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Optimization of treatment of chlorinated volatile organic compound mixtures in constructed wetlands: vegetation and substrate effects

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OPTIMIZATION OF TREATMENT OF CHLORINATED VOLATILE ORGANIC COMPOUND MIXTURES IN CONSTRUCTED WETLANDS: VEGETATION AND SUBSTRATE EFFECTS

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

Microcosm and mesocosm scale experiments were carried out to investigate the effects of vegetation and substrates on the performance of engineered wetland systems (EWSs) meant to treat mixtures of volatile organic compounds (cVOCs). The experiments were conducted with a view to optimizing contributions of these wetland components. *Phragmites communis* and *Typha latifolia*, the commonest wetland plant species, were compared in mesocosm scale EWSs. Eight common wetland plant species were compared using microcosm experiments based on the potential of their rootmatter to enhance treatment of cVOC mixtures. Twelve common wetland plants were similarly compared but on the basis of their aboveground (AGB) biomass contribution. With respect to substrates, a BionSoil/peat/sand mixture was assessed for its ability to support dechlorination of cVOC mixtures. In addition, a mixture of compost materials and sand (row crop compost (RCC)/soil builder compost (SBC)/sand) was evaluated by comparing it to the BionSoil/peat/sand mixture. This study also investigated the effect of salinity on the treatment of cVOC mixtures in EWSs and effects of cVOC initial concentrations and loading rates on EWS performance. Under normal operating conditions, after being inoculated with a dehalorespiring microbial culture, *Phragmites*- and *Typha*-planted EWS units performed comparably, removing completely 1,1,2,2-TeCA and TCE and their daughter products within mainly 18 cm of their bed depths. Before the inoculation, incomplete dechlorination and breakthrough occurred. After a long period of exposure to high concentration cVOCs, *Phragmites* plants weakened and died while *Typha* plants flourished. Microcosm experiments based on both rootmatter and AGB indicated that
different species of wetland plants can differ in their influence on dechlorination of cVOCs. Based on microcosm and mesocosm experiments, the compost/sand mixture performed better than the BionSoil/peat/sand mixture, demonstrating that the former is a better substrate for EWSs. Salinity was found to have a negative effect on treatment of cVOCs. Nonetheless, the salinity problem is manageable through proper design. Pollutant initial concentration and loading rates were found to influence the performance of EWSs. Increasing loading rates and initial concentrations resulted in a decrease in dechlorination rates. As such, loading rates and initial concentrations are important design considerations.
CHAPTER 1: INTRODUCTION

1.1 General Introduction and Rationale of the Study

Much as groundwater pollution is wholly undesirable, some cases are worse than others because of their comparatively more adverse impacts. These deserve a higher priority in both severity assessment and remediation planning. Groundwater pollution by chlorinated VOCs is a special case because chlorinated VOCs: 1) are ubiquitous worldwide at and in the vicinity of the sites where they were originally used, discarded, or accidentally spilled (Löffler et al., 2003); 2) have adverse public health and environmental impacts, including toxic and even carcinogenic effects (Mohr, 2004); 3) exhibit complex fate and transport characteristics in the environment, hence difficult to contain; and 4) are difficult to treat by conventional waste treatment methods (Häggblom and Gosset, 2003).

In many places within and outside the United States of America, groundwater and soils are contaminated by chlorinated VOCs emanating from accidental spills, storage and disposal sites, and from sites where they were used, mainly as solvents and degreasing agents (Löffler et al., 2003; Häggblom and Gosset, 2003). Recognizing present and potential health and environmental impacts from this situation, efforts are put on remedial measures with a view to forestalling further pollution and reversing the pollution that has already occurred. Treatment of the polluted groundwater and decontamination of polluted sites are especially important because many chlorinated VOCs are toxic and some are either suspected or confirmed carcinogens.

Despite this grim picture, the problem has been recognized as evidenced by the technologies and techniques that are continually being developed and employed to
remediate chlorinated VOC pollution. However, conventional remediation technologies have shortcomings, which hamper both their widespread use and efficacy. Most have high acquisition costs and are expensive as well as involving complex operation and maintenance schemes. Also, because of their technical complexity and energy intensiveness, many are not versatile enough for application in the field. Additionally, many are not effective enough on their own. The foregoing and other demerits of conventional remediation technologies are reviewed at length in the literature (Reed et al., 1995; Kadlec and Knight, 1996; Kadlec et al., 2000).

Engineered wetland systems (EWSs) have many merits in connection with waste management in general and remediation in particular. Since they employ natural treatment processes, EWSs do not suffer from the shortcomings of the conventional remediation technologies (Reed et al., 1995; Kadlec and Knight, 1996; Kadlec et al., 2000). As a result, interest in EWSs as a suitable alternative technology for treating chlorinated VOCs is increasing (Pardue et al., 2000; Kassenga, 2003). This is in addition to the use of EWSs to treat domestic wastewater, which is a well established and amply documented practice.

Although considerable research has been done on applicability of wetlands to the treatment of chlorinated VOCs, many knowledge gaps still remain. Notably, although some design guidelines exist for conventional EWSs, there is less coverage on EWSs intended for treating chlorinated VOCs. Also, the roles and influences of various components of EWSs such as media and vegetation are not fully understood or remain controversial.

This study addressed some of the identified knowledge gaps in the wetlands technology. It specifically focused on: 1) the feasibility of co-treatment of different
chlorinated VOCs in EWSs; 2) comparative influence of different types of vegetation on EWS performance at both plant and root matter scales; and 3) the feasibility of using different compost materials as alternative wetland media.

EWS units vegetated with different types of plants were used to assess the co-treatability of different VOCs and to compare effects of different plants on performance efficiency of EWSs. EWS units packed with different types of media were used to assess the suitability of alternative wetland media. On the whole, this study was meant to shed more light on the efficacy of EWSs for treating chlorinated VOCs and provide additional inputs for improving the pertinent design and operation guidelines.

1.2 Review of Background Literature

1.2.1 Application of Engineered Wetland Systems (EWSs) to Treatment of Chlorinated VOCs

Applications of the wetlands technology can generally be grouped into: 1) conventional/mainstream applications and 2) emerging/novel applications. The two categories differ in terms of both design approaches and the treatment mechanisms for which each is optimized. EWSs for treating chlorinated VOCs belong to the latter category. Conventional applications of EWSs are well established and covered widely in the literature (Reed et al., 1995, Kadlec and Knight, 1996; Kadlec et al., 2000; Mitsch and Gosselink, 2000). Probably owing to their novelty, coverage on the emerging applications of EWSs is harder to come by in the literature.

There are also several technologies that are related to EWSs in terms of treatment processes and components that are used to treat chlorinated VOCs. These are invariably identified by names that suggest their main structural or process features. They include in-situ bioremediation, reactive walls, monitored natural attenuation, in-situ reactive
zones, and phytoremediation (Nyer, 1996; Suthersan, 2002; La Grega et al., 2000; Suthersan and Pyne, 2005).

The concept of treatment of chlorinated VOCs in wetlands has been described by Pardue et al. (2000) as one in which the chlorinated VOCs are sequentially dechlorinated via reductive processes or biodegraded via methanotrophic processes to harmless end products as they pass through the organics-rich wetland substrate. Invariably, the key processes are aided by a combination of other wetland processes such as retardation and plant uptake and metabolism (Pardue et al., 2000). Reductive dechlorination is defined by Löffler et al. (2003) as the replacement of a chlorine substituent by hydrogen. Notably, many highly chlorinated environmental pollutants that are resistant to oxygenolytic and hydrolytic dechlorination can be reductively dechlorinated (Löffler et al., 2003).

Evidently, there are not many reported cases that have fully applied the concept described by Pardue et al. (2000). Nonetheless, the concept is strongly supported by field observations reported by Lorah et al. (1997) in a study carried out in a natural wetland at Aberdeen Proving Grounds (Maryland). Kassenga et al. (2003) conducted successful bench scale and microcosm studies for treating chlorinated VOCs in line with this concept as a prelude to pilot scale studies, which are being followed by full-scale implementation of the concept at sites in Massachusetts, North Carolina, and Louisiana. The bench scale study provided input data for the pilot scale study. The data covered geotechnical and hydrodynamic aspects and degradation rates. Related, though less extensive, studies on treatment of chlorinated VOCs in wetlands include that by Wang et al. (2004), in which removal of carbon tetrachloride was observed to take place through both uptake and dechlorination under field conditions in wetlands vegetated with poplar. Newman et al. (1997) also observed significant TCE removal through uptake and bio-
transformation by poplar. Newman et al. (1997) further observed that hybrid poplar plants were able to resist toxicity effects of TCE. Aitchison et al. (2000) have reported on successful removal of a range of chlorinated VOCs including TCE through uptake by poplar trees in engineered wetland and hydroponic systems at different system scales.

1.2.2 Treatability of Chlorinated VOCs in EWSs

There is a considerable number of wetland related chlorinated VOC treatability studies reported in the literature, but most of them are at microcosm scale and were conducted under highly controlled conditions. Nonetheless, the reported studies signify the feasibility of and provide an insight into the wetlands technology. Since in many previously reported cases dechlorination of PCE and TCE stopped at the VC (a human carcinogen) stage (McCarty, 1997), the isolation of Dehalococcoides ethenogenes strain 195 by Maymo-Gatell et al. (1997) was an important milestone in the wetlands technology development. Following the isolation and characterisation of D. ethenogenes strain 195, several different bacteria with comparable dechlorination capabilities have been isolated and characterized. D. ethenogenes strain 195 and the other similar microorganisms can dehalogenate cis-1,2-DCE or VC to ethene (McCarty, 1997). D. ethenogenes is described by Löfler et al. (2003) as an unusual bacterium with an extremely narrow substrate range which can only be grown with hydrogen as the electron donor and chloroethenes (PCE, TCE, cis-DCE, and 1,1-DCE) or 1,2-DCA as electron acceptors. Notably, VC and trans-1,2-DCE do not support growth, but VC is dechlorinated cometabolically to ethene at low rates (Löfler et al., 2003)

Although in the chlorinated VOCs treatment wetland concept described by Pardue et al. (2000) emphasis is put on anaerobic processes, even aerobic processes have some merits (Alvarez-Cohen and Speitel, 2001). After all, numerous aerobic bacteria and fungi
use halogenated compounds as carbon and energy sources for growth (Häggblom and Bossert, 2003). Semprini (2001) argues that anaerobic dehalogenation is most applicable to high chlorinated VOC concentrations near contamination sources while aerobic cometabolism is more suited to more dilute portions of pollution plumes. Sequential anaerobic/aerobic processes have also been used to treat PCE, TCE, \textit{cis}-1,2-DCE, \textit{trans}-1,2-DCE, 1,1-DCE, and VC completely to ethene (McCue et al., 2003). Aerobic cometabolism may be favored however, on plant roots where large methanotrophic populations occur.

Aerobic cometabolic treatment has not found much favour among practitioners of bioremediation because of its complexity, as it requires the addition of both oxygen and the cometabolic substrate (Semprini, 2001). On the other hand, in anaerobic dehalogenation, microorganisms can grow with the chlorinated solvents as electron acceptors and H$_2$ commonly as an electron donor, and complete dehalogenation is achieved (Semprini, 2001). It is notable that for some of the most highly halogenated compounds no effective aerobic degradation processes are known, but efficient anaerobic processes readily occur (Mohr, 2004).

Reductive dehalogenation is largely credited to a class of bacterial enzymes referred to as reductive dehalogenases which carry out the crucial function of removing halogen substituents from many chlorinated organic compounds (Habashash et al., 2004). Reductive dehalogenation is considered the predominant process in anaerobic transformation of halogenated compounds (Häggblom and Bosset, 2003). Halogenated compounds can be used for the following three different metabolic functions in anaerobic bacteria: 1) as carbon or energy source or both, 2) as substrate for metabolic activity, and 3) as terminal electron acceptor in a anaerobic respiration process (Holliger et al., 2003).
Alvarez-Cohen and Speitel (2001) assert that a wide range of chlorinated solvents can be microbially degraded under aerobic conditions by means of cometabolic reactions. Isalou and Sleep (1998) have demonstrated the feasibility of anaerobic degradation of high concentrations of PCE to ethene in column experiments using inocula from an anaerobic digester with methanol as the electron donor.

Lorah et al. (1997) point out that highly chlorinated VOCs like TCE, PCA, and CF are microbially degraded easily through reduction reactions because the carbon atoms have a relatively high oxidation state. Lorah et al. (1997) have also reported field evidence of complete biodegradation of TCE and 1,1,2,2-TeCA through a reductive dehalogenation reaction referred to as hydrogenolysis for TCE and a combination of hydrogenolysis, dichloroelimination, and dehydrochlorination for 1,1,2,2-TeCA. The reductive dechlorination process has also been observed and confirmed by Arnold et al. (2002).

Vogel et al. (1987), who are also cited by both Lorah et al. (1997) and Arnold et al. (2002), describe the reductive dechlorination of interest here as: 1) reductive hydrogenolysis, whereby there is an input of two electrons, and a hydrogen atom replaces a chlorine atom; and 2) dichloroelimination, whereby two chlorine atoms may be removed from two adjacent C-atoms with the subsequent formation of a double bond between the carbon atoms. TCE can result directly from degradation of 1,1,2,2-TeCA through dehydrochlorination (Lorah et al., 1997; Eekert and Schraa, 2001; Arnold et al., 2002). The third type of reductive dechlorination is dehalochlorination which is a slow process, and hence least important for degradation of 1,1,2,2-TeCA (Eekert and Schraa, 2001). House (2002) has reported complete degradation of 1,1,2,2-TeCA to ethane in microcosm experiments conducted under anaerobic conditions.
1.2.3 Hydrological, Hydrodynamic, and Geotechnical Aspects of Engineered Wetland Systems (EWSs)

Important hydrologic aspects of wetlands include water availability, water level or stage, and evapotranspiration rates and their variation. These are important because they are the primary driving forces influencing wetland development, structure, function, and persistence (NRC, 2001). Accurate hydrological information is the key to the assessment of contaminant dynamics and the design of environmental remediation strategies (Xu et al., 2004).

The hydrodynamic aspects pertain to the transport of water through the wetland and factors (such as hydraulic conductivity) that influence it. These are important because they control flow characteristics and retention of contaminants in the wetland and hence treatment effectiveness. They additionally dictate pumping pressure requirement and wetland bed fluidization potential. Important geotechnical aspects in the context of wetlands are media grain size \((d_{10}, U_c)\), density, and porosity. These influence a myriad of aspects, including fluidization potential, hydraulic conductivity; tortuosity, and suitability of the bed for wetland plants.

Tracer tests yield important hydrodynamic information on wetlands. Notably, they give data on distribution of flow and travel times of contaminants, which in turn influence the efficiency and effectiveness of a treatment system (Schmid et al., 2004). The use of tracer tests to evaluate the hydraulics and solute transport characteristics of engineered treatment systems is a well established practice (Goebes and Younger, 2004). Van Genuchten and Wierenga (1976) observe that displacement (akin to tracer) studies have become important tools for predicting the movement of various solutes through soil, providing important information on the behaviour of the chemical (diffusion, dispersion,
anion exclusion, adsorption or exchange processes) and about the medium itself (pore size distribution, aggregation).

Tracers include salts like KBr, dyes like Rhodamine WT, and radioactive substances like tritium (Schmid et al., 2004). There are several preferred tracers. Salts are convenient and widely used tracers (Schimid et al., 2004). Chloride salt tracers have been used widely in tracer studies (Ours et al., 1997). Electrical conductivity has been used successfully as a tracer in full scale engineered wetlands by Charazenc et al. (2003) and in an experimental system by Goebes and Younger (2004). In these studies, electrical conductivity was used as a surrogate measure for salt concentrations.

Bromide (Br⁻) has traditionally been considered an ideal hydrological tracer due to its relatively non-reactive nature and low concentration in soils (Xu et al., 2004). Nonetheless, a study by Xu et al. (2004) also suggests that Br⁻ can be taken up by *Typha latifolia* and *Phragmites australis*. Lin et al. (2003) who have carried out studies to compare rhodamine WT and bromide as tracers in engineered wetlands, have observed that rhodamine WT can only be suitably used in wetlands with a short retention time (<week) because the chemical is prone to irreversible sorption in the wetland substrate.

It is noteworthy that hydrodynamic characteristics of engineered wetlands are affected by scale effects (Ahn and Mitsch, 2002), which decrease with the scale. This observation is important with respect to the use of results from, for example, a bench scale study to implement a full scale EWS.

1.2.4 The Role and Influence of Wetland Media on EWS Performance

Selection of wetland media is important because the media: 1) provide habitat and substrates (including nutrients and essential elements) for microorganisms involved in wetland processes; 2) dictate hydrodynamic and geotechnical characteristics (porosity,
hydraulic conductivity, bulk density) of the wetlands; 3) provide anchorage for wetland plants and control the availability of nutrients and essential elements required by plants; and 4) control physical and the chemical characteristics of the wetland (e.g., pH, redox potential, heat transfer, chemical constituents), which in turn directly influence the wetland’s treatment efficiency and dictate the suitability of the wetland for various microbial populations that take part in treatment processes. Reinforcing the above assertions, Mitsch and Gosselink (2000) point out that the substrate is important to the overall function of a wetland.

Since different media types are made up of different constituent materials, they are bound to exert different physical, chemical, and biological influences in a wetland. By the same token, different wetland media should have different initial microbial community populations and compositions. For example, a wetland medium that is poor in organic matter is expected to support a microbial community that is less effective than those found in media that are richer in organic content. These assertions are supported by findings reported in the literature. Kassenga et al. (2003) observed higher biodegradation of cis-1,2-DCE and 1,1,1-TCA in a sand/peat/Bion Soil mixture than in a sand/peat mixture at both microcosm and mesocosm scales. The superior performance of the sand/peat/Bion Soil mix was attributed to mainly the influence of the high organic content of Bion Soil, a commercially available compost product.

With respect to hydrodynamic and geotechnical characteristics, the fact that wetland media such as humidified bog peat have dual porosities (Ours et al., 1997) has important performance implications with respect to hydraulic conductivity and sorption in the wetland.
Soil organic matter content has an influence on partition of organic compounds (Rutherford et al., 1992). Taking advantage of differing properties of different wetland media, Kao et al. (2000) successfully used peat as a bio-barrier for in-situ removal of TCE and PCE. Kao et al. (2000) cite advantages of peat as a medium as: richness in carbon, sufficient carbon bio-availability for reductive dechlorination, easy availability, relatively low acquisition cost, and easy handling. Karapanagioti et al. (2000) demonstrate that sorption, which is an important process in wetlands, is greatly influenced by heterogeneity of organic matter in the substrate.

Signifying the need for selecting media in line with treatment objectives, Warith et al. (1999) have used an in-situ microbial filter (IMF) for remediation of naphthalene, while Ho and McKay (1998) used peat to remove dye.

1.2.5 The Role and Influence of Wetland Plants in EWSs

Wetland plants can be selected on the basis of: 1) their roles in enhancing treatment effectiveness (Mitsch and Gosselink, 2000), including modifying geotechnical properties and biological and chemical characteristics of the wetland bed; 2) aesthetic/scenic and landscape value (Mitsch and Gosselink, 2000; Tanner, 2001; Mara, 2005); 3) ecological value (as wildlife habitats or repository of flora and fauna) (Mitsch and Gosselink, 2000; Tanner, 2001); 4) individual plant robustness relative to its environment (growth potential and survivability) (Kadlec et al., 2000); 5) relative cost of planting and maintenance (Kadlec et al., 2000); and 6) potential end uses of harvested wetland plants (for example as raw materials for ornamental objects) (Mbuligwe, 2004). For EWS designers, treatment effectiveness may be the most important criterion for selecting wetland plants, but for managers and other stakeholders, the other criteria may be as or more important.
Different wetland plants are expected to differ in their direct roles and influences on processes occurring in their spheres of influence owing to their different ecological attributes (Bardgett, 2005). The presence of living roots can enhance microbial activities such as litter decomposition and mineralization through root exudates which are used by soil microorganisms (Lambers et al., 1998). While it is generally acknowledged that plants are an important component of wetlands (Kadlec et al., 2000; Tanner, 2001), many reported findings on roles and influences of wetland plants give conflicting views. Collins et al. (2004) assert that plants have significant treatment performance effects because they affect bacterial assemblages. Nungesser and Chimney (2001) and Greenway and Woolley (2001) have reported that plant types have a significant influence on wetland performance. Mara (2005) dismisses the role of wetland plants as insignificant, pointing out that in the UK, unplanted rock filters perform as efficiently as the planted EWSs. It may be pointed out that the rock filters referred to by Mara (2005) did not treat chlorinated VOCs, but were used for municipal wastewater.

With respect to treatment of dye-rich wastewater, it has been observed that plants give EWS a performance advantage which differs among plant types (Mbuligwe, 2005). With respect to anaerobically pre-treated domestic wastewater, wetland plants enhance treatment effectiveness significantly, but the performances do not differ among plant species (Mbuligwe, 2004). Kadlec et al. (2000) argue that wetland plants influence treatment performance but different plants do not differ significantly in this respect. Having analyzed organic matter removal data, Allen et al. (2002) assert that plant species influence the performance of wetlands. On the other hand, Da Motta-Margues et al. (2001) argue that wetland plants have a significant influence only in wetlands treating
high pollution loads. The foregoing discussions suggest that the question on the roles and influences of wetland plants is not yet settled.

1.3 Hypotheses and Objectives of the Study

The overall objective of the research for this dissertation was to investigate the treatment of mixtures of chlorinated VOCs, compare the influence of vegetation types on performance of EWSs, and assess the feasibility of using compost materials as alternative wetland media. Specific objectives and corresponding approaches are outlined below.

1.) Evaluation of treatment of mixtures of chlorinated VOCs in engineered wetland soils.

2.) Comparison of treatment efficiencies of EWSs vegetated with *Typha latifolia* and *Phragmites communis* and comparison of influence of different species rootmatter on degradation of VOCs in wetland soils.

3.) Assessment of effectiveness of compost materials as wetland bed materials in EWSs treating chlorinated VOCs.

The hypothesis tested with respect to objective 1 was that more than one chlorinated VOCs can be treated simultaneously in engineered wetland soils as observed in some natural wetlands contaminated with chlorinated VOCs. The cVOCs of interest were co-treated in microcosms constructed using the soil earmarked as bed material for the EWSs. A comparison was made between single compound treatment and cVOC mixture treatment.

For objective 2, two hypotheses were considered. The first was that engineered wetland systems vegetated with *Typha latifolia* and *Phragmites communis* have comparable chlorinated VOC removal efficiencies owing to similarities of growth patterns and root systems of the two wetland plants. Two sets of bench-scale engineered
wetland systems, one vegetated with *Typha latifolia* and another planted with *Phragmites communis* were ran side by side to compare the two types of wetland plants. Feed water containing chlorinated VOCs of interest was pumped through each wetland unit. The treatment performance of each set of wetlands was monitored using modeling and analysis approaches developed for the mixture studies. Other aspects like aboveground biomass and evapotranspiration rates were also monitored as an additional basis for comparing the two types of plants.

The second hypothesis for objective 2 was that rootmatter from candidate wetland plants differ in their stimulation of degradation of chlorinated VOCs in engineered wetland soils because of differences in the physiological, structural, and other attributes of the plant root matter. Freshly obtained rootmatter from different wetland plants (*n* = 8) were used together with an engineered wetland soil to construct different microcosm treatments. A common chlorinated cVOC TCE was used as a test pollutant. The degradation of the test chlorinated VOC was monitored to compare the efficiencies of the different treatments.

For objective 3, two hypotheses were considered. The first of these was that compost materials have physical-chemical and biological attributes that support the processes required to degrade chlorinated VOCs to harmless end products because of their organic origin and suitable geotechnical properties if properly processed. Sets of microcosms were constructed using as substrates the test compost materials separately and in combination. The efficiency of these treatments in degrading chlorinated VOCs was compared to those of microcosms constructed using BionSoil alone and a mixture of BionSoil/peat/sand.
The second hypothesis for objective 3 was that engineered wetland systems packed with a compost materials/sand mixture can remove chlorinated VOCs as effectively as the ones packed with a BionSoil/peat/sand mixture because of their comparable physical – chemical characteristics and organic content. Two sets of wetland units, one packed with the compost materials/sand mixture and another packed with the BionSoil/peat/sand mixture (as control) were operated in an upflow mode. Both sets of wetlands were fed with chlorinated VOC containing water and monitored over time to evaluate the efficacy of the compost materials in comparison with the control substrate.

### 1.4 Scope and Organization of the Dissertation

The temporal scale of this dissertation spans over five years of continuous as well as intermittent experiments and monitoring of experimental EWS units. Scale-wise, the study ranged from microcosm scale treatability experimental set ups carried out in the laboratory to mesocosm scale experimental EWSs set up in the greenhouse.

The central theme of this dissertation, which is treatment of mixtures of chlorinated volatile organic compounds, is reflected throughout the dissertation, but given special emphasis in Chapter 2. The rest of the dissertation is organized around the dissertation’s two sub-themes of vegetation and substrate effects on EWS performance. The theme on vegetation effects on EWS performance is covered in Chapters 3 through 6. Chapter 3 compares cVOC treatment performance efficiencies of EWS units vegetated with *Phragmites communis* to those of the units vegetated with *Typha latifolia*. Chapter 4 compares rootmatter of different wetland plant species with respect to their influence on cVOC treatment. Chapter 5 makes a comparative assessment of effects of salinity on cVOC treatment in EWS units vegetated with *Phragmites communis* and *Typha latifolia*. 
Chapter 6 compares cVOC treatment influences of aboveground biomass of different wetland plant species.

The theme on substrate effects is covered in Chapters 7 and 8. Chapter 7 discusses the characterization and comparative assessment of a number of compost materials as alternative EWS media. It additionally compares EWS units packed with a mixture of compost materials to those packed with a mixture of peat and processed animal waste. Chapter 8 also compares EWS units packed with different media but with a dual focus on effects of pollutant initial concentration and hydraulic and pollutant loading rates.

Chapter 9 wraps up the dissertation by drawing out the key issues from the findings presented in the main body of the dissertation. It also identifies knowledge gaps related to the themes of the dissertation and, on their basis, recommends areas for further research.
2.1 Introduction

The need to treat chlorinated volatile organic compounds (cVOCs) such as 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA) and trichloroethylene (TCE) and their daughter products encountered in the environment arises from their public health and environmental implications. The presence of these chemicals in groundwater and soil in many parts of the world reinforces this need. Much on the extent of pollution of groundwater and soil by these chemicals and their potential as well as actual health and environmental effects is widely reported in the literature (Löffler et al., 2003; Mohr, 2004).

The interest in the treatability of cVOCs stems from the fact that many of them exhibit varying degrees of recalcitrance in both the natural and engineered environments. Notably, both the rates and extents of their degradation depend greatly on, among other things, the substrates available in the treatment medium (Lorah and Voytek, 2004). The need to control the available substrates and other factors that influence the treatability of cVOCs so as to achieve their complete degradation contributes to the interest in the application of engineered wetland systems (EWSs) in the treatment of cVOCs. The chief advantage of the engineered over the natural wetland systems is the fact that EWSs can be optimized to achieve specific treatment goals on a case by case basis.

Wetland bed materials are arguably the most important component of EWSs. Among other functions, wetland bed materials: 1) provide habitats and substrate (energy and carbon source) for the microorganisms that facilitate the treatment of the target
pollutants; 2) provide structural support and nutrients for wetland plants; 3) depending on their chemical compositions, they dictate the chemistry of the EWSs; 4) depending on their composition, they control the fate and transport processes of the target pollutants in the EWSs; and 5) depending on their origin and pre-use handling, they dictate the initial microbial community composition and population size in the EWSs. As such, bed materials for EWSs need to be engineered to optimize for the above and other functions depending on available constituent materials and their cost-effectiveness. This includes selection and testing of suitable constituent materials, mix design and blending of the selected materials, and evaluation of different mixes to select the most suitable.

This study focused on the co-treatability of 1,1,2,2-TeCA and TCE in a wetland bed material designed and produced specially for use in upflow EWS units. Although many studies have reported on treatment of cVOCs, including 1,1,2,2-TeCA and TCE, to our knowledge, few have focused on treatability of mixtures of these cVOCs. The co-treatability of cVOCs in general is an issue of interest because each of these compounds does not usually occur alone. For example, when parent compounds degrade, the degradation products likely coexist with the parent compounds. Also, cVOCs are known to exhibit negative competitive effects when biodegraded simultaneously (Pon et al., 2003; Aulenta et al., 2005; Yu et al., 2005). The specific interest in the co-treatability of 1,1,2,2-TeCA and TCE arises from the fact that the two compounds usually occur together at contaminated sites of interest (Lorah et al., 1997; Lorah et al., 2001). Most importantly, results from this study along with those from previous studies (Kassenga et al., 2003; Pardue, 2005) and subsequent studies (Chapter 3) were meant to provide planning and design information for a wetland technology application project at Aberdeen Proving Ground (ABG) (Superfund Site, Edgewood, Maryland). A plume of
1,1,2,2-TeCA and TCE together with their daughter products underlies the landfill and discharges into the Bush River (Chesapeake Bay) (Pardue, 2005). The landfill itself contains unexploded munitions and other materials, which makes conventional remediation of the landfill site difficult (Pardue, 2005).

This study was carried out as a prelude to similar mesocosm scale studies. The main hypothesis tested in this study was that 1,1,2,2-TeCA and TCE could effectively be co-treated in the engineered wetland bed material at both low and high concentration levels to produce innocuous end products ethene and ethane. The novelty of this study hinges on the fact that: 1) it used a specially engineered substrate, which can be produced in large quantities for use in EWSs; 2) it assessed both the co-treatability of 1,1,2,2-TeCA and TCE and its effect on their kinetics at high as well as low concentrations; and 3) it assessed effects of disruptions in treatment system operation resulting from non-supply or a reduction in the concentrations of the cVOCs treated. Transient contaminant concentration changes can occur in the field due to hydrological factors, resulting in dilution or concentration of pollutants being treated.

2.2 Materials and Methods

2.2.1 Engineered Wetland Soil Design and Characterization

The wetland soil was designed and engineered specially for the treatment of cVOCs in upflow EWSs. The need to design a wetland bed material arose from the fact that naturally available materials seldom have all the required properties. For example, whereas it has excellent geotechnical properties, sand lacks organic matter. On the other hand, most potential constituents that are rich in organic matter have a low bulk density. Potential constituent materials were identified and tested, and suitable ones used to prepare a mix design. The most compatible and suitable constituent materials were
blended to achieve desirable final product properties, including organic content, bulk density, and hydraulic conductivity. Following this procedure, the wetland soil for this study was blended using BionSoil (Dream Maker Dairy, Cowlesville, NY)/Latimer peat (Latimer’s Peat Moss Farm, West Liberty, OH)/sand mixture (37.5%/37.5%/25% w/w). The first two components were included to improve the organic content as well as the initial microbial community structure in the soil. Sand was used to improve the geotechnical and hydrodynamic properties of the medium. The basic characterization data for the engineered wetland soil are shown in Table 2.1.

**Table 2.1: Engineered wetland soil basic characterization data**

<table>
<thead>
<tr>
<th>Characterization parameter</th>
<th>Mean value and standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated moisture content (%)</td>
<td>62.03±3.05</td>
</tr>
<tr>
<td>In-situ moisture content (%)</td>
<td>32.10±3.36</td>
</tr>
<tr>
<td>Saturated bulk density (kg/L)</td>
<td>1.0247±0.0096</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>55.34±0.94</td>
</tr>
<tr>
<td>Organic fraction (%)</td>
<td>21.82±0.86</td>
</tr>
<tr>
<td>Hydraulic conductivity (cm/s)</td>
<td>$8.3 \times 10^{-4}±1.31\times10^{-4}$</td>
</tr>
</tbody>
</table>

Determination of the parameters given in Table 2.1 followed standard procedures and protocols which have been reported elsewhere (Kassenga, 2003; Kassenga et al., 2003). Additional characterization data for BionSoil and Latimer peat have been reported in Kassenga (2003) and Kassenga et al. (2003).

It is evident from Table 2.1 that the engineered soil had the best of characteristics of all its constituents with respect to the functions and operation of the envisaged EWSs.
For example, it had better geotechnical and hydraulic properties than either BionSoil or peat (high bulk density and hydraulic conductivity). Also, it had a higher organic content and porosity than sand. Moreover, it had a potentially richer and more suitable initial microbial community than sand, BionSoil, or peat alone.

The fact that the bulk density of the engineered soil was only slightly higher than that of water is not a serious problem for vegetated EWSs provided full design flow rates are not implemented at commissioning time, if they exceed the critical flow rate. Pumping of full design flow rates should wait until wetland plant root systems have developed sufficiently. Our experience shows that a two month period is sufficient for *Typha latifolia* to develop a sufficiently interwoven root system to enable the EWS bed to behave like a monolithic structure and resist not only stratification, fluidization and wash out of bed constituents, but also resist uplift forces (Chapter 3). As such, the hydraulic loading rate can be increased gradually from one below the critical value to the full value in about three months. The critical loading rate for the engineered soil used in this study, based on the data shown in Table 2.1, is 1.76 cm/d. In practice, an initial hydraulic loading rate of 1 cm/d (which, with a safety factor of more than 1.5, is safe enough) can be doubled every two weeks, reaching a value of 8 cm/d in two months.

### 2.2.2 Sorption Experiments

Sorption experiments were carried out for the individual as well as the combination of all the test compounds in 40 mL VOA vials (ICHEM) with 5 g of the engineered wetland soil and sodium azide as the biocide. Six concentration levels covering the concentration range of interest were used to generate data points for each sorption isotherm. Each data point was obtained using triplicate vials adding up to 18 vials per isotherm. Dilutions of the test chemicals were made at 1:1, 1:2, 1:5, 1:10, and
1:20 using an electrolyte solution (0.01 M CaCl₂). After adding the test soil and filling with chemical solution and capping, the vials were shaken on a reciprocating shaker at 20°C for 48 h. They were then centrifuged after which the supernatant was withdrawn and analyzed for the test compounds using EPA Method 8260 B.

2.2.3 Microcosm Set-up and Treatments

The anaerobic microcosm study was carried out using 160 mL serum bottles. All the bottles contained the same type of blended soil. The 160 mL bottle volume was apportioned as follows: blended wetland medium (plus inoculum where applicable), 54 mL; dionized (DI) water containing the test chemicals, 81 mL; and head space filled with nitrogen gas, 25 mL. The bottles were sealed with rubber stoppers and secured with aluminum crimp caps to ensure air tightness for maintaining anaerobic conditions in the bottles. All the treatments were prepared under oxygen free conditions in an anaerobic chamber. Incubation was done under static conditions in the dark at 25°C.

Six treatments prepared in triplicates (a total of 18 microcosm bottles) were adopted as follows: Treatment 1, wetland soil and DI water inoculated with 10 mL of slurry containing *Dehalococcoides ethenogenes* (DHC) (Kassenga et al., 2004) and spiked with low concentration 1,1,2,2-TeCA only; Treatment 2, wetland soil and DI water inoculated with 10 mL of slurry containing DHC and spiked with low concentration 1,1,2,2-TeCA and TCE; Treatment 3, wetland soil and DI water inoculated with 10 mL of slurry containing DHC and spiked with high concentration 1,1,2,2-TeCA; Treatment 4, wetland soil and DI water inoculated with 10 mL of slurry containing DHC and spiked with high concentration 1,1,2,2-TeCA and TCE; Treatment 5, wetland soil and DI water spiked with high concentration 1,1,2,2-TeCA only plus 3.8 mL of 3.7% formalin (killed control); Treatment 6, non-inoculated wetland soil and DI water. The inoculum was
cultured in the same medium as the test wetland soil (Bion Soil/Latimer peat/sand). The test chemicals were reagent grade and were purchased from Sigma-Aldrich.

2.2.4 Experimental Procedures and Monitoring

This study was carried out in three phases separated by pauses. Phase 1 of the study was initiated by the first spiking of the test chemicals into the microcosm bottles immediately after the microcosms had been set up. After the first phase of the study, all the treatments were subjected to partial starvation. This was effected by intermittently re-spiking the treatments with low doses of 1,1,2,2-TeCA and TCE (about 0.1 mg/L for the low concentration and about 1 mg/L for the high concentration treatments) for a period of two weeks. To initiate the next phase of the study, the treatments were re-spiked with the appropriate doses of the chemicals to attain the desired concentrations.

After the second phase of the study, all the four treatments were again partially starved by sustaining them on doses of low concentration 1,1,2,2-TeCA and TCE for two months before they were re-spiked with the chemicals for the next phase of the study. An important additional difference between Phases 2 and 3 experimental runs is that in Phase 3, both the low and high concentration levels were increased slightly. This was done in order to see whether it would affect the performance of the treatments owing to toxicity and inhibitory effects. Under field conditions, changes in the concentrations of pollutants can occur due to influences of hydrological and hydrogeological conditions. For example, precipitation can result in dilution while excessive evapo-transpiration can result in an increase in concentrations of the pollutants. With specific reference to the site of interest in this study, Pardue (2005) has observed that seepage of contaminated water can be transient.
Aqueous and gaseous samples were taken from all the bottles at appropriate time intervals for analysis to monitor the performance of the treatments. Aqueous phase samples were withdrawn from the bottles using micro-syringes whereas gas phase samples were taken from the head space using gas-tight syringes. The performance was monitored based on concentrations of the parent compounds and their daughter products plus methane, ethene, and ethane. Methane was monitored because it is an indicator of the dominance of methanogenic conditions, which are conducive for reductive dechlorination of cVOCs. Methane can also be used to gauge the intensity of general microbial activity in the course of treatment of cVOCs. Additionally, methane data can help to discern other processes that might impact on cVOC degradation, including reverse methanogenesis (anaerobic oxidation of methane) (Moran et al., 2007). Ethene and ethane were monitored because they are the expected innocuous end products of complete dechlorination of chlorinated ethenes and ethanes. Hydrogen concentrations were monitored because hydrogen is an important electron donor in reductive dechlorination processes. Often it can be the limiting factor in these processes, and as such its concentration can serve as an indicator of the viability of these processes (Kassenga et al., 2004).

2.2.5 Analytical Work

Aqueous phase samples were analyzed for cVOCs using EPA Method 8260B using GC/MS (Agilent Technologies 6890N Network GC System, Agilent Technologies 5973 Network Mass Selective Detector) coupled to an AquaTek 70 Autosampler® (Teledyne Tekmar) and Velocity XPT® purge and trap sample concentrator (Teledyne Tekmar). Methane, ethene, and ethane gases were analyzed using GC/FID. 1 mL of head space sample was injected into the gas chromatography with flame ionization detector
(Agilent 5890 Series II) equipped with a 2.4 m x 0.32 mm ID column packed with Carbopack b/1% Sp-(Supelco, Bellefonte, PA). The column 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. Analytical standards and surrogate for the cVOCs were obtained as mixtures from Supelco Inc.

Hydrogen was analyzed using a reduction gas analyzer (Trace Analytical, Menlo Park, CA) equipped with a reduction gas detector. Head space samples were injected into a 1 mL gas sampling loop prior to being separated using 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 the carrier gas. The carrier gas was first passed through a catalytic combustion converter (Trace Analytical, Menlo Park, CA) to remove traces of H₂. Methane, ethene, and ethane calibration gases were obtained from Gas Products (Baton Rouge, LA).

2.3 Statistical and Mathematical Analysis

The data obtained from sorption experiments were modeled using both the linear sorption model (Equation 2.1) and the Freundlich sorption model (Equation 2.2).

\[
q_s = K_d C_e \quad [2.1]
\]

\[
q_s = K_F C_e^N \quad [2.2]
\]

where \( q_s \) = mass of the chemical sorbed per unit mass of the engineered soil [MM⁻¹], \( K_d \) = linear model sorption distribution coefficient [L³M⁻¹], \( C_e \) = equilibrium concentration [ML⁻³], \( K_F \) = Freundlich sorption constant [L³M⁻¹]N, and \( N \) = Freundlich exponent. The retardation factor was calculated using Equation 2.3.
\[ R = 1 + \frac{K_d \rho_b}{n} \]  

[2.3]

where, \( R \) is the retardation factor, \( K_d \) = distribution coefficient (L/kg), \( \rho_b \) = bulk density of the soil (kg/L), and \( n \) = porosity.

The cVOC data were evaluated for statistical integrity using the appropriate tests (\( t \)-tests, ANOVA). Regression analysis of 1,1,2,2-TeCA degradation data used Equation 2.4.

\[ [E] = [E]_o e^{-k_e t} \]  

[2.4]

where \([E]\) = the concentration of 1,1,2,2-TeCA (moles/L), \( k_e \) = the reaction rate constant \([T^{-1}]\), \( t \) = time \([T]\), and the subscript ‘o’ refers to initial conditions. Recognizing that the degradation rate of TCE resulting from the dehydrochlorination of 1,1,2,2-TeCA was dependent on the degradation of both 1,1,2,2-TeCA and TCE itself, the pertinent data were modeled according to Equation 2.5.

\[
\frac{d[C]}{dt} = k_E [E]_o e^{-k_e t} - k_C [C]
\]  

[2.5]

where \([C]\) = concentration of TCE (moles/L) and \( k_C \) = the actual rate of TCE degradation \([T^{-1}]\). Equation 2.5 was solved to yield Equation 2.6 which was used to model the TCE data.

\[
[C] = \frac{k_E [E]_o}{k_C - k_E} e^{-k_e t} + \left( [C]_o - \frac{k_E [E]_o}{k_C - k_E} \right) e^{-k_C t}
\]

[2.6]

Use of Equation 2.6 for regression analysis of 1,1,2,2-TeCA and TCE data to determine the actual (as opposed to the apparent) reaction rate constant of TCE was done using SigmaPlot (SPSS Inc., Chicago IL). The apparent kinetic rate constant for TCE was determined using Equation 2.4, but with \([E]\) and \([E]_o\) replaced by \([C]\) and \([C]_o\), respectively.
2.4 Results and Discussion

2.4.1 Sorption Experiment Results and Retardation Coefficient Data

Results of the sorption experiments are presented in Table 2.2 along with retardation coefficient data derived from them.

Chloroform was tested along with 1,1,2,2-TeCA and TCE and their daughter products (Table 2.1) for comparison because it is a pollutant that is often found at cVOC contaminated sites.

