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Enhanced bioremediation of 1,1,2,2-tetrachloroethane in wetland soils

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ENHANCED BIOREMEDIATION OF 1,1,2,2-TETRACHLOROETHANE IN WETLAND SOILS

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ABSTRACT

This research investigated the capability of wetland soils to function as a medium for the degradation of 1,1,2,2-TeCA and its daughter products, and the influence of different hydrogen donors and nutrients on this process. The rate constants calculated for this study, show that removal of 1,1,2,2-TeCA is occurring in the microcosms receiving hydrogen donors as well as the biotic controls. The parent compound was undergoing complete degradation to ethene, which was confirmed by ethene analysis, as well as monitoring the intermediate compounds. The analysis for the degradation products of 1,1,2,2-tetrachloroethene is important in determining the overall potential for successful degradation of the parent compound. The major biotic daughter products of TeCA are the 1,2-dichloroethene isomers, vinyl chloride and ethene. The mass balance of the study had recoveries within 40% of the initial injection.

From the data collected in this study, it can be shown that enhanced bioremediation can be a viable strategy for the improvement of the soil degradation phase of wetland remediation processes. The addition of hydrogen donors to the wetland soils showed the ability to support a complete degradation process in the deepsoil. By amending the system with a slow release hydrogen donor such as butyrate and introducing a known dechlorinating culture, early success could be achieved until over time a well established dechlorinator population is produced.

CHAPTER 1
INTRODUCTION

The release of chlorinated hydrocarbons into the environment has resulted in extensive groundwater contamination by these compounds (Burris et al., 1996). Nationwide surveys of drinking water have suggested that there is widespread appearance of at least trace levels of these carcinogenic compounds in the drinking water (Butler and Hayes, 1998). These contaminated waters can often be found in areas where the groundwater discharges into already existing wetlands, or where a wetland could be constructed that would receive the contaminated plume. The current typical pump and treat methods of remediation for these compounds can be quite expensive and disruptive to these contaminated ecosystems. It is has been suggested that natural attenuation is the most promising in situ treatment for the remediation of these chemicals (Lorah et al., 1997).

Natural attenuation is "the biodegradation and/or chemical destruction or stabilization of contaminants and can be an important tool for stabilizing or remediating a contaminated site." Biodegradation, abiotic degradation volatilization, sorption and dispersion are all possible components of natural attenuation (Stiber et al., 1999). In organically contaminated sites, biodegradation is typically the process responsible for breakdown of the contaminants because the biological reactions generally have higher degradation rates than the abiotic processes. Sorption and volatilization both remove the contaminant from the water phase but do not directly impact the breakdown of the contaminant. Dispersion may lower the contaminant concentration as it moves through a system but it does not affect the total contaminant mass (Lorah et al., 1997). Natural attenuation can be applied to systems in cases

where some combination of these various processes result in contaminant removal that is sufficient enough to reduce all hazardous compounds in the groundwater to below regulatory standards before the groundwater flow is released into the environment.

In certain sites, natural attenuation is not completely effective due to some limitation on the processes contributing to the contaminant removal from the environment. At these sites, enhanced natural attenuation is a promising solution for bioremediation of sites including chlorinated solvent contaminated aquifers (Fennel et al., 2001). For example, it has been shown to successfully degrade chlorinated ethenes in groundwater by the addition of an electron donor to the system for (Morse et al., 1998).

Studies of the potential for natural attenuation to occur in wetland systems have been conducted at the Aberdeen Proving Ground (APG) J-Field and Canal Creek sites (Lorah et al., 1997). 1,1,2,2-Tetrachloroethane is one of several chlorinated organic solvents that are now contaminating some of the world's groundwater systems (Lorah et al., 1999). It is a prominent contaminant at the APG sites where, along with trichloroethylene, it was used as a solvent for the cleaning of chemical weapon components. The solvents seeped into the groundwater and having specific gravities greater than water settled to the bottom of the aquifer present under the site. The 1,1,2,2-TeCA (PCA), considered a dense non-aqueous phase liquid (DNAPL), there slowly dissolves into the moving water, creating a contaminated plume that flows toward and out into the Chesapeake Bay. This plume, however, travels upward through wetlands along the edges of the bay. Here it is hypothesized that if the wetland conditions are satisfactory then none of the PCA in the plume, nor any toxic

products formed from its degradation, will survive to reach the Chesapeake (Lorah et al., 1997).

It is believed that chlorinated hydrocarbons are degraded through one or more of three possible pathways all of which are referred to as reductive dechlorination: hydrogenolysis, dichloroelimination, and dehydrochlorination (O'loughlin et al., 1999). Chen et al. (1996), showed all three of these pathways simultaneously occurring from 1,1,2,2-TeCA under methanogenic conditions using municipal digester sludge. Beginning with 1,1,2,2-tetrachloroethane any of these three processes may take place. Dehydrochlorination is an abiotic elimination reaction that transforms PCA into TCE. Dichloroelimination is the other process that converts the parent compounds from ethanes to ethenes. Dichloroelimination results in the release of two adjacent chlorine atoms and the formation of a double bond between the two carbons. Either PCA or 1,1,2-TCA is a potential recipient of this reaction forming cis-1,2-DCE/trans-1,2-DCE or vinyl chloride respectively (Lorah and Olsen, 1999). Hydrogenolysis will potentially reduce the PCA or TCE all the way down to ethane or ethene respectively under the correct conditions (Smatlak and Gossett, 1996). A result of microbial activity, this reaction replaces a chlorine atom with a hydrogen atom and can occur sequentially until all chlorine atoms are removed (Lorah and Olsen, 1999).

The purpose of this thesis is to investigate the capability of wetland soils to function as a medium for the degradation of 1,1,2,2-TeCA and its daughter products, and the influence of different hydrogen donors on this process. The scope of this research is as follows: to conduct several preliminary microcosm studies to develop a general understanding of how the microcosms and various nutrients will react; to

follow up the first studies with a more detailed study that will incorporate knowledge learned in the earlier and to synthesize this information into conclusions that will help contribute to the understanding of the soil microbial degradation portions of both enhanced bioremediation and constructed wetlands.

This thesis is organized as follows: Chapter 1 provides an introduction, background and objectives for the study. Chapter 2 presents a review of existing literature for natural attenuation and conventional treatments, sorption, degradation of PCA, and enhanced bioremediation. Chapter 3 outlines the methods used to conduct the research in this thesis and the materials used in the process. In Chapter 4 the results of studies demonstrating bioremediation capabilities of the wetland soils and its indigenous microbes are presented along with the effects brought about by supplementing the system with an acclimated microbial culture known to fully degrade 1,1,2,2-tetrachloroethane to ethene. These studies included the addition of different hydrogen donors in an attempt to optimize the reducing environment in the microcosms. Also presented are the general conclusions of the research and recommendations about future research and applications.

CHAPTER 2
LITERATURE REVIEW

2.1 Conventional Treatment vs. Natural Attenuation

Over the years, the most common method of treating contaminated groundwater has been some conventional pump and treat technology. In this technology, groundwater is extracted via a collection system of groundwater wells, pumps and manifolds. After collection, groundwater is treated for VOCs using some type of physical-chemical treatment system. Problems have always existed with this approach. The main problem is the low removal efficiency associated with the technology. Common difficulties in groundwater remediation are slow desorption of contaminants to liquid phase, persistent non-aqueous phase liquids (NAPLs), and site heterogeneity (Sahoo and Smith, 1998). In many cases the amount of water actually being pumped is less than anticipated. There are also problems taking care of NAPLs and predicting the rate limiting desorption of contaminants from soil organic matter (SOM) (Sahoo and Smith, 1997). Pump and treat options are problematic in systems with highly organic soils because of the tendency of the contaminants to sorb onto the organic matter and greatly decrease the removal efficiency of the pump and treat system. If this technology is to be applied in wetlands, there is a strong possibility that surface water will be pumped along with the contaminated groundwater (Lorah et al., 1997).

Remediation of contaminated groundwater by pump and treat systems is not only costly and time consuming, but in many cases these technologies can only serve to contain the contaminant plumes and do not actually succeed in remediating the system to proposed health standards (Stiber et al., 1999). Because of the lack for a

more cost effective alternative, solidification/stabilization is the most commonly used alternative to remediation at U.S. Superfund sites (Hwang and Batchelor, 2000). While this approach does not remove contamination from the site, it is designed to prevent release of the contaminants into the environment. This technology involves the solidification of the area surrounding the source contamination by either incorporating cement into the system resulting in the contaminant being trapped within the cement matrix or by the injection of polymer type compound that effectively cuts off the waterflow from the area surrounding the source. Degradative solidification/stabilization is a newer approach that combines the immobilization and degradation of contaminants. In such a system, the contaminants can be retained in the system until enough time has elapsed for degradation to occur and thereby preventing any environmental releases (Hwang and Batchelor, 2000). Contaminant movement is prevented by physical barriers and chemical binding to the cement sites in the concrete matrix (Kulik and Kersten, 2002).

Air sparging is another established method of removing contamination from groundwater, though it is only an option for volatile organic compound (VOC) contamination. The technology this implicates is fairly simple. Air is commonly injected into either the contaminated source zone or along an outer plume area to induce volatilization of the contaminant. This works because many of the hydrocarbon based contaminants in groundwater are at least semi volatile and will partition into the air phase being passed through the contaminated zone. While success in removing the contaminants is expected, reappearance of the compound in the aquifer as well as tailing off of the removal efficiency commonly occurs (Rabideau et al., 1999). Two

other problems this approach might incur would be the generally expensive cost of moving air and possibly the health risk of releasing toxic compounds into the air. Another undesirable result of pump and treat methods is the usual necessity of building above ground structures for use in the treatment process and the potential dewatering of a wetland system (Lorah et al., 1997; McNab et al., 2000).

Iron and palladized-iron cathode systems have been shown to quickly remove carbon tetrachloride and TCE from contaminated water and this reaction occurs fast enough that it is feasible for above ground applications. A canister system is set up that contains a platinum anode and an iron cathode. As contaminated water is passed through this system, the chlorinated hydrocarbons are degraded. The reduction may occur by either direct or indirect mechanisms. The direct mechanism involves either electron tunneling or the formation of a chemisorption complex between the hydrocarbon and the cathode. Indirect reduction may occur because of the presence of atomic hydrogen at the cathode surface. The use of the Pd addition at a concentration of 1 mg/m^2 of cathode increases the reaction rates by a factor of 3 (Li and Farrell, 2000). This brings the degradation rates up to a matter of minutes or even seconds. These reaction values could make it possible for treatment to take place in a small reactor that could be inserted into well bores. Another advantage to the catalytic reactions is that complete degradation is not dependent on the absence of dissolved oxygen in the system (McNab et al., 2000). However, the efficiency of this operation dwindles over time and the reaction mechanisms are not understood well enough to reach a long-term solution (Li and Farrell, 2000).

