THE EFFECT OF CYCLODEXTRIN ON REDUCTIVE DECHLORINATION

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ABSTRACT

Microcosms were constructed from aquifer sediment samples taken from an actively degrading chlorinated solvent contaminated site located in Virginia Beach, Virginia. The objective of this study was to determine if and how the addition of cyclodextrin (CD) affects reductive dechlorination of chlorinated ethenes. After chlorinated solvent degradation rates were established in anaerobic and aerobic microcosms, 100 mg/L of CD solution was added for a period of 21 days. CD was then removed after 26 days to simulate the degradation response of the aquifer in a post CD injection environment. Degradation rates were determined by analyzing PCE, TCE, and *cis*-DCE concentration data over the various phases of the experiment.

Results from this study indicated that chlorinated solvent degradation could be either impaired or facilitated by the addition of CD. CD appeared to stimulate one anaerobic microcosm (IY-2c) where daughter production had not previously occurred. The activity of this microcosm was greatly enhanced by the addition of CD (0 uM/day to 13.89 uM/day). However, biotransformation of PCE in another anaerobic microcosm in which reductive dechlorination was occurring, ceased after the addition of CD (IY-1a). In a third group of microcosms the rate and extent of reductive dechlorination was greatly enhanced by the addition of CD.

The effect of adding CD was also found to be highly dependent on the redox conditions in the microcosm, specifically if the conditions were strongly reducing. The most active microcosms, found in the Aerobic Group, also had the lowest ferrous iron concentrations (3.57 mg/L for BY-1a, 2.25 mg/L for BY-1b, and 0.41 mg/L for BY-1c). The microcosm (IY-2b) that showed no daughter production had the highest level of ferrous iron (44.22 mg/L). This study presents a qualitative approach to the affect of CD on MNA.

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INTRODUCTION

Contamination of groundwater by chlorinated ethenes, which includes tetrachloroethene (PCE) and trichloroethene (TCE), is relatively common due to the historic and widespread use of these compounds as industrial solvents, dry cleaning agents, and degreasers. Chlorinated ethenes are of particular concern because the degradation products of PCE and TCE, which are DCE (dichloroethene) and VC (vinyl chloride), are more toxic than the original contaminant (US EPA OSWER, 1999; Wiedemeier et al. 1999; NRC, 2000). VC, in particular, is a known carcinogen (US EPA OSWER, 1999; Wiedemeier et al. 1999; NRC, 2000).

One factor complicating remediation of chlorinated solvents at contaminated sites is that they originate as DNAPLs (dense nonaqueous phase liquids), which tend to pool on the bottom of an aquifer due to their high density relative to water (Bizzigotti et al. 1997). Furthermore, DNAPLs are relatively insoluble in water and they are expected to persist in the subsurface for decays and even beyond (Bizzigotti et al. 1997). Another factor is that dissolved phase chlorinated ethene plumes are relatively recalcitrant to microbially-based transformation processes.

Recent research has demonstrated that under certain environmental conditions, chlorinated ethenes can be biodegraded or biotransformed by intrinsic microbial communities present in aquifers (Chapelle, 2001). At sites where highly reducing conditions exist in the groundwater, it has been shown that the attenuation capacity may be sufficient to meet site-specific regulatory-based remediation goals using Monitored Natural Attenuation (MNA), usually in combination with source zone treatment (Chapelle and Bradley, 1998).

MNA is the use of the indigenous microbial population to degrade contaminants in the subsurface (NRC, 2000). The EPA has recommended this type of treatment as a long-term finishing step for many remediation strategies due to economic savings and the effectiveness of indigenous microorganisms to remove small quantities of contaminants (Wiedemeier et al. 1999). MNA is particularly useful once systems such as pump-and-treat can no longer effectively remove contaminants (Wiedemeier et al. 1999).

Source treatment control is a means of removing or reducing the source mass and concentration of contamination using physical, chemical, thermal, or biological treatment strategies by either in-situ or ex-situ means (US EPA OSWER, 1999; Wiedemeier et al. 1999; La Grega et al. 2001; US EPA OSWER, 2001). Traditionally pump and above ground treatment, commonly known as pump-and-treat, has been used to remediate chlorinated ethane plumes at the source area by physically pumping out contaminated water and treating it at the surface (Blanford et al. 2001). However, due to the pooling of DNAPLs on the bottom of aquifers and their insolubility in water, pump-and-treat is very expensive, time consuming, and does not necessarily fully remediate chlorinated ethane sites. One reason why pump-and-treat can sometimes be moderately effective is because these chlorinated solvents sorb, or adhere, to the sediment, making removal difficult. Following pump-and-treat, MNA is commonly used as a final remediation strategy to remove any residual contaminant.

To increase the effectiveness of pump-and-treat, solubility-enhancing agents have been used to improve the removal of chlorinated ethenes by increasing the solubility of the contaminant in water, thus allowing the contaminant to be more easily removed (US EPA ORD, 1996). Traditionally surfactants and cosolvents have been used for this purpose (US EPA ORD, 1996). Surfactants and cosolvents, however, can negatively impact an aquifer's microbial population (US EPA ORD, 1996; Lowe et al. 1997; Wiedemeier et al. 1999; La Grega et al. 2001).

Recently, several studies have shown that the addition of cyclodextrin (CD) as a solubility-enhancing agent to treat chlorinated solvents and other DNAPLs can be an effective combination with groundwater extraction systems (McCray et al. 1999; McCray and Brusseau, 1999; Blandford et al. 2001; McCray et al. 2000). The addition of CD enhances the solubility of chlorinated solvents like PCE, thus making them readily removable from the contaminated aquifer. Unlike traditional complexing agents such as surfactants and cosolvents commonly used to enhance pump-and-treat, cyclodextrin is nontoxic to humans and is also biodegradable (Ko et al. 1999).

In the past, the chemical cyclodextrin has been studied in many fields (weightlifting, medical, pharmaceutical, etc.) and has a variety of commercial uses (Hedges, 1998). Unfortunately, little research is available for its applicability as a subsurface complexing agent. One study found that cyclodextrin might have a beneficial use as a solubility-enhancing agent in

subsurface remediation because it has been shown to act as a traditional surfactant by increasing the solubility of non-aqueous phase liquids (Ko et al. 1999). Along with this claim, the fact that cyclodextrin is nontoxic and biodegradable, while many subsurface surfactants are toxic and resist degradation, is another reason CD should be studied.

Research on the effects of cyclodextrin on the degradation rates of chlorinated solvents and microbial populations will aid in evaluating the feasibility of this remediation technology. CD application has the potential of reducing the amount of time and money necessary to treat contaminated sites remediated with technologies such as pump-and-treat. Although CD may reduce the expense to remediate a site, its benefits are nullified if MNA can no longer be effective. MNA can be an efficient and cost effective method of remediation that its loss would greatly increase remediation costs. Thus understanding the effects of CD on degradation capabilities is vital for the remediation industry.

The objective of this research was to investigate the effect of CD on microbial degradation of PCE and its ability to enhance or decrease MNA capabilities. This was accomplished by conducting microcosm studies with a reductive dechlorinating microbial population in laboratory microcosms. CD was then added with an anaerobic solution in some vials and with an aerobic solution in other vials to simulate the injection of an oxygenated solution into an anaerobic aquifer. CD was then removed from the microcosms and the degradation rate of PCE after the aquifer conditions returned to the preinjection environment was examined.

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AUTHOR'S PREFACE

This work is presented in the Virginia Tech manuscript format. Chapter I is a review of the literature pertaining to subsurface remediation and utilization of cyclodextrin and Chapter II is a separate manuscript formatted for journal submission. Chapter II documents in detail the effect of cyclodextrin on the reductive dechlorination of PCE and other chlorinated ethenes derived from the breakdown of PCE. This research was laboratory based which allowed researchers to evaluate the effects through a microcosm study.

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CHAPTER I
LITERATURE REVIEW

CHLORINATED ETHENES

Due to the historic widespread use of chlorinated ethenes (tetrachloroethene, PCE, and trichloroethene, TCE) as industrial solvents, dry cleaning agents, and degreasers, PCE and TCE are ranked third and first, respectively, as the most frequently detected groundwater contaminants (NRC, 1994). The most common chlorinated ethenes and their respective molecular weights are PCE (165.8 g/mol), TCE (131.4 g/mol), dichloroethene (DCE) (96.9 g/mol) and vinyl chloride (VC) (62.5 g/mol). These chemicals are relatively recalcitrant and difficult to degrade using traditional remediation techniques such as pump-and-treat (US EPA OUST, 1995; US EPA OSWER, 1999; Wiedemeier et al. 1999; NRC, 2000) because they degrade under unique environmental conditions, sorb to soil particles, collect in pore spaces as a nonaqueous phase liquid (NAPL), and mix with other contaminants as they sink to the bottom of the aquifer (US EPA OSWER, 1999; Wiedemeier et al. 1999). As a result, pump-and-treat is commonly used only for containing a groundwater plume that includes dissolved chlorinated solvents.

Chlorinated solvents are degraded naturally by microorganisms through direct, reductive, and cometabolic processes (Wiedemeier et al. 1999; Madigan et al. 2000). Under strictly-anaerobic conditions, reductive dechlorination is thought to be the most critical mechanism for attenuating chlorinated ethenes in groundwater. Figure 1-1 shows the degradation pathways of PCE to its final form as ethene. Because reductive dechlorination is one of the few pathways that can fully degrade chlorinated ethenes, care must be taken to ensure that the degradation of PCE and TCE goes to completion (i.e., transformation to carbon dioxide and ethene), because some of the daughter products (such as *cis*-DCE and VC) are more toxic than the original contaminant (US EPA OSWER, 1999; Wiedemeier et al. 1999; NRC, 2000). Some researchers have discovered that the lesser dechlorinated substances, such as DCE and VC, can degrade aerobically, while PCE and TCE generally require more reduced conditions for degradation to occur (Wiedemeier et al. 1999; Madigan et al. 2000; NRC, 2000).

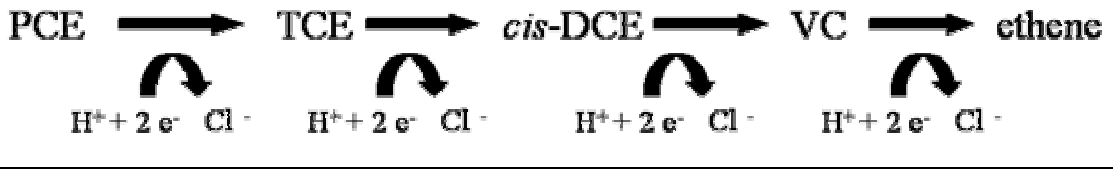

Figure 1-1. PCE Degradation Pathway under Reductive Dechlorination. Figure adapted from Wiedemeier et al. (1999).

Biologically, PCE and TCE may serve as electron acceptors while other chlorinated ethenes (DCE and VC) can be used as electron donors or acceptors. Researchers have found that chlorinated ethenes are used as electron acceptors through the halorespiration process of reductive dechlorination (Wiedemeier et al. 1999). Reductive dechlorination is observed when the chlorine atom is replaced by a hydrogen atom (also known as dechlorination) and is then used by microorganisms for anaerobic respiration (Madigan et al. 2000). Halorespiration is a microbial process driven by the use of hydrogen as the electron donor (Vogel et al. 1987).

Cometabolism is a process by which microorganisms degrade a substance for energy, which in the process also simultaneously degrades another compound without obtaining energy from its degradation (Wiedemeier et al. 1999; Madigan et al. 2000). Cometabolism occurs both aerobically and anaerobically (Chapelle, 2001). In respect to chlorinated ethenes, this occurs anaerobically while a process such as methanogenesis is occurring (Wilson and Wilson, 1985; Chapelle, 2001). Cometabolism is not likely to attribute to MNA due to a slow degradation rate because the compound is indirectly degraded (Chapelle, 2001).

Another degradation process applicable to chlorinated ethenes is direct oxidation. During direct oxidation, DCE and VC are oxidized to carbon dioxide under either aerobic or anaerobic conditions without the accumulation of intermediates such as ethene (Bradley and Chapelle, 1996, 1997, 1998a, and 1998b; Bradley et al. 1998). Anaerobic direct oxidation, however, has recently been considered and is still controversial. Unfortunately, the specific factors involved with direct oxidation are not well known. A more detailed discussion of all of the processes involved in chlorinated ethane degradation can be found in the Chapelle (2001).

MONITORED NATURAL ATTENUATION (MNA)

Monitored natural attenuation (MNA) is a relatively recent and developing technology that has been successfully used to manage and control chlorinated ethene plumes. MNA is the use of natural processes (physical, chemical, and/or biological) as a remediation strategy. The

goal of MNA is to reduce the mobility, toxicity, and the mass or volume of the contaminant along with degrading contaminants in the subsurface without disturbing the subsurface (US EPA OSWER, 1999; Wiedemeier et al. 1999; NRC, 2000). In general, microbial degradation is the driving force of this remediation strategy (Wiedemeier et al. 1999). Usually MNA is used in conjunction with other remediation strategies (i.e., pump-and-treat) or is initiated to remove the remaining residual contamination following the completion of other complex and highly expensive remediation processes (US EPA OSWER, 1999). MNA can be useful for recalcitrant compounds such as chlorinated ethenes, which may not fully degrade when other remediation techniques are used.

Since the early 1990s, MNA has become a more common remediation option for contaminated sites in the United States. In fact, MNA is commonly incorporated as part of the remediation strategy at many contaminated U.S. Department of Defense sites (NRC, 2000). The U.S. Air Force, for example, employs MNA at more than 80% of its 1,500 jet fuel contaminated sites (NRC, 2000). More specifically, Dover Air Force Base is using MNA for removal of chlorinated solvents at Area 6 (Davis et al. 2002; Witt et al. 2002). MNA is also being used as a remediation option for 84 U.S. Army contaminated sites (NRC, 2000). In addition widespread usage of MNA at government-owned sites, some industrial plants use natural attenuation to treat chlorinated solvent plumes created by their manufacturing processes (Wiedemeier et al. 1999). Furthermore, Ferrey et al. (2001) reported the use of MNA at an industrial site in St Paul, Minnesota.

One reason for the increasing interest in MNA is the substantial economic cost associated with site restoration. In comparison to other remediation techniques MNA has cost advantages. In Houston, Texas, for instance, the French Limited site saved approximately $12 million by using MNA instead of traditional source removal techniques combined with pump-and-treat (NRC, 2000). Another reason for MNA's increasing popularity stems from a 1994 National Research Council report which claimed that of 77 groundwater contamination sites reviewed, pump-and-treat was highly unlikely to fully remediate the majority of the sites identified (NRC, 2000).

CONTAMINANT SOURCE TREATMENT

When cleaning up contaminant plumes, it is critical to first identify and remediate the contaminant source. In theory, source treatment control is a means of effectively removing or reducing the source mass and concentration of contamination (US EPA OSWER, 1999; Wiedemeier et al. 1999; US EPA OSWER, 2001). Table 1-1 includes some of the common types of sources treatment. A more extensive list of possible source treatments can be found in an EPA report (2001). The most common technologies used for source treatment are incineration, excavation, solidification/stabilization, soil vapor extraction, thermal desorption, and pump-and-treat (US EPA OSWER, 2001). Source treatment methods can be either in-situ or ex-situ. In-situ treatment processes are becoming increasingly popular because traditional ex-situ processes such as excavation and pump-and-treat are expensive (US EPA OUST, 1995; US EPA OSWER, 2001). In particular, pump-and-treat, which is commonly used for the remediation of groundwater, can be very expensive and not necessarily very effective (US EPA OUST, 1995). Source treatment technologies involve a variety of strategies including physical, chemical, thermal, and biological treatment (La Grega et al. 2001; US EPA OSWER, 2001). Other contamination strategies include source control, a process where the contaminated material is isolated by inserting subsurface barriers and capping. A common example of an isolating source control is a municipal landfill. EPA's Treatment Technologies for Site Cleanup: Annual Status Report (2001) further explains the usage and types of technologies based upon numerous federal sites including RCRA corrective action sites, U.S. Department of Defense and U.S. Department of Energy (DOE) sites, and projects within the Superfund program.

One consequence of source removal with respect to MNA is that both ex-situ and in-situ source removal techniques can negatively affect naturally occurring degradation. For instance, in an anaerobic subsurface, oxygen introduced through source treatment may change the aquifer geochemistry. Also, the alteration of subsurface conditions can inhibit the necessary microbial degraders. For example, Ferrey et al. (2001) found that use of a vacuum vaporizer well to remove chlorinated aliphatic compounds introduced oxygenated water into the aquifer. This resulted in an inhibition of the microbial populations utilized for natural attenuation, which previously were degrading the chlorinated solvent plume under anaerobic conditions (Ferrey et al, 2001).

Table 1-1. Common Source Treatment Strategies[1]

Source Treatment Technology	Type of Treatment
Excavation/ Incineration	Physical
Solidification/ Stabilization	Physical
Soil Vapor Extraction	Physical
Capping	Physical
Soil Flushing	Physical
Containment	Physical
Chemical Oxidation	Chemical
Chemical Treatment	Chemical
Neutralization	Chemical
Bioremediation	Biological
Thermal Desorption	Thermal

1. Source: EPA's Treatment Technologies for Site Cleanup: Annual Status Report (2001)

PUMP-AND-TREAT

Pump-and-treat is a mature remediation technology used for the hydraulic containment of aqueous phase contaminant plumes. Traditionally chlorinated solvent contaminant plumes have been remediated by the use of pump-and-treat (US EPA OUST, 1995; US EPA ORD, 1996; Wiedemeier et al. 1999; La Grega et al. 2001). Researchers have shown that the pump-and-treat methodology can alter subsurface conditions. First, by controlling how much water is removed from or added to the aquifer, pump-and-treat processes can change groundwater flow rates resulting in decreased rates of natural attenuation due to decreased contact time between degrading microorganisms and contaminated groundwater. However, pump-and-treat processes prevent further migration of the contaminant source (Chapelle et al. 1998). Pumping also artificially reduces the chlorinated solvent contaminant concentration and introduces oxygen through the materials pumped into the aquifer (Wiedemeier et al. 1999). The artificial reduction in contamination is shown by an initial drop in contaminant level followed by a stabilization of the contaminant concentration. After the pump-and-treat system is shut off, however, the

contaminant rebounds to a higher concentration than present before shutting off the pump-and-treat system. This process is known as the tailing and rebound phenomena (Wiedemeier et al. 1999; La Grega et al. 2001). The persistence of compounds such as chlorinated solvents following pump-and-treat source treatment has commonly been recorded at the survey of a majority of 19 EPA treated sites along with a majority of 77 source treated sites surveyed by the NRC (Wiedemeier et al. 1999; NRC, 2000). Inadequate remediation through engineered techniques has led to the dependence on alternative strategies for treating the residual chlorinated solvent contamination in the aquifer.

ENHANCED TREATMENT STRATEGIES

Methods to enhance pump-and-treat technology are used to increase the chlorinated solvent removal efficiency because both MNA and pump-and-treat can take decades to reduce chlorinated solvent concentrations to acceptable levels. These enhancements include physical, chemical, and biological methods of altering the subsurface chemistry to increase the chlorinated solvent removal (US EPA ORD, 1996).

Biological enhancement is conducted by adding substances such as nutrients to the pump-and-treat process. The goal of adding nutrients is to promote microbial growth in the subsurface, which should ultimately enhance biodegradation (US EPA ORD, 1996). Physical enhancements include the addition of air or inducing fractures in the subsurface to increase pump-and-treat effectiveness (US EPA ORD, 1996). The chemical enhancement method utilizes the introduction of one or more reagents to chemically modify the contaminants. These chemical enhancements could, for instance, make the contaminants more or less soluble. Reagents such as cosolvents and surfactants are primarily used to alter the properties of the chemical by increasing its solubility (US EPA ORD, 1996). When used together, surfactants and cosolvents have been found to increase the removal efficiency more than when used separately (Lowe et al. 1997). Detailed information about the types and the specific characteristics of the enhancements, in particular the chemical enhancements, are found in US EPA ORD (1996) and Lowe et al. (1997).

Although treatment enhancements can promote conditions that increase chlorinated solvent removal, problems such as oxygenation of anoxic plumes and the inability of the enhancements to reach contaminants due to the heterogeneous, complex environment still exist (US EPA ORD, 1996; Lowe et al. 1997; Wiedemeier et al. 1999). Additionally, the variable and

slow partitioning of DNAPLs is another limitation (Lowe et al. 1997). Biological enhancements can also be ineffective and highly dependent on the complexity of the microbial communities degrading the contaminant. Furthermore, biological enhancements can also negatively impact or inhibit the existing microbial communities. Chlorinated solvents in particular are known for complex degrading communities requiring specific conditions for biodegradation that can easily be disrupted (Lowe et al. 1997).

Another problem is that cosolvents and surfactants usually are toxic to the naturally degrading microbial populations (US EPA ORD, 1996; Lowe et al. 1997; Wiedemeier et al. 1999; La Grega et al. 2001). Therefore, the introduction of these chemicals into the subsurface could potentially inhibit MNA chlorinated solvent removal efficiency. Another concern expressed by field experts and researchers is that because these chemicals can decrease the interfacial tension between chlorinated solvents and water and thus increase the solubility, the contaminants then become mobile in groundwater, potentially causing expansion of the contaminant plume.

Mobilization of contaminants can also be caused by increased groundwater flow due to pumping (US EPA ORD, 1996). This cause of mobilization is particularly problematic for DNAPLs where pumping can cause vertical mobilization (US EPA ORD, 1996). Vertical mobilization may be prevented by creating large upward gradients or by the presence of an ideal environment such as clay layer without the presence of heterogeneities or cracks (Lowe et al. 1997). It can also be minimized through carefully picking surfactant-cosolvent combinations to minimize the density reduction effects on DNAPLs (Lowe et al. 1997).

Surfactants and cosolvents, which can migrate through the groundwater, also can be nondegradable and toxic to humans (Ko et al. 1999; Lowe et al. 1997). Several surfactants have been found to biologically mimic estrogen, a female hormone. Estrogen, in some forms, has been linked to cancer and other health problems (Lowe et al. 1997). Surfactants and cosolvents have also been found to be highly resistant to biodegradation, particularly in anaerobic environments, which are commonly found in the subsurface and necessary for reductive dechlorination (Sun et al. 1995; Lowe et al. 1997).

Although chemical enhancements in particular can provide effective results in pump-and-treat, a number of potential problems have been noted in the literature. As a result, researchers have recently investigated the efficacy of other substances such as complexing agents that can

induce similar results on DNAPLs without the hazards found through surfactant and cosolvent use. Cyclodextrin, for example, is a promising complexing agent that has been used as an enhancement in field and laboratory studies (McCray et al. 1999; McCray and Brusseau, 1999; Blanford et al. 2000; McCray et al. 2000). These studies have found that cyclodextrin can successfully enhance the solubility of low solubility compounds without negatively effecting the subsurface. Researchers have also conduced that cyclodextrin is biodegradable and nontoxic.

THE USES OF CYCLODEXTRIN IN SOCIETY

Cyclodextrin (CD) was first discovered by Villers in 1891 and was isolated as a product of the starch-digesting bacteria, *Bacillus amylobacter* (Bender et al. 1978; Szejtli, 1998). Originally, Villers named the substance cellulosine due to its likeness to cellulose (Szejtli, 1998). Cyclodextrins have also been called Schardinger dextrins, cycloamyloses, and cycloglucans (Bender et al. 1978). In 1904, Schardinger isolated a starch-digesting organism called *Bacillus macerans* while trying to determine the microorganisms involved in food poisoning. He found *Bacillus macerans* to produce harmless crystalline dextrins, now known as cyclodextrins, which he further isolated into the main types of CD (Szejtli, 1998). Schardinger's work is considered the basis of cyclodextrin chemistry.

Cyclodextrins are typically defined as a doughnut or conical cylinder shaped complex sugars containing a cavity that houses inclusion compounds (Bender et al. 1978; Bizzigotti et al. 1997; Szejtli, 1998). This interior hole is relatively hydrophobic, allowing complexation with hydrophobic substances. The exterior surface of CD is hydrophilic, causing increased solubility in complexed hydrophobic compounds in water. The internal cavity varies in size depending on the type of CD, which allows different sizes of molecules to bind with the cyclodextrin.

There are three distinct types of CD organic species: alpha (α) beta (β), and gamma (γ). These symbols represent the number of glucose units in the molecule and are 6, 7, and 8 respectively. Numerous CD derivatives have been created to enhance the properties of the original CD (Bender et al. 1978; Hedges, 1998). For example, modifications of the hydroxyl group on the outer surface of the CD can be used to increase or decrease the solubility of the CD. Hydroxypropyl-beta-CD (HPβ-CD), which was used in this research, enhances the solubility of β-CD by replaced the hydroxyl group with a hydroxypropyl group (Hedges, 1998). A more in-

depth description of CD modification and the types of CD can be found in Bender and Komiyama (1978), Hedges (1998), and in Szejtli (1998).

From the 1930s through the 1960s little research was completed on cyclodextrins due to one study that found CDs to be promising for industrial applications, but toxic and expensive (Szejtli, 1998). Later toxicological work revealed that the toxicity claim was false. As a result, cyclodextrin research again in the 1970s and continued through the present, which is shown by a logarithmic increase over time in CD related articles and patents.

Today, uses for CD range from medical and pharmaceutical to cosmetic and household uses. CDs are used to enhance or mask the qualities or substances in question and are even being incorporated into products for commercial use (Hedges, 1998). For example, researchers have found that, in some cases, CD successfully inhibits the HIV virus (Leydet et al. 1998). The following examples are just a few of the uses for CD (Hedges, 1998):

(1) Prevention of rashes due to poison ivy
(2) Enhanced delivery of steroids such as hydrocortisone
(3) Solubilization and renaturing of denatured proteins
(4) Prevention of browning in juices
(5) Removal of large lipids such as ear wax
(6) Control of fragrance release in laundry sheets

The cost of CD has dropped through the years as it has become more economic to manufacture due to an increase in scientific knowledge and improved technology. Originally in 1970 one of the three main types of CD, beta (β) -CD, cost approximately $2,000 per kg. Now it only costs several dollars per kg for technical grades (Szejtli, 1998).

CYCLODEXTRIN AS A SOLUBILIZATION AGENT

Experimentally, CD has been shown to solubilize many types of hydrophobic contaminants commonly found at polluted sites. Two types of CD that have effectively removed explosives from contaminated military areas are Hydroxy-propyl β-CD and randomly methylated β-CD (Jozefaciuk et al. 2001). Wang and Brusseau (1995b) found CD to enhance the solubility of naphthalene, pyrene, anthracene, acenaphthene, phenanthrene, and fluoranthene, which are types of polycyclic aromatic compounds (PAHs). Cyclopentanol was also used in this study to further enhance the solubility effect of CD. Several studies were also conducted determining

that CD effectively enhanced remediation of mixed waste containing contaminants ranging from organic solvents to heavy metals (Brusseau et al. 1994; Wang and Brusseau, 1995a; Brusseau et al. 1997).

Several pilot and laboratory studies have suggested that adding cyclodextrin (in the form of HPβ-CD) to aquifers containing chlorinated solvents and other DNAPLs is an effective method of enhancing pump-and-treat (McCray et al. 1999; McCray and Brusseau, 1999; Blanford et al. 2000; McCray et al. 2000). Theoretically, the injection of CD would enhance the solubility of chlorinated solvents in groundwater (e.g., PCE), thus making them readily removable from the contaminated aquifer during pumping. Adding CD to the contaminant source zone would potentially decrease the necessary remediation time relative to the time of remediation associated with other technologies.

McCray et al. (1999) performed a pilot scale field study comparing a normal pump-and-treat water flush to a CD enhanced or complexing sugar flush (CSF). The amount removed for each of the several NAPLs tested by the flushing was greatly enhanced by CD addition. In fact, for TCE, the removal amount that occurred in ten days of CSF would have approximately taken more than a year of water flushing (McCray et al. 1999). Concentrations of TCE removed from an aquifer extraction well increased three fold due to the addition of a cyclodextrin (Blanford et al. 2000). The same study found the TCE removal rate to return to six percent of the previous removal rate during the CD pulse when the pulse ceased.

EFFECTIVENESS OF CD FOR CHLORINATED SOLVENT REMOVAL

All remediation strategies described previously rely on CD as a solubilizing or complexing agent. Although CD enhances the solubility of compounds in a manner similar to surfactants and cosolvents, some researchers have demonstrated that both surfactants and cosolvents are markedly more effective than CD in increasing contaminant solubility, which can actually mobilize the contaminant (McCray and Brusseau, 1998; McCray et al. 1999**)**. Studies conducted both in field and laboratory settings have shown that, unlike surfactants, which can exhibit retardation and mass loss effects due to pore exclusion and adsorption, CD is not lost to either of these mass sinks and thus displays conservative transport (Brusseau et al. 1994; McCray and Brusseau, 1999).

A column experiment conducted by Boving et al. (1999) found that the solubility of PCE was increased 35 times the original solubility due to cyclodextrin addition. Also TCE, which is more soluble than PCE, experienced a 9.5 increase in solubility due to cyclodextrin addition. Another experiment found that the solubility of organic chemicals increased linearly along with increased CD concentrations (Wang and Brusseau, 1993; McCray and Brusseau, 1998).

IMPACT OF CYCLODEXTRIN USE ON THE EFFICACY OF MNA

Following the use of pump-and-treat, a chlorinated solvent contaminated site is often subjected to MNA as a final remediation strategy. The EPA has recommended this type of treatment as a long-term finishing step for many remediation strategies due to economic savings and the effectiveness of indigenous microorganisms to remove small quantities of contaminants (Wiedemeier et al. 1999). MNA is particularly useful once systems such as pump-and-treat can no longer effectively remove contaminants efficiently (Wiedemeier et al. 1999). In the cases where a solubilization agent such as CD is used in conjunction with pump-and-treat, MNA would still be a final remedy for long-term remediation.

A thorough literature review revealed that the effect of CD, during and following a CSF, on the in-situ microbial degradation of chlorinated ethenes has not been well studied. Theoretically, the introduction of CD into a chlorinated ethene-contaminated aquifer may enhance microbial degradation due to: (1) increased solvent bioavailability caused by the CD (i.e., the microorganisms will have greater access to the contaminant) and (2) the inherent toxicity of the chemical contaminant upon the microorganism is reduced (contaminant's inclusion in the CD structure, possibly minimizing microorganism exposure to the contaminant's toxicity) (Schwartz and Bar, 1995).

Olah et al. (1988) looked at the effects of ß-CD on removing pesticides from wastewater to improve its quality. In 1985, a study was conducted using ß-CD to research the microbial degradation of phenol in wastewater (Schwartz and Bar, 1995). In 1992, Wildenauer patented the use of beads containing ß-CD to increase the degradation of a variety of substances (Schwartz and Bar, 1995). Wang et al. (1998) also showed that HPβ-CD was resistant to degradation and nontoxic to phenanthrene degraders for several months. Schwartz and Bar (1995) also looked specifically at the effect of ß-CD on the toluene degrader, *Pseuodmonas*

putida and found its effects to be benign. Each of these studies found that CD had a positive affect on microorganisms and biodegradation was enhanced.

Conversely, potential negative impacts of CD usage include (1) degradation of the cells of microorganisms (i.e., CD may extract the microorganism's cell components compromising MNA capabilities) (Schwartz and Bar, 1995), (2) degradation of CD by the microorganisms (some microorganisms use starches as food sources, inhibiting the effectiveness of CD to interact with contaminants) (Saha and Zeikus, 1992), (3) reduced reductive dechlorination rates or partial or complete inhibition due to the presence of oxygen (i.e., CSF can introduce oxygen into the aquifer), and (4) inclusion of microorganisms instead of the contaminants in the CD structure (this could affect microbial reductive dechlorination capacities as well).

Although CD can be inert to some cells, CD can compromise the integrity of the cell membrane by extracting some of its components (Schwartz and Bar, 1995). This is particularly relevant to MNA, where cell viability and the structural integrity of CD are necessary for successful long-term remediation of MNA. Some microorganisms grow on starch, and since CD is a derivative of this substance, this could indicate the ability of the microbe to degrade the CD structure. Saha and Zeikus (1992) have also reported microorganisms that secrete cylclodextrinases, a CD degrading enzyme. CD degradation would compromise its solubility enhancement properties.

In the case of chlorinated solvents, injecting oxygenated water with CD as part of the remediation regime to enhance pump-and-treat could possibly endanger MNA in the long term. The oxygenated water could inhibit the anaerobic microorganisms responsible for the reductive dehalogenation of PCE, TCE, and the chlorinated daughter products (DCE, VC) (He et al. 2002).

Since CD increases the solubility of low solubility compounds by their inclusion into the CD structure, there is also a potential for microorganisms to be drawn into the hydrophilic hole of CD. Inclusion of reductive dechlorinators in the CD structure could impact their activity and hence reductive dechlorinating capacity. The possibility of microbial inclusion in the CD structure also leads to the potential for the degrading microorganisms to be removed during the CSF as CD is pumped through the aquifer thereby decreasing the capacity of MNA.

Although the injection of CD during pump-and-treat has proven to be effective in enhancing the chlorinated ethene solubility in a manner similar to surfactants and cosolvents, CSF could also cause a concentration of chlorinated ethenes that is toxic to the reductive

dechlorinators. The inclusion of PCE in the CD structure could also make the chlorinated ethene less available for biodegradation. Even though CD injections will likely increase chlorinated solvent removal in the short term, the effectiveness reductive dechlorination could be reduced, ultimately preventing remediation or increasing the time until the site is completely remediated.

Once CD is removed from the aquifer, conventional pump-and-treat may continue, and it is expected that MNA will play a role in the final remediation strategy. However, whether or not the reductive dechlorinators can rebound after the impact of CD introduction is unknown. Also, whether or not the rate of reductive dechlorination will increase, decrease, or maintain the same rate found prior to CD introduction is unknown.

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CHAPTER II

THE EFFECT OF CYCLODEXTRIN ON REDUCTIVE DECHLORINATION

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ABSTRACT

Microcosms were constructed from aquifer sediment samples taken from an actively degrading chlorinated solvent contaminated site located in Virginia Beach, Virginia. The objective of this study was to determine if and how the addition of cyclodextrin (CD) affects reductive dechlorination of chlorinated ethenes. After chlorinated solvent degradation rates were established in anaerobic and aerobic microcosms, 100 mg/L of CD solution was added for a period of 21 days. CD was then removed after 26 days to simulate the degradation response of the aquifer in a post CD injection environment. Degradation rates were determined by analyzing PCE, TCE, and *cis*-DCE concentration data over the various phases of the experiment.

Results from this study indicated that chlorinated solvent degradation could be either impaired or facilitated by the addition of CD. CD appeared to stimulate one anaerobic microcosm (IY-2c) where daughter production had not previously occurred. The activity of this microcosm was greatly enhanced by the addition of CD (0 uM/day to 13.89 uM/day). However, biotransformation of PCE in another anaerobic microcosm in which reductive dechlorination was occurring, ceased after the addition of CD (IY-1a). In a third group of microcosms the rate and extent of reductive dechlorination was greatly enhanced by the addition of CD.

The effect of adding CD was also found to be highly dependent on the redox conditions in the microcosm, specifically if the conditions were strongly reducing. The most active microcosms, found in the Aerobic Group, also had the lowest ferrous iron concentrations (3.57 mg/L for BY-1a, 2.25 mg/L for BY-1b, and 0.41 mg/L for BY-1c). The microcosm (IY-2b) that showed no daughter production had the highest level of ferrous iron (44.22 mg/L). This study presents a qualitative approach to the affect of CD on MNA.

