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I am submitting herewith a thesis written by Charles Michael Morrissey entitled "Dechlorination of High Concentrations of Tetrachloroethene in Microcosms and in a Laboratory Sand Column." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Environmental Engineering.

R. Bruce Robinson, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor
and Dean of The Graduate School

Dechlorination of High Concentrations of Tetrachloroethene in Microcosms and a Laboratory Sand Column

A Thesis
Presented for the
Master of Science
Degree
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Charles Michael Morrissey
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DEDICATION

I dedicate this thesis to the memory of my father, Peter Charles Morrissey, Jr. He was a career military man who served our country in three wars: World War II, Korea, and Vietnam. He always encouraged hard work and perseverance. This thesis required a lot of both. Thanks.

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ABSTRACT

High concentrations (up to 88 mg/L) of tetrachloroethene (PCE) were shown to be dechlorinated in anaerobic microcosms and in a laboratory sand column operated under anaerobic, methanogenic conditions. Initial experiments were conducted in microcosms prepared using sediment from two sites known to have been contaminated with chlorinated hydrocarbons. Anaerobic sand column experiments were performed using an inoculum from the secondary anaerobic digester of a local municipal sewage treatment plant. Experiments that attempted to couple the anaerobic and aerobic processes were carried out in a single sand column in which anaerobic and aerobic zones were established. All experiments monitored the disappearance of PCE and the formation and degradation of PCE dechlorination products. Acetate was provided as the primary substrate. Results of this study add to the body of knowledge necessary to evaluate *in situ* bioremediation as an alternative for clean up of sites where high concentrations of PCE are present in groundwater.

The extent of PCE dechlorination in anaerobic sediment microcosms varied depending upon the source of the sediments. PCE removal rates in active sediment microcosms averaged 1.58 µmole/L/day and proceeded stepwise to trichloroethene (TCE) and cis-1,2-dichloroethene (CIS). No vinyl chloride (VC) was detected in any sediment microcosms.

PCE dechlorination in the anaerobic sand column occurred, after a lag time, at influent PCE concentrations of 32 and 88 mg/L. Dechlorination proceeded at a minimum volumetric removal rate of 160 µmole/L/day. At the higher PCE concentration, dechlorination proceeded through TCE to both CIS and trans-1,2-dichloroethene (TRANS). At the lower influent PCE concentration, VC and traces of ethene (ETH) were

formed.

Methane content of the column offgas varied from 40 to 60%. Acetate utilization in the column was rapid and complete. Electron balance calculations indicated that ~1% of the reducing power of acetate was used in the reductive dechlorination of PCE.

During operation of the sand column in a combined anaerobic/aerobic mode, methanogenesis was sustained in the lower portion of the column while a mixed culture of methanotrophs was inoculated into the upper portion. Methanotrophs proved difficult to establish in the sand column environment. However, detection of both the CIS and TRANS epoxides showed that small amounts of the dichloroethene (DCE) isomers were degraded by the methanotrophs.

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LIST OF ABBREVIATIONS

A	activity in solution; cross-sectional area
BES	bromoethanesulfonic acid
c_{in}	influent concentration
c_r	resident concentration
C	Centigrade
C_i	concentration of i^{th} species
CAC	chlorinated aliphatic compound
CE	chloroethane
CERCLA	Comprehensive Environmental Response Compensation and Liability Act
CIS	cis-1,2-dichloroethene
cm	centimeter
COD	chemical oxygen demand
D	dispersion coefficient
DCE	dichloroethene
DOE	U. S. Department of Energy
E	measured electrode potential
E_0	reference electrode potential
ECD	electron capture detector
ETH	ethene
EPA	U. S. Environmental Protection Agency
FID	flame ionization detector
g	gram
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry

LIST OF ABBREVIATIONS (continued)

IC	ion chromatography
ISA	ionic strength adjustor
K_{oc}	organic carbon distribution coefficient
K_{ow}	octanol-water partition coefficient
KCMC	Kansas City Mixed Culture
L	liter; total length of column
M	molar
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
min	minute
mL	milliliter
mM	millimolar
MMO	methane monooxygenase
mmol	millimole
mN	millinormal
mV	millivolt
MSB	M-area settling basin
N	normal
NATE	nutrient and trace element
NHP	New Hope Pond
OLF	Oil Landfarm
ORNL	Oak Ridge National Laboratory
P	Peclet number
PCE	perchloroethylene (tetrachlorothene)

LIST OF ABBREVIATIONS (continued)

ppm	parts per million
Q	volumetric flow rate
R	retardation factor
RAMM	revised anaerobic mineral medium
RCRA	Resource Conservation and Recovery Act
s	adsorbed concentration
S	electrode slope
SDWA	Safe Drinking Water Act
SRB	sulfate-reducing bacteria
ss	stainless steel
t	time
TCA	trichloroethane
TCD	thermal conductivity detector
TCE	trichloroethylene
TRANS	trans-1,2-dichloroethene
v	average pore-water velocity; bulk velocity
VC	vinyl chloride
VOA	volatile organic analysis
VOC	volatile organic compound
x	distance along column
Z_i	charge of the i^{th} species
γ	activity coefficient
γ_s	rate constant for zero-order production in the solid phase
γ_w	rate constant for zero-order production in the liquid phase

LIST OF ABBREVIATIONS (continued)

θ	volumetric water content
μ_s	rate constant for first-order decay in solid phase
μ_w	rate constant for first-order decay in liquid phase
µeq	microequivalent
µL	microliter
µm	micrometer
µM	micromolar
µmol	micromole
µS	microsiemen
ρ	porous medium bulk density

1. INTRODUCTION

1.1 BACKGROUND

Tetrachloroethene, also known as tetrachloroethylene or perchloroethylene (PCE), is a common industrial chemical widely used as a solvent for metal cleaning and dry-cleaning. PCE is also used for processing and finishing in the textile industry, as an extraction solvent, as a veterinary anthelmintic, as a heat-exchange fluid, in grain fumigation, and in the manufacture of fluorocarbons (Verschueren, 1983). A survey revealed that as much as 10 percent of industrial effluents in the United States contain PCE (Staples et al., 1985). In 1990, United States industry produced 291,000,000 pounds of PCE (United States Tariff Commission, 1991).

The United States Environmental Protection Agency (EPA) has determined that ingestion of PCE can cause cancer in humans. Therefore, EPA has classified PCE as a Category I chemical under the Safe Drinking Water Act (SDWA) (Federal Register, 1991a). Standard EPA practice sets a maximum contaminant level goal (MCLG) of zero for any Category I chemical. EPA must set the maximum contaminant level (MCL) in drinking water as close to the MCLG as technically possible. The MCL for PCE is 5 µg/L (Federal Register, 1991b). The Resource Conservation and Recovery Act (RCRA) and the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) also regulate PCE in the environment.

PCE is a frequently detected contaminant in drinking water supplies and in contaminated groundwater at Superfund sites. The EPA water supply survey detected PCE in approximately 10 percent of the wells surveyed at concentrations up to 69 µg/L (Westrick et al., 1984). Other studies of domestic, industrial, municipal, and irrigation wells have produced results similar to the EPA study (Fusillo et al., 1985; Goodenkauf

and Atkinson, 1986). CERCLA investigations have identified PCE as a major groundwater contaminant at many Superfund sites, including Department of Energy (DOE) facilities (Davis et al., 1988; Dresen et al., 1989). PCE contamination is a result of improper waste disposal methods and accidental spillage. Data show that groundwater at some DOE sites is contaminated with PCE at several milligrams-per-liter (Davis et al., 1988; Dresen et al., 1989). Groundwater data from the Savannah River Site, presented in Table 1.1, illustrate the presence of high PCE concentrations in groundwater at a major DOE facility. The DOE is committed to remediating such sites and supports research into innovative technologies that destroy contaminants effectively and cost-efficiently (U.S. Department of Energy, 1994).

Table 1.1. PCE concentrations in selected wells at the Savannah River Site M Area Settling Basin.

Well[1]	PCE range (mg/L)	Well[1]	PCE range (mg/L)
MSB-3A	470	MSB-11F	1.03 - 2.09
MSB-4A	1.8 - 2.5	MSB-12B	1.12 - 1.41
MSB-9A	1.27 - 3.71	MSB-16C	0.6 - 5.92
MSB-9B	0.634 - 83.6	MSB-22	91 - 130
MSB-9C	43.5 - 988	MSB-24A	5.16 - 7.50
MSB-10C	14.5 - 56.7	MSB-27A	9.09 - 86.3
MSB-11C	<0.001 - 14.6	MSB-31C	69.6 - 123
MSB-11D	1.76 - 1.96	MSB-34B	0.51 - 1.36

Source: Davis, H. A., D. K. Martin, and J. L. Todd. 1989. Savannah River Site environmental report for 1988, WSRC-RP-89-59-1, Volume II.

[1]MSB = M-area settling basin.

Volatile organic compounds, such as PCE, are usually removed from groundwater by traditional methods such as air stripping and carbon adsorption (Weber and Smith, 1986). Such methods result in the transfer of the contaminant(s) from one environmental medium to another without ultimate destruction. Recently, innovative treatment methods that can destroy organic contaminants have attracted much interest for application to chlorinated solvent removal from groundwater (Fredrickson et al., 1993). Such methods include bioremediation, oxidation using Fenton's reaction (Takemura et al., 1994), and reduction in the presence of zero-valence metals (Liang et al., 1995)

Biological treatment of contaminated groundwater involves the use of native or specially adapted microorganisms either *in situ* or in above ground bioreactors. Researchers have investigated the removal of chlorinated solvents, including PCE, from water under aerobic, denitrifying, sulfate reducing, and methanogenic conditions (Hinchee et al., 1994). Experiments have been conducted in laboratory microcosms, bioreactors, and in the field (Dobbins et al., 1995; Hinchee and Olfenbuttel, 1991a; Hinchee and Olfenbuttel, 1991b; Semprini et al., 1990). However, the majority of these studies have used low concentrations, in the microgram-per-liter range, of the chlorinated solvents.

Research has demonstrated that microorganisms such as the methane-consuming bacteria (methanotrophs) and some toluene-oxidizing organisms mineralize trichloroethene (TCE), dichloroethene (DCE), and vinyl chloride (VC) to carbon dioxide, water, and chloride ion through a cometabolic process. Cometabolism is defined as the fortuitous biodegradation of an organic substrate by a microbe that cannot use the substrate for growth. The microbe must rely on other compounds for carbon and energy (Thomas and Ward, 1989). The methanotrophs use methane for growth, and coincidentally metabolize the chlorinated compound in the process. Figure 1.1 depicts the proposed reaction sequence leading from trans-1,2-dichloroethene (TRANS) to CO_2.

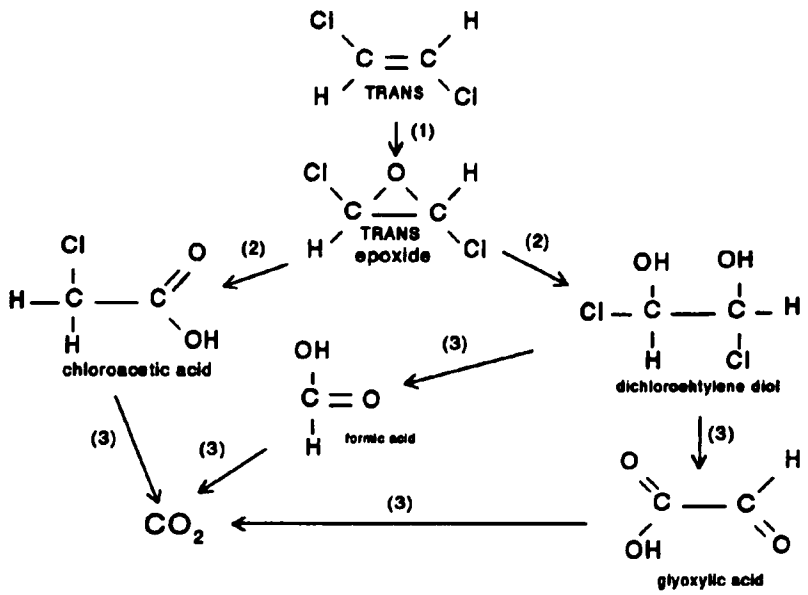


Figure 1.1. TRANS epoxide formation and degradation pathway. Adapted from Little et al. (1988). (1) methanotrophs; (2) spontaneous; (3) heterotrophs.

A similar reaction scheme is proposed for TCE, cis-1,2-dichloroethene (CIS), and VC. The first step involves the insertion of oxygen into the TRANS molecule by the methane monooxygenase (MMO) enzyme present in methanotrophs. This initial reaction forms an epoxide that is characteristic of MMO mediated cometabolic degradation.

Anaerobic bacteria, such as methane producers (methanogens) and sulfate reducers, can dechlorinate PCE to TCE, DCE, and VC, when provided a carbon source such as acetate or methanol. Reductive dechlorination is the name given to this process (Vogel and McCarty, 1985; Bagley and Gossett, 1990). Figure 1.2 shows the sequence of reductive dechlorination reactions for the complete dechlorination of PCE to ethylene (ETH). Most research indicates that the rate of these dechlorination reactions decreases as the number of chlorine atoms decreases (*i.e.*, the rate of conversion of PCE to TCE is much faster than the rate of formation of ETH from VC). Also, of the three possible DCE

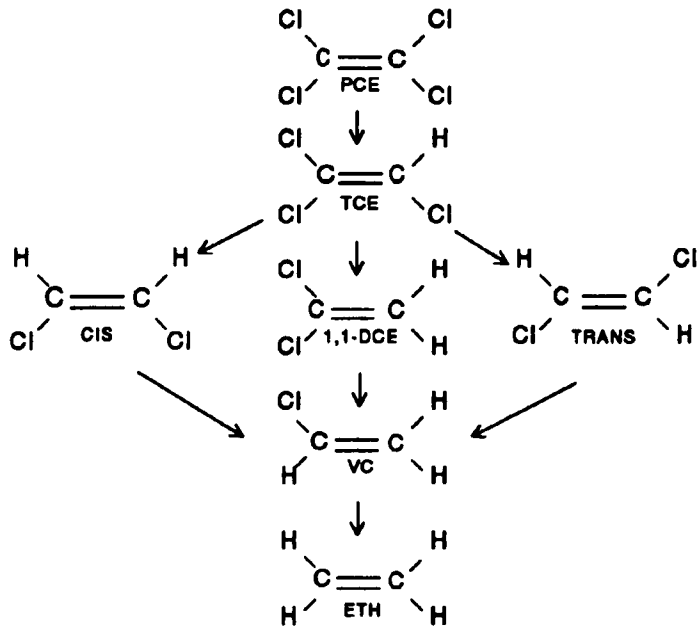


Figure 1.2. Reductive dechlorination pathway for PCE. After Mohn and Tiedje (1992).

isomers formed from TCE, CIS tends to predominate in both laboratory and field studies.

Electrons necessary for the reductive dechlorination process are provided by the carbon source. In the case of methanogenic use of acetate, electron flow is diverted from the normal reduction of acetate to methane to the reductive dechlorination reactions (Figure 1.3).

Because bioremediation can destroy organic contaminants, it is considered a preferred treatment option under CERCLA. Ultimately, bioremediation may prove to be more cost efficient than the commonly used methods of groundwater treatment, especially if the *in situ* option is viable (Lee et al., 1988). Bioremediation generally requires less mechanical equipment than other options and reduces or eliminates disposal costs. Such costs are significant when treatment options such as liquid-phase carbon adsorption and air stripping with offgas carbon adsorption are selected. The

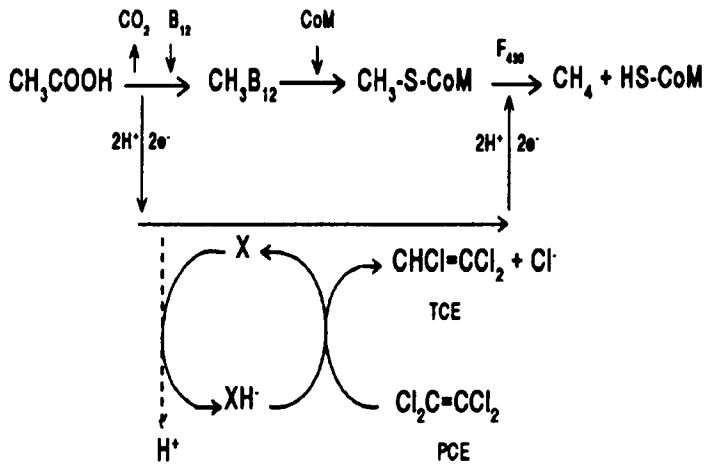


Figure 1.3. Proposed mechanism for electron transfer from the electron donor (acetate) to PCE during methanogenesis and reductive dechlorination. Adapted from Fathepure and Boyd (1988b). X is an unidentified electron carrier. CoM is coenzyme M, a methyl group carrier. F_{430} is a Ni-containing coenzyme associated with the methyl reductase enzyme.

public may view biological processes as more natural, and thus more acceptable, treatment alternatives. Public acceptability is an important factor because CERCLA requires the involvement of the affected community in decisions concerning cleanup alternatives.

1.2 OBJECTIVES

Most previous research has focused on low concentrations of PCE, generally in the microgram-per-liter range, which represent concentrations found in some drinking water supplies. Any *in situ* biological process designed for use at DOE sites must consider the effectiveness of dechlorination when concentrations of PCE are in the milligram-per-liter range. One aim of this research was to test the hypothesis that PCE can be degraded in an anaerobic, methanogenic environment at concentrations up to 88

mg/L. Simulated groundwater flow conditions in an inoculated, nutrient-fed, laboratory-scale sand column were used to test this hypothesis. Also, this research attempted to test the hypothesis that complete dechlorination of PCE may be possible by following anaerobic conditions with aerobic, methanotrophic conditions. PCE dechlorination products can degrade under methanotrophic conditions to CO_2 and H_2O.

In order to meet the objectives of this research, the following specific experiments were conducted:

1. Anaerobic microcosms were prepared using sediments from sites known to have been contaminated with chlorinated organic solvents. Acetate as a carbon source, a nutrient solution, and PCE were added. These microcosms were used to develop analytical techniques appropriate for following the reductive dechlorination of PCE and the appearance of PCE dechlorination products.

2. Aerobic microcosms were prepared using a mixed methanotrophic culture isolated from groundwater. These microcosms were fed nutrients, CH_4, and TRANS or CIS. The primary purpose for preparing these microcosms was to detect and positively identify TRANS and CIS epoxides.

3. A continuous-flow, anaerobic sand column simulated groundwater movement in a homogeneous, saturated, sandy aquifer contaminated with a high PCE concentration. The column was inoculated with supernatant from a municipal anaerobic digester. The reductive dechlorination of PCE at two influent concentrations, 32 and 88 mg/L, was observed along the length of the column.

4. Anaerobic, methanogenic dechlorination of PCE was coupled, in the same column, with aerobic, methanotrophic degradation of the less-chlorinated anaerobic degradation products. The possibility of completely dechlorinating PCE *in situ* was investigated using this column arrangement. The ideal result would have been the

complete mineralization of PCE. Other investigators have studied combined anaerobic/aerobic treatment systems. However, they have focused on separating the anaerobic and aerobic stages into different reactors designed to simulate an above ground biological reactor system (Fathepure and Vogel, 1991). One of the objectives of this research was to combine the anaerobic and aerobic processes in the same reactor. This arrangement better simulated an *in situ* remediation scheme.

2. LITERATURE REVIEW

Literature identifying the class of compounds generally referred to as volatile organic compounds (VOCs) as groundwater contaminants began to appear about 1980. Included among the VOCs are the chlorinated aliphatic compounds (CACs), such as PCE. The EPA's groundwater supply survey, conducted in 1981 and 1982, identified PCE as one of the five most frequently detected VOCs in finished water of groundwater origin (Westrick et al., 1984). These findings prompted investigations, often sponsored by the EPA, into technologies for removal of VOCs from drinking water, wastewater, and groundwater (Clark et al., 1988; Dworkin and Cawley, 1988; U.S. EPA, 1989). One of the technologies that has shown potential for removal of VOCs from groundwater is biological treatment. A number of different biological treatment techniques, including activated sludge, various completely mixed reactor systems, and fixed film processes such as trickling filters, anaerobic filters, and expanded and fluidized beds, have been investigated for their effectiveness in treating VOCs (Carter and Jewell, 1993; Chu and Jewell, 1994; Namkung and Rittmann, 1987; Phelps et al., 1991; Zitomer and Speece, 1993).

At least two problems exist with the biological degradation of PCE. First, methods using aerobic organisms known to degrade TCE, CIS, TRANS, and VC have not proved effective on the more highly chlorinated PCE (Janssen et al., 1988; Henson et al., 1989; Fogel et al., 1986). Second, reductive dechlorination of PCE results in the accumulation of degradation products, especially VC, which are as hazardous or more hazardous than the parent compound. Some current research suggests that anaerobic biological systems can dechlorinate PCE to the innocuous product ethene. However, the last steps in this conversion (DCE to VC to ETH) may be too slow to be practical (Freedman and

Gossett, 1989; DiStefano et al., 1991).

To avoid these problems, several researchers have proposed a two-stage process (Lee et al., 1988; Fathepure and Vogel, 1991; Zitomer and Speece, 1993). In the first stage, reductive dechlorination would reduce PCE to less-chlorinated compounds under anaerobic conditions. The second stage would involve an aerobic cometabolic process. The less-chlorinated products of the reductive dechlorination stage would be mineralized to CO_2, H_2O, and chloride. The overall effect would be the complete degradation of PCE to innocuous products.

This literature review summarizes research conducted on the following topics relevant to the biological degradation of PCE:

- physicochemical properties of the CACs
- physical, chemical, and biological fate of PCE and related compounds in groundwater
- laboratory studies conducted into the anaerobic and aerobic biological degradation of PCE

2.1 PHYSICOCHEMICAL PROPERTIES OF PCE, TCE, CIS, TRANS, AND VC

When studying biological degradation of the CACs, it is important to be aware of potential removal mechanisms that are purely physicochemical in nature. Volatilization and sorption are two such mechanisms that are involved in the removal of all the CACs studied in this research. These processes are accounted for by using Henry's law constants and distribution coefficients, respectively. Tables 2.1 through 2.5 present properties of the five CACs frequently dealt with when studying biological degradation of PCE.

Table 2.1. Physicochemical Properties of PCE.[a]

Formula	$Cl_2C=CCl_2$
Molecular weight	165.83
Water solubility (mg/L)	150
Henry's law constant (dimensionless @ 25°C)	0.723
Henry's law constant (atm m^3 water/m^3 air)	1035
Log K_{ow}	2.53
Log K_{oc}[b]	2.56
Specific gravity (20°C / 20°C)	1.623
Vapor pressure (torr @ 25°C)	19
Boiling point (°C)	121.4
Mobility class[c]	moderate

[a] Verschueren, 1983, unless otherwise noted.
[b] Smith and Dragun, 1984.
[c] Fetter, 1988.

Table 2.2. Physicochemical Properties of TCE.[a]

Formula	$Cl_2C=CHCl$
Molecular weight	131.39
Water solubility (mg/L)	1100
Henry's law constant (dimensionless @ 25°C)	0.392
Henry's law constant (atm m^3 water/m^3 air)	544
Log K_{ow}	1.48
Log K_{oc}[b]	2.10
Specific gravity (20°C / 20°C)	1.466
Vapor pressure (torr @ 25°C)	77
Boiling point (°C)	86.7
Mobility class[c]	moderate

[a] Verschueren, 1983, unless otherwise noted.
[b] Smith and Dragun, 1984.
[c] Fetter, 1988.

Table 2.3. Physicochemical Properties of CIS.[a]

Formula	ClHC=CHCl
Molecular weight	96.94
Water solubility (mg/L)	3500
Henry's law constant (dimensionless @ 25°C)	0.167
Henry's law constant (atm m^3 water/m^3 air)	160
Log K_{ow}	1.48
Log K_{oc}[b]	1.77
Specific gravity (25°C liquid)	1.27
Vapor pressure (mm Hg @ 25°C)	215
Boiling point (°C)	60.3
Mobility class	[c]

[a] Verschueren, 1983, unless otherwise noted.
[b] Smith and Dragun, 1984.
[c] Not listed in Fetter, 1988.

Table 2.4. Physicochemical Properties of TRANS.[a]

Formula	ClHC=CHCl
Molecular weight	96.94
Water solubility (mg/L)	6300
Henry's law constant (dimensionless @ 25°C)	0.384
Henry's law constant (atm m^3 water/m^3 air)	429
Log K_{ow}	1.48
Log K_{oc}[b]	1.77
Specific gravity (25°C liquid)	1.27
Vapor pressure (mm Hg @ 25°C)	336
Boiling point (°C)	47.5
Mobility class[c]	very high

[a] Verschueren, 1984, unless otherwise noted.
[b] Smith and Dragun, 1984.
[c] Fetter, 1988.

Table 2.5. Physicochemical Properties of VC.[a]

Formula	$H_2C=CHCl$
Molecular weight	62.5
Water solubility (mg/L)	2670
Henry's law constant (dimensionless @ 20°C)	1.137
Henry's law constant (atm m^3 water/m^3 air)	355,000
Log K_{ow}	0.60
Log K_{oc}[b]	0.91
Specific gravity	0.908
Vapor pressure (torr @ 20°C)	2580
Boiling point (°C)	-13.9
Mobility class	[c]

[a] Verschueren, 1984, unless otherwise noted.
[b] Smith and Dragun, 1984.
[c] Not listed in Fetter, 1988.

The CACs are of low solubility in water, relatively dense, very volatile, and quite mobile in groundwater environments. These properties make the identification of particular removal mechanisms difficult both in the field and in the laboratory. Low solubility means that there is a potential for two liquid phases to exist, and volatility results in a contaminated vapor phase. High density means that a spill of sufficient magnitude is likely to move downward through an aquifer until a confining layer is reached. The low K_{oc} values indicate that relatively little retardation of the compounds by sorption onto soil organic matter will occur.

