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Treatment of chlorinated volatile organic compounds using wetland systems

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TREATMENT OF CHLORINATED VOLATILE ORGANIC COMPOUNDS USING WETLAND SYSTEMS

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Civil and Environmental Engineering

by

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ABSTRACT

Bench-scale continuous vertical flow column and microcosm studies were conducted to investigate the attenuation potential of chlorinated volatile organic compounds (CVOCs) in constructed wetland soil mixtures prepared from peat, compost and sand and in a pristine natural freshwater wetland soil. The study also determined geotechnical properties of potential synthetic peat mixtures for construction of treatment wetlands for CVOCs. Trichloroethene (TCE), cis-1,2-dichloroethene (cis-1,2-DCE) and 1,2-dichloroethane (1,2-DCA) were the main test chemicals used during the studies. Based on geotechnical and sorption characteristics, two mixtures (one comprised of sand and peat and the other comprised of sand, peat and compost product) were selected out of ten potential soil mixtures for column and sorption studies as the most promising materials for construction of the wetland. Faster removal kinetics and higher sorption potential were observed in peat/compost/sand mixture than in peat/sand mixture for all pollutants tested. Degradation kinetics of cis-1,2-DCE and 1,2-DCA were observed to be fastest under methanogenesis followed by sulfate-and sulfite-reducing conditions. Detection of the 16S rDNA gene of Dehalococcoides sp. via DNA extraction, PCR amplification, cloning and sequencing was directly correlated with complete dechlorination of TCE and cis-1,2-DCE to ethene. Two steady-state solute transport models were used to model TCE attenuation in natural and constructed wetland mesocosms, one equation had a dispersion term whereas another equation ignored the dispersion effects. Both models were able to capture the spatial concentration trends of TCE in the soil columns equally well. However, the model without the dispersion term underestimated the TCE removal rate.
constant by a factor of at least two compared with the other model. TCE attenuation rate constants were observed to be significantly faster in the constructed wetland columns than in the natural wetland columns. Model simulations indicated that attenuation of TCE in the constructed wetland columns was controlled by biodegradation whereas both sorption and biodegradation were equally important removal mechanisms in the natural wetland columns. The results of this study may be useful in establishing design information for treatment wetlands for CVOCs.
CHAPTER 1: INTRODUCTION

1.1 Background and Rationale of the Study

Contamination of groundwater by chlorinated aliphatic hydrocarbons such as trichloroethylene (TCE), tetrachloroethylene (PCE) and 1,1,1-trichloroethane (1,1,1-TCA) throughout the world has mainly been caused by accidental spills and leaks from chemical disposal and storage facilities (Wiedemeier et al., 1996; Kao and Lei, 1999; Hendrickson et al., 2002). These pollutants may cause adverse health effects following exposure (Wiedemeier et al., 1996; EPA, 2002). The maximum contaminant level (MCL) for protection of human health and the environment for isomers of dichloroethene (DCE), PCE, 1,2-dichloroethane (1,2-DCA) and TCE is 5 µg/L whereas that of vinyl chloride (VC), a proven carcinogenic, is 2 µg/L (EPA, 2002).

Contamination of groundwater by chlorinated solvents is one of the most difficult problems to solve and, as such, the problem has attracted the attention of many researchers and professionals in the environmental protection field. In the last three decades, there have been a number of technological innovations for remediation of halogenated volatile organic compounds (VOCs) in groundwater, including pump and treat systems, air stripping, air sparging, carbon adsorption, flameless thermal oxidation, catalytic oxidation, natural attenuation, in-situ air sparging with horizontal wells, and methane enhanced bioremediation using horizontal wells. Others are reactive walls or cells, UV oxidation, six-phase soil heating, dynamic underground stripping of volatile organic compounds, vacuum vapor extraction, enhancement with hydrogen peroxide, and phytoremediation to mention a few of them (Boulding, 1995; Hoffmann, 1997). Pump
and Treat (P&T) systems were once the standard approaches for remediation of halogenated VOCs in groundwater. Today, environmental professionals realize that P&T systems rarely achieve their goal of complete site cleanup, especially for chlorinated solvents and they have lengthy time requirements and prohibitive maintenance and operation costs (Duba et al., 1996; Hoffmann, 1997; Kao and Lei, 1999). Therefore, more economic and less expensive approaches are desirable for groundwater remediation to provide for long-term control of contaminated groundwater. One of the promising cost-effective approaches for remediation of aquifers contaminated with chlorinated solvents is natural attenuation using natural and constructed wetlands (Lorah et al., 1997; Lorah and Olsen, 1999; Pardue et al., 2000). Natural treatment systems such as natural and constructed wetlands are similar to conventional treatment systems in that both require energy to operate. However, energy for conventional treatment systems is typically of a nonrenewable fossil fuel. Natural treatment systems require the same amount energy input for every kilogram of pollutant degraded as conventional systems, but the source for energy is the sun, wind, rain, soil and biomass. The driving cost for a natural treatment system is the amount of land required, whereas the driving cost of a conventional treatment system is the amount of energy required (Kadlec and Knight, 1996).

Wetlands are defined as “lands transitional between terrestrial and aquatic systems where the water table is usually at or near the surface or the land is covered by shallow waters” (Knight and Kadlec, 1996). The multiple functions and value of wetlands have been widely recognized during the last three decades (Pardue, 1992; Knight and Kadlec, 1996). Among their ecological importance as transition zones between land and
water and as habitats with great diversity and heterogeneity, natural and constructed wetlands are used extensively for water quality improvement (Knight and Kadlec, 1996). Initially, wetlands were employed mainly to treat point-source wastewater, followed later by an increased emphasis on nonpoint-source urban and agricultural runoff (Schulz and Peall, 2001).

While the fate and retention of nutrients and sediments in wetlands are understood quite well, the same cannot be claimed for chlorinated VOCs (Lorah and Olsen, 1999). Only a few studies refer to the potential of wetlands for removal of chlorinated VOCs, probably the most notable one is the field and laboratory study on degradation of chlorinated VOCs in a freshwater tidal wetland at Aberdeen Proving Ground by Lorah and co-workers (Lorah et al., 1997; Lorah and Olsen, 1999). Results of this study (to be discussed in detail later in this chapter) suggested that organic matter dominated wetlands have natural attributes, which enable them to attenuate chlorinated VOCs effectively. Another study conducted by Pardue (1992) investigated the capacities of mineral and organic matter dominated wetlands to assimilate hexachlorobenzene. He found that freshwater marsh soil was more capable of degradation of hexachlorobenzene than bottomland hardwood soil (Pardue, 1992), thus implying that a bottomland hardwood wetland system is likely to suffer more ecological damage than a freshwater marsh system in case of pollution due to spillage of hexachlorobenzene.

Since wetlands have a high ability to retain and process carbonaceous material, it seemed reasonable that constructed wetlands may be used for treatment of groundwater contaminated with halogenated VOCs. Although treatment of halogenated solvents using natural wetland systems may not gain acceptance in the immediate future (as it was the
case for domestic wastewaters) it is still worth to determine assimilative capacities of wetland systems for the solvents in case of contamination (Pardue, 1992).

1.2 Basic Treatment Concept of Chlorinated Hydrocarbons in Constructed Wetland

Removal of VOCs has been shown to be remarkably fast in natural peat wetlands bordering the Aberdeen Proving Grounds, MD (Lorah et al., 1997; Lorah et al., 1999). Attenuation of high concentration of trichloroethylene (TCE) and 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA) has been observed as groundwater flows through the base of these marshes and then upward to the surface as Figure 1.1 illustrates. Important fate mechanisms include sorption of VOCs onto peat, reductive dechlorination of VOCs by halorespiring organisms in the anaerobic portion of the marsh and cometabolism of chlorinated VOCs by methanotrophic bacteria in aerobic portions of the marsh (the root rhizosphere). Based on these field observations, a concept for chlorinated VOCs removal by constructed wetland may be formulated.

Contaminant fate and transport within a wetland system are complex dynamic processes involving countless interactions among various entities and parameters. A combination of natural attenuation processes are involved in reduction of contaminant concentrations, mobility, or toxicity in wetlands. Possible natural attenuation processes include biodegradation, abiotic chemical transformation, volatilization, sorption, dispersion, plant uptake and metabolism. Biodegradation is commonly the primary destructive process for organic groundwater contaminants, because abiotic chemical reactions occur at a slower rate than biologically mediated reactions for most contaminants (Lorah et al., 1997; Lee et al., 1999).
Sorption and volatilization are nondestructive processes that reduce dissolved concentrations by transferring the contaminants to another media (sediment and air, respectively). Dispersion, which consists of molecular diffusion and mechanical mixing, results in a decrease in contaminant concentrations but not in a net loss of contaminant mass (Wiedemeier et al., 1999). Accounting for attenuation of a contaminant by all these processes and their interactions, if possible, would be a very difficult undertaking. In
view of this, simplification of this system was deemed necessary in investigation of fate and transport of contaminants within a wetland system in identification of crucial parameters to be considered in designing constructed treatment wetlands for groundwater contaminated with chlorinated solvent. A simplified conceptual model of the treatment wetland for chlorinated solvents is shown in Figure 1.2. Chlorinated organics in the groundwater plume will reach the base of the wetland where conditions are more anaerobic, possibly iron-reducing or sulfate reducing. Under these conditions, reductive dechlorination, accelerated by carbon leaching from the wetland, will begin to produce lower chlorinated daughter products. As the water reaches the methanogenic overlying peat, reductive dechlorination processes will be accelerated, completely converting the parent compounds. In addition, the highly organic soil will slow the movement of the compounds relative to water resulting in longer detention times and thus, more complete degradation.

As the water moves up into the plant root zone, the lower chlorinated daughter products will pass through a high surface area rhizosphere populated by methanotrophic bacteria. At this location the daughter and remaining parent products may be degraded to CO₂. Plant uptake will act in a complementary way to remove any remaining parent or daughter compounds. The mass of target organics will decrease as the water moves closer to the surface. Very low concentrations of chlorinated organics should reach the surface thus presenting little risk to the wetland itself. Chlorinated solvents-contaminated groundwater will be introduced by uniform distribution, through a piping network installed at the bottom of the wetland. The purpose of this design is to create a uniform,
vertical flow, through the wetland sediment. Total water depth can be controlled via an outlet control device (weir or orifice), ensuring complete saturation of the entire sediment of the wetland.

Based on this concept, several questions need to be addressed before embarking on a full-scale constructed treatment wetland (CTW). What is an appropriate substrate that will allow the passage of the design flow of water in an upflow mode? Which contaminant fate and transport processes are important in attenuation of VOCs? What are the VOC sorption properties of the selected substrate? Which wetland substrate material will favor halorespiring bacteria over other bacterial groups in competition for hydrogen?
What is the degradation potential of VOCs in the selected substrate? Does it produce conditions favorable to reductive biodegradation reactions after flooding and what length of time is needed before biodegradation can be observed? What will be degradation kinetics of VOCs under different reducing sedimentary environments? Finally, which organisms are responsible for mediating reductive dechlorination reactions? These questions were addressed in the current study by conducting geotechnical measurements, sorption potential, batch microcosm and continuous flow soil column studies, and molecular analysis of 16S ribosomal DNA from wetland soils. The study was conducted to establish some information, which may be useful for the design process of constructed treatment wetlands for chlorinated ethenes and ethanes.

1.3 Transformation of Chlorinated Aliphatic Compounds

The anaerobic transformation of chlorinated ethenes and ethanes has been extensively studied. Research described in this dissertation focused on a number of chlorinated aliphatic compounds including TCE, cis-1,2-dichloroethene (cis-1,2-DCE), 1,2-DCA, 1,1,1-TCA and VC. TCE is one of the most common organic groundwater pollutants in the United States (Wiedemeier et al., 1996). Field studies and laboratory evidence for dechlorination of TCE in the absence of oxygen have been widely reported (for example, Lorah et al. 1997, Ballapragada et al., 1997, and Haston and McCarty, 1999).

For anaerobic dechlorination, the chlorinated VOCs serve as electron acceptors. Each dechlorination step requires two electrons, and therefore an electron donor is necessary to complete the reaction (Ballapragada et al., 1997). Thus, for dechlorination to occur, an electron donor must be provided, and numerous organic substrates such as
propionate, butyrate, and benzoate have been shown to serve this function, perhaps by being fermented to hydrogen that serves as the primary electron donor for dehalogenation (Wiedemeier et al., 1996; Ballapragada et al., 1997; Fennel et al., 1997; Fennel et al., 1998; Yang et al., 1998; Haston and McCarty, 1999).

Given an appropriate electron donor under anaerobic conditions, TCE is reductively dechlorinated stepwise through, \( cis-1,2 \)-DCE, vinyl chloride (VC), and then to ethene (Haston and McCarty, 1999). The intermediate VC is a known human carcinogen, and thus its formation and persistence is of special concern.

Chlorinated solvents may also be biodegraded through cometabolism. In cometabolism, the degradation of the compound is catalyzed by an enzyme cofactor that is fortuitously produced by organisms for other purposes (Wiedemeier et al., 1996). The compound serves as neither an electron acceptor nor a primary substrate in a biologically mediated redox reaction. Cometabolism is, however, not nearly as important a degradation mechanism for chlorinated VOCs in the saturated zone of most aquifers as in reductive dechlorination (McCarty and Semprini, 1994).

Some chlorinated VOCs may also be degraded through direct biological oxidation. Less oxidized compounds such as isomers of DCE can be used as primary substrate under aerobic conditions (Wiedemeier et al., 1996). VC has been reported to be more rapidly degraded through aerobic biodegradation than through reductive dehalogenation (Wiedemeier et al., 1996; Maymó-Gatell et al., 2000).

According to Haston and McCarty (1999), the rate of dehalogenation of a given chlorinated VOC may be influenced by a number of factors, including the concentrations of both the electron donor and acceptor (chlorinated VOC), potential toxicity of the
chlorinated VOC itself, and competitive inhibition. The rate of dechlorination may also be influenced by the prevailing reduction-oxidation (redox) reactions (Lovley et al., 1994) and the rate of production of hydrogen from the soil substrate (Cord-Ruwisch et al., 1988; Yang and McCarty, 1998) because hydrogen is thought to be the primary electron donor for most of the dechlorinating bacteria (Ballapragada et al., 1997).

The extent of reductive dechlorination depends on the types of organisms responsible for mediating dechlorination reactions (Holliger et al., 1993; McCarty, 1997; Maymó-Gattel et al., 1997). Only one microorganism, a recently discovered microbial species, *Dehalococcoides ethenogenes* strain 195 described by Maymó-Gattel et al. (1997) has been isolated in pure culture that can dechlorinate PCE and TCE completely to ethene. Other microbial species that have been isolated in pure culture are able to dechlorinate PCE and TCE only to the *cis*-1,2-DCE and VC stages. This is especially undesirable in cases where anaerobic degradation is the only contaminant removal mechanism because VC is a human carcinogen that poses a greater risk to human health than either PCE or TCE (Holliger et al., 1993; McCarty, 1997). In view of this, the success of remediation of contaminated sites under predominantly anaerobic conditions depends on the availability of organisms, which are capable of dechlorinating the target contaminants all the way to innocuous chlorine-free products.

In the research reported in this dissertation, fate and transport processes of chlorinated solvents in natural and constructed wetland systems and geotechnical properties of the wetland soils were investigated. The potential of natural and constructed wetlands to assimilate chlorinated solvents and the geotechnical characteristics of the wetland soils were compared and contrasted. Organisms responsible for dechlorination
were identified using a 16S ribosomal DNA gene-based detection method. The results of the study will provide useful data for formulation of design considerations for treatment wetlands for groundwater contaminated with chlorinated VOCs.

1.4 Objectives

The overall objective of this dissertation was to investigate the potential of natural and constructed wetlands for remediation of groundwater contaminated with chlorinated aliphatic compounds. The study involved monitoring of the transport and fate of chlorinated VOCs in wetland soil columns to obtain information necessary to provide future design parameters for treatment wetlands for chlorinated VOC-contaminated groundwater. The research work also involved conducting wetland microcosm (batch) studies and measurement of geotechnical properties of wetland soils. The specific objectives were as follows:

(i.) Determination of the kinetics of degradation of chlorinated VOCs in natural and constructed wetland mesocosms and microcosms under methanogenic conditions.

(ii.) Determination of sorptive capacities of natural and constructed wetland soils.

(iii.) Evaluation of the potential for fluidization and determination of transport parameters in natural and constructed wetland soils.

(iv.) Determination of the degradation kinetics of cis-1,2-DCE and 1,2-DCA in constructed wetland soil microcosms under methanogenic, sulfite- and sulfate-reducing conditions and the associated H₂ concentration levels and bioenergetics of the terminal electron accepting processes.

(v.) Identification of the organisms responsible for reductive dechlorination of the chlorinated solvents.
This dissertation was prepared in a journal format. All chapters are being submitted to peer-reviewed journals. Individual chapters contained in this dissertation serve to answer the research questions and address the research objectives for which this study was intended. Chapter 1 presents the rationale for conducting the study, research questions and objectives of the study for which this dissertation was prepared. Chapter 2 gives sorption and biodegradation potentials and hydraulic characteristics of two candidate soil substrate mixtures for construction of an upflow treatment wetland for chlorinated solvents (cis-1,2-DCE and 1,1,1-TCA, VC, chloroethene and 1,2-DCA) at a Superfund site. A 1-D mass transport model was used to determine the effective wetland bed depths required to meet the National Primary Drinking Water Regulations for the target contaminants for different scenarios. The results of this paper have been published in *Ecological Engineering* (The Journal of Ecotechnology) (Kassenga et al., 2003).

Transformation kinetics of cis-1,2-DCE and 1,2-DCA in two constructed wetland soils under anaerobic and aerobic conditions and identification of organisms involved in anaerobic reductive dechlorination reactions using a PCR based molecular analysis approach are covered in Chapter 3. Under anaerobic conditions, cis-1,2-DCE was observed to be transformed significantly faster than 1,2-DCA in both types of soils, whereas the opposite was true under aerobic conditions.

In Chapter 4, hydrogen dynamics during anaerobic transformation of cis-1,2-DCE and 1,2-DCA and the effects of 2-bromoethanesulfonate (an inhibitor of methanogenesis) on the degradation kinetics of the test chemicals and the associated hydrogen concentrations are investigated and presented. During continuous dechlorination, significantly lower hydrogen concentrations were observed for cis-1,2-DCE as compared
with 1,2-DCA. 2-bromoethanesulfonate did not inhibit dechlorination but the inhibitor slowed down degradation kinetics of the test chemicals.

Dechlorination kinetics of 1,2-DCA and cis-1,2-DCE and the associated hydrogen concentrations and the energetics of terminal electron acceptor processes (TEAPs) under sulfite-reducing, sulfate-reducing and methanogenic conditions are covered in Chapter 5. Kinetics of transformation of 1,2-DCA and cis-1,2-DCE were observed to be fastest under methanogenic conditions followed by sulfate-and sulfite-reducing conditions and the test chemicals could not be concomitantly dechlorinated under methanogenic conditions. Thermodynamic calculations and hydrogen data were used to discern the terminal electron accepting processes (TEAPs) occurring in the microbial systems.

Chapter 6 presents the results on investigation of inherent potential of the freshwater natural wetland sediments and the effectiveness of the constructed wetland soil to attenuate TCE using saturated continuous-flow columns. TCE sorption potentials and hydraulic and geotechnical properties of the soils were also investigated. TCE monitoring data in the soil columns were modeled using two different contaminant mass transport models and compared. One transport model disregarded dispersion while the other model incorporated it. Biodegradation processes observed in the soil columns and the associated hydrogen concentrations were confirmed using microcosms studies. Organisms responsible for degradation of TCE past cis-1,2-DCE in the soil columns were identified. Ignoring dispersion in the contaminant transport model underestimated degradation kinetic constants of TCE. Significantly higher TCE degradation kinetics were observed in the constructed columns than in the natural wetland mesocosms in which breakthroughs of TCE and degradation products were observed throughout the
study period. The results indicate that TCE attenuation potential of the natural wetland is limited and that constructed treatment wetland is a more promising alternative for remediation of aquifers contaminated with chlorinated solvents.

Chapter 7 summarizes major findings and important conclusions from this dissertation. Implications of the results, unsolved problems and recommendations for future research are also presented in this chapter.
CHAPTER 2: TREATMENT OF CHLORINATED VOLATILE ORGANIC COMPOUNDS IN UPFLOW WETLAND MESOCOSMS

2.1 Introduction

Chlorinated VOCs are suspected carcinogens that are common groundwater pollutants and pose special treatment problems (Wiedemeier et al., 1996). Natural treatment systems including treatment wetlands may provide a cost-effective alternative for contaminated groundwater, either by intercepting groundwater plumes or by serving as treatment for pumped groundwater (Pardue et al., 2000). Removal of volatile organic compounds (VOCs) has been shown to be remarkably fast in natural peat wetlands bordering the Aberdeen Proving Grounds, MD (Lorah et al., 1997; Lorah and Olsen, 1999a,b). Attenuation of high concentration of trichloroethene (TCE) and 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA) has been observed as groundwater flows through the base of these marshes and then upward to the surface. Important fate mechanisms include sorption of VOCs onto peat, reductive dechlorination of VOCs by microorganisms in the anaerobic portion of the marsh and cometabolism of chlorinated VOCs by methanotrophic bacteria in aerobic portions of the marsh (the root rhizosphere) (Lorah et al., 1997). The efficiency of these reactions suggests that an upflow treatment wetland could be designed to mimic behavior of these natural systems. A preliminary design approach for treatment wetlands for VOCs has been published (Pardue et al., 2000).

In a treatment wetland system for VOCs, contaminated groundwater would be pumped into the wetland bed in an upflow mode to minimize volatilization. Chlorinated

---

organics in the groundwater plume would be applied to the base of the wetland where conditions are anaerobic. Under these conditions, reductive dechlorination of chlorinated VOCs is possible. Efficient reductive dechlorination has been observed in wetland soils for a number of contaminants (Parsons et al., 1984; Parsons and Barrio-Lage, 1985; Barrio-Lage et al., 1986; Pardue et al., 1996; Jackson and Pardue, 1998; Lorah et al., 1997; Lorah and Olsen, 1999a,b). As the water moved upwards, sorption of VOCs onto the highly organic soil would retard the movement of the compounds resulting in longer detention times (Lorah et al., 1997). Within the plant root zone, the lower chlorinated daughter products would pass through a high surface area rhizosphere populated by methanotrophic bacteria (King, 1994; Calhoun and King, 1997). At Aberdeen Proving Ground, microcosm studies indicated that daughter and remaining parent products could be degraded to CO₂ via this process (Lorah and Olsen, 1999a). Plant uptake would act in a complementary way to remove any remaining parent or daughter compounds (Burken and Schnoor, 1998). The mass of target organics would decrease as the water moves closer to the surface. Very low concentrations of chlorinated organics should reach the surface, thus presenting little risk to wetland biota.

Before embarking to a full-scale treatment wetland, it was necessary to conduct bench-scale studies to investigate the feasibility of using constructed wetlands for treatment of VOCs. The overall objective of the bench-scale studies was to provide information for establishing the feasibility of a pilot-scale treatment wetland for a Superfund site in Connecticut. The specific objectives of the study were as follows: (1) to assess the hydrodynamics of VOC-contaminated water flowing through peat beds in a wetland mesocosm, (2) to measure sorption potential of candidate peats, (3) to measure
degradation potential of target compounds in candidate peat substrate mixtures in a bench-scale treatment wetland, (4) to correlate degradation in bench-scale wetlands with natural attenuation parameters, and (5) to confirm evidence of biodegradation processes observed in the bench-scale soil columns and to examine transformation patterns of representative chlorinated ethanes and ethenes using anaerobic microcosms.

2.2 Materials and Methods

- Candidate Substrate Mix Selection Approach

Ten substrate mixtures were prepared for hydraulic conductivity measurements based on the results of previous geotechnical testing (dry bulk density, moisture content, organic carbon content, grain size analysis, and pH). The objective of this testing was to evaluate which materials would be suitable for bench-scale studies. Results of hydraulic conductivity tests were used to select two substrate mixtures for measurement of sorption and degradation potentials of target compounds in a bench-scale treatment wetland.

Geotechnical testing was performed on the four basic candidate substrates Bion Soil (Dream Maker Dairy, Cowlesville, NY), a product derived from agricultural waste; and three commercially available peats; Premier (Premier Horticulture Ltd., Dorval, Quebec, Canada), Latimer (Latimer's Peat Moss Farm, West Liberty, OH) and Worcester (Worcester Peat Co., Inc., Cherryfield, ME). Hydraulic conductivity was determined using the falling head method (Charbeneau, 2000). Table 2.1 presents a summary of geotechnical characteristics of the tested substrates.

Fine to medium sand was used for preparing the mixtures. Four additional mixes (7 through 10) were developed based on the following general criteria: (1) the overall mix should result in a volume-averaged density greater than 62.4 pounds per cubic foot (pcf)
(1 kg/L) and preferably above 75 pcf (1.2 kg/L) (to prevent flotation of the material in water and to withstand an induced upward hydraulic gradient), (2) the overall mass-averaged total organic content (TOC) should be greater than 20% and preferably nearing 50% (typical organic carbon content of a mature wetland peat) suitable for significant microbial activity; and (3) the overall mass-averaged pH should be in the neutral range near pH 7. The composition and rationale for each mixture is presented in Table 2.2.

Table 2.1: Summary of substrate characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bion Soil</th>
<th>Latimer</th>
<th>Premier</th>
<th>Worcester</th>
<th>Sand (estimated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated Density at 5 psi surcharge (pcf)</td>
<td>34.6</td>
<td>8.9</td>
<td>14.2</td>
<td>8.3</td>
<td>100</td>
</tr>
<tr>
<td>TOC %</td>
<td>57.1</td>
<td>82.5</td>
<td>96.5</td>
<td>99.6</td>
<td>0</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>2.9</td>
<td>5.3</td>
<td>2.9</td>
<td>7</td>
</tr>
<tr>
<td>D10 (mm)</td>
<td>0.002</td>
<td>0.15</td>
<td>0.15</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Saturated Moisture Content (%)</td>
<td>125.4</td>
<td>602.4</td>
<td>349.3</td>
<td>591.8</td>
<td>-</td>
</tr>
<tr>
<td>Saturated Unit Weight (pcf)</td>
<td>78</td>
<td>66</td>
<td>63.8</td>
<td>57.4</td>
<td>120</td>
</tr>
</tbody>
</table>

1 pound per cubic feet (pcf) = 0.016 kg/L

- Sorption Isotherms

Sorption experiments were done using 40 mL VOA vials (ICHEM) containing the Superfund site water, sodium azide as a biocide, and 12 g (dry weight) of peat. Except for TCE no other VOC was added to the site water prior to sorption studies. Six points of the isotherm were developed across the range of water concentrations of VOCs. Isotherm points were generated by diluting the site water with an electrolyte solution (0.01 M CaCl₂) using dilutions of 1:1, 1:2, 1:5, 1:10 and 1:20. Three replicate vials were used for each isotherm point for a total of 18 vials. Simultaneous measurement of partitioning of TCE, cis-1,2-DCE, 1,1,1-TCA and 1,1 dichloroethane (1,1-DCA) was performed.
Samples were shaken on a reciprocating shaker at 20°C for 48 hours, centrifuged, and the supernatant analyzed for target compounds using EPA Method 8260B, as described below.

Table 2.2: Summary of selected substrate mixtures and rationale

<table>
<thead>
<tr>
<th>Mix</th>
<th>Bion Soil * (%)</th>
<th>Latimer * (%)</th>
<th>Premier * (%)</th>
<th>Sand * (%)</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Determine permeability of Bion Soil.</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>Evaluate the potential effect of adding sand on Bion Soil permeability.</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>Determine permeability of Latimer.</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>80</td>
<td>-</td>
<td>20</td>
<td>Evaluate the potential effect of adding sand on Latimer permeability.</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>Determine permeability of Premier.</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>80</td>
<td>20</td>
<td>Evaluate the potential effect of adding sand on Premier permeability.</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>40</td>
<td>-</td>
<td>20</td>
<td>Evaluate permeability of the Bion Soil and Latimer mix.</td>
</tr>
<tr>
<td>8</td>
<td>40</td>
<td>-</td>
<td>40</td>
<td>20</td>
<td>Evaluate permeability of the Bion Soil and Premier mix.</td>
</tr>
<tr>
<td>9</td>
<td>70</td>
<td>-</td>
<td>30</td>
<td>-</td>
<td>Evaluate permeability of Bion Soil with Premier amendment for increased TOC.</td>
</tr>
<tr>
<td>10</td>
<td>70</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>Evaluate permeability of Bion Soil with Latimer amendment for increased TOC.</td>
</tr>
</tbody>
</table>

* All values are percent by volume.

- **Bench-Scale Wetland Apparatus**

  Column experiments were performed in three 60 cm long, 15 cm diameter glass columns with 5 sampling ports equipped with Mininert Valves (VICI Precision Sampling,
Baton Rouge, LA) along their lengths (Figure 2.1). Soil column apparatus and accessories were made from Viton, Teflon, stainless steel, and glass to avoid adsorption of VOCs. The experiment was set-up in a temperature controlled greenhouse at 26±3 °C. Three mesocosms were constructed, two with the candidate peats (Mix 4, a combination of Latimer peat and sand, and Mix 7, a combination of Latimer peat, sand and Bion Soil) and one with a clean autoclaved sand. The sand column was used as a reference column since neither sorption nor degradation of VOCs was expected to occur in the column primarily due to the absence of organic matter. The mesocosms were glass cores packed to a uniform bulk density. Column and flow characteristics are shown in Table 2.3. A 1-cm layer of fine gravel was placed at the bottom for even distribution of flow through the column. Mix 4 and Mix 7 columns were planted with 5 young Scirpus americanus shoots approximately 6 inches in length. To avoid introducing organic matter, the sand column was not planted. Glass wool was inserted in the inner side of sampling ports to minimize dead zones and clogging of sampling ports. The sides of the columns were wrapped with aluminum foil to keep off light for minimization of growth of photosynthetic organisms.

Groundwater from a Superfund site contaminated with chlorinated ethenes and chlorinated ethanes was introduced through 2-mm i.d. stainless steel tubing in an upflow mode. Contaminated groundwater was shipped from the site on a weekly basis under refrigerated conditions to minimize losses of VOCs during transportation. The water was neither treated nor contaminants added prior to introduction into the columns.

The peat columns were fully saturated and flooded to a depth of 10 cm above the level of the packing medium (see Figure 2.1). A pump (Cole Parmer Model No. 7523-40) with Viton tubing was used to convey the site groundwater through the column.
Table 2.3: Physical characteristics of columns and flow data

<table>
<thead>
<tr>
<th>Characteristics/Parameters</th>
<th>Soil Column</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mix 4</td>
</tr>
<tr>
<td><strong>Column characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Length (cm)</td>
<td>60</td>
</tr>
<tr>
<td>Diameter (i.d.) (cm)</td>
<td>15</td>
</tr>
<tr>
<td>Total volume ($V_T$) (cm$^3$)</td>
<td>10,603</td>
</tr>
<tr>
<td>Bulk density, ($\rho_b$) (g/cm$^3$)</td>
<td>1.20</td>
</tr>
<tr>
<td>Media density ($\rho$) (g/cm$^3$)</td>
<td>$0.34 \pm 0.09$</td>
</tr>
<tr>
<td>Media total mass (g)</td>
<td>3605</td>
</tr>
<tr>
<td>Media porosity ($\varepsilon$) (cm$^3$/cm$^3$)</td>
<td>$0.76 \pm 0.04$</td>
</tr>
<tr>
<td>Pore volume ($V_p$) (cm$^3$)</td>
<td>8,058</td>
</tr>
<tr>
<td><strong>Flow data</strong></td>
<td></td>
</tr>
<tr>
<td>Pore velocity ($u_p$) (cm/s)</td>
<td>$1.87 \times 10^{-4}$</td>
</tr>
<tr>
<td>Hydraulic residence time ($\tau$)$^b$ (day)</td>
<td>9.3</td>
</tr>
<tr>
<td>Measured flow rate $Q$ (cm$^3$/min)</td>
<td>$0.60 \pm 0.09$</td>
</tr>
<tr>
<td>Hydraulic conductivity ($k$) (cm/s)</td>
<td>$4.95 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

$^a$ Typical value for medium sand (Bouwer, 1978; Kasenow, 1997)

$^b$ Hydraulic residence time; $\tau = V_p/Q$

Headloss (based on pressure differences between the bottom and top of the core) and the actual flow rate were measured every 7 days in the peat cores. These measurements were used to estimate pore velocity and calibrate a mass transport equation to describe system performance. Actual flow rates were determined by measuring the volume of water applied to each core in a specified duration of time.

Influent solutions were refrigerated throughout the experiment at about 4 °C to minimize volatilization. In addition, a floating cap was used to prevent the development of a headspace in the bottle. The influent concentrations were monitored three times a
Figure 2.1: Schematic diagram of the bench-scale wetland treatment system.
week to ensure that changes in concentration were minimal. Influent samples for all column experiments were collected from the delivery side of the pump. Samples for analysis of chlorinated VOCs were collected by withdrawing porewater from the axial center of the soil columns with a prerinsed gastight 10 mL Hamilton glass syringe fitted with luer-lock and injected into 2 mL glass vials with liquid facing Teflon lined caps. Six samples were taken for each core, one from the inlet tube, one from 5 sampling ports located along the length of the columns (Figure 2.1), and one sample from the effluent (5 cm below the standing water level on top of the core). Sampling of the ports for the first three months was performed at seven days intervals, thereafter the sampling interval was increased to between two and three weeks. The column experiments were run continuously for 320 days with brief periods of interruptions during maintenance of the soil column systems.

- **Anaerobic Microcosm Experiments**

  Following the conclusion of the mesocosms studies, the Latimer peat and sand mixture (Mix 4) and the Latimer peat, sand and Bion Soil mixture (Mix 7) soil were removed from the glass cores minimizing disturbance. The bottom 10 cm of each core was sectioned and used to construct anaerobic microcosms. The bottom section was selected for construction of microcosms because more than 50% of all contaminants were removed within 10 cm from the core bottom in both Mix 4 and Mix 7 soil columns.

  Construction of anaerobic microcosms was done under a nitrogen atmosphere in a glove-bag by homogenizing and placing soil in 160 mL serum bottles using a 1.5:1 volumetric ratio of groundwater to wetland soil (Lorah et al., 1997). All reaction mixtures were sealed with Teflon-lined rubber septa and aluminum crimp seals and incubated in an
inverted position under static conditions at 25 °C in the dark, which was the approximate temperature of the groundwater during the soil column experiments. Resazurin (0.0002%) was added as a redox indicator. Three replicates were prepared for each treatment. Initially, groundwater contaminated primarily with cis-1,2-DCE and 1,1,1-TCA from the Superfund site was used for the experiments. After all the contaminants were reduced to below detection, one compound representative of chlorinated ethanes (1,2-dichloroethane (1,2-DCA)) and one representative of chloroethenes (1,2-cis-DCE) were selected for further investigation of transformation kinetics and biodegradation routes. Although 1,2-DCA was not one of the prominent contaminants in the groundwater used in initial tests, the chemical was, however, chosen because it can dechlorinate via hydrogenolysis and dichloroelimination mechanisms. cis-1,2-DCE and 1,2-DCA at a concentration of about 5 mg/L each were consecutively spiked three times into each microcosm for a total of nine runs per treatment. This was done to further determine degradation patterns and mechanisms of biodegradation of the contaminants. Temporal monitoring of concentrations of parent compounds and daughter products was done after each spike until the concentrations of the contaminants dropped below the analytical method detection limit. Stock solutions of cis-1,2-DCE and 1,2-DCA were made from neat chemicals and added to the microcosms using gas-tight syringes to give the planned initial dissolved concentrations. Abiotic controls were included in the study to monitor for non-biological losses of the test chemicals. Microcosms were prepared as described above, and were adjusted to contain 1% formaldehyde.

Samples were withdrawn from the bottles and immediately analyzed without storage for chlorinated ethenes and ethanes, ethene, ethane and methane at intervals
ranging from 6 to 24 hours. Biodegradation rates were calculated by a first-order approximation. When significant losses (greater than 5 percent) were observed in the abiotic microcosms, these losses were subtracted from the concentration in the non-abiotic microcosms before the first-order rate constant was calculated (Lorah et al., 1997).

- **Analytical Procedures**

  VOCs were analyzed using EPA Method 8260B using a purge and trap apparatus to concentrate and introduce the sample into the gas chromatograph-mass selective detector (Agilent 6890 gas chromatograph-5972A mass selective detector). Daily blanks, calibration checks and tunes were run to assure that the analytical method was in control. Recovery of dibromofluoromethane (Supelco Inc., Bellefonte, PA) surrogate injected into every sample also ensured that no gross dilution errors or leaks occurred in the GC-MS system.

  Porewater (25 mL) from the wetland cores was collected using a gastight Hamilton syringe for analysis of methane, ethene, and ethane. The water was transferred to a 25 mL glass vial fitted with a liquid facing Teflon cap. A headspace was created (10 mL) by displacing water using ultra pure nitrogen with a gastight syringe. The vial was heated using a hot plate at a temperature of 90°C to establish equilibrium. Henry’s Law constants are well known at these higher temperatures. Methane, ethene and ethane in porewater were measured using GC-FID. A subsample (3 mL) of gas in the headspace was withdrawn using a gastight syringe and injected into the GC-FID (Agilent 5890 Series II) equipped with a 2.4 m × 0.32 mm ID column packed with Carbopack B/1% SP-1000 (Supelco Inc., Bellefonte, PA). The column temperature was held at 40°C.
isothermally for 6.5 min, and the injector and detector temperatures were 375 and 325°C respectively. The carrier gas was ultra high purity nitrogen at a flow rate of 12 mL/min.

Measurement of temperature, pH, conductivity, oxidation-reduction potential (ORP), total dissolved solids, dissolved oxygen and conductivity was performed using a multimeter (Ultrameter by Myron L Company, Carlsbad, CA). Spectrophotometric methods were attempted for measurement of nitrite, nitrate, ferrous iron, ferric iron, chloride, sulfate and sulfide but suffered from the small volumes of water available. Hydrogen was analyzed using a reduction gas analyzer (RGA-5, Trace Analytical, Palo Alto, CA). Organic acids (lactate, formate, succinate, acetate, propionate, butyrate and benzoate) were analyzed using high-pressure liquid chromatograph (HP 1090 Series II Liquid Chromatograph).

Analysis of dissolved gaseous constituents (ethene, ethane, methane, hydrogen and oxygen), temperature, pH, conductivity, oxidation-reduction potential, total dissolved solids, and conductivity required withdrawals of large volumes (> 25 mL) of porewater, which would have substantially changed the spatial distribution of the VOCs. Due to this sample volume limitation, composite samples had to be strategically collected instead of sampling from each port. Two composite samples were collected by withdrawing 8 mL of porewater from each of the top two and bottom three sampling ports, representing the “root zone” and “below root zone” sections of the cores, respectively.

- **Chemicals**

Analytical standards and surrogate for the VOCs were obtained as mixtures or neat liquids from Supelco Inc. (Bellefonte, PA). Methane, ethane and ethene calibration
gases were obtained from Gas Products (Baton Rouge, LA). Other chemicals used in this study were reagent grade and were purchased from various vendors.

- **Data Modeling**

  The potential for fluidization of the wetland bed was evaluated as part of the design analysis. Fluidization may result in short-circuiting of water from the bottom distribution layer to the wetland surface and if sufficiently severe, potentially result in failure of the treatment wetland. Fluidization occurs when the difference in pressure forces between the lower and the upper layers exceed the gravitational force of the substrate mass. Under such conditions, the effective stress through the substrate will effectively become zero and fluidization may occur. The potential for fluidization was evaluated by considering the effective stress within the substrate bed and estimating the maximum flow rate to prevent fluidization. Effective stress, the portion of the total stress carried by the pore water, maintains peat stability. The reduction of effective stress resulting from upward seepage can be calculated as:

\[
\sigma = z(\gamma_{\text{sat}} - \gamma_w) - iz\gamma_w \tag{2.1}
\]

where \( z \) [L] is the depth of the substrate bed, \( \gamma_{\text{sat}} \) [M/L^3] is saturated unit weight of the substrate, \( \gamma_w \) [M/L^3] is unit weight of water, \( \sigma \) [M/L^2] is the effective stress and \( i \) [L/L] is the hydraulic gradient. A critical gradient, \( i_{cr} \) [L/L] can be defined by setting the effective stress equal to zero. This results in:

\[
i_{cr} = \frac{(\gamma_{\text{sat}} - \gamma_w)}{\gamma_w} \tag{2.2}
\]

Critical flow per unit surface area of the wetland, \( q_{cr} \) [L/T] is calculated as follows:

\[
q_{cr} = k i_{cr} \tag{2.3}
\]
where \( k \) [L/T] is the hydraulic conductivity of the wetland bed substrate.

The sorption distribution coefficient \( K_d \) [L\(^3\)/M] in a linear sorption model can be described by

\[
K_d = \frac{q_e}{C_e}
\]  
(2.4)

where \( q_e \) is the mass of chemical sorbed per unit mass of soil [M/M] and \( C_e \) [M/L\(^3\)] is the equilibrium concentration. Nonlinear isotherms can be described by the Freundlich equation:

\[
q_e = K_F C_e^N
\]  
(2.5)

where \( K_F \) [L\(^3\)/M] is the Freundlich sorption constant and \( N \) is the Freundlich exponent.

The organic carbon content normalized distribution coefficient \( K_{oc} \) [L\(^3\)/M] is described by

\[
K_{oc} = K_d f_{oc}
\]  
(2.6)

where \( f_{oc} \) is the fraction organic carbon content of the sample.

The linear partition coefficient of a compound \( K_p(VOC) \), can be calculated from known linear partition coefficient of another compound \( K_p(\text{known}) \), using the rearranged form of the classic Karickhoff relationship (Karickhoff, 1981):

\[
K_p(VOC) = \frac{K_p(\text{known}) \times K_{ow}(VOC)}{K_{ow}(\text{known})}
\]  
(2.7)

where \( K_{ow} \) [L\(^3\)/M] is the octanol-water partition coefficient.

A mass transport model was used to describe the movement of VOCs through the bed. The model is based on the following 1-D lumped first order kinetic reaction equation:

\[
C = C_0 e^{-kR\nu/v}
\]  
(2.8)
where \( C \) [M/L^3] is the concentration of the pollutant at a vertical distance, \( x \) [L], \( C_0 \) [M/L^3] is the initial concentration, \( k \) [T^{-1}] is a lumped temporal removal rate constant, \( R \) is the retardation coefficient (unitless) and \( v \) [L/T] is the seepage velocity.

The retardation coefficient \( R \) is calculated as follows

\[
R = \left( 1 + \frac{\rho_p}{\eta} K_d \right)
\]  \hspace{1cm} (2.9)

where \( \rho_p \) [M/L^3] is the bulk density of the medium, \( \eta \) is the porosity, \( K_d \) [L^3/M] is the linear distribution coefficient.

The mass transport model (Eq. 2.8) assumes that the effect of molecular diffusion/dispersion on contaminant transport is insignificant, a reasonable assumption for homogenous medium; that a degradation rate constant is available that integrates the biodegradation occurring in the aqueous and sorbed phases; and the system is at steady state.

The spatial lumped removal rate constant \( k_s \) [L^{-1}] can be calculated from the first order kinetic reaction,

\[
C = C_0 e^{-k_s x}
\]  \hspace{1cm} (2.10)

Other parameters have been defined above. It is worth noting that for a given contaminant, the spatial degradation rate constant does not account for sorptive and hydraulic properties of the substrate.