Natural attenuation involves both bioremediation and phytoremediation. Bioremediation is the destruction of contaminants by the microorganisms in the soil matrix. Phytoremediation involves the uptake of contaminants into plant species whose root zones come in contact with the contaminated water plume or aerobic degradation by methanogens and methanotrophs around the root zones of the plants (Lorah et al., 1997). It is believed that some wetland vegetative species are capable of dechlorination of chlorinated ethenes. With the high level of vegetative biomass present in wetlands, this process could be of interest to natural attenuation capabilities of wetland systems (Pardue, 2002). The diverse populations of microorganisms and redox conditions present in wetlands make them prime locations for the natural attenuation of VOC contaminated groundwater (Lorah et al., 1997). Natural attenuation has been shown to be successful in the removal of VOC's from contaminated groundwater plumes (Eganhouse et al., 2001, Maymo-Gatell et al., 2001). This success hinges on the ability of the system to degrade the parent compound completely to ethene and/or ethane and not release any of the highly toxic daughter products into the environment (Smatlak and Gossett, 1996).

The EPA completed a directive in 1999 that states monitored natural attenuation is "an appropriate remediation option for contaminated soil and groundwater under certain circumstances." It defines natural attenuation as "a variety of physical, chemical, or biological processes that, under favorable conditions, act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil or groundwater. These in situ processes include biodegradation; dispersion; dilution; sorption; volatilization; radioactive decay; and

chemical or biological stabilization, transformation, or destruction of contaminants" (Stiber et al., 1999).

In-situ remediation technologies involving bioaugmentation and biostimulation are gaining popularity because of the potential for a cost-effective and hopefully more successful alternative to the often-costly conventional treatment methods (Bradley and Chapelle, 1998). Bioaugmentation is the addition of known dechlorinating microbial populations to the subsurface region in order to degrade a contaminated waterflow (Harkness et al., 1999). Biostimulation involves the addition of organic acids and nutrients to the soil to provide indigenous microbial dechlorinators with a favorable environment for dechlorination of contaminants (Morse et al., 1998). It is hoped that these technologies will provide a permanent solution to contaminated sites so very little work or costs will be associated with the project after the initial involvement (Smatlak and Gossett, 1996). Bioremediation, when implemented correctly, shows promise of being more ecologically unobtrusive than conventional pump and treat technologies (Lorah et al., 1997).

2.2 Sorption

At the base of chemical transport, bioavailability, and degradation rates of organic compounds lies the sorption of the chemical to the soil matrix (Pignatello and Xing, 1996). Once introduced into the environment, groundwater and soil, nearly all fate processes of the organic compound are regulated by its location. There are three basic locations for the compound to be present: the liquid phase, attached to the soil particle surface, or inside the minute pore spaces of the soil particle. Each location

will affect the observed properties of the compound with regard to its ability to be remediated.

The primary soil characteristic determining the sorption ability of organic compounds is presence of soil organic matter. Sorption of the sorbate to the sorbent occurs linearly as long as there are available sites (Xing et al., 1996). In wetland systems there is generally plenty of available sorption sites for the organic compounds to attach. However, many researchers have observed non-equilibrium conditions in natural systems that make linear approaches to sorption calculations impractical (Culver et al., 1997). More recent work by Xing and Pignatello (1997), suggest that the surficial adsorbed phase is merely an intermediate point in the movement from the water phase to the absorbed phase in the intraparticle pore spaces. This hypothesis would support the nonlinear sorption characteristics seen in many natural systems.

While the greatest percentage of sorption occurs within a relatively short timeframe, complete equilibrium may take a nearly indefinite time to occur. For most modeling purposes, it has been considered acceptable to treat the quickly sorbed fraction as complete sorption because of the near impossibility of waiting for true complete sorption to occur. This is a dangerous assumption since the change in the extent of sorption between short contact times and longer contact times can range from around 30 percent up to a ten-fold increase in sorption (Pignatello and Xing, 1996.) As a compound is left in contact with the soil matrix, it is believed to become less available to microorganisms over time (Hatzinger and Alexander, 1997)

Desorption is, naturally, the opposite of the sorption process. Here the compound in question moves back into the liquid phase from the soil surface or

intraparticle pore spaces. In many cases desorption has been observed to occur in two different phases: a fast phase and slow phase. The rate-limiting phase is the movement out of the intraparticle pore spaces, which is typically known as the slow phase (Rugner et al., 1999). In many studies only quick phase desorption is taken into consideration since the slow phase desorption may take many months or even years to reach equilibrium and in most cases small amounts of compound exist in the surface phase or the intraparticle pore spaces after a couple of months.

Sorption properties of wetland soils are of particular interest when considering the remediation potential of wetland systems. The movements of the compounds through the wetland soils are dependant on the sorption/desorption properties (Shin and Pardue, 2002). Sorption is primarily controlled by the organic carbon in the soil (Lorah et al., 1997). Sorption coefficients for VOCs have been shown to be markedly higher in peat soils high in organic matter than other soil types with lower organic content (Moore et al., 2002). This leads to potential retention times of VOCs in wetlands to be 4 to 10 times higher than the residence time of the water even using the most conservative estimates (Pardue, 2002).

2.3 Natural Attenuation of 1,1,2,2-Tetrachlorethane

Hydrogenolysis, dichloroelimination, and dehydrochlorination are the three degradation reactions that are thought to exist for 1,1,2,2-Tetrachloroethane. O'loughlin et al. (1999), state that these three types of reactions are also the primary degradation pathways for chlorinated ethenes which make up some of the 1,1,2,2-TeCA daughter products. Hydrogenolysis is the sequential replacement of chlorine atoms by hydrogen atoms via reductive dechlorination. This is usually driven by

microbial activity. Dichloroelimination is the simultaneous release of two chlorine atoms and a hydrogen, which results in the formation of an alkene. Dehydrochlorination is an abiotic elimination reaction that results in the formation of TCE from PCA. Studies have shown that the biotic reactions found in natural systems can effectively degrade TeCA levels to below detection limits (Lorah and Olsen, 1999). Figure 2.1 shows a diagram of the potential pathways of degradation for 1,1,2,2-tetrachlorethane. Smatlak and Gossett (1996), showed that a microbial enrichment culture was able to degrade PCE to ethene over a two day period showing that microbial rates of degradation are indeed unlikely to be a limiting factor in natural systems if proper hydrogen donor levels are present. It is assumed that all these processes are results of biodegradation since abiotic reactions typically occur slower than biologically mediated ones (Lorah et al., 1997).

It should not be assumed that natural systems would mimic the well-defined biological cultures laboratory studies sometimes use. While most complete mineralization of TCE via a chloroethene intermediate result in ethene and ethane in laboratory studies, these two products cannot account for all of the original compound in natural systems. In these instances it is possible for methane and/or carbon dioxide to be formed as well (Bradley and Chapelle, 1999). More recent work noting that methane is accumulating during degradation of VC under methanogenic conditions suggests that methanogens may play a role in this process even if they are not responsible for the removal of the parent compound (Bradley and Chapelle, 2000).

In systems where the chlorinated compound is not being completely dechlorinated or where the rate of dechlorination is not enough to adequately reduce levels of the compound, the release of intermediate compounds such as vinyl chloride

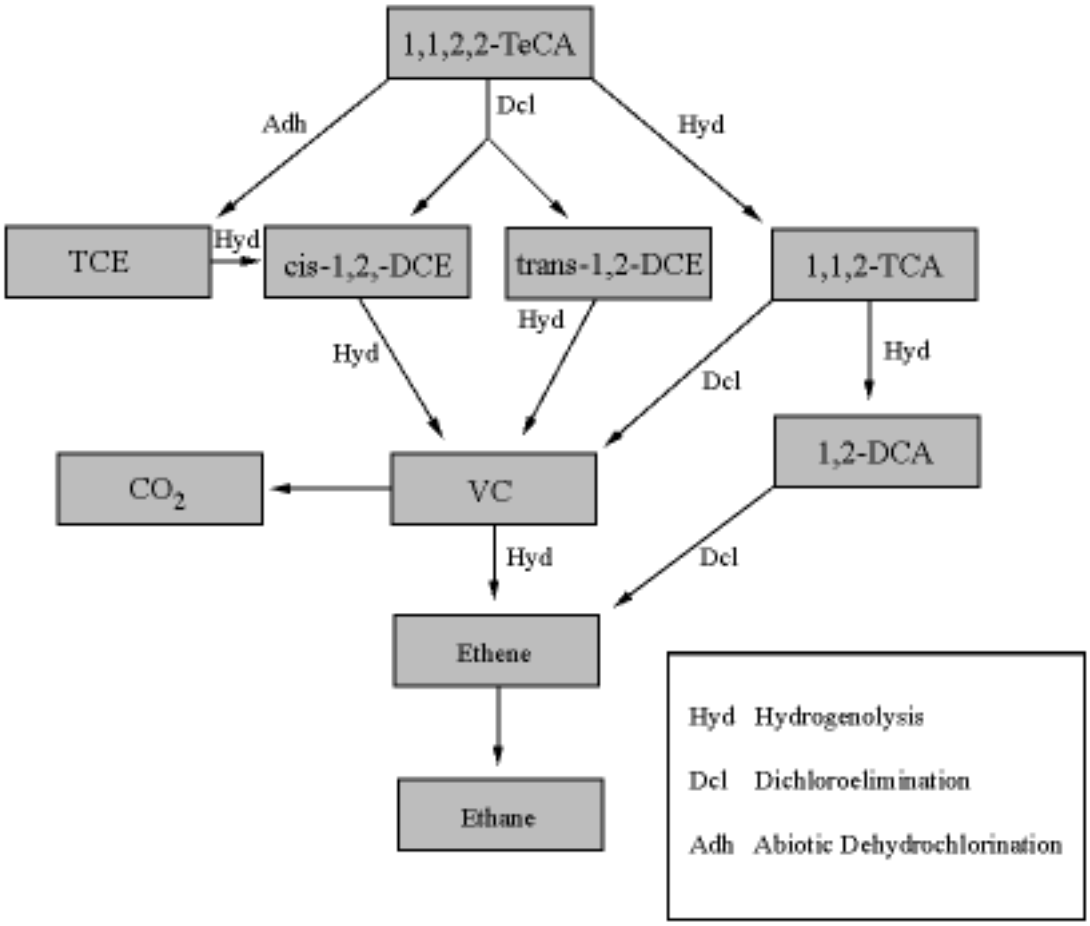

Figure 2.1. Degradation pathways of 1,1,2,2-tetrachloroethane

and *cis*-1,2-dichloroethene can occur. By enhancing the system through supply of electron donors, acceptors or nutrients, there is potential to avoid the need for implementation of pump and treat or other ex-situ technologies (Harkness et al., 1999). Difficulties can arise in achieving complete degradation of the lower daughter products because they are more resistant to dechlorination due to their relatively

reduced state (Bradley and Chapelle, 1998). Ballapragada et al. (1997), claim that the rate-limiting step of chlorinated ethene degradation is the transformation of VC to ethene. This is supported to some extent by the half velocity coefficients measured by Haston and McCarty (1999). In the degradation of PCE to ethene via reductive dechlorination, they found the maximum degradation rates to be 77 + 5, 59 + 11, 14 + 3, and 13 + 3 μM/day and the half velocity coefficients (K_s) of PCE, TCE, cDCE and VC at 25^{o}C to be 0.11 $\pm$ 0.04, 1.4 $\pm$ 0.9, 3.3 $\pm$ 2.2, and 2.6 $\pm$ 1.9 μM respectively. While not supporting the removal of VC to be the rate-limiting step, this data does suggest that the more chlorinated compounds are more quickly degraded in general.