One should exercise caution in the use of property data such as are presented in tables 2.1 through 2.5. Different sources often present quite different values for solubility and the various distribution coefficients.

2.2 PHYSICAL, CHEMICAL, AND BIOLOGICAL FATE OF PCE *IN SITU*

Transport of PCE in groundwater depends upon groundwater flow velocity, dispersion, volatilization, sorption and desorption to the aquifer solids, temperature, availability of oxygen, pH, abiotic transformation, and the presence of microorganisms capable of transforming PCE. Several studies have examined the fate of PCE in groundwater environments. Some researchers have studied the fate of PCE from a theoretical perspective (Tursman and Cork, 1992; Bouwer, 1992; Morgan and Watkinson, 1989) while others have examined field data (Lesage at al., 1990; Major et al., 1991; McNab and Narasimhan, 1994; Milde et al., 1988). This section reviews studies that have considered the fate of PCE in groundwater from both perspectives.

These studies show that PCE can be quite mobile in groundwater. Abiotic reactions that might lead to the removal of PCE are quite slow. Biological reactions appear to be more important than physical or chemical reactions in reducing groundwater contamination from PCE and related contaminants. Many groundwaters are anaerobic, and anaerobes capable of PCE dechlorination are widespread. Methanotrophs capable of degrading the less-chlorinated PCE products also appear to be common and are readily stimulated by adding oxygen and methane to groundwater. Field investigations show that biological reductive dechlorination is a naturally occurring process that has been observed in many areas where PCE has contaminated groundwater. Reductive dechlorination of PCE occurs under methanogenic conditions, under sulfate reducing conditions, and perhaps under denitrifying conditions. Products of the reductive dechlorination of PCE include TCE, CIS, TRANS, VC, and ETH. The CIS isomer of DCE generally predominates over TRANS.

Smith and Dragun (1984) summarized what was then known about

transformation, in groundwater, of several CACs. Included in their review were the compounds of interest in this study. In their discussion of sorption, mobility, and volatilization, Smith and Dragun present theoretical calculations which show that a saturated subsoil containing 1% organic carbon would have 17% of the total TCE in solution. The presence of such a large fraction of compound in solution, as opposed to sorbed to the soil, lead to the conclusion that CACs are quite mobile in groundwater. They also point out that, since groundwater is not in direct contact with the atmosphere, volatilization is unlikely to be a major mechanism for removal of CACs from groundwater.

Smith and Dragun's review of the literature led them to conclude that the primary microbial degradation products of CACs in groundwater are the result of reductive dechlorination. They reached this conclusion despite the fact that field results are sometimes conflicting. Such conflicting field data indicate the importance of local site conditions. They make an important point that should be kept in mind when considering an *in situ* bioremediation project, *i.e.*, the high rates of microbial degradation observed under controlled laboratory conditions are unlikely to be matched in the field. Degradation in the field, whether physical, chemical, or biological, will depend upon soil conditions, pH, types of microorganisms present, temperature, groundwater chemistry, and other environmental conditions.

An overview of VOC biodegradation in groundwater (Pfaender, 1990) also makes the point that the physicochemical properties of the VOCs result in fairly rapid movement in groundwater. Rapid movement of the VOCs occurs in the absence of biological degradation. The rate and extent of degradation will be determined by local environmental conditions.

Pfaender points out that in most aquifers the oxygen concentration and rate of oxygen recharge are low. Thus, growth of anaerobic microorganisms is favored. The

reports he reviewed led him to the conclusion that anaerobic bacteria with the ability to degrade PCE, as well as other halogenated VOCs, are fairly widespread in the environment. Also present among the subsurface microorganisms are the aerobic, methane-consuming methanotrophs. These organisms exist in the environment in association with the anaerobic, methane-producing methanogens. Methanotrophs possess the capacity for degrading some VOCs, although not the highly-chlorinated PCE. Sulfate-reducing organisms are also present in the subsurface and have shown some ability to degrade VOCs. The fact that sulfate has been shown to inhibit methanogenesis, probably due to competition between sulfate reducers and methanogens, may complicate VOC degradation in any *in situ* remediation scheme. Despite this potential drawback, Pfaender suggests that a remediation scheme for VOCs in the subsurface might involve a sequential anaerobic process for the more highly chlorinated compounds followed by an aerobic process for the less chlorinated compounds. Regardless of the biological approach taken, whether it be pump and treat in above-ground reactors or *in situ* treatment, biological methods offer advantages over physical-chemical treatment methods of lower cost and final destruction of the contaminant. However, the success of *in situ* biological methods will depend upon bringing the right combination of microorganisms and environmental conditions together at the same site, a task that may prove difficult.

In an overview of biotic and abiotic reactions of PCE and related compounds, Vogel and coworkers (Vogel et al., 1987) conclude that most abiotic reactions that lead to degradation of CACs are slow. However, abiotic reactions can be significant in the time frame of slow groundwater movement. Although abiotic transformations can be important, biological processes are often the most significant factors in bringing about CAC removal from the groundwater environment. They discuss, from a fundamental

thermodynamic point of view, reactions such as hydrolysis, dehydrohalogenation, and epoxidation that are possible for CACs. They also consider the conditions under which each reaction type is likely to occur.

CACs may be either oxidized or reduced depending upon environmental conditions. The more halogenated CACs, such as PCE, are more oxidized and are, therefore, likely to be reduced. This reduction generally involves the replacement of a chlorine atom with a hydrogen atom and is termed hydrogenolysis. Such reductions are usually only observed under strictly anaerobic conditions and are mediated by the electron acceptors sulfate or, in the case of methanogenesis, carbon dioxide. Some studies have also shown reduction of CACs under the less stringent anoxic conditions of denitrification (Bae et al., 1990; Bouwer and McCarty, 1983). The less-chlorinated CACs can undergo oxidation usually via epoxidation. The epoxides thus formed are generally short lived and spontaneously degrade to alcohols.

Lesage (Lesage et al., 1990) studied an area of a landfill in Canada where solvents and other liquid wastes were disposed of by incineration in trenches. Included in the compounds disposed were PCE and related chlorinated aliphatic solvents. Incomplete incineration led to contamination of the underlying aquifer. Uncontaminated groundwater upgradient of the disposal unit contained 2-6 mg/L of oxygen and had E_h values greater than 500 mV. Groundwater in the contaminant plume had nondetectable oxygen and E_h values of -40 to +200 mV. These latter values are indicative of sulfate reducing conditions.

Degradation products of PCE including TCE, CIS, TRANS, 1,1-DCE, and VC were detected in the contaminant plume. Comparison of data over a five year period showed an increase in the lesser chlorinated compounds relative to the more highly chlorinated compounds. PCE decreased from 10% to 3% of the total chlorinated ethanes plus

ethenes while TCE increased from 22% to 37%. CIS, TRANS, and VC were reported at 0.5, 0.2, and 1.0% of the total, respectively, at the end of the five year period. Only TRANS was reported at the beginning of the study period at 0.1%. Lesage and coworkers concluded that *in situ* biotransformation of highly chlorinated compounds was occurring under sulfate-reducing conditions. They based their conclusion on the presence of lesser chlorinated compounds typically observed in laboratory studies. This study also provides field evidence that reductive dechlorination occurs at common groundwater temperatures (9° C to 10° C in this case).

Major and coworkers (Major et al., 1991) report field and laboratory evidence for the complete dechlorination of PCE to ethene. The authors studied groundwater contamination at an old chemical transfer facility in Canada where PCE spills had occurred. They detected PCE and PCE reductive dechlorination products in site groundwater. CIS was the predominant DCE isomer found. Also, both methanol and acetate were detected in the groundwater at concentrations ranging from 5 to 810 mg/L. There was a strong spatial relationship among PCE, ETH, methanol, and acetate. This provided evidence that methanol and acetate were promoting PCE dechlorination.

Methane and sulfate were present in the groundwater throughout the site. Sulfate-reducing bacteria (SRB) and methanogenic organisms were detected in sediments but did not comprise a significant portion of the microbial biomass. The low SRB and methanogen biomass indicated that other anaerobes were involved in the *in situ* dechlorination of PCE. The authors speculate that acetogenic microorganisms that produce acetate from methanol were active in the dechlorination of PCE to ETH. Since hydraulic conductivities at the site were determined to be too low for successful implementation of pump and treat strategies and bacterial reductive dechlorination was evident, the authors suggested that *in situ* anaerobic biotransformation might be exploited

as a remediation strategy.

Stanford researchers (Semprini et al., 1990) conducted a well-controlled field demonstration of *in situ* biodegradation of TCE, CIS, TRANS, and VC at the Moffett Air Force Base in California. They stimulated indigenous methanotrophs in a shallow aquifer by injecting O_2- and CH_4-containing groundwater. Within two meters of the injection well (one to two day travel time) they observed decreases in all four CACs. These decreases were attributable to biotransformation. The less-chlorinated CACs were degraded to the greatest extent. Also, TRANS was degraded more readily than CIS. Degradation occurred only with CH_4 addition, thus indicating that CH_4 utilization was required for biotransformation of the CACs. The addition of nitrogen and phosphorous was not necessary. They were able to detect, identify, and partially quantify the TRANS epoxide intermediate. However, it appeared that the TRANS epoxide represented only a few percent of the total TRANS degraded.

The same group of Stanford researchers (McCarty et al., 1991) initiated a groundwater investigation at a Superfund site in Michigan. Groundwater at this site was contaminated with high concentrations of TCE, CIS, and VC. Some TRANS was detected, but concentrations were much lower than CIS. High concentrations of CH_4 were observed and ETH was present. Therefore, it appeared that natural anaerobic dechlorination was occurring under methanogenic conditions. They also made chemical oxygen demand (COD) measurements and determined that natural organic matter was acting as the electron donor for reductive dechlorination. Their data allowed them to estimate that from 15 to 56% of the reducing power of the COD went into dechlorination. The remainder went to CH_4 production. They concluded that the significant amount of TCE reduction occurring at this site indicated that reduction of TCE to ETH could be stimulated by adding methanol or acetate as additional electron acceptors.

The successful test at the Moffett Field site led the authors to propose a full scale test of methanotrophic, *in situ* bioremediation at the Michigan site. They proposed injecting CH_4 and O_2 into the groundwater to enhance VC removal.

Work performed by McCarty's group at Stanford led him to propose (McCarty, 1991) that the best remediation strategy for groundwater contaminated with chlorinated solvents may be to wait for natural biological proceeses to accomplish the task. However, if there is no evidence that natural biological processes are occurring, addition of appropriate microorganisms and/or nutrients should be considered. McCarty points out that the hydrogeology of the particular site may limit the effectiveness of any *in situ* bioremediation design. He proposed that the most effective *in situ* treatment scenario for high concentrations of PCE may involve enhanced anaerobic dechlorination followed by aerobic, methanotrophic degradation. Whatever the alternative chosen, the main advantages of biological methods are minimal disruption of the subsurface and lower costs. This is particularly true for anaerobic processes because no O_2 and fewer nutrients are required. Also, anaerobic systems will result in less biomass buildup and thus less chance of restricting flow in the aquifer. Proposals for *in situ* bioremediation projects should be site specific and preceded by detailed laboratory studies.

McNab and Narasimhan (1994) analyzed groundwater data from the Lawrence Livermore National Laboratory and verified that, under highly oxidizing groundwater conditions, reductive dechlorination does not occur to any significant extent. Both PCE and TCE were detected at parts-per-million levels. Some CIS and TRANS were also present, but VC was never detected . The presence of O_2 and the speciation of manganese and chromium confirmed oxidizing conditions. They showed that TCE:PCE ratios were not increasing with time. Therefore, there was no evidence for significant reductive dechlorination. They also suggest that their data validate theoretical predictions

that the chlorinated ethenes are highly resistant to hydrolysis and elimination reactions.

Although several researchers have suggested exploiting biological reductive dechlorination as an *in situ* groundwater remediation strategy, there do not appear to be any examples of this actually being done. In contrast, examples of successful *in situ* bioremediation using biostimulated methanotrophs do appear in the literature. All the studies that propose the *in situ* bioremediation option stress the importance of detailed hydrogeological investigations and laboratory studies before proceeding.

Researchers seem to agree that *in situ* biological remediation offers the advantages of lower cost and complete destruction when compared to above-ground physical-chemical treatment options. The best strategy for remediating some PCE-contaminated groundwater may be to wait for natural biological processes to do the job.

2.3 LABORATORY STUDIES

Wilson and McNabb (1983) described the identification of large numbers of microorganisms in the subsurface and reflected on the prospects for biotransformation of several common groundwater contaminants. They postulated that degradation of TCE or PCE under aerobic conditions was not possible, and that transformation under anaerobic conditions was possible but probably incomplete. This section describes anaerobic and aerobic studies that have been conducted in the decade since the Wilson and McNabb report. These studies show that PCE can indeed be dechlorinated under anaerobic conditions. Anaerobic dechlorination of PCE is usually incomplete, sometimes stopping at CIS or VC, but occasionally proceeds to the completely dechlorinated products ethene and ethane. Numerous studies have now shown that PCE is recalcitrant under aerobic, methanotrophic conditions. However, the anaerobic dechlorination products of PCE have been shown to be degradable aerobically by methanotrophs. These findings have led

some researchers to speculate on the possibility of combining anaerobic, methanogenic dechlorination of PCE with an aerobic, methanotrophic process for complete mineralization of highly chlorinated compounds such as PCE.

2.3.1 Anaerobic Processes

Anaerobic bacteria can dechlorinate PCE to TCE, DCE, and VC by the process of reductive dechlorination described earlier. Electrons necessary for reductive dechlorination have been provided by a number of different carbon sources. Acetate and methanol have generally led to the most rapid and complete dechlorination of PCE. Many researchers have dealt with the complicating problems of sorption and volatilization losses.

2.3.1.1 Microcosms

Early investigations (Bouwer et al., 1981) led researchers to conclude that PCE and related compounds were not biodegradable even under anaerobic conditions. However, by the mid-1980's, studies began to show that CACs, particularly PCE and TCE, could be degraded under anaerobic conditions when appropriate electron donors were available (Kleopfer et al., 1985; Parsons et al., 1984; Parsons et al., 1985). The process of reductive dechlorination was identified as the mechanism for the stepwise dechlorination observed. In general, these early studies used parts-per-billion to low parts-per-million levels of PCE or TCE as the starting contaminant. Usually, the reductive dechlorination process proceeded to the stage of VC. CIS was identified as the predominant DCE isomer formed.

Researchers discovered wide variability in the dechlorinating potential among soils and sediments from different locations and among individual microcosms. Such

variability was attributed to the history of exposure of the soil or sediment to contaminants, slight differences in microcosm preparation that led to differences in redox potential, electron donor availability, presence of appropriate microorganisms, or other unknown factors critical to the reductive dechlorination process.

Debate arose among researchers as to the organisms responsible for reductive dechlorination of CACs. Some researchers (Barrio-Lage et al., 1987) suggested that acetate decarboxylating methanogens were not responsible for TCE degradation. This conclusion was based on observations that PCE was reductively dechlorinated to TCE, DCE, and VC in microcosms in which methanogenic conditions were seldom observed (Barrio-Lage et al., 1990). Reductive dechlorination was apparently occurring under sulfate-reducing conditions.

Further work investigated the ability of pure cultures of methanogens to carry out reductive dechlorination (Fathepure et al., 1987). Such work showed that methanogens are capable of dechlorinating PCE to TCE and that the rate of the dechlorination increased directly with the methane production rate. The important role that methanogens play in the reductive dechlorination process was confirmed by studies using mixed cultures containing methanogens (Fathepure and Boyd, 1988a). Use of the methanogenic inhibitor, bromoethane sulfonic acid (BES), inhibited the dechlorination of PCE. These studies also compared the effect of the electron donor on the reductive dechlorination process. Methanol was found to be a more effective electron donor than acetate. Researchers hypothesized that electrons generated in the process of sythesizing methane were diverted to the reductive dechlorination process. Some even speculated that dechlorination of CACs *in situ* could be enhanced simply by stimulating methanogenesis (Fathepure and Boyd, 1988b).

Further research confirmed that CACs could be dechlorinated under both

methanogenic and sulfate-reducing conditions. In experiments with mixed cultures of methanogens and nonmethanogenic fermenters, Baek and Jaffé (1989) confirmed the correlation between methane production and dechlorination. However, their work also showed that the nonmethanogenic fermenters enhanced, and may have been necessary for, reductive dechlorination under methanogenic conditions.

As work on the anaerobic biodegradation of PCE and related compounds progressed, experiments indicated that PCE could actually be completely dechlorinated to ETH under methanogenic conditions (Freedman and Gossett, 1989). However, although dechlorination to the level of VC was rapid, the production of ETH from VC was very slow. This work confirmed that added electron donor was necessary for dechlorination to proceed at a reasonable rate and that methanol appeared to be the best choice. Also confirming other work was the observation that inhibition of methane production interrupted reductive dechlorination.

Investigations in the early 1990s confirmed that reductive dechlorination can occur under sulfate-reducing conditions (Pavlostathis and Zhuang, 1991). As is the case for methanogenesis, other microorganisms may be required to work in concert with sulfate reducers to accomplish reductive dechlorination. Reductive dechlorination under sulfate-reducing conditions also requires electron donors. However, the best donors under sulfate-reducing conditions may be different from those proven most effective under methanogenic conditions.

Recent work has shown that high concentrations (~55 mg/L) of PCE can be completely dechlorinated to ETH without the buildup of VC (DiStefano et al., 1991). This research was conducted under non-methanogenic, anaerobic conditions. Methanol was converted to acetate and, in the process, electrons were used for reductive dechlorination.

The microcosm studies described here, as well as numerous others, show that PCE can be dechlorinated under both sulfate-reducing and methanogenic conditions. Dechlorination has been to shown to be a sequential process from PCE→TCE→DCE→VC. Some researchers have demonstrated complete dechlorination to ETH. Each transformation step in the reaction sequence proceeds at a slower rate than the previous one. Reductive dechlorination of PCE has been shown to occur rapidly at temperatures ranging from 20°C to 35°C. The conditions of incubation and the observed results from the anaerobic microcosm studies reviewed are presented in Table 2.6.

For significant degradation to occur, a primary electron donor must be provided to the microcosms. Acetate and methanol are the two most common electron donors used in the studies reviewed. The results obtained in the microcosm studies do not clearly identify either acetate or methanol as a preferred electron donor for reductive dechlorination.

Results from these studies also demonstrate the importance of careful microcosm preparation. Volatilization losses were a recurring problem with such highly volatile compounds and affected researchers' ability to perform mass balances. Therefore, the importance of identifying degradation products and not simply observing contaminant loss from a microcosm is clear.

Table 2.6. Summary of anaerobic microcosm studies.

reference	inoculum	CAC	conc. (µg/L)	added e^- donor	conditions[a]	temp. (°C)	dechlorination rate	dechlorination products
Bouwer et al., 1981	mixed methanogenic culture	PCE, TCE	10, 30, 100	methanol[b]	M	35	no degradation after 16 weeks	none measured
Kleopfer et al., 1985	soil	TCE	2 mg/kg of soil	soybean meal	A	23	0.3 µg DCE/kg soil/day	1,2-DCE
Parsons et al., 1984	freshwater sediments	PCE	1600 - 4100	methanol[b]	S	25	300 nmol PCE/L/day	TCE, CIS, VC, trace of TRANS, 1,1-DCE
Parsons et al., 1985	sediment	PCE	4200	methanol[b]	S	25	300 nmol TCE/L/day	TCE, CIS, trace of TRANS
Barrio-Lage et al., 1986	sediment	CIS, TRANS, 1,1-DCE	5000	none	A	25	0.853 - 3.57 x 10^{-4} hr^{-1} (highest for TRANS)	VC, CE (from the CIS microcosm only)
Barrio-Lage et al., 1987	muck, calcareous bedrock, soil	TCE	5000	acetate[c]	S	25	20 - 30 nmol TCE/L/day	CIS, some TRANS and VC
Barrio-Lage et al., 1990	soil (clay, ~4% organic carbon)	VC	2000	methanol, acetate	M	25	$t_{½}$ = 57 yrs.	small amount of CO_2
Fathepure et al., 1987	pure cultures of methanogens	PCE	1000	methanol	M	37	0.5 - 0.8 nmol TCE/mg protein/day	TCE
"	strain DCB-1	PCE	"	pyruvate	"	"	2.34 nmol TCE/mg protein/day	TCE
"	mixed culture	PCE	730	3-chloro-benzoate	"	"	340 nmol TCE/L/day	beyond TCE but unspecified
Fathepure and Boyd, 1988b	anaerobic sludges	PCE	1000	methanol[b]	M	37	250 - 445 nmole TCE/L/day	TCE
Fathepure and Boyd, 1988a	pure cultures of methanogens	PCE	1000	acetate, methanol	M	37	—	TCE (only a small fraction of PCE was dechlorinated)
"	strain DCM	"	3000	methanol	"	"	120 nmol TCE/L/day	TCE
"	"	"	"	trimethyl-amine	"	"	188 nmol TCE/L/day	TCE

Table 2.6. (continued)

reference	inoculum	CAC	conc. (µg/L)	added e^- donor	conditions[a]	temp. (°C)	reaction rate	dechlorination products
Scholz-Muramatsu et al., 1990	biomass from a lab reactor fed benzoate	PCE	saturation	benzoate	A	30	0.4 mmol PCE/L/day[d]	DCE
Baek and Jaffé, 1989	mixed methanogens + fermenters	TCE	~8[e]	formate, acetate, glucose	M	35	1.4 nmol TCE/L/day	CE (main), VC, 1,1-DCE, 1,2-DCE (no isomer specified)
"	"	"	"	methanol, formate	"	25	[e]	CE (minor), VC, 1,1-DCE, 1,2-DCE (no isomer specified)
"	methanogens	"	"	formate	"	35	----	none
Freedman and Gossett, 1989	mixedculture from a lab reactor	PCE, TCE	~500 - 1000	methanol, H_2, formate, acetate, glucose	M	35	~3.6 µmole PCE/L/day (best with CH_3OH as the e^- donor)	TRANS, VC, ETH
Pavlostathis and Zhuang, 1991	contaminated soil	TCE	74 - 9800	acetate + lactate	S	20	0.0018 - 0.2 µmole TCE/L/day	CIS
"	"	"	"	"	"	35	213 µmole TCE/L/day	"
DiStefano et al., 1991	enrichment cultures	PCE	55,000	methanol	A	35	275 µmole PCE/L/day	ETH

[a] M = methanogenic; S = sulfate reducing; A = unspecified anaerobic.
[b] Solvent used to dissolve the CAC before adding to the microcosm ∴ not intentionally added as an e- donor but present in sufficient quantity to act as such.
[c] Added to some of the microcosms in an attempt to increase biomass but had no effect on dechlorination.
[d] Calculated from data presented.
[e] Not clear from information presented.

2.3.1.2 Columns and Reactors

Successful experiments have led to the installation of bioreactors for chlorinated solvent removal at the pilot plant and field scale (Donaldson et al., 1993; White, T., Ciba-Geigy Corporation, Greensboro, N.C., personal communication, Sept. 12, 1991). Most of this work has focused on above ground reactors. However, researchers have recently directed their efforts toward the *in situ* degradation of chlorinated organic contaminants (Roberts et al., 1990; Thomas and Ward, 1989; McCarty, 1991).

Several column studies have been conducted to observe the removal of PCE in a simulated groundwater environment under methanogenic conditions (Bouwer and McCarty, 1983; Vogel and McCarty, 1985; Bouwer and Wright, 1988). Columns were typically inoculated with digested sewage sludge and packed with glass beads to minimize the effects of sorption. Most were conducted at ~2 hr. hydraulic retention times and at room temperature. Influent PCE ranged from a few micrograms-per-liter to 20 mg/L and was often one of several contaminants applied to the columns. Acetate was the preferred carbon source. The ability to reductively dechlorinate PCE under methanogenic conditions was confirmed in these column studies. Unfortunately, TCE, DCE, and VC were often produced but not quantified. Also, some of the studies relied solely on the disappearance of PCE as confirmation of biological dechlorination.

Column studies have also been conducted under sulfate-reducing and denitrifying conditions (Bouwer and Wright, 1988; Cobb and Bouwer, 1991). Some studies showed reductive dechlorination of PCE in columns operated under sulfate-reducing conditions while others did not. No removal of PCE under denitrifying conditions was observed.

The anaerobic column studies confirm that PCE can be reductively dechlorinated under methanogenic conditions with acetate as a carbon source (electron donor). They also show the importance of observing the formation of the reductive dechlorination

products so that adequate information can be obtained about the rate and extent of the reductive dechlorination process. Most of these studies showed that a large percentage of PCE was dechlorinated, but the removal of PCE could not always be accounted for in the dechlorination products.

2.3.2 Aerobic Processes

This review of the literature of microbial degradation of the CACs under aerobic conditions focuses on methanotrophic degradation. Although organisms that use toluene or phenol as a primary substrate are capable of degrading some CACs (Nelson et al., 1990), methane utilizing microorganisms are of primary interest to this research.

Research has been unable to show PCE degradation by methanotrophs. However, methanotrophic degradation of the reductive dechlorination products of PCE has been well studied. Researchers have concluded that the MMO enzyme responsible for the methanotroph's ability to utilize methane can fortuitously degrade certain CACs. However, methanotrophs apparently cannot obtain energy for growth from the CACs. This process of fortuitous transformation is termed cometabolism.

In general, the rate of cometabolic transformation of CACs decreases with an increase in the number of chlorine atoms on the molecule. For example, research shows that TCE methanotrophic degradation occurs at a much slower rate than does DCE degradation. Compounds saturated with chlorines, such as PCE, are not degraded by methanotrophs. Literature results are conflicting regarding the relative rates of CIS and TRANS degradation, but TRANS is generally reported to be more readily degraded under methanotrophic conditions than CIS.

The following two sections summarize research that demonstrates methanotrophic degradation of TCE, CIS, and TRANS. Experiments involving

microcosms, soil columns, and bioreactors are discussed.