Keywords: Cyclodextrin; Chlorinated solvents; Reductive Dechlorination; PCE; TCE; DCE

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INTRODUCTION

Due to the historic and widespread use of chlorinated solvents as dry cleaning agents, degreasers, and industrial solvents contamination of groundwater by chlorinated ethenes is quite common. Chlorinated ethenes-tetrachloroethene (PCE), trichloroethene (TCE), DCE dichloroethene (DCE), and vinyl chloride (VC)-are difficult to breakdown when in the environment. These chemicals are difficult to remove because (1) they are derived from DNAPLs (dense nonaqueous phase liquids) which tend to pool on the bottom of an aquifer and can also remain in the subsurface for more than 100 years if left untreated, and (2) dissolved phase chlorinated solvent plumes are relatively recalcitrant to remediation and microbial degradation in aerobic aquifers (Bizzigotti et al. 1997).

Traditionally pump and above ground treatment, commonly known as pump-and-treat, has been used to remove chlorinated solvent plumes at the source area by physically pumping out contaminated water and sending it through a treatment process at the surface (Blanford et al. 2001). However, due to DNAPL pooling and their insoluble nature, pump-and-treat can be very expensive, time consuming, and does not guarantee total chlorinated solvent removal. As a result, remediation experts have focused on combining pump-and-treat with the naturally occurring degradation, monitored natural attenuation (MNA).

To increase the effectiveness of pump-and-treat, surfactants and cosolvents may be injected in the source zone to increase the solubility of chlorinated solvents (US EPA ORD, 1996). Cyclodextrin (CD), a complexing agent that enhances solubility, has been proposed and tested to enhance pump-and-treat. However, unlike traditional surfactants and cosolvents, cyclodextrin is nontoxic to humans and is also biodegradable (Ko et al. 1999). While some studies have shown that the addition of cyclodextrin (CD) is effective when combined with groundwater extraction systems, it is unknown if and to what extent the addition of CD affects the indigenous microorganism population (McCray et al. 1999; McCray and Brusseau, 1999; Blandford et al. 2001; McCray et al. 2000).

Although CD injection during pump-and-treat could enhance long-term remediation of source zones, it could reduce MNA effectiveness following pump-and-treat, thereby increasing the time of or inhibiting complete remediation. To investigate this concern, an experimental study was undertaken to:

(1) Establish an active reductive dechlorinating population in microcosms and PCE mass loss rate
(2) Determine the effect of CD injection on the rate of reductive dechlorination of PCE
(3) Determine changes in the microbial response and PCE mass-loss rates following a CD injection

MATERIALS AND METHODS

Site Description and Sample Collection

In July 2001 groundwater and aquifer sediment samples were collected at a site located on the Naval Amphibious Base Little Creek (NABLC) site in Virginia Beach, VA. The NABLC is currently an active military base. The site has a history of chlorinated solvent contamination due to former presence of a laundry and dry cleaning facility. Approximately 200 gallons of dry cleaning waste containing PCE were discharged into a storm sewer between 1973 and 1978 (Rectanus, 2000). The PCE plume is located in an unconfined aquifer of the Columbia Group geological formation, which is comprised of fine to medium sized sand and fine clay and silt. Figure 2-1 shows the location for sample collection at the NABLC as well as total chlorinated ethene concentrations. The water table is approximately two meters below the land surface.

Groundwater samples were collected for chemical analysis and for construction of microcosms in the Environmental Engineering Laboratory (ENEL) at Virginia Tech. The groundwater samples were analyzed onsite for sulfides, dissolved oxygen (DO), and iron. Samples for both elements were filtered using 0.45 μm filter membranes. Iron and sulfide concentrations were determined using a HACH DR/700 Colorimeter (Loveland, CO). DO was determined using a CHEMets kit (CHEMetrics, Calverton, VA) in which samples were visually compared to standards included with the kit to determine the corresponding DO concentrations

Gas samples were also collected onsite from each well for dissolved hydrogen concentration measurements using the bubble-sampling method (Chapelle et al. 1995). A 1.0 mL gas sample was collected from each well using a bubble sampler and a gas tight syringe. The samples were then transported to a nearby lab and analyzed using a reduced gas detector (RGD) from Trace Analytical (Menlo Park, CA). The RGD has a detection limit of 0.05 μg/L. Groundwater samples with headspace were also collected from each well and later analyzed at

the ENEL for methane, ethene, and ethane gas analysis using the HP (Hewlett Packard) 5890A flame ionization detector (Wilmington, DE) equipped with a Carbosieve S-III Carbon Molecular Sieve packed column (Supelco of Bellefonte, PA). Groundwater samples were also collected in 4 mL VOC vials, transported on ice in a cooler, and were then analyzed at the ENEL on a purge and trap system for chlorinated ethenes.

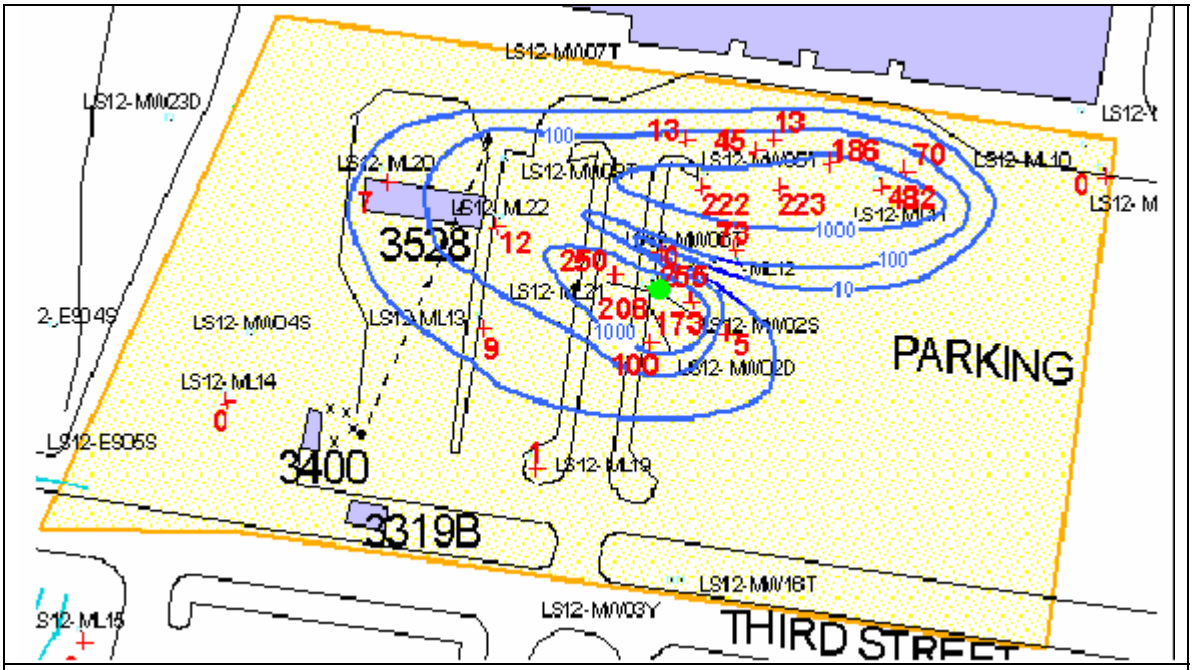

Figure 2-1. NABLC Sampling Site Plan Showing Volatile Organic Compound (VOC) Plume Migration. Solid blue lines indicate subsurface VOC concentration boundaries. The lime green circle indicates the approximate location (multilevel sampler 12) of onsite sampling for microcosm construction. This figure was extracted from a report produced by CH2MHILL (2002).

Aquifer sediment samples were collected from the Columbia Group at a depth of two to three meters below the surface for use in microcosm construction. Sediment samples were collected using a Geoprobe™ drill equipped with a hollow-stem sampling probe. Figures 2-2 shows the Geoprobe™ rig used for sample collection. The sediment sample was collected in a sterilized acetate liner, which is shown in Figure 2-3. The acetate liner was sterilized prior to use by soaking the liner in a 10 ppm (part per million) chlorine solution for 10 minutes and then rinsed with distilled water. The acetate liner was then inserted into the hollow stem sampling probe as shown in Figure 2-3 to the desired depth. Following sample collection, the liner was capped at both ends to minimize oxygen exposure to the sediment and was weighed, labeled, and

sealed in a plastic bag, which was then filled with nitrogen. The sediment sample was then placed in a cooler with ice for transport to the ENEL.

Figure 2-2. Geoprobe™ Drill Equipped with a Hollow Stem Sampling Probe at the Sediment Collection Point. This collection point is located at multilevel sampler 12.

Figure 2-3. Acetate Liner (left) and Hollow-Stem Sampling Probe (right) used for Sediment Collection

Groundwater samples were also collected for microcosm construction. The groundwater used for the microcosms was collected from multi-level samplers onsite at the same depth as the sediment. The groundwater was collected in a carboy and transported under ice to the ENEL for use in microcosm development. Once at the ENEL, both the groundwater and sediment were stored at 5°C until used in microcosm construction.

Experimental Matrix

The study consisted of three phases or experiments designed to address the research objectives. Experiment 1 was created to establish rates of chlorinated solvent degradation. The most active microcosms from Experiment 1 were then split into three and used to create the microcosms in Experiment 2. The second phase of the research was needed to establish PCE degradation and daughter production rates. Experiment 3 consisted of testing the effect of CD of microbial degradation and the changes in microbial degradation patterns following the removal of CD.

Experiment 1

The control microcosms were created at the start of Experiment 1 to allow comparison of PCE loss rates between abiotic and live microcosms with and without the presence of a biostimulant (proprionate). The experimental matrix is summarized in Table 2-1. Three controls were created for this purpose. The abiotic control provided the rate of PCE loss in the presence of sediment in the absence of microbial degradation. The sediment control indicated the rate of microbial activity in the presence of soil absent of any additives. The liquid control was constructed to measure the PCE losses in the absence of microorganisms and sediment.

All of the microcosms in Experiment 1 were created so that anaerobic conditions existed. This experiment was conducted to evaluate and quantify PCE biotransformation in intrinsic and enhanced environments. The additives that were introduced to each microcosm are shown in Table 2-1. Proprionate was added to the microcosms labeled as “B” (biostimulated) to determine the effect of the addition of an electron donor on microbial degradation. Microcosms created without the addition of an electron donor are identified by an “I” (intrinsic).

Table 2-1. Experiment 1 Microcosm Matrix

Microcosm Type	*Microcosm ID*	*Microcosm Additive*	
		PCE	Proprionate
Control Microcosms	AC	X	
	SC		
	LC	X	
CD Experimental Microcosms	I-1	X	
	I-2	X	
	I-3	X	
	I-4	X	
	I-5	X	
	I-6	X	
	B-1	X	X
	B-2	X	X
	B-3	X	X
	B-4	X	X
	B-5	X	X
	B-6	X	X

AC = abiotic control, SC = soil control, LC = liquid control, I = intrinsic, and B = biostimulated.

Experiment 2

Table 2-2 illustrates the experiment matrix of Experiment 2. The four microcosms displaying active degradation of PCE from Experiment 1 (B-1, B-2, I-1, and I-2) were each divided into three parts to create the microcosms for Experiment 2. Figure D-2 in Appendix D provides the exact details of the separation of Experiment 1 microcosms to create the Experiment 2 microcosms. In Experiment 2, the added electron donor was changed from proprionate to yeast in an effort to further enhance reductive dechlorination..

The microcosm IDs of Experiment 2 microcosms were related to the IDs of the active microcosms in Experiment 1. The ID (letter and number for example B-1) used to identify the Experiment 1 microcosm was used in the Experiment 2 microcosm name. The addition of yeast to every microcosm in Experiment 2 is signified by the present of "Y" in the microcosm name. Since each Experiment 1 microcosm was divided into three parts and the same name was used to identify Experiment 2 microcosms, a, b, and c were also added to the microcosm name to identify each new Experiment 2 microcosm.

The abiotic control from Experiment 1 was also divided into three parts for Experiment 2 to create the three abiotic controls (AC-1, AC-2, and AC-3 in Table 2-2). Sodium azide was also added to each of the controls at this time to ensure that no microbial growth occurred. The abiotic controls were then designated by the letters "AC" and the numbers 1, 2, or 3.

Table 2-2. Experiment 2 Microcosm Matrix

Microcosm Type	*Microcosm ID*	*Microcosm Additive*		
		PCE	**Yeast**	**Sodium Azide**
Control Microcosms	AC-1	X		X
	AC-2	X		X
	AC-3	X		X
	SC			
	LC	X		
CD Experimental Microcosms	IY-1a	X	X	
	IY-1b	X	X	
	IY-1c	X	X	
	IY-2a	X	X	
	IY-2b	X	X	
	IY-2c	X	X	
	BY-1a	X	X	
	BY-1b	X	X	
	BY-1c	X	X	
	BY-2a	X	X	
	BY-2b	X	X	
	BY-2c	X	X	

AC = abiotic control, SC = soil control, I = intrinsic, Y = yeast, B = biostimulated, and Y = yeast.

Experiment 3

Once degradation was reestablished, the Experiment 2 microcosms were divided into three groups (Anaerobic Group One, Anaerobic Group Two, and the Aerobic Group) to conduct the experiments shown in Table 2-3. The last group of three microcosms (called Group Four in Table 2-3) were not used in Experiment 3 due to relatively low PCE loss rates during Experiment 2.

Experiment 3 consisted of exposing the microcosms to CD and also monitoring the microcosms following the removal of CD. The IDs in Table 2-3 explain what conditions and additives are present in the microcosm. Each group of three microcosms contains two microcosms with CD and one microcosm without CD. This setup allows comparison within

each condition scenario or group of the changes due to the CD addition. The Anaerobic Group One microcosms were designed to simulate the effect of CD on microbial degradation without the presence of oxygen. The Anaerobic Group Two microcosms were designed to simulate the effect of CD on an environment where PCE was shown to degrade, but without the production of daughter products (TCE, *cis*-DCE, VC). Finally, the Aerobic Group microcosms were designed to simulate the effect of CD in the presence of oxygen on an actively degrading microbial community. The details of the actual microcosm construction, additives, and sampling for each matrix are included in the sections below.

Table 2-3. Experiment 3 Microcosm Matrix

Microcosm Group	*Microcosm ID*	*Microcosm Additive*			
		PCE	**Yeast**	**CD**	**Oxygen**
Anaerobic Group One	IY-1a-NC	X	X	X	
	IY-1b-N	X	X		
	IY-1c-NC	X	X	X	
Anaerobic Group Two	IY-2a-N	X	X		
	IY-2b-NC	X	X	X	
	IY-2c-NC	X	X	X	
Aerobic Group	BY-1a-A	X	X		X
	BY-1b-AC	X	X	X	X
	BY-1c-AC	X	X	X	X
Group Four	BY-2a-N	X	X		
	BY-2b-N	X	X		
	BY-2c-N	X	X		

I = intrinsic, B = biostimulated, Y = yeast, N = anaerobic, A = aerobic, and C=CD added.

Microcosm Construction

During the construction of the Experiment 1 microcosms, the aquifer sediment was first extruded in an anaerobic glove box. Approximately one inch was removed from each end of the liner as waste and the remaining soil was then homogenized in preparation for microcosm creation. Groundwater collected from the site was autoclaved for 15 minutes at 121°C and then cooled in the glove box. To construct microcosms, 150 grams (g) of aquifer sediment and 180 mL of groundwater were placed into 250 mL beakers modified to fit a Mininert™ valve. Figure 2-4 depicts the modified flask setup used for Experiment 1 microcosms. Once constructed, all of the microcosms were covered and stored in the dark at 20°C.

Figure 2-4. Schematic of the Modified Flask Setup (right) for Experiment 1 Microcosms and the Serum Vial Setup (left) for Experiment 2 and 3 Microcosms.

The microcosms for Experiment 2 contained approximately 47 g of soil and 60 mL of autoclaved groundwater in 100 mL serum vials (shown in Figure 2-4) with Mininert^{TM} valves. After 58 days the Experiment 2 microcosms were bioaugmented with 5 g of sediment from another group of laboratory microcosms constructed using site sediment to address inactivity (Rectanus, 2000).

CD and oxygen were introduced in the microcosms as described below in the supplement section. After 21 days of CD exposure, the water was removed from the microcosms and was replaced with non-CD water. The non-CD water had the same additives (minus CD) for each microcosm given in Table 2-2. For Experiment 1 and 2, each microcosm was shaken for ten seconds following purge and trap sampling. The Experiment 3 microcosms were shaken each day for approximately ten seconds to ensure that all of the soil was exposed to the CD solution.

Controls

At the time of the initial microcosm construction at the end of July 2001, aquifer sediment for the abiotic control was autoclaved for at least ten cycles (30 minutes per cycle at 121°C) and then returned to the glove box. After autoclaving, soil was added to autoclaved water to complete the abiotic control microcosm. The sediment control microcosm was created by adding soil (not autoclaved) and autoclaved water. Autoclaved water was added to a modified flask to create the liquid control. The control microcosms contained the same amount of soil (150 g) and/or water (180 mL) throughout the entire experimental period as initially established.

Microcosm Additives

Initially PCE was added to Experiment 1 the microcosms at a concentration of approximately 18 mg/L. In Experiments 2 and 3 PCE was added to the microcosms at a concentration of approximately 15 mg/L. The microcosms were respiked with PCE when the PCE levels fell below approximately 3 mg/L or when the microcosms were opened.

After microcosm construction, resazurin and cysteine were added to the microcosms according to standard microbial procedures for oxygen indication and reducing potential, respectively (Demain and Solomon, 1986). Resazurin is a solution that changes color based upon the oxygen concentrations present in the microcosm. Cysteine is an acid solution (which must be neutralized before addition) that reduces the oxygen level in the microcosms. Cysteine was not added to the new Experiment 2 or 3 microcosms (Tables 2 and 3) due to concerns that it may inhibit reductive dechlorination. All supplements were added to the respective microcosms any time the water was replaced.

Proprionate was also added as an electron donor source to the Experiment 1 microcosms at 0.1 mL as determined by the method given in Fennell et al. (1997). For Experiments 2 and 3, yeast extract was added (200 mg/L concentration) to the microcosms to further encourage microbial activity. The microcosms were periodically replenished with both the proprionate and yeast approximately once per month so that a consistent amount of electron donor source was present in the microcosms.

Sodium azide at a 0.05% concentration by volume was added to the abiotic control microcosms in Experiment 2. This addition ensured that the abiotic controls remained sterile at the time of separation for Experiment 2.

At the start of Experiment 3 CD was added to select microcosms as indicated in Table 2-3. CD was added at a concentration of 10.0% by volume to the water and then introduced into the microcosms.

DO (dissolved oxygen) was initially added to the aerobic group microcosms of Experiment 3 (Table 2-3) at a concentration of 8.0 mg/L. This DO level was achieved by bubbling air for approximately 30 minutes via an aquarium filter pump attached to a 0.45 μm filter. During the bubbling period, the DO level was monitored by using a DO probe. Due to the rapid usage of DO in the aerobic microcosms, 5.0 mL of water at approximately 12.8 mg/L DO was later added to the microcosms to maintain an aerobic environment. This concentrated DO level was achieved by pressurizing the water to enhance the DO levels that naturally could be added to the water.

Microcosm Sampling Procedures

Sampling of the microcosms typically occurred every seven days. Both the headspace gas and the liquid inside the microcosms were analyzed for gas production and chlorinated ethene degradation. Liquid analysis was conducted on a purge and trap system (type of gas chromatogram) to measure chlorinated ethene concentrations (described in more detail in the next section). Using a gas tight syringe, a 0.2 mL liquid sample was withdrawn from each microcosm and injected into a 5.0 mL distilled water solution. This combined mixture was then injected into the purge and trap unit. Between each sample, the gas tight syringe was rinsed 3 times with methanol and once with distilled water to eliminate contamination between samples.

Headspace samples were analyzed for carbon dioxide and methane. Gas samples of 1.0 mL were withdrawn using a gas tight syringe in duplicate approximately every two weeks from each microcosm until day 110, which included the time during Experiment 1 and part of Experiment 2. The samples were then analyzed on the TCD (method described in Technical Analysis).

Every other week, the tops of the microcosms were opened to relieve gas pressure and prevent microcosm explosion by removing 1 mL of headspace gas from each microcosm. Due to

the frequent opening and sampling of the microcosms during Experiment 3, headspace sampling was not performed, as it would not provide an accurate indication of the gas levels present in the microcosms. The microcosms were then "burped" every week to relieve the gas pressures in the microcosms.

Liquid samples from the CD-spiked microcosms from the CD Experimental Matrix were analyzed on the HPLC (as described below) to quantify changes in CD concentration. Using a 1.0 mL gas tight syringe, 0.5 mL samples were withdrawn from the respective microcosms following liquid sample collection. These liquid samples were stored at 4°C until testing was conducted. Periodically samples from non-CD microcosms were withdrawn for comparison.

Following the removal of the CD-enhanced water during Experiment 3, pH, DO, and Fe (II) readings were taken from each microcosm containing CD and from the Aerobic Biotic control microcosms. The Fe (II) concentration was determined by a HACH kit as described in the Site Description and Sample Collection section (Method 8146). For comparison purposes, samples were also collected for iron analysis on the ICP (iron detection limit of 0.006 mg/L). To determine the pH of each microcosm, pH paper was used. A DO probe was used to determine the DO level in each microcosm. The microcosms were then tested in the same manner after a period of two weeks following the removal of the CD-enhanced water and the addition of non-CD water.

Technical Analysis Methods

The purge and trap system used for liquid sampling contained a 16 port Model 2016 Tekmar auto sampler which fed into a Tekmar 3000 Purge and Trap Concentrator (Tekmar Company, Cincinnati, OH). The concentrator then fed into a Tremetric 9001 gas chromatogram (GC) (Tremetrics Inc., Austin, TX) containing a 6C RTX Volatiles megabore capillary column (Restek, Bellefonte, PA). The GC was connected to a Tracor 1000 Hall detector (Tracor Instruments, Austin, TX).

Once a sample was injected into the auto-sampler, the sample was purged with helium for approximately 11 minutes to strip off all of the volatile chlorinated ethenes. Next the volatiles were condensed on a Tenex silica gel charcoal trap. Following the purging period, the trap was heated to 230°C for ten minutes with a two minute desorbing time period. The chlorinated ethenes were then carried via helium gas (25 mL/min) into the GC. To separate the ethenes and

identify results, an increased temperature program was used on the GC. The temperature program was given as follows:

- The temperature was held at 35°C for five minutes.
- The temperature then rose at 6 °C per minute until 95°C was achieved.
- The temperature was immediately increased at a rate of 25°C per minute until the final temperature of 225°C was achieved.
- There was no holding time for the temperature for either of the two rate increases.

The ethenes were then sent to the Hall detector, which had a hydrogen gas flow of 25 mL per minute and maintained a temperature of 842°C. Results were recorded on a chromatogram for each sample. The detection limit for each chlorinated ethene was approximately 1 µg/L.

Standard checks, distilled water, and duplicate run samples (in a 5.0 mL distilled water solution) were also a part of every run to ensure QA/QC. Calibration curves for PCE, TCE, *cis*-DCE, and VC were periodically created to ensure proper chlorinated ethene detection

Headspace sampling was conducted using the GC series 580 thermal conductivity detector (TCD) (Gow-Mac Instrument Company, Bridgewater, NJ). The TCD used helium as the carrier gas at a flow rate of 20 mL/min. Gas samples were transported via helium gas to the Haysap D column (Supelco, Bellefonte, PA) size 100/120 to analyze carbon dioxide and methane. The permanent gases column was four inches long with an outer diameter of ¼ inch. The column was maintained at a 32°C temperature. The detector was maintained at a 70°C temperature and the injector was kept at 90°C. The detector current was maintained at 150 amps. Each sample ran for approximately nine minutes. The detection limit for methane on the TCD is 1.40×10^{-6} moles/injection or 0.1 % by mole. The detection limit for carbon dioxide is 5.29×10^{-7} moles/injection or 0.1 % by mole. Standard samples from a Scotty II gas mix containing 5.0% carbon dioxide and 3.99% methane were injected on a regular basis to ensure quality ensurance/quality control (QA/QC).

CD analysis was completed on a HP 1090 Liquid Chromatograph (HPLC) (Agilent, Wilmington, DE). Liquid samples were identified using an HP Series 1100 Refractive Index detector (Hewlett Packard). Samples were sent via the eluent of nanopure water at a flow rate of 1mL/min to the Bio-Rad Aminex HPX-87P column (Hercules, CA) (size 300 mm x 7.8 mm). The samples were then baked at an oven temperature of 85°C for analysis. Each sample ran for

15 minutes based on an injection volume of 20 μL. The detection limit of CD on the HPLC is 0.02 % or 0.002 g/L. Standard solutions were also run every sampling session to ensure proper calibration. A calibration curve was created based on varying known CD concentrations.

RESULTS AND DISCUSSION

The results and discussion section was separated into subsections to describe the findings of each phase of the research (Experiment 1, 2, 3, and Sediment and Liquid Control Microcosms). The discussion of Experiment 3 was further divided into the microcosm groups that make up each experiment (Controls, Anaerobic Group 1 through 3, and Aerobic Group). Results of the abiotic controls were discussed in the Experiment 1 and 2 sections. Appendices were created for each subsection to include all the data collected for each experiment and microcosm. Headspace gas collection was only conducted during Experiment 1 and for part of Experiment 2 (until 110 days from the initial microcosm construction). Molar conversions (of PCE to TCE or *cis*-DCE) are used to quantify and qualify chlorinated ethene daughter production.

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Sediment and Liquid Control Microcosms

The liquid control demonstrated that a mass balance of PCE was maintained and chemical and volatilization losses were insignificant throughout Experiment 1, 2, and 3. A PCE loss rate of 0.02 uM/day was calculated for the liquid control microcosm. This relatively low PCE loss rate showed that negligible PCE loss can be attributed to volatilization. The analytical data for all of the control microcosm tests are found in Appendix D.

Analytical analyses indicated that the sediment control microcosm was absent of PCE and other chlorinated solvents throughout the experiments. This finding showed that no significant levels of chlorinated solvents were inherently present in the soil or water of the microcosms. Absence of chlorinated solvents also indicated that the indigenous microorganisms were not naturally (without chlorinated solvent injection) producing chlorinated solvents.

Gas sampling found both methane and carbon dioxide in the headspace of the sediment control. However, during the period of gas sampling, no methane and minimum carbon dioxide (between 10^{-7} and 10^{-8} mol/L) was detected in the liquid control. Methane concentration of the sediment control was 10^{-6} mol/L at the start of Experiment 1 and rapidly dropped to 10^{-8} mol/L

and remained at that concentration throughout the experimental period. The concentration of carbon dioxide gas dropped from 10^{-6} mol/L to 10^{-7} mol/L. The sediment control gas data indicated that both methane and carbon dioxide were present at low levels in the presence of PCE.

Experiment 1 Microcosms

In Experiment 1, PCE loss combined with chlorinated ethene daughter production was only observed in four of the twelve microcosms. The results of the eight inactive microcosms will not be discussed in detail in this section because they were not used in Experiments 2 and 3. The calculated PCE loss rates and gas production results for all of the microcosms used in Experiment 1 are included in Appendix A. The results of the four active microcosms are described below.

Figure 2-5 shows the mass loss patterns of PCE in microcosms I-1 and I-2 in comparison to the results in the abiotic control (AC) and the PCE loss rates are given in Table 2-5. Two mass loss phases are shown in Figure 2-5 starting at day 157 when microcosms I-1 and I-2 were producing daughter products following a PCE respike. The two microcosms showed consistent PCE loss rates (3.22 and 2.74 uM/day, respectively) and *cis*-DCE production rates. Following a PCE respike at day 222, the PCE loss rate decreased in both microcosms, most notably in I-1. Both PCE loss rates were greater than the baseline abiotic control degradation rate (0.42 uM/day). Abiotic losses may be due to chemical degradation or sorption.

The reduced rates following the second PCE respike at day 222 may be due to removal of sediment from I-1, I-2, B-1, and B-2 to bioaugment the eight inactive microcosms. The removed soil in I-1, I-2, B-1, and B-2 was replaced with the same amount of soil from the inactive microcosms. The introduction of different microbial communities due to sediment transfer may have caused competition between the different microorganisms. The dominant microbial community may have not been the original reductive dechlorinating community, leading to the reduced degradation rates following bioaugmentation. For each respike, both PCE and cysteine were injected into the microcosm. Following the removal of cysteine from the microcosms in Experiment 2, activity was enhanced. Based upon this, cysteine may also have reduced or inhibited microbial degradation.

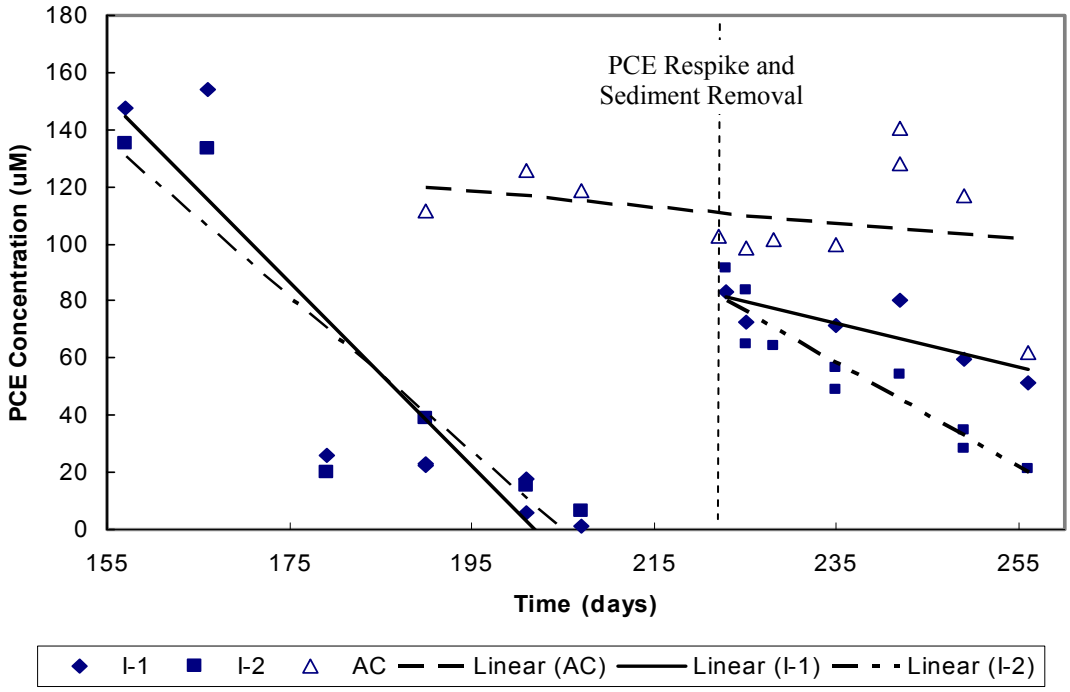

Figure 2-5. Experiment 1 PCE Data and Zero Order Loss Trendlines for I-1, I-2, and AC.

Table 2-4. Experiment 1 PCE Zero Order Loss Rates (uM/day)

Microcosm Conditons	Microcosms				
	I-1	I-2	B-1	B-2	AC
Initial Rate	3.22	2.74	0.07	0.86	0.42
Rate After PCE Respike	0.76	1.82	0.76	1.09	

As shown in Figure 2-6, *cis*-DCE was produced throughout Experiment 1 in both I-1 and I-2. For I-1, *cis*-DCE was produced at a rate of 0.67 uM/day prior to the use of the microcosm for bioaugmentation. This was approximately a 5:1 conversion from PCE to *cis*-DCE. The low conversion rate may be due to the routine purging of gas from the microcosms, which resulted in *cis*-DCE loss. Following the respike and sediment removal, *cis*-DCE production decreased drastically to a rate of 0.02 uM/day. TCE production began at this time at a rate of 0.29 uM/day, which was a 9:1 conversion from PCE to TCE.

In I-2 there initially was minimal conversion from PCE to *cis*-DCE (*cis*-DCE production rate of 0.01 uM/day). Following the PCE respike and sediment removal, there was an increase in the *cis*-DCE production rate, and *cis*-DCE was converted from PCE at about 6.7:1 although there

was no production of TCE. The lack of direct conversion in the microcosms can be due to several processes (chemical degradation, sorption, etc.) occurring in the microcosms along with reductive dechlorination.

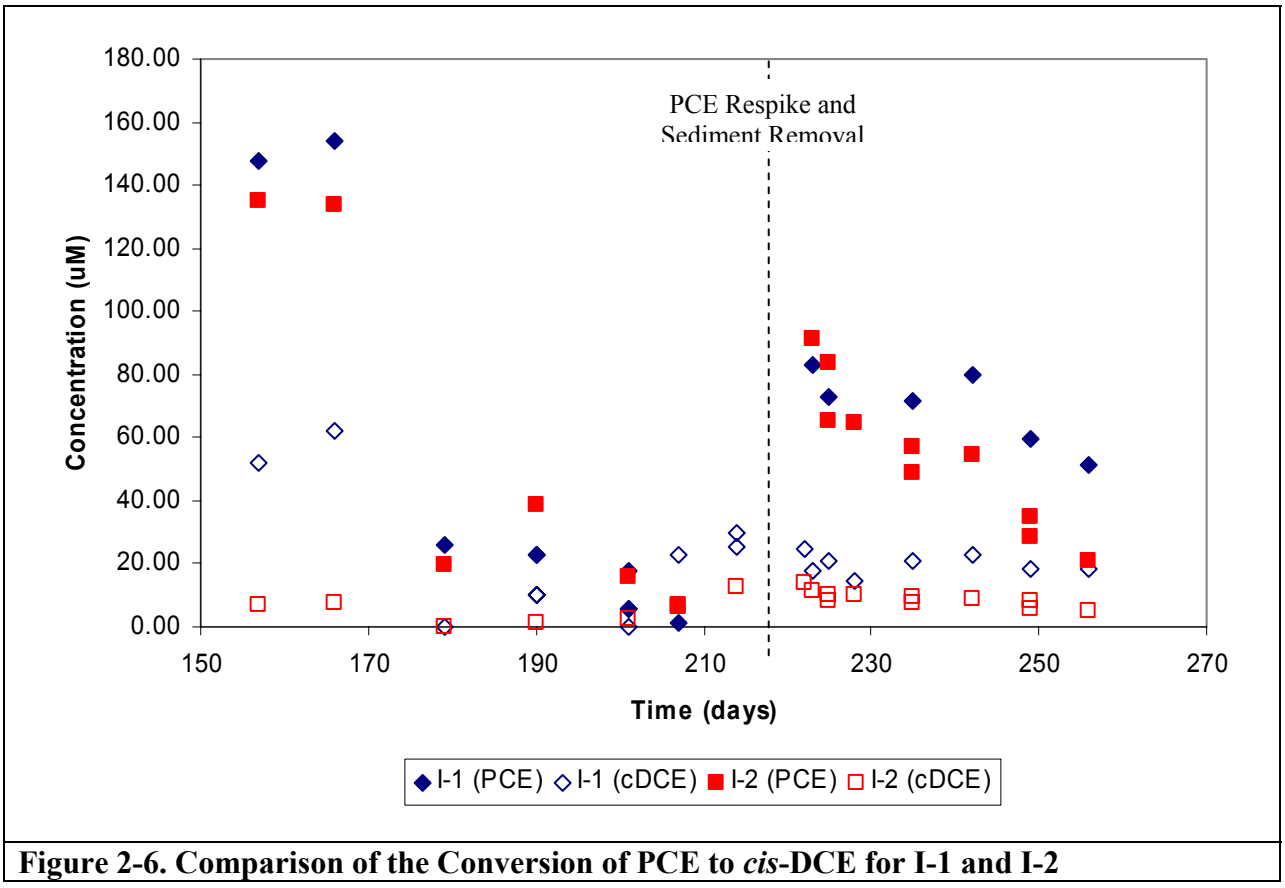

Figure 2-6. Comparison of the Conversion of PCE to *cis*-DCE for I-1 and I-2

In contrast to I-1 and I-2, the active biostimulated microcosms (B-1 and B-2) had less consistent PCE loss rates (Table 2-4) and daughter production patterns. The data used to determine the PCE loss rates for the biostimulated microcosms is plotted in Figure 2-7. Microcosm B-2 had higher PCE loss rates than the abiotic control throughout the sampling period and the B-1 PCE loss rate was also greater than the control rate after the second PCE respike. In the period before the first respike, PCE degradation only occurred in the B-2 microcosm. The low concentrations of PCE in B-1 at the first respike are due to laboratory error, resulting in an artificially low loss rate. The initial low concentrations of PCE in B-1 may also attribute to the very low PCE loss in B-1. Following the second PCE respike and use of these microcosms for bioaugmentation of the inactive microcosms, both biostimulated microcosms

showed an increased rate of PCE loss. These rates however were still lower than the PCE loss rates of I-1 and I-2. Perhaps opening these microcosms for sediment removal stimulated PCE degradation.

