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ANAEROBIC BIODEGRADATION OF MIXTURES OF CHLORINATED SOLVENTS

by

Vasilios Kaseros

A thesis submitted in conformity with the requirements
For the degree of Master of Applied Science
Graduate Department of Civil Engineering
University of Toronto

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ABSTRACT

The widespread contamination of groundwater with mixtures of chlorinated hydrocarbons poses a health threat and an obligation to the landowner to remediate this threat. Organic solvents such as tetrachloroethene (PCE) and carbon tetrachloride (CT) are frequently found on contaminated sites and as suspected carcinogens must be removed from the environment. The objective of this study was to determine the susceptibility of mixtures of PCE and CT to biological reductive dechlorination.

Using microcosms and a continuous column the impact of CT degradation on the degradation of a PCE was examined. Using an ethanol/PCE enrichment culture the effect of CT and its degradation products, chloroform (CF) and dichloromethane (DCM), on the degradation of PCE was examined. Various concentrations of CT were applied (0.5 mg/L to 5.0 mg/L) along with 5 mg/L PCE and monitored over time.

In microcosms 5 mg/L CT totally inhibited the degradation of PCE. Even when all the CT had been transformed to CF, DCM and CO₂ and after the concentration of CF was reduced to 0.5 mg/L the PCE was still recalcitrant in excess of 160 days. Full PCE degradation to cis-DCE did occur after the bottles were purged with nitrogen (to remove the chlorinated compounds) and PCE was applied in the absence of CT. In the column studies the addition of 0.5 mg/L CT totally suspended PCE degradation for 24 days and within an additional 10 days all the incoming PCE was degraded to mostly (ETH) and some (VC), with degradation seemingly unaffected by the presence of CF. When the concentration of CT was doubled to 1.0 mg/L and again to 2.0 mg/L, PCE degradation was significantly slowed, however, the degradation capacity returned with increasing swiftness.

The biodegradation of organic solvents can be an effective way to eliminate possible human health threats, however extra care must be taken when dealing with mixtures of organic solvents. A modified approach may be required for effective degradation of mixtures of contaminants.
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INTRODUCTION

1.0 INTRODUCTION

1.1 Groundwater Contaminated with Chlorinated Aliphatic Hydrocarbons

Contamination of groundwater with chlorinated aliphatic hydrocarbons is a widespread and common occurrence due to their extensive use in dry cleaning, metal degreasing and a variety of other industrial processes. Organic solvents including tetrachloroethene (PCE) and carbon tetrachloride (CT) are among the most frequently found chlorinated contaminants in soil and groundwater (Abelson, 1990; Westrick et al., 1984). Uncontrolled releases of these solvents resulted in their introduction to the land, water and air. Spills and fugitive emissions can occur before, during and after their intended use from accidental spillage, discharge of industrial and municipal liquid effluents and inadequate disposal methods. Once released into the environment they can form dense nonaqueous phase liquids (DNAPL) which may slowly dissolve into flowing groundwater causing a long term source of contamination which may be transported substantial distances (Mackay and Cherry, 1989). Both PCE and CT 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 at a maximum contamination level of 5μg/L (Pontius, 1992).

Since these solvents and their chlorinated degradation products are considered toxic and are suspected carcinogens, their interactions in the environment must be fully understood and remedial actions must be taken to limit human health effects. Two general methods of removing these contaminants from the subsurface are physical/chemical processes and biological processes. Physical/chemical processes such as adsorption to granulated activated carbon and air stripping accomplish this by
removing and transferring the contaminant to another medium, which would require further treatment to detoxify the contaminant. Biological treatment however, has the capability for complete detoxification of the contaminant to environmentally benign products.

PCE has not been observed to degrade under aerobic conditions, however anaerobic degradation has been reported for both pure cultures (Fathpure et al., 1987; Neumann et al., 1994), and mixed cultures (DiStefano et al., 1991; DeBruin et al., 1992) under a variety of different reactor configurations. CT is also susceptible to anaerobic degradation (Criddle et al., 1990a).

Reductive dechlorination of PCE proceeds stepwise to trichloroethene (TCE), dichloroethene isomers (DCEs), vinyl chloride (VC) and ethene (ETH) (Freedman and Gossett, 1989). CT degradation proceeds to chloroform (CF), dichloromethane (DCM) and CT is known to also degrade to CO2. Each step of reductive dechlorination involves electron and hydrogen additions and chlorine elimination. Unlike oxidation reactions, the rate of reductive dechlorination decreases with less halogenated compounds. Reduction reactions involve electron transfer to the halogenated substrate and are more favorable with highly halogenated compounds resulting in persistence of less chlorinated compounds under anaerobic conditions. This poses a potential problem, since the conversion of VC to ETH may require a long time (Vogel and McCarty, 1985). Dechlorination to VC, which is often the final step, is especially important since VC is a more toxic substance than its parent compound and is less susceptible to sorption to soil allowing for greater contaminant mobility.
INTRODUCTION

There has been a great amount of research on the susceptibility of isolated solvents to reductive dechlorination. However, there has been little work examining the potential for reductive dechlorination of mixtures of chlorinated solvents which may better represent real life situations since, frequently, there are mixtures of chlorinated contaminants (Thomas and Ward, 1989) present in the subsurface at contaminated sites.

1.2 Purpose

The objective of this study was to determine if CT and its breakdown products (CF and DCM) would inhibit the anaerobic biodegradation of PCE. Studies were conducted in microcosms and in a continuous flow column to assess acclimation to PCE and CT, inhibition of PCE degradation, and effect on electron donor utilization.

1.3 Significance

PCE and CT pose a threat to human health and thus must be treated to limit exposure. Unlike physical/chemical processes, biodegradation allows for complete detoxification to harmless species. Chloroform is a chlorinated byproduct of CT degradation and is a known inhibitor of anaerobic systems (Hickey et al., 1987). The presence of CF may result in the complete inhibition of PCE biodegradation or may result in the accumulation of its less chlorinated byproducts. Interactions between these solvents may have an effect reducing the viability of anaerobic PCE biodegradation for specific sites containing mixtures of chlorinated species. These interactions may not allow the bacteria, indigenous or enhanced (bioaugmentation) to perform dechlorination successfully.
2.0 LITERATURE REVIEW

2.1 Reductive Dechlorination

Highly chlorinated compounds such as PCE and CT are susceptible to a series of sequential electron and hydrogen additions and chlorine elimination under appropriate conditions referred to as reductive dechlorination (hydrogenolysis). Fortunately, reductive dechlorination can lead to environmentally harmless products (Freedman and Gossett, 1989). The rates of degradation vary widely for different parent compounds and for less chlorinated by-products, with rates usually higher for more chlorinated compounds. Many researchers have found incomplete dechlorination of PCE to DCE's and VC, and of CT to CF and DCM. These products are, however, not the desired outcome of dechlorination. Less chlorinated products are just as harmful if not more so than their more chlorinated parent material. Thus, reductive dechlorination is considered complete only when harmless compounds are produced such as ethene (ETH), ethane or CO₂. Figure 2.1 demonstrates the pathways for anaerobic degradation of PCE and CT.