2.3.2.1 Microcosms

Early studies (Bouwer et al., 1981) indicated that neither PCE nor TCE were degradable under aerobic conditions. However, work in the mid-1980s (Fogel et al., 1986; Little et al., 1988; Wilson and Wilson, 1985) focused on the discovery that TCE could be degraded by organisms grown on methane, methanol, or propane. This work led to the hypothesis that TCE degradation was a cometabolic process that involved epoxidation by the MMO enzyme. Indications were that degradation occurred when organisms were actively using a growth substrate, such as methane, and diminished when substrate was depleted.

Further experiments conducted under methanotrophic conditions investigated the degradation of PCE, TCE, CIS, and TRANS (Janssen et al., 1988; Henson et al., 1989; Uchiyama et al., 1989; Fogel et al., 1986; Fogel et al., 1987). Results confirmed that degradation was more rapid for the less chlorinated compounds. Degradation of the CACs, except for PCE, occurred at concentrations in the tens-of-milligrams per liter range without inhibiting growth of the methanotrophs. Cometabolic degradation via epoxide intermediates was confirmed. When methane was provided as the carbon source, it was required, along with oxygen, for CAC degradation to proceed. Inhibition of methane utilization inhibited CAC removal. TCE appeared to be toxic at high concentrations, but low part-per-million levels were readily degraded. Results consistently showed that the less chlorinated CACs such as CIS, TRANS, and VC were much more readily degradable than the more highly chlorinated compounds. Table 2.7 summarizes the results of Fogel and coworkers. These data are typical of results obtained under methanotrophic conditions except for some debate as to the relative degradability of CIS versus TRANS.

Table 2.7. Summary of data from Fogel et al. (1986).

compound	initial concentration (ppb)	final concentration (ppb)	time (hr)	degradation rate (nmole/hr/mg protein)
VC	540	0	23	32
1,1-DCE	630	200	48	7
CIS	660	0	50	11
TRANS	650	0	50	11
TCE	650	200	96	2.5
PCE	200	230	190	0

Recently, researchers have investigated the problem of competitive inhibition between methane and the CACs for the MMO enzyme (Alvarez-Cohen and McCarty, 1991). This work has led to ideas for using "resting" cells of methanotrophs in the absence of methane to carry out the transformation of TCE. Successful experiments have been conducted with such cells and have shown no inhibition at TCE concentrations up to ~20 mg/L. Other recent work (Broholm et al., 1993) has shown that methanotrophic conversion of TCE can occur at typical groundwater temperatures. However, since not all of the cultures studied showed the ability to degrade TCE, the presence of an indigenous methanotrophic population does not guarantee that TCE will be degraded at a particular site.

2.3.2.2 Columns and Reactors

A column study by Wilson and Wilson (1985) was apparently the first work that demonstrated methanotrophic degradation of TCE. This study was built on earlier work that showed that the MMO enzyme of methanotrophs could oxidize and dechlorinate

halogenated methanes. Also, propane utilizing organisms had been shown to epoxidate ethene. Building on this earlier work, Wilson and Wilson exposed an unsaturated soil column to a stream of natural gas in air. They were able to demonstrate >95% removal of TCE from the influent water in a two day residence time.

The work of Wilson and Wilson was followed by a large number of soil column and bioreactor studies that addressed methanotrophic degradation of many CACs (Mayer et al., 1988; Moore et al., 1989; Strandberg et al., 1989; Leahy et al., 1989; Strand et al., 1990; Arvin, 1991; Hicks et al., 1991; Strand et al., 1991). These studies confirmed that PCE was not degradable by methanotrophs. They also showed, as did the methanotrophic microcosm studies, that the fewer the chlorines attached to a compound, the faster and to a greater extent will the compound be degraded by the MMO enzyme. A common finding in these column studies was that TRANS was more readily degradable than CIS.

Two of these studies (Moore et al., 1989 and Arvin, 1991) reported the detection and positive identification of epoxide intermediates in the methanotrophic degradation of CIS and TRANS. Detection of epoxides conclusively demonstrates that the degradation is biological. Epoxides do not form under physical or chemical degradation mechanisms. The CIS and TRANS epoxides were the only CAC epoxides stable enough to be detected by conventional gas chromatography and mass spectrometry techniques.

Some data show that higher rates of degradation are achieved under richer methane conditions. However, degradation was also achieved under methane starvation conditions. These column studies also generated a variety of information concerning the inhibitory effects of copper and the potential problems associated with the use of hydrogen peroxide as an oxygen source.

Researchers familiar with the anaerobic work proposed that a combined

anaerobic methanogenic/aerobic methanotrophic process could completely mineralize highly chlorinated compounds such as PCE and TCE (Moore et al., 1989). However, some researchers point out that the need to supply methane to the subsurface will present great difficulties in designing an *in situ* bioremediation process. The work of Hicks (Hicks et al., 1991) suggests the possibility that dissolution of methane and oxygen in the influent water supplied to the subsurface, coupled with the development of methanogenic zones in the subsurface, may provide an adequate methane supply to support methanotrophic activity. Table 2.8 summarizes results from the methanotrophic column studies reviewed herein.

Table 2.8. Summary of methanotrophic column studies.

compound	conc. (µg/L)	t_r (hrs.)	% removal	k[c] (day^{-1})	reference
CIS	4000	5	31	129 ± 16	Arvin, 1991
TCE	1200	"	23	86 ± 13	"
TRANS[d]	2000	"	97	8200 ± 1400	"
TCE	150	48	95	[e]	Wilson and Wilson, 1985
TCE	1000	~0.8	>50	23 - 66	Strandberg et al., 1989
"	20,000	"	~40	—	"
TRANS	1000	"	>90	86 - 500	"
TCE	<55	1.5	400[f]	130	Strand et al., 1991
"	<1000	"	60 ± 5	9.6	"
TCE	<3000	[g]	[h]	0.14 - 1.22[i]	Strand et al., 1990
TCE	50	[j]	20	—	Mayer et al., 1988
PCE	700	[k]	0	—	Leahy et al., 1990
TCE	41 - 400	1.25 - 2.50[l]	44 - 98	—	Hicks et al., 1991
TRANS	1000	24	—	—	Moore et al., 1989

[a] Hydraulic retention time.
[b] (concentration in - concentration out)/(concentration in) x 100 unless otherwise noted.
[c] Reaction rate constant. First order unless otherwise noted.
[d] TRANS based on limited data and should be taken only as an order of magnitude estimate.
[e] One order of magnitude reduction in 24 hrs.
[f] Maximum volumetric rate observed, µg/L/hr.
[g] Two-liter, suspended growth, closed reactor.
[h] TCE < detection limit after 3 - 5 days.
[i] µg TCE/mg VSS/hr.
[j] Columns operated as sequential batch reactors.
[k] Rotating biological contactor operated in batch mode.
[l] Darcy velocity (Q/A), m/day.

2.3.3 Combined Anaerobic/Aerobic Systems

Researchers recognize that there are advantages and limitations to both anaerobic and aerobic biological treatment systems for the treatment of CACs. Methanogenic conditions have been shown to lead to the rapid dechlorination of PCE and TCE but often result in the buildup of VC. Under methanotrophic conditions, VC and DCE are readily converted to CO_2, but PCE is recalcitrant. The findings reviewed in the previous sections have led researchers to propose combining the two treatment systems into a single process train either in above ground reactors or *in situ*. Work on such systems is still at the laboratory scale of development and the available data are limited.

Experiments have been conducted in dual-column systems composed of an anaerobic reactor followed by an aerobic reactor. Columns have been packed with glass beads and inoculated with sewage (Fathepure and Vogel, 1991) and packed with soil or sand and fed landfill leachate (Dienemann et al., 1990). An "aquifer simulator" was constructed that could be made alternately anaerobic and aerobic (Fogel, 1988).

In the study performed by Fathepure and Vogel (1991), PCE and TCE were fed to the anaerobic column along with several other organic contaminants. They found that ~80% of the influent PCE and ~30% of the influent TCE were dechlorinated in the anaerobic column when operated at a hydraulic retention time of 37½ hrs. After passage through the aerobic column, which was supplied both O_2 gas and H_2O_2, an overall removal of ~96% of the influent TCE was observed at a total hydraulic retention time of 1.65 days. They confirmed that acetate and methanol could both serve as electron donors for the reductive dechlorination process.

Very little quantitative information is available from these studies. Researchers have observed the expected dechlorination of PCE, generally present in microgram-per-liter influent concentrations, to TCE and TCE to DCE in the anaerobic columns. Fogel

(1988) also observed the DCE epoxide after switching his "aquifer simulator" to the aerobic mode. However, conclusive demonstration that combining the two systems can achieve complete mineralization of PCE is lacking.

3. METHODOLOGY

3.1 EXPERIMENTAL DESIGN

Experiments conducted during this research fall into four major categories: microcosm experiments, anaerobic column experiments, combined anaerobic/aerobic column experiments, and bromide tracer experiments. The equipment and techniques used for each category of experiment are described in detail in Sections 3.1.1 through 3.1.4.

3.1.1 Microcosms

Anaerobic microcosms were prepared in 40 mL volatile organic analysis (VOA) vials (ICHEM part no. IRS236-0040) to which mineral medium and sediment were added. The vials were purchased precleaned and fitted with 0.125 in. teflon/silicone septa. Sediment from two sources was used in preparing the microcosms: the waste oils and solvents landfarm at the Oak Ridge Y-12 plant and New Hope Pond, a holding pond for Y-12 plant effluents. Groundwater monitoring in these areas shows the presence of a variety of volatile organic compounds including PCE. Sediment from both sites was collected by pushing a piece of galvanized pipe into the sediment and then forcing the collected material into a sample bottle. The sediment-containing bottles were stored at room temperature in a N_2-purged atmosphere.

The medium used to prepare microcosms was the revised anaerobic mineral medium (RAMM) of Shelton and Tiedje (1984). Medium ingredients are listed in Table 3.1. In addition to those ingredients listed in the table, 50 mg/L of yeast extract and 1 g/L of sodium acetate were added to the medium. Yeast extract served to replace the

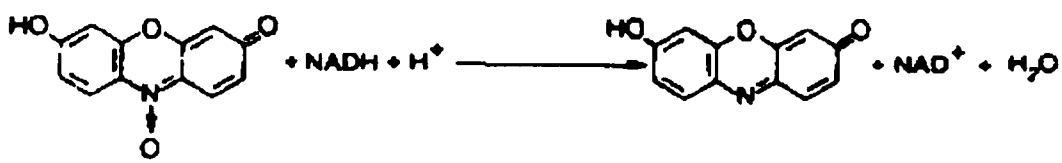
Table 3.1. Composition of the revised anaerobic mineral medium (RAMM).

Compound	Amount per Liter
phosphate buffer:	
KH_2PO_4	0.27 g
K_2HPO_4	0.35 g
mineral salts:	
NH_4Cl	0.53 g
$CaCl_2 \cdot 2H_2O$	75 mg
$MgCl_2 \cdot 6H_2O$	100 mg
$FeCl_2 \cdot 4H_2O$	20 mg
trace minerals:	
$MnCl_2 \cdot 4H_2O$	0.5 mg
H_3BO_3	0.05 mg
$ZnCl_2$	0.05 mg
$CuCl_2$	0.03 mg
$Na_2MoO_4 \cdot 2H_2O$	0.01 mg
$CoCl_2 \cdot 6H_2O$	0.5 mg
$NiCl_2 \cdot 6H_2O$	0.05 mg
Na_2SeO_3	0.05 mg
add after boiling:	
$NaHCO_3$	1.2 g
$Na_2S \cdot 9H_2O$	0.5 g

Source: Shelton, D. R. and J. M. Tiedje. 1984. General method for determining anaerobic biodegradation potential. Applied and Environmental Microbiology 47(4):850-857.

vitamin preparation called for by Shelton and Tiedje. It also provided some unknown complex carbon sources. Acetate served as the primary carbon source and electron donor. The use of many different compounds as primary carbon sources is described in the literature, but acetate is the most commonly used when studying the dechlorination of PCE. Redox potential of the medium was ~-400 mV.

Preparation of 1 L of medium was accompolished by adding KH_2PO_4 (0.27 g) and K_2HPO_4 (0.35 g) to 1 L distilled water. The pH was adjusted to ~7 with 1 N NaOH. Twenty milliliters of the mineral salts solution and 5 mL of the trace mineral solution were added followed by 1 mL of 1 g/L resazurin (a redox indicator) solution, 50 mg yeast extract, and 1 g sodium acetate. The medium was then placed on a hot plate and heated to boiling while purging with an N_2/CO_2 mixture. After boiling and cooling, 1.2 g of $NaHCO_3$ and 0.5 g $Na_2S{\cdot}9H_20$ were added. Stabilization of the pH at ~7 was accomplished by adjusting the N_2:CO_2 ratio in the purge gas while monitoring the pH with a probe suspended in the medium. Resazurin in the medium remained colorless as long as oxygen was excluded and turned the medium pink if oxygen was present. This provided a very sensitive visual indication of the redox status of the medium during preparation and during the experiments. The mechanism of action of resazurin is shown by the following equation (Liu, 1983):



RESAZURIN
(BLUE 610 nm)

RESORUFIN
(PINK 580 nm)

Blue resazurin is irreversibly converted to the pink resorufin. Subsequently, there is a reversible color change to the colorless dihydroresorufin.

Initially, medium and sediment were added to the vials by the anaerobic pipette techniques of Owen (Owen et al., 1979). These rather laborious procedures proved unnecessary for achieving anaerobic conditions in the vials. Therefore, the vials were routinely filled by pumping medium into them with a peristaltic pump while simultaneously purging the vials with a mixture of ~90% N_2 and ~10% CO_2. Slight adjustments to the N_2:CO_2 ratio were necessary to maintain the medium pH, and thus the initial pH in the vials, at ~7.0. The pH of the medium was continuously monitored while filling the vials. The sediment microcosm preparation technique is depicted in Figure 3.1.

Microcosms were prepared by completely filling the 40 mL vials. This resulted in no initial partitioning of PCE into a headspace and helped assure that initial conditions in the vials were as anaerobic as possible.

Aerobic, methanotrophic microcosms were prepared using the nutrient and trace element (NATE) medium of Wittenberry (Table 3.2). The macronutrients were prepared by adding 10 g $MgSO_4 \cdot 7H_2O$, 2 g $CaCl_2$, 10 g KNO_3, and 1 g NH_4Cl to 990 mL deionized water. The trace element solution was then prepared by adding 10 mg each of $MnSO_4 \cdot H_2O$, $CoCl_2$, and MoO_3 and 70 mg $ZnCl_2$ to 1 L of deionized water. Ten milliliters of the trace element solution was added to 990 mL of the macronutrient solution. This 10X NATE was stored under refrigeration and diluted 1:10 with deionized water prior to use. Twenty milliliters of the phosphate buffer and 10 mL of the $FeCl_3$ solution were added to the 1X NATE to complete the medium preparation. Table 3.2 lists the concentrations in the final preparation. Copper was omitted from the medium because research has shown that Cu^{+2} interferes with the monooxygenase enzyme involved in chlorinated hydrocarbon degradation by methanotrophs.

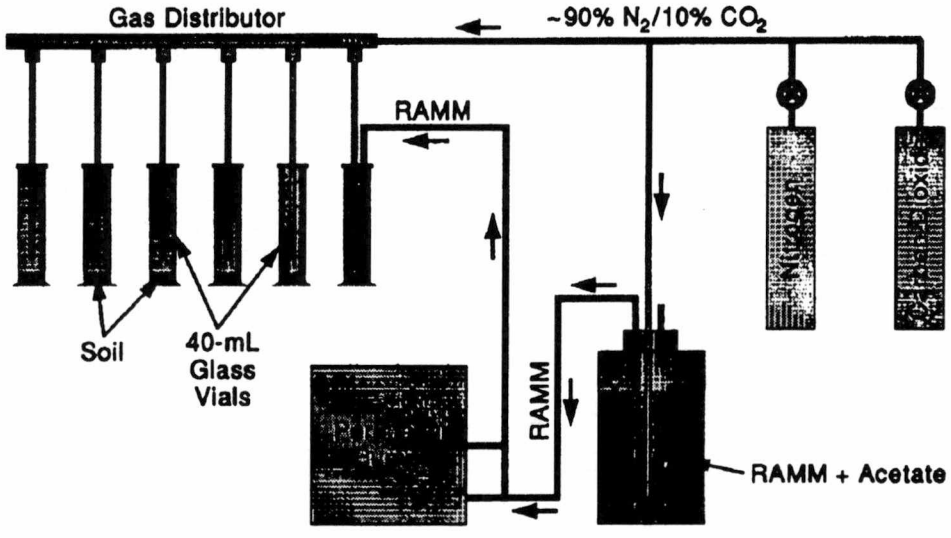


Figure 3.1. Anaerobic soil microcosm preparation technique.

Table 3.2. Composition of the nutrient and trace element medium (NATE).

Compound	amount per liter
macronutrients:	
$MgSO_4 \cdot 7H_2O$	971 mg
$CaCl_2$	194 mg
KNO_3	971 mg
NH_4Cl	97 mg
micronutrients:	
$MnSO_4 \cdot H_2O$	0.01 mg
$ZnCl_2$	0.07 mg
$CoCl_2$	0.01 mg
MoO_3	0.01 mg
other:	
PO_4 buffer	1%
$FeCl_3$	2.62 mg

Source: Wittenberry, R. K., K. C. Phillips, and J. F. Wilkinson. 1970. Enrichment, isolation, and some properties of methane-utilizing bacteria. Journal of General Microbiology, 61, 205-218.

A mixed methanotrophic culture, designated the Kansas City Mixed Culture (KCMC), isolated from groundwater at the Kansas City DOE facility was maintained on nutrient agar in a 3% methane atmosphere. Samples from the agar surface were transferred, as needed, to fresh medium in 250 mL glass bottles. A few cubic centimeters of CH_4 were added to each bottle daily, and the methanotrophs were allowed to grow until the medium became quite cloudy (typically 2 or 3 days). The contents of each bottle were then centrifuged in 50 mL aliquots. The packed cells from each aliquot were resuspended in 25 mL of fresh NATE medium in 40 mL VOA files. More methane was added to each vial, and the cultures were allowed to stabilize for ~24 hours before the VOC of interest was added.

3.1.2 Anaerobic Column

The primary purpose of this research was to study the dechlorination of high concentrations of PCE in a simulated *in situ* process. Therefore, one of the first tasks was to design an aquifer simulator such that microorganisms and nutrients could be added and samples of liquid and gas collected. A borosilicate glass column 5 cm in diameter and 70 cm long (Spectrum Medical Instruments, Inc., Los Angeles, CA) was available from use in previous experiments. The column was fitted with teflon end caps and five sampling ports. Sampling ports 1 and 5 were located 15 cm from the column ends, and the ports were spaced 10 cm apart. Therefore, the distances along the column length were 15, 25, 35, 45, and 55 cm for ports 1 through 5, respectively. Samples could also be withdrawn from the influent sampling/mixing bulb located before the entrance to the column. Dow Corning 3140 RTV sealant was applied around the outside of the teflon end caps. This stopped liquid leakage that developed over time probably because of the deteriorating condition of the teflon threads.

Sampling ports were constructed to accommodate 13 mm, screw-on Mininert valves (Alltech catalog no. 95246). These valves provided access to the column contents while maintaining a leak-proof seal. The liquid contents of the column were sampled using a 1 mL gas-tight Hamilton syringe with a 3 in. stainless steel, leur-lock needle. The needle was inserted through the septum of the Mininert valve, and ~1 mL was drawn into the needle. This liquid sample also contained gas bubbles that were expelled by inverting the syringe and gently tapping to force the bubbles to the needle end. Enough liquid was then expelled from the syringe to leave a 0.5 mL sample that was immediately injected into a headspace sampling vial for gas chromatography analysis.

The column was wet packed with a coarse, angular, sandblasting sand (Moire Industrial Sand) after sieving through U.S. standard sieve sizes 20 (0.85 mm) and 30 (0.6 mm) (W.S. Tyler, Inc.). Sand retained by the #30 sieve was used as packing material. Packing was accomplished by adding ~100 cm^3 of sand at a time, with water, and compacting by tamping with a metal rod. Fines were removed by pumping water through the column until the effluent was free from obvious suspended particulate. The column design thus represented an ideal, homogeneous, isotropic, saturated, sandy aquifer.

The column design and operational components for the anaerobic experiments are depicted in Figure 3.2. All experiments, except the initial hydraulic analysis, were conducted with the column operated in a saturated, upflow mode. Original plans were to conduct the experiments with the column in a horizontal orientation. However, when microorganisms were added to the column, sand was forced away from the upper surface of the glass, probably because of gas production within the column. Since the dislodging of part of the sand from the walls of the column presented a major hydraulic disturbance, degradation experiments were conducted in an upflow mode. All tubing was either

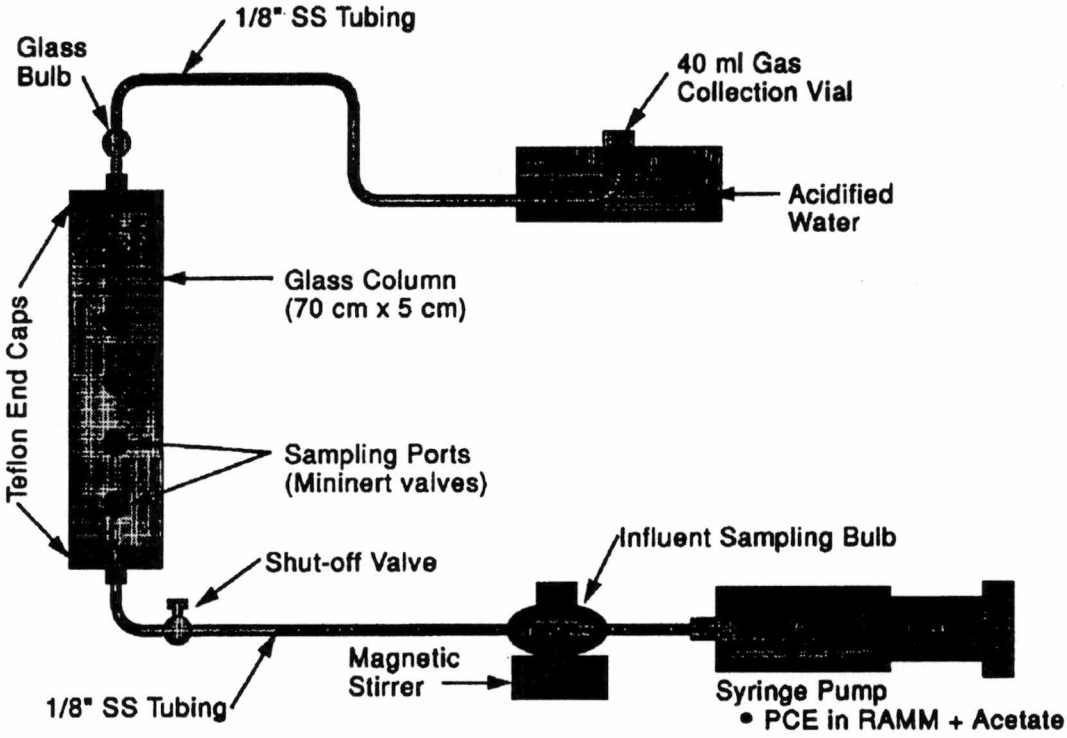


Figure 3.2. Experimental arrangement for the anaerobic column PCE dechlorination experiments.

1/8" stainless steel or Viton. The influent syringe pump (Sage Instruments model 355) was adjusted (3%, x1000 scales) so that the flow rate through the column would be similar to that which occurs in a natural groundwater system. A 100 mL liquid/gas syringe (SGE model 100MAXLLGT) was used with the influent pump. A typical flow rate through the column was 2 mL/hr. This resulted in an approximate 10 day hydraulic retention time.

Anaerobic mineral medium was prepared in a 1 L glass bottle with a rubber stoppered top through which inlet gas, exhaust gas, and liquid lines were inserted. The medium was purged with a N_2/CO_2 gas mixture such that anaerobic conditions and the desired pH were maintained. Medium was pumped into the influent syringe with a Pharmacia Model P-3 peristaltic pump through a 5 µm Gelman syringe filter. The syringe filter was used in order to minimize the presence of particulates in the influent syringe.

Anaerobic digester sludge was obtained from a primary anaerobic digester at the Oak Ridge (Tennessee) Sewage Treatment Plant. This digester is operated between 34° C and 36° C at a hydraulic retention time of ~35 days. A 1 L glass bottle was filled directly from a tap on the digester, transported to the lab immediately, and allowed to settle before use.

The influent PCE solution was prepared in the influent syringe by adding the desired amount of the neat liquid (Aldrich, HPLC grade) using a 10 µL Hamilton syringe (#1701, Hamilton, Reno, NV). The PCE was added through the open tip of the influent syringe. A magnetic stir bar was placed in the syringe before filling with medium. This PCE-containing solution was allowed to stir for at least one hour in the syringe before connecting to the influent lines. Dissolution was assumed to be complete when no obvious globules of neat PCE were observable in the syringe.

The influent mixer/sampling port was constructed from the bulb of a 20 mL volumetric pipette. The ends were removed from the pipette, and a threaded opening

was added in the center of the bulb in order to attach a Mininert sampling valve. A small magnetic stir bar was placed inside the bulb to facilitate mixing (Figure 3.2).

Column effluent was routed through the bulb of a 2 mL volumetric pipette in order to provide visual indication of the color, and thus the redox state, of the effluent liquid. As previously mentioned, the medium contains resazurin, a redox indicator, that is pink when oxidized and colorless when reduced. The stainless steel effluent line was submerged in acidified water and placed underneath an inverted, 40 mL VOA vial filled with the acidified water. Effluent gas was collected in the vial by displacement of the acidified water. Effluent liquid was allowed to escape into the acidified water container.

3.1.3 Combined Anaerobic/Aerobic Column

This experiment was conducted to simulate a combined anaerobic/aerobic *in situ* treatment scheme that might result in the complete mineralization of PCE. The combined system was simulated in a single column. The experiment attempted to split the column into a lower anaerobic zone and an upper aerobic zone by providing oxygenated NATE medium at the midpoint of the column. Otherwise, the column was operated and sampled exactly as it was during the anaerobic column experiments. Figure 3.3 depicts the experimental arrangement.

