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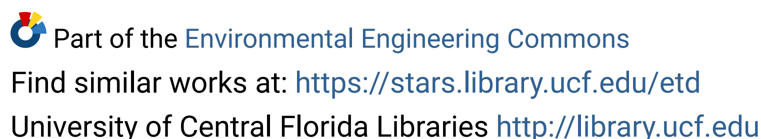

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DEGRADATION OF HALOGENATED ALIPHATIC COMPOUNDS IN SEQUENTIAL ANAEROBIC/AEROBIC METHANOGENIC AND HOMOACETOGENIC ENVIRONMENTS

by

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B.S. University of Central Florida, 1996

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
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ABSTRACT

The objective of this study was to utilize an alternating anaerobic/aerobic sequence to biologically transform perchloroethylene to non-hazardous end products such as ethylene, CO_2 and H_2 using a single microbial consortia in a methanogenic and/or a homoacetogenic environment followed by an aerobic methanotrophic environment. Reductive dechlorination of PCE and TCE to cDCE and VC in an anaerobic environment is typically carried out by methanogens, sulfidogens, or homoacetogens but often (e.g. in-situ) leads to an accumulation of daughter compounds (cDCE, VC) which are more toxic than their parent compounds (PCE, TCE). Furthermore, PCE is resistant to degradation in aerobic environments while VC and cDCE are readily oxidized co-metabolically by aerobic methanotrophic bacteria, among others.

In order to achieve complete mineralization of chlorinated solvents using a biotic system, an anaerobic/aerobic treatment strategy was investigated. This strategy has been accomplished successfully at a lab scale with anaerobic and aerobic reactors in series, and in-situ anaerobic zones with downgradient aerobic zones have been proposed in the field. In contrast, the focus of this research was to expose single mixed microbial consortia to sequential anaerobic/aerobic treatments in order to determine if reductive dechlorination could be sustained following aerobic phases of treatment. If possible this would imply that the anaerobic and aerobic zones (in-situ) or reactors (ex-situ) would not necessarily have to be spatially separated. In pure or dilute cultures where soil material is not present strict anaerobes would typically not resume metabolic activity if exposed to frequent aerobic phases of treatment. However in aquifer material or reactors with large floc/granules it might be possible due to the protection of anaerobic micro-environments as a result of diffusion limitations. Microcosms contained in sealed 120-mL serum bottles were

used to generate experimental data including autoclaved abiotic controls with mercuric chloride. Inocula for these microcosms come from a several sources, including anaerobic digester sludge, soils, and contaminated aquifers. Once an experimental microcosm showed signs of reductive dechlorination, an aerobic treatment was implemented. The anaerobic phase of the microcosm was interrupted with a short duration aerobic phase. Headspace air or hydrogen peroxide addition was used to supply oxygen.

Analytical data from the experiments indicated that anaerobic reductive dechlorination was readily accomplished during anaerobic phase experiments as PCE was sequentially dechlorinated to TCE and then to cDCE as reported in previous research reported by others in the literature. Additionally, a few mixed consortia microcosms showed evidence of further reductive dechlorination to VC and ethylene. During the sequential environment experiments, analytical data also indicated that reductive dechlorination also resumed after an aerobic sequence utilizing hydrogen peroxide as an oxidizer in the microcosm. No conclusive evidence was observed to indicate aerobic degradation of cDCE during any of the aerobic phase treatments. This was probably due to the inocula not containing methanotrophs.

This thesis is dedicated to my father, Larry W. Hoxworth.

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I want to take this opportunity to acknowledge my sources of guidance and support that has allowed me to conduct and finish this research project.

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LIST OF ACRONYMS/ABBREVIATIONS

1,1 DCE	1,1 Dichloroethylene
BES	Bromoethanesulfonate
cDCE	cis-1,2 Dichloroethylene
HACs	Halogenated Aliphatic Hydrocarbon
PCE	Tetrachloroethylene
TCE	Trichloroethylene
tDCE	trans-1,2 Dichloroethylene
VC	Vinyl Chloride

CHAPTER 1   INTRODUCTION

1.1 Background

Tetrachloroethene or perchloroethene (PCE) is a chlorinated solvent that is widely used in the dry cleaning industry, metal degreasing, and numerous other industrial processes (Fetter, 1993). Decades of its use, improper handling, and uncontrolled disposal have resulted in widespread contamination in the soil and groundwater (Westrick at al., 1984, Abelson, 1990). Due to the carcinogenic and toxic characteristics of PCE and its natural degradation products (Ensley, 1991), the EPA has listed PCE as a hazardous pollutant under the Comprehensive Environmental Response, Compensation and Liability Act, and the Resource Conservation and Recovery Act. Additionally, PCE is regulated under the Safe Drinking Water Act Amendments of 1986 with a maximum contaminant level (MCL) of 5 μg/L. The cleanup of contaminated groundwater aquifers to meet these regulatory requirements has resulted in substantial public interest in potential remediation technologies and techniques.

PCE and trichloroethene (TCE) contaminated groundwater aquifers under anaerobic conditions were observed to be accumulating dechlorinated transformation by-products (Beeman et al., 1994). The phenomenon of reductive dechlorination has subsequently been demonstrated in laboratory, field, microbiological study under a variety of anaerobic metabolisms, including methanogenic, homoacetogenic (DiStefano et al., 1991), and sulfidogenic (Bagley and Gossett, 1990). Reductive dechlorination has been observed in laboratory and field studies to transform PCE by sequentially replacing the chlorine molecules with hydrogen molecules resulting in transformation by-products TCE, cis-1,2 dichloroethene (cDCE), trans-1,2 dichloroethene

(tDCE), 1,1 dichloroethene (1,1DCE), vinyl chloride (VC), and ethylene. The complete mineralization of PCE to ethylene has been reported by some methanogenic bacteria (Freedman and Gossett, 1989). However, the rate of transformation decreases with each molecular chlorine substitution such that PCE and TCE are dechlorinated at relatively more rapid rates then cDCE and VC. Transformations beyond cDCE and VC are difficult to achieve and typically require slow rates of transformation. The dechlorination of VC to ethylene is the rate limiting step (Freedman and Gossett, 1989; Tandoi et al., 1994). The result is typically partial reductive dechlorination of PCE to VC in in-situ groundwater situations where low temperature conditions ($<15^{o}C$) further diminish reductive dechlorination activity (Freedman and Gossett, 1989; Tandoi et al., 1994). Because VC is a known human carcinogen and a serious public health threat (Easter and Von Bung, 1994), the use of reductive dechlorination to remediate PCE contaminated groundwater aquifers is severely limited.

Due to the lower oxidation state of TCE, DCE, and VC, various aerobic bacteria have been observed to co-metabolically degrade these halogenated aliphatic hydrocarbons (HACs) aerobically by methane, phenol, and toluene-oxidizing bacteria (Dolan and McCarty, 1995; Hopkins and McCarty, 1995). The high oxidation state of the PCE molecule has been shown to be resistant to aerobic biodegradation (Fogel et al., 1986). This paradox has resulted several research efforts attempting to combine anaerobic reductive dechlorination of PCE and TCE with aerobic co-metabolic degradation of the resultant reductive dechlorination by-products DCE and VC (McCarty, 1991; Kastner, 1991; Zitomer and Speece, 1993). This strategy is known as sequential anaerobic/aerobic environment treatment. Due to the known intolerance of oxygen to anaerobic bacteria, particularly methanogens (Madigan et al., 1997), most sequential

environmental research is focused on separated anaerobic and aerobic treatment processes by space, either with reactors in series or treatment zones in-situ.

Sulfate-reducing anaerobic bacteria have been shown to be more aero-tolerant than methanogens (Madigan et al., 1997) which leaves open the possibility that a single microbial consortia utilizing sulfidogenic and/or other anaerobic/facultative bacteria and aerobic bacteria can continue reductive dechlorination with low aerobic exposure events to stimulate aerobic bacteria such as methanotrophs to complete the PCE mineralization. Furthermore, oxygen diffusion limitations may possibly permit methanogens to survive aerobic treatments in microanaerobic zones. Methanogens could ideally provide a methane substrate for methanotrophs within the microbial consortia. This opens the possibility that a single treatment zone or reactor with alternating anaerobic/aerobic phases of treatment could completely mineralize PCE. Such a strategy, if practical, would have clear advantages to spatially separated treatments, particularly for in-situ groundwater treatment.

The purpose of this research project was to investigate the feasibility of using a non-sulfate reducing anaerobic environment (methanogenic/homoacetogenic) and aerobic methanotrophic environment in a single microbial consortia to biodegrade PCE. This research was accompanied by an investigation of a sulfate-reducing anaerobic/methanotrophic aerobic treatment strategy (McCue, 1999).

1.2 Statement of Objectives

This study investigated the effects of several anaerobic/aerobic treatment strategies upon the ability of a single microbial consortia to dechlorinate PCE and its reductive declorination

daughter products (TCE, cDCE, VC) in methanogenic/homoacetogenic environments. The research objectives included:

1. Develop and maintain a low sulfate methanogenic reactor to sustain a methanogenic metabolism to serve as a source of innocula for anaerobic dechlorination experiments.
2. Create a microcosm series that reductively dechlorinates PCE in methanogenic and homoacetogenic environments.
3. Conduct aerobic treatment experiments with two injection strategies (hydrogen peroxide and air into microcosm headspace) to the anaerobic microcosms that demonstrate successful reductive dechlorination of PCE to investigate the effect on further chloroethene degradation.

CHAPTER 2 LITERATURE REVIEW

2.1 Anaerobic Reductive PCE Dechlorination

Research into reductive dechlorination of PCE is well established with numerous studies and experiments completed since the early 1980s. Reductive dechlorination of PCE dehalogenates the PCE molecule (C_2Cl_4) sequentially by replacing the chlorine atoms attached to the double-bonded carbon atoms with hydrogen atoms. The result is a reductive dechlorination pathway where PCE is dechlorinated to TCE (C_2HCl_3), TCE is dechlorinated to one of three DCE ($C_2H_2Cl_2$) isomers (cDCE, tDCE, and 1,1DCE), DCE to VC (C_2H_3Cl), VC to ethylene (C_2H_4).

Each step in the reductive dechlorination pathway reduces the oxidation potential of the chlorinated ethene. Reductive dechlorination of PCE to TCE and then to cDCE appears to be relatively favorable with many studies in the laboratory and the field consistently demonstrating this natural phenomenon. Reductive dechlorination of VC to ethene has been reported in a few laboratory and field studies (Freedman and Gossett, 1989; DiStefano et al., 1991; Tandoi et al., 1994; and Maymo-Gatell et al. 1997). The conversion of VC to ethene appears to be the rate-limiting step with VC accumulation occurring in these studies (Freedman and Gossett, 1989; Tandoi et al. 1994; and Haston and McCarty, 1999).

Many field studies have been conducted as the proliferation of sites identified with active natural biological degradation have been identified. Most current anaerobic dechlorination studies in the field focus on providing electron donors to the site to lower the redox potential in the contaminated area and accelerate the naturally occurring anaerobic processes. Recirculation

amendments (Fam et al. 2000), hydrogen injection (Newell et al. 2000), and vegetable oil injection (Boulicault et al. 2000, Wadill et al. 2002) are a few examples of these recent efforts. Dechlorination remains limited by VC to ethene transformations with most studies continuing to evaluate the longer term potential of these technologies (Boulicault et al. 2000, Wadill et al. 2002).

There are five identified metabolic groups of PCE-dechlorinating bacteria that have been noted in research literature; methanogenic, acetogenic, facultative anaerobic, Gram-positive halorespirators, and Gram-negative halorespirators (Damborsky, 1999). The following sections describe the metabolisms of the bacteria and summarize documented research using these metabolisms to reductive dechlorinate PCE.

2.1.1 Methanogenic PCE Dechlorination

Methanogenic bacteria are a group of highly specialized obligate anaerobes that produce methane (CH_4), typically from H_2 and CO_2 by H_2-utilizing methanogens or from acetate by acetoclastic methanogens (Madigan, et al. 1997). H_2-utilizing methanogens use CO_2 as their terminal electron acceptor which is reduced to CH_4 in reaction 2.1 (Madigan et al. 1997):

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \qquad (2.1)$$

Most species of methanogens studied in pure cultures are capable of the H_2 utilization metabolism (Zehnder, 1988). Acetoclastic methanogens convert acetate (CH_3COO^-) to methane according to the following reaction (Madigan et al. 1997):

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \qquad (2.2)$$

Besides acetate and H_2/CO_2 substrates, a few other substrates can be utilized in methanogenesis,

including methanol, formate, methyl mercaptan, and methylamines (Madigan et al. 1997). Methanogenesis is dependent upon other microorganisms to produce these specific compounds from more complex organic substrates (Madigan, et al. 1997). PCE is not a substrate for methanogens and is believed to be cometabolically reduced during methanogenesis.

Much of the pioneering PCE reductive dechlorination research in the early 1980s was focused on methanogenic environments. Bouwer and McCarty (1983) reported that PCE could be degraded under methanogenic conditions with acetate as the primary carbon source. Using radioactive carbon-14 in the PCE molecule, they identified TCE as the first dechlorination product of PCE biotransformation.

Parsons et al. (1984) observed the successive transformation of PCE to TCE, cDCE and tDCE, and VC in microcosms inoculated with Floridian swamp muck with methanol as the carbon and energy source. Vogel and McCarty (1985) reported the partial mineralization of PCE to CO_2 through radiotracer studies. The result of the column study confirmed that PCE can be transformed by reductive dechlorination to TCE, DCEs and VC under methanogenic conditions. Freedman and Gossett (1989) demonstrated that PCE could be further dechlorinated to ethylene. The PCE, TCE, DCE isomer, VC pathway of reductive dechlorination under methanogenic conditions was confirmed by numerous researchers investigating kinetic rates and pure bacteria culture studies (Barrio-Lage et al. 1986; Parsons et al. 1985; Freedman and Gossett, 1989).

2.1.2 Homoacetogenic PCE Dechlorination

Homoacetogenic bacteria are obligate anaerobes that produce acetate during anaerobic respiration utilizing CO_2 as a terminal electron acceptor (Madigan et al., 1997). Homoacetogens

will form acetate according to the following reaction (Drake, 1994):

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \qquad (2.3)$$

Use of the term homoacetogens to imply that these bacteria solely synthesize acetate is a misnomer, as many homoacetogens will synthesize acetate under favorable environmental conditions, but can also produce other reduced end products as the energy-conserving, growth supporting mechanisms for the cell (Drake, 1994).

Homoacetogens are ecologically closely interrelated with methanogens. The Homoacetogens' role in the anaerobic conversion of complex polymers to methane has only started to be appreciated in recent decades (Drake, 1994). Along with H_2 utilizing methanogens and sulfate-reducing bacteria, homoacetogens compete for the limited available H_2 in the environment being produced by syntrophic fatty acid oxidizers (Madigan et al., 1997).

Cometabolic reductive dechlorination has been linked to H_2 utilizing homoacetogens since the early 1990s (DiStefano et al., 1991). A few researchers have isolated strains of homoacetogenic bacteria identified as dechlorinators. Egli et al. (1988) observed dechlorination of PCE to TCE by *Acetobacterium woodii* WB1 in a resting cell assay with 5 mmol/L fructose in the reaction medium (Damborsky, 1999). Terzenbach and Blaut, (1994) tested the ability of eight homoacetogenic strains of bacteria for PCE dechlorination potential and found one of the strains, *Sporomusa ovata*, capable of PCE dechlorination to TCE. Methanol was utilized as the carbon and energy source during the experiments on *S ovata*. The rate of PCE dechlorination was found to be dependent on the substrate concentration and was confirmed to be present when both CO_2 and methanol were available for acetogenesis (Terzenbach and Blaut, 1994).

2.1.3 Facultative Anaerobic PCE Dechlorination

Only one study was found in the literature demonstrating facultative organisms within an anaerobic environment responsible for PCE dechlorination. This may be more indicative of a lack of focus in researching and isolating facultative bacteria capable of PCE dechlorination, so the potential utility of these organisms in reductive dechlorination remains very speculative.

Sharma and McCarty (1996) isolated two facultative anaerobic PCE dechlorinating bacteria. A previously unclassified facultative MS-1 bacteria was isolated from an enrichment of aquifer material from a PCE contaminated site (Damborsky, 1999). The MS-1 bacteria reductively dechlorinated PCE to cDCE in the absence of oxygen, nitrate, and high concentrations of fermentable compounds (Damborsky, 1999). The second facultative microorganism, *Pantoea agglomerans*, was isolated from blood and also dechlorinates PCE to cDCE (Sharma and McCarty, 1996).

2.1.4 Halorespiring PCE Dechlorination

Reductive dechlorination is typically observed as a cometabolic reaction associated with an anaerobic metabolism (Maymo-Gatell et al. 1997). However, more recent research has demonstrated reductive chlorination by halorespiring microorganisms that use halogenated compounds as electron acceptors for energy conservation and growth (Damborsky, 1999). One promising study by Maymo-Gatell et al. (1997) isolated a halorespiring eubacterium, *Dehalococcoides ethenogenes*, without a close affiliation to any known groups that can dechlorinate PCE to ethene.

Several sulfate-reducing bacteria have also been identified as halorespiring bacteria (Damborsky, 1999). Studies have shown that sulfate-reducing bacteria can also dechlorinate PCE. Sulfate-reducing bacteria are competitors with methanogens in many subsurface environments, sharing similar ecological niches. Bagley and Gossett (1990) investigated PCE dechlorination in sulfate-reducing mixed cultures and demonstrated PCE could be degraded to TCE and cDCE under sulfidogenic conditions with a slower kinetic rate than comparable methanogenic studies. Fathepure et al (1987) authored an early study documenting successful dechlorination of PCE by sulfate-reducing bacteria. Fathepure worked with pure cultures of two different types of sulfate-reducing bacteria. One of the two bacteria was shown to successfully dechlorinate tetrachloroethylene (PCE) to trichloroethylene (TCE) in a sulfate-reducing environment. DiStefano et al. (1991) reported PCE mineralization to ethylene with methanogenesis inhibited under high PCE concentrations.

Pavlostathis and Zhuang (1991) used contaminated soil from a hazardous waste disposal site to innoculate experimental microcosms with lactate and acetate electron donors in a high sulfate concentration environment. Dechlorination of TCE to cDCE was documented in long-term (281 days) incubation cultures and in higher temperature enriched cultures. TCE transformation rates for the long-term incubation culture were between 1.8×10^{-3} and 2×10^{-1} µmol/day. The highest observed transformation rate for TCE in enriched cultures at 35°C was 213 µmol/day.

One field-scale application with sulfate-reducing bacteria has been reported (Beeman et al., 1994). An aquifer contaminated with PCE, TCE, and DCE was treated in situ by pumping water to the surface, supplementing the water with magnesium sulfate, and recharging the sulfate-rich water towards recovery wells. This technique of enriching sulfate-reducing bacteria

resulted in the dechlorination of both PCE and TCE. PCE was dechlorinated to TCE and to DCE in the first year of operation resulting in increased DCE and VC concentrations. After two years of operation, the PCE, TCE, DCE, and VC concentrations were degraded below detection limits.

2.2 Aerobic Degradation of Chlorinated Ethenes

Aerobic microorganisms known as methanotrophs degrade TCE cometabolically in the presence of methane (as an electron donor) by the action of a mono-oxygenase enzyme (Wilson and Wilson, 1985). In contrast, methanotrophic degradation of PCE has been reported to be minimal (Fogel et al., 1986). Both methanotrophic cometabolism (Fogel et al., 1986; Nelson et al., 1994; Roberts et al., 1989) and heterotrophic cometabolism (Davis et al., 1990) have been used to degrade vinyl chloride aerobically. Aerobic mineralization of VC to CO_2 and H_20 has been demonstrated (Davis et al., 1990), as well as the use of VC as the sole carbon and energy source for microbial growth (Hartmans et al., 1985). However, detailed kinetics of VC degradation remain to be defined (Nelson, et al., 1994).

A field evaluation of the feasibility of stimulating methanotrophic degradation of chlorinated compounds (including TCE, cDCE, tDCE, and VC) has been conducted (Semprini et al., 1991) in a shallow confined aquifer at the Moffett Field Naval Air Station. A laboratory-scale microcosm bottle experiment was performed with a methanotrophic mixed culture to determine net growth rates of the mixed culture at varying chlorinated aliphatic hydrocarbon concentrations. The pilot study focused on determining the effects of competitive inhibition of cometabolism of CAHs and methane oxidation by methanotrophic culture.

2.3 Sequential Environment Chlorinated Ethene Degradation

A number of researchers have proposed using more than a single environment to dechlorinate PCE because of the advantages of reductive environments for highly chlorinated compounds, and oxidative environments for their less chlorinated intermediates (Fathepure et al., 1991; Kastner, 1991; McCarty, 1991). The few combined processes studied exclusively relied on two separate microbial populations which cannot exist under the same environmental conditions, confining all technologies to two-stage systems (i.e. two injection sites or reactors) for both ex-situ and in-situ applications (Fathepure et al., 1991). This is largely because most of these technologies rely exclusively on a series of cometabolic biotransformations by first a methanogenic community (non-aerotolerant), followed by a methanotrophic community (aerobic).

2.3.1 Laboratory-Scale Experiments

Several researchers have performed laboratory scale research using sequential environments. All laboratory techniques found during this literature review except one (Innis and Ketchum, 1997) attempted sequential treatment with two separate microbial environments with the effluent of the anaerobic treatment being pumped into a second vessel (column or reactor) for the aerobic treatment.

Inniss and Ketchum (1997) showed that an aerobic/facultative mixed culture in sequencing batch reactors could dechlorinate PCE during anaerobic periods. During the experiment, 90 to 95% of 10 mg/L PCE was reductively dechlorinated to cDCE during the bench-scale study. PCE was degraded in the absence of oxygen and as the dominant electron

acceptor in the same reactor vessel as short aerobic periods were introduced (Inniss and Ketchum, 1997).

Isalou (1998) conducted a laboratory-scale column experiment with sequential anaerobic/aerobic environments in two separate columns in series. An anaerobic microbial community developed in the first column from anaerobic digester sludge over a 2.5 year period. The first column demonstrated sustained dechlorination of up to 120 mg/L PCE to vinyl chloride. The effluent of the anaerobic column was pumped to an aerobic second column with a methanotrophic culture developed from a local pond. The second column degraded VC to ethene but exhibited inhibition to degradation with continued exposure to high concentrations of VC in the methanotrophic column influent. The second column was required to be operated in a pulsing fashion to allow methanotroph growth rates to balance the VC toxicity effects.

2.3.2 Field-Scale Experiments

Several studies were found in the literature involving research of in-situ anaerobic/aerobic biological degradation of PCE/TCE. Typical of these studies is an upgradient anaerobic zone with an anoxic transition to an aerobic zone. PCE/TCE is dechlorinated to DCE and or VC in the anaerobic zone and the plume of degraded contaminants migrates to a natural or artificial aerobic zone where the contaminants are aerobically degraded.

In one such study, sequential anaerobic and aerobic biodegradation was observed of TCE and TCA at a field site in Sacramento, California. A former lagoon on the site was a source area for TCE and TCA. The source area was under anaerobic conditions. Immediately downgradient of the source area the aquifer was under aerobic conditions. Reductive dechlorination of TCE

and TCA to ethene and ethane was observed (Cox et al., 1995). The natural state of this aquifer appears to be aerobic with the presence of contaminants in the source area creating an anaerobic zone through depletion of oxygen.

In another pilot study, the two treatment zones (anaerobic and aerobic) were enhanced artificially with an electron donor amendment injected into the anaerobic treatment zone and oxygen addition in the aerobic treatment zone (Turpie et al., 2000) were being used to test biodegradability of a landfill leachate plume with chlorinated solvents, petroleum hydrocarbons, and arsenic. Persistent cDCE was the focus of the anaerobic treatment zone. This documented study showed increased dechlorination rates of the cDCE to VC in the anaerobic treatment zone.

CHAPTER 3 METHODS AND MATERIALS

3.1 Analytical Techniques

3.1.1 Chlorinated Solvent Analysis

Following procedures established in EPA Method 624 (40 CFR, Part 136, Appendix A), liquid phase chlorinated solvents were analyzed using a Tekmar Co. (Cincinnati, OH) purge-and-trap system fitted with a Vocarb 3000 trap and a Hewlett Packard (Palo Alto, CA) gas chromatograph (GC) Model 5890 equipped with a flame ionization detector (FID) and a 60-meter long Vocol capillary column with 0.25-mm inner diameter (Supelco Inc., Bellefonte, PA). A 5-mL sample aliquot (unfiltered) was transferred from its experimental microcosm to a purge vial on the purge and trap. Helium was bubbled through the sample for 11 minutes at 22°C in order to volatilize any halogenated aliphatic compounds present in the liquid sample. A Vocarb 3000 trap captured these freshly volatilized analytes. The desorb time from the trap was four minutes at 250°C and the bake time was seven minutes at 260°C. After the completion of the bake cycle, the volatilized sample was routed to the gas chromatograph. The flow rate of the carrier gas, helium, through the capillary column was approximately 1 mL/min. The temperature of the gas chromatograph oven was programmed to begin sample analysis at 60°C for three minutes, to increase at a rate of 15°C/min to 180°C for 8 minutes, and hold at 180°C for an additional three minutes. The resultant peaks separated by the capillary column were analyzed by utilizing a HP 3393A integrator.

Calibration curves for PCE, TCE, and the DCE isomers were developed by creating stock solutions of the various analytes from reagents (99%+ purity) purchased from Fisher Scientific (Pittsburgh, PA) and Aldrich Chemical Company (Milwaukee, WI). The stock solutions, dissolved in methanol, varied in concentration from 5000 mg/L to 2000 mg/L, depending upon the analyte in question. In all cases, the coefficient of determination values for the calibration curves were greater than 0.99. Calibration curves were conducted in the concentration ranges of 0.5 ppm to 12 ppm. The quality of the data was assured through measurements of precision (duplicates of matrix samples) and accuracy (percent recovery calculations of matrix samples). Response factors against TCE for vinyl chloride and ethylene were developed on another GC equipped with an FID (Ruiz, 1998).

Samples were withdrawn from sealed experimental microcosms with a 21.5-gauge needle and a 5-mL gas-tight Hamilton Luer-lock syringe (Fisher Scientific). The samples were then injected immediately into the purge vials on the purge and trap. The liquid samples were not filtered because it was feared that filtering these samples would volatilize analytes present within the sample. Since samples were analyzed immediately, no sample storage protocols were necessary.

3.1.2 Volatile Fatty Acid Analysis

Liquid samples were analyzed for acetic and propionic acids following Supelco Bulletin 856B. A Shimadzu (Columbia, MD) gas chromatograph model 14-A equipped with a flame-ionization detector (FID) was utilized to conduct the analysis. A 3-mm i.d. glass column with 60/80 Carbopack C/0.3% Carbowax 20M/0.1%H_3PO_4 packing (Supelco Inc., Bellefonte, PA)

was used to separate the various volatile fatty acids (VFAs). Helium at approximately 30 mL/min was used as the carrier gas. The injection port and the detector were maintained at 200°C. The oven of the gas chromatograph was programmed to begin sample analysis at 105°C and to remain there for 2 minutes, to increase temperature at a rate of 5°C/min to 150°C for 9 minutes, and to hold at 150°C for an additional 2 minutes, resulting in a total run time of 13 minutes. The sample injection volume was 2µL. A Shimadzu auto sampler AOC-20i injected the samples into the gas chromatograph. A Shimadzu Chromatopac CR501 integrated the resultant peaks that were separated by the gas chromatograph.

Calibration curves were established for acetic and propionic acids by using pure reagents purchased from Fisher Scientific (Pittsburgh, PA) and neat standards purchased from Supelco (Bellefonte, PA). Calibration curves typically had coefficient of determination values greater than 0.99 and were conducted in the concentration range of 6 to 1000 ppm. A calibration curve with at least four points was developed with each sampling event. Standards were stored at 4°C and fresh standards were prepared every two weeks. For the purposes of quality control, 10% of all matrix samples were replicates and an additional 10% of all matrix samples were spiked duplicates to allow calculation of percent recoveries.

All samples were filtered with a 0.45-µm membrane filter prior to analysis. Samples were withdrawn from sealed microcosms using a 21.5-gauge needle and a 5-mL gas-tight Luer-lock syringe. A filter holder with a Luer-lock adapter was then attached to the 5-mL gas-tight Luer-lock syringe. After filtration, the filtrate was collected directly into 1.5-mL gas chromatography vials. The vials were then sealed with teflon-lined butyl rubber septum and screw caps and stored at 4°C. Immediately prior to analysis, 150µL of 3% H_3PO_4 was added to each sample using a 250-µL syringe in order to drop the pH to approximately 3. The vial was

recapped after acidification and analyzed. This sample acidification allowed for better analyte separation by the column packing. The reason that H_3PO_4 was not added prior to any extended storage was because both acetic and propionic acids are more volatile at low pH values. The H_3PO_4 was added immediately before sample analysis.

3.1.3 Biogenic Gas Analysis

Microcosm headspace samples and anaerobic reactor off-gas samples were monitored for biogenic gas production. Biogenic gases include methane (CH_4), carbon dioxide (CO_2), and hydrogen sulfide (H_2S). A Shimadzu gas chromatograph model 14-A equipped with a thermal conductivity detector (TCD) was utilized to conduct the analysis. The column used for this analysis was 15 ft x 1/8" i.d. stainless steel support packed with 60/80 Carboxen 1000 (Supelco Inc., Bellefonte, PA). The carrier gas, helium, was maintained at a flow rate of approximately 30 mL/min through the column. The injection port was set at 175°C and the TCD oven was kept at 220°C. The oven of the gas chromatograph was programmed to begin sample analysis at 40°C and to remain there for 5 minutes, to increase at a rate of 20°C/min to 220°C for 9 minutes, and to hold at 220°C for an additional 10 minutes, resulting in a total run time of 24 minutes. A sample volume of 1 mL was used during the analysis. A Shimadzu Chromatopac CR501 integrated the resultant peaks that were separated by the gas chromatograph.

Calibration curves for CH_4 and CO_2 were established by using 99% pure gases purchased from Scott Specialty Gases (Plumsteadville, PA). Calibration curves typically had coefficient of determination values greater than 0.99. Tedlar bags were filled with the standard gases directly from the original cylinders. A 1-mL gas tight syringe was used to withdraw the standard gases

from Tedlar bags. Standard gases were immediately injected into the gas chromatograph after withdrawal from the Tedlar bag. At least one standard was injected and analyzed each time the instrument was used. Establishing a calibration curve for H_2S, however, was problematic. After the completion of this research project, it was determined that the cause of the problem was that H_2S sorbs to stainless steel columns. The column was replaced with a glass column with identical packing material (Supelco Inc., Bellefonte, PA) after this research project was terminated.

Headspace samples were withdrawn from microcosms by using a 1-mL gas tight syringe. Samples were immediately injected into the gas chromatograph after withdrawal from the microcosm. No sample preservation techniques were needed.

3.2 Experimental Methods

3.2.1 Experimental Microcosms

3.2.1.1 Microcosm Components

To explore the effect of anaerobic/aerobic sequencing upon dechlorination of halogenated aliphatic hydrocarbons in a methanogenic environment, a large number of microcosm batch experiments were created. Each microcosm was contained within a 120-mL amber glass Wheaton serum bottle. The serum bottles were crimp sealed with aluminum caps and PFTE-lined grey butyl rubber septum.

A typical methanogenic microcosm contained the following basic components: 92 mL buffer solution, 5-mL of liquid containing biomass from the methanogenic reactor (see Section 3.2.2), 1-mL trace metal solution, 1-mL vitamin solution, 1-mL 5 g/L $Na_2S \cdot 9H_2O$ (a reducing agent), and 0.5 µL pure PCE. Table 3.1 shows the contents of the buffer solution.

Table 3.1: Buffer Solution Components

Component	*Concentration (mg/L)*
$NH_4H_2PO_4$	230
$(MgCO_3)_4 \cdot Mg(OH)_2 \cdot 5H_2O$	120
$CaHPO_4$	60
K_2HPO_4	170
$NaHCO_3$	250

The purpose of the buffer solution was to provide a phosphate buffering system for a low sulfate concentration experimental matrix. This solution focused on a low chloride basal media formulation in order to facilitate chloride mass balances (Hirl, 1997). In these microcosms the presence of chlorides in a chloride-free media lends support to the dechlorination of halogenated aliphatic compounds (HACs).