Modeling of cis-1,2-DCE and VC monitoring data in anaerobic microcosms was done using pseudo sequential dechlorination first-order kinetic models,

First step: \([\text{DCE}]_t=\text{[DCE]}_0 e^{-k_1 t}\)  \hspace{1cm} (2.11)

Second step: \([\text{VC}]_t=\frac{k_2}{k_2-k_1} \left( e^{-k_1 t} - e^{-k_2 t} \right)\)  \hspace{1cm} (2.12)
where \([\text{DCE}]_t [\text{M/L}^3]\) is the concentration of \(\text{cis}-1,2\)-DCE at any time \(t\); \([\text{DCE}]_0 [\text{M/L}^3]\) is the initial concentration of \(\text{cis}-1,2\)-DCE; \([\text{VC}]_t [\text{M/L}^3]\) is the concentration of VC at any time \(t\); \(k_1 [\text{T}^{-1}]\) and \(k_2 [\text{T}^{-1}]\) are the pseudo first-order reaction rate constants for \(\text{cis}-1,2\)-DCE and VC degradation, respectively.

### 2.3 Results and Discussion

- **Hydraulic Characteristics**

  Table 2.4 summarizes the hydraulic conductivity results and computes critical flows achievable without causing fluidization. Based on critical flow values presented in Table 2.4, Mix 4 (Latimer peat and sand mixture) and Mix 7 (Latimer peat, Bion Soil and sand) were selected as the most promising substrate mixtures for construction of the wetland bed. These mixtures were selected because they had the highest critical flow, implying that they are least prone to fluidization. Mixture 4 had an average hydraulic conductivity of \(4.95 \times 10^{-4} \text{ cm/s}\) with a critical flow of \(39.5 \text{ gpm/acre} (368 \text{ L/min/ha})\). Mix 7 had an average hydraulic conductivity of \(3.02 \times 10^{-4} \text{ cm/s}\) with a critical flow of \(36.8 \text{ gpm/acre} (344 \text{ L/min/ha})\). Therefore, sorption and column experiments were conducted for these mixes to determine their sorptive and chlorinated solvent degradation capabilities.

  Peat materials in general have low bulk density and hydraulic conductivity (as low as \(1.06 \times 10^{-7} \text{ cm/s}\)) (Lorah et al., 1997). Densities less than that of water are not uncommon for peat materials, for example Worcester, one of the peat materials used in this study had a saturated bulk density of \(0.92 \text{ kg/L}\). Low hydraulic conductivity will allow unreasonably low flows to pass through the wetland bed and peat material with saturated density lower than that of water will have a negative critical flow according to
Eq. 2.1 and will, therefore, float on water. For these reasons, peat materials were mixed with sand to increase their bulk densities and hydraulic properties. For example mixing Latimer peat (80% V/V) and sand (20% V/V) increased the bulk density and hydraulic conductivity of the original peat material by a factor of 1.2 and 3.5 respectively. The resulting organic carbon content of the mixtures was lower than that of “pure” materials by factors ranging between 1.7 and 3. However, the values of organic matter content of the mixtures were generally comparable to those of mature wetland peat (> 20%) as Table 2.4 shows.

- **Sorption and Retardation**

  Batch adsorption experiments were performed using the selected substrate mixtures (Mix 4 and Mix 7) as sorbates to determine the potential for retardation of the contaminants in the wetland soil columns. Isotherm data were fit by both a Freundlich model (Eq. 2.5) and a linear partitioning model (Eq. 2.4). Table 2.5 summarizes results of sorption experiments for the substrate mixtures. The Karickhoff relationship (Eq. 2.7) was utilized to predict the partitioning of VC due to its low concentration in the site groundwater. Both the linear and Freundlich models fit the data well ($r^2 > 0.85$). Based on these fits, there does not appear to be any reason to select the more complex, 2-parameter Freundlich model. Partition coefficients of all contaminants for Mix 7 (peat, sand and Bion Soil) were significantly higher than those for Mix 4 (peat and sand alone). This is attributed to the effect of the organic Bion Soil. Surprisingly, organic carbon content normalized distribution coefficients of some pollutants (notably 1,1-DCA and cis-1,2-DCE) for Mix 7 are also appreciably higher than those of Mix 4 (Table 2.5). The nature of organic matter has been reported to play a significant role in the sorptive behaviors of
Table 2.4: Summary results of hydraulic conductivity tests

<table>
<thead>
<tr>
<th>Mix</th>
<th>Composition</th>
<th>Volume Averaged Saturated Bulk Density, ( \rho_{\text{sat}} ) (pcf)</th>
<th>Submerged Unit Weight (1), ( \rho_{\text{sub}} ) (pcf)</th>
<th>Critical Hydraulic Gradient (2) (ft/ft)</th>
<th>Mass Averaged TOC (%)</th>
<th>Mass Averaged pH</th>
<th>Critical Flow (3) (gpm/acre)</th>
<th>Critical Flow/S.F.=1.5 (gpm/acre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 Bion (%) Latimer (%) Premier (%) Sand (%)</td>
<td>78</td>
<td>15.6</td>
<td>0.25</td>
<td>57.1</td>
<td>7.4</td>
<td>1.99E-05</td>
<td>3.20</td>
</tr>
<tr>
<td>2</td>
<td>80 Bion (%) Latimer (%) Premier (%) Sand (%)</td>
<td>80</td>
<td>86.4</td>
<td>24</td>
<td>0.38</td>
<td>33.1</td>
<td>7.2</td>
<td>2.17E-05</td>
</tr>
<tr>
<td>3</td>
<td>100 Bion (%) Latimer (%) Premier (%) Sand (%)</td>
<td>62.5</td>
<td>0.1</td>
<td>0.002</td>
<td>82.5</td>
<td>2.9</td>
<td>1.43E-04</td>
<td>0.147</td>
</tr>
<tr>
<td>4</td>
<td>80 Bion (%) Latimer (%) Premier (%) Sand (%)</td>
<td>74</td>
<td>11.6</td>
<td>0.19</td>
<td>21.7</td>
<td>5.9</td>
<td>4.95E-04</td>
<td>59.03</td>
</tr>
<tr>
<td>5</td>
<td>100 Bion (%) Latimer (%) Premier (%) Sand (%)</td>
<td>63.8</td>
<td>1.4</td>
<td>0.02</td>
<td>96.5</td>
<td>5.3</td>
<td>2.96E-05</td>
<td>0.43</td>
</tr>
<tr>
<td>6</td>
<td>80 Bion (%) Latimer (%) Premier (%) Sand (%)</td>
<td>75</td>
<td>12.6</td>
<td>0.20</td>
<td>35</td>
<td>6.4</td>
<td>3.68E-05</td>
<td>4.76</td>
</tr>
<tr>
<td>7</td>
<td>40 Bion (%) Latimer (%) Premier (%) Sand (%)</td>
<td>80.2</td>
<td>17.8</td>
<td>0.29</td>
<td>29</td>
<td>6.8</td>
<td>3.01E-04</td>
<td>55.19</td>
</tr>
<tr>
<td>8</td>
<td>40 Bion (%) Latimer (%) Premier (%) Sand (%)</td>
<td>80.7</td>
<td>18.3</td>
<td>0.29</td>
<td>33.9</td>
<td>6.9</td>
<td>5.68E-05</td>
<td>10.68</td>
</tr>
<tr>
<td>9</td>
<td>70 Bion (%) Latimer (%) Premier (%) Sand (%)</td>
<td>73.5</td>
<td>11.1</td>
<td>0.18</td>
<td>63</td>
<td>7.1</td>
<td>4.72E-06</td>
<td>0.53</td>
</tr>
<tr>
<td>10</td>
<td>70 Bion (%) Latimer (%) Premier (%) Sand (%)</td>
<td>73.3</td>
<td>10.9</td>
<td>0.17</td>
<td>59.6</td>
<td>7</td>
<td>9.37E-06</td>
<td>1.05</td>
</tr>
</tbody>
</table>

Notes:
1) Submerged unit weight calculated as Saturated Density-Water Density = \( \rho_{\text{sat}} - 62.4 \) pcf
2) Critical hydraulic gradient = \( (\rho_{\text{sat}} - \rho_{\text{water}})/\rho_{\text{water}} = \rho_{\text{sub}}/62.4 \) pcf.
3) Critical flow = (Average hydraulic Conductivity [cm/s]) \times (Critical hydraulic gradient [cm/cm]) \times (641395.28 gpm/acre) \times (1 acre)

S.F. = safety factor

1 pcf = 0.016 kg/L
1 gpm/acre = 9.35L/min./ha
Table 2.5: Summary of sorption results and retardation coefficients of contaminants

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sorption isotherm models</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Linear</td>
<td>Freundlich</td>
<td></td>
<td></td>
<td>$R^2$</td>
</tr>
<tr>
<td></td>
<td>Parameter values</td>
<td>Parameter values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$K_p$ and $K_{oc}^+$</td>
<td>$r^2$</td>
<td>$K_F$</td>
<td>$N$</td>
<td>$r^2$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(L/kg)</td>
<td></td>
<td>(L/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mix 7</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis-1,2 dichloroethene</td>
<td>8.02 (27.66)</td>
<td>0.948</td>
<td>10.69</td>
<td>0.88</td>
<td>0.953</td>
<td>7.32</td>
</tr>
<tr>
<td>Trichloroethene</td>
<td>4.62 (15.93)</td>
<td>0.911</td>
<td>10.97</td>
<td>0.56</td>
<td>0.963</td>
<td>4.64</td>
</tr>
<tr>
<td>1,1-dichloroethene</td>
<td>4.67 (16.10)</td>
<td>0.850</td>
<td>4.27</td>
<td>0.68</td>
<td>0.900</td>
<td>4.68</td>
</tr>
<tr>
<td>Chloroethane*</td>
<td>3.32 (11.45)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.62</td>
</tr>
<tr>
<td>1,1,1-trichloroethane</td>
<td>7.06 (24.34)</td>
<td>0.997</td>
<td>7.34</td>
<td>0.92</td>
<td>0.996</td>
<td>6.56</td>
</tr>
<tr>
<td>Vinyl chloride*</td>
<td>0.26 (0.89)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.20</td>
</tr>
<tr>
<td><strong>Mix 4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis-1,2 dichloroethene</td>
<td>4.13 (19.03)</td>
<td>0.940</td>
<td>7.71</td>
<td>0.76</td>
<td>0.952</td>
<td>3.51</td>
</tr>
<tr>
<td>Trichloroethene</td>
<td>2.88 (13.27)</td>
<td>0.932</td>
<td>5.71</td>
<td>0.66</td>
<td>0.950</td>
<td>2.75</td>
</tr>
<tr>
<td>1,1-dichloroethene</td>
<td>1.34 (6.18)</td>
<td>0.850</td>
<td>1.26</td>
<td>0.69</td>
<td>0.891</td>
<td>1.81</td>
</tr>
<tr>
<td>Chloroethane*</td>
<td>1.00 (4.61)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.40</td>
</tr>
<tr>
<td>1,1,1-trichloroethane</td>
<td>4.43 (20.41)</td>
<td>0.974</td>
<td>4.43</td>
<td>1.00</td>
<td>0.974</td>
<td>3.69</td>
</tr>
<tr>
<td>Vinyl chloride*</td>
<td>0.11 (0.51)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.07</td>
</tr>
</tbody>
</table>

* Organic carbon content normalized distribution coefficients ($K_{oc}$) are shown in brackets.
* Partition coefficient computed using Karickhoff relationship.
† Calculated using Eq. 2.9.

sediments. Therefore, differences in the quality of organic matter apart from organic matter content could account for the observed differences in sorption potential of the investigated substrate mixtures (Rutherford et al., 1992; Karapanagioti et al., 1997; Werth and Reinhard, 1997). Based on parameter values ($K_p$, $K_F$ and $K_{oc}$) for both models the
results demonstrate that Mix 7 had a higher sorption capability for all contaminants than Mix 4.

Retardation coefficients of the contaminants for VOCs in the substrate mixtures range from slightly above 1 to approximately 7 (Table 2.5). Computed retardation coefficients for target VOCs are very similar to those reported by Lorah et al. (1997) from “natural” Canal Creek wetland peat ($R$’s ranging from 6-10) from Aberdeen Proving Ground, MD. From Table 2.5 it can be observed that Mix 7 has significantly higher retardation coefficients than Mix 4 for all contaminants.

- **Fate of VOCs in Mesocosms**

  In general, two groups of compounds were detected in the site groundwater, chlorinated ethenes ($cis$-1,2-DCE and VC) and chlorinated ethanes (1,1,1-TCA, 1,1-DCA, and chloroethane (CA)). TCE (a parent compound of $cis$-1,2-DCE and VC) was below the detection limit (10 µg/L at this dilution) in the site water. These five compounds represent the bulk of the chlorinated compounds detected. Results were somewhat skewed by differences in the influent concentrations from the batches of groundwater from the site. This is illustrated in profiles of concentrations measured in the sand core (Figure 2.2). It is expected that this variability is a natural result of the extraction process at the wells and will also be a factor in a pilot and full-scale treatment wetland systems. Observations of VOCs fate in the cores are grouped into two time periods: a start-up period during the first 10-12 weeks where concentrations throughout the core were variable and a period of apparent equilibrium after 10-12 weeks. The entire monitoring period spanned 45 weeks.
Concentrations observed up to a distance of 37.5 cm from the core bottom in the sand core were very similar to influent concentrations (Figure 2.2). Decreases in concentrations of the contaminants in the top 22.5 cm of the sand column were probably due to direct volatilization losses due to the highly permeable nature of sand.

Low or negligible concentrations were measured at the effluent (standing water at the top of the core) in the sand column indicating how rapidly volatilization can remove those compounds that arrive at the surface. Measuring VOCs concentrations in the standing water at the wetland surface would be a poor indicator of performance since similar concentrations were measured in both peat and sand cores. Degradation daughter products of the VOCs were not detected in the sand column. The above observations indicate that negligible biodegradation and little sorption was occurring in the sand column, as expected. Since concentrations were very similar to influent concentrations (at least up to a distance of 37.5 cm), the lack of bias in the sampling procedures was also demonstrated. The distribution of cis-1,2-DCE and CA in the core containing Mix 4 (Latimer peat and sand) is shown in Figure 2.3. After the ninth week, decreases in concentrations were observed in the column containing Mix 4 that were attributed to biodegradation, as observed from the sharp drop in cis-1,2-DCE (Figure 2.3). This was after the initial contaminant “front” had passed through much of the column. Subsequent measurement of cis-1,2-DCE in this core were low (<1 mg/L) above the midpoint of the core despite similar influent concentrations. Increased VC along the flow path was also detected during this period as Figure 2.4 shows. CA concentrations also showed decreases in concentration (Figure 2.3) that were not expected from sorption alone.
Figure 2.2: Porewater concentration profiles of chloroethane and cis-1,2-dichloroethene in sand core for selected weeks.
Figure 2.3: Porewater concentration profiles of chloroethane and cis-1,2-dichloroethene in Mix 4 core for selected weeks.
In addition, ethane, a daughter product of CA was detected in composite samples collected from the top three sampling ports (data not shown). The appearance of these daughter products was used to identify the onset of biodegradation.

The distribution of cis-1,2-DCE and CA in the core containing Mix 7 (peat, sand and compost) is shown in Figure 2.5. A similar onset of biodegradation was observed from 10-12 weeks after initiating the experiment as seen in Mix 4. cis-1,2-DCE was observed to decrease with distance from the inlet whereas concentration of VC (a daughter product of cis-1,2-DCE degradation) was noted to increase. Remarkably, by week 12 over 90% of the cis-1,2-DCE was degraded to VC between the inlet and the first sampling point, a distance of only 7.5 cm. A representative profile of this trend is presented as Figure 2.4. Similar trends were observed for CA in Mix 7 although decreases in concentration were less pronounced. Mass balancing of contaminants in the system was complicated because it was difficult to differentiate between the biodegraded and sorbed fractions of the contaminants.

Despite the promising evidence of biodegradation, breakthrough of chloroethenes and chloroethanes was observed. Breakthrough of cis-1,2-DCE was transient in the mixture containing Latimer peat and sand alone (Mix 4). Once evidence for biodegradation was observed, breakthrough of cis-1,2-DCE ceased. For the chloroethanes (1,1-DCA and CA), breakthrough occurred for both mixtures even late in the study.

Concentrations in the overlying water for these compounds ranged from 0.35 to 2.76 mg/L for CA and from 0.35 to 1.08 mg/L for 1,1-DCA. These concentrations are higher than those observed in the standing water of the sand column probably due to higher volatilization rate in the sand core due to the absence of vegetation cover coupled
Figure 2.4: Sample plots of porewater concentration profiles of *cis*-1,2-dichloroethene and vinyl chloride in Mix 4 and Mix 7 for week 33.
Figure 2.5: Porewater concentration profiles of chloroethane and cis-1,2-dichloroethene in Mix 7 core for selected weeks.
with higher permeability of sand. This indicates a deeper wetland bed is necessary to fully treat these compounds.

Data for target organics were fit to the first order kinetic reaction equations (Eq. 2.8 and 2.10). A software package (Table Curve 2D Version 4; SPSS, Inc.) was used to fit data to the degradation equations to determine the lumped temporal and spatial removal rate constants. Removal rate constants were determined from VOC monitoring data collected after contaminant plumes have broken through the columns (for cis-1,2-DCE, Mix 4 = 13 days; Mix 7 = 28 days) and the onset of biodegradation, by that time equilibrium was assumed. The VOC data fit reasonably well ($r^2 > 0.8$) with the first order kinetic reaction equation as representative plots in Figure 2.6 demonstrate. Table 2.6 summarizes the removal rate constants. As expected, the degradation rate of chlorinated solvents increased with increasing number of chlorine atoms for both mixes. This observation is consistent with the fact that under anaerobic conditions the more chlorinated aliphatic hydrocarbons are dehalogenated faster than the less chlorinated ones (Fathepure et al., 1997; Haston and McCarty, 1999; Kao and Lei, 2000). It is, however, worth noting that these removal rates may be lower for the actual field conditions since the temperature at which the experiments were conducted (26°C) is higher than the field temperatures, especially during cold seasons. However, temperature correction coefficients for reductive dechlorination reaction rates are yet to be established. As with other biological reactions, rate constants may decrease by a factor of 2 with every 10°C drop in temperature.

Mean values of removal rate constants of cis-1,2-DCE and 1,1,1-TCA were found to be statistically higher in the Mix 7 column at $\alpha = 0.05$. The measured first order
temporal removal rate constants (day⁻¹) for cis-1,2-DCE are two orders of magnitude higher than field measurements in aquifers (0.0012 to 0.0017 day⁻¹) reported by Wiedemeier et al. (1996). In addition, first-order removal rate constants of cis-1,2-DCE (0.009 to 0.026 day⁻¹) and 1,1,1-TCA (0.003 to 1.73 day⁻¹) in anaerobic microcosms for various studies reported by Wiedemeier et al. (1999) are much lower than those reported in the present study. Rate constants determined by batch and column experiments are not expected to be the same since batch experiments are conducted under static conditions whereas column experiments are done under advective and dispersive flow conditions similar to those in the field (Porro et al., 2000).

The ability of Mix 7 to degrade cis-1,2-DCE and 1,1,1-TCA more efficiently than Mix 4 may be due to several factors. A difference in microbial activity attributed to the presence of the Bion soil is the likeliest explanation. Mix 7 was capable of supporting plant growth much better than Mix 4. The number of plant stems in the Mix 7 cores after 32 weeks was 136 compared with 31 in the Mix 4 cores. At the end of week 20, plant roots had extended to the bottom of the cores in both soil substrate mixtures.

- **Redox Conditions in the Cores**

  Results for measurement of temperature, pH, total dissolved solids, oxidation-reduction potential and conductivity did not clarify differences in biodegradation rates observed between the cores. Redox potentials for Mix 7 varied between -103 to -80 mV while those in Mix 4 ranged from -175 to -145 mV. These ranges of redox potential suggest that anaerobic reduction of chlorinated solvents in the peat substrate mixtures is feasible (Wiedemeier et al., 1999). Higher TDS (397 – 2703 mg/L) and conductivity
Figure 2.6: Experimental data and curve fit of cis-1,2-dichloroethene for week 16 for Mix 7 and Mix 4.
(564 - 3400 µS) measurements observed in Mix 7 indicate that Bion Soil possesses some salt content.

Table 2.6: Temporal and spatial removal rate constants

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>Influent concentration range, $C_0$</th>
<th>Removal rate constants (26 ± 3 °C)</th>
<th>Mix 7</th>
<th>Mix 4</th>
<th>Mix 7</th>
<th>Mix 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Temporal $(k)^*$ Spatial $(k)_s^+$</td>
<td>Temporal $(k)^*$ Spatial $(k)_s^+$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(mg/L) [day$^{-1}$] (cm$^{-1}$)</td>
<td>(mg/L) [day$^{-1}$] (cm$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis-1,2 DCE</td>
<td>25</td>
<td>0.85 – 13.67</td>
<td>1.00 – 12.40</td>
<td>0.84 ± 0.36</td>
<td>0.41 ± 0.15</td>
<td>0.37 ± 0.13</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>1,1,1-TCA</td>
<td>25</td>
<td>0.02 – 1.14</td>
<td>0.12 – 1.08</td>
<td>6.52 ± 3.12</td>
<td>2.80 ± 1.34</td>
<td>1.48 ± 0.42</td>
<td>0.42 ± 0.11</td>
</tr>
</tbody>
</table>

$n =$ Number of observation sets
* Calculated using Eq. 2.8
+ Calculated using Eq. 2.10

Composite samples from the root zone and below root zone for analysis of dissolved gases (ethene, ethane, methane and hydrogen) and organic acids were collected three times (week 5, week 12 and week 29). Results from the dissolved gas measurements indicate that methanogenic conditions were operating in the columns. Methane concentrations varied across a wide range. Concentrations were near the detection limit of 0.008 mg/L in the root zone of Mix 4 and Mix 7 early in the experiment. The highest concentrations measured were 0.74 mg/L in Mix 7 and 0.31 mg/L in Mix 4 from composite samples taken from the lower sections of the cores. Methane concentrations were consistently higher in Mix 7. Since reductive
dechlorination reactions are most commonly associated with methanogenic conditions, redox conditions in the columns were therefore favorable for degradation of VOCs (Ballapragada et al., 1997). The detection of ethene and ethane, final degradation products of chloroethenes and chloroethanes correlates well with what was observed with the VOCs. Composite samples detected ethene as high as 220 µg/L in Mix 4 and Mix 7 columns. Since the composite samples are subject to dilution, it is expected that actual concentrations were higher. Trace amounts of hydrogen (<10 µg/L) were detected in pore water samples collected from both Mix 4 and Mix 7 columns. Organic acids were not detected from the analyzed samples at a detection limit of 0.5 mg/L.

- **Anaerobic Microcosms**

Anaerobic microcosms were used to confirm that removal rates were due to anaerobic biodegradation in the lower portion of the bed as demonstrated by the VOC profiles. The mixed cultures were able to degrade 1,2-DCA under anaerobic conditions without a lag, suggesting robust indigenous bacterial populations (Figure 2.7). The redox indicator remained colorless throughout the study demonstrating that low redox conditions were maintained in the microcosms. Ethene was the primary product of degradation since other potential 1,2-DCA possible metabolites (notably VC, CA and ethane) were below the detection limit of the instrument (10 µg/L at these dilutions). 1,2-DCA was therefore dechlorinated mainly to ethene in essentially a single step, reductive dihaloelimination reaction (Klečka et al., 1998). As shown in Figure 2.7 anaerobic biodegradation of 1,2-DCA started almost immediately after incubation; no lag period was evident prior to disappearance of the parent compound in all substrate mixtures. After 5 days, over 95% of the parent compound was degraded in the active microcosms
of Mix 7, whereas over 90% of the initial 1,2-DCA concentrations were recovered in the killed controls within the same period. In Mix 4 microcosms, the proportion of 1,2-DCA degraded in Mix 4 was 75% for live treatments and 96% was recovered in formaldehyde-amended treatments after 5 days. Detection of ethene coincided with the onset of the disappearance of 1,2-DCA thus suggesting that the formation of ethene was indeed due to biological activity because it was not detected in killed controls. Amounts of ethene quantified in the active microcosms at the end of the incubation period were consistent with the stoichiometric conversion of DCA to ethene. 

$ cis $-1,2-DCE degraded almost immediately after incubation without a noticeable lag period (Figure 2.8). Degradation of $ cis $-1,2-DCE proceeded with little accumulation of VC. It can furthermore be observed from Figure 2.8 that VC conversion to ethene started even before $ 1,2-cis $-DCE was completely depleted, thus suggesting that the mixed cultures could also metabolize VC.

Repeated doses of $ cis $-1,2-DCE showed the same trend. Similar DCE degradation trends were also observed in Mix 4, however, the degradation kinetics were slower than in Mix 7. Pseudo first-order degradation rate constants of DCE and DCA in Mix 4 were calculated and found to be $1.1 \pm 0.12$ day$^{-1}$ (half life, $t_{1/2} = 0.63 \pm 0.07$ days) and $0.31 \pm 0.05$ day$^{-1}$ ($t_{1/2} = 2.2 \pm 0.34$ days), respectively. In contrast, degradation rate constants of DCE and DCA in Mix 7 were, respectively, $1.7 \pm 0.19$ day$^{-1}$ ($t_{1/2} = 0.41 \pm 0.05$ days) and $0.77 \pm 0.18$ day$^{-1}$ ($t_{1/2} = 0.90 \pm 0.14$ days).

Degradation rate constants of VC were determined using Eq. 2.12 using a software package Sigma Plot 6.0 (SPSS Inc., Chicago IL), which employs a Marquardt-Levenberg algorithm to find the parameters of interest from the observed data.
Degradation rate constants of VC in Mix 4 and Mix 7 were found to be $0.59 \pm 0.15 \text{ day}^{-1}$ ($t_{1/2} = 1.17 \pm 0.31 \text{ days}$) and $1.12 \pm 0.22 \text{ day}^{-1}$ ($t_{1/2} = 0.62 \pm 0.13 \text{ days}$) respectively.

Figure 2.7: Temporal trends of 1,2-DCA degradation in anaerobic microcosms of Mix 7

Differences in degradation rate constants in Mix 4 and Mix 7 were found to be statistically significant ($\alpha = 0.05$) for all test compounds. Therefore, results of microcosm experiments confirmed the observations made during the column studies that Mix 7 was more effective in attenuation of the target compounds than Mix 4. It can also be inferred from the results of degradation kinetic studies of the test compounds in the microcosms that biodegradation was probably the most important removal mechanism of VOCs in the mesocosms when compared with sorption, plant uptake and volatilization. Degradation
rate constants for cis-1,2-DCE in the microcosms are at least higher by a factor of two than those observed in soil columns. This may be due to lack of mass transfer limitations in the more homogeneous microcosms.

Figure 2.8: Temporal trends of cis-1,2-dichloroethene in anaerobic microcosms of Mix 7.

- Wetland Bed Depth

Based on the findings of the study, effective wetland bed depths required to meet National Primary Drinking Water Regulations (NPDWRs) (EPA, 2002) of cis-1,2-DCE (70 µg/L), VC (2 µg/L) and that of 1,1,1-TCA (200 µg/L) for different scenarios were calculated using the first-order mass transport equation (Eq. 2.8). Calculations were based on removal rate constants at 25°C (temperature at which the studies were conducted) and
15°C (typical temperature of groundwater at the Superfund site during cold seasons). For calculating the wetland depth, removal rate constants observed in the soil columns were halved to account for a decrease in microbially mediated degradation reactions in the wetland bed as a result of temperature drop during cold seasons. Since VC is a proven carcinogen, the effective depth required for its removal was also calculated based on the following assumptions: (1) removal rate constants of VC in the soil columns are half those determined in anaerobic microcosms since removal rate constants of cis-1,2-DCE in soil columns were approximately half those found in anaerobic microcosms; (2) removal rate constants during cold seasons (groundwater temperature 15°C) are lower by a factor of two than those observed in column studies (26°C); and, (3) cis-1,2-DCE is stoichiometrically transformed into VC at the bottom immediately after entering the wetland. The calculations assumed a loading of 15 gpm/acre, about half of the critical flow for both substrate mixtures and the loading utilized in laboratory testing to minimize chances of bed fluidization. The wetland bed depth calculation results are summarized in Table 2.7.

Table 2.7 demonstrates that for all scenarios and contaminants, wetland beds constructed from the mixture containing peat, compost and sand (Mix 7) will be shallower and most likely more cost-effective than the ones constructed from peat and sand alone (Mix 4). For this groundwater composition, VC appears to be the design contaminant, requiring more bed depth to remove observed contaminants to meet the National Primary Drinking Water Regulations. The combined stimulation of the removal rate and the retardation coefficient by the addition of the compost material argues strongly for a peat-compost-sand recipe for the upflow treatment wetland.
Table 2.7: Effective wetland bed depths required to meet MCL of VOCs for different scenarios

<table>
<thead>
<tr>
<th>Compound</th>
<th>Maximum Concentration, $C_0$ (mg/L)</th>
<th>Removal rate constant $(R)_{26°C}$ (day$^{-1}$)</th>
<th>Effective wetland bed depth$^*$ ($cm$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$26°C$</td>
</tr>
<tr>
<td>Mix 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Peat:sand:compost)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis-1,2-DCE</td>
<td>13.67</td>
<td>0.84</td>
<td>7</td>
</tr>
<tr>
<td>VC</td>
<td>13.67</td>
<td>0.56</td>
<td>120</td>
</tr>
<tr>
<td>Mix 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Latimer:sand)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis-1,2-DCE</td>
<td>13.67</td>
<td>0.37</td>
<td>35</td>
</tr>
<tr>
<td>VC</td>
<td>13.67</td>
<td>0.30</td>
<td>250</td>
</tr>
</tbody>
</table>

$^*$ Calculated using Eq. 2.8. National Primary Drinking Water Regulations’ Maximum Concentration Level of cis-1,2-DCE and VC are respectively 70 µg/L and 2 µg/L. Design flow of the treatment wetland system is 15 gpm/acre (160 L/min./ha).

2.4 Conclusion

The present study has attempted to address some unknown aspects of the treatment wetland-VOC concept including determination of sorption potential of the candidate peat/compost/sand mixtures, degradation kinetic reaction rates of target contaminants within the bed, the lag time for degradation to begin after system start-up and the hydrodynamic behavior of the peat bed. Other unknowns include the actual field degradation rates and the seasonally and spatial variability of degradation processes due
primarily to weather changes (especially temperature and precipitation). These latter questions will only be answered in the pilot study itself because the present work was not meant to replicate a well-developed treatment wetland and did not mimic actual field conditions.

Although breakthroughs of VC were not observed in the bench-scale wetland mesocosms, wetland bed depths required to meet the MCL for VC were found to be unreasonably high because they were calculated using very conservative assumptions. It is, therefore, anticipated that more reasonable bed depths will be obtained using the actual field degradation rates from the pilot study.

However, the present study has shed light on some potential design, construction and operational problems that may be encountered in the process of developing the treatment wetland concept into a full-scale groundwater treatment system for chlorinated solvents. For example, breakthrough of some target compounds (especially chloroethanes) may occur in the pilot plant during system start-up as it did during the bench-scale studies and this problem needs to be properly addressed in the design of the pilot system and eventually in the full-scale treatment system. Notwithstanding the observed limitations of the study, results from the present work have suggested that treatment wetland may potentially be a technically and economically viable option for treatment of groundwater contaminated with chlorinated solvents.
3.1 Introduction

Field and laboratory studies have demonstrated that wetlands are capable of rapid natural attenuation of chlorinated VOCs. The mechanisms observed were microbial anaerobic reductive dechlorination for highly chlorinated compounds and direct biological oxidation and cometabolism mechanism for less-chlorinated compounds (Pardue, 1992; Lorah et al., 1997; Lorah and Olsen 1999; Pardue et al., 2000; Kassenga et al., 2002; Kassenga et al., 2003). These studies further suggested that treatment wetlands could be cost-effective alternatives to conventional pump and treat remediation methods in cleaning-up aquifers contaminated with chlorinated organic compounds.

Reductive dechlorination is an important biological process for bioremediation of sediments and aquifers contaminated with halogenated aliphatic and aromatic compounds (Wiedemeier et al., 1999; Suthersan, 2002). Reductive dechlorination may either be a cometabolic or respiratory process carried-out by halorespiring bacteria, which are capable of reducing organochlorine compounds (Wiedemeier et al., 1999; Löffler et al., 1999; Suthersan, 2002). Since rates of the cometabolic dechlorination processes observed in natural environments are generally low, they may not be as important as the respiratory processes (Wiedemeier et al., 1999). Respiratory processes use halogenated hydrocarbons as electron acceptors in energy metabolism and, hence, for growth in a process known as halorespiration (Löffler et al., 1999; Hendrickson et al., 2002). Respiratory processes are likely to be major contributors in the bioremediation of anaerobic contaminated soils (Hendrickson et al., 2002). Wetland soils are predominantly anaerobic because of limited
diffusion and rapid depletion of oxygen due to abundance of organic substrates for microbial respiration (Lorah and Olsen, 1999). Highly anaerobic conditions, microbial diversity, and abundant supply of electron donors make wetland soils very conducive for supporting reductive dechlorination reactions (Pardue, 1992; Lorah and Olsen, 1999).

A wide range of chlorinated solvents can be microbially transformed under aerobic conditions. However, with the exception of trichloroethene (TCE) (Alvarez-Cohen et al., 2001; Arp et al., 2001), the more-chlorinated solvents (such as perchloroethene (PCE), tetrachloroethane (TeCA) and trichloroethane (TCA)) have not been shown to be susceptible to aerobic degradation, while many of their anaerobic degradation daughter products (e.g. vinyl chloride (VC), 1,2-dichloroethane (1,2-DCA) and isomers of dichloroethene (DCE)) can be aerobically transformed either via direct oxidation, cometabolism or both (Wiedemeier et al., 1999). In direct oxidation, the chlorinated solvent (e.g. VC and 1,2-DCA) is used as a primary growth substrate, whereas, in aerobic cometabolism, transformation reactions of a chlorinated solvent (the secondary substrate) are catalyzed by enzymes from microorganisms routinely degrading another compound (the primary substrate or cometabolite) that yield no carbon or energy benefits to the transforming cells (LaGrega et al., 1994; Alvarez-Cohen et al., 2001). Therefore, in aerobic cometabolism, a growth substrate must be available to provide for growth, provide an energy source, and induce production of the cometabolic enzymes (Alvarez-Cohen et al., 2001). Direct oxidation normally occurs relatively faster in comparison to cometabolism (Alvarez-Cohen et al., 2001). Since dissolved oxygen is normally limited in groundwater systems, aerobic transformation of chlorinated solvents must be engineered by oxygen addition (Wiedemeier et al., 1999). In wetlands, however,
aerobic biodegradation of chlorinated organics may occur in the rhizosphere without exogenous oxygen addition due to the higher microbial activities found in the rhizosphere, an ample supply of readily degradable carbon co-substrates leaking from the plant, and the leakage of oxygen into the rhizosphere from the root (Pardue et al., 2000).

Cis-1,2-dichloroethene (cis-1,2-DCE) and 1,2-DCA, the test chemicals used in the present study can be degraded in the environment through both abiotic and biological processes (Vogel et al., 1987; Lee et al., 1999). However, abiotic transformation mechanisms normally proceed at much lower rates than biological degradation processes and therefore may not be very important in the removal of cis-1,2-DCE and 1,2-DCA in aquifer systems (Vogel et al., 1987; Lee et al., 1999; Wiedemeier et al., 1999; Suthersan, 2000). Biologically-mediated transformation of cis-1,2-DCE and 1,2-DCA typically proceed at faster rates than abiotic transformation provided that organisms that are capable of transforming the chemicals are present, the right types of substrates are available at sufficient amounts, and environmental conditions are appropriate.

The test chemicals may be degraded both aerobically and anaerobically (Vogel et al., 1987; Klečka et al., 1998; Lee et al., 1999; Lorah et al., 1999; Dyer et al., 2000). 1,2-DCA is the only chlorinated ethane that has been shown to be aerobically degradable (Wiedemeier et al., 1999). 1,2-DCA can be transformed directly via aerobic oxidation to 2-chloroethanol, which may further be hydrolytically converted to ethanol. Under aerobic conditions, 2-chloroethanol may further be transformed into chloroacetaldehyde, chloroacetic acid, and glycolade (Dolfing and Janssen, 1994; Wiedemeier et al., 1999; Lee et al., 1999). Dyer et al. (2000) reported that 1,2-DCA can be used as a growth substrate by aerobic bacteria, such as Xanthobacter, Ancylobacter and Pseudomonas
strains, which completely oxidize 1,2-DCA to carbon dioxide, water, and chloride. cis-1,2-DCE may be aerobically transformed by methanotrophic microorganisms, via co-metabolism using methane as an electron donor (Bradley and Chapelle, 1998; Wiedemeier et al., 1999; Hage et al., 2001). Methanotrophs possess an enzyme, methylmonooxygenase (MMO) that will attack chlorinated ethenes oxidizing them through various intermediates to CO₂ (Wiedemeier et al., 1999; Pardue et al., 2000). Aerobic transformation of cis-1,2-DCE has been demonstrated in field pilot tests (Cornuet et al., 2000) and in laboratory microcosms (Lorah et al., 1997; Bradley and Chapelle, 1998). However, aerobic transformation products of cis-1,2-DCE were not reported in these studies; the disappearance of cis-1,2-DCE in live treatments, recovery of [1,2-¹⁴C]DCE radioactivity as ¹⁴CO₂ and the recovery of the chemical in sterilized controls were considered as evidence of microbial transformation.

Under anaerobic conditions cis-1,2-DCE may be sequentially dechlorinated completely to ethene via vinyl chloride (Vogel et al., 1987; Ballapragada et al., 1997; Yang and McCarty, 1998). Two mechanisms of 1,2-DCA microbially-mediated reductive dechlorination have been reported by various researchers. These are hydrogenolysis, which involves two sequential replacement of a chlorine atom by hydrogen to form chloroethane (CA) and finally ethane, and dihaloelimination in which two adjacent chlorine atoms are removed and replaced by a carbon double bond to form ethene (Holliger et al., 1990; Wild et al., 1995; Lorah and Olsen, 1999; Dyer et al., 2000). Both aerobic and anaerobic degradation of cis-1,2-DCE and 1,2-DCA release inorganic chloride (Klećka et al., 1998; Lee et al., 1999).
Isolation of microorganisms that both couple reductive dechlorination to growth and are capable of completely degrading chlorinated solvents to innocuous end-products has been a subject of intensive investigation in recent years. Except for a recently discovered microbial species, *Dehalococcoides ethenogenes* strain 195 by Maymó-Gatell et al. (1997) which can dechlorinate PCE and TCE completely to ethene, no organism has been isolated in pure culture that can completely dechlorinate chlorinated ethenes. Other known microorganisms such as *Dehalobacter restrictus* (Fennell and Gossett, 1997), *Dehalospirillum multivorans* (Smatlak and Gossett, 1996), *Desulfomonile tiedje* (Ballapragada et al., 1997) and *Desulfitobacterium frapperi* (Maymó-Gatell et al., 2001) have been shown to be able to dechlorinate PCE and TCE to the cis-1,2-DCE and VC stages only, which is undesirable, because VC is a human carcinogen that poses a greater risk to human health than either PCE or TCE (Holliger et al., 1993; McCarty, 1997). Accumulation of cis-1,2-DCE at many chlorinated ethene sites is suspected to be due to the prevalence of organisms that can reduce PCE or TCE as far as cis-1,2-DCE over organisms that can completely transform PCE and TCE into ethene (Wiedemeier et al., 1999). The recently reported finding that *D. ethenogenes* appear to be widely distributed in the environment (Hendrickson et al., 2002) implies that there is a good potential for using *D. ethenogenes* in remediation of contaminated sites. *D. ethenogenes*, therefore, presents a promising candidate for bioremediation (Maymó-Gatell et al., 1997; Löffler et al., 2000; Fennel et al., 2001; Hendrickson et al., 2002). It has, however, been observed that the reduction of VC does not appear to support the growth of *D. ethenogenes* (Flynn et al., 2000), the organism is reported to transform VC via a fortuitous mechanism (Adamson and Parkin, 2000). The difficulty in cultivating *D. ethenogenes* and the finding
that extract of an anaerobic sludge community has been shown to sustain the growth of *D. ethenogenes* suggests that the organism relies on biochemical collaboration with other microorganisms and that it benefits significantly by growing in a diverse microbial community (Richardson et al., 2002).

This is the first of a two paper series on microbial transformation of cis-1,2-DCE and 1,2-DCA in two candidate soil mixtures of peat/sand/compost for construction of a treatment wetland for chlorinated aliphatic compounds at a Superfund site. Objectives of the study for which the present paper was prepared were (1) to determine and compare degradation kinetics of cis-1,2-DCE and 1,2-DCA in different sections of two types of constructed wetland soil columns under anaerobic and aerobic conditions, (2) to examine the pathways of transformation of the test chemicals under anaerobic conditions, and (3) to identify organisms responsible for anaerobic reductive dechlorination of the test chemicals by molecular analysis using polymerase chain reaction (PCR) based detection of 16S rDNA primers specific for *Dehalococcoides* sp.

### 3.2 Materials and Methods

- **Selection of the Test Chemicals**

  1,2-DCA, representing chlorinated ethanes and 1,2-*cis*-DCE representing chlorinated ethenes were selected for determination of degradation kinetics of the target pollutants at a Superfund site for which the present study was conducted.

- **Chemicals**

  Neat solutions of cis-1,2-DCE and 1,2-DCA (Supelco Park, Bellefonte, PA) were used to prepare stock solutions. Analytical standards and surrogates for the VOCs were obtained as mixtures or neat liquids from Supelco (Bellefonte, PA). Methane, ethane and
ethene for calibration were also obtained from Supelco (Bellefonte, PA). Resazurin and formaldehyde were procured from Sigma Chemical Co. (Saint Louis, MO). Other chemicals used in this study were reagent grade and were purchased from various vendors.

- **Soil Preparation**

Two 60-cm soil columns were removed from glass cores minimizing disturbance. The cores were utilized in previous bench scale studies on treatment of chlorinated solvents in up-flow wetland systems (see Chapter 2 and Kassenga et al., 2003). The soil columns were then sectioned and used to construct anaerobic and aerobic microcosms. One wetland soil column was comprised of *Latimer peat* (Latimer's Peat Moss Farm, West Liberty, OH), *Bion Soil* (Dream Maker Dairy, Cowlesville, NY) and sand mixed at a ratio of 1.3:1.1:1 (Bion Soil:Latimer:sand) by weight abbreviated as PSB. Another soil column was prepared from *Latimer peat* and sand (1.3:1 w/w) referred to as PS. These mixing ratios for the column soil mixtures were determined from previous studies (Kassenga et al., 2003). These soil mixtures were identified as the most promising materials for construction of a treatment wetland bed for chlorinated aliphatic compounds at a Superfund site (Kassenga et al., 2003).

The bottom, middle and top sections of the soil columns were respectively sliced at following intervals from the base of the soil columns; 0 to 10; 20 to 30; and 40 to 50 cm. Soils collected from these sections were used to construct aerobic and anaerobic microcosms.
Microcosm Experiments

Biodegradation was investigated under both anaerobic and aerobic conditions in microcosms constructed with combinations of the soil mixtures and deionized water spiked with the test chemicals.

