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SEQUENTIAL TRANSFORMATION OF CARBON TETRACHLORIDE, PERCHLOROETHYLENE, AND 1,1,1-TRICHLOROETHANE USING METHANOGENIC AND METHANOTROPHIC CONDITIONS

by

Sarah Jane Christ

A thesis submitted in partial fulfillment
of the requirements for the Master of
Science degree in Civil and Environmental Engineering
in the Graduate College of
The University of Iowa

July 1996

Thesis supervisor: Professor Gene F. Parkin

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

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has been approved by the Examining Committee for the thesis requirement for the Master of Science degree in Civil and Environmental Engineering at the July 1996 graduation.

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ABSTRACT

A methanogenic-methanotrophic system has been established to assess the capability of sequential treatment to transform the chlorinated aliphatics perchloroethylene (PCE), carbon tetrachloride (CT) and 1,1,1-trichloroethane (1,1,1-TCA). Experiments compared the transformation of PCE alone and in a mixture of the target compounds. A comparison of acetate and iron as electron donors was also made. Data from the anaerobic systems have shown that PCE transformation is inhibited when in a mixture with CT and 1,1,1-TCA compared to when it is fed alone. When iron is the electron donor, PCE and 1,1,1-TCA are transformed without an acclimation period. CT was completely transformed regardless of the electron donor used. The acetate-fed system required approximately 60 days to acclimate to 1,1,1-TCA and 70 days for acclimation to PCE. Although the sequential system has been constructed, sampling from the aerobic column was not done before the thesis project ended. More investigation is required to determine the ability of the sequential system to degrade PCE, CT, and 1,1,1-TCA.

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CHAPTER 1
INTRODUCTION AND OBJECTIVES

Chlorinated Aliphatics

Chlorinated aliphatic hydrocarbons (CAHs) are pollutants of major concern today. Primarily used as industrial solvents, they are components of landfill leachate, hazardous waste sites, and industrial wastewaters, all of which pose a serious threat to surface and ground water systems. It is estimated that there are at least 36,000 sites contaminated with CAHs today, and 1290 of these are on the National Priorities List (NIOSH, 1996). To compound the problem, CAHs exist in subsurface material in many phases. Most are dense, non-aqueous phase liquids (DNAPLs), which sink below the water table to the lowest level of an aquifer. As the DNAPL passes through the ground water, a portion dissolves. Additionally, some will sorb to soil material, and some will volatilize and exist as a gas in the vadose-zone soil air. This multiplicity of phases necessitates the use of a variety of technologies to remediate a contaminated site.

Health Effects

Perchloroethylene (PCE), carbon tetrachloride (CT) and 1,1,1-trichloroethane (1,1,1-TCA) are among the most common CAH contaminants in the United States today. All have been used as solvents in the dry cleaning industry and in other industrial processes. CT was also used as a fire extinguishing agent and an insecticide. 1,1,1-TCA was one of the most widely

used solvents due to its high solvency and low flammability. It is estimated that over 50,000 workers are occupationally exposed to such industrial CAHs and that more that one million people may be exposed to surface and ground water or ambient air concentrations (NIOSH 1978).

PCE and CT are suspected carcinogens (OSHA). Although not suspected to be a carcinogen, 1,1,1-TCA depletes the ozone layer, and is a strong narcotic and depressant of the central nervous system. In industrial settings, direct hand contact with 1,1,1-TCA has reportedly caused distal paresthesias (prolonged numbness of extremities) and peripheral neuropathy (House et al. 1996). Volatilized PCE has been shown to cause acute hepatotoxicity in humans at levels higher than 100 ppm (Hake and Stewart, 1977) and may cause liver damage at airborne levels as low as 16 ppm (Brodkin et al. 1995). This amount of PCE is lower than the Occupational Safety and Health Administration (OSHA) limit of 100 ppm, and indicates the hazard associated with exposure to such PCE and other CAHs even at very low levels.

Chronic effects of all three compounds include liver and kidney damage, and heart disorders. Acute effects include dizziness, irregular heart beat, liver and kidney damage, eye and lung irritation, and fetal disorders. Ecological concerns include a low growth rate in plants, fish kills and other widespread animal deaths, along with shortened lifespan, low birth rates, a change in appearance and behavior of animals, and a potential for bioaccumulation (OSHA). Appendix A.1 contains important data on PCE, CT, 1,1,1-TCA and many of their metabolites.

Current and Future Treatment Technologies

Soil vacuum extraction (SVE) is commonly used to remove CAH vapor from the vadose-zone. Air stripping techniques and granular activated carbon adsorption (GAC) are employed to clean contaminated ground water. These processes do not provide a terminal solution to the contaminant problem; rather, CAHs are transferred to the another phase, unless the waste gas is first treated by GAC, catalytic combustion, or incineration (Wilson, 1994). Furthermore, these strategies can only gradually remediate a large contaminant plume. As the subsurface water is cleaned, more contaminant will desorb off soil material or dissolve out of the NAPL phase, thus recontaminating the water. The NAPL phase can be removed using deep well fracturing, but the process is very difficult, since this phase exists at the deepest aquifer locations, and is very hard to locate. Furthermore, once the pure product is recovered, a disposal problem still exists.

Bioremediation, which uses indigenous or engineered microorganisms to degrade contaminants, offers the potential for complete destruction to inert products. Several emerging technologies demonstrate the viability of microbes to remediate contaminated sites *in-situ*. In field-scale applications, bioventing supplies indigenous, vadose-zone microorganisms with necessary oxygen via injection wells (Wilson, 1994). An extraction well recovers any volatilized CAHs, and reinjects them into the ground for further transformation. Another field-scale study successfully stimulated indigenous methanotrophic and phenol-utilizing populations by injecting dissolved oxygen (DO) and methane or phenol to the biostimulation zone (Roberts et al., 1990 and Semprini et al., 1991). Microbes cometabolized several CAHs,

including vinyl chloride (VC), *cis*- and *trans*-1,2-dichloroethane (1,2-DCE), and trichloroethylene (TCE).

Although field-scale studies indicate the feasibility of bioremediation to treat contaminated sites, much more information is necessary before treatment schemes can be expanded to full-scale operations (McCarty and Semprini, 1994).

Sequential Systems

Sequential treatment of industrial wastewaters has gained appeal as an effective process over the past decade (Zitomer and Speece, 1993). Anaerobic pretreatment has been recognized as a capable unit operation for high-strength waste streams. Other specialized sequences effectively enhance sludge settling or promote nitrogen and phosphorus removal. Recently, it has been demonstrated that sequential systems are also capable of mineralizing highly chlorinated compounds. Highly chlorinated aliphatics can be transformed to less chlorinated metabolites in an anaerobic step, which can then be mineralized in an aerobic step. Appendix A.1 shows the aerobic and anaerobic potential of PCE, CT, 1,1,1-TCA and many of their metabolites of interest.

Suspended growth reactors demonstrated the degradative capacity of a sequential, anaerobic-methanotrophic system (Long et al., 1993). The effluent from an anaerobic batch reactor was fed to an aerobic batch reactor to produce sequential conditions. CT, PCE, and hexachloroethane (HCA) were transformed in the anaerobic reactor. VC, DCM, and *cis*-1,2-dichloroethylene (c-1,2-DCE) were detected in the anaerobic effluent, and were subsequently degraded in the aerobic reactor.

A two-stage biofilm successfully degraded a mixture of hexachlorobenzene (HCB), PCE, and CT. Conversion to carbon dioxide was 23, 49, and 61% for HCB, PCE, and CT, respectively (Fathepure et al., 1993). A multiple-electron acceptor biofilm column, using aerobic, denitrifying, and sulfate-reducing zones, removed more than 99% of CT and 56% of 1,1,1-TCA from a mixture of twelve halogenated aliphatics and benzenes (Cobb and Bouwer, 1991).

Hypothesis and Objectives

The hypothesis of this research is that a sequential system could successfully mineralize a mixture of PCE, 1,1,1-TCA, and CT. These parent compounds, under anaerobic conditions, should be transformed into lesser chlorinated metabolites, which should in turn be mineralized under aerobic conditions. The general idea is to use the methane produced from an anaerobic column as the energy source for methanotrophic bacteria. One way to do this would be to feed acetate to the anaerobic column. Another strategy would be to use elemental iron as a support media for the anaerobic column. Research in our laboratory (Weathers, 1995) has shown that when the iron is oxidized, hydrogen is produced, which will serve as the energy source for methane-producing bacteria.

The specific objective of this research were to:

1. Establish three sequential, biofilm-column systems consisting of an anaerobic column containing glass beads and fed acetate or containing iron, and an aerobic column fed the effluent from the anaerobic column and H_2O_2 as an oxygen source;

2. Assess the effect of a mixture of CAHs on the removal of an individual CAH by feeding PCE alone to one glass-bead-column system and a mixture of PCE, CT, and 1,1,1-TCA to the other glass-bead-column system;
3. Compare acetate-fed systems to iron-containing systems;
4. Identify to the extent possible the products of transformation of PCE, CT, and 1,1,1-TCA, and attempt to do mass balances on the systems;
5. Assess the strategy of controlling the acetate fed to the anaerobic column as a means of controlling the CH_4 fed to the aerobic column.

CHAPTER 2
LITERATURE REVIEW

Overview: PCE, CT, and 1,1,1-TCA

PCE, CT, and 1,1,1-TCA, along with their various metabolites, comprise the bulk of CAHs that have been used extensively in the past in industrial and commercial applications. Due to improper storage and disposal techniques, theses compounds are now a predominant contaminant in ground and surface water environments. In subsurface environments, these compounds can be transformed into a number of lesser-chlorinated metabolites. PCE dechlorinates to trichloroethylene (TCE), *cis*- and *trans*-1,2-dichloroethylene (*cis*- and *trans*-1,2-DCE), 1,1-dichloroethylene (1,1-DCE), vinyl chloride (VC), and ethylene. CT is reduced to chloroform (CF), dichloromethane (DCM), and chloromethane (CM). 1,1,1-TCA is reduced to 1,1-dichloroethane (1,1-DCA), and chloroethane. Additionally, 1,1,1-TCA can be transformed abiotically to acetic acid (HAc). Pertinent data for these compounds, including their solubilities and maximum contaminant limits (MCL's) are provided in Table A.1. Figure 2.1 summarizes some common pathways of transformation (Vogel et al., 1987; McCarty and Semprini, 1994).

Transformation Reactions

There are two basic categories of transformation: those that require electron transfer to complete the reaction (oxidation and reduction) and those that do not (substitution or dehydrohalogenation). Substitution and

dehydrohalogenation are more common in abiotic environments; reduction and oxidation are usually biologically mediated in natural systems (Vogel et al., 1987). Although abiotic reactions are generally much slower than biotic, both are important in ground water systems where flowrates are slow and residence times range on the order of years to decades (Schnoor, 1995). Biotic reactions occur more rapidly, but are often nutrient and substrate limited in the subsurface.