It is widely accepted that vinyl chloride is a common degradation product of the 1,1,2,2-TeCA reactions. Though vinyl chloride is a very toxic compound and readily formed by degradation of more chlorinated alkanes and alkenes, not much is known about the ultimate fate of this known carcinogen. Barrio-Lage et al. (1990), found four different transformations of VC in anaerobic conditions: reductive dechlorination to ethylene, mineralization to methane, formation of chloromethane most likely followed by dechlorination, and bio-oxidation to CO_2 when acetate and citrate were present. The mineralization of this late forming daughter product is essential in remediation efforts because VC is listed as a priority pollutant by the United States EPA and is a known carcinogen (Bradley and Chapelle, 1996).

A number of halorespiratory microorganisms have been isolated that are able to use the chlorinated hydrocarbons as electron acceptors and gain energy from this. Research has uncovered several halorespirers that dechlorinate tetrachloroethene to Trichloroethene and finally to cis-1,2-dichloroethene (*cis*-1,2-DCE). These

halorespirers include *Dehalobacter restrictus* (PER-K23) (Hollinger et al., 1998), *Dehalobacter sp.* (strain TEA) (Wild et al., 1996), *Dehalospirillum multivorans* (Scholz-Muramatsu et al., 1995) *Desulfuromonas ethenica* (TT4B) (Krumholz et al., 1996), and *Desulfitobacterium sp.* (strain PCE1) (Gerritse et al., 1996).

The only microorganism isolated in pure culture that is able to completely dechlorinate PCE to ethene is *Dehalococcoides ethenogenes* strain 195 (Maymo-Gatell et al., 1997). *Dehalococcoides*, like other halorespirers, uses H_2 as an electron donor. It has been shown to exhibit zero-order kinetics in degrading PCE to vinyl chloride (VC) with minimal accumulation of the intermediates. Strain 195 is then capable of dechlorinating VC to ethene, which is the ultimate goal of chlorinated ethene remediation efforts, as a first-order reaction after the PCE in the system has been completely removed. Other organisms similar to *Dehalococcoides* exist as has been shown by comparisons of 16S rDNA sequences taken from several cultures, but strain 195 remains the only isolated strain capable achieving complete dechlorination (Hendrickson et al., 2002). *Dehalococcoides* strain 195 has been found at groundwater sites that show complete degradation of chlorinated ethenes. This suggests that the presence of this halorespirer is critical for full dechlorination of the contaminant at these contaminated (Hendrickson et al., 2002).

2.4 Other Fate Processes

Other methods of enhanced bioremediation of chlorinated hydrocarbons exist. Lesage et al. (1998), studied the possible pathways induced by Vitamin B_{12} and titanium citrate additions to chlorinated ethenes. The titanium citrate serves to reduce the Vitamin B_{12} to a state where the chlorinated compound can bind to the cobalamine

present in the Vitamin B_{12} (Woods et al., 1999). It is thought to be possible that by choosing the correct type and amount of titanium chelate for this remediation strategy, that a pathway can be selected that eliminates the sequential formation of the chlorinated intermediates and results immediately in ethene and acetylene (Lesage et al., 1998). Iron mediated dechlorination has received increasing attention as another method of treating groundwater containing chlorinated organic compounds. Its been shown to be effective in treating aquatic systems and is now being tested in pilot and field scale applications at various sites (Farrell et al., 2000). Humic-Metal complexes, found naturally in aquatic and terrestrial systems, have also been shown to degrade TCE. In the presence of Ni-Aldrich humic acid complexes, TCE was able to fully degrade in less than 23 h, while Cu-Aldrich complexes only resulted in 60% removal of TCE after 150 h. These results suggest that transition metal-humic acid complexes may represent another class of natural electron mediators (O'loughlin et al., 1999).

2.5 Enhanced Bioremediation

Chlorinated solvents can be sequentially dehalogenated to ethene in natural systems where the right conditions are present. Sometimes these conditions occur naturally, but other times intervention is necessary (Yang et al., 1998). Enhanced bioremediation has the potential to degrade chlorinated ethenes by the addition of an electron donor and/or nutrient amendment to the aquifer. It is also conceivable that wetlands receiving discharge of chlorinated solvents can hold microbial populations capable of dechlorinating the contaminants in the discharge. One of the most promising methods for determining the suitability for enhanced bioremediation is RABITT, which is an acronym for reductive anaerobic biological in-situ treatment

technology. This technology seeks to find the best hydrogen donor for the specific site in question that will optimize the ability of the dechlorinators to out compete the methanogens (Morse et al., 1998).

The RABITT technology is a four phase process encapsulating various aspects of biostimulation feasibility studies. The first phase is a review of any existing site data to develop a conceptual model for the site and see if it is a candidate for this technology. The second phase is a test plot within the contaminated plume for more detailed site characterization. If it is decided to proceed, the third phase involves laboratory microcosm studies to determine if RABITT application at a site is a possibility. The last phase is a field test of the application developed during the first 3 phases (Morse et al., 1998).

The microcosm studies test the ability of various hydrogen donors and nutrients to stimulate dechlorinating microbes in the site soil. Microcosms are set up that contain soil and groundwater from the potential application site. These microcosms are then amended with the different treatment combinations and are analyzed for successful degradation of the site contaminant. If a successful treatment exists, then a pilot scale field test can be conducted (Morse et al., 1998).

In pilot scale tests, contaminated water from the site is pumped out and amended with the selected treatment. It is then reinjected into the test plot where it flows through the plot past an array of testing wells that monitor the treated water. The data from this test is analyzed and a final decision can then be made on the potential of success for RABITT at the site (Morse et al., 1998).

Earlier studies have suggested the ability of reductive dechlorinating microbes to utilize lower levels of H_2 than methanogens. If this is true, then it may be possible to select for dechlorinating microbes in a system by the delivery method of the H_2. It is hypothesized that electron donors that only release H_2 under low hydrogen conditions would be able to successfully select for dechlorinators over methanogens (Fennel et al., 1997). This information is supported by work from Smatlak and Gossett (1996). They conclude that the nearly 1 order of magnitude difference in K_s values between dechlorinators and methanogens suggests that an electron donor that releases a slow, steady, low level of H_2 can favor the dechlorinating microorganisms over methanogenic communities. From their studies, butyric acid was able to act as such an electron donor and gives hope to future studies comparing even more donors searching for an even more efficient option for selection of dechlorinators (Smatlak and Gossett, 1996). A study using a benzoate acclimated microbial population showed that the best results occurred in the situation when H_2 levels were 2 and 11 ηM. The 2 ηM value is that which is necessary to be above the threshold for the degradation of *cis*-1,2-DCE. At higher ranges, the H_2 concentrations are high enough to support a methanogenic population that has the potential to outcompete the dehalogenators (Yang and McCarty, 1998).

Up flowing groundwater like that commonly found in wetland systems can contribute to successful remediation of VOC compounds. Because of the difficulty of removing the more reduced daughter products formed from the mineralization of 1,1,2,2-TeCA in an anaerobic environment, it is beneficial to have a remediation

strategy that follows anaerobic treatment with an aerobic treatment, which has been shown to readily degrade the DCE and VC (Bradley and Chapelle, 1998).

Bioaugmentation has also been shown to be a viable means of enhanced bioremediation. In one particular study using Dover soil columns, the indigenous microbial population was only able to degrade TCE to *cis*-1,2-DCE in the columns. After amending the columns with a TCE-dechlorinating microbe, the TCE was degraded to ethene within a span of 30 days. After the microbial population had become established in the columns, the 4 mg/L influent stream was fully degraded within the first few centimeters of the column. This shows considerable promise for the application of bioaugmentation in natural systems (Harkness et al., 1999).

Generating considerable interest for use in in-situ remediation applications are organisms that are able to utilize chlorinated hydrocarbons as respiratory electron acceptors. These dechlorination steps require 2 electrons for the removal of each chlorine present. This makes the presence of an electron donor crucial for the anaerobic mineralization of these compounds (Fennel et al., 1997). Among the numerous possibilities existing for electron donors, some of those that have shown promise are: lactate, butyrate, methanol, propionate, acetate, glucose, formate, crotonate and ethanol. It is hypothesized that different electron donors contribute differing levels of success to different microbial populations due primarily to the rate of release of hydrogen molecules into the system (Ballapragada et al., 1997).

Before implementing a full-scale enhanced bioremediation project, it is important to know how the indigenous microbes will react to the proposed treatment, because the site-to-site variability can result in complete dissimilarity of remediation

properties (Flynn et al., 2000). It is also critical to know what the fate of the introduced electron donor in the system is since it is possible that other organisms in the system might be outcompeting the dechlorinators for the hydrogen donor. It is common to find microorganisms that can reduce the more chlorinated compounds like TCE and TeCA at many sites, but the organisms that can further dechlorinate the compounds to VC or ethene are less commonly present (Fennel et al., 2001). In fact, the only bacteria known to reduce TCE past cDCE in a pure culture is *Dehalococcoides ethenogenes* strain 195. While this strain readily degrades *cis*-1,2-DCE at concentrations high enough to make it suitable for remediation, it is inhibited by the presence of chloroform that is found in pure *cis*-1,2-DCE solutions (Fennel et al., 2001; Maymo-Gatell et al., 2001). Typically the compounds are less likely to be degraded as the chlorine atoms in the molecule are replaced by hydrogen. This is true even though it is shown using Gibbs free energy estimates that there is energy available from all reductive dechlorination steps involving chlorinated ethenes (Flynn et al., 2000). The approach taken by engineers to develop a remediation strategy for a particular site hinges tightly on the knowledge of the system that is best obtained with small scale field studies carried out at the site in question before a full-scale operation is commenced (Fennel et al., 2001).

2.6 Remediation of Wetland Environments

Removal of 1,1,2,2-tetrachloroethane is the first important step in the bioremediation of a TeCA contaminated site. The rate at which the TeCA can be removed is extremely important in designing a remediation approach for any site. As with all degradation processes, there will be a rate-limiting step involved. For the

degradation of TCE it has been shown to be VC (Ballapragada et al., 1997). If these removal rates are not high enough, bioremediation may not be a feasible process for the site in question (Smatlak and Gossett, 1996). However, in wetland soils with high organic carbon content such as those used in this study, the residence time of the compound is markedly increased allowing for much slower removal rates to be practical. The slow movement through groundwater systems of TeCA, while good for wetland applications, is a cause for difficulty in using the traditional pump and treat methods (Lorah et al., 1997). The process becomes inefficient when the compound slowly dissolves into the water and then is further retained by sorption onto soil particles (Sahoo and Smith, 1998).

Wetland environments present an ideal situation for the application of enhanced bioremediation, particularly biostimulation. The release of chlorinate hydrocarbon groundwater plumes into wetland systems is a common occurrence. About 75 percent of RCRA and Superfund sites are located within a half mile of a surface water body and many of these have wetlands associated with them (Lorah et al., 2002). At these sites, removal of the contaminant as the groundwater flows through the wetland has been observed (Pardue, 2002). Because wetlands are able to retard the movement of VOCs to a great extent, the possibilities for the application of wetland remediation technologies are numerous (Kassenga and Pardue, 2002).

Not all wetlands are capable of naturally treating the contaminated plumes that are discharged into them. Incomplete degradation of the parent compound or escape of toxic daughter compounds may occur (Lorah et al., 2002). By injecting hydrogen donors like butyrate into the microbial environment, degradation rates of the parent

compound can be increased. Biostimulation can result in successful dechlorination where the natural system was unable to achieve such (Smatlak and Gossett, 1996).