Table 2.2: Summary of sorption experiment results and retardation coefficient data

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Linear model</th>
<th>Freundlich model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_d$ (L/kg)</td>
<td>$K_F$ (L/kg)$^N$</td>
</tr>
<tr>
<td>Chloroform</td>
<td>8.0</td>
<td>9.70</td>
</tr>
<tr>
<td>TCE</td>
<td>23.1</td>
<td>23.70</td>
</tr>
<tr>
<td>cis-1,2-DCE</td>
<td>8.3</td>
<td>7.67</td>
</tr>
<tr>
<td>Chloroform</td>
<td>7.9</td>
<td>7.44</td>
</tr>
<tr>
<td>TCE</td>
<td>20.5</td>
<td>19.06</td>
</tr>
<tr>
<td>cis-1,2-DCE</td>
<td>10.2</td>
<td>12.45</td>
</tr>
<tr>
<td>1,1,2,2-TeCA</td>
<td>10.7</td>
<td>14.60</td>
</tr>
</tbody>
</table>

$^a$Calculated using Equation 2.3; $^b$ Results of sorption experiments involving all the chemicals simultaneously.

A number of noteworthy observations are evident from Table 2.2. First, the Linear model was as good a fit for the sorption data as well as the Freundlich model. As such, the simpler Linear model can be used instead of the Freundlich model in subsequent application of the sorption experiment results. Second, the distribution
coefficient \((K_d)\) values based on the Linear model are closely comparable to the ones obtained using the Freundlich model. This observation supports the earlier assertion regarding the applicability of the Linear model in place of the Freundlich model.

Table 2.2 suggests that the coefficients of distribution of chloroform and TCE decrease only slightly when the two compounds undergo sorption in competition with other compounds. In contrast, (although it is hard to explain) sorption of \textit{cis} \(-1,2\)-DCE seems to increase when it occurs in the presence of other compounds. It can also be observed that TCE undergoes sorption more than twice as readily as either chloroform or \textit{cis}-1,2-\textit{DCE}.

2.4.2 1,1,2,2-TeCA and TCE Treatment in Phase 1 Experimental Run

Results of treatment of 1,1,2,2-TeCA and co-treatment of 1,1,2,2-TeCA and TCE in the engineered soil during the first phase of this study (Phase 1) are shown in Figures 2.1 through 2.10.

It is evident from Figure 2.1 that degradation of 1,1,2,2-TeCA (low concentration) did not result in an accumulation of daughter products. The concentration of ethene, which increased with time in parallel with the removal of 1,1,2,2-TeCA, indicates that 1,1,2,2-TeCA degraded completely. 1,1,2,2-TeCA is known to degrade completely through the TCE pathway whereby hydrochlorination of 1,1,2,2-TeCA to TCE is abiotic and through the 1,1,2-TCA pathway whose first step (hydrogenolysis) as well as the subsequent steps are are biotic (Chen et al., 1996; Lorah and Olsen, 1999; Aulenta et al., 2005; Hunkeler et al., 2005: Aulenta et al., 2006).

The TCE pathway leads to the production of ethene, whereas the 1,1,2-TCA pathway can produce either ethene or ethane or both (Chen et al., 1996; Lorah and Olsen, 1999; Aulenta et al., 2005; Hunkeler et al., 2005; Aulenta et al., 2006).
Figure 2.1: Phase 1 treatment of low concentration 1,1,2,2-TeCA (Treatment 1) in the engineered wetland soil

Figure 2.2: Variation of aqueous phase gas concentrations in during Phase 1 treatment of low concentration 1,1,2,2-TeCA (Treatment 1)
Figure 2.3: Phase 1 treatment of low concentration 1,1,2,2-TeCA and TCE (Treatment 2) in the engineered wetland soil

Figure 2.4: Variation of aqueous phase gas concentrations during Phase 1 treatment of low concentration 1,1,2,2-TeCA and TCE (Treatment 2)
Figure 2.5: Phase 1 treatment of high concentration 1,1,2,2-TeCA (Treatment 3) in the engineered wetland soil

Figure 2.6: Variation of aqueous phase gas concentrations in during Phase 1 treatment of high concentration 1,1,2,2-TeCA (Treatment 3)
**Figure 2.7:** Phase 1 treatment of high concentration 1,1,2,2-TeCA and TCE in the engineered wetland soil

**Figure 2.8:** Variation of aqueous phase gas concentrations during Phase 1 treatment of high concentration 1,1,2,2-TeCA and TCE (Treatment 4)
Figure 2.9: Phase 1 treatment of high concentration 1,1,2,2-TeCA in the engineered wetland soil (killed control).

Figure 2.10: Variation of aqueous phase methane gas concentration during Phase 1 treatment of high concentration 1,1,2,2-TeCA in the killed control (Treatment 6). For comparison, the corresponding profile in the live control (devoid of cVOCs) is also shown.
In the backdrop of the preceding observation, the accumulation of ethane just slightly out of step with the production trend of ethene (Figure 2.2), suggests that 1,1,2,2-TeCA degraded through the 1,1,2-TCA or through both the 1,1,2-TCA and TCE pathways. However, additional experiments on 1,1,2,2-TeCA and TCE degradation with the same type of substrate and inoculum (data not shown) showed that, as the cVOCs were being depleted, ethene, which was originally present disappeared, whereas ethane, which was originally absent, appeared. Most importantly, in the supplementary experiments ethane production was mostly observed after the depletion of the cVOCs. This suggests that ethane was not formed directly from the transformation of the cVOCs. The decrease in ethene concentration accompanying the increase in concentration of ethane towards the end of the experimental run (around day 16 in Figures 2.1 and 2.2) supports the foregoing arguments. The initial concentration of ethane (at the beginning of the experimental run) is also likely due to transformation of ethene and was probably in the inoculum which was cultured and sustained using TCE. It is noteworthy that the initial concentration of ethene was almost zero, but increased in the course of cVOC dechlorination.

The decrease in the concentration of methane evident in Figures 2.2, 2.4, 2.6, and 2.8 during degradation of 1,1,2,2-TeCA was clearly a result of dilution or consumption that took place almost in lock-step with dechlorination. In the absence of evidence for the occurrence of dilution, it is logical to attribute the observed drop in methane levels to consumption that was most apparent during cVOC dechlorination. Therefore, the decrease in methane levels was likely caused by its consumption during reverse methanogenesis. Anaerobic oxidation of methane is a well known phenomenon (Hinrichs and Boetius, 2002; Moran et al., 2007; Chanton et al., 2008). Reverse methanogenesis
can take place if there are H₂ consumers, mainly sulfate reducing bacteria (SRB), but also nitrate-, Mn(IV)-, and Fe(III)-reducers (Konhauser, 2007). The reverse methanogenesis processes mediated by SRB are exemplified by reactions represented by Equations 2.7 and 2.8 (Konhauser, 2007):

\[
4H_2 + SO_4^{2-} \rightarrow HS^- + OH^- + 3H_2O \quad (-152 \text{ kJ}) \quad [2.7]
\]

\[
CH_4 + 3H_2O \rightarrow 4H_2 + HCO_3^- + H^+ \quad (+136 \text{ kJ}) \quad [2.8]
\]

Equations similar to Equations 2.7 and 2.8 can be written for the other electron acceptors. It can be argued that, in this study, dechlorination of 1,1,2,2-TeCA, TCE, and their daughter products, which made use of H₂ as the main electron donor, reduced H₂ levels, creating conducive conditions for reverse methanogenesis.

The non-accumulation of 1,1,2,2-TeCA daughter products evident in Figure 2.1 has several implications of practical significance. Most importantly, a treatment system exhibiting such a behavior would obviate the potential health and environmental effects from the daughter products such as vinyl chloride (VC), which are more harmful than the parent compounds. VC, which can form from \textit{cis}-1,2-dichloroethylene (\textit{cis}-1,2-DCE) through the TCE pathway, is a proven carcinogen unlike 1,1,2,2-TeCA or TCE (McCarty, 1997).

In Figure 2.3, the apparent lag in TCE degradation (up to day 6) was likely caused by the addition of TCE formed from 1,1,2,2-TeCA that degraded through the TCE pathway. The presence of 1,1,2-TCA indicates that some 1,1,2,2-TeCA degraded via the 1,1,2-TCA pathway. Comparing Figures 2.2 and 2.4, it is evident that the decrease in methane concentration is far greater in Figure 2.4 than in Figure 2.2. This probably signifies the effects of co-treatment. It can be argued that the decrease in methane
concentration in Figure 2.4 resulted from the sum of the effects of degradation of both 1,1,2,2-TeCA and TCE.

In Figure 2.5, the profile of 1,1,2,2-TeCA concentration suggests that in this treatment (high concentration) the cVOC degraded at a slower rate than in the low concentration treatment (Figure 2.1). Also, unlike in the case of the low concentration treatment (Figure 2.1), in Figure 2.5 treatment of 1,1,2,2-TeCA alone resulted in an observable build up of TCE. It is, however, also evident that the TCE build up depleted even before the depletion of 1,1,2,2-TeCA. This indicates that TCE and its daughter products degraded at rates that were higher than those of 1,1,2,2-TeCA. The presence of TCE also shows that a significant amount of 1,1,2,2-TeCA degraded via the TCE pathway.

From an engineering design point of view, the depletion of the daughter products ahead of their parent compound suggests that treatment systems can be designed on the basis of the parent compound and still be effective across the whole range of treatment needs. This also implies that such systems can be designed even when the specific degradation pathways of the parent compounds of interest are not known with a high degree of certainty.

The absence of 1,1,2-TCA in Figure 2.5 suggests that it degraded faster than its rate of production from 1,1,2,2-TeCA. This also means the degradation products of 1,1,2-TCA degraded at faster rates than its own. These assertions, however, do not preclude the possibility that 1,1,2-TeCA did not form at all or that only a small immeasurable amount of it was produced. The low degradation rate of 1,1,2,2-TeCA in Figure 2.5 is attributable to inhibitory or toxic effects resulting from its relatively high concentration (Aulenta et al., 2005).
Comparing Figures 2.2, 2.4, 2.6, and 2.8, it is apparent that the decrease in methane concentration was proportional to the quantity of cVOC treated. The magnitudes of the decrease in methane concentration were in this order: Figure 2.8 > Figure 2.6 > Figure 2.4 > Figure 2.2. It is prominently evident that the decrease in methane concentration accompanying the dechlorination of 1,1,2,2-TeCA and its daughter products was only a temporary effect. Figure 2.6 (and Figure 2.5) is a noteworthy special case of this observation. In Figure 2.6, methane concentration was at its lowest on day 10. Then it recovered, reaching a level higher than the initial one even before 1,1,2,2-TeCA and TCE had been depleted. More specifically, methane production recovered to more than 75% of its initial concentration even before 50% of 1,1,2,2-TeCA had degraded (around day 16) and in spite of the accumulation of TCE. The recovery of methane exhibited in Figure 2.6 suggests that in the long term, methane production in a treatment system such as this may not be significantly affected by the presence and degradation of 1,1,2,2-TeCA and TCE and their daughter products.

With respect to all of the treatments, the rapid recovery of methane levels paralleling the culmination of dechlorination processes suggests that the decrease in methane levels preceding the recovery must have been caused by methane consumption that occurred in tandem with the dechlorination of the cVOCs. The fact that where methane consumption during cVOC dechlorination was most rapid, the recovery was also apparently rapid (for example, Figure 2.8), whereas the opposite was true for the cases where the decline in methane levels was slow (for example, Figure 2.2), suggests that some feedback mechanism linked to methane production was involved. It can even be argued that the dechlorination of the cVOCs did not disrupt the production of methane, but rather triggered or accelerated its consumption as discussed earlier.
Although it is reported that 1,1,2-TCA can form vinyl chloride (VC) through dichloroelimination and dichloroethenes (DCEs) via dehydrochlorination (Lorah and Olsen, 1999; Aulenta et al., 2005; Aulenta et al., 2006), these were not observed in this study. Supplementary experiments on TCE and 1,1,2-TCA showed that degradation of 1,1,2-TCA did not produce detectable quantities of either VC or DCEs, while TCE did. Therefore, the cis-DCE observed in Treatment 4 (Figure 2.7) is likely from the degradation of TCE. It is noteworthy that, as observed with respect to Figure 2.5, in Figure 2.7 1,1,2,2-TeCA was depleted much later than its daughter products, including TCE which was present at a high concentration at the beginning of the experimental run.

The presence of TCE in Figure 2.9 (killed control treatment) suggests that some 1,1,2,2-TeCA degraded through dehydrochlorination (Lorah and Olsen, 1999; Aulenta et al., 2005; Aulenta et al., 2006). Evidently, this process was not as extensive in the killed control as in the biotic treatments (Treatments 1 – 4). The inefficiency of dehydrochlorination in the killed control treatment is probably due to differences in environmental conditions that were not monitored. It can be inferred from this that microorganisms are capable of modifying the conditions favorably (in favor of 1,1,2,2-TeCA degradation).

The production of methane in the killed control treatment (Figure 2.10) suggests that the biocide used did not actually kill all the microorganisms. The pattern of methane concentration (in spite of the effect of the biocide), which is different from the live treatments (Treatments 1 – 4), reinforces the notion advanced earlier that in the other treatments methane was consumed during dechlorination of 1,1,2,2-TeCA and TCE and their daughter products.
It is important to point out that in a control treatment to which cVOCs were not added (data not shown), methane concentration consistently increased with time. The increase in methane concentration with time in the live control treatment to which cVOCs were not added can be attributed to the fact that, unlike in Treatments 1 – 4, reverse methanogenesis, which would have consumed methane, did not take place because the absence of the dechlorination processes reduced the demand for H\(_2\), leading to a build up in H\(_2\). In turn, the build up of H\(_2\), made reverse methanogenesis unfavorable (Konhauser, 2007). The summary of aqueous phase hydrogen concentration data corresponding to Figures 1 – 10 is given in Table 2.3; it bears out the preceding arguments regarding reverse methanogenesis.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Treatment descriptions</th>
<th>H(_2) (nM), (STD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 1</td>
<td>Low concentration 1,1,2,2 TeCA + DHC</td>
<td>3.188±1.626</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>Low concentration 1,1,2,2-TeCA + TCE +DHC</td>
<td>2.648±1.372</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>High concentration 1,1,2,2 TeCA + DHC</td>
<td>5.525±2.765</td>
</tr>
<tr>
<td>Treatment 4</td>
<td>High concentration 1,1,2,2-TeCA + TCE +DHC</td>
<td>3.801±1.948</td>
</tr>
<tr>
<td>Treatment 5</td>
<td>High concentration 1,1,2,2-TeCA killed control</td>
<td>11.338±2.331</td>
</tr>
<tr>
<td>Treatment 6</td>
<td>Live control (without cVOCs)</td>
<td>3.294±2.212</td>
</tr>
</tbody>
</table>

For each treatment, n = 18; STD = standard deviation

In Table 2.3, the high concentration of hydrogen in the killed control treatment implies that the failure of this treatment to degrade the cVOCs was not caused by a lack
of an electron donor. This also reinforces the notion that the biocide did not kill all the microorganisms in this treatment.

With respect to the statistics pertinent to Table 2.3, the presence of the cVOCs did not have a significant negative impact on hydrogen production irrespective of the concentrations of the cVOCs (P<0.05). On the one hand, this points to the resilience of hydrogen producers, while on the other it reaffirms the potential of the engineered soil as a bed material for EWSs intended to treat cVOCs.

The remarkably higher hydrogen concentration in the killed control than in the other treatments is probably due to a reduction in consumption due to effects of the biocide used, leading to accumulation. The fairly comparable levels of hydrogen in most treatments suggest that its production could have partly been spurred by or linked to its consumption through feedback mechanisms. The fact that the higher cVOC concentration treatments did not pull down hydrogen levels more than the lower concentration treatments suggests that availability of hydrogen was not the limiting factor in the dechlorination processes. It also indicates that the hydrogen producers were not significantly affected by the concentrations or overall quantities of the cVOCs treated.

2.4.3 1,1,2,2-TeCA and TCE Treatment in Phase Two Experimental Run

Results of treatment of 1,1,2,2-TeCA and co-treatment of 1,1,2,2-TeCA and TCE in the engineered soil during the second phase of this study (Phase 2 experimental run) are shown in Figures 2.11 – 2.14.

An important observation regarding the 1,1,2,2-TeCA degradation profiles shown in Figures 2.11 and 2.13 is the non-accumulation of degradation products in Treatments 1 and 3 (1,1,2,2-TeCA alone) irrespective of the initial concentration of the chemical.
Figure 2.11: Phase 2 treatment of low concentration 1,1,2,2-TeCA alone (Treatment 1) in the engineered wetland soil

Figure 2.12: Phase 2 treatment of low concentration 1,1,2,2-TeCA and TCE (Treatment 2) in the engineered wetland soil
Figure 2.13: Phase 2 treatment of high concentration 1,1,2,2-TeCA alone (Treatment 3) in the engineered wetland soil

Figure 2.14: Phase 2 treatment of high concentration 1,1,2,2-TeCA and TCE (Treatment 4) in the engineered wetland soil
Another noteworthy observation, which applies to all of the treatments (Figures 11 – 14), is that in all the treatments, including the ones in which 1,1,2,2-TeCA was treated together with TCE and other degradation products of 1,1,2,2-TeCA, the degradation products were depleted earlier than 1,1,2,2-TeCA itself. As discussed with respect to Phase 1 of this study, the complete degradation of daughter products before the parent chemical is desirable because it can reduce prediction errors in design.

On the whole, Figures 2.11 and 2.13 indicate that, regardless of the initial concentrations, treatment of 1,1,2,2-TeCA and TCE together has the potential to produce transient accumulations of daughter products. This indicates, as would logically be expected, that treatment of mixtures of cVOCs is more demanding than treatment of single compounds. However, the fact that in this case the accumulation of the daughter products did not last long precludes any of its undue serious effects.

Concluding from the preceding and previous discussions, it is apparent that a treatment system designed to remove the parent compounds completely would be able to remove the daughter products completely along with the parent compounds. It is additionally reasonable to assume that the mechanisms and pathways of 1,1,2,2-TeCA degradation in Phase 2 were the same as in Phase 1 due to the similarities in patterns of degradation profiles between the corresponding treatments.

2.4.4 1,1,2,2-TeCA and TCE Treatment in Phase Three Experimental Run

Results of treatment of 1,1,2,2-TeCA and co-treatment of 1,1,2,2-TeCA and TCE in the engineered soil during the third phase of this study (Phase 3 experimental run) are shown in Figures 2.15 – 2.18. It is evident from Figures 2.15 – 2.18 that the 1,1,2,2-TeCA degradation profiles for Phase 3 were similar to those for Phase 2. In Phase 3 too,
Figure 2.15: Phase 3 treatment of low concentration 1,1,2,2-TeCA alone (Treatment 1) in the engineered wetland soil

Figure 2.16: Phase 3 treatment of low concentration 1,1,2,2-TeCA and TCE (Treatment 2) in the engineered wetland soil
Figure 2.17: Phase 3 treatment of high concentration 1,1,2,2-TeCA alone (Treatment 3) in the engineered wetland soil.

Figure 2.18: Phase 3 treatment of high concentration 1,1,2,2-TeCA and TCE (Treatment 4) in the engineered wetland soil.
in both cases, treatment of 1,1,2,2-TeCA alone did not result in an accumulation of any daughter products regardless of the initial concentration of 1,1,2,2-TeCA. Notably, the initial concentration of 1,1,2,2-TeCA in Figure 2.17 was 2.8 times higher than in Figure 2.15 (Treatment 1 Phase 3), 5 times higher than in Figure 2.13 (Treatment 3 Phase 2), and 15 times higher than in Figure 2.11 (Treatment 1 Phase 2).

Although in Treatment 4 (For 1,1,2,2-TeCA and TCE) (Figure 2.18), the daughter products of 1,1,2,2-TeCA did not disappear as rapidly as in Treatments 1 and 3 (for 1,1,2,2-TeCA alone), the accumulation of the daughter products was low. Again, the daughter products degraded faster than 1,1,2,2-TeCA. Another important observation with respect to Figures 2.16 and 2.18 is that, although in Treatments 2 and 4 (1,1,2,2-TeCA and TCE) the daughter products were detected, they degraded more rapidly than 1,1,2,2-TeCA and complete degradation of 1,1,2,2-TeCA itself took only slightly longer than in Treatments 1 and 3 (1,1,2,2-TeCA alone).

The accumulation of daughter products in the treatments containing both 1,1,2,2-TeCA and TCE can be attributed to effects of unfavorable competition among parent and daughter cVOCs involved (Pon et al., 2003; Aulenta et al., 2005; Yu et al., 2005). Conversely, the non-accumulation of the daughter products in the treatments containing 1,1,2,2-TeCA alone was partly due to less negative competitive effects. On the other hand, the overall high rate of removal of the daughter products than 1,1,2,2-TeCA in all the treatments was due to the degradation rates of the daughter products being higher than those of 1,1,2,2-TeCA. It can also be argued that degradation of 1,1,2,2-TeCA through several diverse mechanisms and pathways resulted in low concentration of many daughter products instead of high concentration of only a few of them. Conceivably,
many low concentration daughter products would not accumulate as easily and would not be as toxic or inhibitory as few high concentration daughter products.

The fact that VC was only detected in the treatment for Figure 2.18 implies that in the other cases VC was not detected because it did not accumulate rather than because the pathways of 1,1,2,2-TeCA dechlorination did not involve the production of VC.

2.4.5 Comparison of Treatment Efficiencies of Different Treatments and the Effect of Transient Treatment Disruptions

Regression analysis of 1,1,2,2-TeCA and TCE degradation data for the different treatments during Phases 1 - 3 of this study are summarized in Table 2.4.

**Table 2.4**: Comparison of 1,1,2,2-TeCA degradation in the different treatments treating 1,1,2,2-TeCA and TCE during Phases 1 – 3 of the study

<table>
<thead>
<tr>
<th>Wetland soil treatments</th>
<th>First order rate constants (first stage for 1,1,2,2-TeCA and second stage for TCE) (d⁻¹) (R²)</th>
<th>Time to 100% removal (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TeCA</td>
<td>TCE (Appar.)</td>
</tr>
<tr>
<td>Phase 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC TeCA</td>
<td>0.185 (0.883)</td>
<td>17</td>
</tr>
<tr>
<td>LC TeCA+TCE</td>
<td>0.066 (0.981) 0.098 (0.896) 0.185 (0.896)</td>
<td>11</td>
</tr>
<tr>
<td>HC TeCA</td>
<td>0.089 (0.760)</td>
<td>25</td>
</tr>
<tr>
<td>HC TeCA+TCE</td>
<td>0.083 (0.916) 0.104 (0.900) 0.210 (0.900)</td>
<td>25</td>
</tr>
<tr>
<td>Phase 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC TeCA</td>
<td>0.112 (0.935)</td>
<td>38</td>
</tr>
<tr>
<td>LC TeCA+TCE</td>
<td>0.083 (0.939) 0.147 (0.966) 0.152 (0.966)</td>
<td>38</td>
</tr>
<tr>
<td>HC TeCA</td>
<td>0.102 (0.962)</td>
<td>38</td>
</tr>
<tr>
<td>HC TeCA+TCE</td>
<td>0.060 (0.984) 0.142 (0.968) 0.183 (0.968)</td>
<td>47</td>
</tr>
<tr>
<td>Phase 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC TeCA</td>
<td>0.113 (0.906)</td>
<td>48</td>
</tr>
<tr>
<td>LC TeCA+TCE</td>
<td>0.096 (0.959) 0.198 (0.991) 0.317 (0.991)</td>
<td>42</td>
</tr>
<tr>
<td>HC TeCA</td>
<td>0.040 (0.975)</td>
<td>100</td>
</tr>
<tr>
<td>HC TeCA+TCE</td>
<td>0.030 (0.985) 0.119 (0.995) 0.144 (0.995)</td>
<td>110</td>
</tr>
</tbody>
</table>

LC, low concentration; HC, high concentration; NA, no product accumulation throughout the experiment; App, apparent. Note that removal time is based on sampling day when the respective chemical was not detected. Products refer to intermediate products of the parent compound.
Based on the 100% cVOC removal data given in Table 2.4, on the whole, the degradation products took a shorter period of time (0 – 42 d) than 1,1,2,2-TeCA (11 – 110 d) to be removed completely. Also, TCE, which was the most prominently detected daughter product of 1,1,2,2-TeCA, had higher degradation rates (0.144 – 0.317 d\(^{-1}\)) than 1,1,2,2-TeCA (0.030 – 0.185 d\(^{-1}\)).

A statistical comparison of the degradation rate data shown in Table 2.4, based on ANOVA and \(t\)-tests, indicate that, on the whole, the degradation rates were not significantly different (\(P \leq 0.05\)) among the three phases. This was the case in spite of the fact that, in Phase 3 1,1,2,2-TeCA took longer to degrade completely. Evidently, the three phases were even more comparable in terms of complete removal of the daughter products. This suggests that the partial and transitory starvation the of cVOC dechlorinators did not have a significant adverse effect.

A statistical comparison (\(t\)-test) of the degradation rate values of 1,1,2,2-TeCA between the low initial concentration treatments and the high initial concentration treatments showed that the degradation rates were significantly different. The 1,1,2,2-TeCA degradation rates for the low initial concentration treatments were on average 32.7% higher than those for the high initial concentration treatment. Moreover, the degradation rate of 1,1,2,2-TeCA was generally inversely correlated to the initial concentration of 1,1,2,2-TeCA. This suggests that the initial concentration of 1,1,2,2-TeCA has an influence on its rate of degradation, and as such is an important design consideration. This assertion is supported by mesocosm EWS study results (Chapter 8) and is in agreement with observations made by Kadlec (2000), Carleton (2002), and Kadlec and Wallace (2009).
A statistical analysis of 1,1,2,2-TeCA degradation rate values shows that the degradation rates of 1,1,2,2-TeCA for the 1,1,2,2-TeCA plus TCE co-treatment treatments were not significantly lower than the ones for the 1,1,2,2-TeCA alone treatments at $P = 0.05$, but they were significantly lower at $P = 0.10$. The degradation rates of 1,1,2,2-TeCA in the 1,1,2,2-TeCA alone treatments were 6.7 - 64.3% (average = 29.7%, SD $\leq$ 20.5%) higher than the ones for the 1,1,2,2-TeCA+TCE co-treatment treatments. In spite of co-treatment of 1,1,2,2-TeCA with TCE having an apparent negative effect on the degradation rate of 1,1,2,2-TeCA, the reduced 1,1,2,2-TeCA degradation rates were still high enough for most practical applications. As such, co-treatment of 1,1,2,2-TeCA with TCE is practically as feasible as treatment of 1,1,2,2-TeCA alone.

2.5 Application of the Study Results

In addition to its intended primary use as a bed material for EWSs treating cVOCs, the engineered soil discussed in this study can be used as a bioreactive component of remediation systems for removal of cVOCs from groundwater or leachate. Several possibilities exist; one of them is the use of the material as the biologically active component of a permeable reactive barrier (PRB) or trench. It is reasonable to expect that a PRB or trench using the engineered soil can effect removal of 1,1,2,2-TeCA and other cVOCs in rates and extents comparable to the ones reported in this study, if the soil is properly inoculated and maintained under anaerobic conditions.

Warith (1996) has derived a very simple expression for computing the width of a PRB required for complete removal of cVOCs based on a number of factors and assumptions. He considered the fact that the migration of cVOCs through a bioreactive wall is mainly influenced by advection and dispersion mechanisms. Further arguing that
the contribution of dispersion as a transport mechanism compared to that of advection is minimum, Warith (1996) simplified his original transport equations and came up with Equation 2.9, which is applicable to the data obtained in this study.

\[
\frac{\delta x}{\delta t} = -\frac{V_s}{R}
\]

[2.9]

It is obvious in Equation 2.9 that \(\delta x\) is the same as the width, \(W\) of a PRB using the engineered soil, while \(\delta t\) is the same as \(t_{100\%R}\) for a specific initial concentration of 1,1,2,2-TeCA as defined earlier. The negative sign indicates that the flux of cVOCs is downgradient. Therefore, the width of the wall can be calculated using Equation 2.10.

\[
W = \frac{t_{100\%R} \times V_s}{R}
\]

[10]

where \(W\) = effective width of the PRB or trench occupied by the engineered soil [L], \(t_{100\%R}\) = time to 100% removal of the cVOC of interest [T]; \(V_s\) = seepage velocity of groundwater or leachate to be treated [LT\(^{-1}\)]; \(R\) = retardation factor. In connection with the use of Equation 2.10, it should be pointed out that the width and seepage velocity are considered the main parameters of PRB design (Ahmad et al., 2007).

Using the retardation coefficient data (for 1,1,2,2-TeCA) from Table 2.2 and cVOC \(t_{100\%R}\) data from Table 2.4, widths of PRBs or trenches can be estimated as shown in Table 2.5 for the worst case scenarios for both 1,1,2,2-TeCA and its daughter products for 1,1,2,2-TeCA only and 1,1,2,2-TeCA plus TCE. It should be borne in mind that the given widths are effective only for the respective or lower initial concentration of the parent compounds or daughter products.

The results shown in Table 2.5 are conservative because the \(t_{100\%R}\) data are based on the sampling day when the chemical of interest was not detected in the samples. For example, for \(t_{100\%R} = 100\) d, the last sampling day when 1,1,2,2-TeCA was detected was
$t_{n-1} = 8.3$ d. This means the width of the wall given in Table 2.5 is overdesigned by between 0 and 8.4%. For $t_{100\%R} = 35$ d, $t_{n-1} = 24$ d, hence the magnitude of the overdesign error is between 0 and 45.8%. The magnitude of the overdesign error can be reduced by making $\Delta t = t_{100\%R} - t_{n-1}$, which represents the resolution of the data, as small as possible. This can in turn be achieved through increasing the sampling frequency. Except for the fact that it is costly, conservative design avails a desirable design safety factor.

Table 2.5: Estimation of permeable reactive barrier (PRB) effective width for complete removal of 1,1,2,2-TeCA using the engineered soil

<table>
<thead>
<tr>
<th>Pollutants</th>
<th>Worst case scenario input data</th>
<th>Pollutants</th>
<th>Worst case scenario input data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_O$ (mg/l)</td>
<td>$t_{100%R}$ (d)</td>
<td>$V_s$ (cm/d)</td>
</tr>
<tr>
<td>Parent cVOCs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC TeCA only</td>
<td>11.449</td>
<td>100</td>
<td>6.37</td>
</tr>
<tr>
<td>HC TeCA + TCE</td>
<td>11.827</td>
<td>110</td>
<td>6.37</td>
</tr>
<tr>
<td>Daughter products</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC TeCA only</td>
<td>4.03</td>
<td>35</td>
<td>6.37</td>
</tr>
<tr>
<td>HC TeCA + TCE</td>
<td>11.83</td>
<td>42</td>
<td>6.37</td>
</tr>
</tbody>
</table>

$HC = high\ concentration; \ LC = low\ concentration. \ C_O = initial\ concentration\ of\ the\ cVOC\ of\ interest; \ t_{100\%R} = time\ to\ 100\%\ removal\ of\ cVOCs. \ Groundwater\ velocity = 2 – 5\ cm/d,\ hence\ with\ porosity\ of\ 0.55,\ average\ seepage\ velocity\ through\ the\ wall = 6.37\ cm/d.$

It is evident from Table 2.5 that the requirements for the worst case scenarios for the parent compounds are far bigger than those for the daughter products. Notably, the engineered soil does not have to extend more than 34 cm of the PRB or trench. Widths of PRBs reported in the literature are in the range 30 - 45 cm (Ahmad et al., 2007). Ahmad et al. (2007) have also presented a design approach for PRBs based on organic mulch.
The mesocosms scale EWS, for which this study was meant to serve as a prelude, was to be operated at an initial hydraulic loading rate of 2.56 cm/d. Given a porosity of 0.55 for the engineered soil, the seepage velocity would be 4.65 cm/d. Using Equation 2.10, the effective depth, $H_E$ of the upflow mesocosm scale EWS for the worst case scenario when treating a mixture of 1,1,2,2-TeCA and TCE (Table 2.5) would be:

$$H_E = 110 \times 4.65 \times (d)/20.92 = 24.45 \text{ cm} \approx 25 \text{ cm}.$$ 

It should be pointed out that an effective depth of 25.5 cm was adopted for the mesocosm scale EWS units that were set up in the greenhouse to follow up on this study (Chapter 3). Commercially available glass aquaria that could accommodate this depth were acquired for this purpose.

As discussed in greater depth in Chapter 8, an increase in the hydraulic loading rate, depending on its magnitude, would likely result in breakthrough of the cVOCs. As reported in another study (Chapter 3), the depth of 25.5 cm turned out to be sufficient for the complete removal of 1,1,2,2-TeCA and TCE and the accompanying degradation products over wide ranges of pollutant and hydraulic loading rates. Among other things, in a way this validates the depth estimation approach used in this study.

### 2.6 Conclusions

This study was carried out to evaluate the treatability of a mixture 1,1,2,2-TeCA and TCE and effects of transitory starvation in a substrated earmarked for an upflow engineered wetland system (EWS) for treating chlorinated ethenes and ethanes. The study served as a prelude to mesocosm scale studies. Results from this study were also used to estimate key dimensions of mesocosm scale EWS units that were used in a subsequent follow up study. On the whole, treatability of the mixture of 1,1,2,2-TeCA and TCE was found to be feasible. On the other hand, transitory starvation of the microorganisms
responsible for treatment of the cVOCs was found to have practically negligible effects. Other important conclusions that can be drawn from this study are outlined below.

- 1,1,2,2-TeCA and TCE as well as their daughter products degraded completely within 110 days for the high concentration treatments and within 48 days for the low concentration treatments.

- Generally, its daughter products were degraded faster than 1,1,2,2-TeCA. This obviates risks imminent from daughter products like vinyl chloride which are more harmful than their parent chemicals.

- 1,1,2,2-TeCA degraded mainly through dehydrochlorination to TCE followed by reductive dechlorination of TCE to cis-1,2-DCE, cis-1,2-DCE to vinyl chloride (VC), and VC to ethene.

- Generally, 1,1,2,2-TeCA degraded more slowly at higher initial concentrations. Therefore, pollutant initial concentrations are an important EWS design consideration.

- Dechlorination rates of 1,1,2,2-TeCA in treatments containing 1,1,2,2-TeCA alone were only slightly higher than the ones for treatments in which 1,1,2,2-TeCA and TCE were co-treated. Therefore, co-treatment of 1,1,2,2-TeCA with TCE is practically as feasible as treatment of either 1,1,2,2-TeCA or TCE alone.

- Concentrations of hydrogen gas (the electron donor) were comparable in all treatments except the killed control treatment in which it was higher and more stable. As such, differences in dechlorination rates among the treatments are not attributable to the electron donor availability.

- Methane gas concentration decreased in parallel with the dechlorination of 1,1,2,2-TeCA and its daughter products. Methane levels recovered to pre-
dechlorination levels immediately upon depletion of the cVOCs. The decrease in methane concentration during dechlorination is attributable to its consumption in reverse methanogenesis processes.
3.1 Introduction

Environmental pollution problems and public health implications of chlorinated volatile organic compounds (cVOCs) are widely recognized and reported in the literature. Serious concern on implications of cVOCs stems from their widespread presence in groundwater in many parts of the world (Häggblom and Gosset, 2003; Löffler et al., 2003) and the severity of their effects since many are toxic and some (such as vinyl chloride (VC)) are carcinogenic (McCarty, 1997; Mohr, 2004). Another reason for concern stems from the difficulty in both containing cVOC pollution to minimize spreading and cleaning up contaminated sites. Challenges to effective and efficient in-situ and ex-situ treatment of cVOC-contaminated groundwater and soil include the relative recalcitrance of some of these compounds (McCarty, 1997). For example, whereas TCE biodegrades relatively easily, its daughter product VC is known to be fairly resistant to both biotic and abiotic degradation. An additional challenge pertains to the need to modify the often unsuitable site attributes and engineer the treatment systems to achieve the chemistry, substrate composition and availability, and microbial community structure suitable for the target cVOCs.

In spite of the difficulty and complexity of these challenges, many success stories have been reported. Studies on systems for treating cVOCs ranging from laboratory scale to field scale remediation have been reported (Kassenga, 2003; Kassenga et al., 2003 Lorrah and Voytek, 2004; Aulenta et al., 2006). Notably, Pardue et al. (2000) have developed, tested, and implemented successfully an upflow EWS for treating cVOC
contaminated groundwater and soil. Studies on the development and testing of this treatment system concept have been reported in previous publications (Kassenga, 2003; Kassenga et al., 2003). Other researchers have also reported studies on cVOC treatment systems based on concepts similar to the one developed by Pardue et al. (2000). Nonetheless, there are knowledge and practical experience gaps regarding the effectiveness and efficiency of EWS with respect to treatment of mixtures of cVOCs. The lacking knowledge and experience are an important issue because cVOCs do often occur in mixtures. Notably, even degradation of single cVOCs results in mixtures of parent chemicals and daughter products. Treatment of mixtures of cVOCs is different from treatment of single chemicals in that interactions among several chemicals can result in either desirable or undesirable outcomes. For example, some chemicals exhibit negative competitive effects when treated together (Aulenta et al., 2005; Yu et al., 2005).

Another knowledge and experience gap that needs to be addressed pertains to the role and influences of wetland plants on the performance of EWSs. In this respect, whether or not EWSs vegetated with different plant species are comparable, is a very important issue. This is because different wetland plant species are expected to influence the soil they are grown in and the microorganisms they support differently because of their different ecological traits (Bardgett, 2005). In turn, the differences in the influence of the different wetland plant species on soil and soil microorganisms is expected to translate into differences in EWS performance differences.

It is important to note that the issue of the comparability of EWSs vegetated with different plant species has multidimensional importance that spans beyond performance. This is mainly because the multifaceted nature and applicability of wetlands give rise to different sets of, occasionally irreconcilable, wetland plant selection criteria among
different stakeholders. For example, while both *Phragmites communis* and *Typha latifolia* are the clear favorite wetland plants for EWSs, there is also no doubt that there are mixed feelings about them; they are liked by some and despised by others. *Phragmites communis* is considered an invasive and hence undesirable plant in most of Eastern North America (Mitsch and Gosselink, 2007). Its being a rapid colonizer makes *Typha latifolia* suitable for EWSs. However, the same trait makes it a “bully” when it grows among other plant species, which it practically edges out. For this reason, *Typha latifolia* is also considered undesirable by some wetland managers (Mitsch and Gosselink, 2007). It is important to point out as well that water budget is an important additional consideration when selecting wetland plants since different types of plants exhibit different evapotranspiration rates.

This study focused on *Phragmites communis* and *Typha latifolia* because: 1) they are generally the commonest wetland plants in the USA and the world over, which gives them priority over other wetland plants; 2) wetland technology practitioners generally prefer these plants to the others, most likely because of having more experience with them than with the others; and 3) these plants are native to many parts of the USA and ubiquitous worldwide, which increases the potential applicability and comparability of the results from this study with others from around the world.

The main objectives of this study were: 1) to assess the feasibility of treating mixtures of cVOCs in EWSs, 2) to compare the performance efficiencies of EWS units vegetated with *Phragmites communis* and *Typha latifolia*, and 3) to test an EWS inoculation approach as well as assess the effect on performance of inoculation of the EWS units with a halorespiring bacterial culture. A mixture of 1,1,2,2-TeCA and TCE was used as a test cVOC mixture because the two cVOCs are common groundwater and
soil contaminants and often occur together. A more detailed discussion on this issue is presented in Chapter 2.

3.2 Materials and Methods

3.2.1 Experimental Set-up and Characteristics

The experimental set up used in this study was as schematically illustrated in Figures 3.1 and 3.2.

![Figure 3.1: Schematic lay out plan of four mesocosm scale engineered wetland system units for co-treating 1,1,2,2-TeCA and TCE. Note that the discharge line from each pump delivers the influent solution to each EWS unit through three inlets in an upflow mode.](image)

To facilitate control of feed water flow, the experimental system was designed to be operated as a set of four independent engineered wetland system (EWS) units as shown in Figure 3.1. Each EWS unit comprised one feed water reservoir, one pumping system, and one upflow EWS unit.

The feed water reservoir consisted of a cylindrical container with a capacity of 37.8 L. At the planned flow rate of 14.4 L/d, this storage volume would be sufficient for at least 2.5 d of operation. However, the feed water in the reservoir was replenished daily...
to minimize head space with a view to minimizing volatilization of the cVOCs. As illustrated in Figure 3.2, the feed water reservoir had an outer removable fixed lid and a floating lid to minimize volatilization of the cVOCs from the feed water. The removable outer lid was made of steel to make it heavy for tightness to minimize air exchange between the outside and inside of the reservoir. In contrast, the floating lid was made of Styrofoam to enable it to float, but lined with Teflon to minimize sorption. The rest of the reservoir was made of steel for structural strength, but coated with a special metallic paint for corrosion protection and minimization of sorption.

![Diagram](image)

**Figure 3.2:** Schematic section of mesocosm scale engineered wetland system unit for co-treating 1,1,2,2-TeCA and TCE

The pumping system comprised a peristaltic pump and Tygon tubing for suction of the feed water from reservoir and controlled discharge into the EWS units. The feed water was pumped through each EWS unit in an upflow manner in order to minimize volatilization of the influent and enhance anaerobic conditions by reducing contact between the influent and the air.
Each of the four EWS units was constructed using a rectangular aquarium measuring 90.2 cm long, 30.5 cm wide, and 25.5 cm deep (to the highest sampling port). The depth of the EWS units was selected based on findings from a previous study (Chapter 2). All the four EWS units were set up side by side in the greenhouse so as to be able to control environmental conditions (temperature, humidity, precipitation, and light) and ensure that all EWS units were exposed to the same conditions. The EWS units were covered with aluminum foil to prevent the growth of photosynthetic organisms and were kept fully water-saturated for the whole duration of the study.

Three parallel inlets installed through the bottom of each EWS unit were provided to facilitate uniform distribution of flow across the wetland unit bed. The connection for the effluent discharge was installed near the top. The effluent discharge line terminated in a covered container to minimize unaccounted for effluent losses that could introduce errors in the water balance. Only losses from the EWS bed through evapotranspiration were deliberately not controlled.

Sampling ports were provided in an array of three equidistant vertical rows and five horizontal rows spaced at different heights from the bottom of the EWS units (a total of 15) (Figures 3.1 and 3.2). Biopsy needles were installed to serve as sampling ports to facilitate convenient withdrawal of pore water samples from the EWS bed. Each biopsy needle was secured in place by a rubber plug inserted in a hole drilled through the side of the EW unit. Stainless steel connectors for inlets were similarly secured in place.

3.2.2 Experimental Materials and Wetland Plants

3.2.2.1 Wetland Bed Material Mix Design and Blending

The four EWS units were packed with a bed material blended using three constituents: BionSoil, a processed animal waste (Dream Maker Dairy, Cowlesville, NY),
Latimer peat (Latimer’s Peat Moss Farm, West Liberty, OH), and commercial sand from Home Depot (Baton Rouge, LA). The three bed material components were blended at a ratio of BionSoil (37.5% w/w), Latimer peat (37.5% w/w), and sand (25% w/w). This ratio was selected based on treatability and geotechnical studies reported by Kassenga et al. (2003) and treatability studies carried out as a component of this dissertation (Chapter 1). Each component of the blended bed material was intended to serve a specific purpose as discussed elsewhere (Kassenga et al., 2003; Chapter 1).