Anaerobic microcosms

Since sectioning of the soil columns was done in the ambient atmosphere, soil slices used for construction of anaerobic microcosms were shaved under a nitrogen atmosphere in a glove bag (I2R, Cheltenham, PA) to minimize the effects of exposure of anaerobic organisms to air. The interiors of the soil columns were used for construction of anaerobic microcosms. Construction of anaerobic microcosms was done under a nitrogen atmosphere in a glove bag (I2R, Cheltenham, PA) by homogenizing and packing soil from each section in 160 mL serum bottles using a 1.5:1 volumetric ratio of groundwater to wetland sediments according to Lorah et al. (1996). All reaction mixtures were sealed with Teflon-lined rubber septa and aluminum crimp seals and incubated in an inverted position under static and light-excluded conditions at 25 °C, which was the approximate temperature of the groundwater during the soil column experiments. Resazurin (0.0002%) was added as the redox indicator. Initially, groundwater contaminated mainly with cis-1,2-DCE and 1,1,1-trichloroethane (1,1,1-TCA) from a Superfund site was used for the experiments. When the initial doses were degraded, the bottles were consecutively respiked three more times by adding cis-1,2-DCE and 1,2-DCA stock solutions to a final concentration of about 60 µM each, after first withdrawing an equivalent volume of water. Temporal monitoring of concentrations of the parent compounds and degradation daughter products and methane were done after each spike until the concentrations of the
parent compounds and degradation daughter products had dropped below the detection limits of the analytical methods (5μg/L). Abiotic controls were included in the study to monitor for non-biological losses of the test chemicals. For the abiotic control, reaction mixtures were prepared as described above, and then they were subsequently adjusted to contain 1% formaldehyde. Samples were withdrawn from the bottles and immediately analyzed without storage for chlorinated ethenes and ethanes, ethene, ethane and methane. To ensure reproducibility, triplicates were used in each experiment and, whenever feasible, experiments were repeated at least two times.

Microcosms were neither amended with electron donors nor nutritional supplements to support microbial growth unlike in other studies reported in literature in which pure culture or enrichment mixed cultures were used.

Aerobic microcosms

Aerobic biodegradation kinetics of cis-1,2-DCE and 1,2-DCA were evaluated in bottom, middle and top sediment slurries as described above for anaerobic microcosms. Microcosms were constructed under ambient air in 160-mL glass bottles using 35 mL of deionized water spiked with the test chemicals and 25 mL of soil slurry (Lorah et al., 1997). The bottles were sealed with Miniinert Valves (VICI Precision Sampling, Baton Rouge, LA) that are designed to allow repeated withdrawals of headspace samples from the same bottles. Resazurin (0.0002%) was added as the redox indicator.

High purity methane (99.0%) was added to the headspace of the microcosms at a concentration of 1 percent to provide a substrate for methanotrophic bacteria according to Lorah et al. (1997). Abiotic controls to distinguish abiotic removal of the test chemicals from microbial reactions were constructed by the addition of 1% formaldehyde into each
of the above treatments. Triplicate microcosms and one abiotic control were prepared for each soil section to ensure reproducibility.

Microcosms were stored in the dark without agitation at 25 °C, which was the approximate temperature of the groundwater during the soil column experiments as stated earlier. Approximately every 3 days, oxygen gas was added to all microcosms at a concentration of 10 percent by volume in the headspace for the purposes of maintaining aerobic conditions. Samples were withdrawn from the headspace once or twice a week as deemed appropriate and analyzed for chlorinated VOCs and methane by direct injection into GC/MS and GC/FID, respectively. Sample withdrawals for headspace analysis were done prior to the injection of methane and oxygen to eliminate dilution of the test chemicals. Aqueous concentrations of the test chemicals were calculated using dimensionless Henry coefficients of 0.16 and 0.04 for cis-1,2-DCE and 1,2-DCA respectively at 25°C (LaGrega et al., 1994). Analyses were not conducted for potential degradation products of 1,2-DCA under aerobic conditions, such as 2-chloroethanol, ethanol, chloroacetaldehyde, chloroacetic acid, and glycolate (Lee et al., 1999).

- **Analytical Procedures**

VOCs were analyzed using EPA Method 8260B using a purge and trap apparatus to concentrate and introduce the sample into a gas chromatograph-mass selective detector (Agilent 6890 gas chromatograph-5972A mass selective detector). The GC was equipped with a 30 m × 0.25 mm × 0.25 μm film thickness, Agilent 5MS (5% Phenyl Methyl Siloxane) capillary column (Palo Alto, CA). A thermal desorption trap (VOCARB 3000; Supelco, Bellefonte, PA) was employed in the purge and trap apparatus. The samples were purged for 11 min with ultra-high-pure helium at a flow rate of 35 mL/min,
desorbed for 0.5 min and baked for 13 min at 225°C. The GC column temperature program was -80°C for 1 min ramped to 20°C at 15°C/min then ramped to 80°C at 10°C/min and finally to 220°C at 20°C/min. The injector and detector temperatures were 250°C and 280°C respectively. Helium was used as a carrier gas at a flow rate of 2.1 mL/min. Daily blanks, calibration checks and tunes were run to assure that the analytical method was in control. Recovery of surrogates injected into every sample also ensured that no gross dilution errors or leaks have occurred in the GC/MS system.

Methane was measured using GC/FID. 1 mL of gas in the headspace was withdrawn using a gas tight syringe and injected into the gas chromatography with flame ionization detector (Agilent 5890 Series II) equipped with a 2.4 m × 0.32 mm ID column packed with Carbopack b/1% SP-1000 (Supelco, Bellefonte, PA). The column temperature was held at 50°C isothermally for 6.5 min, and the injector and detector temperatures were 375 and 325°C respectively. The carrier gas was ultra high purity nitrogen at a flow rate of 12 mL/min.

Redox potential and pH were determined using Ultrameter 6P (Myron L Company, Carlsbad, CA). The instrument was standardized in commercially prepared buffer solution (pH = 7.00) (Hach Co., Loveland, CO). Redox sensitive constituents (sulfate, nitrate, manganese, and ferric iron) were analyzed using the liquid chromatography (Dionex LC-20, Dionex Corp., Sunnyvale, CA).

• Analysis of Kinetic Data

The limitations of the first-order approximations in describing kinetics of transformation of substrates are well recognized (Bradley and Chapelle, 1998; Widermeier et al., 1999; Suthersan, 2000; Pavlostathis and Prytula, 2000). However, the
implementation of more advanced models such as the Michaelis-Menten kinetic model and others is difficult since these models involve biodegradation parameters that cannot easily be determined in soils containing complex microbial systems normally found in the field (Wiedemeier et al., 1999). Therefore, biodegradation of the test chemicals was modeled with first-order kinetics, assuming that the inherent limitations of the model will equally affect the model output results from the kinetic data of the contaminants, thus minimizing the chances of bias in comparing kinetic constants. Furthermore, to minimize the chance of errors associated with using the first-order model, variations in initial concentrations of the test chemicals were minimized during the kinetic studies as suggested by previous researchers (Bradley and Chapelle, 1998).

Reductive dechlorination models for cis-1,2-DCE and 1,2-DCA were formulated by accounting for both the consumption of the parent compounds and the simultaneous production of less chlorinated compounds. The models were finally formulated by solving sets of ordinary differential equations analytically. The formulated models were used to calculate the rates of transformation of the parent compounds and intermediate degradation products.

The anaerobic biotransformation pathway of cis-1,2-DCE observed in the current study was as follows:

\[ \text{cis-1,2- DCE} \xrightarrow{k_1} \text{VC} \xrightarrow{k_2} \text{ETH} \]

The following set of differential equations applies to the first-order reactions of the pathway illustrated above for the transformation of cis-1,2-DCE:

\[ -\frac{d[DCE]}{dt} = k_1[DCE] \]  \hspace{1cm} (3.1)
The above ordinary differential equations (Eq. 3.1, Eq. 3.2 and Eq. 3.3) were solved analytically and their solutions are, respectively, as follows:

\[
\frac{d[VC]}{dt} = k_1[DCE] - k_2[VC] \tag{3.2}
\]

\[
\frac{d[ETH]}{dt} = k_2[VC] \tag{3.3}
\]

where \([DCE]_t, [VC]_t\) and \([ETH]_t\) are the concentrations of cis-1,2-DCE, VC and ethene at any time \(t\) \([DCE]_0\) is the initial concentration of cis-1,2-DCE, and \(k_1\) and \(k_2\) are degradation rate constants of transformation of cis-1,2-DCE and VC, respectively. The degradation rate constant of VC \((k_2)\) may be calculated using either Eq. 3.5 or Eq. 3.6.

Experimentally, 1,2-DCA was observed to be dechlorinated through two pathways:

\[1,2\text{-DCA} \xrightarrow{k_1} \text{ETH (dihaloelimination)}\]

\[1,2\text{-DCA} \xrightarrow{k_1} \text{CA} \xrightarrow{k_2} \text{ETHANE (consecutive hydrogenolysis)}\]

Since chloroethane (CA) was not produced in appreciable amounts, the biotransformation of 1,2-DCA by hydrogenolysis was therefore assumed to be a single-step process similar to dihaloelimination. In view of this, the equations and solutions of the dihaloelimination pathway also apply to the hydrogenolysis mechanism, thus:

\[-\frac{d[DCA]}{dt} = k_1[DCA] \tag{3.7}\]

From material balance of reactants and products,
\[
[\text{ETH,ETHANE}]_t = [\text{DCA}]_0 - [\text{DCA}]
\]  

Solutions to the dihaloelimination/ hydrogenolysis differential equations are as follows:
\[
[\text{DCA}]_t = [\text{DCA}]_0 e^{-k_1 t}
\]  

\[
[\text{ETH,ETHANE}]_t = [\text{DCA}]_0 (1 - e^{-k_1 t})
\]

where \([\text{DCA}]_t\) and \([\text{ETH,ETHANE}]_t\) are respectively the concentrations of 1,2-DCA and ethene/ethane at any time \(t\), \([\text{DCA}]_0\) is the initial concentration of 1,2-DCA, \(k_1\) is degradation rate constant of transformation of 1,2-DCA.

Kinetic rate constants of transformation of the test chemicals were estimated using the above kinetic models and the time course data of the reactants and degradation daughter products by non-linear regression performed using the program Sigma Plot 6.0 (SPSS Inc., Chicago IL), which employs a Marquardt-Levenberg algorithm to calculate the kinetic constants of interest.

For each chemical, the characteristic half-life period \((t_{1/2})\) was calculated from the degradation rate constant \(k\), using the following equation:
\[
t_{1/2} = -\frac{(\ln 2)}{k}
\]

- **Detection of Dehalococcoides 16S rDNA Sequences**

To determine if *Dehalococcoides* sp. were present in the various microcosms, a PCR-based detection method was used. DNA extracted from the bulk community was amplified by PCR using two different sets of primers previously reported to be specific to this group of bacteria (Hendrickson et al., 2002). Detection of the appropriate size PCR products was used as direct evidence that these organisms were present in the microcosms. To verify that PCR products were of the expected sequence, a subset of the PCR products were cloned and sequenced. The methods for the various steps in this process are summarized in the following subsections.
DNA Extraction

Samples for DNA extraction were collected by pouring soil slurry from serum bottles into sterile cryogenic vials. Sufficient slurry was then transferred to a sterile microcentrifuge tube to result in 0.25 to 0.4 g of wet soil, and DNA extraction from pelleted soil samples was performed using a MoBio Ultraclean Soil DNA Isolation Kit (Salona Beach, CA) according to the manufacturer’s instructions with the following modifications. The kit protocol was amended so that a Biospec Mini-Beadbeater 3110BX (Biospec Products Inc., Bartlesville, OK) was used in place of the MoBio Vortex Adapter (Salona Beach, CA). The beadbeater was operated for 3 minutes at 4,800 rpm. A detailed description of the DNA extraction procedure is shown in Appendix A. Because of the high humic acid content of the soil samples, prior to DNA extraction, the samples were treated with Polyvinylpolypyrrolidone (PVPP) (Agros Organics, Geel, Belgium) as described in Appendix B. As an additional step to remove humic acid impurities, two additional washes of the S4 solutions were added (for a total of 3 washes, instead of the 1 wash recommended by the manufacturer’s standard protocol).

PCR amplification

PCR amplification was performed using an Eppendorf MasterTaq kit (Brinkmann Instruments, Inc., Westbury, NY), which includes Taq DNA Polymerase (5U/µL), 10X Taq Buffer with Mg²⁺ and 5x TaqMaster PCR Enhancer (Brinkmann Instruments, Inc., Westbury, NY). A 2.5 mM solution of each dNTP (mix 10 mM total) was obtained from Applied Biosystems (Forster City, CA). The 5x TaqMaster PCR Enhancer often required heating at 60°C to completely dissolve the components.
A mastermix was made by the addition of 63.5 µL 18 Mega Ohm water, 15 µL of 5x TaqMaster PCR Enhancer (Brinkmann Instruments, Inc., Westbury, NY), 10 µL of 10X Taq Buffer with Mg$^{2+}$, 8 µL of the 10 mM dNTP mix, 0.5 µL of the Taq DNA Polymerase and 1-µL of each primer (forward and reverse) per sample if all samples required the same primers, otherwise the primers from the mastermix were added individually.

For each sample to be amplified, 99 µL of the mastermix was placed in a 500 µL sterile PCR reaction tube, and then 1 µL of the extracted DNA was added. The contents were mixed and then centrifuged for 1 minute at 13,000 rpm. PCR amplification was then conducted using an Eppendorf Thermocycler (Eppendorf GmbH, Hamburg, Germany) under the following conditions previously reported by Hendrickson et al. (2002): 2 minutes of denaturation at 95°C, followed by 30 cycles of 1 minute of denaturation at 94°C, 1 minute of annealing at 55°C and 1 minute of extension at 72°C. Samples not analyzed immediately were stored at 0 to 4 °C until analysis.

**Primers**

Two sets of primers (arbitrarily referred to as DE Primer Set 1 and DE Primer Set 2) specific to variable regions of the 16S rDNA present in *Dehalococcoides* group bacteria (Hendrickson et al., 2002) were used in this study. In cases where no PCR products were detected using these primers, a nested approach was used in which bacterial DNA was first amplified using a set of universal primers (see Table 3.1), and then the products of this initial amplification were amplified using the *Dehalococcoides* specific primers. Table 3.1 lists the primers used in this study. All primers were synthesized, purified, desalted, and lyophilized by AlphaDNA (Montreal, Quebec,
Canada). Primers obtained from Alpha DNA were reconstituted with TE buffer (10 mM Tris, 1 mM EDTA, pH = 8) to make 50 µM primer stock solutions that were stored at -20°C prior to use.

Table 3.1: Designation, positions, and sequences of primers used

<table>
<thead>
<tr>
<th>Primer</th>
<th>bp coordinates</th>
<th>Primer sequence</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DE Primer Set 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fp DE 1</td>
<td>1–17</td>
<td>5’GATGAACGCTAGCGGCG3’</td>
<td>1,377</td>
</tr>
<tr>
<td>Rp DE 1377</td>
<td>1385–1366</td>
<td>5’GGTTGGCACATCGACTCAA3’</td>
<td></td>
</tr>
<tr>
<td><strong>DE Primer Set 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fp DE 946</td>
<td>946–963</td>
<td>5’AGTGAACCGAAAGGGAAA3’</td>
<td>266</td>
</tr>
<tr>
<td>Rp DE 1212</td>
<td>1220–1199</td>
<td>5’GGATTAGCTCCAGTTACACTG3’</td>
<td></td>
</tr>
<tr>
<td><strong>Universal Primer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8F</td>
<td>8–27</td>
<td>5’AGAGTTTGATCCTGGCTCAG3’</td>
<td>1,502</td>
</tr>
<tr>
<td>1492R</td>
<td>1510–1492</td>
<td>5’GGTACCTTTGTTACGACTT3’</td>
<td></td>
</tr>
</tbody>
</table>

For the DE primer sets, the coordinates are the *Dehalococcoides ethenogenes* strain 195 16S rDNA base position coordinates, and for the universal primer the coordinates are the *E. coli* base position coordinates.

Detection of PCR Products

PCR products were analyzed using 1 µL samples of the PCR reactions in conjunction with an Agilent 2100 Bioanalyzer and corresponding DNA labchip kits (Agilent Technology, Willington, DE).

Cloning and Sequencing

To verify that PCR products produced using *Dehalococcoides* specific primers were the desired target sequence, a representative from each sample was cloned and sequenced. The sample representatives were the 1377 base pair fragments of peat/sand core bottom soil (PS-B2) and peat/sand/Bion Soil core bottom soil (PSB-B1), and the 266 base pair fragment of BSB core bottom soil (PSB-B1). A TOPO TA Cloning Kit for
Sequencing (with pCR4-TOPO®) with One Shot® TOP10 Chemically Competent *E. Coli* (Invitrogen, Carlsbad, CA) was used to clone each sample, following a modification of the manufacturer protocol (see Appendix C). DNA was extracted from clones, reamplified by PCR using the same primers as were used to generate the PCR product used for cloning and then purified using UltraClean™ PCR Clean-up Kit (MoBio, Carlsbad, CA). A sequencing reaction was performed on the purified products using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Forester City, CA), using minor modifications of the manufacturer’s protocol (see Appendix D). For sequencing reactions using the 266 base pair (bp) fragment, only the 946f primer listed in Table 3.1 was used. To sequence the 1377 bp amplicons, two sequencing reactions were performed, one using the 1f primer listed in Table 3.1 and the other using the 774f primer described by Hendrickson et al. (2002) which has a sequence of 5’GGGAGTATCGACCCTCTC3’ and corresponds to a variable region of the 16S rDNA of *Dehalococcoides* bacteria. Sequencing was performed using an ABI 377 Automated DNA Sequencer. For the 1377 bp PCR product, the partial sequences generated using the individual primers (1f and 774f) were then joined.

The sequences were edited using BioEdit software (North Carolina State University, Raleigh, NC) and were subsequently compared to sequences in the National Center for Biotechnology Information (NCBI) database. The sequences were aligned with the top three reference species from the NCBI database along with two closest matches to previously cultured organisms. A pair-wise comparison of the aligned sequences was made, and a similarity matrix was produced.
3.3 Results and Discussion

- **Transformation of 1,2-cis-DCE under Anaerobic Conditions**

  Terminal electron accepting conditions in the microcosms were observed to be methanogenic as evidenced by high methane concentration (>1000 µM) and the fact that other redox-sensitive constituents like nitrate, sulfate and ferric iron were not detected in appreciable amounts. The measured redox potentials of between -150 and -120 mV indicated that conditions in the microcosms were highly reducing and therefore conducive for reductive dechlorination (Wiedemeier et al., 1999).

  Throughout the study, the redox indicator remained colorless indicating that anaerobic conditions were indeed maintained in the microcosms as also supported by the values of redox potential (-150 to -120 mV). Wetland soil microbial populations were able to degrade cis-1,2-DCE under anaerobic conditions without a noticeable lag period, probably because they were acclimated with the substrate for a long exposure period prior to the start of the microcosm studies (see Figure 3.1). The concentration of cis-1,2-DCE was observed to decrease while that of VC initially increased to a maximum. Thereafter, the concentration of VC started to decrease with concomitant accumulation of ethene. It can be observed from Figure 3.1 that VC conversion to ethene started even before 1,2-cis-DCE had been completely depleted, thus suggesting that the mixed culture could also metabolize VC. Repeated doses of cis-1,2-DCE showed the same trend in all live microcosm, but the rates of degradation kinetics were different in microcosms constructed from slurries obtained from different sections of the soil columns. Comparison of the temporal concentration trends in the live to abiotic controls indicates that indeed biodegradation was a major removal mechanism of cis-1,2-DCE (Figure 3.1).
Figure 3.1: Anaerobic degradation of cis-1,2-DCE in microcosm experiments constructed with bottom sediments of PSB soil mixture.

Methane did not increase appreciably above its initial concentration during dechlorination of cis-1,2-DCE indicating that methanogenesis was either inhibited or occurred at a very low rate.

Temporal trends of concentrations of cis-1,2-DCE and degradation daughter products and the non-linear fit of the data to Eq. 3.4 through Eq. 3.6 are shown in Figure 3.2 and Figure 3.3. The reasonable agreement between the experimental data and these equations suggests that the pseudo-first order reaction kinetics model described the
transformation of cis-1,2-DCE in the mixed cultures reasonably well as attested by the relatively high values of coefficients of determination ($R^2$). A minor drawback of the pseudo-first-order sequential dechlorination model as Figure 3.2 and Figure 3.3 illustrate is that it slightly under-predicts depletion rates of the intermediates of transformation of chlorinated aliphatic organic compounds after attaining their maximum values as Pavlostathis and Prytula (2000) also observed.

Fitting the cis-1,2-DCE degradation monitoring data for bottom soils of PSB and PS cores to the rate laws (Eq. 3.4 through Eq. 3.6) resulted in the kinetic rate constant of VC of $0.025 \pm 0.006 \text{ hr}^{-1}$ (Figure 3.2) and $0.018 \pm 0.009 \text{ hr}^{-1}$ (Figure 3.3) respectively. Transformation rate constants of VC in the other live microcosms were similarly estimated and found to be lower by factors ranging from 2 to 4 compared to those of cis-1,2-DCE. These results affirm the notion that rates of reductive dechlorination tend to decrease as the number of chlorine substituents decreases (Vogel et al., 1987; Wiedemeir et al., 1999; Suthersan, 2000).

- **Transformation of cis-1,2-DCE under Aerobic Conditions**

  The color of the redox indicator was monitored and observed to remain pink throughout the study, suggesting that aerobic conditions were indeed maintained in the microcosms. To evaluate the role of methane in aerobic degradation of the test compounds by methanotrophic bacteria, methane was not added into the microcosms during the first four weeks after incubation. Comparable concentrations of cis-1,2-DCE were observed in abiotic and live controls prior to addition of methane (Figure 3.4). cis-1,2-DCE started to disappear in live microcosms after addition of methane (Figure 3.4),
Figure 3.2: Concentrations of cis-1,2-DCE, vinyl chloride, and ethene versus time for PSB bottom soil. Data points represent experimentally measured values, bars indicate the range of results for individual bottles and lines represent model fits.
**Figure 3.3**: Concentrations of *cis*-1,2-DCE, vinyl chloride, and ethene versus time for PS bottom soil. Data points represent experimentally measured values, bars indicate the range of results for individual bottles and lines represent model fits.
Figure 3.4: Time courses of cis-1,2-DCE and 1,2-DCA aerobic transformation in bottom slurries of PSB. Data points represent means of results from analysis of triplicate microcosms; bars indicate the range of results for individual bottles.
indicating that methane was probably utilized during the transformation of the chemical. No chlorinated anaerobic degradation products of cis-1,2-DCE were observed in the microcosms as would be expected because aerobic microorganisms are known to degrade cis-1,2-DCE to carbon dioxide and water through the formation of short-lived, unstable intermediates releasing chloride during the cis-1,2-DCE biotransformation process (Lorah et al., 1997; Lee et al., 1999). Concentrations of cis-1,2-DCE did not decrease significantly in abiotic controls, indicating microbially mediated transformation of cis-1,2-DCE in live controls.

In the current study, the mechanism of oxidation may have been direct or co-metabolic oxidation as suggested in Bradley and Chapelle (1998), Wiedemeier et al., (1999) and Suthersan (2000). Co-metabolic oxidation of cis-1,2-DCE using methane as a cosubstrate under aerobic conditions has been demonstrated (Bradley and Chapelle, 1998). Wiedemeier et al., (1999) reported that cis-1,2-DCE has been observed to be more amenable to aerobic co-metabolism than direct oxidation. In addition, Arp et al. (2001) reported that cis-1,2-DCE is not susceptible to direct aerobic metabolism. It is, therefore, likely that cis-1,2-DCE transformation was via methanotrophic co-metabolism rather than through direct oxidation in the present study since the contaminant started to disappear after methane was injected into the microcosms. These results indicate that the microbial community in the bottles was capable of transforming cis-1,2-DCE aerobically in the presence of methane.

• Transformation of 1,2 DCA under Anaerobic Conditions

The redox indicator in the 1,2-DCA amended microcosms remained colorless throughout the study, thus suggesting that anaerobic conditions were maintained. The
mixed cultures were able to degrade 1,2-DCA under anaerobic conditions without a lag, suggesting robust indigenous bacterial populations (Figure 3.5). After about 100 hours, over 95% of the parent compound was degraded in the soil slurries prepared from the bottom section of the PSB core, whereas about 94% remained in the abiotic controls within the same period. Production of ethene coincided with the onset of disappearance of 1,2-DCA whereas no ethene was detected in abiotic controls (Figure 3.5), thus indicating that the formation of ethene was indeed due to microbial transformation of 1,2-DCA. Amounts of ethene quantified in the active microcosms at the end of the incubation periods were reasonably consistent with the stoichiometric conversion of 1,2-DCA to ethene (Figure 3.5). Similar trends were observed in the other live microcosms but the final degradation product was either ethene or ethane depending on the transformation mechanism involved. The first-order model for 1,2-DCA dechlorination via dihaloelimination fitted the kinetic data fairly well as Figure 3.6 shows.

VC, one of the possible intermediate degradation products of 1,2-DCA (Maymó-Gatell et al., 2001) was not detected in any microcosms. Chloroethane, another possible intermediate metabolite of 1,2-DCA was detected in trace amounts (< 2 µM), which is about 3% of the initial 1,2-DCA concentration. These results indicate that two mechanisms were involved in the transformation of 1,2-DCA. In the first mechanism, DCA was dechlorinated mainly to ethene in essentially a single step reductive dihaloelimination reaction, whereas in the second mechanism the chemical was transformed through consecutive hydrogenolysis to ethane via chloroethane as an intermediate. Holliger and co-workers (1990) also observed the above two reductive
dechlorination mechanisms of 1,2-DCA in their study involving cell suspensions of methanogenic bacteria. In the current study, consecutive hydrogenolysis was observed in

![Reaction kinetics of 1,2-DCA dechlorination in bottom sediments of PSB injected with three approximately equal consecutive doses of the chemical.](image)

**Figure 3.5:** Reaction kinetics of 1,2-DCA dechlorination in bottom sediments of PSB injected with three approximately equal consecutive doses of the chemical.

about 80% of the live microcosms whereas dihaloelimination was observed in the remaining live controls (Table 3.2), which indicates that the former dechlorination mechanism was more preferred by dechlorinating organisms than the latter transformation pathway. Surprisingly, dechlorinating organisms appeared to prefer hydrogenolysis to dihaloelimination although the latter route is energetically more favorable than the former (Dolfing, 1999).
Chloroethane was probably not an important intermediate degradation product of 1,2-DCA in the current study since trace amounts of CA were detected in agreement with findings of Lee et al. (1999), but in contrast with observations made by Holliger and co-workers (1990) in which concentrations of CA of between 12 and 30% of the initial 1,2-DCA concentrations were detected. It is possible that CA was not detected in appreciable amounts in the present study because initial concentrations of 1,2-DCA used were lower (approximately 60 μM) compared with those used in the studies conducted by Holliger and co-workers (1990) of between 246 and 356 μM. Differences in enzymatic activities of bacteria involved in dechlorination reactions of 1,2-DCA may also explain the differences in relative amounts of metabolic products observed in the present study (Holliger et al., 1992).

**Table 3.2**: Degradation kinetic rate constants, half-lives and transformation routes of cis-1,2-DCE and 1,2-DCA for PS and PSB under anaerobic conditions

<table>
<thead>
<tr>
<th>Section</th>
<th>Biodegradation rate constant, $K$ (hr$^{-1}$)</th>
<th>Half-life, $t_{\frac{1}{2}}$ (days)</th>
<th>1,2-DCA biodegradation mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1,2-cis-DCE</td>
<td>1,2-DCA</td>
<td>1,2-cis-DCE</td>
</tr>
<tr>
<td><strong>PS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom (B)</td>
<td>0.046 ± 0.005</td>
<td>0.013 ± 0.002</td>
<td>0.63 ± 0.07</td>
</tr>
<tr>
<td>Middle (M)</td>
<td>0.039 ± 0.003</td>
<td>0.009 ± 0.002</td>
<td>0.74 ± 0.08</td>
</tr>
<tr>
<td>Top (T)</td>
<td>0.034 ± 0.006</td>
<td>0.003 ± 0.001</td>
<td>0.85 ± 0.19</td>
</tr>
<tr>
<td><strong>PSB</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom (B)</td>
<td>0.071 ± 0.008</td>
<td>0.032 ± 0.008</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td>Middle (M)</td>
<td>0.059 ± 0.006</td>
<td>0.008 ± 0.001</td>
<td>0.49 ± 0.06</td>
</tr>
<tr>
<td>Top (T)</td>
<td>0.048 ± 0.008</td>
<td>0.007 ± 0.003</td>
<td>0.60 ± 0.11</td>
</tr>
</tbody>
</table>

* Number of experimental runs per column section = 9
In both soil mixtures and in all live microcosms, 1,2-DCA was observed to be consumed at an increasing rate over time as a sample plot (Figure 3.5) illustrates. This suggests that the chemical supported microbial growth. For example, the first order degradation rate constant of 1,2-DCA in the bottom sediments of PSB core was observed
to increase in successive experimental runs for the same dosage of the test chemical as follows: $0.023 \pm 0.002$, $0.034 \pm 0.001$ and $0.047 \pm 0.003$ hr$^{-1}$. Maymó-Gatell et al. (2001) also observed that 1,2-DCA is capable of supporting growth in an experiment in which Dehalococcoides ethenogenes strain 195 was incubated in an enriched growth medium.

Methane was repeatedly and consistently observed to increase during degradation of 1,2-DCA (Figure 3.5). Holliger et al. (1990) observed an increase in amounts of 1,2-DCA dechlorination products formed as a result of stimulation of methanogenesis, but the opposite occurred when methanogenesis was inhibited. Lee et al. (1999) also observed simultaneous disappearance of 1,2-DCA and accumulation of ethane and methane in microcosms constructed from aquifer materials. The findings of Holliger et al. (1990), Lee et al. (1999) and observations made in the present study suggest that the observed phenomena are a result of simultaneous methanogenesis and 1,2-DCA dechlorination, perhaps mediated by methanogenic bacteria with comparable physiological characteristics. Wild et al. (1995) reports that hydrogenotrophic and aceticlastic methanogenic bacteria are capable of reductive dechlorination of 1,2-DCA. Holliger et al. (1992) have demonstrated that both dihaloelimination and hydrogenolysis mechanisms of 1,2-DCA dechlorination can simultaneously be catalyzed by purified methyl-CoM reductase, a key enzyme of methanogenesis. This could be the reason for the observed concomitant 1,2-DCA dechlorination and methanogenesis in the current study since it appears that a common enzyme catalyzes these metabolic reactions. It is not surprising then, that 1,2-DCA can be transformed to carbon dioxide and methane by some methanogenic organisms (Bouwer and McCarty, 1983).
• Transformation of 1,2 DCA under Aerobic Conditions

Like in the aerobic cis-1,2-DCE experiments, methane was not added into the microcosms during the first four weeks of the aerobic degradation experiments of 1,2-DCA, and during this period the concentration of 1,2-DCA remained unchanged. Five days after methane was added into the microcosms 1,2-DCA started to disappear. The observed decrease in concentration of 1,2-DCA was primarily attributable to biological activity, because the recovery of 1,2-DCA in abiotic control microcosms was more than 95%. Furthermore, since contaminant concentrations remained unchanged during the first four weeks prior to the addition of methane, disappearance of 1,2-DCA in the microcosms can circumstantially be attributed to microbial processes and not to abiotic losses. Since ethene, ethane and chloroethane were not detected, the decrease in 1,2-DCA concentration can be ascribed to aerobic rather than anaerobic transformation. 1,2-DCA may be transformed via both direct aerobic oxidation and cometabolism with methane serving as a cosubstrate (Wiedemeier et al., 1999; Arp et al., 2001) but it is not entirely clear which of these two routes was involved during degradation of 1,2-DCA in the current study. Degradation of 1,2-DCA following addition of methane, however, strongly suggest that cometabolism played a role.

• Comparison of Degradation Kinetics of the Test Chemicals

It can generally be observed from Table 3.2 that under anaerobic conditions, degradation rate constants of cis-1,2-DCE transformation for both types of substrates decreased with height from the bottom of the soil columns. ANOVA results for cis-1,2-DCE for both types of soils showed that differences in anaerobic degradation rate constants in the soil column sections were statistically significant ($P > 0.05$). Results of t-
tests showed that differences in degradation rate constants of \textit{cis}-1,2-DCE in bottom, middle and top sections of the soil columns were also statistically significant. Degradation rate constants in PSB microcosms for \textit{cis}-1,2-DCE were found to be significantly higher compared to those in PS microcosms ($P < 0.05$).

Results of t-tests showed that 1,2-DCA degradation rate constants of bottom soils under anaerobic conditions were significantly higher than those of middle and top soils for both soil mixtures. However, reaction rate constants of middle and top soils were not significantly different for both soil mixtures ($P < 0.05$). PSB soil mixture had significantly higher 1,2-DCA degradation rate constants than PS mixture for bottom soils. Kinetic constants of middle and top sections of the soil mixtures were found to be statistically similar ($P > 0.05$).

Half-lives of 1,2 DCA for both soil mixtures under anaerobic conditions are shown in Table 3.2. Half-lives of 1,2-DCA have been determined previously by various researchers. Half-lives of 1,2-DCA observed under field conditions along groundwater flow paths reported by Klečka et al. (1999) ranged between 1 year and 30 years. In another study on intrinsic bioremediation of chlorinated organic compounds at a chemical manufacturing site conducted by Lee and co-workers (1990), half-lives estimated for 1,2-DCA degradation under field conditions ranged from 64 to 165 years. Obviously these reported half-life values for 1,2-DCA degradation are significantly higher than those found in the present study. It should however be pointed out that interpretation and comparison of dechlorination rates reported by different studies conducted under widely varying experimental conditions need to be done with some caution. The current study was conducted under static conditions following mixing and homogenization using a
mixed culture whereas as the reported studies were conducted under advective and dispersive conditions. The rates found in the present study are higher than those reported in the field studies mentioned above probably because microcosms create a relatively protected, stable environment for the microorganisms. A lack of mass transfer limitations may also account for higher degradation rates in microcosms. Another reason could be that soil substrates used in the current study have higher organic carbon contents (21.7 to 29%) and consequently higher microbial activity compared with aquifer sediments, which normally have lower organic matter content with some as low as 0.005% (Wiedemeier et al., 1999).

The observation that under anaerobic conditions bottom soil microcosms had the highest degradation rates for both contaminants in both soil mixtures supports the findings from up-flow column studies (Kassenga et al., 2003), which showed that over 90% of contaminants were removed within 7.5 cm from the inlet of the columns. Higher bacterial population density due to availability of an abundant supply of contaminants immediately downstream of the inlet to the columns could be the main reason for higher degradation rates in the bottom section compared to the other sections. The fact that concentrations of parent compounds decreased with increasing height from the soil column inlets could also explain the observed differences in the kinetic rates in the soil sections since anaerobic biotransformation of parent compounds yields more energy for bacterial growth than their degradation daughter products (Dolfing, 1999).

Under aerobic conditions, transformation rates of 1,2-DCA (0.22 to 0.28 day\(^{-1}\)) were higher by a factor of at least five compared to those of cis-1,2-DCE (0.03 to 0.05 day\(^{-1}\)) in both soil mixtures. Kinetics of degradation of the test chemicals at different
depths of the soil columns was found to be statistically comparable ($P < 0.05$) for both soil mixtures. Based on kinetic constants, 1,2-DCA was found to be more amenable to aerobic degradation in both soil mixtures than cis-1,2-DCE.

Kinetic constants of aerobic transformation of cis-1,2-DCE in the present work compare well with those observed by Lorah et al. (1997) for wetland sediments (0.05 to 0.09 day$^{-1}$). Lower degradation rates of cis-1,2-DCE were observed under aerobic compared to under anaerobic conditions in agreement with findings of Lorah et al. (1997) and Cornuet et al. (2000). However, aerobic degradation rates of the less chlorinated solvents (cis-1,2-DCE, VC and 1,2-DCA) are generally thought to be faster than their anaerobic reductive dechlorination rates (Vogel et al., 1987; Wiedemeier et al. 1999; Suthersan, 2000) and this is in contrast with the observations made for cis-1,2-DCE during the current work and findings of Lorah et al. (1997) and Cornuet et al. (2000).

- **Detection of Dehalococcoides sp. in Anaerobic Soil Slurries**

Soil slurry samples from two microcosms of bottom, middle and top sections for each type of soil incubated under anaerobic conditions were tested for the presence of Dehalococcoides 16S rDNA sequences. DNA from the microbial communities were extracted and tested for the presence of Dehalococcoides 16S rDNA using 2 primer sets (Table 3.1). The PCR Dehalococcoides assays performed with community DNA from all samples yielded amplified products of the anticipated size for each Dehalococcoides PCR primer set used. Figure 3.7 shows an example of the PCR products detection results for some of the samples analyzed. An example electropherogram is presented in Figure 3.8. All samples tested positive for the presence of the Dehalococcoides 16S rDNA sequences for both primer sets used.
**Figure 3.7:** Detection of *Dehalococcoides* 16S rDNA sequences in PS and PSB microcosms. Lane L – Ladder; lane 1 – PS Bottom; lane 2 – PS Middle; lane 3 – PS Top 1; lane 4 - PSB Bottom 1; lane 5 – PSB Middle 3; lane 6 – PSB Top 1.

The 1377 base pair fragments of PS and PSB bottom soil and the 266 base pair fragment of PSB bottom soil were cloned, sequenced, and compared to databases of previously reported microorganisms. All of the 1377 and the 266 base pair fragments were each 99.75% similar, by pair wise comparison, to the same three uncultured *Dehalococcoides* entries in the database: AF529119 (uncultured *Dehalococcoides* sp.)
Figure 3.8: Electropherogram showing the detection of *Dehalococcoides* 16S rDNA sequences in PSB Middle sediments microcosms.

Clone FTLM182), AF357918 (*Dehalococcoides* *sp.* FL2) and AF388546 (uncultured *Dehalococcoides* *sp.* Clone DHC-nftx). Clones in the present study were 98.2 and 99.7% similar to *Dehalococcoides* *ethenogenes* Strain 195 and strain CBDB1, respectively. The closest 16S rDNA match to all of the clones in the study described herein is a strain referred to as CBDB1, which was previously described by Adrians et al. (2000). Strain
CBDB1 is a highly specialized bacterium that stoichiometrically dechlorinates 1,2,3-trichlorobenzene (TCB), 1,2,4-TCB, 1,2,3,4-TeCB, 1,2,3,5-tetrachlorobenzene (TeCB) and 1,2,4,5-TeCB to dichlorobenzenes or 1,3,5-TCB. For CBDB1, presence of chlorobenzene as an electron acceptor and hydrogen as an electron donor is apparently essential for growth, and indicates that strain CBDB1 meets its energy needs by a dehalorespiratory process. According to their 16S rRNA gene sequences, strain CBDB1 is closely related to *Dehalococcoides ethenogenes* strain 195 and several uncultivated bacteria form a new bacterial cluster, of which strain CBDB1 is the first, so far, to thrive on a purely synthetic medium.

Differences between the sequences of the cloned 1377 bp fragment of 16S rDNA determined in the study described in this chapter and that of *Dehalococcoides ethenogenes* strain 195 and the three closest NCBI matches for uncultured microorganisms are shown in Table 3.3. The first column of the table lists the base position in the 16S rDNA sequence for *Dehalococcoides ethenogenes* Strain 195 to provide a frame of reference. Positions not listed in Table 3.3 were identical to Strain 195 in the other three sequences. A “+” symbol indicates an insertion in sequence relative to strain 195 (after the specified base number). A “-” symbol indicates a deletion. Base numbers shown in bold text in the first column of Table 3.3 indicates difference between strain 195 and those detected in cloned DNA sequences reported by Major et al., (2002).

The 266 base pair fragment of PSB-B1 was also cloned, sequenced, and compared to the NCBI database. The sequence originating from PSB-B1 was aligned with the top three sequences in the database that produced significant alignments. The sequences
Table 3.3: 16S rDNA sequence differences from 1377 bp partial 16S rDNA sequences determined in this study relative to *Dehalococcoides ethenogenes* Strain 195\(^a\) and the closest sequence matches reported in the NCBI database.

<table>
<thead>
<tr>
<th>Strain 195 base number</th>
<th>Strain 195 AF004928</th>
<th>AF529119, AF357918, AF388546</th>
<th>PS-B2 AFxxxxxx</th>
<th>PSB-B1 AFxxxxxx</th>
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<td>1341</td>
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</table>

\(^a\) Base number: base position in the 16S rDNA sequence for *Dehalococcoides ethenogenes* Strain 195. Positions not listed were identical to Strain 195 in the other three sequences. “+”: insertion in sequence relative to strain 195 (after specified base no.). “-”: deletion in sequence relative to other sequences. Bold: indicates difference between strain 195 and that detected in cloned DNA sequences reported by Major et al. (2002).
were of AF388548, AF388535 and AF388532, all described in the NCBI as uncultured *Dehalococcoides* group microorganisms. The sequences of the aligned 232 base pairs were identical in a pairwise comparison.

The results of the PCR-based detection technique, thus, indicate that microorganisms closely related to *Dehalococcoides ethenogenes* were present in both the PS and PSB microbial communities and that they were distributed throughout the middle, bottom, and top. While it seems that *Dehalococcoides* species were likely important elements in the PS and PSB communities that dechlorinate *cis*-1,2-DCE completely to ethene, it is likely that other organisms were also involved, either directly or indirectly, in converting *cis*-1,2-DCE to ethene. For example, some bacterial species such as *Desulfovibrio* can ferment substrates such as lactate to produce hydrogen, the only known electron donor for *Dehalococcoides* species (Richardson et al., 2002).

### 3.6 Conclusion and Significance of the Results

From kinetic studies it was found that while anaerobic degradation was observed to be an important removal process for *cis*-1,2-DCE, both aerobic and anaerobic degradation processes were observed to be comparably effective in the removal of 1,2-DCA in both soil mixtures. Since methane production and oxidation occur across the O$_2$ gradient within the rhizosphere in wetland systems, the coupled anaerobic dechlorination and aerobic cometabolism oxidation (Pardue et al., 2000) may particularly be a more important fate mechanism for 1,2-DCA compared to *cis*-1,2-DCE in a constructed wetland.

Complete dechlorination of *cis*-1,2-DCE to ethene was most likely mediated by *Dehalococcoides sp*. Therefore, knowledge of consortia members, especially those whose
presence correlates with dechlorination activity, can potentially be valuable in aiding the
detection and where possible cultivation of the relevant dechlorinating populations. Since
*D. ethenogenes* and the same types of methanogens were detected in both types of soil,
the differences in sizes of microbial populations may explain the significant
dechlorination rates in the soil mixtures. Therefore, kinetic data suggest that the
peat/sand/Bion Soil mixture was able to support the growth of *Dehalococcoides* and other
bacterial groups better than peat/sand mixture only due to the effect of the Bion Soil (the
only ingredient that differentiated the two soil mixtures). Mixture of peat/sand/Bion Soil
is therefore a more promising material for construction of a treatment wetland for
chlorinated aliphatic compounds than its counterpart.