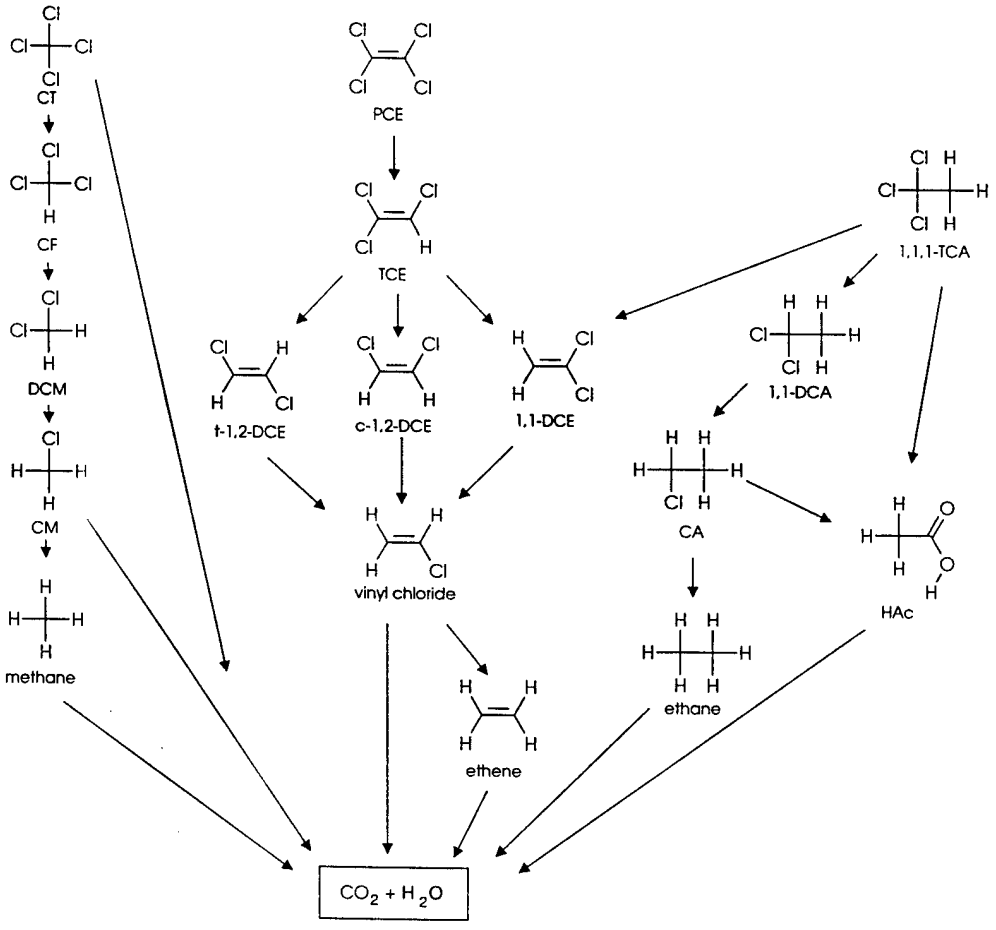

Figure 2.1. Common Pathways of CAH Mineralization (after Vogel et al., 1987).

Substitution

Substitution is the removal of a molecular substituent via a nucleophilic attack on an organic bond. In aquatic environments, water is generally the nucleophile; however, OH^-, NO_3^-, SO_4^{2-}, HS^-, HCO_3^-, and HPO_4^{2-} may also be substituted (Sawyer et al., 1994). Halogenated aliphatic compounds have been shown to undergo substitution in water (hydrolysis) in the absence of catalysts (Vogel et al., 1987). When a CAH is hydrolized, an alcohol is formed and hydrogen chloride is released:

$$R\text{–}CH_2Cl + H_2O \rightarrow R\text{–}CH_2OH + HCl \qquad (1\text{-}1)$$

The alcohol can then be further transformed through other reactions. Substitution becomes more likely with a smaller number of chlorine atoms on the molecule: a dichlorinated alkane is more likely to undergo substitution than a trichlorinated alkane, and so on (Vogel et al., 1987)

Dehydrohalgenation

Dehydrohalogenation is an elimination reaction that is common for saturated CAHs. It is characterized by the release of HX (where X is the halogen atom) and the formation of a carbon-carbon double bond. For CAHs, hydrogen chloride is a product of the reaction:

$$CCl_3CH_3 \rightarrow CCl_2{=}CH_2 + HCl \qquad (1\text{-}2)$$

The oxidation states of the carbon atoms involved in the reaction change: the oxidation state decreases with the loss of the halogen, and increases with the loss of hydrogen. Therefore, there is no net change of oxidation state for the molecule, and the reaction is considered an elimination.

Dehydrohalogenation becomes more likely with a greater number of chloride atoms on the molecule. A trichlorinated compound is more likely to be transformed by this mechanism than a dichlorinated compound. Monohalogenated compounds do not undergo dehydrohalogenation (Vogel et al., 1987).

Oxidation and Reduction

Oxidation and reduction reactions utilize the transfer of electrons to transform organic compounds. Common environmental electron acceptors include O_2, NO_3^-, SO_4^{2-}, and CH_4. Transition metals, such as manganese, nickel, iron, and cobalt also make excellent electron acceptors and donors. Photochemical oxidants, such as hydrogen peroxide or ozone, cause rapid oxidation of CAHs when added to ground water systems.

Primary Substrate Utilization

Biotransfomation usually occurs through two different processes: CAHs may be used as primary substrates to supply microbes with carbon and energy required for growth, or they may be cometabolized (McCarty and Semprini, 1994). Recent studies have also shown that new types of microbes have evolved that are capable of utilizing PCE and TCE as terminal electron acceptors (Hollinger et al., 1993). Use as a primary substrate is the normal pathway for organic decomposition in nature, and is applied in treatment

schemes for municipal and industrial wastewater. Few CAHs, however, have been shown to serve as primary substrates for many microbial populations. DCM is one of the few compounds that can be utilized, and has been shown to be mineralized under anaerobic or aerobic conditions by typical municipal digester sludge microorganisms (Freedman and Gossett, 1991; Rittmann and McCarty, 1980) or by pure cultures of *Pseudomonas* and *Hyphomicrobium* (Brunner et al. 1980). Additionally, VC has been utilized by microbes under aerobic conditions (Hartmans and de Bont, 1992). Current literature indicates that the only CAHs metabolized as primary substrates are lesser-chlorinated one- and two-carbon compounds. Furthermore, the organisms that are capable of CAH primary substrate utilization may not be ubiquitous in the environment. It appears that cometabolism, not primary substrate utilization, is the major biological transformation pathway for CAHs in the environment.

Cometabolism

Cometabolism is the fortuitous transformation of organic compounds in the presence of another organic compound which is used as the primary energy source (Brock et al., 1994). Microbes express enzymes with loose substrate specificity that catalyze degradation reactions (Vogel and McCarty, 1987). Microbes do not benefit from cometabolism, and may even produce a by-product that is toxic. For cometabolism to occur, an active culture that is capable of expressing the necessary enzymes and cofactors must be present. The proper substrate must be present in order for microbes to express such enzymes. Additional nutrients must be available to sustain growth of the microbial population.

Several different enzyme systems catalyze transformation of CAHs via oxidation and reduction. Oxygenase enzymes systems catalyze the oxidation of CAHs. For example, monooxygenase enzyme systems containing cytochrome P_{450} are common in mammals (Sligar and Murray, 1986). Methanotrophic bacteria contain methane monooxygenase (MMO), which catalyzes the oxidation of some CAHs. Several organisms contain toluene dioxygenase (TDO) or toluene monooxygenase (TMO) enzyme systems, which are induced by a variety of substrates (toluene, benzene, phenol). Nitrifying bacteria contain ammonia monooxygenase (AMO). CAHs can also serve as electron acceptors (transformation by reduction) in anaerobic environments. For example, dehalogenations may be mediated by glutathione (Bonse et al., 1975), or haloperoxidase enzymes. Methanogens do not contain cytochrome P_{450}; instead, the have a nickel-containing enzyme and/or cofactors like F_{430} used for anaerobic reduction.

When CAHs are transformed biologically, complete mineralization is not often obtained. For transformation to inert end products to occur, other reactions, both abiotic or biotic, are necessary. Aerobic biotic reactions are generally hydroxylations, where an OH^- group is substituted onto the molecule, or epoxidations, where an oxygen atom is incorporated into an unsaturated carbon-carbon bond. Anaerobic biotic processes are usually hydrogenolysis reactions, where a chlorine is replaced by a hydrogen, or dihaloelimination, where two adjacent chlorine atoms are removed and a double bond is formed between the carbon atoms (Vogel et al., 1987; McCarty and Semprini, 1994).

Anaerobic Transformation

Bouwer et al. (1981) demonstrated that one- and two-carbon CAHs could be successfully degraded under methanogenic conditions. Further study indicated that such low-molecular-weight compounds could be transformed at trace concentrations, and that cultures had the ability to acclimate in the presence of potentially inhibitory or toxic compounds like CT. Reductive dehalogenation was suggested at the primary transformation pathway for CT to CF, DCM, and CO_2 (Bouwer and McCarty, 1983).

The fate of 1,1,1-TCA has also been investigated by many researchers. 1,1,1-TCA can be abiotically transformed, either to 1,1-DCE via dehydrohalogenation, or to acetic acid through hydrolysis. Once 1,1-DCE is formed, it can undergo reductive dehalogenation to VC, and subsequent transformation to ethylene and CO_2. 1,1,1-TCA can be reductively dehalogenated to 1,1-DCA, and subsequently, CA, and then abiotically hydrolyzed to ethanol, which is readily mineralized (Vogel and McCarty, 1987). The abiotic steps may be very slow, with half-lives ranging from months to years (Vogel et al., 1987; Cline and Delfino, 1989).

The transformation of chlorinated ethylenes, such as PCE, TCE, and further metabolites, has also been studied extensively. PCE may be reductively dechlorinated to TCE, and subsequently to *cis*- and *trans*-1,2-DCE. Both dichlorinated isomers can be transformed to VC which is mineralized to CO_2 and ethylene (Freedman and Gossett, 1989; DiStefano et al., 1991; Bouwer and McCarty, 1983). Studies indicate that the formation of VC is the rate limiting step; VC accumulates and may have a toxic effect on microbes.

A diverse consortia of microorganisms exist in a symbiotic relationship to produce methanogenic conditions. Acetogens transform the primary electron donors (such as methanol, ethanol, lactate, benzoate, etc.) to acetate and hydrogen, a reaction that is not thermodynamically favorable. Hydrogenotrophic methanogens consume hydrogen and CO_2 to produce methane. Aceticlastic methanogens consume acetate to produce methane. In early studies (Bouwer et al., 1981; Bouwer and McCarty, 1983; Brunner et al., 1980), methanogenic mixed cultures transformed CAHs at low levels. Acclimation seemed to have a positive effect on the ability to transform CAHs. High concentrations of PCE seemed to inhibit methanogenesis, and acetogenesis became the predominant pathway for PCE dechlorination (DiStefano et al., 1991). It has been suggested that hydrogen is the primary energy source that many dechlorinating microbes use, and that electron donors which can contribute to hydrogen production should be used to dechlorinate large amounts of PCE and other chlorinated ethylenes (DiStefano et al., 1992). In other words, a portion of the hydrogen produced by acetogenic organisms is diverted to dechlorination, not methanogenesis.

When four different electron donors (methanol, ethanol, lactic acid, and butyric acid) were compared for their ability to sustain anaerobic reductive dechlorination, butyric acid was the most effective dechlorinating substrate and methanol the worst (Fennel et al., 1995). Methanol stimulated methanogenesis, which apparently resulted in less complete dechlorination. A separate study observed only minimal methane production in a glucose-fed culture that transformed TCE to ethylene (Wild et al., 1995). Incidentally, when 1,1,1-TCA was added along with TCE, both acetogenesis and

methanogenesis were inhibited. Since methanogenic mixed cultures have been shown to transform 1,1,1-TCA (Vogel and McCarty, 1987; Egli et al., 1987), an inhibitory effect due to the interaction of the two compounds may have occurred.

Studies indicate that there may be two different pathways for PCE transformation in mixed cultures. Acetogenesis may supply hydrogen to dechlorinating microorganisms that compete with hydrogenotrophic methanogens; or, a methanogenic consortia may reductively dehalogenate CAHs after an adaptation period.

Table 2.1 shows the half reactions for some common electron donors, including hydrogen. Although hydrogen is one of the most thermodynamically favorable energy sources for methanogenesis, its practical use is hampered by its low solubility, which is approximately 1.6 mg/L at 20°C, based on a Henry's constant of 6.83×10^4 atm/mol (Metcalf and Eddy, 1991). An effective method of making hydrogen available for biological use is by using zero-valent metals.