Acetic and propionic acid were also added to the methanogenic media in concentrations of 500 ppm and 400 ppm, respectively. These VFAs served as organic carbon sources, energy sources, and electron donors for the bacteria. This initial concentration of VFAs was selected because it was the same concentration fed to the methanogenic reactor which served as the

inoculum source for these experiments (please see Section 3.2.2 for further information about the methanogenic reactor). With the VFAs added, the pH of the methanogenic media was adjusted to 7.0 using a potassium hydroxide (1 N) solution.

The trace metal solution and the vitamin solution were both necessary for bacterial growth. Anaerobes, particularly methanogens, are sensitive to the absence of trace metals (Speece, 1996). The composition of both the trace metal solution (Table 3.2) and the vitamin solution (Table 3.3) were adopted from Atlas (1993) and similar to those used by other researchers (Pavlostathis and Zhuang, 1991).

Table 3.2: Trace Metal Solution Components

Component	*Concentration (mg/L)*
$MgSO_4 \cdot 7H_2O$	30
Nitrilotriacetic acid	15
NaCl	10
$MnSO_4 \cdot H_2O$	5
$FeSO_4 \cdot 7H_2O$	1
$CoCl_2 \cdot 6H_2O$	1
$CaCl_2$	1
$ZnSO_4 \cdot 7H_2O$	1
$CuSO_4 \cdot 5H_2O$	100
$AlK(SO_4)_2 \cdot 12H_2O$	100
H_3BO_3	100
$Na_2MoO_4 \cdot 2H_2O$	100

Please note that trace metal and vitamin concentrations listed in Tables 3.2 and 3.3 are not the actual concentrations found within the microcosm. Only 1 mL of both the trace metal and vitamin solutions are contained within the 100-mL microcosm liquid volume, so the actual concentration within the microcosm was approximately 1% of the tabulated amount. The combination of buffer, vitamin, trace metal solutions, and injected PCE mass resulted in a microcosm composition presented in Table 3.4.

Table 3.3: Vitamin Solution Components

Component	*Concentration (mg/L)*
Pyridoxine HCl	100
Thiamine HCl	50
Riboflavin	50
Nicotinic acid	50
Calcium pantothenate	50
p-Aminobenzoic acid	50
Thiotic acid	50
Biotin	20
Folic acid	20
Vitamin B-12	1

Table 3.4: Typical Methanogenic Microcosm Components

Component	*Concentration*
$NH_4H_2PO_4$	212 mg/L
$(MgCO_3)_4 \cdot Mg(OH)_2 \cdot 5H_2O$	110 mg/L
$CaHPO_4$	55.2 mg/L
K_2HPO_4	156 mg/L
$NaHCO_3$	230 mg/L
$MgSO_4 \cdot 7H_2O$	300 μg/L
Nitrilotriacetic acid	150 μg/L
NaCl	100 μg/L
$MnSO_4 \cdot H_2O$	50 μg/L
$FeSO_4 \cdot 7H_2O$	10 μg/L
$CoCl_2 \cdot 6H_2O$	10 μg/L
$CaCl_2$	10 μg/L
$ZnSO_4 \cdot 7H_2O$	10 μg/L
$CuSO_4 \cdot 5H_2O$	1000 μg/L
$AlK(SO_4)_2 \cdot 12H_2O$	1000 μg/L
H_3BO_3	1000 μg/L
$Na_2MoO_4 \cdot 2H_2O$	1000 μg/L
Pyridoxine HCl	1000 μg/L
Thiamine HCl	500 μg/L
Riboflavin	500 μg/L
Nicotinic acid	500 μg/L
Calcium pantothenate	500 μg/L
p-Aminobenzoic acid	500 μg/L
Thiotic acid	500 μg/L
Biotin	200 μg/L

Component	*Concentration*
Folic acid	200 μg/L
Vitamin B-12	10 μg/L
Na_2S9H_2O	50 mg/L
PCE	8.11 mg/L

3.2.1.2 <u>Microcosm Creation Technique</u>

Microcosms were constructed in 120-mL Wheaton amber glass serum bottles crimp sealed with PFTE butyl-lined septum tops. Initial liquid volume for the microcosms was 100 mL with 20 mL of headspace. Prior to microcosm creation, all 120-mL serum bottles and PFTE lined grey butyl septum were heat sterilized in an autoclave at 121°C and 15 psig for 20 minutes. The serum bottles were capped with foil during sterilization. The septa were wrapped in foil during sterilization. The methanogenic media with the VFAs added and the reducing agent, $Na_2S{\cdot}9H_2O$, were capped with foil and also heat sterilized in an autoclave prior to microcosm creation. Filter sterilization was not practical for the volume of media generated to conduct the experiment.

The microcosms were created on a laboratory countertop exposed to the atmosphere. A propane-fed Bunsen burner was lit during microcosm creation in order to create an upward convection current to prevent air-borne microorganisms from landing inside the microcosms. After breaking the foil seal on a sterilized serum bottle, 92 mLs of sterilized methanogenic media were added to the serum bottle through the use of 50-mL aseptic polystyrene disposable pipets. A mixture of trace metal and vitamin solutions (1 mL each), filter sterilized with a 0.2-μm

membrane (Pall Gelman Laboratories, Ann Arbor, MI), was next added to the serum bottle with 3-mL aseptic disposable syringes. This solution was filter sterilized, not autoclaved, because high temperatures would denature the vitamin solution. The next component added was 0.5 µL of pure PCE, resulting in an initial liquid PCE concentration of 8.1 mg/L. The reducing agent, 1 mL of a 5000 mg/L $Na2S \cdot 9H_2O$ solution, was added immediately prior to the 5-mL addition of the inoculum. Inoculum was provided from the methanogenic anaerobic reactor (see Section 3.2.2). After the inoculating bacteria were added to the serum bottle, the microcosm was capped with a sterilized PFTE lined grey butyl septum and sealed using a Wheaton crimp sealer purchased from Fisher Scientific (Pittsburgh, PA).

Abiotic controls were created in a slightly different fashion. After the addition of the methanogenic media, trace metal and vitamin solution, reducing agent, and biomass, the abiotic controls were capped with foil and autoclaved (121°C, 15 psig) for 30 minutes. After cooling, 0.5 µL of pure PCE and 1 mL of 2000 mg/L $HgCl_2$ were added to each abiotic control. A concentration of 20 mg/L $HgCl_2$ has been shown in the literature to be an effective biocide. The abiotic control microcosms were finally sealed with PFTE lined grey butyl septa and crimp sealed after the addition of PCE.

3.2.2 Methanogenic Anaerobic Reactor Creation

An anaerobic reactor capable of supporting a methanogenic metabolism was established in order to have a source of bacteria with which to inoculate experimental microcosms. The initial seed source for this methanogenic reactor came from the header of an anaerobic digester sludge dewatering unit from the Conserve II Water Reclamation Facility, Orlando, FL. The

anaerobic digester at the Conserve II Water Reclamation Facility was maintained at a temperature between 93° and 97° F and a detention time between 21 and 28 days. The methanogenic reactor was fed as a continuous batch reactor with consistent weekly organic substrate introduced in either one or two feeding events per week. A liquid level of 15 liters was maintained in the methanogenic reactor. The methanogenic reactor was fed a media similar in all respects to the methanogenic media used in the creation of microcosm experiments outlined in Section 3.2.1.1. Weekly loadings consisted of 1 L of methanogenic media with 1000 ppmv acetic acid and 800 ppmv proprionic acid. Acetic and propionic acids were selected as the primary electron donors for the methanogenic reactor. Acetic acid was included to promote acetoclastic methanogenic microorganisms. Propionic acid was selected to provide an electron donor to encourage the growth of fermenters and other anaerobic microorganisms in the consortia resulting in production of H_2 for H_2-utilizing methanogens. The feed solution was sparged with nitrogen gas for at least fifteen minutes prior to adding it to the methanogenic reactor. The methanogenic reactor was maintained at room temperature (about 22°C) and had a solids retention time of 45 days. A stir bar and magnetic stirrer provided continuous mixing of the media during the experiment. Biogenic gases were continuously vented from the reactor using a glass water-seal airlock.

3.2.3 Sequential Environment Microcosm Experiment

The sequential anaerobic/aerobic environment microcosm experiment consisted of 42 biotic microcosms, 4 abiotic autoclaved and $HgCl_2$-dosed control microcosms, and 6 biotic control microcosms dosed with 50mM of BES to suppress methanogenic activity (Pavlostathis

and Zhuang, 1991). Table 3.5 summarizes the sequential environment study organization. The 46 biotic microcosms consisted of 12 microcosms to be used as anaerobic environmental controls and 30 microcosms to be used with a sequenced anaerobic and aerobic environment. The 30 microcosms consisted of 3 sets of 10 microcosms using different aerobic environmental strategies. Two of the aerobic environmental strategies utilized hydrogen peroxide addition into the microcosm liquid phase to initiate the aerobic environment. The third aerobic environmental strategy utilized the injection of atmospheric air into the microcosm headspace to initiate the aerobic environment. Abiotic control microcosms were autoclaved and dosed with 20 mg/L

Table 3.5: Summary of Sequential Environment Microcosm Study

Microcosm Series	*Duration*	*Purpose*	*Label*	*Number of Serum Bottles*
Anaerobic	0 to 132 days	Determine time frame of completed reductive dechlorination.	BIO	12
Anaerobic w/ BES	0 to 132 days	Determine reductive dechlorination caused by non-methanogens	BES	6
Anaerobic/ Aerobic w/ 15 ppm H_2O_2	35 to 132 days	Determine effectiveness of subsequent aerobic phase initiated with a lower H_2O_2 concentrations.	LOW	10
Anaerobic/ Aerobic w/ 75 ppm H_2O_2	35 to 132 days	Determine effectiveness of subsequent aerobic phase initiated with a higher H_2O_2 concentrations.	HIGH	10
Anaerobic/ Aerobic w/ Air	84 to 132 days	Determine effectiveness of subsequent aerobic phase initiated with an air injection to microcosm headspace.	AIR	10

$HgCl_2$. Reductive dechlorination was not observed in three of four abiotic controls. Reductive dechlorination in the one abiotic control bottle was attributed to ineffective autoclaving. Further investigation has determined that in sulfate-reducing environments, mercuric ions (Hg^{+2}), which are typically converted into highly toxic methylmercury and dimethylmercury by bacteria in most anaerobic environments, are instead precipitated as HgS when in the presence of H_2S (Madigan, et al., 1997). Due to the use of sodium sulfide as a reducing agent during microcosm creation, H_2S was present in all of the microcosms. The low solubility of HgS apparently prevented $HgCl_2$ from being an effective biocide. The results of this experiment are discussed in detail in Section 4.2.

3.2.4 Methanogenic Microcosm Experiments Inoculated with Aquifer Soil Core

A core sample from an aquifer contaminated with TCE from the eastern coast of Florida was obtained on October 15, 1997 for the purpose of inoculating a series of methanogenic microcosms. The depth of the core sample was between 39 and 40 feet. The contaminated soil sample was delivered in two-inch inner diameter stainless steel cylinders with plastic caps on both ends. These steel cylinders were placed within ziplock bags filled with nitrogen immediately after removal. The steel cylinders were then placed in an ice-filled cooler and transported to UCF.

The microcosm series inoculated with the contaminated soil sample was created on October 17, 1997. A portable anaerobic glove box was purchased in order to increase the chances of successful transfer of bacteria from the contaminated soil sample to the experimental microcosms. All items necessary for microcosm creation were placed within the anaerobic glove

box immediately prior to the filling of the glove box with nitrogen gas. The anaerobic glove box was purged with nitrogen gas for approximately 30 minutes with vents open in order to displace the air initially present within the glove box. After 30 minutes, the vents for the glove box were closed. At this point, the plastic caps covering the stainless steel container were opened and three scoops of contaminated soil were placed within each microcosm. After completion, the core sample microcosms were maintained at room temperature (approximately 22° C) and kept in the dark. In all other respects, the creation of this microcosm series was similar to the steps outlined in Section 3.2.1.

The microcosms inoculated from the contaminated soil were monitored for chlorinated solvents between the date of their creation on October 17, 1997 and June 15, 1998. No evidence of PCE transformation was ever detected within the core sample microcosms. It is possible that the native bacteria present within the core sample were compromised during sample transfer from the field to UCF. It is also possible that the high levels of PCE in the microcosms was toxic to the native bacteria.

3.2.5 Anaerobic Environment Microcosm Experiments

To develop technique and parameters for reductive dechlorination in anaerobic environments, two anaerobic microcosm studies were conducted prior to the sequential environment microcosm experiment described in Section 3.2.3. Microcosm composition and creation was identical to methods described in Section 3.2.1. The results of these experiments are discussed in detail in Section 4.1.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Anaerobic Environment Microcosm Experiments

Two anaerobic experiments were performed to determine sampling intervals and gather other data to assist the performance of the sequential environment experiments (See Section 3.2.5). Previous anaerobic experiments had been run to develop the anaerobic microcosm construction and GC analysis techniques. These series showed that the sequential dechlorination would proceed very rapidly (typically within 72 hours) after a variable acclimation period. Also they had shown that an initial equilibration time existed in which the contaminant dissolved thoroughly into the microcosm solution. Sampling the microcosm immediately after creation typically resulted in significantly underreported PCE concentrations. Due to the limitation of the microcosm size, estimating the time interval required to reductively dechlorinate PCE to cDCE was critically important to minimize the required sampling of the microcosms prior to commencing the aerobic phase of the experiment. The purpose of these experiments was to determine good time frames for performing initial and subsequent GC sampling during the initial anaerobic phase. Understanding the duration of the equilibration and subsequent dechlorination kinetics allowed maximizing the utility of each microcosm sample and minimizing the disruption of the microcosm anaerobic environment prior to initiation of the aerobic phase of the experiment.

Testing during these studies demonstrated that anaerobic environment microcosms could reliably achieve sequential reductive dechlorination of PCE to TCE and finally to cDCE. However, the dechlorination rates and acclimation times were determined to be highly variable.

4.1.1 ME series Anaerobic Experiment

The first preliminary anaerobic experiment performed was designated the methanogenic echo (ME) microcosm series. The ME series consisted of 24 biotic anaerobic microcosms, labeled ME-1 to ME-24, and 12 abiotic anaerobic controls, labeled MEC-1 to MEC-12. Analytical results were not reported on the ME-22 and MEC-11 bottles due to an equipment malfunction during the GC analysis. The innocula source for the ME microcosm series was the methanogenic anaerobic reactor described in Section 3.2.2. This microcosm series was initiated on March 14, 1998.

See Figure 4.1 for chlorinated solvent partitioning results for the biotic anaerobic microcosms and Figure 4.2 for the abiotic anaerobic controls. Analytical results are tabulated in Table A.2 in Appendix A. The evidence supported sequential reductive dechlorination in several bottles from PCE to TCE to cDCE to vinyl chloride and possibly to ethylene. This was the only experiment performed that showed reductive dechlorination beyond cDCE. Vinyl chloride concentrations were calculated based on a gas chromatograph response factor for vinyl chloride relative to TCE that was developed by Nancy Ruiz in previous work (Ruiz, 1998). The microbial consortia in this series appeared to be unusually active; reductive dechlorination was observed in the bottles within six days of creation. This was an unusual occurrence as samples taken on the

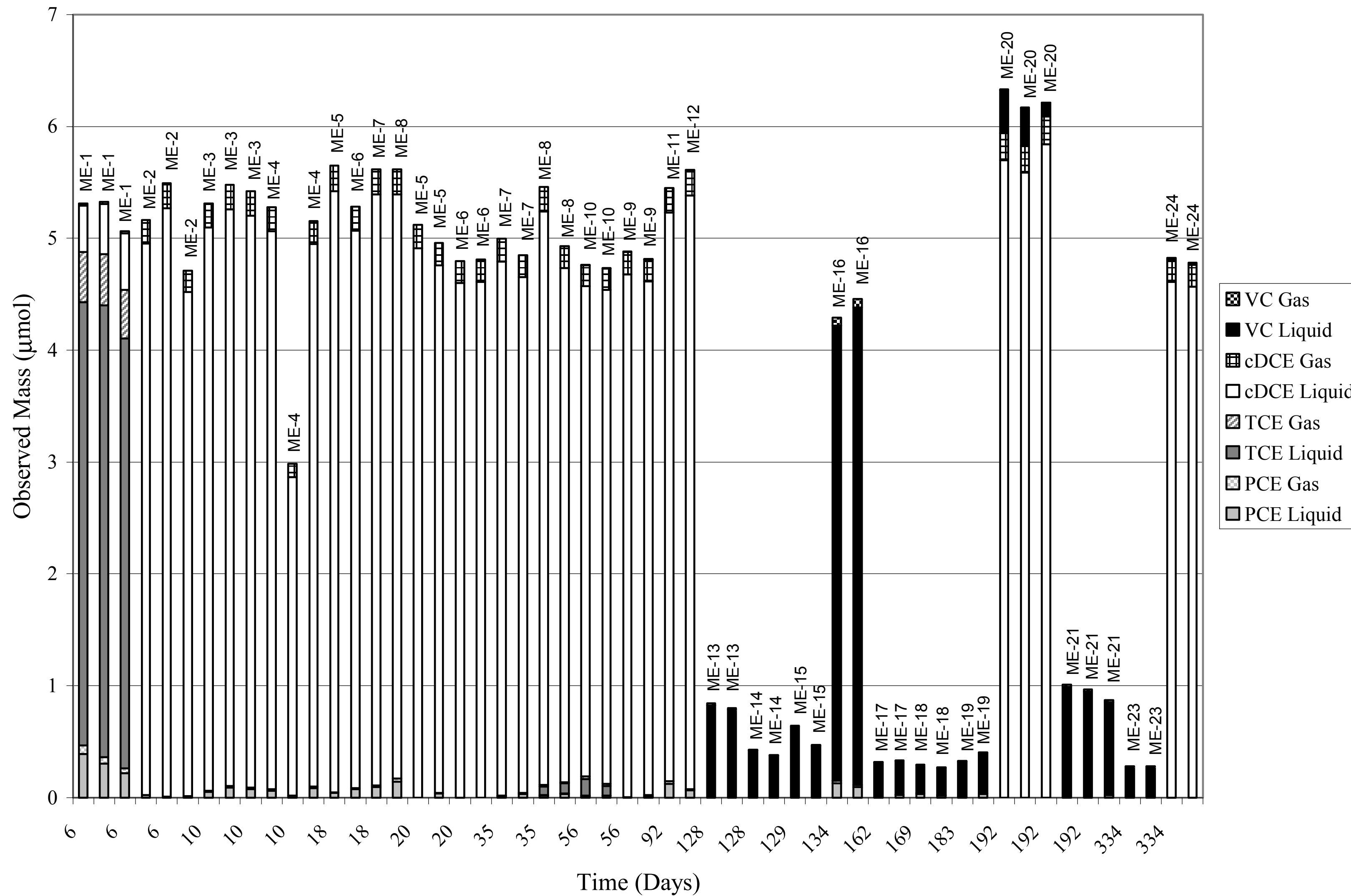

Figure 4.1: ME Series Anaerobic Microcosm Chlorinated Solvent Partitioning

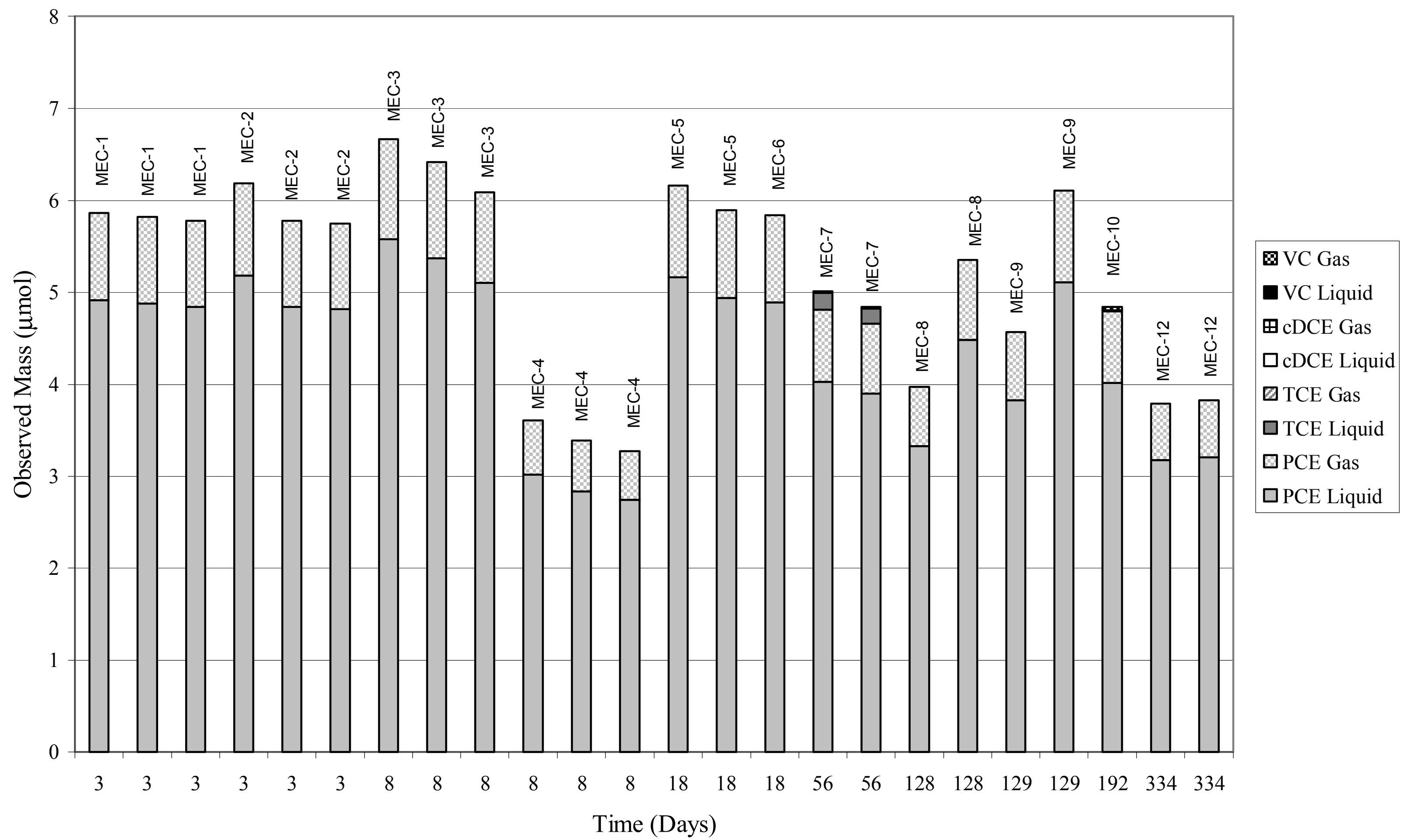

Figure 4.2: ME Series Abiotic Control Microcosm Chlorinated Solvent Partitioning

sixth day of the experiment were intended to provide initial PCE concentrations in the bottle after equilibration. This was the shortest observed acclimation period. An apparent end to the reductive dechlorination at cDCE appeared to occur in the bottles within ten days of the start of the experiment. The experiment was monitored infrequently while the next series of experiments attempted to more closely identify parameters which could assist development of a protocol for sampling the sequential environment study (see MI series in Section 4.1.2.)

Disappearance of cDCE in nine of eleven bottles was noted four months into the experiment (See Figure 4.1). The low observed mass indicated a complete transformation of cDCE to ethylene, sorption, or a physical loss of contaminant from the microcosms. A minor detection of vinyl chloride was noted in the bottles suggesting that a conversion to vinyl chloride occurred prior to transformation to ethylene. In one bottle (ME-16), the majority of chlorinated mass was detected as vinyl chloride (see day 134). The presence of vinyl chloride in the ME-16 bottle strongly suggests that reductive dechlorination had proceeded from cDCE to vinyl chloride in the nine bottles versus physical loss. However, a possibility exists that the vinyl chloride actually was lost through the septum after transformation. Two of the eleven bottles (ME-20 and ME-24) showed little to no transformation of cDCE. Bottle ME-20 had a minor detection of vinyl chloride. The absence of further reductive dechlorination in bottles ME-20 and ME-24 as compared to the other bottles is puzzling as the experimental conditions were identical in all bottles. The continued presence of cDCE in ME-20 and ME-24 supports that cDCE transformation in the other bottles was not due to physical loss of this analyte.

Additionally, ethylene was detected in all of the bottles with vinyl chloride detections. No ethylene detections were noted in samples from the other bottles during this experiment. It appears that the absence of detectable chlorinated contaminant was a result of a transformation

versus a physical loss. Unfortunately, ethylene was not evaluated quantitatively with our analytical technique.

The abiotic controls showed no dechlorination of PCE to other transformation products (see Figure 4.2). Based on the absence of transformation products within the abiotic controls, the dechlorination observed in the biotic microcosms can be attributed to biological activity. The low observed mass of bottle MEC-4 observed on Day 8 of the experiment suggests the possibility that this bottle had not reached equilibrium yet. However, variability of the initial volume of PCE injection into the microcosm may also be the cause.

4.1.2 MI series Anaerobic Experiment

The second preliminary anaerobic experiment performed was designated the methanogenic india (MI) microcosm series. The MI series consisted of 24 biotic anaerobic microcosms, labeled MI-1 to MI-24, and 8 abiotic anaerobic controls, labeled MIC-1 to MIC-8. The MI-24 bottle was accidentally destroyed prior to sampling and analysis. Additionally, 8 biotic anaerobic microcosms were created with the BES methanogenic inhibitor, labeled BMI-1 to BMI-8. Six methanogenic suppressed abiotic controls were also created, labeled BMIC-1 to BMIC-6. The BMI-8 and BMIC-6 bottles were accidentally destroyed prior to sampling and analysis. As in the ME series, the innocula source for the MI microcosm series was the methanogenic anaerobic reactor described in Section 3.2.2. This microcosm series was initiated on June 12, 1998. See Figures 4.3 to 4.4 for chlorinated solvent liquid and gas phase partitioning results.

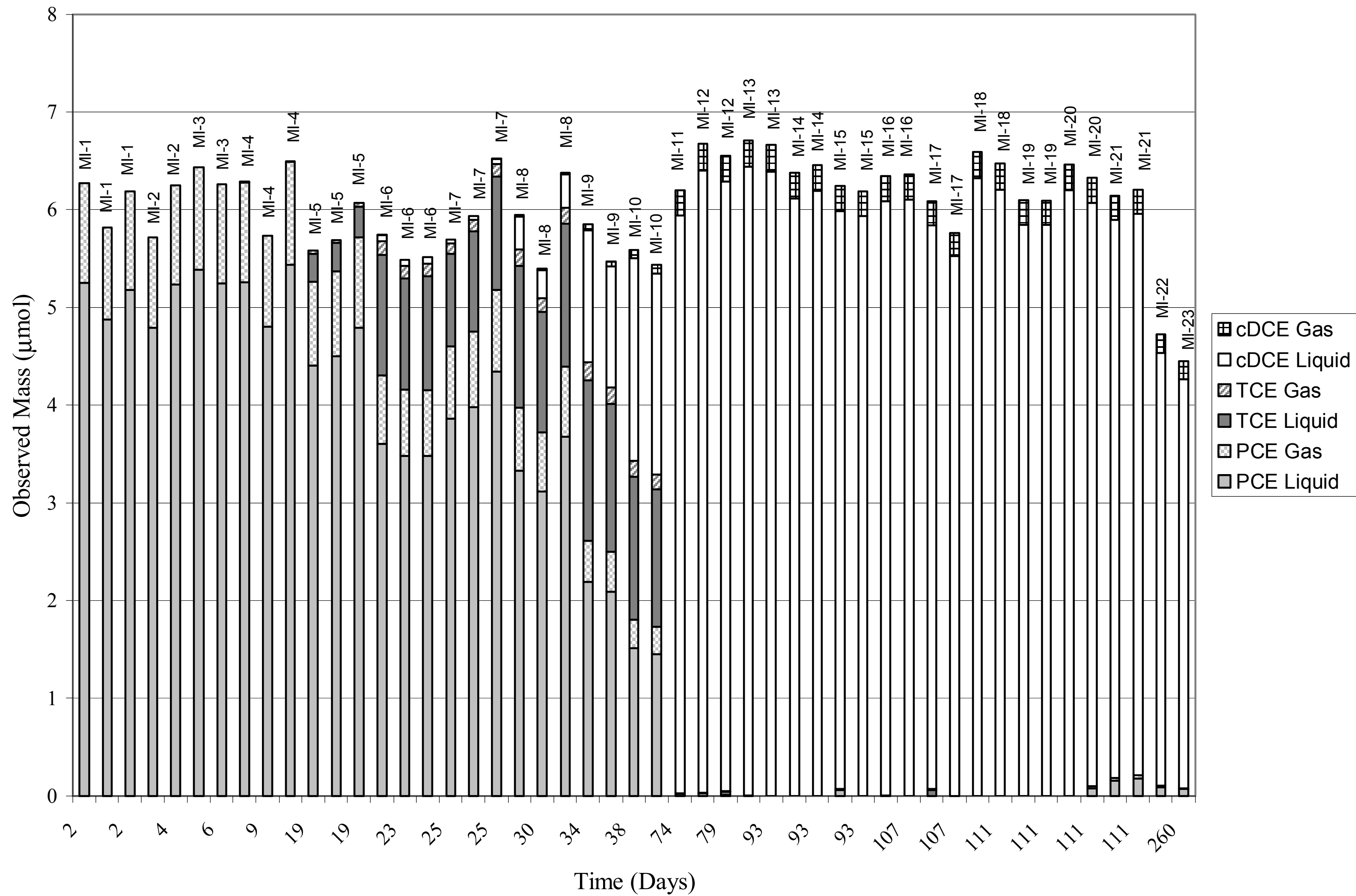

Figure 4.3: MI Series Anaerobic Microcosm Chlorinated Solvent Partitioning

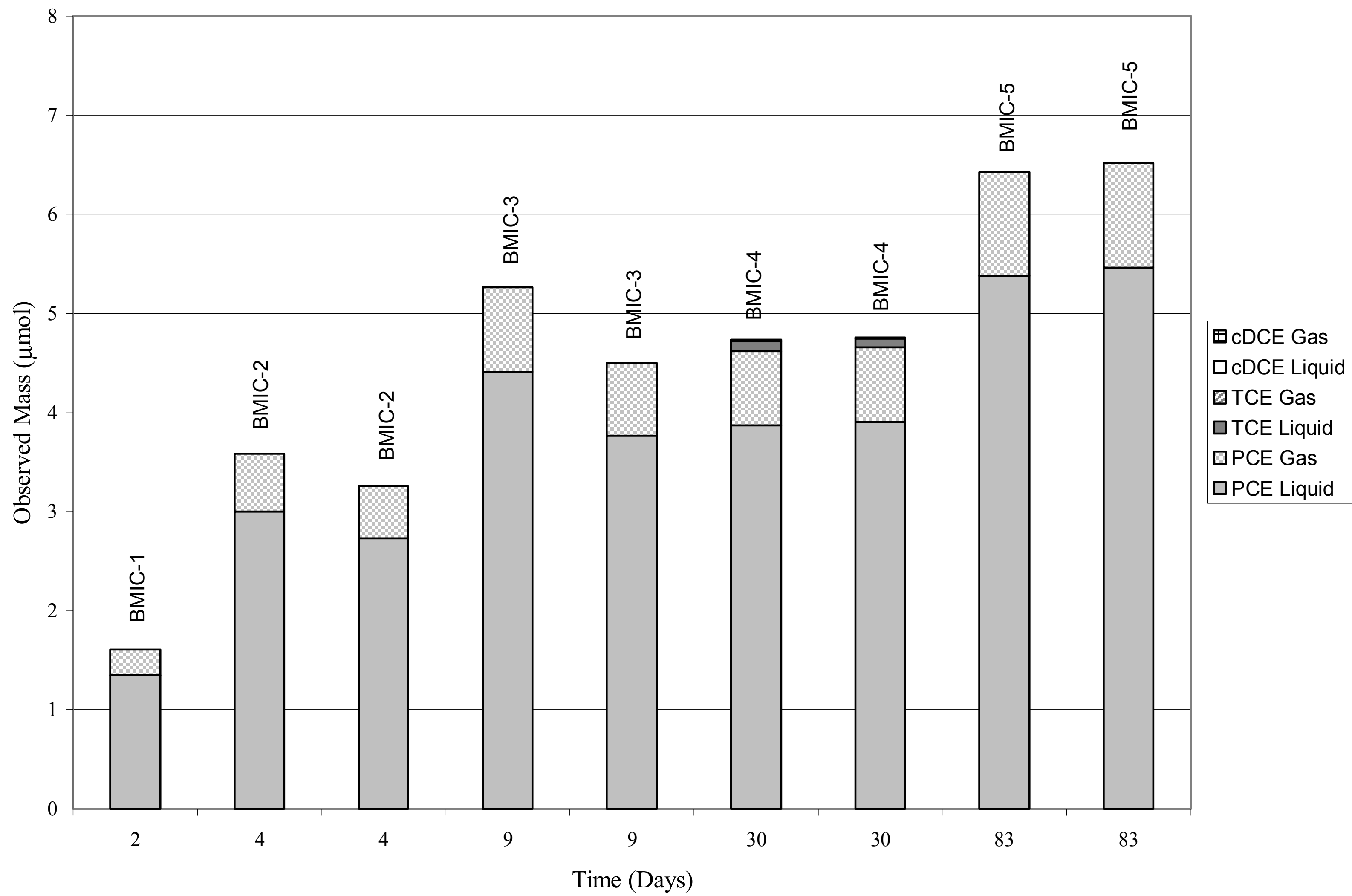

Figure 4.4: MI Series BES Suppressed Abiotic Control Microcosm Chlorinated Solvent Partitioning

This anaerobic experiment was more typical of those performed in the study than the ME series. Transformation of the PCE by reductive dechlorination to cDCE was the only observed transformation. A slower and more delayed transformation also occurred in the MI series versus the ME series. Reductive dechlorination was not observed until Day 19 of the experiment. Transformation was slower in this series, though GC equipment difficulties precluded an accurate assessment of completion of the reductive dechlorination to cDCE. No evidence was found of further reductive dechlorination to vinyl chloride or ethylene through day 260 of the experiment. The microbial consortia appeared to be considerably less active than the consortia in the ME series. This is likely due to the fact that the anaerobic methanogenic reactor serving as the innocula source of the ME series had received a fresh addition of digester sludge only 10 days prior to the start of the experiment. The MI series was started three months after the addition.

