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Characterization of Microbial Processes that Degrade Chlorinated Solvents in a Constructed Wetland Using Organic Acid and Inorganic Anion Concentration Profiles

Chad B. BonDurant

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CHARACTERIZATION OF MICROBIAL PROCESSES THAT DEGRADE CHLORINATED SOLVENTS IN A CONSTRUCTED WETLAND USING ORGANIC ACID AND INORGANIC ANION CONCENTRATION PROFILES

THESIS

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AFIT/GEM/ENV/04M-03

DEPARTMENT OF THE AIR FORCE
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Wright-Patterson Air Force Base, Ohio

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THESIS

Presented to the Faculty

Department of Systems and Engineering Management

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In Partial Fulfillment of the Requirements for the Degree of Master of Science in Engineering Management

Chad B. BonDurant, BS

Captain, USAF

March 2004

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Abstract

Chlorinated solvents have been used in industrial cleaning and degreasing processes in the United States since the early 1900s, and their induction into the environment increased significantly with the growth of industrial processes over the past century. PCE, TCE and their daughter products have been associated with a number of human health concerns and are currently the most common contaminants found in groundwater in the United States. Wetlands possess characteristics necessary for the complete degradation of chlorinated ethenes by microorganisms via anaerobic and aerobic regions that foster the necessary oxidation-reduction conditions.

Organic acid and inorganic anion concentrations were evaluated in samples taken from a constructed wetland at Wright-Patterson Air Force Base, Ohio during the summer and fall of 2003. These analytes are indicative of redox conditions in the subsurface and suggest the occurrence of microbial activities that degrade chlorinated ethenes to innocuous end products. Organic acid concentrations decreased by 100% from July 2003 to fall 2003. Combined with data collected previously during the months of December and January, this suggested that changing seasons and temperature fluctuations have a significant influence on microbial metabolisms. Nitrate and sulfate reduction above stratum C indicated mildly reducing conditions in the lowest stratum that became more highly reducing in the upper two strata. Based on the changing analyte concentrations throughout the wetland cell over several seasons, it was evident that the appropriate subsurface conditions existed for the reductive dechlorination of chlorinated ethenes.
Acknowledgements

I thank God for granting me some insight into His great creation. I doubt I will ever fully understand the complexities of the scientific wonders that take place in wetlands, but I have learned to search for answers confidently and not to be intimidated by what is unknown to me.

I am grateful to my wife for her encouragement and faith in me when I had little of my own. I admire her work ethic and persistence and am inspired by her will to succeed.

I would like to express much thanks to my thesis advisor, Dr. Mike Shelley, for his patience with me during this effort. I learned more practical knowledge from our discussions than I did from reading the literature. Thanks also to my committee members for their instruction and guidance. Thanks to Dr. Amon for the additional time he spent providing hands-on experience with the wetlands. A special thanks to Jason Lach for getting me started in the lab.

Finally, I am honored to have shared the past 18 months with my 22 classmates. It has been humbling to be surrounded by such greatness and I am proud to serve alongside each of them. Thanks to Kevin Mares and Teresa Sobolewski for the discussions and laughter that we shared in the lab.

Chad B. BonDurant
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Characterization of Microbial Processes That Degradate Chlorinated Solvents in a Constructed Wetland Using Organic Acid and Inorganic Anion Concentration Profiles

1. Introduction

Overview

The purpose of this effort was to continue to develop an understanding of the microbial degradation processes occurring in a vertical flow wetland constructed to remediate perchloroethene (PCE), trichloroethene (TCE), and their daughter products in low concentrations in groundwater. Data collected in this study was compared to that of previous research efforts to improve the understanding of the probable microbial and chemical mechanisms of chlorinated ethene remediation occurring in the constructed wetlands at Wright-Patterson Air Force Base (WPAFB), Ohio. Ion chromatography was used to analyze groundwater samples for low-molecular weight, mono-carboxylic acids and inorganic ions that are indicative of microbiological processes associated with dehalogenation. Measurements of analyte concentrations, pH, temperature, dissolved oxygen, oxidation-reduction potentials, and a number of other measurements may suggest the location, character, and extent to which these degradation processes are taking place.

Background

Chlorinated solvents have been used in industrial cleaning and degreasing processes in the United States since the early 1900s, and their production has increased significantly with the growth of industrial processes over the past century (Pankow and Cherry, 1996). PCE, TCE and
their daughter products of dichloroethene (DCE) isomers and vinyl chloride (VC) have been associated with a number of human health concerns including cancer, coma, liver and kidney disease, prevention of blood clotting, and nerve damage (Agency, 2003). The intense use of these chlorinated solvents over time has resulted in their introduction into groundwater sources via leaky storage tanks, spillage, and improper disposal. As a result, PCE and TCE are the most common contaminants found in groundwater in the United States (McCarty, 1997). Realizing the detrimental impacts of this contamination, Congress passed the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) in 1980 and the Resource Conservation and Recovery Act (RCRA) in 1986 to eliminate the discharge of chlorinated solvents into drinking water sources. Due to the prevalent use of chlorinated ethenes and their effects on human health, it is imperative to understand the microbial and chemical degradation processes that transform PCE and TCE to its harmless end product, ethene.

Chlorinated ethenes are denser than water and are referred to as dense non-aqueous phase liquids (DNAPLs) when released as bulk material into saturated soils (Pankow, 1996). This higher density combined with a low solubility makes it easier for them to move downward through groundwater to the bottom of an aquifer. Once they reach the aquitard, DNAPLs are immobile. As groundwater flows past these trapped DNAPLs, small portions of the contaminant will partition into the water and form a plume in the direction of groundwater flow. Although solubility is very low, it takes only a minute concentration to violate Maximum Contaminant Levels (MCLs) for drinking water. Even after it has solubilized into the groundwater, solid materials in the aquifer do not effectively retard the DNAPL because DNAPLs do not partition well into soils (Pankow, 1996). These combined characteristics of chlorinated ethenes make them extremely difficult to remediate.
While DNAPLs enter groundwater relatively easily, it is much more difficult to locate and remediate them. According to the National Research Council (1997), there are three categories of chlorinated ethene remediation technology: 1) solidification, stabilization, and containment; 2) extraction of the contaminant from the subsurface and separation from the media; 3) biological and chemical reactions that destroy or transform the contaminant. The most common of these remediation technologies is extraction, and the majority of contaminated sites are treated by effective pump-and-treat methods. However, this technology is costly and requires an intense investment of energy and maintenance that can range in the millions of dollars. With an estimated 7,300 contaminated sites in the Department of Defense (DoD) alone, the overall cost of remediation using extraction methods is in the hundreds of billions of dollars.

**Natural Remediation**

The high cost of physically removing chlorinated solvents from water sources has led to the pursuit of less expensive options, and in-situ bioremediation methods have shown promise. There are chemical and microbiological processes at work in groundwater systems that have the ability to break down chlorinated solvents such as PCE and TCE into harmless end products like carbon dioxide (CO$_2$) and water (Lee et al., 1998). This presents the possibility of using processes that occur naturally for chlorinated solvent remediation versus expensive extraction methods. The potential for tremendous monetary savings has warranted investigation into the specifics of these natural attenuation processes.

There are several complex processes involved with the biodegradation of chlorinated aliphatic hydrocarbons in the natural environment. Halorespiration occurs when the chlorinated solvent acts as an electron acceptor and replaces a chlorine atom with a hydrogen atom (Wiedemeier et al., 1997). This reductive dechlorination removes chlorines from the
contaminant but only occurs under anaerobic conditions. Chlorinated solvents may also act as electron donors providing an energy source for certain microorganisms. This occurs predominantly in aerobic conditions and is most often associated with the less-chlorinated solvents like vinyl chloride (Norris, 1994). Co-metabolism occurs when enzymes that are produced by organisms from other processes subsequently catalyze chlorinated solvent degradation (Wiedemeier, 1997). It is observed mostly in aerobic environments and the rate of co-metabolism increases as the degree of chlorination decreases (Vogel, 1987). Methane monooxygenase is an example of an enzyme that has been shown to co-metabolize chlorinated hydrocarbons (Chapelle, 2001).

With the continual exchange of electrons providing energy for microbes, oxidation-reduction (redox) chemistry is an important concept in chlorinated hydrocarbon transformation. PCE and TCE generally require reducing conditions suitable for methanogenesis before they will transform to ethene (McCarty, 1996). Such conditions require the presence of enough organic substrate to reduce all of the oxygen, nitrate, iron, and sulfate before dechlorinating bacteria will successfully compete to reduce chlorinated aliphatic hydrocarbons. A study of these concentrations in a subsurface wetland should indicate the potential for bioremediation of chlorinated compounds (McCarty, 1996).