Mixing of the separate bed material components to obtain a uniform mixture was done before packing. It was done in small batches that were manageable and whose uniformity was easy to control and check. Packing of the blended EWS bed material into the EWS unit was done carefully to minimize segregation of the constituents. The blended EWS bed material was transferred into the EWS unit in small portions. After packing, the EWS bed material was visually inspected for uniformity and discrepancies corrected. The resulting engineered wetland bed material had characteristics given in Table 3.1.

After inspection of the packed bed material, all the EWS units were covered with aluminum foil to prevent growth of photosynthetic organisms. Immediately after that, tap water was pumped into each unit to saturate the bed material. The water was pumped into rather than poured onto the bed in order to avoid segregation of the bed constituents. The flow rate was also kept low to avoid fluidization of the bed as well as segregation of the bed constituents. Consequently, the initial filling of the EWS units took a long time (days). After filling, each EWS bed was kept fully saturated with water by continued pumping to replace the water lost through evaporation.
Table 3.1: Engineered wetland bed material basic characterization data and bed specifications

<table>
<thead>
<tr>
<th>Characterization parameter</th>
<th>Mean value and standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated moisture content (%)</td>
<td>62.03±3.05</td>
</tr>
<tr>
<td>In-situ moisture content (%)</td>
<td>32.10±3.36</td>
</tr>
<tr>
<td>Saturated bulk density (kg/L)</td>
<td>1.2470±0.0096</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>55.34±0.94</td>
</tr>
<tr>
<td>Organic fraction (%)</td>
<td>21.82±0.86</td>
</tr>
<tr>
<td>$d_{10}$ (cm)</td>
<td>0.002 – 0.15</td>
</tr>
<tr>
<td>Hydraulic conductivity (cm/s)</td>
<td>$8.3 \times 10^{-4}±1.31\times10^{-4}$</td>
</tr>
<tr>
<td>Bed effective length (cm)</td>
<td>90.17</td>
</tr>
<tr>
<td>Wetland effective width (cm)</td>
<td>30.48</td>
</tr>
<tr>
<td>Bed effective depth (cm)</td>
<td>25.50</td>
</tr>
<tr>
<td>Bed surface area (cm$^2$)</td>
<td>2748.4</td>
</tr>
<tr>
<td>Bed total volume (cm$^3$)</td>
<td>70083.7</td>
</tr>
<tr>
<td>Bed pore volume (cm$^3$)</td>
<td>38546.0</td>
</tr>
</tbody>
</table>

$^a$ Adopted from Kassenga et al. (2003).

3.2.2.2 Wetland Plant Selection

*Phragmites communis* (common reed) and *Typha latifolia* (cattail) were selected to be evaluated as alternative wetland plants in this study generally because they are two of the commonest and ubiquitous wetland plants. In connection with wetland systems used for treatment, *Phragmites* and *Typha* are the clear favorite wetland plant species (Mitsch and Gosselink, 2003&2007) and a considerable amount of literature is available on them. More specifically, *Phragmites communis* was selected because it has been found to flourish in areas contaminated with cVOCs and there have been indications that it enhances processes that treat cVOCs (Lorah et al., 1999, Pardue et al. 2000; Pardue, 2005). On the other hand, *Typha latifolia* is preferred by potential users of the wetland technology because it supports habitats suitable for diverse fauna species unlike the more woody *Phragmites communis*. The hypothesis that *Typha latifolia* could be as effective as *Phragmites communis* in terms of treatment functions was inferred from the fact that their
root systems bear some similarities. Comprehensive information on *Phragmites communis* and *Typha latifolia* along with other major wetland plants is presented in Correll and Correll (1972). Though less comprehensively, Mitsch and Gosselink (2000&2007), Kadlec et al. (2000), and Kadlec and Wallace (2009) provide some information on these wetland plant species too.

### 3.2.2.3 Feed Water – Synthetic Groundwater

In this study, a solution of 1,1,2,2-TeCA and TCE was used as the synthetic groundwater. The target concentrations for the two compounds in the feed water were selected to bear a ratio of 2:1. The two chemicals were selected because they often occur together at cVOC contaminated sites in both soil and water. The two compounds also produce degradation products that are common at many cVOC contaminated sites in the USA. The ratio of 2:1 was selected because the two chemicals occur at that concentration ratio at the remediation site of interest.

Fresh batches of feed water were prepared for replenishing the feed water storage reservoir daily just prior to use. They were prepared from stock solutions which were in turn prepared from neat 1,1,2,2-TeCA and TCE (Supelco, Bellefonte, PA). At any time during this study, the exact concentrations of 1,1,2,2-TeCA and TCE depended on the phase of the study. The initial target concentrations were 1 mg/L and 0.5 mg/L for 1,1,2,2-TeCA and TCE, respectively.

### 3.2.3 Experimental Methods and Procedures

#### 3.2.3.1 Wetland Bed Characterization

Sorption experiments were carried out for the individual as well as the combination of all the test compounds in 40 mL VOA vials (ICHEM) with 5 g of the engineered wetland soil and sodium azide as the biocide. Six concentration levels
covering the concentration range of interest were used to generate data points for each sorption isotherm. Each data point was obtained using triplicate vials adding up to 18 vials per isotherm. Dilutions of the test chemicals were made at 1:1, 1:2, 1:5, 1:10, and 1:20 using an electrolyte solution (0.01 M CaCl₂). After adding the test soil and filling with chemical solution and capping, the vials were shaken on a reciprocating shaker at 20°C for 48 h. They were then centrifuged after which the supernatant was withdrawn and analyzed for the test compounds using EPA Method 8260 B, which is described later.

The coefficients of hydraulic conductivity of the wetland media were determined using the Falling-head permeability test described elsewhere (Pardue, 2005).

3.2.3.2 EWS Commissioning and Initial Operation

Commissioning of the EWS units started with calibration of the pumps and flow adjustment to ensure that all the pumps were pumping the same amount of flow through the EWS units and were delivering the desired discharges. Flow adjustment was done regularly throughout the study to account for changes in hydraulic resistance of the bed over time. The wetland bed was conditioned by gradual increase in flow rate without exceeding the critical seepage flow rate for the unplanted beds (Kassenga et al., 2003). This was meant to avoid fluidization and stratification of the beds, and flushing out of fines.

The next commissioning stage was hydrodynamic characterization of the bed (tracer study). To prepare the EW beds for the tracer study, the beds were flushed by pumping through them plain water continuously until the background concentration of chloride, which was to be used as the conservative tracer, was negligible. Flushing lasted two weeks.
3.2.4 EWS Hydrodynamic Characterization – Tracer Study

Sodium chloride (common salt) was selected to be used as the conservative tracer because it is reasonably reliable and yet inexpensive and easy to work with. The suitability of sodium chloride as a tracer is backed by the fact chloride salts have been widely used in tracer studies (Ours, et al., 1998). In fact, salts in general are widely used as tracers (Schimid, 2004). To make the tracer solution, reagent grade sodium chloride was dissolved in water to a final concentration of 220 mg/L. The resulting solution was used as the feed water. It was pumped through each EWS unit continuously at a constant rate of 11.2 liters per day (0.467 L/hour). Although all the EWS units were fed with the feed water containing the tracer, for convenience, only one unit was used for the tracer study. Water samples were taken from the sampling port located at the height of 25.5 cm at convenient predetermined intervals and analyzed immediately for chloride.

3.2.5 Preparation and Planting of Wetland Plants

*Phragmites communis* and *Typha latifolia* plants used in this study were bought from Hydra Aquatica (Tijeras, NM 8705 USA). The seedlings that were bought had been growing in nurseries prior to being shipped. Only seedlings of the right age for transplanting were selected. All the plants were shipped and delivered in separate containers for each type of plant and each container had separate slots for each seedling to facilitate their separation during transplanting.

Immediately upon delivery from the supplier, the seedlings were transplanted to large plastic containers kept in the greenhouse. A starting separation distance of about 10 cm between seedlings was adopted to allow the plants to grow and develop their root systems freely. All the plants were grown in a mixture of a commercial potting soil (Miracle Gro) and sand (3:1 w/w). Sand was mixed with the potting soil because media
for engineered wetland systems incorporate sand to improve their geotechnical and hydrodynamic properties.

All the seedlings were grown under the same conditions and for the same length of time before use. The plants were allowed to grow to maturity so as to ensure adequate development of their root systems. Transplanting of seedlings from the nurseries to the EWS units was done as soon as enough seedlings were ready. Seedlings were assigned to EWS units at random. Twelve wetland plants (shoots) were planted in each unit, which is a plant density of about 43 plants/m². This high plant density was selected deliberately to accelerate the growth of wetland plants in the EWS units and consequently jumpstart the operation of EWS units and the study.

3.2.6 Water Budget Assessment and Monitoring

Water budgets in the wetlands are very important because the presence of water is what differentiates a wetland system from a land ecosystem. Moreover, water is the main determining factor of wetland processes. The computation of hydraulic and pollutant loading rates as well as evapotranspiration rates was based on the following water balance considerations:

\[
\frac{\Delta V}{\Delta T} = (Q_{IN} - Q_{EF}) + (Q_{IF} - Q_{SE}) + (Q_{PR} - Q_{ET}) + \frac{\Delta V_s}{\Delta T} \quad [3.1]
\]

where \(\Delta V_s/\Delta T\) = change in storage of water in the EWS unit [L³T⁻¹], \(\Delta V_s\) = storage volume change [L³], \(\Delta T\) = time increments (time interval corresponding to specific volumes of influent and effluent measured) [T], \(Q_{IN}\) = inflow rate [L³T⁻¹], \(Q_{EF}\) = effluent flow rate, \(Q_{IF}\) = infiltration rate [L³T⁻¹], \(Q_{SE}\) = seepage or leakage rate [L³T⁻¹], \(Q_{PR}\) = precipitation rate [L³T⁻¹], \(Q_{ET}\) = evapotranspiration rate [L³T⁻¹]. Since the EWS units had no leak and were screened from rain, \(Q_{SE} = 0\) and \(Q_{PR} = 0\). Furthermore, all the EWS
units were operated in the same hydrologic regime, which means there was no net change
of storage, hence $\Delta V_S/\Delta T = 0$. As such, Equation 3.1 can be simplified into Equation 3.2
considering:

$$ Q_{EF} = (Q_{IN} - Q_{EF}) = \frac{\Sigma V_{IN}}{\Sigma (\Delta T)} - \frac{\Sigma V_{EF}}{\Sigma (\Delta T)} $$

$$ Q_{EF} = \frac{1}{\Sigma (\Delta T)} (\Sigma V_{IN} - \Sigma V_{EF}) \quad \text{[3.2]} $$

where $V_{IN}$ = volume of influent [L], $V_{EF}$ = volume of effluent [L]. On account of Equation
3.2, the water balance in each wetland system unit was monitored by keeping records of
quantities of water pumped into each EWS unit, quantities of effluent discharged by each
EWS unit, and the pertinent time intervals. The influent flow rates were based on
quantities of water actually pumped into the wetlands while the effluent flow rates were
based on quantities of water that actually came out of the wetland system.

3.2.7 Aboveground Biomass Monitoring

The aboveground biomass productivity was monitored on the basis of the total
number of wetland plants (shoots) in the each wetland system unit. All wetland plant
shoots (old and new) were counted manually during each counting session.

3.2.8 EWS Inoculation with a Halorespiring Microbial Culture

The inoculum for all the four wetland system units were cultured in 160 mL
capacity serum bottles. They were themselves inoculated with portions of slurry known
to contain the bacterium *Dehalococcoides ethenogenes*, which is capable of degrading
TCE to ethene. Six (6) serum (inoculum) bottles were prepared so that one and a half
inoculum bottles would be available for inoculating each of the four wetland system
units.
The total volume of each inoculum bottle was apportioned as follows: BionSoil (30 mL), Latimer peat (30 mL), *Dehalococcides ethenogenes*- containing slurry (22.5 mL), DI water (52.5 mL), and head space (the remaining volume).

The inoculum bottles were spiked with a solution of 1,1,2,2-TeCA/TCE (2:1 concentration ratio) two times and monitored for both the aqueous phase and gaseous phase degradation products from May 17 to May 27, 2004. The spiking of the 1,1,2,2-TeCA/TCE solution into the inoculum bottles was done after it was observed that the two compounds had degraded completely. The starting concentrations of both 1,1,2,2-TeCA and TCE in the inoculum bottles were increased from 1 mg/L and 0.5 mg/L for 1,1,2,2-TeCA and TCE, respectively during the first spiking to 5 mg/L and 2.5 mg/L for 1,1,2,2-TeCA and TCE, respectively in the last spiking.

The inoculum was delivered into each of the four wetland system units through the three bottom (lowest level) sampling ports using 60 mL syringes. About 203 mL of inoculum was fed into each of the wetland system unit. Inoculation of all the wetland system units was completed in about four hours and was done in accordance with the following order: EWS unit 1 (*Typha* 1), EWS unit 2 (*Phragmites* 1), EWS unit 3 (*Typha* 2), EWS unit 4 (*Phragmites* 2). After inoculation the wetland system units continued to be fed with 1,1,2,2-TeCA/TCE solution as was the case before inoculation.

### 3.2.9 EWS Routine Operation and Maintenance

Routine operation and maintenance aspects included the activities done to ensure that the study was executed successfully and smoothly. These activities are documented here because operation and maintenance are an important practical aspect of every treatment technology. Most importantly, some of the needs pointed out here apply to experimental as well as full scale EWS systems. The operation and maintenance needs
were: 1) replenishment of the feed water (1,1,2,2-TeCA + TCE solution or tracer solution); 2) monitoring and adjustment of flow rates; 3) care of wetland plants, including killing of pests; 4) checking for and repairing leaks; 5) checking for and clearing blockages; 6) replacing worn out tubing; 7) measurement of inflow and outflow for water balance accounting; 8) collection and proper disposal of effluent; and 9) inspection for and repair of structural damage to the EWS units, for example, cracks.

3.2.10 Sampling and In-situ Analysis

Samples of pore water for the tracer study were taken from the sampling port located at the height of 25.5 cm above the bottom. Other samples were taken from all the other sampling ports (a total of 15 per EWS unit). Samples from the same EWS unit were always taken at the same time and analyzed together. Samples from EWS units that were compared were taken at the same time and analyzed together in sets of corresponding sampling ports. All samples were analyzed immediately after collection, which obviated the need for sample treatment and storage. Samples for pH and temperature monitoring were analyzed on site. The rest of the samples were taken to the laboratory.

3.2.11 Analytical Work

Aqueous phase samples were analyzed for VOCs using EPA Method 8260B using GC/MS (Agilent Technologies 6890N Network GC System, Agilent Technologies 5973Network Mass Selective Detector) coupled to an AquaTek 70 Autosampler® (Teledyne Tekmar) and Velocity XPT® purge and trap sample concentrator (Teledyne Tekmar). Methane, ethene, and ethane gases were analyzed using GC/FID. 1 mL of head space sample was injected into the gas chromatography with flame ionization detector (Agilent 5890 Series II) equipped with a 2.4 m x 0.32 mm ID column packed with
Carbopack b/1% Sp-(Supelco, Bellefonte, PA). The column 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. Analytical standards and surrogate for the eVOCs were obtained as mixtures from Supelco Inc.

Hydrogen was analyzed using a reduction gas analyzer (Trace Analytical, Menlo Park, CA) equipped with a reduction gas detector. Head space samples were injected into a 1 mL gas sampling loop prior to being separated using 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 the carrier gas. The carrier gas was first passed through a catalytic combustion converter (Trace Analytical, Menlo Park, CA) to remove traces of H₂. Methane, ethane, and ethane calibration gases were obtained Gas Products (Baton Rouge, LA). Analysis of methane, ethene, and ethane in the liquid phase was done using the methods described by Kassenga et al. (2003).

Chloride in the tracer study samples was analyzed using a spectrometer instrument (Hach Co., USA) in accordance with the manufacturer’s instructions. All tracer study samples were analyzed immediately after collection.

3.3 Data Analysis and Modeling

3.3.1 Sorption Data Modeling

The data obtained from sorption experiments were modeled using both the linear sorption model (Equation 3.3) and the Freundlich sorption model (Equation 3.4).

\[ q_s = K_d C_e \]  \hspace{1cm} [3.3]

\[ q_s = K_F C_e^N \]  \hspace{1cm} [3.4]
where \( q_s \) = mass of the chemical sorbed per unit mass of the engineered soil [MM\(^{-1}\)], \( K_d \) = linear model sorption distribution coefficient [L\(^3\)M\(^{-1}\)], \( C_e \) = equilibrium concentration [ML\(^{-3}\)], \( K_F \) = Freundlich sorption constant [L\(^3\)M\(^{-1}\)]\(N\), and \( N \) = Freundlich exponent. The retardation factor was calculated using Equation 3.5.

\[
R = 1 + \frac{K_d \rho_b}{n}
\]  

[3.5]

where, \( R \) is the retardation factor, \( K_d \) = distribution coefficient (L/kg), \( \rho_b \) = bulk density of the soil (kg/L), and \( n \) = porosity.

### 3.3.2 Tracer Study Data Analysis

For the purpose of determining the coefficient of hydrodynamic dispersion, \( D \) the tracer study data can be transformed to give the scaled time parameter, \( \zeta \), which is defined by Equation 3.6 (Charbeneau, 2000).

\[
\zeta = \frac{T - 1}{\sqrt{2T}}
\]  

[3.6]

where \( T = tv/L \), the characteristic time, \( t \) = time (s), \( v \) = velocity (cm/s), \( L \) = characteristic length (depth) of the wetland (cm). The coefficient of hydrodynamic dispersion can be estimated using Equation 3.7. Equation 3.7 makes use of the plot that relates the time – scaled parameter to the relative concentration of the tracer.

\[
D = \frac{vL}{4} (\zeta_{0.84} - \zeta_{0.16})^2
\]  

[3.7]

To model the tracer study results, suitable solutions to the general differential equation for one-dimensional flow transport (Equation 3.8) were considered.

\[
\frac{\partial c}{\partial t} + v \frac{\partial c}{\partial x} = D \frac{\partial^2 c}{\partial x^2}
\]  

[3.8]
where \( c = \) concentration, \( x = \) distance of the column, \( t = \) time, and \( D = \) coefficient of dispersion.

Four types of transport models, which are discussed at length in the literature (Charbeneau, 2000), were found to be suitable for processing the tracer study data. The models are conveniently identified by Charbeneau (2000) as Type 1 Model (Equation 3.9), Type 2 Model (Equation 3.10), Type 3 Model (Equation 3.11), and Simple Model (Equation 3.12).

\[
\frac{c}{c_o} = \frac{1}{2} \left( \text{erfc} \left( \frac{x-vt}{\sqrt{4Dt}} \right) + \exp \left( \frac{xv}{D} \right) \text{erfc} \left( \frac{x+vt}{\sqrt{4Dt}} \right) \right) \tag{3.9}
\]

Type 1 Model (Equation 3.9) assumes that inlet concentration is constant, and the boundary conditions are of the form \( c(0,t) = c_o \), where \( c_o \) is the concentration in the source reservoir (Charbeneau, 2000).

\[
\frac{c}{c_o} = \frac{1}{2} \left( \text{erfc} \left( \frac{x-vt}{\sqrt{4Dt}} \right) + \frac{4v^2t}{\pi D} \exp \left( -\frac{(x-vt)^2}{4Dt} \right) - \left( 1 + \frac{vx}{D} + \frac{v^2t}{D} \right) \exp \left( \frac{vx}{D} \right) \text{erfc} \left( \frac{x+vt}{\sqrt{4Dt}} \right) \right) \tag{3.10}
\]

Equation 3.10 assumes that the flux from the source reservoir is constant, and as such the flux out of the reservoir is only due to advection. The pertinent boundary condition is given by \( qc_o = qc - nD \frac{\partial c}{\partial x} \bigg|_{x=0} \), where \( q \) = seepage velocity and \( n \) = porosity (Charbeneau, 2000).

\[
\frac{c}{c_o} = \frac{1}{2} \left( \text{erfc} \left( \frac{x-vt}{\sqrt{4Dt}} \right) - \exp \left( \frac{vx}{D} \right) \text{erfc} \left( \frac{x+vt}{\sqrt{4Dt}} \right) \right) \tag{3.11}
\]

Equation 3.11 assumes that the mass (of the chemical of interest) is introduced at the origin at a constant rate, but it can diffuse both upstream and downstream.
Additionally, mass is advected downstream. The pertinent boundary condition is given by

\[ \int_{-\infty}^{\infty} nc(x,t)dx = q c_o t \] (Charbeneau, 2000).

\[
\frac{c}{c_o} = \frac{1}{2} \left( \text{erfc} \left( \frac{x-vt}{\sqrt{4Dt}} \right) \right)
\]  \[3.12\]

For Equation 3.12 (Simple Model), the column is taken 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 solution constituents from mixing.

### 3.3.3 Analysis and Modeling of cVOC Removal Data

The cVOC data were statistically processed and summarized. They were evaluated for statistical integrity using the appropriate tests (\( t \)-tests, ANOVA). 1,1,2,2-TeCA degradation data were fit to a pseudo-first order kinetic model (Equation 3.13):

\[ [E] = [E_o] e^{-k_E x} \]  \[3.13\]

where \([E]\) = molar concentration of 1,1,2,2-TeCA (moles/L), \( k_E \) = 1,1,2,2-TeCA removal rate constant (cm\(^{-1}\)), \( x \) = distance (cm), and the subscript ‘o’ refers to initial conditions. Recognizing that the degradation rate of TCE resulting from the dehydrochlorination of 1,1,2,2-TeCA was dependent on the degradation of both 1,1,2,2-TeCA and TCE itself, the pertinent data were modeled according to Equation 3.14.

\[
\frac{d[C]}{dt} = k_E [E_o] e^{-k_E x} - k_C [C]
\]  \[3.14\]

where \([C]\) = molar concentration of TCE (moles/L) and \( k_C \) = the actual rate of TCE degradation [cm\(^{-1}\)]. Equation 3.14 was solved to yield Equation 3.15 which was used to model the TCE data.

\[
[C] = \frac{k_E [E_o]}{k_C - k_E} e^{-k_E t} + \left( [C_o] - \frac{k_E [E_o]}{k_C - k_E} \right) e^{-k_C t}
\]  \[3.15\]
Use of Equation 3.15 for regression analysis of 1,1,2,2-TeCA and TCE data to determine the actual (as opposed to the apparent) reaction rate constant of TCE was done using SigmaPlot (SPSS Inc., Chicago IL) with a few mathematical manipulations. The apparent kinetic rate constant of TCE was determined using Equation 3.13 but with \( [E] \) and \([E_0]\) replaced by \([C]\) and \([C_0]\), respectively.

### 3.4 Results and Discussion

#### 3.4.1 EWS Bed Characterization

Results of the sorption experiments are presented in Table 3.2 along with retardation coefficient data derived from them. It is noteworthy that, chloroform was tested along with the other major test compounds because it was deemed of interest although not as much as 1,1,2,2-TeCA and TCE.

**Table 3.2: Summary of sorption experiment results and retardation coefficient data**

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Linear model</th>
<th>Freundlich model</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( K_d ) (L/kg)</td>
<td>( K_F ) (L/kg) ( N )</td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>8.0</td>
<td>9.70</td>
<td>0.8591</td>
</tr>
<tr>
<td>TCE</td>
<td>23.1</td>
<td>23.70</td>
<td>0.9572</td>
</tr>
<tr>
<td>cis-1,2-DCE</td>
<td>8.3</td>
<td>7.67</td>
<td>0.9954</td>
</tr>
<tr>
<td>Chloroform(^b)</td>
<td>7.9</td>
<td>7.44</td>
<td>1.0305</td>
</tr>
<tr>
<td>TCE(^b)</td>
<td>20.5</td>
<td>19.06</td>
<td>0.9019</td>
</tr>
<tr>
<td>cis-1,2-DCE(^b)</td>
<td>10.2</td>
<td>12.45</td>
<td>0.8420</td>
</tr>
<tr>
<td>1,1,2,2-TeCA(^b)</td>
<td>10.7</td>
<td>14.60</td>
<td>0.8085</td>
</tr>
</tbody>
</table>

\(^a\) Calculated using Equation 3; \(^b\) Results of sorption experiments involving all the chemicals simultaneously.

#### 3.4.2 EWS Hydrodynamic Characterization

Results of analysis of samples of the effluent from the EWS unit used for the tracer study during the tracer study period are illustrated in Figure 3.3.
**Figure 3.3:** Wetland tracer study results. Relative concentration = chloride concentration at any time/chloride concentration in the influent.

**Figure 3.4:** Relation between the wetland time – scaled parameter and the relative concentration of the tracer

With reference to Figure 3.3, the time to the passage of the center of the plume of the tracer or pollutant gives the average retention time ($t_R$) of the tracer, and as such also
gives the linear velocity of the tracer front or pollutant plume. In this study, based on the NaCl breakthrough curve shown in Figure 3.3, \( t_R = 3.87 \text{ d} \). The tracer velocity, \( V_R = \frac{H_C}{t_T} = \frac{25.5}{3.87} = 6.59 \text{ cm/d} \), where \( H_C = \text{EWS height}. \) Given the EWS soil porosity of 55\%, flow rate of 11.12 L/d, and EWS unit plan area of 2748 cm\(^2\), the seepage velocity, \( V_S = 6.56 \text{ cm/d} (7.592 \times 10^{-5} \text{ cm/s}) \).

After transformation using Equation 3.6, the data for Figure 3.3 were plotted to relate the relative concentration to the time scaled parameter as shown in Figure 3.4.

Figure 3.4 was used to determine the time scaled parameter values corresponding to \( C/C_0 = 0.84 \) and \( C/C_0 = 0.16 \). By interpolation, these were found to be -0.1166 and -0.6111, respectively. Using Equation 3.7, the coefficient of hydrodynamic dispersion was determined as follows:

\[
D = \frac{7.592 \times 10^{-5} \times 25.5}{4} (-0.11658 - -0.61108)^2 = 1.24 \times 10^{-4} \text{cm}^2/\text{s}
\]

The coefficient of hydrodynamic dispersion can also be defined in terms of its diffusion and mechanical dispersion components (Equation 3.16).

\[
D = \pi D_m + a_L v \tag{3.16}
\]

where \( D_m = \text{coefficient of molecular diffusion (cm}^2/\text{s}), \tau = \text{tortuosity, } a_L \text{ dispersivity (cm), and } v = \text{seepage velocity (cm/s).} \) Using Equation 3.16, the dispersivity was found to be 1.094 cm. The hydraulic residence time, \( t_R \) in the wetlands was calculated as follows:

\[
t_R = \frac{\text{Volume of EWS bed}}{\text{Porosity} \times \text{flow rate}} = \frac{\text{Effective EWS bed depth}}{\text{seepage velocity}} = \frac{25.5 \text{ cm}}{7.592 \times 10^{-5} \text{ cm/s}} \approx 3.89 \text{ days}
\]

The hydraulic retention time obtained above compares well with the one obtained using the tracer study data. To see how well simulation results for the four models
assessed in this study compare with the tracer study results, tracer study results were plotted alongside the simulation results for each model as shown in Figures 3.5 – 3.8.

**Figure 3.5:** Comparison of tracer study results and Simple Model results

**Figure 3.6:** Comparison of tracer study results and Type 1 Model results
**Figure 3.7:** Comparison of tracer study results and Type 2 Model results

**Figure 3.8:** Comparison of tracer study results and Type 3 Model results
To further quantify the comparison of how well the four models represent the tracer study results, a correlation analysis was done between simulation results of each model and the tracer study results. The results of the comparison are given in Table 3.3.

**Table 3.3:** Comparison of model simulation results and tracer study results

<table>
<thead>
<tr>
<th>Mode type</th>
<th>Goodness of fit ($R^2$)</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple Model</td>
<td>0.9630</td>
<td>2</td>
</tr>
<tr>
<td>Type 1 Model</td>
<td>0.9624</td>
<td>4</td>
</tr>
<tr>
<td>Type 2 Model</td>
<td>0.9896</td>
<td>1</td>
</tr>
<tr>
<td>Type 3 Model</td>
<td>0.9625</td>
<td>3</td>
</tr>
</tbody>
</table>

In Table 3.3, simulation results of Type 1 Model and Type 3 Model were practically identical. On the whole, all the four models fitted to the experimental tracer study data can represent well the behavior of the EWS. However, the Simple Model is a preferable option because it can represent the EWS just as well as the other three models despite its apparent mathematical and computational simplicity.

### 3.4.3 EWS Water Budget and Hydraulic and Contaminant Loading Rates

Tables 3.4 and 3.5 summarize water budgets for the EWS units studied for the period following planting of the wetland plants.

**Table 3.4:** Pumping and evapo-transpiration rates of 1,1,2,2 –TeCA/TCE feedwater in wetlands at the beginning of wetland plant growth (July 2004)

<table>
<thead>
<tr>
<th>Wetland type</th>
<th>Loading rate (LR)</th>
<th>Effluent flow rate</th>
<th>Evapo-transpiration rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(L/d)</td>
<td>(mm/d)</td>
<td>(L/d)</td>
</tr>
<tr>
<td><strong>Typha</strong> 1</td>
<td>12.47</td>
<td>45.4</td>
<td>11.73</td>
</tr>
<tr>
<td><strong>Typha</strong> 2</td>
<td>12.28</td>
<td>44.7</td>
<td>11.25</td>
</tr>
<tr>
<td><strong>Phragmites</strong> 1</td>
<td>12.66</td>
<td>46.1</td>
<td>10.65</td>
</tr>
<tr>
<td><strong>Phragmites</strong> 2</td>
<td>13.23</td>
<td>48.1</td>
<td>11.25</td>
</tr>
</tbody>
</table>

Hydraulic loading rate (influent flow rate), mm/d = L m$^{-2}$ d$^{-1}$. ET (%) = (ET/LR) x 100.
It is evident from Table 3.4 that the *Phragmites* vegetated wetland system units exhibited higher evapo-transpiration rates (ET) than the *Typha* vegetated ones. These results make sense because they are consistent with the biomass productivity data during this period. ET rates depend, to a large extent, on the above-ground biomass quantity. The proportions of the leafy surface area (which affects ET) to the total above ground plant surface area were slightly higher for *Phragmites* than *Typha* at the early growth stage of the plants.

The ET rates shown in Table 3.5, which are for the advanced stage of wetland plants growth, are generally higher for *Typha* wetland system units (23.1 – 27.0% of the loading rate) than for *Phragmites* (13.4 – 14.2%). This is understandable because at this stage the ratio of the leafy surface area to the total surface area was higher for *Typha* than for *Phragmites* plants. Notably, the trunk (stem) of a mature *Phragmites* plant is a hard reed (like that of a bamboo plant), while that of *Typha* is more grass-like. Additionally, during the advanced growth stage, *Typha* plants have considerably more biomass per shoot than *Phragmites* plants. It was also observed during this stage that, while *Typha* plants remained wholly green, *Phragmites* plants had many dead leaves. As such, *Typha* plants were more active in contributing to ET compared to *Phragmites* plants.

**Table 3.5:** Pumping and evapo-transpiration rates of 1,1,2,2-TeCA/TCE feedwater in wetlands during advanced wetland plant growth (end of September 2004)

<table>
<thead>
<tr>
<th>Wetland type</th>
<th>Loading rate (LR) (L/d)</th>
<th>Effluent flow rate (L/d)</th>
<th>Evapo-transpiration rate (L/d)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Typha</em> 1</td>
<td>13.30</td>
<td>48.3</td>
<td>10.23</td>
<td>3.07</td>
</tr>
<tr>
<td><em>Typha</em> 2</td>
<td>8.85</td>
<td>32.2</td>
<td>6.46</td>
<td>2.39</td>
</tr>
<tr>
<td><em>Phragmites</em> 1</td>
<td>14.06</td>
<td>51.2</td>
<td>12.17</td>
<td>1.89</td>
</tr>
<tr>
<td><em>Phragmites</em> 2</td>
<td>15.48</td>
<td>56.3</td>
<td>13.28</td>
<td>2.20</td>
</tr>
</tbody>
</table>

Hydraulic loading rate (influent flow rate), mm/d = L m⁻² d⁻¹. ET (%) = (ET/LR) x 100.
ET data for October 2005 were 32.4 – 36.3% for *Typha* and 14.1 – 19.2% for *Phragmites*. This shows that ET from the *Typha* EWS units increased while that from the *Phragmites* units remained almost the same. At the same time, the ET rates for *Typha* EWS units were more than twice as high as those for the *Phragmites* units. These observations attest to the earlier assertions regarding ET rates from the two types of plants. The observations also indicate that the plant area that is effective for ET increases faster in *Typha* than in *Phragmites* plants. Since the sizes of the EWS units planted with the two types of plants were the same, the bulk of the differences in ET rates between the EWS units is attributable to differences in characteristics (structural, physiological, and morphological differences) of the two wetland plant species.

At 2.8 – 13.0 mm/d, the ET data shown in Tables 3.4 and 3.5 are lower than those reported in the literature. Disregarding differences in growth stages of the plants, the lower ET rates in this study are probably due to this study being conducted in an enclosed space (greenhouse). Ranieri (2003) has reported ET rates of 21 – 32 mm/d with summer peaks of up to 40 mm/d for *Phragmites* planted wetlands in Italy.

Everything else being equal, judging from the ET rate data, it is likely that *Typha* plants can lose more cVOCs present in the water being treated through ET than *Phragmites* plants, and as such can contribute to air pollution much more easily. On the other hand, very high ET can potentially cause increases in concentration (loss of water unaccompanied by loss of contaminant) if the cVOCs are retained in the wetland bed material due to sorption. This can lower the apparent performance of the EWS.

### 3.4.4 Aboveground Biomass Growth and Response to Exposure to cVOCs

Aboveground wetland plant biomass production is important because two of the most important benefits of wetlands are their roles as sources of food and as ecological
habitats. Both of these are related to aboveground biomass quantity and regeneration rates. Figure 3.9 illustrates the trends of the aboveground biomass (ABG) production in the wetlands for this study. The number of stems (shoots) is used as a surrogate for the total quantity of biomass since, for each wetland plant species under specific growing conditions, biomass quantity is directly proportional to the number of stems (shoots).

It should be pointed out that the monitoring of the aboveground biomass in the wetlands continued even after day 82, but counting of the wetland plants stopped because it became increasingly difficult with increase in the number of wetland plants.

![Figure 3.9: Variation of wetland plants biomass in aquarium wetlands for the first 82 days after transplanting](image)

**Figure 3.9:** Variation of wetland plants biomass in aquarium wetlands for the first 82 days after transplanting

It is evident from Figure 3.9 that the number of *Phragmites communis* shoots was consistently higher and increased at a higher rate than that of *Typha latifolia*. The fact that the patterns of biomass increase for both types of wetland plants did not change after commencement of TCE pumping suggests that the presence of TCE in the wetlands did
not have a discernible adverse effect on the productivity of the wetland plants. Monitoring of the wetland plant productivity beyond the data for Figure 3.9 (data not shown) confirms this notion. This implication has ecological importance.

A variety of additional information can be gleaned from Figure 3.9, apart from the response of productivity of the plants to exposure to cVOCs. This includes an indication of how well replicated EWS units were within each treatment (each type of plant). The coefficients of correlation between the number of wetland plants were $R^2 = 0.9104$ for the *Typha* EWS units and $R^2 = 0.9866$ for the *Phragmites* units. This suggests that other factors that influence wetland performance, especially with respect to substrate, were comparable. This further indicates that the performance of the EWS units would be well replicated between similar treatments. Also, since the EWS units were designed and set up in exactly the same way, packed with the same bed materials, and allocated to the treatments randomly (*Typha* 1 and *Typha* 2, *Phragmites* 1 and *Phragmites* 2), the good replication likely applies to between different treatments (*Phragmites* versus *Typha*) as well as between similar treatments (*Typha* versus *Typha* or *Phragmites* versus *Phragmites*). Notably, the degree of how well replicated the EWS units can be is influenced by the degree of replication of substrates, hydrology and hydraulics of operation, growth and productivity of wetland plants, and environmental conditions.

The fact that the number of *Phragmites* shoots increased faster than the number of *Typha* shoots suggests that *Phragmites* plants can get established and cover a wetland bed faster than *Typha* plants. Also, since there is a direct correlation between aboveground biomass (AGB) growth and below-ground biomass (BGB) growth, it can be inferred that *Phragmites* root systems develop faster and as such a *Phragmites* EWS is likely to mature faster. While this may be true at very early stages of growth, based on this study,
it is completely not true at later stages of plant growth. While *Phragmites communis* increased mostly in number, *Typha latifolia* increased in both number and size. Shoots of *Typha* were thicker and their leaves longer than those of *Phragmites* plants. *Typha* shoots had a base diameter of up to 28 mm (area = 615.6 mm$^2$) while for *Phragmites* the corresponding figure was 8 mm (area = 50.3 mm$^2$). The largest base size observed for non-circular *Typha* shoots was 102 mm long and 19 mm wide (area = 1938 mm$^2$). Root systems of both plants developed and grew fast, but those of *Typha* grew faster, reaching the bottom of the EWS units earlier (more than 25.5 cm deep) than those of *Phragmites*. Root systems of both types of plants reached and covered the bottom of the EWS units within the 92 days covered by Figure 3.9.

In addition to growing faster, *Typha* shoots turned out to be stronger and remained so even after being exposed to cVOCs for a long time. Even after four years of exposure to cVOCs it was impossible to uproot or split off *Typha* plants by hand. When pulled by hand, *Phragmites* plants easily broke off at the base. After four years of operation of the EWS units and exposure to cVOCs, the sections of *Phragmites* shoots at the water level were rotten and so weakened that the upper and lower portions were only kept together by thin threads of what remained of the stem. Consequently, *Phragmites* shoots broke off at the water level at the slightest pull. It is possible that *Phragmites* plants were weakened by being subjected to both high water levels and exposure to cVOCs.

Apparently due to the intricately interwoven nature and structural strength of the root systems of *Typha* plants, sustained attempts to pull the plants off the bed by the base of the shoots gave the impression of the whole bed lifting up instead of the shoots breaking off. The same efforts with *Phragmites* plants resulted in the shoots breaking off, as explained earlier. Attempts to pull the *Phragmites* plant root system resulted in only
small chunks of roots coming off the bed. Since *Phragmites* plants are generally strong (USDA – NRCS, 2008), the weakness of their shoots and root systems observed in this study must have been induced to by exposure to cVOCs. This suggests that *Typha latifolia* species is more robust with respect to cVOC exposure than *Phragmites communis* species. It is noteworthy that, after pumping cVOC laden water through the EWS units for 4 years, *Typha* plants maintained their evergreen status and continued to grow vigorously, with new shoots replacing old ones continually.

During the monitoring period represented by Figure 3.9, the wetland plant density increased from 43.7 shoots/m² to an average 145.6 shoots/m² for the *Typha* EWS units. The corresponding figures for *Phragmites* units were 43.7 shoots/m² and 338.4 shoots/m². The increase in number of shoots of wetland plants when they are not constrained, before overcrowding sets in, can be taken to be exponential, \( N/N_o = \exp(r_G t) \) where \( N = \) number of shoots at time \( = t \), \( N_o = \) initial number of shoots, \( r_G = \) rate of increase of number of shoots \((d^{-1})\). Based on this model, the rate of increase of *Typha* shoots was 0.0147 \( d^{-1} \) \((R^2 = 0.8750)\) while that of *Phragmites* was 0.0244 \( d^{-1} \) \((R^2 = 0.9771)\).

In the *Phragmites* beds, the growth and regeneration process practically stopped not long after collecting the last batch of samples whose results are reported in this study. No new shoots were being produced and the mature shoots started wilting and losing their green color. To test further the regeneration and recovery capabilities of the two species of wetland plants and assess the effect of harvesting the AGB, we harvested all the AGB from one EWS unit of each set. The *Typha* EWS unit re-established its AGB to the pre-harvesting level within three months. The *Phragmites* EWS unit did not recover at all, probably because of the failure of the plant to withstand adverse effects of the
cVOCs after being weakened by harvesting in addition to the effects of the exposure to cVOCs.

### 3.4.5 Pre-inoculation EWS Performance

Contaminant cVOC monitoring results obtained prior to inoculation of the EWS units with bacteria capable of completely dechlorinating 1,1,2,2-TeCA and TCE are shown in Figures 3.10 – 3.17. For comparison purposes, monitoring results for four different periods are shown. The periods are: December 2003, just after commissioning

![Figure 3.10: Variation of concentrations of cVOCs with height in EWS units planted with *Typha* sp. 1 (*Typha* 1, top; *Typha* 2, bottom). Each data point is a mean of three values. The three values are analysis results for samples taken from three parallel sampling points. Data are from December 2003.](image-url)
the EWS units, between January 31 and February 11 2004, between 25 – 28 April 25\textsuperscript{th} – 28\textsuperscript{th}, and between May 10\textsuperscript{th} and 13\textsuperscript{th} 2004. Results for monitoring pH variation in the EWS units are presented in Figure 3.18.

![Diagram](image1)

**Figure 3.11:** Variation of concentrations of cVOCs with height in EWS units planted with *Phragmites* sp. 1 (*Phragmites* 1, top; *Phragmites* 2, bottom). Each data point is a mean of three values. The three values are analysis results for samples taken from three parallel sampling points. Data are from December 2003.

During December 2003 (Figures 3.10 through 3.14), which was two months after planting the wetland plants, pumping was set at a low rate of 3 mL/minute to avoid breakthrough of TCE and 1,1,2,2-TeCA as well as their degradation products. The flow
Figure 3.12: Variation of concentrations of cVOCs with height in EWS units planted with *Typha* sp. 1 (*Typha* 1, top; *Typha* 2, bottom). Each data point is a mean of three values. The three values are analysis results for samples taken from three parallel sampling points. Data are from January 31 – February 2004.
Figure 3.13: Variation of concentrations of cVOCs with height in EWS units planted with *Phragmites* sp. 1 (*Phragmites* 1, top; *Phragmites* 2, bottom). Each data point is a mean of three values. The three values are analysis results for samples taken from three parallel sampling points. Data are from January 31 – February 2004.
**Typha wetland unit 1**

![Graph showing VOCs concentration (mg/L) vs. height of wetland (cm) measured from the bottom for Typha wetland unit 1.](image)

**Typha wetland unit 3**

![Graph showing VOCs concentration (mg/L) vs. height of wetland (cm) measured from the bottom for Typha wetland unit 3.](image)

**Figure 3.14:** Variation of concentrations of cVOCs with height in EWS units planted with *Typha* sp. Each data point is a mean of three values. The three values are analysis results for samples taken from three parallel sampling points. Data are from April 2004.