Use of Elemental Iron as an Electron Donor

Daniels et al. (1987) demonstrated that methanogens can use iron (Fe^0) as an energy source for growth. When Fe^0 is immersed in water, it is oxidized; subsequently, water-derived protons are reduced, forming a layer of hydrogen on the surface of the iron. Methanogens consume this hydrogen, catalyzing the oxidation of even more metal:

$$4Fe^0 + 8H^+ \rightarrow 4Fe^{2+} + 4H_2$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

$$4Fe^0 + 8H^+ + CO_2 \rightarrow 4Fe^{2+} + CH_4 + 2H_2O$$

Other metals that may be good electron donors for methanogenesis are aluminum (Al^0) and zinc (Zn^0); however, zinc (Zn^0), copper (Cu^0), nickel(Ni^0), and cobolt (Co^0) may be inhibitory to some methanogens (Belay and Daniels, 1990).

Table 2.1. Half Reactions for Electron Donors of Interest.

Electron Donor	$\Delta G°(W)$ kcal/equiv.
Acetate	-6.609
$\frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O \rightarrow \frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^-$	
Ethanol	-7.592
$\frac{1}{12}CH_3CH_2OH + \frac{1}{4}H_2O \rightarrow \frac{1}{6}CO_2 + H^+ + e^-$	
Lactate	-7.873
$\frac{1}{12}CH_3CHOHCOO^- + \frac{1}{3}H_2O \rightarrow \frac{1}{6}CO_2 + \frac{1}{12}HCO_3^- + H^+ + e^-$	
Methanol	-8.965
$\frac{1}{6}CH_3OH + \frac{1}{6}H_2O \rightarrow \frac{1}{6}CO_2 + H^+ + e^-$	
Hydrogen	-9.670
$\frac{1}{2}H_2 \rightarrow H^+ + e^-$	

Source: Sawyer, C. N., P. L. McCarty, and G. F. Parkin. "Chemistry for Environmental Engineers." McGraw-Hill, Inc. New York, 1994, 4th ed.

Elemental metals have also been used abiotically to dechlorinate CAHs. Helland et al. (1994) demonstrated that CT can be dechlorinated to CF and DCM by zero-valent iron under anoxic and oxic conditions. Gillham and O'Hannesin (1994) showed no appreciable degradation of DCM, although they did observe transformation of thirteen other CAHs, including CT, CF, PCE, DCE-isomers, and VC. With each successive dehalogenation, subsequent transformations proceeded more slowly.

There are three major reduction pathways in an Fe^0-H_2O system. Electrons may be transferred directly from the Fe^0 surface to the adsorbed CAH (oxidative dissolution of Fe^0). Ferrous iron, produced through corrosion, is also capable of causing dehalogenation. Hydrogen, also a product of corrosion, can cause dehalogenation, but requires a catalyst to be effective. It has been suggested that oxidative dissolution is the dominant pathway for reductive dehalogenation (Matheson and Tratnyek, 1994). It may be possible, then, to sustain a methanotrophic culture with the H_2 produced from corrosion of Fe^0 and still achieve abiotic reductive dehalogenation without competition between abiotic and biotic mechanisms for corrosion products.

Aerobic Transformation

Although aerobic transformation of lesser-chlorinated CAHs has been accepted for quite some time, it is a recent discovery that more highly-chlorinated compounds, such as TCE, are also susceptible to biological oxidation by organisms that utilize methane for growth (Wilson and Wilson, 1985). Methane monooxygenase (MMO) enzymes, which have a relaxed substrate specificity, normally oxidize methane to carbon dioxide through the following pathway:

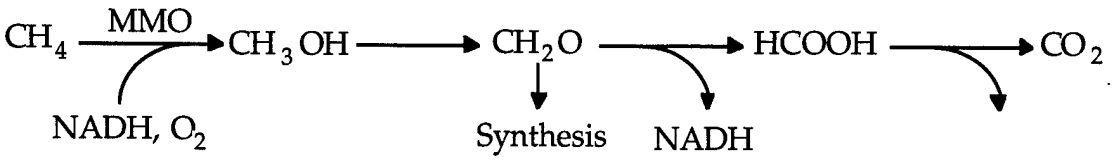

Figure 2.2. Methane Oxidation Via MMO (after McCarty and Semprini, 1994).

MMO also oxidizes TCE to form an epoxide (Little et al., 1988; Alvarez-Cohen and McCarty, 1991a,b), which is very unstable, and can be further transformed to a variety of compounds, including carbon dioxide, carbon monoxide, and many acids (Miller and Guengerich, 1982). Oxygenase enzymes have to potential to initiate transformations of most CAHs, the primary exceptions being CT and PCE (McCarty and Semprini, 1994). While unsaturated carbon chains form epoxides via oxidation, saturated chains gain hydroxyl groups, resulting in unstable products that are easily degraded.

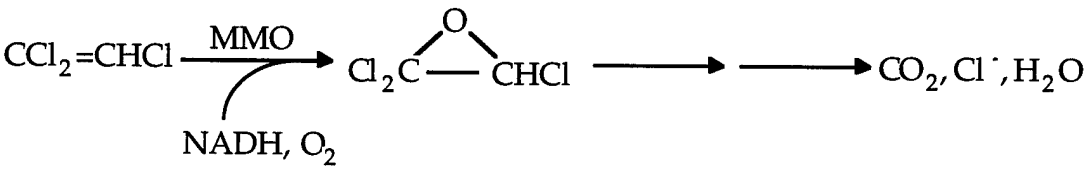

Figure 2.3. TCE Epoxidation Using MMO (after McCarty and Semprini, 1994).

Other studies have shown similar TCE transformation using propane oxidizers (Wackett et al., 1989), toluene oxidizers (Nelson et al., 1987; Wackett and Gibson, 1988; Arcangeli et al., 1995), and ammonia oxidizers (Arciero, 1989).

Archangeli et al. (1995) demonstrated a cross-inhibition phenomenon between toluene and TCE, where the presence of toluene affects the kinetics of TCE degradation, and vice versa. Semprini et al. (1991) showed the same phenomenon between TCE and methanotrophic organisms. Competition for the same enzyme to degrade TCE and the primary substrate (competitive inhibition) may explain this interaction. If competitive inhibition is a major mechanism in aerobic degradation, then more knowledge is needed to determine optimum concentrations of primary substrate to maximize CAH transformation.

Inhibition and toxic effects may also be caused by by-products of transformation. TCE and chloroform (CF) by-products significantly decreased transformation rates of the parent compounds in methanotrophic resting cells (Alvarez-Cohen and McCarty, 1991), even thought parent compounds did not have a toxic effect. In mammalian systems, TCE epoxides bind irreversibly to DNA (Henschler, 1985), resulting in toxic effects.

Mixtures

Little is known about the possible inhibitory effects caused by mixtures of CAHs, or due to the build up of a mixture of metabolites. TCE and CF by-products significantly decreased transformation rates of the parent compounds in methanotrophic resting cells (Alvarez-Cohen and McCarty, 1991), indicating that transformation products can produce a toxic effect on the microbial community even when the parent compounds do not. Other studies have shown that VC may only be transformed in methanogenic systems when all of the parent compound, PCE, has been degraded (Tandol et al. 1994). Thus, the parent compound or a by-product may exhibit inhibitory

effects upon overall degradation rates. Additionally, a one-carbon compound, such as CF, may inhibit transformation of a two-carbon compound. The presence of DCM and CF have been shown to decrease the degradation of TCA (Hughes and Parkin, 1991).

There may also be positive interactions in mixtures. Hughes and Parkin (1992) demonstrated enhanced CF transformation when fed along with DCM. Also, more rapid acclimation to and faster transformation of DCM occurred when it was fed in a mixture along with CF and TCA.

The effects of mixtures is an area of research that has not been explored to a great extent. Since many contaminated sites contain mixtures of chlorinated compounds, more knowledge is required to understand the nature of their interrelations.

Sequential Systems

Table A.1 summarizes the transformation potential of CT, PCE, and 1,1,1-TCA and their metabolites under aerobic and anaerobic conditions. Upon close examination of this table, a general trend becomes clear. The more highly-chlorinated a compound is, the more amenable it is to anaerobic degradation (reduction). Likewise, the fewer chlorine atoms on the CAH, the more amenable it is to aerobic degradation (oxidation). Therefore, interest has grown regarding the feasibility of sequential anaerobic/aerobic systems to completely mineralize highly chlorinated CAHs.

Research to date indicates the potential of sequential systems. In a sequential biofilm study, PCE was reductively dechlorinated by a methanogenic consortia to *cis*-1,2-DCE and small amounts of TCE, chloroethane, and chloride (Gerritse et al., 1995). Subsequently, *cis*-1,2-DCE

was transformed by methanotrophic bacteria. Chlorinated ethylene disappearance ranged between 86% and 100%. Another study reported successful degradation of a mixture of hexachlorobenzene (HCB), PCE, and CT when introduced with acetate as a primary substrate under continuous flow conditions (Fathepure et al., 1993). Anaerobic columns were allowed a 4-6 week adaptation period after CAH addition began. Complete conversion to carbon dioxide was 23, 49, and 61% for HCB, PCE, and CT, respectively.

Summary

Evidence suggests that highly chlorinated CAHs, like PCE, CT, and 1,1,1-TCA, are converted under anaerobic conditions to less chlorinated products, such as TCE, 1,2-DCE, VC, CF, DCM, and 1,1-DCA, which tend to accumulate. There are some notable exceptions (DiStefano et al., 1991; Fennel et al., 1995; Tandol et al., 1994) where PCE has been completely transformed to ethylene using hydrogen as an electron donor. Unfortunately, studies have not confirmed complete mineralization of other CAHs, like CT and 1,1,1-TCA in anaerobic environments.

Under aerobic conditions, however, organisms containing oxygenase enzymes can rapidly mineralize lesser chlorinated metabolites. Thus, a sequential anaerobic-aerobic system has potential advantages for treatment of mixtures of highly chlorinated CAHs. This study was undertaken to provide information on the feasibility of such a system to transform, and possibly mineralize, a mixture of PCE, CT, and 1,1,1-TCA.

CHAPTER 3
MATERIALS AND METHODS

This chapter discusses the chemicals, procedures, and systems used to study the transformation of PCE, CT, and 1,1,1-TCA in a sequential methanogenic/methanotrophic system. All experiments were performed in biofilm columns which had been seeded from stock culture reactors maintained in our laboratory.

Reactor Systems

Three sequential, biofilm column systems were established to assess the transformation of PCE, CT, and 1,1,1-TCA. Each system consisted of two columns: a primary, anaerobic column containing a methanogenic enrichment, and a secondary aerobic column, containing a methanotrophic enrichment.

In the first system, termed System A, glass beads were the support media for biomass in both columns. Acetate was added as a carbon and energy source and PCE (≈ 5 μM) was added in the feed solution. The second system, System B, was identical to System A, except that a mixture of PCE, CT, and 1,1,1-TCA (≈ 5 μM of each) was added. In the third system, System C, steel wool replaced glass beads as the support media in the anaerobic column. Steel wool also provided Fe^0 as an energy source. A mixture of PCE, CT, and 1,1,1-TCA (≈ 5 μM of each) was fed to System C. H_2O_2 was used to supply oxygen to all aerobic columns. Table 3.1 summarizes the system conditions.

Table 3.1. System Conditions.

