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Renovation of Ammonia Contaminated Produced Water Using Constructed Wetlands

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RENOVATION OF AMMONIA CONTAMINATED PRODUCED WATER USING CONSTRUCTED WETLANDS

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment of the
Requirements for the Degree
Doctor of Philosophy
Environmental Engineering and Science

by
Alex Beebe
August, 2013

Accepted by:
Dr. James Castle, Committee Chair
Dr. Fred Molz
Dr. John Rodgers, Jr.
Dr. Eric Snider
Abstract

Pilot-scale wetland treatment systems were designed and constructed to evaluate renovation of simulated oilfield produced water contaminated with ammonia (20 mg/L ammonia-N). A process-based pilot-scale constructed wetland was designed to meet specific biogeochemical conditions for conversion of ammonia to nitrogen gas through microbial nitrification and denitrification. The process-based constructed wetland treated the simulated produced water to meet stringent discharge requirements (less than 1.2 mg/L ammonia-N). Clinoptilolite, a zeolite mineral, was evaluated for use in constructed wetlands to increase ammonia sorption and nitrification activity. Clinoptilolite increased wetland ammonia sorption capacity and served as a microbial carrier for nitrifying bacteria when ranges of conditions (e.g. hydrosoil redox and equilibrium ammonia concentration) were met. Vertical tracer tests performed on bench-scale constructed wetlands demonstrated that plant transpiration enhances transport of water and dissolved constituents though the hydrosoil, where biogeochemical conditions for treatment reactions including denitrification occur. Evapotranspiration measured using a small, 2 m² lysimeter was compared with evapotranspiration previously reported for large-stand wetlands (greater than 1 hectare) to compare differences in evapotranspiration water loss expected between pilot-scale and full-scale constructed wetlands. Although water loss by evapotranspiration from the pilot-scale wetland ($K_c = 2.54$) was greater than reported from large-stand wetlands ($K_c = 1.0$), performance differences predicted using a one-dimensional analytical model were negligible for treatable constituents ($k = 1.2$ d$^{-1}$). This
research demonstrates that constructed wetlands offer a solution to treating ammonia in produced water to meet surface discharge criteria and beneficial use guidelines.
Dedication

This work would not be possible without the loving support and encouragement I have received from so many people. Thank you to all of my friends who have stood by me through my journey including David, Brent, Jarrod, and the rest of the Clemson Foothills Church. I am so grateful for all of my family members who have encouraged me along the way including Alicia, Rich, Kevin, Jill, Allison, and many many others. I am especially thankful for the advice and financial support given to me by my parents, Don and Julie. I also know that my dissertation is a product of the loving support I have received from my wife Rachel who has never once doubted that I could complete this. Her patience and devotion to my studies is truly a testament of her unyielding love. Thank you everyone, and I love all of you!
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CHAPTER 1: INTRODUCTION
1.1 Background and Approach

During production of oil and natural gas from subsurface formations, large quantities of water are brought to the surface as a byproduct. In 2007 alone, a combined 21 billion barrels of water (1 barrel = 42 U.S. gallons) were generated from the nearly 1 million active wells in the United States, representing one of the nation’s largest waste-streams (Allen and Rosselot, 1994; Clark and Veil, 2009). On average, 7 barrels of water are generated for each barrel of oil produced from active wells (Lee et al., 2002). Due to prolonged contact with host rock formations and hydrocarbons, this produced water may contain chemical and physical constituents of concern that hinder its ability to meet stringent discharge or beneficial use criteria. Therefore, cost effective management strategies are of paramount importance to oil companies (Fillo et al., 1992; Ray and Engelhardt, 1992; Jackson and Myers, 2002; USGS, 2002; Veil et al., 2004; Johnson et al., 2008; Clark and Veil, 2009; Alley et al., 2011).

Current and proposed technologies for treating produced water include oil-water separation, membrane filtration, ion exchange, deionization, distillation, evaporation, and constructed wetlands (Veil et al., 2004; Xu et al., 2008; Ahmadun et al., 2009; Davis et al., 2009). Constructed wetland treatment systems (CWTSs) offer the ability to treat produced waters (Kadlec and Srinivasan, 1995; Ji et al., 2007; Johnson et al., 2008; Rodgers and Castle, 2008; Horner et al., 2011) and operate at low costs provided that adequate land area is available (as low as 0.001$/bbl; Jackson and Myers, 2002). In addition, CWTSs are resistant to changes in system conditions and can treat a variety of constituents of concern simultaneously (Kadlec and Srinivasan, 1995; Lim et al., 2001;
Rodgers and Castle, 2008). The ability of CWTSs to remove ammonia in wastewaters has been demonstrated in previous studies with mixed results (Gersberg et al., 1983; Gersberg et al., 1984; Crites et al., 1997; Platzer, 1999; Huddleston et al., 2000; USEPA, 2000; Riley et al., 2005; Crites et al., 2006); however, no study has been performed to develop and evaluate ammonia treatment performance of a constructed wetland specifically designed to promote the biogeochemical conditions that control nitrification and denitrification (e.g. pH, alkalinity, dissolved oxygen, organic carbon, etc.). By designing constructed wetlands to specifically target the biogeochemical conditions that control nitrification and denitrification, more consistent and effective ammonia treatment is expected.

Research represented by this dissertation included several important aspects related to the ability of CWTSs to renovate produced water contaminated with ammonia. Major objectives of this research were: (1) design and evaluate a pilot-scale, process-based CWTS, (2) evaluate clinoptilolite for use in CWTSs, and (3) investigate the effects of evapotranspiration on CWTS treatment. These objectives were achieved through the use of pilot- and bench-scale CWTSs, laboratory experiments, and computer simulations.