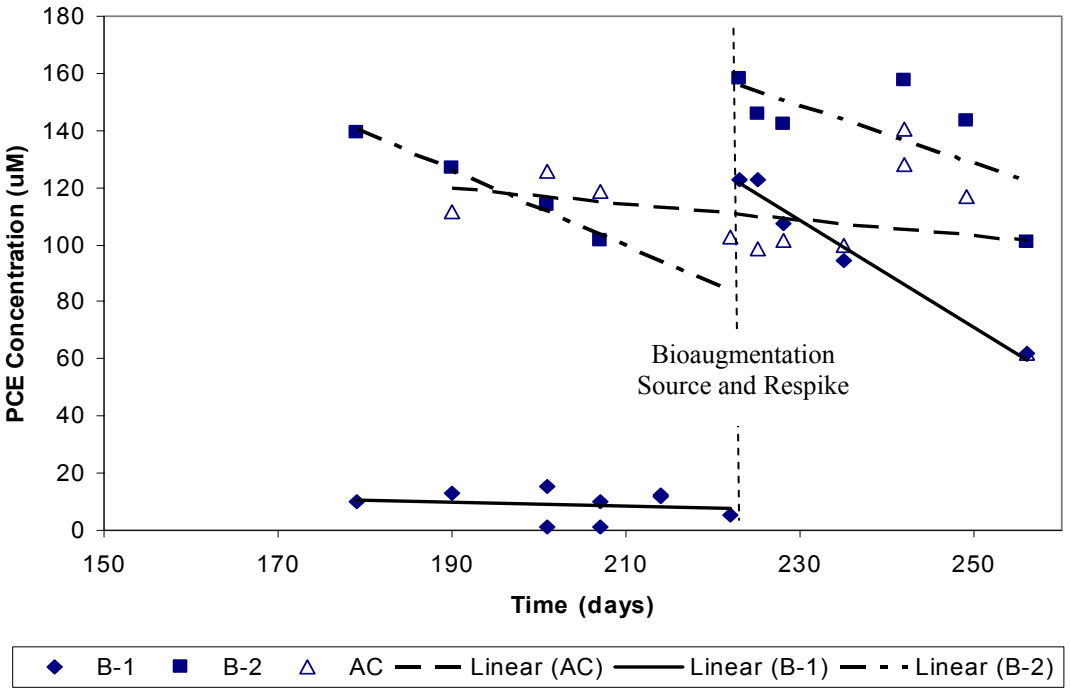

Figure 2-7. Experiment 1 PCE Data and Zero Order Loss Trendlines for B-1, B-2, and AC

In B-1 there was no daughter production until after the bioaugmentation and PCE respike event. At the time only a minor amount TCE was produced (0.0036 uM/day) and the PCE-TCE conversion was 20:1. Before the respike and bioaugmentation in B-2, *cis*-DCE was produced at a rate of 0.14 uM/day (a conversion of 6:1 from PCE). There was no *cis*-DCE production in B-2 after the respike, but TCE was produced at a rate of 0.17 uM/day. The conversion of PCE to TCE in B-2 was about 5:1 during this period.

In the abiotic control (AC) for Experiment 1, similar to the sediment control microcosm, no methane gas production was detected. There was also minimum carbon dioxide production with the concentrations consistently being 10^{-8} mol/L. Based on the rate data in Table 2-4, it appears that the lack of methane gas production indicated weak reducing conditions or virtually no microbial degradation of PCE.

In the active intrinsic microcosms, there appeared to be a stronger, more direct conversion of PCE degradation to daughter production (mostly *cis*-DCE although there was some TCE) when low concentrations of gas were present in the microcosms at slightly higher concentrations than found in the control microcosms. Both carbon dioxide and methane gas concentrations were between 10^{-7} mol/L and 10^{-8} mol/L in I-1 and I-2 throughout the experiments, with the lower concentrations occurring after the respike and sediment removal. Of the biostimulated microcosms, the only gas concentrations that appear to be above the background concentrations found in the control microcosms are found in B-1 (10^{-2} to 10^{-3} mol/L), which was less active.

Interestingly, the microcosms that showed no daughter production during PCE degradation (data not shown) had substantially higher methane concentrations (10^{-2} to 10^{-3} mol/L). It is also interesting that minimal to no carbon dioxide production occurred in these inactive microcosms. The gas data for all microcosms, including the inactive microcosms mentioned in this paragraph, is found in Appendix B.

Chlorinated solvent degradation patterns in this study were similar to those observed in other research. Results in this study were found be similar to Rectanus (2000), which determined that soil from the same U.S. Navy site contained microorganisms capable of reductive dechlorination. In particular, the microcosms in this study appeared to stop reductive dechlorination at the intermediate product *cis*-DCE, while VC production was observed by Rectanus (2000). In other wells, there was no indication of VC production. The rates conducted in this study were two to nine times higher than the rates of Rectanus (2000). The rate increase may be due to the use of proprionate to encourage reductive dechlorination in these experiments.

Experiment 2 Microcosms

The data for Experiment 2 figures begin at day 58 following their creation from Experiment 1 microcosms because no data trends were apparent until the microcosms were bioaugmented. The abiotic control microcosms however, were not bioaugmented, so the data in the figures begin at time zero. The graphs in Experiment 3 are a continuation of Experiment 2 graphs. This is reflected in the continuation of the timeline from Experiment 2 for Experiment 3 graphs. The tables for Experiment 2 and 3 rates are given in this section.

Abiotic Controls

In Experiment 2, the PCE loss rates for the three abiotic controls (AC-1, AC-2, AC-3) ranged from 0.24 to 0.34 uM/day (Table 2-5). These rates could be lower due to the injection of sodium azide at their creation. Following a PCE respike at the start of Experiment 3, two of the three abiotic controls (AC-2 and AC-3) had lowered degradation rates, and AC-1 had an enhanced PCE degradation rate relative to Experiment 2. Graphs of the abiotic control data in comparison to the experimental microcosms are found in Figures 2-8 through 2-10.

The iron (Fe^{+2}) concentrations in mg/L and pH readings for the abiotic controls of Experiments 2 and 3 are shown in Table 2-6. The pH and iron concentrations were determined for the abiotic controls to compare conditions between the control and experimental microcosms.

Table 2-5. Zero Order PCE Loss Rates (uM/day) of the Abiotic Controls for Experiments 2 and 3

Microcosm Conditions	Microcosms		
	AC-1	AC-2	AC-3
Experiment 2	0.40	0.59	0.44
Experiment 3	0.53	0.11	0.21

Table 2-6. Iron (Fe+2) Concentrations and pH Values for the Abiotic Control Microcosms

Microcosm Name	Fe^{+2} Concentration (mg/L)	pH
AC-1	2.58	6.7
AC-2	3.13	6.7
AC-3	9.22	6.7

Anaerobic Group 1

The Anaerobic Group 1 microcosms consisted of the three microcosms IY-1a, IY-1b, and IY-1c split from the Experiment 1 microcosm I-1. The PCE data trends of the three Anaerobic Group 1 microcosms compared to AC-1 are shown in Figure 2-8. The PCE loss rates in the IY-1 microcosms were greater than the rates of the abiotic control (0.40 uM/day). For approximately the first 44 days no daughter products were detected and minimal PCE degradation occurred in each of the microcosms. Following bioaugmentation and PCE respiking (day 58 in Experiment 2), two ananerobic group one microcosms consistently degraded PCE and produced measurable

amounts of daughter products. The PCE loss rates for Experiment 2 (pre CD) are given below in Table 2-7. Microcosm IY-1b had the highest PCE loss rate (1.17 uM/day). IY-1c had the lowest PCE loss rate (0.67 uM/day), similar to the loss rate found in AC-1. Microcosm IY-1a exhibited a higher rate of PCE mass loss (0.86 uM/day) than IY-1c.

Table 2-7. Zero Order PCE Loss Rates (uM/day) of the Anaerobic Group 1 Microcosms for Experiments 2 and 3

Microcosm Conditions	Microcosms		
	IY-1a	IY-1b (no CD added)	IY-1c
pre CD	0.86	1.17	0.67
CD	0.06	0.98	1.05
post CD	0.00		0.00

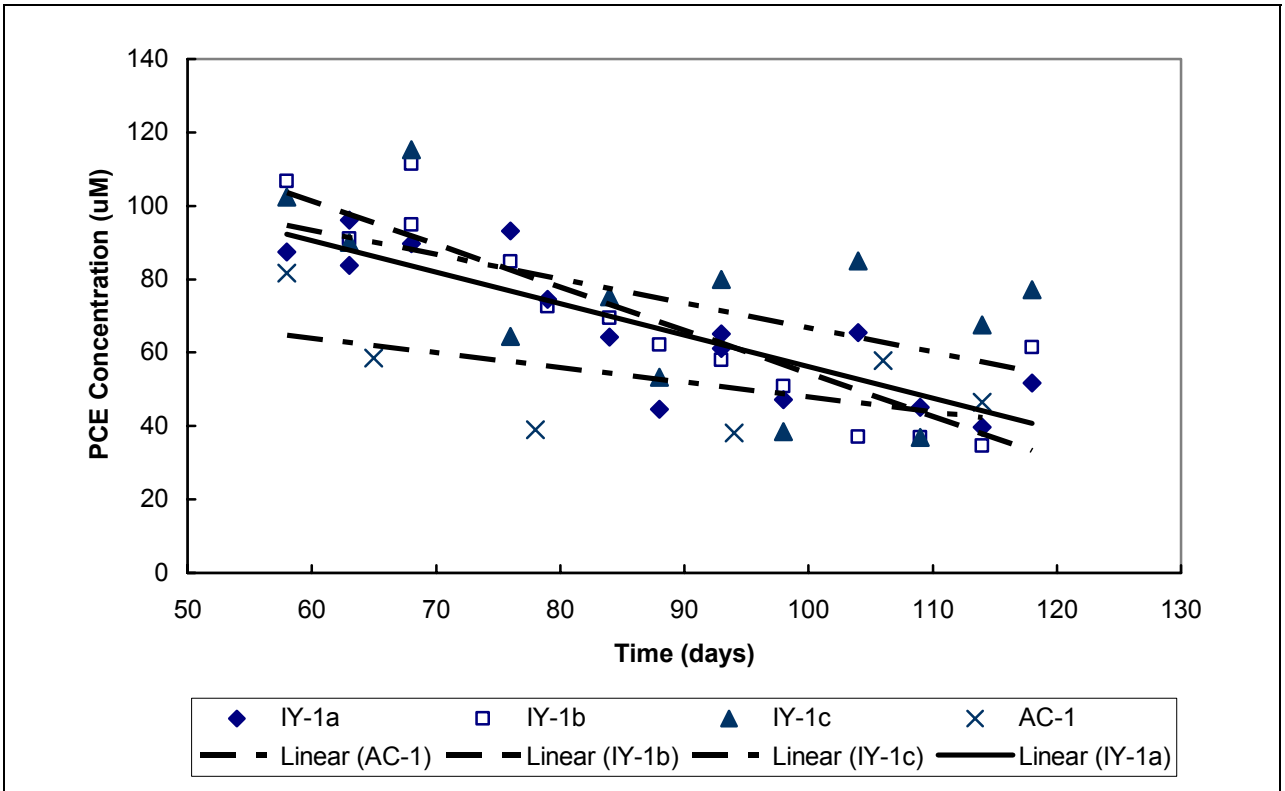

Figure 2-8. Experiment 2 PCE Data and Zero Order Loss Trendlines for IY-1a, IY-1b, IY-1c, and AC-1

In IY-1b, both TCE (.01 uM/day) and *cis*-DCE (.06 uM/day) were produced in this microcosm. For IY-1b there was a low conversion from PCE to TCE of 16.7:1. *Cis*-DCE was converted from PCE only at a conversion of 33.3:1. IY-1a had the highest daughter production

rates with 0.06 uM/day of TCE and 0.44 uM/day of *cis*-DCE. There was a 3:1 conversion of PCE to TCE and a 2:1 conversion of PCE to *cis*-DCE. There was no daughter production in IY-1c.

Anaerobic Group 2

PCE concentration data for the Anaerobic Group 2 microcosms, consisting of IY-2a, IY-2b, and IY-2c, compared to AC-2, are shown in Figure 2-9. The live microcosm data showed PCE degradation at higher rates than that of AC-2 (0.59 uM/day). The Experiment 2 PCE loss rates are given below, as the preCD rates in Table 2-8. Although the PCE loss rates shown in Table 2-8 are similar to the results for the IY-1 group (Table 2-7), this group of microcosms consistently had no daughter production throughout Experiment 2. These differences between groups could be the result of the dominance of different microbial populations.

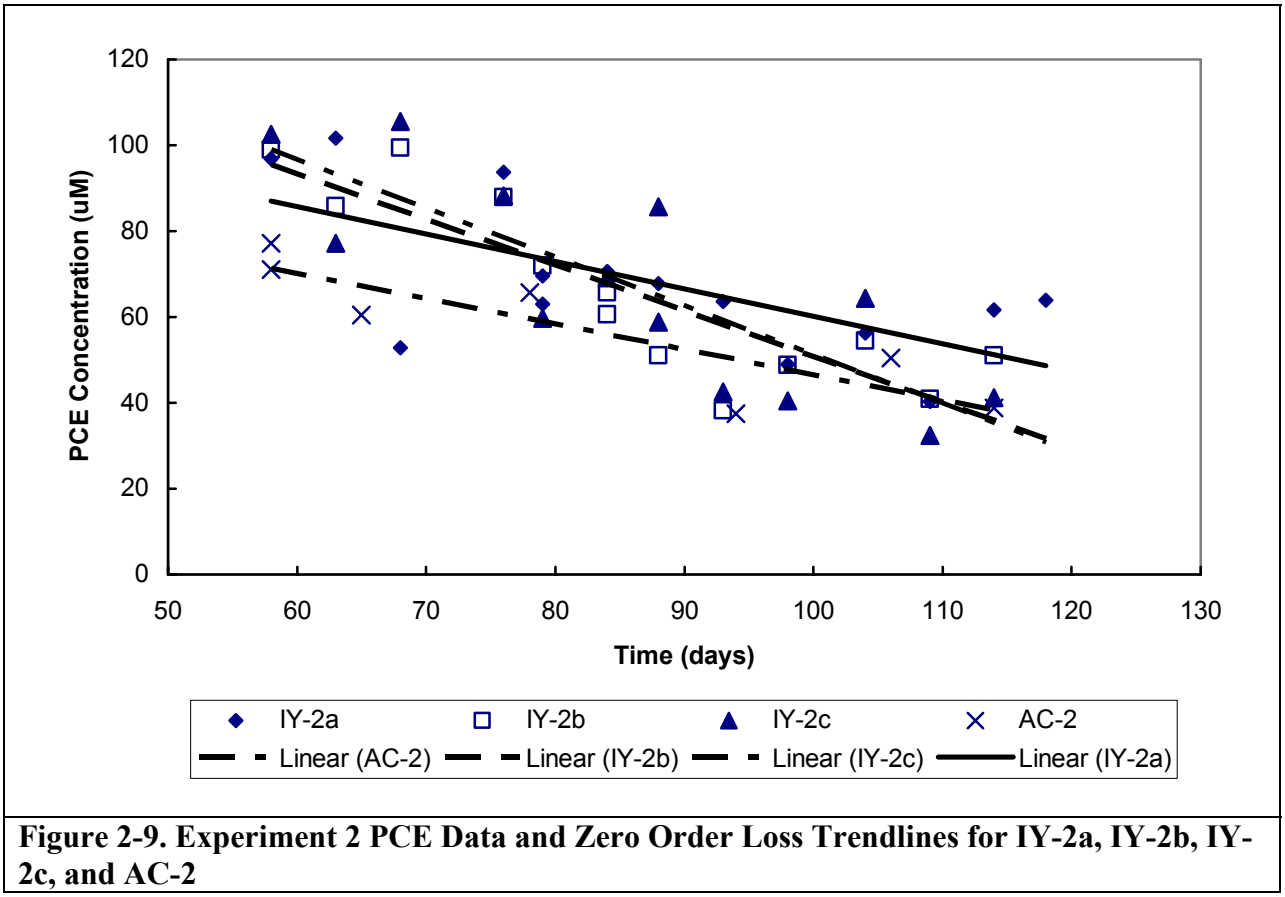

Figure 2-9. Experiment 2 PCE Data and Zero Order Loss Trendlines for IY-2a, IY-2b, IY-2c, and AC-2

Table 2-8. Zero Order PCE Degradation Loss Rates (uM/day) of Anaerobic Group 2 Microcosms for Experiments 2 and 3

Microcosm Conditions	Microcosms		
	IY-2a (no CD added)	IY-2b	IY-2c
pre CD	0.64	1.06	1.14
CD	0.49	0.11	26.30
PCE respike		1.39	0.00
post CD			1.29

Anaerobic Group 3

Anaerobic Group 3 consisted of BY-2a, BY-2b, and BY-2c. The microcosms in this group had consistent PCE degradation rates. There was sporadic production of TCE in microcosms in BY-2a and no TCE production in BY-2b. Due to the inconsistent activity in these three microcosms, they were not used for Experiment 3. They were however respiked with PCE at the beginning of Experiment 3. The rates and experimental results for the three microcosms of this group are found in Appendix B. Figure 2-10 shows the PCE concentrations in the Anaerobic Group 3 throughout Experiments 2 and 3.

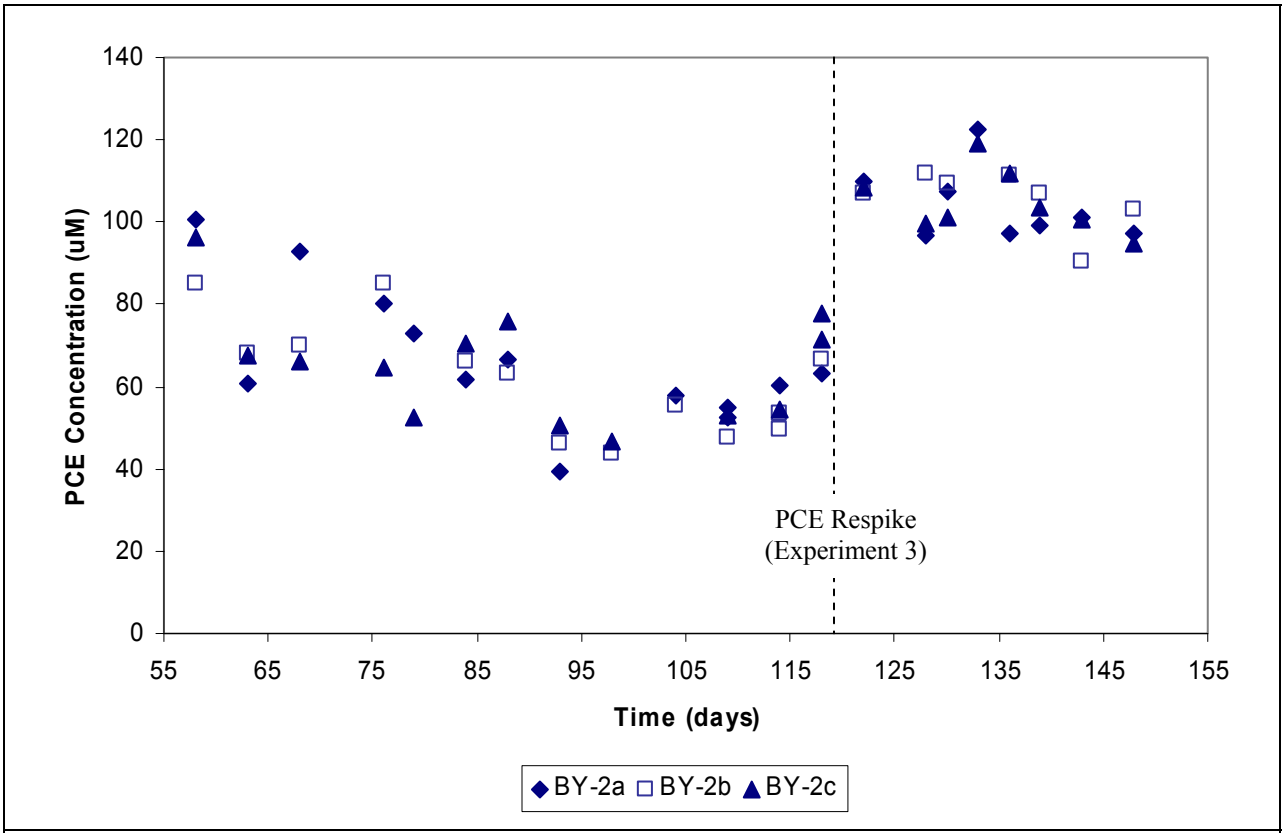

Figure 2-10. PCE Data for Anaerobic Group 3 Microcosms for Experiments 2 and 3

Aerobic Group

The Aerobic Group consisted of the microcosms BY-1a, BY-1b, and BY-1c. The data of Aerobic Group microcosms used to create the Experiment 2 PCE loss rates were compared below to AC-3 in Figure 2-11. As shown in the figure, PCE mass loss in the three live microcosms was similar and demonstrated a higher rate relative to A-3. The PCE loss rates of the Aerobic Group microcosms are shown in Table 2-9 under the "preCD" heading. The three microcosms in the Aerobic Group displayed PCE mass loss and two microcosms had consistent TCE production (BY-1a and BY-1c) throughout Experiment 2. All three of the Aerobic Group microcosms had similar PCE loss rates. BY-1c, was the most active microcosm with vigorous daughter production and had the highest PCE degradation rate of 0.87 uM/day. BY-1b had the lowest PCE loss rate (0.66 uM/day) and no consistent daughter production.

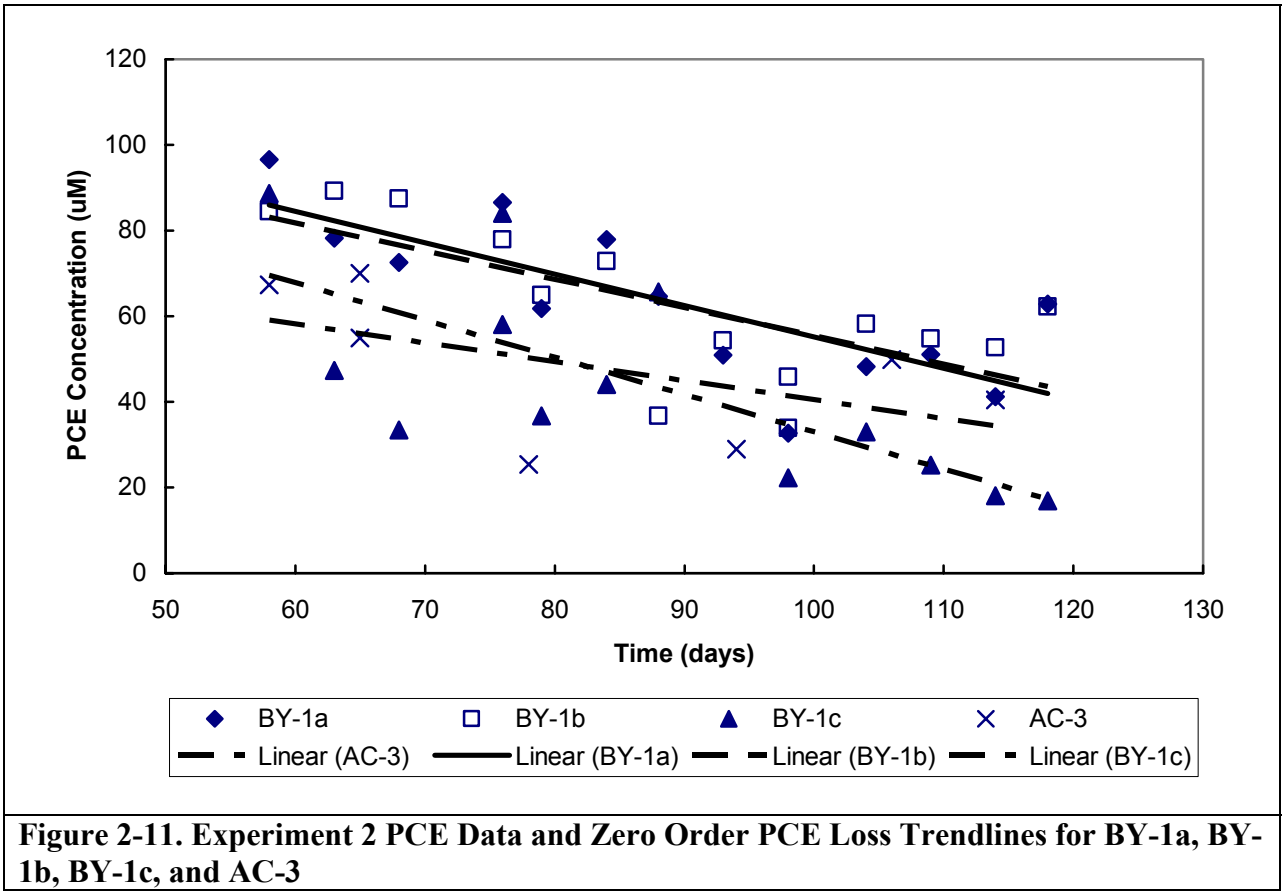

Figure 2-11. Experiment 2 PCE Data and Zero Order PCE Loss Trendlines for BY-1a, BY-1b, BY-1c, and AC-3

Similar to the results shown in Anaerobic Group 1, the Aerobic Group microcosms that produced daughter products showed the highest PCE loss rates. TCE was produced in BY-1a at a rate of 0.05 uM/day. TCE was also produced in BY-1c at a rate of 0.44 uM/day.

Table 2-9. Zero Order PCE Loss Rates (uM/day) of Aerobic Group Microcosms for Experiments 2 and 3

Microcosm Conditions	Microcosms		
	BY-1a (no CD added)	BY-1b	BY-1c
preCD	0.73	0.66	0.87
CD	5.44	20.37	28.51
respike (DO)	2.37	7.99	16.63
respike 2 (DO)	0.09	5.47	0.92
post CD & DO	2.03	78.16	76.92
PCE respike		4.24	10.70

Gas Production

Similar to the Experiment 1 data, the methane concentrations measured in each of the three abiotic controls were approximately zero. The carbon dioxide concentration for each of the controls was in range of 10^{-8} mol/L concentration. These concentrations for all the controls provide the baseline of gas concentrations of the microcosms created and used in the Experiments 2 and 3.

Interestingly all of the Anaerobic Group 1 microcosms had similar gas concentrations. Methane production was negligible in all of these microcosms. The last day of gas sampling (110 days after microcosm construction), the methane concentrations in each of the microcosms was measured at 10^{-7} mol/L range. These methane concentrations were considerably above the background concentrations in the control microcosms (approximately 0 mol/L). The methane concentrations in IY-1c, although lower than the concentrations in the other two microcosms (in the 10^{-10} mol/L range), began earlier, giving readings at day 46. The carbon dioxide gas concentration in each of these microcosms was 10^{-8} mol/L, which is similar to the background concentrations found in the abiotic controls.

The methane gas concentrations in all three microcosms of the Anaerobic Group 2 increased from 10^{-9} mol/L to 10^{-8} mol/L, which is higher than the background concentrations found in the control microcosms (0 mol/L). The carbon dioxide gas concentrations were

consistently less than 10^{-9} mol/L. These concentrations are actually slightly lower than the baseline concentrations found in the abiotic controls (in the order of 10^{-8} mol/L). The production o f methane is consistent with the conditions needed for reductive dechlorination.

Methane gas concentrations for all three of the microcosms of the Aerobic Group were consistently between 10^{-9} and 10^{-10} mol/L. However there were a few readings of methane concentrations in BY-1a between 10^{-8} and 10^{-7} mol/L. This concentration is slightly higher than the background concentration found of zero. The carbon dioxide concentrations increased in all three microcosms from 10^{-9} to 10^{-8} mol/L, which are higher than the background carbon dioxide concentrations found in the abiotic controls. Of the three groups discussed to this point, the most active microcosms (found in the Aerobic Group) have similar methane readings, which are all about the same concentration range, which is above the values found in the abiotic controls. The carbon dioxide readings of the Aerobic Group however are higher than the concentration ranges found in the other microcosm groups.

Experiment 3 Microcosms

The following results describe the changes in PCE mass loss rates upon the addition and removal of cyclodextrin from the microcosms. Due to the constant opening and sampling of the microcosms during this experiment, gas sampling was not conducted. Data collected for the Experiment 3 microcosms are found in Appendix C.

The initial concentration of the CD solution was between 71 and 73 g/L or 7 % v/v basis. Following the removal of CD from the microcosms to which a CD solution was added, a residual amount of CD was present, which varied between 7.5 and 11 g/L in all the microcosms.

Anaerobic Group 1

When CD was added to IY-1a, the PCE loss rate decreased and daughter production ceased. Following the removal of CD from this microcosm, PCE mass loss also ceased. The rates for each of the Anaerobic Group One microcosms were included in Table 2-7. The amount of CD present in the microcosm during Experiment 3 maintained approximately the same concentration (72.75 g/L) throughout the CD testing period.

The pH and DO concentration (1.2 mg/L) for this microcosm were determined following the removal of the CD solution from the microcosm. The pH for IY-1a, determined during both

the times of the presence and removal of CD from the microcosm, was found to be 6.0 in both cases. The measured iron (Fe^{+2}) concentration was 13.22 mg/L. Following the removal of CD from the microcosm, the PCE loss rate decreased to zero and there still was no daughter production. These results suggest that CD and/or the onset of iron reducing conditions may have decreased the PCE loss rates.

When CD was added to the IY-1c microcosm, the PCE loss rate increased from 0.67 to 1.05 uM/day. The iron concentration was measured as 9.83 mg/L. The pH was determined to be 6 and there was a minimal DO concentration. Following CD removal, the PCE loss rate ceased and there was no daughter production. Figure 2-12 shows the PCE loss rates for the Anaerobic Group 1 throughout Experiment 3.

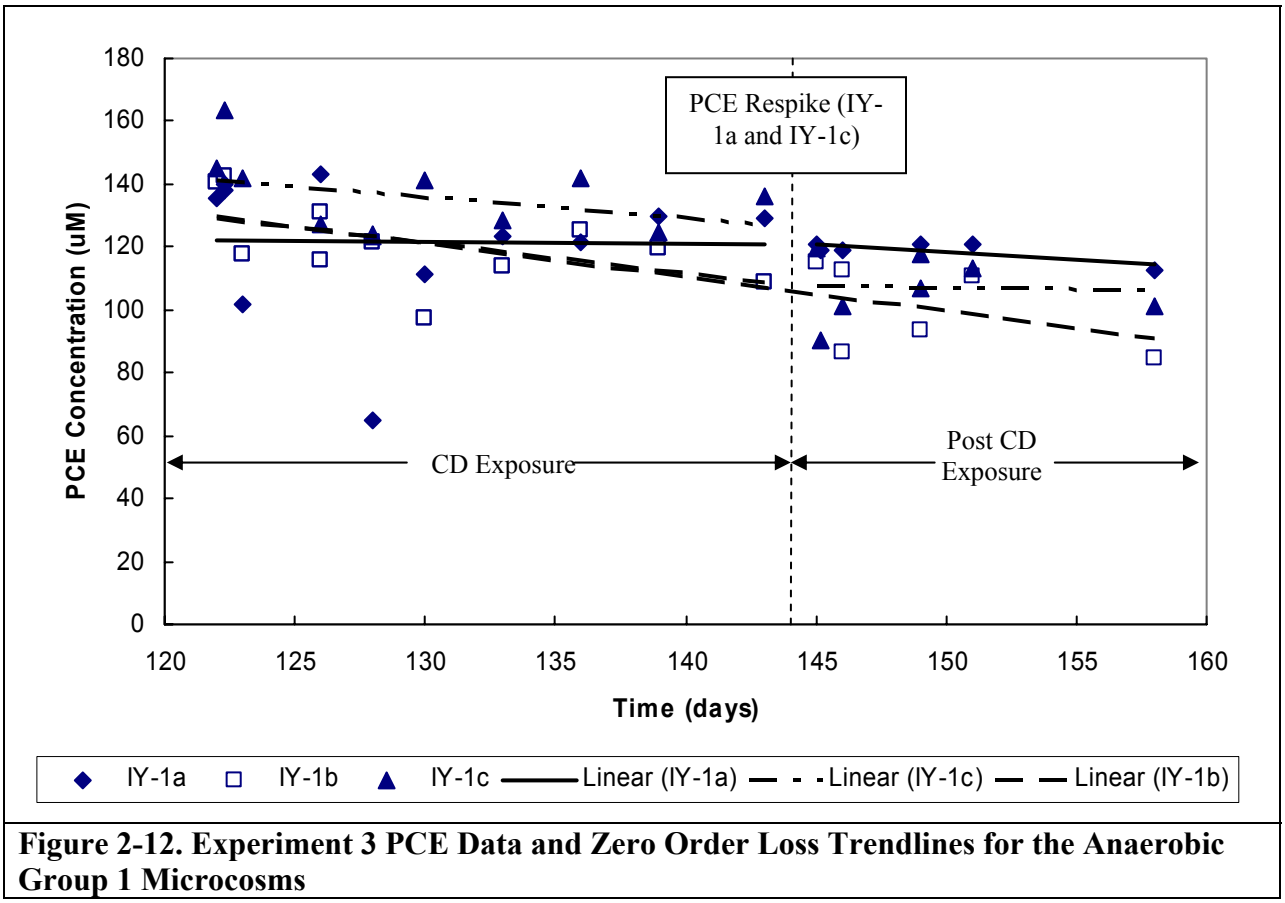

Figure 2-12. Experiment 3 PCE Data and Zero Order Loss Trendlines for the Anaerobic Group 1 Microcosms

To determine the effect of CD on the microcosms, IY-1b did not have CD added. PCE was respiked into the microcosm at the beginning of Experiment 3. The PCE mass loss also decreased (1.17 to 0.98 uM/day) in this microcosm from Experiment 2 to Experiment 3 resulting

in a PCE loss rate of 0.98 uM/day. In addition, the TCE (0.01 to 0.08 uM/day rates) substantially increased and cis-DCE (0.06 to 0.04 uM/day rates) slightly decreased following the PCE respike.

Since both IY-1a and IY-1c had similar gas concentrations in Experiment 2 and very similar pH readings, only the iron reading indicates a difference between the two microcosms. IY-1c had a slightly lower ferrous iron concentration and a larger PCE degradation rate, had no daughter production. In these microcosms, the presence of ferrous iron in solution may have changed the interactions between CD and the PCE found in solution. The iron particle itself may have been included in the CD structure or changes in the chemistry due to the higher amount of iron may have caused a chemical reaction which reduced the effectiveness of CD.