System	A	B	C
Anaerobic Column	media = glass beads e^- donor = acetate CAH = PCE	media = glass beads e^- donor = acetate CAH = PCE, CT, 1,1,1-TCA	media = steel wool e^- donor = Fe^0 CAH = PCE, CT, 1,1,1-TCA
Aerobic Column	media = glass beads O_2 source = H_2O_2	media = glass beads O_2 source = H_2O_2	media = glass beads O_2 source = H_2O_2

System Design

Figure 3.1 is a schematic of the column systems used in all experiments. Glass columns (1.5 in. O.D. x 12 in.) were constructed by the glass blower at the University of Iowa Chemistry Department by combining two threaded glass connectors (Ace Glass). Columns were filled with 3 mm borosilicate glass beads (Fisher Scientific) or steel wool (Rhodes/American, Chicago, IL) and sealed with threaded adapter plugs and FETFE O-rings (Ace Glass). Teflon PFTE tubing (Cole-Parmer) was used to minimize sorption. 100-mL glass syringes (SGE) on syringe pumps (Harvard Apparatus, #22) were used to add the feed solution. Syringes were connected to tubing using a female luer and 2-way switch valve (Hamilton). To ensure gas-tight connections, a flangeless ferrule/nut system (Fisher Scientific) was used to connect all tubing to valves and columns. Sample ports consisted of 2-mL glass in-line vials (Omnifit) and a 1/2 inch diameter Mininert valve (Supelco). H_2O_2 was added using 50-mL glass syringes (SGE) and a (Harvard

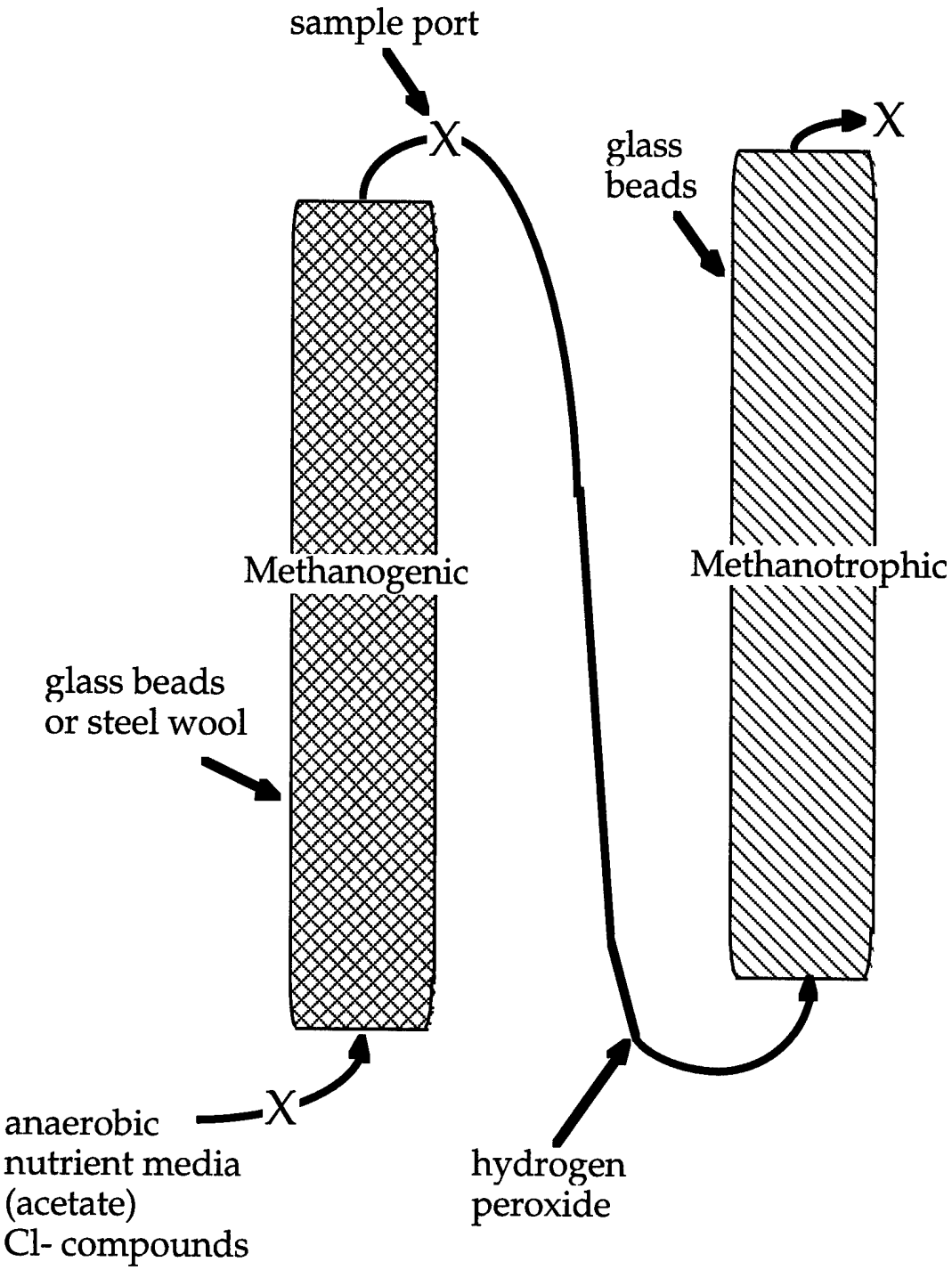

Figure 3.1. Experimental System Schematic (X = sample port).

Apparatus, #22) syringe pump. Syringes were connected to tubing in the same manner as the feed syringes. The H_2O_2 line was connected to the system between the two columns and after the mid-way sampling port using a 3-way fitting and flangeless ferruels (Cole-Parmer) to ensure a gas-tight connection.

The anaerobic nutrient feed solution was pumped to the anaerobic columns at a rate of 0.52 mL/hr. The H_2O_2 feed was 0.18 mL/hr, and the net flow rate through the aerobic columns was 0.70 mL/hr. The void volume of the glass bead columns before seeding with microbial cultures was 70 mL ± 2 mL. Based on the void volume, the maximum HRT for the anaerobic glass bead columns was 5.6 days and the maximum HRT for the aerobic glass bead columns was 4.2 days. The void volume of the steel wool column was 130 mL and the maximum HRT was 10 days. All systems were kept at a temperature of 20°C.

Culture Establishment

Before experiments could begin, healthy columns had to be established. All columns were filled with nutrient media, and then seeded by withdrawing 400 mL of liquid from stock culture reactors and injecting it into the columns. Columns were not disturbed for one day to allow biofilm growth to begin, and then nutrient media, including acetate to the acetate-fed column, was pumped into the systems.

Methane production was monitored for several months after the anaerobic columns were initially seeded. The columns reached steady state, producing approximately 15.0 mg/L CH_4 in the acetate-fed columns and 12.0 mg/L CH_4 in the iron-fed column, after nearly four months of operation. The biofilms might have reached steady-state sooner, but the systems were

severely shocked when the heating system in the laboratory failed, and the temperature dropped to 4°C for more than six hours. Acetate consumption was also monitored in Systems A and B; effluent acetate levels were below detection limits, indicating complete conversion of the primary substrate.

Stock Culture Reactors

Column systems were seeded from two stock cultures maintained in the laboratory. Methanogenic and methanotrophic cultures were grown and maintained in 9.5-L, magnetically stirred glass reactors. The methanogenic culture had been developed previously (Weathers, 1995). The reactor had a nominal cell suspension volume of 8 L and was operated at 20°C with an average 40 day HRT such that 400 mL of liquid was removed every other day and replaced with 400 mL fresh media. The methanotrophic culture was enriched from a 1 L sample taken from the Coralville Marsh, Coralville, IA. The sample was mixed with 1 L of aerobic media (Table 3.2) in the reactor. The headspace (7.5 L) was sealed from the outside atmosphere using two 1-L respirator bottles filled with 1 L of water. The headspace was flushed with 100% oxygen gas for 15 minutes. Methane gas (100%) was added to adjust the headspace gas concentration to 10%/90% methane/oxygen (Alvarez-Cohen and McCarty, 1991). Because the headspace is initially pressurized with methane and oxygen, all of the water is in the second respirator bottle. As CH_4 is consumed, CO_2 is produced. A vial at the top of the reactor holds a NaOH solution that scrubs CO_2, causing an overall drop in headspace volume. As the volume drops, the water level in the second respirator bottle drops and rises into the first bottle, indicating biological activity. The reactor was operated at 20°C on an average 12 day HRT by removing 500 mL of liquid

every third day and adding 500 mL of fresh media, which was buffered to 6.8 ± 0.1 using K_2HPO_4.

Feed Solutions

The methanogenic stock culture was maintained using Hawkeye media (Weathers, 1995). The methanotrophic stock culture was maintained using a nutrient solution from Fogel et al., (1986). A list of ingredients for each solution is shown in Table 3.2. In order to supply adequate nutrients to both the methanogenic and methanotrophic cultures, the sequential column system was fed Hawkeye media, supplemented by additional nutrients, based on Fogel et al. (1986), that were fed through the H_2O_2 line (Table 3.2). PCE, CT, and 1,1,1-TCA were added directly to the feed syringes from aqueous stock solutions.

The feed solution for Systems A and B was buffered with $NaHCO_3$ to a pH of 6.8 ± 0.1. Glacial acetic acid was added (420 mg/L) as the carbon and energy source. H_2O_2 was added (260 mg/L) to provide oxygen to the aerobic columns. This value was based on information obtained from literature (Morgan and Watkinson, 1992; American Petroleum Institute, 1987) and adjusted experimentally to compensate for peroxide spent oxygenating the anaerobic column effluents.

The feed to System C was buffered with $NaHCO_3$ to a pH of 6.2 ± 0.1. The pH was lower in the iron-fed column to compensate for a pH increase associated with the anaerobic oxidation of Fe^0 in water (Matheson and Tratnyek, 1994). Bicarbonate also served as the carbon source. H_2O_2 was added (260 mg/L) to provide oxygen to the aerobic column.

Chemicals and Stock Solutions

The parent CAHs used in this study were PCE, CT and 1,1,1-TCA (Fisher Scientific). Aqueous stock solutions were prepared by adding approximately 5 mL of each chemical to 25 mL of nano-pure water in 36-mL serum bottles sealed with Teflon-lined rubber septa and aluminum crimp caps. Methanol stocks were made of all target chemicals and suspected metabolites (Fisher Scientific). Suspected metabolites were trichloroethylene (TCE), *cis*- and *trans*-1,2-dichloroethylene (*cis*-- and *trans*-1,2-DCE), vinyl chloride (VC), chloroform (CF), dichloromethane (DCM), chloromethane (CM), 1,1-dichloroethane (1,1-DCA), 1,1-dichloroethylene (1,1-DCE), and chloroethane (CA). All aqueous and methanol stocks were stored at 4°C until ready for use. Other chemicals included glacial acetic acid (Malinkrodt), methane gas (Air Products, Inc.), oxygen gas (University of Iowa General Stores), steel wool (Rhodes/American, Chicago, IL). The chemical composition of steel wool, as reported by the manufacturer, was (in %): Fe (52), Si (30), C (16), Mn (1.25), P (0.7), and S (0.05).

Experimental Design

Experiments were divided into three phases: acclimation, anaerobic column studies, and sequential column studies. Target compounds were added to the feed solutions at approximately 5 μM-levels. Transformation was analyzed using GC headspace techniques. When all systems began transforming appreciable levels of the target compounds, the aerobic columns were connected.

Table 3.2. Nutrient Media.