NATE medium supplied at the column midpoint was prepared at twice the normal strength to allow for dilution. A Braun-Melsangen syringe pump was used to inject the nutrients into the middle port of the column. This syringe pump was equipped with a 50-mL, liquid/gas syringe (Hamilton model 1050TLL) and operated at a flow rate similar to that of the influent syringe pump. The luer-lock needle attached to the syringe was sealed at the end and had six, approximately equally spaced holes drilled into it in an attempt to insure a uniform distribution of flow across the column.

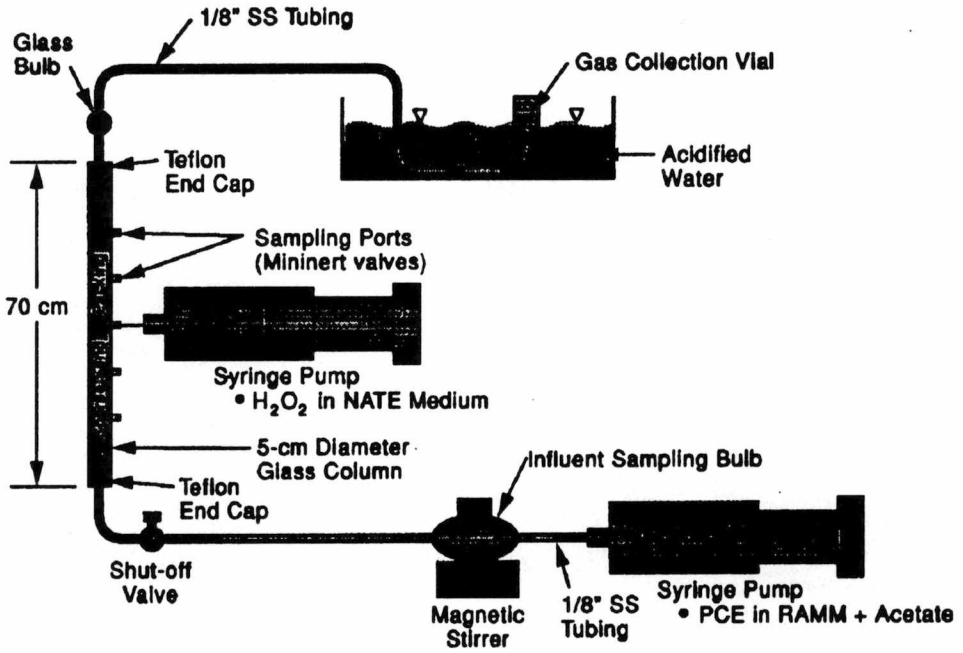


Figure 3.3. Experimental arrangement for the combined anaerobic/aerobic column experiment.

NATE medium was prepared for use in this experiment by adding 1 mL of the $FeCl_3$ and 2 mL of the phosphate buffer to 100 mL of 2X NATE. One-tenth milliliter of resazurin solution and 83 µL of 30% H_2O_2 were then added to give ~250 mg/L H_2O_2. This oxygenated NATE was then added to a 50 mL syringe. Flow into the column at port 3 was started at a rate of 1.5 to 2 mL/min using the syringe pump.

The KCMC mixed methanotrophic culture was prepared as described for the aerobic microcosm experiments. Methanotrophs were added to the column by centrifuging two 100 mL cultures of KCMC in NATE, combining the pellets, and resuspending in fresh NATE. Aliquots were then injected into the column at port 3. Before beginning the experiment, port 3 was alternately purged with 3% CH_4 in air and supplied with NATE for several days in an attempt to establish a healthy methanotrophic population in the upper half of the column. Despite these efforts, very little obvious methanotrophic growth was observed except for some pinkish growth on the column wall near port 3.

3.1.4 Bromide Tracer Experiments

Tracer experiments with the column were performed before adding microbes or medium. These experiments were performed to judge the effectiveness of the column packing technique and to determine column porosity. Bromide is an easily measured conservative tracer commonly used in laboratory and field experiments.

Sodium bromide was added to the column using either the Pharmacia peristaltic pump or the SAGE syringe pump. Low flow rates were established with the syringe pump at about the same rate (~2 mL/min) as used in the anaerobic column experiments. Faster flow rates of 5 to 8 mL/min were set with the peristaltic pump. A solution of 0.01 M KBr in deionized water was used as the tracer. Initially, 0.01 M KCl was added to the

column before the KBr to minimize the effects of diffusion. However, data analysis revealed no difference in the calculated porosity or flow velocity regardless of the presence of KCl.

Samples of the column effluent were collected using an ISCO Retriever 33 fraction collector (ISCO model #68-2180-001). Two tubes per minute were collected at the higher flow rates, and one tube per hour was collected at the slowest flow. Bromide concentrations were determined using an ORION ion-specific bromide electrode with an ORION Research microprocessor ionalyzer model 901 as described in Section 3.2.3.

Data were analyzed using the concentration, distance, and time fitting model (CXTFIT) of Parker and van Genuchten (1984). Effluent bromide concentration data at discrete time intervals were input, and the model fit the data to determine average pore-water velocity and dispersion coefficients. Pore-water velocity was used with column length and dispersion coefficient to calculate a Peclet number (vL/D) as a measure of column packing efficiency (*i.e.*, no channeling effects). Details of the mathematical formulation and use of the model are discussed in the results section.

3.2 ANALYTICAL TECHNIQUES

The major analytical techniques utilized in the experiments performed during this research are described in Sections 3.2.1 through 3.2.4. Four techniques were used: gas chromatography for VOCs and fixed gases, gas chromatography/mass spectral analysis for confirmation of the DCE epoxides, bromide ion analysis using a specific ion electrode, and ion chromatography to identify acetate.

3.2.1 Gas Chromatography

3.2.1.1 Volatile Organic Compounds

The choice of an analytical technique for the VOCs was made after a literature search and an inventory of the available equipment. The most common analytical techniques described in the literature involve liquid-liquid extraction followed by gas chromatography, usually using an electron capture detector (ECD). Various extractants employed include carbon disulfide, hexane, dodecane, and pentane. However, the desire to avoid waste generation from the use of an organic extractant and the necessity of identifying several VOCs in the same liquid sample led to the use of a headspace gas chromatography technique employing a flame ionization detector (FID).

A Perkin-Elmer Sigma 2000 gas chromatograph (GC) equipped with both a ^{63}Ni ECD and an FID was available. This instrument was also equipped with a head space analyzer (Perkin-Elmer model HS-6). The Sigma/HS-6 system (Figure 3.4) consists of a thermostatically controlled magazine mounted to an injection port of the GC. The HS-6 magazine can hold six specially designed head space vials (Perkin-Elmer part #B010-4240). The glass head space vials are ~6 mL in volume and are sealed by crimping a teflon-backed butyl rubber septa (Perkin-Elmer part #B010-4239) in place. Sample is added to the vials, the vials are sealed and inserted into the magazine, and an appropriate equilibration temperature from 40°C to 190°C is selected. The temperature control system is able to maintain the temperature constant to within 0.1°C. When equilibrium is reached, a sample of the head space is injected onto the GC column by pushing the magazine upward until the vial septum is punctured by the HS-6 injection needle. Simultaneously, a relay contact starts the control unit program. An injection time of five seconds is electronically controlled by the HS-6 analyzer (injection time can be changed to 15 or 30 sec. by rearranging two internal jumpers within the HS-6 control

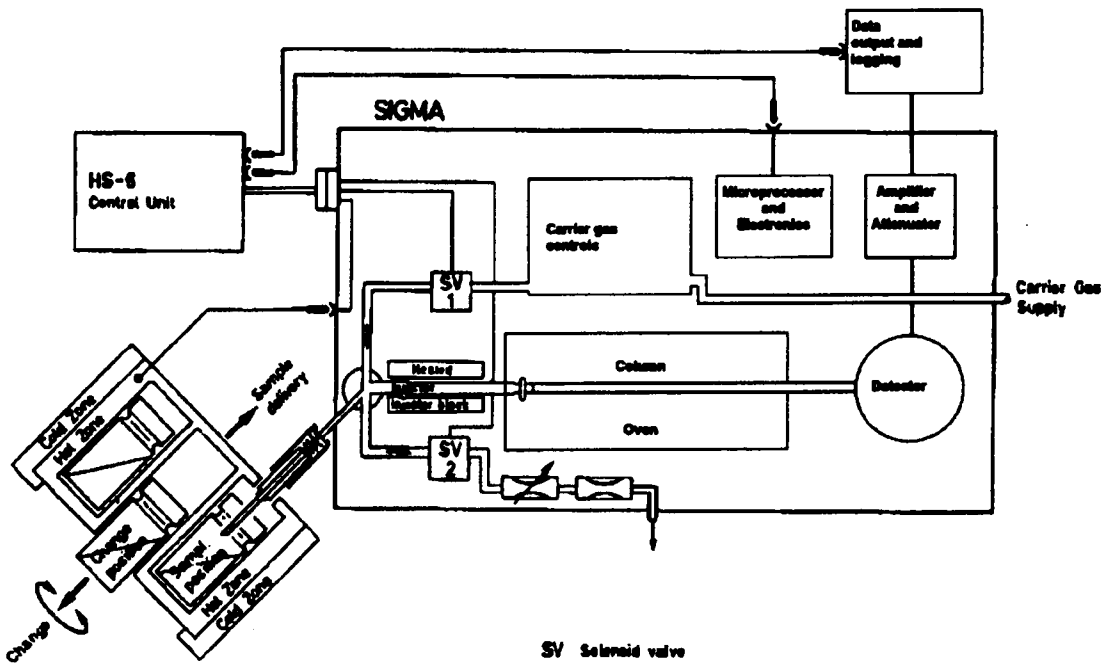


Figure 3.4. Operational diagram of the HS-6 headspace sampling unit with the Sigma 2000 gas chromatograph (Perkin-Elmer. HS-6 head space sampler for the SIGMA series and F22 gas chromatographs: operator's manual. Perkin-Elmer Corporation, Norwalk, Conn.)

unit). A portion of the sample vial's head space contents are swept out of the vial by the carrier gas and onto the column. The control system is designed to provide a reproducible volume of head space injected. Carrier gas is automatically redirected to the column by the control switch immediately after injection. Reliable analyses with this system depend upon the pressure in the vial reaching that of the pressure at the head of the GC column. Normally, this is accomplished by selecting an appropriate pressurization time on the HS-6 control unit. However, low head pressures (2 to 5 psig) are used with capillary GC columns. Therefore, the high vapor pressures exerted by the VOCs in the samples used in these experiments were sufficient to eliminate the need for a pressurization time, *i.e.* the HS-6 control unit was set to an infinite pressurization time and the manual inject feature was used.

The GC column selected for use in these experiments was a Supelco VOCOL™ column. This column is 0.53 mm i.d., has a 3 μm film thickness, and is 30 m long (Supelco #2-5320). A temperature program was set to begin at 40° C, hold for 4 min, and ramp at 4° C/min to 96° C for a total run time of 18 min. The FID range was set to 1 (*i.e.*, the most sensitive range). The GC injector temperature was 240° C, the detector temperature was 250° C, carrier gas pressure setting was 8 psig, while the FID air and H_2 were set at 20 psig. When used with the FID and N_2 carrier gas, at a flow rate of 4 cm^3/min, this column produced an excellent separation of PCE, TCE, CIS, TRANS, and VC. Methane, which is not retained by the column, eluted at 1.7 min. thereby verifying the 4 cm^3/min carrier gas flow rate. Retention times were ~5.0 min. for TRANS, ~7.0 min. for CIS, ~10.8 min. for TCE, and ~16.4 min. for PCE. Figure 3.5 shows a typical GC output of a standard mixture of VOCs. The TRANS and CIS epoxides appeared at about 8.6 min. and 14.6 min., respectively.

A sample volume of 500 μL was selected. This volume provided a sufficient mass

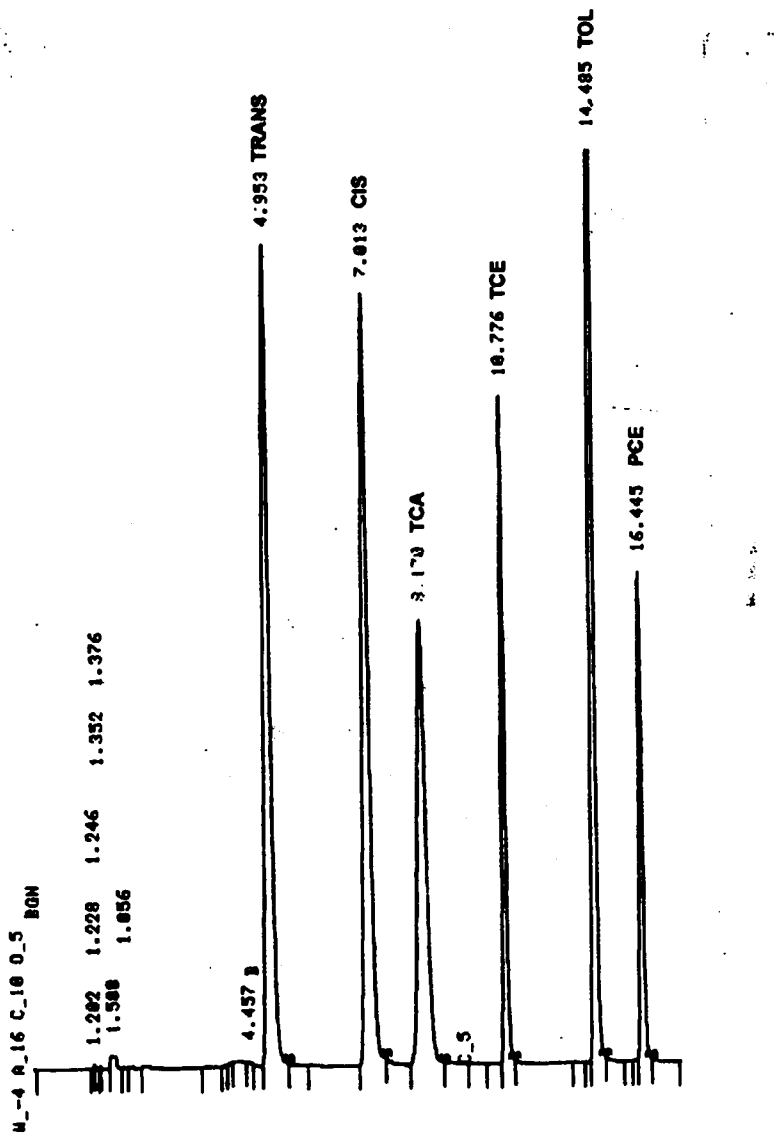


Figure 3.5. Typical GC output of a standard mixture of VOCs separated by the Supelco VOCOL™ capillary column using the Perkin-Elmer HS-6 system.

of VOC in the HS-6 vial headspace for analysis while allowing repeated sampling of the microcosms. Sampling such a small volume relative to the total volume of liquid in the sand column minimized the hydraulic disturbance to the column.

The HS-6 controller was set to infinite pressurization time, 50° C or 70° C, and manual inject. These settings allowed for immediate injection of headspace gas onto the column once the HS-6 vial was pierced. This proved to be necessary to avoid double peaks in the chromatograph that would be caused by premature injection of headspace if a pressurization time were selected. The vapor pressure of the VOCs of interest proved to be sufficient to allow injection onto the column without the need for any pressurization time. The inject button of the HS-6 controller was hit immediately after the GC needle penetrated the vial septum.

Concentrations of VOCs were determined by comparison of GC peak areas using a Perkin-Elmer LCI-100 Laboratory Computing Integrator. Solutions containing known concentrations of PCE, TCE, CIS, and TRANS were prepared in order to standardize the gas chromatographic output. Freshly prepared RAMM was pumped into 250 mL clear glass, septum-capped bottles (ICHEM) using the Pharmacia P-3 pump. A 5 µm syringe filter was used in order to minimize the amount of sulfide precipitate introduced into the standard bottle. Each bottle contained a small stirring bar. The bottles were filled to overflowing to exclude headspace. An appropriate amount of the neat liquid of the desired compound was injected through the septum. Bottles were placed on a magnetic stirrer for several hours before analysis. Stirring continued until no visible globules of the neat liquid could be observed. Three, 1 mL, gas-tight syringes were used to remove 500 µL aliquots in triplicate. Each aliquot was then injected through the septum of an HS-6 vial. This provided triplicate, 500 µL standard samples for analysis.

3.2.1.2 Fixed Gases

Analyses for the fixed gases CH_4, CO_2, N_2, O_2 present in the microcosm headspace or column offgas were performed using a Perkin Elmer gas chromatograph model 3920B. This GC was equipped with both an FID and a thermal conductivity detector (TCD). The Perkin-Elmer 3920B used He as a carrier gas at a flow rate of 20 mL/min. The TCD detector current was set to 150 mA and the temperature at 150°C. Regardless of the analysis, the 3920B oven was operated isothermally at 50°C and the injector, detector, and interface were set at 150°C. A Hewlett-Packard model 3390A integrator was used for quantification of peak areas. Typical integrator settings were 0.16 peak width at a threshold value of -2.

Three different GC columns were used depending upon the gas(es) of interest. All three were stainless steel, packed columns. Gas samples were injected manually through the GC septum using a gas-tight syringe. Typically, 200 µL was injected using a 500 µL, Pressure-Lok®, gas-tight syringe (Series A-2, Dynatech Precision Sampling Corp., Baton Rouge, LA).

A molecular sieve 5 Å 60/80 mesh packing was used in a stainless steel GC column 6 ft. long by ⅛ in. diameter (Alltech #5605PC) for the separation of N_2 and O_2. These gases, as well as CH_4, were detected by the TCD. Molecular sieves are synthetic zeolites that separate molecules based on molecular diameter and configuration (McNair and Bonelli, 1969). Peaks eluted in the order of O_2, N_2, and CH_4 at ~0.8 min., ~1.5 min., and ~2.5 min., respectively. The molecular sieve 5 Å column absorbs CO_2.

A PORAPAK® Q column (Alltech #2701PC) was also used with the TCD. PORAPAK® is a porous polymer of ethylvinylbenzene cross-linked with divinylbenzene that forms a structure with a distinct pore size designed for separation of compounds with a molecular weight less than 300 (McNair and Bonelli, 1969). PORAPAK® Q cannot

separate N_2 from O_2. Elution times were 1.5 min. for N_2/O_2, 2.1 min. for CH_4, and 3.5 min. for CO_2. A calibration gas (Scotty II) consisting of 4.5% CH_4, 15% CO_2, 7% CO, and 7% O_2 in N_2 by volume was used. Analysis provided sample gas composition as a percentage by volume.

The FID was used to detect CH_4 in conjuction with a PORAPAK® R (Alltech #2707PC) column. The injector, detector, and interface were maintained at 150° C and the GC oven at 50° C. Carrier gas flow rate was ~30 mL/min. A typical injection volume was 50 µL using a gas-tight syringe. This analysis was used only to determine the presence or absence of CH_4. Therefore, no standard curve was prepared. Ethene could also be detected with this column and detector arrangement. Methane eluted at ~0.8 min and ethene at ~2.5 min.

3.2.2 CIS and TRANS Epoxide Identification

The CIS and TRANS epoxides were positively identified by the ORNL Analytical Chemistry Division using gas chromatography/mass spectrometry (GC/MS). Liquid samples of the methanotrophic microcosms to which CIS or TRANS had been added were analyzed with the GC/MS system. Retention times of the unknown peaks noted using the Perkin-Elmer Sigma 2000 GC that exhibited the same retention time as peaks from the GC/MS system were then used to identify the DCE epoxides in later microcosm and column experiments.

Standard preparations of DCE epoxides are not commercially available, therefore no quantification of the epoxides was possible. Epoxides are typically very unstable compounds and are very difficult to detect and quantify. Fortunately for this research, DCE epoxides are sufficiently stable that detection is possible. Unfortunately, TCE or PCE epoxides have extremely short half-lives (on the order of milliseconds) and could not

be detected with the equipment available.

3.2.3 Bromide Ion Analysis

Bromide analysis was conducted using an ORION specific ion electrode with the ORION model 901 microprocessor analyzer. Bromide standards of 0.01, 0.001, 0.002, and 0.004 M were prepared, and the response of the electrode was plotted versus bromide ion concentration. The measured electrode potential is related to Br concentration by the Nernst equation:

$$E = E_0 - S \log(A)$$

Where E = measured electrode potential
E_0 = reference electrode potential (a constant)
A = activity of species in solution (Br) = ($[C_{Br}]$
S = electrode slope (~59 mV @ 20° C)

The procedure involved sampling the sand column effluent, adding an ionic strength adjustor (ISA), swirling to mix, and inserting the bromide and reference electrodes into the solution. Readings were taken when the microprocessor output stabilized and were compared to the standard curve for quantification. If the background ionic strength ($\frac{1}{2}\sum C_i Z_i^2$) is high and constant relative to the sensed ion concentration, then (is constant, and A is directly proportional to C. Addition of the ISA ($NaNO_3$) insures the high background ionic strength.

3.2.4 Acetate Ion Chromatography

An analytical method was necessary for the detection and quantification of the primary carbon source acetate. Data analysis involved comparing acetate removal to PCE dechlorination and methane production.

Acetate analysis was first attempted by gas chromatography using the Perkin-

Elmer 3920B with the FID. Although GC techniques for acetate analysis are frequently reported in the literature, detection and quantification proved difficult. Therefore, an ion chromatography (IC) method was chosen for acetate analysis.

The IC system consisted of a Waters Model 510 high performance liquid chromatography pump and a Waters Model 430 conductivity detector with readout in microsiemens (μS). The separatory column, a Waters IC-PAK ion exclusion column (Waters #10295), was 7.8 mm in diameter by 15 cm long. The eluent chosen was 1 mN H_2SO_4. The system was operated at a flow rate was 1.0 mL/min which resulted in an elution time for acetate of ~5 min. The detector settings were typically: 0.01 gain, 50 range (x10 = 500, *i.e.*, 500 μS full scale), temperature on (maintained at 35° C), and + polarity.

The H_2SO_4 eluent was prepared from 1 N standardized acid using deionized water as a diluent. The diluted acid was filtered and degassed through a 0.45 μm filtration unit before use. One milliliter samples were collected with a syringe from the microcosms or the column sampling ports and filtered through a 5 μm syringe filter into a glass vial. Approximately 0.5 mL of each sample was taken up in a plastic syringe equipped with a 0.45 μm filter and injected through the injection port of the ion chromatograph. The injection port was equipped with a 20 μL sample loop. Detector output was recorded on a chart recorder operated at 0.5 cm/min at an input range of either 10, 5, or 1 mV depending upon the sensitivity desired. Peak heights of samples were compared to peak heights produced by known concentrations of acetate.

4. RESULTS AND DISCUSSION

4.1 MICROCOSM EXPERIMENTS

4.1.1 Anaerobic Microcosms

The rationale for preparing anaerobic microcosms was to develop sampling and analytical techniques for CACs, acetate, and methane, demonstrate reductive dechlorination of PCE, identify reductive dechlorination products, and estimate rates of PCE removal and product formation. Data obtained from the microcosm experiments regarding which dechlorination products formed, the rate of PCE removal, gas production, and acetate utilization would then be applied toward optimizing the conditions under which the anaerobic column would be operated.

Sediments used in preparing microcosms were from two sites at the DOE Y-12 facility. Sediment samples from different locations around the oil landfarm (OLF) were designated OLF-1, OLF-2, and OLF-3. These sediments were collected from the "oil pit" sludge and were typically sticky, wet, clayey, and clumpy. Sediments collected from the New Hope Pond (NHP) area were identified as NHP-4 and NHP-5 and were dry and sandy.

Data presented in the following two sections show that reductive dechlorination of PCE was observed in NHP microcosms but not in OLF microcosms. The dechlorination always terminated with CIS. Reductive dechlorination was not observed in autoclaved controls. Acetate utilization was not complete, and methane formation was not consistently observed.

As described in the Methodology section, anaerobic microcosms were typically composed of ~1 g of sediment added to a 40 mL VOA vial. The vial was completely filled

with RAMM by the previously described technique. Finally, 1 µL of neat PCE was added to give a target initial PCE concentration of 40 mg/L (240 µM).

4.1.1.1 Oil Landfarm Sediment Microcosms

Figure 4.1 shows the fate of PCE in the OLF-2 sediment microcosms. Error bars reflect the standard deviation among the three individual microcosms. Other OLF microcosms produced similar results. After an initial rapid loss (based upon a calculated, not measured, initial PCE concentration of 244 µM), PCE gradually declined in OLF-2 microcosms over the ~5 months of monitoring. The initial loss can be accounted for as sorption to a low-organic-carbon content sediment. Using the K_{oc} for PCE (Table 2.1) and assuming an organic carbon content of 0.1% for the OLF sediment, 5.2 µmole of PCE would be sorbed to the sediment. This compares to the ~4 µmole estimate obtained using the first measured liquid-phase PCE concentration. The initial loss may also be the result of volatilization losses, leakage from the vials, or other physical losses. Since the initial point on the graph was calculated from the volume of PCE added, it is possible that attempting to deliver 1 µL of a dense liquid such as PCE with a 10 µL Hamilton syringe resulted in inaccurate measurements that are reflected in this initial loss.

No PCE dechlorination products were observed in any OLF microcosms. After the initial rapid loss, PCE disappeared from OLF-2 microcosms with a zero-order rate constant of 0.46 µmole/L/day. This gradual decline in PCE without the formation of reductive dechlorination products is most likely the result of sorption and volatilization losses and was not the result of biological activity since no reductive dechlorination products were observed. Leakage from the vials over time is indicated by comparing the solid line in Figure 4.1 (the predicted aqueous-phase concentration due to redistribution between liquid and headspace based upon Henry's Law) to the measured data points.