The second chapter of this dissertation focuses on the design of a pilot-scale, process-based CWTS constructed to promote the biogeochemical conditions necessary for microbial transformation of ammonia to nitrogen gas. Ranges of biogeochemical conditions under which microbial nitrification and denitrification have been observed in previous studies of natural and artificial systems were identified as targeted ranges for the CWTS design. Amendments including aeration, sucrose, and crushed oyster shells were
added to the CWTS to promote the targeted ranges, which were monitored during the study. Ammonia treatment performance of the CWTS was evaluated on the basis of removal extents, efficiencies, and first-order rate coefficients.

The third chapter of this dissertation focuses on the ability of clinoptilolite, a naturally occurring zeolite mineral, to enhance ammonia sorption and nitrification activity in CWTSs. An ammonia Freundlich sorption isotherm was determined for clinoptilolite using data collected from a serial batch sorption experiment. The isotherm was used to determine masses of clinoptilolite loaded into two pilot-scale CWTSs for increased ammonia treatment through enhanced sorption capacity. Samples of the clinoptilolite were retrieved from the CWTSs after 50 days and tested for the presence of nitrifying bacteria to determine if the clinoptilolite served as a microbial carrier.

The fourth chapter of this dissertation focuses on effects of evapotranspiration on treatment performance in CWTSs. The process-based CWTS used in the second chapter of this dissertation was converted into a lysimeter for measuring evapotranspiration and determining the crop coefficient for pilot-scale wetlands. The pilot-scale crop coefficient was compared with crop coefficients determined previously for large-stand wetlands (greater than 1 hectare) to predict differences in evapotranspiration between pilot-scale and full-scale CWTSs. Performance differences attributed to water loss caused by evapotranspiration were predicted using a first-order, one-dimensional tank-in-series model derived from the wetland water balance and law of mass conservation. The ability of plant transpiration to vertically transport constituents through the hydrosoil was investigated using vertical tracer tests.
1.2 Disseration Organization

This dissertation consists of five chapters including the Introduction (Chapter 1) and Conclusions (Chapter 5). The body of this dissertation consists of three chapters formatted as stand-alone manuscripts for submission to scientific journals for peer review and publication. The manuscripts and their targeted journals are:


Chapter 3: Treatment of Ammonia in Pilot-scale Constructed Wetland Systems with Clinoptilolite, submitted to Journal of Environmental Chemical Engineering

Chapter 4: Effects of Evapotranspiration on Treatment Performance in Constructed Wetlands: Experimental Studies and Modeling, prepared for submission to Wetlands

Collectively, these manuscripts provide information on treatment techniques for renovating waters contaminated with ammonia through the use of CWTSs, particularly for scaling from pilot- to full-size systems.
1.3 References


CHAPTER 2: BIOGEOCHEMICAL PROCESS-BASED DESIGN FOR TREATING AMMONIA USING CONSTRUCTED WETLAND SYSTEMS

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2.1 Abstract

Constructed wetlands have been used to treat ammonia with varying degrees of success. This research aims to improve the design of ammonia-treating constructed wetlands by targeting key biogeochemical conditions needed for microbial nitrification and denitrification of ammonia. A pilot-scale constructed wetland was designed to meet targeted ranges of dissolved oxygen concentration, hydrosoil redox potential, pH, alkalinity, and organic carbon availability needed for nitrification and denitrification. Design features included mechanical aeration, sucrose addition, and crushed oyster shell addition. Ammonia-N concentrations in the constructed wetland decreased from approximately 20 mg/L to non-detectable levels (<0.1 mg/L) during three of four treatment months. Measured biogeochemical conditions within the constructed wetland indicate that nitrification and denitrification occurred outside some of the targeted ranges for conditions. Results from this study demonstrate the advantages of designing a constructed wetland to target biogeochemical conditions that promote nitrogen removal pathways for improved ammonia treatment. In addition, the results of this study suggest that constructed wetlands designed for ammonia treatment are resilient to wider ranges of biogeochemical conditions than previously studied natural or engineered systems.
2.2 Introduction

During production of oil and natural gas from subsurface formations, large quantities of water are brought to the surface as a byproduct. In 2007 alone, a combined 21 billion barrels of water (1 barrel = 42 U.S. gallons) were generated from the nearly 1 million active wells in the United States, representing one of the nation’s largest waste-streams (Allen and Rosselot, 1994; Clark and Veil, 2009). Current and proposed technologies for treating produced water include oil-water separation, membrane filtration, ion exchange, deionization, distillation, evaporation, and constructed wetlands (Veil et al., 2004; Xu et al., 2008; Ahmadun et al., 2009; Davis et al., 2009). In produced waters containing multiple dissolved constituents that require treatment (e.g. dissolved solids, metals, inorganic and organic compounds), a sequential treatment consisting of several treatment steps, each targeting removal of different constituents, can be used. In a previous study by Ganesh et al. (2006), produced water from an oilfield in San Ardo, CA containing constituents of concern including dissolved solids (7,000 mg/l TDS), temperature (190 °F), boron (25 mg/l), ammonia (20 mg/l ammonia as N), and organics (75 mg/l TOC) was targeted for treatment using a sequential pilot-scale treatment system containing a water softening unit, cooling tower, and reverse osmosis. The system was effective in abating scaling compounds, total dissolved solids, temperature, boron, and organics to regulatory levels required for beneficial use as aquifer recharge water; however, outflow concentrations of ammonia remained in excess of the required concentration (>5 mg/L as N; Ganesh et al., 2006). Proposed treatment methods for ammonia removal from produced water include biological oxidation with aerated lagoons
or biodisks, ion exchange using zeolites, and electrodialysis (Beyer et al., 1979; Palmer et al., 1981; de Lima et al., 2009); however, operational costs of these methods can limit their practicality for treating large volumes of produced water (Jackson and Myers, 2002).