Faster anaerobic degradation kinetics of the target contaminants indicates higher
bacterial growth rates at the bottom of the wetland bed, which may cause clogging of
pore spaces due to biofilm growth, leading to failure of the constructed wetland system.
Isalou et al., (1998) observed 89% reduction in porosity for the bottom section (from 0 to
15 cm) of sand columns used for PCE degradation over a period of 2.5 years due to
biofilm growth. Therefore, clogging of pore spaces due to biofilm growth in bottom
sediments may be a critical consideration in designing a treatment wetland for chlorinated
solvents.
CHAPTER 4: BIODEGRADATION OF CIS-1,2-DICHLOROETHENE AND 1,2-DICHLOROETHANE IN CONSTRUCTED WETLAND SOILS: II. HYDROGEN THRESHOLDS

4.1 Introduction

Contamination of soil and groundwater with chlorinated organic solvents is a widespread and serious environmental problem, which has received much attention in recent decades (Suthersan, 2002; Wiedemeier et al., 1999). Anaerobic dehalogenation is recognized as a useful method for remediation of sites contaminated with chlorinated aliphatic organic compounds, either naturally through intrinsic remediation (natural attenuation) or through engineered approaches because it results in complete dehalogenation to innocuous end products, ethene and ethane and is also more cost-effective than the traditional pump-and-treat systems (Yang and McCarty, 1998; Wiedemeier et al., 1999; Löffler et al., 1999; Suthersan, 2002).

Among the factors affecting the practical application of dehalogenation, competition for electron donors between dehalogenators and other microorganisms in anaerobic mixed environments is perhaps one of the most important factors (Smatlak and Gossett, 1996; Ballapragada et al., 1997; Yang and McCarty, 1998). Several researchers suggest that the microbial reductive dechlorination of chlorinated ethenes depends on the presence of molecular hydrogen as the actual electron donor, either directly available or produced from the fermentation other substrates (Ballapragada et al., 1997; Suthersan, 2002). At least seven of the strains of dechlorinating bacteria that have so far been isolated including *Dehalobacter restrictus* (Fennell and Gossett, 1997), *Dehalospirillum multivorans* (Smatlak and Gossett, 1996), *Desulfomonile tiedje* (Ballapragada et al.,
Desulfitobacterium frapperi (Maymó-Gatell et al., 2001) and a recently discovered strain, Dehalococcoides ethenogenes Strain 195 (Maymó-Gatell et al., 2000) have been observed to use a very limited set of substrates including hydrogen. Therefore, it is most likely that hydrogen could be the primary donor for most of the dechlorinating bacteria (Ballapragada et al., 1997).

Natural wetlands have been observed to be capable of attenuating chlorinated solvents without human intervention (Pardue, 1992; Lorah et al., 1997). In a recent study, Kassenga et al. (2003) observed high dechlorination rates of chlorinated volatile organic compounds in bench-scale constructed wetland mesocosms. Since natural processes are involved in attenuation of the contaminants, these studies suggest that natural and engineered wetland systems may potentially offer a cost-effective alternative to treatment of sites contaminated with chlorinated solvents. High organic carbon content and the abundance of microbial diversity (Pardue, 1992; Lorah and Olsen, 1999) make natural wetland soils conducive for production of electron donors such as hydrogen and volatile fatty acids such as propionate, butyrate and acetate (Westermann, 1994; Conrad, 1999) necessary for driving microbial reductive dechlorination reactions (Lorah and Olsen, 1999). The fact that peat is rich in organic carbon, and among other components it consists of acetate and propionate (Kao and Lei, 2000), which under appropriate geochemical conditions may ferment to produce high levels of H₂ (Conrad, 1999) it may be a good candidate for construction of treatment wetlands for chlorinated volatile organic compounds. Since hydrogen has been observed to be an important electron donor during reductive dechlorination (Ballapragada et al., 1997), it is therefore important to
investigate H\textsubscript{2} dynamics in wetland soils to better understand factors that affect its consumption and production.

H\textsubscript{2} is produced through the action of many fermentative organisms in the natural environment and it is consumed by methanogens, nitrate-reducing bacteria, sulfate-reducing bacteria, and ferric-reducing bacteria, among others (Conrad, 1999). Members of these bacterial groups compete for hydrogen with halorespirers and, as such, competition can limit reductive dechlorination due to the limited supply of hydrogen (H\textsubscript{2}) in anaerobic systems (Lovley and Klug, 1983; Lovely and Phillips, 1987; Lovley and Goodwin, 1988; Fennel and Gossett, 1998; Yang and McCarty, 1998). Cord-Ruwisch et al. (1988) reported that the outcome of competition for traces of H\textsubscript{2} depends on threshold of concentration for H\textsubscript{2} rather than kinetic parameters.

According to Lovley et al. (1994), H\textsubscript{2} may be a useful tool for analyzing the redox conditions in groundwaters. In their study, H\textsubscript{2} concentrations associated with the various terminal electron-accepting reactions in aquatic sediments were as follows: methanogenesis, 7-10 nM; sulfate reduction, 1-1.5 nM; Fe(III) reduction, 0.2 nM; Mn(IV) and nitrate reduction, less than 0.05 nM (Lovley et al., 1994). The results suggested that each terminal electron-accepting reaction has a unique threshold H\textsubscript{2} concentration associated with it, which is primarily dependent upon the physiological characteristics of the H\textsubscript{2}-consuming microorganisms and the energy yield from hydrogen oxidation as affected by the electron acceptor used (Table 4.1). Therefore, the H\textsubscript{2} threshold is inversely related to changes in Gibbs’ free energy and the electrochemical potential of the H\textsubscript{2}-consuming reaction, i.e. organisms using acceptors associated with greater energy production (more electrochemically positive) have lower hydrogen
Table 4.1: Energetics of hydrogen-utilizing reactions examined during this study

<table>
<thead>
<tr>
<th>Process</th>
<th>Reaction</th>
<th>$^a\Delta G_r^{o'}$ (kJ/mol H₂)</th>
<th>$^b\varepsilon^{o'}$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanogenesis</td>
<td>$\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$</td>
<td>-48.6</td>
<td>-162.2</td>
</tr>
<tr>
<td>cis-1,2-DCE reduction</td>
<td>$\text{C}_2\text{H}_2\text{Cl}_2 + \text{H}_2 + \text{H}^+ \rightarrow \text{C}_2\text{H}_3\text{Cl} + \text{Cl}^- + 2\text{H}^+$</td>
<td>-156.9</td>
<td>+397.9</td>
</tr>
<tr>
<td>VC reduction</td>
<td>$\text{C}_2\text{H}_3\text{Cl} + \text{H}_2 + \text{H}^+ \rightarrow \text{C}_2\text{H}_4 + \text{Cl}^- + 2\text{H}^+$</td>
<td>-167.0</td>
<td>+451.3</td>
</tr>
<tr>
<td>1,2-DCA reduction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dihaloelimination</td>
<td>$\text{C}_2\text{H}_4\text{Cl}_2 + \text{H}_2 + \text{H}^+ \rightarrow \text{C}_2\text{H}_4 + 2\text{Cl}^- + 3\text{H}^+$</td>
<td>-205.6</td>
<td>+651.3</td>
</tr>
<tr>
<td>Consecutive hydrogenolysis</td>
<td>$\text{C}_2\text{H}_4\text{Cl}_2 + 2\text{H}_2 + \text{H}^+ \rightarrow \text{C}_2\text{H}_5\text{Cl} + \text{Cl}^- + 4\text{H}^+$</td>
<td>-132.4</td>
<td>+272.0</td>
</tr>
<tr>
<td></td>
<td>$\text{C}_2\text{H}_4\text{Cl}_2 + \text{H}_2 + \text{H}^+ \rightarrow \text{C}_2\text{H}_6 + \text{Cl}^- + 2\text{H}^+$</td>
<td>-169.4</td>
<td>+463.7</td>
</tr>
<tr>
<td>Sum (Consecutive hydrogenolysis)</td>
<td>$\text{C}_2\text{H}_4\text{Cl}_2 + 3\text{H}_2 + 2\text{H}^+ \rightarrow \text{C}_2\text{H}_6 + 2\text{Cl}^- + 6\text{H}^+$</td>
<td>-144.7</td>
<td>+335.7</td>
</tr>
</tbody>
</table>

$^a\Delta G_r^{o'}$ values were calculated according to Dolfing and Janssen (1994): organic substrate + H₂ → organic product + H⁺ + Cl⁻. Aqueous $\Delta G_r^{o'}$ values were used for organic substrates at 25 °C, pH 7. $\Delta G_r^{o'}(\text{H}^+) = -39.9$ kJ/mol; $\Delta G_r^{o'}(\text{Cl}^-) = -131.3$ kJ/mol. Thermodynamic data on solutes are from Dolfing and Janssen (1994) and Stumm and Morgan (1981).

$^b\varepsilon^{o'}$ calculated according to Löffler et al. (1990) $\varepsilon^{o'} = (\Delta G_r^{o'} / -0.193) \cdot 414$ (redox potential of H₂ = -414mV).

Concentration thresholds than organisms using electron acceptors that yield less energy from H₂ oxidation (Yang and McCarty, 1998; Löffler et al., 1999). According to this approach, the steady-state threshold concentrations of H₂ for the electron accepting reactions of interest in the present study (Table 4.1) will be expected to follow the following order (from least to most energetically favorable TEAP): methanogenesis > cis-1,2-DCE dechlorination > 1,2-DCA dechlorination (dihaloelimination degradation...
mechanism); or methanogenesis > 1,2-DCA dechlorination (consecutive hydrogenolysis degradation route) > cis-1,2-DCE dechlorination.

A number of studies have focused on the degradation of 1,2-DCA (for example, Wild et al. (1995); Lee et al. (1999); and Maymó-Gatell, et al. (1999)) but few (if any) have evaluated hydrogen thresholds relative to dechlorination of 1,2-DCA. Yang and McCarty (1998) investigated competition for H₂ within a chlorinated solvent dehalogenating anaerobic mixed culture using cis-1,2-DCE as one of the test chemicals. However, this study was conducted under conditions far removed from most actual field conditions and it did not replicate the competitive nature for H₂ observed in natural sedimentary environments since exogenous hydrogen precursors were added during the experiments. Moreover, no study has been reported on H₂ concentrations associated with the transformation of cis-1,2-DCE and 1,2-DCA under inhibited methanogenesis by 2-bromoethanesulfonate (BES).

This is the second part of the two-part paper series. The first part of the paper covered the kinetics of degradation, mechanisms involved in the transformation of the test compounds (cis-1,2-DCE and 1,2-DCA) and PCR detection of the 16S rDNA gene of Dehalococcoides sp. (see Chapter 3). The main objectives of the present study was to evaluate hydrogen concentrations during dechlorination of cis-1,2-DCE and 1,2-DCA and to establish if higher hydrogen concentration levels would favor methanogenesis over dechlorination as observed in previously reported studies. The paper also examines the effects of BES, an inhibitor of methanogenesis on degradation kinetics of the test chemicals, on H₂ concentration and on TEAPs.
4.2 Materials and Methods

- **Chemicals**

  Neat solutions of *cis*-1,2-DCE and 1,2-DCA (Supelco Park, Bellefonte, PA) were used to prepare stock solutions. Analytical standards and surrogate for the VOCs were obtained as mixtures or neat liquids from Supelco Inc. (Supelco Park, Bellefonte, PA). Resazurin, formaldehyde, and BES were procured from Sigma Chemical Co. (Saint Louis, MO). Hydrogen standards were prepared using 10 ppm hydrogen (BOC Group Inc., Baton Rouge, LA). Other chemicals used in this study were of reagent grade and were purchased from various vendors.

- **Soil Preparation**

  One wetland soil column was comprised of *Latimer peat* (Latimer's Peat Moss Farm, West Liberty, OH), *Bion Soil* (a compost product) (Dream Maker Dairy, Cowlesville, NY) and sand mixed at a ratio of 1.3:1.1:1 (Bion Soil:Latimer:sand) by weight. The second soil column was prepared from *Latimer peat* and sand (1.3:1 w/w) alone. These soil mixtures were identified as the most promising materials for the construction of treatment wetland bed for chlorinated aliphatic compounds (Kassenga et al., 2003).

  The bottom, middle and top sections of the soil columns were respectively sliced at following intervals from the base of the soil columns; 0 to 10; 20 to 30; and 40 to 50 cm. Soils collected from these sections were used to construct anaerobic microcosms. Since sectioning of the soil columns was done in an ambient atmosphere, the soil slices used for construction of microcosms were shaved under a nitrogen atmosphere in a glove bag (I2R, Cheltenham, PA) to minimize the effects of exposure to air of anaerobic
organisms. The interiors of soil columns were used for construction of anaerobic microcosms.

**Anaerobic Microcosms**

Construction of anaerobic microcosms was done in a nitrogen atmosphere in a glove bag (I2R, Cheltenham, PA) by homogenizing and packing soil from each section in 160 mL serum bottles using a 1.5:1 volumetric ratio of groundwater to wetland sediments according to Lorah et al. (1996). Volume of headspace in serum bottles was 20 mL. All reaction mixtures were sealed with Teflon-lined rubber septa and aluminum crimp seals and incubated in an inverted static position in the dark at 25 °C, which was the approximate temperature of the groundwater during the soil column experiments. Resazurin (0.0002%) was added as the redox indicator. Microcosms were not amended with hydrogen donors or nutritional media.

Initially, groundwater contaminated mainly with *cis*-1,2-DCE and 1,1,1-tetrachloroethane (1,1,1-TCA) from a Superfund site was used for the experiments. After all the contaminants were reduced to below detection (5 µg/L), *cis*-1,2-DCE and 1,2-DCA at a concentration of about 60 µM were consecutively spiked three times into the microcosms to determine degradation kinetics of the contaminants. Temporal monitoring of concentrations of parent compounds and degradation daughter products, H₂ and methane were done after each spike until the concentrations of the parent compounds and degradation daughter products dropped below the detection limit of the analytical methods. Monitoring of H₂ and methane continued for four more weeks after the disappearance of the parent compounds and degradation daughter products to establish the thresholds of H₂ under methanogenesis. Samples were withdrawn from the bottles
and immediately analyzed without storage for chlorinated ethenes and ethanes, ethene, ethane, hydrogen and methane.

Treatments used during the study were as follows: (1) soil slurries from the bottom, middle and top sections of both types of soils (peat/sand and peat/sand/Bion Soil) amended with *cis*-1,2-DCE and 1,2-DCA; (2) soil slurries from the middle of peat/sand/Bion Soil core amended with the test chemicals and BES; (3) soil slurries from the middle of peat/sand/Bion Soil core amended with BES and (4) soil slurries from the middle of peat/sand/Bion Soil core without the test chemicals and BES. Abiotic controls were included for each of the above treatment to monitor for non-biological losses of the test chemicals. For abiotic controls, reaction mixtures were prepared as described above and adjusted to contain 1% formaldehyde. To ensure reproducibility, triplicate serum bottles were used in each experiment, and, whenever feasible, experiments were repeated at least two times.

- **Analytical Procedures**

VOCs were analyzed using EPA Method 8260B using a purge and trap apparatus to concentrate and introduce the sample into the gas chromatograph-mass selective detector (Agilent 6890 gas chromatograph-5972A mass selective detector). The GC was equipped with a 30 m × 0.25 mm × 0.25 μm film thickness, Agilent 5MS (5% Phenyl Methyl Siloxane) capillary column (Palo Alto, CA). A thermal desorption trap (VOCARB 3000; Supelco, Bellefonte, PA) was employed in the purge and trap apparatus. The samples were purged for 11 min with ultra-high-pure helium at a flow rate of 35 mL/min, desorbed for 0.5 min and baked for 13 min at 225°C. The GC column temperature program was -80°C for 1 min ramped to 20°C at 15°C/min then ramped to
80°C at 10°C/min and finally to 220°C at 20°C/min. The injector and detector temperatures were 250°C and 280°C respectively. Helium was used as a carrier gas at a flow rate of 2.1 mL/min. Daily blanks, calibration checks and tunes were run to assure that the analytical method was in control. Recovery of surrogates injected into every sample also ensured that no gross dilution errors or leaks have occurred in the GC/MS system.

Methane was measured using GC/FID. 1 mL of gas in the headspace was withdrawn using a gas tight syringe and injected into the gas chromatography with flame ionization detector (Agilent 5890 Series II) equipped with a 2.4 m × 0.32 mm ID column packed with Carbopack b/1% SP-1000 (Supelco, Bellefonte, PA). The column temperature was held at 50°C isothermally for 6.5 min, and the injector and detector temperatures were 375 and 325°C respectively. The carrier gas was ultra high purity nitrogen at a flow rate of 12 mL/min.

Hydrogen was analyzed using reduction gas analyzer (Trace Analytical, Menlo Park, CA) equipped with a reduction gas detector. Headspace samples were injected into a 1-mL gas sampling loop and were separated with a molecular sieve analytical column (Trace Analytical, Menlo Park, CA) at an oven temperature of 40 °C, ultra-high-purity nitrogen (BOC Gases, Baton Rouge, LA) was used as a carrier gas after it was passed through a catalytical combustion converter (Trace Analytical, Menlo Park, CA) to remove traces of H₂. The detection limit for H₂ under these conditions was 1 ppb. Aqueous H₂ concentrations were calculated using the following equation adopted from Löfler et al. (1999): [H₂,aq.] = LP/RT, where [H₂,aq.] is the aqueous concentration of H₂ (in moles per liter); L is the Ostwald coefficient for H₂ solubility (0.01913 at 25°C); R is
the universal gas constant (0.0821 liter·atm·K⁻¹·mol⁻¹); $P$ is the pressure (in atmospheres); and $T$ is the temperature (K). All hydrogen data are reported as the aqueous phase concentration unless otherwise stated. Hydrogen was sampled at a sufficient interval of time (8 to 72 hours) to ensure complete equilibrium during the continuous production and consumption of aqueous hydrogen in the soil slurries. In addition, Lovley and Goodwin (1988) reported that dissolved $H_2$ comes into equilibrium with gaseous $H_2$ within 20 minutes even without shaking sediments.

Organic acids (lactate, formate, succinate, acetate, propionate, butyrate and benzoate) were analyzed using high-pressure liquid chromatograph (Agilent 1090 Series II Liquid Chromatography). Redox sensitive substances, sulfate, nitrate and ferric iron were analyzed using the liquid chromatography (Dionex LC-20, Dionex Corp., Sunnyvale, CA).

- **Statistical Analysis**

  Statistical analysis included ANOVA (hydrogen data), linear regression (hydrogen, methane, ethane and ethene calibration curves), and non-linear regression using a first-order decay model (VOCs monitoring data). Results were considered statistically significant if $P \leq 0.05$.

4.3 Results and Discussion

- **Degradation Kinetics**

  Since other redox-sensitive compounds such as sulfate, nitrate and ferric iron were not detected, reducing conditions in all microcosms were predominantly methanogenic as indicated by high concentrations of methane (>1000 µM).
Pseudo-first order degradation rate constants (Table 4.2) of the test compounds in microcosms constructed from bottom, middle and top sections of the soil columns were found to be statistically different ($P < 0.05$) for both soil mixtures (see Chapter 3). Higher degradation rates were observed in bottom sediments followed by middle and top sediments for all the test compounds in both peat/sand and peat/sand/Bion Soil microcosms (Table 4.2). Statistically higher dechlorination rates of the test chemicals were observed in peat/sand/Bion Soil compared to those in peat/sand microcosms.

Indigenous microorganisms in the microcosms were able to sustain dechlorination reactions at relatively high rates for about 16 months under advective-dispersive conditions in the soil columns (Kassenga et al., 2003) and for over 12 months under static conditions in serum bottles without exogenous hydrogen and nutritional supplements. Since neither external hydrogen donor nor nutritional supplements were used, it appears that H$_2$-utilizing metabolic reactions in the microcosms were sustained by fermentation of natural organic carbon present in the soil mixtures.

- **H$_2$ Threshold Concentrations**

One of the complications derived from using headspace measurements instead of aqueous measurements of H$_2$ and methane in batch fed systems is interphase mass-transfer limitations between the headspace and the liquid (Smatlak and Gossett, 1996). Any rate of change in the headspace concentration due to air sampling will necessarily mean disequilibrium between the phases. Gas-phase will always lag behind aqueous-phase concentrations because of gas-to-liquid mass-transfer limitations especially if the sampling interval is not sufficiently long for equilibrium to reestablish. For aquatic
Table 4.2: Summary results of anaerobic degradation kinetics of *cis*-1,2-DCE and 1,2-DCA for peat/sand (PS) and peat/sand/Bion Soil (PSB) soil mixtures and the associated hydrogen threshold values

<table>
<thead>
<tr>
<th>Section</th>
<th>*Biodegradation rate constant, $K$ (hr$^{-1}$)</th>
<th></th>
<th>$^1$Hydrogen threshold (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1,2-<em>cis</em>-DCE</td>
<td>1,2-DCA</td>
<td>1,2-<em>cis</em>-DCE dechlorination</td>
</tr>
<tr>
<td>PS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom</td>
<td>0.046 ± 0.005</td>
<td>0.013 ± 0.002</td>
<td>1.84 ± 1.50</td>
</tr>
<tr>
<td>Middle</td>
<td>0.039 ± 0.003</td>
<td>0.009 ± 0.002</td>
<td>2.12 ± 1.24</td>
</tr>
<tr>
<td>Top</td>
<td>0.034 ± 0.006</td>
<td>0.003 ± 0.001</td>
<td>2.26 ± 1.64</td>
</tr>
<tr>
<td>PSB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom</td>
<td>0.071 ± 0.008</td>
<td>0.032 ± 0.008</td>
<td>2.49 ± 1.08</td>
</tr>
<tr>
<td>Middle</td>
<td>0.059 ± 0.006</td>
<td>0.008 ± 0.001</td>
<td>2.62 ± 0.45</td>
</tr>
<tr>
<td>Top</td>
<td>0.048 ± 0.008</td>
<td>0.007 ± 0.003</td>
<td>2.39 ± 1.58</td>
</tr>
</tbody>
</table>

* Number of experimental runs per section = 9

$^1$ Hydrogen thresholds were determined based on at least 16 observations
sediments, Lovley and Goodwin (1988) report that it takes less than 20 minutes for dissolved H₂ to come into equilibrium with gaseous H₂ under static conditions and when sediments are shaken, equilibrium is reached within an even shorter period. Since headspace sampling in the present study was performed at intervals ranging between 8 to 72 hours, it was assumed that there was no mass-transfer limitation on H₂ and other gaseous parameter data (i.e., gas and liquid phases were at equilibrium).

Steady-state hydrogen kinetics in anaerobic sediments is reached when the rate of hydrogen consumption equals the rate of hydrogen production (Mazur and Jones, 2001). On this basis, estimates of the H₂ threshold for methanogenesis and cis-1,2-DCE and 1,2-DCA dechlorination were determined for the mixed culture in the present study. Hydrogen concentration thresholds for methanogenesis and dechlorination were evaluated by determining minimum H₂ contents obtained in the absence and in the presence of the test compounds, respectively. Hydrogen was allowed to drop to and rest at the physiological threshold, eliminating the need for mass-transfer corrections of H₂ data. During the determination of threshold concentrations of H₂, the fates of 1,2-DCA and cis-1,2-DCE were closely monitored in all microcosms to ensure that the consumption of H₂ was not limited by the availability of electron acceptor (Löffler et al., 1999). To insure this, the test chemicals were respiked before they had been completely depleted.

- **H₂ Concentrations in the Presence and Absence of cis-1,2-DCE Dechlorination**

Representative plots of temporal trends of cis-1,2-DCE dechlorination and concentrations of H₂ and methane are presented in Figure 4.1. Aqueous H₂ concentration levels decreased drastically from about 45 nM to approximately 2 nM within just 8 hours
Figure 4.1: Time course of dechlorination of cis-1,2-DCE and associated H₂ and methane concentrations in bottom soil of peat/sand soil mixture. Data points represent means of results from analysis of triplicate microcosms; bars indicate the range of results for individual bottles.
after cis-1,2-DCE was spiked into the microcosms. Thereafter it stayed nearly constant at 2.71 ± 0.73 nM during the period when dechlorination was occurring. After cis-1,2-DCE was completely degraded to ethene, H₂ concentration increased gradually to levels comparable to the initial concentrations. These observations indicate that reductive dechlorination of cis-1,2-DCE was driven by H₂. Comparable H₂ concentration levels were observed even after all cis-1,2-DCE was transformed to VC indicating that dechlorination of VC was also driven by H₂. This probably is not surprising because Dehalococcoides sp., organisms suspected to play an active role in the dechlorination of cis-1,2-DCE in the present study (see Chapter 3) are also capable of metabolizing VC (Maymó-Gattel et al., 1999; Hendrickson et al., 2002). A drastic drop in H₂ level after cis-1,2-DCE was spiked into the serum bottles also provided a circumstantial evidence that Dehalococcoides sp. were responsible for mediating the dechlorination reactions since these organisms have been observed to use H₂ as a sole electron donor (Maymó-Gattel et al., 1999; Hendrickson et al., 2002). Monitoring results revealed that methane concentration remained nearly constant in the microcosms during cis-1,2-DCE dechlorination period (Figure 4.1). This observation indicates that methanogenesis either ceased or proceeded at a significantly low rate during the cis-1,2-DCE and VC dechlorination periods because methane started to accumulate after these chemicals were completely transformed. Comparable trends were also observed in other microcosms which actively dechlorinated cis-1,2-DCE except that the time to reach steady-state H₂ concentration differed depending on the degradation rate of the substrate. During dechlorination of cis-1,2-DCE H₂ threshold concentrations ranged from 2.07 ± 0.17 for peat/sand and 2.50 ± 0.09 nM for peat/sand/Bion Soil (n = 20, ± standard error of the
estimate. Threshold concentrations of H₂ in the present study during dechlorination of cis-1,2-DCE are comparable to those reported by Yang and McCarty (1998) of between 2 and 11 nM but significantly higher than those observed by Löfler et al. (1999) ranging from 0.27 to 0.31 nM. It should however, be noted that Yang and McCarty (1998) and Löfler et al. (1999) used exogenous precursors of H₂ and gaseous H₂ in their studies whereas in this work H₂ was produced by fermentation of organic matter by natural organisms in the microcosms. Moreover, Löfler et al. (1999) used pure and mixed cultures in their studies unlike in the present study in which a dechlorinating and methanogenic mixed culture was involved. Differences in the compositions of microbial systems may partly account for the observed differences in threshold concentrations of H₂. H₂ threshold values, observed at different rates of cis-1,2-DCE dechlorination, were not significantly different, thus suggesting that the H₂ threshold concentration is independent of the kinetics of degradation of chlorinated solvents as Yang and McCarty (1998) also observed.

Steady-state threshold concentrations of H₂ in the present study were noted to be reached faster at higher dechlorination rates (i.e., higher rates of H₂ consumption) without influencing the final H₂ threshold concentrations consistent with observations made by Löfler et al. (1999). Lovely and Goddwin (1988) also noted that the steady-state H₂ threshold concentrations are controlled by the physiological characteristics of the H₂-consuming organisms and are independent of kinetics of the H₂-consuming reactions.

Reported values of physiological threshold of H₂ for methanogens vary widely from 5 to 95 nM (for example, Carr and Hughes, (1998), Yang and McCarty, (1998); Wiedemeier et al., (1999) and Löfler et al., (1999)). However, most of the studies have
reported H$_2$ threshold values for methanogens higher than 10 nM. H$_2$ concentrations during dechlorination of \textit{cis}-1,2-DCE were significantly lower than the reported physiological threshold of H$_2$ for methanogenesis (Table 4.2). This could, therefore, be the main reason for a nearly constant concentration level of methane observed during the dechlorination period (Figure 4.1) indicating that dechlorinators outcompeted methanogens for H$_2$. These observations are in agreement with the findings of Yang and McCarty (1998), who observed that methanogenesis ceased and dechlorination continued in the presence of \textit{cis}-1,2-DCE once the H$_2$ concentration was lower than 11 nM. Löffler et al. (1999) also observed very low H$_2$ concentration in the presence of \textit{cis}-1,2-DCE and other chlorinated compounds which could not support methane production. However, the observations of the present work are in contrast to findings of Chiu and Lee (2001) who observed accumulation of methane during the reduction of TCE (a parent compound of \textit{cis}-1,2-DCE) indicating that dechlorination and methanogenesis were occurring simultaneously. However, Chiu and Lee (2001) did not report H$_2$ data associated with TCE dechlorination, which would have possibly shed some light on the reasons for the observed concomitant TCE dechlorination and methanogenesis. In the absence of \textit{cis}-1,2-DCE, H$_2$ was consumed to thresholds ranging from 31 to 43 in peat/sand/Bion Soil and between 31 and 51 nM in peat/sand, suggesting that methanogenesis was the dominant TEAP (Table 4.2).

Since initial H$_2$ concentrations (35 to 206 nM) before \textit{cis}-1,2-DCE was spiked were significantly higher than the reported physiological threshold of H$_2$ for dechlorinators (Ballapragrada et al., 1997; Yang and McCarty, 1998; Löffler et al., 1999), results from this study seem to differ with the findings from previous studies that suggest
methanogenesis will be favored over dechlorination at high H\textsubscript{2} concentrations (for example, Smatlak et al. (1996), Ballapragada et al. (1997) and Mazur and Jones (2001)). These results suggest that the initial levels of hydrogen did not have an impact on the reductive dechlorination of \textit{cis}-1,2-DCE possibly because the steady state concentrations of hydrogen in the present study were controlled by the type of bacteria mediating the reduction of \textit{cis}-1,2-DCE independent of the rate of hydrogen production as opposed to what has been reported by other studies (Smatlak et al., 1996; Fennell et al., 1997; Ballapragada et al., 1997; Carr and Hughes, 1998). The findings of the present work are consistent with the observations made in a study on the long-term effects of high concentrations of H\textsubscript{2} on reductive dechlorination of PCE reported in Suthersan (2002). In this study the ability of four fermentable substrates (chosen on the basis of their rates of fermentation) to sustain long-term PCE dechlorination was demonstrated. Despite difference in the resulting partial pressures of H\textsubscript{2} which ranged from 1×10\textsuperscript{-5} to 3×10\textsuperscript{-3} atm. ([H\textsubscript{2}]\textsubscript{aq.} = 7.82 to 2345.7 nM), no long-term effect on dechlorination of PCE was observed. Both active dechlorination and methanogenesis were observed at high (though unspecified) levels of H\textsubscript{2}. However, when H\textsubscript{2} levels fell, dechlorination continued at a slower rate but methanogenesis ceased entirely (Suthersan, 2002).

- **H\textsubscript{2} Concentrations in the Presence and Absence of 1,2-DCA Dechlorination**

Temporal trends of 1,2-DCA dechlorination and concentrations of H\textsubscript{2} and methane are shown in Figure 4.2. Unlike in \textit{cis}-1,2-DCE amended microcosms (Figure 4.1), H\textsubscript{2} concentration did not change much after dechlorination of 1,2-DCA started and that during the period of continuous dechlorination of 1,2-DCA methane accumulated (Figure 4.2). This is probably because H\textsubscript{2} concentration (Table 4.2) in the microcosms
Figure 4.2: Time course of dechlorination of 1,2-DCA and associated H$_2$ and methane concentrations in bottom sediments of peat/sand/Bion Soil mixture. Data points represent means of results from analysis of triplicate microcosms; bars indicate the range of results for individual bottles.
amended with 1,2-DCA was sufficiently high to support both 1,2-DCA dechlorination and methanogenesis, as opposed to the relatively low H2 concentrations observed during the transformation of cis-1,2-DCE, which could sustain the dechlorination reaction only. Hydrogen thresholds were statistically indistinguishable for bottom, middle and top slurries of both types of soils, although degradation kinetic rate constants of 1,2-DCA in these slurries were different (Table 4.2). Based solely on thermodynamic considerations, concomitant dechlorination of 1,2-DCA and methanogenesis is not feasible since H2 concentrations during the reduction of 1,2-DCA would be expected to be lower compared to those under methanogenesis. Therefore, reductive dechlorination of 1,2-DCA would be expected to competitively exclude methanogenesis for H2 contrary to the observations made in the present study.

Two different reductive dechlorination mechanisms for 1,2-DCA transformation were observed in the present study; as previously identified by Holliger et al., (1990). These are dihaloelimination which involves transformation of 1,2-DCA to ethene and consecutive hydrogenolysis in which 1,2-DCA was reduced to ethane via chloroethane as an intermediate (Holliger et al., 1990). Holliger et al., (1990) also observed that methanogenic cell suspensions grown on H2/CO2 had higher 1,2-DCA conversion rates and activity relative to methane formation than those grown on methanol and acetate. Holliger et al. (1992) have demonstrated that both mechanisms of 1,2-DCA dechlorination (dihaloelimination and hydrogenolysis) can simultaneously be catalyzed by purified methyl-CoM reductase, a key enzyme of methanogenesis. In addition, four strains of hydrogenotrophic and acetoclastic methanogenic bacteria (Methanosarcia barkeri (DSM 2948), Methanococcus mazei (DSM 2053), Methanobacterium
thermoautotrophicum (DSM 2133) and Methanothrix soehngenii (DSM 2139)) have been reported to degrade 1,2-DCA (Holliger et al., 1990). In a study conducted by Belay and Daniels (1987) all three hydrogenotrophic methanogenic bacteria used in the experiment were able to transform 1,2-DCA but none could degrade cis- and trans-1,2-DCE. Based on the transformation mechanisms involved and degradation daughter products observed in other studies, it is most likely that hydrogenotrophic methanogens were responsible for dechlorination of 1,2-DCA but not cis-1,2-DCE in the present study. This could probably partly explain the differences in H2 threshold concentrations observed during the transformation of the test chemicals and concomitant degradation of 1,2-DCA and methanogenesis since H2 thresholds depend not only on the potential energy yield of the TEAP but also on the type of organisms mediating the metabolic reaction (Lovely et al., 1994). The fact that H2 threshold concentrations in the presence and absence of 1,2-DCA were comparable (Table 4.2) indicate that possibly organisms with similar physiological characteristics were responsible for mediating both dechlorination of 1,2-DCA and methanogenesis.

Hydrogenolysis dechlorination mechanism involves the sequential replacement of a chlorine atom by hydrogen, whereas in dihaloelimination two adjacent chlorine atoms are removed and replaced by a carbon double bond to form ethene (Vogel et al., 1983; Dolfing, 1999a; Lorah and Olsen, 1999). Based on thermodynamic considerations, dihaloelimination yields about 60% more Gibbs free energy than hydrogenolysis (Table 4.1), thus implying that the former dechlorination mechanism is energetically more favorable than the latter mechanism (Dolfing, 1999a). Since reducing equivalents (notably H2) are generally in short supply in microbial systems, when microbes have the
option of selecting between various electron acceptors and degradation pathways they
generally use the energetically most favorable redox couple (Dolfing, 1999a). In the
present study 1,2-DCA dechlorination through hydrogenolysis was observed in about
80% of the laboratory microcosms (see Chapter 3) in which methanogenesis was also
found to be active although this mechanism yields less energy than dihaloelimination.
Dolfing, (1999a) hypothesized that under conditions of abundant supply of reducing
equivalents, dechlorinating systems will tend to use the hydrogenolysis pathway for
chloroethane degradation, although this pathway is less energetically favorable than
dihaloelimination but allows the microbes to consume more reducing equivalents. In
view of this, highly methanogenic conditions in the constructed wetland soils with an
abundant supply of H₂ (aqueous H₂ concentrations of between 35 and 226 nM) may have
prompted the 1,2-DCA dechlorinating organisms to use the hydrogenolysis mechanism
rather than the dihaloelimination pathway in the present study.

- **General Observations**

  Since in the present study reducing conditions were predominantly methanogenic
it is reasonable to assume that H₂ threshold concentrations observed after addition of cis-
1,2-DCE reliably indicated that dechlorination was the dominant TEAP. However, during
dechlorination of 1,2-DCA it was difficult to discern the dominant TEAP based solely on
H₂ measurements since dechlorination and methanogenesis were occurring
concomitantly. This is perhaps one of the main shortcomings of using H₂ concentration
data only in evaluation of dominant TEAPs since this approach assumes competitive
exclusion of TEAPs (Jacobsen and Postma, 1999), which is not always the case as
observed in the current study.
Among other factors, the hydrogen threshold concentrations have been reported to depend on the thermodynamics of the terminal electron-accepting reaction (Lovely and Klug, 1983; Lovely and Phillips, 1987; Lovley and Goodwin, 1988; Löffler et al., 1999). However, physiological H₂ threshold is different from thermodynamic H₂ threshold (i.e., the H₂ concentrations below which the free energies of the terminal electron accepting reaction cease to be negative). Thermodynamic considerations suggest that organisms mediating 1,2-DCA dechlorination will have a higher affinity (or lower H₂ concentration threshold) than methanogens (Table 4.1). According to the conventional thermodynamic thinking, the H₂-consuming reaction that has the more favorable energetics will dominate in mixed populations. In addition, the energetics of the H₂-electron acceptor couple will determine how low the threshold for H₂ will be (Fennell, 1998). H₂ concentrations recorded during dechlorination of 1,2-DCA seem to defy this conventional thermodynamics thinking because both dechlorination and methanogenesis were observed to occur concurrently.

It was repeatedly demonstrated that organisms that were responsible for dechlorination of 1,2-DCA could not pull down H₂ levels as low as those which mediated cis-1,2-DCE reduction. In view of this, the H₂ threshold for dechlorination may be dependent on the type of organisms mediating the dechlorination reaction and should therefore not always be considered to be the same for all chlorinated compounds as sometimes implied (Yang and McCarty, 1998). Predicting the predominant TEAP using H₂ threshold for dechlorination in methanogenic conditions may sometimes be misleading. For example, Carr and Hughes (1998) and Chiu and Lee (2001) reported simultaneous occurrence of dechlorination (high energy yield TEAP) and
methanogenesis (low energy yield TEAP) contrary to the expectations from the model that assumes competitive exclusion for H₂ between TEAPs with different potential energy yields (Lovely et al., 1988). Based on the findings of the present work and those by Carr and Hughes (1998) and Chiu and Lee (2001), using hydrogen thresholds as indicators of halorespiratory physiology as suggested by Löffler et al. (1999) may be ambiguous in certain circumstances.

Soil mixtures in the present study were able to produce high concentration levels of H₂ (between 35 and 226 nM), especially the mixture containing Bion soil. Except for small amounts of acetic acid (< 2 µM), other organic acids (formic, benzoic, lactic, propionic, butyric) were not detected probably because they did not accumulate as they were fermented to H₂ as quickly as they were being produced. These results suggest that H₂ was indeed the main electron donor that drove the TEAPs.

- **Effects of BES on Dechlorination Kinetics, Methanogenesis and H₂ Concentrations**

  To understand the possible role of methanogenic bacteria with regard to dechlorination kinetics of the test compounds, a comparative study using the methanogenesis inhibitor, 2-bromoethanesulfonic acid (BES) was conducted. The inhibitory mechanisms of methanogenesis by BES are detailed elsewhere (Löffler et al., 1997).

  BES did not completely inhibit dechlorination but rather slowed down the kinetics of degradation of the test compounds (Figure 4.3 and Figure 4.4). Pseudo-first order degradation rate constants of cis-1,2-DCE and 1,2-DCA in the presence of BES were 0.18 ± 0.02 and 0.14 ± 0.04 day⁻¹, respectively, whereas degradation rates in microcosms without BES were 0.94 ± 0.07 day⁻¹ for cis-1,2-DCE and 0.21 ± 0.04 day⁻¹ for 1,2-DCA.
Figure 4.3: Time course of dechlorination of 1,2-DCA in the presence of BES and associated H₂ and methane concentrations in middle soil of peat/sand/Bion Soil mixture. Data points represent means of results from analysis of triplicate microcosms; bars indicate the range of results for individual bottles.
Figure 4.4: Time course of dechlorination of cis-1,2-DCE in the presence of BES and associated H$_2$ and methane concentrations in middle soil of peat/sand/Bion Soil mixture. Data points represent means of results from analysis of triplicate microcosms; bars indicate the range of results for individual bottles.
Chiu and Lee (2001) observed that dechlorination of TCE was not completely inhibited by BES as previously observed by Löffler et al. (1997). A close examination of the plots of time courses of dechlorination of TCE in the presence and absence of BES in Chiu and Lee (2001) revealed that BES not only changed the dechlorination pathway but it also slowed down the kinetics of degradation of TCE in agreement with the findings of the present work. However, the magnitude of reduction in dechlorination rates of TCE in Chiu and Lee (2001) was not reported.

Inhibition of methanogenesis by BES was demonstrated by nearly constant concentrations of methane in the headspace (Figure 4.3 and Figure 4.4). The fact that H$_2$ concentrations (Figure 4.3 and Figure 4.4) were significantly higher than the reported ranges of physiological H$_2$ threshold concentrations for methanogenesis rules out the possibility that methane production was limited by the availability of H$_2$. Elimination of methanogenesis by BES probably directly affected consumption and production of substrates for methanogenesis (Conrad and Klose, 2000; Chiu and Lee, 2001) notably methane and H$_2$ in the present work. Levels of H$_2$ in BES amended microcosms containing the test compounds were observed to be statistically higher than those without BES. The accumulation of H$_2$ due to inhibition of methanogenesis, one of the important sinks of H$_2$ may partly explain higher H$_2$ concentrations observed in slurries amended with 1,2-DCA and BES since methanogenesis and 1,2-DCA dechlorination were observed to occur simultaneously in slurries without BES. However, reasons for higher H$_2$ concentrations in the presence of cis-1,2-DCE and BES were unclear. It is possible that partial inhibition of reductive dechlorination and other unidentified H$_2$-consuming reaction caused an accumulation of H$_2$. Interestingly, H$_2$ threshold concentrations in
microcosms containing the test compounds and BES (i.e., BES plus cis-1,2-DCE and BES plus 1,2-DCA) were statistically the same ($P < 0.05$). Higher H$_2$ concentrations than those normally reported for methanogenesis (6 to 95 nM) were observed in microcosms amended with BES only (data not shown) indicating that the observed concentration levels of H$_2$ in the absence of the test chemicals were indeed due to methanogenesis.

Accumulation of ethene and inhibition of methane production were observed in microcosms containing soil slurries and BES only, whereas ethene was not detected and methane was observed to accumulate in microcosms without both BES and test compounds (data not shown). This indicates that ethene was probably produced by degradation of BES since some hydrogenotrophic methanogens have been observed to form ethene from BES (Belays and Daniels, 1987; Holliger et al., 1990). Belays and Daniels (1987) observed concurrent production of methane and ethene by two Methanobacterium strains in tubes amended with 107 µM BES, and partial inhibition was observed at 2,124 µM BES. In the same study, two Methanococcus species were fully inhibited by 1 µM BES, indicating that the potency of BES is dependent on the applied dosage of the inhibitor as well as the type of methanogenic bacteria involved. It is worth noting that the concentration of BES (30 mM) used in the present work was higher by a factor of at least 6 compared to concentrations used by Holliger et al. (1990) and Belays and Daniels (1987) in their studies. It could therefore be inferred from the foregoing discussion that slurries in the present study probably contained hydrogenotrophic methanogens, which while not being able to be completely inhibited by BES were capable of transforming the compound into ethene.
Since mechanisms of inhibition of dechlorination by BES are yet to be understood (Löffler et al., 1997; Chiu and Lee, 2001) speculation of possible reasons for the partial inhibition of \textit{cis}-1,2-DCE and 1,2-DCA based on the observations made during the present study is in order. Some methanogenic bacteria have been reported to recover from BES inhibition as fast as 8 hours after incubation (Belay and Daniels, 1987). The same cells were observed to grow normally on transfer into BES-containing medium, thus indicating adaptation (Belay and Daniels, 1987). Hydrogenotrophic methanogenic bacteria, \textit{Methanosarcina barkeri}, which has been confirmed to be capable of transforming 1,2-DCA (Holliher et al., 1990) was one of the methanogens reported by Belay and Daniels (1987) to be able to recover from BES inhibition. The possibility that organisms in the present work were able to transform the test compounds albeit at slower rates because they were either partially inhibited or they were able to recover from BES inhibition can, therefore, not be completely ruled-out. Although monitoring of BES concentrations was not done, it is possible that dechlorination of the test compounds and BES transformation concurrently occurred as evidenced by the observed higher concentrations of ethene than the levels expected from stoichiometric conversion of the test chemicals (Figure 4.3 and Figure 4.4). Transformation of BES possibly caused the reduction of the potency of BES over time. Reduction in the effectiveness of BES over time may probably explain the faster degradation rate of 1,2-DCA observed after the test chemical was spiked again (Figure 4.3). From the foregoing discussion it is important to note that although BES has been traditionally used as a potent inhibitor of methanogenic organisms, its action may differ from one strain to another for the same applied dosage,
thus using it to attribute dechlorination activities to methanogens may in certain
conditions be misleading as Löffler et al. (1997) and Chiu and Lee (2001) also observed.