2.1.1 Reductive Dechlorination of PCE

Highly chlorinated compounds such as PCE are resistant to aerobic degradation (Mohn and Tiedje, 1992) while their less chlorinated products are susceptible to aerobic degradation. However, anaerobic degradation of PCE has been widely documented. Using enrichment cultures Freedman and Gossett (1989) first observed anaerobic PCE degradation to ETH. There was, however, a large production and accumulation of VC. It was later elucidated that the presence of more chlorinated compounds inhibited the
Anaerobic PCE degradation

\[
\begin{align*}
2H & \text{ HCl} \quad 2H & \text{ HCl} \\
\text{PCE} & \rightarrow \text{TCE} & \text{TCE} & \rightarrow \text{cis-1,2-DCE} & \text{cis-1,2-DCE} & \rightarrow \text{VC} & \text{VC} & \rightarrow \text{ETH}
\end{align*}
\]

Anaerobic CT degradation

\[
\begin{align*}
2H & \text{ HCl} & 2H & \text{ HCl} \\
\text{CT} & \rightarrow \text{CF} & \text{CF} & \rightarrow \text{DCM} \\
\text{CO}_2 & & \text{CO}_2
\end{align*}
\]

Figure 2.1: Pathways for anaerobic degradation of PCE and CT.

conversion of VC to ETH and only when the more chlorinated compounds were removed was there significant production of ETH (DiStefano et al., 1991).

Many researchers have found different end products of PCE degradation. PCE to TCE has been observed (Fatherpure et al., 1987), PCE to cis-DCE (Bagley and Gossett, 1990), PCE to trans-DCE and VC (Parsons et al., 1984), PCE to ETH (DiStefano et al., 1991), PCE to ethane (de Bruin et al., 1992) and even to CO\textsubscript{2} (Vogel and McCarty, University of Toronto
1985). CO₂ production was verified with ¹⁴C but accounted for only 24% of the initial labeled PCE. CO₂ production has not been confirmed in other studies and should be considered a very minor degradation pathway for PCE degradation.

Using a sulfate-reducing mixed culture Bagley and Gossett (1990) were able to degrade PCE to cis-DCE with equal dechlorination capability with methanogenesis and with methanogenesis inhibited using 2-bromoethansulfonate (BES). This suggests sulfate-reducing bacteria may have been conducting reductive dechlorination in this system. DiStefano et al. (1991) reported complete dechlorination to ETH in the absence of methanogenesis using a methanol-PCE enrichment culture. In this case, methanogenesis was inhibited by high concentrations of PCE. Previously methanogenesis was linked with PCE dechlorination (Fathepure and Boyd, 1988).

Sharma and McCarty (1996) isolated a facultative aerobe (Strain MS-1) with characteristics of the family Enterbacteriaceae from a PCE contaminated aquifer. This organism was able to reductively dechlorinate PCE to cis-DCE in a defined medium during growth on a variety of carbon sources, but only in the absence of oxygen, nitrate and high concentrations of fermentable carbon sources. The strain was closely related to Enterobacter agglomerans, also reported in this study to be capable of mediating the reductive dehalogenation of PCE.

Maymo-Gatell et al. (1997) isolated a new bacterium (Dehalococcoides ethenogenes strain 195) with the capability of complete PCE degradation to ethene, using H₂ as an electron donor. This has been the first isolated organism with the ability of complete conversion of PCE to ETH.
Strain PCE1 was isolated from a culture with the capability of complete dechlorination of PCE to ethene. However, strain PCE1 did not have the capability for complete dechlorination to ethene. Strain PCE1 was capable of degradation of PCE to TCE and small amounts of cis-DCE and trans-DCE (Gerritse et al., 1996).

Reductive dechlorination not only requires the proper environmental conditions, but also requires a source of electrons. These electrons are thought to be used during every step of reductive dechlorination. A variety of donors have been examined under various conditions with conflicting results. It is thought that H₂ is used directly by dechlorinators during reductive dechlorination. It has also been postulated that dechlorinators can out compete methanogens at low H₂ partial pressures (Smatlak et al., 1996). This would indicate that donors which, when fermented, produce a low partial pressure of H₂, such as butyric and propionic acids, which are utilized slowly producing a slow and steady stream of H₂ would allow for more efficient degradation of chlorinated compounds.

2.1.2 Reductive Dechlorination of CT

Highly chlorinated compounds such as CT are resistant to aerobic degradation, however, under anaerobic conditions CT can be degraded. Using anaerobic digester sludge and low concentrations of electron donors (humic acid, acetic acid and glucose) Doong and Wu (1996) were able to enhance the rate of reductive dechlorination of CT by altering the biomass and electron donor levels. The rate of reductive dechlorination of CT positively correlated with concentrations of acetic acid used as the electron donor.
They concluded initial biomass level and increased electron donor concentrations had the effect of increasing the rate of reductive dechlorination.

Jin and Englande (1997a) studied the effect of oxidation-reduction potential (ORP) and dissolved oxygen (DO) on the biodegradation of CT. Using *Escherichia coli* K-12 they found no evidence of degradation at high oxygen levels (initial DO=7.6mg/L) while degradation was found at lower DO levels (initial DO=1.4mg/L). Similar results were found for ORP. As the free electron activity of the system was reduced the amount of CT degradation increased. Criddle *et al.* (1990) found using $^{14}$C there was no evidence of CT transformation under high oxygen levels (3.6 mmol/bottle), however under lower oxygen levels (0.34 mmol/bottle) $^{14}$C carbon dioxide was recovered with little CF production.

In another study Jin and Englande (1997b) found two possible pathways for CT degradation. One pathway involved one-electron transfer leading to CF formation (Vogel *et al.*, 1987), followed by CF biodegradation by *P. cepacia*. A second pathway involved two-electron reduction of CT, leading to the production of a dichlorocarbene radical followed by spontaneous hydrolysis to formate which could be oxidized to carbon dioxide (Criddle *et al.*, 1990b).

### 2.2 Electron Donors

#### 2.2.1 Anaerobic Degradation of Ethanol

Ethanol is a rapidly fermented substrate with can serve both as an electron donor and as a carbon source. During ethanol degradation both acetic and propionic acids are produced along with $H_2$, $CH_4$ and $CO_2$.
Equation 1 represents ethanol degradation to acetic acid and hydrogen. Equation 2 represents ethanol degradation to propionic acid and acetic acid. Equation 3 represents propionic acid degradation to acetic acid and hydrogen. Equation 4 represents acetic acid degradation to methane and carbon dioxide. The degradation of ethanol to acetic acid and propionic acids has been observed by many researchers (Schink et al, 1987; Laanbroek et al, 1982).