**Wetlands**

The natural wetland environment possesses the characteristics necessary for the complete degradation of chlorinated aliphatic hydrocarbons. While the deeper, anaerobic regions of the wetland foster transformation of more highly-chlorinated contaminants like PCE and TCE, the aerobic zones near the surface are conducive to the degradation of DCE and VC to ethene. This phenomenon has been observed in natural wetlands at the Aberdeen Proving Grounds in
A constructed research wetland at the Air Force Institute of Technology (AFIT) at Wright-Patterson Air Force Base (WPAFB), Ohio uses water from an aquifer contaminated with PCE. The water is pumped from the aquifer into a subsurface flow wetland and flows vertically through anaerobic and aerobic zones to the surface. These zones consist of hydric soil and are penetrated by roots from surface vegetation. While data collected from water samples has demonstrated removal of chlorinated solvents, the chemical and biological processes occurring in the wetland are still not well-understood.

There are some organic acids and inorganic ions that can help identify when and where aerobic and anaerobic conditions might exist. Nitrate and sulfate, for example, are electron acceptors and indicate what redox conditions are prevalent in an environment. Organic acids function as by-products and substrates in the ecosystem and can indicate the presence of biological activity. Acetate is an especially significant organic acid and substrate for diverse microbial processes, especially in an anaerobic environment (Seagren and Becker, 1999). An analysis of the organic acid and anion distribution in the constructed wetlands at WPAFB and comparison with those distributions previously determined will enhance the understanding of developing biodegradation processes taking place.

**Research Questions**

1. What inorganic ions and low-molecular weight mono-carboxylic organic acids are currently prominent in each layer of wetland cell 1?

2. Does the comparative analysis of organic acid and inorganic anion concentrations during the same time of year indicate significant deviations from previous findings?
3. Does the comparative analysis of organic acid and inorganic anion concentrations during different times of year suggest changes in microbial behavior?

4. What microbial metabolic processes seem to be occurring in each layer based on the anion and organic acid concentrations?

5. Do these hypothesized metabolic activities correlate with the observed behavior of chlorinated aliphatic compounds in the wetland?
II. Literature Review

Natural attenuation of chlorinated ethenes occurs via several biotic and abiotic processes. While some chlorinated aliphatic hydrocarbons are broken down by chemical reactions that do not involve microorganisms (that is, abiotic processes), biotic processes have demonstrated the most significant potential for implementing bioremediation techniques. Chlorinated solvents transform to innocuous end products via three primary metabolic pathways: direct oxidation, reductive dehalogenation, and co-metabolism (“Natural Attenuation”, 2003). Oxidation-reduction (redox) reactions are fundamental to these microbial metabolic processes whereby microorganisms transfer electrons between substrates to obtain energy for growth and reproduction (Chapelle, 2001). The conditions of the subsurface environment are determined by biogeochemical cycles in which microbial metabolisms interact with naturally occurring chemicals like oxygen, carbon, nitrogen, and iron. It is common to classify an aquifer macroscopically as either aerobic or anaerobic depending on the amount of dissolved oxygen available to microorganisms; however, a heterogeneous consortium of both aerobic and anaerobic bacteria is more likely affecting the transformation of chlorinated organic solvents (Lee et al., 1998).

Biogeochemical Cycles

The chemicals required for microbes to survive in subsurface environments - oxygen, carbon, hydrogen, nitrogen, iron, and sulfur – pass through the microbial and botanical food chain via biogeochemical cycles (Chapelle, 2001). Their presence and availability in an ecosystem correspond with the particular redox reactions taking place. Biogeochemical cycles
are essential to the survival of subsurface microorganisms and to the success of chlorinated organic degradation.

The restricted availability of oxygen is one of the most significant characteristics of an aquatic ecosystem. Oxygen is produced by photosynthesis in green, chlorophyllic plants where energy from the sun transforms carbon dioxide and water into organic carbon and oxygen. Oxygen is balanced on a global scale (Chapelle, 2001), as the amount of oxygen consumed via respiration is equal to the amount produced by photosynthesis. However, groundwater ecosystems that are isolated from sunlight may exhibit an oxygen deficiency, and the rate of oxygen diffusion is too slow to allow a sufficient supply from the atmosphere. Oxygen is therefore consumed by respiring microorganisms more rapidly than it is replenished by photosynthesis or by dissolved oxygen in water flows. In such cases, the system becomes anaerobic. Anaerobic conditions are more prevalent in deeper groundwater and aquatic sediments because oxygen has been consumed. Shallow aquifers and surface water are more often exposed to oxygen in the atmosphere and are more likely to have higher concentrations of dissolved oxygen. This is significant because microbial metabolic processes are sensitive to oxygen availability.

Carbon is the chemical basis for all life. It can maintain various oxidation states and can efficiently store and release energy (Chapelle, 2001). Plants absorb energy from the sun and form reduced organic carbon compounds by photosynthesis. Chemical energy is released when these compounds are aerobically oxidized back to carbon dioxide. Under anaerobic conditions, not all of the reduced compound can be transformed to CO₂. Carbon is an important substrate and energy source in microbial environments.
Reduced organic carbon provides energy to microorganisms that fix nitrogen. Nitrogen is present in the atmosphere in its elemental form (N₂), and it is fixed into NH₃ and converted to organic and inorganic forms by bacteria. Nitrogen fixing bacteria can be either aerobic or anaerobic but require large amounts of metabolic energy to carry out the fixation of N₂ to NH₃. NH₃ is oxidized under aerobic conditions to NO₂ and eventually to NO₃ by nitrifying bacteria. These oxidizing reactions are energy-yielding steps. The cycle of nitrogen is complete when denitrification occurs. Under anaerobic conditions, denitrification is associated with anaerobic respiration and this reaction can also yield energy from reduced carbon compounds. In organisms carrying out these metabolic steps, NO₃ replaces O₂ as a final electron acceptor in respiration. NO₃ is converted to NO₂ and eventually into the gases N₂O or N₂. Denitrification needs a reduced carbon source for reactions that is often supplied by the plant community. NO₃ and NH₃ produced by these processes are generally considered the primary plant nutrients. NO₂ is toxic in high concentrations. However, it is not typically produced in high amounts, as it is rapidly reduced to other products in anaerobic conditions or oxidized back to NO₃ under aerobic conditions. A great variety of bacteria carry out these reactions. Because a particular species may be responsible for only one chemical step, it takes a robust microbial community to make this cycle work. Since the cycle requires both aerobic and anaerobic conditions, the microbial community must be spatially organized into zones where these conditions are prevalent.

In the absence of oxygen and nitrate in the subsurface environment, there are a variety of microorganisms that will use ferric iron (Fe(III)) as an electron acceptor. Fe(III) is reduced anaerobically to more soluble ferrous iron (Fe(II)) and may release phosphates that have been bound by Fe(III) oxyhydroxides. The phosphates and Fe(II) are mobilized and may return to the aerobic zone where Fe(II) can be reoxidized to Fe(III) oxyhydroxides (Chapelle, 2001).
Oxidation of ferrous iron yields small amounts of energy, and bacteria must oxidize a great deal of Fe(II) to gain a significant amount of energy. Large quantities of ferric iron are produced and react with H₂O to produce insoluble oxyhydroxides. These oxyhydroxides are often responsible for clogging wells and water lines. Drilling wells into anaerobic zones that have high concentrations of Fe(II) provides an immediate source of oxygen for this oxidation and clogging to occur. Sulfate is next in the order of affinity for electron acceptors. Microbes will reduce sulfate to sulfide to provide sulfur for protein synthesis. Microbes will also reduce sulfate as an electron acceptor to oxidize carbon and hydrogen to produce hydrogen sulfide (Chapelle, 2001).

In a constructed wetland, the presence of one or more of these compounds may suggest which metabolic activities have occurred and indicate the potential for the oxidation or reduction of the chemical compounds in this study. For instance, high concentrations of dissolved oxygen may indicate that aerobic bacteria are active if they are not limited by nutrients. A region high in nitrate might indicate that 1) aerobic conditions are converting NO₂ to NO₃, 2) aerobic conditions are preventing denitrification, or 3) NH₃ produced by N₂ fixation is being oxidized to NO₃.

**Redox Reactions**

For every oxidation reaction, there is a complementary reduction reaction and vice versa. An oxidation reaction occurs when a compound loses an electron, while a reduction reaction involves gaining an electron. The combination of both concurrent reactions is commonly referred to as a redox reaction. The compound that loses an electron (reducing agent) is oxidized by the compound gaining an electron (oxidizing agent), and the oxidizing agent is simultaneously reduced by the reducing agent. The reducing agent is commonly referred to as the electron donor and the oxidizing agent is the electron acceptor. Redox reactions are critical
to the microbial degradation of chlorinated organics because these reactions provide the energy required for microbes to grow and reproduce. Many of the chlorinated contaminants in groundwater have the potential to be electron donors or electron acceptors depending on the redox conditions. For example, the carbons in PCE are in their most highly oxidized state due to chloride. Since PCE cannot be oxidized any further, it can only be transformed to a more reduced state by replacing a chloride molecule with a hydrogen molecule. This occurs only in reducing environments. Microorganisms in groundwater use a number of different electron acceptors for their metabolisms, and they will use the substrates that yield the greatest energy benefits. For instance, oxygen is the terminal electron acceptor in aerobic environments because it has the highest affinity for microbial metabolisms in this environment. When chlorinated solvents compete as electron acceptors, they may not be degraded by bacteria if more beneficial electron acceptors are available. The oxidation-reduction potential (ORP) is the measurement of the electrical potential for chemicals to be electron donors or electron acceptors. Redox reactions segregate into certain zones based on the most beneficial electron acceptor (Chapelle, 2001), and ORP data collected from samples in a constructed wetland may be a good predictor of which biochemical processes are occurring in a particular groundwater region. This may help identify homogeneous regions within a constructed wetland.