**Phragmites wetland unit 2**

![Graph showing VOCs concentration (mg/L) vs. height of wetland (cm) measured from the bottom for Phragmites wetland unit 2.](image)

**Phragmites wetland unit 4**

![Graph showing VOCs concentration (mg/L) vs. height of wetland (cm) measured from the bottom for Phragmites wetland unit 4.](image)

**Figure 3.15:** Variation of concentrations of cVOCs with height in EWS units planted with *Phragmites* sp. Each data point is a mean of three values. The three values are analysis results for samples taken from three parallel sampling points. Data are from April 2004.
Figure 3.16: Variation of concentrations of cVOCs with height in EWS units planted with *Typha* sp. Each data point is a mean of three values. The three values are analysis results for samples taken from three parallel sampling points. Data are from May 2004.

Figure 3.17: Variation of concentrations of cVOCs with height in EWS units planted with *Phragmites* sp. Each data point is a mean of three values. The three values are analysis results for samples taken from three parallel sampling points. Data are from May 2004.
rate was increased to about 10 mL/minute after inoculation. This flow was maintained for the rest of the study period.

During the April 25th – 28th 2004 monitoring period (Figures 3.14 and 3.15) the EWS units were fed the synthetic contaminated groundwater prepared to achieve a target concentration of 1.5 mg/L for 1,1,2,2-TeCA and 0.75 mg/L for TCE. During the May 10th - 13th 2004 monitoring period (Figures 12 – 14) the EWS units received a target concentration of 2 mg/L for 1,1,2,2-TeCA and 1 mg/L for TCE.

During the December 2003 monitoring period, in both the Phragmites- and Typha-planted EWS units, concentrations of TCE and 1,1,2,2-TeCA decreased rapidly from the bottom of the wetland where the influent TCE and 1,1,2,2-TeCA was added.

Figure 3.18: Variation of pH with height of wetland
(Figure 3.10). At 18 cm from the influent inlet, concentrations of parent compounds were below detection. Low concentrations of cis-1,2 DCE and 1,2-dichloroethane (1,2-DCA) were observed. During this initial period of operation of the EWS units, sorption and potentially plant uptake, were the likely fate processes operating. It is noteworthy that during this monitoring period very little breakthrough of cVOCs was observed.

During the January – February monitoring period in the Typha EWS units, TCE and 1,1,2,2-TeCA removal profiles were similar to those of December 2003 results (Figure 3.12). Evidently, during this period, 1,2-DCA was not detected but low concentrations of cis-1,2-DCE were observed. It can be noted that in one of the two Typha-planted EWS units, TCE increased slightly as 1,1,2,2-TeCA decreased, 3.5 cm above the influent location. This suggests that 1,1,2,2-TeCA was partly eliminated abiotically through dehydrochlorination directly to TCE (Lorrah and Olsen, 1999; Lorrah and Voytek, 2004; Aulenta et al., 2006). Apparently, dehydrochlorination of 1,1,2,2-TeCA occurred within the gravel layer near the inlet. In addition to the above, in the second Typha-planted EWS units, more elevated cis-1,2-DCE concentrations were observed, a potential dechlorination product of both TCE and 1,1,2,2-TeCA. Vinyl chloride was not observed and concentrations of trans-1,2-DCE and 1,2-DCA were also below detection. A slight increase in 1,1,2,2-TeCA in the overlying water was also observed, an indication of another transport process. cis-1,2-DCE concentrations observed did not account for the loss of all of the parent TCE and 1,1,2,2-TeCA, indicating that sorption and plant uptake processes may also have been involved.

During the January – February monitoring period, in the Phragmites-planted EWS units, 1,1,2,2-TeCA and TCE were also removed (Figure 3.13). In Phragmites,1, levels of cis-1,2-DCE were much more elevated, reaching concentrations as high as 0.3
mg/L midway up the mesocosm. Whereas observed concentrations still did not account for the loss of parent TCE and 1,1,2,2-TeCA completely, dechlorination was more prevalent in this EWS unit than the *Typha*-planted units. In the *Phragmites* 2 EWS unit, similar trends were observed. TCE and 1,1,2,2-TeCA decreased rapidly and *cis*-1,2-DCE increased along the flowpath (Figure 3.13). Evidently, concentrations of *cis*-1,2-DCE in *Phragmites* 2 EWS units were not as high as observed in *Phragmites* 1, but still the monitoring results were more indicative of reductive biodegradation processes. Some increased concentrations of 1,1,2,2-TeCA were observed further up in the EWS unit bed than conventional sorption retardation concepts would predict. Although short-circuiting through macropores in the wetland is possible, we attribute this to plant uptake and translocation to the upper parts of the plant and discharge to the surface water.

During the April 2004 monitoring period (Figures 3.14 – 3.15), the EWS units removed completely both 1,1,2,2-TeCA and TCE within the first 10 cm of the wetland bed (Figure 3.14 and 3.15). In both *Typha* and *Phragmites*-planted EWS units, *cis*-1,2-DCE was the only metabolite observed. Some breakthrough of *cis*-1,2-DCE was observed in both the *Phragmites* and *Typha* EWS units and complete dechlorination of TCE and 1,1,2,2-TeCA were not observed.

During this monitoring period prior to the inoculation of the EWS units with the dehalorespiring bacterial culture, TCE and 1,1,2,2-TeCA were easily removed from the influent water in the EWS units. Some breakthrough of parent 1,1,2,2-TeCA was observed but this was likely due to transport through the plants followed by release in the overlying surface water. Concentrations of *cis*-1,2-DCE were observed in EWS units planted with both *Typha* and *Phragmites* but concentrations of *trans*-1,2-DCE, 1,2-DCA, vinyl chloride, ethene and ethane were very low. This indicates that biodegradation of
TCE was occurring but potentially not 1,1,2,2-TeCA. Dechlorination of 1,1,2,2-TeCA would produce trans-1,2-DCE along with cis-1,2-DCE or alternately, trichloroethanes, dichloroethanes and chloroethanes (Lorah and Olsen, 1999). None of these dechlorination products were produced at any significant concentrations within the bed. The increase in TCE concentrations observed in some EWS units near the inlet can be attributed to abiotic elimination of 1,1,2,2-TeCA as pointed out earlier, although pH conditions need to be basic for this reaction to occur (Cooper et al., 1987). Profiles of pH measurement results in Figure 3.18 indicate that only a small portion of the bed was favorable for dehydrochlorination of 1,1,2,2-TeCA. Notably, 1,1,2,2-TeCA has half-lives less than a day at pH >8 (Cooper et al., 1987).

3.4.6 Post-inoculation EWS Performance

On May 27th, 2004, we inoculated all the four EWS units with a culture containing organisms previously observed to completely dechlorinate TCE and chloroethanes (Kassenga et al., 2004). Kassenga et al. (2004) have published characteristics of the Dehalococcoides population detected in the culture.

Contaminant cVOC monitoring results obtained after inoculation of the EWS units are shown in Figures 3.19 – 3.22. For comparison purposes, the monitoring results are shown for September 2004 and January 2005. Average data for dissolved gas concentrations in the EWS units for the same period are given in Table 3.6.

In contrast with the situation prior to inoculation, shortly after inoculation, vinyl chloride, ethene and ethane began to be detected in both Typha and Phragmites-planted EWS units (Figures 3.19 – 3.22 and Table 3.6). Notably, vinyl chloride, ethane and ethene were observed in samples taken from heights equal to and above 18 cm. These results imply that complete dechlorination of both 1,1,2,2-TeCA and TCE took place in
all the EWS units, and this in turn also implies that the inoculation of the EWS units with
*Dehalococcoides ethenogenes* was successful.

![Graph showing variation of 1,1,2,2-TeCA, TCE, and VC concentrations with height in mesocosm wetland vegetated with *Typha latifolia* (cat tail) after inoculation with *Dehalococcoides ethenogenes*. The data are from September 2004. The data are based on means for 3 sampling ports.](image)

**Figure 3.19:** Variation of 1,1,2,2-TeCA, TCE, and VC concentrations with height in mesocosm wetland vegetated with *Typha latifolia* (cat tail) after inoculation with *Dehalococcoides ethenogenes*. The data are from September 2004. The data are based on means for 3 sampling ports.

During the January 2005 monitoring period, dechlorination products that were detected were primarily ethene and ethane (Table 3.6). Low levels of vinyl chloride were observed in *Typha* 1 and elevated ethene and ethane observed in *Phragmites* EWS units. Despite some differences observed in their efficiency and effectiveness, in both the *Phragmites*- and *Typha* planted EWS units, complete removal of parent and daughter chlorinated VOCs were observed over travel distances of about 10 cm.
Figure 3.20: Variation of 1,1,2,2-TeCA, TCE, and VC concentrations with height in mesocosm wetland vegetated with *Phragmites communis* (common reeds) after inoculation with *Dehalococcoides ethenogenes*. The data are from September 2004. The data are based on means for 3 sampling ports.

Table 3.6. Typical dissolved gas concentrations in the EWS units after inoculation with a dehalorespiring culture.

<table>
<thead>
<tr>
<th>Height above bottom (cm)</th>
<th>Methane (ug/L)</th>
<th>Ethane (ug/L)</th>
<th>Ethene (ug/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Typha</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1518.20</td>
<td>32.23</td>
<td>71.76</td>
</tr>
<tr>
<td>10.5</td>
<td>725.65</td>
<td>6.10</td>
<td>3.65</td>
</tr>
<tr>
<td>18</td>
<td>787.36</td>
<td>2.10</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>25.5</td>
<td>9.30</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td><strong>Phragmites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1607.97</td>
<td>115.84</td>
<td>405.18</td>
</tr>
<tr>
<td>10.5</td>
<td>931.55</td>
<td>7.81</td>
<td>79.91</td>
</tr>
<tr>
<td>18</td>
<td>702.59</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>25.5</td>
<td>66.68</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>
Figure 3.21: Variation of concentrations of cVOCs with height in EWS units planted with *Typha* sp. 1 (*Typha* 1, top; *Typha* 2, bottom). The data are from January 2005. Each data point is a mean of three values. The three values are analysis results for samples taken from three parallel sampling points.
Figure 3.22: Variation of concentrations of cVOCs with height in EWS units planted with *Phragmites* sp. 1 (*Phragmites* 1, top; *Phragmites* 2, bottom). The data are from January –2005. Each data point is a mean of three values. The three values are analysis results for samples taken from three parallel sampling points.

3.5 Conclusions

This study has demonstrated that co-treatment of 1,1,2,2-TeCA and TCE in upflow engineered wetland systems (EWSs) packed with BionSoil/peat/sand mixture
(37.5:37.5:25 w/w) and vegetated with either *Phragmites communis* or *Typha latifolia* is feasible. The experimental mesocosm EWS units were able to dechlorinate completely 1,1,2,2-TeCA and TCE plus their daughter products at both low and high initial concentration levels.

Before inoculation of the EWS units with a microbial culture containing *Dehalococcoides ethenogenes*, a dehalorespiring bacterial species, incomplete transformation of 1,1,2,2-TeCA and TCE was observed. After inoculation, complete dechlorination of both 1,1,2,2-TeCA and TCE started occurring. At the peak of efficiency of the EWS units complete removal of 1,1,2,2-TeCA and TCE and their daughter products occurred within the first 18 cm of the EWS beds. This demonstrates the effectiveness of the bed material chosen to retard the cVOCs and support enhanced microbial activity.

Under normal operating conditions, which characterize EWSs operating under non-extreme conditions, both wetland plant species did not show any discernible signs of being affected by their long term exposure to 1,1,2,2-TeCA and TCE and their degradation products. Both *Phragmites communis* and *Typha latifolia* were practically unperturbed by long term exposure to cVOCs, plant diseases, pests, and heat in the greenhouse. However, over time, *Phragmites communis* plants showed clear signs of not being able to cope with the stress they were subjected to. Also, after harvesting, *Phragmites communis* plants failed to re-establish to the pre-harvesting levels. In contrast, *Typha latifolia* plants reestablished quickly and covered their beds to their pre-harvesting levels within three months. In conclusion, although both *Phragmites communis* and *Typha latifolia* performed well with respect to treatment of 1,1,2,2-TeCA and TCE, due to its better resistance to the cVOCs and other stresses, *Typha latifolia* is a
better wetland plant species than *Phragmites communis*, especially for EWSs intended to treat cVOCs.

This study has also demonstrated the effectiveness of a simple EWS inoculation technique whereby the inoculum separately enriched for cVOC treatment was diluted and delivered into the bottom of the EWS beds using syringes inserted through the lowest level sampling ports. Arguably, the technique can easily be adapted to the requirements of full scale EWSs.
4.1 Introduction

In the context of engineered wetland systems (EWSs), the roles of plants pertain mainly to: 1) enhancement of structural (physical) strength with respect to reinforcing EWS beds, including preventing bed material segregation and erosion and resisting bed uplift forces; 2) enhancement of physical-chemical processes in EWS beds, including filtration, and sorption (Olson et al., 2003); 3) provision of habitats for soil micro-fauna coupled with supporting the pertinent biochemical processes (Mitsch and Gosselink, 2000; Olson et al., 2003; Bardgett, 2005); 4) modification and control of the chemistry of the EWS bed, including controlling soil pH and oxidation – reduction potential (Gregory, 2006); 5) modification and enrichment of EWS beds with organic matter (Kadlec et al., 2000); 6) direct uptake of pollutants along with nutrients from water being treated in the EWS beds; 7) hydraulic and hydrodynamic modification of flow of water being treated through modifying such aspects as hydraulic conductivity, flow distribution, and flow regime (et al., 2003); 8) provision of ecological functions that are not necessarily related to the treatment function of EWSs, including provision of habitats for above-ground macro-fauna (Kadlec et al., 2000; Mitsch and Gosselink, 2000); and 9) aesthetic and scenic quality improvement of EWSs and their neighbourhood.

Wetland plants are able to play the roles listed above through their aboveground biomass (AGB) and below-ground biomass (BGB) components. EWS AGB is made up of shoots and leaves, while the BGB is made up of roots and rhizomes. Although both the AGB and BGB are important and indispensable components of wetland plants (all plants
for that matter), in the context of EWSs treating contaminated water, the BGB plays a more important role. The BGB is responsible for most (1 – 7) of the functions listed above some of which it performs almost exclusively without the contribution of the AGB.

Wetland plant roots are important in EWS beds because of the roles they play after they die as well as when they are growing. They provide exudates and excretions that are useful for microorganisms (Negri et al., 2003; Olson et al., 2003). Some of the microorganisms that use the exudates and excretions play various functions that mediate processes responsible for the treatment of pollutants of interest (Olson, et al., 2003). The usefulness of roots extends beyond their death, especially with respect to microorganisms that feed on labile constituents released by senescent and dead root components (Bardgett, 2005). Similarly to the functions of whole plants, wetland plant roots also provide favourable habitats for microorganisms that mediate the treatment of pollutants of interest (Kadlec et al., 2000). Notably, this function has been shown to be effective even with artificial plants (Collins, 2004), which means dead as well as growing roots can provide for this function effectively. Leigh et al. (2002) have showed conclusively that, after death, fine roots of mulberry can serve as a source of substrate for PCB-degrading bacteria.

The decomposition of roots also releases carbon and other substances some of which are readily degradable, enhancing microbial activity that contributes to the treatment of pollutants (Olson et al., 2003; Badgett, 2005). Root exudates, which include low molecular weight organic compounds such as carbohydrates, amino acids, and organic acids, leak passively from roots while root lysates, which include phenolic compounds and cellular debris, form following the autolysis of root cells (Hutchinson et al., 2003). Yi and Crowley (2007) observed that planted *Apium graveolens* culture and its
crushed root tissues did not differ in promoting removal of pyrene and benzo[a]pyrene from contaminated soil. Summing up the preceding discussions, plant roots play an important role in EWS because they can serve as sorption media, reaction sites for contaminants, and habitats as well as action sites for useful microorganisms. They can also serve as sources of substrates needed by useful microorganisms.

In the context of plant physiology, the main function of plant roots is the acquisition of plant mineral nutrients and water from the soil (de Kroon, 2007). This function of roots is exploited by remediation system designers in a way that it facilitates the uptake of pollutants from soil. Studies that demonstrate this, especially for individual plants, abound (Fismes et al., 2002; Shukla et al., 2007; Bialowiec et al., 2007; Rao et al., 2007; Lui et al., 2007). Plants are expected to differ in the way they carry out this function because of differences in quantity as well as quality of the resources they produce, which is in turn due the fact that basic ecological traits differ significantly among different plant species (Bardgett, 2005). For example, whereas some plants produce and release compounds that are desirable to microorganisms, others produce and exude unpalatable or outright toxic compounds. It is also noteworthy that individual plant species influence microorganisms within their spheres of influence under field as well as laboratory conditions (Bardgett, 2005).

Since a growing body of literature suggests that different plant species differ in their potential to enhance remediation or treatment of pollutants (Hutchinson et al., 2003), it makes sense to expect roots to contribute to the observed differences given their roles in plants. As such, it is reasonable to expect rootmatter from different wetland species to differ in its potential to enhance pollutant treatment processes. Although a number of studies on the influence of rootmatter on treatment of pollutants have been
reported (for example, Lin, 2003; Alvarez, 2006; Kim et al., 2006; Osem et al., 2007; Rao et al., 2007), each of these studies covered only one or two plant species. Moreover, none of these studies covered cVOCs in the context of EWSs. Liu et al. (2007) used many wetland plant species, but focused on metal uptake.

The main objective of this study was to determine whether rootmatter enhances dechlorination of cVOCs in engineered wetland soils, and whether this capacity differed significantly among different wetland plant species. Since roots are an important part of wetland plants, especially with respect to pollutant uptake, provision of habitats for useful microorganisms, and enhancement of microbial activity and pollutant transformation processes, evaluation of rootmatter of different plant species is an important part of the assessment of each wetland plant as a whole. The overall assessment of wetland plants with respect to EWS performance can widen the spectrum of wetland plant options beyond the more popular few. This is important for advancing the frontiers of wetland and phytoremediation technologies.

4.2 Materials and Methods

4.2.1 Treatment Selection and Descriptions

Treatments adopted for this study were mainly dictated by the types of wetland plants pre-selected for comparison in the study. Eight wetland plants were selected to represent more than 73 identified wetland plants. The 73 wetland plants were identified as suitable on the basis of their: 1) universality and widespread availability in the USA, and 2) acceptability among practitioners and application in wetland restoration projects.

The selected treatments involved rootmatter from the following: 1) Phragmites communis (common reed or reed grass); 2) Typha latifolia (common cattail or broad-leaved cattail); 3) Scirpus validus (soft stem bulrush); 4) Scirpus americanus (three
.square rush); 5) *Carex nebraskensis* (Nebraska sedge); 6) *Juncus effusus* (common rush); 7) *Eleocharis palustris* (creeping spike rush); and 8) *Scirpus californicus* (giant bulrush (also known as tule). Treatment 9 was the active control (engineered soil without rootmatter) and Treatment 10 was the killed control (engineered soil without rootmatter, but plus a biocide. Treatments 1 through 9 were inoculated with a microbial culture known to contain *Dehalococcoides ethenogenes* (DHC), which can dechlorinate TCE completely to ethene.

### 4.2.2 Wetland Plant Acquisition and Pre-use Care and Preparation

The wetland plants used as sources of rootmatter were bought from Hydra Aquatica (Tijeras, NM 8705 USA). All the plants had been growing in nurseries prior to being shipped. Only seedlings of the right age for transplanting were selected. All the plants were shipped and delivered in separate containers for each type of plant and each container had separate slots for each seedling to facilitate their separation during transplanting.

Immediately upon delivery from the supplier, the seedlings were transplanted to large plastic containers kept in the greenhouse. A starting separation distance of about 10 cm between seedlings was adopted to allow the plants to grow and develop their root systems freely. All the plants were grown in a mixture of a commercial potting soil (Miracle Gro) and sand (3:1 w/w). Sand was mixed with the potting soil because media for engineered wetland systems incorporate sand to improve their geotechnical and hydrodynamic properties.

All the seedlings were grown under the same conditions and for the same length of time before use. The plants were allowed to grow to maturity so as to ensure adequate development of their root systems.
4.2.3 Microcosm Set-up and Monitoring

The microcosms were constructed using 160 mL serum bottles. For the treatments containing rootmatter, the total volume of each serum bottle was apportioned as follows: 46 mL, DHC inoculum (diluted from 15 mL of original inoculum slurry for easy handling); 16.5 mL, wetland soil (a blend of BionSoil/Latimer peat/sand (37.5:37.5:25 w/w)); 20 mL rootmatter; 52.5 mL, DI water and TCE solution; and 25 mL head space filled with nitrogen gas. The rootmatter from different wetland plant species occupied different volumes, and as such the balance was filled with DI water.

Freshly harvested rootmatter was flushed with tap water to remove soil sticking to it. Then it was rinsed with DI water to remove the tap water and all remaining traces of soil. Thereafter it was taken to the laboratory and placed in the anaerobic chamber ready for weighing and loading.

For the active control treatment, the total volume was apportioned as follows: 46 mL, DHC inoculum; 36.5 mL, wetland soil; 52.5 mL DI water together with TCE solution; and 25 mL, head space filled with nitrogen gas. The killed control treatment volume was apportioned as follows: 36.5 mL wetland soil; 98.5 mL, DI water and TCE solution plus 3.8 mL of formalin (biocide); and 25 mL head space filled with nitrogen gas. The volume of DI water was adjusted to accommodate the biocide.

All preparatory work immediately preceding the loading of the microcosm bottle constituents was done in the anaerobic chamber filled with nitrogen gas. The rootmatter was weighed and loaded first. Ten (10) grams of root matter (wet weight equivalent to 5 g dry weight) was used per bottle and three bottles were used per treatment. After loading all contents, including an appropriate quantity of pre-prepared TCE solution, each bottle was topped with DI water to make up for any volume shortfall to a final volume of 135
mL, leaving 25 mL as head space filled with nitrogen gas. Then, stoppers with Teflon-lined rubber septa and aluminum crimp seals were put in place. The microcosms were incubated at room temperature (25°C) under static conditions in the dark to exclude light. Aqueous phase sampling for TCE degradation monitoring was done using a micro-syringe. Gaseous phase samples were withdrawn using a gas tight syringe.

4.2.4 Aqueous and Gas Phase Sample Analysis

Aqueous phase samples were analyzed for VOCs using EPA Method 8260B. Methane, ethene, and ethane gases were analyzed using GC/FID. 1 mL of head space sample was injected into the gas chromatography with flame ionization detector (Agilent 5890 Series II) equipped with a 2.4 m x 0.32 mm ID column packed with Carbopack b/1% Sp-(Supelco, Bellefonte, PA). The column 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 a reduction gas analyzer (Trace Analytical, Menlo Park, CA) equipped with a reduction gas detector. Head space samples were injected into a 1 mL gas sampling loop prior to being separated using 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 the carrier gas. The carrier gas was first passed through a catalytic combustion converter (Trace Analytical, Menlo Park, CA) to remove traces of H₂.

4.2.5 Wetland Plant Rootmatter Moisture and Organic Content Analysis

The roots intended for moisture and organic content analysis were acquired and initially processed in the same way as the rootmatter intended for the microcosm experiments described earlier. To remove the excess water remaining on the roots after
flushing to remove soil clinging to them, the roots were placed on a sieve to drain off the water. Repeated visual inspection of the roots was done to determine when all the excess water had drained off. The moisture and organic content analysis was done according to the Standard Methods for the Analysis of Water and Wastewater (APHA et al., 1998). Drying was done at 103°C while combustion was done at 550°C.

4.3 Statistical and Mathematical Analysis of TCE Data

Statistical analysis of TCE dechlorination data for the different rootmatter treatments was done using both ANOVA and paired t-tests to determine if there were any significant differences among the treatments. To compare the influence of the different wetland plant species rootmatter on the degradation of TCE, TCE degradation rate constants were determined by regression analysis of TCE dechlorination monitoring data based on first – order kinetics (Equation 4.1):

\[ C = C_o e^{-k_c t} \]  

where \( C \) = the concentration of TCE [ML\(^{-3}\)], \( k_c \) = the reaction rate constant [T\(^{-1}\)], \( t \) = time [T], and the subscript ‘o’ refers to initial conditions (the beginning of an experimental run.

4.4 Results and Discussion

4.4.1 Rootmatter Moisture and Organic Matter Content Analysis Results

The results of analysis of moisture and organic matter contents of the wetland plant rootmatter used in this study are summarized in Table 4.1. A summary of aqueous phase hydrogen concentration analysis data corresponding to the treatments for Figures 4.1 – 4.10 is given in Table 4.3. Table 4.4 summarizes a quantitative comparison of the performance of the different wetland plant rootmatter microcosms with respect to
dechlorination of TCE and its daughter products on the basis of their rate constant, half-life ($t_{1/2}$), and 100% removal time ($t_{100\%R}$) data.

**Table 4.1:** Wetland plant root-matter moisture and organic content analysis results

<table>
<thead>
<tr>
<th>Test wetland plant species – scientific and common names</th>
<th>Moisture content (%)</th>
<th>Organic content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phragmites communis</em> (common reed)</td>
<td>88.93</td>
<td>87.27</td>
</tr>
<tr>
<td><em>Typha latifolia</em> (common cattail)</td>
<td>87.62</td>
<td>87.46</td>
</tr>
<tr>
<td><em>Scirpus validus</em> (soft-stem bulrush)</td>
<td>84.69</td>
<td>87.14</td>
</tr>
<tr>
<td><em>Scirpus americanus</em> (three square rush)</td>
<td>82.78</td>
<td>86.13</td>
</tr>
<tr>
<td><em>Carex nebraskensis</em> (Nebraska sedge)</td>
<td>80.74</td>
<td>85.57</td>
</tr>
<tr>
<td><em>Juncus effusus</em> (common rush, corkscrew rush)</td>
<td>76.69</td>
<td>81.30</td>
</tr>
<tr>
<td><em>Eleocharis palustris</em> (creeping rush)</td>
<td>82.78</td>
<td>90.11</td>
</tr>
<tr>
<td><em>Scirpus californicus</em> (giant bulrush or tule)</td>
<td>76.67</td>
<td>77.79</td>
</tr>
<tr>
<td><em>Juncus balticus</em> (Baltic rush, wire rush)</td>
<td>75.20</td>
<td>92.95</td>
</tr>
<tr>
<td><em>Scirpus acutus</em> (hard-stem bulrush)</td>
<td>77.17</td>
<td>78.98</td>
</tr>
<tr>
<td><em>Carex emoryi</em> (Emoryi’s sedge)</td>
<td>78.03</td>
<td>91.26</td>
</tr>
<tr>
<td><em>Scirpus pungens</em> (Common three-square)</td>
<td>77.22</td>
<td>75.55</td>
</tr>
<tr>
<td>Engineered soil (reported in Chapters 2 and 3)</td>
<td>62.03</td>
<td>21.82</td>
</tr>
</tbody>
</table>

Organic content values are based on dry weight. Engineered soil constituents: BionSoil/Latimer peat/sand (37.5:37.5:25 w/w). Roots of *Juncus balticus, Scirpus acutus, and Carex emoryi* were tested for comparison purposes even though they were not used in this study.
It can be observed from Table 4.1 that, on the whole, the moisture and organic contents of the rootmatter of the plant species used in this study were comparably high, even though, at 75.55%, the organic content of *Scirpus pungens* was the lowest. The highest organic content was that of *Juncus balticus* at 92.95%. The organic content values of all of the plant species were significantly higher than that of the engineered soil used in other experiments previously (Chapters 2 and 3) and EWS substrates tested and used by Kassenga et al. (2003). Unsurprisingly, the values were also higher than those for natural peat (Mitsch and Gosselink, 2003), commercial peat, and BionSoil (Kassenga et al., 2003). Considering the classification of wetland soil on the basis of its organic content (Mitsch and Gosselink, 2000; 2007), the organic contents of all of the wetland plant species rootmatter tested in this study were at the high end of the scale. For a wetland soil to be classified as organic rather than mineral soil, the minimum organic content is 20% (Mitsch and Gosselink, 2007).

Despite the differences among wetland species, the high organic content of each plant species rootmatter indicates that each plant species produces rootmatter that has a high sorption potential in addition to being a good substrate for microorganisms. These two qualities make each of the rootmatter types tested a good constituent of EWS bed material. The fact that the organic matter contents of the rootmatter tested were all high implies that organic matter quantity was not a limiting factor and as such would not explain differences among species observed in the dechlorination of TCE in this study.

### 4.4.2 Effects of Wetland Plant Rootmatter on Biodegration of TCE

Figures 4.1 – 4.10 show results of monitoring for TCE dechlorination in the microcosms containing the eight different wetland plant rootmatter types described
earlier. Table 4.2 summarizes results of the final mass balance of TCE dechlorination in both the aqueous and gas phases in each treatment assessed in this study.

**Figure 4.1:** Dechlorination of TCE in *Phragmites communis* (common reed) microcosm

It is evident from Table 4.2 that the final mass balance for TCE dechlorination does not provide a complete account of every mole of TCE dechlorinated in each treatment. Nonetheless, it provides suitable and sufficient information to draw meaningful conclusions, especially with respect to the final harmless products ethene and ethane. Notably, although the killed control lost 16.34% of its TCE content, it did not produce any dechlorination products. The loss is likely due to sorption.
Figure 4.2: Dechlorination of TCE in *Typha latifolia* (common cattail) microcosm

Figure 4.3: Dechlorination of TCE in *Scirpus validus* (soft stem bulrush) microcosm
Figure 4.4: Dechlorination of TCE in *Scirpus americanus* (three square rush) microcosm

Figure 4.5: Dechlorination of TCE in *Carex nebraskensis* (Nebraska sedge) microcosm
Figure 4.6: Dechlorination of TCE in *Juncus effusus* (common rush) microcosm

Figure 4.7: Dechlorination of TCE in *Eleocharis palustris* (creeping spike rush) microcosm
Figure 4.8: Dechlorination of TCE in *Scirpus californicus* (giant bulrush tule) microcosm

Figure 4.9: Dechlorination of TCE in live control microcosm
Figure 3.10: Degradation of TCE in abiotic control microcosm

On the whole, the microcosms for the rootmatter-amended treatments (Figures 4.1 – 4.8) and the live control treatment (Figure 4.9) produced ethene as the final innocuous end product of TCE dechlorination. It is also evident that ethane formed during dechlorination of TCE even though in most of the treatments (Figures 4.3 – 4.9) it appeared mostly towards the end of the experiment when TCE and its daughter products had been depleted. The *Phragmites communis* and *Typha latifolia* rootmatter-amended treatments (Figures 4.1 and 4.2) seem to be the exception to this generalization. It is apparent that ethene production in the *Phragmites communis* rootmatter-amended treatment (Figure 4.1) was not well correlated with the cVOC dechlorination trends. So was the case in the *Typha latifolia* rootmatter-amended treatment (Figure 4.2), especially during the first 55 hours. It is evident that in both Figures 4.1 and 4.2, prior to the 55th hour, ethane was being consumed rather than produced or consumed faster than it was
Table 4.2: Final mass balance of TCE dechlorination (aqueous and gas phases)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Initial (10^-7 moles)</th>
<th>Final (10^-7 moles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCE</td>
<td>DC</td>
</tr>
<tr>
<td>Phragmites communis</td>
<td>21.54</td>
<td>0</td>
</tr>
<tr>
<td>Typha latifolia</td>
<td>24.33</td>
<td>0</td>
</tr>
<tr>
<td>Scirpus validus</td>
<td>22.17</td>
<td>0</td>
</tr>
<tr>
<td>Scirpus americanus</td>
<td>21.42</td>
<td>0</td>
</tr>
<tr>
<td>Carex nebraskensis</td>
<td>19.08</td>
<td>0</td>
</tr>
<tr>
<td>Juncus effusus</td>
<td>21.44</td>
<td>0</td>
</tr>
<tr>
<td>Eleocharis palustris</td>
<td>27.60</td>
<td>0</td>
</tr>
<tr>
<td>Scirpus californicus</td>
<td>23.10</td>
<td>0</td>
</tr>
<tr>
<td>Live control</td>
<td>23.16</td>
<td>0</td>
</tr>
<tr>
<td>Killed control</td>
<td>19.80</td>
<td>16.34</td>
</tr>
</tbody>
</table>

DC, cis-1,2-DCE; VC, vinyl chloride; ETH, ethene; ETA, ethane.

being produced. The lack of correlation between cVOC dechlorination and ethane production profiles, which is apparent in Figures 4.1 – 4.9, and the fact that production of ethane as an end product of TCE dechlorination is not supported by the literature, suggests that ethene was indeed the end product of TCE dechlorination. The production of ethene as the final product of TCE dechlorination is supported by similar studies reported in the literature such as Chen et al. (1996), Lorah et al. (1997), Kassenga (2003), Kassenga et al. (2003), Hunkeler et al., 2005; Aulenta et al., 2005 & 2006; and Heimann et al. (2007).
Table 4.3: Aqueous phase hydrogen concentration levels during treatment of TCE in different microcosm treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Treatment descriptions</th>
<th>H₂ (nM), (STD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 1</td>
<td><em>Phragmites communis</em> (common reed)</td>
<td>5.818±3.665</td>
</tr>
<tr>
<td>Treatment 2</td>
<td><em>Typha latifolia</em> (common cattail)</td>
<td>6.397±2.355</td>
</tr>
<tr>
<td>Treatment 3</td>
<td><em>Scirpus validus</em> (soft-stem bulrush)</td>
<td>5.867±2.888</td>
</tr>
<tr>
<td>Treatment 4</td>
<td><em>Scirpus americanus</em> (three square rush)</td>
<td>6.811±2.292</td>
</tr>
<tr>
<td>Treatment 5</td>
<td><em>Carex nebraskensis</em> (Nebraska sedge)</td>
<td>6.691±1.928</td>
</tr>
<tr>
<td>Treatment 6</td>
<td><em>Juncus effusus</em> (common rush, corkscrew rush)</td>
<td>5.870±2.388</td>
</tr>
<tr>
<td>Treatment 7</td>
<td><em>Eleocharis palustris</em> (creeping rush)</td>
<td>7.947±2.355</td>
</tr>
<tr>
<td>Treatment 8</td>
<td><em>Scirpus californicus</em> (giant bulrush or tule)</td>
<td>6.969±2.029</td>
</tr>
<tr>
<td>Treatment 9</td>
<td>Live control (biotic control)</td>
<td>2.519±0.318</td>
</tr>
<tr>
<td>Treatment 10</td>
<td>Killed control (abiotic control)</td>
<td>120.666±178.970</td>
</tr>
</tbody>
</table>

For each treatment, n = 21; STD = standard deviation

Contrasting with the live treatments, the abiotic control (Figure 4.10) did not exhibit any transformation of TCE let alone produce any measurable quantities of TCE daughter products, despite the loss of TCE discernible from Figure 4.10. These observations indicate that TCE dechlorination in the rootmatter – amended and live control treatments were mediated by dechlorinating bacteria.

It is evident from Figures 4.1 – 4.9 that dechlorination of TCE produced similar daughter products in all the rootmatter – amended treatments and the live control. It can be inferred that TCE was first transformed into *cis*-1,2-DCE, which was in turn transformed into vinyl chloride (VC). VC was transformed into ethene. Based on these
observations, the main pathway for TCE transformation can be summarized as: TCE→cis-1,2-DCE→VC, which is in agreement with what has been reported in the literature for similar studies involving TCE, cis-1,2-DCE, and VC (Kassenga and Pardue, 2006; Heimann et al., 2007). It is also the pathway expected to be exhibited by the transformation of these cVOCs when their dechlorination is mediated by microbial cultures containing Dehalococcoides ethenogenes like the one used in this study for which previous findings have been reported by Kassenga et al. (2003; 2004) and Kassenga and Pardue (2006). Other recent studies that have reported on the role of Dehalococcoides sp. and similar halorespirers with respect to dechlorination of cVOCs include Lorah et al. (2001), Kassenga (2003), Lorah and Voytek (2004) and Heimann et al. (2007).

The apparent similarity in TCE dechlorination pathways and mechanisms exhibited by all the treatments (Figures 4.1 – 4.9 and Table 4.2) suggests that the performance differences among the treatments were mainly pertinent to the efficiency rather than the mechanisms of the dechlorination processes. Since the only difference among the treatments was the rootmatter source plant species, it is reasonable to attribute the dechlorination efficiency differences to the influence of the rootmatter source plant species. As observed earlier, plant species differ in their basic ecological traits, and these influence the quantity as well as quality of the resources they produce (Bardgett, 2005).

Comparing the results of rootmatter organic content analysis (Table 4.1) and Figures 4.1 – 4.8, it is apparent that the organic content of the rootmatter tested did not have a discernible influence on TCE dechlorination. The lack of correlation between the organic content and the efficiency and effectiveness of dechlorination of a treatment is
attributable to the fact that all of the treatments had more than the minimum organic content required to sustain dechlorination, as pointed out earlier.

Table 4.4: Quantitative comparison of performance of different wetland plant root matter microcosms

<table>
<thead>
<tr>
<th>Treatment/rootmatter source plant species</th>
<th>Lag time (h)</th>
<th>Rate constant $(d^{-1})(t_{1/2},h)$</th>
<th>Time to 100% removal (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TCE</td>
</tr>
<tr>
<td><em>Phragmites communis</em></td>
<td>0</td>
<td>0.355(46)(0.28)</td>
<td>163</td>
</tr>
<tr>
<td><em>Typha latifolia</em></td>
<td>0</td>
<td>0.293(57)(0.35)</td>
<td>163</td>
</tr>
<tr>
<td><em>Scirpus validus</em></td>
<td>0</td>
<td>0.259(64)(0.39)</td>
<td>163</td>
</tr>
<tr>
<td><em>Scirpus americanus</em></td>
<td>0</td>
<td>0.305(55)(0.34)</td>
<td>163</td>
</tr>
<tr>
<td><em>Carex nebraskensis</em></td>
<td>0</td>
<td>0.245(68)(0.36)</td>
<td>189</td>
</tr>
<tr>
<td><em>Juncus effusus</em></td>
<td>0</td>
<td>0.401(42)(0.32)</td>
<td>132</td>
</tr>
<tr>
<td><em>Eleocharis palustris</em></td>
<td>0</td>
<td>0.322(52)(0.32)</td>
<td>161</td>
</tr>
<tr>
<td><em>Scirpus californicus</em>^c</td>
<td>87-132</td>
<td>0.199(84)(0.44)</td>
<td>189</td>
</tr>
<tr>
<td>Live control^c</td>
<td>87-132</td>
<td>0.214(79)(0.42)</td>
<td>189</td>
</tr>
</tbody>
</table>

^aFor each treatment the fraction in parentheses next to the $t_{1/2}$ value is the $t_{1/2}/t_{100\%R}$ for TCE. ^bThe figures in parentheses in the VC column are concentrations of VC remaining at the respective sampling times. ^cThe lag time is given as a range because its end did not coincide with any sampling time.

The apparent insignificant influence of organic matter content of rootmatter on TCE dechlorination can also be interpreted to mean that the differences in TCE dechlorination efficiency among the different treatments were more due to quality (type, form, and relative quantities of the organic constituents) rather than the absolute total quantity of the organic matter. In this respect, it can be recalled that, whereas some plants
release in their surroundings compounds that are useful and preferred by microorganisms, others release unpalatable or unwanted compounds (Bardgett, 2005).

Although there are not many reported studies in the literature that focused on effects of rootmatter on dechlorination of cVOCs and even fewer that attempted to differentiate influences of different rootmatter types by their source plant species, there are clear indications that rootmatter source plant species influences dechlorination. Lin (2003) observed that the dechlorination rate of 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB) increased with quantities of *Typha latifolia* rootmatter used. The quantities of rootmatter of *Typha latifolia* correlated well with both quantities of organic acids detected in the treatments and 1,2,3,4-TeCB dechlorination rate constant values for the treatments. This implies that the presence of roots influenced the increase in organic acid quantities, which in turn influenced dechlorination processes in the treatments amended with the roots. This role of roots with respect to the production of organic acids is widely reported in the literature (for example, Olson et al., 2003; Bardgett, 2005; Kim et al., 2006; Yi and Crowley, 2007) for various treatment contexts.

Plant inputs to soil, which differ in their proportions among plant species, are characterized into three carbon classes that reflect the ease with which they can be degraded (Bardgett, 2005; Gregory, 2006): 1) labile low molecular weight fraction (sugars, organic acids, etc), which are a soluble easily degradable resource for rhizosphere microorganisms; 2) intermediate fraction (including cellulose and hemicellulose); and 3) the most recalcitrant fraction, which is made up of mainly the structural material lignin. Notably, plant root exudates and rhizodeposition belong to the first of the three classes of plant soil inputs listed above. Root exudates are not only themselves substrates but can also enhance the decomposition of other organic substrates
in the soil (Gregory, 2006). Conceivably, this is beneficial to rhizosphere microorganisms, and serves to explain even more why rootmatter influences transformation of pollutants by rhizosphere microorganisms.

Lin (2003) observed first-order 1,2,3,4-TeCB dechlorination rates of between 0.014 d\(^{-1}\) (for a treatment containing 1 g of rootmatter) and 0.63 d\(^{-1}\) (for a treatment containing 5 g of rootmatter). Alvarez (2006) reported first-order aged 1,2,3,4-TeCB dechlorination rates of 0.113 d\(^{-1}\) for treatments amended with *Phragmites communis* rootmatter and 0.097 d\(^{-1}\) for treatments amended with *Typha latifolia* rootmatter. Kim et al. (2006) observed that plant roots of *Medicago sativa* L. (alfalfa) significantly influenced bacterial community structure in diesel – contaminated soil. Kim et al. (2006) further observed that the presence of diesel also affected the bacterial community structure but this effect was more pronounced in the presence than in the absence of the plant roots. Considering this, it is not surprising that Miller and Cramer (2005) have observed that the availability of carbon in the rhizosphere is a major factor controlling soil microflora.

Olson et al. (2003) have reported that higher levels of biphenyl-utilizing bacteria were found in soils amended with orange (*Citrus sinensis*) peels, *Eucalyptus* leaves, pine needles (*Pinus* spp.), and ivy leaves (*Hedera* spp.). This means not only do plant compounds enhance microbially - mediated transformation of pollutants, but these compounds work effectively even when not alive. Yi and Crowley (2007) attributed the ability of four plants to enhance pyrene degradation significantly to their containing substantial quantities of linoleic acid. They singled out linoleic acid as the main substance responsible for stimulating PAH degradation. Lin (2003) correlated enhanced 1,2,3,4-
TeCB dechlorination with elevated release of acetic acid and propionic acid by *Typha latifolia* rootmatter.