The abiotic controls showed no transformation of PCE to dechlorination by-products (Figure 4.5). The initial reduced mass observed in MIC-1, MIC-2, MIC-3 bottles in the first six days of the experiment appear to be due to non-equilibrium conditions in the bottles. This is consistent with the observations made in the ME series abiotic controls. The MIC-8 bottle showed a 53% reduction in PCE mass from the initial injection mass on Day 258 of the experiment. With no evidence of any transformation products in the MIC-8 bottle, the loss of mass is possibly due to a low contaminant injection mass or physical loss from the microcosm over the 37 weeks of the experiment.

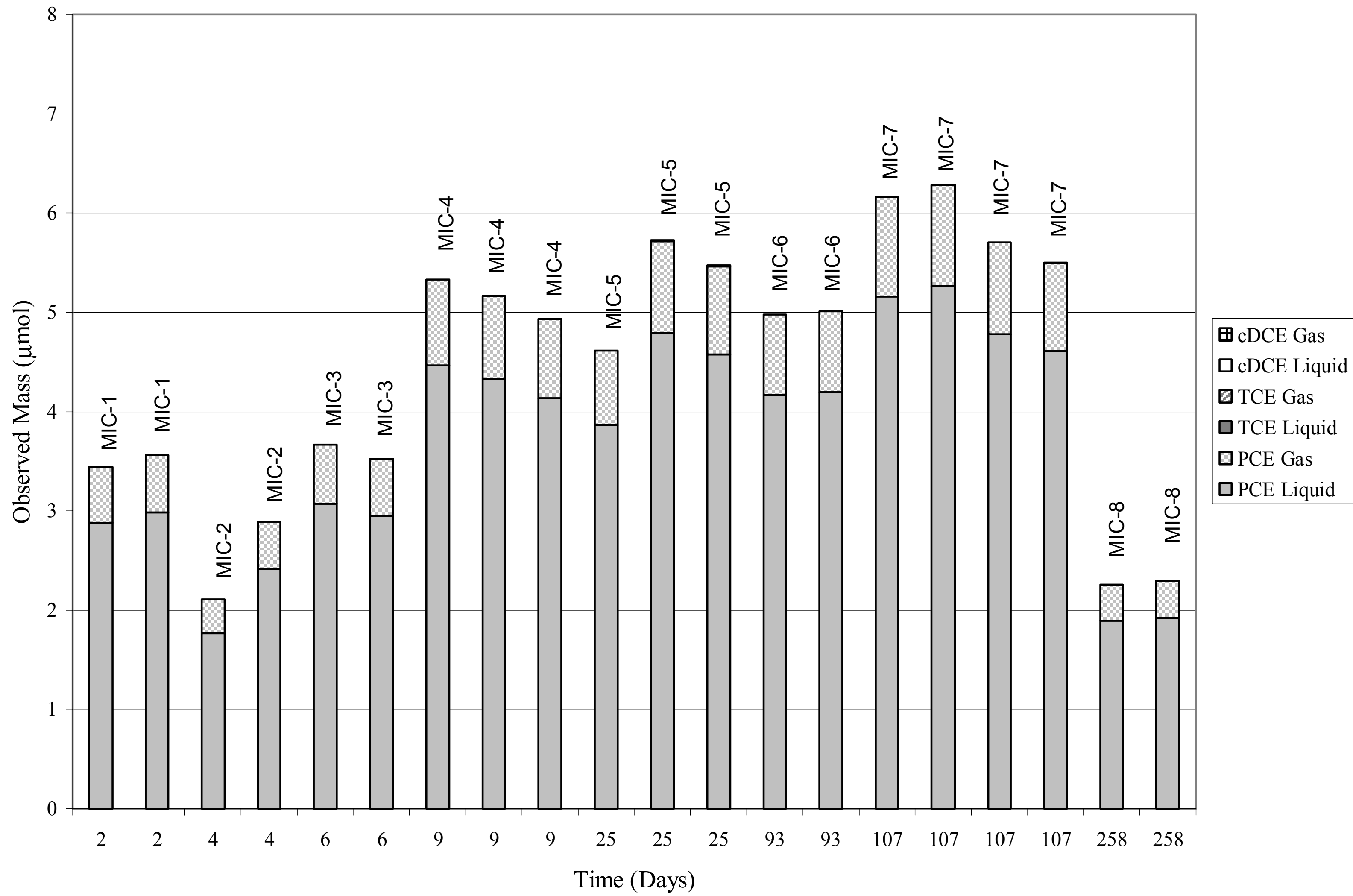

Figure 4.5: MI Series Abiotic Controls Microcosm Chlorinated Solvent Partitioning

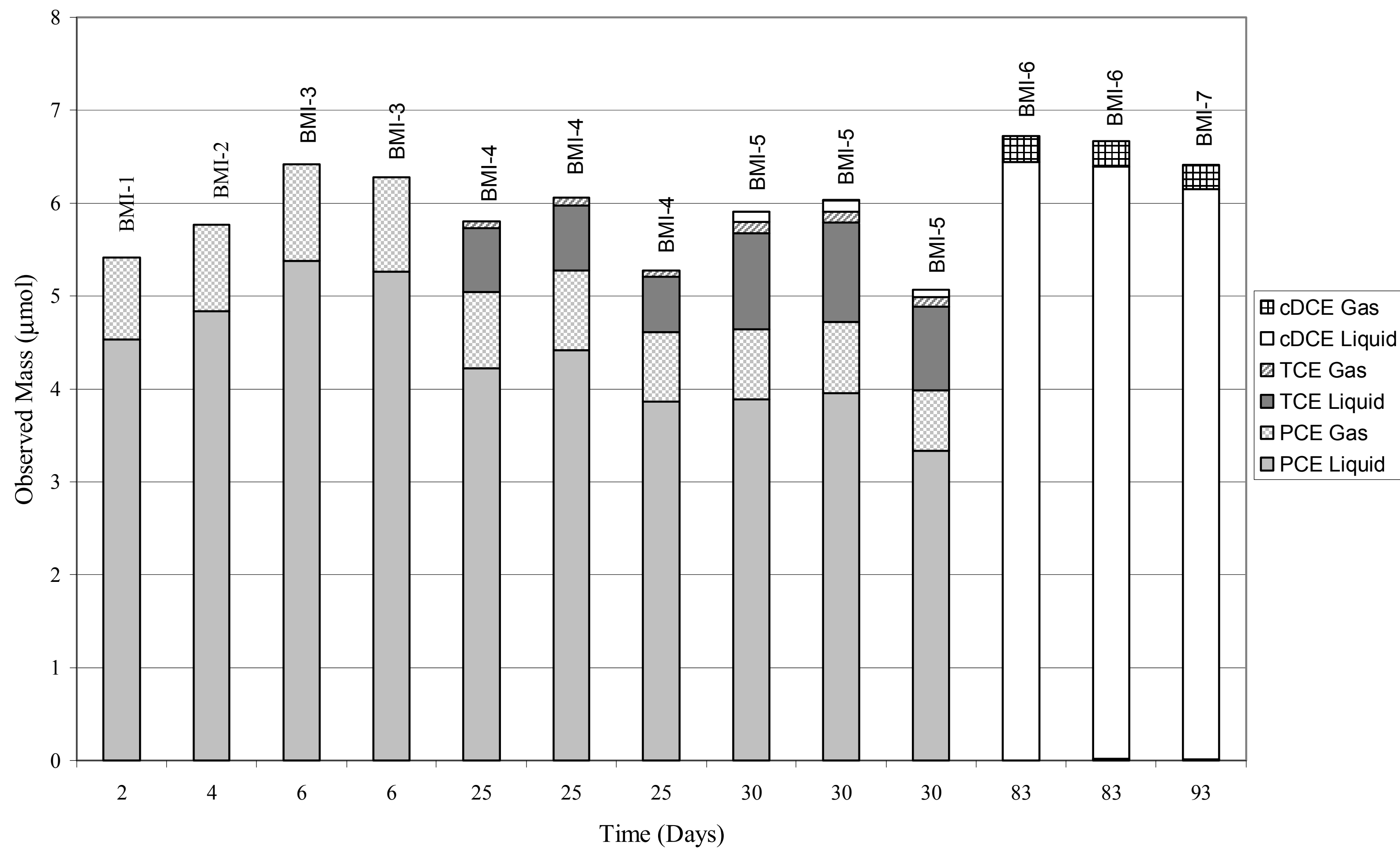

Figure 4.6: MI Series BES Suppressed Microcosm Chlorinated Solvent Partitioning

The methanogenic suppressed biotic microcosms (Figure 4.6) showed similar performance to the unsuppressed biotic microcosms (Figure 4.3). The BMI-4 and BMI-5 bottles show very similar rates of dechlorination as bottles MI-6, MI-7, and MI-8 bottles. Complete dechlorination to cDCE has also occurred in the methanogenic suppressed microcosms by day 83 of the experiment. This suggests that methanogenic activity is not the sole contributor to the reductive dechlorination process occurring in these experiments. The abiotic controls with the methanogenic inhibitor showed no transformation of PCE to dechlorination byproducts (Figure 4.4). The small detection of TCE found in BMIC-4 on day 30 is likely due to residual TCE from calibration work on the GC performed prior to the analysis performed that day.

4.2 Sequential Environment Microcosm Experiment

The sequential anaerobic/aerobic environment microcosm experiment consisted of 42 biotic microcosms, 4 abiotic autoclaved and $HgCl_2$-dosed control microcosms, and 6 biotic control microcosms dosed with 50mM of BES to suppress methanogenic activity (Pavlostathis and Zhuang, 1991). Table 4.1 summarizes the sequential environment study organization.

The 42 biotic microcosms consisted of 12 microcosms to be used as anaerobic environment controls and 30 microcosms to be used with a sequenced anaerobic and aerobic environment. The 12 anaerobic environment controls were labeled BIO-1 to BIO-12. The 30 sequenced environment microcosms consisted of 3 sets of 10 microcosms using different aerobic environment strategies. Two of the aerobic environment strategies utilized hydrogen peroxide addition into the microcosm liquid phase to initiate the aerobic environment. Two different hydrogen peroxide concentrations were used to evaluate this technique. The first hydrogen

peroxide series used a 75-mg/L H_2O_2 concentration during the aerobic phase (labeled HIGH-1 to HIGH-10). The second series used a 15-mg/L H_2O_2 concentration during the aerobic phase (labeled LOW-1 to LOW-10). The third aerobic environment strategy utilized the injection of atmospheric air into the microcosm headspace to initiate the aerobic environment (labeled AIR-1 to AIR-10).

Table 4.1: Summary of Sequential Environment Microcosm Study

Microcosm Series	*Duration*	*Purpose*	*Label*	*Number of Serum Bottles*
Anaerobic	0 to 132 days	Determine time frame of completed reductive dechlorination.	BIO	12
Anaerobic w/ BES	0 to 132 days	Determine reductive dechlorination caused by non-methanogens	BES	6
Anaerobic/ Aerobic w/ 15 ppm H_2O_2	35 to 132 days	Determine effectiveness of subsequent aerobic phase initiated with a lower H_2O_2 concentrations.	LOW	10
Anaerobic/ Aerobic w/ 75 ppm H_2O_2	35 to 132 days	Determine effectiveness of subsequent aerobic phase initiated with a higher H_2O_2 concentrations.	HIGH	10
Anaerobic/ Aerobic w/ Air	84 to 132 days	Determine effectiveness of subsequent aerobic phase initiated with an air injection to microcosm headspace.	AIR	10

Abiotic control microcosms were autoclaved and dosed with 20 mg/L $HgCl_2$. Reductive dechlorination was not observed in three of four abiotic controls. Reductive dechlorination in the one abiotic control bottle was attributed to ineffective autoclaving. Further investigation has determined that in sulfate-reducing environments, mercuric ions (Hg^{+2}), which are typically converted into highly toxic methylmercury and dimethylmercury by bacteria in most anaerobic environments, are instead precipitated as HgS when in the presence of H_2S (Madigan, et al., 1997). Due to the use of sodium sulfide as a reducing agent during microcosm creation, H_2S was present in all of the microcosms. The low solubility of HgS apparently prevented $HgCl_2$ from being an effective biocide. The results of the methanogenic/aerobic environment experiments are summarized in Table 4.2.

Table 4.2: Summary of Results from Microcosm Studies

Microcosm Series	*Experimental Time Frame*	*Complete Transformation of PCE to cDCE*	*CH_4 Production* (1)	*CO_2 Production* (1)
Anaerobic	0 to 132	35 to 63 days	100 %	100 %
Anaerobic w/ BES	0 to 132	<81 days	4 %	70 to 78 %
Anaerobic/ Aerobic w/ 15 ppm H_2O_2	35 to 132	42 to 81 days	0 to 220 %	0 to 370 %
Anaerobic/ Aerobic w/ 75 ppm H_2O_2	35 to 132	Maximum converted to cDCE = 60% (Bottle #1 only)	0 to 47 %	0 to 1200 %
Anaerobic/ Aerobic w/ Air	84 to 132	35 to 63 days (Same as anaerobic series)	Not Observed	Not Observed

(1) CH_4/CO_2 production relative to non-suppressed anaerobic microcosms.

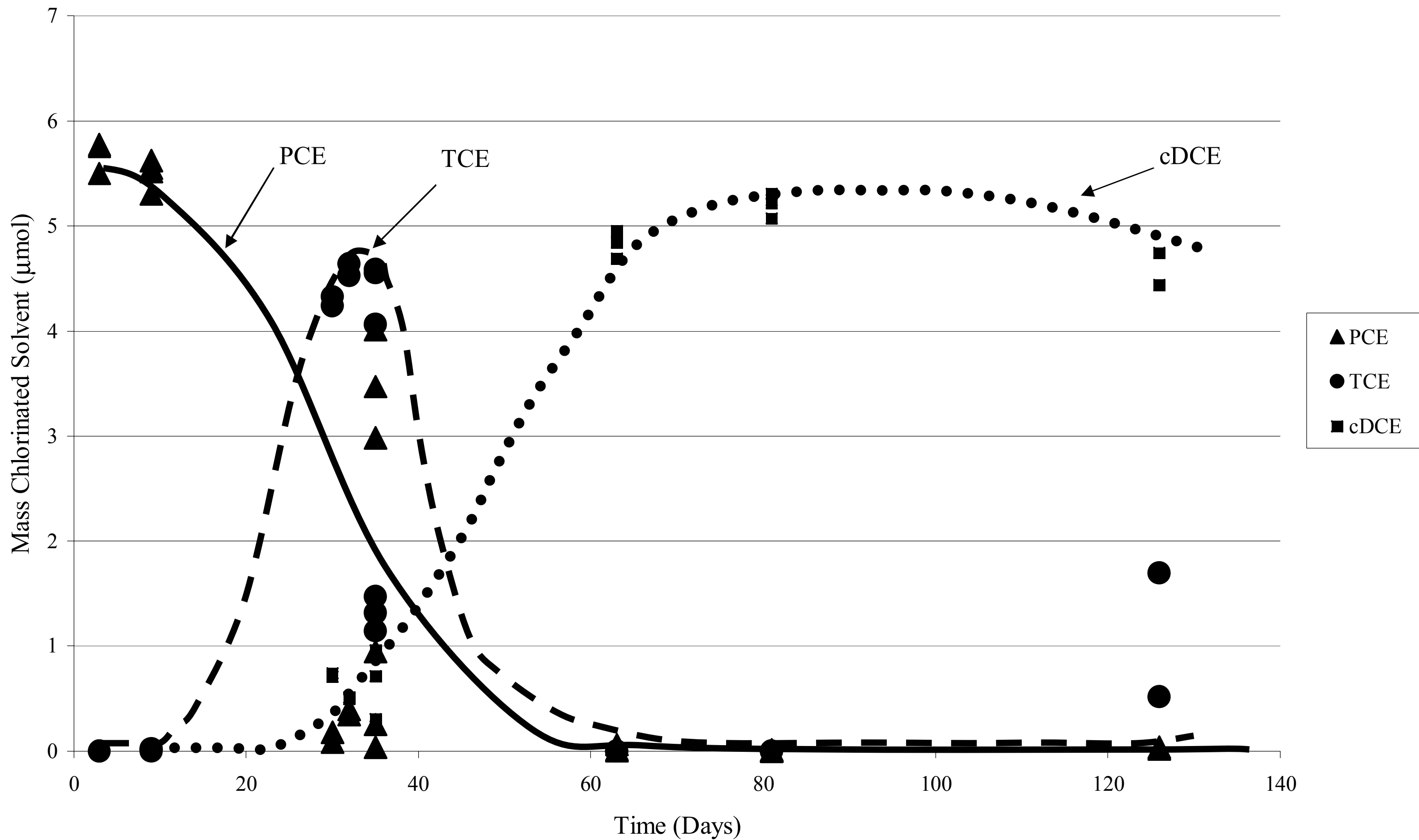

Figure 4.7: Biotic Anaerobic Control Microcosm Chlorinated Solvent Molar Content

4.2.1 Anaerobic Microcosms

The results for the non-BES suppressed microcosm series are shown in Figure 4.7. Reductive dechlorination daughter products of PCE appeared in the anaerobic microcosms by Day 30 of the experiment. At this point, the data indicated that transformation of PCE to TCE was occurring. Chlorinated solvent measurements performed on Day 35 indicated that partial transformation of TCE to cDCE had also occurred. Complete transformation of PCE to cDCE was observed by Day 63 for microcosms which remained anaerobic. Quantitatively, an overall PCE to cDCE transformation rate of 9.3×10^{-2} μmol/day from day 9 to day 63 was observed in the non-BES suppressed anaerobic microcosms. An overall average chloride release rate of 1.8×10^{-1} μmol/day was calculated (based on chlorinated solvent disappearance) in the anaerobic microcosms.

Chlorinated solvent partitioning for the non-BES suppressed microcosms are shown in Figure 4.8. Conservation of chlorinated solvent mass appears to be consistent through the study in bottles unaffected by prior puncture of the septa due to sampling. Any differences in observed masses between the bottles may be attributed to the accuracy of the GC analytical technique and variability of the initial PCE injection volumes.

Methane gas production was detected within a few days of the start of the experiment. Methane gas production averaged 32 μmol/day during the first 63 days of reductive dechlorination and net CO_2 gas production averaged 5.5×10^{-1} μmol/day. After 63 days, the increasing pressure in the headspace made it difficult to obtain quantitative information on the headspace gas.

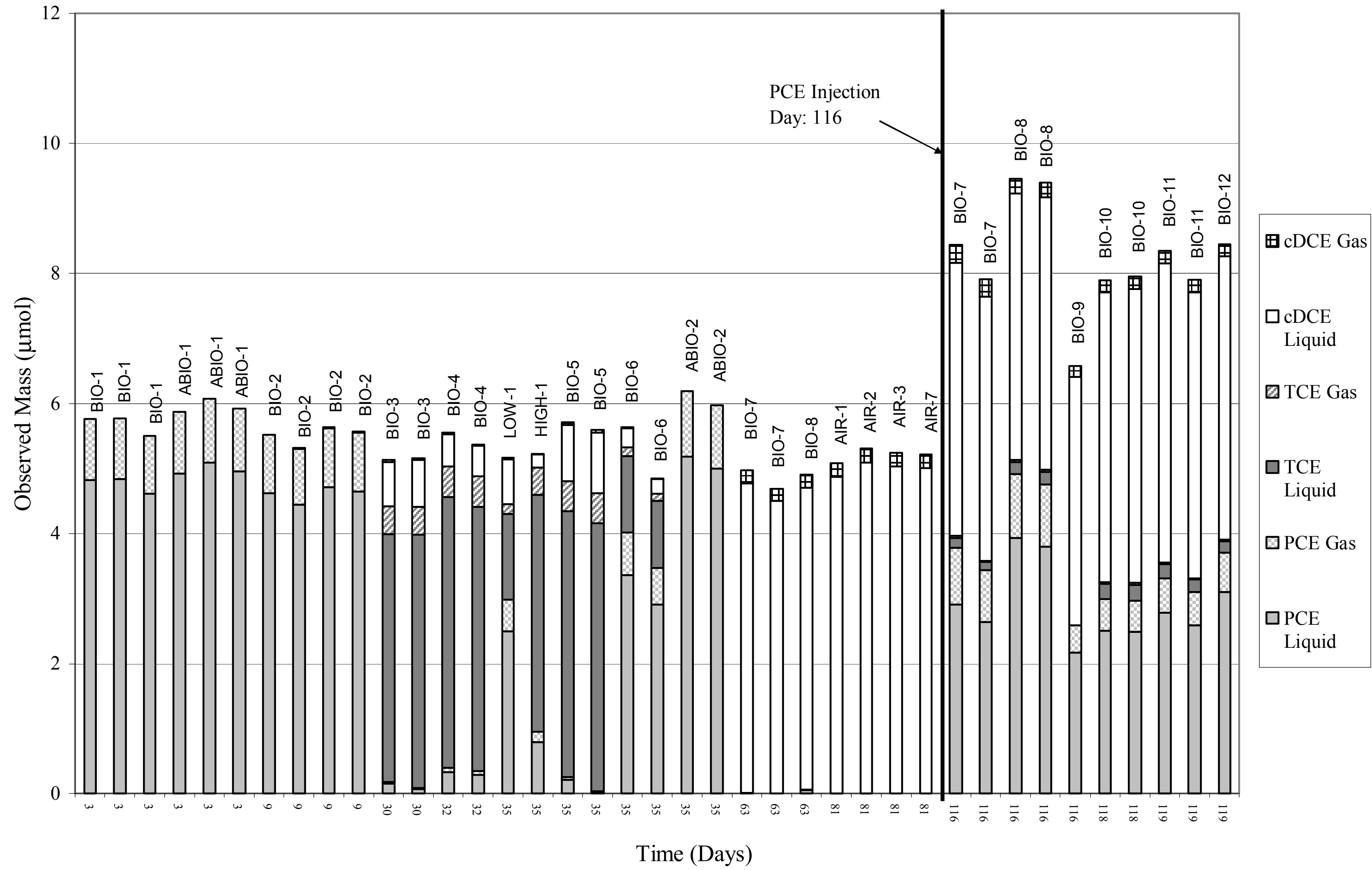

Figure 4.8: Biotic Anaerobic Control Chlorinated Solvent Partitioning

From day 63 on, VFA analysis performed on the anaerobic microcosms consistently showed over 98% of proprionate had been consumed from the start of the experiment. Acetate reduction varied between 34% and 99% for microcosms sampled on either day 63 or day 81 of the experiment. The VFA and biogenic gas data support the occurrence of both proprionate fermentation and acetoclastic methanogenesis in the anaerobic phase of the experiment. H_2 utilizing methanogenesis and homoacetogenic activity probably occurred also but their relative contribution to the overall metabolism could not be assessed.

Table 4.3: Volatile Fatty Acid Concentrations in Biotic Anaerobic Microcosms

Microcosm I.D.	*Time (day)*	*Acetate (ppm)*	*Δ Acetate*(2) *(ppm)*	*Proprionate (ppm)*	*Δ Proprionate*(3) *(ppm)*
BIO-7	63	34	-466	4.8	-395.2
BIO-8	63	6.8	-493.2	4.3	-395.7
AIR-1	81	310	-190	0.0	-400
AIR-2	81	330	-170	0.7	-399.3
AIR-3	81	230	-270	0.0	-400
AIR-4	81	180	-320	0.0	-400
BES-1(1)	81	380	-120	140	-260

(1) - Biotic anaerobic control dosed with 50 mM BES for suppression of methanogenesis.
(2) - Initial microcosm acetate concentration = 500 ppm.
(3) – Initial microcosm proprionate concentration = 400 ppm.

Table 4.3 shows volatile fatty acid analysis which was performed intermittently during the experiment. Equipment malfunctions with the gas chromatograph during the first two months of the experiment prevented a more complete and meaningful VFA analysis from being

performed. Microcosms had an initial acetate concentration of 500 ppm and proprionate concentration of 400 ppm. VFA analysis performed on BIO-7 and BIO-8 bottles on Day 63 of the experiment shows consumption of most of the proprionic and acetic acid had occurred prior to day 63 of the experiment. VFA analysis performed on bottles AIR-1 through AIR-4 prior to air injection indicated 40 to 60% of the acetate was still not consumed in these bottles over a longer time frame than the BIO-7 and BIO-8 bottles. The difference in acetate consumption rates is possibly due to homoacetogenic activity that may have followed the consumption of acetate observed by day 63 of the experiment. The data shown here are inconclusive. Additional VFA data are presented in Section 4.2.2.2 (Table 4.4). The BES suppressed microcosm (BES-1) showed the smallest change in proprionate and acetate concentrations of all the anaerobic microcosms.

All BES suppressed microcosms reductively dechlorinated the PCE to cDCE by day 81. The transformation was accomplished prior to day 81 of the experiment. The normalized chloride release rate was at least 1.2×10^{-2} μmol/day for the BES suppressed versus 1.8×10^{-2} μmol/day for the non-BES suppressed microcosms. Methane production was 3% to 4% and CO_2 was two-thirds of that produced in the non-BES suppressed anaerobic controls. Reduced proprionate and acetate utilization compared to the non-BES suppressed microcosms was observed also. These data suggested that methanogenesis may not have been the primary metabolism responsible for reductive dechlorination in this experiment or certainly that other metabolisms (e.g., homoacetogenic, fermentative) participated in that phenomena.

The results of biogenic gas monitoring of the biotic anaerobic microcosms are shown in Figure 4.9 and Figure 4.10. The biogenic gases CH_4 and CO_2 were determined analytically by use of a gas chromatograph. The biotic anaerobic controls exhibited methane production within

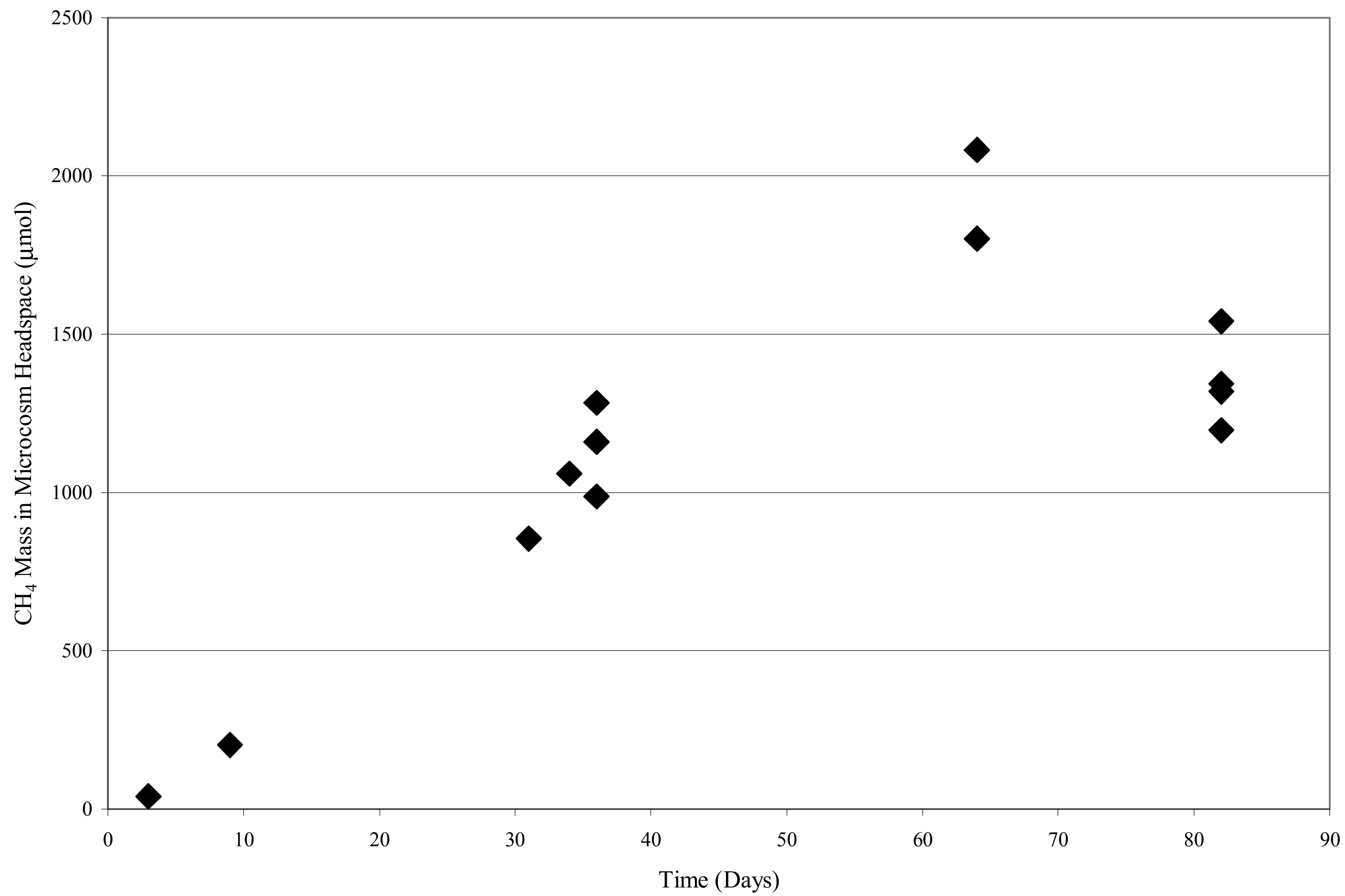

Figure 4.9: CH_4 Gas Production in Biotic Anaerobic Control Microcosms

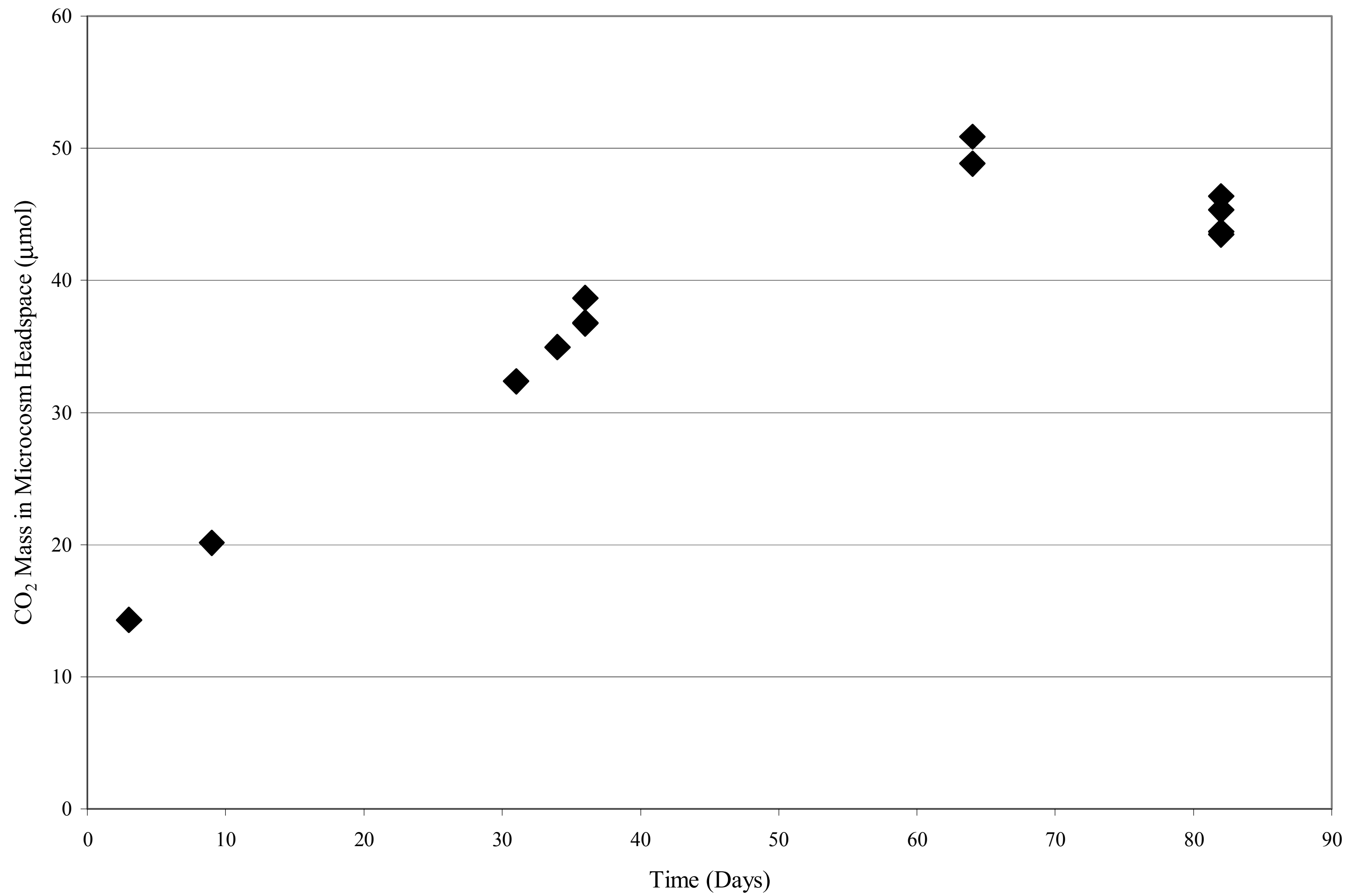

Figure 4.10: CO_2 Gas Production in Biotic Anaerobic Control Microcosms

a few days of the start of the experiment. The methane gas concentration in the headspace of the microcosms increased through Day 63. This methane increase corresponds to the time frame of PCE dechlorination to cis-DCE. Methane gas was produced at an average rate of 32 μmol/day during the reductive dechlorination process. All biotic anaerobic microcosms showed detectable methane concentrations that increased during the experiment through Day 63. Methane concentrations in the headspace decreased after Day 63 of the experiment. A possible cause for the observed decrease in the methane mass is that methanogenesis was inhibited by the higher methane concentrations already present in the experimental matrix. Additionally, some methane gas may have leaked from the headspace of the microcosm over the longer time frame. More probable, the methane mass loss reflects variability between individual microcosms. Estimated gas pressure in the microcosm headspace on Day 63 of the experiment was approximately 2 atmospheres. Estimated gas pressure in the microcosm was calculated based on the observed N_2 concentration in the gas sample and an assumption that atmospheric N_2 mass was conserved in the microcosm head space. Carbon dioxide gas also increased in the biotic anaerobic controls until Day 63. The average production rate of CO_2 was 5.5×10^{-1} μmol/day during the first 63 days of the experiment. CO_2 gas and CH_4 gas production occurred at a relatively stable rate during the reductive dechlorination process. Biogenic gas observations support the presence of active methanogenic organisms in the microcosms during reductive dechlorination.