Chemical (all values in mg/L)	Hawkeye Media (Weathers, 1995)	Aerobic Media (from Fogel et al., 1986)	Peroxide line supplement
NH_4Cl	400		2508
KCl	400		
$MgCl_2 \bullet 6H_2O$	400		
$Na_2S \bullet 9H_2O$	300		
$(NH_4)_2HPO_4$	80		
$FeCl_2 \bullet 4H_2O$	40	3.0	
$CaCl_2 \bullet 2H_2O$	25	20	175
$(NaPO_3)_6$	10		
Cysteine	10		
KI	2.5	40	
$CoCl_2 \bullet 6H_2O$	2.5	0.1	
$MnCl_2 \bullet 4H_2O$	0.5	0.02	
NH_4VO_3	0.5		
$ZnCl_2$	0.5	0.07	
$NaMoO_4 \bullet 2H_2O$	0.5	0.03	
H_3BO_3	0.5	0.02	
$NiCl_2 \bullet 6H_2O$	0.5	0.02	
$MgSO_4$		200	778
H_3PO_4		184	
$NaNO_3$		1000	
KH_2PO_4		160	622
Na_2HPO_4			716

Analytical Methods

Chlorinated Compound Analysis

PCE, CT, 1,1,1-TCA and their metabolites were detected using gas chromatography on an HP 5890A gas chromatograph equipped with HP Chemstation software. Higher-chlorinated compounds (PCE, TCE, CT, CF,

1,1,1-TCA, and 1,1-DCA) were detected using an electron capture detector (ECD), and lesser-chlorinated compounds (*cis*-and *trans*-1,2-DCE, VC, 1,1-DCE, DCM, CM, CA) were detected using a Flame ionization detector (FID). 1-mL samples were drawn from the sample ports and injected into 2-mL screw-top glass vials with open top caps and Teflon-faced silicone septa (Wheaton). Sample vials were inverted to minimize volatilization losses, and allowed to equilibrate for approximately 30 minutes before analysis. After equilibration, a 250-µL headspace sample was drawn using a Dynatech Series A Headspace Syringe (Supelco) and injected onto the GC.

The GC/ECD contained a 30 m capillary column with a 1.0 µm DB-5 stationary phase (Supelco). The carrier gas was N_2, at a flow rate of 2-3 mL/min. The ECD had a 60 mL/min N_2 make-up gas. The oven temperature was 35°C. The inlet temperature was 150°C and the detector temperature was 250°C.

The GC/FID was equipped with a 30 m, 0.53 mm ID capillary column (Supelco) with a DB-Wax stationary phase at a flow of approximately 12 mL/min N_2. The FID air flow rate was 300 mL/min, H_2 flow was 30 mL/min, and the make-up gas was 15 mL/min. The oven temperature was 35°C. The inlet and detector temperatures were 150°C and 250°C, respectively.

Unfortunately, the GC/FID analysis was hampered by the presence of methane. It was very difficult to qualify VC, CA, DCM, and CM, whose retention times were very close to methane. a new analytical technique may be necessary in order to quantify these lesser-chlorinated compounds.

Concentrations were quantified from a calibration curve of concentration versus peak area using a minimum seven external standards,

ranging from 1.0 to 1000 μg/L CAH. External standards were treated like samples. Detection limits were approximately 6.5 x 10^{-3} μM for PCE, CT, and 1,1,1-TCA, approximately 8 x 10^{-3} μM for TCE and CF, and approximately 10 x 10^{-3} μM for 1,1-DCA.

Methane Analysis

Methane concentrations were determined by GC/FID analysis. 1-mL samples were collected and analyzed in the same manner as for the chlorinated compounds. Concentrations were quantified from a calibration curve of concentration versus peak area using a minimum four external standards ranging from 1.04 to 10.4 mM CH_4. External standards were treated like samples. The detection limit for this method was approximately 0.625 mM.

pH

The pH of the influent solutions was adjusted before fed into the system. The pH of the anaerobic column effluent and the system effluent was monitored at least once per week using a Beckman model F 72 pH meter and combination electrode (Fisher Scientific).

Acetate

Acetate was measured using a Gilson HPLC system with Gilson 712 software control. Samples were filtered through a 0.45 μm filter and acidified with formic acid. A 2-μL sample was injected onto a PRP-x300 column (Hamilton) with a 0.001 M H_2SO_4 eluent at 1.0 mL/min utilizing UV detection at 210 nm. External standards were not prepared for this qualitative analysis. The detection limit was approximately 1 mg/L.

CHAPTER 4
RESULTS AND DISCUSSION

The goals of this research were to establish a series of sequential biofilm column systems and study the transformation of PCE, CT, and 1,1,1-TCA. The methanotrophic column in each system would utilize the methane in the anaerobic column effluent as a primary substrate. Acetate would be compared to elemental iron as an energy source. The impact of mixtures on the transformation of PCE would also be studied. Although the entire system has been established, the thesis project ended before all of the "bugs" had been worked out, and mineralization of CAHs has not been studied. This chapter summarizes the work that has been accomplished so far. Chapter 6 discusses the limitations of this project and recommendations for future work.

System Development and Acclimation

Before CAHs Were Added

After construction and seeding with the methanogenic enrichment culture, anaerobic columns were fed continuously without CAHs from July 15, 1995 until November 10, 1995 to allow them to reach steady-state with respect to methane production. Methane levels were monitored intermittently during this time, but data was overwritten on a computer spreadsheet and lost. On November 10, a constant-temperature control unit failed, causing the temperature to drop from 20°C to 4°C for more than six hours. Columns were allowed an additional two months of growth, until

January 14, 1996, before methane levels were measured again. Figure 4.1 shows methane concentrations before and after addition of CAHs began. Methane levels, recorded from January 14, 1996 to February 15, 1996 were 16 ± 1.9 mg/L for System A, 18 ± 5.8 mg/L for System B, and 11 ± 0.7 for System C. Appendix A.2 contains all methane data.

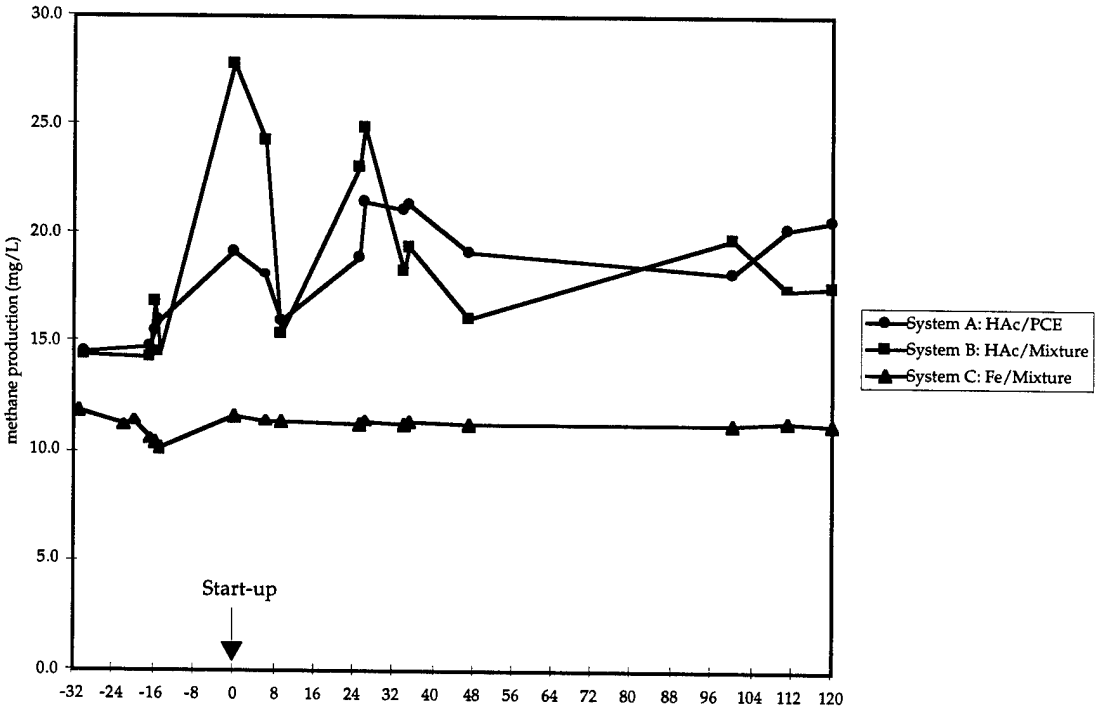

Figure 4.1. Methane Production by Anaerobic Columns.

After CAHs Were Added

CAHs were added to the feed solution on February 15, 1996, after a month of "steady" methane production. This is noted on Figure 4.1 as the Start-up date (day 0). On day 0, methane levels in the acetate-fed systems

increased noticeably. If anything, levels should have decreased. Hughes (1992) showed a decrease in acetate utilization which indicated a drop in methane production in a similar system when a mixture of CAHs was fed. Additionally, this fluctuation was not detected in the effluent from System C, which rules out sampling or analytical errors. It is possible that something in the feed to the acetate systems changed, which increased the production of methane. More acetate may have been inadvertently fed , for example, or the addition of CAHs may have altered the feed solution. It is also possible that the presence of CAHs in the column is causing the fluctuation. It is more pronounced in System B, which may indicate that the presence of a mixture has more of an effect than PCE alone. Finally, it is possible that, after columns were shocked on November 10, they had not quite reached steady-state again before CAH addition began.

Methane levels after CAHs were added were 20 ± 1.8 mg/L for System A, 20 ± 3.4 mg/L for System B, and 11 ± 0.1 mg/L for System C. Methane data are given in Appendix A.2.

Aerobic Columns

Two methanotrophic cultures were developed. The first was destroyed on September 5, 1995, when the reactor system failed, killing the entire population. A vial containing an NaOH solution, which scrubbed CO_2 from the headspace gas, contaminated the culture and raised the pH to 14. Biomass color turned from pink to yellow, and methane consumption stopped completely. The system flaw was corrected and a new culture was established, which was used to seed the aerobic column in each system.

Aerobic columns were seeded on the 67th day after CAH addition, and sequential systems were connected in series on day 74. Sampling ceased at this point due to physical problems with the systems. Flow lines began clogging intermittently, possibly due to higher pressures in each system with the added column, or because biomass or other solid particles were building up along the inside of Teflon tubing. This problem cannot be explained, but seems to be occurring less frequently. Systems have not clogged since day 105.

Additionally, the anaerobic columns were accidentally contaminated by the aerobic columns on day 81. Anaerobic feed syringe pumps had been shut off in order to remove the syringes and fill them with fresh feed solution. The Mininert valves that connected the syringes to the systems had not been closed, and approximately 5 mL of liquid drained out of the systems and back into the syringes. Aerobic columns were refilled and disconnected from the system for 1 day. Anaerobic feed syringes were filled with fresh media, and the anaerobic effluent was redirected to a waste container for one day to allow any contaminated liquid to wash out. The systems were reconnected the following day, and sampling did not begin again until day 119.

Due to these physical problems and malfunctions, methane consumption in the aerobic columns has not yet been analyzed. The appearance of the columns indicates good health and growth, but no data are available to corroborate this.

Effect of Mixtures on Transformation of PCE

To study the effect of a mixture of CAHs on the transformation of PCE in the anaerobic columns, PCE was added to the methanogenic column in System A and a mixture of PCE, CT, and 1,1,1-TCA was fed to the

methanogenic column in System B. All compounds were fed at concentrations of approximately 5 μM of each CAH (860 mg/L PCE, 800 mg/L CT, and 720 mg/L 1,1,1-TCA). Acetate was the electron donor in both systems. Figure 4.2 shows the behavior of PCE in both systems. All data are summarized in the Appendices A.3 and A.4.

PCE did not appear in high concentrations in either system until after day 20. PCE should have appeared much earlier, since the maximum HRT in both columns was 5.6 days. This may indicate sorption of CAHs to biomass or other column material, although Hughes (1992) showed that sorption does not significantly impact similar laboratory studies using CAHs.