**Chlorinated Solvent Oxidations**

**Direct Oxidation** is not strictly an aerobic process; terminal electron acceptors such as nitrate, iron, and sulfate will oxidize some chlorinated solvents anaerobically. However, under aerobic conditions, chlorinated ethenes are mineralized to carbon dioxide and water with O₂ as the terminal electron acceptor. The chlorinated ethene acts as the carbon and energy source for aerobic bacteria such as *Mycobacterium sp.* and *Rhodococcus sp.* that will mineralize vinyl
chloride to CO₂ and H₂O (Lee et al., 1998). The more chlorines that are present in an organic compound, the more oxidized it becomes. Thus, the oxidation potential decreases as the number of chlorines increases (Pankow and Cherry, 1996). While VC is the least chlorinated and least oxidized ethene and has the greatest tendency for oxidation, PCE is the most highly chlorinated ethene and has not demonstrated any potential to degrade aerobically. The consumption of dichloroethene (DCE) by aerobic microorganisms indicates that it can also be aerobically degraded as a primary substrate to support microbial metabolism (Bradley and Chapelle, 2000).

**Co-metabolic oxidation** occurs when microorganisms produce enzymes during the oxidation of hydrocarbons as part of their normal microbial metabolic processes. Although chlorinated ethenes are not part of the microorganisms’ metabolisms, the presences of these enzymes in the same ecosystem will fortuitously degrade partially chlorinated solvents. The microorganisms that produce the enzymes do not benefit from this coincidental oxidation. For example, methanotrophic bacteria oxidize methane by the enzyme methane monooxygenase. This monooxygenase enzyme then oxidatively degrades TCE. PCE is the most highly-oxidized chlorinated ethene and will not transform via this process (Lee et al, 1998). Cometabolic oxidation due to methanotrophs rarely occurs naturally in groundwater systems because significant concentrations of methane and dissolved oxygen are not commonly found together. However, bioremediation techniques that deliver substantial amounts of oxygen and a hydrocarbon substrate to the subsurface microorganisms have been successful (Lee at al. 1998).

**Chlorinated Solvent Reductions**

**Direct dehalorespiration** is a form of anaerobic respiration that occurs when microorganisms take advantage of energy released during reductive dechlorination and conserve that energy for growth. The chlorines in a chlorinated organic compound are sequentially
replaced by hydrogen. Dehalorespiring bacteria use the chlorinated compound as an electron acceptor just as aerobes use oxygen as the terminal electron acceptor in aerobic oxidation (McCarty, 1997). Microorganisms use this electron transfer to gain energy for growth and reproduction. Dehalorespiration takes place when the compounds involved in the dechlorination reaction are thermodynamically favorable to microbial metabolism. Holliger et al. (1999) have identified a number of anaerobic bacteria that are capable of reducing chlorinated ethenes. *Dehalobacter restrictus* uses hydrogen as an electron donor and PCE or TCE as an electron acceptor to gain metabolic energy. *Dehalospirrillum multivorans* is another dehalorespiring bacteria that reduces PCE to TCE and then to DCE; however, it is less discrete regarding electron donors and acceptors. Although it uses hydrogen and PCE for dehalorespiration, it also uses other donors like formate and pyruvate and acceptors such as fumarate and selenate that have been shown to inhibit PCE reduction. *Desulfuromonas chloroethenica* reduces PCE and TCE to DCE, but PCE is not an energy-yielding electron acceptor for *D. chloroethenica* and is most likely reduced co-metabolically (Holliger et al., 1999). While a multitude of these organisms will partially dechlorinate PCE, almost none have shown the ability to degrade PCE past its di-chlorinated daughter product. Perhaps the most encouraging discovery is the ability of *Dehalococcoides ethenogenes* to completely reduce PCE to ethene under anaerobic conditions. It is currently the only known anaerobe that will completely reduce PCE to ethene using hydrogen as the electron donor (Holliger et al., 1999). *D. ethenogenes* does not respond to alternate electron donors or acceptors, so it is unlikely that their presence will impact the ability of *D. ethenogenes* to reductively dechlorinate PCE and TCE to ethene. This discovery may lead to enhanced reductive dehalogenation processes that will completely reduce halogenated organics anaerobically to innocuous end products. Holliger et al. (1999) have also identified
mixed cultures that will completely dechlorinate PCE to ethene. This is encouraging since most bacteria do not exist in a pure state in the natural environment but depend on cultural diversity for survival. However, mixed cultures are much more difficult to isolate, cultivate, and exploit than are pure cultures. Bacteria that reductively dechlorinate ethenes are found almost everywhere in the environment. A great number of these bacteria have already been identified, and there are likely many more remaining to be discovered. Due to the progress already achieved and the promising ability of organisms like *D. ethenogenes* to completely reduce PCE to ethene, the continued research and identification of new microorganisms holds great promise regarding the future of environmental remediation via reductive dehalogenation.

**Co-metabolic reductive dehalogenation** occurs when anaerobic microorganisms such as methanogens, acetogens, and sulfate reducers produce cofactors during normal metabolic activity that also mediate dechlorination of halogenated organics. The microorganisms that produce the cofactors do not receive any benefit from the co-metabolic dechlorination, and the chlorinated solvents are only partially dechlorinated – usually to DCE (Gossett and Zinder, 1997). This relatively slow and incomplete transformation appears to be of little significance except in ecosystems with high concentrations of organics and highly active methanogenic or sulfidogenic respiration (Lee et al., 1998). Wetlands and landfills may provide environments capable of significant levels of co-metabolic reductive dehalogenation that can effectively remove chlorinated solvents from groundwater.

**Electron Donors**

Dehalorespiration occurs when microorganisms take advantage of the energy released from the electron exchange between a donor and an acceptor. During the reductive dechlorination of ethenes, chlorines are sequentially replaced with hydrogen.
Though hydrogen is not always the only electron donor, it appears to be the donor most often used by dechlorinating bacteria. Ballapragada et al. (1997) have evaluated the importance of hydrogen in reductive dechlorination by evaluating the response to various electron donors in a fluidized bed reactor (FBR). Using PCE as the electron acceptor and acetate, lactate, propionate, and H₂ as electron donors, each substrate was added to the FBR for evaluation over a twenty-four hour period. Adding PCE alone to the FBR and then combining PCE with acetate yielded similar results during the first nine hours: less than ten percent of the PCE was dechlorinated. However, adding H₂ to both mixtures after nine hours resulted in immediate transformation of PCE to DCE, VC, and ethene. Similar results were achieved by adding lactate and propionate as electron donors to the FBR. During each twenty-four hour test period, approximately eighty percent of the PCE was transformed to ethene (Ballapragada et al., 1997), even when adding H₂ after the first nine hours of very little activity. This suggests that H₂ and other electron donors are essential for reductive dechlorination to occur.

While H₂ effectively promotes dechlorination, it is also one electron donor used by methanogens. Hence, there is potential for competition and the inhibition of reductive dechlorination. Ballapragada et al. (1997) explain that the competitive advantage depends on relative cell yields, affinity for a common electron donor, and maximum specific utilization rates. At H₂ partial pressures less than 100 parts per million (ppm), dechlorinators maintain the
competitive advantage in all three categories. Since H₂ rarely reaches partial pressures greater than 100 ppm in methanogenic environments, it is likely that dechlorination will not be impacted by competition from methanogens (Ballapragada et al., 1997). Smatlak et al. (1996) also explain that a substrate that ferments slowly to H₂ such as butyrate or propionate is conducive to complete dechlorination. Dechlorinating bacteria can use hydrogen at lower levels than methanogens, and methanogenesis yields to dechlorination.

Organic acid data collected from a constructed wetland may be helpful in determining which microbial metabolic processes are occurring. The presence of organic acids could indicate that they are forming as a result of plant activity and fermentation of organics by bacteria, but they are not being consumed and may not be acting as electron donors. It is also possible that they are being consumed at a slower rate than they are being produced. Certain organic acids may indicate that prime conditions exist for chlorinated ethene degradation. For example, butyrate and propionate generate hydrogen more slowly than other organic acids (Lee, 1998), and their presence may suggest that dechlorinators are able to use them for reduction of chlorinated aliphatic hydrocarbons.