4.2.2 Anaerobic/Aerobic Microcosms (H_2O_2)

On day 35 of the experiment, a 3 percent H_2O_2 solution was injected into 2 sets of 10 non-BES suppressed microcosms. At this point, parallel and identical microcosms not receiving

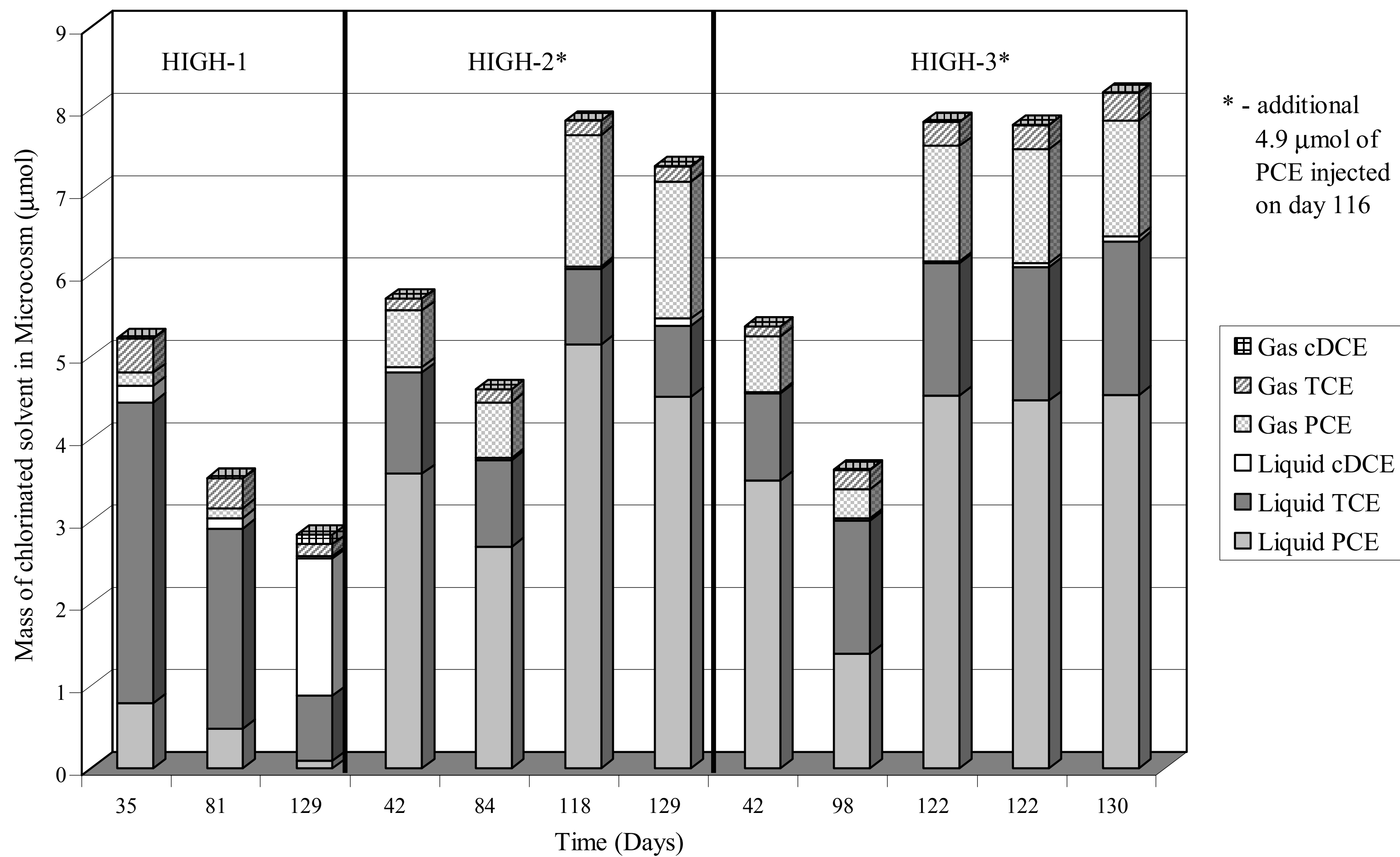

Figure 4.11: Hydrogen Peroxide (75 ppm) Experiment, Selected Data

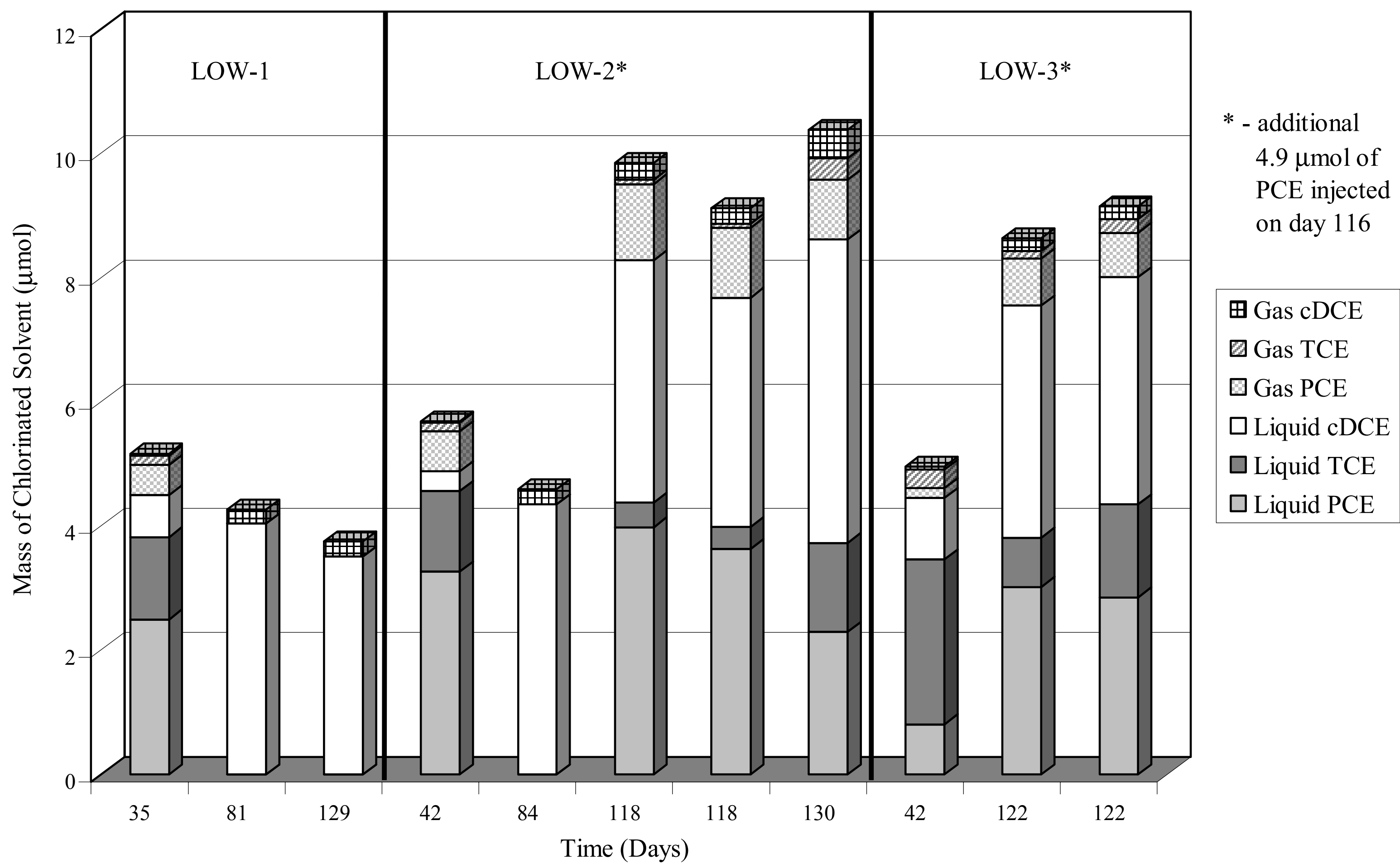

Figure 4.12: Hydrogen Peroxide (15 ppm) Experiment, Selected Data

H_2O_2 showed that 50% to 95% of the PCE had been transformed to mostly TCE with some cDCE. Ten microcosms were injected to produce an overall H_2O_2 concentration of 15 mg/L (LOW-1 through LOW-10). The 15-mg/L H_2O_2 concentration corresponds to 0.375 meq/L O_2. The other ten microcosms were injected to produce an overall H_2O_2 concentration of 75 mg/L (HIGH-1 through HIGH-10). The 75-mg/L H_2O_2 concentration corresponds to 1.874 meq/L. Four of the 10 bottles in each series were also injected with $HgCl_2$ to establish an abiotic control but $HgCl_2$ was not an effective biocide. This effectively invalidated the control microcosms (i.e., bottles LOW-7 through LOW-10 and HIGH-7 through HIGH-10) in this experiment.

Following the H_2O_2 injection on day 35 of the experiment, the microcosms were then sampled over the following 12 weeks to check for indications of aerobic biodegradation (e.g., methanotrophic cometabolism) of the cDCE or continued reductive dechlorination of PCE or TCE. On day 116, selected bottles (4 of 6) were injected with additional PCE to determine if further reductive dechlorination was possible at the end of the experiment.

Some of the chlorinated solvent data for the H_2O_2 experiments are shown in Figures 4.11 and 4.12. The 75-ppm injection substantially inhibited the reductive dechlorination process while the 15-ppm injection bottles continued to reductively dechlorinate PCE and TCE to cDCE. However, no conclusive evidence of mineralization of the PCE daughter products was found during the experiment to show successful aerobic biodegradation had occurred. This was because the inocula probably did not contain methanotrophs. However, the experiments did show that anaerobic reductive dechlorination could be recovered following periodic aerobic conditions in the 15-ppm bottles only. The microcosms were initially reduced with a Na_2S solution. The original S^{-2} concentration in the microcosm was calculated to be 0.374 meq/L to ensure soluble O_2 in solution was reduced prior to injection of the innocula. A majority of the sulfide

concentration was assumed to be utilized to reduce the soluble O_2 at the beginning of the experiment. Since the initial O_2 concentration in the medium was likely less than saturation, it is likely that sufficient S^{-2} (0.1875 meq/L) remained in solution at the time of the 15-ppm hydrogen peroxide addition to completely consume the soluble O_2 generated from the peroxide decomposition.

4.2.2.1 75-ppm Hydrogen Peroxide Injection (HIGH)

Chlorinated solvent data for the high concentration (75 ppm) hydrogen peroxide injection are shown in Figure 4.11 and 4.13. HIGH-1 just prior to the H_2O_2 injection on day 35 shows only 17% of the HAC mass remains as PCE. The PCE has been largely converted to TCE with less than 5% converted to cDCE. Then on day 81 (46 days later), HIGH-1 was resampled and showed little change. During this same time frame, identical anaerobic microcosms that didn't receive H_2O_2 converted all the PCE to cDCE. Reductive dechlorination resumed between day 81 and 129 and by day 129 about 65% of the PCE mass had been converted to cDCE.

Inhibition of reductive dechlorination was also apparent in HIGH-2. However, unlike HIGH-1, reductive dechlorination did not resume before the end of the experiment. Both HIGH-2 and HIGH-3 received a second 0.5-µL (4.9-µmol) PCE injection on day 116 of the experiment. HIGH-3 showed some additional reductive dechlorination after the 75-ppm H_2O_2 addition, although not as dramatic as that seen in HIGH-1. In HIGH-3, overall HAC mass dropped by approximately 33% while the TCE mass increased by 52% between day 42 and day 98 of the experiment, but further reductive dechlorination is not evident between day 98 and day 130.

PCE transformation to cDCE was less than 20% in HIGH-4 through HIGH-6, supporting the inhibition of the reductive dechlorination mechanism after H_2O_2 injection. However on day 130, HIGH-6 showed about 70% of the total PCE mass was transformed to TCE and another 8% to cDCE after a second 0.5 µL PCE injection on day 116. HIGH-6 supported the presence of continued reductive dechlorination after the 75 ppm H_2O_2 injection.

4.2.2.2 15-ppm Hydrogen Peroxide Injection (LOW)

Chlorinated solvent data for the low concentration (15 ppm) hydrogen peroxide injection is shown in Figure 4.12 and Figure 4.14. Unlike the high H_2O_2 concentration experiment, reductive dechlorination recovered in all 6 of the microcosm bottles. LOW-1 was sampled immediately prior to the H_2O_2 injection on day 35 of the experiment. Similar to the anaerobic control bottles, Bottle #1 showed partial transformation of the PCE with 45% of the PCE transformed to TCE or cDCE. By day 81 of the experiment, cDCE was the only detectable chlorinated solvent remaining in LOW-1. This result was confirmed by LOW-2 which showed partial PCE transformation (33% of PCE mass transformed to TCE or cDCE) 7 days after the H_2O_2 injection (day 42) and only cDCE detectable on day 84. LOW-3 was sampled 7 days after the injection and showed 82% of PCE mass transformed to TCE or cDCE. Resampling LOW-3 at the end of the experiment [two weeks after a PCE injection of 0.5 µL (an increase of 4.9 µmol) on day 116) showed a cDCE concentration increase of 270%. Only 3.8 µmol of PCE was observed in bottle LOW-3 implying that 23% of the PCE injected day 116 had been reductively dechlorinated. LOW-1, LOW-2, and LOW-3 provided strong evidence that reductive dechlorination continued after a hydrogen peroxide injection of 15 ppm. In addition, LOW-4 and

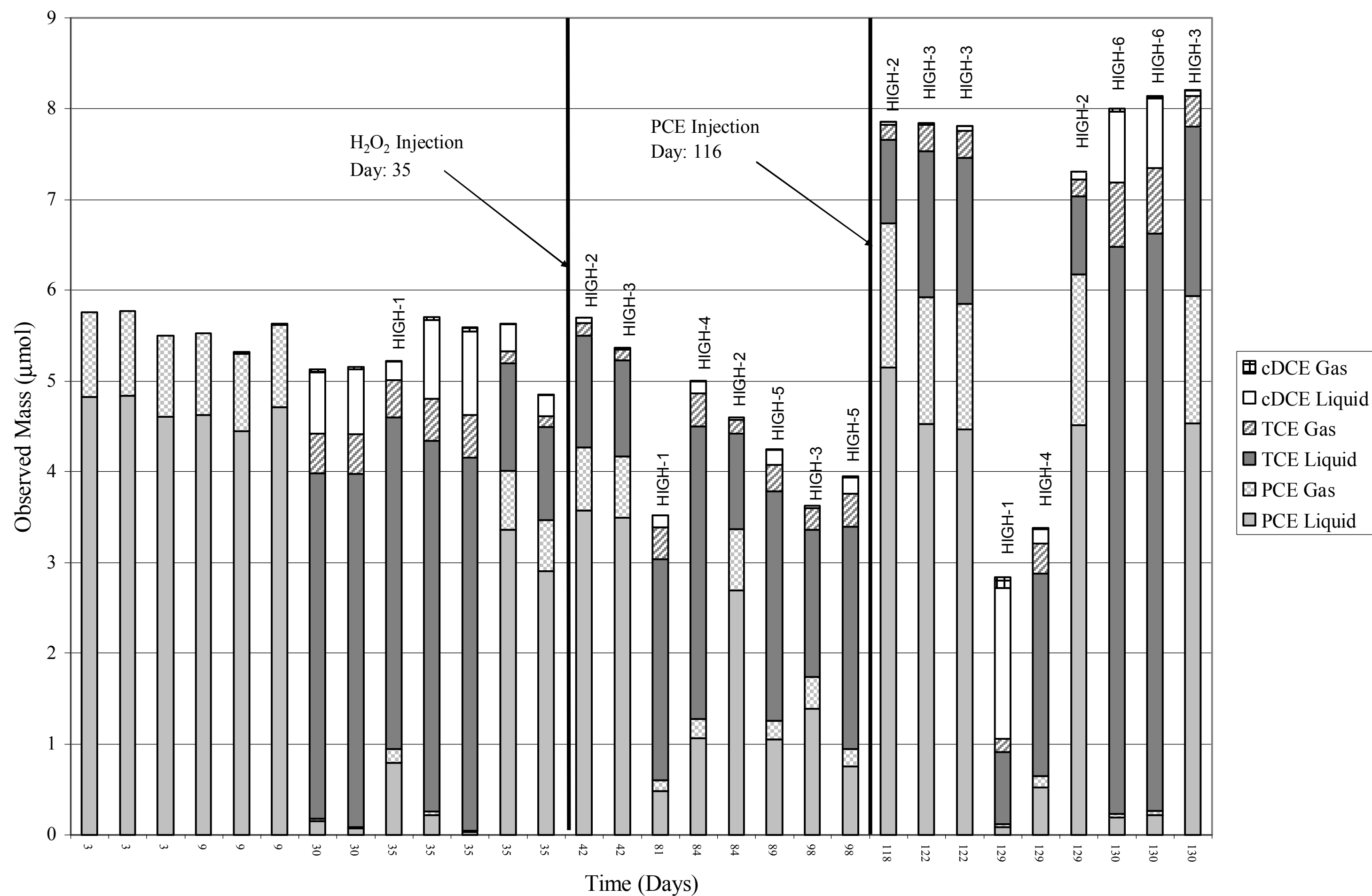

Figure 4.13: High Concentration H_2O_2 Addition (75 ppm) Chlorinated Solvent Partitioning

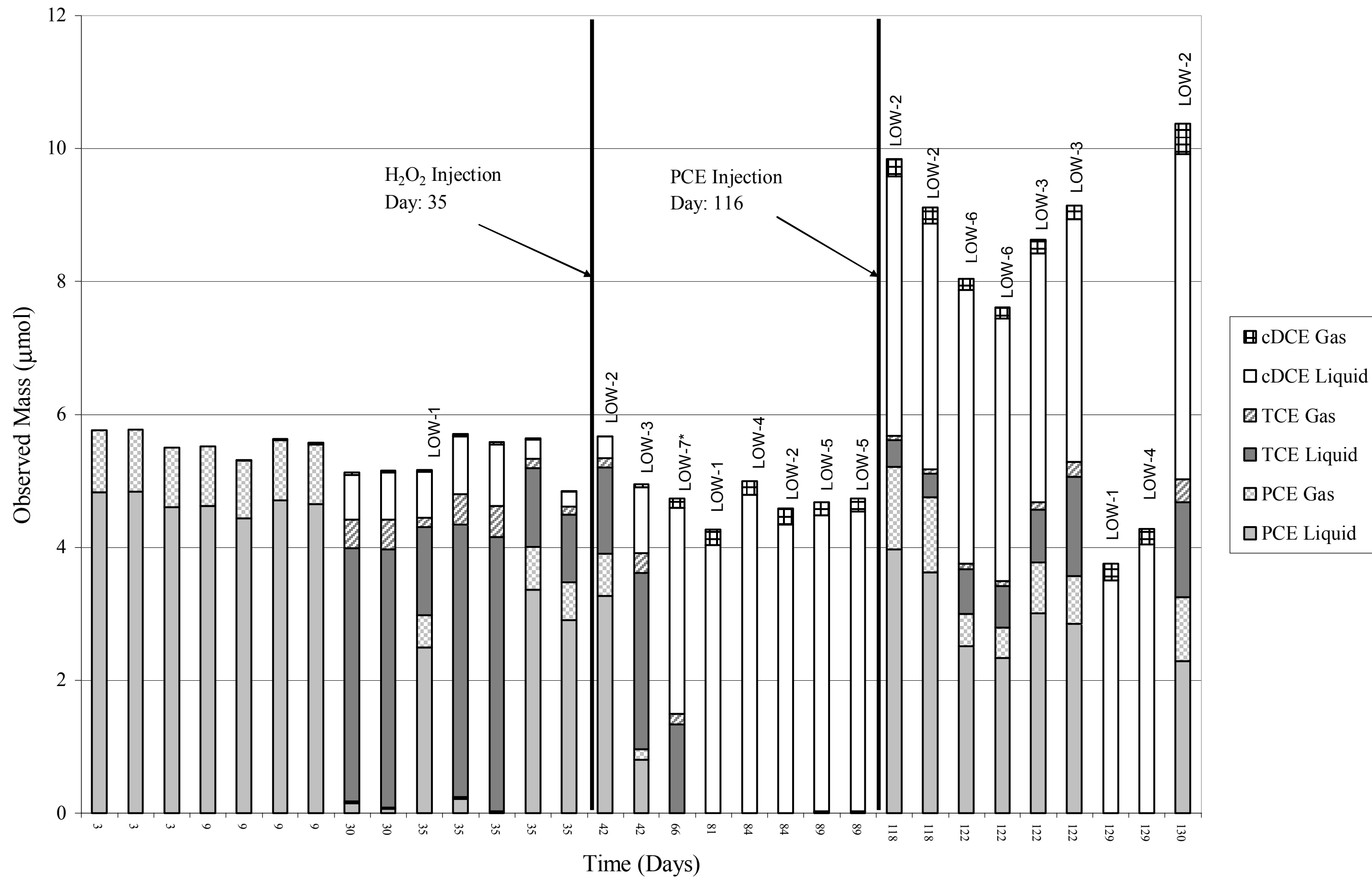

Figure 4.14: Low Concentration H_2O_2 Addition (15 ppm) Chlorinated Solvent Partitioning

LOW-5 were sampled on days 84 and 89 respectively with the only detectable chlorinated solvent species being cDCE (Figure 4.14). With complete transformation of PCE to cDCE, the bottles LOW-4 and LOW-5 provide corroborating evidence for resumed reductive dechlorination following H_2O_2 injection. LOW-6 was only sampled at the end of the experiment [two weeks after a second 0.5 μL PCE injection (an increase of 4.9 μmol) on day 116] and showed 57% of the injected PCE mass had transformed to cDCE.

Higher CH_4 gas production was observed in the 15-ppm H_2O_2 experiment as compared to the 75-ppm H_2O_2 experiment. After H_2O_2 addition on day 35 of the experiment, CH_4 generation continued in the 15 ppm microcosms with CH_4 mass increasing by 150% in LOW-1 and 89% in LOW-2. There was an overall increase in CH_4 mass in the 75-ppm microcosms of 20 to 40%. There was a correlation between continued CH_4 production and the resumption of reductive dechlorination when the CH_4 data were compared with the chlorinated solvent data. The 15-ppm experiments showed higher CH_4 production and better resumption of reductive dechlorination following H_2O_2 injection. This indicated methanogens and presumably other anaerobes tolerated 15 ppm better than 75 ppm.

The shutdown in reductive dechlorination at 75 ppm coincided with a shutdown of CH_4 production and an increase in CO_2 production over that of identical anaerobic microcosms. Continued reductive dechlorination at 15 ppm coincided with continued CH_4 production at perhaps a slightly reduced rate and steady CO_2 production similar to identical anaerobic microcosms. VFA data from samples drawn from the H_2O_2 experiments on days 84 and 89 indicated that reductive dechlorination was inhibited and proprionate fermentation was shut down or inhibited by the 75 ppm H_2O_2 addition. Proprionate fermentation appeared to have

continued in the 15 ppm H_2O_2 microcosms along with reductive dechlorination after H_2O_2 injection.

Table 4.4: Volatile Fatty Acid Utilization in Hydrogen Peroxide Experiment

Microcosm I.D.	*Time (day)*	*Acetate (ppm)*	*Δ Acetate[(2)] (ppm)*	*Proprionate (ppm)*	*Δ Proprionate[(3)] (ppm)*
BIO-7	63	34	-466	4.8	-395
BIO-8	63	6.8	-493	4.3	-396
AIR-1	81	310	-190	0.0	-400
AIR-2	81	330	-170	0.7	-399
AIR-3	81	230	-270	0.0	-400
AIR-7	81	180	-320	0.0	-400
BES-1[(1)]	81	380	-120	140	-260
HIGH-4	84	240	-403	97	-303
HIGH-5	89	150	-350	98	-302
HIGH-7[(4)]	84	220	-280	68	-332
HIGH-9[(4)]	89	170	-330	78	-322
LOW-2	84	280	-220	0.0	-400
LOW-4	84	27	-473	0.0	-400
LOW-5	89	340	-160	0.4	-399
LOW-7[(4)]	84	240	-260	0.3	-400
LOW-9[(4)]	89	5.7	-494	1.1	-398

(1) - Biotic anaerobic control dosed with 50 mM BES for suppression of methanogenesis.
(2) - Initial microcosm acetate concentration = 500 ppm.
(3) – Initial microcosm proprionate concentration = 400 ppm.
(4) – Microcosms dosed with HgCl2 on day 35 of experiment.

Table 4.4 shows the results of volatile fatty acid analysis for the hydrogen peroxide experiment combined with VFA data presented in Table 4.3. Samples were drawn from the hydrogen peroxide experiments on days 84 and 89 of the experiment. Samples taken from the high concentration hydrogen peroxide experiment microcosm appear to indicate that reductive dechlorination was inhibited and proprionate fermentation was shut down or inhibited by the 75-ppm hydrogen peroxide addition. Proprionate fermentation appears to have continued in the low concentration hydrogen peroxide microcosms along with reductive dechlorination after day 35. Acetate concentrations appear to fluctuate during the experiment. This possibly indicates that an acetoclastic metabolism was prevalent at the start of the experiment and was surpassed by homacetogenic and/or CO_2 respiring methanogenesis after acetate concentrations were consumed.