Constructed wetland treatment systems (CWTSs) offer the ability to treat produced waters (Kadlec and Srinivasan, 1995; Ji et al., 2007; Johnson et al., 2008; Rodgers and Castle, 2008; Horner et al., 2011) and operate at low costs provided that adequate land area is available (as low as 0.001$/bbl; Jackson and Myers, 2002). In addition, CWTSs are resistant to changes in system conditions and can treat a variety of constituents of concern simultaneously (Kadlec and Srinivasan, 1995; Lim et al., 2001; Rodgers and Castle, 2008). The ability of CWTSs to remove ammonia in wastewaters has been demonstrated in previous studies with mixed results (Gersberg et al., 1983; Gersberg et al., 1984; Crites et al., 1997; Platzer, 1999; Huddleston et al., 2000; USEPA, 2000; Riley et al., 2005; Crites et al., 2006); however, no study has been performed to develop and evaluate ammonia treatment performance of a constructed wetland specifically designed to promote the biogeochemical conditions that control nitrification and denitrification (e.g. pH, alkalinity, dissolved oxygen, organic carbon, etc.). By designing constructed wetlands to specifically target the biogeochemical conditions that control nitrification and denitrification, more consistent and effective ammonia treatment is expected.

The objectives of this research were to (1) identify targeted ranges of biogeochemical conditions for microbial nitrification and denitrification of ammonia
from previous studies of nitrogen transformations in natural and artificial systems, (2)
design and construct a process-based pilot-scale CWTS to promote targeted ranges of
biogeochemical conditions for microbial nitrification and denitrification, and (3) measure
and compare biogeochemical conditions and ammonia removal performance between the
process-based pilot-scale CWTS and a generic pilot-scale CWTS based on conventional
CWTS design features used in previous studies. Achieving these objectives provides a
process-based CWTS design that may offer a more cost-effective and robust option for
managing produced waters containing ammonia. The impetus for this investigation was
to determine design criteria for improving ammonia removal from oil field produced
waters using CWTSs. However, the results of this study have application to treating other
waters contaminated with ammonia.

2.3 Materials and Methods

2.3.1 Targeted Conditions for Ammonia Treatment

Studies focused on the fate and transport of ammonia and ammonium
(collectively termed ammonia in this study) in aqueous environments were reviewed to
identify potential removal pathways including volatilization, sorption, microbial
assimilation, plant uptake, and microbial transformation. Volatilization may contribute to
wet and dry deposition of ammonia into surrounding watersheds (Asman, 1994; Poach et
al., 2002); and sorption, microbial assimilation, and plant uptake can allow ammonia to
be subsequently cycled back into CWTSs (Wittgren and Mæhlum, 1997; Kadlec, 2005;
Kadlec and Wallace, 2009). Microbial transformation of ammonia to nitrogen gas
through nitrification and denitrification was selected as the targeted removal process.
Ranges of biogeochemical conditions under which microbial nitrification and
denitrification have been observed in previous studies of natural and artificial systems
were identified as targeted biogeochemical conditions for the process-based CWTS
design.

2.3.2 Pilot-scale CWTS Construction

Two free-water surface pilot-scale CWTSs were designed and constructed. One
system (process system) was designed based on identification of targeted ranges of
dissolved oxygen concentration, hydrosoil redox potential, pH, alkalinity, and organic
carbon availability. The second system (generic system) was constructed to match the
design of CWTSs used to treat ammonia in other studies (Gersberg et al., 1986;
Huddleston et al., 2000; Riley et al., 2005) and did not contain specific design features
other than hydrosoil and plant selection to promote targeted biogeochemical conditions
for microbial transformation of ammonia. Each of the two systems consisted of four
wetland cells (Figure 2.1). The systems are described in Section 3.2.

2.3.3 CWTS Performance

Using the methods listed in Table 2.1, biogeochemical conditions (e.g. pH, redox,
dissolved oxygen, and alkalinity) were monitored throughout the experiment (March
through June of 2010) to determine if the two systems were capable of promoting the
targeted conditions for microbial transformation of ammonia to nitrogen gas.

Water samples were collected from the retention basin (inflow) and the outflow of
each of the wetland cells during four sampling periods between March and June, 2010.
The samples were stored in 50mL Nalgene centrifuge tubes and immediately transported
to the laboratory for analysis of ammonia and nitrate. System removal rate coefficients \( (d^{-1}) \) for ammonia were calculated assuming first-order kinetics (Kadlec and Wallace, 2009) using the integrated form of the first-order rate coefficient law:

\[
\text{Removal rate coefficient } (k_1) = -\frac{\ln(C_0/C_t)}{t} \quad \text{Eqn. 1}
\]

Where \( C_0 \) is initial inflow ammonia concentration (mg/L ammonia-N); \( C_t \) is system outflow ammonia concentration (mg/L ammonia-N) at time \( t \), and \( t \) is the time (days) corresponding to the system HRT. System removal efficiencies were calculated for ammonia using the initial inflow concentration and final outflow concentration:

\[
\text{Removal efficiency } (\%) = \frac{C_0 - C}{C_0} \times 100 \quad \text{Eqn. 2}
\]

Where \( C \) is system outflow ammonia concentration (mg/L ammonia-N), also defined as the removal extent. Removal rate coefficients, efficiencies, and extents were determined for each of the four sampling events. Performance results from the two systems were compared.