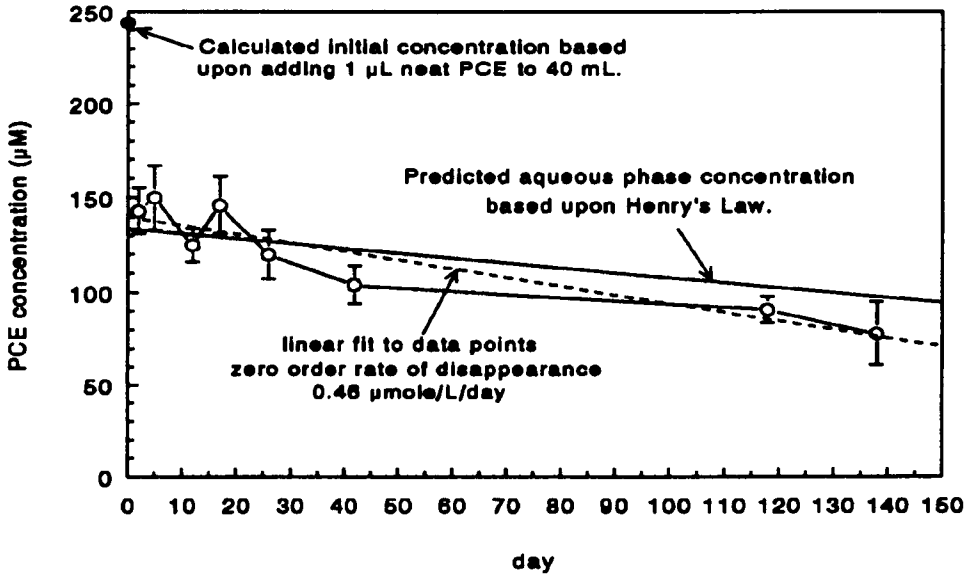


Figure 4.1. Oil landfarm sediment microcosms (OLF-2). Dashed line is a linear fit of the data excluding the initial steep decline.

No methane was detected in any OLF-2 microcosms. However, some methane production was observed in all microcosms prepared from OLF-1 and OLF-3 sediments. Therefore, low numbers of methanogens may be present in specific locations at the oil landfarm site. Methane production in the microcosm studies was not quantified. Only the presence or absence and a relative comparison of methane among microcosms was of interest.

4.1.1.2 New Hope Pond Sediment Microcosms

Figure 4.2 shows the decline in PCE concentration over 4 months in the autoclaved NHP-4 sediment microcosms. Triplicate sediment microcosms were prepared after autoclaving the NHP-4 sediments on three consecutive days. The initial decrease in PCE concentration over the first 30 days was probably the result of the same physical

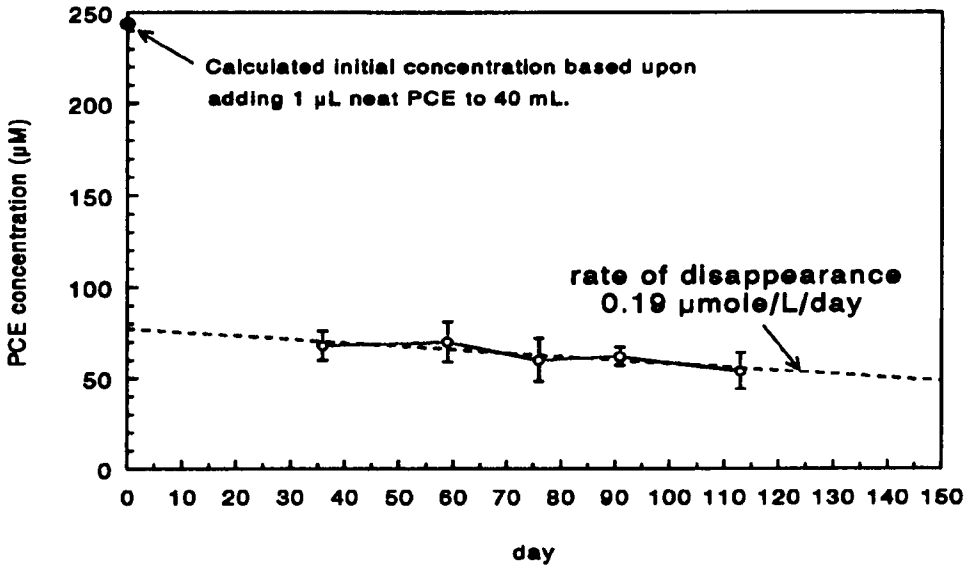


Figure 4.2. Autoclaved New Hope Pond sediment microcosms. Initial PCE concentration of 40 mg/L (1 µL PCE to 40 mL RAMM). 1g of sediment.

removal mechanisms and possible errors described for the initial PCE loss observed in the OLF-2 microcosms. Unfortunately, no PCE concentration measurements were made during the first few days of incubation. However, the fact that no PCE dechlorination products were observed is of primary importance. The rate of PCE disappearance, 0.19 µmole/L/day, was determined by ignoring the initial calculated data point of 244 µM PCE. This rate of PCE disappearance provides a reference data point for comparing physical losses expected in all microcosms to the biologically-mediated disappearance of PCE in unautoclaved sediment microcosms.

Figures 4.3 through 4.5 show data from individual microcosms in the NHP-4 series. PCE was added to each of these microcosms at a target initial liquid phase concentration of 244 µM. These triplicate microcosms showed a very similar pattern of

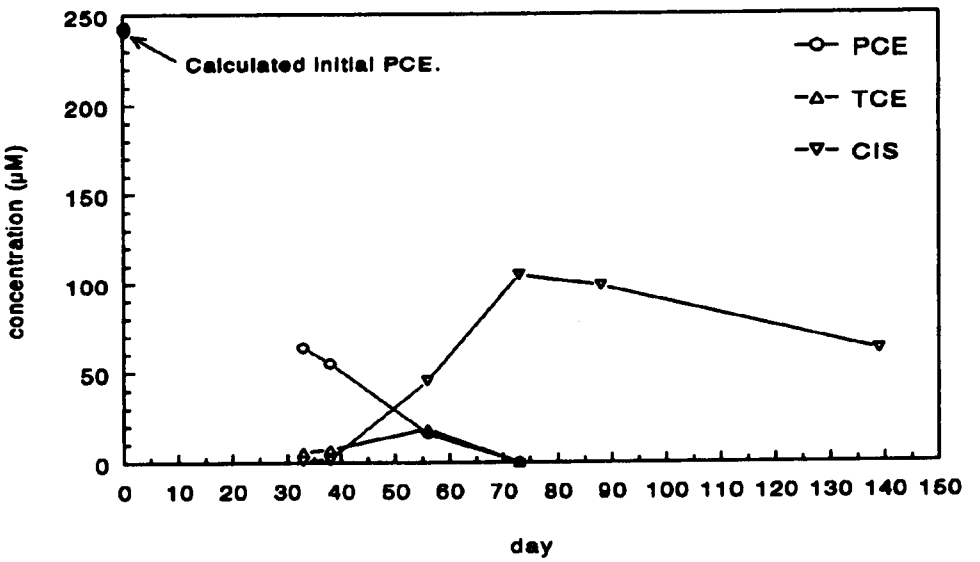


Figure 4.3. New Hope Pond sediment microcosm NHP-4-1. Initial PCE concentration of 40 mg/L (1 µL PCE to 40 mL RAMM). 1 g of sediment.

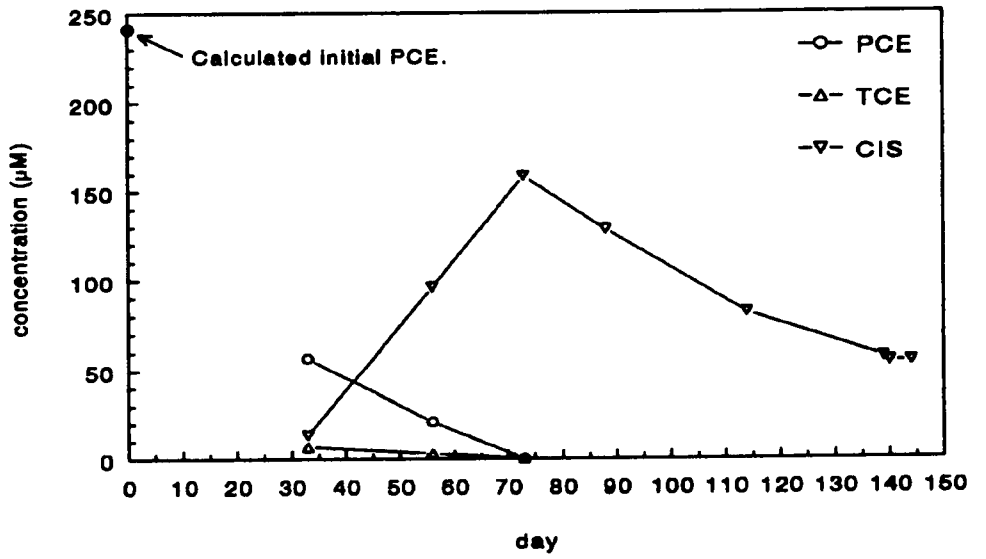


Figure 4.4. New Hope Pond sediment microcosm NHP-4-2. Initial PCE concentration of 40 mg/L (1 µL PCE to 40 mL RAMM). 1 g of sediment.

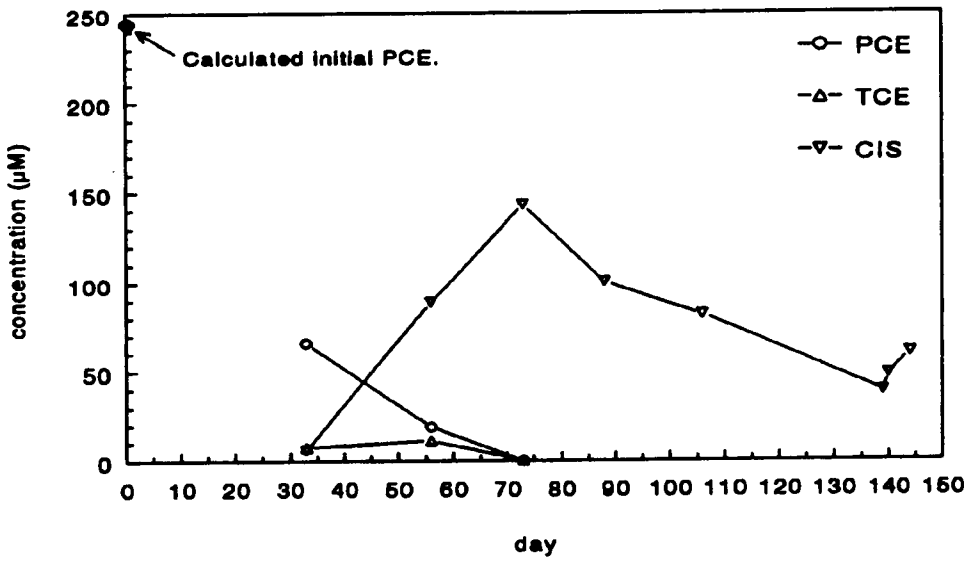


Figure 4.5. New Hope Pond sediment microcosm NHP-4-3. Initial PCE concentration of 40 mg/L (1 µL PCE to 40 mL RAMM). 1 g of sediment.

PCE removal and TCE and CIS formation. All three showed the transient nature of TCE. Each microcosm produced CIS but no TRANS. No VC was detected in any of the microcosms over the five months of monitoring. Ignoring the initial steep decline in PCE, removal rates in unautoclaved NHP-4 sediment microcosms ranged from 1.43 µmole PCE/L/day to 1.67 µmole PCE/L/day. The decline in the total micromoles of PCE, TCE, and CIS present in the microcosms' liquid phase reflects redistribution between the liquid phase and the headspace after each sampling, sorption to the sediment, and volatilization losses through the vial cap that was repeatedly punctured.

Figure 4.6 displays the average data obtained from sampling the three NHP sediment microcosms, NHP-4-1, NHP-4-2, and NHP-4-3. The average rate of disappearance of PCE from the NHP-4 microcosms was 1.59 ± 0.14 µmole/L/day. The

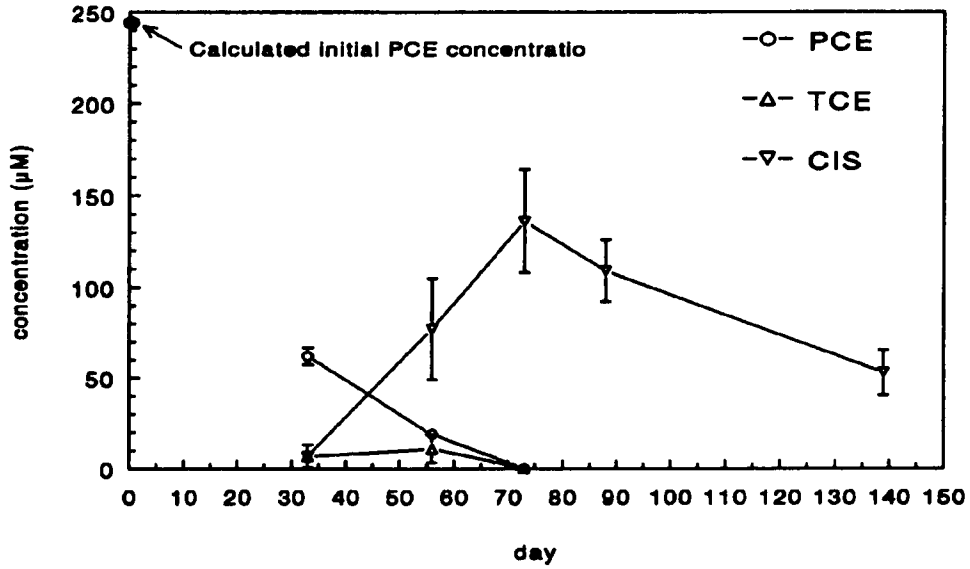


Figure 4.6. Average of New Hope Pond sediment microcosms NHP-4-1,2,3. Initial PCE concentration of 40 mg/L (1 µL PCE to 40 mL RAMM). 1g of sediment added to each microcosm.

data show good reproducibility of PCE dechlorination among microcosms. All three microcosms show a similar pattern of PCE decline and TCE and CIS production as well as similar timing in the formation of the dechlorination products.

Figure 4.7 shows the results from triplicate NHP-4 microcosms where PCE was added but no acetate. Although measurable amounts of TCE and CIS were produced, PCE was not completely removed after three months of incubation. Yeast extract has been shown to provide some reducing power (Tandol et al., 1994). Thus, the yeast extract added to the RAMM was the likely source of electrons for PCE reduction in the absence of acetate. The rate of disappearance of PCE, calculated excluding the initial rapid loss in these microcosms, averaged 0.41 µmole/L/day.

This series of microcosms without added acetate was the only group of NHP-4 microcosms that were sampled in the first day after adding PCE. Figure 4.7 confirms that

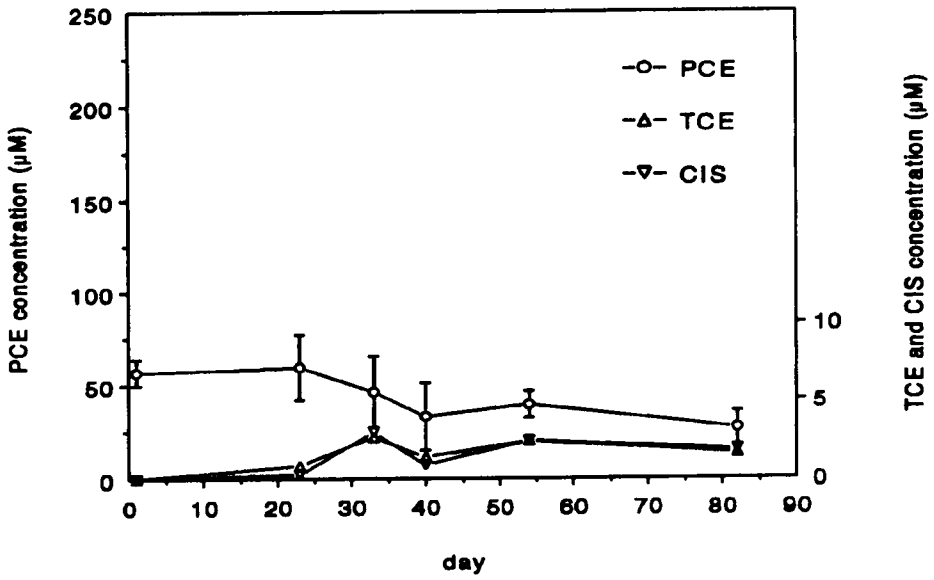


Figure 4.7. Average of three New Hope Pond sediment microcosms prepared without acetate (50 mg/L of yeast extract present in RAMM).

the initial loss of PCE is rapid and not accompanied by the formation of detectable dechlorination products.

Acetate utilization in the NHP-4 series microcosms was variable. Table 4.1 shows the percent of acetate removed in each of the microcosms after 2½ months of incubation. Since acetate utilization was similar in both the PCE-free microcosms and in microcosms to which PCE was added, the high concentration of PCE did not appear to be toxic to the acetate-utilizing organisms present. Acetate used in the autoclaved microcosm indicates that either not all of the sediment microorganisms were destroyed by autoclaving or that the micrcosms were contaminated during or after preparation with organisms capable of using acetate. Since no special precautions were taken to keep the autoclaved microcosms sterile, microbial contamination is not surprising.

Although methane production in the microcosms was not quantified, relative

Table 4.1. Acetate removal in NHP-4 microcosms.

microcosm type	% removal in triplicate microcosms	average ± standard deviation
autoclaved controls	51,6,30	29 ± 22
no PCE added	57,34,78	56 ± 22
PCE added	40,58,52	50 ± 9

comparisons were made using GC area units obtained when methane was detected in headspace samples. Methane in the headspace of NHP-4 vials prepared without PCE was greater than that produced in either vials prepared with PCE or in vials autoclaved and fed PCE. However, the presence of methane in vials that contained PCE was not noted until ~4 months after incubation (after the added PCE was no longer detected). A relatively small amount of methane was detected in the NHP-4 vials prepared without acetate.

Results obtained from microcosms prepared with the New Hope Pond area sediment sample designated NHP-5 were variable. One NHP-5 microcosm removed PCE rapidly and completely to the level of CIS, one showed no dechlorination products produced at all, and a third showed the buildup of low concentrations of TCE and CIS.

Table 4.2 summarizes PCE removal rates calculated from the anaerobic microcosm experiments performed. PCE dechlorination in the anaerobic microcosms depended upon the source of sediment used to prepare the microcosms. Dechlorination to CIS was consistently observed in microcosms prepared from NHP-4 sediments and occurred, but not consistently, in microcosms prepared from NHP-5 sediments. Dechlorination was not observed in microcosms prepared from oil landfarm sediments. Physical losses of PCE occurred in all sediment microcosms and were relatively

Table 4.2. Comparison of PCE disappearance rates in anaerobic microcosms.[a]

microcosm[b]	acetate?	autoclaved?[c]	rate[d] (µmole/L/day)
OLF-1	Yes	No	0.26 ± 0.08
OLF-2	Yes	No	0.46 ± 0.09
OLF-3	Yes	No	0.12 ± 0.05
NHP-4	Yes	No	1.58 ± 0.14
NHP-4	No	No	0.41 ± 0.08
NHP-4	Yes	Yes	0.19 ± 0.10
NHP-5	Yes	No	0.43 ± 0.70[e]
NHP-5	Yes	Yes	0.20 ± 0.04

[a] Disappearance rates presented in this table represent an overall rate that includes all physical, chemical, and biological mechanisms that may be active. Only in the unautoclaved NHP-4 microcosms and in one of the unautoclaved NHP-5 microcosms were PCE dechlorination products detected.
[b] OLF = Y-12 Oil Landfarm; NHP = New Hope Pond.
[c] Autoclaved on 3 consecutive days. Autoclaved OLF microcosms were prepared but only sampled once at the end of the experiment. No reductive dechlorination products were observed in autoclaved OLF microcosms.
[d] Average rate of disappearance of PCE from triplicate microcosms ± 1 standard deviation.
[e] One microcosm converted PCE to CIS.

consistent among microcosms. PCE and its dechlorination products were readily separated and detected when liquid was withdrawn from microcosms with a gas-tight syringe and analyzed by headspace gas chromatography. Sufficient information was obtained from the anaerobic microcosms to gain confidence that the analytical and sampling procedures would work well in the column studies. Although sufficient quantitative information that could explain completely the mechanisms of PCE loss from the anaerobic microcosms was not obtained, such microcosms can be used as screening tools for further study. The NHP-4 microcosms demonstrated that high concentrations (~40 mg/L) of PCE could be dechlorinated by reductive dechlorination through TCE to CIS. The buildup of CIS is in agreement with many other studies cited in the literature review. Other researchers have also had problems with unquantifiable physical losses.

More microcosms should have been prepared so that repeated sampling of individual microcosms could have been avoided. Numerous punctures of the septa undoubtedly enhanced the opportunity for leakage from the vials. However, sufficient sediment was not available to prepare the number of microcosms that would have been necessary.

4.1.2 Aerobic Microcosms

The primary purpose for preparing aerobic microcosms was to identify CIS and TRANS epoxides. Figure 4.8 shows the results obtained when TRANS was incubated for 5 days with the mixed culture of methanotrophs designated KCMC. TRANS was removed from the microcosms with a first order rate constant of 0.0071 hr^{-1}. A new peak appeared at 8.6 min. into the chromatogram. The mass spectrum of the species that produced this peak was similar to that reported by Arvin (1991) for the TRANS epoxide (Figure 4.9). Autoclaved controls showed no loss of TRANS and no indication of epoxide formation over the five days of the experiment. Methanotrophic activity was confirmed by a decrease in headspace CH_4 content from ~35% to ~8% after five days. Biological activity was further indicated by an increase in headspace CO_2 from 1.5% to 7% over the five days. Autoclaved controls showed a reduction of methane content to about 15%, while carbon dioxide decreased to less than 1%. Such losses were consistent with leakage through the septa of the microcosm vials.

Results obtained from the incubation of KCMC with CIS are displayed in Figure 4.10. Results were similar qualitatively to those obtained from the TRANS microcosms. The first order removal rate obtained for CIS is about twice that of TRANS. However, the amount of biomass present was not quantified in either the TRANS or CIS microcosms. Therefore, degradation rates are not directly comparable.

The CIS epoxide peak appeared at 14.6 min. into the GC run. Mass spectral data

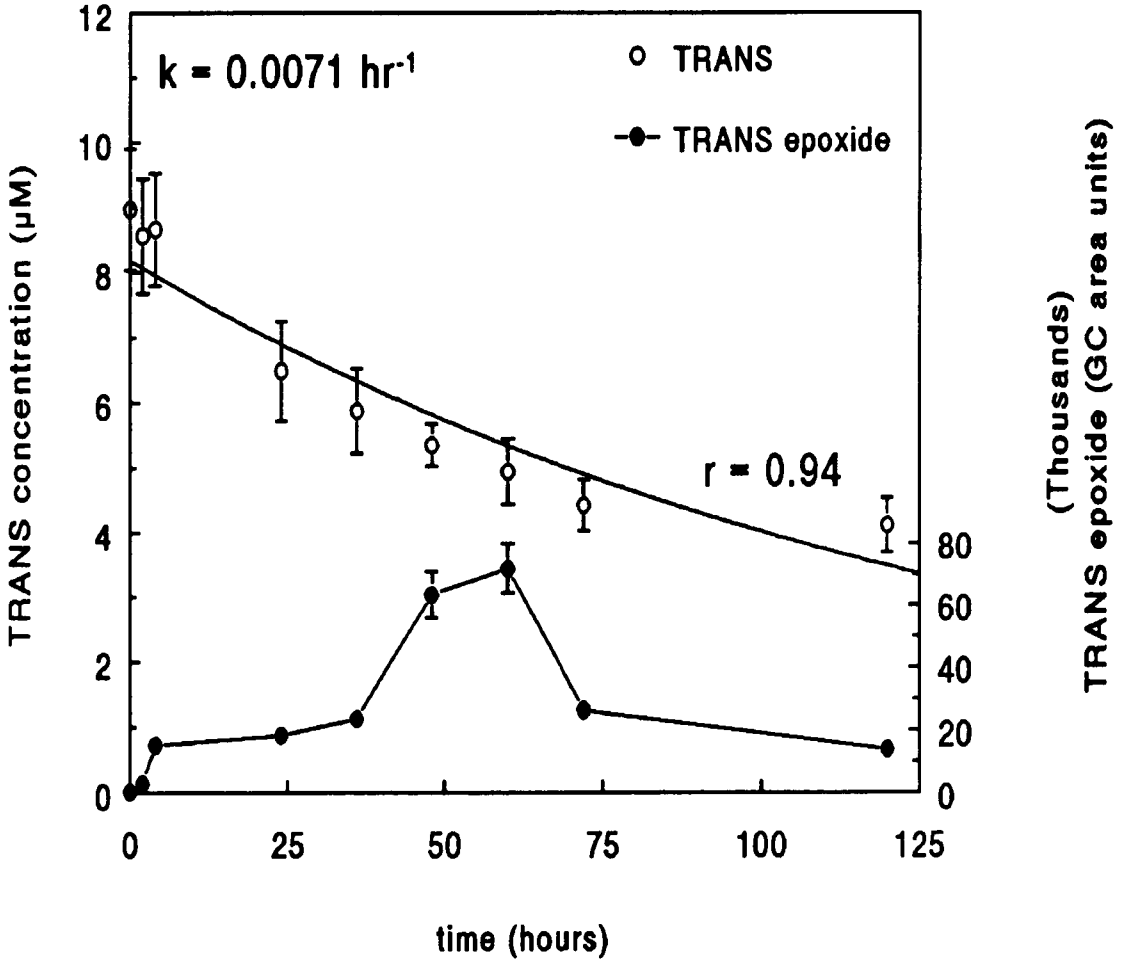


Figure 4.8. TRANS degradation and epoxide formation in KCMC microcosms.