Anaerobic Group 2

Prior to the addition of CD (Experiment 2), there was no daughter production occurring with the PCE mass loss in any of the microcosms in this group. When CD was added to the microcosms in this experiment, there were mixed results in the mass loss and production rate patterns. The mass loss and production of chlorinated solvents for this group of microcosms are summarized in Table 2-8. Figure 2-13 shows the concentrations used to determine the rates in Table 2-8. CD was added to both IY-2b and IY-2c. In IY-2b, the PCE degradation rate decreased (from 1.06 to 0.11 uM/day) during the CD exposure period as compared to the pre-CD exposure rate found in Experiment 2. Following the removal of CD, the PCE loss rate once again increased in this microcosm.

The ferrous iron concentration found in this microcosm was very high at 44.44 mg/L. The pH of the microcosm was determined to be 6.2 and the DO concentration once again was minimal. The CD concentrations during the CD exposure period were maintained at the same concentration throughout the experiment. As in the other microcosms exposed to CD, there were low concentrations of CD remaining following the removal of the CD solution from the microcosms.

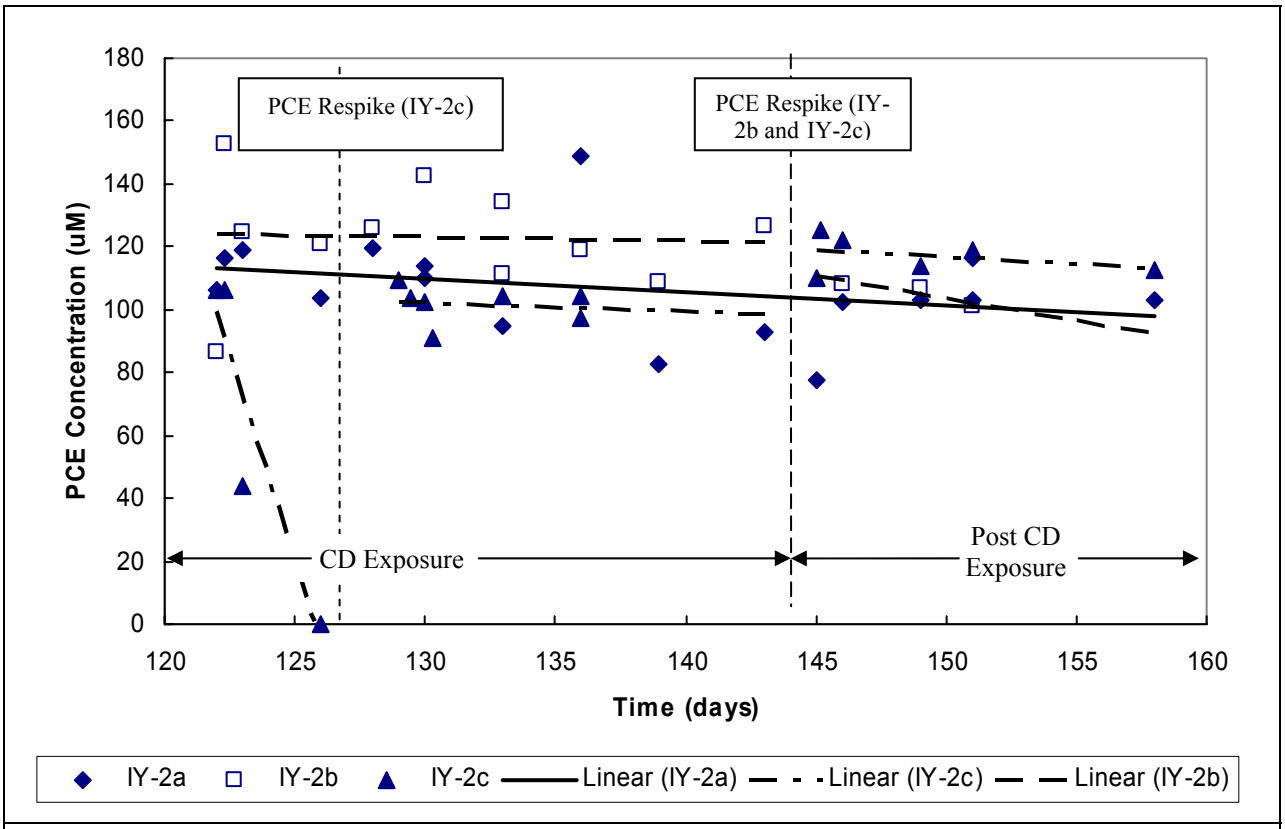

Figure 2-13. Experiment 3 PCE Data and Zero Order Loss Trendlines for the Anaerobic Group 2 Microcosms

IY-2c demonstrated enhanced in the PCE loss during the CD exposure period. During this part of Experiment 3, there was also a high amount of TCE production (rate of 13.85 uM/day, approximately a 1:1 conversion) corresponding to the PCE loss in this microcosm. The degradation and production rates were so great that the PCE was completely degraded in approximately 4.5 days. The microcosm was respiked with PCE prior to the removal of the CD solution from the microcosm. After the PCE respike, the PCE mass loss decreased to approximately zero and TCE production ceased. There was, however, a slight production of *cis*-DCE (rate of 0.01 uM/day) at this time.

After the removal of the CD solution, the PCE loss rate resumed at 1.29 uM/day, but there was no renewed daughter production in this microcosm. Similar to the above-mentioned microcosms, the concentration of CD remained consistent in the microcosms during the CD exposure period. Following the removal of the CD solution and its replacement with a non-CD liquid solution, there was a small amount of CD present in the liquid phase of the microcosm.

The ferrous iron concentrations for this microcosm were determined to be high at approximately 38.33 mg/L. The pH of the microcosm was 6 and the amount of DO in solution was minimal.

IY-2a was not exposed to CD during Experiment 3. The PCE loss rate decreased slightly (from 0.64 to 0.49 uM/day) between the preCD period of Experiment 2 and the PCE respike that occurred at the beginning of Experiment 3. Throughout Experiment 2 and 3, there was no daughter production in this microcosm.

Although IY-2b and IY-2c appeared to have similar microcosm conditions with relatively high ferrous iron concentrations, similar pH readings, and similar gas conditions, the microcosms reacted differently in the presence of CD. In IY-2b, exposure to CD appeared to decrease the PCE degradation rate, which significantly rebounded following the removal of CD. Initially, IY-2c seemed to be enhanced by the presence of CD with a substantial increase in PCE mass loss and TCE production in IY-2c stopped although a slight production of *cis*-DCE (0.01 uM/day) occurred during this respike period. This significant decrease in rates may be attributed to the fact that CD has a finite chlorinated solvent enhancing solubility, which may have been attained with the large increase in chlorinated solvent presence during the respike period. The return of PCE loss rate following the removal of CD to approximately the same level as the rate present prior to CD exposure may suggest that CD does not hinder the microcosms from returning to the conditions present prior to CD exposure. Both IY-2b and IY-2c have iron reading significantly higher than concentrations present in any of the other microcosms.

Aerobic Group

The Aerobic Group microcosms had the most significant reactions to CD introduction, showing the highest rates of PCE mass loss and daughter product generation in comparison to all the other microcosms used for Experiment 3. Each of the three microcosms in this group were exposed to oxygen and respiked with PCE at the beginning of Experiment 3. CD was added to microcosms BY-1b and BY-1c. Then PCE loss rates for each microcosm are found as CD and post CD in Table 2-9. Note that the PCE loss rates for BY-1a were the same during and after CD exposure because CD was not added to this microcosm and the conditions were not changed (i.e. PCE was not respiked and the microcosms were not "opened"). To maintain aerobic conditions, the three microcosms were injected with a saturated DO solution two times following the initial

creation of aerobic conditions in the microcosms. This respiking of DO was needed due to the rapid usage of oxygen in the three microcosms.

Initially, BY-1a, which was not exposed to CD during this experiment (as shown in Figure 2-14), had an enhanced PCE loss rate in comparison to Experiment 2 (from 0.73 uM/day in Experiment 2 to 5.44 in Experiment 3). The TCE production rate also increased from 0.05 uM/day in Experiment 2 to 0.75 uM/day (which is a PCE to TCE conversion of 25.6:1) in Experiment 3. However following this initial rate increase, the PCE loss rate and TCE production rates decreased with each addition of DO solution following the initial introduction of the DO solution. This is shown in Table 2-9 by the respike (DO) and respike 2 (DO) rows. The ferrous iron concentration of BY-1a was determined to be 6.66 mg/L. The pH was measured at 7.2 with DO concentration maintaining a minimal concentration.

BY-1b experienced a significant increase in the PCE loss rate and daughter production rate following the PCE respike, oxygen introduction, and CD addition. The PCE loss rate increased from 0.66 uM./day in Experiment 2 to 20.37 uM/day in Experiment 3 immediately following oxygen and CD introduction. The TCE production also increased. Due to the rapid use of oxygen and PCE, the microcosm was respiked with CD along with PCE after approximately six days of beginning Experiment 3. As shown in Figure 2-15, with each of the two additions of oxygen, the PCE loss rate decreased. The TCE production rate decreased between the initial introduction and CD and respike (DO+PCE) (2.25 to 0.69 uM/day) and then decreased again with the third introduction of DO to the microcosm (respike 2 (DO) (0.50 uM/day). Immediately following the removal of CD and oxygen, the PCE degradation rate increased to a rate (78.16 uM/day, a 11:1 PCE to TCE conversion) higher than any PCE loss rate previously found in the microcosm. The TCE production rate also was enhanced to a higher concentration than found in the microcosm giving a PCE to TCE conversion of 1.3:1 (a TCE production rate of 9.09 uM/day). The usage of PCE following the CD solution removal occurred in less than one day, which is faster than ever occurred previously in this microcosm. There was also now production of *cis*-DCE in this microcosm at a rate of 1.56 uM/day, or a PCE to *cis*-DCE conversion of 12.3:1.

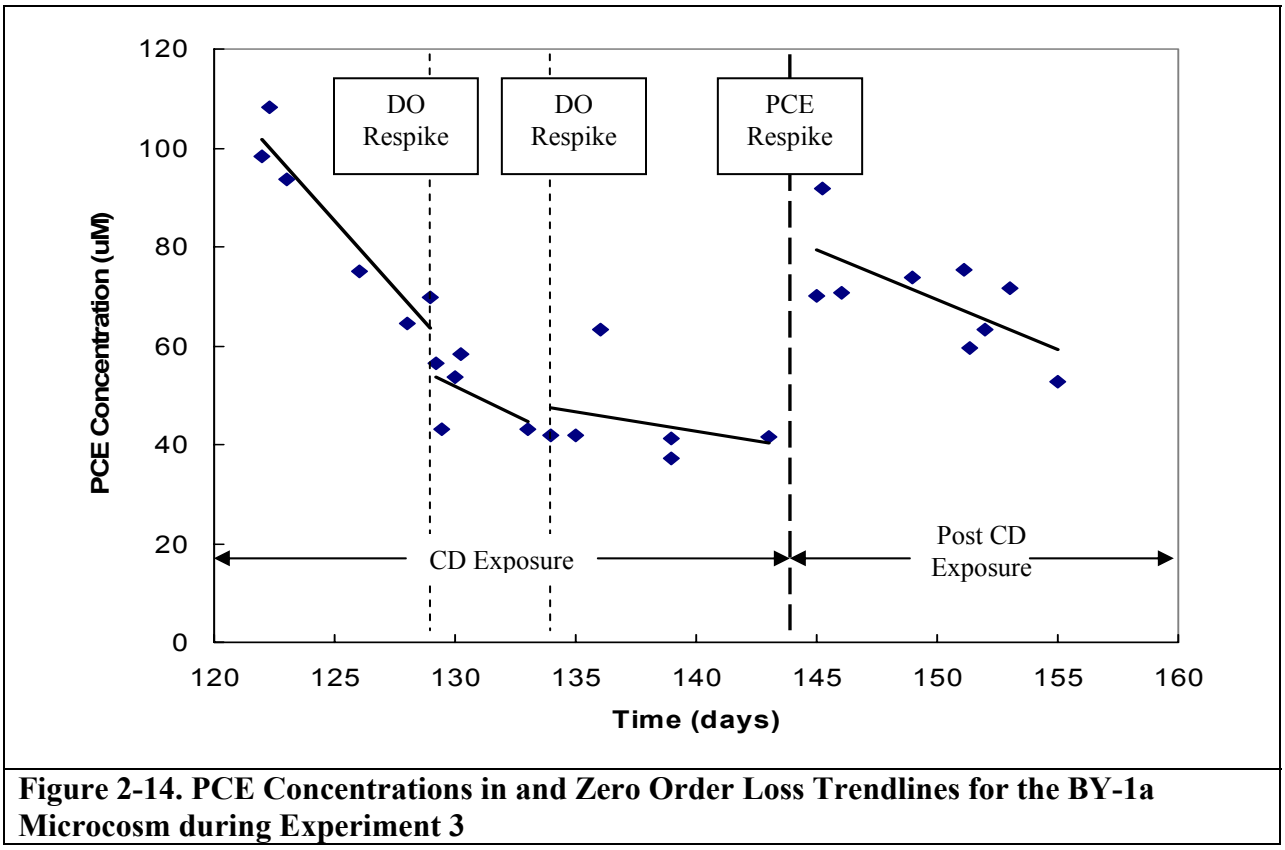

Figure 2-14. PCE Concentrations in and Zero Order Loss Trendlines for the BY-1a Microcosm during Experiment 3

Following the rapid usage of PCE in BY-1b after CD was removed; the microcosm was respiked with PCE. Following the PCE respike, cis-DCE production ceased, the TCE production rate decreased to an amount lower than the initial rate created during CD introduction to the microcosm (1.62 uM/day). PCE loss also decreased (4.24 uM/day) to a rate lower than found during CD introduction. However, this rate of mass loss is still higher than the degradation rate that occurred prior to CD exposure (0.66 uM/day). The PCE loss rate and TCE production is less than that found in BY-1c. Both the PCE loss and TCE production rates, however, are greater the rates found in BY-1a, which was only exposed to oxygen. It appears that while exposure to DO decreased mass loss and production rates, the introduction of CD enhances mass loss and production rates, even following the removal (although to a lesser extent) of CD from the microcosms.

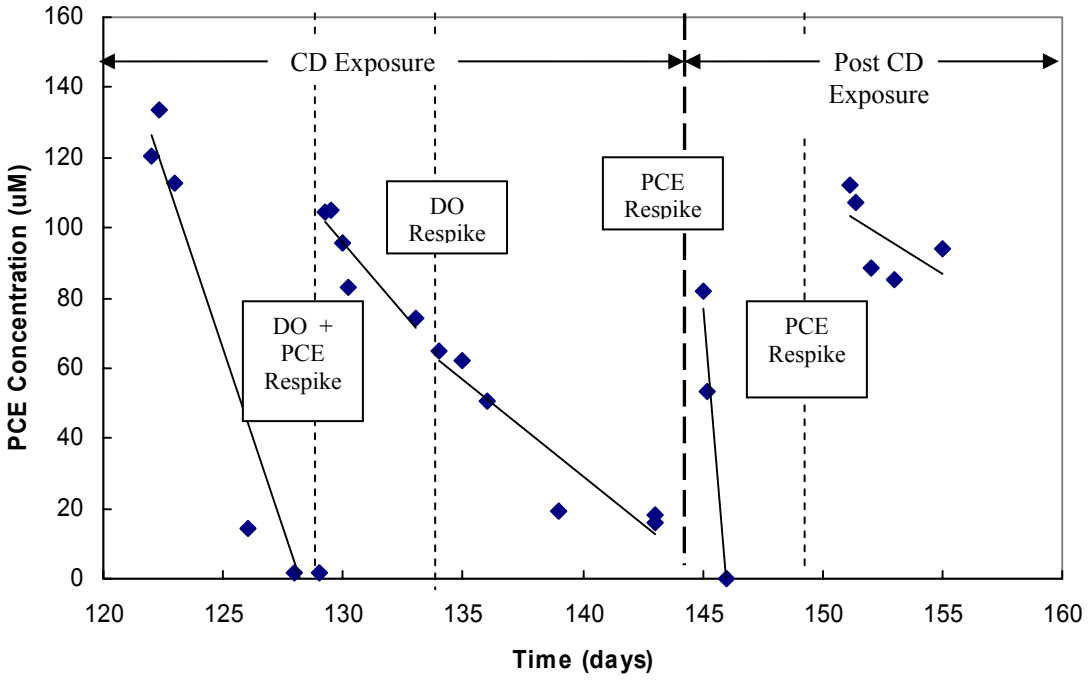

Figure 2-15. PCE Concentrations in and Zero Order Loss Trendlines for the BY-1b Microcosm during Experiment 3

As with the previous microcosm, several tests were conducted to determine the conditions present in the BY-1b. The pH of the microcosm was determined to be 6.5 with a minimal DO concentration, which is similar to the results found in the other microcosms of Experiment 3. The iron concentration present in the microcosm is 2.248 mg/L, which is significantly lower than the iron concentrations found in all the microcosms of Experiment 3 with the exception of BY-1a and BY-1c. The low concentrations of iron in comparision to the other microcosms along with the enhanced degradation and production, suggest that iron-reducing conditions were present in the other microcosms, where PCE mass loss rates were lower.

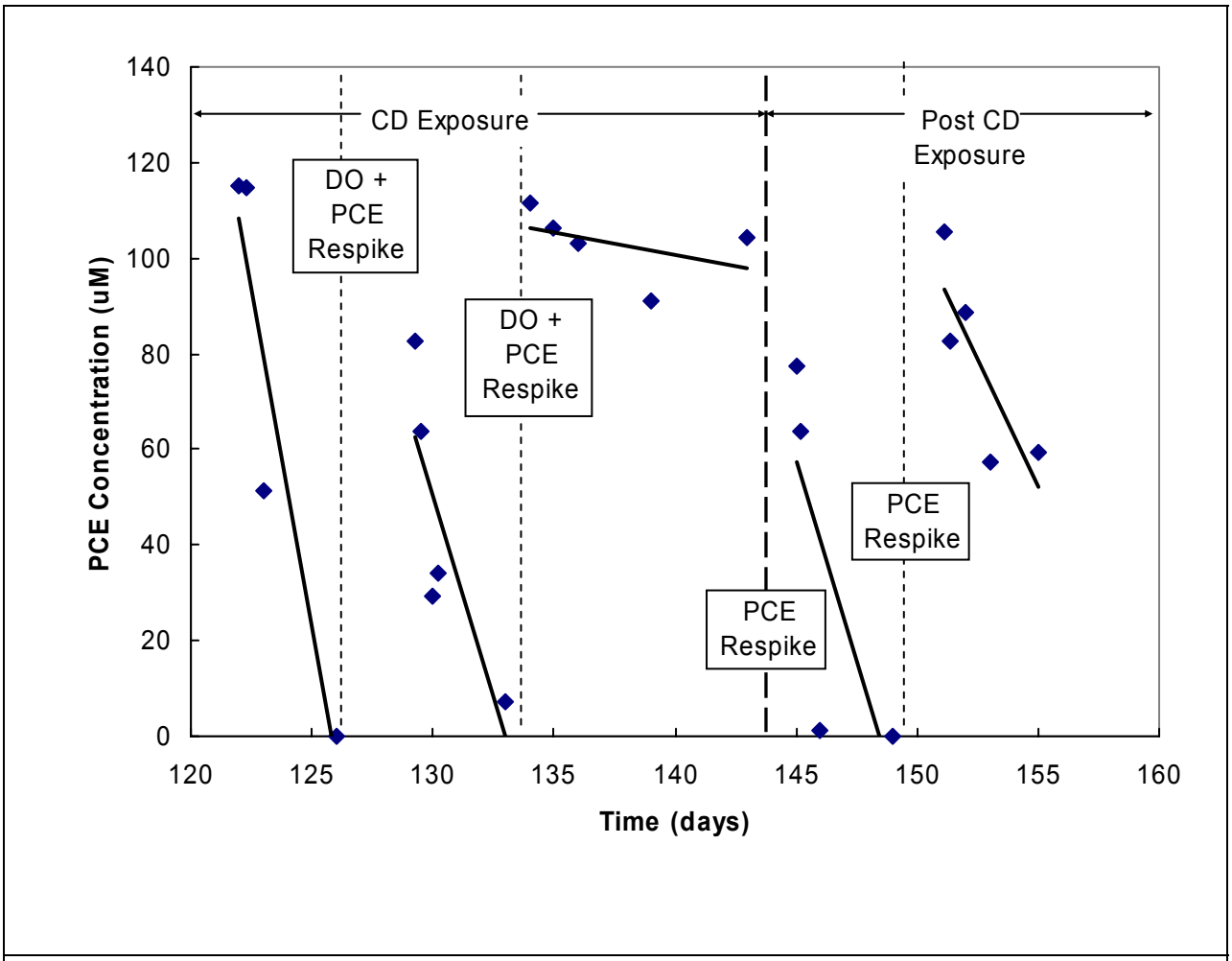

Figure 2-16. PCE Concentrations in and Zero Order Loss Trendlines for the BY-1c Microcosm during Experiment 3

Results of the CD concentration during the CD exposure phase of Experiment 3 are shown in Figure 2-17. As shown in this figure, the amount of CD in the microcosm decreased during the CD experiment (from approximately 72 g/L to 59 g/L of CD in solution which gives a degradation rate of 0.69 g/L/day). This CD loss is due to the dilution of the CD solution with each addition of supersaturated DO to the microcosm. Once the dilution is accounted for, CD loss is negligible. Following the removal of the CD solution, there were still trace amounts of CD present at a consistent concentration. Although the microcosm was initially created with a 10% (100 g/L) CD solution, all the microcosms to which CD was added had CD concentrations in the 7% v/v (70 g/L) concentration. The concentration of CD following the removal of the CD solution was around 8 g/L.

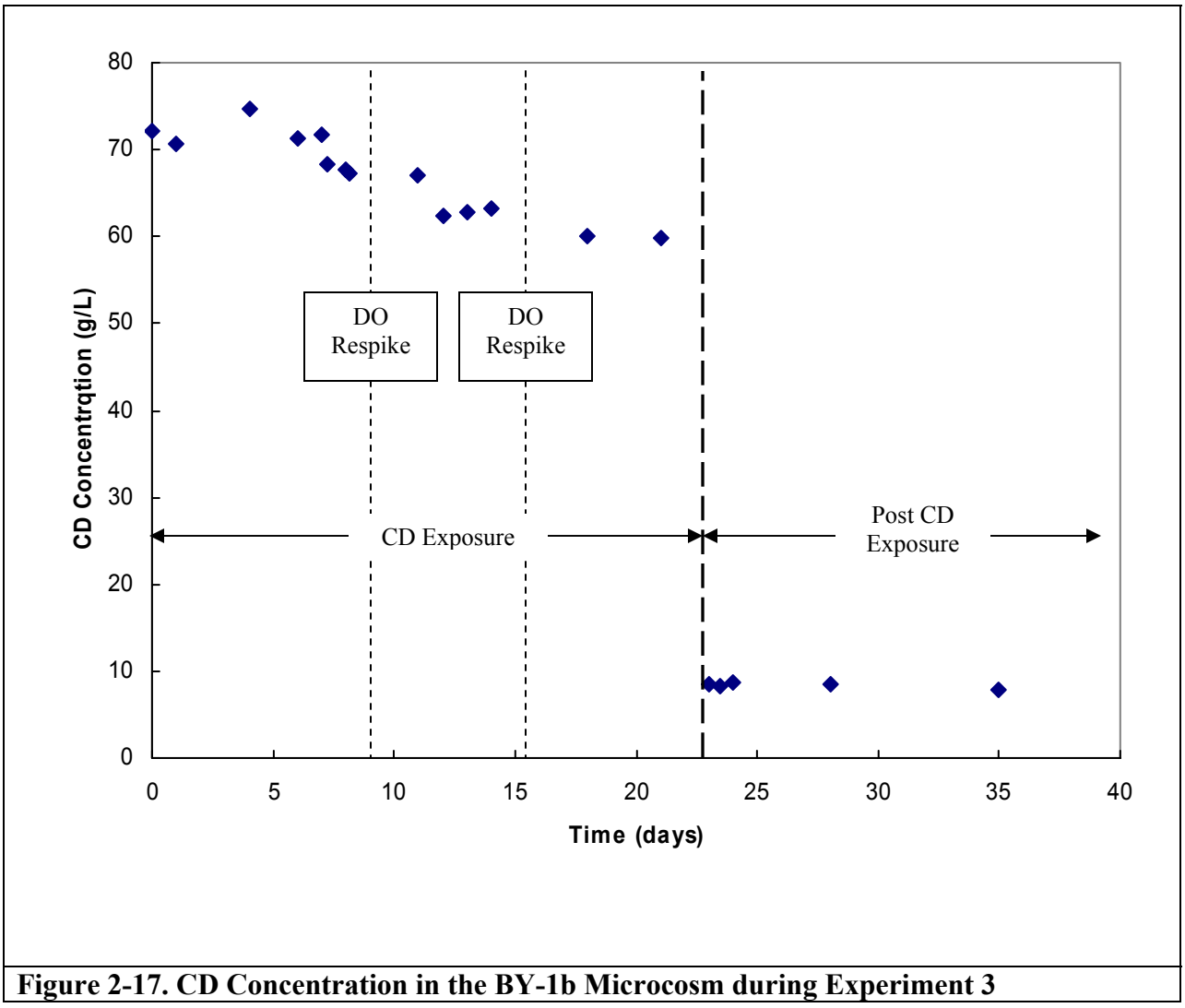

Figure 2-17. CD Concentration in the BY-1b Microcosm during Experiment 3

The microcosm BY-1c (Figure 2-16) showed the greatest enhancement of reductive dechlorination due to CD exposure. The effect of CD and DO on the loss and production patterns is shown by the rate changes represented in Figure 2-18. The introduction of CD and DO, along with PCE caused an enhancement in the PCE loss rate (from 0.66 to 28.51 uM/day). The TCE production rate also greatly increased to 16.73 uM/day and *cis*-DCE production was initiated at a production rate of 0.85 uM/day. Following a respike of PCE along with the addition of a concentrated DO solution at day 129, PCE mass loss decreased to 16.63 uM/day and *cis*-DCE production ceased. TCE production however, slightly increased to a rate of 17.81 uM/day.

Although the PCE loss rate was decreased from the initial enhancement due to CD introduction, the loss rate was still high enough to quickly consume PCE. Five days later, BY-1c

was once again respiked with PCE and DO (called respike 2 (DO) on Table 2-9) and the PCE loss rate was decreased to 0.92 uM/day, which is lower than any loss rate during CD exposure, but slightly higher than the PCE loss rate of the microcosm before CD exposure (0.87 uM/day). TCE production also significantly decreased to a rate of 0.86 uM/day, was still higher than the initial TCE production rate (0.44 uM/day) in the microcosm. Production of *cis*-DCE, however, was renewed with a production rate of 0.29 uM/day.

Following the removal of CD and DO from BY-1c, the PCE loss and daughter production rates were increased. The PCE loss rate increased to 76.92 uM/day, which is greater than any of the loss rates previously occurring in the microcosm. The TCE production rate also increased from 0.86 to 9.58 uM/day. This rate is greater than the TCE production rate that what was present in the microcosm before CD introduction (0.44 uM/day). *Cis*-DCE production increased following the removal of CD and DO at a production rate of 1.17 uM/day.

The PCE was used in approximately one day in this microcosm following the removal of the CD and DO solution. The consumption of PCE in one day is faster than had ever previously occurred in this microcosm. The microcosm was then respiked with PCE (labeled as PCE Respike on Table 2-9) to continue to determine the effects of the removal of CD and DO. Following the PCE respike, PCE degradation drastically decreased to a degradation rate of 10.70 uM/day, which is higher degradation rate than what was found in the microcosm prior to CD and DO exposure. TCE production also decreased to a production rate of 7.54 uM/day, which is also higher than the initial TCE production rate of 0.44 uM/day. Production of *cis*-DCE also ceased at this time.

Ferrous iron concentration of 0.41 mg/L was present in the microcosm. The pH of the microcosm was determined to be 6.5 and there was a minimal amount of oxygen in the microcosm. The microcosm with the lowest concentration of iron also had the highest PCE loss and daughter production rates.

Similar to BY-1b, the amount of CD in solution in BY-1c also slightly declined in this microcosm (see Figure 2-18). The CD concentration decreased from 70.75 g/L to 56.24 g/L, which give a loss rate of 0.68 g/L/day. This loss of CD, like in BY-1b, is due to the dilution of the CD solution following the injection of the supersaturated DO solutions. Once dilution was accounted for, the CD loss is negligible. Following the removal of CD from the microcosm, the residual CD concentration was determined to be approximately 7.3 g/L.

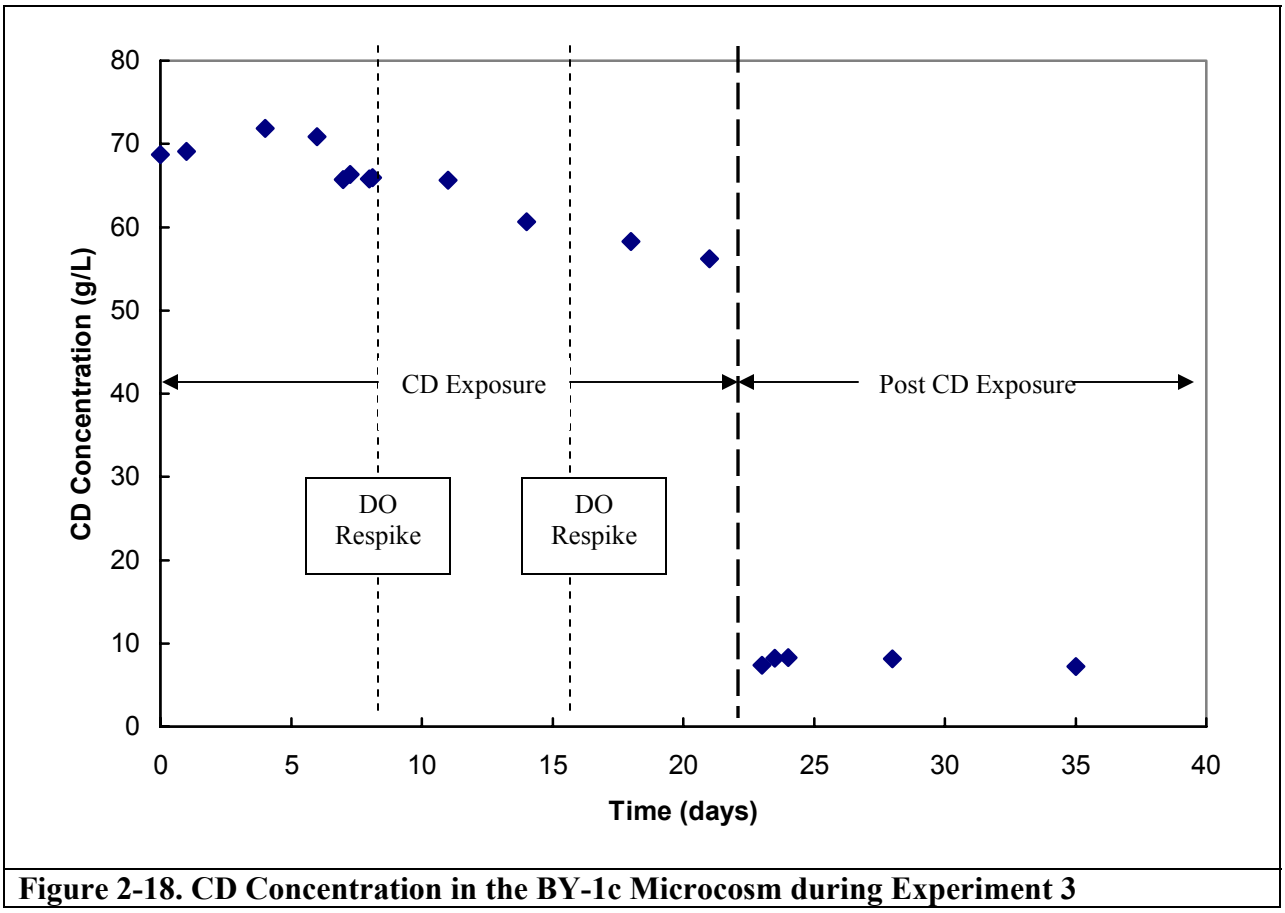

Figure 2-18. CD Concentration in the BY-1c Microcosm during Experiment 3

Prior to the start of Experiment 3, each of these microcosms had produced TCE, although only microcosms BY-1a and BY-1c had TCE production. The initiation of Experiment 3, with the oxygen and PCE addition, caused enhanced PCE loss and TCE production. BY-1b and BY-1c, which were introduced to CD along with PCE and DO at the beginning of Experiment 3, had significantly higher TCE production and PCE loss rates than BY-1a. In BY-1c, the most active microcosm of the group since their construction, the introduction of CD also seemed to stimulate the production of *cis*-DCE. *Cis*-DCE was not produced in BY-1a and did not occur in BY-1b until later (the second respike of DO) in the CD phase of the experiment.

Although CD appeared to enhance the mass loss and production patterns of the microcosm, the continued exposure to oxygen seems to have slowed down the PCE loss and TCE/ *cis*-DCE production rates in the microcosms after an initial rise. Although DO was added several times to the microcosms, the rapid usage of oxygen may have minimized the sediment and hence microbial exposure to oxygen. This usage of oxygen would explain why the mass loss

and production patterns were progressively slowed with each oxygen injection and the decreased consumption of oxygen with each DO injection. The rapid oxygen usage may be due to the presence of yeast, which is sometimes added to reduce the conditions of the microcosm. The steady decrease of PCE loss and TCE production rates was shown in BY-1a.

Both BY-1b and BY-1c had a steady decline in PCE loss during the continued exposure to oxygen, there was, however, a slight increase in the TCE production rates in both microcosms. Since PCE was spiked into the microcosms several times, could have led to a build up of TCE production, even if the PCE loss rate was lowered. The buildup of TCE may also have accounted for the production of *cis*-DCE in BY-1c during the last respike of DO (respike 2 (DO+PCE) from Table 2-9) in the microcosm. Interestingly, the most active microcosms in the entire experiment, BY-1b, and BY-1c, degraded CD during the progression of Experiment 3. This group of microcosms, with the greatest PCE loss and TCE/ *cis*-DCE production rates, also has the lowest concentration of ferrous iron present in all the microcosms.

Elevated iron concentrations in several microcosms are most likely attributed to the dominant microbial population; microcosms where increased iron concentrations were detected most likely have a dominant iron–reducing bacteria population. Chapelle (2001) explained that the reducing capability of the soil or terminal electron accepting processes (TEAPs) determines which microbial population is dominant and is actively degrading substances. The dominating TEAP was not experimentally determined in this study; however, future research should consider this observation by looking at aqueous and gas concentrations (oxygen, ferric iron, ferrous iron, sulfate, manganese, methane).

The presence of elevated iron concentrations is one reason why reductive dechlorination did not readily occur in some microcosms. Elevated iron concentrations were indications that a dominant iron bacteria population existed. Iron-reducing bacteria have not been shown to carryout reductive dechlorination processes. Furthermore, the dominance of iron bacteria might have inhibited reductive dechlorinating organisms from populating.

Elevated ferrous iron concentrations in some microcosms may have caused “abiotic” PCE mass loss as observed by Szecsody et al. (2000) and Lee and Batchelor (2002) in respect to TCE; this is known as hydrogenolysis. This may explain why there were high PCE loss rates in microcosms particularly those without daughter production in comparison to the rate of daughter production.