The Canadian waterweed (*Elodea canadensis*) plant and its cell-free extracts (dehalogenases) can degrade many compounds including TCE and PCE (Wolfe and Hoehmer, 2003). Many other plant-derived enzymes that can degrade or transform pollutants have been reported by Wolfe and Hoehmer (2003). Apart from the effects of enhancement of microbial activity through the provision of readily degradable substrates, the plants tested in this study probably enhance dechlorination of TCE and its daughter products due to an enzyme or enzymes similar to the one found in *Elodea Canadensis*. In such a case, the differences in the dechlorination enhancement capacity could be due to differences in enzymes possessed or differences in levels of the same enzymes in the different wetland plant species.

It is noteworthy that the influence of plant species on uptake and transformation of contaminants applies to even heavy metals. Liu et al. (2007) reported that accumulation of Cd, Pb, and Zn was influenced by wetland plant species. Mertens et al. (2007) observed that poplar trees took up more quantities of Cd and Zn than other species.

Table 4.4 confirms in quantitative terms the trends exhibited by Figures 4.1 – 4.9. Table 4.4 shows clearly that differences in influence of rootmatter of different plant species were exhibited in terms of parent cVOC dechlorination rates and lengths of time needed for complete dechlorination of the parent as well as the daughter product cVOCs. The differences in TCE dechlorination rates are especially remarkable when the best treatment (k = 0.401 d⁻¹) is compared to the worst treatment (k = 0.199 d⁻¹) or the live control (k = 0.214 d⁻¹). It is also clearly evident from Table 4.4 that the *Juncus effusus*
rootmatter treatment was in every respect superior to the other treatments. Its TCE dechlorination rate was the highest while its 100% removal times ($t_{100\% R}$) were the shortest for TCE (132 h), cis-1,2-DCE (161 h), and VC (187 h). In addition, *Juncus effusus* was one of only two treatments that achieved complete dechlorination of all parent and daughter product cVOCs by the end of the monitoring period.

Referring to Table 4.4 again, it is notable that the *Scirpus validus* treatment achieved complete dechlorination of TCE and its daughter products in 187 h (just like the *Juncus effusus* treatment) despite the *Scirpus validus* treatment’s remarkably lower first-order TCE dechlorination rate constant value of 0.259 d$^{-1}$ versus 0.401 d$^{-1}$ for *Juncus effusus*. Although the TCE dechlorination rate constant of the *Phragmites communis* treatment was slightly higher than that of the *Typha latifolia* treatment, the latter exhibited a lower concentration of VC at the end of the monitoring period. Therefore, it is reasonable to conclude that the two treatments were comparable.

Both ANOVA and paired $t$-tests of the data for Figures 4.1 – 4.9 and Table 4.4 indicated that wetland plant rootmatter had a significant ($p<0.05$) mainly positive influence on TCE dechlorination. In addition, the influence of rootmatter from different wetland species differed significantly ($p<0.05$). This is not wholly surprising because, as observed by Bardgett (2005), different plant species influence soils differently. The fact that one rootmatter – amended treatment did not enhance cVOC dechlorination favourably is not surprising either. Li and Crowley (2007) screened 43 species of plants, but rootmatter of only four of them stimulated PAH degradation significantly.

With reference to Table 4.3, it is important to note that the concentrations of H$_2$ in the different rootmatter-amended treatments were practically comparable even though they measurably differed. Most importantly, there is no correlation between H$_2$
concentration levels in Table 4.3 and cVOC removal data (removal rate and \( t_{100\%R} \)) in Table 4.4. For example, the *Juncus effusus* rootmatter-amended treatment, which performed best, had a lower mean H\(_2\) concentration than that of the *Scirpus californicus* rootmatter-amended treatment whose performance was the worst. It is also noteworthy that the hydrogen levels observed in this study were higher than thresholds for most microbially - mediated biogeochemical processes that are important in remediation and nutrient cycling, including chlororespiration (Yang and McCarty, 1998; Löffler, et al., 1999). Yang and McCarty (1998) observed that dehalogenators compete best against methanogens and acetogens when the concentration of H\(_2\) is kept within the range 2 – 11 nM, which was the case for all live treatments in this study. In view of the above, the differences in the efficiency and effectiveness of dechlorination observed among the treatments monitored in this study cannot be attributed to electron donor deficiency *per se* just as they cannot be attributed to differences in TCE dechlorination mechanisms and pathways. Nonetheless, the H\(_2\) concentration in the live control, at more than a factor of two lower, was significantly lower than all the rootmatter - amended treatments. This suggests that rootmatter enhanced H\(_2\) production, enabling it to remain high despite consumption by dechlorinators in addition to methanogens and acetogens. It is noteworthy that the concentration of H\(_2\) in the live control hovered close to the lower end of the suitable concentration range reported by Yang and McCarty (1998).

The high concentration of H\(_2\) in the killed control can be attributed to the fact that the concentration of H\(_2\) progressively increased with time due to non-use, which is consistent with the cVOC monitoring results. The high H\(_2\) concentration standard deviation for this treatment is a testament to this assertion; the low H\(_2\) concentration at the beginning of the experiment and the high H\(_2\) concentration at the end were
remarkably different. The high H₂ concentration in the killed control also implies that hydrogen producers were practically not affected by the biocide. It additionally indicates that a deficiency in H₂ was not the cause of the killed control’s failure to dechlorinate TCE.

Some additional interpretations of the data presented in Table 4.4, especially those related to the half-life (\(t_{1/2}\)) and 100% removal time (\(t_{100\%R}\)) data for TCE is useful at this point. The maximum error in the \(t_{100\%R}\) value due to poor data resolution (too long a sampling interval) for each treatment is given by: \(\Delta t_{100\%R} = t_n - t_{n-1}\), where, \(t_n\) is the sampling time when TCE was not detected and \(t_{n-1}\) is the sampling time preceding the one when TCE was not detected.

Based on the source data for Table 4.4, the calculated \((t_{100\%R})/2-t_{1/2}\) values ranged between 17.5 and 35.5 h, while the \(\Delta t_{100\%R}\) values ranged between 24 and 37 h. In addition, \((t_{100\%R})/2-t_{1/2} = 35.5\) h corresponds to \(\Delta t_{100\%R} = 37\) h, while \((t_{100\%R})/2-t_{1/2} = 17.5\) h corresponds to \(\Delta t_{100\%R} = 24\) h. If the zero-order model were applicable, then for each treatment the difference between the observed and the model based half-life value would be fully attributable to poor resolution of the \(\Delta t_{100\%R}\) data. The fact that \(t_{1/2}/t_{100\%R} < 0.50\) for all of the treatments monitored implies that the use of the first order (rather than zero order) TCE dechlorination model to compare the effectiveness of the treatments monitored in this study is justified. Obviously, the use of the first-order model would be more justifiable with increasing values of \(t_{1/2}/t_{100\%R}\).

It is instructive to see how the model – derived TCE dechlorination rate data and \(\Delta t_{100\%R}\) data correlate across the different rootmatter-amended treatments monitored in this study (Figure 4.11).
Evidently, the rate constant and $\Delta t_{100\%R}$ data correlate reasonably well ($R^2 = 0.8282$). As expected, the observed TCE $\Delta t_{100\%R}$ and dechlorination rate constant values correlate in an inverse manner. The reasonably high correlation between the two sets of data validates the use of the first-order kinetic model to assess the influence of different plant species rootmatter on TCE dechlorination. This is in addition to the fact that the correlation attests to the notion that the influence of rootmatter from different plant species on cVOC dechlorination is both quantifiable on the individual species basis and predictable across the different species.

![Graph](image)

**Figure 4.11:** Correlation between TCE dechlorination rates and 100% removal times across different treatments
4.5 Conclusions

This study has demonstrated that wetland plant rootmatter has a significant influence on dechlorination of cVOCs in engineered soils. The influence is attributable to the ability of roots of most plant species to enhance microbial activity through availing readily usable carbon sources and nutrients plus the provision of habitats and action sites for useful microorganisms. It has also been shown that rootmatter of different wetland plant species differ in their ability to influence cVOC dechlorination. This is attributed to different biological traits of different species of wetland plants. In this study, the TCE dechlorination rate for the rootmatter of the best performing wetland plant species was twice as high as that of the worst performing wetland plant species’ rootmatter.

In addition to the above, *Juncus effusus* rootmatter had the most significant positive influence on cVOC dechlorination. This is judged from the treatment’s TCE dechlorination rate constant, which was the highest (twice as high as the worst case and almost twice as high as the live control) and cVOC dechlorination completion times, which were the shortest for both TCE and its daughter products *cis*-1,2-DCE and VC. Based on the influence of rootmatter alone, *Phragmites communis* and *Typha latifolia*, which are the commonest and most popular wetland plant species used in EWSs, are comparable in effectiveness as alternative wetland plants for EWSs intended to treat cVOCs.
CHAPTER 5: EFFECTS OF SALINITY ON TREATMENT OF CHLORINATED VOLATILE ORGANIC COMPOUNDS IN ENGINEERED WETLAND SYSTEMS

5.1 Introduction

Factors that influence the performance efficiency and effectiveness of engineered wetland systems (EWSs) intended to treat chlorinated volatile organic compounds (cVOCs) and other pollutants can be classified under: 1) system design and implementation factors; 2) operation and maintenance factors; 3) system intrinsic properties and characteristics depending on its components; 4) influent water characteristics such as nature, concentrations, and toxicity of its constituents; and 5) external environment factors such as climatic factors.

For EWSs and similar treatment and remediation systems, consideration of the factors identified above must be made with the needs and influences of the treatment media and vegetation in mind. This is because these are the most important and influential components of any EWSs (Chapters 1, 2, 7, and 8). In this context, one of the most important environmental factors that can influence the performance of EWSs and limit their application is salinity. Salinity stress is an important consideration because: 1) salinity affects useful microorganisms and vegetation directly and indirectly through influencing the chemistry of the soil (media) (Sairam et al., 2006) and 2) it is a widely prevalent problem (Miguel et al., 2005). Both of the above have relevance to EWSs. The first directly affects the treatment media, wetland vegetation, and microorganisms that mediate treatment processes in EWSs. The second limits spatial or geographical application of EWSs.
In the context of EWSs, the importance of salinity is reflected in EWS design and implementation needs; in some cases salinity necessitates the adoption of plants that are specially adapted to high salinity. For example, Wu et al. (2008) recommend *Aegisseras carmiculatum* (mangrove) for conventional wetlands because of its salt tolerance. EWSs can be exposed to high salinity when they are used in coastal environments (Pardue, 2005). Another exposure route is treatment of high salinity contaminated water such as acid mine drainage (AMD).


The main objective of this study was to evaluate the effects of salinity on the performance of EWSs vegetated with *Phragmites communis* (common reed) and *Typha latifolia* (cattail) treating cVOCs. *Phragmites communis* and *Typha latifolia* are the most commonly utilized wetland plants in wetlands used for treating wastewater. *Phragmites communis* is known to be effective in enhancing cVOC dechlorination (Lorah et al., 1997, Lorah and Olsen, 1999; Pardue, 2005), while *Typha latifolia* is preferred by potential managers of wetlands (Pardue, 2005). The need for this study stemmed from the fact that EWSs for treating cVOCs are likely to be exposed to high salinity, which can influence their performance and effectiveness. A review of the available literature revealed a shortage of this kind of information. This study also evaluated genetic
differences in bacteria colonizing roots and bulk soil at different depths in the EWS units treating cVOCs under high salinity conditions.

5.2 Materials and Methods

5.2.1 Experimental Set-up Description

The experimental set up used in this study was as schematically illustrated in Figures 5.1 and 5.2. This set-up had been being used for treating 1,1,2,2-TeCA and TCE under negligibly low salinity conditions previously (Chapter 3) for over seventeen months.

As shown in Figure 5.1, the experimental system was designed to be operated as a set of four independent engineered wetland system (EWS) units so as to facilitate influent flow control. Each EWS unit was made up of one feed water reservoir, one pumping system, and one upflow EWS unit. Descriptions of each EWS unit components, including specification of bed materials, and pumping system are detailed in Chapter 3.

**Figure 5.1:** Schematic lay out plan of four mesocosm scale engineered wetland system units for co-treating 1,1,2,2-TeCA and TCE. Note that the discharge line from each pump delivers the influent solution to each EWS unit through three inlets in an upflow mode.
The feed water reservoir had a capacity of 37.8 L. Although this storage capacity would be sufficient for at least 2.5 d of operation at a planned flow rate of 14.4 L/d, it was replenished daily to minimize head space with a view to minimizing volatilization of the cVOCs. The pumping system comprised a peristaltic pump and Tygon tubing for suction of the feed water from reservoir and controlled discharge into the EWS units. The feed water was pumped through each EWS unit in an upflow manner in order to minimize volatilization of the influent and enhance anaerobic conditions by minimizing contact between the influent and the air.

Each EWS unit was constructed using a rectangular glass container measuring 90.2 cm long, 30.5 cm wide, and 25.5 cm deep. All the four EWS units were set up side by side in the greenhouse so as to be able to control environmental conditions and ensure that they were exposed to the same conditions. Three parallel inlets installed through the bottom of each EWS unit were provided to facilitate uniform distribution of flow across the wetland unit bed. The connection for the effluent discharge was installed near the top.

**Figure 5.2:** Schematic section of mesocosm scale engineered wetland system unit for co-treating 1,1,2,2-TeCA and TCE
A total of 15 Biopsy needles were installed (as shown in Figures 5.1 and 5.2) to serve as sampling ports to facilitate convenient withdrawal of pore water samples from the EWS bed. Each biopsy needle was secured in place by a rubber plug inserted in a hole drilled through the side of the EW unit. Stainless steel connectors for inlets were similarly secured in place.

The bed material for each EWS unit was blended using three constituents: BionSoil, a processed animal waste (Dream Maker Dairy, Cowlesville, NY), Latimer peat (Latimer’s Peat Moss Farm, West Liberty, OH), and commercial sand from Home Depot (Baton Rouge, LA). The mix design for the bed material components was: BionSoil (37.5% w/w), Latimer peat (37.5% w/w), and sand (25% w/w). The basis of the mix design and bed material characterization data are given in Chapters 1 - 3.

Both Phragmites communis and Typha latifolia were used because this study was meant to compare the two wetland plant species. The two were selected because they are two of the commonest and ubiquitous wetland plants (Chapter 3). More specifically, Phragmites communis was selected because it has been found to flourish in areas contaminated with cVOCs and there have been indications that it enhances processes that treat cVOCs (Lorah et al., 1997; Lorah and Olsen, 1999; Pardue, 2005; Chapter 3). On the other hand, Typha latifolia is preferred by potential users of the wetlands technology because it supports habitats suitable for diverse fauna species unlike the more woody Phragmites communis (Chapter 3).

5.2.2 Specifications and Preparation of Sulfate Containing Feed-water

The commercially available Instant Ocean® sea salt (Aquarium Systems, Inc., Mentor, OH 44060 USA) was used as the source of sulfate. The salt is certified to be free of both nitrate and phosphorus. Apart from the salt components, Instant Ocean® sea salt
contains major and trace elements necessary for the health of marine fish and invertebrates.

To prepare the salt feed water for pumping through the wetland units, a predetermined quantity of sea salt sufficient for one batch of feed water was dissolved in water and stirred until it dissolved completely. The resulting homogeneous sea salt solution was poured into the feed water preparation container. Then, a predetermined quantity of a premixed solution of 1,1,2,2-TeCA and TCE was added followed by water. Stirring was done as each component of the feed water was being added. Every time a new batch of feed water was needed, a fresh solution of salt water was prepared and used immediately.

According to its specifications, about 36 g of Instant Ocean® sea salt is enough to prepare 1 liter of full strength sea water. For achieving the objectives of this study, 10% full strength salt water was selected. Batches of feed water were prepared in volumes of 20 liters. As such, every batch of feed water (10% strength sea water) was prepared using 72 g of Instant Ocean® sea salt.

5.2.3 Experimental System Operation and Monitoring

Overall, the study was accomplished in three serial phases. First, all the EWS units were operated under salinity–free conditions. Next, they were operated under a low level of salinity. Finally, they were operated under a high level of salinity. Each phase was preceded by a sufficiently long period of time to allow for stabilization of the EWS units. The performance of each of the EWS was monitored and evaluated on the basis of treatment of the eVOCs, reduction in sulfate concentration during treatment in the EWS units, and effects of salinity on the wetland plants. Sulfate concentration was used as the surrogate for salinity.
Pumping of the saltwater through the wetland units started at midday on September 5th 2005, beginning with a low concentration. Pumping of the high sulfate concentration feed water started at midday on October 14th 2005. The beds of the four EWS units were kept saturated with water during the whole study period to maintain anaerobic conditions in the bulk of the EWS beds.

Samples of pore water for analysis were taken through the sampling ports shown in Figures 5.1 and 5.2 (a total of 15 per EWS unit). Samples from the same EWS unit were always taken at the same time and analyzed together. Samples from EWS units that were compared were taken at the same time and analyzed together in sets of corresponding sampling ports. All samples were analyzed immediately after collection, which obviated the need for sample preservation and storage.

5.2.4 Analytical Work

Aqueous phase samples were analyzed for VOCs following EPA Method 8260B using GC/MS (Agilent Technologies 6890N Network GC System, Agilent Technologies 5973 Network Mass Selective Detector) coupled to an AquaTek 70 Autosampler® (Teledyne Tekmar) and Velocity XPT® purge and trap sample concentrator (Teledyne Tekmar). Analytical standards and surrogate standards for the cVOCs were obtained as mixtures from Supelco Inc. Sulphate in the feedwater samples was analyzed using a Hach spectrometer (Hach Co., USA) in accordance with the manufacturer’s instructions. All samples were analyzed immediately after collection.

5.2.5 Microbial Community Analysis

5.2.5.1 Soil and Rootmatter Sample Collection and Preparation

At the end of the experiments on the evaluation of salinity effects on the treatment of chlorinated volatile organic compounds in the EWS units described earlier, samples of
soil and rootmatter were collected from the EWS units. A core of the middle one third of the EWS bed (one for each wetland species) comprising both roots and soil was taken. The section of interest was the one located above the middle inlet (see Figures 5.1 and 5.2) and adjacent to the middle row of the sampling ports (see Figures 5.1 and 5.2). The core was loosened and removed from rest of the EWS bed by cutting the bed using a sharp knife at the one third and two thirds marks along its length across its full height from the top to the bottom, and across its width from the front to the back. The core removed from each EWS bed was sectioned across its height into three equal parts: bottom, middle, and top sections. Each of the three sections was separated into rootmatter and bulk soil samples.

Rootmatter samples were rinsed by carefully shaking them in saline phosphate-buffer (7 mM Na₂HPO₄, 3 mM NaH₂PO₄, 130 mM NaCl [pH 7.2]) to remove adhering soil particles. Soil samples were obtained from the bulk soil between the roots. Rootmatter in bulk soils was removed to avoid cross-contamination. All the samples were handled with great care all the time during processing to avoid cross-contamination.

5.2.5.2 DNA Extraction from Soil and Rootmatter Samples

Total genomic DNA of bulk soils and rootmatter was extracted from 250 mg of the samples (on a dry basis) using the MoBio UltraClean Soil DNA Isolation kit (MoBio Laboratories, Inc., Solana Beach, CA) with a modified version of the manufacturer's protocol as follows: the soil was rinsed by a washing buffer (120 mM sodium phosphate, pH 8.0) three times (Lee et al., 1996) before the first step of the manufacturer's protocol. Following extraction, the purified DNA was resuspended in 50 µL of solution S5 (MoBio Laboratories, Inc.) and stored at –20°C until PCR amplification.
5.2.5.3 16S rDNA Amplification and DGGE Analysis

For denaturing gradient gel electrophoresis (DGGE) analysis, variable regions V3 through V5 of 16S rDNA (corresponding to positions in *Escherichia coli*) were amplified with forward primer 341F-clamp (specific for members of the *Bacteria*) and reverse primer 907R (universal primer) (Muyzer et al., 1998). The PCR reactions for both sets of primers contained 0.2 mM of each primer, 0.2 mM of each deoxynucleotide, 2 mM MgCl₂, 1U of Taq polymerase (Promega, Madison, WI), 1 µL of DNA extract, and 1×PCR reaction buffer in a final volume of 50 µL. PCR amplification was performed with a Bio-Rad iCycler as follows: 95°C for 7 min, followed by 35 cycles of 95°C for 45 s, 55°C for 45 s, and 72°C for 30 s and a final extension at 72°C for 10 min. DGGE analysis of 16S rDNA fragments was performed using DCodeTM universal mutation detection system (BioRad Laboratories, Hercules, CA). Gels (16 cm×16 cm) consisted of 6% acrylamide/bis-acrylamide (37.5:1) and a denaturant gradient of 40–60%. The 100% denaturant was 7M urea and 40%[vol/vol] deonized formamide. Electrophoresis was performed in 1×TAE buffer (0.04 M Tris base, 0.02 M sodium acetate, and 1 mM EDTA; pH 7.4) at 60°C and 65V for 15 h. Approximately 700 ng (bacteria) of PCR product from samples were applied to individual lanes in the gel. Gels were stained for 10 min with ethidium bromide (EtBr) staining, destained in water for 10 min and analyzed using the Gel Doc 2000 system (BioRad Laboratories, Hercules, CA) and Quantity One software (BioRad Laboratories, Hercules, CA). This software carries out a density profile analysis of each lane, detect the bands, and calculates the relative contribution of each band to the total band intensity in the lane. Then the software identifies the bands occupying the same position in the different lanes of the gel. A dendrogram relating band pattern similarities was automatically calculated with the Dice coefficient, without band weighting.
(consideration of band density) by the unweighted pair group method using arithmetic averages (UPGMA) in this software.

5.3 Mathematical Analysis and Modeling of Chemical Analysis Data

The eVOC data were statistically processed and summarized. 1,1,2,2-TeCA degradation data were fit to a pseudo-first order kinetic model (Equation 5.1):

\[ [E] = [E]_0 e^{-k_E x} \]  

where \([E]_0\) = the concentration of 1,1,2,2-TeCA (moles/L), \(k_E\) = the reaction rate constant (cm\(^{-1}\)), \(t\) = distance up the EWS unit (cm), and the subscript ‘o’ refers to initial conditions. Recognizing that the degradation rate of TCE resulting from the dehydrochlorination of 1,1,2,2-TeCA was dependent on the degradation of both 1,1,2,2-TeCA and TCE itself, the pertinent data were modeled according to Equation 5.2.

\[ \frac{d[C]}{dx} = k_E [E]_0 e^{-k_E x} - k_C [C] \]  

where \([C]\) = concentration of TCE (moles/L) and \(k_C\) = the actual rate of TCE degradation (cm\(^{-1}\)). Equation 5.2 was solved to yield Equation 5.3 which was used to model the TCE data.

\[ [C] = \frac{k_E [E]_0}{k_C - k_E} e^{-k_E x} + \left( [C]_0 - \frac{k_E [E]_0}{k_C - k_E} \right) e^{-k_C x} \]  

Use of Equation 5.3 for regression analysis of 1,1,2,2-TeCA and TCE data to determine the actual (as opposed to the apparent) reaction rate constant of TCE was done using SigmaPlot (SPSS Inc., Chicago IL) with a few mathematical manipulations. The apparent kinetic rate for TCE was determined using Equation 5.1 but with \(E\) and \(E_0\) replaced by \(C\) and \(C_0\), respectively.
The performance effectiveness of the EWS units was quantified on the basis of both the cVOC removal rate constant data and the EWS bed depths required to achieve complete removal of the cVOCs and their daughter products.

5.4 Results and Discussion

5.4.1 1,1,2,2,-TeCA and TCE Treatment Performance Results

Figures 5.3 and 5.4 illustrate results of treatment of the water containing 1,1,2,2-TeCA and TCE in the EWS units prior to commencing to feed them saline water containing sulfate as well as 1,1,2,2-TeCA and TCE.

Figures 5.5 and 5.6 illustrate the performance of the EWS units after commencing to pump through them the water containing 1,1,2,2-TeCA and TCE and a low level of salinity (monitored based on sulfate). Figures 5.8 and 5.9 illustrate the performance of the EWS units after commencing to pump through them water containing 1,1,2,2-TeCA and TCE and a high level of salinity.

Table 5.1 shows a summary of quantitative measures for comparing the performance efficiencies of the EWS units under salinity free operating conditions. Table 5.2 provides the same information for the period when the EWS units were operated under low salinity. Table 5.3 gives a corresponding summary for the period when the EWS units were operated under a high level of salinity. Figure 5.7 illustrates profiles of sulfate concentrations in pore water in the EWS beds when the EWS units were operated under a low level of salinity. Figure 5.10 provides the same information for the period when the EWS beds when the EWS units were operated under a high level of salinity.

Figures 5.3 – 5.6, 5.8 – 5.9 and Tables 5.1 – 5.3 suggest that treatment of 1,1,2,2-TeCA and TCE in both the Phragmites and Typha EWS units was affected by salinity. The effects manifested by way of accumulation of 1,1,2,2-TeCA and TCE dechlorination

140
products and the persistence of both 1,1,2,2-TeCA and TCE and their daughter products. As a result, the depth at which 1,1,2,2-TeCA was still detected in the EWS beds was higher at high salinity than at low salinity. These observations are clearly evident if, for example, Figures 5.8 and 5.9 (high salinity) are compared to Figures 5.3 and 5.4 (low salinity).

**Figure 5.3:** 1,1,2,2-TeCA and TCE removal in *Typha* EWS units when operated under salinity free conditions. Each data point is a mean of results of analysis of three different replicates.
Figure 5.4: 1,1,2,2-TeCA and TCE removal in Phragmites EWS units when operated under salinity free conditions. Each data point is a mean of results of analysis of three different replicates.

In quantitative terms, Tables 5.1 – 5.3 show a general reduction in removal rate constants, which were most marked for 1,1,2,2-TeCA. Tables 5.1 – 5.3 also show an increase in the heights at which complete removal ($H_{100\%R}$) of 1,1,2,2-TeCa and TCE and their daughter products were observed. In the Phragmites vegetated EWS units, the rate constant for 1,1,2,2-TeCA removal was reduced from 0.239 cm$^{-1}$ under salinity free conditions to 0.190 cm$^{-1}$ under low salinity conditions, to 0.185 cm$^{-1}$ under high salinity conditions. The corresponding figures for the Typha vegetated EWS units were 0.584 cm$^{-1}$, 0.479 cm$^{-1}$, and 0.276 cm$^{-1}$.
Table 5.1: Comparison of performances of *Phragmites communis* and *Typha latifolia* EWS units under the influence of salinity free operating conditions

<table>
<thead>
<tr>
<th>EWS unit</th>
<th>Chemical</th>
<th>Rate constant (cm⁻¹)</th>
<th>$H_{100%R}$ (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phragmites communis</em></td>
<td>1,1,2,2-TeCA</td>
<td>0.2386 (R²=0.9901)</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0.1917 (R²=0.9564)</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>cis-1,2-DCE</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1,1,2-TCA</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Trans-1,2-DCE</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td><em>Typha latifolia</em></td>
<td>1,1,2,2-TeCA</td>
<td>0.5842 (R²=0.9999)</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0.3666 (R²=0.9994)</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>cis-1,2-DCE</td>
<td>-</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1,1,2-TCA</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Trans-1,2-DCE</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

$H_{100\%R}$, height at which 100% removal of the cVOC was achieved. The degradation rate constant of TCE is the apparent rather than the actual. Degradation rate constants for the other cVOC were not evaluated due low levels of these compounds. The reported $H_{100\%R}$ values represent the highest of those observed among replicates.

Under salinity free conditions, the highest $H_{100\%R}$ value was 18.0 cm for both the *Phragmites* and *Typha* EWS beds. At low salinity conditions, this rose to 25.5 cm for both sets of EWS units. At the highest salinity loading rate, the *Phragmites* EWS units sustained breakthrough of vinyl chloride (VC). Notably, the *Typha* EWS units did not exhibit breakthrough of the parent or daughter products through out this study.

Judged on the basis of the daughter products detected during treatment of the cVOCs, a number of generalizing observations can be made. Evidently, the pathways of dechlorination of 1,1,2,2-TeCA were similar between the *Phragmites* and *Typha* EWS units as well as among the phases (different salinity levels). The latter assertion implies that the pathways did not change with increase in salinity. It can be argued that the failure
to detect dechlorination daughter products such as vinyl chloride and chloroethane under salinity free conditions was simply because they degraded faster than they were generated. Apparently, the dechlorination rates of the daughter products were reduced by salinity, leading to their accumulation. We have previously observed in microcosm experiments (Chapters, 2, 6, and 7) non-accumulation of dechlorination products of 1,1,2,2-TeCA and TCE at low concentrations or during treatment of single cVOCs, and accumulation at higher concentrations or during treatment of more than one cVOC.

Figure 5.5: 1,1,2,2-TeCA and TCE removal in Typha EWS units when operated under low salinity conditions. Each data point is a mean of results of analysis of three different replicates.
It can be inferred from the preceding observations that salinity changed the dechlorination rates rather than the pathways. The 1,1,2,2-TeCA dechlorination pathways exhibited in this study are similar to those reported in the literature (Lorah et al., 1997; Lorah and Olsen, 1999; Lorah and Voytek, 2004; Aulenta et al., 2005&2006; Hunkeler et al., 2006). The pathways observed in this study are also the same as the ones observed in our other microcosm and mesocosm studies (Chapters 2, 3, 4, 6, 8). Results from this and other similar studies (Lorah et al., 1997; Lorah and Olsen, 1999; Lorah and Voytek, 2004; Aulenta et al., 2005&2006; Hunkeler et al., 2006) indicate the following likely
pathways for 1,1,2,2-TeCA and TCE dechlorination: 1) 1,1,2,2-TeCA→TCE→cis-1,2-DCE and trans-1,2-DCE→VC→ethene; 2) 1,1,2,2-TeCA→1,1,2-TCA→VC→ethene; 3) 1,1,2,2-TeCA→1,2,2-TCA→1,2-DCA→chloroethane→ethane; and 4) 1,1,2,2-TeCA→cis-1,2-DCE and trans-1,2-DCE→VC→ethene.

Figure 5.7: Sulfate concentration profile in Typha latifolia and Phragmites communis EWS units when they were operated under a low level of salinity. Each data point is a mean of results of analysis of three different replicates.
Table 5.2: Comparison of performances of *Phragmites communis* and *Typha latifolia* EWS units under the influence of low salinity operating conditions

<table>
<thead>
<tr>
<th>EWS unit</th>
<th>Chemical</th>
<th>Rate constant (cm⁻¹)</th>
<th>$H_{100%R}$ (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phragmites communis</em></td>
<td>1,1,2,2-TeCA</td>
<td>0.1904 (R²=0.9702)</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0.1341 (R²=0.8444)</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>cis-1,2-DCE</td>
<td>-</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>-</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>-</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>1,1,2-TCA</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Trans-1,2-DCE</td>
<td>-</td>
<td>18</td>
</tr>
<tr>
<td><em>Typha latifolia</em></td>
<td>1,1,2,2-TeCA</td>
<td>0.4791 (R²=0.9998)</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0.2812 (R²=0.9968)</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>cis-1,2-DCE</td>
<td>-</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>-</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>1,1,2-TCA</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Trans-1,2-DCE</td>
<td>-</td>
<td>18.0</td>
</tr>
</tbody>
</table>

$H_{100\%R}$, height at which 100% removal of the cVOC was achieved. The degradation rate constant of TCE is the apparent rather than the actual. Degradation rate constants for the other cVOC were not evaluated due low levels of these compounds. The reported $H_{100\%R}$ values represent the highest of those observed among replicates.

Despite the effects of high salinity, even at the highest salinity level, characterized by sulfate concentrations of 275 mg/L, the *Typha* EWS units were able to completely remove the cVOC parent and daughter products within 25.5 cm of their bed depths. For the *Phragmites* EWS units, only VC broke through. These findings are in agreement with those reported by Aulenta et al. (2007). They observed effective dechlorination activity under sulfate reducing conditions, and concluded that it was not necessary for contaminated water to be sulfate free for a cVOC remediation or treatment system to be effective. These observations demonstrate the feasibility of using EWSs to treat cVOCs at high salinity levels.

At low salinity (Figure 5.7), sulfate concentration was reduced by 72.2% in both the *Phragmites* and *Typha* EWS units. At the high salinity level (Figure 5.10), the
Phragmites EWS units reduced sulfate by 41.8%, while the corresponding figure for the Typha units was 40%. These sulfate reduction data would seem to suggest that sulfate removal decreased with salinity. That cannot be further from the truth, for at the low salinity level the Phragmites EWS units removed 1696 mg m\(^{-2}\) d\(^{-1}\) but removed 6000 mg m\(^{-2}\) d\(^{-1}\) at the highest salinity level. The Typha EWS units removed 1696 mg m\(^{-2}\) d\(^{-1}\) of sulfate at the low salinity level but removed 5739 mg m\(^{-2}\) d\(^{-1}\) at the highest level of salinity. Removal of sulfate observed in this study can be attributed to sulfate reducing bacteria (SRB).

![Diagram](image)

**Figure 5.8:** 1,1,2,2-TeCA and TCE removal in *Typha* EWS units when operated under high salinity conditions. Each data point is a mean of results of analysis of three different replicates.
The effects of salinity on the performance of the EWS units observed in this study can be partly be explained by influences of competition or unfavorable interactions between dechlorination and reduction of alternative electron acceptors such as sulfate brought about by high salinity. Aulenta et al. (2007) observed that in the presence of alternative electron acceptors such as sulfate and nitrate, dechlorination was significantly
slowed down due to the majority of electrons being consumed by the side reactions. This occurred to the detriment of the dechlorination processes.

Figure 5.10: Sulfate concentration profile in *Typha* and *Phragmites* EWS units when operated under high salinity conditions. Each data point is a mean of results of analysis of three different replicates.

The performance effects of salinity can also be attributed to influences of accumulated toxic compounds that result from reduction of sulfate and denitrification (Okutman Tas and Pavlostathis, 2008). Some of these effects are known to even result in microbial community changes (Weber et al., 2008) apart from affecting wetland plants. Salinity induces stress on plants, affecting their development and productivity (Suiram et al., 2006; Botella et al., 2005). Yilmaz (2007) observed a reduction in nickel
accumulation by *Lemna gibba* which was related to an increase in salinity. Okutman Tas and Pavlostathis (2008) observed that nitrate concentrations above 50 mg N/L resulted in accumulation of toxic compounds that interfered with treatment of compounds of interest. Wu et al. (2008) argue that wetland plants such as *Typha latifolia* (one of the species used in this study) have little tolerance for salinity and, therefore, cannot survive salt stress for a long time. Nonetheless, the *Typha latifolia* used in this study withstood the salt stress for a long time (years) without exhibiting any discernible signs of harm.

**Table 5.3:** Comparison of performances of *Phragmites communis* and *Typha latifolia* EWS units under the influence of high salinity operating conditions

<table>
<thead>
<tr>
<th>EWS unit</th>
<th>Chemical</th>
<th>Rate constant (cm⁻¹)</th>
<th>$H_{100%R}$ (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phragmites communis</td>
<td>1,1,2,2-TeCA</td>
<td>0.1847 ($R^2=0.9994$)</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0.2063 ($R^2=0.9904$)</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td><em>cis</em>-1,2-DCE</td>
<td>-</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>-</td>
<td>&gt;25.5</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1,1,2-TCA</td>
<td>-</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>Trans-1,2-DCE</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Typha latifolia</td>
<td>1,1,2,2-TeCA</td>
<td>0.2761 ($R^2=0.9860$)</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0.4554 ($R^2=0.9998$)</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td><em>cis</em>-1,2-DCE</td>
<td>-</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>-</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>-</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>1,1,2-TCA</td>
<td>-</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>Trans-1,2-DCE</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

$H_{100\%R}$, height at which 100% removal of the cVOC was achieved. The degradation rate constant of TCE is the apparent rather than the actual. Degradation rate constants for the other cVOC were not evaluated due low levels of these compounds. The reported $H_{100\%R}$ values represent the highest of those observed among replicates.

### 5.4.2 Microbial Community Genetic Similarity Analysis

Figure 5.11 shows a negative image of ethidium bromide-stained DGGE profiles of PCR-amplified 16S rRNA fragments obtained by using universal bacterial primers.
Figure 5.12 shows a cluster analysis presenting the genetic similarity of the microbial community profiles obtained by PCR-DGGE of bacteria.

**Figure 5.11:** Negative image of ethidium bromide-stained DGGE profiles of PCR-amplified 16S rRNA fragments obtained by using universal bacterial primers. S1, *Phragmites* - top soil; S2, *Phragmites* – middle soil; S3, *Phragmites* – bottom soil; S4; *Typha* – top soil; S5, *Typha* – middle soil; S6, *Typha* – bottom soil; R1, *Phragmites* – top rootmatter; R2, *Phragmites* – middle rootmatter; R3, *Phragmites* – bottom rootmatter; R4, *Typha* – top rootmatter; R5, *Typha* – middle rootmatter; R6, *Typha* – bottom rootmatter.

It is evident from Figures 5.11 and 5.12 that A, B, C and D were the dominant bands of the DGGE patterns of rootmatter samples, with the exception R3. On the other hand, bands E and F were the dominant bands in bulk soil samples (Figure 5.12). Figures
5.11 and 5.12 suggest that bacteria that colonized the roots and the bulk soil in the beds of both the *Phragmites* and *Typha* EWS units were generally different. The bacteria that colonized rootmatter at the bottom of the *Phragmites* EWS unit bed (R3) were the exception; they were more closely related to the bacteria that colonized the bulk soil, interestingly, in the middle of the *Typha* EWS unit bed. It appears that the bacteria that colonized roots of *Phragmites communis* (A1) were different from those that colonized

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**Figure 5.12:** Cluster analysis presenting the genetic similarity of the microbial community profiles obtained by PCR-DGGE of bacteria. S1, *Phragmites* - top soil; S2, *Phragmites* – middle soil; S3, *Phragmites* – bottom soil; S4, *Typha* – top soil; S5, *Typha* – middle soil; S6, *Typha* – bottom soil; R1, *Phragmites* – top rootmatter; R2, *Phragmites* – middle rootmatter; R3, *Phragmites* – bottom rootmatter; R4, *Typha* – top rootmatter; R5, *Typha* – middle rootmatter; R6, *Typha* – bottom rootmatter.
roots of *Typha latifolia* (A2). This may partly explain why the *Typha latifolia* EWS units performed better than the *Phragmites communis* EWS units in this study.

The observed trend of different bacteria colonizing roots versus bulk soil is not unexpected given the differences between these sites and because of the way roots interact with soil microorganisms (Gregory, 2006). On the whole, because of availability of more readily biodegradable substrates in the vicinity of roots (Bardgett, 2005), more microorganisms are expected to colonize roots than the bulk soil. Rhizodeposition is the main attraction of microorganisms to roots (Gregory, 2006). The difference in bacteria that colonized roots versus the bulk soil can be interpreted as signifying the importance and usefulness of wetland plants. It can be argued that the presence of wetland plants brings about or enhances the presence of microorganisms that would otherwise be absent or whose populations in the EWS bed would be low.

### 5.5 Conclusions

This study assessed effects of salinity on the treatment of chlorinated volatile organic compounds (cVOCs) in engineered wetland systems (EWSs) vegetated with *Phragmites communis* and *Typha latifolia*. A number of major conclusions that can be made based on the findings from this study are outlined below.

- Salinity negatively affected the performance of EWSs and the effects seemed to increase with salinity. In the *Phragmites* vegetated EWS units, the rate constant for 1,1,2,2-TeCA removal was reduced from 0.239 cm\(^{-1}\) under salinity free conditions to 0.190 cm\(^{-1}\) under low salinity conditions, to 0.185 cm\(^{-1}\) under high salinity conditions. The corresponding figures for the *Typha* vegetated EWS units were 0.584 cm\(^{-1}\), 0.479 cm\(^{-1}\), and 0.276 cm\(^{-1}\).
Despite the conclusion that salinity negatively affects the performance of EWSs treating cVOCs, the removal rates of the cVOCs even under the influence of salinity are high enough to make the use of EWSs to treat cVOCs feasible. However, the performance effect of salinity must be taken into consideration during design.

*Typha latifolia* is a better wetland plant species than *Phragmites communis* with respect to treating cVOCs in EWSs at both low and high salinity levels. The performance superiority of *Typha latifolia* was even more pronounced under salinity free conditions. This wetland plant also flourished well under high salinity as well as salinity – free conditions.

Within the range of salinity used in this study, removal of sulfate in both the *Phragmites communis* and *Typha latifolia* vegetated EWS units increased with salinity. The *Phragmites communis* EWS units removed 1695.5 mg SO$_4^{2-}$/m$^2$ d$^{-1}$ at low salinity ($C = 45$ mg SO$_4^{2-}$/L), but removed 5999.5 mg SO$_4^{2-}$/m$^2$ d$^{-1}$ at high salinity ($C = 275$ mg SO$_4^{2-}$/L). The *Typha latifolia* EWS units removed 1695.5 mg SO$_4^{2-}$/m$^2$ d$^{-1}$ at low salinity but removed 5738.6 mg SO$_4^{2-}$/m$^2$ d$^{-1}$ at high salinity.

Based on genetic similarity analysis, for the most part, bacteria that colonized roots in EWS beds differed from those that colonized the soil away from the roots. Also, bacteria that colonized the roots of *Phragmites communis* and *Typha latifolia* exhibited more similarities within rather than between the two wetland plant species.
CHAPTER 6: INFLUENCE OF WETLAND ABOVEGROUND BIOMASS TYPE ON TREATMENT OF CHLORINATED VOLATILE ORGANIC COMPOUNDS

6.1 Introduction

Shoots and leaves constitute the bulk of the aboveground biomass (AGB) of non-tree wetland plants commonly used in engineered wetland systems (EWSs). The AGB constitutes practically one half of a terrestrial or wetland plant biomass. The remaining half is made up of the root system – the belowground biomass (BGB). Therefore, wetland plant AGB is an important aspect of EWSs. The wetland plant AGB of most interest in connection with EWSs is the one that can serve as a source of useful substrates and their precursors. These include cellulose, amino acids, sugars, proteins, nucleic acids, and lignin. Cellulose is the main component of plant tissues (Badgett, 2005), and as such it is the principal component of wetland plant detritus.

In natural and engineered wetland systems, dead shoots and leaves continually fall to the ground where they subsequently decompose under the influence of macro-fauna and micro-fauna as well as physical and chemical processes. Decomposing wetland plants recycle organic material to the wetland medium, thereby modifying its biological, physical, and chemical characteristics (Kadlec and Knight, 1996; Badgett, 2005; Mitsch and Gosselink, 2007). In hydroponic systems and EWSs using coarse aggregate or crushed rock (materials which are mineral in nature) as media, decomposing plants have a significant influence on characteristics of the media, and consequently the performance of these engineered systems. Notably, the decaying plants may be the only source of solid undissolved organic material for hydroponic treatment systems. In their study on the effect of wetland vegetation harvesting on the performance of wetland systems, Álvarez
and Bécarez (2008) showed that decaying AGB is a very significant and important source of organic carbon, nitrogen, and phosphorus. However, in conventional EWSs intended to remove nutrients and biochemical oxygen demand (BOD), decaying wetland vegetation is an additional load. Álvarez and Bécarez (2008) have shown that this burden can be reduced by harvesting the wetland plants.