2.4 Results

2.4.1 CWTS Conditions for Ammonia Treatment

Key biogeochemical controls for microbial nitrification and denitrification identified through a literature review include dissolved oxygen concentration, redox potential, pH, alkalinity, temperature, and organic carbon availability (Andersen, 1977; Gambrell and Patrick, 1978; Knowles, 1982; Gujer and Boller, 1984; Szwerinski et al., 1986; USEPA, 1993; Kirmeyer et al. 1995; Odell et al., 1996; Holt et al. 2000, Van Haandel and Van der Lubbe, 2007; Gerardi, 2010). Growth of nitrifying bacteria has been observed in previous studies under oxidizing conditions with dissolved oxygen.
concentrations greater than 2.0 mg/L and redox greater than 100 mV (Odell et al. 1996, Gerardi, 2010), pH between 6.6 and 8.7 (USEPA 1993, Odell et al. 1996), alkalinity greater than 50 mg/L as CaCO₃ (Gujer and Boller, 1984; Szwerinski et al. 1986), and temperature between 8 and 30 °C (Kirmeyer et al. 1995, Holt et al. 2000). Growth of denitrifying bacteria has been observed under reducing conditions with dissolved oxygen concentration less than 0.2 mg/L and redox less than 50 mV (Knowles, 1982; Van Haandel and Van der Lubbe, 2007; Gerardi, 2010), pH between 7 and 8 (Knowles 1982; Van Haandel and Van der Lubbe, 2007), alkalinity greater than 35 mg/L as CaCO₃ (Van Haandel and Van der Lubbe 2007), temperature between 5 and 40 °C (Andersen, 1977; Van Haandel and Van der Lubbe, 2007), and a continuous carbon supply (Odell et al. 1996; Van Haandel and Van der Lubbe 2007). Previously observed biogeochemical conditions for microbial nitrification and denitrification were identified as targeted biogeochemical conditions (Table 2.2) and provided the basis for design of the process system.

2.4.2 Pilot-scale CWTS Construction

Eight 265-L Rubbermaid® containers (cells) located outdoors in Clemson, South Carolina, were filled to a depth of approximately 45 cm with sandy, fluvial sediment collected from nearby Eighteen Mile Creek and divided into two groups or systems (process system and generic system) of four cells each (Figure 2.1). The cells from each system were connected using poly-vinyl chloride (PVC) pipes with adjustable overflow levels to control water depth and arranged to allow gravity flow from cell to cell. Each cell was planted with approximately 20 broadleaf cattails (*Typha latifolia*) collected from
Cattails were selected because they have been used to promote ammonia and nitrogen treatment in constructed wetlands in previous studies (Gersberg et al., 1986; Huddleston et al., 2000; Riley et al., 2005). The first cell in each of the two systems was connected by Fluid Metering, Inc. ® piston pumps to a 5,678-L polypropylene carboy retention basin containing ammonia-contaminated water. The pumps delivered 45 mL/minute to the process system for a nominal hydraulic retention time (HRT) of 2 days per cell and 90 mL/minute to the generic system for an HRT of 1 day per cell. The extended HRT for the process system was used to determine the maximum extent of ammonia removal.

The process system was designed to promote biogeochemical conditions for microbial transformation of ammonia to nitrogen gas in a three step process: (1) nitrification of ammonia to nitrite, (2) nitrification of nitrite to nitrate, (3) and reduction of nitrate to nitrogen gas (denitrification). Because nitrification and denitrification require different geochemical conditions (Gambrell and Patrick, 1978; Odell et al., 1996; Stumm and Morgan, 1996; Gerardi, 2010), the design featured amendments arranged to promote oxidizing conditions in the first cell and reducing conditions in the last three cells, thus allowing nitrification and denitrification to operate sequentially through the system.

Specific amendments to the process system included aeration to enhance dissolved oxygen concentration for nitrification, sucrose to serve as an electron donor and promote reducing conditions for denitrification, and crushed oyster shells (CaCO₃) to stabilize pH and raise alkalinity. Aeration was supplied to the first cell of the process system by a submerged, slotted PVC pipe connected to a 1/3 horse-power air pump in order to
increase dissolved oxygen to targeted concentrations. Organic carbon was supplied to the second cell of the process system using an FMI® pump delivering 0.9 mL/minute of a 20 mg/mL solution of sucrose (20 mg sucrose per mg ammonia-N loaded). Crushed oyster shells were added to the process system at a rate of 50 g per cell every two weeks.

The two systems acclimated while receiving a mixture of municipal water (i.e. tap water) and ammonium chloride salt formulated to simulate produced water from the San Ardo oil field, California, USA (20 mg/L ammonia-N, Ganesh et al., 2006). To address nutrient requirements of the macrophytes and microbes, nitrogen-free fertilizer (Osmocote®) was added to the hydrosoil during acclimation.

2.4.3 CWTS Performance

Explanatory parameters measured in both systems from March-June 2010 (Table 2.3) indicate that some but not all of the targeted biogeochemical conditions were met. Both systems operated within the targeted temperature ranges for nitrification (8 – 30 ºC) and denitrification (5–40 ºC).

For the process system, dissolved oxygen concentration in the aerated cell met the targeted concentration for nitrification (> 2.0 mg/L) during all four sampling periods, but the targeted concentration for denitrification (< 0.2 mg/L) was not met by any cells during any sampling periods. The lowest dissolved oxygen concentration (0.69 mg/L) was measured in the sucrose amended cell during the month of May. Hydrosoil redox potential did not meet the target for nitrification (> 100 mV) in any cells, but did meet the target for denitrification (< 50 mV) in all cells during all sampling periods. pH was outside of the targeted range for nitrification (6.6-8.7) in all cells except for the third cell
in May and did not meet the targeted range for denitrification (7.0 – 8.0). Alkalinity met the targeted concentrations for nitrification (> 50 mg/L as CaCO₃) and denitrification (> 35 mg/L as CaCO₃) in the last three cells, and hardness increased from inflow to outflow as a result of calcium release from the crushed oyster shells.

For the generic system, all cells met the targeted concentration of dissolved oxygen for nitrification (> 2.0 mg/L) during all four sampling periods, but did not meet the targeted concentration for denitrification (< 0.2 mg/L). Hydrosoil redox did not meet the target for nitrification (> 100 mV) in any cells, but met the target for denitrification (< 50 mV) in all cells except the third cell in March and the fourth cell in June. pH was outside of the targeted range for nitrification (6.6-8.7) and denitrification (7.0 – 8.0) in all cells. Alkalinity did not meet the targeted concentrations for nitrification (> 50 mg/L as CaCO₃) or denitrification (> 35 mg/L as CaCO₃).