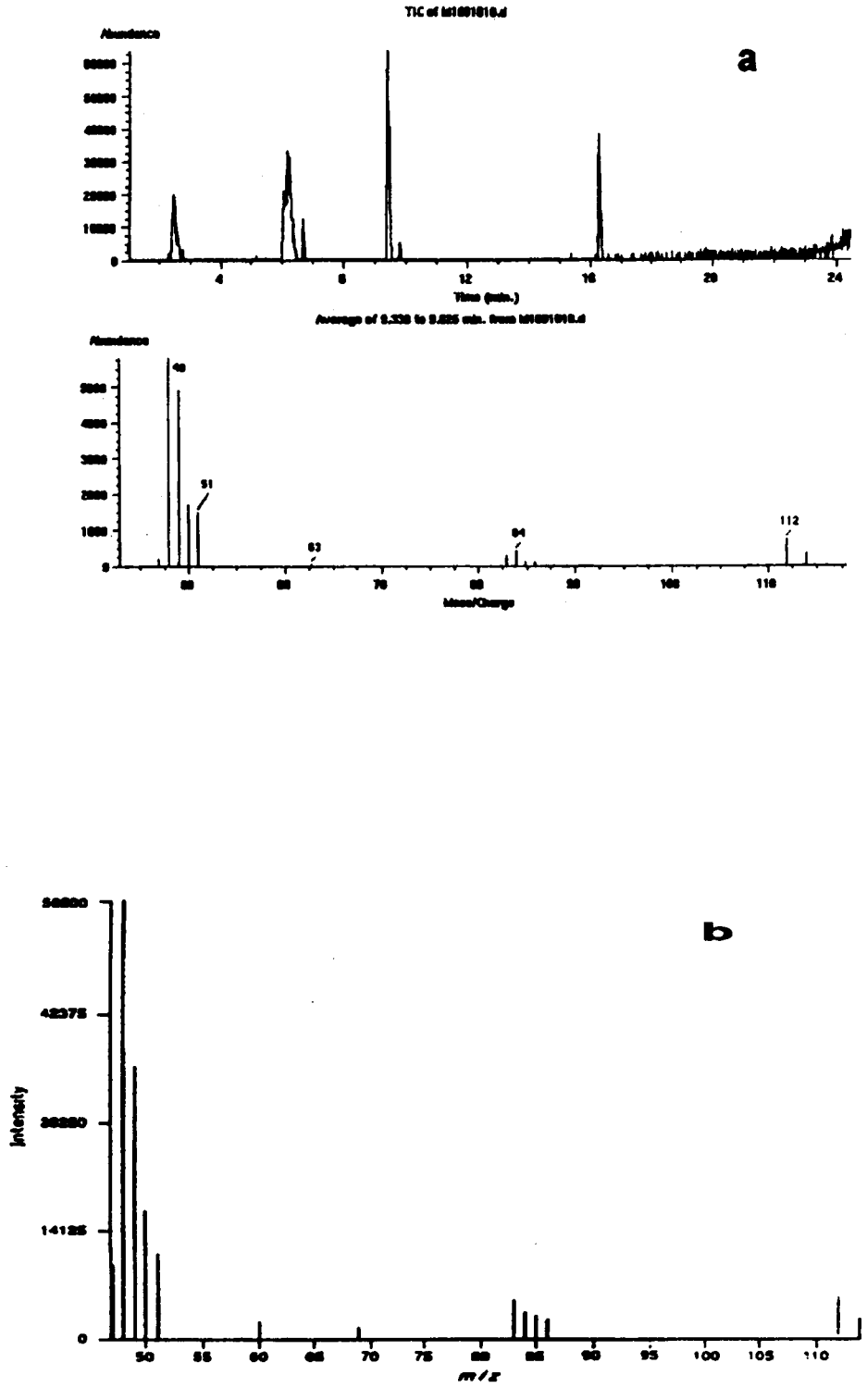


Figure 4.9. Mass spectra of the compound identified as the TRANS epoxide. (a) Results obtained in this work. (b) Data reported by Arvin (1991).

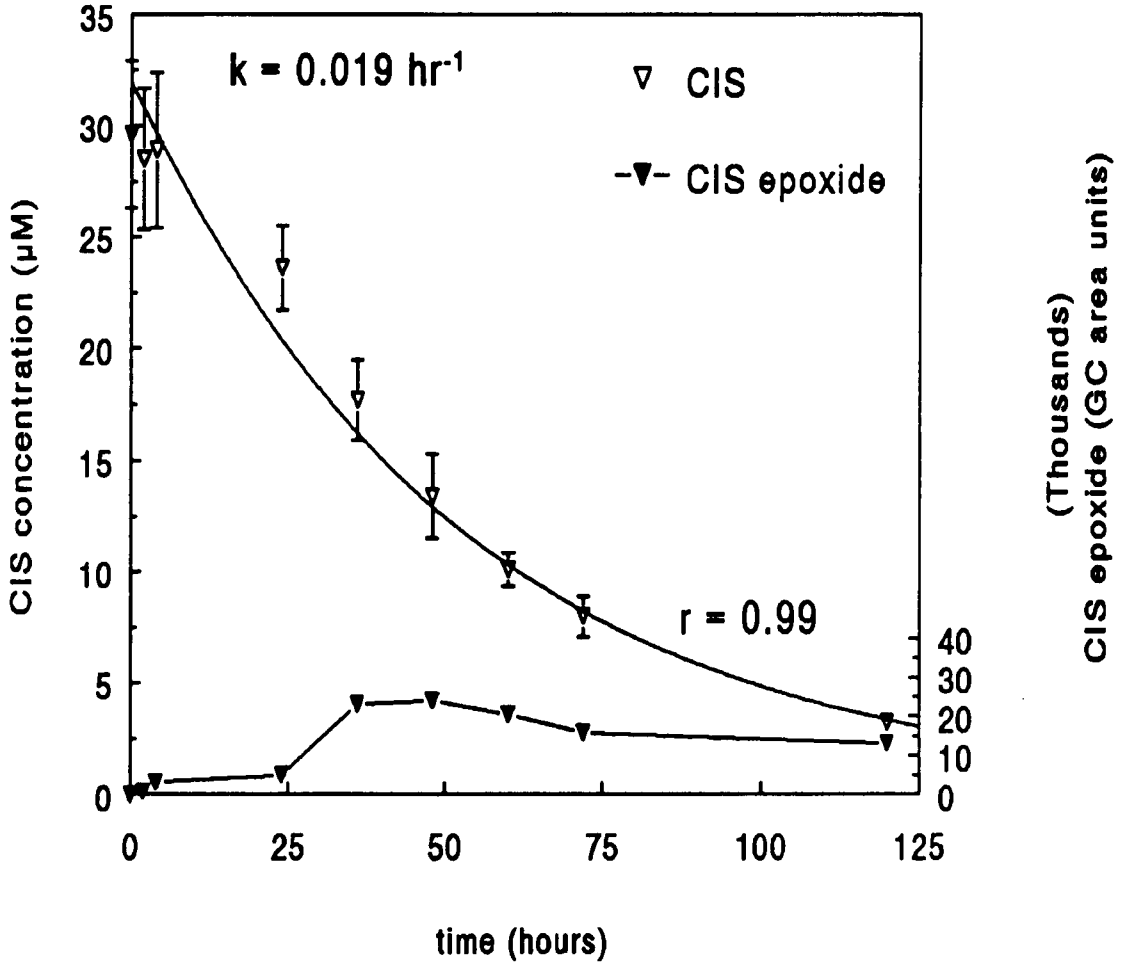


Figure 4.10. CIS disappearance and epoxide formation in KCMC microcosms.

for this peak did not closely match that published in Arvin's 1991 article. However, since this was the only new peak that appeared in the chromatogram, its appearance coincided with CIS disappearance, and the 14.6 min. retention time is consistent with that reported by Arvin, a peak at 14.6 min. was assumed to identify the CIS epoxide.

4.2 CONTINUOUS-FLOW COLUMN EXPERIMENTS

Column experiments performed as part of this research included the use of a conservative tracer, bromide, to investigate the hydraulic properties of the column. PCE was pumped through the column before inoculation with microrganisms to study retardation and/or abiotic reaction of PCE in the column. The first column experiments performed after inoculation were designed to investigate the dechlorination of PCE under anaerobic, methanogenic conditions. Anaerobic column experiments were conducted at a flow rate representative of groundwater flow. Two, relatively high influent concentrations of PCE were studied: 88 mg/L and 32 mg/L. Experiments were also conducted under combined anaerobic/aerobic conditions after inoculation of a methanotrophic culture into the upper portion of the column. Combined anaerobic/aerobic experiments were performed at an influent PCE concentration of 32 mg/L. Anaerobic/aerobic conditions were investigated to test the hypothesis that the less-chlorinated PCE degradation products produced under anaerobic conditions could be degraded by methanotrophs. The ideal result would be anaerobic, reductive dechlorination of PCE followed by the complete mineralization of the less-chlorinated reductive dechlorination products to CO_2 and H_2O.

Results obtained from the bromide tracer, the anaerobic column, and the anaerobic/aerobic column experiments are described in the next sections. Results are compared to similar experiments reported in the literature when possible and appropriate.

4.2.1 Bromide Tracer Experiments

The computer program CXTFIT (Parker and van Genuchten, 1984) was used to determine the dispersion coefficient (D) for bromide and the average pore-water velocity (v) for the sand column at various flow rates. Peclet numbers,

$$P = \frac{vL}{D},$$

were then calculated from the v and D values determined by CXTFIT. Peclet numbers were used to determine the efficiency of packing of the sand column, *i.e.* a well-packed column will have minimal preferential flow or wall channeling. Peclet numbers greater than ~35 to 40 indicate a well packed column in which little or no preferential flow exists (Dunnivant, et al. 1992). CXTFIT was then used to calculate a retardation factor, R, for PCE using the results obtained from the conservative tracer experiments.

CXTFIT fits concentration, distance, and time data to the classical advection-dispersion equation for a one dimensional, steady flow, homogeneous, single reactive species system. The advection-dispersion equation described in Parker and van Genuchten is:

$$\frac{\rho}{\theta}\frac{\partial s}{\partial t} + \frac{\partial c_r}{\partial t} = D\frac{\partial^2 c_r}{\partial x^2} - v\frac{\partial c_r}{\partial x} - \mu_w c_r - \frac{\mu_s \rho}{\theta} s + \gamma_w + \frac{\gamma_s \rho}{\theta}$$

Where:

c_r = volume averaged resident concentration in the liquid phase, M/L^3
s = adsorbed concentration per unit mass of solid phase, M/M
x = distance along the column, L
t = time, T
D = dispersion coefficient, L^2/T
v = average pore-water velocity, L/T
ρ = porous medium bulk density, M/L^3

θ = volumetric water content, L^3/L^3
μ_w = rate constant for first order decay in the liquid phase, T^{-1}
μ_s = rate constant for first order decay in the solid phase, T^{-1}
γ_w = rate constant for zero order production in the liquid phase, $M/(L^3T)$
γ_s = rate constant for zero order production in the solid phase, T^{-1}

For the nonreactive, nonadsorbing bromide tracer, the equation reduces to:

$$\frac{\partial c_r}{\partial t} = D\frac{\partial^2 c_r}{\partial x^2} - v\frac{\partial c_r}{\partial x}$$

Where the initial condition,

$$c_r(x,0) = c_i$$

and the following boundary conditions apply:

for a continuous tracer input,

$$@\ x = 0, \left(c_r - \frac{D}{v}\frac{\partial c_r}{\partial x}\right)\Bigg|_{x=0} = c_{in}(t)$$

for a pulse tracer input,

$$@\ x = 0, \left(c_r - \frac{D}{v}\frac{\partial c_r}{\partial x}\right)\Bigg|_{x=0} = \begin{cases} c_0, & 0<t<t_0 \\ 0, & t>t_0 \end{cases}$$

and

$$@\ x = L, \frac{\partial c_r}{\partial x}(L,t) = 0$$

For a nonreactive, adsorbing species the advection-dispersion equation is:

$$\frac{\varrho}{\theta}\frac{\partial s}{\partial t} + \frac{\partial c_r}{\partial t} = D\frac{\partial^2 c_r}{\partial x^2} - v\frac{\partial c_r}{\partial x}$$

assuming linear adsorption where

$$s = kc_r$$

and the retardation factor, R, is defined as

$$R = 1 + \frac{\rho k}{\theta}$$

the equation becomes

$$R\frac{\partial c_r}{\partial t} = D\frac{\partial^2 c_r}{\partial x^2} - v\frac{\partial c_r}{\partial x}$$

Four bromide tracer experiments were performed using either a continuous input or a slug input of 0.01 M (800 mg/L) KBr at flow rates of 5, 1, 0.3, and 0.033 mL/min. The latter flow rate (2 mL/hr) corresponds to the flow rate used in the anaerobic and anaerobic/aerobic column experiments. Figures 4.11 through 4.14 plot reduced concentration vs. time breakthrough curves for each bromide tracer experiment conducted. The CXTFIT program fit the experimental distance, concentration and time data to the advection-dispersion equation and calculated a < and D for the conservative (R=1) bromide tracer. PCE movement through the column was also followed at the same flow rates (except at 1 mL/min) in order to determine a D and R for PCE. Samples for PCE concentration measurements were collected at the sampling ports along the length of the column. Therefore, no plots of PCE effluent concentration similar to Figures 4.11 through 4.14 could be presented. The CXTFIT program can use concentration data at any point along the flow path to solve the advection-dispersion equation. Column porosity, 2, was calculated from each bromide tracer study using the following equation:

$$\theta = \frac{\textit{bulk velocity } (v = Q/A)}{\textit{average pore water velocity } (v \textit{ CXTFIT})}$$

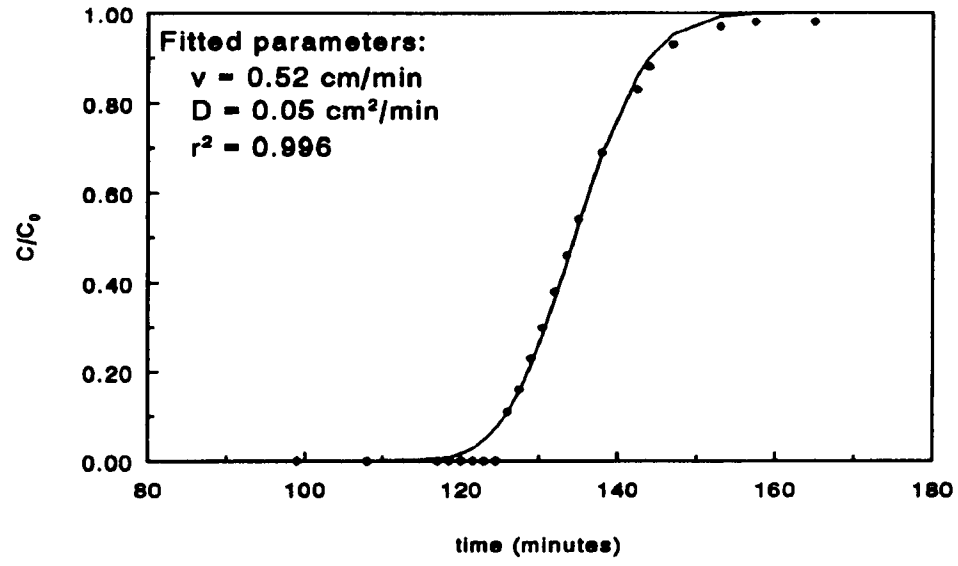


Figure 4.11. Continuous input bromide tracer experiment at 5 mL/min.

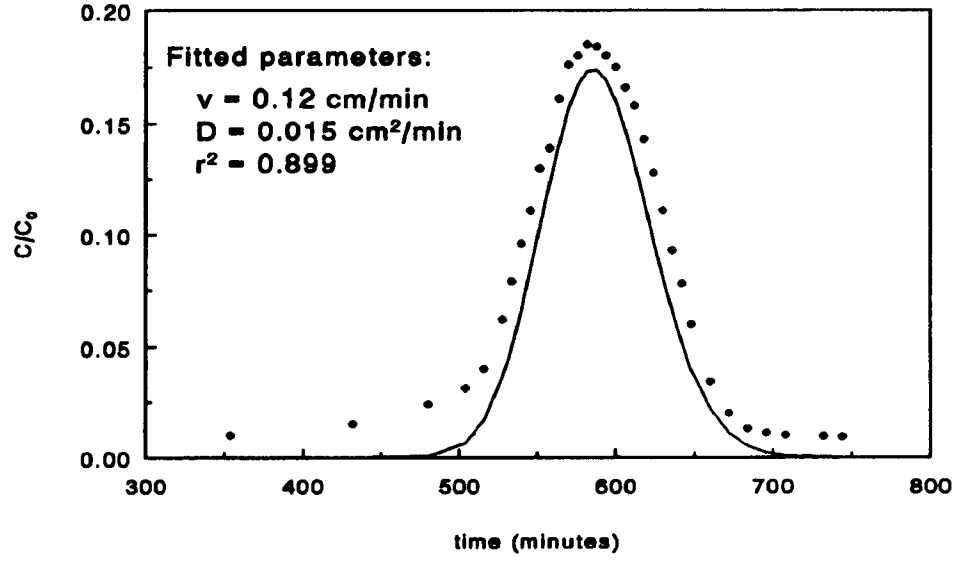


Figure 4.12. Slug input bromide tracer experiment at 1 mL/min.

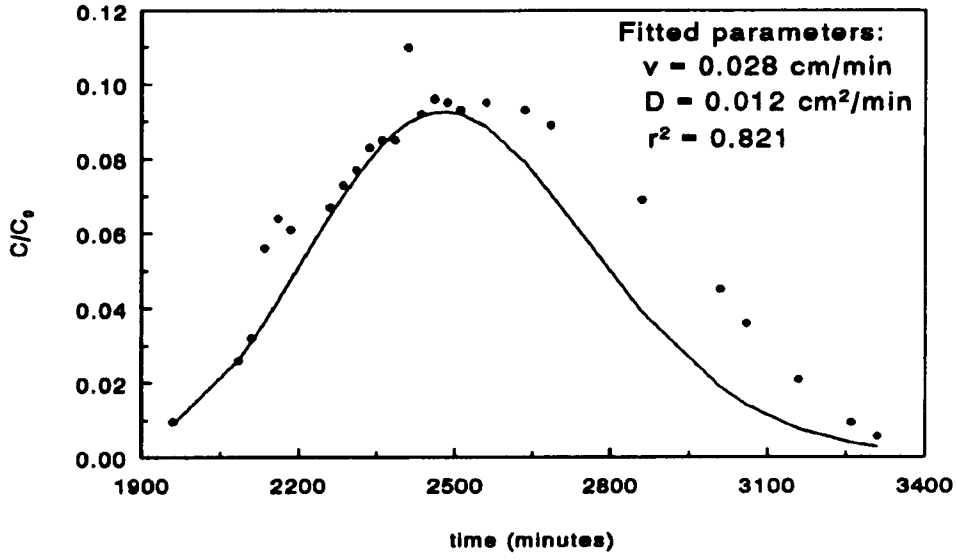


Figure 4.13. Slug input bromide tracer experiment at 0.3 mL/min.

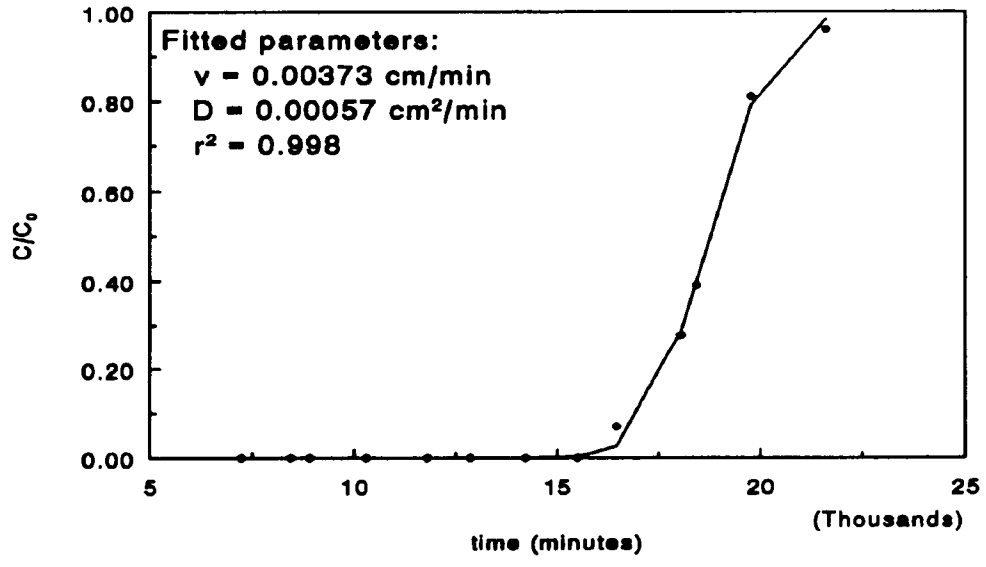


Figure 4.14. Continuous input bromide tracer experiment at 2 mL/hr.

Column porosity determined from the CXTFIT data was compared to the porosity determined by gravimetric means. Table 4.3 shows the results obtained from the CXTFIT simulations at the four flow rates.

Table 4.3. Experimental results from bromide and PCE tracer studies.

flow rate (mL/min)	v (cm/min)	D_{Br} (cm^2/min)	P	D_{PCE} (cm^2/min)	R_{PCE}	θ^a (%)
5.0	0.52	0.051	710	0.2	1.4	50
1.0	0.12	0.015	560	[b]	[b]	54
0.30	0.028	0.012	160	0.034	1.2	43
0.033	0.0037	0.00057	450	0.0075	0.91	46

[a] Porosity determined by weight = 42%.
[b] PCE parameters not determined at this flowrate.

These data indicated a well packed column with a porosity of 48% ± 5%. This relatively high porosity is not surprising given the size and homogeneity of the sand packing. There was no indication that channelling or wall effects complicated the interpretation of the movement of PCE or its dechlorination products through the column. The R_{PCE} values indicated little or no retardation of PCE through the column. However, these data were determined before inoculation of the column with microbes. Therefore, the possiblity of retardation of PCE by sorption onto biomass in the column was not evaluated quantitatively. Data presented in the next section indicate that sorption to biomass was not significant.

4.2.2 Anaerobic Column Experiments

Anaerobic column experiments were started by inoculating the sand column with 10% sludge as described in the Methodology section. Acetate containing RAMM was pumped through the column for 5½ weeks before PCE was added to the influent. Acetate utilization and gas production and composition were monitored throughout the period of column operation. After the addition of PCE to the influent, its removal and the formation of reductive dechlorination products were followed along the length of the column. The next sections describe results obtained from experiments conducted at two influent PCE concentrations, 88 mg/L and 32 mg/L.

4.2.2.1 Acetate Utilization

Acetate concentration in the column influent, at each column port, and in the column effluent was measured by ion chromatography as described in the Methodology section. Figure 4.15 depicts the changes in acetate concentration over time along the length of the column. After an acclimation period, the rate of acetate use increased with time until an apparent steady-state was reached. At the steady-state, acetate removal from the column was almost complete by port 1. Remaining acetate concentrations at ports 1 and 2 were typically <2% and <1% of the influent, respectively. A similar acetate removal profile was maintained throughout the course of the experiments at both 88 mg/L and 32 mg/L influent PCE.

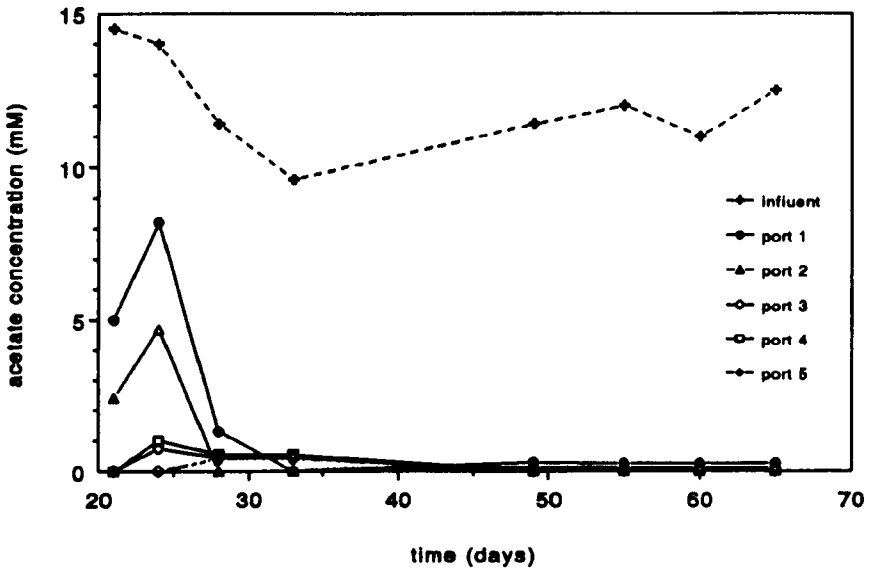


Figure 4.15. Concentration changes of acetate with time in the anaerobic sand column.

4.2.2.2 Gas Production

Gas production in the sand column was measured and its composition analyzed as described in the Methodology section. Gas could be collected within 24 hrs. of inoculation and startup. However, the amount of gas collected was only 3 or 4 mL/day for the first few days of operation. Initial methane composition of the offgas was less than 10%. After this initial period of relatively low gas production, gas was collected at a rate of ~15 to ~20 mL/day. Methane content was typically 30% to 40% after the startup phase. Figure 4.16 shows gas production during experiments at an influent PCE concentration of 32 mg/L. Day zero on this plot refers to the start of PCE addition at this concentration. The column had already been in operation for the anaerobic experiments at 88 mg/L PCE. Therefore, no startup period of low gas production is observed in Figure 4.16.