2.2.2 Anaerobic Degradation of Yeast Extract

Yeast extract (YE) has been used to stimulate anaerobic degradation of chlorinated solvents, as a source of micronutrients, and as a source of slowly available reducing equivalents (Fennell et al, 1997). Fennell et al. (1997) used ethanol, ethanol with fermented yeast extract (FYE) and ethanol with a surrogate FYE (SFYE) to determine the role of YE. The SFYE was a blend of volatile fatty acids (VFA) expected to contribute reducing equivalents comparable to FYE, but without the micronutrients found in FYE. Ethanol alone produced a large amount of reducing equivalents initially, allowing for dechlorinators and methanogens to initiate activity rapidly and robustly. As the reducing equivalents were transformed methanogenesis ceased and dechlorination continued at a much slower rate. It was thought propionic acid consumption, which occurs at low H₂ partial pressures (10⁻⁵ atm) provided the reducing equivalents for
dechlorination. The methanogens could not compete with dechlorinators for hydrogen at partial pressures that low. Ethanol with FYE resulted in a similar trend. There was a lot of activity initially, with less activity later on as the reducing equivalents from ethanol degradation were consumed. The FYE did however result in a more gradual decrease in the degradation rate. The FYE served as a slow release source of reducing equivalents. Ethanol with SFYE yielded results similar to ethanol with FYE. The VFA present in YE provide a gradual release of reducing equivalents at low H₂ partial pressures. Butyric acid (a component of FYE) is readily degraded at H₂ partial pressures below 10⁻⁴.⁹ atm providing reducing equivalents. This results in more reducing equivalents available for a longer time at lower levels and allows for a shift in the competitiveness of methanogens to dechlorinators for available H₂.

2.3 CF Inhibition of Anaerobic Systems

Chloroform is produced by anaerobic CT biodegradation. CF is susceptible to aerobic degradation to CO₂ and to anaerobic degradation to DCM and CO₂ in both methanogenic and nonmethanogenic cultures. CF however, is a known inhibitor of many anaerobic systems including methanogenic systems (Bagley and Gossett 1995). Using a methanogenic methanol enrichment culture and cultures of Methanosarcina barkeri 227, Bagley and Gossett (1995) studied chloroform degradation. Methanol consumption was inhibited by CF in both methanogenic methanol enrichment culture and Methanosarcina barkeri 227 culture. Methanol additions stimulated CF removal in both systems. In the enrichment culture methanol consumption rates increased as remaining CF
concentrations decreased, giving some insight into the inhibition. It is not known what effect CF may have on organisms over the long term.

A methanogenic culture using acetic acid as the primary substrate dechlorinated CF to DCM, which was further transformed to CO₂. However, the presence of CF at any concentration inhibited the degradation of acetic acid (Gupta et al., 1996a). In a similar study, CF dechlorination to DCM was achieved using a sulfate-reducing culture with acetic acid as the primary substrate. Acetic acid consumption was not inhibited with CF concentrations as high as 29.3μM (Gupta et al., 1996b).

Yang and Speece (1986) studied the conversion of acetate to methane in anaerobic digestion over prolonged periods of time. They identified and assessed factors which affected toxicity response including concentration of toxicant, solids retention time, biomass concentration, toxicant exposure, cell age, toxicant administration pattern and temperature, using gas production as a measure of methanogenesis. Higher biomass concentrations and younger cells had the ability to recover sooner than lower biomass concentration systems and older cells. The exposure time to CF was directly proportional to recovery time. Gradually increasing the chloroform concentrations resulted in a culture which could tolerate 15 mg/L CF, while unacclimated cultures were inhibited at CF concentrations of 0.5 mg/L. Temperature was also found to have an affect on methanogenesis, cultures at 35°C had no significant reduction in gas production after the addition of CF. However, at 25°C, gas production dropped to 50% of normal and required 15 days for complete recovery.
3.0 METHODS AND MATERIALS

3.1 Anaerobic Microcosm Studies

Microcosm studies were conducted to examine the effect of CT on the degradation of PCE and EtOH and on acetic acid and propionic acid production and consumption. Microcosm studies were conducted in 250 mL screw-cap bottles sealed with Teflon® Mininert® valves. The total volume in all bottles was determined by adding distilled water to the bottles. The mass of the empty bottle and the mass of the bottle filled with water was recorded with the difference being the mass of water required to fill the bottle. This mass was converted to a volume correcting for the temperature of the water. The bottles were then dried, capped and pressure tested at 15 psi for 48 hours to ensure integrity. Finally the bottles were autoclaved and filled with 175 mL of medium while sparging with nitrogen to ensure anaerobic conditions. Throughout the study the bottles were stored under quiescent conditions at room temperature (22°C) in the dark. The bottles were kept inverted to minimize losses and stored in a fume hood. The microcosms were maintained on a semicontinuous basis with sampling every 2 to 3 days with fresh medium added after every third sampling. Duplicate bottles were inoculated with 10% of a first generation PCE/EtOH enriched consortium obtained from a previous study (Lalonde, 1997). Bottles A - 1 and A - 2 were amended with 5 mg/L PCE, 100 mg/L EtOH and 50 mg/L yeast extract (YE). Bottles B - 1 and B - 2 were amended with 5 mg/L CT, 100 mg/L EtOH and 50 mg/L YE. Bottles C - 1 and C - 2 were amended with 5 mg/L PCE, 5 mg/L CT, 100 mg/L EtOH and 50 mg/L YE. Control bottles D - 1 and D - 2 were set up to verify bottle integrity and as abiotic controls. These bottles
contained the same culture except the bottles were autoclaved after the media was added to sterilize the media. These bottles contained only 5 mg/L PCE and 5 mg/L CT.

3.2 Anaerobic Continuous Column

A laboratory scale column filled with natural screened sand (n=0.35, porosity and $f_{oc}=0.1$, fraction organic carbon) was used to investigate anaerobic degradation of PCE and CT mixtures. The stainless steel column was 2 m tall and had an inner diameter of 10 cm giving a void volume of 5.5 L. Sampling ports equipped with Teflon® Mininert® valves were positioned every 10 cm beginning 5 cm from the inlet, with additional ports at the top and bottom of the column. An anaerobic basal medium and chlorinated compound solution containing PCE, CT and EtOH were simultaneously pumped into the column (5.0 L/day) at a rate to produce the desired inlet concentrations. The basal medium solution was pumped via a diaphragm pump (electronic metering, positive displacement pump, Pulsatron) with a back pressure regulator. The chlorinated compound solution was delivered via a peristaltic pump (Masterflex). Calculated concentrations of the compounds were compared to measured concentrations throughout the operation as an integrity control against any losses. All lines leading to and from the column were ¼” Teflon® tubing or size 13 Viton® tubing. A schematic diagram of the column and all additional components is shown in Figure 3.1. Samples were withdrawn from the column using Hamilton gas-tight syringes.
3.2.1 Basal Medium Solution

A 20 L glass vessel was used to prepare and hold the basal medium. The container was filled with 20 L of distilled water then purged with nitrogen for 30 minutes. During this time the ingredients were weighed and added to the water. After all the

---

Figure 3.1: Schematic Diagram of the Anaerobic Column
ingredients were added the nitrogen was switched to a 90% N\textsubscript{2} / 10% CO\textsubscript{2} mix, purged for an additional 10 minutes and then capped and held under a pressure of 3 psi to maintain anaerobic conditions. Appendix A contains the recipe and a full preparation procedure for the anaerobic basal medium.