Increased iron concentrations may also have interfered with the cyclodextrin chlorinated solvent solubility enhancing properties by replacing the inclusion of chlorinated solvents in each CD molecule. Unfortunately, the effect of iron on the microbial activity cannot be confirmed since the specific controls were not in place and additional experiments were not conducted.

PCE loss rates varied between microcosms even though identical oxygen conditions existed. These differences are most likely attributable to micro-scale differences in the sediment and microorganism population. For instance, some microcosms in the anaerobic group appeared to have the same non-oxygenated conditions and iron concentrations, were similarly exposed to CD, and also produced the same amount of gas, but showed varying PCE degradability results. This variability is analogous to previous remediation research where PCE loss has been found to be dependent on the dominant microbial population and micro-scale soil conditions (Wiedemeier et al., 1999; Chapelle, 2001). Soil conditions on a single site may have a very heterogeneous composition depending on the surrounding geologic conditions.

CONCLUSIONS

The objective of this study was threefold: (1) to determine the impact of CD on MNA and whether reductive dechlorination was enhanced, decreased, or maintained the same rate due to CD exposure (2) to study the effect of introducing an oxygenated CD solution on MNA and the corresponding reductive dechlorination rates (3) to evaluate the changes in MNA (i.e. reductive dechlorination) following the removal of CD. This research indicates that CD affected degradation and production rates of chlorinated solvents. CD appeared to both positively and negatively impact the microcosms. Quantitative results stating whether or not this affect is positive could not be determined due to differences between microcosms (i.e., iron concentrations and microbial activity).

CD appeared to rapidly promote PCE loss in some microcosms while the addition of CD was less effective in others. CD appeared to stimulate one anaerobic microcosm (IY-2c) where daughter production had not previously occurred. However, another anaerobic microcosm in which reductive dechlorination was occurring, ceased after the addition of CD (IY-1a). Before reductive dechlorination ceased, this microcosm showed a decrease in PCE loss rates. Other microcosms, which had demonstrated only PCE loss prior to CD introduction, showed both

increases and decreases in the PCE loss rates, without daughter production after the addition of CD.

In the Aerobic Group microcosms the oxygen was rapidly depleted and only PCE loss hindered the PCE loss rates once DO depletion occurred. The initial rate increases in the CD-exposed Aerobic Group microcosms may be attributed to CD, specifically, with the inclusion of yeast into the CD structure, making the yeast available for the microorganisms during oxygen exposure.

The addition of the additive yeast may also have helped consume oxygen and drive the microcosms to reducing conditions. This effect of yeast could also cause enhanced activity in the presence of oxygen. The presence of yeast, however, by altering the microcosm's response following oxygen injection, could cause differences in results of batch study, such as this one, to an actual field study where there are no additives naturally present in the environment and the groundwater flows would flush the oxygenated CD solution through the aquifer.

The most active reductive dechlorinating microcosms also had low levels of iron. The present of high iron in some microcosms could indicate that different TEAPs are dominant, which would change the PCE degradation and daughter production rates in the system. The iron itself could also be degrading PCE and causing daughter production. Ferrous iron may also interfere, due to a chemical reaction or bonding to the CD molecule, with the CD-chlorinated ethene interactions.

As mentioned above, batch studies may lead different results than field studies due to the scale of the experiment. The constantly changing conditions in an aquifer due to groundwater flow in a natural system, also can lead to varying results between lab and full-scale experiments. The microcosms of this batch study exemplify micro-scale differences can drastically change the results in microcosms created under the same conditions.

Additional research is warranted to investigate in-situ conditions that affect CD and microbial degradation interactions. Several factors may be influencing chlorinated solvent degradation and may not necessarily be consistent throughout similar microcosms. In the absence of a complete understanding of subsurface interactions and the affect of CD addition, a more quantitative study with stricter variable controls must be conducted. In particular, the effect of iron and oxygen concentrations should be studied as they relate to CD addition. Although it

appears that CD is impacting MNA, the exact result (positive or negative) of this exposure was not determined by this study.

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Appendix A
Experiment 1 Data

Active Microcosms

Table A-1 Control A Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	PCE	TCE	cDCE	PCE	TCE	cDCE
25-Oct-01	0	23.76743	0	0	143.3241	0	0
		10.47665	0	0	63.17705	0	0
29-Oct-01	3	0	0	0		0	0
		2.650561	0	0	15.98361	0	0
01-Nov-01	6	0	0	0		0	0
		2.195581	0	0	13.23995	0	0
04-Nov-01	9	0	0	0		0	0
		1.949187	0	0	11.75413	0	0
09-Nov-01	14	0	0	0		0	0
		1.690637	0	0	10.195	0	0
12-Nov-01	17	0	0	0	0	0	0
		0	0	0	0	0	0
17-Nov-01	22	0	0	0	0	0	0
		0	0	0	0	0	0
26-Nov-01	31	0	0	0	0	0	0
		0	0	0	0	0	0
03-Dec-01	37	0	0	0	0	0	0
		0	0	0	0	0	0
15-Dec-01	49	0	0	0	0	0	0
		0	0	0	0	0	0
21-Dec-01	55	213.7032	0	0	1288.689	0	0
		193.5786	0	0	1167.332	0	0
04-Jan-02	69	0	0	7.47037	0	0	77.05384
		0	0	7.957558	0	0	82.07899
17-Jan-02	82	0	0	0.518924	0	0	5.352496
		0	0	0.403588	0	0	4.162848
24-Jan-02	89	2.488125	0.852011	5.669381	15.00407	6.484594	58.47737
		3.396286	0.396687	5.731682	20.48053	3.019153	59.11998
07-Feb-02	103	0	0	6.276725	0	0	64.74188
		0	0	7.47098	0	0	77.06013
22-Feb-02	118	0	0	5.363451	0	0	55.32182
		0	0	5.044848	0	0	52.03557
22-Mar-02	146	0	0	4.449897	0	0	45.89888
		0	0	4.33499	0	0	44.71367
02-Apr-02	157	27.86563	0	5.394528	168.0373	0	55.64238
		21.07021	0	4.727427	127.0591	0	48.7615
11-Apr-02	166	28.66241	0	6.206251	172.8421	0	64.01497
		22.43781	0	5.844141	135.3061	0	60.27995
24-Apr-02	179					0	0
		4.349919	0	0	26.2312	0	0
05-May-02	190					0	
		3.810976	0	0.975533	22.98122	0	10.06223
05-May-02	190					0	
dup run		3.735269	0	0.980644	22.52469	0	10.11495
16-May-02	201						
		2.945117	0.120209	0.565413	17.75986	0.914901	5.832008

Table A-2 Control A duplicate Chlorinated Ethene Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	PCE	TCE	cDCE	PCE	TCE	cDCE
25-Oct-01	0	74.27506	0	0	447.8988	0	0
		42.66903	0	0	257.3059	0	0
29-Oct-01	3	19.06598	0	0	114.9731	0	0
		22.0036	0	0	132.6877	0	0
01-Nov-01	6	24.27389	0	0	146.3782	0	0
		8.449287	0	0	50.9515	0	0
04-Nov-01	9	26.32311	0	0	158.7355	0	0
		22.89588	0	0	138.0684	0	0
09-Apr-01	14	24.33249	0	0	146.7315	0	0
		17.66236	0	0	106.5088	0	0
12-Nov-01	17	39.01536	0	0	235.2732	0	0
		19.96423	0	0	120.3897	0	0
17-Nov-01	22	4.892247	0	0	29.50158	0	0
		7.433103	0	0	44.82363	0	0
26-Nov-01	31	3.194311	0	1.130799	19.26256	0	11.66373
			0	1.49211		0	15.39051
03-Dec-01	37	11.97559	0	0	72.21607	0	0
		8.084493	0	0	48.75169	0	0
15-Dec-01	49	0	0	0.949569	0	0	9.794424
		0	0	0.265101	0	0	2.734415
21-Dec-01	55	3.636092	0	0.652404	21.92662	0	6.729288
			0	0.552465		0	5.698456
04-Jan-02	69	0	0	n/a	0	0	n/a
		0	0	n/a	0	0	n/a
17-Jan-02	82	0	0	0.954965	0	0	9.850073
		0	0	0	0	0	0
23-Jan-02	89	3.294426	0.545079	0.779514	19.86628	4.148559	8.040366
		2.280141	0.841916	0.83612	13.74987	6.40776	8.624241
07-Feb-02	103	0	0	0.625792	0	0	6.454794
		0	0	0.845865	0	0	8.724757
22-Feb-02	118	0	0	0.781407	0	0	8.059892
		0	0	0.482226	0	0	4.973968
22-Mar-02	146	0	0	0.890107	0	0	9.181091
		0	0	0.718944	0	0	7.415616
02-Apr-02	157	28.85145	0	0.737455	173.9821	0	7.606545
		16.00604	0	0.611579	96.52077	0	6.308189
11-Apr-02	166	28.25816	0	0.827388	170.4044	0	8.534169
		16.00604	0	0.611579	96.52077	0	6.308189
24-Apr-02	179					0	0
		3.303311	0	0	19.91986	0	0
05-May-02	190					0	0
		6.428345	0	0.228285	38.76467	0	2.354663
16-May-02	201					0	0
		2.580767	0	0.537696	15.56272	0	5.546115
22-May-02	207					0	0
		1.056512	0.273768	1.358279	6.371054	2.083631	14.0101

Table A-3 Control B Chlorinated Ethene Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	PCE	TCE	cDCE	PCE	TCE	cDCE
25-Oct-01	0	59.21105	0	0	357.0587	0	0
		74.14536	0	0	447.1167	0	0
30-Oct-01	5	81.98127	0	0	494.3694	0	0
		66.63992	0	0	401.8568	0	0
02-Nov-01	7	65.93206	0	0	397.5883	0	0
		53.65327	0	0	323.5438	0	0
05-Nov-01	10	56.92849	0	0	343.2943	0	0
		38.69619	0	0	233.3486	0	0
09-Nov-01	14	51.6936	0	0	311.7265	0	0
		40.66394	0	0	245.2146	0	0
12-Nov-01	17	45.15455	0	0	272.2942	0	0
		38.95918	0	0	234.9345	0	0
17-Nov-01	22	44.83684	0	0	270.3783	0	0
		35.05547	0	0	211.394	0	0
26-Nov-01	31	41.4913	0	0	250.2038	0	0
		35.03374	0	0	211.263	0	0
03-Dec-01	37	36.31129	0	0	218.967	0	0
		38.1836	0	0	230.2575	0	0
15-Dec-01	49	42.41611	0	0	255.7807	0	0
		40.59544	0	0	244.8015	0	0
21-Dec-01	55	39.16283	0	0	236.1625	0	0
		32.44916	0	0	195.6773	0	0
04-Jan-02	69	27.83162	0	0	167.8323	0	0
		39.55513	0	0	238.5282	0	0
Time Zero							
17-Jan-02	82	0	0	2.642271	0	0	27.25395
		0	0	3.331434	0	0	34.36239
24-Jan-02	89	0	0	0	0	0	0
		0	0	0	0	0	0
07-Feb-02	103	2.106516	0	0	12.70286	0	0
			0	0		0	0
22-Feb-02	118	0	0	0	0	0	0
		0	0	0	0	0	0
22-Mar-02	146	2.802161	0	0	16.89779	0	0
		2.022794	0	0	12.198	0	0
02-Apr-02	157	0	0	0	0	0	0
		0	0	0	0	0	0
11-Apr-02	166	0	0	0		0	0
		0.129547	0	0	0.781205	0	0
24-Apr-02	179						0
		1.659392	0.148059	0	10.00659	1.126864	0
05-May-02	190						0
		2.182183	0.129595	0	13.15916	0.986339	0
16-May-02	201					0	0
		2.504527	0	0	15.10298	0	0
16-May-02	201					0	0
dup run		0.242077	0	0	1.459787	0	0

Table A-4 Control B duplicate Chlorinated Ethene Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	PCE	TCE	cDCE	PCE	TCE	cDCE
25-Oct-01	0	60.8134	0	0	366.7211	0	0
		77.1	0	0	464.9339	0	0
30-Oct-01	5	65.356	0	0	394.1146	0	0
		71.1265	0	0	428.9124	0	0
02-Nov-01	7	61.601	0	0	371.4708	0	0
		46.9442	0	0	283.0862	0	0
05-Nov-01	10	30.111	0	0	181.5776	0	0
		47.8336	0	0	288.4496	0	0
09-Nov-01	14	81.5565	0	0	491.8079	0	0
		66.2878	0	0	399.7335	0	0
12-Nov-01	17	33.5539	0	0	202.3392	0	0
		62.1715	0	0	374.9109	0	0
17-Nov-01	22	40.5653	0	0	244.62	0	0
		47.1834	0	0	284.5285	0	0
26-Nov-01	31	64.4262	0	0	388.5074	0	0
		51.712	0	0	311.8376	0	0
03-Dec-01	37	52.8252	0	0	318.5502	0	0
		52.2834	0	0	315.2831	0	0
15-Dec-01	49	0	0	4.608026	0	0	47.52992
		0	0	2.100187	0	0	21.66257
Time Zero (since container broke)							
21-Dec-01	55	60.1659	0	0	362.8169	0	0
		52.5366	0	0	316.8097	0	0
04-Jan-02	69	68.3361	0	0	412.085	0	0
		61.4119	0	0	370.3304	0	0
17-Jan-02	82	0	0	6.051345	0	0	62.41718
		0	0	5.001728	0	0	51.5908
24-Jan-02	89	0	0	0	0	0	0
		0	0	0	0	0	0
07-Feb-02	103	0.28093	0	0	1.694078	0	0
			0	0		0	0
22-Feb-02	118	0	0	0	0	0	0
		0	0	0	0	0	0
22-Mar-02	146	0	0	0	0	0	0
		0	0	0	0	0	0
02-Apr-02	157		0	0		0	0
		3.49308	0	0	21.06421	0	0
11-Apr-02	166	11.6637	0	0	70.33542	0	0
		2.23876	0	0	13.50035	0	0
24-Apr-02	179						
		23.1035	1.14065	1.788493	139.3206	8.681374	18.44758
05-May-02	190						
		21.0394	1.05237	2.105759	126.8735	8.009513	21.72005
16-May-02	201						
		2.05232	1.12285	25.11956	12.37606	8.545932	259.0981
22-May-02	207						
		18.8607	1.29414	2.624349	113.7353	9.849637	27.0691

Table A-5 Control A and B and Corresponding Duplicates Chlorinated Ethene Degradation and Production Rates

Control A (I-1)			
	PCE	TCE	cDCE
before augmentation	-0.8802		0.6675
after augmentation	-0.7554	0.2943	0.0205
Control A dup (I-2)			
	PCE	TCE	cDCE
before augmentation	-2.7352		0.0051
after augmentation	-1.8154		-0.1434

Control B (BB) (B-1)			
	PCE	TCE	cDCE
initial	-2.74777		
after augmentation	-1.8634	0.0036	
Control B dup (B-2)			
	PCE	TCE	cDCE
before augmentation	-2.7434	-0.0521	0.1442
after augmentation	-1.0942	0.1671	-0.0616

Table A-6 Gas Data for Control A and Duplicate

BioIntrin						BioIntrin dup					
date	Days	Methane		CO2		date	Days	Methane		CO2	
		ppm	mol/L	ppm	mol/L			ppm	mol/L	ppm	mol/L
2/6/2002	102	6.29E-02	3.93E-06	3.86E-02	8.78E-07	2/2/2002	102	3.36E-03	2.10E-07	1.29E-03	2.94E-08
2/20/2002	116	1.27E-02	7.96E-07	4.04E-02	9.19E-07	2/20/2002	116	1.27E-02	7.96E-07	5.90E-03	1.34E-07
2/26/2002	122	4.87E-02	3.05E-06	3.92E-02	8.90E-07	2/26/2002	122	1.42E-02	8.89E-07	7.17E-03	1.63E-07
3/22/2002	146	1.22E-02	7.63E-07	1.87E-02	4.25E-07	3/22/2002	146	2.50E-03	1.56E-07	1.44E-03	3.27E-08
3/29/2002	153	1.72E-02	1.07E-06	3.38E-02	7.68E-07	3/29/2002	153	1.07E-02	6.66E-07	8.63E-03	1.96E-07
4/11/2002	166	1.02E-02	6.35E-07	2.22E-02	5.03E-07	4/11/2002	166	9.20E-03	5.75E-07	6.61E-03	1.50E-07
4/24/2002	179	1.22E-02	7.63E-07	2.74E-02	6.24E-07	4/24/2002	179	1.08E-02	6.75E-07	8.08E-03	1.84E-07
5/17/2002	202	3.50E-04	2.19E-08	2.14E-03	4.87E-08	5/17/2002	202	3.29E-04	2.06E-08	1.11E-03	2.53E-08
5/22/2002	207	2.75E-04	1.72E-08	2.08E-03	4.72E-08	5/22/2002	207	3.08E-04	1.93E-08	1.01E-03	2.29E-08
5/29/2002	214	2.80E-04	1.75E-08	2.00E-03	4.56E-08	5/29/2002	214	3.89E-04	2.43E-08	8.98E-04	2.04E-08
6/5/2002	221	2.76E-04	1.73E-08	1.83E-03	4.15E-08	6/5/2002	221	5.02E-04	3.14E-08	5.19E-04	1.18E-08
6/12/2002	228	2.54E-05	1.59E-09	1.18E-03	2.67E-08	6/12/2002	228	1.32E-05	8.24E-10	2.25E-04	5.12E-09
6/19/2002	235	2.50E-05	1.56E-09	1.44E-03	3.28E-08	6/19/2002	235	0.00E+00	0.00E+00	3.03E-04	6.89E-09
6/26/2002	242	1.11E-04	6.95E-09	5.63E-03	1.28E-07	6/26/2002	242	0.00E+00	0.00E+00	1.94E-03	4.41E-08
7/10/2002	257	1.20E-05	7.52E-10	2.04E-03	4.63E-08	7/10/2002	257	0.00E+00	0.00E+00	6.09E-04	1.38E-08

Table A-7 Gas Data for Control B and Duplicate

BioBiostim						Bio Biostim dup					
date	Days	Methane		CO2		date	Days	Methane		CO2	
		ppm	mol/L	ppm	mol/L			ppm	mol/L	ppm	mol/L
2/6/2002	102	1.09E-01	6.80E-06	6.40E-02	1.45E-06	2/2/2002	102	2.63E-04	1.64E-08	2.62E-02	5.95E-07
2/20/2002	116	5.76E-02	3.60E-06	6.61E-02	1.50E-06	2/20/2002	116	5.37E-04	3.35E-08	3.06E-02	6.96E-07
2/26/2002	122	6.67E-02	4.17E-06	1.85E-02	4.20E-07	2/26/2002	122	3.46E-04	2.16E-08	3.18E-02	7.23E-07
3/22/2002	146	5.50E-02	3.44E-06	6.09E-02	1.38E-06	3/22/2002	146	2.71E-04	1.70E-08	2.69E-02	6.12E-07
3/29/2002	153	4.28E-02	2.67E-06	2.04E-02	4.64E-07	3/29/2002	153	2.91E-04	1.82E-08	1.62E-02	3.69E-07
4/11/2002	166	3.33E-02	2.08E-06	8.33E-03	1.89E-07	4/11/2002	166	2.43E-04	1.52E-08	1.23E-02	2.79E-07
4/24/2002	179	5.16E-02	3.23E-06	9.40E-03	2.14E-07	4/24/2002	179	3.06E-04	1.91E-08	1.59E-02	3.60E-07
5/17/2002	202	1.20E-02	7.52E-07	1.67E-03	3.80E-08	5/17/2002	202	7.00E-05	4.38E-09	3.04E-03	6.91E-08
5/22/2002	207	9.51E-03	5.94E-07	1.44E-03	3.28E-08	5/22/2002	207	5.92E-05	3.70E-09	2.58E-03	5.85E-08
5/29/2002	214	9.26E-03	5.79E-07	1.58E-03	3.58E-08	5/29/2002	214	5.84E-05	3.65E-09	2.78E-03	6.33E-08
6/5/2002	221	9.16E-03	5.73E-07	1.83E-03	4.16E-08	6/5/2002	221	5.94E-05	3.71E-09	2.90E-03	6.59E-08
6/12/2002	228	0.00E+00	0.00E+00	1.44E-03	3.27E-08	6/12/2002	228	8.29E-06	5.18E-10	1.28E-03	2.91E-08
6/19/2002	235	0.00E+00	0.00E+00	1.66E-03	3.76E-08	6/19/2002	235	6.73E-06	4.21E-10	2.32E-02	5.27E-07
6/26/2002	242	4.28E-02	2.67E-06	5.93E-03	1.35E-07	6/26/2002	242	0.00E+00	0.00E+00	4.27E-03	9.71E-08
7/10/2002	257	3.38E-06	2.11E-10	1.52E-03	3.47E-08	7/10/2002	257	0.00E+00	0.00E+00	1.63E-02	3.70E-07

Inactive Microcosms

Table A-8 CD1 and Duplicate Chlorinated Solvent Concentrations (uM and ppm)

CD 1				CD 1 dup		
Time (days)	PCE (uM)	PCE (ppm)		Time (days)	PCE (uM)	PCE (ppm)
103	32.13289	5.3285964		103	26.6931342	4.426522444
118	31.43103	5.2122084		118	25.532065	4.23398234
146	26.98993	4.4757404		146	24.95954339	4.139041081
157	26.6979	4.4273124		157	25.94843871	4.303029592
166	32.04376	5.3138168		166	28.2410446	4.683212425
179	28.76891	4.770748		179	21.99523051	3.647469076
179	1.192558	0.1977619		190	22.21530932	3.683964745
190	29.70477	4.9259414		201	29.2236413	4.846156437
201	27.4521	4.5523816		201	0	0
207	22.28364	3.6952968		207	24.98487091	4.143241143
bioaugment and respike				bioaugment and respike		
222	104.426	17.316958		222	121.6597221	20.17483172
222	114.8631	19.04775		225	114.2779623	18.95071449
225	112.3943	18.638352		225	111.5064934	18.4911218
228	98.76649	16.378447		228	108.9078814	18.06019398
235	101.2307	16.787084		235	115.4332518	19.14229615
242	124.6718	20.674329		242	109.4370092	18.14793923
242	118.7832	19.697824		249	136.7189038	22.67209583
249	125.42	20.798399		249	118.6967222	19.68347744
256	108.8298	18.047251		256	107.6450098	17.85077198
263	98.95399	16.40954		263	111.3727676	18.46894606
277	77.37976	12.831886		277	114.7181199	19.02370583
291	129.9069	21.542454		291	137.3354676	22.77434059
319	120.7952	20.031465		319	84.84509475	14.06986206
319	121.5602	20.158321		333	111.8262722	18.54415072

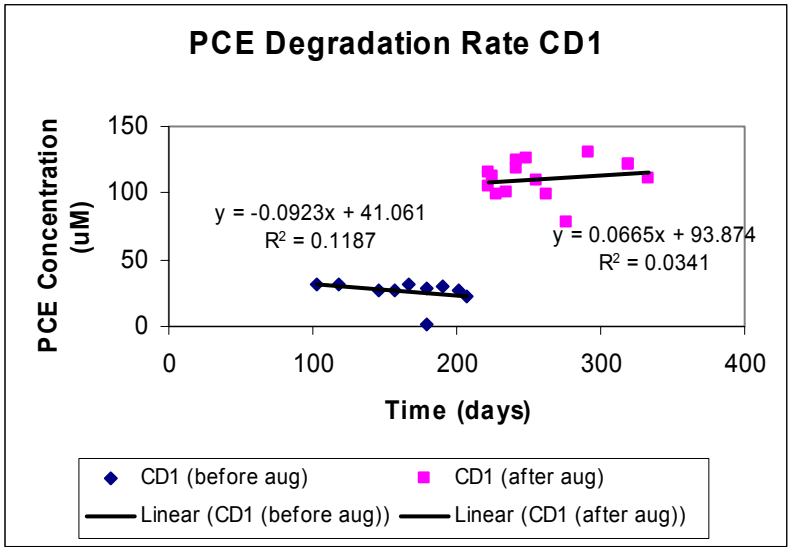

Figure A-1 CD 1 PCE Degradation Rate

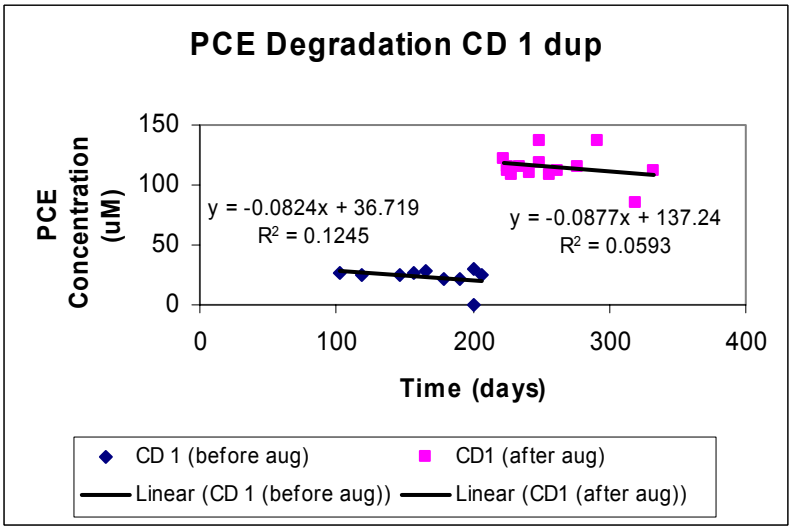

Figure A-2 CD 1 Duplicate PCE Degradation Rate

Table A-9 CD2 and Duplicate Chlorinated Solvent Concentrations (uM and ppm)

CD 2				CD 2 dup		
Time (days)	PCE (uM)	PCE (ppm)		Time (days)	PCE (uM)	PCE (ppm)
103	11.84698	1.964584459		103	1.537944	0.255037172
118	0	0		118	8.169854	1.354806824
146	5.901657	0.978671791		146	1.922026	0.318729529
157	0	0		157	0.574118	0.190412122
166	3.418212	0.566842116		166	0.747543	0.247930167
179	11.1862	1.855007713		179	0	0
179	10.2242	1.695478918		190	2.080387	0.344990582
190	13.17243	2.184383774		201	22.6591	3.757559244
201	12.41554	2.05886886		207	17.47113	2.897237988
207	10.55603	1.750506349		207	1.99509	0.330845715
bioaugment and respike						
222	172.1642	28.54999047		207	1.608293	0.266703194
				bioaugment and respike		
225	101.7445	16.87228555		222	117.6473	19.50945659
228	101.717	16.867728		225	100.4058	16.65030014
228	103.9298	17.23468689		228	104.012	17.2483051
235	76.54998	12.69428319		228	113.512	18.82369461
242	102.5219	17.00120906		235	106.4214	17.64785676
249	94.42519	15.65852945		242	128.0581	21.23588062
249	67.72909	11.23151418		249	115.5576	19.16292133
256	72.63316	12.0447565		256	98.42257	16.32141544
263	83.2927	13.81242793		263	106.9784	17.74023165
277	58.38509	9.681998817		277	120.4485	19.97397019
291	45.24754	7.503399304		291	127.2411	21.10039396
318	53.26332	8.832655596		333	44.95256	7.454482802
333	44.95256	7.454482802				

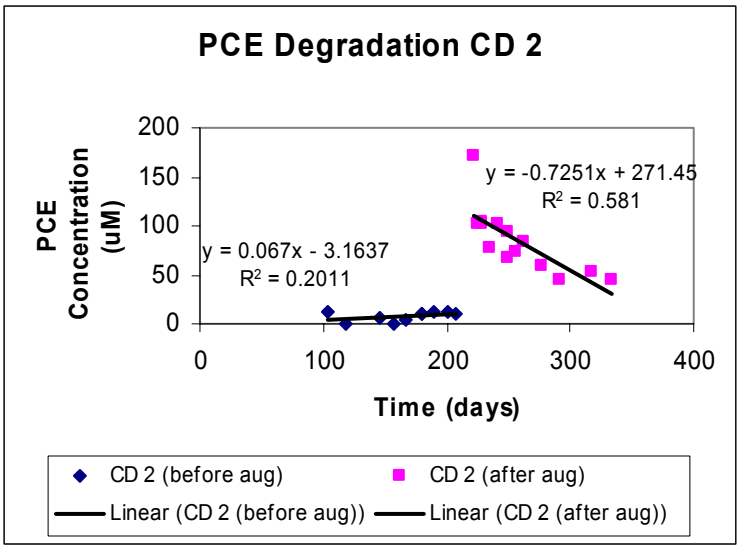

Figure A-3 CD 2 PCE Degradation Rate

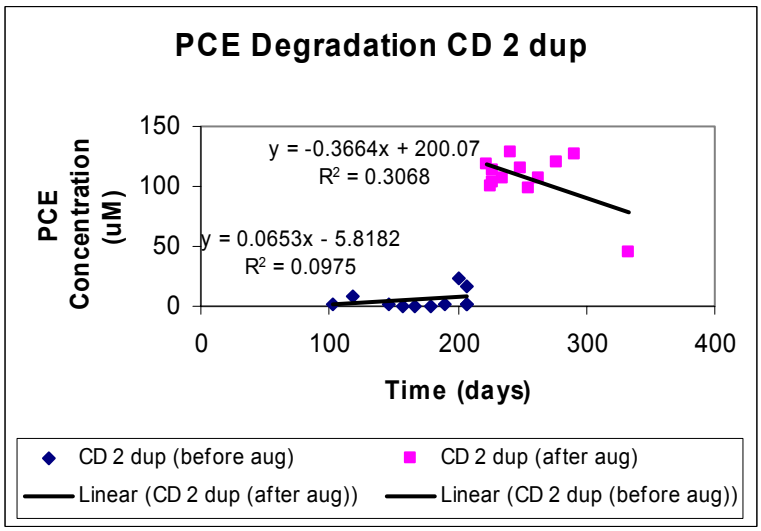

Figure A-4 CD 2 Duplicate PCE Degradation Rate

Table A-10 CD3 and Duplicate Chlorinated Solvent Concentrations (uM and ppm)

CD 3				CD 3 dup		
Time (days)	PCE (uM)	PCE (ppm)		Time (days)	PCE (uM)	PCE (ppm)
103	3.587547	0.594923		103	24.65605	4.0887124
118	1.030932	0.1709594		118	19.77278	3.2789206
140	25.63139	4.2504531		146	21.13036	3.5040469
146	14.0839	2.3355338		157	23.19665	3.8467006
157	6.490214	1.0762721		166	20.76545	3.4435351
166	3.286545	0.5450077		179	18.37101	3.0464645
179	1.02875	0.1705975		190	1.861355	0.3086685
179	24.70367	4.0966094		201	1.861355	0.3086685
190	1.903565	0.3156681		207	1.861355	0.3086685
				bioaugment and respike		
190	0.912695	0.1513523		214	120.7963	20.031653
201	0	0		222	121.201	20.098766
207	0	0		225	96.3397	15.976013
respike						
214	102.192	16.946495		225	114.1738	18.933438
222	104.7749	17.374822		228	107.015	17.7463
225	48.07956	15.946065		235	96.56602	16.013543
228	101.5673	16.842904		235	106.17	17.606165
235	106.5428	17.667994		242	112.0348	18.578726
242	164.4391	27.268941		249	117.171	19.430472
242	124.3602	20.622654		256	101.4774	16.828003
249	123.9919	20.561574		263	112.8504	18.713988
256	96.70296	16.036253		277	123.2061	20.431274
263	48.26461	16.007441		291	131.1348	21.746081
277	98.52018	16.337601		319	145.4136	24.113933
291	116.9181	19.388536		333	105.9624	17.571737

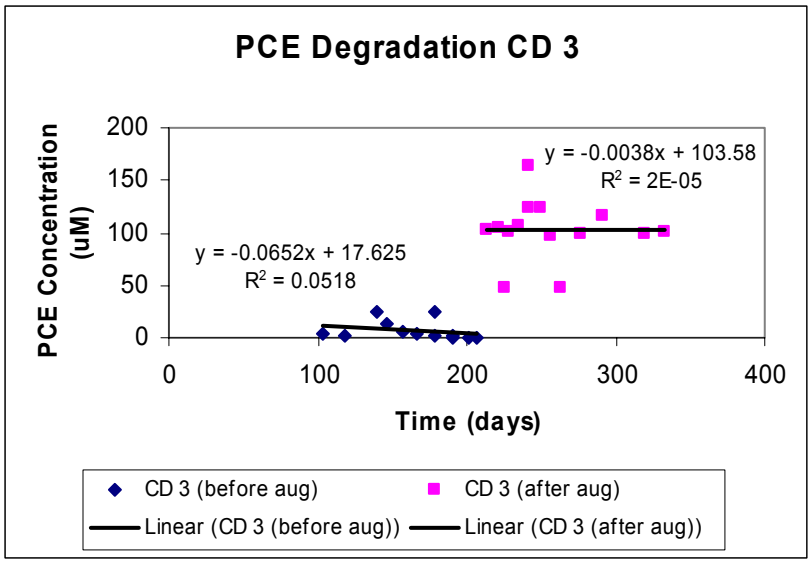

Figure A-5 CD 3 PCE Degradation Rate

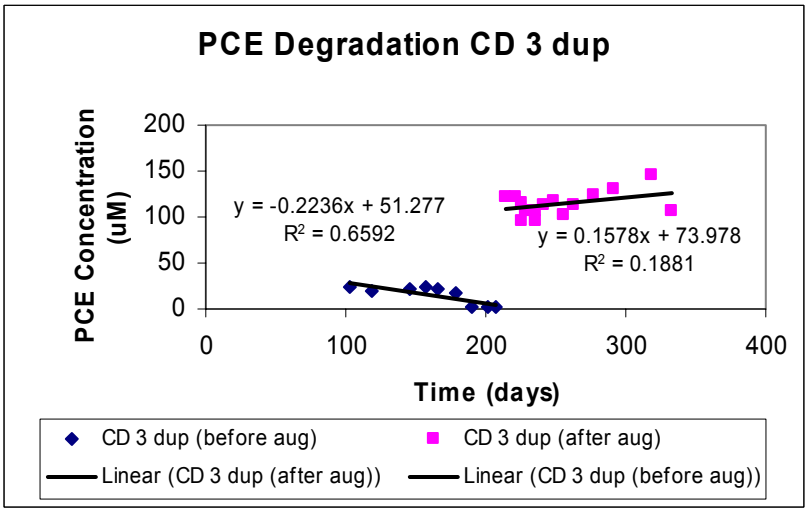

Figure A-6 CD 3 Duplicate PCE Degradation Rate

Table A-11 CD 4 and Duplicate Chlorinated Solvent Concentrations (uM and ppm)

CD 4				CD 4 dup		
Time (days)	PCE (uM)	PCE (ppm)		Time (days)	PCE (uM)	PCE (ppm)
103	15.02912	2.8614448		103	16.4912	2.7347353
118	13.54211	2.1231136		118	16.85772	2.7955163
146	15.88085	2.3682612		146	21.79544	3.6143371
157	16.51776	2.8987819		157	22.68562	3.7619572
166	10.05525	2.5794978		166	19.1826	3.1810499
179	4.994724	1.5108498		179	18.83984	3.1242112
190	2.717015	1.8022502		190	17.79727	2.9513217
201	0.431708	0		201	9.836326	2.4467369
				respike		
201	0.431708	0.2863604		207	40.51341	10.077509
207	0.275358	0		214	109.6521	18.183612
respike						
207	49.17865	0.1826505		222	103.292	18.510991
214	79.82498	32.438528		225	101.6495	15.746844
222	54.87341	20.510978		228	105.5982	17.966213
222	47.7143	15.887654		235	123.7742	17.05647
225	48.23289	15.762198		242	140.2958	23.994493
228	48.45673	16.23164		242	126.8714	22.536005
235	53.2785	15.910677		249	112.5913	19.542169
242	59.74355	19.430019		256	89.69735	17.799853
249	53.96088	20.199076		256	102.0045	11.94917
256	49.50738	15.594255		263	121.7441	21.881649
				CD added		
263	56.56717	17.244982		269	112.3087	18.496015
263	59.46167	20.277153		277	107.9544	18.752276
CD added						
269	55.31271	19.164965		284	105.9767	17.051866
277	53.60619	17.525063		291	120.3698	18.096356