Products of biodegradation of dead plant biomass support enhanced microbial activities (Badgett, 2005) while the resulting matrix of high organic content wetland medium serves as a good sorption medium. Several studies carried out in our research group have shown that wood-based compost materials are good substrates for EWSs that treat chlorinated volatile organic compounds (cVOCs) (Chapters 7 and 8). These materials are capable of supporting vigorous microbial activity with resultant production of large quantities of methane in parallel with the dechlorination of the cVOCs. This suggests that compost materials derived from decaying wetland biomass, which are also rich in cellulose just like the wood based compost, can make a good EWS substrate. This assertion is further supported by the role that decaying plant AGB plays in modifying soil, which results in the enhancement of soil biota. Plants modify soil food webs through their litter and root exudates that they deposit in the soil (Badgett, 2005).

Owing to the differences in characteristics among wetland plants, it is conceivable that wetland substrates derived from different wetland plant species would differ in pollutant removal potential. After all, different plant species influence the soil in which they grow differently (Badgett, 2005). Litter quality controls respiration patterns of decomposers (Manzoni et al., 2008), which implies that different plant species can influence the composition of biota in their spheres of influence differently. Many studies reported in the literature indicate that different plant species differ in their potential to
enhance remediation or treatment of pollutants (Hutchinson et al., 2003). With specific reference to the effect of the AGB on waste treatment, it is known, for example, that litter from plants supplies organic carbon to denitrifying bacteria and as such can influence denitrification (Bastviken et al., 2007).

Studies on the influence of plants on in-situ remediation of polluted sites and ex-situ treatment of pollutants, especially those carried out under the auspices of phytoremediation are numerous. Good examples are many studies reported in a volume by McCutcheon and Schnoor (2003), which is wholly devoted to phytoremediation. Other recent studies include those reported by Liu et al. (2007) and Mertens et al. (2007). However, no studies have focused on the effect of the AGB on EWSs; they instead focused on the effect of the whole plant or roots on the treatment of pollutants. More specifically, none of the studies reported in the literature has focused on the effect of wetland plant ABG on dechlorination of cVOCs.

In line with the preceding discussion, the main objective of this study was two fold: 1) to investigate the influence of wetland plant AGB on dechlorination of cVOCs and 2) to compare the effectiveness and efficiency of AGB from different wetland plant species commonly used in wetland restoration and potentially suitable for EWSs intended to treat cVOCs. This study together with several others (Chapters 2, 3, and 4) were meant to contribute answers to the larger question on the influence of wetland plants on the performance of EWSs treating cVOCs.

6.2 Materials and Methods

6.2.1 Treatment Selection and Descriptions

The wetland plant species used in this study were selected to represent more than 73 identified wetland plants. The 73 wetland plants were selected as suitable based on: 1)
universality and widespread availability in the USA and 2) acceptability among practitioners and application in wetland restoration projects. The study was carried out in microcosms using the following treatments: Treatment 1, *Phragmites communis* (common reed) AGB (10 g) plus compost inoculum (5 g); Treatment 2, *Typha latifolia* (common cattail) AGB (10 g) plus compost inoculum (5 g); Treatment 3, *Scirpus validus* (softstem bulrush) AGB (10 g) plus compost inoculum (5 g); Treatment 4, *Scirpus americanus* (three square rush) AGB (10 g) plus compost inoculum (5 g); Treatment 5, *Carex nebraskensis* (Nebraska sedge) AGB (10 g) plus compost inoculum (5 g); Treatment 6, *Juncus effusus* (common rush) AGB (10 g) plus compost inoculum (5 g); Treatment 7, *Eleocharis palustris* (creeping spike rush) AGB (10 g) plus compost inoculum (5 g); Treatment 8, *Scirpus californicus* (giant bulrush or tule) AGB (10 g) plus compost inoculum (5 g); Treatment 9, *Juncus balticus* (Baltic rush, wire rush) AGB (10 g) plus compost inoculum (5 g); Treatment 10, *Carex emory* (Emoryi’s sedge) AGB (10 g) plus compost inoculum (5 g); Treatment 11, *Scirpus pungens* (common three square) AGB (10 g) plus compost inoculum; Treatment 12, compost mixture - Control 1; Treatment 13, α-cellulose - Control 2. The AGB consisted of dead (and fallen) wetland stems and leaves collected from microcosm wetlands (one for each wetland plant species). The microcosm wetlands had been being nurtured in the greenhouse for more than two years. The compost used in the Control 1 and as inoculum was made up of a mixture of wood – based compost: row crop compost (RCC), soil builder compost (SBC), and sand (37.5:37.5:25 w/w). This compost mixture was adopted as an inoculum because it had been found to be suitable in previous studies (Chapter 7). The AGB for *Phragmites communis* included leaves only because stems of *Phragmites* plant are less amenable to microbial decomposition than those of the other wetland species.
6.2.2 Wetland Plant Biomass Acquisition and Pre-use Preparation

The seedlings for wetland plants used as sources of the AGB were originally bought from Hydra Aquatica (Tijeras, NM 8705 USA). All the seedlings had been growing in the supplier’s nurseries prior to being shipped. Only seedlings of the right age for transplanting were selected. The seedlings were shipped and delivered in separate containers for each plant species and each container had separate slots for each seedling to facilitate their separation during transplanting.

Immediately upon delivery from the supplier, the seedlings were transplanted to the greenhouse microcosm wetlands. A starting separation distance of about 10 cm between seedlings was adopted to allow the plants to grow and develop their root systems freely. The wetland plants were grown in a mixture of a commercial potting soil (Miracle Gro) and sand (3:1 w/w). Sand was mixed with the potting soil because media for EWSs often incorporate sand to improve their geotechnical and hydrodynamic properties (Chapters 2, 3, 6 and 7).

All the wetland plants were grown under the same conditions and for the same length of time before use. They were allowed to grow to maturity so as to ensure adequate development of their biomass both belowground and aboveground. Sufficient soil depth in the microcosm wetlands and space were provided to ensure this.

6.2.3 Microcosm Set-up and Monitoring

The microcosms were constructed using 160 mL serum bottles. For each of the treatments containing wetland plant AGB, 10 g of the AGB plus 5 g of compost inoculum were added into the serum bottle first. Then, DI water was added to fill the bottle to a final volume of 135 mL. The remainder of the bottle’s volume was left as headspace filled with nitrogen gas. 1,1,2,2-TeCA solution was added later separately, as described
later. When adding the 1,1,2,2-TeCA solution, an equal volume of DI was withdrawn from each bottle using a syringe under anaerobic conditions. For Control 1 (compost mixture), 15 g of the compost mixture was used instead of 5 g (which was the case with the AGB amended treatments). For Control 2 (α-cellulose), 10 g of α-cellulose and 5 g of compost inoculum were used. The remainder of the volumes for the controls was apportioned in a manner similar to the one for the AGB treatments.

To put each wetland plant species AGB in the form suitable for this study, freshly collected AGB was reduced in size, first by cutting with a pair of scissors, and finally by grinding using a pestle and mortar. Liquid nitrogen was poured on the AGB being ground to make the grinding easier and more effective. Once the grinding was complete, the ground AGB of each wetland species was weighed and placed in appropriate microcosm bottles. Three replicate microcosm bottles were used per each treatment. After the AGB from all the wetland species had been ground and placed in the microcosm bottles, the bottles were transferred to an anaerobic chamber for further processing. The anaerobic chamber was filled with nitrogen gas. In the anaerobic chamber, the compost inoculum was added to each treatment. Control 1 (compost mixture) was prepared in the anaerobic chamber from the beginning to the end to minimize exposure of the inoculum to aerobic conditions. The addition of the DI water to bring to 135 mL the final volume in each microcosm bottle was done in the anaerobic chamber as well. After all the contents had been placed in each bottle, rubber septa and aluminum crimp seals were put in place, ensuring air-tightness. The microcosms were statically incubated at room temperature (25°C) in the dark.

Initially, the microcosms were incubated for 31 days without 1,1,2,2-TeCA to monitor the evolution of ethene from the wetland AGB as discussed in greater detail.
later. Monitoring for 1,1,2,2-TeCA dechlorination commenced on day 31 immediately after spiking the cVOC into the microcosm bottles and continued until day 174. The addition of 1,1,2,2-TeCA into the microcosm bottles was done as described earlier.

6.2.4  **Aqueous and Gas Phase Sample Analysis**

Aqueous phase samples were analyzed for VOCs using EPA Method 8260B. Methane, ethene, and ethane gases were analyzed using GC/FID. 1 mL of head space sample was injected into the gas chromatography with flame ionization detector (Agilent 5890 Series II) equipped with a 2.4 m x 0.32 mm ID column packed with Carbopack b/1% Sp-(Supelco, Bellefonte, PA). The column 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.

6.2.5  **Wetland Aboveground Biomass Moisture and Organic Content Analysis**

The AGB intended for moisture and organic content analysis was apportioned from the one used in the microcosms. As such, it was acquired and initially processed in the same way as the AGB intended for the microcosm experiments described earlier. The moisture and organic content analysis was done according to the Standard Methods for the Analysis of Water and Wastewater (APHA et al., 1998). Drying was done at 103°C while combustion was done at 550°C. It was carried out immediately after the preliminary preparations were completed without storage.

6.3  **Statistical and Mathematical Analysis**

Statistical analysis of 1,1,2,2-TeCA dechlorination data for the different AGB treatments was done using both ANOVA and paired t-tests to determine if there were any significant differences among the treatments. To compare the influence of the different wetland plant species AGB on the degradation of 1,1,2,2-TeCA, 1,1,2,2-TeCA
degradation rate constants were determined by regression analysis of 1,1,2,2-TeCA
dechlorination monitoring data based on first- order kinetics (Equation 6.1). Appropriate
statistical corrections were also done.

\[ E = E_0 e^{-kCt} \]  

where \( E \) = the concentration of 1,1,2,2-TeCA \([\text{ML}^{-3}]\), \( k_C \) = the reaction rate constant \([\text{T}^{-1}]\),
\( t \) = time \([\text{T}]\), and the subscript ‘o’ refers to initial conditions (the beginning of an
experimental run.

6.4 Results and Discussion

6.4.1 Aboveground Biomass Moisture and Organic Content Analysis Results

Results of analysis of moisture and organic matter contents of the AGB of the
wetland plant species used in this study are summarized in Table 6.1.

Clearly, the moisture content values shown in Table 6.1 indicate very dry AGB,
which makes sense because only dead (and expectedly dry) wetland plant AGB was used
in this study. These moisture content values, which ranged between 8.46 and 11.10%,
contrast sharply with those for rootmatter which ranged between 75.20 and 88.93%
(Chapter 4). Notably, the AGB of each wetland plant species assessed was even drier
than the engineered soil (moisture content = 62.03%, constituents: BionSoil/Latimer
peat/sand \([37.5:37.5:25 \text{ w/w}]\); Chapters 2 and 3). On the other hand, the AGB’s organic
content, ranging between 75.33 and 87.75%, was comparable to that of rootmatter
(Chapter 4), which was in the range 75.5 – 92.55%. In contrast, the engineered soil
(Chapters 2 and 3) had an organic content of only 21.82%. The organic content of the
AGB of each of the wetland plant species assessed was far higher than the minimum for
an organic wetland soil. A wetland soil is classified as organic (rather than mineral) if its
organic content is at least 20% (Mitsch and Gosselink, 2000&2007).
Table 6.1: Wetland aboveground-biomass moisture and organic content analysis results

<table>
<thead>
<tr>
<th>Test wetland plant species</th>
<th>Moisture content (%)</th>
<th>Organic content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phragmites communis</em> (common reed)</td>
<td>10.67</td>
<td>85.84</td>
</tr>
<tr>
<td><em>Typha latifolia</em> (common cattail)</td>
<td>8.65</td>
<td>87.75</td>
</tr>
<tr>
<td><em>Scirpus validus</em> (soft-stem bulrush)</td>
<td>9.26</td>
<td>76.86</td>
</tr>
<tr>
<td><em>Scirpus americanus</em> (three square rush)</td>
<td>9.52</td>
<td>80.25</td>
</tr>
<tr>
<td><em>Carex nebraskensis</em> (Nebraska sedge)</td>
<td>9.09</td>
<td>82.46</td>
</tr>
<tr>
<td><em>Juncus effusus</em> (common rush, corkscrew rush)</td>
<td>10.75</td>
<td>80.88</td>
</tr>
<tr>
<td><em>Eleocharis palustris</em> (creeping rush)</td>
<td>10.49</td>
<td>81.54</td>
</tr>
<tr>
<td><em>Scirpus californicus</em> (giant bulrush or tule)</td>
<td>9.41</td>
<td>80.33</td>
</tr>
<tr>
<td><em>Juncus balticus</em> (Baltic rush, wire rush)</td>
<td>10.43</td>
<td>81.02</td>
</tr>
<tr>
<td><em>Scirpus acutus</em> (hard-stem bulrush)</td>
<td>8.46</td>
<td>80.52</td>
</tr>
<tr>
<td><em>Carex emoryi</em> (Emoryi’s sedge)</td>
<td>11.10</td>
<td>82.64</td>
</tr>
<tr>
<td><em>Scirpus pungens</em> (Common three-square)</td>
<td>10.42</td>
<td>75.33</td>
</tr>
</tbody>
</table>

Organic content values are based on dry weight. *Scirpus acutus* rootmatter was tested for comparison purposes even though it was not used in this study.

The ratio of AGB organic content (this chapter) to BGB organic content (Chapter 4) (AGB-OC/BGB-OC ratio) for each wetland species was generally less though close to 1.0. The across the species range was 0.872:1 (*Juncus balticus*) to 1.033:1 (*Scirpus californicus*) (mean = 0.957:1). This implies that treatment enhancement differences between AGB and BGB are due to the quality and accessibility of the organic content rather than the gross quantity.
The organic content of the AGB (Table 6.1) weakly but positively correlated with that of BGB (rootmatter) whose effect on dechlorination of cVOCs is discussed in Chapter 4. In the BGB study, the *Scirpus pungens* rootmatter - amended treatment had the lowest organic content of all the AGB amended treatments. This is evidently consistent with the results presented in Table 6.1. Root and shoot biomass (AGB and BGB) of rice cultivars is known to positively correlate with carbon exudation, which implies that carbon exudation is driven by plant biomass (Aulakh et al., 2001).

### 6.4.2 Effect of Wetland Aboveground Biomass on Dechlorination of cVOCs

The odd-numbered figures between and including Figures 6.1 and 6.25 illustrate the dechlorination trends of 1,1,2,2-TeCA and its daughter products in the different treatments. The even-numbered figures between and including Figures 6.2 and 2.26 show the corresponding gas production trends before and during 1,1,2,2-TeCA dechlorination. Table 6.2 summarizes results of a quantitative comparison of the wetland plant AGB microcosms with respect to dechlorination of 1,1,2,2-TeCA and its daughter products. An inventory of the main daughter products observed is also given.

Wetland plants, like other plants, during their growth produce and store ethene, a plant hormone that regulates growth and other plant functions (Gregory, 2006; Wu et al., 2006). Plants can also take ethene from the soil (Wang and Yamauchi, 2006). Ethene production is enhanced when plants are flooded (Lambers and Colmer, 2005; Wang and Yamauchi, 2006) which is often the case with wetland plants. As such in this study, it was hypothesized that some of the ethene stored in the wetland plant AGB would be released during 1,1,2,2-TeCA dechlorination processes and end up being confused with that resulting from complete dechlorination of 1,1,2,2-TeCA. This could happen because at least one of 1,1,2,2-TeCA anaerobic dechlorination pathways produces ethene as the
Figure 6.1: Profile of 1,1,2,2-TeCA degradation in *Phragmites communis* microcosm

Figure 6.2: Liquid phase gas concentration profiles before and during 1,1,2,2-TeCA treatment in *Phragmites communis* AGB microcosm. 1,1,2,2-TeCA was spiked on day 31.
Figure 6.3: Profile of 1,1,2,2-TeCA degradation in *Typha latifolia* microcosm

Figure 6.4: Liquid phase gas concentration profiles before and during 1,1,2,2-TeCA treatment in *Typha latifolia* AGB microcosm. 1,1,2,2-TeCA was spiked on day 31.
**Figure 6.5:** Profile of 1,1,2,2-TeCA degradation in *Scirpus validus* microcosm

**Figure 6.6:** Liquid phase gas concentration profiles before and during 1,1,2,2-TeCA treatment in *Scirpus validus* AGB microcosm. 1,1,2,2-TeCA was spiked on day 31.
Figure 6.7: Profile of 1,1,2,2-TeCA degradation in *Scirpus americanus* microcosm

Figure 6.8: Liquid phase gas concentration profiles before and during 1,1,2,2-TeCA treatment in *Scirpus americanus* AGB microcosm. 1,1,2,2-TeCA was spiked on day 31.
Figure 6.9: Profile of 1,1,2,2-TeCA degradation in Carex nebraskensis microcosm

Figure 6.10: Liquid phase gas concentration profiles before and during 1,1,2,2-TeCA treatment in Carex nebraskensis AGB microcosm. 1,1,2,2-TeCA was spiked on day 31.
Figure 6.11: Profile of 1,1,2,2-TeCA degradation in *Juncus effusus* microcosm

Figure 6.12: Liquid phase gas concentration profiles before and during 1,1,2,2-TeCA treatment in *Juncus effusus* AGB microcosm. 1,1,2,2-TeCA was spiked on day 31.
Figure 6.13: Profile of 1,1,2,2-TeCA degradation in *Eleocharis palustris* microcosm

Figure 6.14: Liquid phase gas concentration profiles before and during 1,1,2,2-TeCA treatment in *Eleocharis palustris* AGB microcosm. 1,1,2,2-TeCA was spiked on day 31
Figure 6.15: Profile of 1,1,2,2-TeCA degradation in *Scirpus californicus* microcosm

Figure 6.16: Liquid phase gas concentration profiles before and during 1,1,2,2-TeCA treatment in *Juncus californicus* AGB microcosm. 1,1,2,2-TeCA was spiked on day 31
**Figure 6.17:** Profile of 1,1,2,2-TeCA degradation in *Juncus balticus* microcosm

**Figure 6.18:** Liquid phase gas concentration profiles before and during 1,1,2,2-TeCA treatment in *Juncus balticus* AGB microcosm. 1,1,2,2-TeCA was spiked on day 31.
**Figure 6.19:** Profile of 1,1,2,2-TeCA degradation in *Carex emoryi* microcosm

**Figure 6.20:** Liquid phase gas concentration profiles before and during 1,1,2,2-TeCA treatment in *Carex emoryi* AGB microcosm. 1,1,2,2-TeCA was spiked on day 31.
Figure 6.21: Profile of 1,1,2,2-TeCA degradation in *Scirpus pungens* microcosm

Figure 6.22: Liquid phase gas concentration profiles before and during 1,1,2,2-TeCA treatment in *Scirpus pungens* AGB microcosm. 1,1,2,2-TeCA was spiked on day 31.
Figure 6.23: Profile of 1,1,2,2-TeCA degradation in compost mix microcosm

Figure 6.24: Liquid phase gas concentration profiles before and during 1,1,2,2-TeCA treatment in compost mix microcosm (Control 1). 1,1,2,2-TeCA was spiked on day 31.
Figure 6.25: Profile of 1,1,2,2-TeCA degradation in α-cellulose microcosm

Figure 6.26: Liquid phase gas concentration profiles before and during 1,1,2,2-TeCA treatment in α-cellulose microcosm (Control 2). 1,1,2,2-TeCA was spiked on day 31.
### Table 6.2: Quantitative comparison of performance of different wetland plant AGB microcosms.

<table>
<thead>
<tr>
<th>Treatment/AGB source</th>
<th>TeCA k (d⁻¹) (E, %)</th>
<th>TCE c-DCE</th>
<th>1,1,2-DCE</th>
<th>1,1-DCA</th>
<th>t-1,2-DCE</th>
<th>1,1-DCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phragmites communis</em></td>
<td>0.019 (94.9)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Typha latifolia</em></td>
<td>0.017 (93.2)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Scirpus validus</em></td>
<td>0.011 (87.0)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Scirpus americanus</em></td>
<td>0.019 (95.6)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Carex nebraskensis</em></td>
<td>0.012 (86.7)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Juncus effusus</em></td>
<td>0.015 (92.9)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Eleocharis palustris</em></td>
<td>0.015 (92.4)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Scirpus californicus</em></td>
<td>0.012 (87.8)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Juncus balticus</em></td>
<td>0.018 (94.3)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Carex emoryi</em></td>
<td>0.017 (93.8)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Scirpus pungens</em></td>
<td>0.012 (86.6)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Control 1 (compost)</td>
<td>0.043 (100)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Control 2 (α-cellulose)</td>
<td>0.003 (45.0)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Control 1, compost mixture: (RCC/SBC/sand); control 2, α-cellulose. *C*₁₂, *cis*-1,2-DCE; *t*-DCE, *trans*-1,2-DCE. “+”, cVOC detected; “-”, cVOC not detected. E (%) = \[\frac{(C_i - C_F)}{C_F}\]x100%, where $C_i$ initial concentration; $C_F$ final concentration.

Final harmless end product (Lora and Voytek, 2004; Aulenta et al., 2005; Aulenta et al., 2006). In view of this, monitoring of ethene as well as methane production before and during dechlorination of 1,1,2,2-TeCA was meant to provide information on the quantity of ethene released by the AGB from the different wetland plants during incubation. This
information was meant to serve as the baseline for estimating the quantity of ethene resulting from dechlorination of 1,1,2,2-TeCA, if it dechlorinated completely.

The 1,1,2,2-TeCA dechlorination profiles depicted in Figures 1 – 23 and summarized in Table 2 demonstrate strong similarities in the dechlorination trends exhibited by the ABG – amended treatments, especially with respect to the dechlorination products.

It is clear from the gas concentration profiles shown in the even-numbered figures between and including Figures 6.2 and 6.22 that the AGB of each of the wetland species released ethene originally present in the AGB as hypothesized in this study. As pointed out earlier, wetland plants produce and store ethene (Gregory, 2006; Lambers and Colmer, 2005; Wang and Yamauchi, 2006; Wu et al., 2006). The fact that the concentrations of ethene changed similarly with time irrespective of the AGB treatment, implies that this phenomenon applied across the wetland plant species, as expected. The absence of ethene in the compost control (Control 1) and the α-cellulose control (Control 2) between day 0 and day 31, which was prior to the spiking of 1,1,2,2-TeCA, reinforces the interpretation that ethene was released by the AGB rather than generated during incubation.

The consistent decrease in ethene concentration with time, which was exhibited by each of the AGB treatments prior to the spiking of 1,1,2,2-TeCA, suggests that ethene was consumed or transformed over time. Under this premise, the stabilization of ethene’s concentration following the spiking of 1,1,2,2-TeCA can be attributed to production through complete dechlorination of 1,1,2,2-TeCA. This also explains the presence of the gas in the compost control (Control 1) in which it was not detected prior to the spiking of 1,1,2,2-TeCA. Chen et al. (1996) observed rapid and complete removal of 1,1,2,2-TeCA
in a complex methanogenic community that had previously not been exposed to 1,1,2,2-
TeCA.

The steep drop in methane gas concentrations evidently exhibited by all of the
AGB amended treatments, which coincided with the commencement of 1,1,2,2-TeCA
dechlorination (after day 31), suggests that the gas was consumed or transformed.
Clearly, the consumption or transformation of methane gas was related to dechlorination
of 1,1,2,2-TeCA and its daughter products. A mere decrease or stoppage of methane
production would have resulted in the leveling off, rather than the observed reduction in
concentration of the gas. Dilution is out of the question because methane was the
dominant gas produced in all of the treatments, including the AGB amended ones.
Methane consumption or transformation mediated by microorganisms under anaerobic
conditions is possible through reverse methanogenesis (Hinrichs and Boetius, 2002;
Moran et al., 2007; Konhauser, 2007; Chanton et al., 2008; Chapter 2). We have observed
what appeared to be evidence of reverse methanogenesis in some of our previous studies
(Chapters 2 and 5).

The 1,1,2,2-TeCA dechlorination profiles illustrated in Figures 6.1 – 6.23
demonstrate strong similarities, especially among the AGB amended treatments and
between these and the compost control (Control 1). Although the observed trends should
have been expected considering that all of the treatments were inoculated with the same
microbial culture as the compost control, the similarities in the dechlorination trends
signify that the AGB of each wetland plant species did not influence the microbial culture
unfavorably. It also suggests that if any changes occurred in the community structure of
the original microbial culture in the inoculum due to the influence of the AGB and
exposure to cVOC, they must have been similar among the AGB treatments and the
compost control. This is especially evident in the similarities in terms of the daughter products detected. In a way, the similarities between the dechlorination profiles of the AGB amended treatments and the compost control imply that the inoculation of the microcosms was comparably successful in each treatment.

Clearly, Table 6.2 gives useful information on the pathways and mechanisms of 1,1,2,2-TeCA dechlorination exhibited in the different treatments and controls during this study. Evidently, the most common of these involved TCE, cis-1,2-DCE, 1,1,2-TCA, 1,1-DCA, trans-1,2-DCE, and 1,1-DCE as daughter products. It is remarkable that VC was not detected in most of the treatments. It is also worth pointing out that the *Scirpus americanus* AGB amended treatment, which performed best, and the *Scirpus validus* AGB amended treatment, which performed worst, exhibited the same species of daughter products. This suggests that the difference in efficiency between the two treatments were likely due to differences in quality of the substrate constituents they availed rather than differences in the microbially mediated dechlorination mechanisms that prevailed.

The fact that the α-cellulose control did not exhibit any evidence of dechlorination (by way of the presence of dechlorination products) despite being inoculated with the same culture as the AGB amended treatments, demonstrates that α-cellulose was not a suitable substrate for the pertinent microorganisms. This observation also reiterates the importance of substrate quality.

The dechlorination products profiled in Figures 6.1 – 6.26 and shown in the inventory in Table 6.2 are typical of 1,1,2,2-TeCA anaerobic transformation daughter products reported in the literature (Chen et al., 1996; Lorrah and Olsen, 1999; Ferguson and Pietari, 2000; Lorrah et al., 2004; Aulenta et al., 2005; Hunkeler et al., 2005; Aulenta et al., 2006).
The presence of ethene in the compost control (Control 1) and the leveling off of ethene concentrations in the AGB amended treatments after a sharp decline (after day 31) suggests that complete dechlorination of 1,1,2,2-TeCA likely occurred, even though to a small extent. In connection with the foregoing argument, it should be recalled that before the respiking of 1,1,2,2-TeCA, ethene was not detected in the compost control. Moreover, consequent to commencement of dechlorination of 1,1,2,2-TeCA, in all of the AGB amended treatments, ethene concentration initially showed a decreasing trend, but leveled off as dechlorination proceeded, suggesting that a source of ethene was counteracting the effect of the ethene consumption or transformation initially observed.

On the whole, 1,1,2-TCA, TCE, and cis-1,2-DCE seem to have quantitatively dominated in most of the treatments included in this study. Based on Table 2.1 and reference to the literature that have reported on transformation of 1,1,2,2-TeCA and its main daughter products (Chen et al., 1996; Lorrah and Olsen, 1999; Ferguson and Pietari, 2000; Lorrah et al., 2004; Aulenta et al., 2005; Hunkeler et al., 2005; Aulenta et al., 2006), a number of inferences can be made regarding how 1,1,2,2-TeCA was transformed in this study. TCE likely formed through dehydrochlorination of 1,1,2,2-TeCA. TCE was microbially - reductively dechlorinated to give cis-1,2-DCE, trans-DCE, and 1,1-DCE. Equally likely, the three DCEs were in turn reductively dechlorinated to give VC, even though VC was not detected. VC was also likely transformed into ethene through reductive dechlorination. 1,1,2-TCA possibly formed directly from 1,1,2,2-TeCA through the microbially mediated reductive dechlorination process. 1,2-DCA and chloroethane are first and second stage products of sequential reductive dechlorination of 1,1,2-TCA. 1,1,2-TCA was also likely partly transformed through dehydrochlorination into the DCEs observed. Direct dichloroelimination of 1,1,2,2-TeCA is likely partly responsible for the
dominant presence of DCEs in all of the AGB amended treatments and the compost control. Dichloroelimination along with reductive dechlorination (hydrogenolysis) are reported to be microbially mediated while dehydrochlorination is usually attributed to abiotic processes (Chen et al., 1996; Lorrah and Olsen, 1999; Ferguson and Pietari, 2000; Lorrah et al., 2004; Aulenta et al., 2005; Hunkeler et al., 2005; Aulenta et al., 2006).

Further with respect to the results shown in Table 6.2, it is noteworthy that the compost control (Control 1) removed 1,1,2,2-TeCA completely in 79 days. In contrast, the α-cellulose control (Control 2) had removed only 45% of 1,1,2,2-TeCA by the last day (day 174) of this study, a total of 143 (174 minus 31) days of incubation.

Comparing the 1,1,2,2-TeCA dechlorination rates exhibited in this study to those reported in other studies, it is obvious that the ones observed in this study are generally lower for all treatments. Notably, 1,1,2,2-TeCA dechlorination rates are lower by more than an order of magnitude compared to the ones observed in microcosms using an engineered wetland soil comprising BionSoil, peat, and sand (Chapter 2 and 3). The observed differences can be attributed to the the engineered soil microcosms being inoculated with a well acclimated dehalorespiring microbial culture. Other studies have used this culture to achieve complete dechlorination of various cVOCs (Kassenga, 2003; Kassenga et al., 2003; Kassenga et al., 2004; Kassenga and Pardue, 2006).

Since the dechlorination rates of 1,1,2,2-TeCA (Table 6.2) were very low, the accumulation of the daughter products observed in this study implies that dechlorination rates of the daughter products must have been even lower. This observation contrasts with what was observed in the engineered wetland soil microcosm study (Chapter 2). In the engineered wetland soil microcosm study, accumulation of the daughter products was minimal even though 1,1,2,2-TeCA was transformed at a rate more than an order of
magnitude higher than in this study. Again, the difference between this study and the engineered wetland soil microcosm study is attributable to the role of the well-acclimated dehalorespiring microbial culture, as pointed out earlier.

It is evident from Figures 6.1 – 6.26 that the wetland plant ABG influenced the treatment of cVOCs and the different AGB species differed in their effectiveness in small but measurably significant ways. The differences are best appreciated by comparing the best performing to the worst performing AGB species. The differences in the cVOC dechlorination effectiveness of AGB from different wetland plant species can be attributed to factors similar to the ones given with respect to the influence of rootmatter on dechlorination of cVOCs, which are discussed at length in Chapter 4. Different plant species produce resources (including biomass content) of different qualities and quantities because of their different basic ecological traits (Bardgett, 2005). Moreover, different plant species differ in their potential to enhance remediation or treatment of pollutants (Hutchinson et al., 2003). Decaying plant components, which include AGB are a source of substrates for microorganisms (Badget 2005; Gregory, 2006; Bastiviken et al., 2007; Manzoni et al., 2008). Conceivably, these substrates and their derivatives can directly or indirectly serve as inputs in cVOC dechlorination processes.

In addition to providing substrates for microorganisms, plants produce other useful exudates, including enzymes. Olson et al. (2003) observed higher levels of biphenyl-utilizing bacteria in soils amended with orange (Citrus sinensis) peels, Eucalyptus leaves, pine needles (Pinus spp.), and ivy leaves (Hedera spp.). Yi and Crowley (2007) observed that linoleic acid, which was obtained from four different plant species, was the main substance responsible for stimulating PAH degradation. With respect to cVOCs, it is worth pointing out that the Canadian waterweed (Elodea
canadensis) plant and its cell-free extracts (dehalogenases) can degrade many compounds including TCE and PCE (Wolfe and Hoehmer, 2003). A more comprehensive list of other plant-derived enzymes that can degrade or transform pollutants has been reported by Wolfe and Hoehmer (2003).

For comparison purposes, the study that assessed the influence of rootmatter (BGB) of different wetland plant species (the same as the ones used in this study) (Chapter 4) indicated a higher degree of resolution of inter-species dechlorination enhancement differences than in this study. It can also be argued that a wetland plant selection based on more discernible performance differences among candidate species is likely to have more significant EWS performance advantages than the one based on less discernible differences. Therefore, if one has to choose between using BGB and AGB performance results, it may be more advantageous to select wetland plants for their cVOC treatment enhancement ability based on BGB rather than AGB performance results.

6.5 Conclusions

This study investigated the influence of AGB of wetland plants on dechlorination of cVOCs. It assessed and compared the effectiveness and efficiency of AGB from eleven different wetland plant species identified as potentially suitable for engineered wetland systems intended to treat cVOCs. The AGB amended treatments were also compared to two controls (compost mixture control and α-cellulose control).

Results from this study show that wetland plant ABG influences the treatment of cVOCs. In addition, different AGB species differ in their cVOC dechlorination effectiveness in small but measurable and significant ways. The AGB-amended treatments outperformed the α-cellulose control by a factor of 3.7 - 6.3, but the compost
control outperformed the AGB-amended treatments by a factor of 2.3 - 3.9. Four of the AGB amended treatments performed best (1,1,2,2-TeCA $k = 0.017 – 0.019$ d$^{-1}$), two exhibited a medium performance ($k = 0.015$ d$^{-1}$), while three performed worst ($k = 0.011 – 0.012$ d$^{-1}$). In spite of their performance differences, all of the AGB amended treatments exhibited similar 1,1,2,2-TeCA dechlorination pathways and produced ethene, which is one of the desired end products of 1,1,2,2-TeCA dechlorination.

The AGB-amended treatments and the compost control generated large quantities of gases, mainly methane, which signifies that they supported more vigorous microbial activity. In contrast, the $\alpha$-cellulose control performed poorly in this respect. This study suggests that selection of wetland plants for their cVOC treatment enhancement ability based on AGB has less resolution than that based on belowground biomass. As such, while AGB based performance results are a useful basis of wetland plant selection, the ones based on BGB are likely to be better.
CHAPTER 7: ASSESSMENT OF COMPOST MATERIALS AS SUBSTRATES FOR ENGINEERED WETLANDS TREATING VOLATILE ORGANIC COMPOUNDS

7.1 Introduction

An engineered wetland system (EWS) designed expressly to treat water contaminated with chlorinated and non-chlorinated VOCs has the following major components: 1) the wetland bed material (substrate); 2) wetland plants; 3) a containment structure for the wetland bed material and vegetation; and 4) a system for delivery, flow control, and distribution of the water to be treated. The first and second are the core components of EWSs, whereas the third and fourth components may not even be unique to EWSs. It can additionally be pointed out that wetland bed materials are more important than wetland plants. Several arguments can be given to support this assertion. First, wetland plants are more dependent on wetland bed materials than the other way around. This is because wetland plants need the bed materials for anchorage and supply of nutrients as well as water (Bardgett, 2005). Second, owing to the fact that it accommodates all the main fate and transport processes of pollutants treated in EWSs, the bed is the main EWS treatment medium. Although vegetation is an important and integral component of EWSs (Chapters 1 - 5), by comparison, its roles are not as indispensable as those of the wetland bed.

The assertion that bed materials are the most important component of EWSs makes it the appropriate target when contemplating improvement of EWS performance. It also reinforces the need to optimize the effectiveness of EWS bed materials. The optimization efforts include exploring better ways of using the already known bed materials and evaluating new alternative materials.
An important attribute of EWS bed materials, which also facilitates the exploitation of other attributes, is the fact that the bed is the most flexible of all EWS components. First, there is a wide selection of materials from which suitable substrates can be chosen. Second, EWS bed materials are the most easily amenable to enhancements that can directly translate into EWS performance improvement. The foregoing arguments are the rationale for the focus on wetland bed materials in this study.

Although every bed material has to have suitable structural and geotechnical, and hydrodynamic attributes, these are not as important as the source of the organic component needed to support EWS treatment processes. The source of the organic component needed to support physicochemical and biochemical treatment processes directly is the most important bed material component. For suitable structural and geotechnical attributes, high density, inert materials are suitable. On the other hand, porous, inert materials are needed to provide suitable hydrodynamic properties. In contrast, materials rich in organic matter are necessary to give the bed the required chemical and biological attributes (Pardue et al., 2000; Pardue, 2005).

Sources of the organic component of EWS bed materials can conveniently be classified as conventional or non-conventional substrate types. Conventional substrate types are the ones that are already widely used in practice. They typically include peat, processed animal waste, and proprietary compost materials. Use of these is widely reported in the literature (Kassenga, 2003; Kassenga et al., 2003; Pardue, 2005). Non-conventional substrates are the ones whose application is just being explored. Their sources are typically organic waste fraction components such as processed solid waste, processed sludge and biosolids, processed wetland plant biomass, processed biomass
from macrophyte ponds, and biogas plant slurry. Undoubtedly, many more non-conventional substrate types could be identified.

Despite the obvious need to enhance performance efficiencies of EWS focusing on EWS bed materials as discussed above, little has been reported in the literature on substrate options and their performance optimization. Notably, knowledge gaps have been observed with respect to performance attributes of both the conventional and non-conventional substrate sources. Therefore, the main objective of this study was to characterize and evaluate four, widely available compost materials for their suitability as substrates for EWSs meant to treat chlorinated volatile organic compounds (cVOCs). The candidate compost materials were compared to one another as well as to other materials that had been previously found to be suitable.

The potential suitability of compost materials as substrates for cVOC treating EWSs was inferred from our research experience coupled with attributes of compost materials as reported in the literature (for example, de Betoldi et al., 1983; Haug, 1993; Diaz et al., 2007; Rudnik, 2008). Composting often enables the transformation of formerly relatively recalcitrant organic materials into forms that make them more amenable to beneficial microbial action. Composting also converts different types of organic material fractions into a form that gives them characteristics similar to those of natural organic soil (de Betoldi et al., 1983). As such, composting makes organic materials more suitable for not only plants but also for microorganisms normally found in the soil. Products of anaerobic composting include organic acids and alcohols (Haug, 1993). The most attractive features of compost as an EWS substrate are: 1) the diversity and ubiquity of its raw materials and 2) the relative ease with which composting can inexpensively be accomplished. Both of these features are important because they
provide assurance of the substrate’s availability cheaply and in large quantities. Availability and cost are critical advantages in the face of expensive and scarce alternatives, especially proprietary ones such as BionSoil. Additional information on compost in the context of EWSs is presented in Chapter 1.

7.2 Materials and Methods

7.2.1 Anaerobic Microcosm Set-up, Treatments, and Experimental Procedures

The microcosms were set up using 160 mL serum bottles. They were prepared under oxygen free conditions in an anaerobic chamber. Before the microcosm treatments were selected, four types of compost materials (row crop compost (RCC), soil builder compost (SBC), aged pine bark (APB), and wood fiber (WF)) were tested and evaluated for their suitability as potential substrates for EWSs intended to treat cVOCs. An additional purpose for evaluating the four types of compost material was to provide a basis for coming up with a set of criteria for deriving suitable substrate mixing ratios. The compost materials were characterized as supplied from their sources. The characterization results are shown in Table 7.1.

It is evident from Table 7.1 that, although porosity values were comparable among the four compost materials, this similarity did not apply to the other parameters. It is also notable that the hydraulic conductivity values of all the compost materials were high enough for the materials to be suitable for EWSs. Unsurprisingly, the organic fraction values were also high enough for the compost materials to be suitable for EWS bed materials. Wetland soils are classified as organic if their organic content is above 20% (Mitsch and Gosselink, 2000&2007). In contrast, the bulk density values were too low for an individual or a mixture of the compost materials alone to be suitable as a bed material for EWSs.
Table 7.1: Basic characterization of compost materials earmarked as EWS bed materials

<table>
<thead>
<tr>
<th>Characterization parameter</th>
<th>Types of wetland media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APB</td>
</tr>
<tr>
<td>Dry density (kg/L)</td>
<td>0.3018</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>59.67</td>
</tr>
<tr>
<td>Saturated bulk density (kg/L)</td>
<td>0.9137</td>
</tr>
<tr>
<td>Initial pH</td>
<td>6.34</td>
</tr>
<tr>
<td>Saturated moisture content (%)</td>
<td>76.5±1.79</td>
</tr>
<tr>
<td>Organic fraction (%)</td>
<td>87.0±0.01</td>
</tr>
<tr>
<td>Hydraulic conductivity (cm/s)</td>
<td>5.33x10⁻²</td>
</tr>
</tbody>
</table>

APB = Age pine bark; WF = Wood fiber; SBC = Soil builder compost; RCC = Row crop compost; CS = commercial sand. pH was measured in mixtures of equal volumes of compost and water.

Based on the evaluation results shown in Table 7.1 and the preceding discussion, the following treatments were selected for this study: Treatment 1, old BionSoil inoculated with 20 mL of slurry containing *Dehalococcoides ethenogenes* (DHC); Treatment 2, row crop compost (RCC) inoculated with 20 mL of slurry containing DHC; Treatment 3, soil builder compost (SBC) inoculated with 20 mL of slurry containing DHC; Treatment 4, an RCC/SBC/sand (37.5%/37.5%/25% w/w) mixture inoculated with 20 mL of slurry containing DHC. The ratio used for this treatment was selected on the basis of past experience and and evaluation of physical chemical characteristics of the
individual compost materials. Treatment 5 was a BionSoil/Latimer/sand (37.5%/37.5%/25% w/w) mixture inoculated with 20 mL of slurry containing DHC. Previous studies had indicated that this was a suitable mixture for upflow EWS beds (Kassenga, 2003; Kassenga et al., 2003). Hence, it was used as a biotic control for the study. Treatment 6 was BionSoil/Latimer/sand (37.5%/37.5%/25%) spiked with a 3.8 mL of biocide formalin. This served as the abiotic control for the study. All six treatments were prepared in triplicates (a total of 18 microcosm bottles).

The microcosm experiments were carried out in two phases. The first phase was meant to evaluate the effectiveness of the compost materials based on trichloroethylene (TCE), a common groundwater pollutant which is well studied. The second phase was meant to mimic the treatment of groundwater from sites of interest. Because it was not feasible to get enough groundwater from the sites to last the whole study period, a synthetic groundwater was used. The synthetic groundwater contained chemicals that represented well the site groundwater constituents. These were: cis-1,2-DCE; 1,1-DCA; 1,1,1-TCA; and chlorobenzene. In the first phase, the microcosm bottles for all of the treatments were spiked with TCE immediately after being set-up. In the second phase, all of the treatments were spiked with the synthetic groundwater. The second phase of the study followed immediately after the first phase. All the treatments were incubated at room temperature under static conditions in the dark. During each phase, aqueous and gas phase samples were regularly withdrawn from the microcosm bottles for analysis.