### 3.2.2 Chlorinated Compound Solution

The concentrations of PCE and CT in the delivery vessel were chosen to keep the liquid concentrations under their respective solubilities of 150 mg/L for PCE and 800 mg/L for CT (in water) (Mackay et al. 1993). A 1 L Tedlar\textsuperscript{®} gas bag was used to maintain a constant headspace avoiding a negative pressure in the 1 L glass vessel as the liquid was removed and to avoid additional losses into the headspace. The Tedlar\textsuperscript{®} bag was purged with nitrogen several times to remove any oxygen and then capped. The glass vessel was also purged with nitrogen for several minutes prior to and during the addition of anaerobic basal medium to maintain anaerobic conditions. After 1 L of basal medium was added the gas bag was connected, sealing the medium from the atmosphere. The PCE, CT and EtOH were then added through a Mininert\textsuperscript{®} valve in neat form and the solution was stirred on a stir plate for a minimum of 5 hours to allow the PCE and CT to dissolve into the liquid and partition into the headspace.
3.2.3 Effluent Treatment System

3.2.3.1 Primary Oxidation Chamber

The liquid and gas effluents were collected in a 4 L vessel which was continuously stirred with a stir plate. The liquid level was maintained at 2.5 L giving a hydraulic retention time (HRT) of 12 hours. Potassium permanganate KMnO₄ (100,000 mg/L) was delivered (0.05 ml/hr) via a syringe pump (Cole-Parmer Instruments, Niles, Illinois) to oxidize the effluent. Gas was allowed to escape periodically through a granulated activated carbon (GAC) filter before emission through a fume vent to the atmosphere, with liquid entering the secondary air stripping chamber.

3.2.3.2 Secondary Air Stripping Chamber

Oxidized effluent was passed through a secondary treatment system to ensure all organics were removed. A 3 L vessel with 2 L liquid volume was purged with compressed air at a high rate (10 PSI), with gas passing through a GAC filter before emission through a fume vent to the atmosphere. The final clean effluent was then disposed into the sanitary sewer.

3.3 Chemicals

Tetrachloroethene (PCE), trichloroethene (TCE), carbon tetrachloride (CT), chloroform (CF) and dichloromethane (DCM) were obtained from (Aldrich Co. 99.5%). 1,1-dichloroethene (1,1-DCE), trans-1,2-dichloroethene (trans-DCE) and cis-1,2-dichloroethene (cis-DCE), were obtained in neat liquid form (1000 or 5000 mg ampouls; Supelco, Inc.). Vinyl chloride (VC) (200μg/ml ampoul; Supelco, Inc.) and ethene was
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purchased from Scott Specialty Gases. Other chemicals used were reagent grade and were obtained from VWR Scientific and Fisher Scientific.

Compressed gases; extra dry air, ultra high purity helium and ultra high purity hydrogen used for gas chromatography and nitrogen and nitrogen/CO₂ for basal media preparation and delivery were all obtained from BOC gases.

3.4 Analytical Methods

3.4.1 Gas Chromatography

Identification and quantification of chlorinated methanes and ethenes and ethanol were accomplished using a gas chromatograph (GC, 5890 Series II Plus, Hewlett Packard, Co.) with a purge and trap concentrator (Tekmar 3000) and an AS16 autosampler. The GC was equipped with a Vocarb capillary column (30 m long x 0.53 mm i.d. x 3.0 μm film thickness; Supelco, Inc.) and a flame ionization detector (FID). Aqueous samples (1 or 2 mL) were purged with helium for 11 min. at 45 mL/min onto a Vocarb 3000 trap (Supelco, Inc.). Desorption occurred at 250°C for 4 min. at 8 mL/min, helium was used for desorption and as the carrier gas 8 mL/min. The trap was baked at 260°C for 6 min. prior to purging the next sample. The oven temperature was held at 35°C for 4 min., then increased at 10°C/min. to 100°C, where it was held for 2 min. Injector and detector temperatures were 240 and 250°C respectively.

Ethene quantification was accomplished using 10 μL headspace injections into a gas chromatograph (GC, 5890 Series II Plus, Hewlett Packard with a Vocarb capillary column (30 m long x 0.53 mm i.d. x 3.0 μm film thickness; Supelco, Inc.) and a flame ionization detector (FID). Helium was used as the carrier gas at 8 mL/min., the oven
temperature was held at 35°C for 4 min., then increased at 10°C/min. to 100°C, where it was held for 2 min. Injector and detector temperatures were 240 and 250 °C respectively.

Acetate and propionate were quantified on a gas chromatograph (GC, 5890 Series II, Hewlett Packard, Co.) equipped with a FID and a packed glass column (2 m x 2 mm TightSpec™, 80/120 Carbopack B-DA/4% CARBOWAX® 20M; Supelco, Inc.) with 1µL on column aqueous injection. Helium was the carrier gas at 45 ml/min. The oven temperature was held isothermal at 175°C with injector and detector temperatures 225°C. Prior to injection, aqueous samples were centrifuged and acidified with 0.03 M oxalic acid.

Methane quantification was accomplished using 10 µL headspace injections into a gas chromatograph (GC, 5890 Series II Plus, Hewlett Pack, Co.) with a Carboxen™ 1006 Plot fused-silica capillary column (30 m long x 0.53 mm i.d.; Supelco, Inc) with a thermal conductivity detector (TCD). Helium was the carrier gas at 2 mL/min., the oven was held at 35°C for 3 min., then increased at 10°C/min. to 140°C. Both injector and detector temperatures were 200°C.

3.4.2 Mass Spectrometry

Identification and quantification of VC was performed using a HP 5890 Series II GC with a Trio 1000 Mass Spectrometer (MS) (Fisons Instruments). 1 mL samples were collected into 2 mL HP vials sealed with Teflon-faced septa. Samples were immediately agitated for 15 min. and then left unagitated a further 15 min. to reach equilibrium between the aqueous and headspace phases. Then, a 10µL headspace sample was injected into a 30 m x 0.53 mm x 3 µm film thickness HP-624 capillary column (Hewlett
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Packard). The MS was operated in the electron impact ionization mode with an electron energy of 70 eV. A full mass scanning range of m/z 50-180 was used at 0.45 s scan time with 0.05 s inter-scan delay. The detector voltage was set at 480 V, and the ion source was maintained at 200 °C. The GC interface temperature (transfer line) was kept at 120 °C. The instrument was tuned with heptacosa as the reference chemical before analysis. Data acquisition and processing were controlled by the Fisons MassLab data system. The carrier gas used was ultra high purity helium at a flow rate of 6 mL/min. The initial oven temperature was 35°C for 4 min. followed by a 8 °C/min. increase to 120 °C and held for 3 min. Injection temperature was 200 °C. All chlorinated ethenes and methanes were identified by their mass spectral fragmentation pattern.