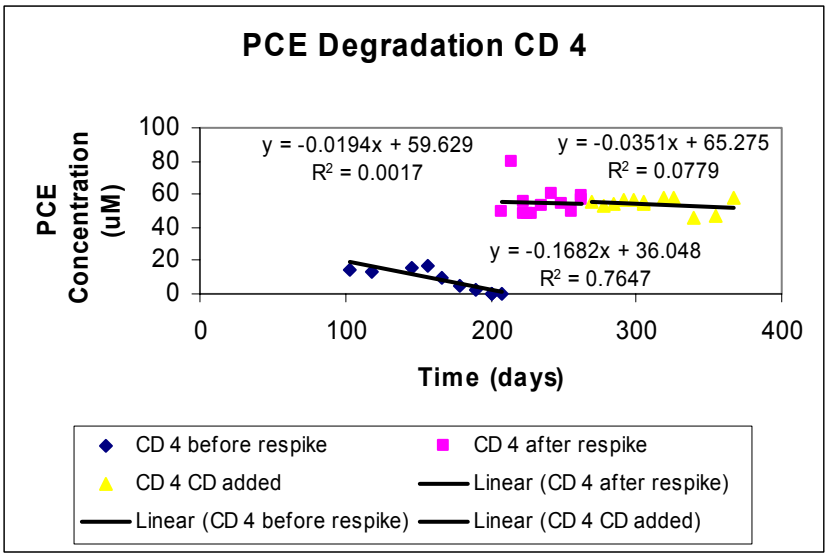

Figure A-7 CD 4 PCE Degradation Rate

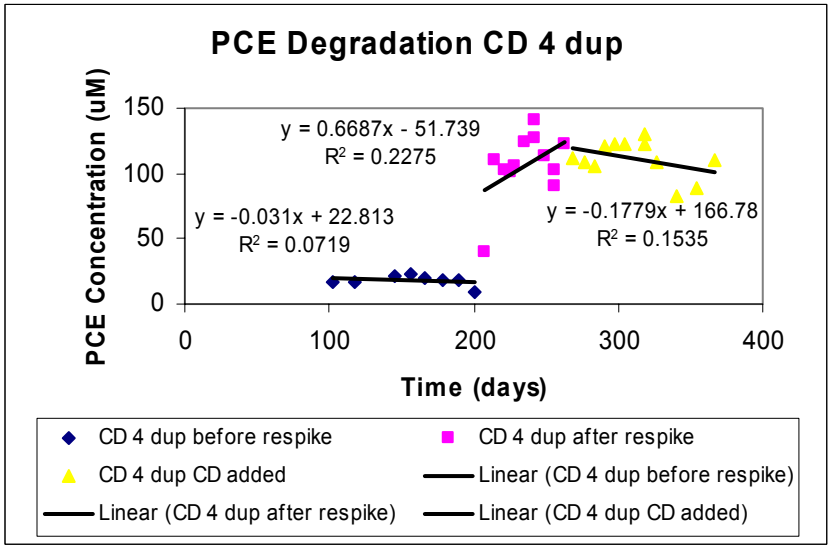

Figure A-8 CD 4 Duplicate PCE Degradation Rate

Table A-12 Gas Data for CD 1 and Duplicate

AnoxIntrin						AnoxIntrin dup					
date	Days	Methane		CO2		date	Days	Methane		CO2	
		ppm	mol/L	ppm	mol/L			ppm	mol/L	ppm	mol/L
2/6/2002	102	0.00E+00	0.00E+00	1.64E-03	3.74E-08	2/2/2002	102	1.30E-04	8.14E-09	3.27E-03	7.43E-08
2/20/2002	116	0.00E+00	0.00E+00	7.30E-03	1.66E-07	2/20/2002	116	1.03E-03	6.44E-08	1.53E-02	3.49E-07
2/26/2002	122	1.03E-03	6.44E-08	1.53E-02	3.49E-07	2/26/2002	122	1.42E-02	8.89E-07	7.17E-03	1.63E-07
3/22/2002	146	1.22E-02	7.63E-07	1.87E-02	4.25E-07	3/22/2002	146	2.50E-03	1.56E-07	1.44E-03	3.27E-08
3/29/2002	153	0.00E+00	0.00E+00	4.93E-03	1.12E-07	3/29/2002	153	2.45E-03	1.53E-07	2.05E-02	4.66E-07
4/11/2002	166	0.00E+00	0.00E+00	2.96E-03	6.73E-08	4/11/2002	166	2.13E-03	1.33E-07	1.27E-02	2.88E-07
4/24/2002	179	7.18E-06	4.49E-10	3.14E-03	7.13E-08	4/24/2002	179	4.16E-03	2.60E-07	2.06E-02	4.67E-07
5/17/2002	202	9.66E-07	6.04E-11	5.72E-04	1.30E-08	5/17/2002	202	1.18E-03	7.37E-08	4.48E-03	1.02E-07
5/22/2002	207	4.06E-07	2.54E-11	4.95E-04	1.13E-08	5/22/2002	207	1.01E-03	6.30E-08	3.95E-03	8.98E-08
5/29/2002	214	0.00E+00	0.00E+00	5.13E-04	1.17E-08	5/29/2002	214	1.07E-03	6.71E-08	4.24E-03	9.64E-08
6/5/2002	221	0.00E+00	0.00E+00	5.65E-04	1.29E-08	6/5/2002	221	1.12E-03	7.01E-08	4.52E-03	1.03E-07
6/12/2002	228	0.00E+00	0.00E+00	2.56E-04	5.82E-09	6/12/2002	228	1.36E-04	8.49E-09	2.39E-03	5.42E-08
6/19/2002	235	0.00E+00	0.00E+00	3.93E-04	8.94E-09	6/19/2002	235	1.48E-04	9.23E-09	2.68E-03	6.09E-08
6/26/2002	242	0.00E+00	0.00E+00	1.70E-03	3.86E-08	6/26/2002	242	9.28E-04	5.80E-08	1.02E-02	2.31E-07
7/10/2002	257	0.00E+00	0.00E+00	9.45E-04	2.15E-08	7/10/2002	257	1.82E-04	1.14E-08	4.43E-03	1.01E-07
7/17/2002	264	0.00E+00	0.00E+00	7.83E-04	1.78E-08	7/17/2002	264	1.37E-04	8.54E-09	4.39E-03	9.98E-08
8/7/2002	285	0.00E+00	0.00E+00	6.94E-04	1.58E-08	8/7/2002	285	1.16E-04	7.26E-09	5.38E-03	1.22E-07
8/13/2002	291	0.00E+00	0.00E+00	6.94E-04	1.58E-08	8/13/2002	291	1.16E-04	7.26E-09	5.38E-03	1.22E-07
8/28/2002	306	0.00E+00	0.00E+00	6.73E-04	1.53E-08	8/28/2002	306	9.01E-05	5.63E-09	3.40E-03	7.73E-08
10/21/2002	361	6.05E-05	3.78E-09	2.53E-03	5.74E-08	10/16/2002	361	1.21E-04	7.55E-09	3.82E-03	8.69E-08
10/30/2002	370	0.00E+00	0.00E+00	1.81E-03	4.11E-08	10/30/2002	370	1.49E-04	9.31E-09	4.27E-03	9.71E-08

Table A-12 Gas Data for CD 2 and Duplicate

AnoxBiostim						AnoxBiostim dup					
date	Days	Methane		CO2		date	Days	Methane		CO2	
		ppm	mol/L	ppm	mol/L			ppm	mol/L	ppm	mol/L
2/6/2002	102	1.21E-05	7.59E-10	3.57E-03	8.12E-08	2/2/2002	102	0.00E+00	0.00E+00	1.04E-02	2.37E-07
2/20/2002	116	3.41E-05	2.13E-09	1.87E-02	4.24E-07	2/20/2002	116	8.35E-06	5.22E-10	1.17E-02	2.66E-07
2/26/2002	122	1.37E-03	8.58E-08	2.45E-02	5.57E-07	2/26/2002	122	2.41E-05	1.50E-09	1.18E-02	2.69E-07
3/22/2002	146	4.71E-05	2.95E-09	2.26E-02	5.13E-07	3/22/2002	146	1.63E-04	1.02E-08	1.11E-02	2.52E-07
4/11/2002	166	3.57E-05	2.23E-09	2.22E-02	5.05E-07	4/11/2002	166	1.77E-04	1.10E-08	1.07E-02	2.44E-07
4/24/2002	179	4.27E-05	2.67E-09	2.63E-02	5.98E-07	4/24/2002	179	2.11E-04	1.32E-08	1.27E-02	2.89E-07
5/17/2002	202	1.77E-04	1.11E-08	6.14E-03	1.39E-07	5/17/2002	202	2.09E-04	1.30E-08	2.99E-03	6.79E-08
5/22/2002	207	1.96E-04	1.23E-08	5.10E-03	1.16E-07	5/22/2002	207	1.83E-04	1.15E-08	2.54E-03	5.76E-08
5/29/2002	214	2.69E-04	1.68E-08	5.82E-03	1.32E-07	5/29/2002	214	2.07E-04	1.29E-08	2.73E-03	6.19E-08
6/5/2002	221	2.96E-04	1.85E-08	5.72E-03	1.30E-07	6/5/2002	221	3.02E-04	1.89E-08	5.82E-03	1.32E-07
6/12/2002	228	3.16E-05	1.97E-09	2.52E-03	5.73E-08	6/12/2002	228	2.32E-05	1.45E-09	1.40E-03	3.19E-08
6/19/2002	235	0.00E+00	0.00E+00	1.33E-03	3.02E-08	6/19/2002	235	9.84E-06	6.15E-10	1.23E-03	2.81E-08
6/26/2002	242	7.15E-06	4.47E-10	2.60E-03	5.91E-08	6/26/2002	242	6.39E-05	4.00E-09	4.76E-03	1.08E-07
7/10/2002	257	2.51E-05	1.57E-09	1.55E-03	3.52E-08	7/10/2002	257	2.15E-05	1.34E-09	2.01E-03	4.57E-08
7/17/2002	264	2.01E-05	1.26E-09	1.44E-03	3.27E-08	7/17/2002	264	2.06E-05	1.29E-09	1.98E-03	4.50E-08
8/7/2002	285	8.27E-06	5.17E-10	3.33E-04	7.56E-09	8/7/2002	285	2.01E-05	1.26E-09	1.95E-03	4.44E-08
8/13/2002	291	8.27E-06	5.17E-10	3.33E-04	7.56E-09	8/13/2002	291	2.01E-05	1.26E-09	1.95E-03	4.44E-08
8/28/2002	306	0.00E+00	0.00E+00	1.02E-03	2.33E-08	8/28/2002	306	8.61E-06	5.38E-10	1.68E-03	3.82E-08
10/21/2002	361	9.49E-06	5.93E-10	2.12E-03	4.81E-08	10/21/2002	361	4.92E-06	3.07E-10	2.22E-03	5.04E-08
10/30/2002	370	0.00E+00	0.00E+00	2.63E-03	5.97E-08	10/30/2002	370	0.00E+00	0.00E+00	5.07E-03	1.15E-07

Table A-13 Gas Data for CD 3 and Duplicate

Oxic Intrin						Oxic Intrin dup					
date	Days	Methane		CO2		date	Days	Methane		CO2	
		ppm	mol/L	ppm	mol/L			ppm	mol/L	ppm	mol/L
2/6/2002	102	4.69E-04	2.93E-08	2.28E-02	5.19E-07	2/2/2002	102	4.41E-03	2.76E-07	3.18E-02	7.23E-07
2/20/2002	116	2.47E-03	1.54E-07	2.83E-02	6.43E-07	2/20/2002	116	4.95E-03	3.09E-07	3.37E-02	7.66E-07
2/26/2002	122	3.62E-03	2.26E-07	2.94E-02	6.68E-07	2/26/2002	122	5.83E-03	3.64E-07	3.48E-02	7.91E-07
3/22/2002	146	1.79E-05	1.12E-09	4.60E-03	1.04E-07	3/22/2002	146	4.83E-03	3.02E-07	2.50E-02	5.68E-07
3/29/2002	152	4.82E-05	3.01E-09	1.32E-02	2.99E-07	3/29/2002	153	4.84E-03	3.03E-07	3.14E-02	7.13E-07
4/11/2002	166	1.12E-04	6.97E-09	1.16E-02	2.64E-07	4/11/2002	166	4.01E-03	2.50E-07	2.32E-02	5.28E-07
4/24/2002	179	1.78E-04	1.11E-08	1.45E-02	3.30E-07	4/24/2002	179	4.98E-03	3.12E-07	2.74E-02	6.23E-07
5/17/2002	202	4.11E-05	2.57E-09	3.02E-03	6.85E-08	5/17/2002	202	1.10E-03	6.88E-08	5.71E-03	1.30E-07
5/22/2002	207	3.39E-05	2.12E-09	2.58E-03	5.87E-08	5/22/2002	207	8.98E-04	5.61E-08	4.85E-03	1.10E-07
5/29/2002	214	3.19E-05	1.99E-09	2.49E-03	5.66E-08	5/29/2002	214	9.33E-04	5.83E-08	5.18E-03	1.18E-07
6/5/2002	221	3.82E-05	2.39E-09	2.94E-03	6.69E-08	6/5/2002	221	9.69E-04	6.06E-08	5.37E-03	1.22E-07
6/12/2002	228	3.02E-05	1.89E-09	2.38E-03	5.42E-08	6/12/2002	228	7.37E-04	4.61E-08	4.29E-03	9.76E-08
6/19/2002	235	3.09E-05	1.93E-09	2.52E-03	5.72E-08	6/19/2002	235	7.98E-04	4.99E-08	4.80E-03	1.09E-07
6/26/2002	242	1.99E-04	1.24E-08	9.35E-03	2.12E-07	6/26/2002	242	4.68E-03	2.92E-07	1.69E-02	3.85E-07
7/10/2002	257	3.55E-05	2.22E-09	3.39E-03	7.70E-08	7/10/2002	257	9.63E-04	6.02E-08	6.28E-03	1.43E-07
7/17/2002	264	2.93E-05	1.83E-09	3.14E-03	7.13E-08	7/17/2002	264	7.93E-04	4.96E-08	5.62E-03	1.28E-07
8/7/2002	285	2.00E-04	1.25E-08	3.26E-03	7.41E-08	8/7/2002	285	6.10E-04	3.81E-08	4.50E-03	1.02E-07
8/13/2002	291	2.00E-04	1.25E-08	3.26E-03	7.41E-08	8/13/2002	291	6.10E-04	3.81E-08	4.50E-03	1.02E-07
8/28/2002	306	1.37E-05	8.53E-10	2.03E-03	4.62E-08	8/28/2002	306	4.07E-04	2.55E-08	3.35E-03	7.62E-08
10/21/2002	361	7.45E-05	4.66E-09	0.00342	7.77E-08	10/21/2002	361	6.05E-04	3.78E-08	0.005044	1.15E-07
10/30/2002	370	8.86E-05	5.53E-09	4.15E-03	9.44E-08	10/30/2002	370	2.46E-05	1.54E-09	4.16E-03	9.45E-08

Table A-14 Gas Data for CD 4 and Duplicate

OxicBiostim						Oxic Biostim dup					
date	Days	Methane		CO2		date	Days	Methane		CO2	
		ppm	mol/L	ppm	mol/L			ppm	mol/L	ppm	mol/L
2/6/2002	102	1.09E-01	6.80E-06	6.40E-02	1.45E-06	2/2/2002	102	2.63E-04	1.64E-08	2.62E-02	5.95E-07
2/20/2002	116	5.76E-02	3.60E-06	6.61E-02	1.50E-06	2/20/2002	116	5.37E-04	3.35E-08	3.06E-02	6.96E-07
2/26/2002	122	6.67E-02	4.17E-06	6.82E-01	1.55E-05	2/26/2002	122	3.46E-04	2.16E-08	3.18E-02	7.23E-07
3/22/2002	146	5.50E-02	3.44E-06	6.09E-02	1.38E-06	3/22/2002	146	2.71E-04	1.70E-08	2.69E-02	6.12E-07
3/29/2002	153	6.04E-02	3.77E-06	8.22E-02	1.87E-06	3/29/2002	153	2.83E-04	1.77E-08	3.33E-02	7.58E-07
4/11/2002	166	5.05E-02	3.16E-06	6.37E-02	1.45E-06	4/11/2002	166	2.32E-04	1.45E-08	2.40E-02	5.45E-07
4/24/2002	179	9.87E-04	6.17E-08	1.71E-03	3.90E-08	4/24/2002	179	3.32E-04	2.08E-08	2.72E-02	6.18E-07
5/17/2002	202	1.28E-02	8.01E-07	1.69E-02	3.84E-07	5/17/2002	202	7.69E-05	4.81E-09	4.77E-03	1.08E-07
5/22/2002	207	1.32E-02	8.26E-07	1.41E-02	3.21E-07	5/22/2002	207	7.59E-05	4.74E-09	4.13E-03	9.38E-08
5/29/2002	214	1.44E-02	8.98E-07	1.52E-02	3.45E-07	5/29/2002	214	4.38E-04	2.74E-08	3.88E-03	8.82E-08
6/5/2002	221	1.76E-02	1.10E-06	1.63E-02	3.71E-07	6/5/2002	221	3.31E-05	2.07E-09	3.95E-03	8.97E-08
6/12/2002	228	1.25E-02	7.83E-07	1.29E-02	2.93E-07	6/12/2002	228	8.07E-04	5.05E-08	3.61E-03	8.21E-08
6/19/2002	235	1.71E-02	1.07E-06	1.53E-02	3.47E-07	6/19/2002	235	6.52E-05	4.07E-09	3.59E-03	8.15E-08
6/26/2002	242	1.07E-01	6.66E-06	5.23E-02	1.19E-06	6/26/2002	242	3.32E-04	2.08E-08	1.22E-02	2.77E-07
7/10/2002	257	2.43E-02	1.52E-06	2.06E-02	4.67E-07	7/10/2002	257	8.66E-03	5.41E-07	2.34E-03	5.31E-08
7/17/2002	264	1.72E-02	1.07E-06	1.73E-02	3.94E-07	7/17/2002	264	4.25E-05	2.66E-09	4.29E-03	9.75E-08
8/7/2002	285	4.55E-03	2.84E-07	6.62E-03	1.50E-07	8/7/2002	285	8.68E-06	5.43E-10	4.47E-03	1.02E-07
8/13/2002	291	4.55E-03	2.84E-07	6.62E-03	1.50E-07	8/13/2002	291	8.68E-06	5.43E-10	4.47E-03	1.02E-07
8/21/2002	298	4.73E-03	2.96E-07	7.30E-03	1.66E-07	8/21/2002	298	7.39E-06	4.62E-10	5.17E-03	1.17E-07
8/28/2002	306	3.97E-03	2.48E-07	6.22E-03	1.41E-07	8/28/2002	306	6.81E-06	4.26E-10	4.26E-03	9.68E-08
10/21/2002	361	6.26E-03	3.91E-07	1.07E-02	2.43E-07	10/21/2002	361	4.87E-06	3.04E-10	7.95E-03	1.81E-07
10/30/2002	370	7.28E-03	4.55E-07	1.50E-02	3.42E-07	10/30/2002	370	9.91E-06	6.20E-10	1.08E-02	2.45E-07

Abiotic Control

Table A-15 Abiotic Control PCE Concentrations (uM and ppm)

		ppm	uM
Date	Time (days)	PCE	PCE
25-Oct-01	0	43.6037	262.94
		46.60273	281.03
30-Oct-01	5	68.85537	415.22
REDO		72.27406	435.83
02-Nov-01	7	37.06194	223.49
		50.41815	304.04
05-Nov-01	9	68.39983	412.47
		50.65785	305.48
09-Nov-01	14	59.28829	357.52
		46.27316	279.04
12-Nov-01	17	70.33336	424.13
		49.91083	300.98
26-Nov-01	31	72.62238	437.93
		43.68357	263.42
03-Dec-01	37	66.26328	399.59
		46.65198	281.32
15-Dec-01	49	56.96557	343.52
		51.08708	308.07
21-Dec-01	55	67.11173	404.70
		43.16557	260.30
04-Jan-02	69	50.55947	304.89
		58.07325	350.20
05-May-02	190	18.53353	111.76
dup			
16-May-02	201	20.84275	125.69
22-May-02	207	19.69068	118.74
29-May-02	214	27.18836	163.95
29-May-02	214	27.68182	166.93
dup run			
06-Jun-02	222	17.03224	102.71
09-Jun-02	225	16.36592	98.69
12-Jun-02	228	16.84562	101.58
19-Jun-02	235	16.56466	99.89
24-Jun-02	242	23.3084	140.56
24-Jun-02	242	21.2755	128.30
dup run			
03-Jul-02	249	19.34337	116.65
10-Jul-02	256	10.23352	61.71

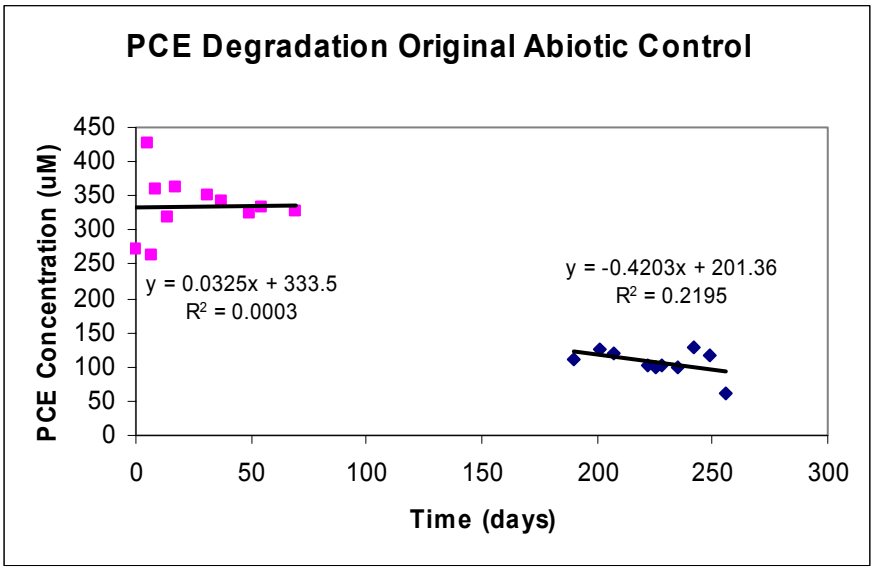

Figure A-9 AC PCE Degradation Rate

Table A-16 Gas Data for AC

A Cntrl					
date	Days	Methane		CO2	
		ppm	mol/L	ppm	mol/L
2/6/2002	102	0.00E+00	0.00E+00	4.18E-03	9.51E-08
2/20/2002	116	1.82E-04	1.14E-08	5.95E-03	1.35E-07
2/26/2002	122	3.27E-05	2.04E-09	4.19E-03	9.52E-08
3/22/2002	146	5.02E-05	3.14E-09	9.03E-03	2.05E-07
4/11/2002	166	2.48E-05	1.55E-09	3.13E-03	7.11E-08
4/24/2002	179	2.86E-05	1.79E-09	3.54E-03	8.04E-08
5/17/2002	202	3.11E-06	1.94E-10	8.22E-04	1.87E-08
5/29/2002	214	2.22E-06	1.39E-10	6.88E-04	1.56E-08
6/12/2002	228	9.54E-07	5.96E-11	6.17E-04	1.40E-08
6/26/2002	242	0.00E+00	0.00E+00	2.98E-03	6.78E-08
7/10/2002	257	1.62E-05	1.01E-09	1.22E-03	2.76E-08

Appendix B
Experiment 2 Data

Table B-1 A-1 Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
14-Jul-02	0	0	0	9.880636	0	0	59.58292
17-Jul-02	3	0	0	13.34139	0	0	80.45218
23-Jul-02	9	0	0	9.165462	0	0	55.27023
31-Jul-02	16	0	0	11.68235	0	0	70.44773
07-Aug-02	23	0	0	11.07476	0	0	66.78381
14-Aug-02	30	0	0	13.11776	0	0	79.10366
21-Aug-02	37	0	0	14.84181	0	0	89.50018
28-Aug-02	44	0	0	13.45388	0	0	81.13056
28-Aug-02	44	0	0	16.18263	0	0	97.58566
11-Sep-02	58	0	0	13.55455	0	0	81.73761
18-Sep-02	65	0	0	9.709298	0	0	58.54971
01-Oct-02	78	0	0.154549	6.468677	0	1.176258	39.00788
17-Oct-02	94	0	0	6.323475	0	0	38.13228
29-Oct-02	106	0	0	9.579621	0	0	57.76772
06-Nov-02	114	0	0	7.706648	0	0	46.47318
begin cd experiment (respike)							
13-Nov-02	121	0	0	20.34654	0	0	122.6952
13-Nov-02	121	0	0	22.25035	0	0	134.1757
dup run							
19-Nov-02	127	0	0	19.79491	0	0	119.3687
05-Dec-02	143	0	0	19.39387	0	0	116.9503
11-Dec-02	149	0	0	19.63645	0	0	118.4131
22-Dec-02	159	0	0	17.0628	0	0	102.8933

Table B-2 A-2 Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	**Time (days)**	**cDCE**	**TCE**	**PCE**	**cDCE**	**TCE**	**PCE**
	Time	cDCE	TCE	PCE	cDCE	TCE	PCE
	(days)	mg/L	mg/L	mg/L	uM	uM	uM
14-Jul-02	0	0	0	12.8352	0	0	77.39972
17-Jul-02	3	0	0	13.79382	0	0	83.18051
23-Jul-02	9	0	0	9.874309	0	0	59.54477
31-Jul-02	16	0	0	12.65669	0	0	76.32327
07-Aug-02	23	0	0	16.96986	0	0	102.3329
14-Aug-02	30	0	0	13.17446	0	0	79.44556
21-Aug-02	37	0	0	10.40722	0	0	62.75836
28-Aug-02	44	0	0	10.60967	0	0	63.97917
11-Sep-02	58	0	0	12.8031	0	0	77.2062
11-Sep-02	58	0	0	11.7716	0	0	70.98595
dup run							
18-Sep-02	65	0	0	10.03473	0	0	60.51213
02-Oct-02	78	0	0	10.89808	0	0	65.71838
17-Oct-02	94	0	0	6.200989	0	0	37.39365
29-Oct-02	106	0	0	8.377501	0	0	50.51861
06-Nov-02	114	0	0	6.44248	0	0	38.84991
begin cd experiment							
13-Nov-02	121	0	0	19.7835	0	0	119.2999
19-Nov-02	127	0	0	15.96465	0	0	96.2712
05-Dec-02	143	0	0	17.29633	0	0	104.3016
11-Dec-02	149	0	0	19.10291	0	0	115.1957
22-Dec-02	159	0	0	17.08378	0	0	103.0198

Table B-3 A-3 Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
14-Jul-02	0	0	0	11.17216	0	0	67.37117
17-Jul-02	3	0	0	13.502	0	0	81.42072
23-Jul-02	9	0	0	11.95649	0	0	72.10087
31-Jul-02	16	0	0	9.431585	0	0	56.87502
07-Aug-02	23	0	0	13.80985	0	0	83.27714
14-Aug-02	30	0	0	13.09043	0	0	78.93888
21-Aug-02	37	0	0	12.05139	0	0	72.67314
28-Aug-02	44	0	0	11.80397	0	0	71.18116
11-Sep-02	58	0	0	11.15808	0	0	67.28625
18-Sep-02	65	0	0	9.106317	0	0	54.91357
18-Sep-02	65	0	0	11.61631	0	0	70.04949
dup run							
02-Oct-02	78	0	0	4.19989	0	0	25.32648
17-Oct-02	94	0	0	4.813749	0	0	29.02822
29-Oct-02	106	0	0	8.269544	0	0	49.8676
06-Nov-02	114	0	0	6.711042	0	0	40.46941
begin cd experiment							
13-Nov-02	121	0	0	18.34878	0	0	110.6481
19-Nov-02	127	0	0	20.01699	0	0	120.7079
05-Dec-02	143	0	0	20.06999	0	0	121.0275
11-Dec-02	149	0	0	19.42204	0	0	117.1202
22-Dec-02	159	0	0	16.85754	0	0	101.6555

Table B-4 IY-1a Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
14-Jul-02	0	0	0	11.8153	0	0	71.2493
14-Jul-02	0	0	0	10.5661	0	0	63.7162
dup run							
17-Jul-02	3	0	0	11.9398	0	0	72.0002
23-Jul-02	9	0	0	9.02324	0	0	54.4126
31-Jul-02	16	0	0	10.5638	0	0	63.7025
07-Aug-02	23	0	0	10.5536	0	0	63.6409
14-Aug-02	30	0	0	11.9212	0	0	71.8883
21-Aug-02	37	0	0	11.0422	0	0	66.5874
28-Aug-02	44	0	0	10.1745	0	0	61.3549
Time Zero---bioaug and respike)							
11-Sep-02	58	0	0	14.5068			87.4801
16-Sep-02	63	0	0	15.9385			96.1136
16-Sep-02	63	0	0	13.8764			83.6783
dup run							
21-Sep-02	68	0	0	14.862			89.6217
29-Sep-02	76	0	0	15.4625			93.2428
01-Oct-02	79	0	0	12.3632		0	74.5534
06-Oct-02	84	0	0.21473	10.656		1.63433	64.2585
10-Oct-02	88	0	0	7.38829			44.5534
15-Oct-02	93	0	0.14456	10.7957		1.10023	65.1011
15-Oct-02	93	0	0.28263	10.1315		2.15108	61.0955
dup run							
20-Oct-02	98	0	0.18758	7.83548	0	1.42767	47.2501
26-Oct-02	104	0.34983	0.40044	10.8443	3.6084	3.04775	65.3942
31-Oct-02	109	0.47668	0.26365	7.47403	4.91672	2.00663	45.0704
05-Nov-02	114	0.53503	0.16379	6.58476	5.51858	1.24661	39.7079
09-Nov-02	118	0.98232	0.55935	8.58639	10.1322	4.2572	51.7782

Table B-5 IY-1b Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
14-Jul-02	0	0	0	9.785366	0	0	59.00842
17-Jul-02	3	0	0	11.65777	0	0	70.29955
23-Jul-02	9	0	0	11.91814	0	0	71.86964
23-Jul-02	9	0	0	7.238598	0	0	43.65072
dup run							
31-Jul-02	16	0	0	10.26029	0	0	61.87235
07-Aug-02	23	0	0	11.87859	0	0	71.6311
14-Aug-02	30	0	0	11.47519	0	0	69.19853
21-Aug-02	37	0	0	10.86818	0	0	65.53807
28-Aug-02	44	0	0	10.9811	0	0	66.21902
Time Zero---bioaug and respike							
11-Sep-02	58	0	0	17.69648			106.7146
16-Sep-02	63	0	0	15.09985		0	91.0562
21-Sep-02	68	0	0.797089	18.48135		6.066589	111.4475
21-Sep-02	68	0	0.579554	15.73371	0	4.410943	94.87858
dup run							
29-Sep-02	76	0.1757	0.775134	14.05246	1.812272	5.899488	84.74015
01-Oct-02	79	0.02528	0.332776	12.05036		2.532735	72.66694
06-Oct-02	84	0.17439	0.350661	11.52798	1.79875	2.668856	69.51683
10-Oct-02	88	0.26394	0.667792	10.30918	2.722484	5.082521	62.16715
15-Oct-02	93	0.32697	0.367474	9.602428	3.372554	2.796816	57.90525
20-Oct-02	98	0	0.495913	8.442597		3.774358	50.91116
26-Oct-02	104	0	0.134878	6.149966			37.08596
31-Oct-02	109	0	0.433379	6.108726		3.298415	36.83728
05-Nov-02	114	0	0.37055	5.751364		2.82023	34.68229
09-Nov-02	118	0.32783	0.674893	10.20197	3.3814	5.136564	61.52066

Table B-6 IY-1c Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
14-Jul-02	0	0	0	11.3846	0	0	68.65207
17-Jul-02	3	0	0	10.0687	0	0	60.71717
23-Jul-02	9	0	0	11.3741	0	0	68.58893
31-Jul-02	16	0	0	9.70503	0	0	58.52396
07-Aug-02	23	0	0	12.3078	0	0	74.21955
07-Aug-02	23	0	0	7.11599	0	0	42.91137
dup run							
14-Aug-02	30	0	0	10.8477	0	0	65.41443
21-Aug-02	37	0	0	10.8466	0	0	65.40818
28-Aug-02	44	0	0	10.8571	0	0	65.47115
Time Zero---bioaug and respike							
11-Sep-02	58	0	0	16.9678	0	0	102.3206
16-Sep-02	63	0	0	14.9403	0	0	90.09428
21-Sep-02	68	0	0	19.1218	0	0	115.3098
29-Sep-02	76	0	0	10.697	0	0	64.50598
01-Oct-02	79	0	0	2.61612	0	0	
06-Oct-02	84	0	0	12.4651	0	0	75.16788
10-Oct-02	88	0	0	8.8282	0	0	53.23645
15-Oct-02	93	0	0	13.2429	0	0	79.85846
20-Oct-02	98	0	0	6.37581	0	0	38.4479
26-Oct-02	104	0	0	14.1026	0	0	85.04252
31-Oct-02	109	0	0	6.13245	0	0	36.98033
05-Nov-02	114	0	0	11.2134	0	0	67.6199
09-Nov-02	118	0	0	12.7879	0	0	77.11458

Table B-7 IY-2a Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
14-Jul-02	0	0	0	10.38871	0	0	62.64676
17-Jul-02	3	0	0	12.13451	0	0	73.17442
17-Jul-02	3	0	0	11.4703	0	0	69.16901
dup run							
23-Jul-02	9	0	0	13.98254	0	0	84.31851
31-Jul-02	16	0	0	11.79017	0	0	71.09792
07-Aug-02	23	0	0	11.19576	0	0	67.51348
14-Aug-02	30	0	0	11.95987	0	0	72.1213
21-Aug-02	37	0	0	12.91668	0	0	77.89107
28-Aug-02	44	0	0	10.4006	0	0	62.71844
Time Zero---bioaug and respike							
11-Sep-02	58	0	0	16.05176	0	0	96.79648
16-Sep-02	63	0	0	16.84912	0	0	101.6048
21-Sep-02	68	0	0	8.757906	0	0	52.81255
29-Sep-02	76	0	0	15.55453	0	0	93.79807
01-Oct-02	79	0	0	11.5291	0	0	69.52362
01-Oct-02	79	0	0	10.44244	0	0	62.97076
dup run							
06-Oct-02	84	0	0	11.7064	0	0	70.59275
10-Oct-02	88	0	0	11.23826	0	0	67.76975
15-Oct-02	93	0	0	10.55246	0	0	63.63421
20-Oct-02	98	0	0	8.10988	0	0	48.90478
26-Oct-02	104	0	0	9.320764	0	0	56.20674
31-Oct-02	109	0	0	6.673811	0	0	40.2449
05-Nov-02	114	0	0	10.21765	0	0	61.61521
09-Nov-02	118	0	0	10.58275	0	0	63.81689