4.4 Conclusion and Significance of the Results

Factors that determine threshold concentration levels of H₂ during dechlorination
reactions are perhaps only partially understood which has led to different reported H₂
threshold values for the same compound. Contrasting observations from different studies
raise the fundamental question as to what factor(s) ultimately control H₂ concentrations
and consequently bioenergetics of dehalogenation reactions in sedimentary environments.
Are these factors thermodynamic or are they related to the physiology of organisms
mediating the TEAP or both? Hoehler et al. (1998) report that in any chemical
transformation, whether biologically mediated or not, thermodynamics represents the
ultimate control. They further suggest that this control is not often expressed because it is
superseded by kinetic considerations or obscured by effects of bacteria operating far from
equilibrium conditions. Mazur and Jones (2001) report that the rate of hydrogen
production, microbial population size, and availability of electron acceptors are all factors
that contribute to the observed H₂ concentrations in anaerobic sediments. Most of these
factors are directly related to the chemistry of the sedimentary environments. Therefore,
the chemistry of the system plays an important role in regulating the concentrations of H₂
and consequently, the bioenergetics of anaerobic sediments. Unfortunately, in most cases
it is difficult to accurately define natural sedimentary environments in terms of the
prevailing metabolic reactions and organisms involved in mediating them (Egli et al.,
1987), which makes clear understanding of factors regulating H₂ concentrations difficult.
No wonder most of the studies reported in literature on H₂ concentrations associated with
various TEAPs are conducted in pure and enrichment cultures, which can easily be defined and manipulated. It should further be remembered that bacteria and methanogenic archaea are very versatile organisms and as such it is sometimes difficult to predict the kinetics of dehalogenation and the associated H₂ concentrations for the same chlorinated solvent under sedimentary environments with different chemistries.

The reasons for higher H₂ concentrations and slower degradation kinetics of cis-1,2-DCE and 1,2-DCA observed in the presence of BES could only be speculated since mechanisms of inhibition of dechlorination by BES are still unclear. Total inhibition of the methane producing methanogens probably resulted into an increase of the H₂ pool whereas BES perhaps partially interfered with enzymatic activities involved in the transformation of the test chemicals thus slowing down the degradation kinetics. The exact mode of inhibition of dechlorination by BES needs further research.

It has been observed that dechlorinating bacteria were physiologically capable of lowering hydrogen concentration to their threshold levels regardless of the initial hydrogen concentration and perhaps independent of the rate of H₂ production as well. These observations are supported by the hypothesis that microbes have the ability to pull down the H₂ level as low as possible until it reaches their threshold value (Jacobsen et al., 1998). Therefore, methanogens were not able to out compete for H₂ organisms responsible for dechlorinating cis-1,2-DCE even when initial H₂ concentration levels seemed to be in favor of methanogenesis rather than dechlorination. On the other hand, 1,2-DCA dechlorination and methane production occurred concomitantly at H₂ concentrations that are normally observed during methanogenesis. Therefore, using hydrogen precursors that are slowly degradable so that they can release hydrogen in a
slow manner (such as proprietary lactate) to maintain the ideal (low) hydrogen concentration for dechlorination as proposed in previous studies (Smatlak et al., 1996; Fennell et al., 1997; Ballapragada et al., 1997; Carr and Hughes, 1998) could in certain circumstances be counter productive since they could limit the amount of reducing equivalents necessary for effective dechlorination of the target compounds. It is, therefore, recommended to conduct studies on degradation kinetics and associated H₂ concentrations for the contaminants of interest mimicking the in situ conditions as much as possible before a decision on the appropriate type of H₂ precursor to be used during the remediation of a contaminated site is reached.

The present study has demonstrated that materials rich in organic carbon such as peat and compost can produce high concentration levels of H₂ necessary for driving reductive dechlorination reactions, this is one of the characteristics which makes them the most promising materials for construction of treatment wetlands for remediation of groundwater contaminated with chlorinated organic compounds.
CHAPTER 5: ENERGETICS OF DECHLORINATION OF CIS-1,2-DICHLOROETHENE AND 1,2-DICHLOROETHANE IN WETLAND SOILS

5.1 Introduction

Chlorinated aliphatic compounds are toxic pollutants of environmental concern, which are found in many groundwater aquifers, sediments, soils and subsurface environments worldwide (Hendrickson et al., 2002). Due to their recalcitrant nature, these pollutants have a tendency of persisting in the environment thus posing a special treatment problem. Therefore, inexpensive remediation methods for chlorinated solvent contamination have to be devised considering the long duration required to cleanup contaminated sites.

Based on field and laboratory studies on natural attenuation of chlorinated volatile organic compounds in a freshwater tidal wetland at Aberdeen Proving Ground, Maryland, natural wetlands have been observed to be capable of assimilating chlorinated organics without human intervention (Lorah et al., 1997). In a recent study conducted by Kassenga et al. (2003), higher attenuation rates of chloroethenes and chloroethanes were observed in continuous flow bench-scale wetland mesocosms constructed using various mixtures of synthetic peat and sand as bed materials. The cited studies thus suggest that natural and engineered wetlands may be promising cost-effective alternatives in remediation of groundwater contaminated with chlorinated organic solvents instead of traditional approaches such as pump and treat methods.

However, potential for attenuation of chlorinated solvents including factors affecting dechlorination activities have to be understood before a decision to use wetlands for remediation of contaminated site is reached. Reducing conditions is one of the
important factors that dictate the fate and kinetics of dechlorination activities in sedimentary environments (Lorah et al., 1997; Wiedemeier et al., 1999) because biodegradation of chlorinated solvents is often linked to the utilization of molecular H\textsubscript{2} as an electron acceptor (Wiedemeier et al., 1999; Suthersan, 2002) and H\textsubscript{2} concentrations in many cases are directly associated with prevailing reducing conditions (Lovley and Klug, 1983; Lovley and Goodwin, 1988).

Therefore, production and utilization of hydrogen is one of the most important factors that control kinetics and thermodynamics of reduction of chlorinated solvents and other electron acceptors such as sulfate, CO\textsubscript{2} and ferric iron, naturally found in groundwater systems (Hoehler et al., 1998; Jacobsen et al., 1998; Conrad, 1999). Hydrogen exhibits a fast turnover (with residence time as short as 0.1 seconds in some cases) and the measured concentration of H\textsubscript{2} in many cases, relates to biogeochemical processes that actually occur in the sampled environment (Hoehler et al., 1998; Jacobsen et al., 1998; Conrad, 1999). In environments free from anthropogenic contaminants such as chlorinated organic compounds, H\textsubscript{2} plays the role of linking oxidation of organic matter to the reduction of inorganic compounds (for example sulfate, and CO\textsubscript{2}) in a process referred to as interspecies hydrogen transfer (Cord-Ruwisch et al., 1988; Hoehler et al., 1998). Terminal metabolic reactions are affected when H\textsubscript{2} concentrations become too low. This may result in inhibition, or in the case of CO\textsubscript{2} reduction possibly reversal of H\textsubscript{2}-based terminal metabolism (Zehnder and Brock, 1979; Hoehler et al., 1994; Hoehler et al., 1998).

It has been well established that bacterial groups, the H\textsubscript{2} consumers and the H\textsubscript{2} producers are limited in their metabolic activities by the H\textsubscript{2} concentration levels in their
natural habitats (Lovely and Klug, 1983; Robinson and Tiedje, 1984; Lovely and Phillips, 1987; Cord-Ruwisch et al., 1988; Lovley and Goodwin, 1988; Fennel and Gossett, 1998; Yang and McCarty, 1998). This limitation is due to the thermodynamics of their metabolic reactions, which are highly sensitive to the H$_2$ levels. Since H$_2$ concentrations in natural habitats are generally low, competition for the limited amount of H$_2$ between microorganisms mediating different terminal electron accepting processes (TEAPs) occurs. The dominant microbial mediated TEAP will limit H$_2$ within a very narrow range, on this basis the H$_2$ concentration may be used as an indicator of the specific TEAP dominating a system by competitively excluding microorganisms mediating other TEAPs (Lovley and Goodwin, 1988; Jacobsen, 1999). Therefore, H$_2$ levels may be a useful tool in indicating the redox conditions if a given TEAP is exceedingly dominant (assuming steady-state conditions and complete competitive exclusion), otherwise interpretation of H$_2$ concentrations may sometimes be ambiguous (Jacobsen and Postma, 1999). Due to limitations of using H$_2$ alone as an indicator of microbiologically mediated TEAPs, Jacobsen et al. (1998) proposed an alternative approach whereby measured TEAPs reactant and products concentrations may be used to determine actual potential in situ energy yield which in turn will indicate the potential for a given hydrogen oxidizing TEAP. This approach can be used to interpret H$_2$ from systems where steady-state and complete exclusion is not achieved (Jacobsen and Postma, 1999). In the present study this approach is extended to chlorinated VOCs degradation under sulfite- and sulfate-reducing conditions and H$_2$/CO$_2$ dependent methanogenesis to evaluate the dominant TEAPs in the time course of VOCs biotransformation to better understand factors affecting the
dechlorination reaction kinetics based on hydrogen concentration and *in situ* bioenergetics of TEAPs.

Dechlorinating organisms have to successfully compete with other H\(_2\) utilizing organisms for the limited amount of H\(_2\) if bioremediation is to be an effective method for cleaning-up a site contaminated with chlorinated solvents. It is apparent from the association of H\(_2\) concentrations with redox chemistry that reductive dehalogenation is mostly favored in methanogenic and sulfate-reducing conditions as Lorah et al. (1997) found. This is because most of the reported values of H\(_2\) threshold values of halorespirers range between 0.05 and 0.1 nM (Yang and McCarty, 1998) whereas those of methanogens (7-10 nM) and sulfate-reducing bacteria (1-1.5 nM) are above this range (Lovely and Goodwin, 1988).

Relative thresholds of H\(_2\) level for biological reduction of various chlorinated aliphatic organic compounds may be predicted based on thermodynamic calculations by assuming standard conditions. However, the relative H\(_2\) threshold values have so far not been demonstrated by thermodynamic calculations based on real *in situ* concentrations of H\(_2\), reactants and metabolites involved in the dechlorination reactions. In addition, a number of studies have focused on the degradation of 1,2-dichloroethane (1,2-DCA) and *cis*-1,2-dichloroethene (*cis*-1,2-DCE) (for example, Holliger et al. (1990), Ballapragada et al. (1997), and Maymó-Gattel et al. (1999)) but few of them (if any) have evaluated hydrogen thresholds relative to dechlorination of 1,2-DCA and *cis*-1,2-DCE and considered the thermodynamics of the involved TEAPs in sulfite- and sulfate-reducing and methanogenic sedimentary environments.
In the present study, dechlorination kinetics of chlorinated aliphatic compounds in synthetic peat/compost/sand mixture under methanogenic and sulfate- and sulfite reducing conditions are investigated using H₂ data in combination with thermodynamics calculations as analytical tools to track the succession of the TEAPs in the sedimentary environment. cis-1,2-DCE and 1,2-DCA were used as test chemicals and the slurry microcosms used during the study contained Dehalococcoides sp., organisms which are capable of transforming chlorinated ethenes all the way to ethene using H₂ as a sole electron donor (Hendrickson et al., 2002) was one of the members of microbial communities.

The main objective of this study was to evaluate the relative biodegradation kinetics, hydrogen thresholds and thermodynamics involved in transformation of cis-1,2-DCE and 1,2-DCA under sulfate- and sulfite-reducing and H₂/CO₂ dependent methanogenic sedimentary aqueous environments using anaerobic laboratory microcosms. Microcosms were constructed from a synthetic soil mixture of peat soil, compost and sand rich in organic carbon. The peat soil/compost/sand mixture used during the current study was identified as a promising material for construction of engineered wetland bed based on previous studies (Kassenga et al., 2003). H₂ and cis-1,2-DCE, 1,2-DCA, sulfite and sulfate and their metabolic products are reported. The data were used to calculate degradation kinetics and the actual Gibbs free energies of the H₂-consuming reactions, which were then used to evaluate if the TEAPs of interest are thermodynamically feasible under given reducing conditions. Results of the present study may be important in predicting the fate of chlorinated ethenes and ethanes in high sulfate
wetland sedimentary environments with mixed microbial communities including *Dehalococcoides sp.*

5.2 Materials and Methods

- **Chemicals**

  Neat solutions of *cis*-1,2-DCE and 1,2-DCA (Supelco Park, Bellefonte, PA) were used to prepare stock solutions. Analytical standards and surrogates for the chlorinated VOCs were obtained as mixtures or neat liquids from Supelco Inc. (Supelco, Bellefonte, PA). Methane, carbon dioxide, ethane and ethylene for calibration were also obtained from Supelco Inc. (Supelco, Bellefonte, PA). Hydrogen standards were prepared using 10 ppmV hydrogen (BOC Group Inc., Baton Rouge, LA). Analytical-grade sodium sulfide and sodium sulfate crystals were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). Sulfite, sulfate, sulfide and chloride standard solutions were obtained from Hach Co. (Loveland, CO). Other chemicals used in this study were reagent grade and were purchased from various vendors.

- **Anaerobic Microcosms**

  Degradation kinetics of *cis*-1,2-DCE and 1,2-DCA were investigated under sulfite- and sulfate-reducing conditions and methanogenic conditions in microcosms constructed with a wetland soil mixture and deionized water spiked with the test chemicals. Microcosms were constructed under a nitrogen atmosphere in a disposable glove-bag from a mixture of *Latimer peat* (Latimer's Peat Moss Farm, West Liberty, OH), *Bion Soil* (Dream Maker Dairy, Cowlesville, NY) and sand mixed at a ratio of 1.3:1.1:1 (Bion Soil:Latimer peat:sand) by weight described in detail elsewhere (Kassenga et al., 2003). The organic carbon content of the soil mixture was ~30%. The
sediments were mixed with deionized water to achieve an approximate 250 g/L slurry. The slurry (145 mL) was dispensed into 160-mL serum bottles that were subsequently sealed with Teflon-lined, butyl-rubber septa and aluminum crimp caps. The microcosms were inoculated with 5 mL of slurry taken from microcosms, which were previously observed to be able to completely degrade the test compounds (see Chapter 3). Resazurin (0.0002%) was added as a redox indicator. Microcosms were kept in the dark at 25 °C for two weeks to allow the systems to reach quasi-steady state with respect to microbial populations (Hoehler et al., 1998).

Microcosms were not amended with hydrogen donor or nutrient media for supporting microbial growth, unlike in other studies reported in literature in which pure and/or enrichment cultures were used. Based on previous dechlorination studies and molecular analysis using the polymerase chain reaction technique and primer-based detection method (see Chapter 3), the slurry used for inoculation of the microcosms was confirmed to contain *Dehalococcoides* sp., organisms capable of degrading *cis*-1,2-DCE completely to ethane via vinyl chloride (VC) and ethene and 1,2-DCA to VC and ETH (Maymó-Gatell et al., 1999; Hendrickson et al., 2002).

The following treatments were used during the experiments; (1) *cis*-1,2-DCE (methanogenic conditions) (2) 1,2-DCA (methanogenic conditions) (3) *cis*-1,2-DCE and 1,2-DCA (methanogenic conditions) (4) 1,2-DCA and sulfite (sulfite-reducing conditions) (5) 1,2-DCA and sulfate (sulfate-reducing conditions) (6) *cis*-1,2-DCE and sulfite (sulfite-reducing conditions) (7) *cis*-1,2-DCE and sulfate (sulfate-reducing conditions) (8) sulfite only (sulfite-reducing conditions) (9) sulfate only (sulfate-reducing conditions) (10) no chemical added (methanogenic conditions). Aqueous stock solutions
of chemicals of interest were added to bottles using a gas-tight syringe to arrive at desired concentrations. Initial concentrations of cis-1,2-DCE and 1,2-DCA in all experiments were approximately 70 µM. To establish soils in which sulfate and sulfite reduction were the predominant TEAPs, sodium sulfate and sodium sulfite were added into serum bottles at final concentration of approximately 2.5 mM from aqueous stock solutions. Abiotic controls were included in the study to monitor for non-biological losses of the test chemicals. For these treatments, reaction mixtures were prepared as described above, and were adjusted to contain 1% formaldehyde. After spiking with appropriate chemicals, the microcosms were incubated in an inverted position under static conditions at 25 °C in the dark. To ensure reproducibility, triplicates were used in each experiment.

Temporal monitoring of concentrations of parent compounds and daughter products was done until the concentrations of the contaminants dropped below the detection limits of the analytical methods (5 µg/L). Samples for measurement of chlorinated ethenes and ethanes, ethane, ethene, methane, carbon dioxide, hydrogen, pH and oxidation-reduction potential (ORP) were withdrawn from the bottles at appropriate intervals of time as determined by the rates of degradation kinetics of test chemicals and analyzed immediately without storage. Sulfate, sulfite, sulfide, hydrogen and methane concentrations were also monitored.

- **Degradation Kinetics**

  Modeling of cis-1,2-DCE and 1,2-DCA monitoring data in anaerobic microcosms was performed assuming that degradation follows first-order kinetics,

\[
C_t = C_o e^{-kt}
\]  

(5.1)
where $C_t$ \([\text{ML}^{-3}]\) is the concentration at any time $t$; $C_o$ \([\text{ML}^{-3}]\) is the initial concentration; and, $k$ \([\text{T}^{-1}]\) is the pseudo first-order reaction rate constant. When significant losses (greater than 5 percent) were observed in the abiotic microcosms, this was subtracted from the concentration in the live microcosms to adjust for abiotic losses before the first-order rate constant was calculated.

- **Thermodynamic Calculations for the TEAPs**

  The amount of Gibbs’s free energy $\Delta G_r$, obtained from a hypothetical biologically mediated reaction \((aA + bB \rightarrow cC + dD)\) for the given environmental conditions was calculated by (Thauer et al., 1977; Dolfing and Harrison, 1992),

  $$\Delta G_r = \Delta G_r^o + RT \ln \frac{[C]^c[D]^d}{[A]^a[B]^b}$$  \(5.2\)

  The value $\Delta G_r^o$ is obtained from the value $\Delta G^o$ by making the appropriate corrections for pH $\approx 7$ and temperature. Table 5.1 presents equations and thermodynamic values used for calculating *in situ* Gibbs free energies for TEAPs of interest in the present study. Thermodynamic values were calculated for 25°C and pH 7 which were the approximate temperature and pH at which the experiments were conducted. Since calculations obtained by use of molar concentrations rather than activities are sufficient for many practical purposes (Sawyer at al., 2003), activity corrections were ignored in the present study.

  The effects of electron acceptors, which potentially can compete with *cis*-1,2-DCE and 1,2-DCA for reducing equivalents, were evaluated by tracking free energy values over time to identify prevailing TEAPs during the course of the experiments based on the threshold $\Delta G_r$ for the reaction. Negative $\Delta G$-values indicate that the reaction is
Table 5.1: Equations and thermodynamic values used for calculating *in situ* Gibbs free energies for H$_2$-oxidizing terminal electron-accepting processes (TEAPs)

<table>
<thead>
<tr>
<th>TEAP</th>
<th>Equation used for calculating $\Delta G_r$</th>
<th>$\Delta G_r^{\circ'}$ (kJ/mol of TEAP)</th>
<th>$\Delta G_r^{\circ'}$ (kJ/mol H$_2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Methanogenesis</strong></td>
<td></td>
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<tr>
<td>HCO$_3^-$ + 4H$_2$(aq) + H$^+$ → CH$_4$(aq) + 3H$_2$O</td>
<td>$\Delta G_r = \Delta G_r^{\circ'} + RT \ln([\text{CH}_4]/[\text{H}_2][\text{HCO}_3^-][\text{H}^+])$</td>
<td>-194.5</td>
<td>-48.6</td>
</tr>
<tr>
<td><strong>Sulfate reduction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SO$_4^{2-}$ + 4H$_2$(aq) + H$^+$ → HS$^-$ + 4H$_2$O</td>
<td>$\Delta G_r = \Delta G_r^{\circ'} + RT \ln([\text{HS}^-]/[\text{H}_2][\text{SO}_4^{2-}][\text{H}^+])$</td>
<td>-222.5</td>
<td>-55.6</td>
</tr>
<tr>
<td><strong>Sulfite reduction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SO$_3^{2-}$ + 3H$_2$(aq) + H$^+$ → HS$^-$ + 3H$_2$O</td>
<td>$\Delta G_r = \Delta G_r^{\circ'} + RT \ln([\text{HS}^-]/[\text{H}_2][\text{SO}_3^{2-}][\text{H}^+])$</td>
<td>-225.7</td>
<td>-75.2</td>
</tr>
<tr>
<td><strong>cis-1,2-DCE dechlorination</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C$_2$H$_2$Cl$_2$ + H$_2$(aq) + H$^+$ → C$_2$H$_4$Cl + Cl$^-$ + 2H$^+$</td>
<td>$\Delta G_r = \Delta G_r^{\circ'} + RT \ln(([\text{C}_2\text{H}_4\text{Cl}][\text{Cl}^-][\text{H}^+]/[\text{H}_2][\text{C}_2\text{H}_2\text{Cl}_2]))$</td>
<td>-156.9</td>
<td>-156.9</td>
</tr>
<tr>
<td><strong>Vinyl chloride dechlorination</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C$_2$H$_3$Cl + H$_2$(aq) + H$^+$ → C$_2$H$_4$ + Cl$^-$ + 2H$^+$</td>
<td>$\Delta G_r = \Delta G_r^{\circ'} + RT \ln(([\text{C}_2\text{H}_4][\text{Cl}^-][\text{H}^+]/[\text{H}_2][\text{C}_2\text{H}_2\text{Cl}]))$</td>
<td>-167.0</td>
<td>-167.0</td>
</tr>
<tr>
<td><strong>Ethene reduction</strong></td>
<td></td>
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<tr>
<td>C$_2$H$_4$ + H$_2$(aq) → C$_2$H$_6$</td>
<td>$\Delta G_r = \Delta G_r^{\circ'} + RT \ln((\text{C}_2\text{H}_6)/[\text{H}_2][\text{C}_2\text{H}_4])$</td>
<td>-116.3</td>
<td>-116.3</td>
</tr>
<tr>
<td><strong>Sum (sequential dechlorination of cis-1,2-DCE)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C$_2$H$_2$Cl$_2$ + 3H$_2$(aq) + 2H$^+$ → C$_2$H$_6$ + 2Cl$^-$ + 4H$^+$</td>
<td>$\Delta G_r = \Delta G_r^{\circ'} + RT \ln(([\text{C}_2\text{H}_6][\text{Cl}^-]^2/[\text{H}_2]^3[\text{C}_2\text{H}_2\text{Cl}_2]))$</td>
<td>-440.0</td>
<td>-146.7</td>
</tr>
</tbody>
</table>
Table 5.1: (Cont’d)

<table>
<thead>
<tr>
<th>TEAP</th>
<th>Equation used for calculating $\Delta G_r$</th>
<th>$\Delta G_r^\circ$ (kJ/mol of TEAP)</th>
<th>$\Delta G_r^\circ$ (kJ/mol H$_2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1,2-DCA dechlorination</strong></td>
<td></td>
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<td></td>
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<tr>
<td><em>Dihaloelimination:</em></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$\text{C}_2\text{H}_4\text{Cl}_2 + \text{H}_2 + \text{H}^+ \rightarrow \text{C}_2\text{H}_4(\text{aq}) + 2\text{Cl}^- + 3\text{H}^+$</td>
<td>$\Delta G_r = \Delta G_r^\circ + R T \ln ([\text{C}_2\text{H}_4][\text{Cl}^-]^2[\text{H}^+]^2/[	ext{H}_2][\text{C}_2\text{H}_4\text{Cl}_2])$</td>
<td>-205.6</td>
<td>-205.6</td>
</tr>
<tr>
<td><em>Consecutive hydrogenolysis:</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{C}_2\text{H}_4\text{Cl}_2 + 2\text{H}_2 + \text{H}^+ \rightarrow \text{C}_2\text{H}_5\text{Cl} + \text{Cl}^- + 4\text{H}^+$</td>
<td>$\Delta G_r = \Delta G_r^\circ + R T \ln ([\text{C}_2\text{H}_6][\text{Cl}^-]^2/[\text{H}_2][\text{C}_2\text{H}_4\text{Cl}_2])$</td>
<td>-264.8</td>
<td>-132.4</td>
</tr>
<tr>
<td>$\text{C}_2\text{H}_5\text{Cl} + \text{H}_2 + \text{H}^+ \rightarrow \text{C}_2\text{H}_6 + \text{Cl}^- + 2\text{H}^+$</td>
<td>$\Delta G_r = \Delta G_r^\circ + R T \ln ([\text{C}_2\text{H}_6][\text{Cl}^-]^2/[	ext{H}_2][\text{C}_2\text{H}_5\text{Cl}])$</td>
<td>-169.4</td>
<td>-169.4</td>
</tr>
<tr>
<td><strong>Sum (Consecutive hydrogenolysis of 1,2-DCA)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{C}_2\text{H}_4\text{Cl}_2 + 3\text{H}_2(\text{aq}) + 2\text{H}^+ \rightarrow \text{C}_2\text{H}_6(\text{aq}) + 2\text{Cl}^- + 6\text{H}^+$</td>
<td>$\Delta G_r = \Delta G_r^\circ + R T \ln ([\text{C}_2\text{H}_6][\text{Cl}^-]^2[\text{H}^+]^4/[\text{H}_2]^3[\text{C}_2\text{H}_4\text{Cl}_2])$</td>
<td>-434.2</td>
<td>-144.7</td>
</tr>
</tbody>
</table>

$\Delta G_r^\circ$ values were calculated according to Dolfing and Janssen (1994): organic substrate + H$_2$ $\rightarrow$ organic product + H$^+$ + Cl$^-$. Aqueous $\Delta G_r^\circ$ values were used for organic substrates at 25 °C, pH 7. $\Delta G_r^\circ$(H$^+$) = -39.9 kJ/mol; $\Delta G_r^\circ$(Cl$^-$) = -131.3 kJ/mol. Thermodynamic data on solutes are from Dolfing and Janssen (1994) and Stumm and Morgan (1981).
exergonic for *in situ* conditions. Values below the threshold $\Delta G_r$ were considered to be favorable.

Jacobsen et al. (1998) report that in steady-state systems a threshold $\Delta G_r$ of about -15 to -7 kJ/mol is observed for a given TEAP, which correspond to the minimum energy necessary for the bacteria to be able to store the gained energy as ATP. This $\Delta G_r$ threshold value should be comparable to most of the TEAPs according to Jacobsen et al. (1998) because it is related to the ATP energy storage system and it has been observed to be similar at least for sulfate reduction. However, variations of this $\Delta G_r$ threshold with TEAP are expected since it is related to the physiology of the specific bacteria. For example, in a number of studies $\Delta G_r$ threshold value for methanogenesis has been observed to be about -20kJ/mol (Hoehler et al., 1998; Conrad, 1999; Richmond et al., 2001). Therefore, except for methanogenesis, a threshold $\Delta G_r$ of about -15 to -7 kJ/mol was used for determining whether a given TEAP is favorable or not in the present study. The above $\Delta G_r$ threshold values were used in the present study to assess the feasibility of TEAPs by comparing it with Gibbs free energies for $H_2$ oxidizing TEAPs calculated from redox-sensitive species.

In the microbial systems studied, a combination of electron acceptors with different redox potentials were present and therefore the systems were not expected to reach a true steady state as long as there were unreacted electron acceptors. In the approach proposed by Jacobsen et al. (1998), measured *in situ* concentrations of $H_2$ and TEAP reactant concentrations are used to calculate Gibbs free energies of the TEAP under the conditions prevailing *in situ*. The calculated free energies may be used to assess an actual potential *in situ* energy yield, representing the potential for a given hydrogen
oxidizing TEAP. This approach may also be used to explain the observed concomitant
TEAPs in some microbial systems. The advantage of this approach is that it can be
applied in systems that have not reached steady-state conditions (Jacobsen et al., 1998).
The microbial systems in the present study were highly dynamic with rapid temporal
changes in reactant concentrations consequently some metabolic reactions notably those
involving chlorinated solvents were completed in just a matter of few days. Therefore,
this approach was found to be more appropriate for assessing the in situ TEAP conditions
than the specific H₂ level approach, which assumes that the TEAPs are mutually
exclusive due to H₂ competition and the system is in a steady-state condition.

Reductive dechlorination of chlorinated compounds such as chloroethenes is a
highly exergonic reaction. It can be noted from Table 5.1 that dechlorination of cis-1,2-
DCE to VC and VC to ethene yield the same amount of energy of about 160 kJ/mol of
Cl⁻. Each dechlorination step yields about 160 kJ/mol of Cl⁻ it therefore follows that the
amount of energy that is available from dechlorination reaction increases with increasing
degree of chlorination of the parent compound (Dolfing, 1999a; Löffler et al., 1999).

Based on Gibbs free energy values per mol of hydrogen oxidized (Table 5.1) 1,2-
DCA is expected to have the lowest threshold for H₂ uptake followed closely by both VC
and cis-1,2-DCE, sulfite, sulfate and CO₂ (Methanogenesis). Concomitant degradation of
cis-1,2-DCE and VC may thermodynamically be feasible since these compounds have
comparable Gibbs free energies under standard conditions.

It is obvious from Table 5.1 that the energy yield for a given metabolic reaction
depends on the chemistry of local environment. For example, pH will be very important
to the $\Delta G_r$ of 1,2-DCA dechlorination—with increasing pH (decreasing $H^+$ activity), the energy available to the bacteria will increase as the $\Delta G_r$ decreases (Table 5.1).

The in situ Gibbs free energies for methanogenesis, sulfate reduction and sulfite reduction, are much sensitive to $H_2$ compared to $cis$-1,2-DCE, 1,2-DCA and VC reduction since they are effective in the third to fourth power (Table 5.1). For example, on day 48 (Table 5.2) if other conditions are held constant, a 50% decrease in $H_2$ will cause methanogenesis to be an energetically unfavorable reaction (a 30% increase in $\Delta G_r$ -value) where as the same change will have an insignificant (a 4% increase in $\Delta G_r$ -value) effect on the energetics of 1,2-DCA dechlorination. Therefore, it is important to accurately determine $H_2$ concentration for thermodynamic calculations as Conrad et al. (1986) also observed. It is also worth noting that $\Delta G_r$ may potentially be influenced by pH (as it may affect the speciation of the reactants and products). Significant pH changes may become particularly important in the case of species with a $pK_a$ close to the water pH (Hoehler et al., 1998), especially $H_2S$ and $H_2CO_3$ in the present study. However, in the present study, extreme pH values were not encountered i.e., most observed pH values were generally circum-neutral ($7.00 \pm 0.4$) in all experiments.

- **Analytical Procedures**

VOCs were analyzed using EPA Method 8260B using a purge and trap apparatus to concentrate and introduce the sample into the gas chromatograph-mass selective detector (Agilent 6890 gas chromatograph-5972A mass selective detector). The GC was equipped with a 30 m × 0.25 mm × 0.25 μm film thickness, Agilent 5MS (5% Phenyl Methyl Siloxane) capillary column (Palo Alto, CA). A thermal desorption trap (VOCARB 3000; Supelco, Bellefonte, PA) was employed in the purge and trap
apparatus. The samples were purged for 11 min with ultra-high-pure helium at a flow rate of 35 mL/min, desorbed for 0.5 min and baked for 13 min at 225°C. The GC column temperature program was -80°C for 1 min ramped to 20°C at 15°C/min then ramped to 80°C at 10°C/min and finally to 220°C at 20°C/min. The injector and detector temperatures were 250°C and 280°C respectively. Helium was used as a carrier gas at a flow rate of 2.1 mL/min. Daily blanks, calibration checks and tunes were run to assure that the analytical method was in control. Recovery of surrogates injected into every sample also ensured that no gross dilution errors or leaks have occurred in the GC/MS system.

Methane, ethene, ethane and carbon dioxide were measured using GC/FID. A subsample of gas in the headspace (1 mL) was withdrawn using a gas tight syringe and injected into the gas chromatography with flame ionization detector (Agilent 5890 Series II) equipped with a 2.4 m × 0.32 mm ID column packed with Carbopack b/1% SP-1000 (Supelco, Bellefonte, PA). The column temperature was held at 40°C isothermally for 6.5 min, and the injector and detector temperatures were 375 and 325°C respectively. The carrier gas was ultra high purity nitrogen (BOC Gases, Baton Rouge, LA) at a flow rate of 12 mL/min.

Hydrogen was analyzed using reduction gas analyzer (Trace Analytical, Menlo Park, CA) equipped with a reduction gas detector. Headspace samples were injected into a 1-mL gas sampling loop and were separated with a molecular sieve analytical column at an oven temperature of 40 °C, ultra-high-purity nitrogen (BOC Gases, Baton Rouge, LA) was used as the carrier gas after it was passed through a catalytical combustion converter (Trace Analytical, Menlo Park, CA) to remove traces of H₂. The detection limit for H₂
under these conditions was 0.01 ppm. Aqueous H₂ concentrations were calculated using the following equation adopted from Löffler et al. (1999): \[ [H_2,\text{aq.}] = \frac{LP}{RT}, \]
where \([H_2,\text{aq.}]\) is the aqueous concentration of H₂ (in moles per liter); \(L\) is the Ostwald coefficient for H₂ solubility (0.01913 at 25°C); \(R\) is the universal gas constant (0.0821 liter·atm·K⁻¹·mol⁻¹); \(P\) is the pressure (in atmospheres); and \(T\) is the temperature (K). Preparation of standards was done using a 10 ppmV H₂ in N₂ (BOC Gases, Baton Rouge, LA). The standards were diluted in serum bottles containing H₂-free N₂ at ambient temperature and pressure. Hydrogen was sampled at a sufficient interval of time (72 to 240 hours) to ensure complete equilibrium during the continuous production and consumption of aqueous hydrogen in the sediment material (Hoehler et al., 1998). In addition before analysis, the bottles were shaken to equilibrate dissolved H₂ with the gas phase. All hydrogen data are reported as the aqueous phase concentration.

Organic acids (lactate, formate, succinate, acetate, propionate, butyrate and benzoate) were analyzed using high-pressure liquid chromatograph (Agilent 1090 Series II Liquid Chromatograph).

Sulfur and chloride were analyzed using colorimetric method with a portable spectrophotometer (DR 2010 Hach Co., Loveland, CO). Whenever possible, samples were immediately analyzed after being collected, otherwise, sulfide samples were filtered and spiked with 4 drops of 2N zinc acetate per 100 mL and stored at 4°C for preservation prior to analysis according to the Standard Methods (APHA, AWWA and WEF, 1998). The HCO₃⁻ concentration was calculated from CO₂ partial pressure in the microcosms headspace using the Henry’s constant for CO₂, the \(pK_a\) for HCO₃⁻ and the \textit{in situ} pH (Conrad et al., 1986). HS⁻ concentration was calculated from the measured values of
hydrogen sulfide using the ionization fraction parameters approach ($pK_{a1} = 7.02$ and $pK_{a2} = 13.9$ at 25°C)(Morel and Hering, 1993). Redox potential and pH were determined using an Ultrameter 6P (Myron L Company, Carlsbad, CA). The instrument was standardized in commercially prepared buffer solution (pH = 7.00) (Hach Co., Loveland, CO).

- **Statistical Analysis**

  Statistical analysis included ANOVA (hydrogen data), linear regression (hydrogen, methane, ethane and ethene calibration curves), and non-linear regression using Eq. 1 (VOCs, sulfite and sulfate degradation monitoring data). Results were considered statistically significant if $P \leq 0.05$.

5.3 Results and Discussion

- **Degradation of cis-1,2-DCE and 1,2-DCA under Methanogenic Conditions**

  Prior to starting the experiments, sediments were analyzed for redox sensitive substances such as ferric iron, nitrate, nitrite, sulfite, sulfate and methane. Except for methane, which was detected at aqueous concentrations in excess of 1000µM in all microcosms, all other redox sensitive compounds were present in trace amounts thus suggesting that before sulfite and sulfate were added, methanogenesis was the most predominant reducing condition in the bottles. Except for acetic acid, which was present in one out of 12 samples analyzed at a concentration of 1.83 µM, other organic acids (formic, benzoic, lactic, propionic, butyric) were not detected probably because they did not accumulate as they were fermented to H$_2$ as quickly as they were being produced. This observation suggests that although methanogenesis could occur by other pathways, but in the present study CO$_2$ reduction via H$_2$ was the major pathway and that H$_2$ was the main source of reducing equivalents for all other TEAPs.
Degradation kinetics of \textit{cis}-1,2-DCE and 1,2-DCA and associated H$_2$ concentrations and Gibbs free energy yields calculated using Eq. 5.2 were investigated individually and simultaneously under methanogenic conditions. Increase in methane concentration, depletion of 1,2-DCA and accumulation of ethene were simultaneously observed in microcosms amended with 1,2-DCA only indicating that dechlorination of 1,2-DCA and methanogenesis were concomitantly occurring as Figure 5.1 illustrates. The observed concomitant dechlorination of 1,2-DCA and methanogenesis was also supported by thermodynamic calculations (1,2-DCA dechlorination, -273 to -270 kJ/mol; methanogenesis, -20.8 to -18.3 kJ/mol).

On the other hand, the onset of dechlorination of \textit{cis}-1,2-DCE coincided with a drastic decrease in H$_2$ concentration from about 55 nM to about 6 nM within 12 hours and stayed nearly constant at 5.62 ± 0.83 nM (± standard error of the estimate) during the dechlorination period (Figure 5.2). This observation is consistent with the hypothesis that microbes have the ability to pull down the H$_2$ level as low as possible until it reaches the threshold value (Jacobsen et al., 1998). Energy yields associated with \textit{cis}-1,2-DCE and VC reduction were calculated and found to range from -166.5 to -155.6 kJ/mol and between -181.9 and -166.4 kJ/mol respectively. Upon depletion of \textit{cis}-1,2-DCE and VC, H$_2$ concentration gradually increased to levels comparable to the initial concentration. During the time of continuous dechlorination of \textit{cis}-1,2-DCE and VC, methane concentration was noted to be nearly constant indicating inhibition of methanogenesis ($\Delta G$ $> 10$kJ/mol).

When spiked together, \textit{cis}-1,2-DCE and 1,2-DCA could not be dechlorinated concurrently as Figure 5.3 illustrates. One of the possible reasons could be that the
Figure 5.1: Time course of 1,2-DCA dechlorination and concentrations of H$_2$, methane and energy yields of the TEAPs.

dechlorination of the two compounds was mediated by different types of organisms such that organisms mediating the degradation of cis-1,2-DCE out-competed for H$_2$ those
which use 1,2-DCA as an electron acceptor by keeping H₂ level too low to make degradation of 1,2-DCA possible. *Dehalococcoides* sp. organisms suspected to be responsible for dechlorination of *cis*-1,2-DCE in the present study has also been reported to degrade 1,2-DCA to ethane (99%) and VC (1%) (Maymö-Gattel et al., 1999). It was, therefore, expected that *Dehalococcoides* sp. would probably have degraded 1,2-DCA and *cis*-1,2-DCE concurrently. Surprisingly it was not the case beside the fact that the total amounts of energy released per mol of H₂ utilized during the complete dechlorination of the test chemicals are comparable (Table 5.1). Complete dechlorination of *cis*-1,2-DCE yields about 146.7 kJ/mol H₂ whereas that of 1,2-DCA via dihaloelimination and consecutive hydrogenolysis pathways yields approximately 206 and 145 kJ/mol H₂ respectively under standard conditions (pH = 7). Therefore based on the energetics of the dechlorination reactions only, it was expected that *Dehalococcoides* sp. will concurrently degrade 1,2-DCA and *cis*-1,2-DCE when both compounds are equally accessible to them. It can be noted from Figure 5.3 that reduction of ethene and 1,2-DCA and methanogenesis concurrently occurred after *cis*-1,2-DCE and VC were completely depleted as also supported by the energy yield values of these TEAPs (ethene, -75 to -74 kJ/mol; 1,2-DCA, -270 to -269 kJ/mol; methanogenesis, -20 to -18 kJ/mol).