3.5 Quality Assurance/Quality Control

To maintain proper quality assurance/control the following practices were employed. A nine point calibration curve using three separate stock solutions was prepared, followed by periodic four point, single stock calibration curve. These curves were compared to curves prepared by different researchers. The calibrations curves were prepared in both distilled water and in the matrix (basal media). Also calibration curves of mixtures of chlorinated ethenes and methanes were prepared. Matrix and matrix + spike were also run every fifth sample to determine accuracy. If samples varied by more than five percent a new calibration curve was prepared.
4.0 RESULTS

4.1 Anaerobic Microcosm Studies

Microcosm studies were performed to elucidate the effects of CT degradation on the degradation of PCE prior to conducting similar studies with the continuous anaerobic column. Using a second generation ethanol/PCE enriched culture the degradation of PCE alone, CT alone, and a mixture of PCE and CT was examined. Previously, this culture had the ability to quickly degrade PCE to cis-DCE (Lalonde, 1997). No production of VC or ETH was observed. Table 4.1 describes the initial conditions for the bottle study. Bottles were amended with additional ethanol and yeast extract at various points throughout the study. Control bottles were also run containing both CT and PCE, in sterilized medium.

Table 4.1: Initial conditions

<table>
<thead>
<tr>
<th>Bottle</th>
<th>CT (Conc. mg/L)</th>
<th>PCE (Conc. mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - 1</td>
<td>N/A</td>
<td>5 mg/L</td>
</tr>
<tr>
<td>A - 2</td>
<td>N/A</td>
<td>5 mg/L</td>
</tr>
<tr>
<td>B - 1</td>
<td>5 mg/L</td>
<td>N/A</td>
</tr>
<tr>
<td>B - 2</td>
<td>5 mg/L</td>
<td>N/A</td>
</tr>
<tr>
<td>C - 1</td>
<td>5 mg/L</td>
<td>5 mg/L</td>
</tr>
<tr>
<td>C - 2</td>
<td>5 mg/L</td>
<td>5 mg/L</td>
</tr>
</tbody>
</table>

- N/A – Not added
- All bottles contained 50 mg/L yeast extract (YE) initially
- All bottles contained 100 mg/L ethanol initially

4.1.1 Effect of CT degradation on PCE degradation

In the microcosms amended with PCE alone all the PCE was degraded to cis-DCE with very little TCE accumulation. The PCE was completely converted to cis-DCE within seven and ten days for bottles A - 1 and A - 2 respectively. Figure 4.1 shows the profile for a representative bottle.
Figure 4.1: PCE degradation in the absence of CT.

Figure 4.2: Donor profile in the absence of CT.
The ethanol (EtOH) was catabolized to acetic (AA) and propionic (PA) acids which were consumed within thirteen days and ten days respectively. Additional ethanol was added when all the donors were exhausted (22 days after the initial ethanol addition) in an attempt to stimulate further degradation beyond cis-DCE. This did not result in detectable degradation of cis-DCE and no VC or ETH was detected. The additional ethanol was converted to acetic and propionic acids, which were also consumed. Figure 4.2 shows the profile for a representative bottle. During the second and third additions of ethanol there was a marked increase in the utilization rate of the acetic and propionic acids. This increase in the rate of utilization could be due to acclimation to the compounds or to an increase in biomass. Fifty days after the initial PCE addition, additional PCE was added to the bottles (Figure 4.1). This time the PCE was completely converted to cis-DCE within one day. Again no degradation beyond cis-DCE was observed for the duration of the study, which lasted 160 days (data shown to day 65 for clarity). An additional attempt on day 65 was made to see if degradation beyond cis-DCE would occur using ethanol, but no cis-DCE degradation was detected (data not shown). No production of VC or ETH was observed and there was no measurable decrease in the mass of cis-DCE in the bottles.

Microcosms containing CT alone degraded the CT to CF, DCM and it was assumed that CO₂ was also produced. Accumulation of CF occurred until all the CT had been converted (32 days after CT amendment). At this time CF concentrations began to decline very slowly (Figure 4.3). DCM concentrations were low throughout the study. CF accounted for about 40% of the initial CT concentration and DCM accounted for about 3% of the initial CT concentration. It was assumed that the rest was converted to
Figure 4.3: CT degradation in the absence of PCE.

Figure 4.4: Donor profile in the presence of CT.
CO₂ (not verified as no ¹⁴C studies were conducted). Ethanol was converted to acetic and propionic acids. However, more time (compared to microcosms with PCE alone) was required to convert the ethanol and there was no sign of acetic and propionic acid degradation. The ethanol utilization rate did increase after the first ethanol addition (Figure 4.4). The second addition of ethanol resulted in an increase in the degradation rate of CT.

In microcosms containing a mixture of PCE and CT, the PCE was completely recalcitrant. At no time were any intermediates, TCE, cis-DCE, VC or ETH detected. Figure 4.5 shows the PCE profile through time for bottles which did not contain CT initially and for bottles which did contain CT initially. CT degradation was seemingly unaffected by the presence of PCE (Figure 4.6) and the degradation of ethanol, acetic acid and propionic acid profiles were similar, with similar trends to bottles containing CT alone (data not shown).

160 days after the CT was degraded there was still no PCE reduction (data not shown). This would indicated that either the initial presence of CT shocked the culture, resulting in recalcitrance of PCE, or the presence of CF or DCM was responsible for the recalcitrance of PCE. CF is a known potent inhibitor of anaerobic systems (especially methanogenic systems) at any concentration (Bagley and Gossett, 1995). Even when the CF and DCM liquid concentrations dropped to 0.5 mg/L and 0.2 mg/L respectively there was no detectable degradation of PCE.
Figure 4.5: PCE degradation with and without CT present.

Figure 4.6: CT degradation with and without PCE present.
4.1.2 Long term effect of CT degradation

In an attempt to identify if the PCE degradation capacity could be restored in a culture which was exposed to CT, CF and DCM for 4 months, bottles C - 1 and C - 2 (from the previous study) were purged with nitrogen to remove any remaining PCE, CF and DCM. These bottles were then spiked with 5 mg/L PCE, 100 mg/L EtOH and 50 mg/L YE and monitored over time. Full PCE degradation to cis-DCE was observed within 10 days, with little TCE accumulation (data not shown).