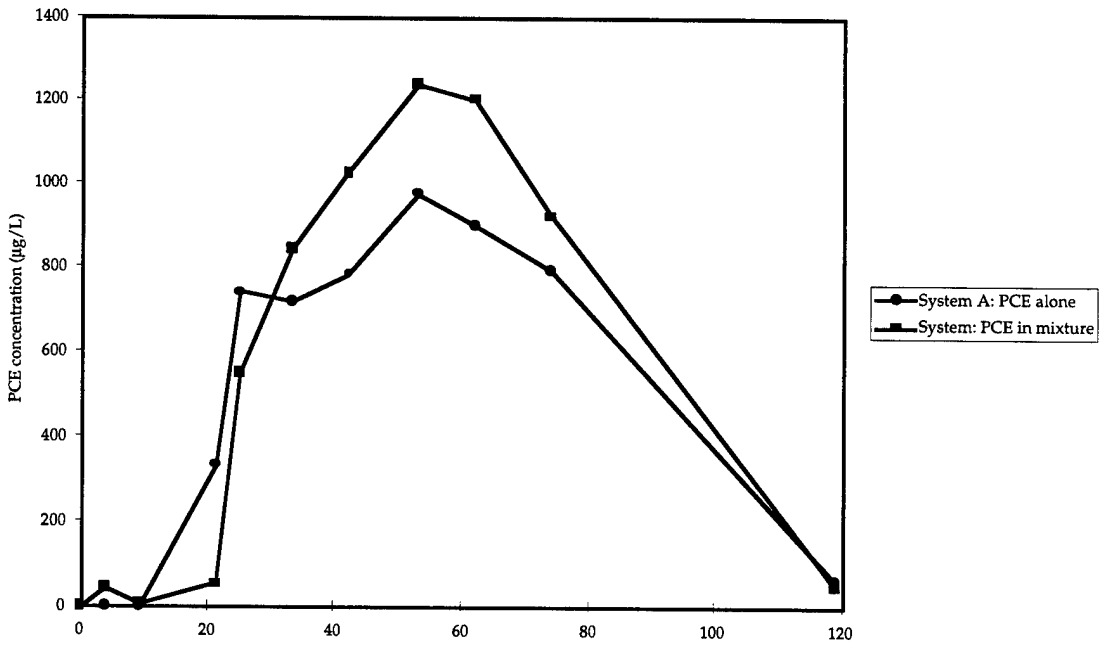

Figure 4.2. Behavior of PCE When Fed Alone versus in a Mixture.

From day 20 until approximately day 30, System B seemed to transform PCE better. After day 30, however, there was more PCE in the effluent of System B. This may indicate an adverse effect of mixtures on the transformation of PCE; however, duplicate columns were not used, so a statistical analysis of the data cannot be made. Trace levels of TCE appeared in both systems, indicating biological transformation of PCE, but other metabolites could not be detected by the analytical methods used. Methane has been produced for the duration of CAH addition, which also indicates biological activity.

On day 53 in the effluent of System A and on day 42 in System B, PCE levels rose higher than the expected influent concentration of approximately 860 μg/L, probably due to a high concentration of PCE in the feed solution. Inconsistent CAH levels in the feed solution were a significant problem for the entire research period. This problem is addressed at the end of the chapter (see "Feed Solutions"). After day 53, PCE levels began decreasing in both systems.

Sampling discontinued after day 74 while anaerobic and aerobic columns were connected in series. Problems with the physical system that prevented sampling were discussed previously. On day 119, effluent PCE levels were 59 μg/L in System A and 49 μg/L in System B.

These preliminary data are inconclusive regarding the effect of mixtures on PCE transformation. Thus far, the presence of CT and 1,1,1-TCA appear to have little impact on the removal of PCE.

Acetate Versus Iron as an Electron Donor

A mixture of PCE, CT, and 1,1,1-TCA was fed to Systems B and C to compare acetate and iron as electron donors. All CAHs were fed at concentrations of approximately 5 μM (860 mg/L PCE, 800 mg/L CT, and 720 mg/L 1,1,1-TCA). Acetate was fed to System B, and steel wool was used as a support media and source of Fe^0 in System C. Figures 4.3 and 4.4 show the behavior of PCE and 1,1,1-TCA in both systems. CT was not detected above detection limits in either system. Raw data sheets are shown in Appendices A.3 and A.4.

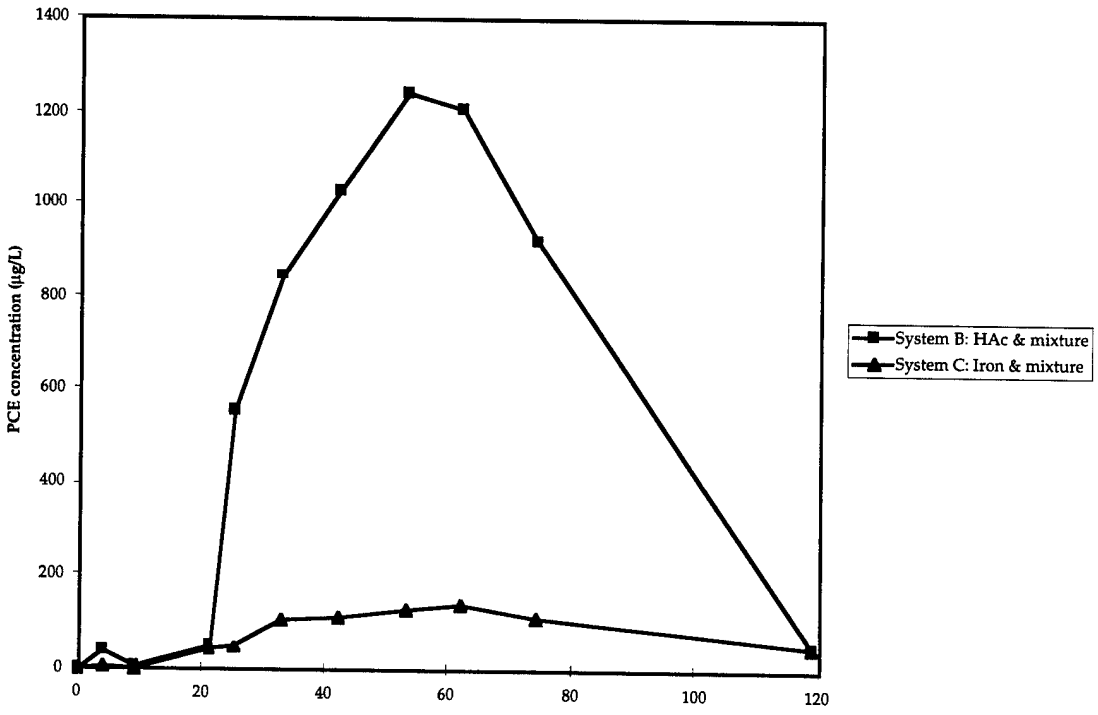

Figure 4.3. Behavior of PCE in Acetate-Fed versus Iron-Fed Systems (CT and 1,1,1-TCA also fed to both systems).

In both systems, PCE began to appear on day 4, but did not begin to increase appreciably until after day 20. The iron-fed system much more effectively removed PCE. Trace levels of TCE were detected in the effluents starting on day 33 in System B, which may indicate biological activity. Trace levels of TCE appeared in the effluent of System C on day 42. Although TCE may be produced via abiotic dehalogenation in System C, methane production is still present, which indicates biological activity in both systems. Detection of subsequent metabolites (*cis*- and *trans*-1,2-DCE, 1,1-DCE, and VC) was limited by current analytical techniques.

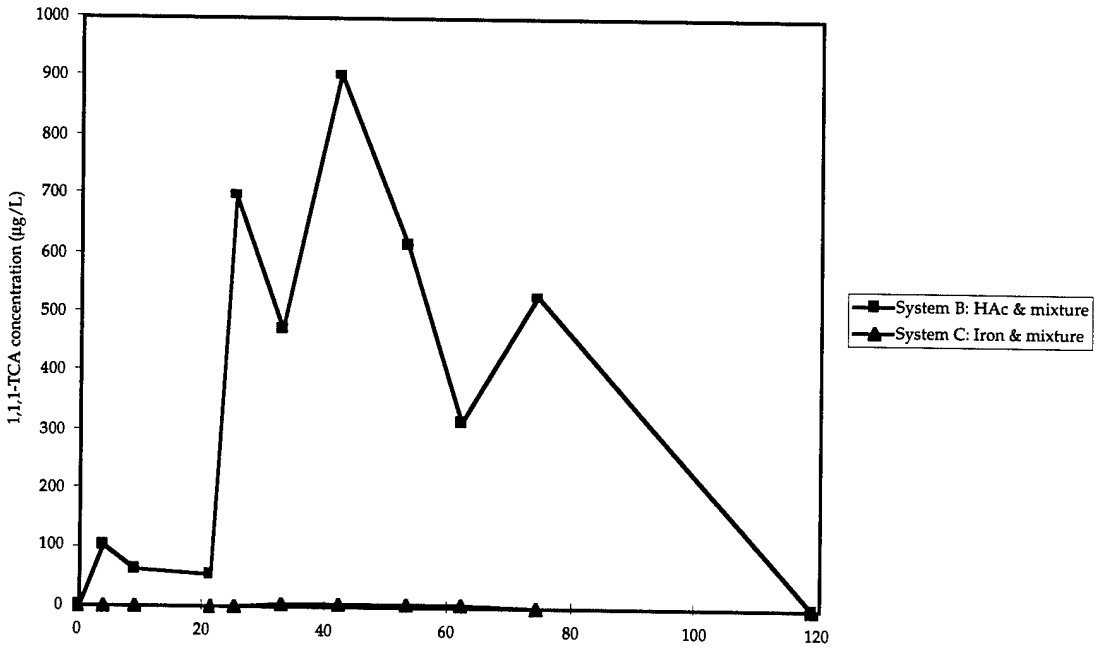

Figure 4.4. Behavior of 1,1,1-TCA in Acetate-Fed versus Iron-Fed Systems (PCE and CT also fed to both systems).

1,1,1-TCA behavior was similar to that of PCE (Fig. 4.4). It appeared in the effluent of the acetate-fed column on day 4, and appeared at trace levels in the iron-fed column effluent on day 33. 1,1,1-TCA was almost completely transformed by the iron-fed system; effluent concentrations were below 3 μg/L, just above the detection limit of 1 μg/L. After day 42, 1,1,1-TCA levels dropped in System B. This was earlier than for PCE, which may indicate that the microbes used in this study need less time to acclimate to 1,1,1-TCA than they do to acclimate to PCE. Methane was produced in both columns, again indicating biological activity. Trace levels of 1,1-DCA were detected, but CA could not be determined analytically.

CT was completely degraded in both systems, and trace levels of CF were detected. Also, DCM was detected in the effluent from the acetate-fed system, but not from the iron-fed system. Although DCM was detected, it could not be quantified using current analytical techniques.

The appearance of TCE, CF, DCM, and 1,1-DCA agrees with earlier studies (Vogel et al., 1987) that identify the same metabolites from PCE, CT, and 1,1,1-TCA.

Removal of PCE and 1,1,1-TCA is greater in the iron-fed column than in the acetate-fed column. This may be due to abiotic dehalogenation, as described by Matheson and Tratnyek (1994), Gillham and O'Hannesin (1994), Helland et al. (1994), and others. This may also indicate the presence of dechlorinating H_2-utilizers in the methanogenic consortia. This information corroborates findings of DiStefano et al., (1991, 1992), Fennel et al., (1995) and others who have found enhanced transformation of PCE using H_2 as the electron donor. 1,1,1-TCA may be transformed by a similar mechanism. CT

was completely transformed by both systems, so it could not be determined which electron donor was more effective.

Feed Solutions

After effluent CAH levels were detected that were above the expected influent values, the concentration of CAHs in the influent was measured for all systems. Table 4.1 shows a sample analysis of feed solutions. Samples were taken when the syringes were filled with new media and again when they were nearly empty. All CAH concentrations were higher than expected in the new media, and lower than expected in the old media. It was hypothesized that CAHs were either not well mixed in the feed syringes, or that a portion of CAH was being removed in the syringe. To improve mixing, Teflon stir bars (1/2 inch, Fisher Scientific) were placed in each syringe, and when syringes were refilled with fresh media, they were inverted

Table 4.1. Analysis of CAHs in Feed Solutions for Fresh and Old Media.