Figure 4.15 and Figure 4.16 show CH_4 gas production for the high concentration (75 ppm) hydrogen peroxide experiment and the low concentration (15 ppm) hydrogen peroxide experiment respectively. Figure 4.17 and Figure 4.18 show CO_2 gas production for the 75-ppm hydrogen peroxide experiment and the 15-ppm hydrogen peroxide experiment respectively. Sustained CH_4 gas production was observed in the 15-ppm hydrogen peroxide experiment as compared to the 75-ppm hydrogen peroxide experiment. After hydrogen peroxide addition on day 35 of the experiment, CH_4 generation continued in the 15-ppm microcosms with CH_4 mass increasing by 150% in LOW-1 and 89% in LOW-2 after the hydrogen peroxide injection. There was an overall increase in CH_4 mass in the in the 75-ppm microcosms of 20 to 40%. CH_4 mass increased by only 38% in HIGH-2 after the hydrogen peroxide injection. There was a correlation between sustained CH_4 production with sustained reductive dechlorination when the CH_4 data

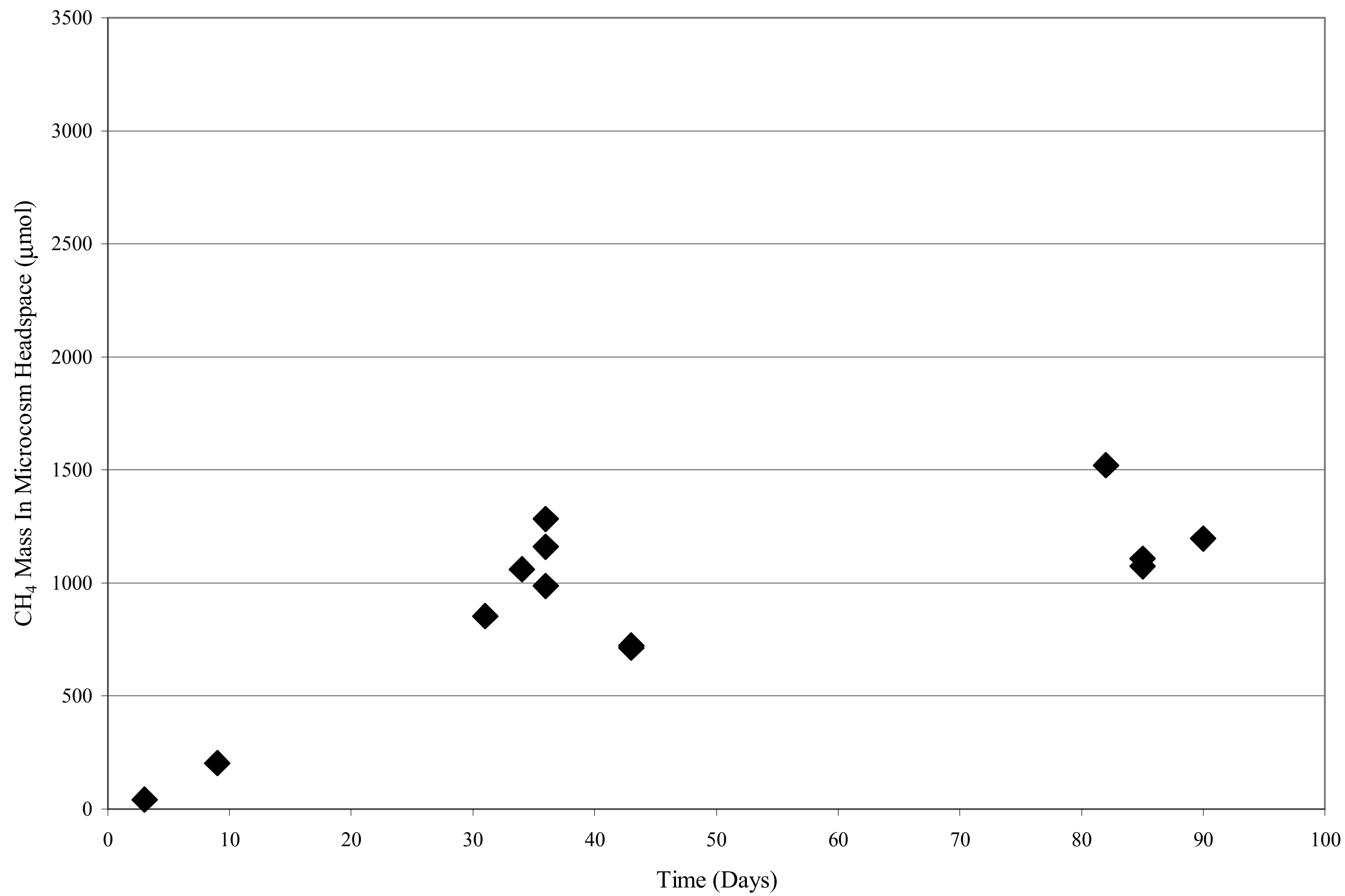

Figure 4.15: CH_4 Gas Production in Hydrogen Peroxide (75 ppm) Experiment

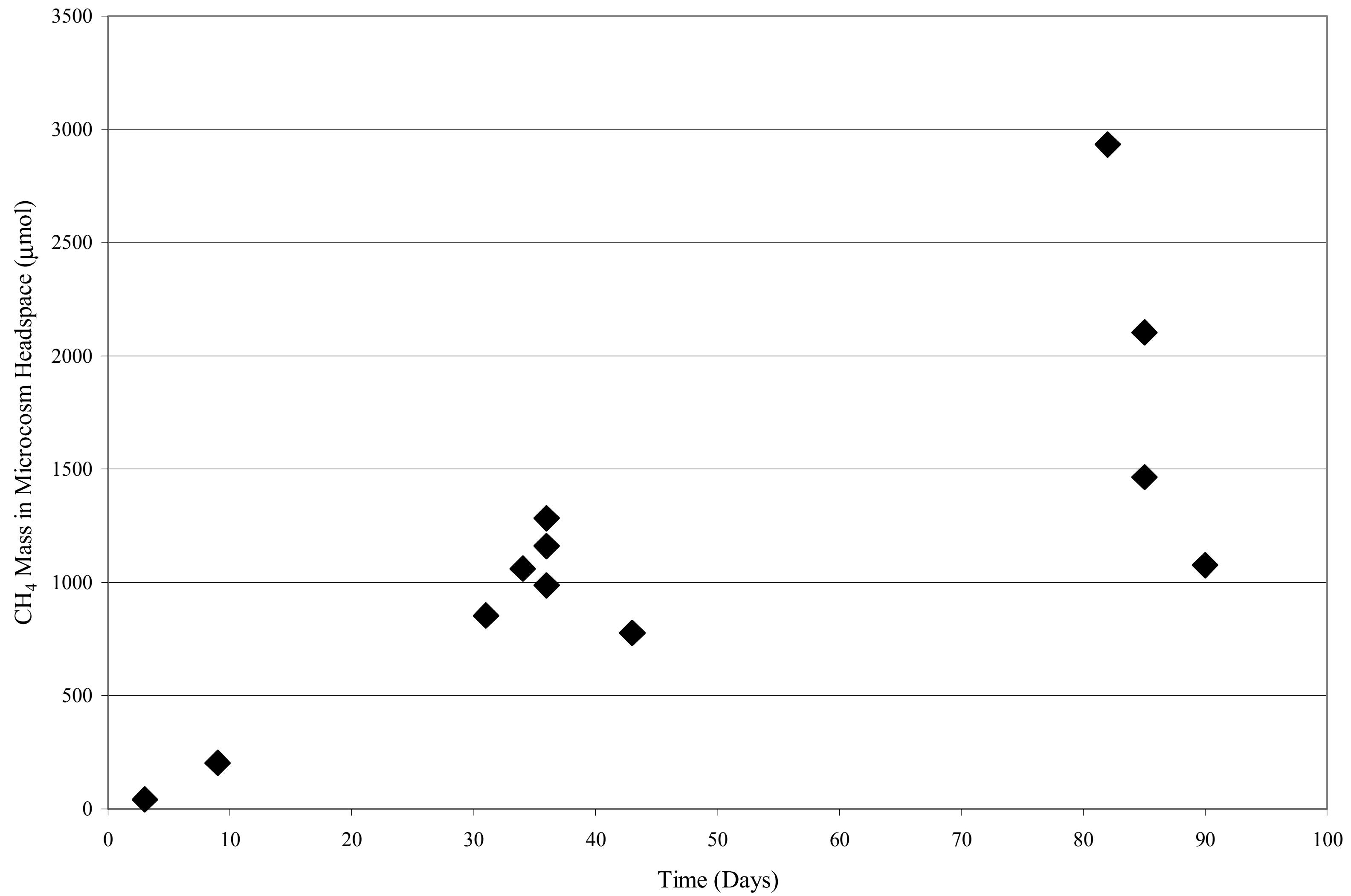

Figure 4.16: CH_4 Gas Production in Hydrogen Peroxide (15 ppm) Experiment

are combined with the chlorinated solvent data showing sustained reductive dechlorination in the 15-ppm microcosms versus the 75-ppm microcosms. The shutdown in reductive dechlorination at 75 ppm corresponded to a reduction in CH_4 production (Figure 4.15) and an increase in CO_2 production (Figure 4.17) over that of biotic anaerobic microcosms (Figure 4.10). Continued reductive dechlorination at 15 ppm corresponded to continued CH_4 production (Figure 4.16) at perhaps a slightly reduced rate and steady CO_2 production (Figure 4.18) similar to biotic anaerobic microcosms (Figure 4.10).

4.2.3 Air Injection Experiments (AIR)

Injection of air into the microcosm headspace was utilized as a second strategy to induce an aerobic phase of the anaerobic/aerobic sequential environment and thereby stimulate methanotrophic bacteria in the microbial consortia to cometabolize the reductive dechlorination daughter products. Each microcosm was allowed to remain anaerobic until day 84 to ensure PCE in the microcosm had been transformed to cDCE. To induce methanotrophic activity, the headspace of each microcosm was vented and flushed with 100 ml of atmospheric air. The vent was removed and each microcosm headspace was injected with 20 ml of 99.99% methane gas as a substrate for the methanotrophic bacteria. The air injection was performed on days 84 and 102 of the experiment to induce the aerobic treatment (methanotrophic) phase of the experiment. The microcosms were then sampled over a one-month period between the first air injection on day 84 and the end of the experiment on day 132. Gas chromatograph analysis for chlorinated solvents was performed periodically to look for indications of aerobic biodegradation of the cDCE or continued reductive dechlorination of PCE or TCE. Additional PCE was added to all bottles on

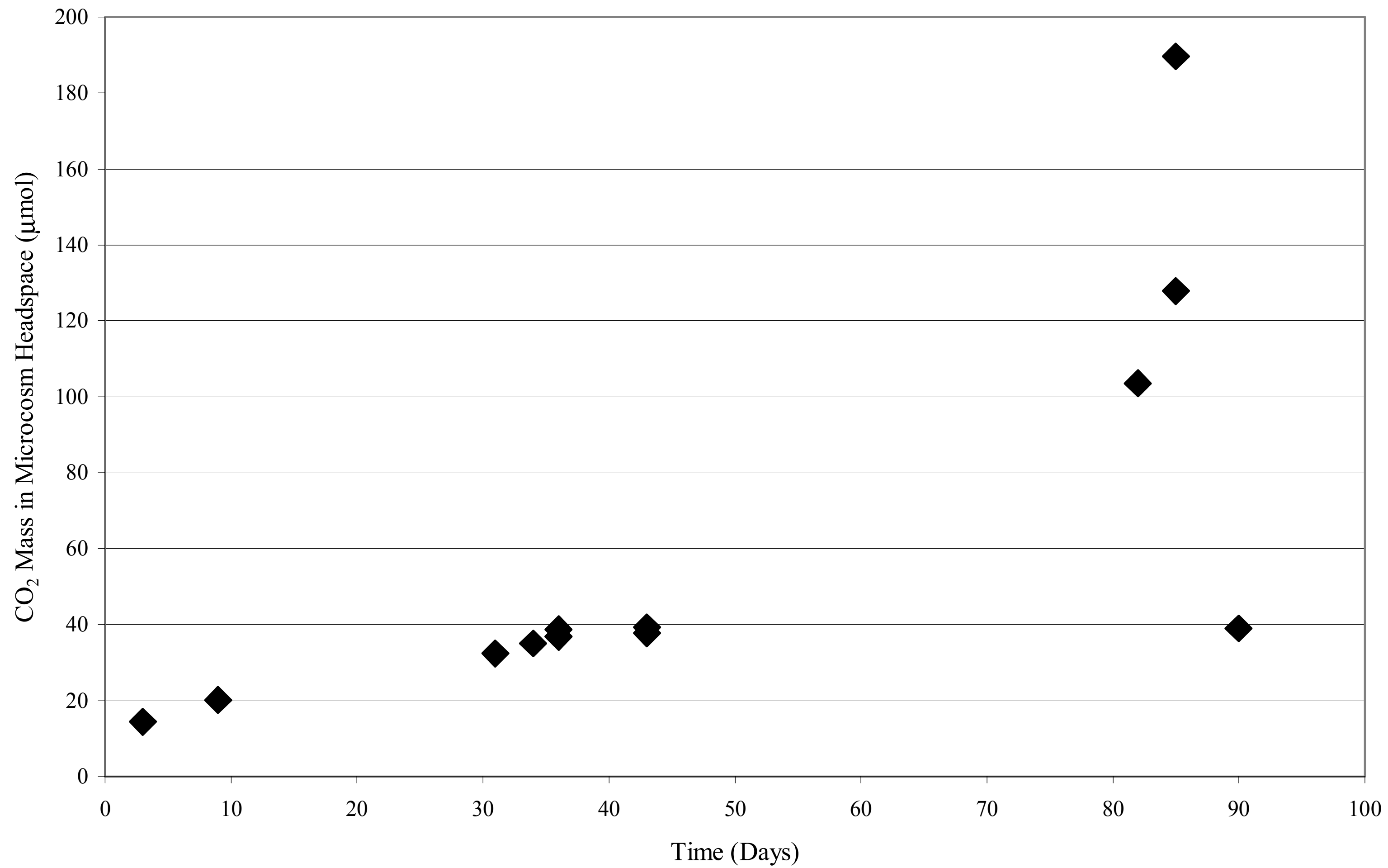

Figure 4.17: CO_2 Gas Production in Hydrogen Peroxide (75 ppm) Experiment

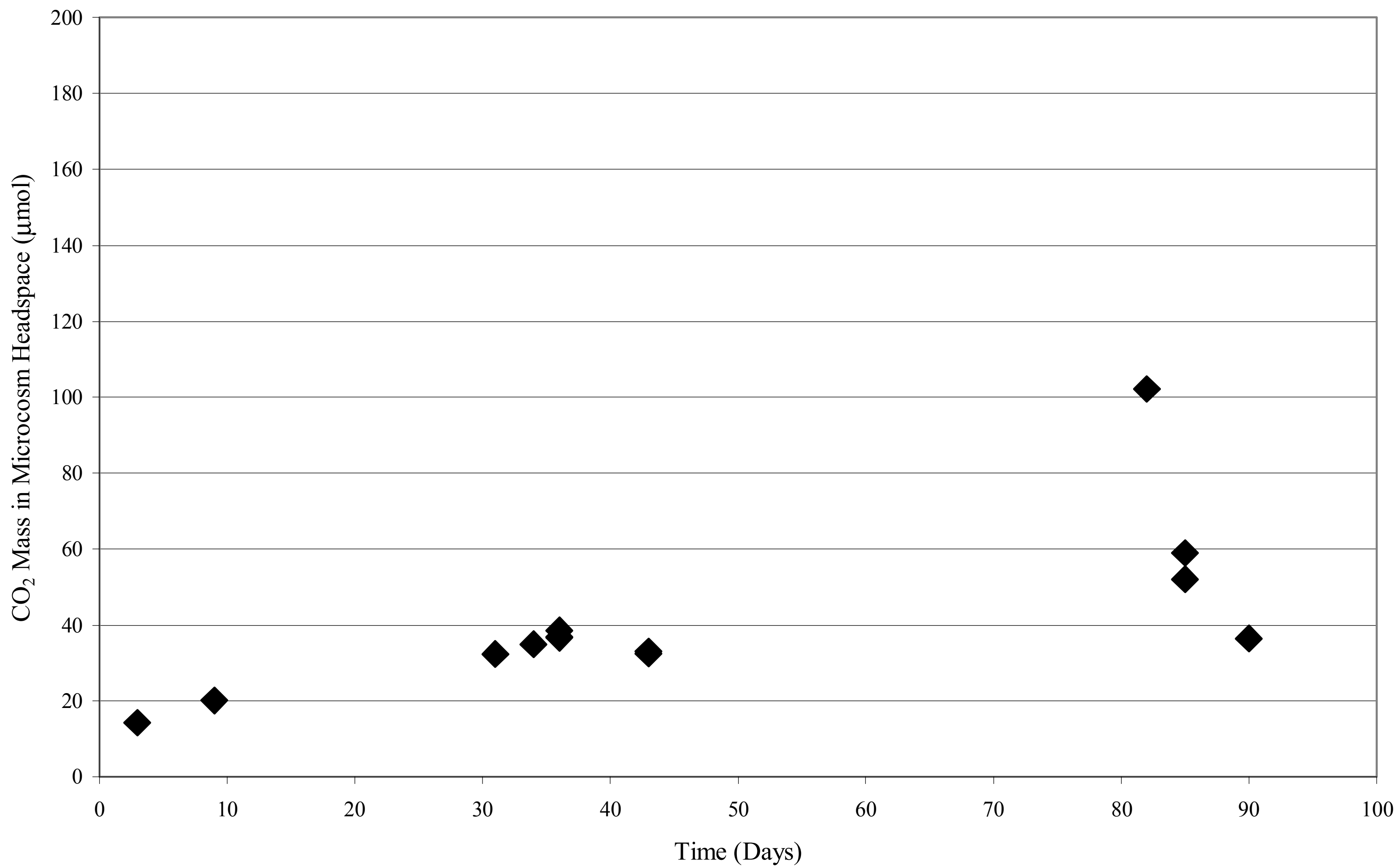

Figure 4.18: CO_2 Gas Production in Hydrogen Peroxide (15 ppm) Experiment

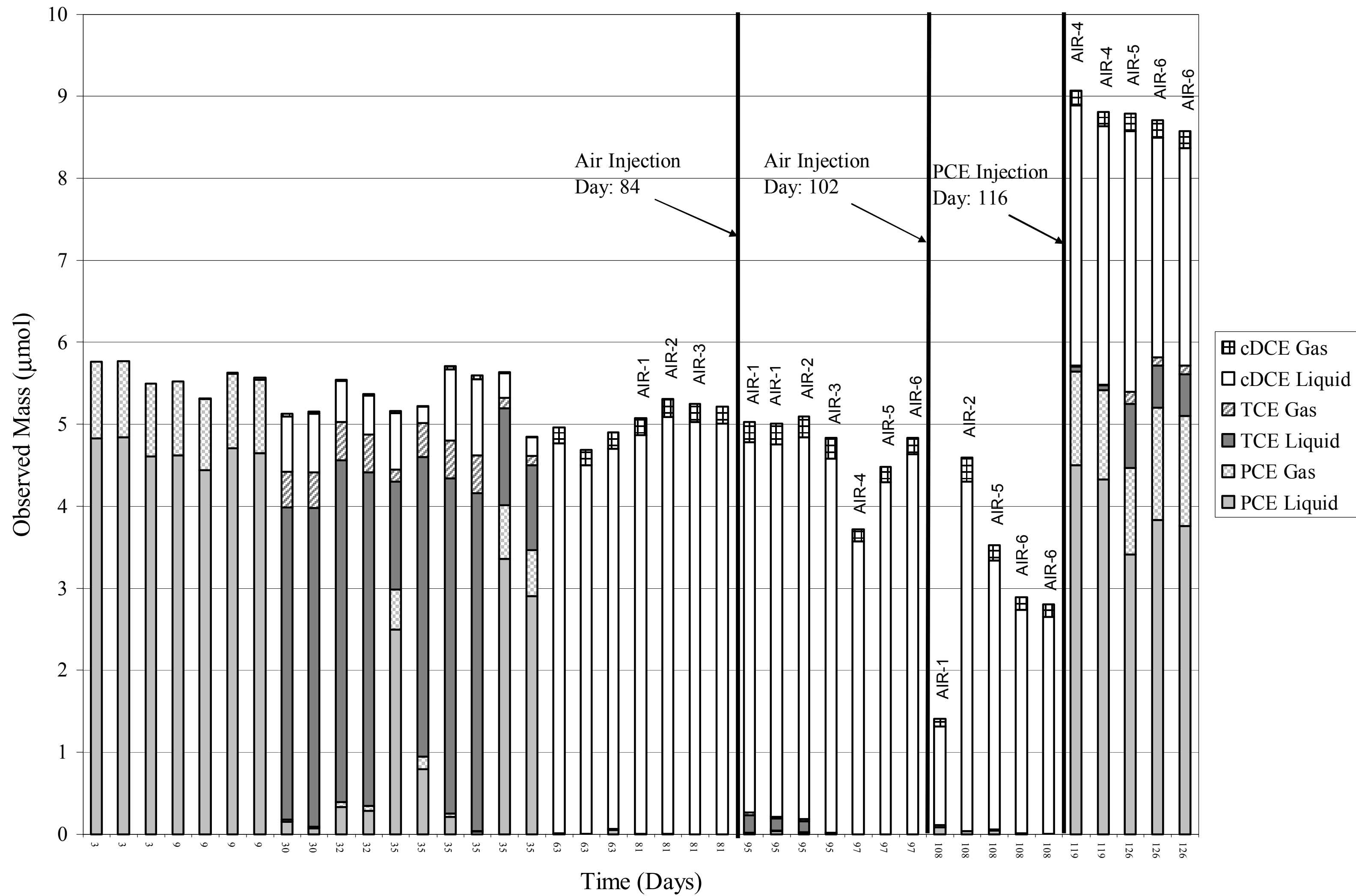

Figure 4.19: CO_2 Gas Production in Hydrogen Peroxide (15 ppm) Experiment

day 116 of the experiment to determine if further reductive dechlorination was possible at the end of the experiment. The air experiments were started seven weeks after the H_2O_2 experiments at which time the PCE had been completely transformed to cDCE. In this condition, further reductive dechlorination of cDCE to VC or ethene is significantly more difficult and unlikely to be observed. Figure 4.19 shows chlorinated solvent concentrations for the air injection experiment. As VC and ethene were never observed, reductive dechlorination was not observed after injection of ambient air on day 84 and day 102 of the experiment. However, additional PCE was injected on day 116 of the experiment to test the microbial consortia for the capability of reductive dechlorination. On day 126 of the experiment (10 days after the PCE injection), samples from two of six microcosms showed detectable concentrations of TCE present. Previous samples from the two bottles on days 97 and 108 of the experiment prior to the PCE addition showed cDCE as the only detectable analyte. The detection of TCE in these microcosms demonstrated that further reductive dechlorination was generated after the ambient air injections intended to induce aerobic degradation. However, no conclusive evidence of mineralization of the PCE daughter products was found during the experiment to show successful aerobic biodegradation had occurred, probably due to the absence of methanotrophs in the inocula. Additionally, this experiment was more difficult to monitor due to losses from the headspace during venting (note reduced mass after day 102 air injection; Figure 4.19). Biogenic gas analysis was not appropriate after the air injection was performed and an unknown amount of chlorinated solvent mass was also lost during the venting.

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

Reductive dechlorination was accomplished in methanogenic and BES-suppressed non-methanogenic environments with similar rates of transformation of PCE to TCE to cDCE. Non-BES suppressed microcosms consistently showed an increase in CH_4 biogenic gas formation indicating the active presence of methanogenic bacteria within these microbial consortia. The data were inconclusive as to the metabolism responsible for all or part of the transformation of PCE, but evidence supports the participation of methanogenic metabolism due to the presence of CH_4 in the unsuppressed microcosms.

However, the reductive dechlorination of PCE to cDCE in the BES-suppressed microcosms and the absence of methane within the headspace of these microcosms supports that non-methanogenic anaerobic metabolisms are present and participating in the reductive dechlorination process, possibly as the primary metabolism responsible for the reductive dechlorination process. Limited VFA data indicated that proprionate fermentation appeared to shutdown after high H_2O_2 concentrations were introduced along with reductive dechlorination. These data suggested that methanogenic microorganisms may not have been the primary metabolism responsible for reductive dechlorination in this experiment or certainly that other metabolisms (e.g., homoacetogenic, fermentative) can take over that function. For these microcosms it would be worthwhile to try anaerobic/aerobic experiments and identify the microbes responsible.

Conclusive evidence of mineralization of the PCE daughter products was not found during the experiment to show successful aerobic biodegradation had occurred. This was because the inocula probably did not contain methanotrophs. However, the experiments did show that

anaerobic reductive dechlorination could be recovered following periodic aerobic conditions. Reductive dechlorination resumed in the microcosm exposed to the lower (15 ppm) H_2O_2 injection and after the air injections. These experiments should be repeated with inocula containing both anaerobes and methanotrophs or in-situ (anaerobes and methanotrophs are common in most soils).

Microcosm sampling and analysis were significantly hindered by the use of 120-mL serum bottles. By necessity, microcosms needed to be discarded due to the volume losses associated with sampling. Further research should be conducted using larger volume microcosms (250 mL) to allow more sampling and analysis of the same consortia. Methanotroph innocula should be introduced to the anaerobic consortia used in these experiments and further microcosm studies conducted to determine the feasibility of the sequential anaerobic/aerobic environment in a single microbial consortia to mineralize PCE. Additionally, the presence of cDCE and PCE are both required at the point of initiation of the aerobic phase of the experiment to allow evaluation of both the aerobic and anaerobic metabolisms simultaneously. This can be accomplished by injection of the appropriate HAC just prior to the aerobic phase. Further hydrogen peroxide injection tests should be performed at concentrations less than 75 ppm as this study has shown that reductive dechlorination appears to be significantly reduced at such high concentrations.

APPENDIX A:
CHLORINATED SOLVENT ANALYSIS AND CALCULATIONS

This appendix contains data analysis and sample calculations for the HACs monitored during this study. The concentrations reported for PCE and its daughter compounds were calculated from calibration curves generated through the analysis of various dilutions of pure standards. A best-fit linear curve with a coefficient of determination in excess of 0.99 was obtained for each of the chlorinated compounds. The area calculated by the integrator from the gas chromatograph was converted into a concentration using these linear curves. The vinyl chloride calibration curve was based upon a TCE response factor developed on a different GC column (Ruiz, 1998). Table A.1 provides the calibration curve slope and intercept for each HAC analyte. The concentration of any given analyte shown in Tables A.2 to A.7 were calculated using the equation A.1.

$$Concentration = \frac{(Area - Intercept)}{Slope} \tag{A.1}$$

Table A.1: Slope and Intercept Values for each HAC Analyte Calibration Curve

HAC Analyte	*Slope*	*Intercept*
PCE	88112	2986
TCE	120979	11822
cDCE	154906	21638
tDCE	239798	17682
1,1DCE	161746	21766
VC(1)	182027	0

(1) VC calibration curve based upon TCE response factor

Table A.2:

ME Microcosm Series Chlorinated Solvent Data

Days from Creation	*GC Analysis Date*	*Sample ID*	*VC Area*	*VC Conc (mg/L)*	*1,1 DCE Area*	*1,1 DCE Conc (mg/L)*	*trans DCE Area*	*trans DCE Conc (mg/L)*	*cis DCE Area*	*cDCE Conc (mg/L)*	*TCE Area*	*TCE Conc (mg/L)*	*PCE Area*	*PCE Conc (mg/L)*
3	3/17/1998	MEC-1	--	0.00	2998	0.00	752	0.00	1716	0.00	1893	0.00	720558	8.14
3	3/17/1998	MEC-1	--	0.00	3859	0.00	854	0.00	2040	0.00	3023	0.00	715185	8.08
3	3/17/1998	MEC-1	--	0.00	1054	0.00	663	0.00	4185	0.00	4247	0.00	709607	8.02
3	3/17/1998	MEC-2	--	0.00	1806	0.00	366	0.00	1460	0.00	8924	0.00	759652	8.59
3	3/17/1998	MEC-2	--	0.00	4970	0.00	518	0.00	5254	0.00	11125	0.00	709635	8.02
3	3/17/1998	MEC-2	--	0.00	3141	0.00	--	0.00	833	0.00	15734	0.03	706654	7.99
8	3/22/1998	MEC-3	--	0.00	855	0.00	--	0.00	1072	0.00	4504	0.00	818393	9.25
8	3/22/1998	MEC-3	--	0.00	912	0.00	--	0.00	990	0.00	2584	0.00	788091	8.91
8	3/22/1998	MEC-3	--	0.00	861	0.00	--	0.00	3395	0.00	3543	0.00	747910	8.45
8	3/22/1998	MEC-4	--	0.00	828	0.00	--	0.00	2128	0.00	2490	0.00	443968	5.00
8	3/22/1998	MEC-4	--	0.00	551	0.00	--	0.00	5419	0.00	5531	0.00	417703	4.71
8	3/22/1998	MEC-4	--	0.00	573	0.00	--	0.00	2705	0.00	3305	0.00	403526	4.55
18	4/1/1998	MEC-5	--	0.00	505	0.00	--	0.00	2826	0.00	4312	0.00	757291	8.56
18	4/1/1998	MEC-5	--	0.00	780	0.00	--	0.00	1330	0.00	2576	0.00	723785	8.18
18	4/1/1998	MEC-6	--	0.00	720	0.00	--	0.00	5120	0.00	6982	0.00	718038	8.12
56	5/9/1998	MEC-7	--	0.00	833	0.00	--	0.00	1856	0.00	41012	0.24	591408	6.68
56	5/9/1998	MEC-7	--	0.00	739	0.00	--	0.00	1463	0.00	38156	0.22	572789	6.47
128	7/20/1998	MEC-11	--	0.00	2528	0.00	--	0.00	672	0.00	1438	0.00	489174	5.52
128	7/20/1998	MEC-11	--	0.00	2978	0.00	2213	0.00	989	0.00	3686	0.00	657896	7.43
129	7/21/1998	MEC-12	--	0.00	10529	0.00	820	0.00	616	0.00	1501	0.00	561899	6.34
129	7/21/1998	MEC-12	--	0.00	19654	0.00	848	0.00	--	0.00	1725	0.00	749954	8.48
192	9/22/1998	MEC-14	--	0.00	6063	0.00	5889	0.00	7188	0.00	13646	0.02	589462	6.66
192	9/22/1998	MEC-14	--	0.00	2625	0.00	2029	0.00	1882	0.00	3831	0.00	272558	3.06
334	2/11/1999	MEC-16	--	0.00	1517	0.00	--	0.00	1140	0.00	1097	0.00	466988	5.27
334	2/11/1999	MEC-16	--	0.00	932	0.00	--	0.00	1085	0.00	1134	0.00	470579	5.31

Days from Creation	*GC Analysis Date*	*Sample ID*	*VC Area*	*VC Conc (mg/L)*	*1,1 DCE Area*	*1,1 DCE Conc (mg/L)*	*trans DCE Area*	*trans DCE Conc (mg/L)*	*cis DCE Area*	*cDCE Conc (mg/L)*	*TCE Area*	*TCE Conc (mg/L)*	*PCE Area*	*PCE Conc (mg/L)*
6	3/20/1998	ME-1	--	0.00	2344	0.00	3501	0.00	83851	0.40	641553	5.21	60012	0.65
6	3/20/1998	ME-1	--	0.00	1864	0.00	1911	0.00	88755	0.43	653987	5.31	47258	0.50
6	3/20/1998	ME-1	--	0.00	1694	0.00	2293	0.00	97210	0.49	623023	5.05	34629	0.36
6	3/20/1998	ME-2	--	0.00	1806	0.00	2168	0.00	761483	4.78	2331	-0.08	5876	0.03
6	3/20/1998	ME-2	--	0.00	1192	0.00	1936	0.00	811210	5.10	1120	-0.09	4157	0.01
6	3/20/1998	ME-2	--	0.00	1531	0.00	1690	0.00	698520	4.37	1478	-0.09	4298	0.01
10	3/24/1998	ME-3	--	0.00	1260	0.00	2090	0.00	778247	4.88	2493	-0.08	10137	0.08
10	3/24/1998	ME-3	--	0.00	1541	0.00	2201	0.00	795691	5.00	3177	-0.07	15736	0.14
10	3/24/1998	ME-3	--	0.00	1461	0.00	2325	0.00	789258	4.96	2887	-0.07	13901	0.12
10	3/24/1998	ME-4	--	0.00	1629	0.00	2402	0.00	771048	4.84	1811	-0.08	11755	0.10
10	3/24/1998	ME-4	--	0.00	954	0.00	931	0.00	448979	2.76	956	-0.09	5109	0.02
10	3/24/1998	ME-4	--	0.00	1424	0.00	2421	0.00	749625	4.70	3235	-0.07	15129	0.14
18	4/1/1998	ME-5	--	0.00	2732	0.00	2750	0.00	828579	5.21	789	-0.09	8658	0.06
18	4/1/1998	ME-6	--	0.00	1358	0.00	2322	0.00	769534	4.83	1438	-0.09	13517	0.12
18	4/1/1998	ME-7	--	0.00	1351	0.00	2369	0.00	814584	5.12	2203	-0.08	16376	0.15
18	4/1/1998	ME-8	--	0.00	2114	0.00	3814	0.00	805695	5.06	5339	-0.05	23906	0.24
20	4/3/1998	ME-5	--	0.00	470	0.00	1507	0.00	758990	4.76	--	-0.10	1110	-0.02
20	4/3/1998	ME-5	--	0.00	942	0.00	1481	0.00	729624	4.57	630	-0.09	8143	0.06
20	4/3/1998	ME-6	--	0.00	599	0.00	1219	0.00	712187	4.46	826	-0.09	897	-0.02
20	4/3/1998	ME-6	--	0.00	877	0.00	2077	0.00	713998	4.47	509	-0.09	2511	-0.01
35	4/18/1998	ME-7	--	0.00	1043	0.00	1408	0.00	738809	4.63	1410	-0.09	4833	0.02
35	4/18/1998	ME-7	--	0.00	1174	0.00	1809	0.00	714447	4.47	1193	-0.09	7724	0.05
35	4/18/1998	ME-8	--	0.00	1546	0.00	4163	0.00	791639	4.97	25177	0.11	5411	0.03
35	4/18/1998	ME-8	--	0.00	1378	0.00	5569	0.00	712041	4.46	26081	0.12	7598	0.05
56	5/9/1998	ME-9	577	0.00	1597	0.00	2120	0.00	723769	4.53	9793	-0.02	3339	0.00
56	5/9/1998	ME-9	1082	0.01	1023	0.00	2675	0.00	710479	4.45	14475	0.02	3956	0.01