4.1.3 PCE degradation with yeast extract

Yeast extract has been shown to decrease the acclimation time of PCE degradation (Lalonde, 1997). The role of yeast extract has also been linked to serving as a source of reducing equivalents and a provider of micronutrients (Fennell et al. 1997). An attempt to clarify the role of yeast extract was made with bottles containing a third generation ethanol/PCE enriched culture (from the previous study). Duplicate bottles containing 5 mg/L PCE and 50 mg/L YE were prepared and monitored similar to the above study. In both bottles complete degradation of PCE to cis-DCE was attained with a rate similar to previous bottles amended with EtOH and YE (data not shown).
4.2 Anaerobic Column Studies

The column was packed with natural screened soil and inoculated with biomass obtained from an anaerobic digester in the Metropolitan Toronto Main Treatment Plant. Prior to inoculation the bacteria were acclimated to ethanol which would serve as the substrate for the duration for the column studies. The column exhibited several stages of PCE degradation including degradation to cis-DCE as the final product (45 days after inoculation), followed by further degradation to VC and ETH (175 days after inoculation). To examine the inhibition effect of CT and its metabolites (CF and DCM), CT was sequentially added to the column. Initially CT was added at 0.5 mg/L and was later increased to 1.0 mg/L and then to 2.0 mg/L.

4.2.1 Degradation of PCE to cis-DCE

Once PCE degradation began it quickly proceeded to complete PCE degradation (5 mg/L) to cis-DCE with no accumulation of TCE observed. Only traces of TCE were detected in the presence of PCE and these disappeared as soon as PCE was fully degraded (Figure 4.7). PCE degradation began at about the midpoint of the column and slowly migrated toward the inlet of the column (Figure 4.8) (Lalonde, 1997). As time proceeded, complete degradation of PCE to cis-DCE was observed by column inlet (Figure 4.9). It was thought degradation was occurring in the tubing leading to the column.

The column also experienced a serious plugging problem leading to excessive pressure at the column inlet (27 psi). The buildup of pressure was possibly due to bacterial growth and/or precipitation of FeS (from the basal medium).
Figure 4.7: Profile of ethenes (95 days after inoculation, PCE to \textit{cis}-DCE stage).

Figure 4.8: PCE profile through time (PCE to \textit{cis}-DCE stage) (from Lalonde, 1997).
To solve the plugging problem, the column was purged with basal medium for 30 days to remove any remaining PCE and cis-DCE. The column was then inverted and the soil up to the second port (15 cm from the inlet) was then removed and re-packed (169 days after inoculation). This procedure remedied the plugging problem for the remainder of the study. After the re-packing the column inlet pressure was reduced to 4.5 psi. Appendix B contains the column pressures over time.

Figure 4.9: Profile of ethenes (145 days after inoculation, PCE to cis-DCE stage).

4.2.1.1 Column Purging

During the one-month column purging neither PCE nor ethanol was added to the column. Nether the less, PCE and cis-DCE were observed in the column for most of the purging cycle. It was speculated the presence of PCE was due to slow PCE desorption from the organics in the soil. The presence of cis-DCE was thought to be due to the
partial degradation of the desorbing PCE, with electrons derived from the YE present in the basal medium.

4.2.2 Degradation of PCE to VC and ETH

Immediately after the column was re-packed and restarted, degradation of cis-DCE to VC and ETH commenced (data not shown). Figure 4.10 shows the ethenes profile 41 days after cis-DCE degradation began. It was also during this stage that there was possibly a lack of electron donor in the upper portion of the column. All the EtOH, AA and PA were consumed by port 3 (Figure 4.11). This also corresponded to a sharp reduction in the degradation rate of VC (Figure 4.10). As time passed the culture apparently acclimated to VC resulting in a greater conversion of PCE to ETH. Figure 4.12 shows VC and ETH at the inlet of the column, in the presence of PCE, TCE and cis-DCE.

![Graph showing ethenes profile](image)

Figure 4.10: Profile of ethenes (41 days after cis-DCE degradation began).
Figure 4.11: Profile of donors (41 days after cis-DCE degradation began).

Figure 4.12: Profile of ethenes (148 days after cis-DCE degradation began).
An attempt was made to stimulate further VC degradation in the upper portion of the column. Additional EtOH was added at port 6 (55 cm from the inlet) resulting in an increase in the production of ETH and in rapid and complete degradation of EtOH, AA and PA. Additional VC degradation also occurred accounting for the increased production of ETH (Figure 4.12). The midpoint EtOH injection was stopped in the hope further degradation of VC would occur over time as the biomass in the upper portion of the column became acclimated to VC.

4.2.3 Effect of CT degradation on PCE degradation

CT was added to the column in 3 stages, while the inlet PCE concentration was kept constant at 5 mg/L. Initially 0.5 mg/L of CT was applied to the column. All the incoming CT was fully degraded, without lag by port 1. Beyond port 2 the concentration of CF stayed relatively constant at 0.15 mg/L. Trace amounts of DCM were produced and were presumably consumed. During this stage DCM was only detected for the first 4 days after adding CT to the column. It is unclear if DCM production stopped or if any DCM produced was immediately converted to CO₂. Figure 4.13 shows the chlorinated methanes profile 24 days after the addition of CT.

PCE degradation was totally inhibited initially, (PCE inhibition identified by a lack of intermediate products) presumably by the presence of CF (Bagley et al. 1998b). Immediately after the addition of CT the PCE concentrations began to increase in the column. The PCE concentrations at the ports approached the inlet concentration however, the inlet concentration was never reached. The long breakthrough time was thought to be due to PCE sorbing to the organics in the soil. Figure 4.14 shows the
temporal PCE profile of the column after the CT was added to the column. Immediately prior to the addition of CT to the column, there was no detectable PCE. As time went on the concentration of PCE increased through the column, until 24 days after CT was first added to the column (Figure 4.14). The PCE concentrations at this time began to rapidly decrease with concurrent production of lesser chlorinated products (Figure 4.15), and by 34 days after the CT was added, there was no PCE detected beyond port 5 (Figure 4.16). Figure 4.17 shows the chlorinated methanes profile 34 days after CT was first added to the column. At this time all the PCE was degraded, mostly to ETH and some VC. The conversion of PCE to ETH and VC was similar to that existing before CT was added to the column. As before, all the EtOH was immediately degraded to AA and PA. The consumption of AA and PA was severely inhibited, presumably by the presence of CF (Figure 4.18). It should be noted there was no change in the donor profile after the PCE began to degrade again.

With a CT concentration of 0.5 mg/L yielding 0.15 mg/L CF there was a pronounced effect on the degradation of PCE. This inhibition was temporary, indicating that the organisms had the ability to adapt or acclimate to CF over 34 days of CT addition. Unlike the time dependent inhibition of PCE, the degradation of AA and PA never resumed.
Figure 4.13: Methanes profile 24 days after the addition of 0.5 mg/L CT.

