence. In the field, it would likely be difficult to deliver nutrients and electron donor to a zone in which the biomass reached the levels present in our column. It likely would not be practically possible to achieve the PCE removal rates of this study in a field application of in situ anaerobic bioremediation of PCE. However, this study has demonstrated the potential for biodegrading concentrations of PCE approaching the solubility limit that might occur in or near source zones of PCE.

Acknowledgments
This research was supported by Imperial Oil, the Environmental Science and Technology Alliance of Canada (ESTAC), and the Natural Sciences and Engineering Research Council of Canada. David M. Bagley provided helpful comments on early drafts of this document.

Literature Cited
(1) Abelsohn, P. H. Science 1990, 250, 73.

Received for review March 26, 1998. Revised manuscript received July 24, 1998. Accepted August 3, 1998.

ES9803052