Days from Creation	*GC Analysis Date*	*Sample ID*	*VC Area*	*VC Conc (mg/L)*	*1,1 DCE Area*	*1,1 DCE Conc (mg/L)*	*trans DCE Area*	*trans DCE Conc (mg/L)*	*cis DCE Area*	*cDCE Conc (mg/L)*	*TCE Area*	*TCE Conc (mg/L)*	*PCE Area*	*PCE Conc (mg/L)*
56	5/9/1998	ME-10	618	0.00	1361	0.00	2643	0.00	679861	4.25	37153	0.21	4797	0.02
56	5/9/1998	ME-10	816	0.00	1100	0.00	4351	0.00	684527	4.28	27657	0.13	5132	0.02
92	6/14/1998	ME-11	--	0.00	1968	0.00	3555	0.00	785274	4.93	4747	-0.06	20665	0.20
92	6/14/1998	ME-12	--	0.00	963	0.00	4263	0.00	818820	5.15	976	-0.09	12139	0.10
128	7/20/1998	ME-13	92834	0.51	2609	0.00	13192	0.00	2927	-0.12	4341	-0.06	3238	0.00
128	7/20/1998	ME-13	88464	0.49	4636	0.00	1906	0.00	2462	-0.12	1942	-0.08	1931	-0.01
128	7/20/1998	ME-14	46891	0.26	3403	0.00	1700	0.00	--	-0.14	2159	-0.08	3645	0.01
128	7/20/1998	ME-14	42039	0.23	2923	0.00	1614	0.00	1614	-0.13	1964	-0.08	1670	-0.01
129	7/21/1998	ME-15	71218	0.39	5853	0.00	668	0.00	1256	-0.13	919	-0.09	878	-0.02
129	7/21/1998	ME-15	52092	0.29	4419	0.00	600	0.00	--	-0.14	834	-0.09	2972	0.00
134	7/26/1998	ME-16	458941	2.54	--	0.00	4661	0.00	8639	-0.08	9446	-0.02	21127	0.21
134	7/26/1998	ME-16	481496	2.67	--	0.00	3680	0.00	--	-0.14	5216	-0.05	16692	0.16
162	8/23/1998	ME-17	35125	0.19	1704	0.00	5026	0.00	5358	-0.11	5424	-0.05	2997	0.00
162	8/23/1998	ME-17	33720	0.19	--	0.00	2922	0.00	3021	-0.12	3513	-0.07	6413	0.04
169	8/30/1998	ME-18	28158	0.16	1722	0.00	2498	0.00	2679	-0.12	9240	-0.02	7915	0.06
169	8/30/1998	ME-18	27215	0.15	2298	0.00	2861	0.00	1716	-0.13	7399	-0.04	5694	0.03
183	9/13/1998	ME-19	36593	0.20	747	0.00	1475	0.00	1542	-0.13	2892	-0.07	2480	-0.01
183	9/13/1998	ME-19	40662	0.23	1300	0.00	2195	0.00	1216	-0.13	3133	-0.07	7363	0.05
192	9/22/1998	ME-20	43166	0.24	1020	0.00	2808	0.00	877074	5.52	1825	-0.08	1127	-0.02
192	9/22/1998	ME-20	38388	0.21	2831	0.00	3874	0.00	860482	5.42	4205	-0.06	826	-0.02
192	9/22/1998	ME-20	13655	0.08	1385	0.00	3728	0.00	898578	5.66	5624	-0.05	--	-0.03
192	9/22/1998	ME-21	111535	0.62	2220	0.00	2262	0.00	2179	-0.13	4579	-0.06	1335	-0.02
192	9/22/1998	ME-21	106917	0.59	2793	0.00	2357	0.00	1741	-0.13	5354	-0.05	2058	-0.01
192	9/22/1998	ME-21	93884	0.52	5740	0.00	6474	0.00	5960	-0.10	14957	0.03	2150	-0.01
334	2/11/1999	ME-23	29757	0.16	592	0.00	836	0.00	587	-0.14	2982	-0.07	4608	0.02
334	2/11/1999	ME-23	30847	0.17	--	0.00	--	0.00	--	-0.14	1595	-0.08	2297	-0.01

Days from Creation	*GC Analysis Date*	*Sample ID*	*VC Area*	*VC Conc (mg/L)*	*1,1 DCE Area*	*1,1 DCE Conc (mg/L)*	*trans DCE Area*	*trans DCE Conc (mg/L)*	*cis DCE Area*	*cDCE Conc (mg/L)*	*TCE Area*	*TCE Conc (mg/L)*	*PCE Area*	*PCE Conc (mg/L)*
334	2/11/1999	ME-24	2030	0.01	--	0.00	1846	0.00	713930	4.47	1341	-0.09	1662	-0.02
334	2/11/1999	ME-24	1923	0.01	635	0.00	1707	0.00	707373	4.43	1505	-0.09	1405	-0.02

Table A.3:

ME Microcosm Series Chlorinated Solvent Partitioning Analysis and Calculations

Days from Creation	*GC Analysis Date*	*Sample ID*	*PCE Liquid phase mass (µmol)*	*TCE Liquid phase mass (µmol)*	*cis-DCE Liquid phase mass (µmol)*	*VC Liquid phase mass (µmol)*	*PCE Gas Phase mass (µmol)*	*TCE Gas Phase mass (µmol)*	*cis-DCE Gas Phase mass (µmol)*	*VC Gas Phase mass (µmol)*	*PCE Initial liquid mass (µmol)*	*Total Liquid mass (µmol)*	*Total Gas Mass (µmol)*	*Unaccounted for mass (µmol)*
3	3/17/1998	MEC-1	4.911	0.000	0.000	0.000	0.952	0.000	0.000	0.000	4.893	4.911	0.952	-0.970
3	3/17/1998	MEC-1	4.874	0.000	0.000	0.000	0.944	0.000	0.000	0.000	4.893	4.874	0.944	-0.926
3	3/17/1998	MEC-1	4.836	0.000	0.000	0.000	0.937	0.000	0.000	0.000	4.893	4.836	0.937	-0.880
3	3/17/1998	MEC-2	5.178	0.000	0.000	0.000	1.003	0.000	0.000	0.000	4.893	5.178	1.003	-1.289
3	3/17/1998	MEC-2	4.836	0.000	0.000	0.000	0.937	0.000	0.000	0.000	4.893	4.836	0.937	-0.880
3	3/17/1998	MEC-2	4.816	0.000	0.000	0.000	0.933	0.003	0.000	0.000	4.893	4.816	0.936	-0.859
8	3/22/1998	MEC-3	5.580	0.000	0.000	0.000	1.081	0.000	0.000	0.000	4.893	5.580	1.081	-1.769
8	3/22/1998	MEC-3	5.373	0.000	0.000	0.000	1.041	0.000	0.000	0.000	4.893	5.373	1.041	-1.522
8	3/22/1998	MEC-3	5.098	0.000	0.000	0.000	0.988	0.000	0.000	0.000	4.893	5.098	0.988	-1.193
8	3/22/1998	MEC-4	3.018	0.000	0.000	0.000	0.585	0.000	0.000	0.000	4.893	3.018	0.585	1.290
8	3/22/1998	MEC-4	2.838	0.000	0.000	0.000	0.550	0.000	0.000	0.000	4.893	2.838	0.550	1.504
8	3/22/1998	MEC-4	2.741	0.000	0.000	0.000	0.531	0.000	0.000	0.000	4.893	2.741	0.531	1.620
18	4/1/1998	MEC-5	5.162	0.000	0.000	0.000	1.000	0.000	0.000	0.000	4.893	5.162	1.000	-1.270
18	4/1/1998	MEC-5	4.933	0.000	0.000	0.000	0.956	0.000	0.000	0.000	4.893	4.933	0.956	-0.996
18	4/1/1998	MEC-6	4.894	0.000	0.000	0.000	0.948	0.000	0.000	0.000	4.893	4.894	0.948	-0.949
56	5/9/1998	MEC-7	4.027	0.184	0.000	0.000	0.780	0.021	0.000	0.000	4.893	4.211	0.801	-0.119
56	5/9/1998	MEC-7	3.900	0.166	0.000	0.000	0.756	0.019	0.000	0.000	4.893	4.065	0.774	0.053
128	7/20/1998	MEC-11	3.327	0.000	0.000	0.000	0.645	0.000	0.000	0.000	4.893	3.327	0.645	0.920
128	7/20/1998	MEC-11	4.482	0.000	0.000	0.000	0.869	0.000	0.000	0.000	4.893	4.482	0.869	-0.458
129	7/21/1998	MEC-12	3.825	0.000	0.000	0.000	0.741	0.000	0.000	0.000	4.893	3.825	0.741	0.326
129	7/21/1998	MEC-12	5.112	0.000	0.000	0.000	0.991	0.000	0.000	0.000	4.893	5.112	0.991	-1.210
192	9/22/1998	MEC-14	4.014	0.011	0.000	0.004	0.778	0.001	0.000	0.000	4.893	4.029	0.779	0.084
192	9/22/1998	MEC-14	1.845	0.000	0.000	0.000	0.357	0.000	0.000	0.000	4.893	1.845	0.357	2.690
334	2/11/1999	MEC-16	3.175	0.000	0.000	0.000	0.615	0.000	0.000	0.000	4.893	3.175	0.615	1.102
334	2/11/1999	MEC-16	3.200	0.000	0.000	0.000	0.620	0.000	0.000	0.000	4.893	3.200	0.620	1.072
6	3/20/1998	ME-1	0.766	3.017	0.035	0.035	0.076	0.448	0.018	0.000	4.893	3.853	0.542	0.498
6	3/20/1998	ME-1	0.586	2.872	0.377	0.377	0.059	0.457	0.019	0.000	4.893	4.211	0.535	0.147
6	3/20/1998	ME-1	3.409	0.272	0.000	0.000	0.042	0.435	0.022	0.000	4.893	3.681	0.498	0.714

Days from Creation	*GC Analysis Date*	*Sample ID*	*PCE Liquid phase mass (μmol)*	*TCE Liquid phase mass (μmol)*	*cis-DCE Liquid phase mass (μmol)*	*VC Liquid phase mass (μmol)*	*PCE Gas Phase mass (μmol)*	*TCE Gas Phase mass (μmol)*	*cis-DCE Gas Phase mass (μmol)*	*VC Gas Phase mass (μmol)*	*PCE Initial liquid mass (μmol)*	*Total Liquid mass (μmol)*	*Total Gas Mass (μmol)*	*Unaccounted for mass (μmol)*
6	3/20/1998	ME-2	3.325	0.255	0.000	0.000	0.004	0.000	0.210	0.000	4.893	3.580	0.214	1.098
6	3/20/1998	ME-2	2.775	0.241	0.000	0.000	0.002	0.000	0.225	0.000	4.893	3.016	0.226	1.650
6	3/20/1998	ME-2	3.985	0.242	0.000	0.000	0.002	0.000	0.193	0.000	4.893	4.227	0.194	0.471
10	3/24/1998	ME-3	4.270	0.175	0.000	0.000	0.009	0.000	0.215	0.000	4.893	4.445	0.225	0.223
10	3/24/1998	ME-3	3.087	0.190	0.000	0.000	0.017	0.000	0.220	0.000	4.893	3.276	0.237	1.379
10	3/24/1998	ME-3	3.059	0.430	0.000	0.000	0.014	0.000	0.218	0.000	4.893	3.489	0.233	1.170
10	3/24/1998	ME-4	3.352	0.323	0.000	0.000	0.012	0.000	0.213	0.000	4.893	3.674	0.225	0.993
10	3/24/1998	ME-4	2.473	0.164	0.000	0.000	0.003	0.000	0.122	0.000	4.893	2.638	0.124	2.130
10	3/24/1998	ME-4	1.726	0.137	0.000	0.000	0.016	0.000	0.207	0.000	4.893	1.863	0.223	2.807
18	4/1/1998	ME-5	2.968	0.335	0.000	0.000	0.008	0.000	0.230	0.000	4.893	3.303	0.237	1.353
18	4/1/1998	ME-6	2.967	0.362	0.000	0.000	0.014	0.000	0.213	0.000	4.893	3.329	0.227	1.337
18	4/1/1998	ME-7	1.013	2.602	0.202	0.202	0.018	0.000	0.226	0.000	4.893	4.019	0.243	0.630
18	4/1/1998	ME-8	0.661	0.996	1.802	1.802	0.028	0.000	0.223	0.000	4.893	5.262	0.251	-0.620
20	4/3/1998	ME-5	0.757	2.020	0.147	0.147	0.000	0.000	0.273	0.000	4.893	3.071	0.273	1.548
20	4/3/1998	ME-5	1.547	1.240	0.295	0.295	0.009	0.000	0.262	0.000	4.893	3.378	0.271	1.244
20	4/3/1998	ME-6	1.605	1.714	0.063	0.063	0.000	0.000	0.255	0.000	4.893	3.445	0.255	1.192
20	4/3/1998	ME-6	0.294	0.547	1.807	1.807	0.000	0.000	0.256	0.000	4.893	4.453	0.256	0.183
35	4/18/1998	ME-7	0.481	1.455	0.110	0.110	0.003	0.000	0.265	0.000	4.893	2.155	0.268	2.469
35	4/18/1998	ME-7	1.141	1.531	0.431	0.431	0.008	0.000	0.256	0.000	4.893	3.534	0.264	1.094
35	4/18/1998	ME-8	1.129	1.465	0.458	0.458	0.004	0.012	0.285	0.000	4.893	3.509	0.301	1.082
35	4/18/1998	ME-8	0.425	1.019	2.238	2.238	0.008	0.013	0.255	0.000	4.893	5.918	0.276	-1.302
56	5/9/1998	ME-9	0.741	0.949	0.622	0.622	0.000	0.000	0.200	0.000	4.893	2.935	0.200	1.758
56	5/9/1998	ME-9	1.710	0.332	0.000	0.000	0.001	0.002	0.196	0.000	4.893	2.042	0.199	2.651
56	5/9/1998	ME-10	0.456	1.539	0.822	0.822	0.002	0.018	0.187	0.000	4.893	3.638	0.208	1.047
56	5/9/1998	ME-10	0.227	0.674	1.921	1.921	0.003	0.011	0.189	0.000	4.893	4.744	0.203	-0.054
92	6/14/1998	ME-11	0.108	0.413	1.721	1.721	0.023	0.000	0.217	0.000	4.893	3.963	0.241	0.688
92	6/14/1998	ME-12	0.042	0.000	2.519	2.519	0.012	0.000	0.227	0.000	4.893	5.081	0.239	-0.428
128	7/20/1998	ME-13	4.033	0.312	0.000	0.000	0.000	0.000	0.000	0.014	4.893	4.345	0.015	0.533

Days from Creation	*GC Analysis Date*	*Sample ID*	*PCE Liquid phase mass (μmol)*	*TCE Liquid phase mass (μmol)*	*cis-DCE Liquid phase mass (μmol)*	*VC Liquid phase mass (μmol)*	*PCE Gas Phase mass (μmol)*	*TCE Gas Phase mass (μmol)*	*cis-DCE Gas Phase mass (μmol)*	*VC Gas Phase mass (μmol)*	*PCE Initial liquid mass (μmol)*	*Total Liquid mass (μmol)*	*Total Gas Mass (μmol)*	*Unaccounted for mass (μmol)*
128	7/20/1998	ME-13	3.418	0.275	0.000	0.000	0.000	0.000	0.000	0.014	4.893	3.693	0.014	1.186
128	7/20/1998	ME-14	3.852	1.297	0.000	0.000	0.001	0.000	0.000	0.007	4.893	5.149	0.008	-0.265
128	7/20/1998	ME-14	3.894	0.420	0.000	0.000	0.000	0.000	0.000	0.007	4.893	4.314	0.007	0.572
129	7/21/1998	ME-15	3.254	0.302	0.000	0.000	0.000	0.000	0.000	0.011	4.893	3.556	0.011	1.326
129	7/21/1998	ME-15	3.292	0.213	0.000	0.000	0.000	0.000	0.000	0.008	4.893	3.506	0.008	1.379
134	7/26/1998	ME-16	2.878	0.327	0.000	0.000	0.024	0.000	0.000	0.071	4.893	3.205	0.095	1.592
134	7/26/1998	ME-16	2.570	0.358	0.000	0.000	0.018	0.000	0.000	0.075	4.893	2.928	0.093	1.871
162	8/23/1998	ME-17	2.045	0.215	0.000	0.000	0.000	0.000	0.000	0.005	4.893	2.259	0.005	2.628
162	8/23/1998	ME-17	3.877	0.404	0.000	0.000	0.005	0.000	0.000	0.005	4.893	4.281	0.010	0.602
169	8/30/1998	ME-18	3.104	0.298	0.000	0.000	0.007	0.000	0.000	0.004	4.893	3.402	0.011	1.480
169	8/30/1998	ME-18	3.931	0.188	0.000	0.000	0.004	0.000	0.000	0.004	4.893	4.119	0.008	0.766
183	9/13/1998	ME-19	2.937	0.598	0.000	0.000	0.000	0.000	0.000	0.006	4.893	3.535	0.006	1.352
183	9/13/1998	ME-19	3.271	0.136	0.000	0.000	0.006	0.000	0.000	0.006	4.893	3.407	0.012	1.473
192	9/22/1998	ME-20	3.297	0.215	0.000	0.000	0.000	0.000	0.243	0.007	4.893	3.512	0.250	1.130
192	9/22/1998	ME-20	3.698	0.202	0.000	0.000	0.000	0.000	0.239	0.006	4.893	3.900	0.245	0.748
192	9/22/1998	ME-20	1.787	0.460	0.000	0.000	0.000	0.000	0.249	0.002	4.893	2.247	0.252	2.394
192	9/22/1998	ME-21	1.972	0.139	0.000	0.000	0.000	0.000	0.000	0.017	4.893	2.111	0.017	2.764
192	9/22/1998	ME-21	5.120	0.210	0.000	0.000	0.000	0.000	0.000	0.017	4.893	5.330	0.017	-0.455
192	9/22/1998	ME-21	3.143	0.120	0.000	0.000	0.000	0.002	0.000	0.015	4.893	3.262	0.017	1.613
334	2/11/1999	ME-23	3.380	0.343	0.000	0.000	0.002	0.000	0.000	0.005	4.893	3.724	0.007	1.162
334	2/11/1999	ME-23	3.345	0.169	0.000	0.000	0.000	0.000	0.000	0.005	4.893	3.514	0.005	1.373
334	2/11/1999	ME-24	2.818	0.278	0.002	0.002	0.000	0.000	0.197	0.000	4.893	3.101	0.197	1.594
334	2/11/1999	ME-24	2.859	0.296	0.000	0.000	0.000	0.000	0.195	0.000	4.893	3.154	0.195	1.543

Table A.4:

MI Microcosm Series Chlorinated Solvent Data

Days from Creation	*GC Analysis Date*	*Sample ID*	*1,1 DCE Area*	*1,1 DCE Conc (mg/L)*	*trans DCE Area*	*trans DCE Conc (mg/L)*	*cis-DCE Area*	*cis-DCE Conc (mg/L)*	*TCE Area*	*TCE Conc (mg/L)*	*PCE Area*	*PCE Conc (mg/L)*
2	6/14/1998	MIC-1	1651	0.00	881	0.00	1769	0.00	2387	0.00	423727	4.78
2	6/14/1998	MIC-1	822	0.00	614	0.00	994	0.00	8313	0.00	438614	4.94
4	6/16/1998	MIC-2	2709	0.00	1693	0.00	5613	0.00	4349	0.00	261187	2.93
4	6/16/1998	MIC-2	3379	0.00	1042	0.00	4928	0.00	4186	0.00	356351	4.01
6	6/18/1998	MIC-3	4690	0.00	585	0.00	2801	0.00	1481	0.00	451782	5.09
6	6/18/1998	MIC-3	1062	0.00	1239	0.00	2447	0.00	987	0.00	434048	4.89
9	6/21/1998	MIC-4	5460	0.00	653	0.00	4699	0.00	2402	0.00	654912	7.40
9	6/21/1998	MIC-4	4515	0.00	539	0.00	1464	0.00	1323	0.00	634919	7.17
9	6/21/1998	MIC-4	3670	0.00	1570	0.00	3627	0.00	5264	0.00	606437	6.85
25	7/7/1998	MIC-5	308	0.00	--	0.00	--	0.00	6227	0.00	567834	6.41
25	7/7/1998	MIC-5	817	0.00	--	0.00	--	0.00	12937	0.01	702533	7.94
25	7/7/1998	MIC-5	684	0.00	--	0.00	--	0.00	12892	0.01	671215	7.58
93	9/13/1998	MIC-6	1244	0.00	738	0.00	1440	0.00	3921	0.00	611956	6.91
93	9/13/1998	MIC-6	3669	0.00	4008	0.00	4060	0.00	8875	0.00	616070	6.96
107	9/27/1998	MIC-7	3928	0.00	3096	0.00	2697	0.00	13903	0.02	756578	8.55
107	9/27/1998	MIC-7	1267	0.00	702	0.00	1092	0.00	8341	0.00	771452	8.72
107	9/27/1998	MIC-7	4778	0.00	4337	0.00	5229	0.00	10593	0.00	700863	7.92
107	9/27/1998	MIC-7	1420	0.00	712	0.00	1367	0.00	9884	0.00	676162	7.64
258	2/25/1999	MIC-13	845	0.00	--	0.00	2876	0.00	1191	0.00	279252	3.14
258	2/25/1999	MIC-13	599	0.00	--	0.00	3291	0.00	1004	0.00	283670	3.19
2	6/14/1998	BMIC-1	775	0.00	273	0.00	1267	0.00	1937	0.00	200134	2.24
4	6/16/1998	BMIC-2	1582	0.00	503	0.00	--	0.00	4593	0.00	441597	4.98
4	6/16/1998	BMIC-2	1739	0.00	1176	0.00	1506	0.00	2705	0.00	401725	4.53
9	6/21/1998	BMIC-3	2866	0.00	3318	0.00	2741	0.00	1620	0.00	647186	7.31
9	6/21/1998	BMIC-3	7415	0.00	1649	0.00	6705	0.00	811	0.00	553319	6.25

Days from Creation	*GC Analysis Date*	*Sample ID*	*1,1 DCE Area*	*1,1 DCE Conc (mg/L)*	*trans DCE Area*	*trans DCE Conc (mg/L)*	*cis-DCE Area*	*cis-DCE Conc (mg/L)*	*TCE Area*	*TCE Conc (mg/L)*	*PCE Area*	*PCE Conc (mg/L)*
30	7/12/1998	BMIC-4	--	0.00	--	0.00	--	0.00	28110	0.13	568176	6.41
30	7/12/1998	BMIC-4	1442	0.00	1098	0.00	1306	0.00	25192	0.11	573372	6.47
83	9/3/1998	BMIC-5	1637	0.00	833	0.00	2281	0.00	3229	0.00	788953	8.92
83	9/3/1998	BMIC-5	2238	0.00	794	0.00	1698	0.00	3950	0.00	800859	9.06
2	6/14/1998	MI-1	1169	0.00	1741	0.00	2105	0.00	3167	0.00	770639	8.71
2	6/14/1998	MI-1	819	0.00	936	0.00	2122	0.00	2566	0.00	715014	8.08
2	6/14/1998	MI-1	1567	0.00	1295	0.00	1415	0.00	3762	0.00	759935	8.59
4	6/16/1998	MI-2	--	0.00	2344	0.00	1693	0.00	2634	0.00	702727	7.94
4	6/16/1998	MI-2	399	0.00	--	0.00	447	0.00	3098	0.00	768437	8.69
6	6/18/1998	MI-3	--	0.00	--	0.00	--	0.00	3954	0.00	790026	8.93
6	6/18/1998	MI-3	--	0.00	984	0.00	385	0.00	3804	0.00	769237	8.70
9	6/21/1998	MI-4	2152	0.00	1111	0.00	2815	0.00	13043	0.01	771132	8.72
9	6/21/1998	MI-4	1184	0.00	1740	0.00	--	0.00	9305	0.00	704983	7.97
9	6/21/1998	MI-4	588	0.00	842	0.00	1254	0.00	13059	0.01	797511	9.02
19	7/1/1998	MI-5	2649	0.00	--	0.00	2121	0.00	57762	0.38	646807	7.31
19	7/1/1998	MI-5	--	0.00	--	0.00	2948	0.00	57896	0.38	659943	7.46
19	7/1/1998	MI-5	--	0.00	--	0.00	3782	0.00	62183	0.42	702596	7.94
23	7/5/1998	MI-6	2292	0.00	--	0.00	31386	0.06	207944	1.62	529614	5.98
23	7/5/1998	MI-6	1653	0.00	1095	0.00	30717	0.06	193025	1.50	511890	5.78
23	7/5/1998	MI-6	2356	0.00	216	0.00	31287	0.06	196559	1.53	511265	5.77
25	7/7/1998	MI-7	1978	0.00	--	0.00	27452	0.04	161815	1.24	566657	6.40
25	7/7/1998	MI-7	--	0.00	--	0.00	27743	0.04	174366	1.34	584894	6.60
25	7/7/1998	MI-7	739	0.00	478	0.00	29598	0.05	195338	1.52	637108	7.20
30	7/12/1998	MI-8	1808	0.00	--	0.00	72430	0.33	242805	1.91	489421	5.52
30	7/12/1998	MI-8	--	0.00	--	0.00	64945	0.28	208247	1.62	458316	5.17
30	7/12/1998	MI-8	2990	0.00	--	0.00	73516	0.33	243814	1.92	540712	6.10

Days from Creation	*GC Analysis Date*	*Sample ID*	*1,1 DCE Area*	*1,1 DCE Conc (mg/L)*	*trans DCE Area*	*trans DCE Conc (mg/L)*	*cis-DCE Area*	*cis-DCE Conc (mg/L)*	*TCE Area*	*TCE Conc (mg/L)*	*PCE Area*	*PCE Conc (mg/L)*
34	7/16/1998	MI-9	522	0.00	10596	0.00	225042	1.31	271728	2.15	323085	3.63
34	7/16/1998	MI-9	517	0.00	1235	0.00	207847	1.20	252033	1.99	308593	3.47
38	7/20/1998	MI-10	2458	0.00	14043	0.00	332108	2.00	244519	1.92	223876	2.51
38	7/20/1998	MI-10	3494	0.00	12525	0.00	330170	1.99	235001	1.84	214858	2.40
74	8/25/1998	MI-11	2008	0.00	2564	0.00	910303	5.74	2824	0.00	5902	0.03
79	8/30/1998	MI-12	6474	0.00	5538	0.00	977784	6.17	12747	0.01	6368	0.04
79	8/30/1998	MI-12	7240	0.00	7869	0.00	958260	6.05	17252	0.04	4319	0.02
93	9/13/1998	MI-13	1073	0.00	582	0.00	987595	6.24	8421	0.00	3428	0.01
93	9/13/1998	MI-13	1043	0.00	672	0.00	981091	6.19	7356	0.00	2419	0.00
93	9/13/1998	MI-14	873	0.00	745	0.00	939904	5.93	7941	0.00	3254	0.00
93	9/13/1998	MI-14	--	0.00	651	0.00	951442	6.00	3674	0.00	2402	0.00
93	9/13/1998	MI-15	2114	0.00	1001	0.00	909964	5.73	1297	0.00	11718	0.10
93	9/13/1998	MI-15	902	0.00	816	0.00	912626	5.75	1782	0.00	986	0.00
107	9/27/1998	MI-16	999	0.00	468	0.00	934833	5.90	12563	0.01	1832	0.00
107	9/27/1998	MI-16	1381	0.00	1974	0.00	937502	5.91	7079	0.00	1743	0.00
107	9/27/1998	MI-17	1731	0.00	807	0.00	888252	5.59	21613	0.08	1622	0.00
107	9/27/1998	MI-17	1505	0.00	856	0.00	851383	5.36	3624	0.00	1584	0.00
111	10/1/1998	MI-18	1157	0.00	778	0.00	970746	6.13	9488	0.00	1461	0.00
111	10/1/1998	MI-18	1437	0.00	1810	0.00	953975	6.02	2349	0.00	2131	0.00
111	10/1/1998	MI-19	906	0.00	1150	0.00	899252	5.67	3553	0.00	3168	0.00
111	10/1/1998	MI-19	879	0.00	675	0.00	898903	5.66	11086	0.00	2725	0.00
111	10/1/1998	MI-20	1129	0.00	834	0.00	952349	6.01	730	0.00	1531	0.00
111	10/1/1998	MI-20	1343	0.00	1328	0.00	918587	5.79	3058	0.00	14997	0.14
111	10/1/1998	MI-21	2895	0.00	3382	0.00	879215	5.54	2615	0.00	25756	0.26
111	10/1/1998	MI-21	1216	0.00	1348	0.00	884467	5.57	3560	0.00	29074	0.30

Days from Creation	*GC Analysis Date*	*Sample ID*	*1,1 DCE Area*	*1,1 DCE Conc (mg/L)*	*trans DCE Area*	*trans DCE Conc (mg/L)*	*cis-DCE Area*	*cis-DCE Conc (mg/L)*	*TCE Area*	*TCE Conc (mg/L)*	*PCE Area*	*PCE Conc (mg/L)*
260	2/27/1999	MI-26	426	0.00	781	0.00	686595	4.29	1667	0.00	15921	0.15
260	2/27/1999	MI-27	--	0.00	1166	0.00	650274	4.06	1240	0.00	13027	0.11
2	6/14/1998	BMI-1	926	0.00	665	0.00	676	0.00	1222	0.00	665552	7.52
4	6/16/1998	BMI-2	1306	0.00	521	0.00	1807	0.00	8506	0.00	709489	8.02
6	6/18/1998	BMI-3	2578	0.00	1230	0.00	760	0.00	3169	0.00	788846	8.92
6	6/18/1998	BMI-3	3692	0.00	4119	0.00	1107	0.00	3061	0.00	771808	8.73
25	7/7/1998	BMI-5	629	0.00	--	0.00	10822	0.00	121257	0.90	620311	7.01
25	7/7/1998	BMI-5	859	0.00	--	0.00	11437	0.00	123921	0.93	648415	7.33
25	7/7/1998	BMI-5	505	0.00	778	0.00	9856	0.00	106339	0.78	567548	6.41
30	7/12/1998	BMI-6	493	0.00	3736	0.00	37821	0.10	177170	1.37	570852	6.44
30	7/12/1998	BMI-6	700	0.00	--	0.00	39575	0.12	181881	1.41	580816	6.56
30	7/12/1998	BMI-6	4610	0.00	--	0.00	33739	0.08	155117	1.18	490949	5.54
83	9/3/1998	BMI-8	1765	0.00	490	0.00	989395	6.25	2056	0.00	3223	0.00
83	9/3/1998	BMI-8	1899	0.00	830	0.00	979139	6.18	3066	0.00	5507	0.03
93	9/13/1998	BMI-9	1554	0.00	835	0.00	942958	5.95	3369	0.00	4917	0.02

Table A.5:

MI Microcosm Series Chlorinated Solvent Partitioning Analysis and Calculations

Days from Creation	*GC Analysis Date*	*Sample ID*	*PCE Liquid phase mass (μmol)*	*TCE Liquid phase mass (μmol)*	*cis-DCE Liquid phase mass (μmol)*	*PCE Gas Phase mass (μmol)*	*TCE Gas Phase mass (μmol)*	*cis-DCE Gas Phase mass (μmol)*	*PCE Initial liquid mass (μmol)*	*Total Liquid mass (μmol)*	*Total Gas Mass (μmol)*	*Unaccounted for mass (μmol)*
2	6/14/1998	MIC-1	2.879	0.000	0.000	0.558	0.000	0.000	4.893	2.879	0.558	1.455
2	6/14/1998	MIC-1	2.981	0.000	0.000	0.578	0.000	0.000	4.893	2.981	0.578	1.333
4	6/16/1998	MIC-2	1.767	0.000	0.000	0.342	0.000	0.000	4.893	1.767	0.342	2.783
4	6/16/1998	MIC-2	2.418	0.000	0.000	0.469	0.000	0.000	4.893	2.418	0.469	2.006
6	6/18/1998	MIC-3	3.071	0.000	0.000	0.595	0.000	0.000	4.893	3.071	0.595	1.226
6	6/18/1998	MIC-3	2.950	0.000	0.000	0.572	0.000	0.000	4.893	2.950	0.572	1.371
9	6/21/1998	MIC-4	4.462	0.000	0.000	0.865	0.000	0.000	4.893	4.462	0.865	-0.434
9	6/21/1998	MIC-4	4.325	0.000	0.000	0.838	0.000	0.000	4.893	4.325	0.838	-0.270
9	6/21/1998	MIC-4	4.130	0.000	0.000	0.800	0.000	0.000	4.893	4.130	0.800	-0.038
25	7/7/1998	MIC-5	3.866	0.000	0.000	0.749	0.000	0.000	4.893	3.866	0.749	0.278
25	7/7/1998	MIC-5	4.787	0.007	0.000	0.928	0.001	0.000	4.893	4.795	0.929	-0.830
25	7/7/1998	MIC-5	4.573	0.007	0.000	0.886	0.001	0.000	4.893	4.580	0.887	-0.574
93	9/13/1998	MIC-6	4.168	0.000	0.000	0.808	0.000	0.000	4.893	4.168	0.808	-0.083
93	9/13/1998	MIC-6	4.196	0.000	0.000	0.813	0.000	0.000	4.893	4.196	0.813	-0.116
107	9/27/1998	MIC-7	5.157	0.013	0.000	0.999	0.001	0.000	4.893	5.170	1.001	-1.279
107	9/27/1998	MIC-7	5.259	0.000	0.000	1.019	0.000	0.000	4.893	5.259	1.019	-1.386
107	9/27/1998	MIC-7	4.776	0.000	0.000	0.925	0.000	0.000	4.893	4.776	0.925	-0.809
107	9/27/1998	MIC-7	4.607	0.000	0.000	0.893	0.000	0.000	4.893	4.607	0.893	-0.607
258	2/25/1999	MIC-13	1.891	0.000	0.000	0.366	0.000	0.000	4.893	1.891	0.366	2.635
258	2/25/1999	MIC-13	1.921	0.000	0.000	0.372	0.000	0.000	4.893	1.921	0.372	2.599
2	6/14/1998	BMIC-1	1.349	0.000	0.000	0.261	0.000	0.000	4.893	1.349	0.261	3.282
4	6/16/1998	BMIC-2	3.002	0.000	0.000	0.582	0.000	0.000	4.893	3.002	0.582	1.309
4	6/16/1998	BMIC-2	2.729	0.000	0.000	0.529	0.000	0.000	4.893	2.729	0.529	1.635
9	6/21/1998	BMIC-3	4.409	0.000	0.000	0.854	0.000	0.000	4.893	4.409	0.854	-0.371