**Electron acceptors**

Anaerobic bacteria are capable of using different electron acceptors for microbial metabolism, and they compete with one another for a source of electrons like hydrogen. Competition for hydrogen and other electron donors among microorganisms is a significant issue concerning reductive dehalogenation (Yang and McCarty, 1998). The nature of chlorinated ethene degradation depends on the reducing conditions in the environment which are determined by the dominant electron acceptor (Chapelle, 2001). Nitrate reducers, iron reducers, sulfate reducers, methanogens, and dechlorinating bacteria all have different affinities for consuming
hydrogen. In anaerobic systems, the order of affinity for hydrogen in descending order is nitrate, iron, sulfate, and carbon dioxide. CO$_2$ is the electron acceptor with the lowest affinity for hydrogen, and H$_2$ concentrations in methanogenic aquifers are relatively high. As nitrate concentrations are depleted, iron reducing conditions may become dominant. Sulfate reducers will take over when iron is depleted, and methanogens will follow sulfate exhaustion (Vogel et al., 1987). Dechlorinating bacteria can successfully compete for electron donors when these other electron acceptors have been exhausted or remain in low concentrations. As chlorinated ethenes become less chlorinated and more reduced, greater concentrations of hydrogen and more strongly reducing conditions are required for them to dechlorinate. Ultimately, the success of reductive dechlorination depends on the presence of dechlorinating microorganisms, a sufficient supply of chlorinated contaminant, an ample organic substrate to donate electrons, and favorable environmental conditions such as temperature and pH (Lee et al., 1998).

Inorganic ion data obtained from a constructed wetland could aid in determining which metabolic pathways are dominating the ecosystem and which, if any, chlorinated ethenes should be degrading. Significant nitrite concentrations in the absence of oxygen might suggest that nitrate-reduction has occurred. Sulfate concentrations that decrease between strata could indicate that sulfate-reducers are active if all the higher-affinity electron acceptors have been depleted.

**Summary**

Chemical compounds are exchanged between the atmosphere and the subsurface aquatic environment via biogeochemical cycles. They transform through a variety of redox reactions that are catalyzed by subsurface aquatic microorganisms. There are consortia of aerobic and anaerobic bacteria that use these compounds as part of their metabolism, and in some instances
they degrade chlorinated organic solvents. Microbially-mediated redox processes tend to segregate into discrete zones based on the terminal electron acceptor (Chapelle, 2001), and these zones may also be influenced by vegetation or other factors such as temperature, pH, and flow paths. This is certainly a consideration when evaluating sample data from a constructed wetland, as values may suggest which redox processes are occurring in a particular region. However, these reactions occur on a micro scale and the sampling technique in this study can only suggest generalities. Mapping the distribution of microbially-mediated redox processes helps to paint a picture for predicting the fate and transport of chlorinated contaminants in groundwater systems (Seagren and Becker, 1999). Highly halogenated compounds are more susceptible to reductive dehalogenation, while less halogenated compounds may also be transformed aerobically. Sequential application of anaerobic and aerobic conditions may be ideal for complete dechlorination (Pankow and Cherry, 1996), and that is why the wetland environment shows much potential for bioremediation of chlorinated organic solvents.
III. Methodology

Overview

Water samples were collected from a constructed wetland located at Wright-Patterson Air Force Base (WPAFB), Ohio during July 2003 and Fall (October and November) 2003. Samples were collected from the influent, effluent, and sixty-six well nests with peizometers at three different depths. Preparation and analysis of samples occurred at the AFIT environmental laboratory also located at WPAFB. Ion chromatography was used to evaluate inorganic ion and organic acid concentrations in each sample. This data was used to determine the suspected redox conditions within the wetland and to suggest a profile for the behavior of chlorinated ethene degradation.

Constructed Wetlands

A 120’ x 60’ wetland cell was constructed at WPAFB in September 2000 to study the behavior of chlorinated solvent degradation via manipulated natural attenuation. It is situated above an aquifer plume that was initially contaminated with approximately 50 parts per billion (ppb) of PCE. The cell is separated from the surrounding environment by an impermeable liner. Water from the contaminated plume is pumped into the subsurface of the wetland through three perforated 3’’ PVC pipes that are buried in a bed of gravel and run the entire length of the wetland cell. The original design specified that the gravel bed should be covered by three 18” layers of soil. However, based on observations from core samples collected during the summer of 2003, it is suspected that the actual depth of each layer is approximately 12”. The first layer (stratum C) is a mixture of ninety percent hydric soil and ten percent wood chips. The two successive 18” lifts (stratum B and stratum A) are hydric soil only. Sixty-six well nests were installed in a grid of eleven rows with six nests per row. Each nest has three piezometers
installed at 9”, 27”, and 45” such that the piezometer screens are uniformly located in the middle of their respective soil layers. Figure 2 shows a cross section of the constructed wetland with a typical nest of piezometers.

![Figure 2: Constructed Wetland Cross Section (Bugg, 2002)](image)

The constructed wetland was planted with seventeen different wetland plant species in Fall 2000. The cell was initially divided into fourteen well-defined plots; however, the more dominant plant species overtook some of the less-competitive plants and the boundary lines between plots have become less obvious. An investigation of redox conditions based on the subsurface water chemistry could indicate that the type and amount of vegetation in certain areas has an impact on the degradation of chlorinated ethenes. Core samples from the summer of 2003 showed evidence that the plant roots extended the full depth of the wetland (Amon, 2003).

**Sampling Procedures**

Extraction, collection, and analysis of wetland samples were consistent with previous research efforts by AFIT graduate students (Kovacic, 2003; Bugg, 2002). A complete sampling run consisted of collecting one sample from each piezometer for a total of 198 samples. Three complete sampling runs were originally scheduled for this research effort to evaluate seasonal
changes in the wetland redox conditions; however, only the July 2003 and Fall 2003 runs were completed. Samples were collected and analyzed by stratum. Inflow and outflow samples were taken each time a stratum was sampled. The uniform layout of the well grid in the wetland provided a realistic and efficient means of statistically analyzing trends in the changing redox conditions (Mac Berthouex and Brown, 2002). Ideally, each sampling point would consistently yield a water sample that would translate into an analyte concentration for statistical evaluation; however, missing data or anomalies could potentially cause skewed data distributions (Gilbert, 1987).

All sixty-six wells in each stratum were purged twenty-four hours prior to collecting samples from that stratum in order to remove stagnant water from the piezometers. Piezometers were purged with a battery-powered peristaltic pump connected to ¼” Teflon tubing. Most wells were pumped dry during purging and were replenished with fresh water for sampling by the following day. Some wells, mostly from stratum C, refilled too rapidly and could not be purged completely. Three well-casing volumes were removed from these piezometers in accordance with United States Environmental Protection Agency recommendations in order to remove any stagnant water (United States, 1992).

The sampling apparatus consisted of a 100mL glass syringe and ¼” Teflon tubing joined by a three-way cock-stop. The length of the tubing was adjusted depending on the depth of the stratum being sampled. Approximately 100mL of sample water was extracted from each piezometer and inserted into a 40mL glass vial on site. The remaining sample was placed in a separate 40mL vial for pH and temperature readings before being discarded. A Cole-Parmer pH 310 Series Portable Meter was used to collect this data. Inflow samples were taken from the influent spigot located inside the well house, and outflow samples were taken from the water
exiting the weir. Appendix A shows a detailed list of piezometer sampling procedures. A sampling run for a single stratum (68 total samples) typically took eight man-hours.

**Laboratory Procedures**

Samples were taken immediately from the field to the AFIT laboratory for analysis and were refrigerated at 3°C prior to preparation. To prevent the organic acids from disappearing from the non-sterile samples, which has been shown to occur after approximately 28 days (Kramer, 1990), samples never sat longer than 48 hours between field extraction and analysis in the Ion Chromatograph. Prior to loading samples into the IC, some 40mL vials with excessive sedimentation were placed in a centrifuge to remove larger soil particles. Samples were spun in an Eppendorf 5810R Centrifuge at 1200 rpm for eight minutes. This precautionary procedure served two purposes: 1) it increased the life of the analytical column in the IC by preventing it from getting clogged with sediment, and 2) it reduced the interference from miscellaneous organic compounds that may co-elute with the organic acids of interest to yield inaccurate system peaks during IC analysis (Chen, 1996). Using a 10mL syringe and hypodermic needles, approximately 5mL of sample was extracted from each 40mL vial. Samples were filtered through a 0.20µm Cole Parmer SFCA syringe filter into a 2mL glass vial. Each 2mL vial was labeled according to its corresponding piezometer and placed in the autosampling tray. A detailed procedure of preparing samples for IC analysis is listed in Appendix B. Once all samples were prepared for IC analysis, the autosampling tray was loaded into the autosampler and the Dionex Analysis Program for Peaknet 6.0 was initiated. A list of the program items and their respective values is located in Appendix C. A Dionex 600 series Ion Chromatograph was used to analyze samples. Refer to Kovacic (2003) and the Dionex manuals and literature
(Dionex, 2003) for additional information on the components and operating procedures for this system.