The treatment goal of < 5 mg/L ammonia-N was met by the process system after 4 days HRT for all months (April-June; Figure 2.2) of performance measurement except for the first month (March). The ammonia treatment goal was not met by the generic system during any sampling periods. Comparison of ammonia removal between two systems at 4 days HRT indicates that the process system consistently outperformed the generic system in terms of removal extents and efficiencies during all sampling periods. 4-day removal extents from April through June were 1.4 to 10.3 mg/L ammonia-N for the process system and 12.6 to 15.0 mg/L ammonia-N for the generic system (Table 2.3). 4-day removal efficiencies were 49.3 to 93.7 % for the process system and 29.6 to 48.6 % for the generic system. First order removal rate coefficients ranged from 0.126 to 1.39 d⁻¹.
for the process system and 0.071 to 0.111 d^{-1} for the generic system (Table 2.4). Outflow nitrate-N concentrations for both systems were below USEPA nitrate-N maximum contaminant level (MCL) of 10 mg/L (USEPA, 1991) during all sampling periods (Table 2.3; Figure 2.3).

2.5 Discussion

Although the process system did not meet all targeted biogeochemical conditions favorable for nitrification and denitrification, the ammonia treatment goal of 5 mg/L ammonia-N was achieved for three of the four sampling periods after 4 days HRT (Figure 2.2), and nitrate concentrations remained less than the USEPA MCL of 10 mg/L nitrate-N (Figure 2.3). The occurrence of nitrification and denitrification inferred from ammonia and nitrate removal data under conditions outside of the targeted biogeochemical ranges suggests that nitrogen removal pathways in the process system are resilient to a wider range of conditions than reported in previous studies. Although microbial nitrification and denitrification were the targeted pathways for ammonia treatment in the process system, other alternative pathways can occur including volatilization, sorption, and plant uptake. However, the microbial pathways are reported to account for up to 90% of ammonia removal in CWTSs (Demin et al., 2001), and formation of nitrate observed in the first two cells of the process and generic systems is not consistent with alternative pathways (Figure 2.3).

A possible explanation for resilience of nitrification and denitrification in the process system to a wider range of biogeochemical conditions is the existence of heterogeneous macro- and micro-environments within individual wetland cells. Previous
studies have demonstrated that both nitrification and denitrification can occur in
environments with bulk biogeochemical conditions outside of the targeted conditions due
to the formation of micro-environments (Killham 1987, Odell et al. 1996). For instance,
denitrification can occur in water treatment systems with bulk aerobic conditions
(dissolved oxygen greater than 2.0 mg/L) within the core of floc bodies where the micro-
environment can promote reducing conditions with redox values less than -200 mV
(Killham, 1987).

The dissolved oxygen concentration measured in surface water of the aerated cell
of the process system (4.57–6.43 mg/L) met the targeted concentration for nitrification (> 2.0 mg/L), but not the targeted concentration for denitrification (< 0.5 mg/L), while the
hydrosoil redox (-254 to -301 mV) met the targeted value for denitrification (< 50 mV),
but not the targeted value for nitrification (> 100 mV). In this case, aerobic conditions in
the water column supported nitrification, while anaerobic conditions in the hydrosoil
supported denitrification, allowing both reactions to occur simultaneously within the
same wetland cell. Simultaneous nitrification in an aerobic zone within the water column
and denitrification in the hydrosoil has been observed previously in natural wetlands
containing *Oryza sativa* (Asian rice), *Pontederia cordata* (Pickerelweed), and *Juncus effuses* (Common rush; Reddy et al, 1989), but not in wetlands containing *T. latifolia*.

In the process system, ammonia removal data indicate that nitrification and
denitrification operated at pH values (5.20-6.61) below the targeted ranges (< 6.6 and
<7.0; respectively). The occurrence of nitrification and denitrification under acidic
conditions in the process system may be the result of attachment of nitrifying and
denitrifying bacteria to submerged shoots, roots, hydrosoil, and other exposed surfaces in the wetland cells. Attached nitrifying bacteria can tolerate extreme pH conditions by forming a protective slime layer (Odell 1996). An experiment by Kilham (1987) demonstrated that nitrifying bacteria attached to glass beads survived in acidic conditions and unattached nitrifying bacteria did not survive. Prosser (1989) demonstrated nitrification in soils having pH below 4.0 and suggested that continued nitrification in acidic conditions can be attributed to bacterial growth and attachment to the surface of soil particles. Growth of nitrifying bacteria on exposed surfaces of submerged shoots, roots, and hydrosoil would likely allow survival in acidic conditions outside of the targeted range.

Freezing temperatures one week prior to the March sampling event likely influenced ammonia treatment performance. Although water temperatures were within the suggested ranges for nitrification and denitrification at the time of March sampling (11–13 °C; Table 2.3), nitrification rates are subject to the Arrhenius equation, which states that reaction rates increase by a factor of two with each temperature increase of 10 °C (Wong-Chong and Loehr, 1975). As temperatures increased from March to June, removal rate coefficients subsequently increased from 0.126 to 1.39 d⁻¹ (Tables 2.3, 2.4).