Days from Creation	*GC Analysis Date*	*Sample ID*	*PCE Liquid phase mass (μmol)*	*TCE Liquid phase mass (μmol)*	*cis-DCE Liquid phase mass (μmol)*	*PCE Gas Phase mass (μmol)*	*TCE Gas Phase mass (μmol)*	*cis-DCE Gas Phase mass (μmol)*	*PCE Initial liquid mass (μmol)*	*Total Liquid mass (μmol)*	*Total Gas Mass (μmol)*	*Unaccounted for mass (μmol)*
9	6/21/1998	BMIC-3	3.766	0.000	0.000	0.730	0.000	0.000	4.893	3.766	0.730	0.396
30	7/12/1998	BMIC-4	3.868	0.102	0.000	0.750	0.012	0.000	4.893	3.970	0.761	0.161
30	7/12/1998	BMIC-4	3.904	0.084	0.000	0.756	0.010	0.000	4.893	3.988	0.766	0.139
83	9/3/1998	BMIC-5	5.379	0.000	0.000	1.042	0.000	0.000	4.893	5.379	1.042	-1.529
83	9/3/1998	BMIC-5	5.460	0.000	0.000	1.058	0.000	0.000	4.893	5.460	1.058	-1.626
2	6/14/1998	MI-1	5.254	0.000	0.000	1.018	0.000	0.000	4.893	5.254	1.018	-1.379
2	6/14/1998	MI-1	4.873	0.000	0.000	0.944	0.000	0.000	4.893	4.873	0.944	-0.925
2	6/14/1998	MI-1	5.180	0.000	0.000	1.004	0.000	0.000	4.893	5.180	1.004	-1.292
4	6/16/1998	MI-2	4.789	0.000	0.000	0.928	0.000	0.000	4.893	4.789	0.928	-0.824
4	6/16/1998	MI-2	5.239	0.000	0.000	1.015	0.000	0.000	4.893	5.239	1.015	-1.361
6	6/18/1998	MI-3	5.386	0.000	0.000	1.044	0.000	0.000	4.893	5.386	1.044	-1.537
6	6/18/1998	MI-3	5.244	0.000	0.000	1.016	0.000	0.000	4.893	5.244	1.016	-1.368
9	6/21/1998	MI-4	5.257	0.008	0.000	1.019	0.001	0.000	4.893	5.265	1.020	-1.392
9	6/21/1998	MI-4	4.804	0.000	0.000	0.931	0.000	0.000	4.893	4.804	0.931	-0.843
9	6/21/1998	MI-4	5.437	0.008	0.000	1.054	0.001	0.000	4.893	5.445	1.055	-1.607
19	7/1/1998	MI-5	4.406	0.289	0.000	0.854	0.033	0.000	4.893	4.695	0.887	-0.689
19	7/1/1998	MI-5	4.496	0.290	0.000	0.871	0.033	0.000	4.893	4.786	0.904	-0.797
19	7/1/1998	MI-5	4.788	0.317	0.000	0.928	0.036	0.000	4.893	5.105	0.964	-1.176
23	7/5/1998	MI-6	3.604	1.234	0.065	0.698	0.140	0.003	4.893	4.903	0.841	-0.851
23	7/5/1998	MI-6	3.483	1.140	0.060	0.675	0.129	0.003	4.893	4.683	0.806	-0.597
23	7/5/1998	MI-6	3.479	1.162	0.064	0.674	0.131	0.003	4.893	4.705	0.808	-0.621
25	7/7/1998	MI-7	3.858	0.944	0.039	0.748	0.107	0.002	4.893	4.840	0.856	-0.803
25	7/7/1998	MI-7	3.982	1.023	0.041	0.772	0.116	0.002	4.893	5.046	0.889	-1.042
25	7/7/1998	MI-7	4.340	1.155	0.053	0.841	0.131	0.002	4.893	5.547	0.974	-1.629
30	7/12/1998	MI-8	3.329	1.453	0.338	0.645	0.164	0.014	4.893	5.120	0.824	-1.052

Days from Creation	*GC Analysis Date*	*Sample ID*	*PCE Liquid phase mass (μmol)*	*TCE Liquid phase mass (μmol)*	*cis-DCE Liquid phase mass (μmol)*	*PCE Gas Phase mass (μmol)*	*TCE Gas Phase mass (μmol)*	*cis-DCE Gas Phase mass (μmol)*	*PCE Initial liquid mass (μmol)*	*Total Liquid mass (μmol)*	*Total Gas Mass (μmol)*	*Unaccounted for mass (μmol)*
30	7/12/1998	MI-8	3.116	1.236	0.288	0.604	0.140	0.012	4.893	4.640	0.756	-0.504
30	7/12/1998	MI-8	3.680	1.459	0.345	0.713	0.165	0.015	4.893	5.485	0.893	-1.485
34	7/16/1998	MI-9	2.191	1.635	1.354	0.425	0.185	0.058	4.893	5.180	0.667	-0.955
34	7/16/1998	MI-9	2.091	1.511	1.240	0.405	0.171	0.053	4.893	4.843	0.629	-0.579
38	7/20/1998	MI-10	1.512	1.464	2.067	0.293	0.166	0.088	4.893	5.043	0.547	-0.697
38	7/20/1998	MI-10	1.450	1.404	2.055	0.281	0.159	0.088	4.893	4.909	0.528	-0.544
74	8/25/1998	MI-11	0.020	0.000	5.918	0.004	0.000	0.253	4.893	5.938	0.257	-1.302
79	8/30/1998	MI-12	0.023	0.006	6.367	0.004	0.001	0.272	4.893	6.396	0.277	-1.781
79	8/30/1998	MI-12	0.009	0.034	6.237	0.002	0.004	0.266	4.893	6.280	0.272	-1.660
93	9/13/1998	MI-13	0.003	0.000	6.432	0.001	0.000	0.275	4.893	6.435	0.275	-1.818
93	9/13/1998	MI-13	0.000	0.000	6.389	0.000	0.000	0.273	4.893	6.389	0.273	-1.769
93	9/13/1998	MI-14	0.002	0.000	6.115	0.000	0.000	0.261	4.893	6.117	0.262	-1.486
93	9/13/1998	MI-14	0.000	0.000	6.192	0.000	0.000	0.265	4.893	6.192	0.265	-1.564
93	9/13/1998	MI-15	0.060	0.000	5.915	0.012	0.000	0.253	4.893	5.975	0.264	-1.347
93	9/13/1998	MI-15	0.000	0.000	5.933	0.000	0.000	0.253	4.893	5.933	0.253	-1.294
107	9/27/1998	MI-16	0.000	0.005	6.081	0.000	0.001	0.260	4.893	6.086	0.260	-1.453
107	9/27/1998	MI-16	0.000	0.000	6.099	0.000	0.000	0.261	4.893	6.099	0.261	-1.467
107	9/27/1998	MI-17	0.000	0.062	5.771	0.000	0.007	0.247	4.893	5.832	0.254	-1.193
107	9/27/1998	MI-17	0.000	0.000	5.525	0.000	0.000	0.236	4.893	5.525	0.236	-0.869
111	10/1/1998	MI-18	0.000	0.000	6.320	0.000	0.000	0.270	4.893	6.320	0.270	-1.698
111	10/1/1998	MI-18	0.000	0.000	6.208	0.000	0.000	0.265	4.893	6.208	0.265	-1.581
111	10/1/1998	MI-19	0.001	0.000	5.844	0.000	0.000	0.250	4.893	5.845	0.250	-1.203
111	10/1/1998	MI-19	0.000	0.000	5.842	0.000	0.000	0.250	4.893	5.842	0.249	-1.198
111	10/1/1998	MI-20	0.000	0.000	6.198	0.000	0.000	0.265	4.893	6.198	0.265	-1.570

Days from Creation	*GC Analysis Date*	*Sample ID*	*PCE Liquid phase mass (μmol)*	*TCE Liquid phase mass (μmol)*	*cis-DCE Liquid phase mass (μmol)*	*PCE Gas Phase mass (μmol)*	*TCE Gas Phase mass (μmol)*	*cis-DCE Gas Phase mass (μmol)*	*PCE Initial liquid mass (μmol)*	*Total Liquid mass (μmol)*	*Total Gas Mass (μmol)*	*Unaccounted for mass (μmol)*
111	10/1/1998	MI-20	0.082	0.000	5.973	0.016	0.000	0.255	4.893	6.055	0.271	-1.434
111	10/1/1998	MI-21	0.156	0.000	5.711	0.030	0.000	0.244	4.893	5.866	0.274	-1.248
111	10/1/1998	MI-21	0.179	0.000	5.746	0.035	0.000	0.245	4.893	5.924	0.280	-1.312
260	2/27/1999	MI-26	0.089	0.000	4.428	0.017	0.000	0.189	4.893	4.516	0.206	0.170
260	2/27/1999	MI-27	0.069	0.000	4.186	0.013	0.000	0.179	4.893	4.255	0.192	0.446
2	6/14/1998	BMI-1	4.534	0.000	0.000	0.879	0.000	0.000	4.893	4.534	0.879	-0.521
4	6/16/1998	BMI-2	4.835	0.000	0.000	0.937	0.000	0.000	4.893	4.835	0.937	-0.880
6	6/18/1998	BMI-3	5.378	0.000	0.000	1.042	0.000	0.000	4.893	5.378	1.042	-1.528
6	6/18/1998	BMI-3	5.262	0.000	0.000	1.020	0.000	0.000	4.893	5.262	1.020	-1.389
25	7/7/1998	BMI-5	4.225	0.688	0.000	0.819	0.078	0.000	4.893	4.913	0.897	-0.917
25	7/7/1998	BMI-5	4.417	0.705	0.000	0.856	0.080	0.000	4.893	5.122	0.936	-1.166
25	7/7/1998	BMI-5	3.864	0.595	0.000	0.749	0.067	0.000	4.893	4.458	0.816	-0.382
30	7/12/1998	BMI-6	3.886	1.040	0.108	0.753	0.118	0.005	4.893	5.034	0.875	-1.017
30	7/12/1998	BMI-6	3.954	1.070	0.119	0.766	0.121	0.005	4.893	5.144	0.892	-1.144
30	7/12/1998	BMI-6	3.339	0.901	0.081	0.647	0.102	0.003	4.893	4.322	0.753	-0.182
83	9/3/1998	BMI-8	0.002	0.000	6.444	0.000	0.000	0.275	4.893	6.446	0.276	-1.829
83	9/3/1998	BMI-8	0.017	0.000	6.376	0.003	0.000	0.272	4.893	6.393	0.276	-1.777
93	9/13/1998	BMI-9	0.013	0.000	6.135	0.003	0.000	0.262	4.893	6.148	0.265	-1.520

Table A.6:

MO Microcosm Series Chlorinated Solvent Data

Days from Creation	*GC Analysis Date*	*Microcosm ID*	*Bottle ID*	*VC Area*	*VC Conc (mg/L)*	*1,1 DCE Area*	*1,1 DCE Conc (mg/L)*	*trans DCE Area*	*trans DCE Conc (mg/L)*	*cis-DCE Area*	*cis-DCE Conc (mg/L)*	*TCE Area*	*TCE Conc (mg/L)*	*PCE Area*	*PCE Conc (mg/L)*
3	11/4/1998	ABIO-1	MOC-1	--	0.00	461	0.00	--	0.00	845	0.00	8266	0.00	721575	8.16
3	11/4/1998	ABIO-1	MOC-1	--	0.00	474	0.00	--	0.00	--	0.00	9547	0.00	745509	8.43
3	11/4/1998	ABIO-1	MOC-1	--	0.00	637	0.00	--	0.00	--	0.00	11667	0.00	727234	8.22
35	12/6/1998	ABIO-2	MOC-2	--	0.00	1868	0.00	1609	0.00	1286	0.00	5374	0.00	760115	8.59
35	12/6/1998	ABIO-2	MOC-2	288	0.00	706	0.00	--	0.00	--	0.00	3213	0.00	733793	8.29
81	1/21/1999	AIR-1	MO-37	--	0.00	1892	0.00	1677	0.00	751415	4.71	1795	-0.08	3986	0.01
95	2/4/1999	AIR-1	MO-37	1000	0.01	1107	0.00	887	0.00	699502	4.38	45471	0.28	5306	0.03
95	2/4/1999	AIR-1	MO-37	1301	0.01	1854	0.00	1230	0.00	704367	4.41	34096	0.18	8647	0.06
108	2/17/1999	AIR-1	MO-37	727	0.00	451	0.00	538	0.00	201847	1.16	3640	0.00	15286	0.14
81	1/21/1999	AIR-2	MO-38	--	0.00	1416	0.00	1496	0.00	785187	4.93	--	0.00	3892	0.01
95	2/4/1999	AIR-2	MO-38	1202	0.01	1218	0.00	1162	0.00	720743	4.51	34325	0.19	5546	0.03
108	2/17/1999	AIR-2	MO-38	--	0.00	1331	0.00	1497	0.00	661037	4.13	2624	0.00	7815	0.05
81	1/21/1999	AIR-3	MO-39	--	0.00	1415	0.00	850	0.00	776954	4.88	286	0.00	2717	0.00
95	2/4/1999	AIR-3	MO-39	1330	0.01	1368	0.00	347	0.00	707187	4.43	7861	0.00	4871	0.02
97	2/6/1999	AIR-4	MO-42	570	0.00	1341	0.00	1365	0.00	557244	3.46	10737	0.00	2419	0.00
119	2/28/1999	AIR-4	MO-42	--	0.00	420	0.00	1157	0.00	498718	3.08	21749	0.08	661292	7.47
119	2/28/1999	AIR-4	MO-42	--	0.00	310	0.00	1098	0.00	495313	3.06	20962	0.08	634782	7.17
97	2/6/1999	AIR-5	MO-43	553	0.00	947	0.00	506	0.00	666520	4.16	5487	0.00	1306	0.00
108	2/17/1999	AIR-5	MO-43	408	0.00	--	0.00	1228	0.00	514848	3.18	2694	0.00	9782	0.08
126	3/7/1999	AIR-5	MO-43	406	0.00	--	0.00	318	0.00	499145	3.08	136387	1.03	501282	5.66
97	2/6/1999	AIR-6	MO-44	660	0.00	939	0.00	468	0.00	717686	4.49	3041	0.00	1000	0.00
108	2/17/1999	AIR-6	MO-44	606	0.00	--	0.00	771	0.00	430673	2.64	1316	0.00	4654	0.02
108	2/17/1999	AIR-6	MO-44	603	0.00	--	0.00	378	0.00	419480	2.57	2254	0.00	3385	0.00
126	3/7/1999	AIR-6	MO-44	497	0.00	404	0.00	--	0.00	423615	2.59	92009	0.66	562905	6.35
126	3/7/1999	AIR-6	MO-44	560	0.00	--	0.00	253	0.00	419421	2.57	92310	0.67	552035	6.23
81	1/21/1999	AIR-7	MO-40	--	0.00	1434	0.00	810	0.00	772811	4.85	265	0.00	3131	0.00
95	2/4/1999	AIR-7	MO-40	1281	0.01	1403	0.00	783	0.00	670045	4.19	13283	0.01	4447	0.02
119	2/28/1999	AIR-7	MO-40	686	0.00	--	0.00	279	0.00	334796	2.02	2274	0.00	23281	0.23
119	2/28/1999	AIR-7	MO-40	--	0.00	328	0.00	670	0.00	534323	3.31	1530	0.00	13966	0.12
95	2/4/1999	AIR-8	MO-41	500	0.00	7931	0.00	8453	0.00	695841	4.35	18381	0.05	2485	0.00

Days from Creation	*GC Analysis Date*	*Microcosm ID*	*Bottle ID*	*VC Area*	*VC Conc (mg/L)*	*1,1 DCE Area*	*1,1 DCE Conc (mg/L)*	*trans DCE Area*	*trans DCE Conc (mg/L)*	*cis-DCE Area*	*cis-DCE Conc (mg/L)*	*TCE Area*	*TCE Conc (mg/L)*	*PCE Area*	*PCE Conc (mg/L)*
119	2/28/1999	AIR-8	MO-41	658	0.00	401	0.00	1110	0.00	557575	3.46	547	0.00	3341	0.00
97	2/6/1999	AIR-9	MO-45	428	0.00	1214	0.00	434	0.00	718398	4.50	4470	0.00	1097	0.00
126	3/7/1999	AIR-9	MO-45	492	0.00	1013	0.00	--	0.00	300426	1.80	186152	1.44	485024	5.47
97	2/6/1999	AIR-10	MO-46	2202	0.01	1353	0.00	605	0.00	706172	4.42	39416	0.23	310	0.00
97	2/6/1999	AIR-10	MO-46	730	0.00	987	0.00	330	0.00	705126	4.41	1809	0.00	432	0.00
108	2/17/1999	AIR-10	MO-46	610	0.00	1235	0.00	1180	0.00	464879	2.86	5431	0.00	2101	0.00
126	3/7/1999	AIR-10	MO-46	662	0.00	--	0.00	--	0.00	478432	2.95	141069	1.07	552825	6.24
81	1/21/1999	BES-1	BMO-1	--	0.00	1066	0.00	613	0.00	769450	4.83	888	0.00	1331	0.00
84	1/24/1999	BES-2	BMO-2	--	0.00	1565	0.00	602	0.00	833193	5.24	673	0.00	4326	0.02
98	2/7/1999	BES-3	BMO-3	--	0.00	695	0.00	355	0.00	701879	4.39	3444	0.00	1448	0.00
98	2/7/1999	BES-3	BMO-3	--	0.00	1196	0.00	574	0.00	711972	4.46	2055	0.00	1810	0.00
98	2/7/1999	BES-4	BMO-4	--	0.00	1192	0.00	334	0.00	712506	4.46	2439	0.00	1246	0.00
129	3/10/1999	BES-5	BMO-8	--	0.00	949	0.00	503	0.00	690261	4.32	2829	0.00	1160	0.00
130	3/11/1999	BES-6	BMO-9	--	0.00	1196	0.00	1166	0.00	720217	4.51	4622	0.00	2120	0.00
130	3/11/1999	BES-6	BMO-9	1012	0.01	1003	0.00	1474	0.00	683267	4.27	11237	0.00	1585	0.00
3	11/4/1998	BIO-1	MO-1	--	0.00	--	0.00	--	0.00	--	0.00	10381	0.00	707861	8.00
3	11/4/1998	BIO-1	MO-1	--	0.00	--	0.00	--	0.00	--	0.00	2209	0.00	709791	8.02
3	11/4/1998	BIO-1	MO-1	--	0.00	346	0.00	--	0.00	--	0.00	828	0.00	676160	7.64
118	2/27/1999	BIO-10	MO-10	--	0.00	449	0.00	800	0.00	690043	4.31	48791	0.31	369057	4.15
118	2/27/1999	BIO-10	MO-10	1686	0.01	386	0.00	2252	0.00	700650	4.38	50388	0.32	366188	4.12
119	2/28/1999	BIO-11	MO-11	323	0.00	903	0.00	1213	0.00	712009	4.46	45998	0.28	408652	4.60
119	2/28/1999	BIO-11	MO-11	759	0.00	1052	0.00	1182	0.00	682448	4.27	42950	0.26	381600	4.30
119	2/28/1999	BIO-12	MO-12	985	0.01	980	0.00	1236	0.00	675684	4.22	40619	0.24	456569	5.15
126	3/7/1999	BIO-13	MO-13	--	0.00	1386	0.00	951	0.00	704861	4.41	85739	0.61	7790	0.05
126	3/7/1999	BIO-14	MO-14	908	0.01	607	0.00	987	0.00	660784	4.13	253885	2.00	5468	0.03
9	11/10/1998	BIO-2	MO-2	--	0.00	506	0.00	--	0.00	--	0.00	11559	0.00	678780	7.67
9	11/10/1998	BIO-2	MO-2	--	0.00	958	0.00	--	0.00	606	0.00	13891	0.02	652106	7.37
9	11/10/1998	BIO-2	MO-2	--	0.00	--	0.00	--	0.00	--	0.00	13579	0.01	690789	7.81
9	11/10/1998	BIO-2	MO-2	--	0.00	2039	0.00	--	0.00	--	0.00	15230	0.03	681914	7.71
30	12/1/1998	BIO-3	MO-3	1159	0.01	1949	0.00	687	0.00	123697	0.66	616794	5.00	25009	0.25

Days from Creation	*GC Analysis Date*	*Microcosm ID*	*Bottle ID*	*VC Area*	*VC Conc (mg/L)*	*1,1 DCE Area*	*1,1 DCE Conc (mg/L)*	*trans DCE Area*	*trans DCE Conc (mg/L)*	*cis-DCE Area*	*cis-DCE Conc (mg/L)*	*TCE Area*	*TCE Conc (mg/L)*	*PCE Area*	*PCE Conc (mg/L)*
30	12/1/1998	BIO-3	MO-3	1289	0.01	1728	0.00	801	0.00	128532	0.69	629730	5.11	13736	0.12
32	12/3/1998	BIO-4	MO-4	--	0.00	1067	0.00	779	0.00	95681	0.48	673980	5.47	51425	0.55
32	12/3/1998	BIO-4	MO-4	--	0.00	934	0.00	440	0.00	93112	0.46	658352	5.34	45411	0.48
35	12/6/1998	BIO-5	MO-5	337	0.00	748	0.00	1070	0.00	151845	0.84	661802	5.37	33950	0.35
35	12/6/1998	BIO-5	MO-5	--	0.00	1201	0.00	1105	0.00	160648	0.90	666740	5.41	7735	0.05
35	12/6/1998	BIO-6	MO-6	323	0.00	1093	0.00	--	0.00	66185	0.29	199338	1.55	494173	5.57
35	12/6/1998	BIO-6	MO-6	552	0.00	822	0.00	--	0.00	55632	0.22	175273	1.35	427532	4.82
63	1/3/1999	BIO-7	MO-7	743	0.00	2020	0.00	758	0.00	734846	4.60	2144	0.00	4770	0.02
63	1/3/1999	BIO-7	MO-7	1366	0.01	1626	0.00	827	0.00	696747	4.36	1289	0.00	3362	0.00
116	2/25/1999	BIO-7	MO-7	--	0.00	838	0.00	1532	0.00	651860	4.07	35538	0.20	428297	4.83
116	2/25/1999	BIO-7	MO-7	--	0.00	589	0.00	886	0.00	630260	3.93	31717	0.16	389059	4.38
63	1/3/1999	BIO-8	MO-8	965	0.01	1661	0.00	1474	0.00	718401	4.50	2666	0.00	10729	0.09
116	2/25/1999	BIO-8	MO-8	--	0.00	755	0.00	1322	0.00	637556	3.98	41496	0.25	576512	6.51
116	2/25/1999	BIO-8	MO-8	886	0.00	976	0.00	2373	0.00	651207	4.06	42378	0.25	557764	6.30
116	2/25/1999	BIO-9	MO-9	--	0.00	929	0.00	1131	0.00	595069	3.70	5035	0.00	320244	3.60
35	12/6/1998	HIGH-1	MO-27	--	0.00	1823	0.00	--	0.00	52388	0.20	592014	4.80	118783	1.31
81	1/21/1999	HIGH-1	MO-27	333	0.00	1007	0.00	267	0.00	40710	0.12	398024	3.19	73328	0.80
129	3/10/1999	HIGH-1	MO-27	--	0.00	2427	0.00	734	0.00	272007	1.62	137846	1.04	15966	0.15
42	12/13/1998	HIGH-2	MO-29	643	0.00	2394	0.00	--	0.00	30748	0.06	207754	1.62	525148	5.93
84	1/24/1999	HIGH-2	MO-29	--	0.00	811	0.00	--	0.00	25862	0.03	178489	1.38	396171	4.46
118	2/27/1999	HIGH-2	MO-29	--	0.00	1581	0.00	--	0.00	26232	0.03	157707	1.21	754947	8.53
129	3/10/1999	HIGH-2	MO-29	--	0.00	1911	0.00	470	0.00	34306	0.08	148591	1.13	662419	7.48
42	12/13/1998	HIGH-3	MO-31	--	0.00	2484	0.00	2988	0.00	23957	0.01	180138	1.39	513397	5.79
98	2/7/1999	HIGH-3	MO-31	--	0.00	1317	0.00	--	0.00	25620	0.03	269056	2.13	206112	2.31
122	3/3/1999	HIGH-3	MO-31	--	0.00	2164	0.00	390	0.00	24484	0.02	267368	2.11	663959	7.50
122	3/3/1999	HIGH-3	MO-31	--	0.00	1941	0.00	302	0.00	29517	0.05	268487	2.12	655499	7.41
130	3/11/1999	HIGH-3	MO-31	--	0.00	2322	0.00	385	0.00	30402	0.06	308944	2.46	664856	7.51
84	1/24/1999	HIGH-4	MO-28	--	0.00	1851	0.00	627	0.00	40973	0.12	525207	4.24	158721	1.77
129	3/10/1999	HIGH-4	MO-28	--	0.00	2777	0.00	803	0.00	46499	0.16	366310	2.93	78488	0.86
89	1/29/1999	HIGH-5	MO-32	--	0.00	2493	0.00	630	0.00	46205	0.16	414722	3.33	156536	1.74

Days from Creation	*GC Analysis Date*	*Microcosm ID*	*Bottle ID*	*VC Area*	*VC Conc (mg/L)*	*1,1 DCE Area*	*1,1 DCE Conc (mg/L)*	*trans DCE Area*	*trans DCE Conc (mg/L)*	*cis-DCE Area*	*cis-DCE Conc (mg/L)*	*TCE Area*	*TCE Conc (mg/L)*	*PCE Area*	*PCE Conc (mg/L)*
98	2/7/1999	HIGH-5	MO-32	--	0.00	2709	0.00	534	0.00	48886	0.18	402170	3.23	112702	1.25
130	3/11/1999	HIGH-6	MO-30	--	0.00	3644	0.00	1605	0.00	139012	0.76	1005592	8.21	30869	0.32
130	3/11/1999	HIGH-6	MO-30	--	0.00	4207	0.00	1985	0.00	136489	0.74	1024018	8.37	34692	0.36
84	1/24/1999	HIGH-7	MO-33	539	0.00	874	0.00	908	0.00	86125	0.42	476734	3.84	5035	0.02
130	3/11/1999	HIGH-7	MO-33	--	0.00	2476	0.00	911	0.00	528656	3.27	10159	0.00	5739	0.03
130	3/11/1999	HIGH-8	MO-34	--	0.00	1446	0.00	554	0.00	106752	0.55	428010	3.44	39934	0.42
129	1/29/1999	HIGH-9	MO-35	--	0.00	1846	0.00	938	0.00	47312	0.17	602982	4.89	7594	0.05
35	12/6/1998	LOW-1	MO-17	--	0.00	1174	0.00	--	0.00	124337	0.66	221632	1.73	367796	4.14
81	1/21/1999	LOW-1	MO-17	--	0.00	1369	0.00	699	0.00	628410	3.92	--	0.00	2251	0.00
129	3/10/1999	LOW-1	MO-17	916	0.01	416	0.00	1521	0.00	548489	3.40	2457	0.00	2982	0.00
42	12/13/1998	LOW-2	MO-19	255	0.00	1405	0.00	770	0.00	69339	0.31	218041	1.70	480513	5.42
84	1/24/1999	LOW-2	MO-19	--	0.00	2616	0.00	695	0.00	674560	4.21	--	0.00	2453	0.00
118	2/27/1999	LOW-2	MO-19	--	0.00	1350	0.00	720	0.00	606911	3.78	75175	0.52	584071	6.59
118	2/27/1999	LOW-2	MO-19	--	0.00	1299	0.00	543	0.00	575974	3.58	68142	0.47	533182	6.02
130	3/11/1999	LOW-2	MO-19	--	0.00	1305	0.00	350	0.00	756038	4.74	239534	1.88	338043	3.80
42	12/13/1998	LOW-3	MO-21	366	0.00	1014	0.00	446	0.00	170312	0.96	433859	3.49	120620	1.34
122	3/3/1999	LOW-3	MO-21	--	0.00	1343	0.00	691	0.00	583198	3.63	138147	1.04	443320	5.00
122	3/3/1999	LOW-3	MO-21	--	0.00	1249	0.00	608	0.00	570343	3.54	250245	1.97	419111	4.72
84	1/24/1999	LOW-4	MO-18	--	0.00	2219	0.00	818	0.00	741333	4.65	--	0.00	846	0.00
129	3/10/1999	LOW-4	MO-18	1516	0.01	1099	0.00	2206	0.00	629579	3.92	6128	0.00	1184	0.00
89	1/29/1999	LOW-5	MO-22	--	0.00	2179	0.00	1431	0.00	691435	4.32	1787	0.00	6187	0.04
89	1/29/1999	LOW-5	MO-22	--	0.00	1818	0.00	1575	0.00	699447	4.38	1469	0.00	6336	0.04
122	3/3/1999	LOW-6	MO-20	--	0.00	1344	0.00	1209	0.00	640198	3.99	119068	0.89	369894	4.16
122	3/3/1999	LOW-6	MO-20	--	0.00	1526	0.00	973	0.00	614703	3.83	112255	0.83	344544	3.88
66	1/6/1999	LOW-7	MO-23	323	0.00	1771	0.00	1551	0.00	487545	3.01	224993	1.76	3426	0.00
66	1/6/1999	LOW-7	MO-23	--	0.00	1332	0.00	750	0.00	97113	0.49	565189	4.57	21712	0.21
84	1/24/1999	LOW-7	MO-23	731	0.00	1609	0.00	1499	0.00	654038	4.08	1052	0.00	3235	0.00
89	1/29/1999	LOW-9	MO-25	--	0.00	1764	0.00	1296	0.00	740467	4.64	2553	0.00	3703	0.01

Table A.7:

MO Microcosm Series Chlorinated Solvent Partitioning Analysis and Calculations

Days from Creation	*GC Analysis Date*	*Microcosm ID*	*Sample ID*	*Microcosm Liquid Volume (mL)*	*PCE Liquid phase mass (μmol)*	*TCE Liquid phase mass (μmol)*	*cis-DCE Liquid phase mass (μmol)*	*PCE Gas Phase mass (μmol)*	*TCE Gas Phase mass (μmol)*	*cis-DCE Gas Phase mass (μmol)*	*PCE Initial liquid mass (μmol)*	*PCE Est. Remaining liquid mass (μmol)*	*Total Liquid mass (μmol)*	*Total Gas Mass (μmol)*	*Unaccounted for mass (μmol)*
3	11/4/1998	ABIO-1	MOC-1	100	4.918	0.000	0.000	0.953	0.000	0.000	4.893	4.893	4.918	0.953	-0.978
3	11/4/1998	ABIO-1	MOC-1	100	5.082	0.000	0.000	0.985	0.000	0.000	4.893	4.893	5.082	0.985	-1.174
3	11/4/1998	ABIO-1	MOC-1	100	4.957	0.000	0.000	0.960	0.000	0.000	4.893	4.893	4.957	0.960	-1.024
35	12/6/1998	ABIO-2	MOC-2	100	5.182	0.000	0.000	1.004	0.000	0.000	4.893	4.893	5.182	1.004	-1.293
35	12/6/1998	ABIO-2	MOC-2	100	5.001	0.000	0.000	0.969	0.000	0.000	4.893	4.893	5.001	0.969	-1.078
81	1/21/1999	AIR-1	MO-37	100	0.007	0.000	4.860	0.001	0.000	0.208	4.893	4.893	4.866	0.209	-0.183
95	2/4/1999	AIR-1	MO-37	94	0.015	0.199	4.243	0.004	0.031	0.251	4.893	4.599	4.457	0.286	-0.144
95	2/4/1999	AIR-1	MO-37	94	0.036	0.132	4.274	0.010	0.021	0.253	4.893	4.599	4.442	0.283	-0.126
108	2/17/1999	AIR-1	MO-37	83	0.070	0.000	0.996	0.030	0.000	0.095	4.893	4.061	1.066	0.125	2.870
81	1/21/1999	AIR-2	MO-38	100	0.006	0.000	5.084	0.001	0.000	0.217	4.893	4.893	5.091	0.218	-0.417
95	2/4/1999	AIR-2	MO-38	94	0.016	0.133	4.376	0.004	0.021	0.259	4.893	4.599	4.526	0.284	-0.210
108	2/17/1999	AIR-2	MO-38	88	0.029	0.000	3.747	0.010	0.000	0.291	4.893	4.305	3.776	0.301	0.228
81	1/21/1999	AIR-3	MO-39	100	0.000	0.000	5.030	0.000	0.000	0.215	4.893	4.893	5.030	0.215	-0.352
95	2/4/1999	AIR-3	MO-39	94	0.012	0.000	4.291	0.003	0.000	0.254	4.893	4.599	4.303	0.257	0.039
97	2/6/1999	AIR-4	MO-42	100	0.000	0.000	3.567	0.000	0.000	0.152	4.893	4.893	3.567	0.152	1.174
119	2/28/1999	AIR-4	MO-42	94	4.235	0.059	2.986	1.135	0.009	0.176	9.785	9.198	7.280	1.321	0.597
119	2/28/1999	AIR-4	MO-42	94	4.064	0.054	2.965	1.089	0.008	0.175	9.785	9.198	7.083	1.273	0.842
97	2/6/1999	AIR-5	MO-43	100	0.000	0.000	4.294	0.000	0.000	0.183	4.893	4.893	4.294	0.183	0.415
108	2/17/1999	AIR-5	MO-43	94	0.044	0.000	3.087	0.012	0.000	0.182	4.893	4.599	3.131	0.194	1.274
126	3/7/1999	AIR-5	MO-43	88	3.001	0.690	2.798	1.057	0.142	0.217	9.785	8.611	6.489	1.417	0.706
97	2/6/1999	AIR-6	MO-44	100	0.000	0.000	4.635	0.000	0.000	0.198	4.893	4.893	4.635	0.198	0.059
108	2/17/1999	AIR-6	MO-44	94	0.011	0.000	2.560	0.003	0.000	0.151	4.893	4.599	2.571	0.154	1.874
108	2/17/1999	AIR-6	MO-44	94	0.003	0.000	2.490	0.001	0.000	0.147	4.893	4.599	2.493	0.148	1.958
126	3/7/1999	AIR-6	MO-44	83	3.180	0.419	2.222	1.374	0.106	0.212	9.785	8.122	5.821	1.691	0.610
126	3/7/1999	AIR-6	MO-44	83	3.119	0.420	2.199	1.347	0.106	0.209	9.785	8.122	5.738	1.662	0.722
81	1/21/1999	AIR-7	MO-40	100	0.001	0.000	5.002	0.000	0.000	0.214	4.893	4.893	5.003	0.214	-0.324
95	2/4/1999	AIR-7	MO-40	94	0.009	0.009	4.059	0.003	0.001	0.240	4.893	4.599	4.077	0.244	0.279
119	2/28/1999	AIR-7	MO-40	88	0.122	0.000	1.835	0.043	0.000	0.143	4.893	4.305	1.957	0.186	2.162
119	2/28/1999	AIR-7	MO-40	88	0.066	0.000	3.004	0.023	0.000	0.233	4.893	4.305	3.070	0.257	0.978
95	2/4/1999	AIR-8	MO-41	100	0.000	0.041	4.490	0.000	0.005	0.192	4.893	4.893	4.531	0.196	0.165
119	2/28/1999	AIR-8	MO-41	94	0.002	0.000	3.355	0.001	0.000	0.198	4.893	4.599	3.357	0.199	1.043
97	2/6/1999	AIR-9	MO-45	100	0.000	0.000	4.640	0.000	0.000	0.198	4.893	4.893	4.640	0.198	0.055
126	3/7/1999	AIR-9	MO-45	94	3.101	1.031	1.745	0.831	0.161	0.103	9.785	9.198	5.877	1.095	2.225

Days from Creation	*GC Analysis Date*	*Microcosm ID*	*Sample ID*	*Microcosm Liquid Volume (mL)*	*PCE Liquid phase mass (μmol)*	*TCE Liquid phase mass (μmol)*	*cis-DCE Liquid phase mass (μmol)*	*PCE Gas Phase mass (μmol)*	*TCE Gas Phase mass (μmol)*	*cis-DCE Gas Phase mass (μmol)*	*PCE Initial liquid mass (μmol)*	*PCE Est. Remaining liquid mass (μmol)*	*Total Liquid mass (μmol)*	*Total Gas Mass (μmol)*	*Unaccounted for mass (μmol)*
97	2/6/1999	AIR-10	MO-46	100	0.000	0.174	4.558	0.000	0.020	0.195	4.893	4.893	4.732	0.214	-0.054
97	2/6/1999	AIR-10	MO-46	100	0.000	0.000	4.551	0.000	0.000	0.194	4.893	4.893	4.551	0.194	0.147
108	2/17/1999	AIR-10	MO-46	89	0.000	0.000	2.627	0.000	0.000	0.195	4.893	4.354	2.627	0.195	1.532
126	3/7/1999	AIR-10	MO-46	83	3.123	0.675	2.525	1.349	0.170	0.240	9.785	8.122	6.323	1.760	0.039
81	1/21/1999	BES-1	BMO-1	100	0.000	0.000	4.980	0.000	0.000	0.213	4.893	4.893	4.980	0.213	-0.300
84	1/24/1999	BES-2	BMO-2	100	0.009	0.000	5.404	0.002	0.000	0.231	4.893	4.893	5.413	0.233	-0.753
98	2/7/1999	BES-3	BMO-3	100	0.000	0.000	4.530	0.000	0.000	0.194	4.893	4.893	4.530	0.194	0.169
98	2/7/1999	BES-3	BMO-3	100	0.000	0.000	4.597	0.000	0.000	0.196	4.893	4.893	4.597	0.196	0.099
98	2/7/1999	BES-4	BMO-4	100	0.000	0.000	4.601	0.000	0.000	0.197	4.893	4.893	4.601	0.197	0.095
129	3/10/1999	BES-5	BMO-8	100	0.000	0.000	4.452	0.000	0.000	0.190	4.893	4.893	4.452	0.190	0.250
130	3/11/1999	BES-6	BMO-9	100	0.000	0.000	4.652	0.000	0.000	0.199	4.893	4.893	4.652	0.199	0.042
130	3/11/1999	BES-6	BMO-9	100	0.000	0.000	4.406	0.000	0.000	0.188	4.893	4.893	4.406	0.188	0.298
3	11/4/1998	BIO-1	MO-1	100	4.824	0.000	0.000	0.935	0.000	0.000	4.893	4.893	4.824	0.935	-0.866
3	11/4/1998	BIO-1	MO-1	100	4.837	0.000	0.000	0.937	0.000	0.000	4.893	4.893	4.837	0.937	-0.882
3	11/4/1998	BIO-1	MO-1	100	4.607	0.000	0.000	0.893	0.000	0.000	4.893	4.893	4.607	0.893	-0.607
118	2/27/1999	BIO-10	MO-10	100	2.505	0.233	4.451	0.485	0.026	0.190	9.785	9.785	7.189	0.702	1.894
118	2/27/1999	BIO-10	MO-10	100	2.486	0.243	4.522	0.482	0.027	0.193	9.785	9.785	7.250	0.702	1.833
119	2/28/1999	BIO-11	MO-11	100	2.776	0.215	4.597	0.538	0.024	0.196	9.785	9.785	7.588	0.759	1.438
119	2/28/1999	BIO-11	MO-11	100	2.591	0.196	4.400	0.502	0.022	0.188	9.785	9.785	7.187	0.712	1.885
119	2/28/1999	BIO-12	MO-12	100	3.104	0.181	4.355	0.602	0.020	0.186	9.785	9.785	7.641	0.808	1.336
126	3/7/1999	BIO-13	MO-13	100	0.033	0.465	4.550	0.006	0.053	0.194	4.893	4.893	5.048	0.253	-0.408
126	3/7/1999	BIO-14	MO-14	100	0.017	1.523	4.256	0.003	0.172	0.182	4.893	4.893	5.796	0.357	-1.261
9	11/10/1998	BIO-2	MO-2	100	4.625	0.000	0.000	0.896	0.000	0.000	4.893	4.893	4.625	0.896	-0.629
9	11/10/1998	BIO-2	MO-2	100	4.442	0.013	0.000	0.861	0.001	0.000	4.893	4.893	4.455	0.862	-0.425
9	11/10/1998	BIO-2	MO-2	100	4.707	0.011	0.000	0.912	0.001	0.000	4.893	4.893	4.718	0.913	-0.739
9	11/10/1998	BIO-2	MO-2	100	4.646	0.021	0.000	0.900	0.002	0.000	4.893	4.893	4.668	0.903	-0.678
30	12/1/1998	BIO-3	MO-3	100	0.151	3.806	0.680	0.029	0.431	0.029	4.893	4.893	4.636	0.489	-0.233
30	12/1/1998	BIO-3	MO-3	100	0.074	3.887	0.712	0.014	0.440	0.030	4.893	4.893	4.673	0.484	-0.265
32	12/3/1998	BIO-4	MO-4	100	0.332	4.166	0.493	0.064	0.471	0.021	4.893	4.893	4.990	0.557	-0.654
32	12/3/1998	BIO-4	MO-4	100	0.290	4.067	0.476	0.056	0.460	0.020	4.893	4.893	4.834	0.537	-0.478
35	12/6/1998	BIO-5	MO-5	100	0.212	4.089	0.867	0.041	0.463	0.037	4.893	4.893	5.168	0.541	-0.816
35	12/6/1998	BIO-5	MO-5	100	0.033	4.120	0.926	0.006	0.466	0.040	4.893	4.893	5.078	0.512	-0.698
35	12/6/1998	BIO-6	MO-6	100	3.362	1.180	0.297	0.651	0.133	0.013	4.893	4.893	4.838	0.798	-0.743

Days from Creation	GC Analysis Date	Microcosm ID	Sample ID	Microcosm Liquid Volume (mL)	PCE Liquid phase mass (μmol)	TCE Liquid phase mass (μmol)	cis-DCE Liquid phase mass (μmol)	PCE Gas Phase mass (μmol)	TCE Gas Phase mass (μmol)	cis-DCE Gas Phase mass (μmol)	PCE Initial liquid mass (μmol)	PCE Est. Remaining liquid mass (μmol)	Total Liquid mass (μmol)	Total Gas Mass (μmol)	Unaccounted for mass (μmol)
35	12/6/1998	BIO-6	MO-6	100	2.905	1.028	0.226	0.563	0.116	0.010	4.893	4.893	4.160	0.689	0.043
63	1/3/1999	BIO-7	MO-7	100	0.012	0.000	4.749	0.002	0.000	0.203	4.893	4.893	4.761	0.205	-0.074
63	1/3/1999	BIO-7	MO-7	100	0.003	0.000	4.496	0.000	0.000	0.192	4.893	4.893	4.498	0.193	0.202
116	2/25/1999	BIO-7	MO-7	89	2.591	0.133	3.735	0.874	0.026	0.278	9.785	8.709	6.458	1.178	1.072
116	2/25/1999	BIO-7	MO-7	89	2.352	0.111	3.607	0.794	0.022	0.268	9.785	8.709	6.070	1.084	1.555
63	1/3/1999	BIO-8	MO-8	100	0.053	0.000	4.640	0.010	0.000	0.198	4.893	4.893	4.693	0.208	-0.009
116	2/25/1999	BIO-8	MO-8	94	3.690	0.175	3.855	0.989	0.027	0.228	9.785	9.198	7.720	1.244	0.234
116	2/25/1999	BIO-8	MO-8	94	3.569	0.181	3.941	0.956	0.028	0.233	9.785	9.198	7.690	1.218	0.290
116	2/25/1999	BIO-9	MO-9	100	2.171	0.000	3.818	0.421	0.000	0.163	9.785	9.785	5.990	0.584	3.211
35	12/6/1998	HIGH-1	MO-27	100	0.792	3.650	0.205	0.154	0.413	0.009	4.893	4.893	4.647	0.575	-0.330
81	1/21/1999	HIGH-1	MO-27	94	0.453	2.284	0.119	0.121	0.357	0.007	4.893	4.599	2.856	0.486	1.258
129	3/10/1999	HIGH-1	MO-27	88	0.078	0.698	1.467	0.028	0.144	0.114	4.893	4.305	2.243	0.285	1.777
42	12/13/1998	HIGH-2	MO-29	100	3.574	1.233	0.061	0.692	0.139	0.003	4.893	4.893	4.867	0.835	-0.809
84	1/24/1999	HIGH-2	MO-29	94	2.529	0.986	0.026	0.678	0.154	0.002	4.893	4.599	3.541	0.834	0.224
118	2/27/1999	HIGH-2	MO-29	88	4.529	0.808	0.027	1.596	0.166	0.002	9.785	8.611	5.363	1.764	1.484
129	3/10/1999	HIGH-2	MO-29	82	3.701	0.706	0.069	1.662	0.185	0.007	9.785	8.024	4.475	1.853	1.695
42	12/13/1998	HIGH-3	MO-31	100	3.493	1.059	0.015	0.677	0.120	0.001	4.893	4.893	4.567	0.797	-0.472
98	2/7/1999	HIGH-3	MO-31	94	1.307	1.521	0.025	0.350	0.238	0.001	4.893	4.599	2.853	0.590	1.156
122	3/3/1999	HIGH-3	MO-31	88	3.981	1.415	0.017	1.402	0.291	0.001	9.785	8.611	5.412	1.695	1.504
122	3/3/1999	HIGH-3	MO-31	88	3.930	1.421	0.046	1.385	0.292	0.004	9.785	8.611	5.397	1.680	1.534
130	3/11/1999	HIGH-3	MO-31	77	3.488	1.439	0.045	1.887	0.455	0.005	9.785	7.534	4.972	2.347	0.215
84	1/24/1999	HIGH-4	MO-28	100	1.066	3.230	0.129	0.207	0.365	0.006	4.893	4.893	4.424	0.577	-0.109
129	3/10/1999	HIGH-4	MO-28	94	0.486	2.096	0.156	0.130	0.328	0.009	4.893	4.599	2.738	0.467	1.394
89	1/29/1999	HIGH-5	MO-32	100	1.051	2.535	0.164	0.204	0.287	0.007	4.893	4.893	3.749	0.497	0.646
98	2/7/1999	HIGH-5	MO-32	94	0.706	2.308	0.171	0.189	0.361	0.010	4.893	4.599	3.185	0.560	0.854
130	3/11/1999	HIGH-6	MO-30	100	0.191	6.252	0.782	0.037	0.707	0.033	9.785	9.785	7.224	0.778	1.783
130	3/11/1999	HIGH-6	MO-30	100	0.217	6.368	0.765	0.042	0.720	0.033	9.785	9.785	7.350	0.795	1.640
84	1/24/1999	HIGH-7	MO-33	100	0.014	2.925	0.429	0.003	0.331	0.018	4.893	4.893	3.368	0.352	1.172
130	3/11/1999	HIGH-7	MO-33	94	0.018	0.000	3.174	0.005	0.000	0.188	4.893	4.599	3.191	0.192	1.215
130	3/11/1999	HIGH-8	MO-34	100	0.253	2.618	0.567	0.049	0.296	0.024	4.893	4.893	3.438	0.369	1.085
129	1/29/1999	HIGH-9	MO-35	100	0.032	3.719	0.171	0.006	0.421	0.007	4.893	4.893	3.922	0.434	0.537
35	12/6/1998	LOW-1	MO-17	100	2.497	1.320	0.684	0.484	0.149	0.029	4.893	4.893	4.500	0.662	-0.270
81	1/21/1999	LOW-1	MO-17	94	0.000	0.000	3.798	0.000	0.000	0.224	4.893	4.599	3.798	0.224	0.576

Days from Creation	*GC Analysis Date*	*Microcosm ID*	*Sample ID*	*Microcosm Liquid Volume (mL)*	*PCE Liquid phase mass (μmol)*	*TCE Liquid phase mass (μmol)*	*cis-DCE Liquid phase mass (μmol)*	*PCE Gas Phase mass (μmol)*	*TCE Gas Phase mass (μmol)*	*cis-DCE Gas Phase mass (μmol)*	*PCE Initial liquid mass (μmol)*	*PCE Est. Remaining liquid mass (μmol)*	*Total Liquid mass (μmol)*	*Total Gas Mass (μmol)*	*Unaccounted for mass (μmol)*
129	3/10/1999	LOW-1	MO-17	88	0.000	0.000	3.087	0.000	0.000	0.240	4.893	4.305	3.087	0.240	0.978
42	12/13/1998	LOW-2	MO-19	100	3.268	1.297	0.318	0.633	0.147	0.014	4.893	4.893	4.883	0.794	-0.784
84	1/24/1999	LOW-2	MO-19	94	0.000	0.000	4.087	0.000	0.000	0.241	4.893	4.599	4.087	0.241	0.271
118	2/27/1999	LOW-2	MO-19	88	3.500	0.351	3.430	1.233	0.072	0.266	9.785	8.611	7.280	1.572	-0.241
118	2/27/1999	LOW-2	MO-19	88	3.193	0.312	3.248	1.125	0.064	0.252	9.785	8.611	6.753	1.441	0.416
130	3/11/1999	LOW-2	MO-19	77	1.766	1.103	3.766	0.955	0.348	0.449	9.785	7.534	6.634	1.753	-0.853
42	12/13/1998	LOW-3	MO-21	100	0.805	2.655	0.990	0.156	0.300	0.042	4.893	4.893	4.450	0.499	-0.056
122	3/3/1999	LOW-3	MO-21	94	2.833	0.747	3.515	0.759	0.117	0.208	9.785	9.198	7.095	1.084	1.019
122	3/3/1999	LOW-3	MO-21	94	2.677	1.410	3.435	0.717	0.221	0.203	9.785	9.198	7.522	1.141	0.535
84	1/24/1999	LOW-4	MO-18	100	0.000	0.000	4.792	0.000	0.000	0.205	4.893	4.893	4.792	0.205	-0.105
129	3/10/1999	LOW-4	MO-18	94	0.000	0.000	3.805	0.000	0.000	0.225	4.893	4.599	3.805	0.225	0.569
89	1/29/1999	LOW-5	MO-22	100	0.022	0.000	4.460	0.004	0.000	0.191	4.893	4.893	4.482	0.195	0.216
89	1/29/1999	LOW-5	MO-22	100	0.023	0.000	4.514	0.004	0.000	0.193	4.893	4.893	4.536	0.197	0.159
122	3/3/1999	LOW-6	MO-20	100	2.511	0.675	4.119	0.487	0.076	0.176	9.785	9.785	7.305	0.739	1.741
122	3/3/1999	LOW-6	MO-20	100	2.338	0.632	3.949	0.453	0.071	0.169	9.785	9.785	6.919	0.693	2.173
66	1/6/1999	LOW-7	MO-23	100	0.003	1.341	3.102	0.001	0.152	0.133	4.893	4.893	4.447	0.285	0.161
66	1/6/1999	LOW-7	MO-23	100	0.128	3.481	0.503	0.025	0.394	0.021	4.893	4.893	4.112	0.440	0.340
84	1/24/1999	LOW-7	MO-23	89	0.002	0.000	3.748	0.001	0.000	0.279	4.893	4.354	3.749	0.279	0.325
89	1/29/1999	LOW-9	MO-25	100	0.005	0.000	4.787	0.001	0.000	0.205	4.893	4.893	4.792	0.205	-0.105

APPENDIX B:
VOLATILE FATTY ACID ANALYSIS AND CALCULATIONS

This appendix contains data analysis and sample calculations for the Volatile Fatty Acids (VFAs) analyzed during this research. The concentrations reported for acetic and propionic acids were calculated from calibration curves generated through the analysis of various dilutions of pure standards. A best-fit linear curve with a coefficient of determination in excess of 0.99 was obtained for each both acetic and propionic acid during each analysis event. A slope and intercept was obtained from the curve for each analysis event. The area calculated by the integrator from the gas chromatograph was converted into a concentration using these linear curves. The concentration acetic and propionic acids shown in Table B.1 was calculated by the equation shown below

$$Concentration = \frac{(Area - Intercept)}{Slope} \tag{B.1}$$

Table B.1:

MO Microcosm Series VFA Analysis and Calculations

Days from Creation	*Microcosm ID*	*Sample ID*	*Acetic Acid Area*	*Acetic Acid Conc (ppm)*	*Propionic Acid Area*	*Propionic Acid Conc (ppm)*	*Acetic Acid Conc (mg/L)*	*Propionic Acid Conc (mg/L)*	*Acetic Acid Conc (mmol/L)*	*Propionic Acid Conc (mmol/L)*	*Microcosm Volume (mL)*	*Acetic Acid Mass (mmol)*	*Propionic Acid Mass (mmol)*
81	AIR-1	MO-37	72150	312.8	--	0.0	328.4	0.0	5.474	0.000	100	0.54740	0.00000
81	AIR-1	MO-37	69537	301.2	--	0.0	316.3	0.0	5.271	0.000	100	0.52710	0.00000
81	AIR-2	MO-38	72488	314.3	1159	0.4	330.0	0.4	5.500	0.005	100	0.55003	0.00053
81	AIR-2	MO-38	79649	346.1	1364	1.0	363.4	1.0	6.057	0.013	100	0.60568	0.00134
81	AIR-3	MO-39	53344	229.3	--	0.0	240.8	0.0	4.013	0.000	100	0.40128	0.00000
81	AIR-3	MO-39	54965	236.5	--	0.0	248.3	0.0	4.139	0.000	100	0.41388	0.00000
81	AIR-7	MO-40	43435	185.3	--	0.0	194.6	0.0	3.243	0.000	100	0.32428	0.00000
81	AIR-7	MO-40	40417	171.9	--	0.0	180.5	0.0	3.008	0.000	100	0.30083	0.00000
81	BES-1	BMO-1	88636	386.0	48526	139.4	405.3	137.7	6.755	1.861	100	0.67550	0.18612
81	BES-1	BMO-1	84694	368.5	50025	143.8	386.9	142.1	6.449	1.920	100	0.64488	0.19199
63	BIO-7	MO-7	36056	33.5	2858	5.3	35.2	5.2	0.586	0.071	100	0.05863	0.00708
63	BIO-7	MO-7	36421	33.8	1207	4.4	35.5	4.3	0.592	0.059	100	0.05915	0.00587
63	BIO-8	MO-8	4982	7.5	962	4.3	7.9	4.2	0.131	0.057	100	0.01313	0.00574
63	BIO-8	MO-8	3416	6.2	1018	4.3	6.5	4.2	0.109	0.057	100	0.01085	0.00574
84	HIGH-4	MO-28	53379	234.6	32972	94.7	246.3	93.6	4.106	1.264	100	0.41055	0.12644
84	HIGH-4	MO-28	53757	236.4	34239	98.5	248.2	97.3	4.137	1.315	100	0.41370	0.13151
89	HIGH-5	MO-32	35542	147.7	34646	96.9	155.1	95.7	2.585	1.294	100	0.25848	0.12937
89	HIGH-5	MO-32	34583	143.7	35273	98.7	150.9	97.5	2.515	1.318	100	0.25148	0.13178
84	HIGH-7	MO-33	49339	216.2	23855	67.6	227.0	66.8	3.784	0.903	100	0.37835	0.09026
84	HIGH-7	MO-33	50285	220.5	24098	68.3	231.5	67.5	3.859	0.912	100	0.38588	0.09119
89	HIGH-9	MO-35	38913	162.0	27404	76.5	170.1	75.6	2.835	1.021	100	0.28350	0.10214
89	HIGH-9	MO-35	41910	174.7	28557	79.7	183.4	78.7	3.057	1.064	100	0.30573	0.10641
84	LOW-2	MO-19	63344	280.2	--	0.0	294.2	0.0	4.904	0.000	94	0.46093	0.00000
84	LOW-2	MO-19	63923	282.8	--	0.0	296.9	0.0	4.949	0.000	94	0.46521	0.00000
84	LOW-4	MO-18	8995	31.8	--	0.0	33.4	0.0	0.557	0.000	100	0.05565	0.00000
84	LOW-4	MO-18	6808	21.8	--	0.0	22.9	0.0	0.382	0.000	100	0.03815	0.00000
89	LOW-5	MO-22	78954	331.7	--	0.0	348.3	0.0	5.805	0.000	100	0.58048	0.00000
89	LOW-5	MO-22	83968	351.7	617	0.8	369.3	0.8	6.155	0.011	100	0.61548	0.00107
84	LOW-7	MO-23	53906	237.0	131	0.7	248.9	0.7	4.148	0.009	89	0.36913	0.00083
84	LOW-7	MO-23	54141	238.1	--	0.0	250.0	0.0	4.167	0.000	89	0.37084	0.00000
81	LOW-9	MO-25	2546	7.9	1126	2.2	8.3	2.2	0.138	0.029	100	0.01383	0.00294
81	LOW-9	MO-25	1517	3.5	--	0.0	3.7	0.0	0.061	0.000	100	0.00613	0.00000

APPENDIX C:
BIOGENIC GAS ANALYSIS AND CALCULATIONS

This appendix contains data analysis and sample calculations for the biogenic gases monitored during this study. The volumes reported for CH_4 and CO_2 were calculated by comparing the area generated for each gas from the 1 mL microcosm headspace sample to the areas produced from the injection of various volumes of pure gas standards onto the gas chromatograph. Calibration curves generated through the analysis of various volumes of pure gas standards. A best-fit linear curve with a coefficient of determination in excess of 0.99 was obtained for each both CH_4 and CO_2. The area calculated by the integrator for the microcosm headspace gas sample was converted into a volume of pure gas using these linear curves. The volume of CH_4 and CO_2 in the 1 mL headspace sample shown in Table C.1 was calculated by the equation shown below.

$$Concentration = \frac{(Area - Intercept)}{Slope} \qquad \text{(C.1)}$$

The mass of gas produced by the experimental microcosm was calculated through the use of the ideal gas law, assuming a pressure of 1 atm and a temperature of 30°C.

Table C.1:

MO Microcosm Series Biogenic Gas Analysis and Calculations

Date from Creation	*GC Analysis Date*	*Sample ID*	*Microcosm ID*	*Headspace Volume*	*CH_4 Area*	*CH_4 Volume (ml)*	*CH_4 mass (μmol)*	*CO_2 Area*	*CO_2 Volume (ml)*	*CO_2 mass (μmol)*
35	12/6/1998	MOC-2	ABIO-2	20	--	0.000	0.000	7489	0.009	7.42
81	1/21/1999	MO-37	AIR-1	20	965308	0.967	777.854	77648	0.033	26.23
95	2/4/1999	MO-37	AIR-1	31	837162	0.839	1045.900	121425	0.047	58.85
81	1/21/1999	MO-38	AIR-2	20	913077	0.915	735.840	79441	0.033	26.71
95	2/4/1999	MO-38	AIR-2	29	808193	0.810	944.635	111142	0.044	51.05
81	1/21/1999	MO-39	AIR-3	20	962216	0.964	775.367	75589	0.032	25.68
95	2/4/1999	MO-39	AIR-3	29	885433	0.887	1034.725	106423	0.042	49.22
97	2/6/1999	MO-42	AIR-4	20	1062496	1.064	856.031	104492	0.042	33.43
97	2/6/1999	MO-43	AIR-5	20	1106769	1.108	891.644	96550	0.039	31.30
97	2/6/1999	MO-44	AIR-6	20	1033340	1.035	832.578	82464	0.034	27.52
81	1/21/1999	MO-40	AIR-7	20	1024814	1.027	825.720	72378	0.031	24.82
95	2/4/1999	MO-40	AIR-7	29	976640	0.978	1141.105	120591	0.047	54.73
95	2/4/1999	MO-41	AIR-8	20	1165016	1.167	938.497	109594	0.043	34.80
97	2/6/1999	MO-45	AIR-9	20	959189	0.961	772.932	67974	0.029	23.64
97	2/6/1999	MO-46	AIR-10	20	1118761	1.120	901.290	98087	0.039	31.71
81	1/21/1999	BMO-1	BES-1	20	5377	0.007	5.695	112463	0.044	35.56
81	1/21/1999	BMO-2	BES-2	20	5754	0.007	5.999	127539	0.049	39.61
3	11/4/1998	MO-1	BIO-1	20	56465	0.058	46.790	41770	0.021	16.61
9	11/10/1998	MO-2	BIO-2	20	266194	0.268	215.494	59743	0.027	21.43
30	12/1/1998	MO-3	BIO-3	20	773927	0.776	623.909	68021	0.029	23.65
33	12/4/1998	MO-4	BIO-4	20	868352	0.870	699.863	65868	0.029	23.07
35	12/6/1998	MO-5	BIO-5	20	851750	0.853	686.509	75031	0.032	25.53
35	12/6/1998	MO-6	BIO-6	20	875350	0.877	705.493	59116	0.026	21.26
63	1/3/1999	MO-7	BIO-7	20	1097785	1.099	884.417	69204	0.030	23.97
63	1/3/1999	MO-8	BIO-8	20	1160177	1.162	934.604	64970	0.028	22.83
81	1/21/1999	MO-27	HIGH-1	26	866303	0.868	907.680	157138	0.059	61.81
42	12/13/1998	MO-29	HIGH-2	20	676794	0.678	545.776	91602	0.037	29.97

Date from Creation	*GC Analysis Date*	*Sample ID*	*Sort Code*	*Headspace Volume*	*CH_4 Area*	*CH_4 Volume (ml)*	*CH_4 mass (μmol)*	*CO_2 Area*	*CO_2 Volume (ml)*	*CO_2 mass (μmol)*
84	1/24/1999	MO-29	HIGH-2	20	720110	0.722	580.619	362898	0.128	102.71
42	12/13/1998	MO-31	HIGH-3	20	702824	0.705	566.714	89974	0.037	29.53
84	1/24/1999	MO-28	HIGH-4	20	878021	0.880	707.641	284572	0.102	81.71
89	1/29/1999	MO-32	HIGH-5	20	1157676	1.159	932.593	93320	0.038	30.43
84	1/24/1999	MO-33	HIGH-7	20	1189992	1.192	958.587	--	0.000	0.00
89	1/29/1999	MO-35	HIGH-9	20	1050054	1.052	846.023	324185	0.115	92.33
35	12/6/1998	MO-17	LOW-1	20	897712	0.899	723.480	65438	0.029	22.96
81	1/21/1999	MO-17	LOW-1	26	1168621	1.170	1223.816	102245	0.041	42.67
42	12/13/1998	MO-19	LOW-2	20	738814	0.741	595.664	74361	0.032	25.35
84	1/24/1999	MO-19	LOW-2	20	974306	0.976	785.092	97711	0.039	31.61
42	12/13/1998	MO-21	LOW-3	20	724481	0.726	584.135	70958	0.030	24.44
84	1/24/1999	MO-18	LOW-4	20	1159320	1.161	933.915	66015	0.029	23.11
89	1/29/1999	MO-22	LOW-5	20	1087688	1.089	876.295	90581	0.037	29.70
84	1/24/1999	MO-23	LOW-7	20	1021395	1.023	822.970	101718	0.041	32.68
89	1/29/1999	MO-25	LOW-9	20	1403711	1.405	1130.501	77858	0.033	26.29

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