Wetland remediation also involves the presence of vegetation. Phytoremediation can supplement the shortcomings of the microbial population when complete microbial degradation of the VOC is not exhibited. Uptake of VOCs by plants as well as aerobic degradation of those is believed to exist (Lorah et al., 1997). Though there is little field evidence of direct plant effects in wetland systems, non wetland plants have been shown to remove VOCs from the system. Laboratory evidence shows that a high percentage of this removal is released into the environment through volatilization (Pardue, 2002).

The indirect effects of the vegetation may play a more important role in wetland remediation. The decay of certain wetland plant species is thought to lead to formation of H2 precursors creating an environment favorable for halorespirers in the wetland. The concentration of these precursors are similar to those used in biostimulation applications (Pardue, 2002).

2.7 Objectives

The primary objective of this study was to analyze the potential of a wetland soil to degrade 1,1,2,2-TeCA and to assess the potential benefits of microbial stimulation by different hydrogen donors. Bioaugmentation was also implemented by adding a known dechlorinating population to the treatment microcosms. By conducting this research, it is hoped to find a treatment that can completely degrade the contaminant within a timeframe consistent with that which might be found in a typical wetland. In doing so, the potential for successful application of biostimulation and

bioaugmentation in wetland systems can be considered in as far as phase 3 of the RABITT technology.

CHAPTER 3
MATERIALS AND METHODS

3.1 Soil Collection and Storage

Soil at a freshwater marsh in Madisonville, LA was collected from average depths of 4 cm, 30 cm, and 60 cm. The collection method consisted of using an open core and sectioning off the appropriate depths. Following the methods outlined by RABITT, mason jars holding a volume of 1 pint were packed with soil in the marsh. After the soil was packed into each jar, the jar was over filled with porewater and sealed off to ensure minimal oxygen would be in contact with the soil. Groundwater for study use was collected from holes that resulted from removing the cores. It was poured into a plastic 5 gallon bottle (Nalgene) and sealed for transportation and storage. Soils and groundwater were stored in a constant temperature room at 10^{o}C prior to use.

3.2 Soil Volume Study

The preliminary soil to water volume ratio study was conducted using 160 mL serum bottles identical to ones selected for the microcosm studies. The goal of this study was to calculate what the maximum ratio of soil to water would provide enough supernatant for sampling during the microcosm study. Different water volumes were added to preweighed serum bottles, which were weighed again to verify porewater volume. The bottles were weighed once again to determine the mass of soil added to each bottle. At this point, the bottles were sealed, shaken and allowed to settle for one day. This was done in order to more closely replicate wetland conditions by maximizing the soil: water ratio achievable in the microcosms.

The maximum wet soil mass for the top soil and deep soil was 60 g and 66 g respectively. By calculating these masses, more accurate soil addition could be achieved than by using any system of volumetric measurements. Also, by using the maximum soil mass possible, the microcosm can more closely achieve the same sorption properties of a natural wetland as well as provide an environment that will better mimic the wetland's ability to support microbial populations.

3.3 Soil Moisture Analysis

Approximately 4g of either top soil or deep soil was placed in a preweighed aluminum tin. This was carried out in triplicate for each soil resulting in three soil samples for each soil depth. The tins were weighed again to get an accurate measure of the wet soil mass in each tin. The tins were then placed in a 100^{o}C oven for a period of 24 hours to completely remove any moisture from the soil after which the tins were weighed once again to measure the difference in mass after moisture removal. From these numbers, the percent water content and percent soil content by mass were calculated. This analysis was carried out on the abiotic microcosm soils after autoclaving occurred in case autoclaving had a significant effect on the soil moisture properties.

3.4 Soil Preparation

A small amount of both the top soil and the deep soil were autoclaved for 30 minutes at 124^{o}C and 20psi in Electric Pressure Steam Sterilizer (Model 25 X, Wisconsin Aluminum Foundry Co. Inc) to kill the indigenous bacteria. These soil samples were then subsequently used in the abiotic control microcosms. After the soils were analyzed for moisture content, 60g of deep soil or 66g of top soil were

added to each bottle. In adding either soil, the process was carried out quickly and then the microcosms were placed immediately in an anaerobic glove bag filled with nitrogen to minimize soil oxygen content.

3.5 Chemicals and Nutrients

Neat 1,1,2,2-tetrachloroethane was obtained from Supelco (99.5% Assay) and Sigma (98% Assay). Standard solutions for GC/MS analysis were prepared from standards obtained from Supelco. All the following nutrient amendment components were obtained from Sigma-Aldrich: Benzoic Acid (99% Assay) $C_7H_5O_2Na$, n-Butyrate Acid (99% Assay) $C_4H_7O_2Na$, DL-Lactic Acid (60% Assay) $C_3H_5O_3Na$, Vitamin B_{12} (99% Assay). Resauzurin (85% Assay) $C_{12}H_6NO_4Na$, an oxygen indicator, was also obtained from Sigma-Aldrich. Yeast Extract used in the study was procured from Becton Dickerson.

3.6 Analytical Equipment and Methods

Samples were analyzed for chlorinated hydrocarbons using a Hewlett Packard 5890 GC/MS. EPA method 8260A was the analytical method used in this study. The column used was HP19091S-433, with a 5% Phenyl Methyl Siloxane fill. The dimensions of this capillary column are 30.0m x 250 microns x 0.25 microns nominal. The flow for through the column was steady at 2.1 mL/min and the total run time was 20.67 minutes. The temperature for the run was variable and the program can be seen in Table 3.1.

Purge and trap of the samples was conducted using a Tekmar 2016 purge and trap autosampler. The column was a Tekmar PT C, which is a lenax/silica gel/charcoal

mixture packed column. The method information for the purge and trap is shown in Table 3.2.

Headspace analyses for methane, ethane, and ethylene were conducted using a Hewlett Packard 5890 GC/FID. The run was a 6.5 minutes at 45^{o}C with an injector temperature of 375^{o}C and a detector temperature of 325^{o}C. The column used was a J&W Scientific 1225032. Its dimensions were 30m x 0.25mm x 0.25 microns.

Table 3.1. Temperature program for GC/MS column

Temperature	**Rate**	**Time (minutes)**
-80^{o}C	0 ^{o}C/minute	0.00 to 1.00
20^{o}C	15 ^{o}C/minute	1.00 to 7.67
80^{o}C	10 ^{o}C/minute	7.67 to 13.67
220^{o}C	20 ^{o}C/minute	13.67 to 20.67

Table 3.2. Method information for purge and trap of samples

Line Temp 100^{o}C	Purge Ready Temp 28^{o}C	Desorb Preheat 220^{o}C
Valve Temp 100^{o}C	Purge Temp 30^{o}C	Desorb Temp 225^{o}C
MCS Line Temp 100^{o}C	Purge Time 11 min	Desorb Time 4 min

3.7 Experimental Equipment

Microcosms were constructed using 125 mL Wheaton serum bottles. The bottles were sealed using Hycar septa and aluminum crimp caps. Hamilton microsyringes were used for microcosm spiking and sampling of microcosm fluid. Gaseous samples were obtained using Hamilton gas tight syringes.

3.8 Experimental Design

The experiment consisted of several sets of preliminary anaerobic degradation studies followed up by a final and more detailed degradation study. The aim was to give a complete picture of how the degradation kinetics of 1,1,2,2-TeCA is affected by different organic acid amendments in fresh marsh soils. The first anaerobic degradation study used a 10 mg/L concentration of 1,1,2,2-TeCA added to the 30 cm deep soil. The secondary degradation studies used the most effective treatments from the first microcosm study and reevaluated them at the other two soil depths with more detailed measurements. The final degradation study was conducted using the knowledge gained in the secondary studies.

3.9 Screening Study of RABITT Treatments

Four liters of a 10 mg/L 1,1,2,2-TeCA stock solution was made in de-aerated nanopure water and allowed to stir for more than 24 hrs to ensure complete dissolution. The solution was stored in 4 L amber glass bottles kept in the refrigerator at 5^{o}C until ready for use. TeCA solutions were kept only short periods before use to minimize TeCA loss before addition to the microcosms.

A soil slurry was created for the microcosm studies. The slurry was prepared by adding predetermined amounts of homogenized marsh soil to each serum bottle and then adding pore water till the fill level was achieved. The soil and water were then shaken till a slurry formed. The homogenized soil was analyzed for water: soil ratio. Approximately 4 g dry weight of 30 cm depth soil was added to each of 27 120-mL serum bottles. This translates into roughly 30 g wet soil in each serum bottle and was

decided on before the soil volume analysis was conducted prior to the detailed kinetic study.

The treatments used in this study were of either a control type or an enhanced type. The first control was an autoclaved abiotic control. Its purpose was to assess abiotic losses in the microcosms over the test period. The second control was the biotic control. This control will analyze the ability of the indigenous organisms to degrade the chemicals without any enhancement. The third control that receives only the yeast extract will determine if the microbial activity was hindered by only a lack of nutrients. The lactate treatments test multiple things. First they assess the functionality of lactate to act as an electron donor. Because it is expected that lactate succeeds in stimulating microbial activity, the nutrient treatments are varied to see if they are necessary for lactate to stimulate degradation. Butyrate and lactate/benzoate mix are included to test slow releasing low-level hydrogen donors which are thought to help the dechlorinators out compete methanogenic microbes (Morse et al., 1998).

Table 3.3. Treatment regime for screening study

Treatment	Individual Donor	Yeast Extract	Vitamin B12
A	None (Abiotic Control)	None	None
B	None (Biotic Control)	None	None
C	None	20mg/L	None
D	None	20 mg/L	0.05 mg/L
E	Lactate 3 mM	None	None
F	Lactate 3 mM	20 mg/L	None
G	Lactate 3 mM	None	0.05 mg/L
H	Lactate 3 mM	20 mg/L	0.05 mg/L
I	Butyrate 3 mM	20 mg/L	0.05 mg/L
J	Lactate/Benzoate (1.5 mM each)	20 mg/L	0.05 mg/L

Stock solution of 4 mg/L 1,1,2,2-TeCA was added to each serum bottle while in an anaerobic glove bag to minimize oxygen content inside the bottles. Bottles were filled until approximately 20 mL of headspace remained in each bottle. Then, the nutrient amendments as well as resazurin, an oxygen indicator, are added to the bottles. Resazurin is colorless at E_H<-110mV and pink/purple at higher levels. If the bottle liquids turn from clear to pink/purple, conditions inside the microcosm have become too oxidizing for the anaerobic degradation processes (Morse et al., 1998). Bottles were then capped and left until time for analysis.

Three different analyses were performed on the bottles. The primary analysis was conducted on a weekly or biweekly basis by GC/MS using a HP5890 method EPA8260-A. This analysis gives information on the amount of 1,1,2,2-TeCA and its degradation compounds present in the liquid phase in the bottles. 20 μL of supernatant from each microcosm bottle was removed by a 100μL syringe and then injected into a syringe containing 5 mL water along with 10 μL EPA method 8260A internal standard and 2.5 μL EPA method 8260A surrogate. This mixture was then loaded onto the purge and trap autosampler and was subsequently analyzed by GC/MS. GC/FID analysis for ethene was conducted by removing 1 mL of headspace gas from the microcosm bottle and injecting it into the sampling loop on the GC/FID. Other analysis was done by GC/FID. The GC/FID analysis was used to quantify the amount of ethane, ethene, and methane present in the headspace. The liquid phase concentrations were then calculated using Henry's Law constants as further described below. Only ethene numbers showed to be significant from our analysis.