The ammonia treatment goal of 5 mg/L ammonia-N was not met by the generic system during any of the sampling periods, and biogeochemical conditions favorable for ammonia treatment through nitrification were not present. Although ranges of dissolved oxygen concentration (1.50–5.64 mg/L) and pH (4.73–6.08) in the generic system were similar to ranges of dissolved oxygen concentration (0.69 – 6.43 mg/L) and pH (5.2–
6.61) in the process system, alkalinity was less in the generic system (6–16 mg/L CaCO₃) than in the process system (8–150 mg/L CaCO₃). Because alkalinity is required for nitrification (7.07 mg CaCO₃ for each mg of ammonia-N oxidized; Ford 1980) and was less than the targeted concentration (<50 mg/L CaCO₃) in all generic system cells during all sampling periods, nitrification was likely limited by the availability of alkalinity. Crushed oyster shells added to the process system provided a continuous source of alkalinity for nitrification. No oyster shells were added to the generic system, and alkalinity was low in the simulated produced water (< 22 mg/L CaCO₃).

Design features of the process system (e.g. addition of aeration, organic carbon, and alkalinity) can be modified for incorporation in full-scale constructed wetlands designed to remove ammonia. Mechanical aeration used for the process system can be replaced with a rock cascade to decrease electricity consumption and operational costs. Organic carbon can be provided through harvesting and application of cattail foliage (Gersberg et al, 1984). Alkalinity can be provided through the selection of hydrosoil material containing carbonate minerals.

2.6 Conclusion

Although not all targeted conditions were met, the process system was able to treat simulated produce water containing 20 mg/L ammonia-N to non-detectable levels (< 0.1 mg/L ammonia-N) during three of the four sampling months. The sequential design of the process system (e.g. aeration followed by organic carbon addition) allowed nitrification to precede denitrification. In contrast, the generic system did not meet the
targeted treatment goal, and ammonia removal was likely limited by the availability of alkalinity. The occurrence of nitrification and denitrification in the process system under biogeochemical conditions outside of the targeted ranges is attributed to the coexistence of an oxidizing zone in the water column and a reducing zone in the hydrosoil, and growth and attachment of bacteria to exposed, submerged surfaces. The difference in treatment performance between the process system and the generic system demonstrates the advantage of designing constructed wetlands to promote biogeochemical conditions favorable for nitrification and denitrification when targeting ammonia for treatment. This work also suggests that nitrification and denitrification operate under a wider range of conditions in constructed wetlands than in previously studied natural and engineered systems.
2.7 Acknowledgement

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2.8 References


Figure 2.1. Schematic diagram of the two pilot-scale CWTSs. Each system consisted of four cells, which were arranged for gravity flow from inflow to outflow. The process system was designed to operate sequentially with nitrification followed by denitrification and included aeration in the first cell and sucrose addition in the second cell. Crushed oyster shells were added to all cells of the process system.

Figure 2.2. Ammonia-N concentrations measured in samples collected from the inflow of each system and the outflow of each cell as a function of hydraulic retention time (A: March; B: April; C: May; D: June). The process system outperformed the generic system in terms of removal extents, efficiencies, and removal rate coefficients throughout the duration of the experiment. The treatment goal of 5 mg/L ammonia-N was met by the process system after 3 days HRT from April through June, but not by the generic system.

Figure 2.3. Nitrate-N concentrations measured in samples collected from the inflow of each system and the outflow of each cell as a function of hydraulic retention time. The increase in nitrate-N concentration during the first days of treatment was consistent with microbial nitrification; however, concentrations remained below the USEPA MCL of 10 mg/L nitrate-N during the experiment. The decrease in nitrate-N concentration in the final cells of each system was consistent with microbial denitrification. Excluded from the graph are sampling periods for each system in which nitrate-N was not detected in all samples.
Figure 2.1.
Figure 2.2.
Figure 2.3.

mg/L Nitrate-N vs. HRT (Days)

- Generic (May)
- × Generic (June)
- ■ Process (April)
- ● Process (May)
- ○ Process (June)
Table 2.1. Analytical methods for determining explanatory and performance parameters in the pilot-scale CWTSs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Direct Instrumentation: Orion Model 420A</td>
<td>0.01 S.U.</td>
</tr>
<tr>
<td>Conductivity</td>
<td>Direct Instrumentation: YSI 30</td>
<td>0.1 μS/cm</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>Standard Method¹: 2320 B Titration</td>
<td>2 mg/L as CaCO₃</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>Direct Instrumentation: YSI Model 52</td>
<td>0.1 mg/L</td>
</tr>
<tr>
<td>Temperature</td>
<td>Direct Instrumentation: YSI Model 52</td>
<td>0.5°C</td>
</tr>
<tr>
<td>Soil Redox</td>
<td>Standard Method¹: 2580B GDT-11 Multi-meter</td>
<td>10 mV</td>
</tr>
<tr>
<td>Ammonia-N</td>
<td>Standard Method¹: 4500-NH₃D Ammonia ISE</td>
<td>0.1 mg/L</td>
</tr>
<tr>
<td>Nitrate-N</td>
<td>Standard Method¹: 4500-NO₃C Cadmium Reduction</td>
<td>1.0 mg/L</td>
</tr>
</tbody>
</table>

¹Standard Methods (APHA, 2005)
Table 2.2. Targeted biogeochemical conditions for microbial nitrification and denitrification.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Pathway</th>
<th>Dissolved Oxygen (mg/L)</th>
<th>Redox (mV)</th>
<th>pH (S.U.)</th>
<th>Alkalinity (mg as CaCO₃/L)</th>
<th>Temperature (°C)</th>
<th>Organic Carbon</th>
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<td>Ammonia</td>
<td>Nitrification</td>
<td>&gt; 2.0</td>
<td>&gt; 100</td>
<td>6.6 – 8.7</td>
<td>&gt; 50</td>
<td>8 – 30</td>
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</tr>
<tr>
<td>Nitrate</td>
<td>Denitrification</td>
<td>&lt; 0.2</td>
<td>&lt; 50</td>
<td>7.0 - 8.0</td>
<td>&gt; 35</td>
<td>5 – 40</td>
<td>Required</td>
</tr>
</tbody>
</table>