(µg/L)	System A	System B			System C		
media	PCE	PCE	CT	1,1,1-TCA	PCE	CT	1,1,1-TCA
fresh	1200	1100	3500	930	1100	3600	920
	(+40%)	(+30%)	(+340%)	(+29%)	(+30%)	(+350%)	(+28%)
old	770	700	330	370	730	210	340
	(-10%)	(-19%)	(-59%)	(-49%)	(-15%)	(-74%)	(-53%)

*Expected values were 860 µg/L PCE, 800 µg/L CT, and 720 µg/L 1,1,1-TCA. Values in parentheses are the percent error from the expected values.

several times before being connected to feed lines. Syringes were washed between fillings, and periodically soaked in an acid bath to remove biomass that may transform CAHs. Stock solutions were also recalibrated.

Sequential Studies

Sequential studies had not been performed by the end of this thesis project. The system has been established, and there is some evidence that methanogenic organisms are capable of transforming PCE, CT, and 1,1,1-TCA. Sequential studies will be performed by other researchers.

CHAPTER 5
SUMMARY AND CONCLUSIONS

Three sequential, biofilm-systems have been established that appear to be acclimated to and capable of degrading PCE, CT, and 1,1,1-TCA. In the anaerobic columns, the effect of mixtures of PCE, CT, and 1,1,1-TCA on the transformation of PCE was assessed. Acetate was compared to elemental iron as an energy source. Transformation products were identified, when possible. The thesis project ended before experiments could begin using the sequential systems. Work will be continued by other researchers.

The following conclusions are drawn from this research:

1. The effect of mixtures on PCE transformation is unclear. Although transformation seemed improved when PCE was fed alone, a statistical analysis could not be made to determine if the difference is significant.
2. When elemental iron provides the electron donor for a methanogenic culture, the transformation of PCE and 1,1,1-TCA appears to be greater than when acetate is used.
3. The following transformation products were identified: TCE from PCE; CF and DCM (when acetate is the electron donor) from CT; and 1,1-DCA from 1,1,1-TCA. Trace levels of TCE, CF and 1,1-DCA were detected. DCM could not be quantified.
4. CT was completely removed regardless of the electron donor. PCE and 1,1,1-TCA appeared to be transformed to a greater extent after

acetate-fed columns had been exposed to the CAHs for a period of time, indicating acclimation of the microbial population.

5. More work is needed before mineralization studies using the sequential system can begin and mass balances can be calculated.

CHAPTER 6
LIMITATIONS OF WORK AND RECOMMENDATIONS FOR FUTURE RESEARCH

The focus of this thesis project was to establish three sequential, biofilm-column systems containing organisms that are capable of degrading CAHs. It was hypothesized that these systems would be able to transform PCE, CT, and 1,1,1-TCA to less chlorinated metabolites in the methanogenic column and achieve complete mineralization in the aerobic column. This hypothesis cannot be tested, although preliminary results do indicate that anaerobic columns may have acclimated to and are now capable of degrading PCE, CT and 1,1,1-TCA and some of their metabolites. Results also indicate a potential difference in transformation ability between systems that are fed acetate versus iron, and systems that are fed PCE alone versus in a mixture with CT and 1,1,1-TCA.

There are several limitations of this work that prevent more definite conclusions from being developed. First, abiotic controls are needed to account for volatilization, leakage in the system, and abiotic transformations of CAHs. Without controls, mass balances cannot be made.

Second, HRTs in the anaerobic columns in Systems A and B are much longer than the expected maximum HRTs. This may indicate sorption of CAHs onto material in the columns, although previous research in our lab (Hughes, 1992) showed that sorption to biomass does not have a significant impact on CAH studies. Control systems may help explain this problem.

Also, the long HRT may simply be due to operator error. Feed solutions may not have contained as much CAH as was thought, or CAHs may have volatilized through leaks in the system.

Third, aerobic columns have only recently been established, and had been operating without system malfunctions for approximately one week prior to the end of the thesis project. Data are not available on their ability to use methane as an energy source. More analysis is required here to prove that the sequential systems are functional.

Fourth, analytical techniques need to be developed to account for all metabolites before mineralization studies can begin. A different type of GC column is currently being investigated. This column may help distinguish less chlorinated metabolites from methane, and may provide better separation between metabolites. Purge and trap analysis may also be employed if headspace techniques cannot identify these metabolites.

Once these limitations have been resolved, mineralization studies using the sequential system can begin. Influent concentrations of PCE, CT, and 1,1,1-TCA could be increased gradually to study changes in the systems due to higher concentrations of the target compounds (e.g. toxic effects, build up of a particular metabolite). The amount of acetate fed to each system could be reduced to determine how little is necessary to maintain methane production that will sustain methanotrophic organisms.

REFERENCES

Alvarez-Cohen, L. and P. L. McCarty (1991). "Product Toxicity and Cometabolic Competitive Inhibition Modeling of Chloroform and Trichloroethylene Transformation by Methanotrophic Resting Cells." Appl. Environ. Technol. 57:1031-1037.

Alvarez-Cohen, L. and P. L. McCarty (1991a). "Effects of Toxicity, Aeration, and Reductant Supply on Trichloroethylene Transformation by a Mixed Methanotrophic Culture." Appl. Environ. Microbiol. 57:228-235.

American Petroleum Institute (1987). "Field Study of Enhance Subsurface Biodegradation of Hydrocarbons Using Hydrogen Peroxide as an Oxygen Source." API Publication No. 4448. Washington, DC.

Arcangeli, J. P., E. Arvin, and H. M. Jensen (1995). "Cometabolic Biodegradation of Trichloroethylene in a Biofilm Reactor." In: Bioremediation of Chlorinated Solvents. Battelle Press, Columbus, OH. pp. 203-212.

Arciero, D., T. Vannelli, M. Logan, and A. B. Hooper (1989). "Degradation of Trichloroethylene by the Ammonia-Oxidizing Bacterium *Nitrosomonas europaea*." Biochem. Biophys. Res. Commun. 159:640-643.

Belay, N. and L. Daniels (1990). "Elemental Metals as Electron Sources for Biological Methane Formation for CO_2." Antoine van Leewenhoek. 57:1-7.

Bonse, G. (1975). "In Vivo Studies of Chlorinated Aliphatic Hydrocarbons." Biochem. Pharmacol. 24:1829-1834.

Bouwer, E. J. and P. L. McCarty (1983). "Transformations of 1- and 2-Carbon Halogenated Aliphatic Organic Compounds Under Methanogenic Conditions." Appl. Environ. Microbiol. 45:1286-1294.

Bouwer, E. J., B. E. Rittmann, and P. L. McCarty (1981). "Anaerobic Degradation of Halogenated 1- and 2-Carbon Organic Compounds." Environ. Sci. Technol. 15:596-599.

Bowen, S. E. and R. L. Balster (1996). "Effects of Inhaled 1,1,1-Trichloroethane on Locomotor Activity in Mice." Neurotoxicology and Teratology. 18:77-81.

Brock, T. D., M. T. Madigan, J. M. Martinko, and J. Parker (1994). Biology of Microorganisms. Prentice-Hall. Englewood Cliffs, NJ.

Brodkin, C. A., W. Daniell, H. Checkoway, D. Echeverria, J. Johnson, K. Wang, R. Sohaey, D. Green, C. Redlich, D. Gretch, and L. Rosenstock (1995). "Hepatic Ultrasonic Changes in Workers Exposed to Perchloroethylene". Occupational and Environmental Medicine. 52:679-685.

Brunner, W. D., Staub, and T. Leisinger (1980). "Bacterial Degradation of Dichloromethane." Appl. Environ. Microbiol. 40:950-958.

Cline, P. V. and J. J. Delfino (1989). "Transformation Kinetics of 1,1,1-Trichloroethane to the Stable Product 1,1-Dichloroethene." In: Biohazards of Drinking Water Treatment. Lewis Publishers, Inc. Chelsea, MI. pp. 47-56.

Cobb, G. D. and E. J. Bouwer (1991). "Effects of Electron Acceptors on Halogenated Organic Compound Biotransformations in a Biofilm Column." Environ. Sci. Technol. 25: 1068-1074.

Daniels, L., N. Belay, B. S. Rajagopal, and P. J. Weimer (1987). "Bacterial Methanogenesis and Growth from CO_2 with Elemental Iron as the Sole Source of Electrons." Science. 237:509-511.

DiStefano, T. D., J. M. Gossett, and S. H. Zinder (1991). "Reductive Dechlorination of High Concentrations of Tetrachloroethene to Ethene by an Anaerobic Enrichment Culture in the Absence of Methanogenesis." Appl. Environ. Microbiol. 57:2287-2292.

DiStefano, T. D., J. M. Gossett, and S. H. Zinder (1992). "Hydrogen as an Electron Donor for Dechlorination of Tetrachloroethene by an Anaerobic Mixed Culture." Appl. Environ. Microbiol. 58:3622-3629.

Egli, C., R. Scholz, A. M. Cook, and T. Leisinger (1987). "Anaerobic Dechlorination of Tetrachloromethane and 1,2-Dichloroethane to Degradable Products by Pure Cultures of *Desulfobacterium ps.* and *Methanobacterium sp.*" FEMS Microbiol. Letters. 43:257-261.

Fathepure, B. Z. and T. M. Vogel (1991). "Complete Degradation of Polychlorinated Hydrocarbons by a Two-Stage Biofilm Reactor." Appl. Environ. Microbiol. 57:3418-3422.

Fennel, D. E., M. A. Stover, S. H. Zinder, and F. M. Gossett (1995). "Comparison of Alternative Electron Donors to Sustain PCE Anaerobic Reductive Dechlorination." In: Bioremediation of Chlorinated Solvents. Battelle Press, Columbus. OH. pp. 9-16.

Fogel, M. M., A. R. Taddeo, and S. Fogel (1986). "Biodegradation of Chlorinated Ethenes by a Methane-Utilizing Mixed Culture." Appl. Environ. Microbiol. 51:720-724.

Freedman, D. L. and J. M. Gossett (1989). "Biological Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene to Ethylene Under Methanogenic Conditions." Appl. Environ. Microbiol. 55:2144-2151.

Freedman, D. L. and J. M Gossett (1991). "Biodegradation of Dichloromethane in a Fixed Film Reactor Under Methanogenic Conditions." In: On-Site Bioremediation Processes for Xenobiotic and Hydrocarbon Treatment. Boston, Mass. pp. 113-133.

Gerritse, J., V. Renard, J. Visser, and G. C. Gottschal (1995). "Complete Degradation of Tetrachloroethene by Combining Anaerobic Dechlorinating and Aerobic Methanotrophic Enrichment Cultures." Appl. Microbiol. Biotechnol. 43:920-928.

Gillham, R. W. and S. F. O'Hannesin (1994). "Enhanced Degradation of Halogenated Aliphatics by Zero-Valent Iron." Ground Water. 32:958-967.

Hake, C. L., and R. D. Stewart. "Human Exposure to Tetrachloroethylene: Inhalation and Skin Contact." Environ. Health Perspect. 21:231-238.

Hartmans, S. and J. A. M. de Bont (1992). "Aerobic Vinyl Chloride Metabolism in *Mycobacterium aurum* L1." Appl. Environ. Microbiol. 58:1220-1226.

Helland, B. R., P. J. Alvarez, and J. L. Schnoor (1995). "Reductive Dechlorination of Carbon Tetrachloride with Elemental Iron." Jrnl. of Haz. Mat. 41:205-216.

Henschler, D. (1985). "Halogenated Alkenes and Alkynes." In: Bioactivation of Foreign Compounds. Academic Press, Inc., New York.

Hollinger, C., G. Schraa, A. J. M. Stams, and A. J. B. Zehnder (1993). "A Highly Purified Enrichment Culture Couples the Reductive Dechlorination of Tetrachloroethene to Growth." Appl. Environ. Microbiol. 59:2991-2997.

House R. A., G. M. Liss, M. C. Wills, and D. L. Holness (1996). "Paresthesias and Sensory Neruopathy Due to 1,1,1-Trichloroethane." Journal of Occupational and Environmental Medicine. 38:123-124.