Prior to analysis of wetland samples with unknown analyte concentrations, standard samples of known concentrations for each analyte were prepared and analyzed in the IC. Stock solutions of 1000mg/L were prepared for each of the analytes listed in Figure 3. Organic acids (lactate, acetate, propionate, formate, butyrate) were prepared together in a 72mL vial, and inorganic ions (chloride, nitrite, nitrate, sulfate, phosphate) were prepared in a separate 72mL vial. These stock solutions were then diluted several times to develop a range of concentrations that might be expected in unknown samples. Calibration curves were developed by running these standards of known concentrations through the IC, and correlation coefficients greater than 99.8% were achieved for each analyte.

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Ret. Time (min)</th>
<th>Cal.Type</th>
<th>No. Points</th>
<th>Rel.Std.Dev. %</th>
<th>Corr.Coeff. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
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<td>Lin</td>
<td>3</td>
<td>12.4932</td>
<td>99.8662</td>
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<td>Acetate</td>
<td>7.60</td>
<td>Lin</td>
<td>3</td>
<td>9.2803</td>
<td>99.9146</td>
</tr>
<tr>
<td>Propionate</td>
<td>8.60</td>
<td>Lin</td>
<td>3</td>
<td>11.3107</td>
<td>99.8773</td>
</tr>
<tr>
<td>Formate</td>
<td>9.90</td>
<td>Lin</td>
<td>3</td>
<td>7.2269</td>
<td>99.9434</td>
</tr>
<tr>
<td>Butyrate</td>
<td>10.65</td>
<td>Lin</td>
<td>3</td>
<td>14.5396</td>
<td>99.9046</td>
</tr>
<tr>
<td>Chloride</td>
<td>14.56</td>
<td>Lin</td>
<td>7</td>
<td>7.7056</td>
<td>99.8993</td>
</tr>
<tr>
<td>Nitrite</td>
<td>15.60</td>
<td>Lin</td>
<td>7</td>
<td>5.7103</td>
<td>99.9512</td>
</tr>
<tr>
<td>Nitrate</td>
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<td>Lin</td>
<td>6</td>
<td>6.5811</td>
<td>99.9451</td>
</tr>
<tr>
<td>Sulfate</td>
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<td>Lin</td>
<td>7</td>
<td>6.2617</td>
<td>99.9373</td>
</tr>
<tr>
<td>Phosphate</td>
<td>26.50</td>
<td>Lin</td>
<td>3</td>
<td>9.2448</td>
<td>99.8392</td>
</tr>
</tbody>
</table>

**Figure 3: Calibration of Target Analyte Standards**

The Peaknet program used these calibration curves to determine the amount of analyte in each of the piezometer samples. At the conclusion of each sample analysis, the Peaknet software generated spreadsheets listing the amount of analyte detected in each sample. While the number of data points was preferably 66 for each stratum (198 total data points per sampling run), several wells did not yield a sample. In some such cases, standard analytes were run during the
IC analysis to validate the analysis procedures and equipment.

**Data Analysis**

Analyte concentrations were extracted from the Dionex Peaknet 6.30 Chromleon program and manipulated using Microsoft Excel and Golden Software Surfer 8.01. Excel was used to organize data by analyte, stratum, and sampling date and to determine mean analyte concentrations in each stratum and in each well nest. Excel was also used to create charts and tables to visualize changes in analyte concentrations over time and between each stratum. Surfer 8.01 was used to create contour maps of each analyte in the wetland cell for the three strata.

Data distributions and contour plots for each analyte were evaluated vertically as they progressed through the three strata. Regional trends were noted and evaluated to see if there were homogeneous areas that may have been created by microbial populations or areas that were possibly affected by vegetation, temperature, pH, or some other environmental factor. Redox processes tend to segregate into discrete zones based on the terminal electron acceptor (Chapelle, 2001). Mean concentrations of each analyte were calculated for each well nest and are included in Appendices Z and AD. Apparent outliers were included in the contour maps in order to observe possible anomalies at a particular well or region, and analyte concentrations surrounding these anomalies were checked to see if a possible trend existed. Data from this effort was compared with similar data from previous efforts to evaluate any possible developments over time and between seasons. Data was also compared to actual chlorinated ethene measurements collected during the same sampling runs by Sobolewski (2004).
IV. Results

Analyte Analysis

Contour maps of analyte concentrations, temperature, and pH showing the relative changes across the wetland cell are included in Appendices D through U. Extreme values and apparent anomalies were included in the contour maps in order to portray any possible trends that may be occurring in a particular area of the wetland cell. Mean concentrations for each analyte were calculated from the inflows and from each stratum to evaluate changes in concentrations as water moved through the strata of the constructed wetland. Data collected during this thesis effort was used in conjunction with data collected by former graduate students (Bugg, 2002; Kovacic, 2003) to establish a more comprehensive picture of seasonal changes in analyte concentrations. No data from stratum C was collected during the July 2003 sampling run. Technical difficulties with the Ion Chromatograph occurred during analysis of Stratum B. It was eventually determined that pressure fluctuations in the GP50 Gradient Pump had been caused by a faulty check valve. The extended time for diagnosis and repair of the IC prevented a timely collection of samples from all three strata during July. During the Fall (October and November) 2003 sampling run, samples were collected from all three strata which aided in seasonal comparisons with previous data. Vertical profiles of the Fall data were developed for an alternate view of analyte behavior between the strata (see Appendices V and W).

**Organic acid** concentrations increased on average between the inflow and the center of stratum A. Only propionate showed a significant decrease through the strata. Average butyrate and lactate concentrations tripled from inflow to stratum A. Changes in the mean stratum concentrations of each analyte are shown in Figure 4.
The high concentrations of lactate (390 ppb) and butyrate (359 ppb) in layer A as opposed to their inflow concentrations (135 and 98 ppb, respectively) indicated that there was an increase in production of organic acids in the wetland. Increases in organic acid concentrations could be attributed to the decay of organic material from plants and roots that were fermented into organic acids. Higher concentrations could also be attributed to a lack of microbial consumption thereby leading to accumulation. However, this seems unlikely, as there are some indications that organic acids were being consumed. Acetate concentrations were lower than all other organic acids. Despite an apparent anomaly in well 9 that skewed that average concentration in stratum A, acetate levels decreased by more than half from stratum B to stratum A.

All remaining organic acids accumulated in the last few rows of well nests in stratum B of the wetland cell (generally in wells 49 through 66). An increased rate of fermentation may

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**Figure 4: Mean Organic Acid Concentrations (ppb), July 2003**

The high concentrations of lactate (390 ppb) and butyrate (359 ppb) in layer A as opposed to their inflow concentrations (135 and 98 ppb, respectively) indicated that there was an increase in production of organic acids in the wetland. Increases in organic acid concentrations could be attributed to the decay of organic material from plants and roots that were fermented into organic acids. Higher concentrations could also be attributed to a lack of microbial consumption thereby leading to accumulation. However, this seems unlikely, as there are some indications that organic acids were being consumed. Acetate concentrations were lower than all other organic acids. Despite an apparent anomaly in well 9 that skewed that average concentration in stratum A, acetate levels decreased by more than half from stratum B to stratum A.

All remaining organic acids accumulated in the last few rows of well nests in stratum B of the wetland cell (generally in wells 49 through 66). An increased rate of fermentation may
have produced higher levels of organic acids in this area. This region is planted with a variety of plant types instead of a single plant species. It is possible that fermenting microorganisms benefited from the higher variety of substrates available from plant decay and produced quantities of organic acids faster than they could be consumed by other microbial communities. It is also possible that a lack of electron acceptors such as nitrate and sulfate inhibited the redox reactions required for microbial metabolisms whereby organic acids are used as electron donors. In this same region of stratum B (that is, wells 49 through 66), there were low concentrations of nitrate and sulfate, which are common electron acceptors in the anaerobic regions of the constructed wetland and could slow the oxidation of organic acids to CO$_2$.