- **Degradation Kinetics of 1,2-DCA, Free Energy and H₂ Thresholds in the Presence of Sulfite and Sulfate**

  The pH and concentrations of reactants and products and the calculated *in situ* Gibbs free energies during transformation of 1,2-DCA under sulfite-reducing conditions are shown in Table 5.2. Gibbs free energies of the H₂-consuming TEAPs shown in Table 5.2 were calculated from the combination of *in situ* aqueous concentrations of H₂,
Table 5.2: Gibbs free energies during sulfite reduction, methanogenesis and 1,2-DCA dechlorination

<table>
<thead>
<tr>
<th>Elapsed time (day)</th>
<th>H$_2$ (nM)</th>
<th>SO$_3^{2-}$ (mM)</th>
<th>H$_2$S (µM)</th>
<th>*HS$^-$ (µM)</th>
<th>CH$_4$ (mM)</th>
<th>CO$_2$ (M)</th>
<th>HCO$_3^-$ (M)</th>
<th>pH</th>
<th>ORP (mV)</th>
<th>CT (mM)</th>
<th>DCA (µM)</th>
<th>C$_2$H$_6$ (µM)</th>
<th>$\Delta G_r$ (kJ/mol of TEAP)</th>
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<tr>
<td>0</td>
<td>162.2(12.3)</td>
<td>2.51</td>
<td>0</td>
<td>0</td>
<td>1.14</td>
<td>1.182</td>
<td>19.14</td>
<td>7.12</td>
<td>-152</td>
<td>2.65</td>
<td>54.36</td>
<td>0</td>
<td>M</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>18.4(3.7)</td>
<td>2.43</td>
<td>0.1</td>
<td>0.05</td>
<td>1.04</td>
<td>1.073</td>
<td>14.12</td>
<td>7.03</td>
<td>-132</td>
<td>2.56</td>
<td>55.09</td>
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<td>-76.74</td>
<td>-1.47</td>
</tr>
<tr>
<td>15</td>
<td>0.51(0.2)</td>
<td>1.81</td>
<td>2.08</td>
<td>0.97</td>
<td>1.16</td>
<td>1.09</td>
<td>12.26</td>
<td>6.96</td>
<td>-153</td>
<td>2.61</td>
<td>52.06</td>
<td>0</td>
<td>-42.37</td>
<td>+34.29</td>
</tr>
<tr>
<td>20</td>
<td>0.45(0.03)</td>
<td>1.62</td>
<td>2.34</td>
<td>1.16</td>
<td>1.07</td>
<td>1.084</td>
<td>13.62</td>
<td>7.01</td>
<td>-136</td>
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<td>51.73</td>
<td>0</td>
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<tr>
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<td>1.56</td>
<td>5.47</td>
<td>2.83</td>
<td>1.06</td>
<td>1.145</td>
<td>15.79</td>
<td>7.05</td>
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<td>4.36</td>
<td>2.08</td>
<td>1.12</td>
<td>1.222</td>
<td>14.33</td>
<td>6.98</td>
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<td>1.15</td>
<td>1.182</td>
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<td>2.55</td>
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<td>1.16</td>
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<td>5.47</td>
<td>2.70</td>
<td>1.18</td>
<td>1.222</td>
<td>15.36</td>
<td>7.01</td>
<td>-153</td>
<td>2.49</td>
<td>12.52</td>
<td>41.07</td>
<td>-20.36</td>
<td>-272.95</td>
</tr>
<tr>
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<td>124.78(45.6)</td>
<td>0.00</td>
<td>8.33</td>
<td>3.50</td>
<td>1.17</td>
<td>1.436</td>
<td>13.38</td>
<td>6.88</td>
<td>-155</td>
<td>2.50</td>
<td>4.89</td>
<td>44.30</td>
<td>-20.87</td>
<td>-268.95</td>
</tr>
<tr>
<td>48</td>
<td>122.08(53.72)</td>
<td>0.00</td>
<td>7.33</td>
<td>3.88</td>
<td>1.21</td>
<td>1.403</td>
<td>20.21</td>
<td>7.07</td>
<td>-152</td>
<td>2.62</td>
<td>0.47</td>
<td>50.90</td>
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<tr>
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<td>4.25</td>
<td>1.26</td>
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<td>7.14</td>
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<td>2.36</td>
<td>0.00</td>
<td>46.39</td>
<td>-21.03</td>
<td>-</td>
</tr>
</tbody>
</table>

* Calculated from $[\text{HS}^-] = [\text{H}_2\text{S}] \times [\text{H}^+] \times 10^{7.02}$

+ Calculated from $[\text{HCO}_3^-] = [\text{H}_2\text{CO}_3^+] \times [\text{H}^+] \times 10^{-6.35}$

() is standard deviation

M = Methanogenesis
SR = Sulfite Reduction
DCAR = 1,2-DCA Reduction
Figure 5.2: Time course of cis-1,2-DCE dechlorination and concentrations of H₂, methane and energy yields of the TEAPs.
Figure 5.3: Time courses of dechlorination of cis-1,2-DCE and 1,2-DCA and reduction of ETH and concentrations of H₂, methane and energy yields of the TEAPs
reactants and products of the terminal electron accepting reactions. The individual values are average values of at least triplicate measurements. The coefficients of variation were highest in hydrogen and hydrogen sulfide samples, but were generally less than ±30 and ±40% respectively. Oxidation-reduction potentials in all microcosms were low (-150 to -120mV) and did not vary much during the experiments as supported also by the redox indicator which remained colorless throughout the study demonstrating that low redox conditions were maintained in the microcosms. It should be noted that background concentrations of chloride were high in the microcosms (Table 5.2). Chloride is suspected to originate from the inoculated slurry to introduce dechlorinating organisms into the microcosms. However, chloride has no significant effect on thermodynamic calculation since changing chloride concentration by four orders of magnitude (other parameters remaining unchanged) resulted into just about 15 and 20 percent change in free energy yields of both cis-1,2-DCE and VC and 1,2-DCA respectively.

Time courses of dechlorination of 1,2-DCA and reduction of sulfite and the corresponding energy yields of the TEAPs are shown in Figure 5.4. 1,2-DCA dechlorination and methanogenesis via H₂/CO₂ were feasible as supported by the Gibbs free energies (Table 5.2) when levels of H₂ concentration were high which coincided with the complete exhaustion of sulfite (Figure 5.4). It is worth noting that despite the favorable thermodynamics, 1,2-DCA degradation started after sulfite was completely depleted. It can also be observed that after day 34, methanogenesis became exergonic and concentration of methane started to increase. These observations suggest that sulfite reduction pulled down H₂ concentration to such low levels that were energetically unfavorable for 1,2-DCA dechlorination and methanogenesis. In another study conducted
Figure 5.4: Time courses of dechlorination of 1,2-DCA, sulfite reduction and concentrations of H₂, methane and energy yields of TEAPs.
under similar conditions (see Chapter 4) and using the same soil substrate as in the present study the thresholds of H₂ during degradation of 1,2-DCA under methanogenic conditions ranged from 30 to 50 nM, comparable to the threshold values normally observed during methanogenesis. Concomitant dechlorination of 1,2-DCA and methanogenesis was also observed.

Although present in small quantities, sulfide indicated that sulfite reduction in the microcosms was active as also supported by the calculated Gibbs free energies (Table 5.2). The concentration of HS⁻ increased as that of sulfite decreased thus suggesting that degradation of the sulfite was indeed occurring. However, there were no apparent reasons for the decrease in concentration of HS⁻ after day 30 in microcosms amended with 1,2-DCA (Figure 5.4).

Based on the Gibbs free energy released per mol of H₂ consumed under standard conditions, 1,2-DCA reduction is indisputably a more energetically favorable reaction and is expected to have a lower H₂ threshold than methanogenesis. Thus, based on hydrogen competition approach, 1,2-DCA reduction and methanogenesis would be thought to be mutually exclusive TEAPs contrary to what was observed in the present study. The reason for concomitant 1,2-DCA reduction and methanogenesis could be that methanogens were responsible for 1,2-DCA reduction since methanogenic bacteria notably *Methanosarcia barkeri* and *Methanococcus mazei* have been observed to degrade 1,2-DCA (Holliger et al., 1990). Holliger et al., (1990) also observed that methanogenic cell suspensions grown on H₂/CO₂ had higher 1,2-DCA conversion rates and activity relative to methane formation than those grown on methanol and acetate thus implying that reduction of 1,2-DCA and methanogenesis were concurrently occurring. It is worth
noting that the reported physiological threshold of H\textsubscript{2} for methanogens ranges from 23 to 100 nM which are comparable to the values observed in the present study during concurrent dechlorination of 1,2-DCA and methanogenesis (Cord-Ruwisch et al., 1988; Carr and Hughes, 1998).

Hoehler et al. (1998) reports that in any chemical transformation, whether biologically mediated or not, thermodynamics represents the ultimate control. This control is however not often expressed because it is superseded by kinetic considerations or obscured by the effects of bacteria operating far from equilibrium conditions. These reasons perhaps could partly explain why 1,2-DCA was dechlorinated at high H\textsubscript{2} concentrations concurrently with CH\textsubscript{4} production contrary to the conventional thermodynamic reasoning. It may, therefore, be inferred from these observations that H\textsubscript{2} threshold depends not only on the potential Gibbs free energy of an electron acceptor but also on the physiological characteristics of organisms that use it as an energy source (Ballapragada et al., 1997; Wiedemeier et al., 1999).

Two mechanisms of 1,2-DCA microbial mediated reductive dechlorination have been reported by various researchers these are hydrogenolysis, which involves the sequential replacement of a chlorine atom by hydrogen, and dihaloelimination in which two adjacent chlorine atoms are removed and replaced by a carbon double bond to form ethene, consequently dihaloelimination requires only 0.5 mol of reducing equivalents per mol of chlorine removed whereas 1 mol of reducing equivalents per mol of chlorine removed is needed for dechlorination via hydrogenolysis. (Lorah and Olsen, 1999; Dolfing, 1999b; Dyer et al., 2000). Based on thermodynamic considerations, dihaloelimination yields about 60% more Gibbs free energy than hydrogenolysis thus
implying that the former dechlorination mechanism is energetically more favorable than the latter mechanism (Dolfing, 1999b). Since reducing equivalents (notably \( \text{H}_2 \)) are generally in short supply in microbial systems, when microbes have the option of selecting between various electron acceptors and degradation pathways they generally use the energetically most favorable redox couple although it is not always the case. This could be the reason that, dihaloelimination was the only pathway used by microbes for dechlorination of 1,2-DCA in the present study perhaps due to highly competitive conditions for \( \text{H}_2 \). In another study conducted by the authors (see Chapter 3) on the degradation of 1,2-DCA under highly methanogenic conditions with abundant supply of \( \text{H}_2 \) (aqueous \( \text{H}_2 \) concentrations of between 35 and 226 nM) dechlorination through hydrogenolysis was observed in about 80% of the laboratory microcosms in which methanogenesis was also found to be active. These observations seem to support the hypothesis that under conditions of abundant supply of reducing equivalents dechlorinating systems will tend to use hydrogenolysis pathway for chloroethanes degradation, although this pathway is less favorable than dihaloelimination but allows the microbes to consume more reducing equivalents (Dolfing, 1999b).

Figure 5.5 shows time courses of 1,2-DCA and sulfate reduction, \( \text{H}_2 \) and methane concentrations and free energy yields of TEAP. For unknown reasons, sulfate reduction started after a lag period of at least 5 days (Figure 5.5). This lag period possibly gave competitive advantage for \( \text{H}_2 \) to 1,2-DCA dechlorinating organisms over sulfate-reducers. At least 50% of 1,2-DCA was dechlorinated before reduction of sulfate started. Hydrogen concentration levels went down after sulfate reduction started and remained constant at 7.73 ± 2.10 nM until sulfate was depleted (day 33). After sulfate was
Figure 5.5: Time courses of dechlorination of 1,2-DCA, sulfate reduction and concentrations of \( \text{H}_2 \), methane and energy yields of TEAPs.
depleted H\textsubscript{2} concentration increased to levels, which were thermodynamically favorable for methanogenesis (Figure 5.5).

- **Degradation Kinetics of cis-1,2-DCE, Free Energy and H\textsubscript{2} Thresholds in the Presence of Sulfite and Sulfate**

  Figure 5.6 shows transformation of cis-1,2-DCE in the presence of sulfite and the associated concentrations of H\textsubscript{2} and the Gibbs free energy yields of the TEAPs. Based on Gibbs free energy yield, VC dechlorination was thermodynamically the most favorable reaction followed closely by cis-1,2-DCE dechlorination, sulfite reduction and methanogenesis (Figure 5.6). When sulfite was nearly exhausted after day 49 (Figure 5.6) methanogenesis seemed to have started to become active albeit at a relatively low free energy yield due to the increase in H\textsubscript{2} concentration. However, for practical purposes at this level of free energy yield (-9 to -2 kJ/mol) methanogenesis cannot be energetically favorable as attested by a nearly constant methane concentration beyond day 49 (Figure 5.6).

  Figure 5.6 suggests that methane production was most likely inhibited by cis-1,2-DCE dechlorination and sulfite reduction. Inhibition of methane production paralleled by a decrease in the steady state concentration of H\textsubscript{2} to a level that provided a positive free energy (\(\Delta G_r > 0\)). It should be noted, however that inhibition of a bacterial process does not necessarily require that \(\Delta G_r > 0\), only that energy yield is too small to support cell multiplication (Hoehler et al., 1998). In addition, positive free energy values (\(\Delta G_r > 0\)) due primarily to low H\textsubscript{2} concentrations do not necessarily indicate that anaerobic oxidation of methane is occurring (Zehnder and Brock, 1979); they simply imply that
Figure 5.6: Time courses of dechlorination of cis-1,2-DCE, sulfite reduction and concentrations of H₂, methane and energy yields of TEAPs
methane production is thermodynamically not feasible. Thermodynamic calculations show that the minimum H\(_2\) level required to make methanogenesis exergonic under \textit{in situ} conditions is 15 nM. However, the H\(_2\) threshold for methanogenesis to be thermodynamically favorable under the \textit{in situ} conditions is about 110 nM. Unlike in the 1,2-DCA/sulfite amended microcosms, methanogenesis did not resume in bottles spiked with \textit{cis}-1,2-DCE and sulfite even after all chlorinated ethenes have been degraded because the experiment was prematurely terminated before all sulfite and ethene (electron acceptors with better energy yields than CO\(_2\)) were completely depleted. It can be noted from Figure 5.6 that after \textit{cis}-1,2-DCE and VC were completely depleted, H\(_2\) concentration started to increase which caused sulfite reduction to become even a more energetically favorable reaction.

Dechlorination of \textit{cis}-1,2-DCE, reduction of sulfate and the associated H\(_2\) and methane concentrations and energy yields of the TEAPs are shown in Figure 5.7. A rapid decrease in H\(_2\) concentration indicates that indeed dechlorination of \textit{cis}-1,2-DCE and reduction of sulfate were supported by H\(_2\). It can be noted from Figure 5.7 that production of ethane from ethene reduction started after VC was completely converted to ethene, this probably because the last step of \textit{cis}-1,2-DCE dechlorination yields less energy than the first two steps (Table 5.1). Since it is most likely that complete dechlorination of \textit{cis}-1,2-DCE was mediated by \textit{D. ethenogenes}, therefore the bacteria will preferentially utilize \textit{cis}-1,2-DCE and VC over ethene when both compounds are equally accessible. On the other hand, \textit{cis}-1,2-DCE and VC were observed to be simultaneously dechlorinated possibly because their yield comparable amount of energy
Figure 5.7: Time courses of dechlorination of cis-1,2-DCE, sulfate reduction and concentrations of H$_2$, methane and energy yields of TEAPs.
as Figure 5.7 shows. Upon depletion of cis-1,2-DCE and VC the dechlorinating organisms had to utilize ethene despite its lower energy yield.

Contrary to the observations made in the present study, it has been reported that dechlorination can be inhibited in sulfate reducing conditions in mixed cultures due to competition for H₂ although Gibbs free energy values suggest that most chlorinated compounds have higher affinity for H₂ than sulfate (Dolfing and Harrison, 1992). Energy yield values observed (-21 to -6 kJ/mol) during active sulfate reduction in the present study compare very well with those observed in laboratory experiments for anoxic sediments by Hoehler et al. (1998) and Jacobsen et al. (1998) in landfill leachate plume but higher (i.e. less negative) than those observed by Conrad et al. (1986) for anoxic lake sediments (-49.2 kJ/mol) at 10 ºC and those reported by Richmond et al. (2001) in subarctic groundwater (-204 to -116 kJ/mol). Besides differences in microbial compositions and chemistries of sedimentary environments, temperatures differences at which the experiments were conducted may also partly account for the observed differences in energy yield values during sulfate reduction since, shifts in temperature may affect the H₂ level and consequently the change in ΔGₚ (Jacobsen et al. 1998). In addition, calculated energy yield values for sulfate reduction (-15 to -6 kJ/mol) during transformation of cis-1,2-DCE and VC suggest that sulfate-reducing organisms were operating close to their thermodynamic threshold.

However, hydrogen thresholds in sulfate-amended microcosms in the present study (Table 5.3) were higher than typical values observed in sulfate reducing environments (1.0 to 1.5 nM) (Lovley et al., 1994). Conrad et al. (1987) also observed H₂ threshold values as high as 9 nM in lake sediments amended with exogenous sulfate six
days after incubation. In view of this, bioenergetics of TEAPs as well as thresholds and
dynamics of H$_2$ may be dependent upon factors that control the production and
consumption (TEAPs) of H$_2$ such as temperature and chemistry of the system, which may
be very different in different microbial systems.

**Table 5.3:** Degradation rate constants and H$_2$ thresholds during dechlorination of cis-1,2-
DCE and 1,2-DCA

<table>
<thead>
<tr>
<th>In the presence of</th>
<th>First-order rate constant, day$^{-1}$</th>
<th>Hydrogen threshold, nM</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis-1,2-DCE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfite</td>
<td>0.05 ± 0.01</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.41 ± 0.12)</td>
<td>(1.84 ± 0.23)</td>
<td></td>
</tr>
</tbody>
</table>
|                   |                                        |                         | Dechlorination of 1,2-
|                   |                                        |                         | DCA started after sulfur was completely depled (Figure 5.4) |
| Sulfate           | 0.28 ± 0.01                           | 0.19 ± 0.06             |         |
|                   | (4.41 ± 0.92)                         | (7.73 ± 2.10)           |         |
|                   |                                        |                         | Sulfate reduction
|                   |                                        |                         | started after a lag period
|                   |                                        |                         | of 5 days in 1,2-DCA
|                   |                                        |                         | amended microcosms
|                   |                                        |                         | (Figure 5.5). |
| cis-1,2-DCE       | -                                      | ND                      |         |
|                   | (2.63 ± 0.29)                         |                         |         |
|                   |                                        |                         | 1,2-DCA was
dechlorinated after complete reduction of
|                   |                                        |                         | cis-1,2-DCE and VC
|                   |                                        |                         | (Figure 5.3). |
| 1,2-DCA           | 0.50 ± 0.02                           | -                       |         |
|                   | (2.63 ± 0.29)                         |                         |         |
| Methanogenic      | 0.62 ± 0.19                           | (0.25 ± 0.02)           |         |
|                   | (5.62 ± 0.83)                         | (50.18 ± 2.91)          |         |

(  ) indicate H$_2$ thresholds

ND: Not dechlorinated

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Therefore, care must be exercised in comparing energy yields and \( \text{H}_2 \) thresholds of TEAPs in different microbial systems. Some of many factors that may contribute to the observed \( \text{H}_2 \) concentrations in anaerobic sediments include the rate of hydrogen production, microbial population size, and availability of electron acceptors (Mazur and Jones, 2001).

Figures 5.1 through 5.7 and Table 5.2 show that microbiologically mediated reduction reactions of the chlorinated solvents are highly exergonic with \( \Delta G \) values significantly below the threshold (-15 to -7 kJ/mol) almost independent of their concentrations. This observation implies that the potential of degradation of chlorinated solvents in the studied microbial systems was high even when present in low concentrations. It is also worthy noting that reduction of 1,2-DCA proceeded with higher energy release than that of \textit{cis}-1,2-DCE and VC, this finding further attests the observations made by Dolfing (1999) that under environmentally realistic conditions, dihaloelimination mechanism of chlorinated ethanes yields more free energy than reductive dechlorination of chlorinated ethanes and ethenes. However, the thermodynamic considerations alone could not provide explanations for concurrent occurrence of dechlorination of 1,2-DCA and methanogenesis and the failure of reduction of 1,2-DCA in the presence of \textit{cis}-1,2-DCE and sulfite.

\( \Delta G_r \) value may be used to measure the distance from equilibrium of the reaction between \( \text{H}_2 \) and the electron acceptor (Jacobsen and Postma, 1999). In general, \( \Delta G_r \) values for sulfite-, sulfate-reduction, and methanogenesis (during active methanogenesis indicated by an increase in methane production) are close to their threshold values suggesting that partial equilibrium was controlling the metabolic reactions of these
TEAPs (Table 5.2 and Figures 5.1 through 5.7). However, for chlorinated solvents the \( \Delta G_r \) values are highly negative and hence far from the threshold thus suggesting that the metabolic reactions of these TEAPs were occurring at non-steady state conditions (Jacobsen and Postma, 1999).

1,2-DCA was not dechlorinated beyond ethene (at least within the period of the study) unlike cis-1,2-DCE which was completely transformed to ethane (via VC and ethene). This circumstantial evidence suggests that organisms involved in dechlorination of 1,2-DCA and cis-1,2-DCE were possibly different. In the microcosms amended with 1,2-DCA only, dechlorination of 1,2-DCA and methanogenesis started concurrently without noticeable lag phase (Figure 5.1) suggesting that probably organisms with comparable physiological and metabolic attributes were responsible for 1,2-DCA dechlorination and methanogenesis.

- **Degradation Kinetics and Hydrogen Thresholds**

  Pseudo-first order degradation rate constants and \( \text{H}_2 \) thresholds observed during dechlorination of the test compounds are shown in Table 5.3. In general sulfite had more effects on dechlorination of both chlorinated solvents than sulfate (Table 5.3).

  In the presence of sulfite and sulfate and under methanogenic conditions, significantly \( (P < 0.05) \) lower hydrogen concentrations were observed in the sediment slurries amended with cis-1,2-DCE as compared to slurries amended with 1,2-DCA (Table 5.3). Hydrogen threshold values in sulfate-amended microcosms are significantly higher than those reported in sulfate-reducing sedimentary environments (1-1.5 nM) in the field (Lovley et al., 1994). However, they are comparable to those observed by Conrad et al. (1987) in anoxic microcosms constructed from eutrophic lake sediments (9
to 17 nM). These differences in H\textsubscript{2} threshold values may be interpreted in terms of differences in chemistry and microbial composition of the systems in which the H\textsubscript{2} levels were measured (Ballapragada et al., 1997). Lack of mass transfer limitations in the more homogeneous microcosm environments as opposed to the field environments may partly explain the observed differences in H\textsubscript{2} levels.

Pseudo first-order reaction rate constants for sulfite- and sulfate-reduction in microcosms amended with 1,2-DCA (sulfite, 0.07 day\textsuperscript{-1}; sulfate, 0.09 day\textsuperscript{-1}) were found to be higher than those in microcosms which actively dechlorinated cis-1,2-DCE (sulfite, 0.02 day\textsuperscript{-1}; sulfate, 0.07 day\textsuperscript{-1}). This observation furthermore suggests that organisms responsible for dechlorination of cis-1,2-DCE were able to compete more effectively for reducing equivalents (H\textsubscript{2}) with bacteria utilizing sulfite and sulfate than those mediating 1,2-DCA reduction as H\textsubscript{2} threshold values also show.

The effects of sulfite and sulfate on dehalogenation rate were also demonstrated by DeWeerd et al. (1991) using resting-cell suspensions of Desulfomonile tiedjei with hydrogen as an electron donor and 3-chlorobenzoate as a test chemical. They observed that addition of sulfite and sulfate to actively dehalogenating cultures of Desulfomonile tiedjei reduced the rate of dehalogenation of 3-chlorobenzoate by 81 and 15 percent respectively. Deweerd et al. (1991) further observed that H\textsubscript{2} consumption rate during dehalogenation of 3-chlorobenzoate in the presence of sulfite was about 30 % higher than that recorded in the presence of sulfate. Notwithstanding the fact that different microbial systems and test chemicals were used, the findings of the present study regarding the effects of the sulfur oxyanions on dehalogenation are in good agreement with observations made by DeWeerd et al. (1991). The reasons for differences in the
dehalogenation rates of the sulfur oxyanions in the DeWeerd et al. (1991) study could not be clearly established. Chemical reactivity of sulfite with the proteins involved in dehalogenation compared with chemically inert sulfate and differences in electron carriers used for electron transfer to the sulfur oxyanions were speculated as possible reasons for the observed differences in dehalogenation rates of 3-chlorobenzoate in the presence of the sulfur oxyanions. In our opinion, significant differences in the effects of sulfite and sulfate on rates of dehalogenation may also partly be explained by thermodynamic considerations since sulfite is energetically more favorable electron acceptor than sulfate assuming that the same type of organisms were responsible for mediating the reduction of the sulfur oxyanions (Table 5.1).

Since dechlorination of 1,2-DCA started after sulfite was completely transformed whereas sulfite and cis-1,2-DCE were concurrently reduced and also the reduction rate of sulfite was higher in the presence of 1,2-DCA than in the presence of cis-1,2-DCE (Table 5.3) it is, therefore, suggested that the presence of sulfite had more effect on the dechlorination of 1,2-DCA compared with the reduction of cis-1,2-DCE. Fortunately sulfite rarely occurs naturally in groundwater sedimentary environments therefore, it is least likely to pose a potential treatment problem of chlorinated solvents in the remediation of contaminated sites.

- **Inhibition of Methanogenesis**

Methanogenesis yields less energy per moles of H₂-consumed than other terminal electron acceptors under standard conditions (Table 5.1). Therefore, to acquire an equivalent energy yield, methanogens require higher H₂ concentrations than other electron acceptors. The differing H₂ concentration requirements could be used by other
bacteria (e.g., dechlorinators, sulfite- and sulfate-reducers) to gain a competitive advantage over methanogens. Thermodynamic inhibition of methanogens can therefore be caused by other organisms, which utilize energetically more favorable electron acceptors by maintaining low H₂ concentrations that cannot support methane production.

Addition of exogenous sulfite, sulfate and cis-1,2-DCE resulted in complete inhibition of H₂-dependent methanogenesis with concomitant decrease of the H₂ concentration to values that are thermodynamically no longer favorable for methanogens (Figure 5.1 to Figure 5.7). For example, before addition of sulfite and cis-1,2-DCE, the ΔGᵣ for methanogenesis was -23.04 kJ/mol, a value which is typical of methanogenic environments. After addition of sulfite and cis-1,2-DCE, the ΔGᵣ increased to values greater than -1.47 kJ/mol, and methanogenesis eventually became inhibited (ΔGᵣ > 0). Achtnich et al. (1995) observed that in the presence of exogenous sulfate, methane production from H₂ in anoxic paddy soil became impossible when the ΔGᵣ had increased to values higher than -17 kJ/mol as also observed in the present study.

Conrad (1999) reports that the steady-state H₂ concentration observed in most methanogenic environment is close to the thermodynamic equilibrium of H₂-dependent methanogenesis. The observed concentration is equivalent to a Gibbs free energy of -23 kJ/mol that is necessary to couple methane production to the generation of 1/3 ATP (Conrad, 1999). Inhibition of methanogenesis will occur if the H₂ concentration decreases below this level. Concentration of H₂ can only be decreased below the threshold of the dominant TEAP if a H₂-consuming reaction with a lower H₂-threshold takes over at a rate that is equal to or greater than that of the prevailing TEAP. However, if the H₂ pool is large enough to satisfy all the required reducing equivalents, more than one TEAP may
concomitantly occur. For example, a H$_2$-consuming reaction with a lower H$_2$ threshold such as sulfate reduction and cis-1,2-DCE dechlorination takes over at a rate higher than that of methanogenesis, methanogenesis will be inhibited. Based on thermodynamic considerations only, methane production may resume only after all electron acceptors with higher potential energy yields have been exhausted as observed in the present study.

To investigate the effects of sulfur oxyanions on methanogenesis, sulfite and sulfate were added into the microcosms to a final concentration of 2.50 mM. Methane production was observed to cease almost immediately after addition of the sulfur oxyanions thus indicating that methanogenesis was inhibited (data not shown). Complete and instantaneous inhibition of H$_2$-dependent methanogenesis by sulfate reducers upon addition of sulfate has been repeatedly demonstrated in laboratory experiments (Conrad et al., 1987; Hoehler et al., 1994; Achnich et al., 1995). However, reasons as to why the resident methanogens should not continue H$_2$ utilization (as it was the case in the present study) albeit at a reduced rate are yet to be clearly established, since complete inhibition can only be achieved after the sulfate reducers have outgrown the methanogenic population. Inhibition of H$_2$-methanogens by exogenous sulfate has been more investigated and is therefore perhaps better understood than inhibition of methanogenesis by dechlorinators. Besides, the failure of methanogens to compete with sulfate reducers for reducing equivalents, rapid inhibition of methanogenesis is also assumed to be due to H$_2$-syntrophic associations consisting of H$_2$-dependent methanogens fueled by sulfate-reducers, which produce H$_2$ during fermentation of organic matter (Conrad et al., 1987; Cord-Ruwisch et al., 1988; Conrad, 1999). Conrad (1999) reports that syntrophic propionate oxidizers that have so far been isolated are able to reduce sulfate. When
sulfate is available, these fermenting bacteria stop H₂ production in favor of sulfide formation. This could be one of the reasons for methanogenesis inhibition in the present study, since background sulfate concentrations in the microcosms were below the detection limit and therefore the population of sulfate-reducers was initially most likely not significantly higher to the extent of depleting exogenous sulfate at the observed rates. Replacement of methanogenic populations by sulfate reducers would require a long-term exposure to these electron acceptors. Since there are no apparent evidence of toxicity effects of sulfate and products of sulfate reduction to methanogens, inhibition of methanogenesis is therefore most likely due to a shift in the energy and electron flow away from methanogens (Conrad et al., 1987).

Inhibition of methanogenesis due to toxicity effects of cis-1,2-DCE and VC on methanogens is highly unlikely because in almost all the experiments methanogenesis consistently resumed almost immediately after cis-1,2-DCE and VC dechlorination reactions were completed. The fact that dechlorination of 1,2-DCA and methanogenesis were observed to concomitantly occur rules out the possibility of toxicity effects of 1,2-DCA on methanogens.

It is worth noting that the measured in situ H₂ concentrations during active methanogenesis in the present study (Table 5.2) correspond to ΔGR values of approximately -20 kJ/mol which is comparable to values generally found in methanogenic environments. Interestingly, the ΔGR values of approximately -23 kJ/mol are equivalent to the energetic threshold of 1/3 ATP or less, thus suggesting that in H₂-dependent methanogenesis operates at its thermodynamic threshold in many cases (for example, Thauer et al. (1977), Achnich (1995), Hoehler et al. (1998), Jacobsen (1998)
and Conrad (1999)). Hoehler et al. (1998) hypothesized that under highly competitive conditions for reducing equivalents, terminal metabolic bacteria are forced to operate at their thermodynamic limits. This hypothesis could be true for methanogenic bacteria in the present study since the calculated changes in $\Delta G_r$ were consistently at the edge of thermodynamic threshold during active methanogenesis.

5.4 Conclusion and Implications of the Results

Degradation kinetics of cis-1,2-DCE and 1,2-DCA were observed to be fastest under methanogenic conditions followed by sulfate-and sulfite-reducing conditions. In view of this, sedimentary environments with sulfite- and sulfate-reducing conditions are more likely to slow kinetics of dechlorination of 1,2-DCA compared to that of cis-1,2-DCE. In a plume containing both contaminants, cis-1,2-DCE and 1,2-DCA may not be simultaneously dechlorinated, the later compound will most likely be degraded after the former compound and its first degradation daughter product have completely been exhausted, thus posing a special bioremediation problem.

Variations in hydrogen concentration affected the thermodynamics of methanogenesis to a greater degree than was true of any other constituent involved in the metabolic reactions. This finding is in line with observations from other studies (Hoehler et al., 1998; Jacobsen et al., 1998). Results of this study have also shown that, in some cases, thermodynamic considerations alone may be insufficient to explain the relationship between the thresholds of H$_2$ and the potential energy yield ($\Delta G'_r$) of TEAPs. Therefore, threshold of H$_2$ for the TEAP may be related not only to the potential energy yield of the TEAP but also to the activity and physiology of organisms mediating the TEAP reaction. In view of this, observations made by Cord-Ruwisch and co-workers (1988) that
hydrogen threshold of an electron acceptor is the same regardless of the type of bacteria mediating the TEAP should be interpreted with some caution in some circumstances.

The present study was done under static conditions whereas convective-dispersive flow conditions are generally dominant in the constructed wetland bed as well as in groundwater sedimentary environments. Spatial and temporal distribution as well as concentrations of H$_2$ and other electron donors and redox species may be significantly different under static and continuous flow conditions. Conditions in microcosms are spatially homogeneous, and temporally changing whereas in continuous flow regimes conditions tend to vary both temporally and spatially (Isalou et al., 1998). For example in a study conducted by Jacobsen et al. (1998) on H$_2$ concentrations in a landfill leachate plume methanogenesis was observed to occur in stagnant zones where H$_2$ levels were suspected to be higher than in the moving water. In view of this, the present study may not have been able to accurately mimic the conditions that are prevailing in the field. In addition, the rates of reduction of the chlorinated solvents as well as the sulfur oxyanions and the interactions between the chemical species and the microbial community may be quite different under continuous flow biofilm conditions than under batch suspended growth conditions used in the present study.

The concept of relative free energy yield in assessment of redox conditions used in the present study has some limitations. Postma and Jacobsen (1996) observed that energy yield may not always predict the kinetics of overall reaction with different TEAPs since fermentation of organic matter to produce small organic molecules such as H$_2$, propionate and acetate (electron donors) is overall rate limiting in sedimentary environments.
Notwithstanding the limitations of the study and the approach used to interpret the experimental data, the present study has confirmed the findings from previous studies that microbial competition for hydrogen-reducing equivalents may not always result in complete exclusion of one process over another and concomitant redox processes may occur (Postma and Jacobsen, 1996; Jacobsen et al., 1998; Jacobsen and Postma, 1999; Jacobsen et al., 1999) thus casting a doubt on the reliability of \( \text{H}_2 \) concentration data only as a redox indicator. In fact Jacobsen and Postma (1999) noted that \( \text{H}_2 \) data was not useful for redox zonation in an aquifer since it showed little correlation to the distribution of TEAPs. They further noted that the \( \text{H}_2 \) concentration is only indicative for the dominant TEAP if the system microbiologically is at steady state, implying that there is no net bacterial growth in the system. In view of this, the competitive exclusion concept may not be applicable in non-steady-state systems, for example, in the present study shifting from sulfite-reduction to 1,2-DCA-dechlorination and methanogenesis after sulfite was depleted, the microbial system should obviously have been in a non-steady state conditions also.

Results and the approach used in this study may be used to predict the potential for dechlorination of 1,2-DCA and \textit{cis}-1,2-DCE and perhaps other chlorinated aliphatic compounds under sulfite, sulfate as well as under other reducing conditions knowing the concentrations of \( \text{H}_2 \), reactants and products and the chemistry of the sedimentary environment. In addition, the dechlorination reaction of the chlorinated solvent of interest must be known and thermodynamic values must be available (Jacobsen et al., 1998).
6.1 Introduction

Groundwater contamination by chlorinated hydrocarbons, such as trichloroethene (TCE) and tetrachloroethene (PCE), is a widespread problem throughout the United States. TCE has been widely used in the dry cleaning industry, as an industrial solvent, and in the production of silicon wafers (Newman et al., 1997; Wiedemeier et al., 1999; Suthersan, 2000). Because of its widespread use and poor handling, storage, and disposal practices, many groundwater systems have been contaminated with TCE thereby, posing a threat to public health and ecosystem viability. Since pump-and-treat systems rarely achieve their goal of complete cleanup of sites contaminated with chlorinated solvents due to their timelessness and prohibitive maintenance and operation costs (Hofman, 1997) more economic and less expensive approaches are desirable for groundwater remediation to provide for long-term control of contaminated groundwater (Duba et al., 1996; Kao and Lei, 1999).

Natural attenuation of chlorinated solvents, specifically via anaerobic reductive dechlorination has been considered as an efficient and cost-effective method for in situ remediation of contaminants such as TCE and PCE. Wetlands have initially been used as municipal wastewater treatment systems for decades (Kadlec and Knight, 1996; Cole, 1998) followed later by application on urban non-point sources and agricultural runoff (Schultz and Peall, 1998). Wetlands may also provide a more cost-effective treatment alternative for chlorinated solvents contaminated groundwater compared with the traditional pump and treat systems (Lorah and Olsen, 1999; Pardue et al., 2000; Pardue et
However, while the fate and retention of nutrients, metals and sediments in wetlands are understood quite well, the same cannot be claimed for chlorinated solvents (Schultz and Peall, 1998; Lorah and Olsen, 1999). In addition, a few (if any) continuous flow column studies have been conducted on attenuation of chlorinated solvents in synthetic sand, peat and compost mixture and natural wetland soils. Most of the continuous flow column studies reported in literature on attenuation of chlorinated organics were conducted using packed sand (e.g., Siegrist and McCarty, 1987; Fogel et al., 1995; Isalou et al., 1998).

Natural wetlands have all the basic elements needed for the attenuation of chlorinated organic compounds including high organic carbon content (>30%) in the sediments to bind the contaminants; high microbial density and diversity in the sediments to biodegrade contaminants; and both anaerobic and aerobic conditions to ensure that contaminants can be fully degraded without accumulation of potentially toxic intermediates such as vinyl chloride (Lorah et al., 1997; Pardue et al., 2000; Suthersan, 2002). Based on field studies, Lorah and Olsen (1999) observed that natural wetlands are capable of removing chlorinated solvents from groundwater. In view of this, treatment of chlorinated solvents using natural wetland systems may be technically feasible and probably cost effective option, however it is worthy to determine the inherent assimilative capacities of these systems in case of contamination. Unlike active engineered remediation methods, natural attenuation of chlorinated organic solvents in wetlands would leave the ecosystem largely undisturbed (Lorah et al., 1999), an important consideration since wetlands are very delicate ecosystems.
Some contaminated groundwater plumes are shallow and intercept wetland ecosystems on the surface in accordance with natural groundwater flow. Aberdeen Proving Grounds have such a groundwater flow regime and have shown remarkable results indicating that chlorinated organic contaminants were degraded to non-detectable levels by natural attenuation without engineering enhancement (Lorah et al., 1997). At many sites, however, conditions do not lend themselves to natural attenuation processes that are significant or robust enough to protect potential receptors; either the processes are not observed at all or the travel time of the contaminants in the subsurface is insufficient for the processes to completely degrade the contaminants. An option is the construction of a treatment wetland on site to passively intercept shallow groundwater or to serve as an upwelling treatment system for pumped groundwater from greater depth. The treatment “concept” is that TCE and other chlorinated VOCs would be reductively dechlorinated to lower chlorinated daughter products or biodegraded via methanotrophic processes to CO₂ as the compounds pass through the highly organic sediment of the wetland. The organic sediments would retard the movement of contaminants relative to groundwater flow, resulting in a longer detention time of the contaminants in the wetland sediments. Plant uptake and metabolism of the compounds in the rhizosphere would provide further treatment.

Advection, dispersion, diffusion and volatilization are other processes that control the transport of chlorinated solvents in wetlands besides sorption, chemical and biological transformations and plant uptake (Wiedemeier et al., 1999). However, biological transformation is the most desirable and perhaps the most important fate process since it involves the destruction of the contaminant. Volatilization is obviously the least desirable
fate mechanism as it merely entails the transfer of a pollutant from aqueous to gaseous environmental compartments. The important contaminant fate and transport processes in wetlands are briefly described hereunder.

- **Reductive Dechlorination**

  An excellent review of the reductive dechlorination of chlorinated solvents is available (Wiedemeier et al., 1999). Briefly, the higher chlorinated ethenes are degraded via chlorine-removal while serving as the electron acceptor during microbial metabolism. The rate and efficiency of these reactions is dependent on the ambient hydrogen (H₂) concentrations that serve as the electron donor. One of the first demonstrations of reductive dechlorination of chlorinated ethenes in the laboratory was performed using wetland sediments from the Everglades in south Florida (Parsons and Lage, 1985). These studies identified dechlorination kinetics of PCE, TCE, 1,1-dichloroethene (1,1-DCE), cis-1,2-dichloroethene (cis-1,2-DCE), and trans-1,2-dichloroethene (trans-1,2-DCE) among others. Other studies by Pardue and others (Pardue et al., 1993; Jackson and Pardue, 1998) have documented the importance of dechlorination of chlorinated benzenes and chlorinated anilines in wetland sediments. Dechlorination of chlorinated ethanes in marsh systems has also been recently reported (Lorah et al., 1999). The conclusion of these studies is that wetlands are ideal environments for reductive dechlorination processes. Wetlands do not possess the limitations often found in aquifers (i.e., low organic carbon, low H₂ concentrations). The effectiveness of a synthetic peat, compost and sand mixture to treat groundwater from a Superfund site contaminated with a number of chlorinated aliphatic compounds was recently demonstrated by Kassenga et al. (2003) using bench-scale columns. In this study, removal rates of chlorinated organics in the
peat/compost/sand mixtures were observed to be faster than those normally observed in natural wetland systems.

- **Direct Biological Oxidation and Cometabolism**

  Less-chlorinated aliphatic solvents (e.g. vinyl chloride, 1,2-dichloroethane, and isomers of DCE) are more susceptible to direct biological oxidation and cometabolism compared with anaerobic reductive dechlorination (Wiedemeier et al., 1999). Research has demonstrated that aerobic biodegradation of chlorinated organics in the rhizosphere, the region immediately adjacent to plant roots, can be dramatically higher than in the bulk soil (Anderson and Walton, 1995). This has been attributed to the higher microbial activities found in the rhizosphere, an ample supply of readily degradable carbon co-substrates leaking from the plant and the leakage of oxygen into the rhizosphere from the root. The influence of the rhizosphere is particularly acute for processes that require both anaerobic and aerobic conditions. Since the rhizosphere has an extremely high surface area, it brings anaerobic zones (the bulk soil) and aerobic zones (the rhizosphere) in close contact. A key fate mechanism of chlorinated solvents that can occur in the rhizosphere is the fortuitous co-metabolism of TCE and daughter products by methanotrophic microorganisms, those that function under aerobic conditions using methane as an electron donor. These organisms possess an enzyme, methyl-monoxygenase (MMO) that will attack chlorinated ethenes oxidizing them through various intermediates to carbon dioxide (Wiedemeier et al., 1999; Suthersan, 2000). Since methane production and oxidation occurs across the oxygen gradient within the rhizosphere in wetland systems, the coupled dechlorination and oxidation may be a particularly important fate mechanism. This has been confirmed by recent laboratory studies (Lorah et al., 1999).
• **Sorption**

Sorption of organic contaminants to solid surfaces (e.g., suspended solids and sediments) is an important fate process because it lowers soluble concentrations and retards the movement of the organic compounds while they move through a porous media thus allowing the biodegradation processes time to go to completion (Lorah et al., 1997; Pardue et. al., 2000). Sorption of organic contaminants in wetlands is often enhanced since the high productivity of these systems results in suspended solids and sediments dominated by organic matter (high organic carbon fraction, $f_{oc}$). The $f_{oc}$ of highly organic marsh soils (peats), for example, can exceed 0.3 as compared to 0.05 for an average mineral-dominated soil or 0.0001 for an aquifer (Lorah et al., 1997; Wiedemeier et al., 1999; Pardue et. al., 2000).

• **Plant Uptake and Metabolism**

While the rhizosphere is the site of enhanced microbial activity, many chlorinated organics can be taken up into plants directly. The movement of organic contaminants from soils/sediment to plants is of interest because of the large transpiration stream in many plants and the diverse enzymatic systems present in plants (Schnoor et al., 1995). Plants have been identified with dehalogenase activity (e.g., *Myriophyllum spicatum*, “parrot feather”) and much interest has been directed recently at phytoremediation schemes for chlorinated solvents (Schnoor et al., 1995; Susarla, et al., 2002). Stimulation of microbial activity and biochemical transformations in the rhizosphere through release of root exudates and plant enzymes may also be important phytoremediation mechanisms for TCE (Susarla et al., 2002). Structure activity relationships for non-ionic organics (e.g., Briggs et al., 1982) suggest that uptake is only effective for compounds with log
$K_{ow}$’s from 0.5 to approximately 4, within the range of the chlorinated solvents (PCE, $K_{ow} = 2.28$, vinyl chloride, log $K_{ow} = 0.60$). Since the mechanism of plant uptake exists and the enzymes have been demonstrated to exist, phytoremediation remains a possible fate mechanism for chlorinated solvents in a wetland system but one that is difficult to quantify and capture in design. However, plant uptake and volatilization, which are also potential TCE attenuation processes, were not covered in the current work.

- **Dispersion**

Hydrodynamic dispersion (molecular diffusion plus mechanical dispersion) dilutes the concentrations of contaminants by mixing with less contaminated or clean groundwater. Because molecular diffusion is the dominant dispersion mechanism only at extremely low groundwater velocities, it is often ignored in groundwater studies (Lorah et al., 1997).