The calculations for the ethene were based upon the EPA guidelines for ethene analysis. First a density factor for ethene in the headspace is calculated as follows:

$$(1)\ \text{Density factor of analyte in headspace} = \frac{\text{MW (g/mole)} * 273(^{o}\text{K})}{22.4\ \text{(L/mole)} * \text{Temp}(^{o}\text{K})}$$

MW= Molecular weight

Temp (^{o}K) = Method temperature used

$$(2)\ \text{Concentration in headspace gas (mg/L)} = \frac{\text{Conc (ppmv)} * \text{Density factor} * \text{Vol}_h}{\text{Vol}_w * 1000}$$

Vol_h = Volume of the headspace

Vol_w = Volume of the water phase

$$(3)\ \text{Concentration in water (mg/L)} = \frac{\text{Conc (ppmv)} * 55.5\ \text{MW}}{\text{Henry's Constant} * 1000}$$

1L of water =55.5 g-mole

The resulting concentrations were then multiplied by the volume of water in the microcosms to obtain total ethene mass for each microcosm.

3.10 Detailed Kinetic Studies

After the screening study was completed, the three best performing treatments were used in more detailed studies. A few preliminary attempts at the detailed studies were carried out until enough confidence was gained in the methods used before a final kinetic study was conducted. These studies placed the 3 best treatments and abiotic and biotic controls in each of two different soil depths. During the preliminary studies, the bottles were amended by the addition of an established dechlorinating microbial population which remained throughout the remainder of the study. Once the

preliminary treatment results were satisfactory, a final TeCA spike was added to the microcosms from the final preliminary study, which were then monitored on an approximately weekly basis for the detailed study.

Table 3.4. Treatment regime for detailed study

Top soil			**Deep soil**		
Treatment	**Individual Donor**	**Yeast Extract**	**Treatment**	**Individual Donor**	**Yeast Extract**
A	None (Abiotic Control)	None	**A**	None (Abiotic Control)	None
B	None (Biotic Control)	None	**B**	None (Biotic Control)	None
G	Lactate 3 mM	20 mg/L	**G**	Lactate 3 mM	20 mg/L
H	Butyrate 3 mM	20 mg/L	**H**	Butyrate 3 mM	20 mg/L
I	Lactate/Benzoate (1.5 mM each)	20 mg/L	**I**	Lactate/Benzoate (1.5 mM each)	20 mg/L

In the detailed study, microcosm bottles were constructed using soil from the same marsh as the other studies, though porewater from the marsh was used this time instead of nanopure filter water. Concentration of the contaminant was determined on a mass contaminant per mass soil basis instead of fluid concentration. The spike was administered using a 1 μL syringe versus preparation of a certain concentration solution. The spiking method was administered to test microcosms for consistency in resulting concentrations with success. Soil content in bottles was increased to the maximum workable level to more closely simulate natural environments as outlined previously in the soil volume study section. Following the previously described methods, 20 μL volume samples were removed from the microcosms using a microsyringe and were then injected into the 5 mL water and internal standard and

surrogate mix. This was loaded on the purge and trap autosampler as before until analyzed by GC/MS. GC/FID analysis was conducted identically to the previous studies.

Estimated amounts of the sorbed fraction of TeCA and its daughter compounds were estimated using experimentally determined sorption data of TCE for the Madisonville soils, which were determined in a study by previous researchers (Kassenga and Pardue, 2002). The linear K_d values for TCE were 44.67 and 37.11 L/kg for top soil and deep soil, respectively. The octanol-water partition coefficients were obtained from the Agency for Toxic Substances and Disease Registry (ATSDR). Using the following equation and K_{ow} values, K_d values were calculated for the observed compounds:

$$K_d\ (VOC) = \frac{K_d\ (TCE) * K_{ow}\ (VOC)}{K_{ow}\ (TCE)}$$

where K_d (VOC) is the distribution coefficient of the compound in question, K_d (TCE) is the experimental distribution coefficient, K_{ow} (VOC) is the octanol-water partition coefficient of the compound in question, and K_{ow} (TCE) is the octanol-water partition coefficient of the experimental compound (Karickhoff, 1981).

Table 3.5. log K_{ow} values for observed VOCs from (ASTDR)

VOC	**log K_{ow}**
TeCA	2.39
TCE	2.42
cDCE	1.86
VC	0.6

Table 3.6. Distribution coefficients for VOCs in Madisonville soil

VOC	Deep soil	Top soil
TeCA	34.63	41.69
TCE	37.11	44.67
cis-1,2-DCE	10.22	12.3
VC	0.56	0.68

Using the K_d values obtained by the calculation described above, the contaminant concentration in the soil phase was calculated using the following linear equation:

$$C_s = K_d * C_a$$

where C_s is the sorbed concentration, C_a is the aqueous concentration and K_d is the distribution coefficient (Karickhoff and Brown, 1979). The resulting K_d values are shown in Table 3.6.

TeCA data obtained by GC/MS was fit to exponential decay equations and then half-lives of the compound was calculated. Using Sigma Plot, an exponential decay equation was fit to describe each microcosm. The results of the triplicate microcosms were then averaged to give one equation to describe each treatment. All equations are in the form $C = C_o * e^{-bt}$ where C is the concentration at time t, C_o is the initial concentration and b is the removal rate constant. To calculate the half-lives, the ratio of C to C_o was set equal to 0.5 and the time calculated as the half-life. Calculations were made using total μmol found in both the sorbed and the aqueous phases.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 TeCA Removal Rate Constants

The rate constants calculated for this study, show that removal of 1,1,2,2-TeCA is occurring in the microcosms receiving hydrogen donors as well as the biotic controls. The key point in all of this is that TeCA is going somewhere besides the water phase of the microcosms. This is important in bioremediation efforts because it limits the amount of the chemical contamination flowing through a groundwater system and potentially reaching a surface water body or a pumping well for drinking water (Lorah et al., 1997). The rate constants shown below in Table 4.1 are the average b values for the equations, shown in section 3.10, describing TeCA removal in the Madisonville soils. Abiotic controls were discounted due to unsuccessful autoclaving. It should be noted that the removal rate for the deep soil biotic control is higher than that of the butyrate, which could signify an overabundance of hydrogen.

Table 4.1. TeCA removal rate constants (day^{-1}) for microcosm studies

	Biotic Control	**Lactate**	**Butyrate**	**Lactate/ Benzoate**
Deep soil	0.0144	0.0073	0.0104	0.0299
Top soil	0.005	0.0159	0.0172	0.0075

The half-lives presented in Table 4.2 give a more useful idea of the necessary residence time for TeCA to be completely degraded in an upflow wetland treatment system. When the half-life numbers are compared to Lorah's study at Aberdeen Proving Grounds, they are slower than the maximum calculated half-lives for those studies (Lorah et al., 1997). While Lorah's numbers ranged from half-lives of 2 to 7

days, this study recorded half-lives of 23 to 138 days. The wetland in Lorah's study had a minimum hydraulic residence time of 2 years so even the slowest treatment from this study would result in a final concentration of approximately 2.6% of the initial concentration (Lorah et al., 1997). This is a very conservative estimate of the final results because hydraulic residence time instead of the TeCA residence time was used. Sorption of the TeCA to the organic carbon in the wetlands should significantly increase the residence time of the compound over that of the water (Pardue, 2002).

Knowing that TeCA is being removed from the water phase, and that volatilization of the TeCA is not a likely significant contributor to this, it must be assumed that the TeCA is being removed by sorption and/or degradation. Using the Kd values calculated for TCE in Madisonville soil shown in Chapter 3, sorption of TeCA does occur at a significant level and will be discussed later with the mass balances. By analyzing the headspace for the production of ethene and the water phase for the production of TeCA's other daughter products it is seen that degradation is also a contributor in the removal rates of TeCA.

Table 4.2. TeCA half-lives (days) for microcosm studies

	Biotic Control	**Lactate**	**Butyrate**	**Lactate/ Benzoate**
Deep soil	47.91	95.61	66.86	23.18
Top soil	137.71	43.50	40.22	92.42

Making the assumption that sorption occurs instantaneously, we can infer that sorption was at equilibrium at the initial sampling which occurred one week after the microcosms had been spiked with TeCA (Pignatello and Xing, 1996). Previous studies

showed that sorption equilibrium could be established for these VOCs within a 24 hour period (Lorah et al., 1997). From this point, removal of TeCA is thought to be a result of its degradation through abiotic processes. The final product in the degradation process, ethene, is a good indicator that TeCA removal is occurring through the degradation process (Fennell et al., 1997).

Butyrate as a hydrogen donor provides slow consistent production of H_2 for the system (Smatlak and Gossett, 1996). In this study as well as one conducted by Fennell, Zinder and Gossett, ethene production resulted from the degradation of chlorinated hydrocarbons over a broad period of time. This is important in achieving complete removal of the contaminant unlike the results of some other hydrogen donors such as ethanol that act quickly and achieve some fast transformation to ethene, then slows down considerably. Also important is that butyrate achieves these results in the presence of other hydrogenotrophs (Fennell et al., 1997). It requires a hydrogen donor that delivers a slow steady supply of H_2 to the system that allows the dechlorinators to be more successful than the methanotrophs since the half –velocity constants with respect to H_2 of the dechlorinators are roughly a tenth of that of the methanotrophs (Smatlak and Gossett, 1996).

Ethene is a final product in the degradation of TeCA (Lorah et al., 1999). It is difficult to gather much specific information from the correlation of the ethene values and the rate constants in the microcosms because of lack of confidence in the actual headspace values of the ethene. It is assumed that the microcosms were under an insignificant amount of pressure and that all calculations are valid at 1 atm pressure.

Ethene results can be seen in the averaged treatment graphs where the production of ethene can be seen in comparison to the removal of TeCA from the systems.

Over a period of months and several sampling periods, many needles are inserted and removed from the microcosms, and in each instance, there is a chance for ethene to escape from the system. Though since the bottles were slightly pressurized, there is little chance of ethene contamination in the system so it seems safe to say that the ethene originates in the bottles. The origin of the ethene in the system can further be supported by the total mass of ethene present in the autoclaved controls. The values there are 0.36 and 0.011 μmol ethene for the deep soil and top soil treatments respectively after a correction for background ethene levels. This is significantly less than the ethene levels of the other treatments, which range from 1.08 to 5.64 μmol after a correction to account for the removal of the background ethene values. Note that ethene is being produced in the deep soil biotic control microcosms which would suggest that either degradation is occurring at a rate too slow to differentiate itself from desorption or there was a problem with one of the microcosms.

Interestingly, the production of ethene is the lowest in the deep soil biotic control among the non-autoclaved treatments. Even though TeCA is being removed at a rate that is not significantly different from the other treatments, the low production of ethene suggests that degradation is not being carried out through the final step of ethene production. This is supported later in Figure 4.4 where a rise in vinyl chloride can be seen in the treatment.

The small sample sizes and similarity in results in this study make it extremely difficult to find statistically significant differences among the different treatments. By

using these rate constants and the standard errors associated with them, the only difference apparent in the study was that the top soil biotic control was significantly different than top soil lactate treatment.