While each of the organic acid analytes amassed in this region of stratum B, their concentrations were substantially lower in the same region of stratum A suggesting that they were consumed between stratum B and stratum A. Microorganisms may have benefited from the higher concentrations of organic acids in this area and they were able to use them as electron donors as part of their metabolisms. The high concentrations of lactate, propionate, and butyrate could have expedited the use of organic acids as electron donors for reductive dechlorination. Studies have shown that these particular analytes rapidly reduce pure concentrations of chlorinated ethenes in the laboratory environment (Ballapragada, 1997). The lack of nitrate and sulfate in the same region of stratum A similar to that of stratum B could mean that the availability of electron acceptors was insufficient for microbial metabolisms to take advantage of the organic acids. It is also possible that the organic acids were consumed by the vegetation in this region. In fact, plants can both ingest and exude organic acids, which is an alternate explanation for both the presence and disappearance of organic acids in this region.
Another peculiar observation was the high concentrations of formate and butyrate near well 50 in stratum A and the high concentration of lactate near well 46. This coincided with higher concentrations of chloride in the same locations of stratum A. However, chloride was excessively high in these areas and a mass balance of complete dechlorination of PCE would only account for a fraction of the chloride concentration present. It is still possible that microorganisms were taking advantage of these higher organic acid concentrations, and they could have been reducing chlorinated ethenes more rapidly in these areas.

**Inorganic Anion** concentrations for July 2003 could be related to several possibilities regarding the destruction of chlorinated ethenes in the constructed wetland. Ion analyte concentrations are shown in Figure 5.

![Bar chart showing inorganic anion concentrations](image)

**Figure 5: Mean Inorganic Anion Concentrations (ppm), July 2003**

<table>
<thead>
<tr>
<th></th>
<th>Chloride</th>
<th>Nitrates</th>
<th>Nitrites</th>
<th>Sucrates</th>
<th>Phosphates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratum A</td>
<td>53.5524</td>
<td>0.7502</td>
<td>6.5286</td>
<td>4.0741</td>
<td>0.1325</td>
</tr>
<tr>
<td>Stratum B</td>
<td>0.0764</td>
<td>13.6251</td>
<td>35.8289</td>
<td>4.7192</td>
<td>0.1552</td>
</tr>
<tr>
<td>Inflow</td>
<td>74.0351</td>
<td>0.0606</td>
<td>0.0334</td>
<td>25.1692</td>
<td>0.0332</td>
</tr>
</tbody>
</table>
Nitrite and nitrate concentrations in stratum B were two of the most interesting analyte behaviors in this sampling run (see Appendices J and K). Average nitrite concentrations were approximately 70 ppm in the first half of the cell (that is, wells 1 – 36) and were undetected in the second half of the cell. This split-cell condition may have been caused by inhibited flow conditions in the subsurface water pipes that feed the wetland. If there was a pressure loss in the latter half of the pipes, the vertical flow would have been significantly less in the second half of the wetland cell where there are almost no nitrite concentrations. If vertical flow conditions were prevalent in the first half of the cell, it is possible that these higher nitrite concentrations are due to nitrate-reducing bacteria below stratum B that were converting nitrate to nitrite. This assumes that there was a concentration of nitrate in stratum C that was available to nitrate-reducers. The disappearance of high levels of nitrite followed almost immediately by the sudden appearance of nitrate could have been caused by horizontal flow conditions in stratum B and a nitrification step occurring in the center of the stratum that was oxidizing nitrite back to nitrate. This suggests that aerobic conditions were present, and plant roots may have supplied O₂ to stratum B. The high nitrate concentration in the second half of the cell is unusual because plant life was active during July. Nitrate is an important nutrient for plants and it is unlikely that they would release enough of it to create the high concentrations in this region. High nitrate levels in wells 42-47 could have also been caused by aerobic conditions. Nitrate is an electron acceptor, but oxygen is higher in the order of affinity for use by microbes. If aerobic microorganisms were taking advantage of an oxygen supply in this region, nitrate would have accumulated until oxygen levels decreased enough so that nitrate could be used by microorganisms. Another explanation for the relationship between nitrite and nitrate in this area is that these analytes were
trapped in a particular step of the nitrification process where nitrate and nitrite were being
continually converted back and forth.

Nitrate and sulfate concentrations decreased as they moved vertically through the
wetland strata. The average inflow concentration of sulfate was 25 ppm and dropped to 4 ppm in
stratum A. Nitrate was 13.6 ppm in stratum B and fell below 1 ppm in stratum A. There was
also a higher concentration of chloride and an ample supply of organic acid electron donors in
the top stratum. With nitrate and sulfate supplies exhausted or available only in low
concentrations, nitrate-reducing and sulfate-reducing bacteria may have yielded to
dechlorinating bacteria that were then able to compete for a hydrogen source and use chlorinated
ethenes as electron acceptors if significant anaerobic regions exist in the upper stratum.

The inflow chloride concentration during July 2003 was 74 ppm and the average
concentration in stratum A was 53.5 ppm. However, almost no chloride was detected in stratum
B. Knowing that the plant roots extend well into stratum B, it is possible that chloride was being
taken up by the plants if they were experiencing a growth spurt. If the plants were not growing
and the subsurface environment was in equilibrium, chloride concentrations should have
appeared in stratum B due to the vertical flow patterns in the cell. Plant tissue can absorb
approximately 3 mmol of chloride per kilogram of dry weight. Depending on the type of
vegetation, this value can be up to 1000 times higher and may partially explain the disappearance
of chloride in the middle layer. Average PCE concentrations in the inflow were around 30 ppb
(Sobolewski, 2004). A mass balance of the reductive dechlorination of PCE all the way to
ethene yields only 25.6 ppb of chloride. This concentration is significantly less than that
observed in stratum A. Hence, the high concentration of chloride in A must have come from an
external source other than from the inflow. One possible suggestion is that the chloride absorbed
by the plant tissue was being released back into the top stratum as leaves and other plant material
died and decayed.

Phosphate concentrations were much lower than other inorganic anions during July 2003.
Phosphate is an important nutrient for plants and microorganisms in the wetland, and it is
possible that the wetland environment was phosphate limited. However, significant phosphate
concentrations have been found in the soil during similar studies, so it is possible that there were
ample phosphate concentrations in the constructed wetland that had not yet solubilized. Plants
are able to solubilize phosphate as needed for growth, and they can adapt to an environment
where phosphate is available only in very dilute solutions.

Data distributions for temperature and pH values from July 2003 are shown in Figure 6. The pH mean was 6.88 and the standard deviation was 0.15.

![Figure 6: pH and temperature (°C), July 2003](image)

There is an optimal pH value for microbial populations at which growth and reproduction are
maximal. Microbial metabolisms become less efficient as the pH moves away from the optimal
value (USFDA, 1992). Aerobic microorganisms prefer pH ranges between 6.5 to 8.5 and
anaerobes are most efficient between 6.7 and 7.4; they will die or become inactive outside these
ranges (Genesis, 2004). Temperature values were cooler in the lower strata and became warmer in the upper strata during July 2003. The opposite occurred during Fall 2003 (see Appendices O and S). Temperature values are significant when evaluating microbial activity because cooler temperatures tend to slow microbial metabolisms while increasing temperatures within limits can increase activity. Figure 6 shows a distribution of pH and temperature values within acceptable ranges for the majority of microorganisms. Hence, microbial activity was not likely hindered by pH or temperature in the constructed wetland during the July sampling run.

During the Fall 2003 sampling run, samples were collected from all three strata; however, sulfate and chloride were the only analytes detected during the IC analysis. No other inorganic anions and no organic acids being evaluated as part of this effort were detected in the wetland samples. Mean analyte concentrations for the Fall sampling run are shown in Figure 7.

![Figure 7: Mean Inorganic Anion Concentrations (ppb), Fall 2003](image-url)

<table>
<thead>
<tr>
<th>Stratum</th>
<th>Chloride</th>
<th>Sulfate</th>
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<td>5.4935</td>
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<tr>
<td>Stratum B</td>
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<td>18.1754</td>
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<tr>
<td>Stratum C</td>
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<td>44.2316</td>
</tr>
<tr>
<td>Inflow</td>
<td>43.2000</td>
<td>33.2000</td>
</tr>
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</table>
Sulfate concentrations were highest in stratum C and decreased in each successive stratum. The average concentrations in strata C, B, and A were 44.2, 18.2, and 5.5 ppb respectively. The inflow concentration was 33.2 ppb. The higher concentration of sulfate in the bottom layer may indicate that the reducing conditions were not strong enough to convert sulfate to sulfide. Also, another electron acceptor with a higher affinity for hydrogen such as iron could have been outcompeting the sulfate-reducing microorganisms. The lower concentrations of sulfate in strata B and A could mean that sulfate-reducers were able to compete successfully for a hydrogen source somewhere above stratum C. More highly reducing conditions in these areas may have been sufficient for sulfate-reduction. Dechlorinating bacteria will not typically compete well for electron donors until sulfate concentrations have been exhausted or become very low, and these conditions will inhibit dechlorination in most cases.