Continuous flow modes of operation are normally encountered in natural wetland systems and would likely be used in a constructed wetland. Continuous flow operation results in very different conditions than those existing in microcosms where addition of chlorinated compounds, electron donor, and micronutrients are performed on a periodic batch basis. Conditions tend to vary spatially in a continuous flow system, in contrast to microcosms where conditions are spatially homogeneous, and temporally changing (Isalou et al., 1998). In addition, in wetlands, biomass is primarily present as a biofilm. Most microcosm studies have involved suspended growth conditions in a water phase (e.g., Freedman and Gossett, 1989; Tandol et al., 1994), with no or relatively small amounts of soil present. The rates of dechlorination of TCE and the interactions between the chemical species and the microbial community may be quite different under
continuous flow biofilm conditions than under batch suspended growth conditions. However, discerning the relative contribution of different contaminants removal mechanisms (such as sorption and biodegradation) in column experiments, if possible would be extremely difficult (Siegrist and McCarty, 1987). From the foregoing discussion, column tests have more advantages compared with batch studies since they are conducted under advective and dispersive flow conditions similar to those in the field (Porro et al., 2000). In view of this, continuous flow column studies were conducted in the present work to mimic conditions normally observed in wetland systems and microcosm studies were merely used to confirm the degradation processes observed in the soil columns.

Peat is rich in carbon, could exhibit sufficient carbon bioavailability for reductive dechlorination to occur, and is readily available and relatively inexpensive (Kao and Lei, 2000). These characteristics make peat a good candidate for construction of wetlands for treatment of chlorinated-solvent contaminated groundwater. However, mechanisms as well as kinetics of removal of chlorinated VOCs in wetland peat material and geotechnical properties of wetland soils should be well understood to arrive at a rational approach for designing a constructed wetland system for treatment of water contaminated with the chlorinated solvents.

The overall objective of the current study was to investigate the inherent potential of a pristine freshwater natural wetland for remediation of water contaminated with chlorinated organics and to monitor transport and fate of chlorinated solvents in constructed wetlands to obtain information necessary to provide future design parameters for constructed treatment wetlands for chlorinated solvents. TCE was used as a test
chemical because it is classified as a priority pollutant by the United States Environmental Protection Agency and is regulated under the Clean Water Act of 1986. National Primary Drinking Water Regulations’ maximum concentration level (MCL) of TCE is 5 µg/L while those of cis-1,2-DCE and VC (degradation daughter products of TCE) are respectively 70 and 2 µg/L (EPA, 2002). The specific objectives of the present study were as follows: (1) to determine degradation kinetics of TCE in natural and constructed wetland soils (2) to measure sorption potential of natural and constructed wetland soils for TCE (3) to determine geotechnical characteristics of the wetland sediments (4) to identify the organisms responsible for reductive dechlorination of TCE in the soil columns, and (5) to confirm evidence of biodegradation processes observed in the soil columns and to examine transformation patterns of TCE using anaerobic microcosms. In principle, TCE attenuation processes that are investigated in the current study include biodegradation and sorption, with emphasis on biodegradation. Dispersion and advection attenuation mechanisms of the contaminant are discussed as part of the hydraulic characterization of the soil columns.

6.2 Materials and Methods

- **Chemical**

  Neat solution of TCE (Supelco, Bellefonte, PA) was used to prepare stock solutions. Analytical standards and surrogates for the VOCs were obtained as mixtures or neat liquids from Supelco (Supelco, Bellefonte, PA). Methane, ethane and ethene calibration gases were also purchased from Supelco Inc. Resazurin, formaldehyde, and 2-bromoethanesulfonic acid (BES) were procured from Sigma Chemical Co. (Saint Louis, MO). Hydrogen standards were prepared using 10 µL/L hydrogen standard (BOC Group
Inc., Baton Rouge, LA). Other chemicals used in this study were reagent grade and were purchased from various vendors.

- **Column Studies**

  Three undisturbed cylindrical natural wetland columns were collected at randomly selected areas of a pristine freshwater marsh located at Madisonville, Louisiana using thin-walled 15-cm-id aluminum cylindrical tubes that minimized compaction. The top 60 cm of soil from each core was removed and transferred to 15-cm-id glass cylinders with 8 equispaced (7.5 cm center-to-center) sampling ports located along their lengths. The soil disturbance and compaction were minimal due to the near-instantaneous placement of the casing and high water content of the soil. Constructed wetland columns were packed with *Bion Soil* (Dream Maker Dairy, Cowlesville, NY), *Latimer peat* (Latimer's Peat Moss Farm, West Liberty, OH), and sand mixed at a ratio of 1.3:1.1:1 (Bion Soil:Latimer peat:sand) by weight determined from previous studies reported in Kassenga et al. (2003) and then visually inspected for uniformity of packing. The constructed wetland mesocosms were planted with the same type of plants (*Scirpus sp.*) as in the natural wetland mesocosms. Plant roots were observed to cover the entire depths of natural wetland soil columns. The columns were covered with aluminum foil to prevent the growth of photosynthetic organisms and were kept fully water-saturated for the duration of the experiment. TCE solution was pumped in an upflow mode through the column at a flow rate of $0.40 \pm 0.05$ mL/min. A schematic diagram of the bench-scale wetland treatment system is shown in Figure 6.1. The column experiments were conducted in a plant growth chamber (Conviron, Controlled Environments Ltd., Winnipeg, Manitoba, Canada) under controlled climatic conditions (temperature = $25^\circ$;
relative humidity = 60%) as Plate 6.1 shows. Attenuation of TCE was monitored by analyzing samples collected at different depths in the cores on at least weekly basis. Influent and effluent flow rates were tracked throughout the experiments for the purpose of water and contaminant mass accounting in the soil columns. Pressure drop across the soil columns was monitored using piezometers. Column experiments were conducted for 18 months.

- **Bromide Breakthrough Experiments**

Bromide was used as a nonreactive, non-degrading tracer to characterize hydrodynamic dispersion during transport through the porous medium. A bromide
Plate 6.1: (a) Wetland soil columns in a plant growth chamber (b) TCE solution container with a floating lead to eliminate headspace.
solution (400 mg/L) was continuously pumped into the columns at a constant rate of about 0.4 mL/min until saturation was attained. Bromide samples were collected at the middle of the column (a distance of 30 cm from the bottom). The experiments started with an initial solute concentration at zero. A 25-cm layer of coarse gravel placed at the bottom of the column was used to uniformly distribute the flow across the cross-sectional area. This boundary condition is described as the flux input. Pore water velocities were determined from the hydraulic conductivity, porosity and hydraulic gradient values using Darcy’s equation.

- **Sorption Studies**

  The constructed wetland soil was assumed to have uniform sorptive and hydraulic characteristics unlike the natural wetland soil, which was spatially heterogeneous. However, stronger variations in the soil characteristics were observed in the vertical direction than in the horizontal direction mainly due to stratification during formation of the natural wetland soil. In view of this, sorption tests were done for different depth intervals for natural wetland sediments. Three 60-cm long soil columns were cored at randomly selected places in the natural wetland and sectioned into ten 6-cm long pieces. Slices of soil from the same depth interval from the three cored soil columns were mixed and the mixture was subjected to a single sorption test. Three replicate sorption tests were conducted for the constructed wetland soil.

  Batch sorption experiments were done using 40 mL VOA vials (I-CHEM, New Castle, DE) containing water spiked with TCE and sodium azide as a biocide and 12 g (dry weight) of wetland soils. Six points of the isotherm were developed across the range of concentrations of TCE. Isotherm points were generated by diluting TCE solution with
an electrolyte solution (0.01 M CaCl₂) using dilutions of 1:1, 1:2, 1:5, 1:10 and 1:20. Three replicate vials were used for each isotherm point for a total of 18 vials. Samples were shaken on a reciprocating shaker at 20°C for 48 hours, centrifuged, and the supernatant analyzed for TCE using EPA Method 8260B. Any TCE missing from the supernatant was assumed to be adsorbed. Mass balance calculations were finally used to determine the sorption partition coefficients.

- **Anaerobic Microcosms**

Biodegradation was investigated under anaerobic conditions in microcosms constructed with wetland soils and deionized water spiked with the test chemical. Construction of anaerobic microcosms was done under a nitrogen atmosphere in a glove bag (I²R, Cheltenham, PA) by homogenizing and packing soil in 160 mL serum bottles using a 1.5:1 volumetric ratio of water to wetland soils according to Lorah et al. (1997). All reaction mixtures were sealed with Teflon-lined rubber septa and aluminum crimp seals and incubated in an inverted position under static and light-excluded conditions at 25 °C, which was the approximate temperature of the water used during the soil column experiments. Resazurin (0.0002%) was added as a redox indicator. Temporal monitoring of concentrations of TCE and degradation daughter products hydrogen, and methane were done until the concentration of the parent compound and degradation daughter products dropped below the detection limits of the analytical methods.

To investigate the role of methanogenic bacteria with regard to dechlorination of TCE, a comparative study was conducted using the methanogenesis inhibitor 2-bromoethanesulfonic acid (BES). BES was added to a final concentration of 30 mM in the bottles. Stock TCE solution was made from the neat chemical (assay > 99.5%) and
added to the microcosms using a gas-tight syringe to give the planned initial dissolved concentration of about 40 µM. Abiotic controls for each soil type were included in the study to monitor for non-biological losses of the test chemical. For the abiotic controls, reaction mixtures prepared as described above were adjusted to contain 1% formaldehyde. Samples were withdrawn from the bottles and immediately analyzed without storage for chlorinated ethenes, ethene, ethane, hydrogen, and methane. To ensure reproducibility, triplicate serum bottles were used in each treatment and, whenever feasible, experiments were repeated at least two times.

- **Detection of Dehalococcoides 16S rDNA Sequences**

To determine if *Dehalococcoides* sp. were present in the soil columns, a PCR-based detection method was used. DNA extracted from the bulk community was amplified by PCR using two different sets of primers previously reported to be specific to this group of bacteria (Hendrickson et al., 2002). Detection of the appropriate size PCR products was used as direct evidence that these organisms were present in the soil columns. To verify that PCR products were of the expected sequence, a subset of the PCR products were cloned and sequenced. The methods for the various steps in this process are summarized in the following subsections.

**DNA Extraction**

Soil samples for DNA extraction were collected from sampling ports located at a distance of 7.5 cm from the bottom of the soil columns using sterilized spatulas and stored in sterile cryogenic vials. Sufficient soil was then transferred to a sterile microcentrifuge tube to result in 0.25 to 0.4 g of wet soil, and DNA extraction from pelleted soil samples was performed using a MoBio Ultraclean Soil DNA Isolation Kit (Salona Beach, CA) according to the manufacturer’s instructions with the following
modifications. The kit protocol was amended so that a Biospec Mini-Beadbeater 3110BX (Biospec Products Inc., Bartlesville, OK) was used in place of the MoBio Vortex Adapter (Salona Beach, CA). The beadbeater was operated for 3 minutes at 4,800 rpm. A detailed description of the DNA extraction procedure is shown in Appendix A. Because of the high humic acid content of the soil samples, prior to DNA extraction, the samples were treated with Polyvinylpolypyrrolidone (PVPP) (Agros Organics, Geel, Belgium) as described in Appendix B. As an additional step to remove humic acid impurities, two additional washes of the S4 solutions were added (for a total of 3 washes, instead of the 1 wash recommended by the manufacturer’s standard protocol).

**PCR amplification**

PCR amplification was performed using an Eppendorf MasterTaq kit (Brinkmann Instruments, Inc., Westbury, NY), which includes Taq DNA Polymerase (5U/µL), 10X Taq Buffer with Mg\(^{2+}\) and 5x TaqMaster PCR Enhancer (Brinkmann Instruments, Inc., Westbury, NY). A 2.5 mM solution of each dNTP (mix 10 mM total) was obtained from Applied Biosystems (Forster City, CA). The 5x TaqMaster PCR Enhancer often required heating at 60°C to completely dissolve the components.

A mastermix was made by the addition of 63.5 µL 18 Mega Ohm water, 15 µL of 5x TaqMaster PCR Enhancer (Brinkmann Instruments, Inc., Westbury, NY), 10 µL of 10X Taq Buffer with Mg\(^{2+}\), 8 µL of the 10 mM dNTP mix, 0.5 µL of the Taq DNA Polymerase and 1-µL of each primer (forward and reverse) per sample if all samples required the same primers, otherwise the primers from the mastermix were added individually.
For each sample to be amplified, 99 µL of the mastermix was placed in a 500 µL sterile PCR reaction tube, and then 1 µL of the extracted DNA was added. The contents were mixed and then centrifuged for 1 minute at 13,000 rpm. PCR amplification was then conducted using an Eppendorf Thermocycler (Eppendorf GmbH, Hamburg, Germany) under the following conditions previously reported by Hendrickson et al. (2002): 2 minutes of denaturation at 95°C, followed by 30 cycles of 1 minute of denaturation at 94°C, 1 minute of annealing at 55°C and 1 minute of extension at 72°C. Samples not analyzed immediately were stored at 0 to 4°C until analysis.

**Primers**

Two sets of primers (arbitrarily referred to as DE Primer Set 1 and DE Primer Set 2) specific to variable regions of the 16S rDNA present in *Dehalococcoides* group bacteria (Hendrickson et al., 2002) were used in this study. In cases where no PCR products were detected using these primers, a nested approach was used in which bacterial DNA was first amplified using a set of universal primers (see Table 6.1), and

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>Primer</th>
<th>bp coordinates</th>
<th>Primer sequence</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DE Primer Set 1</strong></td>
<td>Fp DE 1</td>
<td>1–17</td>
<td>5’GATGAACGCTAGCGCGGCG3’</td>
<td>1,377</td>
</tr>
<tr>
<td></td>
<td>Rp DE 1377</td>
<td>1385–1366</td>
<td>5’GGTTGGCACATCGACTTCAA3’</td>
<td></td>
</tr>
<tr>
<td><strong>DE Primer set 2</strong></td>
<td>Fp DE 946</td>
<td>946–963</td>
<td>5’AGTGAAACGGAAGGGAAA3’</td>
<td>266</td>
</tr>
<tr>
<td></td>
<td>Rp DE 1212</td>
<td>1220–1199</td>
<td>5’GGATTAGCTCCAGTTACACTG3’</td>
<td></td>
</tr>
<tr>
<td><strong>Universal Primer</strong></td>
<td>8F</td>
<td>8–27</td>
<td>5’AGAGTTTGTATCCTGGCTACG3’</td>
<td>1,502</td>
</tr>
<tr>
<td></td>
<td>1492R</td>
<td>1510–1492</td>
<td>5’GGTTACCTTGTACGACTTT3’</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.1: Designation, positions, and sequences of primers used

For the DE primer sets, the coordinates are the *Dehalococcoides ethenogenes* strain 195 16S rDNA base position coordinates, and for the universal primer the coordinates are the *E. coli* base position coordinates.
then the products of this initial amplification were amplified using the *Dehalococcoides*

specific primers. Table 6.1 lists the primers used in the present study. All primers were

synthesized, purified, desalted, and lyophilized by AlphaDNA (Montreal, Quebec, 

Canada). Primers obtained from Alpha DNA were reconstituted with TE buffer (10 mM 

Tris, 1 mM EDTA, pH = 8) to make 50 µM primer stock solutions that were stored at

-20°C prior to use.

Detection of PCR Products

PCR products were analyzed using 1 µL samples of the PCR reactions in conjunction with an Agilent 2100 Bioanalyzer and corresponding DNA labchip kits (Agilent Technology, Willington, DE).

Cloning and Sequencing

To verify that PCR products produced using *Dehalococcoides* specific primers were the desired target sequence, the 1377 base pair fragments of a representative sample (constructed wetland soil column 3) was cloned and sequenced. A TOPO TA Cloning Kit for Sequencing (with pCR4-TOPO®) with One Shot® TOP10 Chemically Competent *E. Coli* (Invitrogen, Carlsbad, CA) was used to clone each sample, following a modification of the manufacturer protocol (see Appendix C). DNA was extracted from clones, reamplified by PCR using the same primers as were used to generate the PCR product used for cloning, and then purified using UltraClean™ PCR Clean-up Kit (MoBio, Carlsbad, CA). A sequencing reaction was performed on the purified products using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Forester City, CA), using minor modifications of the manufacturer’s protocol (see Appendix D). For sequencing reactions using the 266 base pair (bp) fragment, only the 946f primer listed in Table 6.1 was used. To sequence the 1377 bp
amplicons, two sequencing reactions were performed, one using the 1f primer listed in Table 6.1 and the other using the 774f primer described by Hendrickson et al. (2002) which has a sequence of 5’GGGAGTATCGACCCCTC3’ and corresponds to a variable region of the 16S rDNA of *Dehalococcoides* bacteria. Sequencing was performed using an ABI 377 Automated DNA Sequencer. For the 1377 bp PCR product, the partial sequences generated using the individual primers (1f and 774f) were then joined.

The sequences were edited using BioEdit software (North Carolina State University, Raleigh, NC) and were subsequently compared to sequences in the National Center for Biotechnology Information (NCBI) database. The sequences were aligned with the top three reference species from the NCBI database along with two closest matches to previously cultured organisms. A pair-wise comparison of the aligned sequences was made, and a similarity matrix was produced.

- **Analytical Procedures**

  VOCs were analyzed using EPA Method 8260B using a purge and trap apparatus to concentrate and introduce the sample into the gas chromatograph-mass selective detector (Agilent 6890 gas chromatograph-5972A mass selective detector). The GC was equipped with a 30 m × 0.25 mm × 0.25 µm film thickness, Agilent 5MS (5% Phenyl Methyl Siloxane) capillary column (Palo Alto, CA). A thermal desorption trap (VOCARB 3000; Supelco, Bellefonte, PA) was employed in the purge and trap apparatus. The samples were purged for 11 min with ultra-high-pure helium at a flow rate of 35 mL/min, desorbed for 0.5 min and baked for 13 min at 225°C. The GC column temperature program was -80°C for 1 min ramped to 20°C at 15°C/min then ramped to 80°C at 10°C/min and finally to 220°C at 20°C/min. The injector and detector
temperatures were 250°C and 280°C respectively. Helium was used as a carrier gas at a flow rate of 2.1 mL/min. Daily blanks, calibration checks and tunes were run to assure that the analytical method was in control. Recovery of surrogates injected into every sample also ensured that no gross dilution errors or leaks have occurred in the GC/MS system.

Porewater (25 mL) from the wetland cores was collected using a gastight Hamilton syringe for analysis of methane. The water was transferred to a 25 mL glass vial fitted with a liquid facing Teflon cap. A headspace was created (10 mL) by displacing water using ultra pure nitrogen with a gastight syringe. The vial was heated using a hot plate at a temperature of 90°C to establish equilibrium. Henry’s Law constants are well known at these higher temperatures. Methane was measured using GC/FID. 1 mL of gas in the headspace was withdrawn using a gas tight syringe and injected into the gas chromatography with flame ionization detector (Agilent 5890 Series II) equipped with a 2.4 m × 0.32 mm ID column packed with Carbopack b/1% SP-1000 (Supelco, Bellefonte, PA). The column temperature was held at 50°C isothermally for 6.5 min, and the injector and detector temperatures were 375 and 325°C respectively. The carrier gas was ultra high purity nitrogen at a flow rate of 12 mL/min.

Hydrogen was analyzed using reduction gas analyzer (Trace Analytical, Menlo Park, CA) equipped with a reduction gas detector. Headspace samples were injected into a 1-mL gas sampling loop and were separated with a molecular sieve analytical column (Trace Analytical, Menlo Park, CA) at an oven temperature of 40 °C, ultra-high-purity nitrogen (BOC Gases, Baton Rouge, LA) was used as a carrier gas after it was passed through a catalytical combustion converter (Trace Analytical, Menlo Park, CA) to
remove traces of H$_2$. The detection limit for H$_2$ under these conditions was 1 ppb. Aqueous H$_2$ concentrations were calculated using the following equation adopted from Löffler et al. (1999): 
\[ [H_{2,aq}] = \frac{LP}{RT}, \]
where \( [H_{2,aq}] \) is the aqueous concentration of H$_2$ (in moles per liter); \( L \) is the Ostwald coefficient for H$_2$ solubility (0.01913 at 25°C); \( R \) is the universal gas constant (0.0821 liter-atm.K$^{-1}$mol$^{-1}$); \( P \) is the pressure (in atmospheres); and \( T \) is the temperature (K). All hydrogen data are reported as the aqueous phase concentration unless otherwise stated. Hydrogen was sampled at a sufficient interval of time (8 to 72 hours) to ensure complete equilibrium during the continuous production and consumption of aqueous hydrogen in the sediment materials. In addition, it was demonstrated that dissolved H$_2$ comes into equilibrium with gaseous H$_2$ within 20 minutes even without shaking sediments (Lovley and Goodwin, 1988).

Organic acids (lactate, formate, succinate, acetate, propionate, butyrate and benzoate) were analyzed using high-pressure liquid chromatograph (Agilent 1090 Series II Liquid Chromatography). Redox sensitive substances, sulfate, nitrate and ferric iron were analyzed using the liquid chromatography (Dionex LC-20, Dionex Corp., Sunnyvale, CA).

- **Statistical Analysis**

Statistical analysis included t-test (comparison of kinetic constants), linear regression (hydrogen, methane, ethane and ethene calibration curves), and non-linear regression using contaminant transport and first-order decay models (VOCs monitoring data). Results were considered statistically significant if \( P \leq 0.05 \).
6.3 Data Modeling

- **Potential for Fluidization**

  The potential for fluidization of the wetland bed was evaluated as part of the physical characterization of the wetland soils. Fluidization may result in short-circuiting of water from the bottom distribution layer to the wetland surface and if sufficiently severe, potentially result in failure of the treatment wetland (Kassenga et al., 2003). Fluidization occurs when the difference in pressure forces between the lower and the upper layers exceed the gravitational force of the substrate mass. Under such conditions, the effective stress through the substrate will effectively become zero and fluidization may occur (Charbeneau, 2000). The potential for fluidization was evaluated by considering the effective stress within the substrate bed and estimating the maximum flow rate to prevent fluidization. Effective stress, the portion of the total stress carried by the pore water, maintains peat stability. The reduction of effective stress resulting from upward seepage can be calculated as:

  \[ \sigma = z(\gamma_{\text{sat}} - \gamma_w) - iz\gamma_w \]  

  where \( z \) [L] is the depth of the substrate bed, \( \gamma_{\text{sat}} \) [M/L^3] is saturated unit weight of the substrate, \( \gamma_w \) [M/L^3] is unit weight of water, \( \sigma \) [M/L^2] is the effective stress and \( i \) [L/L] is the hydraulic gradient. A critical gradient, \( i_{cr} \) [L/L] can be defined by setting the effective stress equal to zero. This results in:

  \[ i_{cr} = \left( \frac{\gamma_{\text{sat}} - \gamma_w}{\gamma_w} \right) \]  

  Critical flow per unit surface area of the wetland, \( q_{cr} \) [L/T] is calculated as follows:

  \[ q_{cr} = K i_{cr} \]  

  where \( K \) [L/T] is the hydraulic conductivity of the wetland bed substrate.
Sorption

The sorption distribution coefficient $K_d$ [L$^3$/M] in a linear sorption model can be described by:

$$K_d = \frac{q_e}{C_e}$$

(6.4)

where $q_e$ is the mass of chemical sorbed per unit mass of soil [M/M] and $C_e$ [M/L$^3$] is the equilibrium concentration. Nonlinear isotherms can be described by the Freundlich equation

$$q_e = K_F C_e^N$$

(6.5)

where $K_F$ [L$^3$/M] is the Freundlich sorption constant and $N$ is the Freundlich exponent.

The retardation coefficient $R$, defined as the ratio of the average advective groundwater flow velocity to the contaminant transport velocity is calculated as follows

$$R = \left(1 + \frac{\rho_p}{\eta K_d}\right)$$

(6.6)

where $\rho_p$ [M/L$^3$] is the bulk density of the peat, $\eta$ is the porosity, $K_d$ is the linear distribution coefficient.

Transport Models

The limitations of the first-order approximation in describing the kinetics of transformation of substrates are well recognized (Bradley and Chapelle, 1998; Wiedemeier et al., 1999; Suthersan, 2000; Pavlostathis and Prytula, 2000). However, the implementation of more advanced contaminant transport models incorporating other removal kinetics such as the Michaelis-Menten kinetic model and others, which take into account microbial population, substrate levels and electron donor limitations, are difficult to implement in ill-defined systems since they involve biodegradation parameters that cannot be easily determined in complex microbial systems normally found in the field.
(Wiedemeier et al., 1999). For comparison purposes of the removal kinetics in the two types of wetland soils studied in the current study, we assume here that solute decay follows first-order kinetics. It is also assumed that there is no limitation of hydrogen donors since wetland soils rich in organic carbon content were used and that TCE transformation reaction kinetics is not limited by the availability of electron acceptor due to continuous pumping of TCE solution into the soil columns.

- **Determination of Hydrodynamic Dispersion**

Transport in the wetland soil columns was approximated as a one-dimensional process at a constant flow in a homogeneous porous medium. However, the assumption of homogeneity was probably not satisfied for the natural wetland columns since some variations in texture were observed in the vertical profile of the soil. The general differential equation for one-dimensional transport of a conservative tracer in the soil columns is,

\[
\frac{\partial C}{\partial t} + u_x \frac{\partial C}{\partial x} = D_x \frac{\partial^2 C}{\partial x^2} \quad (6.7)
\]

where \( C \) = aqueous phase concentration, \( u_x \) = seepage velocity, \( x \) = distance of the column, \( t \) = time, and \( D_x \) = coefficient of hydrodynamic dispersion. For laboratory column experiments under constant flux conditions, Eq. 6.8 referred to as the simple model (solution to Eq. 6.7) has been found to be appropriate for analysis of one-dimensional column breakthrough (Charbeneau, 2000).

\[
\frac{C}{C_o} = \frac{1}{2} \left( \text{erfc} \left( \frac{x-u_x t}{\sqrt{4D_x t}} \right) \right) \quad (6.8)
\]

In the simple model (Eq. 6.8) the column is considered to have infinite length in both directions, and initially: \( C = C_o \) for \( x < 0 \) and \( C = 0 \) for \( x > 0 \). Additionally, it is
assumed that a barrier at \( x = 0 \) keeps the different solutions from mixing (Charbeneau, 2000).

The solute transport model (Eq. 6.8) was fitted to the bromide effluent data (bromide breakthrough curves) using MATLAB™ software (Version 5.3, The MathWorks, Inc., Natick, MA) on a desktop computer to determine dispersion coefficient \( D_x \), and consequently Peclet numbers, \( P_L \) (\( P_L = Lu_x/D_x \) where \( L \) is the column length). The estimated dispersion coefficient values were then fixed in subsequent simulations of TCE degradation in the soil columns.

- **Contaminant Transport Models**

  Three transport models were used to describe the movement of TCE through the soil columns. The first model is based on the following 1-D lumped first order kinetic reaction equation:

  \[
  C(x) = C_0 e^{-\lambda R x / u_x}
  \]

  where \( \lambda \) \([\text{[T}^{-1}]\) is a lumped temporal removal rate constant. Other parameters have been defined above. Eq. 6.9 assumes that the effect of molecular diffusion/dispersion on contaminant transport is insignificant; that degradation occurs both within the liquid and sorbed phases at about the same rate; Sorption is approximated by the linear sorption model; and the system is at steady-state (Kassenga et al., 2003).

  The one-dimensional partial differential equation describing transient solute transport with first-order decay of the solute is (Wiedemeier et al., 1999)

  \[
  \frac{\partial C}{\partial t} = \frac{D_x}{R} \frac{\partial^2 C}{\partial x^2} - \frac{u_x}{R} \frac{\partial C}{\partial x} - \lambda C
  \]

  Under transient conditions, the solution to Eq. 6.10 is as follows (Schnoor, 1996)
\[ C = \frac{C_o}{2} \exp \left( \frac{(u_x - v)x}{2D_x} \right) \text{erfc} \left( \frac{(R_x - vt)}{2\sqrt{D_x \cdot Rt}} \right) + \frac{C_o}{2} \exp \left( \frac{(u_x + v)x}{2D_x} \right) \text{erfc} \left( \frac{(R_x + vt)}{2\sqrt{D_x \cdot Rt}} \right) \] (6.11)

where \( v = u_x \left( 1 + 4\frac{D_x}{u^2} \right)^{\frac{1}{2}} \)

Boundary condition 1 (BC 1): \( C(0,t) = C_o \) for \( t > 0 \)

Boundary Condition 2 (BC 2): \( \frac{\partial C(x,t)}{\partial x} = 0 \)

Initial Condition (IC): \( C(x,0) = 0 \) for \( x > 0 \)

Under steady-state conditions, the change in contaminant concentration with respect to time becomes zero, resulting in the following modified equation:

\[ \frac{D_x}{R} \frac{\partial^2 C}{\partial x^2} = \frac{u_x}{R} \frac{\partial C}{\partial x} - \lambda C \] (6.12)

The one-dimensional steady-state analytical solutions for Eq. 6.12 under the initial and boundary conditions listed below is (Wiedemeier et al., 1999):

\[ C(x) = C_o \exp \left\{ \frac{x}{2(D_x / R)} \left[ \frac{u_x}{R} - \sqrt{\left( \frac{u_x}{R} \right)^2 + 4\lambda \frac{D_x}{R}} \right] \right\} \] (6.13)

BC 1: \( C(0,t) = C_o \)

BC 2: \( \frac{\partial C(x,t)}{\partial x} = 0 \)

IC: \( C = 0, \ 0 < x < \infty \) at \( t = 0 \).

Dammköhler number, which is defined as the ratio of reaction rate to that of the velocity normalized by a characteristic length was calculated for the two systems to
evaluate the relative importance of degradation rate versus contaminant advection in the soil columns (Wiedemeier et al., 1999). Dammköhler number $D_N$, is given by:

$$D_N = \frac{\lambda L}{u_x}$$

(6.14)

6.4 Results and Discussion

- Hydraulic and Geotechnical Characteristics

Table 6.2 shows geotechnical properties of the wetland soils and pertinent physical characteristics of the column displacement experiments.

Tracer study results (dispersion coefficients)

Figure 6.2 presents the results of typical bromide breakthrough curves. The determined dispersion coefficients (Table 6.2) were approximately two orders of magnitude higher than the free liquid diffusivity of $8.8 \times 10^{-6}$ cm$^2$/s (25°C) for TCE (Powers et al., 1992). Consequently, it was not necessary to make adjustment for the

![Figure 6.2: Fitted and observed bromide elution curves](image_url)
difference in molecular diffusivities of bromide and TCE (Zhao and Thomas, 2000). Therefore, the dispersion coefficients determined for bromide were assumed equivalent for TCE and were used in the reactive contaminant transport equations.

**Table 6.2:** Column characteristics, geotechnical properties of the soils and flow data

<table>
<thead>
<tr>
<th>Characteristics/Parameters</th>
<th>Soil Column</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constructed</td>
<td>Natural</td>
<td></td>
</tr>
<tr>
<td><strong>Column characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (cm)</td>
<td>60</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Diameter (i.d.) (cm)</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Media total volume ($V_T$) (cm³)</td>
<td>10603</td>
<td>10603</td>
<td></td>
</tr>
<tr>
<td>Media saturated bulk density, ($\rho_b$) (g/cm³)</td>
<td>1.25 ± 0.04</td>
<td>1.12 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Media dry bulk density ($\rho$) (g/cm³)</td>
<td>0.57 ± 0.04</td>
<td>0.21 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Media total mass (g)</td>
<td>13254</td>
<td>11875</td>
<td></td>
</tr>
<tr>
<td>Media porosity ($\varepsilon$) (cm³/cm³)</td>
<td>0.72 ± 0.05</td>
<td>0.90 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Pore volume ($V_p$) (cm³)</td>
<td>7634</td>
<td>9543</td>
<td></td>
</tr>
<tr>
<td>Critical flow ($q_{cr}$) (L/day×hectare)</td>
<td>0.34</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td><strong>Flow data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydraulic conductivity ($K$) (cm/s)</td>
<td>$1.3 \times 10^{-4}$</td>
<td>$3.6 \times 10^{-4}$</td>
<td></td>
</tr>
<tr>
<td>Measured flow rate ($Q$) (cm³/min)</td>
<td>0.43 ± 0.09</td>
<td>0.44 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>Dispersion coefficient, ($D_x$) (cm²/s)</td>
<td>$3.70 \times 10^{-4}$</td>
<td>$7.66 \times 10^{-4}$</td>
<td></td>
</tr>
<tr>
<td>Pore velocity ($u_x$) (cm/s)</td>
<td>$4.63 \times 10^{-5}$</td>
<td>$7.31 \times 10^{-5}$</td>
<td></td>
</tr>
<tr>
<td>Peclet number ($P_L$)</td>
<td>3.75</td>
<td>2.86</td>
<td></td>
</tr>
<tr>
<td>Hydraulic residence time ($\tau = Q/V_p$) (day)</td>
<td>12.33</td>
<td>15.10</td>
<td></td>
</tr>
<tr>
<td>Initial concentration</td>
<td>$C_0$</td>
<td>$C_0$</td>
<td></td>
</tr>
</tbody>
</table>

Flow regimes in the column were checked by calculating Reynolds numbers, $R_e$. Reynolds numbers ($R_e = u_x d/\nu$) were calculated using particle sizes ($d$) of $5 \times 10^{-5}$ m for
constructed wetland soil and $2 \times 10^{-5}$ m for natural wetland sediments and a kinematic viscosity ($\nu$) of $3.22 \times 10^{-5}$ m$^2$hr$^{-1}$ (25 °C). Computed $Re$ was found to be $2.6 \times 10^{-3}$ and $1.6 \times 10^{-3}$ for the constructed and natural wetland columns, respectively. Since when $Re < 1$ flow is considered to be laminar (Siegrist and McCarty, 1987), the calculated Reynolds numbers indicate that flow regimes in the columns were highly laminar.

Potential for fluidization and water balance

Column lengths of constructed wetland mesocosms increased by $8.3 \pm 2.1$ % almost immediately after pumping started and then stabilized after about three days. In contrast to observations made in constructed wetland mesocosms, natural wetland columns showed no apparent evidences of fluidization probably because a well-established fabric of roots developed over a number of years was able to hold together soil particles against hydrodynamic forces responsible for fluidization. Natural wetland soil had higher critical flow for prevention of fluidization than constructed wetland soil (Table 6.2). Flow per unit cross-sectional area applied in the soil columns was about 0.42 L/day×hectare, which was higher than the critical flow of 0.34 L/day×hectare without fluidization of the bed for the constructed soil but slightly lower than that of natural wetland soil (0.45 L/day×hectare). This could also be a reason for the observed fluidization of constructed wetland columns.

Based on the measured average influent flow rates (Table 6.2) and the mean measured outflows (Constructed = $0.28 \pm 0.11$ mL/min; Natural = $0.31 \pm 0.12$ mL/min) the mean evapotranspiration rates were calculated and found to be 2.96 and 1.03 mm/day for the constructed and natural wetland soil columns, respectively. Higher plant density in
constructed wetland columns than in natural wetland mesocosms may account for the observed differences in the evapotranspiration rates and consequently effluent flow rates.

- **Sorption Potential**

  Linear sorption distribution coefficient $K_d$, for the constructed wetland soil was found to be $13.5 \pm 1.8$ L/kg based on three determinations. Results of determination of $K_d$ for the natural wetland soil are presented in Table 6.3. Both the linear and Freundlich models fit the TCE sorption data for natural wetland soil reasonably well ($r^2 > 0.86$). Although, Freundlich model fits sorption data slightly better compared with the linear sorption model (Table 6.3), there does not appear to be any reason to select the more complex, 2-parameter Freundlich sorption isotherm. Furthermore, Schwarzenbach and Westall (1981) found that the sorption of non-polar organic compounds (such as PCE and TCE) by aquifer materials for concentrations normally found in natural waters may be

<table>
<thead>
<tr>
<th>Depth Interval (cm)</th>
<th>Linear Parameter values</th>
<th></th>
<th>Freundlich Parameter values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_d$ (L/kg)</td>
<td>$r^2$</td>
<td>$K_F$ (L/kg)</td>
<td>$N$</td>
</tr>
<tr>
<td>0 - 6</td>
<td>44.67</td>
<td>0.932</td>
<td>71.38</td>
<td>0.700</td>
</tr>
<tr>
<td>6 - 12</td>
<td>48.00</td>
<td>0.953</td>
<td>76.70</td>
<td>0.700</td>
</tr>
<tr>
<td>12 - 18</td>
<td>26.30</td>
<td>0.960</td>
<td>44.10</td>
<td>0.700</td>
</tr>
<tr>
<td>18 - 24</td>
<td>14.53</td>
<td>0.925</td>
<td>18.70</td>
<td>0.845</td>
</tr>
<tr>
<td>24 - 30</td>
<td>6.50</td>
<td>0.868</td>
<td>16.45</td>
<td>0.471</td>
</tr>
<tr>
<td>30 - 36</td>
<td>35.34</td>
<td>0.926</td>
<td>55.00</td>
<td>0.718</td>
</tr>
<tr>
<td>36 - 42</td>
<td>27.33</td>
<td>0.940</td>
<td>42.30</td>
<td>0.671</td>
</tr>
<tr>
<td>42 - 48</td>
<td>42.14</td>
<td>0.960</td>
<td>57.79</td>
<td>0.738</td>
</tr>
<tr>
<td>48 - 54</td>
<td>42.20</td>
<td>0.974</td>
<td>58.30</td>
<td>0.734</td>
</tr>
<tr>
<td>54 - 60</td>
<td>37.11</td>
<td>0.949</td>
<td>61.97</td>
<td>0.688</td>
</tr>
</tbody>
</table>
appropriately described by a linear sorption isotherm model. In addition, sorption isotherms were observed to be linear over high concentration ranges of TCE (0.1 to 4 mg/L) indicating that mechanism of sorption into organic matter was partitioning (Lorah et al., 1997). It can be observed from Table 6.3 that there is no clear pattern of variation of $K_d$ in the vertical profile of the natural wetland sediments. Middle layers (12 to 30 cm) seem to have lowest $K_d$ followed by bottom layers (30 to 60 cm) and top layers (0 to 12 cm). Table 6.4 summarizes chemical characteristics of the natural wetland soil in the vertical profile analyzed by Huffman Laboratories, Inc., (Golden, CO). It can be observed from Table 6.4 that organic carbon content decreases with increasing depth. However, the soil organic carbon content trend does not clearly explain the observed variations in sorptive characteristics of the natural wetland soil (Table 6.3) in the vertical profile, since

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(0-2 cm) &lt; 5 yr</th>
<th>(10-12.5 cm) &gt; 20 yr</th>
<th>(&lt;20 cm) &gt; 40 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Constituents</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Carbon (wt %)</td>
<td>27.58</td>
<td>23.14</td>
<td>20.89</td>
</tr>
<tr>
<td>Hydrogen (wt %)</td>
<td>3.93</td>
<td>3.38</td>
<td>2.84</td>
</tr>
<tr>
<td>Oxygen (wt %)</td>
<td>23.32</td>
<td>21.52</td>
<td>18.25</td>
</tr>
<tr>
<td>Nitrogen (wt %)</td>
<td>2.06</td>
<td>1.65</td>
<td>1.27</td>
</tr>
<tr>
<td>Sulfur (wt %)</td>
<td>1.00</td>
<td>1.01</td>
<td>1.52</td>
</tr>
<tr>
<td>Ash (wt %)</td>
<td>42.40</td>
<td>52.42</td>
<td>57.09</td>
</tr>
<tr>
<td>Carbonate carbon (wt %)</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Organic carbon (wt %)</td>
<td>27.58</td>
<td>23.14</td>
<td>20.89</td>
</tr>
<tr>
<td><strong>Ratios</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/H (H/C)</td>
<td>7.02 (0.14)</td>
<td>6.85 (0.15)</td>
<td>7.36 (0.14)</td>
</tr>
<tr>
<td>C/O (O/C)</td>
<td>1.18 (0.84)</td>
<td>1.08 (0.93)</td>
<td>1.14 (0.87)</td>
</tr>
<tr>
<td>O/H (H/O)</td>
<td>5.93 (0.17)</td>
<td>6.37 (0.16)</td>
<td>6.43 (0.16)</td>
</tr>
<tr>
<td>C/N</td>
<td>13.39</td>
<td>14.02</td>
<td>16.45</td>
</tr>
</tbody>
</table>

*Data indicates percentage per g of dry soil*
sorptive capacity is normally expected to decrease with decreasing organic carbon fraction (Lorah et al., 1997; Wiedemeier et al., 1999).

Although the constructed wetland soil had higher organic carbon content (29%) than the natural wetland sediments (mean organic carbon content = 23%) the latter had a higher mean $K_d$ value (32.4 L/kg) than the former (13.5 L/kg). Differences in the quality of organic matter could account for these differences (Werth and Reinhard, 1997). However, higher retardation coefficient of TCE ($R = 11.7$) was observed in constructed wetland soil compared with natural wetland soil ($R = 8.2$) because the constructed wetland soil had a higher dry bulk density and lower porosity than the natural wetland soil (Table 6.2). Calculated retardation coefficients of TCE are in close agreement with those reported by Lorah et al. (1997) from “natural” Canal Creek wetland peat ($R$’s ranging from 6-10) from Aberdeen Proving Ground, MD.

- Degradation Kinetics in Wetland Soil Columns

Redox conditions

Lorah and Olsen (1999) report that the possible transformation route and degradation kinetics of the chlorinated solvents are mainly dependent on the prevailing redox conditions. In view of this, pore water samples at different depth in the soil columns were analyzed for indicators of terminal electron accepting processes including methane, sulfate, nitrate/nitrite, manganese, and ferrous iron. Except for methane, concentrations of other redox-sensitive constituents were found to be either the same as in the influent solution or below the detection limits of the analytical methods. Appreciable amounts of methane were detected in pore water samples thus suggesting that terminal electron accepting conditions in the wetland soils were largely
methanogenic. Higher levels of methane were observed in natural wetland mesocosms (2.25 - 95 mM) than in constructed mesocosms (0.2 - 2.1mM). In addition to this evidence, results from anaerobic microcosm studies (data not shown) indicated that the total concentration of organic acids and methane in natural wetland sediments were higher than in constructed wetland soil. There were however, no apparent spatial or temporal trends of the types and concentrations of organic acids in the soil columns (data not shown). Acetate, formate, lactate, butyrate and propionate were detected at varying concentrations with acetate accounting for more than 50% of the total amount of organic acids measured in all samples. It is worth noting that acetate is one of the last intermediate products before methane is produced in anaerobic metabolism (Ferry, 1993).