Intermediate products between TeCA and ethene were found in the microcosms studied. They link the two compounds together and further support that full degradation is taking place in the microcosms (Lorah et al., 1999). This is important in assuming that the ethene found in the bottles is being produced by the degradation of TeCA and not through outside contamination or some other process occurring within the microcosms

4.2 Degradation Products

The analysis for the degradation products of 1,1,2,2-tetrachloroethene is important in determining the overall potential for successful degradation of the parent compound. The major biotic daughter products of TeCA are vinyl chloride and the 1,2-dichloroethene isomers. In addition to the biotic products, TCE is a common abiotic product formed from TeCA. The VC and 1,2-DCE are also products of TCE hydrogenolysis (Lorah et al., 1999). Because TCE was never present in any significant amount throughout the study, it is assumed that the degradation did not include an abiotic conversion of TeCA to TCE. Production of *cis*-1,2-DCE and *trans*-1,2-DCE was not large enough to compare the ratios of these compounds in order to help confirm this degradation pathway is taking place in the bottles (Lorah et al., 1999). Since vinyl chloride, *cis*-1,2-dichloroethene, and trichloroethylene are also thought to be carcinogens, the presence of any of these constituents in the groundwater is potentially just as harmful as TeCA (Ballapragada et al., 1997). Therefore the

reduction to ethene is the most important step in determining success of the remediation strategy.

Results shown in the following graphs are the mean masses calculated from the triplicate samples for each treatment. Also, results shown are the total μmol in the system and not concentrations. As can be seen in the following graphs, the ethene levels in the bottle rise along with the reduction of the TeCA in treatment. Levels of ethene were normalized to zero to compensate for background ethene levels present in

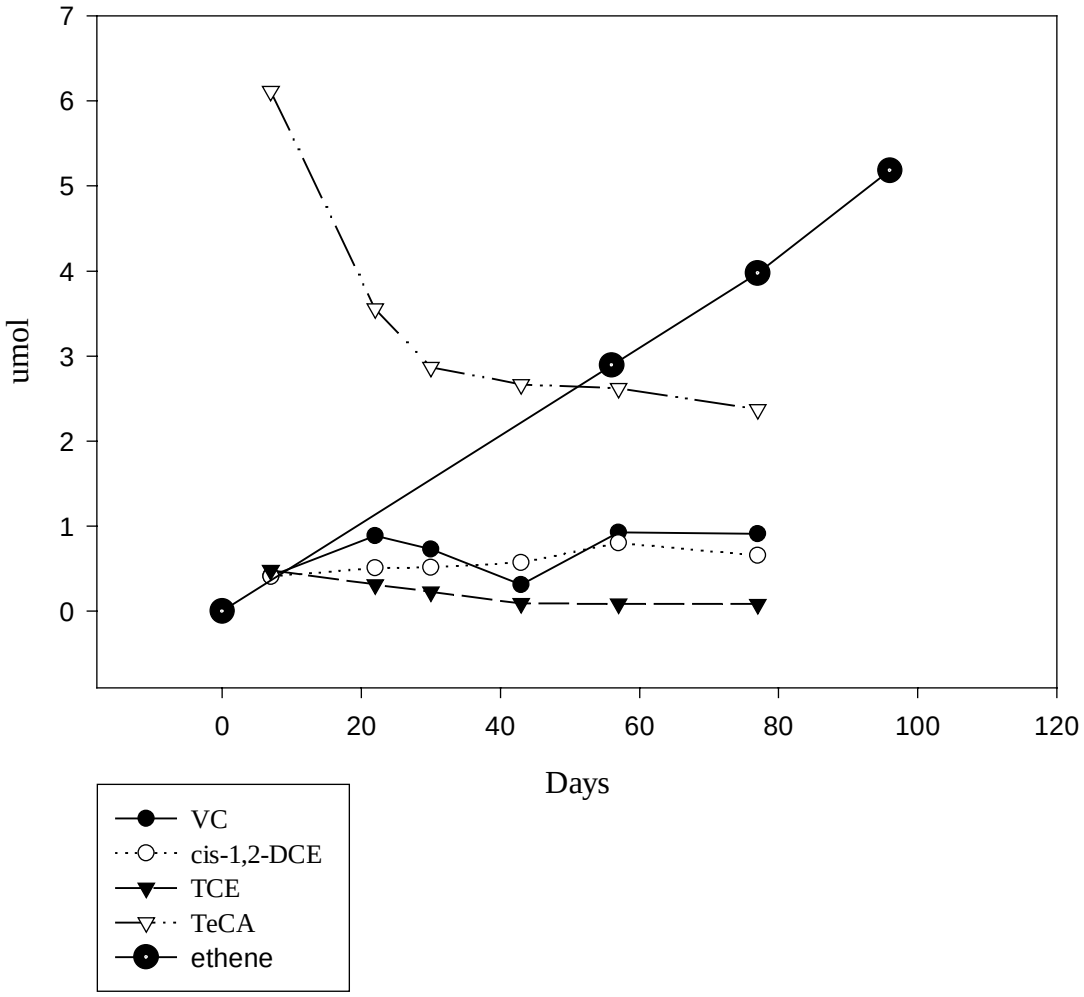

Figure 4.1. Top soil lactate treatment microcosms

the microcosms before the final spike of TeCA. There is also consistent presence of the intermediate daughter products of the degradation process to verify the origin of the ethene. Because there is no buildup of DCE or TCE in the bottles, it can be assumed that neither is a rate-limiting step for these treatments. VC does appear to build up in some treatments suggesting that it might be the rate-limiting step for these treatments, which is consistent with typical degradation by *Dehalococcoides ethenogenes* (Fennell et al., 2001). In treatments where this buildup does not occur, either the treatment is supporting a more diversified microbial population that is more adept at VC removal, or the removal of TeCA is occurring slow enough that VC is not produced at a rate faster than which it can be degraded. Ideally, the VC would be converted to ethene at a rate equal to that at which it is produced thereby reducing the risk of contaminant breakthrough into surface water or other environmental release.

Figure 4.1 above shows an ideal situation where there is no build up of the intermediate daughter products in the system. In a similarly designed microcosm, Fennell et al. (2001), did not see removal of VC and production of ethene. Their microcosm did have Vitamin B_{12} added as well as the yeast and lactate that is present in this study. Apparently the Madisonville soil is well suited to support a microbial population that includes organisms suited for the degradation of VC (Fennell et al., 2001). Though *Dehalococcoides ethenogenes* is the only known organism that fully reduces PCE to ethene, the step from VC to ethene has reported to be rate limiting and does not support growth of the population. The soils here after being spiked with an acclimated microbial population proceeded to fully degrade the VC to TeCA in the system. This was sustained for an extended period as the addition of the

microorganisms occurred several months before the final TeCA spike that was used for calculation of these rate constants.

Figure 4.2 indicates a removal of TeCA. This is supported by the production of VC in the system and a slight increase in ethene mass towards the end of the sampling period. This information presented in Figure 4.2 shows that removal of TeCA alone cannot be used as a sole indicator for successful degradation. The persistence of the vinyl chloride here results in unsuccessful degradation.

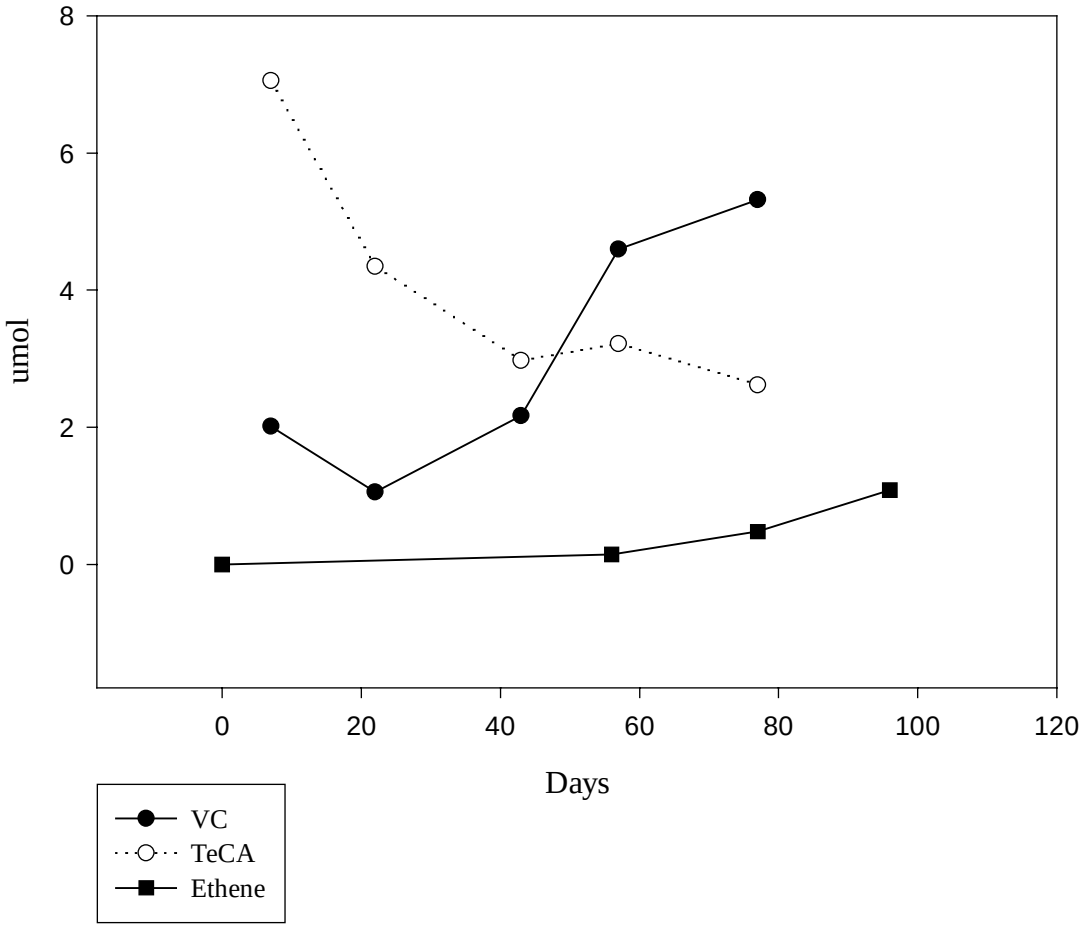

Figure 4.2. TeCA removal vs VC and ethene production for biotic control deep soil treatments

From Figure 4.2 the TeCA is apparently being readily degraded to vinyl chloride, but the final step from VC to ethene was not occurring at a likewise rate. The *cis*-1,2-DCE and TCE values showed no build up of either compound in the microcosms. The lack of ethene production is in contrast to the deep soil amended treatments that all showed production of ethene. The lactate deep soil treatment