Figure 4.14: Temporal PCE profile after 0.5 mg/L CT addition.
Figure 4.15: Ethenes profile 24 days after the addition of 0.5 mg/L CT.

Figure 4.16: Ethenes profile 34 days after the addition of 0.5 mg/L CT.
Figure 4.17: Methanes profile 34 days after the addition of 0.5 mg/L CT.

Figure 4.18: Electron donor profile after the addition of CT.
RESULTS

Once complete PCE degradation capacity was regained after 35 days, the inlet concentration of CT was doubled to 1.0 mg/L. Initially all the incoming CT was fully degraded, without lag, (by port 3) to CF and CO₂ with no detectable DCM produced. DCM production began 15 days after 1.0 mg/L of CT was applied to the column. DCM concentrations began to build up over time (max. conc. observed 0.085 mg/L at the inlet) and then began to decrease, indicating an acclimation to DCM or DCM production stopped. Beyond port 2 the concentration of CF stayed relatively constant at 0.28 mg/L (Figure 4.19).

The increased concentration of CF had a less pronounced affect on the degradation of PCE than the initial addition of CT to the column (Figure 4.20). PCE concentrations increased in the column shortly after the addition of 1.0 mg/L CT and then decreased as time passed. The slow increase in the PCE concentration was presumably due to PCE sorption. This trend indicates that the increase in CF partially inhibited PCE degradation and with time the dechlorinating bacteria acclimated to the increased CF concentration. The second acclimation (to PCE degradation) of 1.0 mg/L CT required less time, (Figure 4.21, 26 days) than the primary acclimation to 0.5 mg/L CT (34 days). The EtOH, AA and PA profiles during this stage of the study were similar to those of stage one. There was no apparent decrease in the degradation rate of EtOH and the concentrations of AA and PA were similar to those seen in stage one (Figure 4.18).
Figure 4.19: Methanes profile 15 days after the addition of 1.0 mg/L CT.

Figure 4.20: Ethenes profile 15 days after the addition of 1.0 mg/L CT.
Figure 4.21: Ethenes profile 26 days after the addition of 1.0 mg/L CT.

The inlet concentration of CT was again doubled to 2.0 mg/L, 40 days after the previous change in CT inlet concentration. DCM production was now greater and immediate resulting in concentrations as high as 0.24 mg/L at the inlet and decreased to zero by port 3. Over time the DCM concentration began to decrease. As before the concentration of CF remained relatively constant above port 2 at 0.36 mg/L (Figure 4.22), this concentration was lower than expected. This could explain why the third acclimation was significantly reduced. Previously doubling the CT concentration resulted in a near doubling of the CF concentration. In this case doubling the CT concentration resulted in only a 22% increase in the concentration of CF. It would seem the culture has become acclimated to CF degradation as seen by both the sharp increase in DCM production and the decreased amount of CF.
As before PCE degradation was partially inhibited and the donor profile remained unchanged from the previous two stages. The inhibition of PCE degradation showed the same trend as the other two stages, however the recovery time was shorter. Figure 4.23 shows the ethenes profile 6 days after the addition of 2.0 mg/L CT and Figure 4.24 shows the ethenes profile 13 days after the addition of 2.0 mg/L CT. It should be noted Figures 4.23 and 4.24 do not include VC data.

Figure 4.25 shows the percentage of PCE remaining in the column at port 3, prior to the addition of CT (day 0) all the PCE was degraded. The first hump after the addition of CT at 0.5 mg/L represents primary acclimation. Primary acclimation required more time than the second acclimation (see second hump when inlet CT concentration was 1.0 mg/L). A full data set for the third hump (third acclimation to 2.0 mg/L) is not available due to analytical problems, however looking at the available data (dashed line) this hump was smaller and narrower.
Figure 4.22: Methanes profile 6 days after the addition of 2.0 mg/L CT.

Figure 4.23: Ethenes profile 6 days after the addition of 2.0 mg/L CT.
Figure 4.24: Ethenes profile 13 days after the addition of 2.0 mg/L CT.

Figure 4.25: Percentage of PCE remaining at Port 3.
5.0 DISCUSSION

5.1 PCE Degradation in the Absence of CT

In this study the degradation of PCE occurred in several stages. Degradation of PCE to *cis*-DCE as the end product was observed in both microcosms and in a continuous column. Further degradation to VC and ETH was also observed in the column presumably due to the growth of organisms capable of degrading *cis*-DCE (Bagley et al., 1998b).

PCE degradation has been observed by many researchers (Fathepure et al., 1987; Neumann et al., 1994; DiStefano et al., 1991; DeBruin et al., 1992) to various end products and it is still unclear why degradation often stops at *cis*-DCE. What is more baffling is why a culture would start degrading *cis*-DCE after six months of *cis*-DCE being the end product. A possible hypothesis lies in the bacteria themselves. Since the bacteria doing the degradation in a mixed culture were not identified general terms will be used. PCE degradation to *cis*-DCE has occurred numerous times, in this study and in numerous other lab and field investigations. We will call these bacteria partial degraders. PCE degradation to ETH also occurs frequently and we will call these bacteria complete degraders. We also have other bacteria that can degrade *cis*-DCE to ETH termed *cis*-DCE degraders. A possible explanation for these differing end products is the partial degraders, the complete degraders, and the *cis*-DCE degraders are not the same bacteria. The presence of the partial degraders may inhibit the complete degraders or the *cis*-DCE degraders from growing and playing a more pronounced role in PCE degradation or the presence of the *cis*-DCE inhibits the *cis*-DCE degraders. In this study *cis*-DCE degradation began six months after PCE degradation to *cis*-DCE began immediately.
following the column purging stage. During this stage basal medium containing various elements and YE was pumped through the column reducing the PCE and cis-DCE concentrations substantially. It is thought this action allowed the complete degraders or the cis-DCE degraders the opportunity to thrive. Why this happened is unknown, it could be due to the fact that the concentration of cis-DCE during the purging stage was extremely low, indicating the presence of cis-DCE inhibited the cis-DCE degraders. Another equally possible explanation may be even more simple dealing with the population size. The bacteria that degrade PCE to ETH or cis-DCE to ETH may not have reached sufficient numbers to perform identifiable degradation. Their numbers could have been increased as a direct result of the purging stage or their numbers may have naturally built up over time (Bagley, 1998, Bagley et al., 1998).

Previously it was reported the degradation of VC to ETH would occur only once the less chlorinated ethenes had been transformed (DiStefano et al., 1991; Isalou, 1998). This culture seems to degrade PCE to VC and ETH simultaneously in the presence of low concentrations of PCE. Concentration however, may play a role in the ETH production. The inlet PCE concentration was only 5 mg/L, while previous studies indicated VC degradation would only occur once high concentrations of the less chlorinated ethenes were transformed.