1Andersen (1977)
2Knowles (1982)
3Szwerinski et al. (1986)
4USEPA (1993)
5Gujer and Boller (1994)
6Holt et al. (2000)
7Kirmeyer et al. (1995)
8Odell et al. (1996)
9Van Haandel and Van der Lubbe (2007)
10Gerardi (2010)
Table 2.3. Ammonia and nitrate analysis and explanatory parameters for March-June sampling periods.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Performance Parameters</th>
<th>Explanatory Parameters</th>
</tr>
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<td></td>
<td>[Ammonia-N] (mg/L)</td>
<td>[Nitrate-N] (mg/L)</td>
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<td></td>
<td></td>
</tr>
<tr>
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</tr>
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<td></td>
</tr>
<tr>
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<td>Gen 2</td>
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<tr>
<td>Gen 3</td>
<td>16.4</td>
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</tr>
<tr>
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<tr>
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<tr>
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</table>

*Gen = generic system cells (e.g. Gen 1 = first cell of generic system); Pro = process system cells
nd = not determined
Table 2.4. Ammonia removal extents, efficiencies, and removal rate coefficients for the generic and process systems.

<table>
<thead>
<tr>
<th>System</th>
<th>Concentration (mg/L ammonia-N)</th>
<th>Removal (%)</th>
<th>Rate Coefficient (d$^{-1}$)</th>
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<td><strong>March</strong></td>
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<td>Inflow</td>
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<tr>
<td>Outflow-Generic</td>
<td>14.3</td>
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<td>Outflow-Process</td>
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<td>&gt;99.6</td>
<td>1.39$^*$</td>
</tr>
</tbody>
</table>

*Concentrations in cells 3 and 4 were identical; therefore removal rate coefficients calculated for 6 days of treatment.
CHAPTER 3: TREATMENT OF AMMONIA IN PILOT-SCALE CONSTRUCTED WETLAND SYSTEMS WITH CLINOPTILOLITE

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3.1 Abstract

Clinoptilolite was investigated as a sorptive medium for use in constructed wetland treatment systems (CWTSs) based on its affinity for ammonia and high surface area for attachment of periphytic biofilms. Results from a batch sorption experiment indicate that the clinoptilolite studied has an affinity for ammonia described by the Freundlich equation \( Q = 0.72C_e^{0.57} \) for equilibrium ammonia-N concentrations from 0.07 to 30.1 mg/L. During a 10-day sampling period, a 0.5-m\(^2\) pilot-scale CWTS planted with *Schoenoplectus californicus* and containing 1,000 g clinoptilolite removed significantly more (\( p = 8.8 \times 10^{-3} \)) ammonia-N (mean outflow 4.5 mg/L, \( \sigma = 4.1 \)) than a control system containing no clinoptilolite (mean outflow 8.6 mg/L, \( \sigma = 2.7 \)). Nitrification was detected in samples of clinoptilolite from the treated CWTS using nitrifying bacteria activity reaction tests (n-BARTs). Ammonia removal was not affected by addition of clinoptilolite to a 0.5-m\(^2\) pilot-scale CWTS planted with *Typha latifolia*, and nitrification activity was not detected in samples of clinoptilolite. These data illustrate that clinoptilolite can increase ammonia removal and nitrifying activity in CWTSs receiving ammonia concentrations equal to or greater than ~6-10 mg/L when conditions required to support nitrification including hydrosoil redox are provided.

Keywords: Constructed Wetland, Ammonia Treatment, Clinoptilolite, Bioregeneration
3.2 Introduction

The ability of constructed wetland treatment systems (CWTSs*) to remove ammonia from impaired waters has been demonstrated in previous studies [1-8]. Specific pathways for ammonia removal in CWTSs include nitrification, plant uptake, and volatilization [3, 6, 8]. Sorptive materials including zeolites and clays have been used to remove aqueous ammonia from water during remediation of ammonia-contaminated waters [9-24]. In the case of ammonia treatment using CWTSs, transfer from the aqueous phase onto a solid phase could enhance performance by concentrating ammonia in areas where nitrifying bacteria (Nitrosomonas spp. and Nitrobacter spp.) may be present, although this hypothesis has not been tested to date.

Clinoptilolite is a readily available, hydrated silicate mineral with the chemical formula (Na, K, Ca)_{2-3}Al_{3}(Al, Si)_{2}Si_{13}O_{36}-12H_{2}O in the group of minerals called zeolites, which have measurable cation exchange capacities [20]. Because of clinoptilolite’s affinity for ammonium ions, several previous studies have investigated its ability to be used as a sorbent of ammonia in contaminated waters [9, 11-18, 18-20, 22-24].

*Abbreviations: CWTS, constructed wetland treatment system; n-BART, nitrifying bacteria activity reaction test; BOD, biochemical oxygen demand; USEPA, United States Environmental Protection Agency; ISE, ion selective electrode; USDA-ARS, United States Department of Agriculture-Agricultural Research Service; HRT, hydraulic retention time; APHA, American Public Health Association; USDOE, United States Department of Energy
Conventional treatment involves placing clinoptilolite in contact with contaminated waters where cation exchange occurs until ammonium saturation is reached. The spent clinoptilolite can then be regenerated using a chemical process involving ammonium ion replacement with sodium ions by exposing the clinoptilolite to sodium chloride brine [13].

Because clinoptilolite is capable of acting as a renewable ammonia ion exchange medium and microbial carrier for nitrifying bacteria [15], addition of clinoptilolite to CWTSs may enhance treatment performance and attenuate fluctuations in inflow concentrations of ammonia. In addition, a cation exchange medium may decrease pH toxicity to nitrifying bacteria by removing excess protons generated during nitrification [10]. Natural zeolites contained within lava sands (chabazite and phillipsite) have been studied for use as filter medium in CWTSs loaded with ammonia, chemical oxygen demand, and phosphate [25]; however, no zeolite has been investigated as a dual purpose sorbent and microbial carrier for nitrifying bacteria in CWTSs. Furthermore, application of clinopitilolite onto hydrosoil of CWTSs to enhance ammonia treatment performance has not been investigated previously.