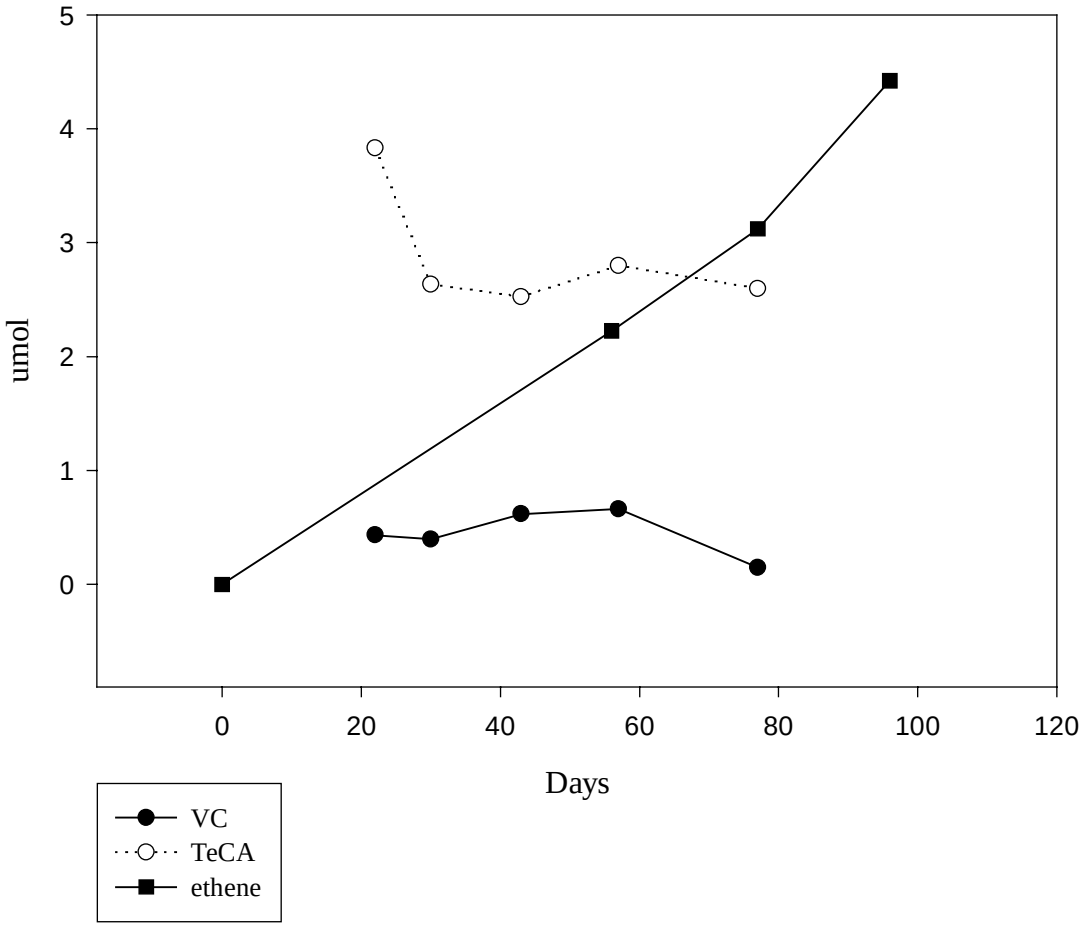

Figure 4.3. TeCA removal vs VC and ethene production in butyrate deep soil treatments

showed some rise in VC but this was on the order of one-third of the values seen in the biotic control. This would suggest that lactate might be the least successful of the

treatments, but that is not really supported by the TeCA removal rate or the high ethene production numbers. Success in a wetland remediation strategy should not all hinge just on the ability of the system to remove the daughter products in the anaerobic soil phase of the treatment plan. The lesser chlorinated ethenes can be aerobically degraded by various oxygenases once the contaminants reach the aerobic zones of the wetland (Burris et al., 1996).

Figure 4.3 shows what would be classified as nearly complete degradation in contrast to Figure 4.2 where ethene production is minimal. The increase in TeCA as

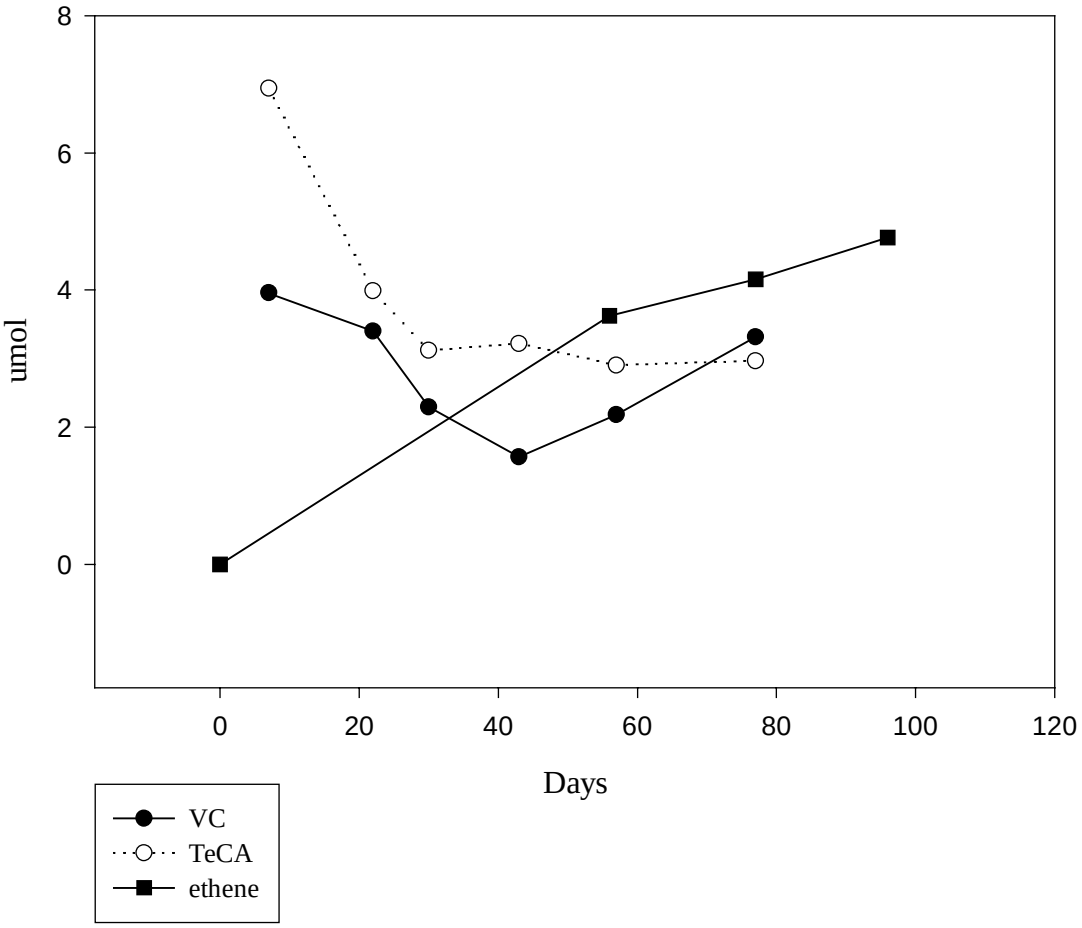

Figure 4.4. TeCA removal vs VC and ethene production in butyrate top soil treatment

VC values approaching zero suggest that the butyrate is allowing the dechlorinators to successfully compete for hydrogen in the system and producing a large enough population to not allow any buildup of VC in the system (Smatlak and Gossett, 1996). The *cis*-1,2-DCE and TCE values are consistently nominal throughout the sampling period showing no build up of either constituent in the butyrate treatments. These results show that this soil and treatment combination is a viable combination to achieve complete degradation of TeCA in a wetland treatment system if an adequate residence time for the TeCA can be achieved. The top soil butyrate treatment shows a situation in which VC removal is not occurring at a fast enough rate to completely degrade the VC at the rate at which it is being produced, yet complete degradation is still occurring. Here, the VC degradation is the rate-limiting step in complete contaminant degradation in this system.

The top soil biotic control also shows complete degradation of 1,1,2,2-tetrachloroethane. Figure 4.5 shows that TeCA and VC are being removed while ethene production is occurring. This is a positive indication that this soil is a good choice for wetland remediation strategies. A natural source of hydrogen donors in this system apparently is providing the dechlorinating bacteria with a sufficient supply of hydrogen to allow them to compete with the other hydrogen utilizing microorganisms which may be present. Values of *cis*-1,2-DCE and TCE are at nominal levels and again show no build up during the sampling period.

The Lactate/Benzoate treatment shown in Figure 4.6 was less successful in terms of VC removal and ethene production in comparison to the biotic control. Levels of *cis*-1,2-DCE and TCE show no signs of build up for this treatment. Because

of the natural ability of the top soil to provide the correct conditions for dechlorination, the additional hydrogen provided by the lactate/benzoate amendment

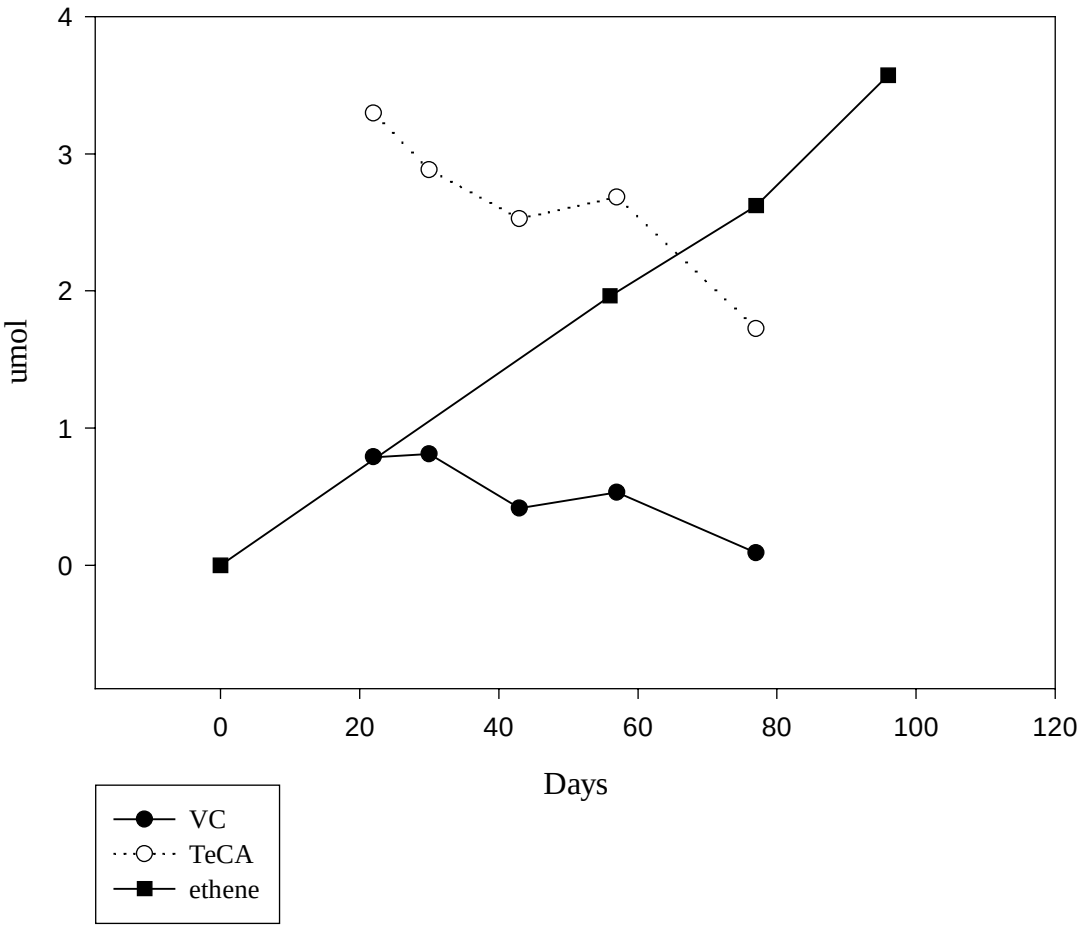

Figure 4.5. TeCA removal vs VC and ethene production for biotic control top soil treatments

might have allowed a large enough methanogen population to grow to out compete the dechlorinators in the system. It has been shown that while dechlorinators are able to out compete methanogens for H_2 at low H_2 concentrations, methanogens begin to gain an advantage over the dechlorinators when H_2 partial pressure reaches and exceeds 20 mg/L (Ballapragada et al., 1997).