7.2.2 Mesocosm Scale EWS Experiments

The EWS units were made of cylindrical glass columns with a diameter of 15 cm, an effective bed height of 45 cm, and an overall height of 60 cm. Each EWS unit had seven sampling ports spaced at 7.5 cm c/c. Each sampling port was fitted with a Biopsy
needle to facilitate sampling. Each biopsy needle was secured in place by a rubber plug inserted in a hole drilled through the side of the EWS unit. Stainless steel connectors for inlets were secured in place in a similar way. The experimental set up is illustrated in Figure 7.1.

**Figure 7.1:** Schematic illustration of the engineered wetland system (EWS) units packed with RCC/SBC/sand (3 units) and Bion Soil/Latimer peat/sand (3 units).
Two different sets of EWS units were used. Set 1 EWS units were packed with the RCC/SBC/sand (37.5%/37.5%/25% w/w) mixture used in the microcosm study. Set 2 EWS units were packed with the BionSoil/peat/sand (37.5%/37.5%/25% w/w) mixture also used in the microcosm study. The porosity of the RCC/SBC/sand mixture was 49%, while that of the mixture of the BionSoil/peat/sand mixture was 55%. All the EWS units were set up in the greenhouse.

Packing of the EWS units with their respective types of bed material was completed on 29 January 2005. Between 29 January and 14 February 2005, the EWS units were flushed by pumping tap water through them. The flushing was meant to initially wet the wetland beds, and subsequently remove fines as well as salts from the bed materials, and moderate pH in the beds. The flushing flow rate was kept much lower (<0.5) than the design flow rate to prevent preferential flow as well as fluidization and the subsequent stratification of the bed material constituents.

Three days into the flushing operation, each EWS unit was planted with *Typha latifolia*. The wetland plant density at planting time was one plant per wetland unit (1 plant/177 cm²). *Typha latifolia* was selected because it had earlier been found to be effective and the preferred wetland species (Chapters 2 and 3). Pumping of site groundwater through each EWS unit started on the 14 February 2005 immediately following the flushing of the EWS beds. The pumping of the site groundwater through each EWS unit was meant to seed the EWS units with microorganisms already acclimated to the pollutants of interest. Pumping of synthetic groundwater, which started in March, 2005, followed immediately after cessation of the pumping of the site groundwater. Sampling started three weeks after beginning the pumping of the synthetic groundwater through the EWS units.
To facilitate the comparison of the RCC/SBC/sand packed EWS units to the BionSoil/peat/sand packed EWS units, the flow rate through each unit was maintained at about 2 L/day. Therefore, the hydraulic retention times in each of the two sets of EWS units were about 1.95 days in the RCC/SBC/sand EWS units and 2.31 days in the BionSoil/peat/sand units.

In the mesocosm scale study (unlike in the microcosm scale study), the EWS units were tested using two different types of synthetic groundwater in two separate phases. Each synthetic groundwater type imitated the composition of groundwater from the site of interest. The chemical constituents of the synthetic groundwater used in the first phase were: cis-1,2-DCE, 1,1-DCA, 1,1,1-TCA, and chlorobenzene. This synthetic groundwater was pumped through each of the EWS units and its phase was executed first.

The second phase tested two different types of synthetic groundwater in parallel. One contained a mixture of methylene chloride and 1,2-DCA, while the other contained a mixture of cis-1,2-DCE and 1,1-DCA. The removal of each type of synthetic groundwater was tested in both RCC/SBC/sand packed and Bion Soil/peat/sand packed EWS unit sets set up and run side by side.

7.2.3 Analytical Work

Aqueous phase samples were analyzed for VOCs using EPA Method 8260B using GC/MS (Agilent Technologies 6890N Network GC System, Agilent Technologies 5973Network Mass Selective Detector) coupled to an AquaTek 70 Autosampler® (Teledyne Tekmar) and Velocity XPT® purge and trap sample concentrator (Teledyne Tekmar). Methane, ethene, and ethane gases were analyzed using GC/FID. One milliliter of head space sample was injected into the gas chromatography with flame ionization detector (Agilent 5890 Series II) equipped with a 2.4 m x 0.32 mm ID column packed
with Carbopack b/1% Sp-(Supelco, Bellefonte, PA). The column 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. Analytical standards and surrogate for the eVOCs were obtained as mixtures from Supelco Inc.

7.3 **Dechlorination Data Analysis and Modeling**

Parent cVOC removal data were fitted to the first-order model (Equation 7.1 for microcosm study data and Equation 7.2 for mesocosm study data) in order to estimate their pseudo-first order removal rate constants.

\[
[C] = [C]_o e^{-k_t t} \quad [7.1]
\]
\[
[C] = [C]_o e^{-k_x x} \quad [7.2]
\]
where \([C]\) is the concentration of cVOC of interest \([\text{ML}^{-3}]\), \(k_t\) is the time-referenced cVOC removal rate constant \([\text{T}^{-1}]\), \(t\) is time \([\text{T}]\), and the subscript ‘\(o\)’ refers to the initial concentrations. On the other hand, \(k_x\) is the distance-referenced cVOC removal rate constant \([\text{L}^{-1}]\), \(x\) is the height \([\text{L}]\) from the bottom of the EWS unit. The subscript ‘\(o\)’ refers to the inlet level of the EWS units at which the distance is zero.

7.4 **Results and Discussion**

7.4.1 **Compost Material Assessment Based on Microcosm Experiments**

7.4.1.1 **Phase 1 – Assessment Based on Treatment of TCE**

Results of treatment of TCE in the different microcosm treatments over a monitoring period of 535 hours (22.3 days) are summarized in Figures 7.2 through 7.7. In each graph, each data point is based on analysis results of triplicates. Regression analysis of TCE degradation data for the different microcosm treatments corresponding to Figures 7.2 – 7.6 based on Equation 7.1 are summarized in Table 7.2.
Figure 7.2: Degradation of TCE in old BionSoil microcosm treatment.

Figure 7.3: Degradation of TCE in RCC microcosm treatment.
Figure 7.4: Degradation of TCE in SBC microcosm treatment.

Figure 7.5: Degradation of TCE in RCC/SBC/sand mix microcosm treatment.
Figure 7.6: Degradation of TCE in BionSoil/peat/sand mix microcosm live control.

Figure 7.7: Degradation of TCE in BionSoil/peat/sand microcosm killed control.
Table 7.2: Regression analysis data for TCE degradation in different microcosm treatments

<table>
<thead>
<tr>
<th>Treatments/engineered wetland system (substrate) bed material type</th>
<th>Rate constant $\text{d}^{-1}, (t_{1/2}, \text{d})$</th>
<th>$t_{100%R}$ (hr)</th>
<th>TCE Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old BionSoil® (BS) + DHC inoculum</td>
<td>0.048 (14.44)</td>
<td>NA</td>
<td>NPA</td>
</tr>
<tr>
<td>Row crop compost (RCC) + DHC inoculum</td>
<td>0.624 (1.11)</td>
<td>134</td>
<td>NPA</td>
</tr>
<tr>
<td>Soil builder compost (SBC) + DHC inoculum</td>
<td>0.432 (1.60)</td>
<td>214</td>
<td>NPA</td>
</tr>
<tr>
<td>RCC/SBC/sand mixture + DHC inoculum</td>
<td>0.336 (2.06)</td>
<td>214</td>
<td>395</td>
</tr>
<tr>
<td>BionSoil® /Latimer/sand mixture + DHC inoculum</td>
<td>0.288 (2.41)</td>
<td>326</td>
<td>326</td>
</tr>
</tbody>
</table>

DHC, *Dehalococcoides ethenogenes*; $t_{1/2}$, half life; NA, not applicable; $t_{100\%R}$, time to 100% removal of parent or daughter product cVOC; NPA, no product accumulation.

In Figure 7.2, only a 68.1% reduction in TCE concentration took place in the 535 hour monitoring period. In the abiotic control (Figure 7.7), the corresponding reduction in TCE concentration was only 18.3%. This low removal of TCE in the abiotic control demonstrates that dechlorination of TCE in the treatments for Figures 7.2 through 7.6 was predominantly (if not wholly) biological. The fact that in these treatments ethene appeared and increased in concentration (data not shown) parallel to the disappearance of the parent and daughter product cVOCs supports this assertion. The fact that intermediate and end products of TCE dechlorination were not observed in the abiotic control suggests that the small loss of TCE in this treatment could have been a result of sorption.

With reference to the effectiveness of TCE dechlorination observed, it is noteworthy that the microcosms for Figures 7.3 through 7.6 exhibited complete
degradation of both TCE and its daughter products cis-1,2-DCE and vinyl chloride over the 535 hour monitoring period.

Refering to the profiles of the parent compound and products in the different treatments corresponding to Figures 7.1 through 7.5, it is noticeable that treatments 1 – 3 did not exhibit accumulation of dechlorination intermediate products cis-1,2-DCE and VC. In contrast, treatments 4 and 5 (Figures 7.5 and 7.6) accumulated at least one of the products. Two different arguments can be given to explain these observations. In the treatment for Figure 7.2, the non-accumulation of the intermediate products was due to the possibility that TCE dechlorination itself proceeded uncharacteristically more slowly than the dechlorination rates of its intermediate products. This can be inferred from the shape of the graph of TCE removal data (Figure 7.2). Evidently, the overall dechlorination rate of cVOCs in this treatment was controlled by the TCE dechlorination step.

The non-accumulation of cVOC intermediate products in treatments 2 and 3 (Figures 7.3 and 7.4), in which TCE dechlorination was rapid, was due to even more rapid dechlorination of the daughter products. Evidently, this high rate of cVOC intermediate product dechlorination was not exhibited by treatments 4 and 5. These inferences regarding causes of accumulation of cVOC intermediate products in the different treatments are supported by dechlorination rate data discussed later with respect to Table 7.2.

Although in treatments 1 – 3 (Figures 7.2 – 7.4), cVOC intermediate products were detected, it is reasonable to say that they were similar to the ones detected in treatments 4 and 5 (Figures 7.5 and 7.6). Kassenga (2003) and Kassenga et al. (2003) have reported the same intermediate and final products for microcosm experiments.
conducted using the same dechlorinating culture as the one used in this study. It is also noteworthy that the non-accumulation of TCE dechlorination intermediate products evident in Figures 7.2 – 7.4 is similar to the one discussed in Chapter 2, which also used the same dechlorinating culture as this study.

In terms of treatment effectiveness, Table 7.2 suggests that RCC was the best type of wetland substrates; it was followed by SBC. Notably, both RCC and SBC are compost materials. In spite of the results shown in Table 7.2, it should be noted that BionSoil is generally a good wetland substrate. The poor performance of old BionSoil treatment in this study can be explained by the fact that old BioSoil such as the one used in this study, tends to exhibit low microbial activity due to exposure to heat and loss of moisture during long term storage. Conceivably, the dryness that develops creates an environment that is hostile for most microorganisms.

It can be argued that the TCE degradation rate of the RCC/SBC/sand mixture was lower than that of either RCC alone or SBC alone partly because of the effect of sand, which is not expected to contribute to the enhancement of TCE degradation. Kassenga (2003) and Kassenga et al. (2003) made similar observations. Sand is an important constituent of wetland bed material because it enhances hydraulics and improves media geotechnical properties such as porosity and bulk density (Chapters 1 and 2).

The TCE dechlorination rates shown in Table 7.2 are generally (for treatments 2 – 4) higher than the ones reported in Chapter 2 for the same cVOC with a BionSoil/peat/sand mixture substrate \( (k = 0.144 – 0.317 \text{ d}^{-1}) \). Evidently, the exceptional treatments in Table 7.2 are the ones that contained old BionSoil \( (k = 0.048 \text{ d}^{-1}) \) and BionSoil/peat/sand mixture \( (k = 0.288 \text{ d}^{-1}) \).
7.4.1.2 Phase 2 – Assessment Based on Treatment of Synthetic Groundwater

Results of treatment of synthetic groundwater in the different microcosm treatments over a period of 2000 hours are summarized in Figures 7.8 through 7.13. In each graph, each data point is based on analysis results of triplicates. Regression analysis of the data for degradation of the major contaminant, cis-1,2-DCE and 1,1-DCA for the microcosm treatments corresponding to Figures 7.8 – 7.12 based on Equation 7.1 are summarized in Table 7.3. A comparison of the lengths of time that complete removal (100% removal) of the monitored VOCs took in treatments 1 through 5 (Figures 7.8 – 7.12) is presented in Table 7.4.

A comparison of initial and final concentrations of VOCs of interest in treatment 6 (Figure 7.13) shows a 21.7% reduction in cis-1,2-DCE concentration over the 1860 hour monitoring period. Corresponding figures for the rest of the VOCs are 24.5% for

![Figure 7.8: Degradation of synthetic groundwater in old BionSoil microcosm treatment.](image-url)
Figure 7.9: Degradation of synthetic groundwater in RCC microcosm treatment.

Figure 7.10: Degradation of synthetic groundwater in SBC microcosm treatment.
Figure 7.11: Degradation of synthetic groundwater in RCC/SBC/sand mixture microcosm treatment.

Figure 7.12: Degradation of synthetic groundwater in BionSoil/peat/sand mixture microcosm active control.
**Figure 7.13:** Degradation of synthetic groundwater in BionSoil/peat/sand mixture microcosm killed control.

**Table 7.3:** Regression analysis data for cis-1,2-DCE and 1,1-DCA degradation in different microcosm treatments

<table>
<thead>
<tr>
<th>Treatments/engineered wetland substrate (bed material) types</th>
<th>First order rate constant (d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1,2-DCE</td>
</tr>
<tr>
<td>Old BionSoil (BS) with DHC inoculum</td>
<td>0.444</td>
</tr>
<tr>
<td>Row crop compost (RCC) with DHC inoculum</td>
<td>0.847</td>
</tr>
<tr>
<td>Soil builder compost (SBC) with DHC inoculum</td>
<td>0.473</td>
</tr>
<tr>
<td>RCC/SBC/sand mix with DHC inoculum</td>
<td>1.390</td>
</tr>
<tr>
<td>BionSoil/peat/sand mix with DHC inoculum</td>
<td>0.770</td>
</tr>
</tbody>
</table>

DHC, *Dehalococcoides ethenogenes*

TCE, 16.0% for 1,1-DCA, 58.8% for 1,1,1-TCA, and 46.0% for chlorobenzene. It can be argued that a large proportion of the reduction in the VOC concentrations observed in
treatment 6 was due to sorption, whose influence is more noticeable and effective at low VOC concentrations. The differences in the extents of the reductions exhibited by the different VOCs are likely to be due to differences in sorption characteristics among the individual VOCs. Notably, the reduction in chlorobenzene in treatment 1 (Figure 7.8) during the 1860 hour period was 82.3%.

**Table 7.4:** Comparison of 100% removal times ($t_{100\%R}$) of monitored VOCs in treatments 1 – 5.

<table>
<thead>
<tr>
<th>Treatments/EWS bed material</th>
<th>100% removal times (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DCA</td>
</tr>
<tr>
<td>Old BS with DHC inoculum</td>
<td>1449</td>
</tr>
<tr>
<td>RCC with DHC inoculum</td>
<td>1677</td>
</tr>
<tr>
<td>SBC with DHC inoculum</td>
<td>1858</td>
</tr>
<tr>
<td>RCC/SBC/sand with DHC inoculum</td>
<td>673</td>
</tr>
<tr>
<td>BS/peat/sand with DHC inoculum</td>
<td>1331</td>
</tr>
</tbody>
</table>

DCA, 1,1-DC; TCA, 1,1,1-TCA; CHB, chlorobenzene; VC, DCE, cis-1,2-DCE; vinyl chloride; CET, chloroethane; INC, incomplete transformation; NPA, no product accumulation.

It is worth pointing out that the TCE observed in Figure 7.13 was carried over from the first phase of the study. As can be observed in Figure 7.13, at the end of the first phase of this study the concentration of TCE in the killed control was still high.

Table 7.3 suggests that out of the five treatments evaluated, the RCC/SBC/sand treatment would be the best candidate for wetland substrates. These results are supported by gas analysis data as well (data not shown). Notably, the RCC/SBC/sand mixture exhibited the most vigorous microbial activity, which was gauged on the basis of overall
gas production. Table 7.4 shows that the RCC/SBC/sand treatment exhibited the shortest 100% removal times ($t_{100%R}$) for 4 out of 6 parent and daughter product VOCs. Notably, this was the best treatment, a result which is in agreement with the dechlorination rate data shown in Table 7.3.

It is further noteworthy that the degradation rate constant data (Table 7.3) for the substrates other than the RCC/SBC/sand mixture are not conclusive, since they give a different verdict for each major contaminant. Nonetheless, it is unequivocally evident that 1,1-DCA exhibited a more recalcitrant behavior than cis-1,2-DCE across the board in all the substrates (Tables 7.3 and 7.4). This suggests that 1,1-DCA is more difficult to treat than cis-1,2-DCE. This observation is in agreement with our experience with these cVOCs with the present and other dehalorespiring microbial cultures and substrates at both microcosm and mesocosm study levels. Kassenga et al. (2004) reported cis-1,2-DCE removal rates of 0.60 – 1.70 d$^{-1}$ in microcosms constructed using a mixture of BionSoil/Latimer peat/sand originally used as bed material for wetlands used to treat cVOCs.

It can be observed from Figures 7.2 – 7.13 and Tables 7.2 and 7.3 that, on the whole, the treatment effectiveness of each wetland medium evaluated was better during the treatment of synthetic groundwater than during the treatment of TCE. Notably, this is despite the fact that the synthetic groundwater contained a larger number of contaminants. This phenomenon is attributable to acclimation of the microorganisms responsible for the VOC treatment processes.

To come up with an overall assessment of the compost materials, the removal rate data given in Tables 7.2 and 7.3 had to be transformed. The removal rate constants of each major VOC for each substrate were normalized against the highest removal rate
constant value for each major contaminant. The overall assessment based on normalization is summarized in Table 7.5.

With reference to Table 7.5, the perfect mean normalized removal rate would be 1 (for the treatment that performed best with respect to all of the major contaminants). Evidently, none of substrates that were assessed performed this well. Nonetheless, it is apparent from Table 7.5 that the RCC/SBC/sand composite medium exhibited the best performance of all of the evaluated substrates. Mainly for this reason, it was selected to be used as a bed material for one set of the mesocosm level engineered wetland units described earlier and whose results are presented in the next section.

Table 7.5: Normalization and overall comparison of VOC removal rate constant data. For each major cVOC, the first column is for the original rate constant value. The second column is for the normalized rate constant value (d⁻¹/d⁻¹).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Removal rate values (first column, d⁻¹; second column, d⁻¹/d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCE</td>
</tr>
<tr>
<td>1</td>
<td>0.002</td>
</tr>
<tr>
<td>2</td>
<td>0.026</td>
</tr>
<tr>
<td>3</td>
<td>0.018</td>
</tr>
<tr>
<td>4</td>
<td>0.014</td>
</tr>
<tr>
<td>5</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Normalized rate = (actual removal rate/highest removal rate for the contaminant of interest). Mean = mean of normalized removal rates for TCE, cis-1,2-DCE, and 1,1-DCA.
7.4.2 Assessment Based on Treatment in Mesocosm Scale EWS Units

7.4.2.1 Phase 1 – Treatment of Chlorobenzene, \textit{Cis}-1,2-DCE, 1,1-DCA, and \textit{Cis}-1,1,1-TCA

Figures 7.14 – 7.21 compare the performance efficiencies of the RCC/SBC/sand and Bion Soil/peat/sand packed EWS units based on treatment of synthetic groundwater containing \textit{Cis}-1,2-DCE, 1,1-DCA, 1,1,1-TCA, and chlorobenzene during weeks 4 - 10 after commissioning the EWS units. Each data point is based on samples taken from triplicate EWS units. Regression analysis of the removal of synthetic groundwater constituent VOCs based on data for Figures 7.14 – 7.21 using Equation 7.2 gave the results summarized in Table 7.6.

Figures 7.14 – 7.21 show that the performance efficiencies of both sets of EWS units were poor. At the beginning, both 1,1-DCA and \textit{Cis}-1,2-DCE broke through. The poor performance at the beginning can be attributed to inadequate acclimation of the microbial communities inoculated in the EWS units. This argument is supported by the fact that the performance of both sets of EWS units improved with time, especially with respect to \textit{Cis}-1,2-DCE removal. The detection of dechlorination products such as vinyl chloride and chloroethane shows that the parent cVOCs were actually being degraded rather than simply being sorbed onto the bed materials.

It is apparent from Table 7.6 that in both types of EWS beds \textit{Cis}-1,2-DCE was removed significantly much more readily than 1,1-DCA. This suggests that 1,1-DCA is more recalcitrant than \textit{Cis}-1,2-DCE, which is in agreement with the results of the microcosm studies discussed in the previous section. It is also supported by studies reported by Semprini et al. (2007) which showed that 1,1-DCA was removed less efficiently than 1,1-DCE in the field. On the whole, these observations imply that, when
other conditions are the same, 1,1-DCA is the more critical cVOC when treating water contaminated with both 1,1-DCA and cis-1,2-DCE.

The dechlorination products observed in this study (Figures 7.14 – 7.21) are typical for these compounds. Reductive dechlorination of cis-1,2-DCE produces vinyl chloride, which is transformed into ethene (Lorah and Olsen, 1999; Lorah and Voyteck, 2004; Hunkeler et al., 2005; Aulenta et al., 2006). Based on the literature (for example, Galli and McCarty, 1989; Suthersan and Pyne, 2005) and findings from our other studies (unpublished) 1,1-DCA was likely reductively transformed into chloroethane, which in turn was transformed into ethane. 1,1,1-TCA was reductively dechlorinated into 1,1-DCA, which was in turn transformed as described above. It is possible that chlorobenzene was reductively transformed into benzene, which was in turn mineralized into water and carbon dioxide.

**Figure 7.14:** Performance of the RCC/SBC/sand EWS units in week 4.
Figure 7.15: Performance of the BionSoil/Latimer peat/sand EWS units in week 4.

Figure 7.16: Performance of the RCC/SBC/sand EWS units in week 5.
Figure 7.17: Performance of the BionSoil/Latimer peat/sand EWS units in week 5.

Figure 7.18: Performance of the RCC/SBC/sand EWS units in week 8.
Figure 7.19: Performance of the wetlands BionSoil/Latimer peat/sand in week 8.

Figure 7.20: Performance of the RCC/SBC/sand EWS units in week 10.
Figure 7.21: Performance of the BionSoil/Latimer peat/sand EWS units in week 10.

Table 7.6: Comparison of degradation rates of major synthetic groundwater constituent VOCs in different wetland media for weeks 4 - 10.

<table>
<thead>
<tr>
<th>Wetland bed types and major cVOCs</th>
<th>First order rate constant range (cm⁻¹)</th>
<th>Regression coefficients ($R^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCC/SBC/sand mix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• cis-1,2-DCE</td>
<td>0.0651-0.2054</td>
<td>0.9522-0.9947</td>
</tr>
<tr>
<td>• 1,1-DCA</td>
<td>0.0303-0.0445</td>
<td>0.8002-0.9707</td>
</tr>
<tr>
<td>BionSoil/Latimer peat/sand mix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• cis-1,2-DCE</td>
<td>0.1263-0.1803</td>
<td>0.7980-0.9517</td>
</tr>
<tr>
<td>• 1,1-DCA</td>
<td>0.0219-0.0434</td>
<td>0.8321-0.9621</td>
</tr>
</tbody>
</table>
7.4.2.2 Phase 2 – Assessment Based on Treatment of Synthetic Groundwater Containing Methylene Chloride and 1,2-DCA

Figures 7.22 -nd 7.25 compare the performance of the RCC/SBC/sand and Bion Soil/peat/sand packed EWS units based on treatment of synthetic groundwater containing methylene chloride and 1,2-DCA during weeks 18 - 26 after commissioning the EWS units. Both sets of EWS units were previously used in the study described in the previous section (section 7.4.2.1). Each data point is based on results from duplicate EWS units. Results of regression analysis of the data for the removal of methylene chloride and 1,2-DCA (Figure 7.22 – 7.25) in the RCC/SBC/sand and Bion Soil/Latimer/sand EWS units based on Equation 7.2 are summarized in Table 7.7.

![Graph: Removal of VOCs in EWS units](image)

**Figure 7.22:** Removal of methylene chloride and 1,2-DCA in the RCC/SBC/sand EWS units in week 18.
Figure 7.23: Removal of methylene chloride and 1,2-DCA in the BionSoil/peat/sand EWS units in week 18.

Figure 7.24: Removal of methylene chloride and 1,2-DCA in the RCC/SBC/sand EWS units in week 26.
Figure 7.25: Removal of methylene chloride and 1,2-DCA in the BioSoil/peat/sand EWS units in week 26.

Table 7.7: Comparison of efficiencies of RCC/SBC/sand and Bion Soil/Latimer/sand EWS units based on removal of methylene chloride and 1,2-DCA (weeks 18 - 26).

<table>
<thead>
<tr>
<th>Wetland media types and major chemicals</th>
<th>First order rate constant range (cm(^{-1}))</th>
<th>Regression coefficients (R(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCC/SBC/sand mix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Methylene chloride</td>
<td>0.1145-0.1638</td>
<td>0.8977-0.9945</td>
</tr>
<tr>
<td>• 1,2-DCA</td>
<td>0.0464-0.0476</td>
<td>0.8934-0.9832</td>
</tr>
<tr>
<td>BionSoil(^R) /Latimer/sand mix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Methylene chloride</td>
<td>0.0340-0.0431</td>
<td>0.9311-0.9599</td>
</tr>
<tr>
<td>• 1,2-DCA</td>
<td>0.0239-0.0256</td>
<td>0.9222-0.9547</td>
</tr>
</tbody>
</table>
It is evident from Figures 7.22 – 7.25 that methylene chloride and 1,2-DCA were removed much more efficiently and effectively than either *cis*-1,2-DCE or 1,1-DCA were removed in the experiment described in the previous section. It is also obvious from Figures 7.22 – 7.25 and Table 7.7 that the RCC/SBC/sand EWS units performed better than the BionSoil/peat/sand units with respect to the removal of both methylene chloride and 1,2-DCA.

The evidently better removal of the cVOCs in this experiment compared to the one discussed in the previous section can partly be attributed to the concentrations of the cVOCs in this experiment being lower (by orders of magnitude) than those for the previous one. This better removal may also partly be attributed to acclimation of the culture inoculated in the EWS units, whose extent and impact increased with time. However, as evident in the discussion presented in the next section, the acclimation of the microbial culture inoculated in the EWS units, was not yet as effective for 1,1-DCA removal as it was for methylene chloride and 1,2-DCA.

It is important to note that this experiment has demonstrated that even very low concentrations of pollutants can be lowered to even lower levels (to below detection limit in the case of the RCC/SBC/sand). This suggests that even drinking water standards for these cVOCs can be achieved using properly designed treatment or remediation systems. It is noteworthy that, with many treatment systems, it is often difficult to completely remove low concentrations of pollutants of interest. This applies to even treatment systems for conventional pollutants. For example, conventional wetland systems cannot remove biochemical oxygen demand (BOD) completely (Kadlec and Knight, 1996; Kadlec and Wallace, 2009). In view of this, results from this study have important practical significance.
Under the conditions similar to the ones that prevailed during this experiment, 1,2-DCA is reductively transformed into chloroethane, which is in turn also transformed into ethane by reductive dechlorination (Lorah and Olsen, 1999; Lorah and Voytek, 2004; Hunkeler et al., 2005; Aulenta et al., 2006). Methylene chloride would be expected to be dechlorinated first into monochloromethane and then methane, in successive steps.

7.4.2.3 Assessment Based on Treatment of Synthetic Groundwater Containing Cis-1,2-DCE and 1,1-DCA

Figures 7.26 – 7.29 compare the performances of the RCC/SBC/sand and BionSoil/peat/sand packed EWS units based on treatment of synthetic groundwater containing cis-1,2-DCE and 1,1-DCA during weeks 19 – 21 after commissioning the EWS units. Both sets of EWS units were previously used in the study for the treatment of cis-1,2-DCE, 1,1-DCA, vinyl chloride, chlorobenzene, and 1,1,1-TCA described in section 7.4.2.1. This experiment was carried out in parallel with the one described in section 7.4.2.2. Each data point is based on results from duplicate EWS units. Results of regression analysis of the data for the removal of cis-1,2-DCE and 1,1-DCA (Figure 7.26 – 7.29) in the RCC/SBC/sand and BionSoil/Latimer/sand - packed EWS units are summarized in Table 7.8. Typical profiles of pH of pore water in the RCC/SBC/sand and BionSoil/peat/sand EWS units during the treatment of the different types of synthetic groundwater (Figures 7.14 – 7.29) are illustrated in Figure 7.30.

It is clearly evident from Figures 7.26 – 7.29 and Table 7.8 that the RCC/SBC/sand EWS units performed better than the BionSoil/peat/sand EWS units, which is consistent with the performance results presented and discussed in the previous two sections. Notably, in both the RCC/SBC/sand and BionSoil/peat/sand EWS units, cis-1,2-DCE was removed completely, which is consistent with the performance results.
for week 8 described earlier. Nonetheless, in the experiment described in this section the complete removal occurred at lower depths (< 30 cm versus < 45 cm).

**Figure 7.26:** Removal of cis-1,2-DCE and 1,1-DCA in the RCC/SBC/sand EWS units in week 19.

**Figure 7.27:** Removal of cis-1,2-DCE and 1,1-DCA in the BionSoil/peat/sand EWS units in week 19.
Figure 7.28: Removal of \textit{cis}-1,2-DCE and 1,1-DCA in the RCC/SBC/sand EWS units in week 26.

Figure 7.29: Removal of \textit{cis}-1,2-DCE and 1,1-DCA in the BionSoil/peat/sand EWS units in week 26.
Table 7.8: Comparison of performance of the RCC/SBC/sand and BionSoil/peat/sand EWS units with respect to removal of cis-1,2-DCE and 1,1-DCA (weeks 19 - 26).

<table>
<thead>
<tr>
<th>Wetland media types and major chemicals</th>
<th>First order rate constant range (cm(^{-1}))</th>
<th>Regression coefficients (R(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCC/SBC/sand mixture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• cis-1,2-DCE</td>
<td>0.1232-0.1399</td>
<td>0.8303-0.9736</td>
</tr>
<tr>
<td>• 1,1-DCA</td>
<td>0.0198-0.0246</td>
<td>0.8804-0.9142</td>
</tr>
<tr>
<td>BionSoil(^R)/Latimer/sand mixture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• cis-1,2-DCE</td>
<td>0.0922-0.0964</td>
<td>0.9135-0.9615</td>
</tr>
<tr>
<td>• 1,1-DCA</td>
<td>0.0090-0.0160</td>
<td>0.7921-0.8584</td>
</tr>
</tbody>
</table>

The removal of 1,1-DCA in both sets of EWS units was not as good as that of cis-1,2-DCE. Although its removal was better in the RCC/SBC/sand than in the BionSoil/peat/sand EWS units, in both cases, 1,1-DCA broke through and its removal rates were remarkably low. The poor removal of 1,1-DCA can be attributed to its relatively high recalcitrance. Our other studies involving 1,1-DCA and cis-1,2-DCE as well as a mixture of other cVOCs indicated that the cVOC was more difficult to degrade when treated in mixtures as well as when treated separately. Although cis-1,2-DCE is known to inhibit transformation of vinyl chloride to ethene (Yu et al., 2005), it is not known whether cis-1,2-DCE can inhibit the dechlorination of 1,1-DCA. Besides, if in this experiment 1,1-DCA transformation was inhibited by the presence of cis-1,2-DCE, then 1,1-DCA dechlorination would increase dramatically above the 15 cm height due to the absence of cis-1,2-DCE. It can be argued that, if suitable microorganisms were present in the EWS units, they would have been able to take advantage of the absence of cis-1,2-DCE.
DCE and act on 1,1-DCA at heights above 15 cm without interference or competition. Evidently, this did not happen in this experiment. Therefore, the dismal performance of the EWS units with respect to 1,1-DCA removal can justifiably be attributed to the cVOC’s relative recalcitrance, which is consistent with the results from our other studies.

It can be speculated that the inocula used in the studies that exhibited poor removal of 1,1-DCA did not contain a microbial population capable of degrading the cVOC. In practice, to enable the EWS units to degrade the cVOC, it would be necessary to reinoculate them with the suitable dechlorinating culture that can completely degrade 1,1-DCA.

It is evident in Figure 7.30 that, whereas the influent groundwater was alkaline, the effluents from both the RCC/SBC/sand and Bion/Latimer/sand EWS units exhibited a pH that was close to neutral. The decrease in pH of the water as it flows through the EWS is expected because biochemical processes that take place in the bed material produce organic acids. Although natural wetlands exhibit a wide range of pH levels, they all lie in the acidic range (Mitsch and Gosselink, 2000; 2007), which is in agreement with our observations.

In spite of slight differences in their pH profiles (Figure 7.30), both the RCC/SBC/sand and BionSoil/peat/sand EWS units maintained pH levels within the range conducive for microbial processes. Both the BionSoil/peat/sand and RCC/SBC/sand EWS units moderated the pH of the influent from high to near neutral levels. Mature wetlands used for wastewater treatment exhibit pH in the range 6.7 – 7.2 (Kadlec and Knight, 1996). In view of this, deficiencies in the performance of the EWS units reported in this study cannot be attributed to less than optimum pH levels.
Figure 7.30: Variation of pH of synthetic groundwater with depth in the wetlands. The neutral line is shown for comparison purposes.

7.5 Conclusions

This study evaluated the suitability of row crop compost (RCC), soil builder compost (SBC), aged pine bark (APB), wood fiber (WF), and an RCC/SBC/sand mixture as bed materials for EWSs meant to treat mixtures of chlorinated volatile organic compounds (cVOCs). These single and composite potential EWS bed materials were compared to a BionSoil/Latimer peat/sand mixture. The study was carried out using both microcosm and mesocosm experiments.

The individual compost materials as well as the RCC/SBC/sand composite medium were found to be effective EWS media comparable to the materials used currently. On the whole, this study showed that the RCC/SBC/sand composite medium
was the best alternative medium of the 5 assessed (including BionSoil/peat/sand, which it is meant to replace). In the microcosm experiments, the RCC/SBC/sand mixture exhibited a TCE removal rate of 0.336 d⁻¹, while the BionSoil/Latimer peat/sand mixture degraded the cVOC at the rate of 0.288 d⁻¹. Similarly, in mesocosm experiments, the RCC/SBC/sand packed EWS units performed better than BionSoil/peat/sand ones. The respective cVOC removal rate constants for the RCC/SBC/sand and BionSoil/peat/sand EWS units were 0.115 - 0.164 cm⁻¹ and 0.034 - 0.043 cm⁻¹ for methylene chloride, 0.046 - 0.048 cm⁻¹ and 0.024 - 0.025 cm⁻¹ for 1,2-DCA, 0.123 - 0.140 cm⁻¹ and 0.092 - 0.096 cm⁻¹ for cis-1,2-DCE, and 0.020 - 0.025 cm⁻¹ and 0.009 - 0.016 cm⁻¹ for 1,1-DCA. This study also showed that 1,1-DCA is more recalcitrant than cis-1,2-DCE in all of the substrates tested.

It can be concluded from this study that compost materials are suitable substrates for EWSs and similar remediation systems for VOC pollution. The study has also demonstrated the utility of engineering substrates so as to achieve desired VOC treatment and remediation goals. It is also evident from this study that, owing to its observed relative recalcitrance, more studies need to be done on removal of 1,1-DCA in EWSs, especially with respect to suitable microbial cultures that can completely dechlorinate it.
CHAPTER 8: EFFECTS OF LOADING RATES, INITIAL CONCENTRATION, AND SUBSTRATES ON TREATMENT OF CHLORINATED VOLATILE ORGANIC COMPOUNDS IN ENGINEERED WETLANDS

8.1 Introduction

The effectiveness of engineered wetland systems (EWSs) treating chlorinated volatile organic compounds (cVOCs) depends on the fate and transport processes which these chemicals encounter in the EWS bed. The fate and transport processes, in turn, dictate the rate and extent of removal of these chemicals in the EWSs. Therefore, it is logical to expect both wetland plants and EWS media to influence the performance of EWSs. This is more so because wetland plants and media influence one another as well as the overall biological, chemical, and physical characteristics of the wetland bed (Mitsch and Gosselink, 2007). Notably, the initial composition and characteristics of an EWS bed is mostly influenced by wetland medium constituents. EWS medium constituents influence the wetland plants as well. The wetland plants, in turn, modify the wetland medium. In so doing, they alter the chemistry and geotechnical as well as hydrodynamic properties of the wetland bed. Depending on the composition of its constituents, the wetland bed material also modifies the structure of the microbial community present in the wetland bed (Mitsch and Gosselink, 2000).

The fate and transport processes that affect cVOC removal in EWSs are similar to the ones that are exhibited in other porous media such as natural soil and aggregate of different sizes. These processes are discussed at length in Schnoor (1996), Charbeneau (2000), Rwamaswami et al. (2005), and Payne et al. (2008). They include advection, dispersion, sorption, biotic degradation, and abiotic degradation. Advection depends on seepage velocity (Schnoor, 1996; Charbeneau, 2000; Payne et al., 2008), which means it
is influenced by the hydraulic loading rate (HLR). Notably, velocity influences the general hydrodynamic behavior of EWSs, including the onset as well as severity of fluidization of the EWS bed. It also influences the occurrence and severity of preferential flows, which can in turn influence the performance of EWS units. Dispersion is directly influenced by concentration gradients (Charbeneau, 2000; Rwamaswami et al., 2005; Payne et al. 2008), which means it can be influenced by the pollutant initial (inlet) concentration (PIC). The PIC can also influence sorption because sorption is influenced by the contaminant concentration.

With respect to biotic contaminant removal processes, it is known that high concentration of contaminants can be toxic to microorganisms responsible for treatment (Bossert et al., 2003). As such, the PIC can influence biotic contaminant degradation processes. The same thing can be said about chemical processes, because chemical reactions are influenced by reactant concentrations.

It is also worth pointing out the importance of the HLR owing to the fact that the extent of degradation or transformation of contaminants in most treatment systems depends on their hydraulic residence time (HRT) (Metcalf and Eddy, 2003). For a treatment system, the HRT is inversely proportional to the HLR. The PLR is influenced by both the HLR and the concentration of the contaminant of interest in the influent while itself influencing both the size and effectiveness of the treatment system.

In spite of the importance of loading rates and initial concentration discussed above, many design equations and guidelines do not account for variations in HLRs, pollutant loading rates (PLRs), and PICs. Even guidelines for EWS meant for conventional pollutants (such as non-toxic organics and nutrients), which are discussed at length in Reed et al. (1995), Kadlec and Knight (1996), Crites and Tchobanoglous
With respect to cVOCs, this situation is attributable to the relative novelty of application of EWSs to the treatment of cVOCs. Therefore, a focus on effects on HLRs, PLRs, and PICs on EWS performance is well poised to make an important contribution to the advancement of the wetland technology, especially with respect to cVOC treatment. The objectives of this study were: 1) to investigate the effects of the HLR, PIC, and PLR on the performance of upflow EWS units treating cVOCs and 2) to compare the performance efficiencies of EWSs packed with row crop compost (RCC)/soil builder compost (SBC)/sand (37.5:37.5:25 w/w) and BionSoil/Latimer peat/sand (37.5:37.5:25) with a view to determining the one that performed better over the selected ranges of HLRs, PICs, and PLRs. The candidate EWS bed materials were identified in earlier studies (Chapters 2 and 7).

8.2 Materials and Methods

8.2.1 Experimental Set-up

This study was accomplished using two sets of identical mesocosm scale EWS units. Figure 8.1 illustrates the main features of the EWS units and the overall experimental set up adopted.

Each EWS unit had an internal diameter of 15 cm and an effective height of 45 cm. Seven sampling ports 7.5 cm c/c (each fitted with a Biopsy needle) were provided along the height of each EWS unit to facilitate withdrawal of pore water samples. The inlet zone (about 7.5 cm deep layer) was made up of a layer of inert gravel to facilitate uniform distribution of the influent flow.
The two sets of the EWS units were set up and operated in parallel. One set of the EWS units was packed with a mixture of row crop compost (RCC), soil builder compost (SBC), and sand (37.5:37.5:25 w/w) (hereinafter called compost) while the other was packed with a mixture of BionSoil (Dream Maker Dairy, Cowlesville, NY), Latimer peat (Latimer Peat Moss Farm, West Liberty, OH), and sand (37.5:37.5:25 w/w) (hereinafter called BionSoil/peat). The wetland bed material components for each mixture were properly blended to achieve homogeneity. The porosity of the compost EWS units was
49% while that of the BionSoil/peat EWS units was 55%. The mix designs for the wetland bed materials were adopted from previous studies (Kassenga et al., 2003; Chapters 2, 3, and 7). The pertinent characterization and specifications are reported elsewhere (Chapters 2, 3 and 7). Each EWS unit was planted with *Typha latifolia*.

The EWS units were commissioned by pumping through them groundwater containing chlorinated ethenes and ethanes plus a culture containing dehalorespiring bacteria (Chapter 7). Before being used in this study the EWS units had been operating for more than three years and had demonstrated effective performance over a wide range of cVOCs, including TCAs and TCE (Chapter 7).

### 8.2.2 Operation and Monitoring

Each of the EWS units was continuously fed a synthetic groundwater containing 1,1,2,2-TeCA and TCE in an upflow manner. The 1,1,2,2-TeCA and TCE bearing synthetic groundwater was meant to mimic the groundwater of interest (Chapters 2 and 3). Monitoring of the performance of the EWS units for this study started at least two years after planting and commissioning the EWS units. Both sets of EWS units were operated under two major phases, one characterized by a constant (a narrow band of) HLR and the other by a constant PIC. Each major phase was accomplished in sub-phases of different but constant PIC or HLRs. First, the HLR was varied in five steps (sub-phases) lasting six weeks, each starting with the highest while the PIC was held constant. Next, the procedure was repeated, but the roles of the HLR and PIC were reversed. The HLR was held constant while the PLR was varied. During each sub-phase, performance monitoring was done by sampling and analyzing pore water from the EWS units at least two times a week. Stabilization of performance of the EWS units (achievement of quasi-steady-state)
between sub-phases took no more than two weeks. Therefore, intensive sampling for evaluation lasted for at least one month.

8.2.3 Analytical Work

Aqueous phase samples were analyzed for eVOCs using EPA Method 8260B using GC/MS (Agilent Technologies 6890N Network GC System, Agilent Technologies 5973Network Mass Selective Detector) coupled to an AquaTek 70 Autosampler® (Teledyne Tekmar) and Velocity XPT® purge and trap sample concentrator (Teledyne Tekmar). Methane, ethene, and ethane gases were analyzed using GC/FID. 1 mL of head space sample was injected into the gas chromatography with flame ionization detector (Agilent 5890 Series II) equipped with a 2.4 m x 0.32 mm ID column packed with Carbopack b/1% Sp-(Supelco, Bellefonte, PA). The column 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.