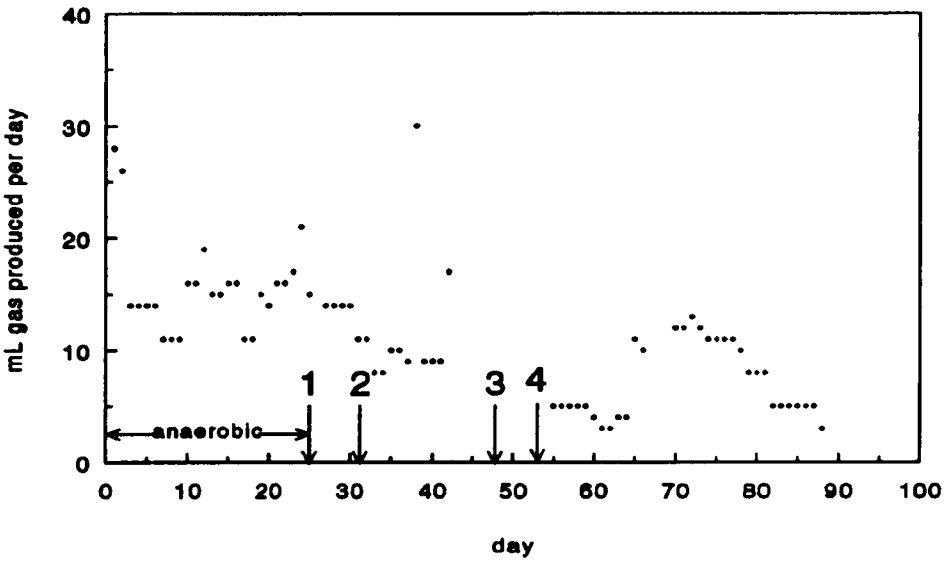


Figure 4.16. Daily gas production in the sand column during the anaerobic and the anaerobic/aerobic column experiments. 1=addition of NATE @ port 3. 2=addition of KCMC culture @ port3. 3=pumping 3% CH_4 in air into port 3. 4=resumed normal influent flow of 2mL/hr of 32 mg/L PCE in RAMM and 2 mL/hr 2X NATE containing 250 mg/L H_2O_2 into port 3.

Based on the stoichiometry of the reaction of acetate conversion to methane and carbon dioxide ($CH_3COOH \rightarrow CH_4 + CO_2$) and an influent acetate concentration of 1000 mg/L at a flow rate of 2 mL/min., one would expect the production of ~18 mL of each gas per day at standard temperature and pressure. Applying a temperature correction by using Charles' Law yields an expected total gas volume of ~39 mL/day at room temperature. About half the expected amount per day was actually collected.

Shelton and Tiedje (1984) suggest that it is appropriate to apply a solubility correction of 0.95 to methane and 0.35 to carbon dioxide to arrive at the expected gas production. Applying these factors to the data obtained here results in a calculated recovery of ~70% of the expected amount (~25 mL) of daily gas production. However, Shelton and Tiedje's solubility correction factors were determined empirically at 35°C.

Since gases are generally more soluble at lower temperatures, higher correction factors may apply. Also, measured acetate influent concentration was generally less than the 1000 mg/L target value and varied. The tortuosity of the gas flow paths made prediction of daily gas collection volumes difficult. Therefore, the amount of gas collected from the anaerobic sand column during these experiments was reasonable and close to the expected amount.

Column offgas data showed that the column was actively methanogenic throughout the course of the experiments. Data from the preceding section on acetate utilization indicate that acetate was the major carbon source for the microorganisms growing in the column. Therefore, PCE dechlorination in the column, described in the next section, occurred under acetate-utilizing, methanogenic conditions.

4.2.2.3 PCE Dechlorination

The first anaerobic column experiment at a target influent PCE concentration of 88 mg/L (530 μM) required several weeks of operation before significant PCE dechlorination was observed. Traces of TCE were observed soon after PCE was added. However, ~7 weeks were required for a significant amount of TCE to build up in the column. Trace amounts of both CIS and TRANS also were detected at this time. Ultimately, PCE was completely removed by the first sampling port. Figures 4.17 through 4.21 show the changes in concentrations of PCE and its reductive dechlorination products over time at each column port. Each data point is the average of two replicate samples.

Maintaining the influent PCE concentration near 88 mg/L proved difficult. Reproducible preparation of the 88 mg/L PCE concentration was subject to numerous sources of error. Such a high concentration required dissolving 6 μL of neat PCE in 100

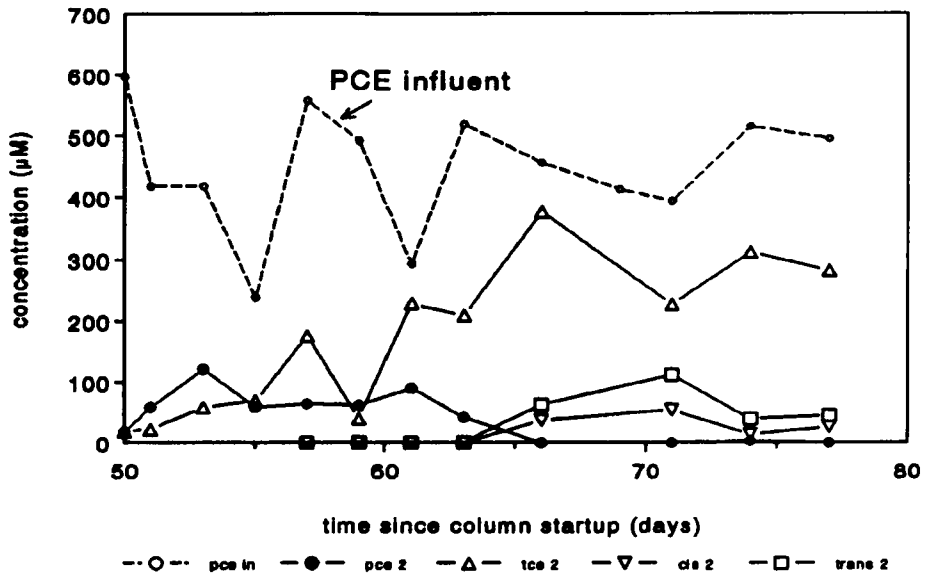


Figure 4.17. Concentrations of PCE and reductive dechlorination products over time at port 1 in the anaerobic sand column. Target influent PCE concentration of 88 mg/L (530 µM).

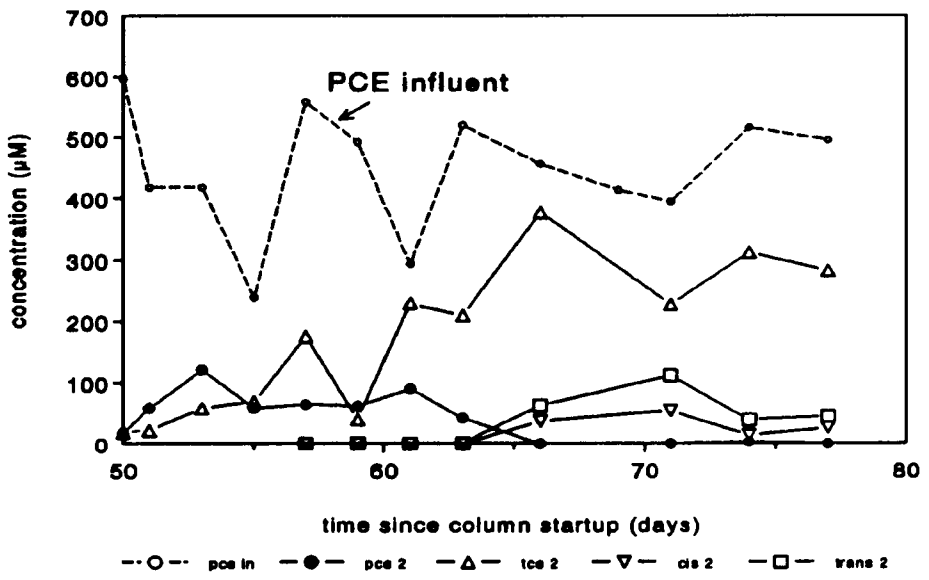


Figure 4.18. Concentrations of PCE and reductive dechlorination products over time at port 2 in the anaerobic sand column. Target influent PCE concentration of 88 mg/L (530 µM).

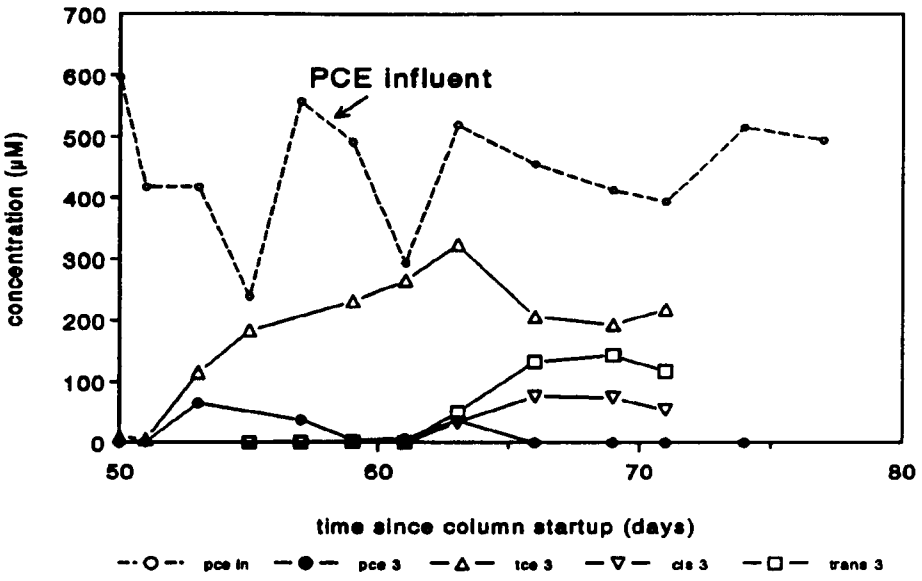


Figure 4.19. Concentrations of PCE and reductive dechlorination products over time at port 3 in the anaerobic sand column. Target influent PCE concentration of 88 mg/L (530 µM).

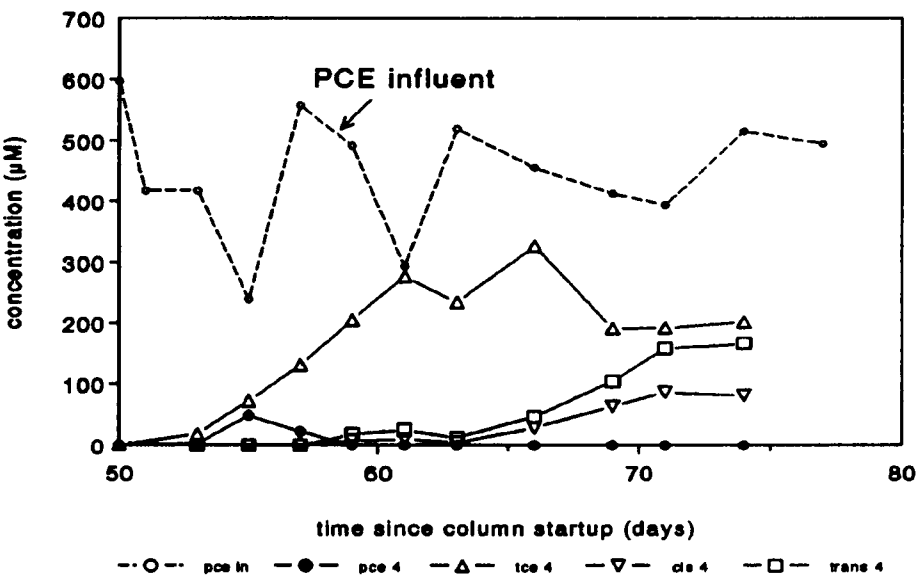


Figure 4.20. Concentrations of PCE and reductive dechlorination products over time at port 4 in the anaerobic sand column. Target influent PCE concentration of 88 mg/L (530 µM).

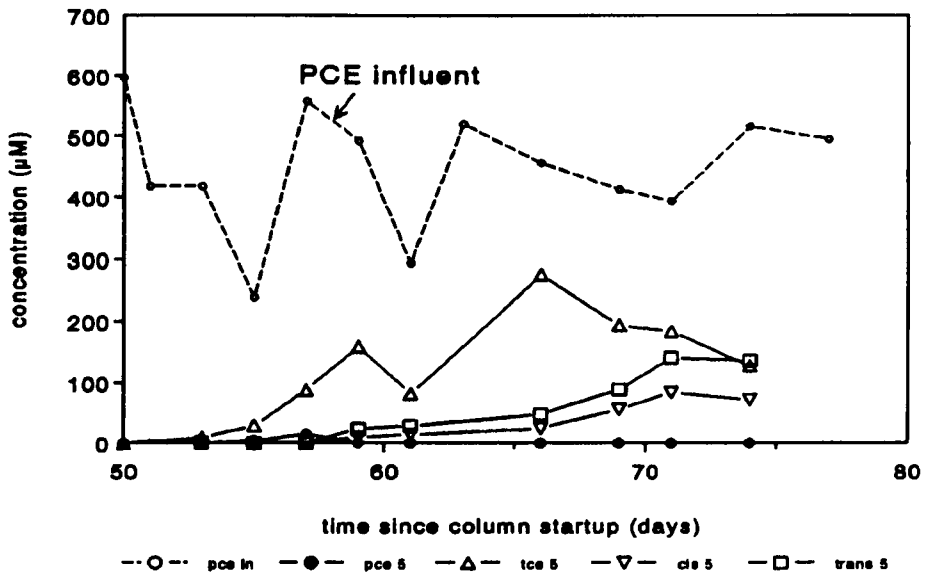


Figure 4.21. Concentrations of PCE and reductive dechlorination products over time at port 5 in the anaerobic sand column. Target influent PCE concentration of 88 mg/L (530 µM).

mL of RAMM. This dissolution required considerable time and vigourous stirring. Complete dissolution was never assured. Slight variations in measuring 6 µL of the dense liquid PCE with a microliter syringe made reproducible preparations difficult. Also, an analytical variability of ±20% between duplicate samples was considered acceptable. Fluctuations in influent concentration will, of course, affect the concentration profile in the column.

TCE was the major dechlorination product detected. Both the CIS and TRANS isomers appeared in significant quantities. TRANS predominated over CIS by an approximately 2:1 molar ratio. No vinyl chloride was detected at any time during the anaerobic experiments at 88 mg/L influent PCE. TRANS was an unexpected dechlorination product based on the results of the microcosm experiments and literature reports. PCE dechlorination in microcosm experiments terminated with the CIS isomer;

no TRANS was detected in any of the microcosms. While some reports in the literature do not distinguish between the DCE isomers formed from PCE dechlorination (Kleopfer et al., 1985; Vogel and McCarty, 1985; Baek and Jaffé, 1989), some report the formation of TRANS (Freedman and Gossett, 1989). However, most reports indentify CIS as the predominant DCE isomer formed during anaerobic dechlorination of PCE (Parsons et al., 1984; Parsons et al., 1985; Barrio-Lage et al., 1987).

Figure 4.22 shows a PCE, TCE, CIS, and TRANS profile obtained two months after PCE was added to the column at the target influent concentration of 88 mg/L. Mass balances between the amount of dechlorination products at any port and the amount of PCE entering the column were not always as good as that depicted in Figure 4.22. Analytical variability and the difficulty of maintaining a steady influent concentration contributed to the problem of achieving a mass balance at any column port at any particular time.

An electron balance between the column inlet and port 1 was determined in order to estimate the electron equivalents being diverted from methane production to reductive dechlorination (Figure 1.3). The balance assumed complete utilization of an influent concentration of 12 mM acetate, an influent flow rate of 50 mL/day, and the production of 25 mL of gas per day that is 40% methane. Given these assumptions and the data shown in Figure 4.22, ~1% of the 4800 microequivalents (μeq) of acetate were used in dechlorinating PCE. This compares favorably to the report of Pavlostathis and Zhuang (1991). However, DiStefano et al. (1991) reported that 31% of the reducing equivalents of methanol were used for dechlorinating PCE in microcosms when PCE was present at an initial concentration similar to that used in this study. Table 4.4 outlines the results of the electron balance calculations.

The data show that ~76% of the reducing power of acetate can be accounted for

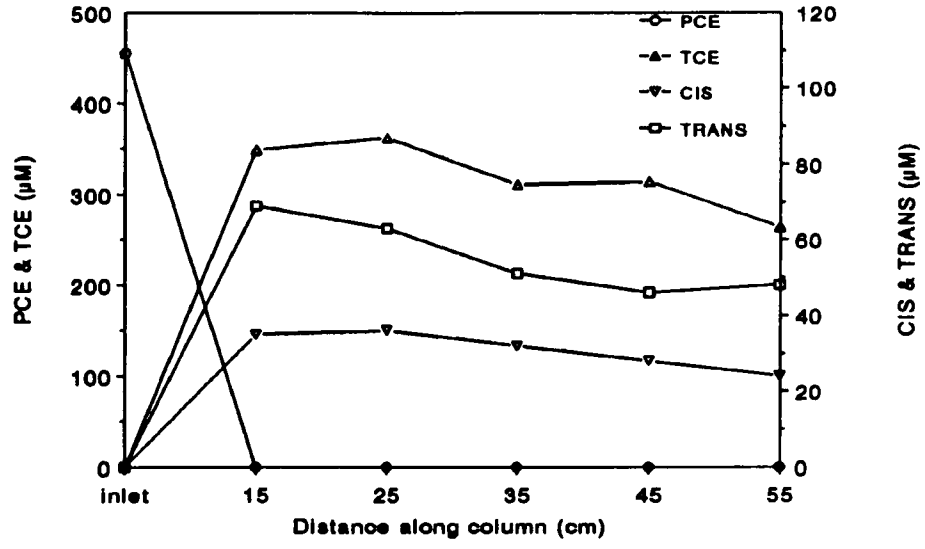


Figure 4.22. PCE dechlorination in the anaerobic sand column @ 88 mg/L (530 µM) influent PCE.

Table 4.4. Electron balance between the inlet and port 1 during the anaerobic column experiments at 88 mg/L (530 µM) influent PCE.[a]

Compound	inlet µmole/L	port 1 µmole/L	µeq/µmole	acetate consumed (µeq/day)	reduced product formed (µeq/day)
acetate	12,000	0	8[b]	4800	
PCE	455	0	0		
TCE	0	349	2[c]		34.9
DCE isomers	0	104	4[d]		20.8
CH_4	0		8[e]		3600

[a] Calculations based on a flow rate of 50 mL/day, influent acetate 12 mM, 25 mL of gas produced per day that is 40% CH_4, ideal gas behavior.
[b] $CH_3COO^- + 3H_2O$ v $CO_2 + HCO_3^- + 8H^+ + 8e^-$
[c] $PCE + 2e^- + H^+$ v $TCE + Cl^-$
[d] $PCE + 4e^- + 2H^+$v $DCE + 2Cl^-$
[e] $CO_2 + 8H^+ + 8e^-$ v $CH_4 + 2H_2O$

by methane production and reductive dechlorination. All of the reducing power of acetate is being used between the column inlet and the first port. Therefore, no reducing power is available for further dechlorination reactions to occur. Thus, the ratios among the dechlorination products remain approximately the same along the remaining length of the column.

A minimum rate of PCE removal in the column can be calculated using the average pore water velocity of 0.0037 cm/min determined from the bromide tracer experiments at the 2 mL/hr flow rate (Table 4.3). Based upon the complete removal of 455 µM PCE between the influent and port 1, the calculated average pore water velocity, and the distance between the influent and port 1 (15 cm), the volumetric rate of removal of PCE is 160 µmole/L/day. This compares to a rate of 275 µmole/L/day calculated by DiStefano and coworkers (DiStefano et al., 1991) in microcosms and 72 µmole/L/day in the biofilm reactor of Fathepure and Tiedje (1994). Since it is unknown exactly where in the first 15 cm of column length that PCE and acetate removal were complete, the actual volumetric removal rate may be much higher than 160 µmole/L/day. This relatively high volumetric rate of PCE dechlorination indicates that removal of high concentrations of PCE under methanogenic conditions is quite feasible. However, additional reducing power above that provided in these experiments is apparently necessary for further dechlorination to occur. Steady gas production and continuing dechlorination indicated that toxicity to the microorganisms at this high PCE concentration was not significant during the course of the experiment.

An experiment with the methanogenic sand column was conducted at a lower influent PCE concentration of 32 mg/L (193 µM). At this lower influent concentration, PCE dechlorination proceeded a step further than it did at 88 mg/L. Within the first 15 cm of the column, significant amounts of VC were produced. Figures 4.23 through 4.27

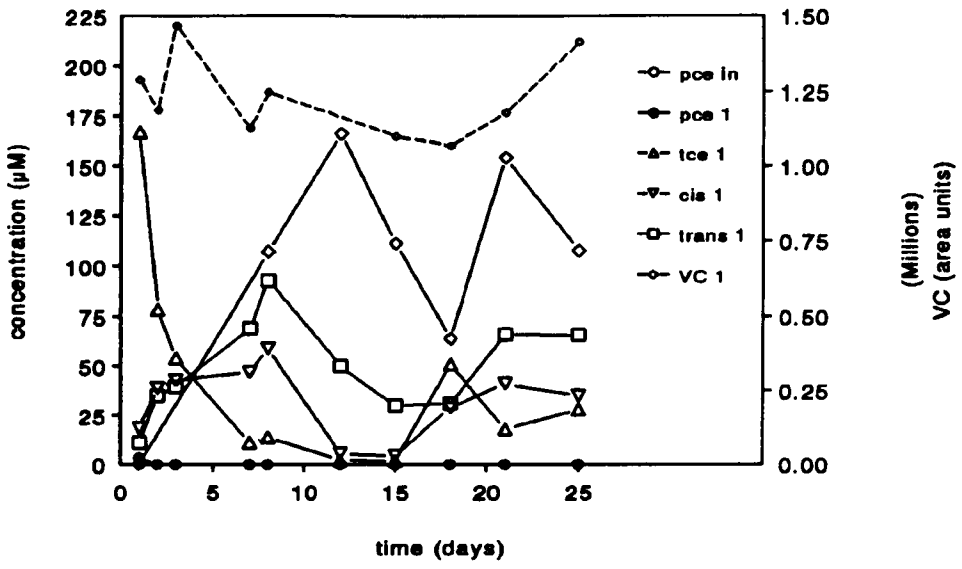


Figure 4.23. Concentrations of PCE and reductive dechlorination products over time at port 1 in the anaerobic sand column. Target influent PCE concentration of 32 mg/L (193 µM).

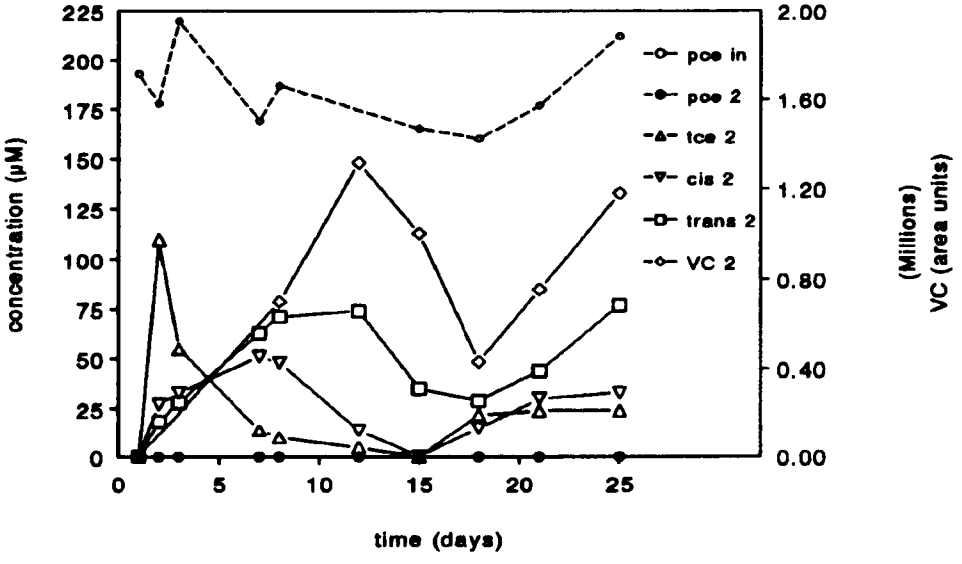


Figure 4.24. Concentrations of PCE and reductive dechlorination products over time at port 2 in the anaerobic sand column. Target influent PCE concentration of 32 mg/L (193 µM).

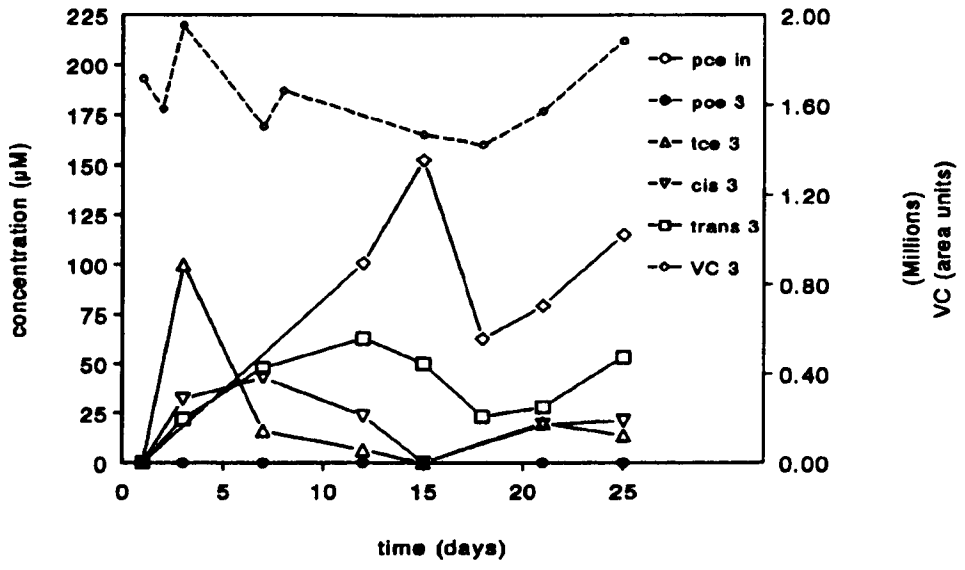


Figure 4.25. Concentrations of PCE and reductive dechlorination products over time at port 3 in the anaerobic sand column. Target influent PCE concentration of 32 mg/L (193 µM).