Table B-8 IY-2b Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
14-Jul-02	0	0	0	13.21968	0	0	79.71828
17-Jul-02	3	0	0	8.960465	0	0	54.03404
23-Jul-02	9	0	0	7.324996	0	0	44.17172
31-Jul-02	16	0	0	10.17171	0	0	61.33816
31-Jul-02	16	0	0	12.27052	0	0	73.9946
dup run							
07-Aug-02	23	0	0	12.70163	0	0	76.59428
14-Aug-02	30	0	0	11.93558	0	0	71.97482
21-Aug-02	37	0	0	10.45789	0	0	63.0639
28-Aug-02	44	0	0	11.14571	0	0	67.21166
Time Zero---bioaug and respike							
11-Sep-02	58	0	0	16.4039	0	0	98.91998
16-Sep-02	63	0	0	14.22182	0	0	85.76144
21-Sep-02	68	0	0	16.47356	0	0	99.34004
29-Sep-02	76	0	0	14.58932	0	0	87.97757
01-Oct-02	79	0	0	11.92599	0	0	71.91697
06-Oct-02	84	0	0	10.04644	0	0	60.58278
06-Oct-02	84	0	0	10.89617	0	0	65.70684
dup run							
10-Oct-02	88	0	0	8.46623	0	0	51.05367
15-Oct-02	93	0	0	6.334251	0	0	38.19725
20-Oct-02	98	0	0	8.084664	0	0	48.75272
26-Oct-02	104	0	0	9.023457	0	0	54.4139
31-Oct-02	109	0	0	6.794007	0	0	40.96971
05-Nov-02	114	0	0	8.461799	0	0	51.02695
09-Nov-02	118	0	0	10.58978	0	0	

Table B-9 IY-2c Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
14-Jul-02	0	0	0	9.66763	0	0	58.29844
17-Jul-02	3	0	0	10.4121	0	0	62.78794
23-Jul-02	9	0	0	9.71949	0	0	58.61118
31-Jul-02	16	0	0	10.0161	0	0	60.40009
07-Aug-02	23	0	0	9.76861	0	0	58.90739
14-Aug-02	30	0	0	7.75182	0	0	46.74556
14-Aug-02	30	0	0	10.0399	0	0	60.54346
dup run							
21-Aug-02	37	0	0	9.3376	0	0	56.30824
28-Aug-02	44	0	0	10.0435	0	0	60.56512
Time Zero---bioaug and respike							
11-Sep-02	58	0	0	17.0072	0	0	102.5582
16-Sep-02	63	0	0	12.7924	0	0	77.14137
21-Sep-02	68	0	0	17.5029	0	0	105.547
29-Sep-02	76	0	0	14.6343	0	0	88.24898
01-Oct-02	79	0	0	9.90259	0	0	59.71534
06-Oct-02	84	0	0	11.4625	0	0	69.12222
10-Oct-02	88	0	0	9.76324	0	0	58.87497
10-Oct-02	88	0	0	14.2029	0	0	85.64736
dup run							
15-Oct-02	93	0	0	7.05006	0	0	42.5138
20-Oct-02	98	0	0	6.69651	0	0	40.38175
26-Oct-02	104	0	0	10.6698	0	0	64.34155
31-Oct-02	109	0	0	5.378	0	0	32.4308
05-Nov-02	114	0	0	6.82116	0	0	41.13348
09-Nov-02	118	0	0	12.8219	0	0	

Table B-10 BY-1a Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
14-Jul-02	0	0	0	12.25621	0	0	73.90831
14-Jul-02	0	0	0	12.44702	0	0	75.05892
dup run							
17-Jul-02	3	0	0	11.34833	0	0	68.43354
23-Jul-02	9	0	0	12.34296	0	0	74.4314
31-Jul-02	16	0	0	12.02697	0	0	72.52591
07-Aug-02	23	0	0	9.9158	0	0	59.79497
14-Aug-02	30	0	0	13.16021	0	0	79.35964
21-Aug-02	37	0	0	11.9899	0	0	72.30235
21-Aug-02	37	0	0	10.63052	0	0	64.1049
duprun							
28-Aug-02	44	0	0	12.03644	0	0	72.583
Time Zero---bioaug and respike							
11-Sep-02	58	0	0	16.02292	0		96.62255
16-Sep-02	63	0	0	12.97983	0		78.27189
21-Sep-02	68	0	0	12.03916	0		72.59941
29-Sep-02	76	0	0	14.364	0		86.61885
01-Oct-02	79	0	0	10.24579	0	0	61.78492
06-Oct-02	84	0	0.22075	12.91239	0		77.86519
10-Oct-02	88	0	0.18938	10.72297	0		64.66243
15-Oct-02	93	0	0.15034	8.447225	0	1.144197	50.93906
20-Oct-02	98	0	0.10948	5.424343	0	0.833247	32.71026
26-Oct-02	104	0	0.18941	7.987186	0	1.441595	48.1649
26-Oct-02	104	0	2.3414	3.872488	0		
dup run							
31-Oct-02	109	0	0.2026	8.452726	0	1.541966	50.97224
05-Nov-02	114	0	0.19439	6.838764	0	1.479506	41.23961
09-Nov-02	118	0	0.26839	10.43001	0	2.042687	62.89577

Table B-11 BY-1b Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
14-Jul-02	0	0	0	10.4752	0	0	63.16832
17-Jul-02	3	0	0	11.53053	0	0	69.53222
23-Jul-02	9	0	0	7.386319	0	0	44.54151
23-Jul-02	9	0	0	8.409018	0	0	50.70867
dup run							
31-Jul-02	16	0	0	11.04635	0	0	66.61248
07-Aug-02	23	0	0	11.4846	0	0	69.25524
14-Aug-02	30	0	0	13.08886	0	0	78.9294
21-Aug-02	37	0	0	11.7728	0	0	70.99317
28-Aug-02	44	0	0	11.09556	0	0	66.90922
Time Zero---bioaug and respike							
11-Sep-02	58	0	0	14.0018	0		84.43469
16-Sep-02	63	0	0	14.79926	0		89.24357
21-Sep-02	68	0	0	14.49416	0		87.40374
29-Sep-02	76	0	0	12.91619	0		77.88811
01-Oct-02	79	0	0	10.76558	0		64.9194
06-Oct-02	84	0	0	12.09014	0		72.90681
10-Oct-02	88	0	3.63988	6.099669	0		36.78266
15-Oct-02	93	0	0	9.017441	0		54.37762
20-Oct-02	98	0	0	5.629824	0		33.94937
20-Oct-02	98	0	0	7.596089	0	0	45.80648
dup run							
26-Oct-02	104	0	0.13488	9.646117	0	1.026545	58.16871
31-Oct-02	109	0	0	9.074368	0	0	54.72091
05-Nov-02	114	0	0	8.741322	0	0	52.71255
09-Nov-02	118	0	0	10.3183	0	0	62.22217

Table B-12 BY-1c Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
14-Jul-02	0	0	0	12.39362	0	0	74.73691
17-Jul-02	3	0	0	8.556776	0	0	51.59969
23-Jul-02	9	0	0	7.843257	0	0	47.29697
31-Jul-02	16	0	0	10.03787	0	0	60.53106
07-Aug-02	23	0	0	10.12092	0	0	61.03188
07-Aug-02	23	0	0	11.87806	0	0	71.62791
dup run							
14-Aug-02	30	0	0	11.63489	0	0	70.16153
21-Aug-02	37	0	0	11.87381	0	0	71.60227
28-Aug-02	44	0	0	9.646929	0	0	58.1736
Time Zero---bioaug and respike							
11-Sep-02	58	0	0	14.68993	0	0	88.58427
16-Sep-02	63	0	2.499846	7.842123	0	19.02615	47.29013
21-Sep-02	68	0	3.250237	5.545369	0	24.73733	33.44008
29-Sep-02	76	0	5.902068	9.635741	0		58.10614
29-Sep-02	76	0	0.126373	13.93389	0		84.02516
dup run							
01-Oct-02	79	0	3.246905	6.086554	0	24.71197	36.70358
06-Oct-02	84	0	4.355775	7.3124	0	33.1515	44.09576
10-Oct-02	88	0	0	10.89838	0		65.72021
15-Oct-02	93	0	0.384844	0.591949	0		
20-Oct-02	98	0	2.293491	3.692697	0	17.45559	22.26797
26-Oct-02	104	0	3.521773	5.476008	0	26.80397	33.02182
31-Oct-02	109	0	3.386674	4.186951	0	25.77573	25.24845
05-Nov-02	114	0	4.162223	2.987362	0	31.67838	18.01461
09-Nov-02	118	0	7.289241	2.784736	0	55.4779	16.79272

Table B-13 BY-2a Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	**Time (days)**	**cDCE**	**TCE**	**PCE**	**cDCE**	**TCE**	**PCE**
14-Jul-02	0	0	0	0	0	0	57.82549
17-Jul-02	3	0	0	0	0	0	65.56364
17-Jul-02	3	0	0	0	0	0	50.2915
dup run							
23-Jul-02	9	0	0	0	0	0	44.24587
31-Jul-02	16	0	0	0	0	0	67.57232
07-Aug-02	23	0	0	0	0	0	70.05702
14-Aug-02	30	0	0	0	0	0	86.78235
21-Aug-02	37	0	0	0	0	0	74.54625
28-Aug-02	44	0	0	0	0	0	62.02212
Time Zero---bioaug and respike							
11-Sep-02	58	0	0	0	0		100.8036
16-Sep-02	63	0	0	0	0		60.52827
21-Sep-02	68	0	0	0	0		92.67801
29-Sep-02	76	0	0	0	0		80.11935
01-Oct-02	79	0	0	0	0		72.94212
06-Oct-02	84	0	0	0	0		61.95484
10-Oct-02	88	0	0	0	0		66.40403
15-Oct-02	93	0	0	0	0		39.25514
20-Oct-02	98	0	0	0	0		
26-Oct-02	104	0	0	0	0	0	58.00838
31-Oct-02	109	0	0	0	0	0	52.43044
31-Oct-02	109	0	0.10601	0	0	0.806834	54.97583
dup run							
05-Nov-02	114	0	0.201775	0	0	1.535697	60.06681
09-Nov-02	118	0	0.136475	0	0	1.038705	63.06284

Table B-14 BY-2b Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
14-Jul-02	0	0	0	12.2019	0	0	73.58101
17-Jul-02	3	0	0	11.2876	0	0	68.06734
23-Jul-02	9	0	0	10.257	0	0	61.85256
31-Jul-02	16	0	0	10.1782	0	0	61.3771
31-Jul-02	16	0	0	9.98619	0	0	60.21944
dup run							
07-Aug-02	23	0	0	9.98668	0	0	60.22241
14-Aug-02	30	0	0	9.84306	0	0	59.35633
21-Aug-02	37	0	0	9.48293	0	0	57.18463
28-Aug-02	44	0	0	8.63147	0	0	52.0501
Time Zero---bioaug and respike							
11-Sep-02	58	0	0	14.122	0	0	85.15923
16-Sep-02	63	0	0	11.2539	0	0	67.86419
21-Sep-02	68	0	0	11.6482	0	0	70.24157
29-Sep-02	76	0	0	14.0947	0	0	84.99479
01-Oct-02	79	0	0	2.07798	0	0	
06-Oct-02	84	0	0	10.9303	0	0	65.91239
10-Oct-02	88	0	0	10.5167	0	0	63.41861
15-Oct-02	93	0	0	7.66206	0	0	46.20431
20-Oct-02	98	0	0	7.22163	0	0	43.54842
26-Oct-02	104	0	0	9.1921	0	0	55.43087
31-Oct-02	109	0	0	7.90973	0	0	47.69782
05-Nov-02	114	0	0	8.85795	0	0	53.41584
05-Nov-02	114	0	0	8.20976	0	0	49.5071
dup run							
09-Nov-02	118	0	0	11.0156	0	0	66.42714

Table B-15 BY-2c Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
14-Jul-02	0	0	0	10.543	0	0	63.57737
17-Jul-02	3	0	0	9.99691	0	0	60.28407
23-Jul-02	9	0	0	10.4802	0	0	63.19829
31-Jul-02	16	0	0	10.9416	0	0	65.98106
07-Aug-02	23	0	0	9.06257	0	0	54.64977
14-Aug-02	30	0	0	11.38	0	0	68.62432
14-Aug-02	30	0	0	10.5398	0	0	63.55792
dup run							
21-Aug-02	37	0	0	9.25115	0	0	55.78695
28-Aug-02	44	0	0	7.62519	0	0	45.98196
Time Zero---bioaug and respike							
11-Sep-02	58	0	0	15.9787	0	0	96.35581
16-Sep-02	63	0	0	11.2364	0	0	67.75844
21-Sep-02	68	0	0	10.9745	0	0	66.17945
29-Sep-02	76	0	0	10.6994	0	0	64.52008
01-Oct-02	79	0	0	8.71305	0	0	52.54203
06-Oct-02	84	0	0	11.6574	0	0	70.29704
10-Oct-02	88	0	0	12.5631	0	0	75.75918
15-Oct-02	93	0	0	8.4076	0	0	50.70013
20-Oct-02	98	0	0	7.75872	0	0	46.78719
26-Oct-02	104	0	0	0	0	0	
31-Oct-02	109	0	0	8.82017	0	0	53.18802
05-Nov-02	114	0	0	9.05991	0	0	54.63373
09-Nov-02	118	0	0.11233	11.8819	0	0.854973	71.65136
09-Nov-02	118	0	0	12.936	0	0	78.00741

Table B-16 Experiment 2 Microcosms Chlorinated Ethene Degradation and Production Rates

BI-1,I			
	PCE	TCE	cDCE
pre CD	-0.8588	0.0622	0.4395
CD	-0.0583		
post CD	0		

BI-2,I	Control		
	PCE	TCE	cDCE
pre CD	-1.1723	0.0089	0.0617
CD	-0.9828	0.0819	0.0438
post CD	-0.9828	0.0819	0.0438

BI-3,I			
	PCE	TCE	cDCE
pre CD	-0.665		
CD	-1.0493		
post CD	0		

BI-1,II	Control		
	PCE	TCE	cDCE
pre CD	-0.6382		
CD	-0.4892		
PCE respike			
post CD	-0.4892		

BI-2,II			
	PCE	TCE	cDCE
pre CD	-1.0643		
CD	-0.1082		
PCE respike	0		
post CD	-1.3922		

BI-3,II			
	PCE	TCE	cDCE
pre CD	-1.1372		
CD	-26.295	13.849	
PCE respike	0	-1.208	0.0089
post CD	-1.2916	-0.9536	

BB-1,I	Control		
	PCE	TCE	cDCE
preCD	-0.7327	0.0457	
CD	-5.4361	0.7489	
respike (DO)	-2.3736	0.1033	
respike 2 (DO)	-0.0861	0.0541	
post CD & DO	-2.0307	-0.2106	

BB-2,I			
	PCE	TCE	cDCE
preCD	-0.6598	-0.0188	
CD	-20.368	2.2525	
respike (DO+ PCE)	-7.9925	0.6904	
respike 2 (DO)	-5.4695	0.4969	
post CD & DO	-78.163	9.0903	1.5576
PCE respike	-4.2399	1.6242	-2.0897

BB-3,I			
	PCE	TCE	cDCE
preCD	-0.8704	0.4389	
CD	-28.514	16.731	0.8518
respike (DO+PCE)	-16.628	17.807	-0.0743
respike 2 (DO+PCE)	-0.9167	0.8604	0.285
post CD & DO	-76.917	9.5787	1.1677
PCE respike	-10.697	7.5426	-2.2644

BB-1,II			
	PCE	TCE	cDCE
preCD	-0.5505	0.0911	
CD	-0.38	0.0008	

BB-2,II			
	PCE	TCE	cDCE
preCD	-0.4707		
PCE respike	-0.6082		

BB-3,II			
	PCE	TCE	cDCE
preCD	-0.352		
PCE respike	-0.1176	0.0689	

Table B-17 Gas Data for Anaerobic Group 1

B. Intrin-1, I						B. Intrin-2, I						B. Intrin-3, I				
date	Days	Methane		CO2		date	Days	Methane		CO2		date	Days	Methane		CO2
		ppm	mol/L	ppm	mol/L			ppm	mol/L	ppm	mol/L			ppm	mol/L	ppm
7/17/2002	3	0.00E+00	0.00E+00	1.56E-04	3.54E-09	7/17/2002	3	0.00E+00	0.00E+00	1.44E-04	3.27E-09	7/17/2002	3	0.00E+00	0.00E+00	1.59E-04
7/23/2002	10	0.00E+00	0.00E+00	2.51E-04	5.71E-09	7/23/2002	10	0.00E+00	0.00E+00	2.63E-04	5.99E-09	7/23/2002	10	0.00E+00	0.00E+00	3.90E-04
7/31/2002	18	0.00E+00	0.00E+00	8.15E-04	1.85E-08	7/31/2002	18	0.00E+00	0.00E+00	8.69E-04	1.97E-08	7/31/2002	18	0.00E+00	0.00E+00	8.67E-04
8/7/2002	25	0.00E+00	0.00E+00	8.63E-04	1.96E-08	8/7/2002	25	0.00E+00	0.00E+00	1.00E-03	2.27E-08	8/7/2002	25	0.00E+00	0.00E+00	1.06E-03
8/13/2002	31	0.00E+00	0.00E+00	8.63E-04	1.96E-08	8/13/2002	31	0.00E+00	0.00E+00	1.00E-03	2.27E-08	8/13/2002	31	0.00E+00	0.00E+00	1.06E-03
8/21/2002	39	0.00E+00	0.00E+00	9.39E-04	2.13E-08	8/21/2002	39	0.00E+00	0.00E+00	1.24E-03	2.82E-08	8/21/2002	39	0.00E+00	0.00E+00	8.94E-04
8/28/2002	46	0.00E+00	0.00E+00	8.55E-04	1.94E-08	8/28/2002	46	0.00E+00	0.00E+00	1.13E-03	2.58E-08	8/28/2002	46	3.66E-07	2.29E-11	7.44E-04
9/18/2002	67	5.53E-06	3.46E-10	1.13E-03	2.56E-08	9/18/2002	67	0.00E+00	0.00E+00	1.44E-03	3.27E-08	9/18/2002	67	1.03E-05	6.44E-10	1.34E-03
10/16/2002	96	0.00E+00	0.00E+00	1.28E-03	2.90E-08	10/16/2002	96	0.00E+00	0.00E+00	1.85E-03	4.19E-08	10/16/2002	96	4.98E-06	3.11E-10	1.54E-03
10/21/2002	101	0.00E+00	0.00E+00	1.07E-03	2.43E-08	10/21/2002	101	0.00E+00	0.00E+00	1.38E-03	3.14E-08	10/21/2002	101	3.33E+03		1.12E-03
10/30/2002	110	2.22E-03	1.39E-07	0.00E+00	0.00E+00	10/30/2002	110	2.71E-03	1.70E-07	0.00E+00	0.00E+00	10/30/2002	110	2.24E-03	1.40E-07	6.29E-06

Table B-18 Gas Data for Anaerobic Group 2

B. Intrin-1,II						B. Intrin-2,II						B. Intrin-3,II		
date	Days	Methane		CO2		date	Days	Methane		CO2		date	Days	Methane
		ppm	mol/L	ppm	mol/L			ppm	mol/L	ppm	mol/L			ppm
7/17/2002	3	0.00E+00	0.00E+00	1.71E-04	3.88E-09	7/17/2002	3	0.00E+00	0.00E+00	1.73E-04	3.92E-09	7/17/2002	3	0.00E+00
7/23/2002	10	0.00E+00	0.00E+00	3.13E-04	7.12E-09	7/23/2002	10	0.00E+00	0.00E+00	2.36E-04	5.37E-09	7/23/2002	10	0.00E+00
7/31/2002	18	0.00E+00	0.00E+00	5.88E-04	1.34E-08	7/31/2002	18	0.00E+00	0.00E+00	1.09E-03	2.47E-08	7/31/2002	18	0.00E+00
8/7/2002	25	0.00E+00	0.00E+00	1.00E-03	2.28E-08	8/7/2002	25	0.00E+00	0.00E+00	1.22E-03	2.78E-08	8/7/2002	25	0.00E+00
8/13/2002	31	0.00E+00	0.00E+00	1.00E-03	2.28E-08	8/13/2002	31	0.00E+00	0.00E+00	1.22E-03	2.78E-08	8/13/2002	31	0.00E+00
8/21/2002	39	0.00E+00	0.00E+00	1.58E-03	3.58E-08	8/21/2002	39	0.00E+00	0.00E+00	1.08E-03	2.46E-08	8/21/2002	39	0.00E+00
8/28/2002	46	0.00E+00	0.00E+00	1.07E-03	2.43E-08	8/28/2002	46	0.00E+00	0.00E+00	8.83E-04	2.01E-08	8/28/2002	46	0.00E+00
9/18/2002	67	9.49E-06	5.93E-10	3.05E-03	6.93E-08	9/18/2002	67	2.27E-05	1.42E-09	1.90E-03	4.31E-08	9/18/2002	67	1.47E-05
10/16/2002	96	6.21E-06	3.88E-10	2.26E-03	5.13E-08	10/16/2002	96	1.57E-05	9.81E-10	2.02E-03	4.59E-08	10/16/2002	96	2.85E-05
10/21/2002	101	7.73E-06	4.83E-10	1.86E-03	4.23E-08	10/21/2002	101	1.85E-05	1.16E-09	1.56E-03	3.55E-08	10/21/2002	101	1.78E-05
10/30/2002	110	8.49E-06	5.31E-10	3.15E-03	7.17E-08	10/30/2002	110	1.65E-05	1.03E-09	3.04E-03	6.90E-08	10/30/2002	110	3.46E-05

Table B-19 Gas Data for Anaerobic Group 3

B. Biostim-1, II						B. Biostim-2, II						B. Biostim-3, II			
date	Days	Methane		CO2		date	Days	Methane		CO2		date	Days	Methane	
		ppm	mol/L	ppm	mol/L			ppm	mol/L	ppm	mol/L			ppm	mol/L
7/17/2002	3	0.00E+00	0.00E+00	1.09E-04	2.48E-09	7/17/2002	3	0.00E+00	0.00E+00	1.02E-04	2.32E-09	7/17/2002	3	0.00E+00	0.00E+00
7/23/2002	10	0.00E+00	0.00E+00	6.42E-04	1.46E-08	7/23/2002	10	0.00E+00	0.00E+00	3.40E-04	7.72E-09	7/23/2002	10	0.00E+00	0.00E+00
7/31/2002	18	0.00E+00	0.00E+00	9.82E-04	2.23E-08	7/31/2002	18	0.00E+00	0.00E+00	9.32E-04	2.12E-08	7/31/2002	18	0.00E+00	0.00E+00
8/7/2002	25	0.00E+00	0.00E+00	1.07E-03	2.42E-08	8/7/2002	25	0.00E+00	0.00E+00	1.14E-03	2.59E-08	8/7/2002	25	0.00E+00	0.00E+00
8/13/2002	31	0.00E+00	0.00E+00	1.07E-03	2.42E-08	8/13/2002	31	0.00E+00	0.00E+00	1.14E-03	2.59E-08	8/13/2002	31	0.00E+00	0.00E+00
8/21/2002	39	0.00E+00	0.00E+00	1.02E-03	2.31E-08	8/21/2002	39	0.00E+00	0.00E+00	1.18E-03	2.67E-08	8/21/2002	39	0.00E+00	0.00E+00
8/28/2002	46	0.00E+00	0.00E+00	9.10E-04	2.07E-08	8/28/2002	46	0.00E+00	0.00E+00	9.74E-04	2.21E-08	8/28/2002	46	0.00E+00	0.00E+00
9/18/2002	67	2.64E-06	1.65E-10	1.82E-03	4.15E-08	9/18/2002	67	1.59E-05	9.95E-10	2.58E-03	5.86E-08	9/18/2002	67	0.00E+00	0.00E+00
10/16/2002	96	0.00E+00	0.00E+00	1.94E-03	4.42E-08	10/16/2002	96	2.01E-05	1.25E-09	2.45E-03	5.57E-08	10/16/2002	96	0.00E+00	0.00E+00
10/21/2002	101	1.49E+04		1.91E-03	4.33E-08	10/21/2002	101	0.00E+00	0.00E+00	1.93E-03	4.38E-08	10/21/2002	101	0.00E+00	0.00E+00
10/30/2002	110	0.00E+00	0.00E+00	2.82E-03	6.42E-08	10/30/2002	110	2.46E-05	1.54E-09	3.31E-03	7.52E-08	10/30/2002	110	0.00E+00	0.00E+00

Table B-20 Gas Data for Aerobic Group

B. Biostim-1,l						B. Biostim-2,l						B. Biostim-3,l			
date	Days	Methane		CO2		date	Days	Methane		CO2		date	Days	Methane	
		ppm	mol/L	ppm	mol/L			ppm	mol/L	ppm	mol/L			ppm	mol/L
7/17/2002	3	1.36E-05	8.48E-10	3.36E-04	7.65E-09	7/17/2002	3	4.71E-06	2.94E-10	3.00E-04	6.81E-09	7/17/2002	3	6.38E-06	3.98E-10
7/23/2002	10	9.66E-06	6.04E-10	1.34E-03	3.04E-08	7/23/2002	10	1.05E-06	6.58E-11	5.83E-04	1.32E-08	7/23/2002	10	0.00E+00	0.00E+00
7/31/2002	18	1.08E-05	6.77E-10	7.06E-04	1.60E-08	7/31/2002	18	4.38E-06	2.74E-10	1.02E-03	2.31E-08	7/31/2002	18	1.67E-06	1.04E-10
8/7/2002	25	1.02E-05	6.38E-10	9.25E-04	2.10E-08	8/7/2002	25	3.83E-06	2.39E-10	1.35E-03	3.08E-08	8/7/2002	25	0.00E+00	0.00E+00
8/13/2002	31	1.02E-05	6.38E-10	9.25E-04	2.10E-08	8/13/2002	31	3.83E-06	2.39E-10	1.35E-03	3.08E-08	8/13/2002	31	0.00E+00	0.00E+00
8/21/2002	39	7.29E-06	4.55E-10	1.35E-03	3.07E-08	8/21/2002	39	3.10E-06	1.94E-10	1.48E-03	3.37E-08	8/21/2002	39	0.00E+00	0.00E+00
8/28/2002	46	6.06E-06	3.79E-10	1.15E-03	2.61E-08	8/28/2002	46	1.68E-06	1.05E-10	1.14E-03	2.59E-08	8/28/2002	46	7.57E-07	4.73E-11
9/18/2002	67	9.08E-06	5.68E-10	1.92E-03	4.36E-08	9/18/2002	67	4.29E-06	2.68E-10	1.78E-03	4.04E-08	9/18/2002	67	8.32E-06	5.20E-10
10/16/2002	96	7.75E-04	4.84E-08	2.36E-03	5.36E-08	10/16/2002	96	1.71E-06	1.07E-10	2.05E-03	4.65E-08	10/16/2002	96	1.32E-05	8.24E-10
10/21/2002	101	3.54E-05	2.21E-09	1.83E-03	4.16E-08	10/21/2002	101	0.00E+00	0.00E+00	1.75E-03	3.99E-08	10/21/2002	101	2.57E-06	1.60E-10
10/30/2002	110	2.91E-03	1.82E-07	3.53E-03	8.02E-08	10/30/2002	110	2.73E-06	1.71E-10	2.87E-03	6.53E-08	10/30/2002	110	1.49E-04	9.30E-09

Table B-21 Gas Data for Abiotic Controls

A Cntrl -1						A Cntrl -2						A Cntrl -3					
date	Days	Methane		CO2		date	Days	Methane		CO2		date	Days	Methane		CO2	
		ppm	mol/L	ppm	mol/L			ppm	mol/L	ppm	mol/L			ppm	mol/L	ppm	mol/L
7/17/2002	3	0.00E+00	0.00E+00	4.14E-04	9.41E-09	7/17/2002	3	0.00E+00	0.00E+00	3.02E-04	6.86E-09	7/17/2002	3	0.00E+00	0.00E+00	3.29E-04	7.48E-09
7/23/2002	10	0.00E+00	0.00E+00	4.38E-04	9.96E-09	7/23/2002	10	0.00E+00	0.00E+00	8.81E-04	2.00E-08	7/23/2002	10	0.00E+00	0.00E+00	6.42E-04	1.46E-08
7/31/2002	18	0.00E+00	0.00E+00	1.20E-03	2.74E-08	7/31/2002	18	0.00E+00	0.00E+00	1.12E-03	2.54E-08	7/31/2002	18	9.15E-07	5.72E-11	1.36E-03	3.09E-08
8/21/2002	39	0.00E+00	0.00E+00	2.00E-03	4.54E-08	8/21/2002	39	0.00E+00	0.00E+00	1.40E-03	3.17E-08	8/21/2002	39	0.00E+00	0.00E+00	1.66E-03	3.78E-08
9/18/2002	67	0.00E+00	0.00E+00	2.78E-03	6.32E-08	9/18/2002	67	0.00E+00	0.00E+00	2.27E-03	5.16E-08	9/18/2002	67	0.00E+00	0.00E+00	2.78E-03	6.31E-08
10/16/2002	96	1.85E-06	1.16E-10	1.92E-03	4.36E-08	10/16/2002	96	0.00E+00	0.00E+00	1.69E-03	3.85E-08	10/16/2002	96	0.00E+00	0.00E+00	1.82E-03	4.14E-08
10/30/2002	110	2.00E-06	1.25E-10	2.02E-03	4.60E-08	10/30/2002	110	0.00E+00	0.00E+00	1.79E-03	4.06E-08	10/21/2002	101	6.87E-06	4.30E-10	1.12E-03	2.55E-08
												10/30/2002	110	0.00E+00	0.00E+00	1.92E-03	4.37E-08

Appendix C
Experiment 3 Data

Table C-1 IY-1a Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
13-Nov-02	122	0.16538669	0	22.5180964	1.7058967	0	135.7902
13-Nov-02	122.33	0.18973141	0	23.1575593	1.95700267	0	139.6464
13-Nov-02	122.33	0.20593162	0	22.9030115	2.12410125	0	138.1114
dup run							
14-Nov-02	123	0	0	16.9149498	0	0	102.0017
17-Nov-02	126	0	0	23.7495095	0	0	143.216
19-Nov-02	128	0	0.55435875	10.7191105	0	4.21919	64.63915
21-Nov-02	130	0	0	18.4868639	0	0	111.4808
24-Nov-02	133	0	0	20.4544516	0	0	123.3459
27-Nov-02	136	0	0	20.1571995	0	0	121.5534
30-Nov-02	139	0	0	21.5585756	0	0	130.0041
04-Dec-02	143	0	0	21.4524619	0	0	129.3642
post cd							
06-Dec-02	145	0	0	20.0482105	0	0	120.8962
06-Dec-02	145.2083	0	0	18.3262489	0	0	
06-Dec-02	145.2083	0	0	19.6903059	0	0	118.7379
dup run							
07-Dec-02	146	0	0	19.7004961	0	0	118.7993
10-Dec-02	149	0	0	20.0106563	0	0	120.6697
12-Dec-02	151	0	0	20.0579018	0	0	120.9546
19-Dec-02	158	0	0	18.6940326	0	0	112.7301

Table C-2 IY-1b Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
13-Nov-02	122	0.37273647	0.86893219	23.3051	3.844626	6.61338	140.536
13-Nov-02	122.33	0.21059963	0.21970453	23.6534	2.17225	1.67216	142.636
14-Nov-02	123	0.05028833	0.29677553	19.5545	0.518704	2.25874	117.919
14-Nov-02	123	0	0.26191465	7.85543			
dup run							
17-Nov-02	126	0.45467478	0.51900081	21.7567	4.689786	3.95008	131.199
17-Nov-02	126	0.19742567	0.65485673	19.2002	2.036366	4.98407	115.783
dup run							
19-Nov-02	128	0.12647127	0.84254598	20.1619	1.3045	6.41256	121.582
21-Nov-02	130	0	0.66813682	16.1526		5.08514	97.4048
24-Nov-02	133	0.19457708	0.67571859	18.8432	2.006984	5.14285	113.63
27-Nov-02	136	0.27022705	0.77288634	20.7815	2.787283	5.88238	125.318
30-Nov-02	139	0.2529211	0.68918862	19.7793	2.608779	5.24537	119.274
04-Dec-02	143	0.4014184	0.83704294	18.0617	4.140468	6.37067	108.917
post cd							
06-Dec-02	145	0.31473244	0.67407478	19.0421	3.246338	5.13034	114.829
07-Dec-02	146	0.3734379	0.74247227	18.6458	3.851861	5.6509	112.439
07-Dec-02	146	0.28548385	0.66564447	14.3385	2.94465	5.06617	86.4652
dup run							
10-Dec-02	149	0.14980827	0.62820958	15.5203	1.545212	4.78126	93.5918
12-Dec-02	151	0.30839509	0.82155809	18.3934	3.180971	6.25282	110.917
19-Dec-02	158	0.24120819	0.59783279	14.0629	2.487965	4.55006	84.8034

Table C-3 IY-1c Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
13-Nov-02	122	0	0	24.00077	0	0	144.7311
13-Nov-02	122.33	0	0	27.0746	0	0	163.2672
14-Nov-02	123	0	0	23.50601	0	0	141.7477
17-Nov-02	126	0	0	21.10706	0	0	127.2813
19-Nov-02	128	0	0	20.60185	0	0	124.2348
19-Nov-02	128	0	0	20.46845	0	0	123.4303
dup run							
21-Nov-02	130	0	0	23.36959	0	0	140.925
24-Nov-02	133	0	0	21.34278	0	0	128.7028
27-Nov-02	136	0	0	23.5499	0	0	142.0123
30-Nov-02	139	0	0	20.6955	0	0	124.7995
04-Dec-02	143	0	0	22.52429	0	0	135.8276
post cd							
06-Dec-02	145	0	0	19.838	0	0	119.6286
06-Dec-02	145.21	0	0	14.98652	0	0	90.37278
07-Dec-02	146	0	0	16.79469	0	0	101.2766
10-Dec-02	149	0	0	17.71208	0	0	106.8086
10-Dec-02	149	0	0	19.54675	0	0	117.8722
dup run							
12-Dec-02	151	0	0	18.74587	0	0	113.0427
19-Dec-02	158	0	0	16.74238	0	0	100.9611

Table C-4 IY-2a Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
13-Nov-02	122	0	0	17.5961563	0	0	106.11
13-Nov-02	122.33	0	0	19.2844437	0	0	116.29
14-Nov-02	123	0	0	19.7469196	0	0	119.079
17-Nov-02	126	0	0	17.1824802	0	0	103.615
19-Nov-02	128	0	0	19.8687593	0	0	119.814
21-Nov-02	130	0	0	18.8451338	0	0	113.641
21-Nov-02	130	0	0	18.2360234	0	0	109.968
dup run							
24-Nov-02	133	0	0	15.7436294	0	0	94.9384
27-Nov-02	136	0	0	24.7107944	0	0	149.013
30-Nov-02	139	0	0	13.6607238	0	0	82.3779
04-Dec-02	143	0	0	15.403164	0	0	92.8853
post cd							
06-Dec-02	145	0	0	12.8477935	0	0	77.4757
07-Dec-02	146	0	0	17.0138382	0	0	102.598
10-Dec-02	149	0	0	17.0774489	0	0	102.982
12-Dec-02	151	0	0	17.1324474	0	0	103.313
12-Dec-02	151	0	0	19.3169526	0	0	116.486
dup run							
19-Dec-02	158	0	0	17.1061913	0	0	103.155