Hughes, J. B. (1992). Anaerobic Biotransformation of Chlorinated Aliphatics: Interactions with Primary Substrate Utilization and Effects of Mixtures. PhD Dissertation, The University of Iowa, Iowa City, IA.

Hughes, J. B. and G. F. Parkin (1992). "The Effect of Mixtures of Xenobiotics and Primary Electron Donor on the Anaerobic Biotransformation of High Concentrations of Chlorinated Aliphatics." Wat. Sci. Tech. 26:117-126.

Little, C. D., A. V. Palumbo, S. E. Herbes, M. E. Lidstrom, R. L. Tyndall, and P. J. Gilmer (1988). "Trichloroethylene Biodegradation by a Methane-Oxidizing Bacterium." Appl. Environ. Microbiol. 54:951-956.

Long, J. L., H. D. Stensel, J. F. Ferguson, S. E. Strand, and J. E. Ongerth (1993). "Anaerobic and Aerobic Treatment of Chlorinated Aliphatic Compounds." Journal of Environmental Engineering. 119:300-320.

Matheson, L. J. and P. G. Tratnyek (1994). "Reductive Dehalogenation of Chlorinated Methanes by Iron Metal." Environ. Sci. Technol. 28:2045-2053.

McCarty, P. L. and L. Semprini (1994). "Ground-Water Treatment for Chlorinated Solvents." In: Handbook of Bioremediation. Lewis Publishers. Boca Raton. pp. 87-116.

Metcalf and Eddy, Inc. (1991). Wastewater Engineering: Treatment, Disposal, and Reuse. McGraw-Hill, New York.

Miller, R. E. and F. P. Guengerich (1982). "Oxidation of Trichloroethylene by Liver Microsomal Cytochrome P-450: Evidence for Chlorine Migration in a Transition State Not Involving Trichloroethylene Oxide." Biochemistry. 21:1090-1097.

Morgan, P. and R. J. Watkinson (1992). "Factors Limiting the Supply and Efficiency of Nutrient and Oxygen Supplements for the *In Situ* Biotreatment of Contaminated Soil and Groundwater." Wat. Res. 26:73-78.

National Institute for Occupational Safety and Health (1978). "Current Intelligence Bulletin 20, Tetrachloroethylene (Perchloroethylene). Cincinnati: DHEW (NIOSH).

Nelson, M. J., S. O. Montgomery, W. R. Mahaffey, and P. H. Pritchard (1987). "Biodegradation of Trichloroethylene and Involvement of an Aromatic Biodegradative Pathway." Appl. Environ. Microbiol. 53:949-954.

Oldenhuis, R., R. L. Vink, D. B. Janssen, and B. Witholt (1989). "Degradation of Chlorinated Aliphatic Hydrocarbons by *Methylosinus trichosporium* OB3b Expressing Soluble Methane Monooxygenase." Appl. Environ. Microbiol. 55:2819-2826.

Rittmann, B. E. and P. L. McCarty (1980). "Utilization of Dichloromethane by Suspended and Fixed-Film Bacteria." Appl. Environ. Microbiol. 39:1225-1226.

Roberts, P. V., G. D. Hopkins, E. M. Mackay, and L. Semprini (1990). "A Field Evaluation of In-situ Biorestoration of Chlorinated Ethenes: Part 1. Methodology and Field Site Characterization." Ground Water. 28:591-604.

Sawyer, C. N., P. L. McCarty, and G. F. Parkin (1994). "Chemistry for Environmental Engineers." McGraw-Hill, New York.

Schnoor, J. L. (1995). "Environmental Modeling: Fate and Transport of Pollutants in Water, Air, and Soil. John Wiley & Sons, Inc. New York, NY.

Schnoor, J. L., et al. (1987). "Processes, Coefficients, and Models for Simulating Toxic Organics and Heavy Metals in Surface Waters." U. S. Environmental Protection Agency, EPA/600/3-87/015.

Semprini, L., G. D. Hopkins, P. V. Roberts, D. Grbic-Galic, and P. L. McCarty (1991). "A Field Evaluation of In-situ Biodegradation of Chlorinated Ethenes: Part 3, Studies of Competitive Inhibition." Ground Water. 29:239-250.

Semprini, L. and P. L. McCarty (1991). "Comparison Between Model Simulations and Field Results for In-situ Birestoration of Chlorinated Aliphatics: Part 1. Biostimulation of Methanotrophic Bacteria.: Ground Water. 29:365-374.

Sligar, S. G. and R. I. Murray (1986). "Oxygenase Enzyme Systems in Mammals" In: Cytochrome P-450, Structure, Mechanism and Biochemistry. Plenum Press. New York. pp. 492-503.

Snoeyink, V. L. and D. Jenkins (1980). Water Chemistry. John Wiley and Sons.

Tandol, V., T. D. DiStefano, P. A. Bowser, F. M. Gossett, and S. H. Zinder (1994). "Reductive Dehalogenation of Chlorinated Ethenes and Halogenated Ethanes by a High-Rate Anaerobic Enrichment Culture." Environ. Sci. Technol. 28:973-979.

Vogel. T. M., C. Criddle, and P. L. McCarty (1987). "Transformation of Halogenated Aliphatic Compounds." Environ. Sci. Technol. 21:722-736.

Vogel, T. M. and P. L. McCarty (1987). "Abiotic and Biotic Transformation of 1,1,1-Trichloroethane Under Methanogenic Conditions." Environ. Sci. Technol. 21:1208-1213.

Wackett, L. P., G. A. Brusseau, S. R. Householder, and R. S. Hanson (1989). "Survey of Microbial Oxygenases: Trichloroethylene Degradation by Propane-Oxidizing Bacteria." Appl. Environ. Microbiol. 55:2960-2964.

Wackett, L. P. and D. T. Gibson (1988). "Degradation of Trichloroethylene by Toluene Dioxygenase in Whole-Cell Studies with *Pseudomonas putida* F1." Appl. Environ. Microbiol. 54:1703-1708.

Weathers, L. J. (1995). Biological and Metallic Iron-Promoted Transformations of Carbon Tetrachloride and Chloroform Under Methanogenic Conditions. PhD Dissertation, The University of Iowa, Iowa City, IA.

Wild, A. P., W. Winkelbauer, and T. Leisinger (1995). "Anaerobic Dechlorination of Trichloroethene, Tetrachloroethene and 1,2-Dichloroethane by an Acetogenic Mixed Culture in a Fixed-Bed Reactor." Biodegradation. 6:309-318.

Wilson, J. T. and D. H. Kampbell (1994). "Bioventing of Chlorinated Solvents for Ground-Water Cleanup Through Bioremediation." In: Handbook of Bioremediation. Lewis Publishers. Boca Raton. pp. 117-129.

Wilson, J. T. and B. H. Wilson (1985). "Biotransformation of Trichloroethylene in Soil." Appl. Environ. Microbiol. 49:242-243.

Zitomer, D. H. and R. E. Speece (1993). "Sequential Environments for Enhanced Biotransformation of Aqueous Contaminants." ES&T Critical Review. 27:226-244.

APPENDIX

Table A.1. Common Chlorinated Aliphatic Hydrocarbons (after McCarty and Semprini, 1994).

Compound	Formula	Aerobic Potential[a]	Anaerobic Potential[a]	Density	Solubility (mg/L)	MCL (µg/L)
Carbon Tetrachloride (CT)	CCl_4	0	XXXX	1.595	800	5
Chloroform (CF)	$CHCl_3$	X	XX	1.485	8200	100
Dichloromethane (DCM)	CH_2Cl_2	XXX		1.325	13,000/ 20,000[c]	5[c]
Chloromethane (CM)	CH_3Cl_2				6450[c]	
1,1,1-Trichloroethane (1,1,1-TCA)	CH_3CCl_3	X	XXXX	1.325	950/4400[c]	200
1,1-Dichloroethane (1,1-DCA)	CH_3CHCl_2	X	XX	1.175	5500/400[c]	
Chloroethane (CA)	CH_3CH_2Cl	XX	b		5740[c]	
Perchloroethylene (PCE)	$CCl_2=CCl_2$	0	XXX	1.625	150/200[c]	5
Trichloroethylene (TCE)	$CHCl=CCl_2$	XX	XXX	1.462	1000/1100[c]	5
cis-1,2-Dichloroethylene (*cis*-1,2-DCE)	CHCl=CHCl	XXX	XX	1.214	400	70
trans-1,2-Dichloroethylene (*trans*-1,2-DCE)	CHCl=CHCl	XXX	XX	1.214	400/600[c]	100
1,1-Dichloroethylene (1,1-DCE)	$CH_2=CCl_2$	X	XX		400[c]	7
Vinyl Chloride (VC)	$CH_2=CHCl$	XXXX	X		90[c]	2

[a]0 - very small if any potential; X - some potential; XX - fair potential; XXX - good potential; XXXX - excellent potential.

[b]Readily hydrolyzed abiotically, with half-life on the order of one month.

[c]Additional data from J. L. Schnoor et al. "Processes, Coefficients, and Models for Simulating Toxic Organics and Heavy Metals in Surface Waters." U. S. Environmental Protection Agency, EPA/600/3-87/015, June 1987.

Table A.2. Methane Production in Anaerobic Columns.

Time (days)	System A (mg/L)	System B (mg/L)	System C (mg/L)
-30	14.5	14.4	11.9
-17	14.8	14.3	10.7
-16	15.5	16.9	10.4
-15	16.0	14.5	10.2
0	19.3	27.8	11.7
Average	16 ± 1.9	18 ± 5.8	11 ± 0.7
6	18.2	24.3	11.5
9	16.0	15.4	11.4
25	19.0	23.0	11.3
26	21.5	25.0	11.4
34	21.1	18.4	11.3
35	21.4	19.4	11.3
47	19.2	16.2	11.3
120	20.7	17.6	11.3
Average	20 ± 1.8	20 ± 3.4	11 ± 0.1

Table A.3. PCE Transformation and Appearance of TCE in System A.

Time (days)	Target Compounds PCE (µg/L)	Metabolites TCE (µg/L)
0	0	0
4	0	0
9	0	0
21	332	0
25	740	tr.
33	720	tr.
42	786	0
53	980	tr.
62	902	tr.
74	792	tr.
119	58.5	0

Table A.4. CAH Transformation and Appearance of Metabolites in System B.

Time	Target Compounds (µg/L)			Metabolites (µg/L)			
(days)	PCE	CT	1,1,1-TCA	TCE	1,1-DCA	CF	DCM
0	0	0	0	0	0	0	0
4	37.9	0.8	103	0	tr.	0	0
9	2.18	0	61.5	0	tr.	0	0
21	51.9	0	52.9	0	0	0	0
25	553	0	697	0	tr.	0	0
33	846	0	471	tr.	tr.	tr.	0
42	1030	0	903	0	tr.	tr.	0
53	1240	0	616	tr.	tr.	tr.	detected
62	1210	0.5	317	tr.	0	tr.	detected
74	924	0.1	529	tr.	0	tr.	detected
119	49.4	0	15.4	tr.	0	tr.	0

DCM was detected, but could not be quantified with the analytical technique used.

Table A.5. CAH Transformation and Appearance of Metabolites in System C.

Time	Target Compounds (µg/L)			Metabolites (µg/L)		
(days)	PCE	CT	1,1,1-TCA	TCE	CF	1,1-DCA
0	0	0	0	0	0	0
4	2.60	12.4	0	0	0	0
9	0	0	0	0	0	0
21	41.3	0	0	0	0	tr.
25	48.1	0	0	0	0	0
33	103	0	2.4	0	tr.	tr.
42	112	0	2.1	tr.	tr.	0
53	130	0	2.4	tr.	tr.	tr.
62	138	tr.	2.0	tr.	tr.	tr.
74	112	tr.	tr.	0	tr.	0
119	45.2	0	0	0	tr.	0