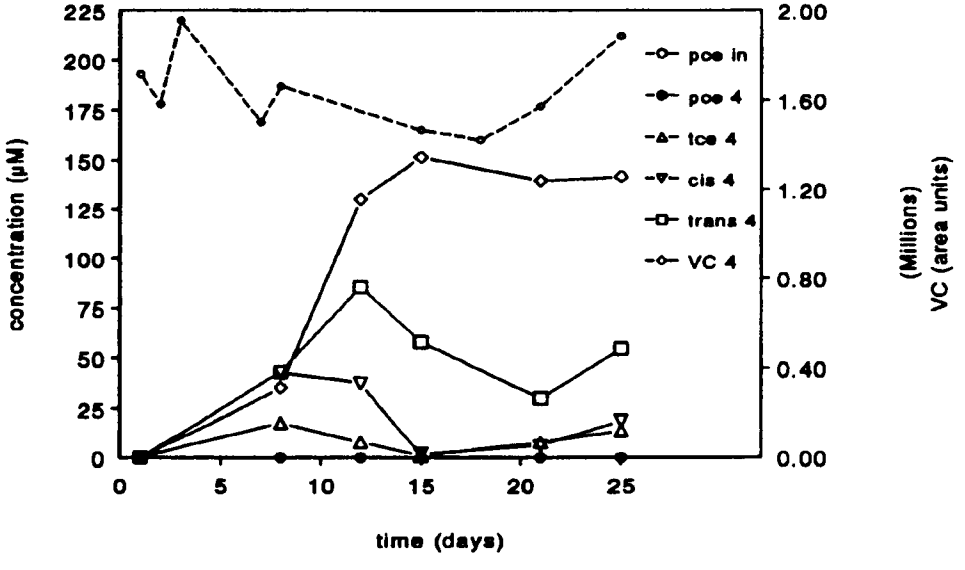


Figure 4.26. Concentrations of PCE and reductive dechlorination products over time at port 4 in the anaerobic sand column. Target influent PCE concentration of 32 mg/L (193 µM).

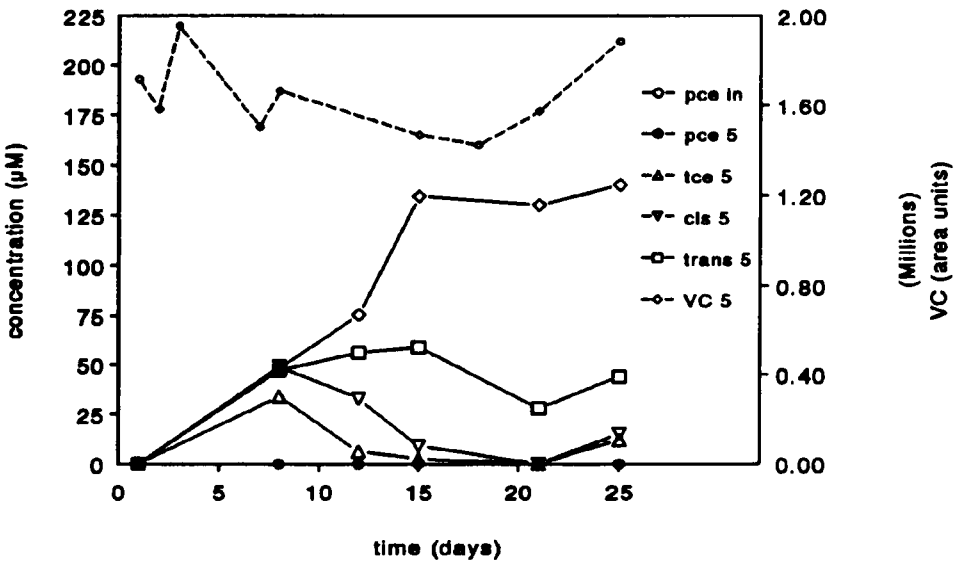


Figure 4.27. Concentrations of PCE and reductive dechlorination products over time at port 5 in the anaerobic sand column. Target influent PCE concentration of 32 mg/L (193 µM).

depict the distribution of PCE and its dechlorination products with time along the length of the column at the 32 mg/L influent PCE concentration. Vinyl chloride is displayed on these figures in GC area units. Amounts of VC formed in the column were not quantified because of the difficulty of standardizing the GC output for VC with the headspace apparatus. Also, an unquantified amount of ethene was detected in the column offgas.

Figure 4.28 is a snapshot of the concentrations along the length of the column on day 25 of the experiment at 32 mg/L influent PCE. This figure shows that the DCE isomers were present at higher concentrations than TCE. It also shows that the 2:1 molar ratio of TRANS:CIS observed in the experiments at 88 mg/L influent PCE was also observed at 32 mg/L influent PCE. Even though VC was not quantified, it was estimated to be the major dechorination product. This estimate was based on the fact that the

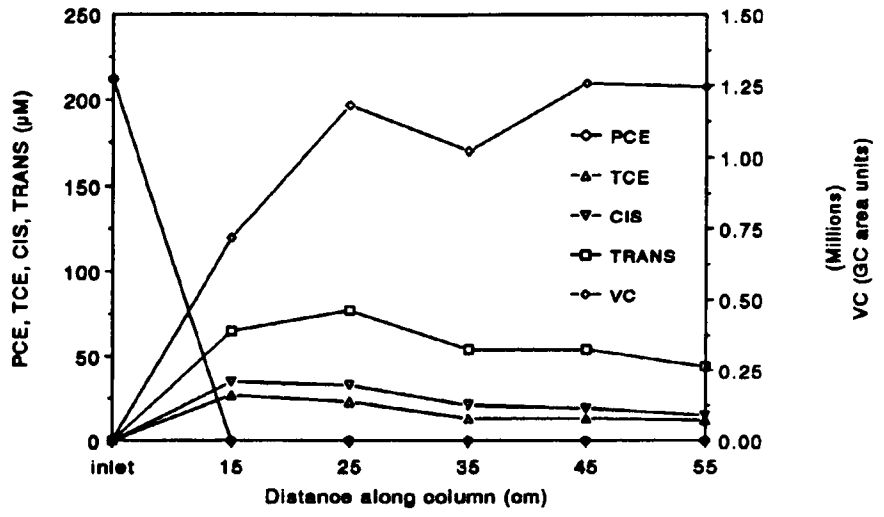


Figure 4.28. PCE dechlorination in the anaerobic sand column @ 32 mg/L (193 µM) influent PCE.

response of the flame ionization detector to each dechorination product was similar. Therefore, a comparison of area units under the peak produced by the GC response indicated that VC was present in the highest concentration. The 2:1 molar ratio of TRANS:CIS observed in the experiments at both influent PCE concentrations indicated that neither DCE isomer was preferentially converted to VC. Other researchers have indicated that the CIS isomer appears to be converted to VC more readily than TRANS (Tandoi et al., 1994).

Electron balance calculations show that that ~1% of the reducing power of acetate was again diverted to reductive dechlorination. Calculations were performed using the same assumptions as discussed in Table 4.4. Since VC was not quantified, the additional assumption was made that the amount of VC was such that the molar sum of TCE, DCE, and VC quantitatively accounted for the moles of PCE in the influent on day 25 (Figure 4.28).

4.2.3 Combined Anaerobic/Aerobic Column Experiments

Combining anaerobic, methanogenic reductive dechlorination of PCE with aerobic, methanotrophic degradation of the dechlorination products proved to be very difficult under the conditions of this study. It was anticipated that methane produced in the lower portion of the column by methanogens would provide the necessary carbon source for growth of methanotrophs in the upper portions of the column. However, methanotrophic growth was never well established. It was expected that growth of methanogens in the column would be obvious by the pink coloration of the KCMC colonies. The only pink growth noted was at port 3, where the methanotrophs and the oxygenated NATE medium were added. Often, cultures added to the column appeared to pass through the column with relative ease. No reduction in methane content of the column offgas was observed as would have been expected if methanotrophs were active. Possible reasons for the apparent lack of methanotrophic growth are that the sand was not an adequate support medium for methanotrophic attachment, oxygen supplied in the NATE medium may not have been sufficient to maintain aerobic conditions in the upper portions of the column, some component of the RAMM that was not utilized by methanogens in the lower portions of the column might have been toxic to methanotrophs, or the hydrogen peroxide added as an oxygen source was toxic to the methanotrophs.

Because the column effluent turned pink (due to resazurin), it was assumed that sufficient oxygen was present for methanotrophic growth. However, the small amount of oxygen necessary to turn resazurin pink would not necessarily mean that sufficient oxygen was continuously available to the methanotrophs. No dissolved oxygen measurements of the effluent were made. The concentration of H_2O_2 supplied at port 3 (250 mg/L) was chosen based upon literature reports where researchers were

investigating the use of peroxide as an oxygen source for bioremediation in general, not specifically for methanotrophs (Hinchee et al., 1991; Spain et al., 1989). Hydrogen peroxide decomposes according to the following equation:

$$H_2O_2 \rightarrow H_2O + \frac{1}{2}O_2$$

The 250 mg/L of H_2O_2 provided could, therefore, theoretically supply ~3⅔ mmole of oxygen per liter. At the 50 mL/day flow rate, this means that ~0.2 mmole of O_2 is supplied, per day, to port 3. The amount of O_2 required for the oxidation of the ~10 mL/day of CH_4 that is produced in the column (~25 mL of gas that is ~40% CH_4) can be calculated based on the following equation:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$

Assuming ideal gas behavior, ~0.45 mmole/day of CH_4 is produced and requires ~0.9 mmole of O_2 for complete oxidation. Therefore, the amount of peroxide supplied is not sufficient for the complete utilization of all the methane produced by the methanogens in the anaerobic portion of the column. All of the O_2 would be consumed between ports 3 and 4. Also, researchers have discovered that the rate of peroxide decomposition can be decreased by the presence of other compounds such as phosphate or sulfide (Aggarwal et al., 1991). However, since methanotrophs are known to thrive at very low oxygen tensions (A. V. Palumbo, Lockheed-Martin Energy Systems, Inc., personal communication, July 19, 1995), the oxygen supplied by the H_2O_2 was assumed to be adequate for growth.

The stoichiometric amount of O_2 required for converting the ~100 μM total DCE present on day 25 (Figure 4.28), based of the equation

$$C_2H_2Cl_2 + 2\frac{1}{2}O_2 \rightarrow 2CO_2 + H_2O + 2Cl^-$$

would be ~0.25 mmole/L. Therefore, the amount of O_2 theoretically supplied by the amount of H_2O_2 added is more than adequate to supply the requirements for methanotrophic oxidation of the CACs present.

Despite the failure to establish prolific methanotrophic growth, experiments were conducted in this anaerobic/aerobic mode. The experimental arrangement was described in the Methodology section. NATE medium was pumped into port 3 with a syringe pump at the same flow rate (~2 mL/hr) as the influent RAMM. Aproximately 50% dilution of the PCE dechlorination products was expected in the absence of degradation by the methanotrophs. Therefore, it was important to have an indicator of biological activity toward the PCE dechlorination products. The ability to detect the DCE epoxides provided such an indicator.

Figure 4.29 shows the effect of dilution on the dechlorination products in the upper half of the column. Bromide tracer pumped into the column at port 3 along with the NATE medium indicated a dilution of ~40% at port 3 and ~50% at ports 4 and 5.

Influent PCE concentration was maintained at 32 mg/L during the anaerobic/aerobic column experiment. The lower portion of the column remained actively methanogenic for at least the first 3 weeks of the experiment. However, after this time, the very dark, reduced lower portion of the column gradually lightened. This presumably occurred as a result of oxygenation caused by diffusion of H_2O_2 into the bottom half of the column. Gas production gradually diminished. The column was operated in the combined anaerobic/aerobic mode for approximately two months before the lower portion of the column appeared to be completely oxygenated.

Despite the apparent minimal methanotrophic growth and the other problems associated with operating the column in the combined anaerobic/aerobic mode, TRANS and CIS epoxides were detected. Small amounts of both epoxides were formed after

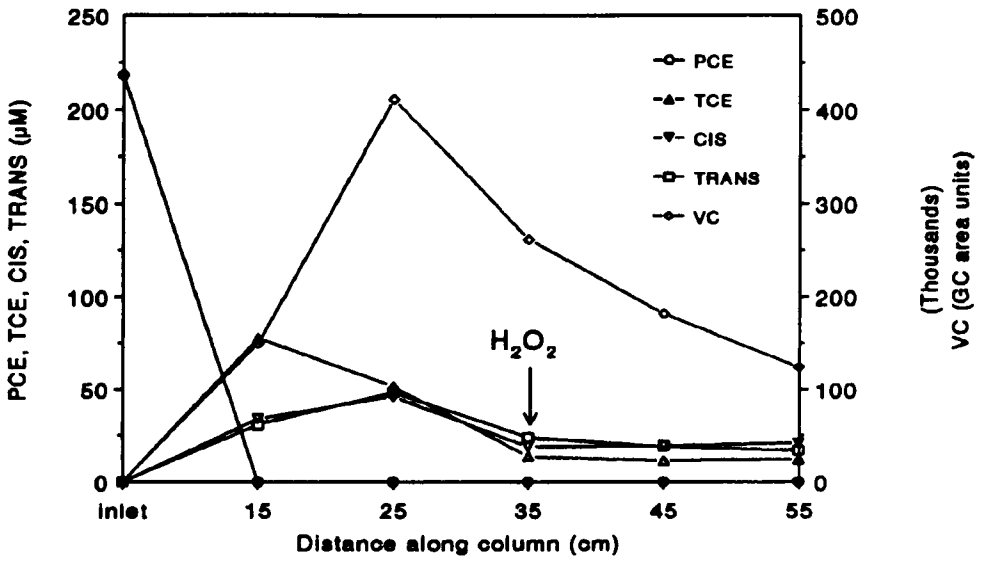


Figure 4.29. PCE dechlorination in the anaerobic/aerobic sand column. Target influent PCE concentration of 32 mg/L. H_2O_2 in 2X NATE added to port 3 at 2 mL/hr.

inoculation of the upper third of the column with methanotrophs. Figures 4.30 through 4.32 show the growth of CIS and TRANS epoxides in the upper portions of the column over the course of the experiment. Day 1 on the graphs marks the initiation of H_2O_2 flow into the column at port 3.

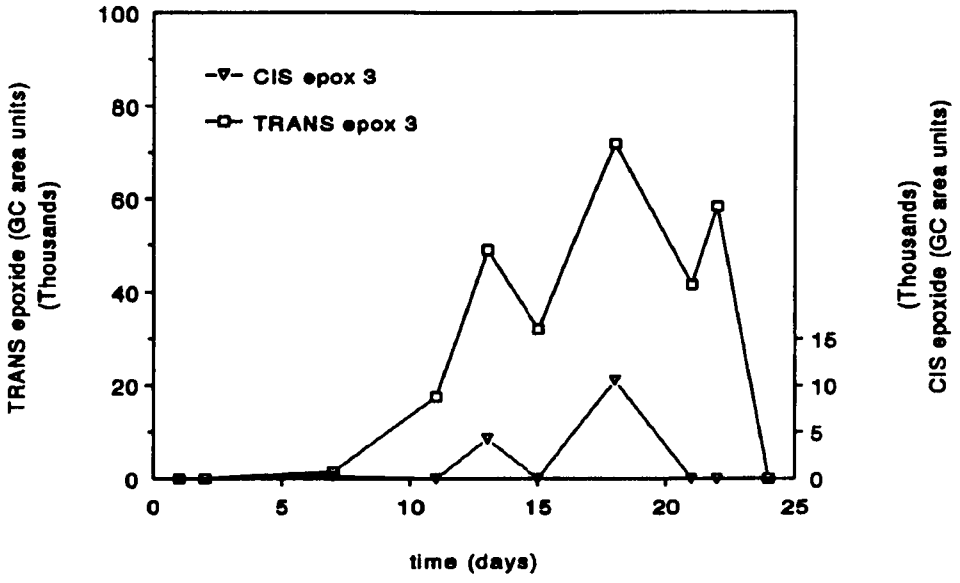


Figure 4.30. Epoxide formation at port 3 in the anaerobic/aerobic sand column with time since the H_2O_2 in NATE was added to port 3 at 2 mL/hr.

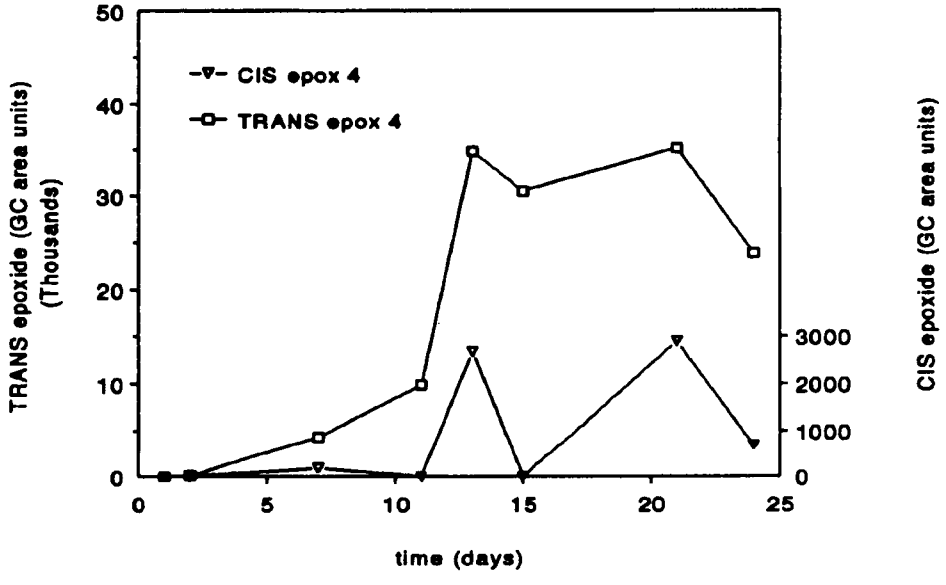


Figure 4.31. Epoxide formation at port 4 in the anaerobic/aerobic sand column with time since the H_2O_2 in NATE was added to port 3 at 2 mL/hr.

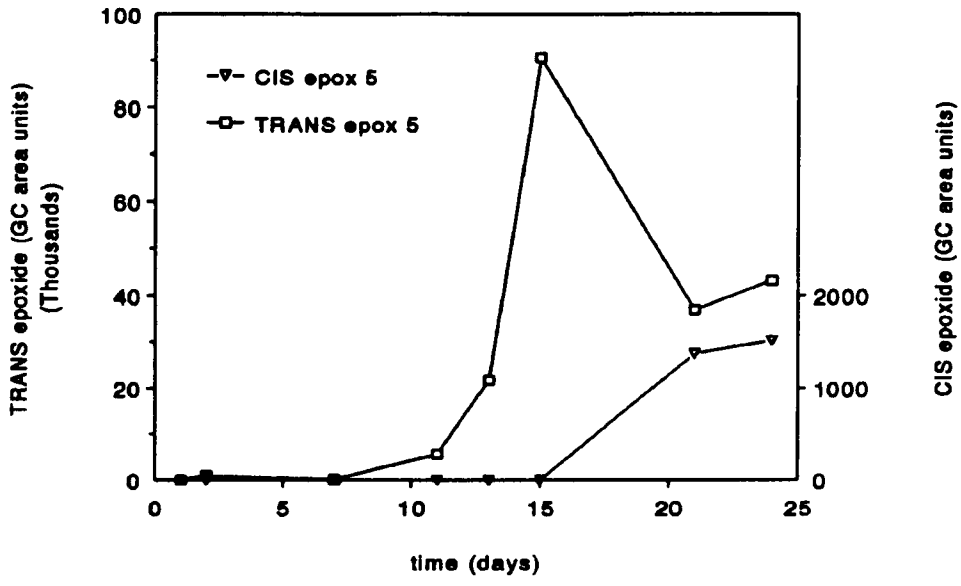


Figure 4.32. Epoxide formation at port 5 in the anaerobic/aerobic sand column with time since the H_2O_2 in NATE was added to port 3 at 2 mL/hr.

5. CONCLUSIONS

Tetrachloroethene (perchloroethene, PCE), a common solvent, is found at high concentrations and as free product in groundwater at Department of Energy sites. PCE is detected frequently at microgram-per-liter levels in groundwater destined for municipal water supplies. The presence of PCE in groundwater above drinking water standards is a public health concern and requires remediation. Commonly used methods for removing PCE from groundwater, air stripping and carbon adsorption, simply transfer the contaminant to another medium. Clearly, a removal mechanism that can completely mineralize PCE to innocuous end products is desirable. Biologically-mediated reductive dechlorination followed by biological oxidation has been proposed as one such mechanism. One of the objectives of this work was to test that hypothesis for high PCE concentrations.

Volatile chlorinated organic compounds were quantified by static headspace gas chromatography of aqueous samples. This technique, not commonly used in previous studies, proved to be a relatively simple procedure. Analyses could be performed using 0.5 mL or less of sample. Liquid-liquid extraction was thus avoided. The use of the flame ionization detector provided a means of readily identifying PCE and its reductive dechlorination products in a single sample. Reproducible preparation of the high concentrations of PCE used in this study proved difficult by the techniques devised for dissolution of neat PCE in mineral medium.

Although the static headspace method did work well, the system required accepting data with a rather large replicate variability (± ~20%). This problem may be unique to this particular headspace GC system that relied on pressure differences between the sample vial headspace and the column head pressure for the injection of

sample onto the GC column. Static headspace systems are available that inject a known amount of sample onto the GC column using an accurate sample loop rather than relying on the pressure difference. The latter system may combine better reproducibility with the ease of sample preparation provided by headspace techniques.

Experiments focused on the dechlorination of high PCE concentrations at room temperature under conditions designed to stimulate anaerobic, methanogenic and aerobic, methanotrophic attack on the PCE molecule and its dechlorination products. Results showed that reductive dechlorination of high concentrations of PCE (~40 mg/L) occurred in anaerobic microcosms under weakly methanogenic conditions. Reductive dechlorination of high PCE concentrations (~32 - ~88 mg/L) also was demonstrated in the simulated groundwater environment of a laboratory sand column under strongly methanogenic conditions. Dechlorination in sediment microcosms depended upon the source of the sediment. In anaerobic microcosms, reductive dechlorination terminated with the CIS isomer of DCE. In the anaerobic sand column, reductive dechlorination led to the formation of both the TRANS and CIS isomers of DCE in a 2:1 molar ratio. At the highest concentration of influent PCE fed to the sand column (~88 mg/L), dechlorination stopped at the DCE isomers and proceeded at a volumetric removal rate of at least 160 µmole/L/day. At this influent PCE concentration, sufficient reducing power was not available from the ~1 g/L of acetate in the feed to carry the reductive dechlorination reaction beyond DCE. Competition apparently occurred between CH_4 production and reductive dechlorination for electrons provided by the electron donor. Calculations demonstrated that only ~1% of the reducing power of acetate was used by the dechlorination reactions. At the lower column influent concentration of 32 mg/L, the reductive dechlorination reactions proceeded to the level of vinyl chloride and beyond; traces of ethylene were detected in the column offgas.

Aerobic microcosms showed that a mixed methanotrophic culture of groundwater origin degraded both CIS and TRANS via epoxide intermediates. This study demonstrated analytical techniques that were capable of detecting and positively identifying the TRANS DCE epoxide. However, mass spectrometric results of the species believed to be the CIS epoxide did not agree well with published results.

Bromide tracer experiments in the sand column showed the column to be well packed, *i.e.*, flow channeling and short circuiting would not complicate the interpretation of contaminant flow. Computer modeling of data obtained from tracer experiments also provided reasonable estimates of the average pore water velocity and column porosity. Little or no retardation of PCE was observed in the clean sand column.

Combining anaerobic, methanogenic dechlorination of PCE with aerobic, methanotrophic degradation of the PCE dechlorination products proved difficult in the experimental arrangement of this study. Conditions established in this work were designed to simulate, as closely as possible, an actual injection of methanotrophs and aerobic mineral medium into an anaerobic groundwater environment. Although the dechlorination products were not completely degraded, methanotrophic activity toward CIS and TRANS was confirmed by the detection of the corresponding epoxides in the aerobic portion of the column. Combining aerobic, methanotrophic degradation with the anaerobic, methanogenic stage requires further study. Particular emphasis needs to be placed upon the response of methanotrophs to hydrogen peroxide concentrations. Also, the production of substances in the anaerobic zone which are then transported to the aerobic zone and may be toxic to methanotrophs should be investigated.

The rapid PCE dechlorination observed in the anaerobic sand column indicates that *in situ* removal of high concentrations of PCE under anaerobic, methanogenic conditions is feasible. The possibility of manipulating electron donor conditions to favor a

desired anaerobic dechlorination product warrants further investigation. Future experiments could involve ways of controlling the anaerobic dechlorination process to stop at DCE. This would avoid the formation of the most dangerous, from a public health standpoint, and difficult to control of the dechlorination products, vinyl chloride.

However, it may be unacceptable to force an aerobic groundwater system anaerobic. The anaerobic nature of many groundwater systems, particularly those that have been heavily polluted, indicates that conditions favorable to PCE dechlorination probably exist in many contaminated groundwaters. Therefore, supplementing particular groundwater systems with appropriate electron donors and nutrients to stimulate PCE dechlorination should be considered.

Even though compounds such as PCE, TCE, CIS, and TRANS can be removed from groundwater under anaerobic or combined anaerobic/aerobic conditions, the economics and success of completely degrading such compounds *in situ* will be site specific. Perhaps focusing on above-ground bioreactors coupled with passive *in situ* systems is most appropriate given the current state of knowledge.

Overall, this research successively met the objective of demonstrating dechlorination of high concentrations of PCE under anaerobic, methanogenic conditions. However, it failed to demonstrate the feasibility of coupling the anaerobic, methanogenic dechlorination process with an aerobic, methanotrophic process to achieve complete dechlorination of PCE and its reductive dechlorination products.

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