Chloride concentrations behaved similarly to sulfate concentrations during the Fall sample, as chloride was highest in stratum C at 44.9 ppb and decreased to 23.6 ppb in stratum B and 16 ppb in stratum A. The influent concentration was 43.2 ppb. These concentrations are the same order of magnitude of values that would be expected from a mass balance of the complete dechlorination of 30 ppb of PCE to ethene (25.6 ppb). However, it is peculiar that the chloride concentration decreased as it moved vertically through the strata. Successive dechlorination of chlorinated ethenes should yield higher chloride concentrations as the contaminants flow vertically through the strata if the subsurface conditions are in equilibrium. Also, if the high concentrations of chloride in stratum C were due to dechlorination, then the sulfate concentrations would not have been significant enough to inhibit dechlorinating bacteria. The sulfate-reducing bacteria may have been releasing enzymes that were fortuitously reducing
chlorinated ethenes co-metabolically, but this is a much slower process than direct
dehalorespiration.

**Seasonal Data Comparison**

One of the most obvious changes between sampling runs in this effort was the complete
disappearance of organic acids from July 2003 to Fall 2003. In a matter of months, all five
organic acids decreased from detectable concentrations in parts per billion to completely
undetectable concentrations. A likely explanation is that the changing seasons had a significant
impact on the availability of substrates for the formation of organic acids. Plants and other
vegetation had died by the time the Fall samples were collected. The exhaustion of decaying
organic matter required for microbial metabolisms could have caused much of the subsurface
microorganisms to become dormant. Temperature is another factor that may have impacted
organic acid concentrations. The average subsurface temperature dropped from 21.3 °C to 12 °C
between sampling runs. Microorganisms are sensitive to temperature fluctuations and
metabolisms can slow significantly with lower temperatures. Organic acid behavior in the
WPAFB constructed wetland from the initial collection of data through the current study is
shown in Appendix AE. Samples were not collected from stratum C during July 2003 and are
not reflected in the chart. There were significantly high concentrations of certain organic acids
during the early development of the wetland cell. Acetate and formate concentrations were
particularly high. The wetland cell was initially seeded with packed plasma containers of
microbial populations prior to installing the soil layers. It is possible that the spikes in these
concentrations were due to hyperactivity in the microorganisms as they adjusted to equilibrium
within the subsurface environment. Organic acid concentrations appeared to become more stable
over time. Figure 8 shows seasonal data on a different scale to reflect more recent behavior of
organic acid concentrations in Stratum A. The July 2003 samples were taken during warmer weather with an average subsurface temperature of 21.3 °C, while the December 2002 and Fall 2003 temperatures were 9.5 °C and 12 °C, respectively.

![Graph showing organic acid concentrations](image)

**Figure 8: Recent Seasonal Organic Acid Behavior – Stratum A**

The concentrations were much higher during the warmer weather, as might be expected due to increased microbial activity during higher temperatures. Stratum B showed a similar trend.

Seasonal changes in inorganic anions are shown in Appendix AF. Sulfate and chloride concentrations were detected in parts per billion during the Fall 2003 sampling run and were much lower than they had been in the past. However, the chloride concentration was uncharacteristically high in stratum A during July 2003 compared to other seasons. Nitrate showed up during every season, but nitrite was only detected during July 2003. Nitrate-reduction was most likely occurring during the warmer seasons when microbes were most active. This is significant because nitrogen is a critical nutrient for vegetation and nitrate can act as an electron acceptor in the direct oxidation of vinyl chloride. Sulfate levels were highest in stratum C and decreased in the upper strata consistently throughout the seasons. This suggests that there are
more-highly reducing conditions in the upper strata than there are in stratum C, as sulfate is not being reduced to sulfide until it reached the upper strata. Between July 2003 and Fall 2003, almost all of the analytes disappeared. The changing seasons and the death of plants and other vegetation may have forced the microorganisms to continually adapt and successively exhaust each of the analytes as they struggled to survive in the rapidly changing subsurface environment.

**Chlorinated Ethene Behavior**

Hypothesized microbial behavior in the constructed wetland based on the analytes in this study were evaluated against actual chlorinated ethene concentrations taken from samples collected during Fall 2003 by Sobolewski (2004). Observations of this data revealed similar behaviors that support some of the hypotheses. High concentrations of PCE and DCE in stratum C coupled with low concentrations of TCE in the same region indicate that PCE was reductively dechlorinated fairly rapidly to DCE. The accumulation of DCE in stratum C suggests that reducing conditions were conducive to PCE reduction; however, DCE, which is lower than PCE in the order of affinity of electron acceptors, requires more strongly reducing conditions than were present in stratum C. This correlates with the higher sulfate concentrations in stratum C that decreased in the upper two strata due most likely to more highly-reducing conditions in these strata. This phenomenon has been observed in the past from ORP data collected by Kovacic (2003). Additionally, mean strata concentrations for DCE decreased in the upper two layers, while mean VC concentrations increased in stratum A (Sobolewski, 2004).
V. Conclusions

Overview

The purpose of this effort was to continue the study of bioremediation of chlorinated ethenes in a constructed wetland by analyzing organic acid and inorganic anion concentrations. The target analytes evaluated in this study are indicative of the microbial processes that have been shown to degrade chlorinated organic solvents to innocuous end products such as CO₂, water, and ethene. Analyte concentrations, temperature, and pH measurements were collected during the summer and Fall of 2003. Comparisons of analyte concentrations were made between these two sampling runs as well as between data collected from previous sampling efforts by Bugg (2002) and Kovacic (2003). Chlorinated ethene concentrations collected simultaneously with this effort by Sobolewski (2004) were also compared to analyte data. These analyses and comparisons were used to answer the research questions that were posed at the onset of this study.

Research question 1 asked which inorganic ions and low-molecular weight monocarboxylic organic acids were currently prominent in each layer. During the July 2003 sampling run, all ten analytes were detected in the wetland cell. The Fall 2003 sampling run yielded only sulfate and chloride in relatively low concentrations compared to other sampling runs. It is suspicious that the concentration data decreased by three orders of magnitude in a matter of months. Although suggestions for this behavior are offered, the validity of this data warrants further review before it is used as part of another study.

Research question 2 asked whether the comparative analysis of organic acid and inorganic anion concentrations during the same time of year indicated significant deviations from previous findings. Data from previous efforts was collected during the months of December and January, and no samples were taken during a comparable timeframe as part of this effort. Although there were seasonal observations that drew comparisons between the Fall data
and previous data from December and January, it was not possible to accurately answer research question 2 during this study.

Research question 3 asked whether the comparative analysis of organic acids and inorganic anions during different times of year suggested changes in microbial behavior in the wetland cell. There were definitely noticeable differences in analyte concentrations during different seasons. The most notable phenomenon was the behavior of organic acids during July 2003 compared to the same analyte concentrations during December 2002 and Fall 2003. Also, the disappearance of all analytes except chloride and sulfate in Fall 2003 showed a significant change in the subsurface environment due to the seasonal impacts on microbial activity.

Research question 4 asked what microbial metabolic processes seem to be occurring in each layer based on the anion and organic acid concentrations. Dechlorination via direct dehalorespiration and co-metabolic reductive dechlorination were likely occurring in some areas of the wetland. Higher nitrate and sulfate concentrations in stratum B may have hindered dechlorination, but their lower concentrations in stratum A and the presence of organic acids in strata A and B indicates that microbial metabolisms were able to take advantage of these chlorinated contaminants. There are other indications that some aerobic processes may have occurred. The accumulation of nitrate in stratum B during July may have been caused by an oxygenated zone that prevented its use as an electron acceptor. Hence, less-chlorinated ethenes such as DCE and VC may have been oxidized in these regions.

Research question 5 asked if these hypothesized metabolic activities correlated with the observed behavior of chlorinated aliphatic compounds in the wetland. In general, chlorinated ethene concentrations decreased from stratum C to stratum A. Based on analyte concentrations observed in these same areas, it is likely that dechlorinating bacteria were degrading them by direct dehalorespiration. Concentrations of the less-chlorinated ethenes such as DCE and VC were small in the upper strata, and these compounds may have been oxidized by aerobic microorganisms that were able to use them as electron donors.
Limitations and Recommendations

While the contour plots of analyte concentrations in the wetland suggest particular behaviors of the subsurface environment, it is risky to base hypotheses on homogeneous flow without knowing the actual flow conditions. A flow analysis would assist in explaining some of the subsurface behavior by attributing some observations to heterogeneous flow patterns.

There are a number of other analytes that would provide additional evidence and more confidence in the hypotheses regarding subsurface microbial behavior. Microorganisms have an affinity for iron as an electron acceptor when it is reduced from Fe(III) to Fe(II). This affinity lies between nitrate and sulfate reduction and would assist in explaining the strength of the present reducing conditions. CO$_2$ and methane concentrations would help identify methanogenic regions where reductive dechlorination has been known to occur when other electron acceptors have been depleted. Oxidation-reduction potentials (ORP) and dissolved oxygen (DO) levels would reveal a great deal of information about the subsurface conditions. However, the YSI sonde that has been used to sample the constructed wetland in the past does not fit in the existing peizometers and can only take readings from the six 2” sampling wells. It would be beneficial to collect ORP data that correlates to the organic acid, inorganic anion, and chlorinated ethene data collected from the 66 peizometers. ORP readings need to be taken often over an extended time period in order to accurately characterize the subsurface soil conditions, as redox conditions can vary weekly and by season (Lewis, 2001).