Table C-5 IY-2b Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
13-Nov-02	122	0	0	14.3493772	0	0	86.5306
13-Nov-02	122.33	0	0	25.3402616	0	0	152.809
14-Nov-02	123	0	0	20.6865352	0	0	124.745
17-Nov-02	126	0	0	20.0887414	0	0	121.141
19-Nov-02	128	0	0	20.8382995	0	0	125.661
21-Nov-02	130	0	0	23.6041452	0	0	142.339
24-Nov-02	133	0	0	22.2345373	0	0	134.08
24-Nov-02	133	0	0	18.4738423	0	0	111.402
dup run							
27-Nov-02	136	0	0	19.7751546	0	0	119.25
30-Nov-02	139	0	0	17.9893454	0	0	108.481
04-Dec-02	143	0	0	21.0144341	0	0	126.723
post cd							
06-Dec-02	145	0	0	17.5095199	0	0	105.587
06-Dec-02	145.21	0	0	17.5205862	0	0	105.654
07-Dec-02	146	0	0	17.9644423	0	0	108.33
10-Dec-02	149	0	0	17.6795403	0	0	106.612
12-Dec-02	151	0	0	16.7518384	0	0	101.018
19-Dec-02	158	0	0	18.2605933	0	0	110.116

Table C-6 IY-2c Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
13-Nov-02	122	0.19571345	16.3389006	18.105531	2.0187	124.3542	109.181
13-Nov-02	122.33	0.17125539	14.346991	17.1871618	1.76643	109.1939	103.643
14-Nov-02	123	0.15963131	13.5676536	17.0158322	1.64653	103.2625	102.61
17-Nov-02	126	0.11137374	14.8915561	15.0587663	1.14878	113.3386	90.8085
19-Nov-02	128	0.25599023	15.591591	17.3002674	2.64044	118.6665	104.325
respike							
20-Nov-02	129	0.13802491	13.4831163	17.2936882	1.42367	102.619	104.286
20-Nov-02	129.48	0.16482309	10.8667746	16.1280135	1.70008	82.70625	97.2563
21-Nov-02	130	0.21447739	15.9191126	19.2915151	2.21225	121.1592	116.333
21-Nov-02	130.25	0.16709278	12.2115655	17.8165292	1.72349	92.94136	107.439
24-Nov-02	133	0	1.50974906	18.2904131	0	11.49059	110.296
27-Nov-02	136	0	1.48008588	20.7620884	0	11.26483	125.201
27-Nov-02	136	0	1.18857907	20.2046852	0	9.046191	121.84
dup run							
30-Nov-02	139	0	1.32296692	18.8335432	0	10.06901	113.571
04-Dec-02	143	0	0.54438759	19.7213366	0	4.143295	118.925
post cd							
06-Dec-02	145	0	1.50974906	18.2904131	0	11.49059	110.296
06-Dec-02	145.21	0	1.48008588	20.7620884	0	11.26483	125.201
07-Dec-02	146	0	1.18857907	20.2046852	0	9.046191	121.84
10-Dec-02	149	0	1.32296692	18.8335432	0	10.06901	113.571
12-Dec-02	151	0	0.54438759	19.7213366	0	4.143295	118.925
19-Dec-02	158	0	0.95894421	18.7046507	0	7.298457	112.794

Table C-7 BY-1a Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
13-Nov-02	122	0	0	16.2859	0	0	98.2085
13-Nov-02	122.33	0	0	17.9476	0	0	108.229
14-Nov-02	123	0	0	15.5355	0	0	93.6832
17-Nov-02	126	0	0.42734498	12.4609	0	3.25249	75.1429
19-Nov-02	128	0	0.55435875	10.7191	0	4.21919	64.6392
20-Nov-02	129	0	0.57939759	11.578	0	4.40975	69.8185
add 5 mL DO h20							
20-Nov-02	129.23	0	0.51377825	10.1437	0	3.91033	61.1695
20-Nov-02	129.48	0	0.44215526	7.7632	0	3.36521	46.8142
21-Nov-02	130	0	0.15453253	9.67852	0	1.17614	58.3641
21-Nov-02	130.25	0	0.45841764	10.4996	0	3.48898	63.3155
24-Nov-02	133	0	0.48350264	7.75909	0	3.6799	46.7894
add 5 mL DO h20							
25-Nov-02	134	0	0.35587998	7.53196	0	2.70858	45.4198
26-Nov-02	135	0	0.41729571	7.51137	0	3.17601	45.2956
27-Nov-02	136	0	0.44230793	11.3309	0	3.36637	68.3284
30-Nov-02	139	0	0.47135689	7.41711	0	3.58746	44.7272
30-Nov-02	139	0	0.81000525	6.64565	0	6.16489	40.0751
dup run							
04-Dec-02	143	0	0.35096452	7.45961	0	2.67117	44.9835
post cd							
06-Dec-02	145	0	0.80009267	11.6031	0	6.08945	69.9696
06-Dec-02	145.21	0	0.78152079	15.2193	0	5.9481	91.7764
07-Dec-02	146	0	0.83752401	11.7096	0	6.37434	70.612
10-Dec-02	149	0	0.72142579	12.2212	0	5.49072	73.6969
12-Dec-02	151.125	0	0.49335486	12.4861	0	3.75489	75.2944
12-Dec-02	151.375	0	0.87308965	9.8951	0	6.64502	59.6701
13-Dec-02	152	0	0.58395092	10.4903	0	4.44441	63.2593
14-Dec-02	153	0	0.35870074	11.8595	0	2.73005	71.5159
16-Dec-02	155	0	0.66785279	8.74214	0	5.08298	52.7175

Table C-8 BY-1b Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
13-Nov-02	122	0	0	19.9492477	0	0	120.2994
13-Nov-02	122.33	0	0	22.1792648	0	0	133.747
14-Nov-02	123	0	0	18.6680301	0	0	112.5733
17-Nov-02	126	0	16.2959627	2.38870227	0	124.027	14.40452
19-Nov-02	128	0	14.706361	0.27360057	0	111.929	1.649886
20-Nov-02	129	0	13.3563279	0.23792884	0	101.654	1.434776
respike and add 5 mL DO h20							
20-Nov-02	129.23	0.17153413	16.2391465	17.3009761	1.76930506	123.595	104.3296
20-Nov-02	129.48	0	17.0341053	17.3886273	0	129.645	104.8582
21-Nov-02	130	0	16.3003456	15.8585533	0	124.061	95.63139
21-Nov-02	130.25	0	12.8041136	13.7414321	0	97.4512	82.86457
24-Nov-02	133	0	16.6578215	12.2671515	0	126.782	73.97426
respike and add 5 mL DO h20							
25-Nov-02	134	0	17.4077566	11.6431591	0	132.489	70.21142
26-Nov-02	135	0	17.2176815	11.1915922	0	131.043	67.48834
27-Nov-02	136	0	15.9348034	9.11505275	0	121.279	54.96625
30-Nov-02	139	0	17.9651065	3.47012702	0	136.731	20.92581
04-Dec-02	143	0	18.1783636	2.87319754	0	138.354	17.32616
04-Dec-02	143	0	22.8338275	3.2434066	0	173.787	19.55862
post cd							
06-Dec-02	145	0	2.6937393	13.6227704	0	20.5019	82.14901
06-Dec-02	145.21	0	6.32670417	8.86064026	0	48.1521	53.43207
07-Dec-02	146	0	14.2025556	0	0	108.095	0
10-Dec-02	149	0.84566061	13.0286945	0	8.7226468	99.1605	0
12-Dec-02	151	0.69893678	12.4978001	0	7.20924993	95.1199	0
post cd(respike)							
12-Dec-02	151.125	1.23735092	13.1518526	18.6233842	12.7627738	100.098	112.3041
12-Dec-02	151.375	1.23498322	13.1195285	17.7792253	12.738352	99.8518	107.2136
13-Dec-02	152	0.97415958	12.2332102	14.702262	10.0480617	93.1061	88.65864
14-Dec-02	153	1.11034363	12.6975179	14.136096	11.452745	96.6399	85.2445
16-Dec-02	155	0.40626407	13.8900379	15.5706982	4.19044939	105.716	93.89554

Table C-9 BY-1c Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
13-Nov-02	122	0	1.0164013	19.0805	0	7.73576	115.06
13-Nov-02	122.33	0	0.5221482	19.0209	0	3.97403	114.701
14-Nov-02	123	0	6.56333678	8.48837	0	49.9531	51.1872
17-Nov-02	126	0.21578529	15.0336464	0	2.225738	114.42	0
19-Nov-02	128	0.35050215	13.691094	0	3.615288	104.202	0
20-Nov-02	129	0.52651745	16.4399578	0	5.430814	125.123	0
respike and add 5 mL DO h20							
20-Nov-02	129.23	0.43064555	12.7297036	13.704	4.441934	96.8849	82.6388
20-Nov-02	129.48	0.35126178	19.993938	10.603	3.623123	152.172	63.9387
21-Nov-02	130	0.40171858	20.5896371	4.86602	4.143564	156.706	29.3434
21-Nov-02	130.25	0.56925242	20.8029953	5.6344	5.871608	158.33	33.9769
24-Nov-02	133	0.38077991	25.1064154	1.16952	3.927591	191.083	7.05255
respike and add 5 mL DO h20							
25-Nov-02	134	0.46958853	22.2071552	18.4654	4.843616	169.017	111.352
26-Nov-02	135	0.24562503	22.6392452	17.6126	2.533523	172.306	106.209
27-Nov-02	136	0.22522239	21.4925639	17.0928	2.323078	163.578	103.074
30-Nov-02	139	0.43948537	20.4241109	15.1237	4.533114	155.446	91.2002
04-Dec-02	143	0.58361177	23.7970641	17.2674	6.019719	181.118	104.127
post cd							
06-Dec-02	145	0	4.88849822	12.8684	0	37.206	77.5998
06-Dec-02	145.21	0	9.26629456	10.5531	0	70.5251	63.6379
07-Dec-02	146	0	17.6337594	0.23096	0	134.209	1.39274
10-Dec-02	149	0.31396056	15.1043836	0	3.238376	114.958	0
12-Dec-02	151	0.56972106	16.0362688	0	5.876442	122.051	0
post cd(respike)							
12-Dec-02	151.125	1.26829947	18.2860315	17.4961	13.082	139.174	105.507
12-Dec-02	151.375	0.898577	17.5798137	13.731	9.268458	133.799	82.8017
13-Dec-02	152	1.11022723	20.7764778	14.7142	11.45154	158.128	88.7308
14-Dec-02	153	0.41248502	18.8410542	9.48903	4.254616	143.398	57.2215
16-Dec-02	155	0.37155722	22.2822558	9.81765	3.832462	169.589	59.2031

Table C-10 BY-2a Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	**Time (days)**	**cDCE**	**TCE**	**PCE**	**cDCE**	**TCE**	**PCE**
13-Nov-02	122	0	0.13647545	18.1946925	0	1.03870503	109.719
19-Nov-02	128	0	0	16.0607596	0		96.8507
21-Nov-02	130	0	0.15071413	17.7795771	0	1.14707461	107.216
24-Nov-02	133	0	0.13636539	20.3029089	0	1.03786737	122.432
27-Nov-02	136	0	0	16.119934	0		97.2076
30-Nov-02	139	0	0.14473002	16.4450371	0	1.10152993	99.168
04-Dec-02	143	0	0.12995701	16.7851846	0	0.98909363	101.219
post cd--nothing done to these							
09-Dec-02	148	0	0	16.0933058	0	0	97.047

Table C-11 BY-2b Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	**Time (da**	**cDCE**	**TCE**	**PCE**	**cDCE**	**TCE**	**PCE**
13-Nov-02	122	0	0	17.73885	0	0	106.97
19-Nov-02	128	0	0	18.5467723	0	0	111.842
21-Nov-02	130	0	0	18.1512322	0	0	109.457
24-Nov-02	133	0	0	20.7105216	0	0	
27-Nov-02	136	0	0	18.4839971	0	0	111.464
30-Nov-02	139	0	0	17.7558969	0	0	107.073
04-Dec-02	143	0	0	15.0072417	0	0	90.4977
post cd							
09-Dec-02	148	0	0	17.0855148	0	0	103.03

Table C-12 BY-2c Chlorinated Solvent Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	cDCE	TCE	PCE	cDCE	TCE	PCE
13-Nov-02	122	0	0.10393827	17.9928904	0	0.79106683	108.502
19-Nov-02	128	0	0.2292035	16.5496743	0	1.7444516	99.799
21-Nov-02	130	0	0.13722103	16.7857444	0	1.04437954	101.223
24-Nov-02	133	0	0.20933751	19.749898	0	1.593253	119.097
27-Nov-02	136	0	0.13520265	18.5006684	0	1.02901784	111.564
30-Nov-02	139	0	0.25647551	17.1709352	0	1.95201696	103.545
04-Dec-02	143	0	0.34728459	16.6649449	0	2.64315843	100.494
post cd							
09-Dec-02	148	0	0.29350566	15.6954216	0	2.23385082	94.6477

Table C-13 Microcosm Characteristics during Experiment 3 (In Presence of CD)

Microcosm Name	Fe (2+)Concentration (mg/L)	pH	DO readings (mg/L)
BI-1,I	13.216	6	1.7
BI-3,I	9.83	6	1.3
BI-2,II	44.22	6.2	1.8
BI-3,II	38.331	6	1.85
BB-1,I	6.658	7.2	2
BB-2,I	9.243	5	2
BB-3,I	11.084	5	2
A Cntrl-1	2.579	6.7	n/a
A Cntrl-2	3.132	6.7	n/a
A Cntrl-3	9.221	6.7	n/a
BB-1,II	3.567	6.5	n/a
BB-2,II	2.248	6.5	n/a
BB-3,II	0.406	6.5	n/a
Average Concent:	11.82576923		

Table C-14 IY-1a Microcosm CD Concentrations during Experiment 3

SampleName	Days After CD Injection	%	g/L
prepceBI,1-I 11-13	-0.5	7.115	71.15
timezeroBI-1-I11-13, 2hrs	0	7.322	73.22
11-14BI-1,I	1	7.193	71.93
11-17BI-1,I	4	7.119282	71.19282
11-19BI-1,I	6	7.142716	71.42716
CDrun28BI-1,I11-21	8	7.384308	73.84308
11-24BI-1,I	11	7.41292	74.1292
11-27BI-1,I	14	7.447921	74.47921
12-1BI-1,I	18	7.187506	71.87506
4-Dec	21	7.429188	74.29188
12/6/2002 (CD Removed)	23	1.05461	10.5461
postcd 12-6	23.5	1.157067	11.57067
7-Dec	24	1.141795	11.41795
post cd512-11,3:45	28	1.104587	11.04587
18-Dec	35	1.050301	10.50301
	average during		
	presence of CD=	72.75384	

Table C-15 IY-1c Microcosm CD Concentrations during Experiment 3

SampleName	Days After CD Injection	%	g/L
prepceBI-3,I	-0.5	7.178	71.78
timezeroBI-3,I	0	7.088	70.88
11-14BI-3,I	1	7.153	71.53
11-17BI-3,I	4	7.145058	71.45058
11-19BI-3,I	6	7.017453	70.17453
CDrun28BI-3,I	8	7.327374	73.27374
11-24BI-3,I	11	7.392227	73.92227
11-27BI-3,I	14	7.346663	73.46663
12-1BI-3,I	18	7.048813	70.48813
12/6/2002 (CD Removed)	21	1.00579	10.0579
4-Dec	23	6.885849	68.85849
postcd	23.5	1.058835	10.58835
7-Dec	24	1.053151	10.53151
post cd5	28	1.05059	10.5059
18-Dec	35	0.989943	9.89943

Table C-16 IY-2b Microcosm CD Concentrations during Experiment 3

SampleName	Days After CD Injection	%	g/L
prepceBI,2-II	-0.5	7.145	71.45
timezeroBI-2,II	0	7.217	72.17
11-14BI-2,II	1	7.258	72.58
11-17BI-2,II	4	7.428946	74.28946
11-19BI-2,II	6	7.182862	71.82862
CDrun28BI-2,II	8	7.397348	73.97348
11-24BI-2,II	11	7.447055	74.47055
11-27BI-2,II	14	7.445403	74.45403
12-1BI-2,II	18	6.888492	68.88492
6-Dec	21	0.937674	9.37674
4-Dec	23	7.394246	73.94246
post cd (CD Removed)	23.5	0.926449	9.26449
7-Dec	24	0.93332	9.3332
post cd5	28	0.921832	9.21832
18-Dec	35	0.891906	8.91906

Table C-17 IY-2c Microcosm CD Concentrations during Experiment 3

SampleName	Days After CD Injection	%	g/L
prepceBI,3-II	-0.5	7.318	73.18
timezeroBI-3,II	0	4.795	47.95
11-14BI-3,II	1	7.203	72.03
11-17BI-3,II	4	7.490575	74.90575
11-19BI-3,II	6	6.003024	60.03024
timezeroBI-3,II?	7	6.30796	63.0796
nightBI-3,II	7.25	7.402328	74.02328
11-21BI-3,II	8	7.387342	73.87342
CDrun28BI-3,II	8.125	7.349545	73.49545
11-24BI-3,II	11	7.359582	73.59582
11-27BI-3,II	14	7.393695	73.93695
12-1BI-3,II	18	7.084478	70.84478
12/6/2002	21	1.027061	
4-Dec	23	7.013677	70.13677
post cd (CD Removed)	23.5	1.085852	10.85852
7-Dec	24	1.088602	10.88602
post cd5	28	1.071501	10.71501
18-Dec	35	0.984249	9.84249

Table C-18 BY-1b Microcosm CD Concentrations during Experiment 3

SampleName	Days After CD Injection	%	g/L
prepceBB,2-I	-0.5	7.214	72.14
timezeroBB,2-I	0	7.211	72.11
11-14BB-2,I	1	7.061	70.61
11-17BB-2,I	4	7.459612	74.59612
11-19BB-2,I	6	7.137368	71.37368
timezero2BB-2,I11-20	7	7.179819	71.79819
nightBB-2,I 11-20	7.25	6.833802	68.33802
11-21BB-2,I	8	6.768945	67.68945
CDrun28BB-2,I	8.125	6.737349	67.37349
11-24BB-2,I	11	6.715703	67.15703
11-25BB-2,I	12	6.232529	62.32529
11-26BB-2,I	13	6.281252	62.81252
11-27BB-2,I	14	6.32433	63.2433
12-1BB-2,I	18	6.003145	60.03145
4-Dec	21	5.975803	59.75803
12/6/2002 (CD Removed)	23	0.84804	8.4804
post cd	23.5	0.824027	8.24027
7-Dec	24	0.867642	8.67642
post cd5	28	0.853293	8.53293
18-Dec	35	0.782152	7.82152

Table C-19 BY-1c Microcosm CD Concentrations during Experiment 3

SampleName	Days Since CD Injection	%	g/L
prepceBB,3-I	-0.5	7.075	70.75
timezeroBB-3,I	0	6.869	68.69
11-14BB-3,I	1	6.908	69.08
11-17BB-3,I	4	7.187589	71.87589
11-19BB-3,I	6	7.083862	70.83862
timezero2BB-3,I	7	6.575191	65.75191
nightBB-3,I	7.25	6.63142	66.3142
11-21BB-3,I	8	6.580063	65.80063
CDrun28BB-3,I	8.125	6.59862	65.9862
11-24BB-3,I	11	6.5639	65.639
11-25BB-3,I	12	3.634765	
11-27BB-3,I	14	6.06451	60.6451
12-1BB-3,I	18	5.827774	58.27774
4-Dec	21	5.623566	56.23566
12/6/2002 (CD Removed)	23	0.735952	7.35952
post cd	23.5	0.821091	8.21091
7-Dec	24	0.83146	8.3146
post cd5	28	0.812167	8.12167
18-Dec	35	0.724015	7.24015

Appendix D
Sediment and Liquid Control Data

Table D-1 Liquid Control PCE Concentrations (uM and ppm)

		ppm	uM
Date	Time (days)	PCE	PCE
06-Jun-02	202	17.4275	105.093
06-Jun-02	202	17.5558	105.866
dup run			
09-Jun-02	205	18.0942	109.113
12-Jun-02	208	16.8364	101.528
19-Jun-02	215	16.4859	99.4142
26-Jun-02	222	21.8191	131.575
03-Jul-02	229	17.9926	108.5
10-Jul-02	236	12.909	
17-Jul-02	243	19.5886	118.124
23-Jul-02	249	16.0408	96.7304
31-Jul-02	257	14.738	88.874
07-Aug-02	264	11.6919	
14-Aug-02	271	19.5501	117.893
21-Aug-02	278	16.5497	99.7993
28-Aug-02	285	18.5225	111.696
11-Sep-02	299	18.2513	110.061
18-Sep-02	306	18.2673	110.157
02-Oct-02	318	13.6282	82.1816
17-Oct-02	333	14.4868	87.3592
05-Dec-02	382	16.51	99.5599
11-Dec-02	388	18.4009	110.962
22-Dec-02	398	19.7558	119.133

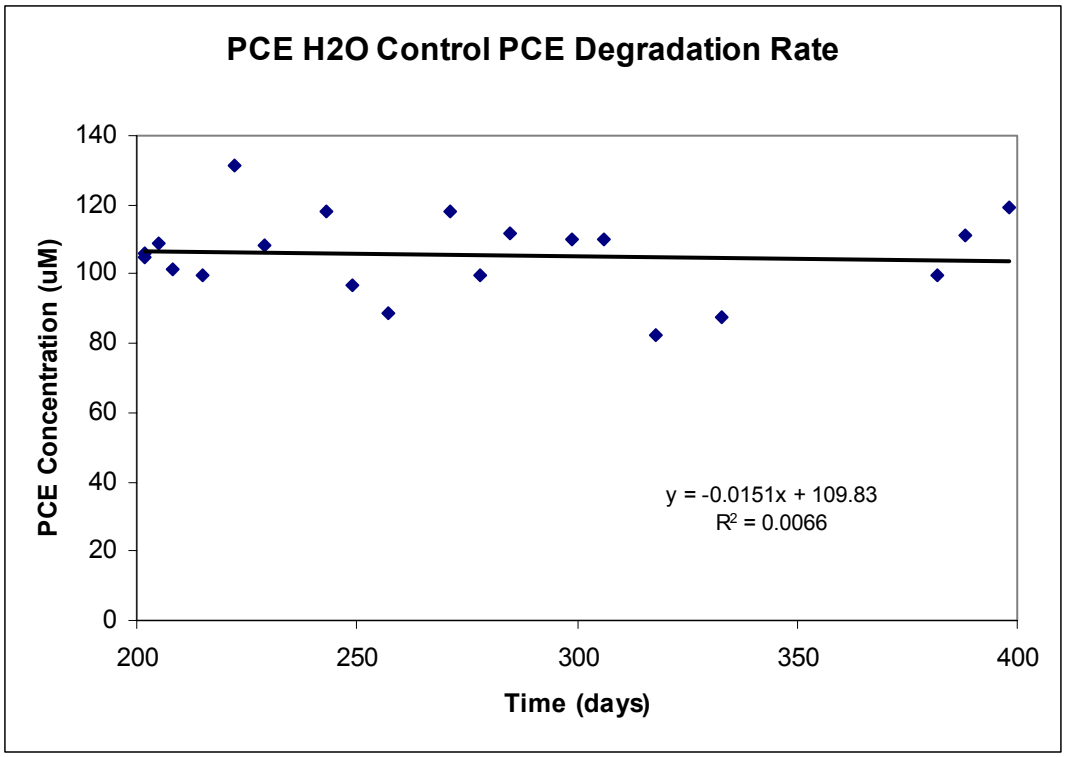

Figure D-1 Liquid Control PCE Degradation Rate

Table D-2 Sediment Control Chlorinated Ethene Concentrations (uM and ppm)

		ppm			uM		
Date	Time (days)	PCE	TCE	cDCE	PCE	TCE	cDCE
24-Apr-02	179	0	0	0.00	0.00	0	0
05-May-02	190	0	0	0.00	0.00	0	0
05-May-02	190	0	0	0.00	0.00	0	0
dup run							
16-May-02	201	0	0	0.00	0.00	0	0
22-May-02	207	0	0	0.00	0.00	0	0
29-May-02	214	0	0	0.00	0.00	0	0
06-Jun-02	222	0	0	0.00	0.00	0	0
12-Jun-02	228	0	0	0.00	0.00	0	0
12-Jun-02	228	0	0	0.00	0.00	0	0
dup run							
19-Jun-02	235	0	0	0.00	0.00	0	0
24-Jun-02	242	0	0	0.00	0.00	0	0
03-Jul-02	249	0	0	0.00	0.00	0	0
10-Jul-02	256	0	0	0.00	0.00	0	0
10-Jul-02	256	0	0	0.00	0.00	0	0
dup run							
17-Jul-02	263	0	0	0.00	0.00	0	0
23-Jul-02	269	0	0	0.00	0.00	0	0
31-Jul-02	277	0	0	0.00	0.00	0	0
07-Aug-02	284	0	0	0.00	0.00	0	0
14-Aug-02	291	0	0	0.00	0.00	0	0
21-Aug-02	298	0	0	0.00	0.00	0	0
21-Aug-02	298	0	0	0.00	0.00	0	0
dup run							
28-Aug-02	305	0	0	0.00	0.00	0	0
11-Sep-02	319	0	0	0.00	0.00	0	0
18-Sep-02	326	0	0	0.00	0.00	0	0

Table D-3 Gas Data for Liquid Control

PCEH2O Cntrl					
date	Days	Methane		CO2	
		ppm	mol/L	ppm	mol/L
2/6/2002	82	0.00E+00	0.00E+00	5.44E-03	1.24E-07
4/11/2002	146	0.00E+00	0.00E+00	6.80E-03	1.54E-07
5/22/2002	187	4.70E-06	2.94E-10	1.52E-03	3.45E-08
6/19/2002	215	0.00E+00	0.00E+00	2.04E-03	4.64E-08
6/26/2002	222	0.00E+00	0.00E+00	7.16E-03	1.63E-07
7/10/2002	237	0.00E+00	0.00E+00	2.55E-03	5.80E-08
7/17/2002	244	1.03E-05	6.41E-10	2.04E-03	4.64E-08
8/7/2002	265	2.57E-06	1.60E-10	1.70E-03	3.85E-08
8/13/2002	271	2.57E-06	1.60E-10	1.70E-03	3.85E-08
8/21/2002	279	2.59E-06	1.62E-10	1.78E-03	4.04E-08
8/28/2002	286	1.55E-06	9.71E-11	1.43E-03	3.24E-08
10/21/2002	340	4.14E-06	2.59E-10	1.93E-03	4.38E-08

Table D-4 Gas Data for Sediment Control

BCntrl					
date	Days	Methane		CO2	
		ppm	mol/L	ppm	mol/L
2/6/2002	102	8.34E-02	5.21E-06	5.90E-02	1.34E-06
2/20/2002	116	5.73E-02	3.58E-06	3.72E-02	8.46E-07
2/26/2002	122	5.69E-02	3.55E-06	6.70E-02	1.52E-06
3/22/2002	146	4.90E-02	3.06E-06	5.46E-02	1.24E-06
3/29/2002	153	5.35E-02	3.34E-06	7.77E-02	1.77E-06
4/11/2002	166	4.38E-02	2.74E-06	5.70E-02	1.30E-06
4/24/2002	179	6.79E-02	4.25E-06	6.79E-02	1.54E-06
5/17/2002	202	1.32E-02	8.27E-07	1.41E-02	3.21E-07
5/22/2002	207	1.09E-02	6.78E-07	1.21E-02	2.74E-07
5/29/2002	214	1.13E-02	7.09E-07	1.30E-02	2.95E-07
6/5/2002	221	1.34E-02	8.40E-07	1.38E-02	3.13E-07
6/12/2002	228	1.18E-02	7.40E-07	1.13E-02	2.57E-07
6/19/2002	235	9.14E-06	5.72E-10	6.99E-03	1.59E-07
6/26/2002	242	1.04E-01	6.52E-06	4.65E-02	1.06E-06
7/10/2002	257	1.21E-03	7.56E-08	1.71E-03	3.88E-08
7/17/2002	264	1.56E-02	9.74E-07	1.39E-02	3.17E-07
7/23/2002	270	1.12E-02	7.00E-07	2.44E-04	5.55E-09
7/31/2002	278	1.10E-02	6.90E-07	2.54E-04	5.77E-09
8/7/2002	285	1.08E-02	6.76E-07	1.14E-02	2.59E-07
8/13/2002	291	1.08E-02	6.76E-07	1.14E-02	2.59E-07
8/21/2002	299	9.07E-03	5.67E-07	1.07E-02	2.43E-07
8/28/2002	306	6.52E-03	4.07E-07	8.60E-03	1.95E-07
9/18/2002	327	6.69E-03	4.18E-07	3.01E-03	6.84E-08
10/16/2002	356	7.26E-03	4.54E-07	2.88E-03	6.55E-08
10/21/2002	361	5.94E-03	3.71E-07	2.56E-03	5.81E-08
10/30/2002	370	1.30E-02	8.12E-07	4.20E-03	9.56E-08

Table D-5 Microcosm Name Conversion Schematic

	Experiment 1 Microcosm Names		Experiment 2 and 3 Microcosm Names	
	Original Name	New Name	Original Name	New Name
Experimental Microcosms	BI (Control A) (Biotic Intrinsic)	I-1	BI-1,I	IY-1a
	BI dup (Control A dup) (Biotic Intrinsic dup)	I-2	BI-2,I	IY-1b
	BB (Control B) (Biotic Biostimulated)	B-1	BI-3,I	IY-1c
	BB (Control B dup) (Biotic Biostimulated dup)	B-2	BI-1,II	IY-2a
	CD1 (Anoxic Intrinsic)	I-3	BI-2,II	IY-2b
	CD1 dup (Anoxic Intrinsic dup)	I-4	BI-3,II	IY-2c
	CD2 (Anoxic Biostimulated)	B-3	BB-1,I	BY-1a
	CD2 dup (Anoxic Biostimulated dup)	B-4	BB-2,I	BY-1b
	CD3 (Oxic Intrinsic)	I-5	BB-3,I	BY-1c
	CD3 dup (Oxic Intrinsic dup)	I-6	BB-1,II	BY-2a
	CD4 (Oxic Biostimulated)	B-5	BB-2,II	BY-2b
	CD4 dup (Oxic Biostimulated dup)	B-6	BB-3,II	BY-2c
Control Microcosms	Biotic Control	SC	A Control-1	AC-1
	PCE-H2O Control	LC	A. Control-2	AC-2
	Abiotic Control	AC	A. Control-3	AC-3

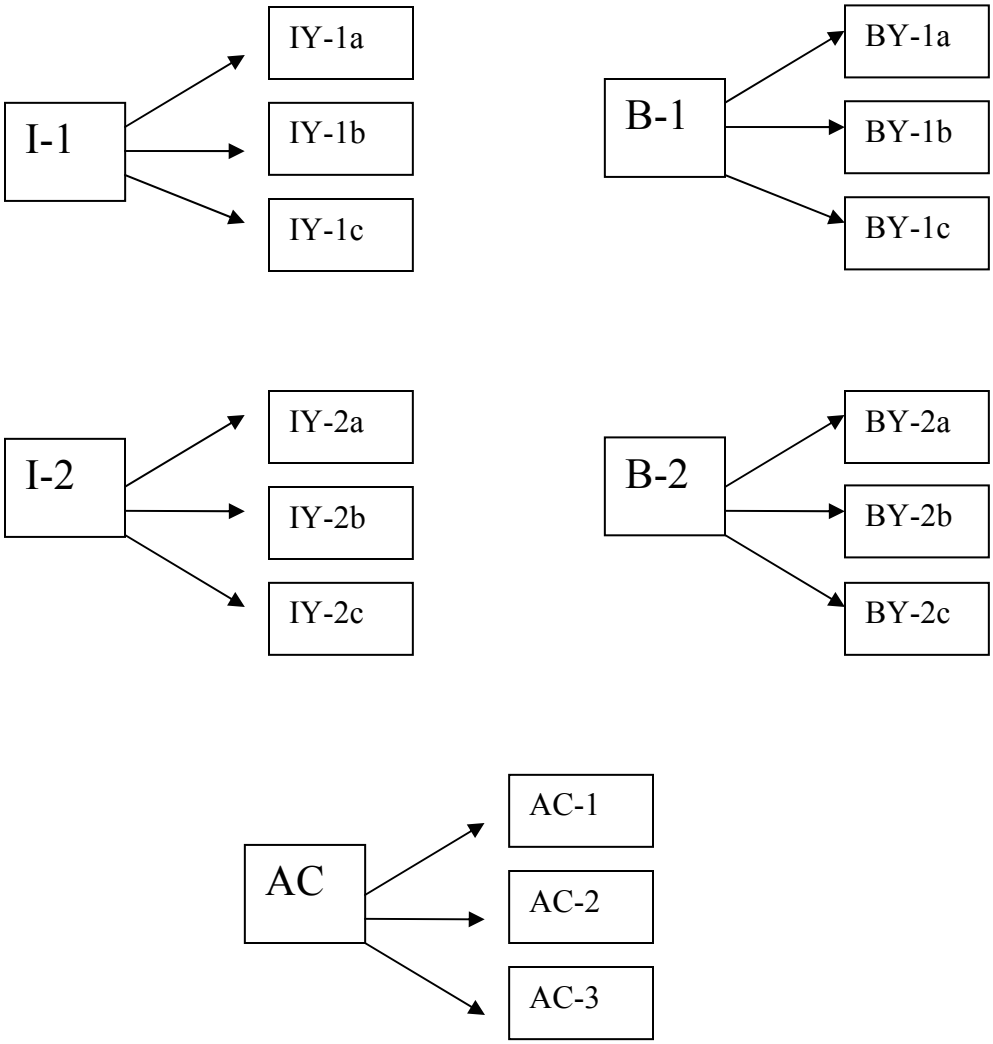

Figure D-2 Microcosm Name Conversion Schematic

VITA

Margaret Faye Cooney was born on June 23, 1978 to Steve and Jane Cooney in Johnstown, Pennsylvania. The first 18 years of her life was spent with her parents and younger siblings, Elizabeth and Stephen, in the rugged mountains around South Fork, PA. During this time, Margaret spent many long hours running, hiking, swimming, and reading in the woods of western Pennsylvania.

Following her 1996 graduation from Forest Hills High School, Margaret attended the Pennsylvania State University (Penn State) at both the Altoona and State College campuses. She pursued a Bachelors of Science degree in Environmental Systems with a minor in Geosciences. As an undergraduate student she served in many organizations including Lion Ambassadors (Penn State Altoona), the Penn State Altoona Pep Band, Society of Environmental Systems Engineers, and was one of the founders of the Altoona section of the Society of Women Engineers. Throughout her collegiate years at Penn State, Margaret gained valuable work experiences. She was a church pianist, a summer high school librarian, and resident assistant at Penn State Altoona. Margaret's most rewarding and challenging professional experiences occurred when she served as an engineering intern for the District Mining Office of the Pennsylvania Department of Environmental Protection and as an undergraduate research assistant for the Mineral Processing Department at Penn State. Margaret graduated from Penn State in the fall 2000.

In the spring of 2001, Margaret began her pursuit of a Masters of Science degree in Civil Engineering, with a strong focus on GeoEnvironmental Engineering under Drs. Mark Widdowson and Madeline Schreiber at Virginia Polytechnic and State University (Virginia Tech). While there, she created reductive dechlorinating microcosms and studied the complexing agent cyclodextrin in the laboratory. The results of this research will be presented in June 2003 at *Battelle's Seventh International Symposium on In-Situ and On-Site Bioremediation* in Orlando, Florida. Following graduation in December 2002, Margaret will continue to pursue her interest in geology, hazardous waste, and subsurface remediation through her work in an ORISE postgraduate research fellowship as an environmental engineer with the United States Army at Aberdeen Proving Grounds in Edgewood, Maryland.