8.3 Data Analysis and Modeling

1,1,2,2-TeCA removal data were modeled according to the first-order kinetic model (Equation 8.1).

\[ [E] = [E]_o e^{-k_e x} \]  

[8.1]

where \([E]\) = molar concentration of 1,1,2,2-TeCA (moles/L) at a height of \(x\) (cm) up the EWS unit, \([E]_o\) = molar concentration of 1,1,2,2-TeCA in the influent into each EWS unit (moles/L), \(k_e = 1,1,2,2\)-TeCA removal rate constant (cm\(^{-1}\)), and \(x\) = distance (cm) up the height of the EWS unit. The removal of TCE was modeled according to Equation 8.2 described elsewhere (Chapters 2 and 3), recognizing the fact that TCE removal depends on 1,1,2,2-TeCA removal as well as on the concentration of TCE in the influent.
\[
[C] = \frac{k_E [E]_O}{k_C - k_E} e^{-k_c x} + \left( [C]_O - \frac{k_E [E]_O}{k_C - k_E} \right) e^{-k_c x}
\]  
[8.2]

where \( k_E \) and \([E]_O \) are the same as in Equation 8.1, \([C] = \) molar concentration of TCE (moles/L) at a height \( x \) above the EWS bottom, \( k_C = \) actual removal rate of TCE (cm\(^{-1}\)), \([C]_O = \) concentration of TCE in the influent to each EWS unit. The apparent removal rate of TCE was determined using Equation 8.1 but with \([E] \) and \([E]_O \) replaced by \([C] \) and \([C]_O \), respectively.

To enable prediction of 1,1,2,2-TeCA removal rates in EWS units operated at different hydraulic loading rates (HLRs) within the range covered in this study, an Arrhenius equation – like model and a two parameter rational equation, which is simpler, were developed. The Arrhenius-like model is represented by Equation 8.3.

\[
k_{HL} = k_{HR} \times \Theta_{HC}^{(H-H_R+\Delta H)}
\]  
[8.3]

where \( k_{HL} = \) contaminant removal rate constant (cm\(^{-1}\)) at the HLR of interest; \( k_{HR} = \) contaminant removal rate constant (cm\(^{-1}\)) at the reference HLR; \( \Theta_{HC} = \) correction factor for the dependence of the contaminant removal rate on the HLR; \( H = \) HLR of interest (cm/d); \( H_R = \) reference HLR (cm/d); and \( \Delta H = \) correction term (error term) for the reference HLR. For predictions of rate constants based on PLR data, the applicable model is in the form of Equation 8.4 for concentration based data (mg/L).

\[
k_{CL} = k_{CR} \times \Theta_{CC}^{(C-C_R+\Delta C)}
\]  
[8.4]

where \( k_{CL} \), \( k_{CR} \), and \( \Theta_{CC} \) correspond to similar terms in Equation 8.3 and \( C \) is the PIC (concentration in the influent). For the mass loading based data (mg m\(^{-2}\) d\(^{-1}\)), the applicable expression is Equation 8.5.

\[
k_{ML} = k_{MR} \times \Theta_{MC}^{(M-M_R+\Delta M)}
\]  
[8.5]
where \( k_{ML}, k_{MR}, \) and \( \theta_{MC} \) correspond to similar terms in Equations 8.3 and 8.4. The rational equation model for predicting the rate constant based on HLR data is given by Equation 8.6.

\[
k_{HL} = \frac{A_H}{H + B_H}
\]  

[8.6]

where \( k_{HL} \) is as defined earlier, \( H \) is the HLR (cm/d) and \( A_H \) and \( B_H \) are concentration-specific constant parameters. A version of Equation 8.6 for predicting rate constants based on PIC data is given by Equation 8.7.

\[
k_{CL} = \frac{A_C}{C + B_C}
\]  

[8.7]

where \( k_{CL} \) is as defined earlier, \( C \) is the initial concentration (mg/L) and \( A_C \) and \( B_C \) are hydraulic loading rate-specific constant parameters. A version of the rational equation that can be used to predict rate constants based on mass loading rate (mg m\(^{-2}\) d\(^{-1}\)) data is given by Equation 8.8.

\[
k_{ML} = \left( \frac{A_M}{M + B_M} \right)^n
\]  

[8.8]

where \( k_{ML} \) is as defined earlier, \( M \) is the mass loading rate (g m\(^{-2}\) d\(^{-1}\)) and \( A_M, B_M, n \) are hydraulic loading rate-specific constant parameters.

An additional comparison between the compost and BionSoil EWS units was done based on the cVOC removal efficiency and breakthrough frequency data. The cVOC removal efficiency was calculated according to Equation 8.9.

\[
E = \left( \frac{(C_i - C_o)}{C_i} \right) \times 100\%
\]  

[8.9]

where \( E \) is the removal efficiency (%), \( C_i \) is the contaminant concentration in the influent,
and $C_e$ is the contaminant concentration in the effluent. The frequency of cVOC breakthrough incidences was calculated using Equation 8.10.

$$f_{BT} = \left( \frac{N_{BTi}}{N_R} \right) \times 100\%$$

[8.10]

where $f_{BT}$ is the frequency of breakthrough incidences (%), $N_{BTi}$ is number of breakthrough incidences observed, and $N_R$ is the total number of analysis replicates. The breakthrough incidence frequency is a useful measure of EWS performance because it directly gauges the absence of the cVOCs of interest in the effluent, hence the absence of the associated environmental and health risks. Besides, where multi-pathway and sequential transformations of parent pollutants are involved, removal rate constants are not easy to compute for all daughter products of interest.

8.4 Results and Discussion

8.4.1 Performance of the EWS Units at Different Hydraulic Loading Rates

Typical results of monitoring the performance of the compost and BionSoil/peat EWS units when operated at hydraulic loading rates of between 3.35 and 22.64 cm/d and a 1,1,2,2-TeCA influent concentration of 25 mg/L are shown in Figures 8.2 – 8.11 and Tables 8.1 – 8.5.

On the whole, the results demonstrate that if the HLR is reduced to a low enough level, it is possible for an EWS to achieve complete removal of influent cVOCs and their daughter products. The results also demonstrate that complete removal of the cVOCs is possible even when the initial concentration is as high as 25 mg/L as was the case in this study. It is furthermore is evident from these results that complete removal of the cVOCs was easier to achieve in the compost than in the BionSoil/peat EWS units. This supports the observations made earlier in Chapter 7 with respect the results of microcosm and mesocosm scale experiments that used these EWS substrates.
Figure 8.2: 1,1,2,2-TeCA removal in compost EWS units operated at HLR = 22.64 cm/d.

Figure 8.3: 1,1,2,2-TeCA removal in BionSoil/peat EWS units operated at HLR = 22.64 cm/d.
Table 8.1: Comparison of the compost and Bion/peat EWS units when operating at a HLR of 22.64 cm/d and 1,1,2,2-TeCA influent concentration of 25 mg/L.

<table>
<thead>
<tr>
<th>Parameter/consideration</th>
<th>Pollutants</th>
<th>Compost units</th>
<th>Bion and peat units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal rates (cm⁻¹)</td>
<td>1,1,2,2-TeCA</td>
<td>0.0465</td>
<td>0.0388</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0.0650</td>
<td>0.0613</td>
</tr>
<tr>
<td>Overall removal efficiencies (%)</td>
<td>1,1,2,2-TeCA</td>
<td>78.61</td>
<td>75.64</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>83.06</td>
<td>81.00</td>
</tr>
<tr>
<td>Parent chemical breakthrough frequency (%)</td>
<td>1,1,2,2-TeCA</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Product breakthrough frequency (%)</td>
<td>cis-1,2-DCE</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1,1-DCA</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>trans-1,2-DCE</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

It is furthermore evident from Figures 8.2 through 8.11 that profiles of removal of 1,1,2,2-TeCA and TCE and their daughter products were similar regardless of the HLR. However, 1,1,2-TCA, 1,1-DCE, and to a much lower extent, 1,2-DCA (profiles not shown) were detected at very high (16.98 – 22.64 cm/d) but not at low HLRs. Even at the high HLRs, the concentration of 1,1,2-TCA was consistently lower than that of TCE while the concentration of 1,1-DCE was consistently lower than concentrations of all the daughter products detected, except for 1,2-DCA whose concentration was even lower. The failure to detect 1,1,2-TCA, 1,1-DCE, and 1,2-DCA at low HLRs was probably
Figure 8.4: 1,1,2,2-TeCA removal in compost EWS units operated at HLR = 22.64 cm/d.

Figure 8.5: 1,1,2,2-TeCA removal in BionSoil/peat EWS units operated at HLR = 22.64 cm/d.
Table 8.2: Comparison of the compost and Bion/peat EWS units when operating at a HLR of 16.98 cm/d and 1,1,2,2-TeCA influent concentration of 25 mg/L.

<table>
<thead>
<tr>
<th>Parameter/consideration</th>
<th>Pollutants</th>
<th>Compost units</th>
<th>Bion and peat units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal rates (cm⁻¹)</td>
<td>1,1,2,2-TeCA</td>
<td>0.0747</td>
<td>0.0620</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0.0129</td>
<td>0.0913</td>
</tr>
<tr>
<td>Overall removal efficiencies (%)</td>
<td>1,1,2,2-TeCA</td>
<td>97.79</td>
<td>96.53</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>98.81</td>
<td>96.58</td>
</tr>
<tr>
<td>Parent chemical breakthrough frequency (%)</td>
<td>1,1,2,2-TeCA</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>50</td>
<td>66.7</td>
</tr>
<tr>
<td>Product breakthrough frequency (%)</td>
<td>cis-1,2-DCE</td>
<td>66.7</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1,1-DCA</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>trans-1,2-DCE</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>66.7</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

because these cVOCs did not accumulate; they were degraded at rates that were faster than they were formed from their parent chemicals. In contrast, the higher HLRs, which resulted in higher overall quantities of cVOCs produced, led to accumulation of 1,1,2-TCA, 1,1-DCE, and 1,2-DCA due to the sheer quantities formed overwhelming the available treatment capacity. It could also be attributable to competition of these compounds with the other cVOCs. Unfavorable results of competitive biotransformation among cVOCs have also been reported by Aulenta et al. (2005). Overall, for both the compost and BionSoil/peat EWS units, the frequency of the breakthrough of the cVOCs
Figure 8.6: 1,1,2,2-TeCA removal in compost EWS units operated at HLR = 22.64 cm/d.

Figure 8.7: 1,1,2,2-TeCA removal in BionSoil/peat EWS units operated at HLR = 22.64 cm/d.
Table 8.3: Comparison of the compost and Bion/peat EWS units when operating at a HLR of 11.32 cm/d and 1,1,2,2-TeCA influent concentration of 25 mg/L.

<table>
<thead>
<tr>
<th>Parameter/consideration</th>
<th>Pollutants</th>
<th>Compost units</th>
<th>Bion and peat units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal rates (cm⁻¹)</td>
<td>1,1,2,2-TeCA</td>
<td>0.1088</td>
<td>0.0797</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0.1440</td>
<td>0.1211</td>
</tr>
<tr>
<td>Overall removal efficiencies (%)</td>
<td>1,1,2,2-TeCA</td>
<td>98.54</td>
<td>93.99</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>100</td>
<td>99.13</td>
</tr>
<tr>
<td>Parent chemical breakthrough frequency (%)</td>
<td>1,1,2,2-TeCA</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Product breakthrough frequency (%)</td>
<td>cis-1,2-DCE</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>1,1-DCA</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>trans-1,2-DCE</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

The removal efficiency for 1,1,2,2-TeCA was higher at the higher HLRs. This is attributable to: 1) overloading of the treatment capacity due to the increase in the quantities of the chemicals to be biodegraded, 2) overwhelming of the sorption capacity of the EWS medium, resulting in lower effective retardation of the cVOCs, and 3) an increase in effects of advection, which depend directly on the HLR (Rwamaswami, 2005; Payne et al., 2008).

Generally, the compost EWS units performed consistently better than the BionSoil/peat EWS units over the whole range of HLRs covered in this study. 1,1,2,2-TeCA removal rates in the compost EWS units were in the range 0.047 – 0.530 cm⁻¹, while the corresponding values for the Bion Soil and peat units were 0.039 – 0.404 cm⁻¹.
Figure 8.8: 1,1,2,2-TeCA removal in compost EWS units operated at HLR = 22.64 cm/d.

Figure 8.9: 1,1,2,2-TeCA removal in BionSoil/peat EWS units operated at HLR = 22.64 cm/d.
Table 8.4: Comparison of the compost and Bion/peat EWS units when operating at a HLR of 6.69 cm/d and 1,1,2,2-TeCA influent concentration of 25 mg/L.

<table>
<thead>
<tr>
<th>Parameter/consideration</th>
<th>Pollutants</th>
<th>Compost units</th>
<th>Bion and peat units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal rates (cm⁻¹)</td>
<td>1,1,2,2-TeCA</td>
<td>0.3777</td>
<td>0.1470</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0.2878</td>
<td>0.2434</td>
</tr>
<tr>
<td>Overall removal efficiencies (%)</td>
<td>1,1,2,2-TeCA</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Parent chemical breakthrough</td>
<td>1,1,2,2-TeCA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>frequency (%)</td>
<td>TCE</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Product breakthrough frequency (%)</td>
<td>cis-1,2-DCE</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1,1-DCA</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>trans-1,2-DCE</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The superior performance of the compost EWS units is attributable to the richer, easily degradable organic matter content and favorable microbial community structure initially present in the constituent compost materials. In previous microcosm experiments, compost treatments outperformed other treatments, including a BionSoil/peat treatment, in terms of supporting cVOC degradation and production of methane and carbon dioxide (exhibiting high pressure and concentration), suggesting that compost materials supported more vigorous methanogenic activity while also supporting dechlorination (Chapter 7).
Figure 8.10: 1,1,2,2-TeCA removal in compost EWS units operated at HLR = 22.64 cm/d.

Figure 8.11. 1,1,2,2-TeCA removal in BionSoil/peat EWS units operated at HLR = 22.64 cm/d.
Table 8.5: Comparison of the compost and Bion/peat EWS units when operating at a HLR of 3.35 cm/d and 1,1,2,2-TeCA influent concentration of 25 mg/L.

<table>
<thead>
<tr>
<th>Parameter/consideration</th>
<th>Pollutants</th>
<th>Compost units</th>
<th>Bion and peat units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal rates (cm⁻¹)</td>
<td>1,1,2,2-TeCA</td>
<td>0.5298</td>
<td>0.4035</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0.2738</td>
<td>0.2768</td>
</tr>
<tr>
<td>Overall removal efficiencies (%)</td>
<td>1,1,2,2-TeCA</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Parent chemical breakthrough frequency (%)</td>
<td>1,1,2,2-TeCA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Product breakthrough frequency (%)</td>
<td>cis-1,2-DCE</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1,1-DCA</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>trans-1,2-DCE</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Conceivably, the vigorous methanogenic activity was due to a higher readily degradable organic content and favorable initial microbial community structure. Composting facilitates the breakdown of recalcitrant organic material fractions such as lignin and hemicelluloses (Insam and de Betoldi, 2007), making them more amenable to microbial action. Arguably, having been acted upon by microorganisms for longer periods of time, BionSoil and peat are likely to have had less readily biodegradable constituents than compost.

The data profiled in Figures 8.2 – 8.10 indicate that 1,1,2,2-TeCA degraded into TCE by dehydrochlorination and into 1,1,2-TCA by hydrogenolyis (Lorah and Olsen,
TCE was transformed into \textit{cis}-1,2-DCE and \textit{trans}-1,2-DCE, both of which were in turn transformed first into vinyl chloride (VC) and eventually into ethene through two successive reductive steps (Chen et al., 1996; Lorah and Olsen, 1999; Aulenta et al., 2005). The higher dominance of \textit{trans}-1,2-DCE suggests that it was formed directly from 1,1,2,2-TeCA by dichloroelimination in addition to being formed from TCE along with \textit{cis}-1,2-DCE by reductive dechlorination.

These transformation mechanisms and pathways of 1,1,2,2-TeCA under methanogenic conditions have been reported by various researchers such as Chen et al. (1996), Lorah and Olsen (1999), Aulenta et al. (2005), and Zhang et al. (2006).

1,1,2-TCA is likely to have been transformed into VC by dichloroelimination and through successive steps of reductive dechlorination into 1,2-DCA, then chloroethane, and finally ethane (Lorah and Voytek, 2004). 1,2-DCA is likely to have also been transformed directly into ethene by dichloroelimination (Lorah and Voytek, 2004; Aulenta et al., 2005; Zhang et al., 2006). The fact that only low levels of 1,2-DCA were detected, coupled with the fact that none of the known intermediate products of 1,2-DCA transformation was detected supports this assertion.

Tables 8.1 through 8.5 support the observations discernible from Figures 8.2 through 8.10. The removal rate constants of 1,1,2,2-TeCA were highest at the lowest HLRs. Evidently, they were higher for the compost EWS units than for the BionSoil/peat units. Also, TCE removal rate constants were higher than those of 1,1,2,2-TeCA, with the resultant non-accumulation of TCE in all the EWS units at all HLRs.

At the lower HLRs, transformation of 1,1,2,2-TeCA was practically the limiting step, which is consistent with results from some of our previous studies (Chapter 2). The insignificant accumulation of daughter products observed can be attributed to the several
different transformation mechanisms and pathways through which 1,1,2,2-TeCA and TCE and their daughter products were transformed. It can be argued that this versatility of the EWS units precluded unfavorable competition among the cVOCs present. From a practical standpoint, the apparent tendency of the transformations of the parent chemicals to be the limiting treatment step is favorable because it provides assurance that treatment systems can be designed on the basis of the parent chemicals without the risk of incidence of breakthrough of their daughter products. The fact that 1,1,2,2-TeCA and TCE are less harmful than their daughter product VC (which is carcinogenic), for example, reiterates the favorable implication of the observation. It is noteworthy that, whereas concentrations of parent chemicals can be known to a reasonably high degree of certainty, the same cannot be said of the daughter products.

An additional noteworthy observation regarding the above is that, at the lowest HLR, 100% removal of TeCA and TCE was achieved at heights as low as 15 cm. At the same time, breakthrough of daughter products did not occur in the compost EWS units and occurred only minimally in the BionSoil/peat EWS units. Also, overall removal efficiencies of 1,1,2,2-TeCA and TCE were higher at the lower HLRs.

8.4.2 Performance of the EWS Units at Different cVOC Loading Rates

Typical results of monitoring the performance the compost and BionSoil/peat EWS units when operated at a hydraulic loading rate of 22.64 cm/d and 1,1,2,2-TeCA influent concentrations of 1.25 – 25 mg/L are shown in Figures 8.12 – 8.17 and Tables 8.6 – 8.9.

Figures 8.12 – 8.17 and Tables 8.6 – 8.8 demonstrate that if the initial concentrations of cVOCs in the influent are kept low enough it is possible to achieve complete removal of the cVOCs and their daughter products in EWSs. Evidently, this
Figure 8.12: Typical profiles of treatment of 1,1,2,2-TeCA in the compost EWS units operated at an influent concentration of 10 mg/L and HLR of 22.64 cm/d.

Figure 8.13: Typical profiles of treatment of 1,1,2,2-TeCA in the BionSoil/peat EWS units when operated at an influent concentration of 10 mg/L and HLR of 22.64 cm/d.
Table 8.6: Comparison of performance of the compost and Bion/peat EWS units when treating 1,1,2,2-TeCA at an influent concentration of 10 mg/L and HLR of 22.64 cm/d.

<table>
<thead>
<tr>
<th>Parameter/consideration</th>
<th>Pollutants</th>
<th>Compost units</th>
<th>Bion and peat units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal rates (cm⁻¹)</td>
<td>1,1,2,2-TeCA</td>
<td>0.0953</td>
<td>0.0526</td>
</tr>
<tr>
<td>Overall removal efficiencies (%)</td>
<td>1,1,2,2-TeCA</td>
<td>98.17</td>
<td>88.50</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>97.96</td>
<td>92.66</td>
</tr>
<tr>
<td>Parent chemical breakthrough frequency (%)</td>
<td>1,1,2,2-TeCA</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>66.67</td>
<td>100</td>
</tr>
<tr>
<td>Product breakthrough frequency (%)</td>
<td>cis-1,2-DCE</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1,1-DCA</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>trans-1,2-DCE</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

was easier with the compost than with the BionSoil/peat EWS units. Figures 8.12 – 8.17 and Tables 8.6 – 8.8 also show clearly, as expected, that the performance of both the compost and BionSoil/peat EWS units was affected significantly by the initial concentration of the cVOCs. Carleton (2002) observed that wetland system removal rate constants and background concentrations had apparent dependence on inlet concentration and HLR. Kadlec (2000) has also observed apparent dependence of pollutant removal rate constants on HLR. Interestingly, contrary to our findings, Bojcevska and Tonderski (2007) have observed an increase in removal rates with loading rates of phosphorus, ammonia, and total suspended solids in free water surface constructed wetlands in Kenya.
Figure 8.14: Typical profiles of treatment of 1,1,2,2-TeCA in the compost EWS units operated at an influent concentration of 5 mg/L and HLR of 22.64 cm/d.

Figure 8.15: Typical profiles of treatment of 1,1,2,2-TeCA in the BionSoil/peat EWS units when operated at an influent concentration of 5 mg/L and HLR of 22.64 cm/d.
Table 8.7: Comparison of performance of the compost and Bion/peat EWS units when treating 1,1,2,2-TeCA at an influent concentration of 5 mg/L and HLR of 22.64 cm/d.

<table>
<thead>
<tr>
<th>Parameter/consideration</th>
<th>Pollutants</th>
<th>Compost units</th>
<th>Bion and peat units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal rates (cm⁻¹)</td>
<td>1,1,2,2-TeCA</td>
<td>0.1292</td>
<td>0.0612</td>
</tr>
<tr>
<td>Overall removal efficiencies (%)</td>
<td>1,1,2,2-TeCA</td>
<td>97.83</td>
<td>96.51</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>100</td>
<td>97.79</td>
</tr>
<tr>
<td>Parent chemical breakthrough frequency (%)</td>
<td>1,1,2,2-TeCA</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0.00</td>
<td>66.67</td>
</tr>
<tr>
<td>Product breakthrough frequency (%)</td>
<td><em>cis</em>-1,2-DCE</td>
<td>66.67</td>
<td>66.67</td>
</tr>
<tr>
<td></td>
<td>1,1-DCA</td>
<td>66.67</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td><em>trans</em>-1,2-DCE</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

The difference between Bojcevska and Tonderski’s (2007) results and ours could be due to differences in the pollutants treated and environmental conditions. It should also be borne in mind that an underloaded (or lightly loaded) treatment system can exhibit an increase in pollutant removal rate with loading rates until it is fully loaded. Other similar studies, with which findings from this study could be compared, are hard come by in the literature. As such, it is difficult to make the much desired additional comparison.

It is notable that, in spite of their different PIC levels, the degradation products (Tables 8.6 – 8.9) were the same for the low as well as the high PIC levels. These results suggest that the parent cVOC degradation pathways and mechanisms were similar.
Figure 8.16: Typical profiles of treatment of 1,1,2,2-TeCA in the compost EWS units operated at an influent concentration of 1.25 mg/L and HLR of 22.64 cm/d.

Figure 8.17: Typical profiles of treatment of 1,1,2,2-TeCA in the BionSoil/peat EWS units when operated at an influent concentration of 1.25 mg/L and HLR of 22.64 cm/d.
Table 8.8: Comparison of performance of the compost and Bion/peat EWS units when treating 1,1,2,2-TeCA at an influent concentration of 1.25 mg/L and HLR of 22.64 cm/d.

<table>
<thead>
<tr>
<th>Parameter/consideration</th>
<th>Pollutants</th>
<th>Compost units</th>
<th>Bion and peat units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dechlorination rates (cm⁻¹)</td>
<td>1,1,2,2-TeCA</td>
<td>0.1503</td>
<td>0.0751</td>
</tr>
<tr>
<td>Overall removal efficiencies (%)</td>
<td>1,1,2,2-TeCA</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Parent chemical breakthrough frequency (%)</td>
<td>1,1,2,2-TeCA</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Products breakthrough frequency (%)</td>
<td>cis-1,2-DCE</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>1,1-DCA</td>
<td>0.00</td>
<td>50.00</td>
</tr>
<tr>
<td></td>
<td>trans-1,2-DCE</td>
<td>0.00</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>0.00</td>
<td>50.00</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>0.00</td>
<td>50.00</td>
</tr>
</tbody>
</table>

In addition, in spite of their differences, the degradation products detected in the compost EWS units were similar to the ones observed in the BionSoil/peat EWS units. Again, this suggests that the dechlorination pathways and mechanisms were similar. The case can largely be explained by the fact that the two sets of EWS units were initially inoculated with the same cVOC degrading bacterial culture.

Based on the products, the pathways exhibited in this study are similar to the ones reported in comparable studies reported in the literature (Chen et al., 1996; Lorah et al., 1999; Kassenga, 2003; Kassenga et al., 2003; Lorah and Voytek, 2004; Aulenta, 2005; Zhang et al., 2006), and as also discussed earlier.
8.4.3 Modeling the Dependence of Pollutant Removal Rate Constants on HLR, PLR, and PIC

The first order pollutant removal rate constant data corresponding to the different hydraulic loading rates (Figures 8.2 – 8.11 and Tables 8.1 – 8.5) can be plotted as shown in Figure 8.18.

![Figure 8.18: Variation of 1,1,2,2-TeCA removal rates with hydraulic loading rates in the compost and BionSoil/EWS units.](image)

It is evident in Figure 8.18 that rate constants were affected by the HLRs mostly at the lower HLR values (<11.32 cm/d) and within this range the performance of the compost and BionSoil/peat EWS units differed significantly. Above this range, the rate constants for the two sets of EWS units were comparable and almost independent of the variations in HLRs. The HLR value of 11.32 cm/d (or thereabouts), which seems like a critical turning point for the relationships between the rate constants and the HLRs, does not seem to have any previously reported hydrodynamic or kinetic significance.
Among other things, Figure 8.18 suggests that hydraulically heavily loaded EWS units can withstand hydraulic shock-loadings more easily than lightly loaded ones. In practice, this means that for EWSs intended to handle variable flows, it is advisable to design them for high hydraulic loading rates and make them taller to compensate for their thinness. This is, arguably, a welcome observation because it weakens one of the main disadvantages leveled against EWSs and most natural waste treatment systems – the demand for large land area. Taller EWSs can have a smaller footprint, resulting in less land area occupancy. However, it should be borne in mind that small, tall EWS units are only feasible with vertical flow EWSs such as the ones used for cVOC treatment.

It is difficult to say whether the whole range of 1,1,2,2-TeCA removal rate constant data observed in this study is comparable to those observed in other studies because findings on performance of EWSs from studies of this nature could not be found in the literature. Nonetheless, the individual rate constants reported in this study compare well with the ones reported by Kassenga (2003) and Kassenga et al. (2003) who used EWS units and substrates similar to the ones used in this study.

Most studies reported in the literature covering cVOC treatment in EWSs do not report HLR, PIC, and PLR as variables. Nonetheless, ranges of HLRs of 3.35 – 22.64 cm/d and cVOC initial concentration of 1.25 – 25 mg/L used in this study cover the whole ranges of HLRs and PICs commonly reported in the literature and encountered in practice. In fact, for the most part, this covered HLRs and PICs higher than the ones commonly reported in the literature. Notably, the EWS units discussed in Chapter 3 were operated at HLRs of 3.22 – 5.63 cm/d and PICs of between around 0.40 to just below 8.00 mg/L. The studies reported by Kassenga (2003) and Kassenga et al. (2003) pertain to HLRs of 3.50 - 3.58 cm/d.
Hydrodynamic data corresponding to Figure 8.18 derived from EWS bed material and hydrodynamic characteristics are summarized in Table 8.9.

**Table 8.9:** Variation of Damköhler, Reynolds, and Peclet numbers with EWS HLRs

<table>
<thead>
<tr>
<th>Hydraulic loading rate (HLR) (cm/d) / (Hydraulic retention time) (d)</th>
<th>Damköhler number  ( \left( D_a = \frac{k_T H_C}{V_s} \right) )</th>
<th>Reynolds number  ( \left( R_e = \frac{V_s d \rho}{\mu} \right) )</th>
<th>Peclet number  ( \left( P_e = \frac{H_C V_s}{D} \right) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compost</td>
<td>BionSoil</td>
<td>Compost</td>
<td>BionSoil</td>
</tr>
<tr>
<td>3.35 (6.58)</td>
<td>48.66</td>
<td>32.01</td>
<td>0.0044</td>
</tr>
<tr>
<td>6.69 (3.30)</td>
<td>34.69</td>
<td>12.03</td>
<td>0.0088</td>
</tr>
<tr>
<td>11.32 (1.95)</td>
<td>9.99</td>
<td>6.52</td>
<td>0.0149</td>
</tr>
<tr>
<td>16.98 (1.30)</td>
<td>6.86</td>
<td>5.07</td>
<td>0.0224</td>
</tr>
<tr>
<td>22.64 (0.97)</td>
<td>4.27</td>
<td>3.17</td>
<td>0.0299</td>
</tr>
</tbody>
</table>

\( k_T \), first-order rate constant \((T^{-1})\); \( t_R \), \( V_s \), seepage velocity \([LT^{-1}]\); \( d \), bed material mean grain size \([L]\); \( \rho \), density of water \([ML^{-3}]\); \( \mu \), dynamic viscosity of water \([ML^{-1}T^{-1}]\); \( H_C \), EWS unit effective height \([L]\); \( D \), coefficient of hydrodynamic dispersion \([L^2T^{-1}]\).

In Table 8.9, the values of the Damköhler number, which in this case compares the influence of dechlorination to the effects of transport processes, show clearly the transition around the HLR value of 11.32 cm/d, which correlates with Figure 8.18, as expected. The fact that Damköhler number values in Table 8.9 are fairly large over the whole range of HLR values used implies that degradation of 1,1,2,2-TeCA during treatment in both the compost and BionSoil/peat EWS units was not overwhelmed by transport processes (Ramaswami et al., 2005). This assertion is borne out by the extensive
transformation of 1,1,2,2-TeCA and its daughter products and limited breakthrough incidences exhibited in this study.

Clearly, the EWS units reported in this study were operated in the predominantly laminar flow regime. In porous media laminar flow is characterized by \( R_E < 1-10 \) (Bear, 1979; 1988). With respect to the Peclet number data, in both the compost and BionSoil/peat EWS units, advective transport processes dominated over the diffusive-dispersive transport processes. At \( P_L \geq 10 \), advection prevails over dispersion, whereas when \( 0.1 < P_L < 10 \), both advection and dispersion are comparably influential (Myers, 1999). More specifically, when \( P_L \geq 10 \), whereby transport by advection dominates that by diffusion – dispersion, the treatment system under consideration approximates the idealized case of a plug flow reactor (PFR) characterized by the absence of mixing (Ramaswami et al., 2005). Therefore, the compost and BionSoil/peat EWS units used in this study operated predominantly as PFRs. EWSs are often designed as PFRs (Reed et al., 1995; Kadlec and Knight, 1996; Crites and Tchobanoglous, 1998; Kadlec et al., 2000; Pardue et al., 2000).

Mathematical modeling of the data for Figure 8.18 using the Arrhenius equation-like model (Equation 8.3) leads to Equation 8.11 for the compost EWS units and Equation 8.12 for the BionSoil/peat EWS units.

\[
k_{HL} = 0.0442 \times 0.857^{(H-22.64)} \quad (R^2 = 0.947) \quad (3.35 \leq H \leq 22.64) \quad [8.11]
\]

\[
k_{HL} = 0.0338 \times 0.869^{(H-22.64)} \quad (R^2 = 0.901) \quad (3.35 \leq H \leq 22.64) \quad [8.12]
\]

Based on the rational equation (Equation 8.6), the data for Figure 8.18 lead to Equations 8.13 and 8.14 for the compost and BionSoil/peat EWS units, respectively.

\[
k_{HL} = \frac{0.9679}{H - 2.7852} \quad (R^2=0.9758) \quad (6.69 \leq H \leq 22.64) \quad [8.13]
\]
\[ k_{HL} = \frac{0.8744}{H - 1.0511} \quad (R^2=0.9804) \quad (3.35 \leq H \leq 22.64) \quad [8.14] \]

In Equations 8.11 – 8.14, \( k_{HL} \) is in cm\(^{-1} \) and \( H \) is in cm. Equations 8.11 – 8.14 apply to PIC levels of about 25 mg/L and below. However, for the lower PIC levels, the equations can lead to overdesign.

The first order removal rate data corresponding to the different contaminant PIC levels (Figures 8.12 – 8.17 and Tables 8.6 – 8.8) can be plotted as shown in Figure 8.19.

![Graph](image)

**Figure 8.19:** Variation of 1,1,2,2-TeCA removal rates with influent cVOC concentrations in the compost and BionSoil/peat EWS units

It is clear from Figure 8.19 that the compost EWS units performed significantly better than the BionSoil/peat EWS units. Evidently, the difference between the performance of the two sets of EWS units decreased with the PIC. Unlike in the case of the HLR (Figure 8.18), in Figure 8.19 the dependence of rate constants seems to be almost equally significant over the whole range of the PICs covered in this study. Notably, this applies to both sets of EWS units although the gradient is considerably steeper for the compost EWS units.
Mathematical modeling of the data for Figure 8.19 using the Arrhenius equation–like model (Equation 8.4) leads to Equation 8.15 for the compost EWS units and Equation 8.16 for the BionSoil/peat EWS units.

\[ k_{CL} = 0.0462 \times 0.9513^{(C-25)} \ (R^2 = 0.9981) \ (1.25 \leq C \leq 25) \]  

[8.15]

\[ k_{CL} = 0.0377 \times 0.9743^{(C-25)} \ (R^2 = 0.9743) \ (1.25 \leq C \leq 25) \]  

[8.16]

Based on the rational equation (Equation 8.7), the data for Figure 8.19 lead to Equations 8.17 and 8.18 for the compost and BionSoil/peat EWS units, respectively.

\[ k_{CL} = \frac{1.5446}{C + 7.601} \ (R^2 = 0.9856) \ (1.25 \leq C \leq 25) \]  

[8.17]

\[ k_{CL} = \frac{1.9760}{C + 26.470} \ (R^2 = 0.9876) \ (1.25 \leq C \leq 25) \]  

[8.18]

In Equations 8.15 – 8.18, \( k_{CL} \) is in cm\(^{-1} \) and \( C \) is in mg/L. Equations 8.15 – 8.18 apply to HLR levels of 22.64 cm/d and below, but for the lower HLR levels they would tend to overdesign.

Mathematical modeling of the data for Figures 8.18 and 8.19 using the Arrhenius equation–like model (Equation 8.4) leads to Equation 8.19 for the compost EWS units and Equation 8.20 for the BionSoil/peat EWS units.

\[ k_{ML} = 0.0381 \times 0.9995^{(M-5660)} \ (R^2 = 0.9349) \ (M \geq 0) \]  

[8.19]

\[ k_{ML} = 0.0326 \times 0.9996^{(M-5660)} \ (R^2 = 0.8860) \ (M \geq 0) \]  

[8.20]

Based on the rational equation (Equation 8.8), the data for Figures 8.18 and 8.19 lead to Equations 8.21 and 8.22 for the compost and BionSoil/peat EWS units, respectively.

\[ k_{ML} = \left( \frac{526.315}{M - 198.421} \right)^{1.299} \ (R^2 = 0.9816) \ (199 \leq M \leq 5660) \]  

[8.21]
\[ k_{ML} = \left( \frac{344.828}{M - 52.345} \right)^{1.149} (R^2 = 0.9832) \quad (53 \leq M \leq 5660) \]  

In Equations 8.19 – 8.22, \( k_{ML} \) is in cm\(^{-1} \) while \( M \) is in mg m\(^{-2}\) d\(^{-1}\). Equations 8.19 – 8.22 apply to a whole range of PLR levels covered in this study whose upper limit is 5660 mg m\(^{-2}\) d\(^{-1}\).

8.5 Conclusion and Recommendations

This study has shown that HLRs, PLRs, and PIC significantly influence EWS performance, and this makes them an important design and operation consideration of EWSs. An increase in HLR from 3.35 to 22.64 cm/d lowered the removal rate constant of 1,1,2,2-TeCA from 0.530 to 0.047 cm\(^{-1}\) for the compost EWS units and from 0.404 cm\(^{-1}\) to 0.039 cm\(^{-1}\) for the BionSoil/peat EWS units. 1,1,2,2-TeCA removal efficiencies were 100% at the HLR of 3.35 cm/d, but not more than 78.6% at the HLR of 22.64 cm/d for all the EWS units. Also, more incidences of breakthrough were observed at higher than lower HLRs. Observations similar to those for the HLR were made with respect to the effects of the PLR and PIC. Other noteworthy observations are outlined below.

- Removal rates of cVOCs are more sensitive to HLR, PIC, and PLR changes at the lower than at the higher end. For the compost EWS units, at the lower end of HLRs an increase in HLR from 3.35 to 11.32 cm/d resulted in a 79.5% decrease in 1,1,2,2-TeCA removal rate while at the higher HLR a doubling of HLR from 11.32 to 22.64 cm/d resulted in only a 11.7% decrease in 1,1,2,2-TeCA removal rate.

- The variation in cVOC removal rates with HLRs, PICs, and PLRs can be modeled according to an Arrhenius equation – like model and a two parameter rational function. In both models, the rate constant is a function of the HLR, PLR, or PIC.
• Compost EWS units performed better than BionSoil/peat EWS units. For example, at the lowest HLR, the compost EWS units exhibited no parent chemical or daughter product breakthrough while the BionSoil/peat EWS units exhibited breakthrough incidences of 1,1-DCA and trans 1,2-DCE.

• Towards the lower end of the HLRs and PICs covered in this study, dechlorination of 1,1,2,2-TeCA was practically the limiting treatment step (as exhibited by minimal accumulation of daughter products). This is favorable because it is an assurance that EWSs can be designed on the basis of the parent cVOCs without the risk of breakthrough of daughter products some of which are more harmful than their parent compounds.

• The fact that the dependence of EWS’ performance on HLRs and PICs was exhibited by both the compost and BionSoil/peat EWS units indicates that it is influenced by factors other than the EWS bed material type.

In the evaluation of the effect of HLR on EWS performance, the PIC was kept constant at 25 mg/L. In the evaluation of the effect of PIC on EWS performance, the HLR was kept constant at 22.64 cm/d. Therefore, studies covering HLRs and PICs that were not covered in this study are recommended in order to provide information on responses of EWSs at these loading rates. The additional studies should cover HLR < 22.64 cm/d and PIC < 25 mg/L, which are more commonly encountered in practice.
CHAPTER 9: CONCLUSIONS AND RECOMMENDATIONS

This study set out to investigate the influence of substrates and wetland plants in the treatment of mixtures of volatile organic compounds (cVOCs) in engineered wetland systems (EWSs). The overall goal was to explore options for optimizing the choice of EWS substrates and vegetation.

Treatment of mixtures of cVOCs in a blended substrate (BionSoil/peat/sand) was found to be feasible. Low and high concentration mixtures of 1,1,2,2-TeCA and TCE degraded completely to ethene and ethane at reasonably high rates. Temporary disruptions in supply of the cVOCs did not have a significant unfavorable impact.

Mesocosm scale EWS units vegetated with *Typha latifolia* and *Phragmites communis* degraded 1,1,2,2-TeCA and TCE and their daughter products completely to ethene and ethane at comparable rates under non-extreme operating conditions, suggesting that the two wetland plant species were equally efficient and effective as EWS plants. However, under extreme conditions, *Typha latifolia* proved to be a better choice than *Phragmites communis*. While *Phragmites communis* weakened and eventually died, *Typha latifolia* continued to flourish even in the face of long term exposure to high concentration cVOCs and other stress inducing influences.

Wetland plant rootmatter was found to influence the treatment of cVOCs and the influence differed among the wetland plant species used. The influence of rootmatter was attributed to species-specific exudates released by roots. The interspecies differences in influence were attributed to ecological traits which differ among plants. Aboveground biomass (ABG) was also found to influence the treatment of cVOCs in a manner similar
to that of rootmatter. However, the influence of ABG was less pronounced and interspecies differences in performance were less resolved.

Salinity reduced the efficiency and effectiveness of treatment of cVOCs in EWSs. The negative influence of salinity on treatment of cVOCs was attributed to toxicity to both plants and microorganisms, and sidereactions such as sulfate reduction which compete with cVOCs for electron donors.

A compost-based, blended substrate consisting of row crop compost (RCC)/soil builder compost (SBC)/sand was significantly better than the BionSoil/peat/sand substrate. The compost-based substrate performed better than the BionSoil/peat one in microcosm and mesocosm experiments. Therefore, the compost-based substrate is a suitable substitute substrate for EWSs intended to treat cVOCs.

Pollutant initial concentrations and hydraulic and pollutant loading rates affected the performance of EWSs. 1,1,2,2-TeCA removal rates in the EWSs decreased with increase in cVOC initial concentration, hydraulic loading rates, and pollutant loading rates regardless of the substrate used (two substrates were tested).

On the whole, of the two wetland substrates tested, the mixture of compost (RCC/SBC/sand) (37.5:37.5:25) is recommended for use in EWSs that are intended to treat cVOCs.

Of the two wetland plant species tested, *Typha latifolia* is recommended because of its fairly good performance in treatment performance tests, and its ability to withstand long term exposure to cVOCs and other adverse conditions. *Typha latifolia* and *Phragmites communis* are the only species that were evaluated on the basis of both mesocosm and and microcosms studies. Therefore, their assessment was more comprehensive, and hence more conclusive. Other wetland plants were tested on the basis
of rootmatter and aboveground biomass only. For these, additional mesocosm scale studies are recommended. Since rootmatter, as a basis for comparing different wetland plant species, gave a better resolution of performance differences among different species, it is a better basis for prioritizing the wetland plant species to be evaluated in the recommended mesocosm studies.

There is clearly a need for an additional insight into the way different wetland plant species influence the performance of EWSs through their influence on microbial communities that mediate cVOC treatment processes. To bridge this knowledge gap, studies that can help to discern differences in profiles of microorganisms that colonize EWSs vegetated with different species of wetland plants are recommended.

It should also be useful to know in detail the microbial community structure changes that are brought about by changes in salinity. The knowledge on microbial community structure changes due to salinity effects is needed to complement the findings from this dissertation with respect to the influence of salinity on the performance of EWSs treating cVOCs.


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VITA

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