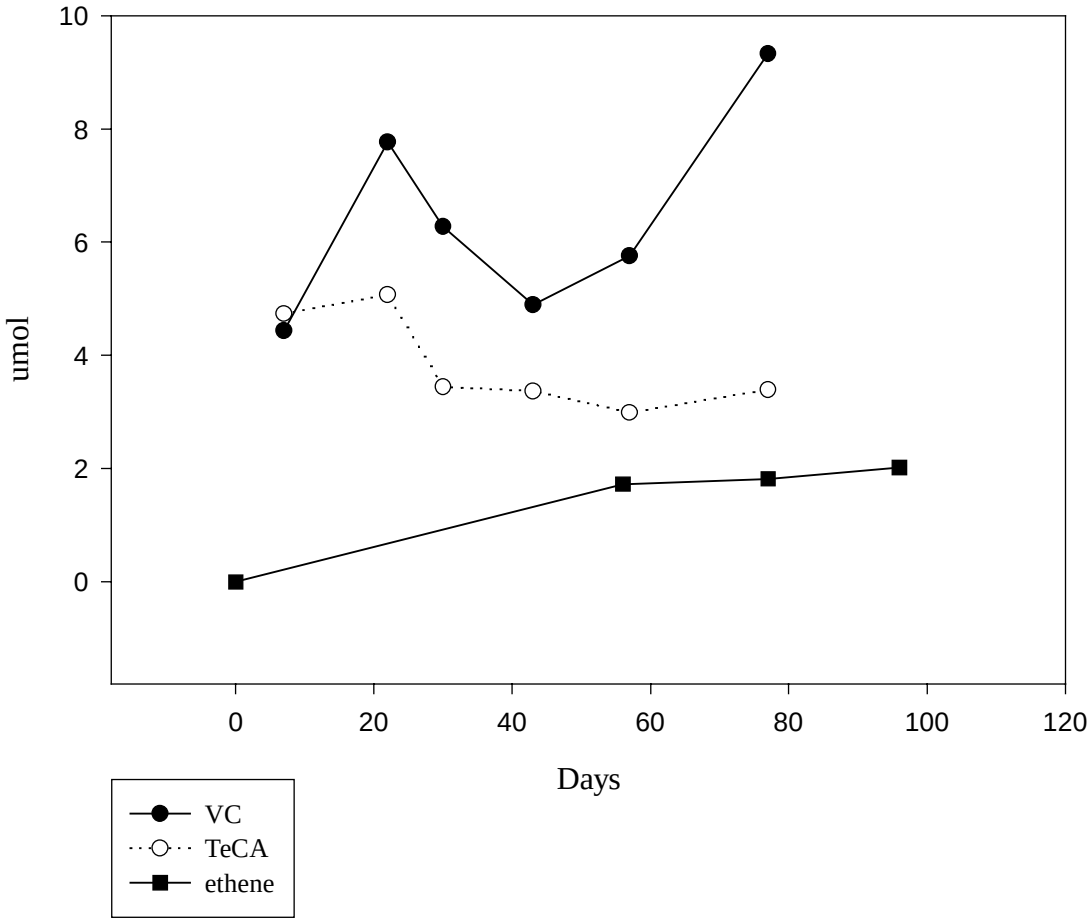

Figure 4.6. TeCA removal vs VC and ethene production for lactate/benzoate top soil treatments

4.3 Mass Balance

Calculating a mass balance for each of the treatments is extremely important when considering the results of this study. Because success is being calculated by the absence or presence of an added compound and/or its daughter products, knowing the percent recovery of this initial contamination is key to having any confidence in the data. If it can be shown that that percent recovery was approximately within 20 percent of 100 percent recovery, then the likelihood that something besides the proposed path of degradation is significantly decreased.

Table 4.3. Percent contaminant recovery in treatment microcosms

Treatment	Biotic Control	Butyrate	Lactate	Lactate/Benzoate
Percent Recovery Top Soil	114.9	141.9	127.4	137.8
Percent Recovery Deep Soil	84.0	140.5	117.2	129.4

From the above table, it can be seen that recovery was mostly within 40% of a perfect mass balance. All but the top soil biotic control was recovered at over 100%, which would indicate that either the calibration of the GC/MS was off, or that there was some consistent experimental or analytical error resulting in inflated recoveries. It is also possible that ethene was being produced by some other source in the microcosms. There appears to be no serious loss of compound throughout the study and therefore the results can be accepted with some level of confidence.

4.4 Biodegradation as a Component of Constructed Wetlands

The breakdown of TeCA in the microcosms supports the notion that biodegradation is a valid treatment strategy for the removal of this contaminant. The field applications of this type of microcosm study can be seen in Lorah's work at Aberdeen Proving Grounds and the RABITT protocol. The field evidence at the Aberdeen Proving Grounds Canal Creek site show parent compounds TeCA and TCE being microbially degraded as the contaminated groundwater flows upward through a natural wetland. As the aerobic water from the aquifer rises through the wetland, the conditions become increasingly reducing until anaerobic conditions prevail in the wetland. Here the parent compounds are anaerobically degraded and are no longer present in the field samples but *cis*-1,2-DCE and VC are present (Lorah et al., 1997).

The purpose of this research was to search for ways to optimize the natural abilities of wetland soils to degrade TeCA under reducing conditions. The RABITT protocol was designed for a full-fledged site specific test and application of enhanced bioremediation, specifically for TCE contaminated groundwater. By combining the RABITT microcosm setup with the knowledge of wetland remediation potential, the Madisonville soil can be analyzed in regards to its suitability to function as a natural soil option for a constructed wetland application.

The ability of the Madisonville soil to support degradation of TeCA in all amended treatments along with its high organic carbon content make it a good candidate for constructed wetland soil based on short term research. Though the TeCA removal rates were slower for the Madisonville soil than Lorah's studies at APG, they were high enough to provide practical degradation rates. It is difficult however to make comparisons between these two studies since the APG soils have been exposed to long term presence of VOCs which have most likely resulted in a well established population of dechlorinators. The natural Madisonville soil exhibited very slow removal rates in the early stages of the microcosm setup, but after the addition of an acclimated microbial culture the second TeCA addition to the soil was readily degraded. It is possible that some of the early problems with degradation in the system might have been due to H_2 levels in the natural soils being high enough to support a methanogenic population that would out compete the dechlorinators (Pardue, 2002). Over time, as the H_2 was used up, the dechlorinators could slowly begin to establish their population. In constructed wetland application, early success of the system would be a major accomplishment. By amending the system with a slow release

hydrogen donor such as butyrate and introducing a known dechlorinating culture, early success could be achieved until over time a well established dechlorinator population is produced.

4.5 Comparison to Existing Research

The microcosm studies conducted in this research offer interesting comparisons to Lorah's field and laboratory studies at Aberdeen Proving Grounds. By looking at the similarities and differences of the results in these studies, the potential success of the Madisonville soil and the enhanced treatments can be analyzed. It must initially be noted that the soil used in the two studies differ significantly in both soil content and in conditions in which the soil is immersed. The differences in the soils will account for some of the variation in the results of the two studies. Table 4.4 outlines some of the key differences between the soils and locations.

The microcosm studies conducted with the APG soil already contained an established microbial population to dechlorinate the TeCA, while the Madisonville study relied on non acclimated indigenous microbes as well as an inoculation of

Table 4.4. Soil comparison of Aberdeen Proving Grounds and Madisonville wetlands

Soil	**APG**	**Madisonville**
Organic Carbon %	6.9-32.6	20.89-27.58
Peat Wetland (Y/N)	Y	Y
Aerobic/Anaerobic	Variable	Anaerobic
Exposure to VOCs (Y/N)	Y	Unknown

microbes from an established culture. The immediate presence of the established culture in the APG microcosms most likely accounts for the higher rate constants

found in those bottles. The Madisonville microcosms would not experience a lag phase due to the inoculation, but the smaller and unestablished population may not have the capacity to degrade the TeCA as rapidly. In both studies, the degradation pathways seem to be the same, and this also fits the field evidence cited from APG. Dichloroelimination of the TeCA resulted in 1,2-DCE. Hydrogenolysis of the 1,2-DCE isomers produces vinyl chloride and further hydrogenolysis of the VC results in the goal product of ethene (Lorah et al., 1997). All data from these studies support this pathway from TeCA to ethene.

The field studies at APG show that the wetland is receiving aerobic water from the aquifer that is showing no indication of TeCA removal. As the water flows upward through the wetland, the conditions become increasingly anaerobic and therefore increasingly reducing. As this change occurs, the TeCA is changed into *cis*-1,2-DCE and VC. In the wetland system, the daughter compounds do not disappear in the anaerobic portion of the wetland, though they were fully removed in the APG anaerobic microcosms within a 34-day period. The daughter products remained until they progressed upwards into the aerobic conditions of the rhizosphere where they were aerobically reduced to ethene (Lorah et al., 1997).

By amending the wetland soils with a slow release hydrogen donor such as butyrate, it is hoped that more of the complete degradation can be accounted for in the anaerobic soil layers of a vertical flow wetland (Smatlak and Gossett, 1996). Hopefully by enhancing the soil to better support dechlorination, *cis*-1,2-DCE and VC levels will not increase along a vertical gradient but will result in a overall decrease in VOC concentrations as the groundwater reaches the release point preventing the

likelihood of breakthrough occurring if conditions were to become less optimal over short time periods. This is especially important in a natural attenuation situation where an existing wetland can be altered to achieve complete degradation in places where the *cis*-1,2-DCE and VC are not being fully degraded before breakthrough (Fennell et al., 2001).

4.6 Interpretation of Results

From the data collected in this study, it can be shown that enhanced bioremediation can be a viable strategy for the improvement of the soil degradation phase of wetland remediation processes. The addition of hydrogen donors to the wetland soils showed the ability to support a complete degradation process in the deep soil. The top soil treatments were also successful in removing the TeCA and converting it to ethene, though it appears that addition of supplemental electron donors may be unnecessary since there was good ethene production in the biotic control. Butyrate has been shown in other studies to be well suited as a hydrogen donor for VOC degradation in wetland soils and the research here supports that idea (Smatlak and Gossett, 1996).

Enhanced bioremediation and natural attenuation of 1,1,2,2-tetrachloroethane appear to be valid options for TeCA remediation. As shown in the comparison of Lorah's APG studies and this research, some sites are naturally able to remediate TeCA Other situations may require enhancement of the system to achieve acceptable results. Based on the findings in this research, the enhancement of a natural system that does not meet the requisite degradation may be a viable alternative to pump and

treat methods provided that any enhancement can be uniformly delivered to the system.

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