TCE attenuation in the soil mesocosms

Figure 6.3 illustrates the concentration profiles of TCE along the column distance for selected weeks. The upward decrease in concentration of TCE and the simultaneous increase in concentration of cis-1,2-DCE indicated that biodegradation was indeed occurring as the TCE solution was flowing through the wetland soil columns (Figure 6.3). cis-1,2-DCE was the only biodegradation product of TCE and was detected in the constructed and natural wetland mesocosms after three and six weeks, respectively after starting the experiments. These periods represent a lag period during which the indigenous bacterial populations were acclimatizing to TCE as a substrate. It has been well established that sometimes degradation follows a lag period, during which catabolically competent consortia are formed (Etienne et al., 2001). This implies that before biodegradation became significant, sorption was probably the main attenuation mechanism of TCE in the columns. After week 10, TCE disappeared within a distance of
7.5 cm from the inlet in the constructed wetland columns, however breakthroughs of *cis*-1,2-DCE at concentrations higher than its MCL (70 µg/L) were sometimes observed. In

**Figure 6.3**: The concentration profiles of TCE and *cis*-DCE as a function of column distance in natural and constructed wetland mesocosms. Data points represent means of results from analysis of triplicate samples.
natural wetland mesocosms, both cis-1,2-DCE and TCE were occasionally detected in effluent samples at concentrations higher than their MCL (Figure 6.3). Other isomers of DCE which can be produced from reductive dechlorination of TCE i.e., trans-1,2-DCE and 1,1-DCE were detected in trace amounts in agreement with previous studies (e.g., Freedman and Gossett, (1989) Lorah et al. (1997) and Isalou et al. (1998)). A decrease in cis-1,2-DCE concentrations above a height of 40 cm in natural wetland soil columns in all weeks and in the constructed wetland mesocosms in week 8 and week 21 (Figure 6.3) could have possibly been due to plant uptake or/and sorption since VC was not detected. Disappearance of cis-1,2-DCE beyond a distance of 15 cm in the constructed wetland columns after week 36 is attributed to biotransformation into VC following inoculation of slurry containing Dehalococcoides sp. organisms as will be discussed later.

Degradation of TCE did not go beyond cis-1,2-DCE in either soil columns during the first 34 weeks of operation. The reasons for accumulation of cis-1,2-DCE in reductive dechlorination are partially understood. Organisms that are capable of degrading TCE and PCE no further than cis-1,2 DCE are diverse and mixed and have been isolated from different sites (Wiedemeier et al., 1996; Maymó-Gatell et al., 2000). However, few organisms that are capable of dechlorinating PCE and TCE past cis-1,2 DCE have been identified and appear to be more nutritionally selective and slower growing than the above organisms (Maymó-Gatell et al., 2000). Therefore, accumulation of cis-1,2-DCE in the present study and at many sites contaminated with chlorinated ethene is suspected to be due to the prevalence of organisms that can reduce PCE or TCE as far as cis-1,2-DCE over organisms that can completely transform PCE and TCE into ethene (Wiedemeier et
al., 1999; Hendrickson et al., 2002; Major, et al., 2002). A recently discovered microbial species, *Dehalococcoides ethenogenes* strain 195 by Maymó-Gatell et al. (1997) is able to dechlorinate PCE and TCE completely to ethene, unlike other known microorganisms such as *Dehalobacter restrictus* (Fennell and Gossett, 1997), *Dehalospirillum multivorans* (Smatlak and Gossett, 1996), *Desulfomonile tiedje* (Ballapragada et al., 1997), and *Desulfitobacterium frapperi* (Maymó-Gatell et al., 2001) which cannot degrade PCE and TCE further than cis-1,2-DCE.

To stimulate degradation of TCE and cis-1,2-DCE, the soil columns were inoculated at a distance of 7.5 cm from the inlet with 30 mL of slurry taken from microcosms, which were previously observed to be able to completely degrade the chemicals. Based on previous dechlorination studies and molecular analysis using the PCR technique (see Chapter 3), the slurry used for inoculation of the soil columns was confirmed to contain *Dehalococcoides sp.*, bacteria capable of degrading TCE completely to ethene via cis-1,2-DCE and vinyl chloride (VC) (Maymó-Gatell et al., 1999; Hendrickson et al., 2002). Inoculation was done in week 34 after the column operations started. Seven days following inoculation, cis-1,2-DCE ceased to accumulate in the constructed mesocosms and VC was detected, which disappeared after a distance of 15 cm from the inlet and no chlorinated ethene was detected in the effluent samples thereafter. Ethene, an innocuous degradation daughter product of TCE was detected in composite samples collected from the constructed wetland columns implying that the observed disappearance of VC was indeed due to biodegradation reactions. On the other hand, biostimulation had less effect on transformation of TCE in the natural wetland mesocosms as compared with their counterparts. Although trace amounts of VC were
detected suggesting that cis-1,2-DCE was being transformed, breakthroughs of TCE and cis-1,2-DCE sometimes at concentrations higher than their MCLs were still observed in the natural wetland columns 12 months following biostimulation.

The influent concentration in the constructed wetland columns was increased initially from about 40 to 80 µM and then from 80 to 160 µM to investigate the ability of the dechlorinating cultures to degrade high concentrations of TCE. Surprisingly, the cultures were able to completely transform elevated concentrations of TCE to ethene within a distance of 15 cm (data not shown) from the inlet thus demonstrating their robustness and versatility.

Detection of *Dehalococcoides* 16S rDNA sequences

Soil samples collected from a distance of 7.5 cm from the inlet of the constructed and natural wetland mesocosms and soil slurry samples from non-inoculated microcosms were tested for the presence of *Dehalococcoides* 16S rDNA sequences. An example electropherogram for some of the samples analyzed is presented in Figure 6.4.

*Dehalococcoides* sp. like sequences were detected in all constructed wetland columns using the two sets of PCR primers specific for *Dehalococcoides* sp. whereas the DNA signatures of the organisms were detected in two out of three natural wetland mesocosms (Table 6.5). VC and trace amounts of ethene were detected in the natural wetland soil columns suspected to contain *Dehalococcoides* sp. suggesting that TCE degradation went past cis-1,2-DCE due to the presence of the organisms. Breakthrough of chloroethenes observed in the natural wetland soil columns following biostimulation may be due to the fact that *Dehalococcoides* sp. population may not have been numerically dominant within the microbial community.
Figure 6.4: Electropherogram showing the detection of *Dehalococcoides* 16S rDNA sequences in (A) Constructed wetland 1 using DE Primer set 1; (B) Constructed wetland 1 using DE Primer set 2 (C); Non-inoculated microcosm using Primer set 2.
Table 6.5: Detection of 16S rDNA sequences in constructed and natural wetland mesocosms and in non-inoculated microcosms

<table>
<thead>
<tr>
<th>Primer</th>
<th>Constructed</th>
<th>Natural</th>
<th>*Non-inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-1</td>
<td>C-2</td>
<td>C-3</td>
</tr>
<tr>
<td>DE Primer set 1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DE Primer set 2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Non-inoculated microcosms were prepared using constructed wetland soil as described above.

The fact that TCE was not dechlorinated past cis-1,2-DCE and that *Dehalococcoides* like 16S rDNA was not detected in non-inoculated constructed wetland soil slurry microcosms (Table 6.5) suggests that *Dehalococcoides* species may be directly responsible for the complete conversion of TCE to ethene in the constructed wetland soil columns. Other bacterial groups may have played an indirect role in dechlorination process such as producing important biochemicals and substrates utilized by *Dehalococcoides* species.

The 1377 base pair fragments of the constructed soil column 3 (C3) was cloned, sequenced, and compared to databases of previously reported microorganisms. Differences between the sequences of the cloned 1377 bp fragment of 16S rDNA determined in the present study and that of *Dehalococcoides ethenogenes* strain 195 and the three closest NCBI matches for uncultured microorganisms are shown in Table 6.6. The 1377 base pair fragment was 99.75% similar, by pair wise comparison, to the three uncultured *Dehalococcoides* entries in the database: AF529119 (uncultured *Dehalococcoides* sp. Clone FTLM182), AF357918 (*Dehalococcoides* sp. FL2) and AF388546 (uncultured *Dehalococcoides* sp. Clone DHC-nfix). The cloned 1377 base pair fragment of the constructed soil column 3 (C3) was found to be 98.2 and 99.7% similar to
Table 6.6: 16S rDNA sequence differences from 1377 bp partial 16S rDNA sequences determined in this study relative to *Dehalococcoides ethenogenes* Strain 195 and the closest sequence matches reported in the NCBI database.

<table>
<thead>
<tr>
<th>Strain 195 base number</th>
<th>Strain 195 AF004928</th>
<th>AF529119, AF357918, AF388546</th>
<th>C3 AFxxxxxx</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td>A</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>144</td>
<td>A</td>
<td>G</td>
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<td>148</td>
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<td>T</td>
<td>T</td>
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<tr>
<td>167</td>
<td>T</td>
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*a* Base number: base position in the 16S rDNA sequence for *Dehalococcoides ethenogenes* Strain 195. Positions not listed were identical to Strain 195 in the other three sequences. “+”: insertion in sequence relative to strain 195 (after specified base no.). “-”: deletion in sequence relative to other sequences. Bold: indicates difference between strain 195 and that detected in cloned DNA sequences reported by Major et al. (2002).
Dehalococcoides ethenogenes Strain 195 and strain CBDB1, respectively. Percent similarities were calculated after putting in gaps in the sequences. The results of the PCR-based detection technique, thus, indicate that microorganisms closely related to Dehalococcoides ethenogenes were indeed present in all constructed wetland columns and two natural wetland mesocosms (Table 6.5).

Kinetic data and molecular analysis results thus suggest that constructed wetland soil was able to support the growth of the inoculated dechlorinating organisms better than natural wetland sediments. Reasons for the discrepancy were not clear. One hypothesis is that the dechlorinating bacteria could not compete for the limited micronutrients with the well-established diverse microbial populations developed over a long period of time (speculated to consist mostly of methanogens) in natural wetland sediments. It should be noted that constructed wetland soil was prepared from dry ingredients, which perhaps initially had lower microbial diversity and populations. It is also possible that constructed wetland soil contained some unidentified essential micronutrients for the growth of the dechlorinating organisms in contrast with natural wetland sediments.

**Role of plants**

An excellent review of the role of plants in remediation of contaminated sites and mechanisms involved in removal of contaminants including TCE from soil by plants is provided in Susarla et al. (2002). TCE and probably its degradation daughter products also may be removed from contaminated soil to a varying extent via phytotransformation, rhizodegradation, phytoextraction, phytovolatilization, phytopumping and rhizofiltration according to the review. Plant characteristics is one of the main factors on which the effectiveness of these phytoremediation mechanisms of TCE removal is dependent on
(Susarla et al., 2002). A number of aquatic plants (e.g., waterweed, parrot feathers and giant duckweed) have been reported to be capable of taking-up and then transforming TCE and PCE into a number of compounds (Schnoor et al., 1995; Newman et al., 1997; Susarla et al., 2002). No study has been reported on removal of TCE by the plant species used in the current study (*Scirpus sp.*). Although no attempt was done to investigate the role of plants in the removal of TCE in the present work however, the possibility that plants were responsible for attenuation of TCE and degradation daughter products albeit at a relatively lower magnitude compared with degradation and sorption cannot be completely ruled-out.

- **Modeling of TCE Removal in the Soil Columns**

  Times required for steady-state conditions to be reached at the middle and at the top of the column were determined for both mesocosms using Eq. 6.11. Transport parameters in Table 6.2 and removal rate constants of TCE determined using the first-order kinetic model were used as inputs to Eq. 6.11. Required time to attain steady-state conditions at the middle (30 cm from the inlet) and at the top of the column (60 cm from the inlet) for both mesocosms was about 80 days (Figure 6.5) which is a significantly short time relative to the anticipated service lifetime of the constructed wetland systems. Therefore, it was found reasonable to use steady-state equations (Eq. 6.9 and Eq. 6.13) to model the TCE data and to determine the wetland bed depth instead of Eq. 6.11, which assumes transient-state conditions and is more complicated to implement.

  It should be noted that Eq. 6.9 ignores dispersion while Eq. 6.13 incorporates the dispersion term. The purpose of using these two models was to investigate whether it is
important to consider dispersion in determining the effective wetland bed depth for removal of target contaminants in designing a constructed treatment wetland. Removal rate constants of TCE in the soil columns were estimated by fitting the mass transport models to monitoring data using a nonlinear regression package Sigmaplot (Version 6, Jandel Scientific, San Rafael, CA) with all other transport parameters fixed. Both models fitted the TCE monitoring data equally well as indicated by exactly the same values of

Figure 6.5: Time required for the TCE plume to attain steady-state conditions at the middle (30 cm from inlet) and at the top (60 cm from inlet) of the column.
goodness-of-fit (Figure 6.6). Figure 6.7 shows temporal trends of degradation rate constants of TCE for the wetland soils. The graph begins after 8 weeks, which is the time when the contaminant front should have reached the top of the core if only sorption was occurring. Removal rate constant of constructed wetland mesocosms increased steadily over time reaching values of $1.28 \pm 0.03$ day$^{-1}$ (using Eq. 6.9) and $2.41 \pm 0.11$ day$^{-1}$

**Figure 6.6**: Degradation of TCE measured in constructed wetland soil columns for week 15. Symbols denote means of results from analysis of triplicate mesocosms (error bars represent one standard deviation), and lines represent fitted mass transport equations. *Note*: The fitted curves of the two equations have coincided.
(using Eq. 6.13) in week 20 after which it leveled off. Removal rate constants in natural wetland columns were significantly lower \((P > 0.05)\) than those in constructed wetland columns for all weeks (Figure 6.7) and remained almost unchanged with time \((0.08 \pm 0.02 \text{ day}^{-1} \text{ based on Eq. 6.9 and } 0.16 \pm 0.06 \text{ day}^{-1} \text{ based on Eq. 6.13}).

**Figure 6.7**: Temporal trends of degradation rate constants of TCE in constructed and natural wetland soil columns. Data points represent means of removal rate constants calculated from analytical results of triplicate mesocosms and error bars represent one standard deviation.
Neglecting dispersion underestimated the removal rate constants of TCE in both soil columns as Figure 6.7 illustrates. Removal rate constants estimated by the model with the dispersion term were two times higher compared with those obtained from the model that ignores dispersion. Thomsen et al. (1999) report that values of the Peclet number, \((P_L) > 5\) generally imply that solute dispersion is minimal in relation to solute transport. The \(P_L\) values for both systems were less than 5 (Table 6.2) indicating that dispersion was probably significant and should therefore not be neglected. Solute dispersivity values were calculated and found to be 8 cm for constructed wetland soils and 10.5 cm for natural wetland soils. Dispersivity values higher than 1 cm suggest a high degree of solute dispersion (Thomsen et al., 1999) in agreement with the measured elongated bromide breakthrough curves (Figure 6.2). Significantly different \((P > 0.05)\) removal rate constants calculated using models with and without dispersion terms indicate that dispersion was probably an important transport mechanism (Figure 6.7).

Values of Dammköhler number for the natural wetland columns varied from 0.4 to 1.3, whereas those for the constructed wetland mesocosms ranged between 1.2 and 19.8. These results signify that the relative importance of contaminant advection over degradation was more pronounced in the natural wetland mesocosms than in the constructed wetland soil columns. An observed temporal increase of Dammköhler number in the constructed wetland mesocosms suggests that as microbial populations became acclimatized to TCE, advection became less important with time as compared to biodegradation reactions. In contrast to the observations made in the constructed wetland mesocosms, Dammköhler numbers in natural wetland columns were nearly constant
(0.75 ± 0.18) after the 12th week indicating that the relative importance of degradation to advection was independent of the time of exposure of the microbial populations to TCE.

Simulation runs to establish the relative importance of biodegradation and sorption in attenuation of TCE were performed using Eq. 6.13. The transport parameters (Table 6.2), sorption data and biodegradation rate constants were used as inputs. During the simulation runs it was assumed that apart from dispersion, sorption and biodegradation, other attenuation processes such as plant uptake, advection and volatilization were insignificant. Simulation results showed that degradation was the most important removal process in the constructed wetland soil columns, whereas in natural wetland mesocosms both degradation and sorption were comparably effective attenuation mechanisms of TCE (Figure 6.8). The combined attenuation effect of sorption and degradation was more pronounced in the natural wetland mesocosms than in the constructed wetland soil columns (Figure 6.8).

Apart from the dissimilarity in organic carbon content and contaminant residence times, they were no other apparent reasons for the differences in TCE degradation kinetics observed in the soil columns. Organic matter content may to some extent account for a remarkable difference in the TCE degradation rates since constructed wetland soil had higher (29%) organic carbon content than its counterpart (23%). An abundant supply of organic carbon is necessary for providing sufficient electron donors required for complete reductive biodegradation of chlorinated solvents (Lorah and Olsen, 1999; Wiedemeier et al., 1999). Lower biodegradation rates in the aquifer sediments have been associated with lower microbial activity due to low organic carbon content (Klečka et al., 1990). Longer contaminant residence time (156 days) in the constructed wetland
columns than in the natural wetland mesocosms (78 days) due to higher sorptive capacity of the synthetic peat/compost/sand mixture compared with the natural wetland soil may partly account for the significant differences in TCE degradation kinetics in the soil columns. Organic acids and hydrogen data did not shed much light on the differences in the kinetics of degradation of TCE between the two types of soils.

![Graph showing Trichloroethene concentration](image)

**Figure 6.8:** Simulation results showing the relative importance of biodegradation and sorption.

- **Anaerobic Microcosms**

  Analysis of dissolved gaseous constituents (ethene, ethane, methane, and hydrogen) and oxidation-reduction potential, required withdrawals of large volumes (> 25 mL) of porewater, which would have substantially changed the spatial distribution of the VOCs in the soil columns. Due to this sample volume limitation, anaerobic
microcosm studies were conducted to confirm evidence of biodegradation processes observed in the soil columns, to examine transformation patterns of TCE, and to determine hydrogen concentrations associated with TCE dechlorination.

Transformation of TCE in constructed soil microcosms started 4 weeks after incubation as supported by the production of cis-1,2-DCE and the decrease in concentration of the parent compound. More than 95% of TCE was recovered in abiotic control microcosms indicating that disappearance of the chemical in active constructed soil microcosms was indeed due to microbial processes and not abiotic losses. By week 6, TCE was completely dechlorinated to cis-1,2-DCE however biodegradation did not go further than cis-1,2-DCE similar to observations made in the soil columns. These results illustrate that biodegradation pattern of TCE in constructed wetland column experiments was in agreement with biodegradation in batch experiments. On the other hand, concentrations of TCE in natural wetland soil in both live and killed microcosms remained almost unchanged and no degradation products were detected up to 90 days (data not shown) after incubation signifying that TCE could not be transformed in the natural wetland soils for unknown reasons.

To stimulate degradation of TCE and cis-1,2-DCE, the microcosms were inoculated with 5 mL of slurry collected from microcosms containing Dehalococcoides sp., organisms capable of transforming TCE completely to ethene (see Chapter 3). Four days after biostimulation, cis-1,2-DCE started to disappear, vinyl chloride crested and then declined as ethene accumulated in active constructed wetland soil microcosms. On the other hand, trace amounts of cis-1,2-DCE were observed in natural wetland microcosms about 2 weeks after inoculating dechlorinating organisms. However, there
was no significant decrease in TCE concentrations as supported by low and nearly constant concentrations of cis-1,2-DCE (data not shown) even after a prolonged incubation period (> 6 months).

Fresh constructed wetland soil microcosms were prepared as described above, inoculated with slurry containing dechlorinating organisms, kept in the darkness at 25 °C for two weeks to allow the systems to reach quasi steady-state conditions with respect to microbial populations and spiked with TCE solution to a final concentration of about 40 µM. Temporal monitoring of concentrations of TCE and daughter products, hydrogen and methane was done until the concentrations of the contaminants dropped below the detection limits of the analytical methods. Transformation of TCE started without a noticeable lag period indicating that organisms were well acclimated with the chemical (Figure 6.9). The sequence of the appearance of intermediates during TCE dechlorination to ethene (Figure 6.9) was consistent with a pathway that includes cis-1,2-DCE and VC. It is worthy noting that cis-1,2-DCE and VC were concomitantly dechlorinated with no accumulation of the latter compound (Figure 6.9) in contrast with the findings of other studies (e.g., Freedman and Gossett, 1989; Tandol et al., 1994). VC is a far more dangerous contaminant compared to the parent compound (Wiedemeier et al., 1999). Like in the constructed wetland soil columns, the first principal degradation product was cis-1,2-DCE, accounting for 99% of the transformed TCE, while both trans-1,2-DCE and 1,1-DCE accounted for less than 1%. The degradation rate constant of TCE in constructed wetland soil microcosms in the current study (0.56 ± 0.06 day⁻¹) is higher than those reported by Lorah et al. (1997) (between 0.1 and 0.3 day⁻¹) for wetland sediments at Aberdeen Proving Ground.
It can be observed from Figure 6.9 that aqueous H₂ concentration level decreased drastically from about 290 nM to approximately 7 nM in just 1 day after TCE was spiked into the microcosms and stayed nearly constant at 6.6 ± 2.1 nM during the dechlorination period. After TCE was completely transformed into ethene, H₂ concentration increased gradually to levels comparable to the initial concentrations (Figure 6.9). These observations indicate that reductive dechlorination of TCE in the microcosms was driven by H₂ as an electron donor in agreement with the findings of previous studies (Freedman and Gossett, 1989; Yang and McCarty, 1998).

To investigate the role of methanogens in dechlorination of TCE, an inhibitor of methanogenesis (BES) was used. The inhibitory mechanism of BES on methanogenesis has been detailed elsewhere (Löffler et al., 1997). cis-1,2-DCE initially accumulated until it reached a concentration approximately equal to the stoichiometric conversion of TCE (Figure 6.10). Eventually, VC and ethene became the principal degradation products. The 3 day delay in cis-1,2-DCE transformation was probably caused by partial and transient inhibition of dechlorinating organisms by BES. At the end of the incubation period, ethene was detected at concentrations higher than that expected from the stoichiometric conversion of TCE, thus suggesting that part of the ethene was likely produced from degradation of BES by methanogens as also observed by Holliger et al. (1990). Hydrogen threshold during active TCE dechlorination in the presence of BES (20.48 ± 5.30 nM) was significantly (P < 0.05) higher compared with that observed in microcosms without BES (6.60 ± 2.1 nM). Higher hydrogen threshold in the presence of BES is probably because elimination of methanogenesis by the inhibitor directly affected consumption and production of substrates for methanogenesis (Conrad and Klose, 2000; Chiu and Lee,
Figure 6.9: Concentrations of TCE, cis-1,2-DCE, VC, ethene, and hydrogen versus time. Data points represent experimentally measured values and bars indicate the range of results for individual serum bottles.
2001) notably methane and H₂ in the current work. In the present study, BES did not completely inhibit but rather it reduced the degradation rate constant of TCE from 0.56 ± 0.06 day⁻¹ in the absence of BES to 0.49 ± 0.08 day⁻¹ in the presence of the inhibitor. These observations are in agreement with the findings of Chiu and Lee (2001) but in contrast with observations made by Löffler et al. (1997) who found that dechlorination of TCE was inhibited in the absence of methanogens thus questioning the reliability of using BES to attribute dechlorination activities to methanogens. However, Freedman and Gossett (1989) observed inhibition of dechlorination of TCE and PCE by BES thus concluding that methanogens played a key role in the biotransformations of the chemicals. The fact that degradation of TCE did not completely stop in the presence of BES provides indirect evidence that methanogens probably did not play a direct role in the biotransformation reaction of TCE in the present work. However, the mechanisms of inhibition of dechlorination by BES are yet to be understood (Löffler et al., 1997; Chiu and Lee, 2001).

- **Comparison of Batch and Column Study Results**

Kinetic data from the batch studies (\( R = 10.38 \) and \( \lambda = 0.56 \) day⁻¹) was used in the transport equation (Eq. 6.13) along with the transport parameters (Table 6.2) to compare results from batch and column studies. Kinetic data for the batch study was calculated using the transport equation (Eq. 6.13) and the batch reaction rate constant and retardation factor as inputs. As Figure 6.11 shows, the simulated batch study kinetic data and column study experimental data compare reasonably well as supported by the value of goodness-of-fit. When the transport equation was fit to the column study experimental data using only the retardation coefficient, \( R \) (Table 6.7), the value of \( R \) was very close to
Figure 6.10: Temporal trends of transformation of TCE in the presence of BES and associated hydrogen concentrations. Data points represent experimentally measured values and bars indicate the range of results for individual serum bottles.
Figure 6.11: Comparison of simulated batch study TCE kinetic data and column study experimental data.

that obtained from the batch sorption study. Similarly, when the transport equation was fit to the experimental data using the removal rate constant, the calculated value was very close to that obtained from the batch experiment (Table 6.7).

When the reaction rate constant and retardation factor were simultaneously calculated by fitting the transport model to the experimental data from the column study, the model results fit the experimental data reasonably well. However, the value of reaction rate constant obtained was lower by a factor of about 3 than that determined
from the batch study whereas the calculated retardation factor was 3 times higher than the batch value (Table 6.7). These results suggest that the model was less sensitive to reaction rate constant and retardation factor probably because the two parameters occur as a ratio in the part of the equation (Eq. 6.13) that is important for the system, while the other parts of the equation are not significantly contributing to the solution.

Table 6.7: Comparison of column and batch studies transport parameters

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<th>Parameter of interest</th>
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<th>Model calculated value</th>
<th>Experimentally determined value</th>
<th>Goodness-of-fit ($r^2$)</th>
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<td>Retardation factor, $R$</td>
<td>$C_o = 38 \mu M$ $D_x = 31.5 \text{ cm}^2/\text{day}$ $\lambda = 0.56 \text{ day}^{-1}$ (batch)</td>
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<td>10.38 (batch)</td>
<td>0.9956</td>
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<td>Removal rate constant, $\lambda$</td>
<td>$C_o = 38 \mu M$ $D_x = 31.5 \text{ cm}^2/\text{day}$ $R = 10.38$ (batch)</td>
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<td>0.56 day$^{-1}$ (batch)</td>
<td>0.9956</td>
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<tr>
<td>Retardation factor, $R$ and removal rate constant, $\lambda$</td>
<td>$C_o = 38 \mu M$ $D_x = 31.5 \text{ cm}^2/\text{day}$ $R = 31.56$ (column) $\lambda = 0.18 \text{ day}^{-1}$ (column)</td>
<td>$R = 10.38$ (batch) $\lambda = 0.56 \text{ day}^{-1}$ (batch)</td>
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Note: $D_x$ was determined from column studies

6.5 Conclusion and Significance of the Results

Complete reductive dechlorination of TCE in the constructed wetland columns was sustained for over 18 months without any amendment in contrast with other studies (e.g., Freedman and Gossett, 1989; Tandol et al., 1994; Isalou et al., 1998) in which exogenous electron donors and nutritional supplements were used to maintain degradation of TCE and PCE. Freedman and Gossett (1989) reported that supplying of electron donor is a major operational cost of anaerobic remediation. In view of this, the constructed wetland soil used in the current work may potentially be a good candidate for construction of treatment wetlands and biobarriers for remediation of aquifers.
contaminated with chlorinated ethenes. From the field feasibility study, Kao and Liu (2000) found that peat biobarriers have the potential to become an environmentally and economically acceptable technology for the bioremediation of groundwater contaminated with chlorinated solvents. However, studies and field experiences on remediation of groundwater contaminated with chlorinated solvents using vertical flow constructed peat wetlands have so far not been reported in literature.

Although, the constructed wetland soil was able to sustain reductive dechlorination for over 18 months without exogenous electron donor and nutritional supplements it is worthwhile to investigate how long can degradation of TCE be maintained before endogenous electron donors and carbon sources are depleted. However, it is expected that leaching/dissolution of carbon into soil from decomposition of detrital plant materials on the top surface of the constructed wetland (Pardue, 1992) will replenish organic carbon spent during the biotransformation reactions of chlorinated solvents thus sustaining the reductive dechlorination processes almost indefinitely.

Chloroethenes monitoring results have shown that microorganisms tend to accumulate in the first 7.5 cm of the columns and rapidly transform the contaminants indicating that the potential for clogging of the media due to biofilm growth is higher near the entrance of the column compared to other column portions. However, no attempt was made to determine the reduction of porosity of the soil in the column due to biofilm growth in the current study. Isalou et al., (1998) observed 89% reduction in porosity for the bottom section (from 0 to 15 cm) of the sand columns used for PCE degradation over a period of 2.5 years due to biofilm growth. Although there were no apparent evidences of clogging of soil due to excessive biofilm in the present study, however, it is
recommended to investigate the reduction of porosity with time, as it appears to be a critical consideration in designing treatment wetlands for chlorinated solvents. Visual inspection revealed that plant roots had covered the entire depth of the constructed wetland soil columns by week 15. Although plant roots have been reported to cause preferential flow, which may in turn cause rapid movement of solutes and consequently failure of treatment wetlands, there were no apparent evidence of the effects of this phenomenon in the constructed wetland mesocosms as VOCs monitoring data showed.

The fact that TCE could not be transformed beyond cis-1,2-DCE indicated that for degradation to go to completion, the right type of organisms must be present among other factors. PCR detection of the 16S rDNA gene of *Dehalococcoides ethenogenes* was directly correlated with dechlorination of TCE beyond cis-1,2-DCE to ethene in the constructed wetland soil mesocosms as opposed to non-inoculated soils, indicating the success of the inoculation strategy in the constructed columns. Results of the present study have therefore indicated that complete degradation of chlorinated ethenes may be achieved by seeding constructed treatment wetlands with *Dehalococcoides sp.* during the system start-up.

Removal rate constants estimated using the transport equation with the dispersion term were found to be significantly higher than those calculated with the equation which ignored dispersion. Therefore, using the transport equation that ignores dispersion in designing a treatment wetland will result in a conservative wetland depth, which may perhaps be uneconomical.

Treatment peat wetlands may potentially be more economic and less expensive option for long-term treatment of groundwater contaminated with chlorinated ethenes as
the findings of the present work have suggested. The results of the present study may provide future design parameters for constructed wetlands for remediation of groundwater contaminated with chlorinated solvents.
CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

Studies were conducted to determine and explore the fundamental processes responsible for the degradation of selected chlorinated ethenes and ethanes within the natural wetland sediment and soil substrate mixtures for construction of an upflow treatment wetland. Hydraulic and geotechnical characteristics of the wetland soils and degradation kinetics of selected chlorinated organics under methanogenic, sulfite- and sulfate-reducing conditions were also determined. The associated hydrogen concentrations and bioenergetics of the terminal electron accepting processes were investigated to understand better the factors that affect degradability of these contaminants in different sedimentary environments.

Two soil mixtures, one a mixture of sand and peat, the other a mixture of sand, peat and Bion Soil, a product derived from agricultural wastes, were selected from ten possible mixtures for construction of an upflow treatment wetland for chlorinated volatile organic compounds (CVOCs) at a Superfund site based on the results of hydraulic and geotechnical testing. On the basis of the results obtained from sorption and biodegradation experiments, the mixture of sand, peat and Bion Soil was found to be superior to the mixture comprised of sand and peat only. The addition of the compost material (Bion soil) stimulated the degradation and enhanced sorptive capacity for chlorinated organics. These results indicate that organic carbon composition is an important factor in selecting bed materials for treatment wetlands for chlorinated organics. Since peat materials are rich in organic carbon and also supply nutrients necessary for microbial growth, they are the most promising candidates for construction of treatment wetlands. The selected mixture (sand, peat and Bion Soil) was further
subjected to kinetic studies to explore its potential for supporting degradation of chlorinated volatile organics under various reducing conditions.

Sequential reductive dechlorination was observed to be the main mechanism of degradation of cis-1,2-DCE whereas 1,2-DCA was predominantly transformed via dihaloelimination and consecutive hydrogenolysis mechanisms. Based on the results of kinetic studies, anaerobic reductive dechlorination was observed to be an important transformation process for cis-1,2-DCE. In contrast, both aerobic and anaerobic degradation reactions were found to be comparably effective in attenuation of 1,2-DCA in the wetland soils. Detection of the 16S rDNA gene of Dehalococcoides sp. via DNA extraction, PCR amplification, cloning and sequencing suggested that the organisms played a role in the complete dechlorination of cis-1,2-DCE and TCE to innocuous product, ethene. Hydrogen and methane monitoring data suggested that dechlorination reactions of chlorinated ethenes (TCE and cis-1,2-DCE) and ethane (1,2-DCA) were possibly mediated by different organisms.

Results of studies on hydrogen concentrations associated with dechlorinating reactions have suggested that dechlorinating organisms are capable of lowering H₂ concentrations to their physiological thresholds independent of the initial H₂ concentration and probably of the rate of its production. Therefore, the use of H₂ precursors that degrade slowly to yield low concentrations of H₂ supposedly to give a competitive advantage to dechlorinating organisms over methanogens as suggested in previous studies may be counterproductive in certain circumstances since it may limit the amount of reducing equivalents necessary for complete degradation of the target compounds.
Degradation kinetics of *cis*-1,2-DCE and DCA were observed to be fastest under methanogenic conditions followed by sulfate- and sulfite-reducing conditions. *cis*-1,2-DCE and DCA could not be concurrently degraded under methanogenic conditions. Transformation of the later compound started after the former compound and its first degradation daughter product (VC) were exhausted. In view of this, among other important factors, the design of constructed wetlands should strictly take into consideration the reducing conditions of the contaminant plume to be remediated and the most difficult to degrade contaminant should dictate the design of the wetland system.

These observations underscore the importance of conducting bench-scale and pilot studies before embarking on a full-scale treatment wetland system because each contaminated site is unique in terms of biogeochemical and hydrological characteristics and many times requires a unique solution.

Constructed wetland soil had a higher potential for attenuation of TCE compared with natural wetland sediments tested in the experiments described in this dissertation. The fate and transport of chlorinated solvents in the constructed wetland mesocosms were affected by microbial transformation to a greater degree than was true of any other attenuation process as exhibited by experimental data and modeling results. On the other hand, both degradation and sorption were observed to be comparably effective attenuation processes of TCE in natural wetland mesocosms. Ignoring dispersion in modeling of fate and transport of contaminants in wetland soils may underestimate the removal rate constants and consequently result in a very conservative effective wetland depth, which perhaps may be uneconomical.
The fact that TCE was breaking through at concentrations higher than its MCL suggests that the natural wetland soil had a limited assimilative capacity for the contaminant. Therefore, laboratory column studies may be useful as a first step in evaluating the inherent potential of natural wetlands for attenuation of chlorinated organic compounds.

The observation that TCE could not be transformed beyond \textit{cis}-1,2-DCE in some microcosms and mesocosms indicate that for degradation to go to completion, the right types of organisms must be present among other factors. PCR based detection of the 16S rDNA gene of \textit{Dehalococcoides sp.} was directly correlated with the complete dechlorination of TCE to ethene in the constructed wetland soil mesocosms as opposed to non-inoculated soils, indicating the success of the inoculation strategy.

In this work, sorption of the test chemicals on sediments was assumed to be linear and irreversible and to reach equilibrium instantaneously. The assumption of linear sorption may have been valid based on isotherm data obtained. However, the validity of the assumptions of irreversibility of sorption and “instantaneous equilibrium” was not verified. For sediments in which biotransformation is a significant attenuation process, the reversible fraction of the contaminant will quickly be taken care of by biodegradation after being reintroduced into the aqueous phase. Kinetic data modeling efforts in this study assumed that degradation occurs both within the liquid and sorbed phases at about the same rate, but this assumption needs to be validated by conducting bioavailability studies of sorbed fraction of the test chemicals in the wetland soils.

Future research should focus on quantifying uptake and metabolism of chlorinated solvents in wetland plants. Efficiencies of these processes perhaps determine the degree
of phytoaccumulation and phytoconcentration, which in turn can increase the potential for wetland animals and human exposures.

Chloroethenes monitoring results from mesocosm studies suggest that microorganisms accumulated in the first 7.5 cm of the columns and rapidly transformed the target contaminant, thus posing a potential problem of clogging of the media by biofilm. Although there was no apparent evidence of clogging of soil due to excessive biofilm, it is, however, recommended to investigate the reduction of porosity over time, as it appears to be a critical consideration in designing a treatment wetland for chlorinated solvents based on previous studies.

Studies reported in this dissertation were conducted under controlled climatic conditions. As such, the actual field degradation rates and the seasonal and spatial variability of degradation processes due primarily to weather changes (especially temperature and precipitation) are unknown. These unknowns can be answered by conducting field pilot studies, because the work described in this dissertation was not meant to replicate a well-developed treatment wetland and did not mimic actual field conditions reasonably well.

Results of the present study suggest that although there are many viable remediation technologies and each contaminated site is unique, the use of a constructed wetland to treat water contaminated with CVOCs may perhaps be a less costly and beneficial option. Findings from this study have shed some light on important considerations in designing constructed treatment wetlands for chlorinated organics.
REFERENCES


APPENDIX A: PROTOCOL FOR REMOVING HUMIC ACIDS FROM SOIL SLURRY SAMPLES USING POLYVINYL PYRROLIDONE (PVP) TREATMENT

1. The bead solution provided by MoBio was emptied from the bead solution tube into a clean microcentrifuge tube. 250 to 400 µL of soil slurry was placed in the bead solution tubes.

2. Approximately 1.5 mL of 1X TE buffer was added to the soil slurry.

3. 0.02 g PVP was added to the soil slurry in TE buffer. It was then vortexed, at maximum speed for 5 seconds.

4. The sample was centrifuged at 5000 rpm for 10 minutes.

5. The supernatant was removed.

6. The bead solution was placed back into the bead solution tube containing the soil pellet and gently vortexed.
APPENDIX B: DNA EXTRACTION FROM SOIL SLURRY SAMPLES USING
THE MOBIO ULTRACLEAN SOIL DNA ISOLATION KIT

1.  60-µL of solution S1 was added to the MicroBead tube and vortexed briefly.
2.  200-µL of solution IRS (Inhibitor Removal Solution) was added.
3.  The MicroBead tube was secured in a Biospec Mini-Beadbeater 3110BX. The
    sample was bead-beaten at 4,800 rpm for 180 seconds.
4.  The MicroBead tube was centrifuged at 11,000 rpm for 30 seconds.
5.  The supernatant was transferred to a clean microcentrifuge tube.
6.  250-µL of solution S2 was added to the supernatant and vortexed for 5 seconds. The
    sample was then placed in a 0 - 4°C refrigerator for 5 minutes.
7.  The tube was centrifuged for 1 minute at 11,000 rpm.
8.  450-µL of supernatant was transferred to a clean 1.9 mL tube, being careful to avoid
    the pellet.
9.  900-µL of solution S3 was added to the supernatant and vortexed for 5 seconds.
10. 700-µL was loaded onto a spin filter and centrifuged at 11,000 rpm for 30 seconds.
     The flow through was discarded. The remaining supernatant was added to the spin
     filter and the centrifuging was repeated. Again, the flow through was discarded.
11. 300-µL of solution S4 was added. The sample was then centrifuged for 30 seconds at
     11,000 rpm. The flow through was discarded. This step was repeated for a total of
     three washes with S4 solution.
12. The sample was again centrifuged at 11,000 rpm for 1 minute.
13. The spin filter was carefully transferred to a new 1.9 mL tube, being careful to avoid
    any splashing.
14. 50-μL of solution S5 was added to the center of the white filter membrane.

15. The sample was centrifuged for 30 seconds at 11,000 rpm.

16. The spin filter was discarded. The extracted DNA was kept frozen at -20 °C until further use.
APPENDIX C: PROTOCOL FOR CLONING

1. The TOPO cloning reaction was initiated by combining and gently mixing 3 µL of PCR product, 1 µL of salt solution (from kit), and 1 µL of TOPO cloning vector (from the kit).

2. The reaction was incubated at room temperature for 5 minutes then placed on ice.

3. 2 µL of the TOPO cloning reaction was added to a vial of chemically competent cells (from the kit).

4. The cells were incubated on ice for 15 minutes.

5. The cells were shocked with heat for 30 seconds in a 42°C water bath, then immediately transferred to ice.

6. 250 µL of SOC medium (from the kit) was added to the cells.

7. The cells were shook for 1 hour in a horizontal shaker, set at 200 rpm, 37°C.

8. 0.05 mg/mL Kanomycin plates were warmed in the shaker for the last 15 minutes of the hour.

9. 150 µL, 50 µL and the remainder of the cells were dispensed and spread on the Kanomycin plates.

10. The plates were incubated overnight at 37°C.

11. Single colonies were picked off the plates with sterilized pipet tips and spread just above the water line of a PCR tube with 25 µL of water.

12. The tubes were vortexed well.

13. The cells were boiled for 10 minutes at 98°C, then immediately placed on ice.
14. The cells were centrifuged for 1 minute at 13,000 rpm immediately prior to the cloning PCR reaction. PCR amplification of the clones was performed using a 50 µL reaction mix with 2.5 U AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA), 1X GeneAmp PCR buffer with 1.5 mM MgCl₂ (Applied Biosystems, Foster City, CA), 0.02 µM of each deoxynuclease triphosphate (Roche, Indianapolis, IN), 5 ng of primers M13f and M13r (Alpha DNA, Quebec, Canada) and 0.5 µL of the boiled cells. The sequences for primer M13f and M13r are GTAAACGACGGCCAGT and GGAAACAGCTATGACCATG, respectively.

15. The cloning PCR was performed on the BioRad iCycler PCR thermal cycler with the following temperature profile: 94°C for 3 minutes, 25 cycles of 52°C for 30 seconds, 72°C for 1 minute, 94°C for 30 seconds, a final extension of 72°C for 7 minutes and hold at 12°C.

16. The PCR products were cleaned up using the UltraClean PCR Clean Up Kit (MoBio, Carlsbad, CA) following the manufacturer’s instructions.
APPENDIX D: PROTOCOL FOR SEQUENCING REACTION

1. The sequencing reaction mix was made by combining 4 µL of Sequencing Reagent (from kit), 2.5 µL of 0.5 µg/µl primer (primer 1f or 774f), 3 µL 18-Mega Ohm water and 1 µL of the cloning PCR product.

2. The reaction was run on a Perkin Elmer GeneAmp 2400 PCR thermal cycler (Toronto, Canada) using the following temperature profile: 94°C for 30 seconds, 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, 60°C for 4 minutes, then hold at 12°C.

3. The sequencing reaction products were precipitated using ethanol and sodium acetate. They were resuspended in 25 µL of HiDi Formamide (Applied Biosystems, Foster City, CA).

4. The samples were sequenced by the ABI Prism 3100 16-Capillary Automated Sequencer (Applied Biosystems, Foster City, CA).

5. The sequences were edited and aligned using the Bioedit Sequence Alignment Editor software package.

6. The BLAST software at the Genbank website (Genbank, National Center for Biotechnical Information; www.ncbi.nlm.nih.gov) was used to determine each fragment’s phylogenetic position and the most closely related organisms based on sequence comparison.
APPENDIX E: LETTER OF PERMISSION


Dr. Gabriel R Kassenga
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Dear Dr Kassenga

ECOLOGICAL ENGINEERING, Kassenga et al, "Treatment of chlorinated volatile organic compounds in upflow wetland mesocosms", (ECOENG 727, accepted for publication)

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Gabriel Roderick Kassenga, a son of Roderick Ulisubisya Kibona and Lena Keenan Kyejo was born on September 9, 1961, in Rungwe, Mbeya, Tanzania. He graduated from Rwegerulila Water Resources Institute, Dar es Salaam in Tanzania with a Full Technician Certificate in Water Resources Engineering in May 1982. Four years later, he graduated from the then Ardhi Institute in Dar es Salaam, Tanzania (now the University College of Lands and Architectural Studies (UCLAS)), in April 1986 with an Advanced Diploma in Public Health Engineering. In 1993, he obtained a Master of Science Degree in Renewable Energy from Carl Von Ossietzky University of Oldenburg, Germany under the Germany Academic Exchange Program (DAAD). Mr. Kassenga is employed by UCLAS (a constituent college of the University of Dar es Salaam, Tanzania) as a senior lecturer in the Department of Environmental Engineering since July 1996. In fall 1998 he enrolled in the doctoral program in the Department of Civil and Environmental Engineering at Louisiana State University and Agricultural and Mechanical College under the Fulbright Junior Staff Scholarship Program. His doctoral program emphasized in environmental engineering. After completing his doctoral program he intends to continue teaching, researching and offering consulting services in the environmental engineering field.