5.2 PCE Degradation in the Presence of CT

CF is a chlorinated by-product of CT degradation and is also a known and potent inhibitor of anaerobic systems (Bagley and Gossett, 1995). The degradation of CT to CF and DCM in this study was shown in both microcosm and column studies to have an
effect on PCE degradation. In microcosms 5 mg/L CT totally inhibited PCE degradation long after (160 days) all the CT was degraded. Even after the CF concentration was reduced to 0.5 mg/L there was still no PCE degradation. Yet after these microcosms were purged and then supplemented with PCE and EtOH the culture was able to quickly degrade the PCE. This indicated the presence of CF was possibly responsible for the PCE inhibition and not a shock caused by CT. It also indicated that CF did not kill the culture or cause a competitive inhibition, it simply suppressed the culture’s ability to perform reductive dechlorination of PCE.

In the column the culture had been exposed to PCE for a little over a year and was acclimated to PCE and EtOH. The culture had the ability to quickly convert PCE to VC and ETH and to convert EtOH to acetic and propionic acids, which were also quickly consumed. Upon the addition of 0.5 mg/L CT there was a dramatic change in the culture’s activity. While the culture retained the ability to degrade EtOH, the ability to degrade acetic and propionic acids was irreversibly inhibited. The inhibition of PCE degradation, however, was transient. After the culture regained the capacity to degrade PCE, it did not regain the capacity to degrade acetic and propionic acid in the presence of CF. This gives some insight to the bacteria responsible for PCE degradation. Aceticlastic methanogenic and the propionate consuming acetogenic organisms, the organisms responsible for degrading acetic and propionic acids, are very likely not directly responsible for PCE degradation in this system as they remained inhibited after PCE degradation resumed.

The initial acclimation to CF required the longest amount of time compared to the subsequent additions of CT and also exhibited the most pronounced inhibition effect of
PCE degradation. Once the culture became acclimated to the presence of CF, an increase in the CF concentration had a less pronounced effect, indicating that once a culture adapts to a situation further adaptation may be more rapid.

The initial concentration of CF may also play an important role in the ability of a culture to degrade PCE. In the microcosm study the culture was exposed to a high concentration of CF (up to 1.50 mg/L) and was unable to regain PCE degradation capacity within 160 days (degradation capacity was regained after purging). In the column CF concentration was much lower, initially 0.15 mg/L and acclimation occurred within one month. The relationship between initial concentration and acclimation period has not been identified, other than an increased CF concentration may require a longer acclimation period. It is not known whether acclimation is at all possible for initially high CF concentrations or how long is required if acclimation is possible. However the fact that 5 mg/L CT seemingly totally inhibited PCE degradation in microcosms is not necessarily an indication of a similar event in a column.

5.3 CT Degradation

CT degradation in both microcosm and column studies occurred without delay, with CT degraded to CF, DCM and CO₂. CF degradation occurred slowly in microcosms and CF degradation in the column was undetectable. CF recalcitrance in the column cannot be due to a lack of electron donor. While CF seemingly inhibited the degradation of acetic and propionic acids, it should be assumed that electrons were available since PCE degradation was occurring throughout the column. The recalcitrance of CF must be due to a different reason, such as the electron donor, EtOH or most likely a lack of
reaction sites. CF is an inhibitor to anaerobic systems and CF tends to deactivate reaction sites (Bagley et al., 1995). In contrast to EtOH degrading systems, methanol degrading organisms produce more coenzymes (F₄₃₀ and cobalamins) resulting in increased available reaction sites for CF degradation (Krone et al., 1989; Wolfe, 1996).

5.4 The Role of Yeast Extract

Yeast extract has been shown to decrease the acclimation time for PCE degradation (Lalonde, 1997). YE also acts as a carbon source and as a source of reducing equivalents for dechlorination (Fennell et al., 1997). H₂ is thought to be the ultimate electron donor for dechlorination. YE contains several VFA’s including butyric acid, which is readily degraded at H₂ partial pressures below 10⁻⁴ atm providing reducing equivalents. It is also thought dechlorinators can out compete methanogens at low partial pressures indicating if the partial pressure can be kept low enough, the available reducing equivalents can be used for their intended purpose, dechlorination. In this column study the bacteria that degrade acetic and propionic acids were inhibited resulting in no reducing equivalents available from acetic and propionic acids. In a bottle study YE alone provided the reducing equivalents for PCE degradation and we can infer that YE also played a role in PCE degradation in the column before and after the addition of CT.
6.0 CONCLUSIONS and RECOMMENDATIONS

1. CF has an inhibitory effect on the degradation of PCE. This inhibition is temporal.

2. The initial inhibition of PCE caused by CF has the most pronounced effect compared to subsequent increases in CF concentrations.

3. The organisms responsible for the degradation of acetic acid, (aceticlastic methanogenic organisms) and propionic acid, (propionate-consuming acetogenic organisms) are not responsible for the degradation of PCE in this system.

4. The presence of PCE has no measurable effect on the degradation of CT.

5. Yeast extract likely served as a slow release, source of reducing equivalents for reductive dechlorination in the column, especially in the presence of CF.

Some interesting results have been obtained in the present work. However, more work is needed, to further identify the role of YE in the degradation of PCE in the presence and absence of CF.

The effect of CF on the degradation of PCE has been shown to an extent. However, the question remains is there an upper limit of CF to which a PCE acclimated culture can acclimate to CF and regain its PCE degradation capacity? Also can a culture previously not acclimated to PCE, acclimate to both PCE and CF?
APPENDIX A

Composition of Basal Medium

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity (per 20 L)</th>
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</thead>
<tbody>
<tr>
<td>NH₄Cl</td>
<td>1.20 g</td>
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<tr>
<td>K₂HPO₄</td>
<td>0.40 g</td>
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<tr>
<td>CaCl₂·2H₂O</td>
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<td>MgCl₂·6H₂O</td>
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<td>FeSO₄·7H₂O</td>
<td>0.30 g</td>
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<tr>
<td>Resazurin</td>
<td>0.02 g</td>
</tr>
<tr>
<td>Trace Metal Solution</td>
<td>200 mL</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>2.00 g</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>20.00 g</td>
</tr>
<tr>
<td>Na₂S·9H₂O</td>
<td>0.60 g</td>
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</table>

Procedure
- Add 20L distilled water and purge with nitrogen for 20 minutes
- During purging add first seven items
- Add the remaining three items after purging is complete
- Switch N₂ to N₂/CO₂ and purge for an additional 10 minutes
- Add HCl/NaOH to achieve pH of 7
- Tighten stopper with clamp

Trace Metal Solution
To 1 L of water add 0.086 g MnSO₄·H₂O; 0.17 g CoCl₂·6H₂O; 0.21 g ZnSO₄·7H₂O; 0.019 g H₃BO₃; 0.05 g NiCl₂·6H₂O; 0.02 g Na₂MoO₄·2H₂O; 0.5 g Nitrilotriacetic Acid.
APPENDIX B

Column Pressure Data

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All values are in PSI.
REFERENCES