Although seasonal data was collected and evaluated as part of this thesis effort, there are additional seasons that have yet to be sampled. Plants and vegetation begin to grow significantly in early spring, and mid-April would be an excellent time to collect wetland samples. The sudden growth and interaction of plants and microorganisms would certainly reveal new suggestions regarding subsurface microbial behavior. Additional data from seasons that have already been sampled will also enhance this study, as this effort is only the third iteration of data collection from the WPAFB constructed wetland.
Conclusion

The behavior of organic acid and inorganic anion concentrations in the subsurface wetland environment of cell #1 at the WPAFB constructed wetland indicates the existence of microbial processes that support degradation of chlorinated ethenes. These processes are occurring slowly, yet they have demonstrated significant changes in the early stages of development. Future efforts that monitor and evaluate samples from this wetland will continue to provide valuable insight regarding these processes.
Appendix A: Piezometer Sample Collection Procedures

1. Purge all piezometers to be sampled 24 hours prior to sampling. Most piezometers will be pumped dry within 20-30 seconds using the battery-powered peristaltic pump. Some piezometers (many from strata C) may recharge too quickly to be completely purged. Remove three well casing volumes from these piezometers.
2. Remove protective PVC cap from piezometer. Insert Teflon tubing until it reaches the bottom of the well.
3. Draw approximately 20mL of water into the 100mL syringe.
4. Turn the cockstop to close the tube valve and open the purge valve.
5. Purge the water from the syringe.
6. Turn cockstop to open the tube valve and close the purge valve.
7. Draw 100mL of sample water into the syringe.
8. Turn the cockstop to close the tube valve and open the purge valve.
9. Purge sample into a 40mL vial until a surface meniscus has formed. Remove any air bubbles and cap the 40mL vial.
11. Turn cockstop to open the tube valve. Insert Teflon tubing into DI water container and draw 50mL of DI water into 100mL syringe.
12. Remove tubing from DI water and continue to draw 50mL of air.
13. Gently agitate air/water mixture to rinse the inside of the syringe.
14. Turn cockstop to open the purge valve. Purge the contents of the syringe.
15. Repeat steps 2-14 for each piezometer sample.

NOTE: Before and after collecting samples, thoroughly rinse all materials that come in contact with the sample with deionized (DI) water.
Appendix B: Sample Preparation for IC Analysis

1. Attach hypodermic needle to 10mL syringe.
2. Insert syringe needle into septum of 40mL vial containing piezometer sample.
3. Insert a separate hypodermic needle into septum as a vent.
4. Draw approximately 5mL of sample from 40mL vial.
5. Remove both needles from 40mL vial.
6. Remove hypodermic needle from 10mL syringe.
7. Attach 0.20 µL syringe filter to 10mL syringe.
8. Insert sample through filter and into 2mL glass autosampler vial. Ensure there is a surface meniscus with no bubbles and cap the 2mL vial.
9. Insert 2mL vial into corresponding location in the autosampler tray
10. Remove and discard the 0.20 µL syringe filter. Discard the remaining sample.
11. Rinse 10mL syringe and both hypodermic needles with DI water.
12. Repeat steps 1-11 for each sample.
## Appendix C: Dionex Analysis Program for Peaknet 6.0

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Appendix D: Acetate, July 2003

ppb Acetate, Stratum A

ppb Acetate, Stratum B
Appendix E: Butyrate, July 2003

ppb Butyrate, Stratum A

ppb Butyrate, Stratum B
Appendix F: Formate, July 2003

ppb Formate, Stratum A

ppb Formate, Stratum B
Appendix G: Lactate, July 2003

ppb Lactate, Stratum A

ppb Lactate, Stratum B
Appendix H: Propionate, July 2003

ppb Propionate, Stratum A

ppb Propionate, Stratum B
Appendix I: Chloride, July 2003

ppm Chloride, Stratum A

ppm Chloride, Stratum B
Appendix J: Nitrate, July 2003

ppm Nitrate, Stratum A

ppm Nitrate, Stratum B
Appendix K: Nitrite, July 2003

ppm Nitrite, Stratum A

ppm Nitrite, Stratum B
Appendix L: Sulfate, July 2003

ppm Sulfate, Stratum A

ppm Sulfate, Stratum B
Appendix M: Phosphate, July 2003

ppm Phosphate, Stratum A

ppm Phosphate, Stratum B
Appendix N: pH, July 2003

pH, Stratum A

pH, Stratum B
Appendix O: Temperature (°C), July 2003

Temperature (°C), Stratum A

Temperature (°C), Stratum B
Appendix R: pH, Fall 2003

pH, Stratum A

pH, Stratum B

pH, Stratum C
Appendix S: Temperature (°C), Fall 2003

Temperature (°C), Stratum A

Temperature (°C), Stratum B

Temperature (°C), Stratum C
Appendix T: Mean Well Nest Analyte Concentrations, July 2003

ppb Acetate

ppb Butyrate
Appendix T: Mean Well Nest Analyte Concentrations, July 2003

ppb Formate

ppb Lactate
Appendix T: Mean Well Nest Analyte Concentrations, July 2003

ppm Nitrate

ppm Nitrite
Appendix T: Mean Well Nest Analyte Concentrations, July 2003

ppm Sulfate

ppm Phosphate
Appendix U: Mean Well Nest Analyte Concentrations, Fall 2003

ppb Chloride

ppb Sulfate
Appendix V: Chloride Vertical Profiles, Fall 2003

Wells 1-6

Wells 7-12
Appendix V: Chloride Vertical Profiles, Fall 2003

Wells 13-18

Wells 19-24
Appendix V: Chloride Vertical Profiles, Fall 2003

Wells 25-30

Wells 31-36
Appendix V: Chloride Vertical Profiles, Fall 2003

Wells 37-42

Wells 43-48
Appendix V: Chloride Vertical Profiles, Fall 2003

Wells 49-54

Wells 55-60
Appendix V: Chloride Vertical Profiles, Fall 2003
Appendix W: Sulfate Vertical Profiles, Fall 2003

Wells 1-6

Wells 7-12
Appendix W: Sulfate Vertical Profiles

Wells 37-42

Wells 43-48
Appendix W: Sulfate Vertical Profiles, Fall 2003

Wells 49-54

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## Appendix X: Stratum A Data, July 2003

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## Appendix Z: Mean Well Nest Data, July 2003

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Appendix AF: Seasonal Inorganic Anion Profiles (ppb)

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Bibliography


Amon, James P. Department of Biological Sciences, Wright State University. Laboratory investigation of WPAFB constructed wetland core samples. November 2003.


Characterization of Microbial Processes That Degrade Chlorinated Solvents in a Constructed Wetland Using Organic Acid and Inorganic Anion Concentration Profiles

Organic acid and inorganic anion concentrations were evaluated in samples taken from a constructed wetland at Wright-Patterson Air Force Base, Ohio during the summer and fall of 2003. These analytes are indicative of redox conditions in the subsurface and suggest the occurrence of microbial activities that degrade chlorinated ethenes to innocuous end products. Organic acid concentrations decreased by 100% from July 2003 to fall 2003. Combined with data collected previously during the months of December and January, this suggested that changing seasons and temperature fluctuations have a significant influence on microbial metabolisms. Nitrate and sulfate reduction above stratum C indicated mildly reducing conditions in the lowest stratum that became more highly reducing in the upper two strata. Based on the changing analyte concentrations throughout the wetland cell over several seasons, it was evident that the appropriate subsurface conditions existed for the reductive dechlorination of chlorinated ethenes.

Chlorinated solvents have been used in industrial cleaning and degreasing processes in the United States since the early 1900s, and their induction into the environment increased significantly with the growth of industrial processes over the past century. PCE, TCE and their daughter products have been associated with a number of human health concerns and are currently the most common contaminants found in groundwater in the United States. Wetlands possess characteristics necessary for the complete degradation of chlorinated ethenes by microorganisms via anaerobic and aerobic regions that foster the necessary oxidation-reduction conditions.

Organic acid and inorganic anion concentrations were evaluated in samples taken from a constructed wetland at Wright-Patterson Air Force Base, Ohio during the summer and fall of 2003. These analytes are indicative of redox conditions in the subsurface and suggest the occurrence of microbial activities that degrade chlorinated ethenes to innocuous end products. Organic acid concentrations decreased by 100% from July 2003 to fall 2003. Combined with data collected previously during the months of December and January, this suggested that changing seasons and temperature fluctuations have a significant influence on microbial metabolisms. Nitrate and sulfate reduction above stratum C indicated mildly reducing conditions in the lowest stratum that became more highly reducing in the upper two strata. Based on the changing analyte concentrations throughout the wetland cell over several seasons, it was evident that the appropriate subsurface conditions existed for the reductive dechlorination of chlorinated ethenes.