The objectives of this investigation were to (1) develop a Freundlich sorption isotherm with ammonia as the sorbate and clinoptilolite to determine sorbent loading masses for a pair of pilot-scale CWTSs (2) measure the immediate effects (sorption) of clinopitilolite addition to pilot-scale CWTSs on ammonia removal to determine if ammonia sorption by clinoptilolite enhances treatment performance, and (3) determine if clinoptilolite is capable of serving as a microbial carrier in constructed wetlands to
support nitrifying bacteria, thereby enhancing treatment capacity. Achieving these objectives provides a fundamental basis for understanding the potential use of clinoptilolite in CWTSs for enhancing ammonia removal by acting as both a sorbent and microbial carrier.

3.3 Materials and Methods

3.3.1 Clinoptilolite Sorption Isotherm

The sorptive capacity of clinoptilolite may vary depending on geologic source and pretreatment [21], so partitioning of ammonia from water at different ammonia concentrations to clinoptilolite was measured using a serial batch sorption experiment. Approximately 3 g of clinoptilolite obtained from River Bend Laboratories, a Division of the Chemtron Corporation based in St. Charles, MO, was weighed and added to each of nineteen 300-mL biochemical oxygen demand (BOD) bottles along with solutions containing a mixture of deionized water and concentrations of ammonia-N varying from 1 to 80 mg/L. The initial concentrations of ammonia-N, prepared from standard solutions, were selected to develop an isotherm over a range of equilibrium concentrations from 0.1 to 30 mg/L. Two control bottles were initiated using only clinoptilolite and no ammonia, and two more were initiated using ammonia solution and no clinoptilolite. All 21 prepared BOD bottles were sealed and placed in a dark area with constant room temperature (20° C) to allow sorption equilibration to occur. Replicate bottles containing 40 mg/L ammonia-N were analyzed for ammonia-N using an ion selective electrode (ISE) at days 3, 5, 7, and 11 to monitor sorption equilibration. The remaining 18 bottles were sampled at day 11 when there was no longer a significant change (as determined by
confidence interval, α=0.05) in ammonia-N concentrations between replicate bottles monitored during the equilibration period. The resulting equilibrium concentrations (C_e) were used to solve for the sorption mass fraction using the following mass balance equation:

\[ q = \frac{V_w(C_o - C_e)}{M_c} \]  

Eqn.1

where q is the sorption mass fraction of ammonia (mg ammonia-N/g clinoptilolite), \( V_w \) is the volume (liters) of ammonia solution exposed to the clinoptilolite (i.e. volume of ammonia solution added to each BOD bottle), \( C_o \) is the initial concentration (mg/L) of ammonia-N in the ammonia water solution, and \( M_c \) is the mass (grams) of clinoptilolite (i.e. mass of clinoptilolite added to each BOD bottle).

Results from Eqn. 1 were used to calculate a Freundlich sorption isotherm by fitting data points for q vs. C_e for each sample (excluding controls). Two methods were used to fit the exponential function. The first method involved linearizing the data by performing log transformations of both sorption values and equilibrium concentrations, and then fitting the log-log transformed data using a linear regression in Excel®. The second method involved generating a best fit using the Excel® spreadsheet [26] available from the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) that includes a solver function to optimize the fit generated using the linear regression. The best fit method produced an optimized Freundlich sorption isotherm, which was used to calculate loading masses of clinoptilolite to be used in pilot-scale CWTSs for a later experiment in this investigation.
3.3.2 Clinoptilolite Addition to Pilot-scale CWTSs

Four pilot-scale CWTSs each consisting of a single wetland cell were prepared for clinoptilolite testing by filling four 265-L Rubbermaid® troughs to a depth of approximately 30 cm with sandy sediment collected from Eighteen Mile Creek located near Clemson, South Carolina. Two systems were planted with approximately 20 broadleaf cattail plants (*Typha latifolia*) each and the remaining two systems were planted with approximately 20 giant bulrush plants (*Schoenoplectus californicus*) each. The two different species of plants were selected because both have been used previously to treat ammonia in CWTS studies [7, 27] and were readily available from an aquaculture pond near the study location. The four systems were placed inside a climate-controlled greenhouse and connected using Teflon® tubing to a 2,080-L detention basin containing ammonia solution (20 mg/L as N). Fluid Metering, Inc.® piston pumps delivered 60 mL/min ammonia solution (nominal 2-day hydraulic retention time) to the two systems containing bulrush. 30 mL/min ammonia solution (nominal 4-day hydraulic retention time) was delivered to the two systems containing cattails. The hydraulic retention times (HRTs) were chosen to maintain detectable outflow concentrations of ammonia-N within the range of the Freundlich sorption isotherm (between 0.1 and 30.1 mg/L ammonia-N) so that loading masses of clinoptilolite could be calculated accurately. The bulrush systems operated with a 4-day HRT for the first 3 months after planting, but the outflow concentration of ammonia-N in both systems was non-detectable. Therefore, HRT was decreased to 2 days so that differences in outflow concentrations of ammonia-N due to
clinoptilolite addition could be detected. A 4-day HRT was maintained for the cattail systems because ammonia-N was detectable throughout the experiment.

After HRT for the two bulrush systems was adjusted, the four systems were allowed to acclimate for five months with the 20 mg/L ammonia-N inflow concentration to promote growth of both the nitrifying bacteria and plants. 20 g of crushed oyster shells were added to each system twice monthly to stabilize pH. During acclimation, outflow ammonia-N concentrations were monitored using an ammonia ISE to determine the equilibrium concentration of ammonia-N for each of the four pilot-scale systems.

The optimized Freundlich sorption isotherm generated from the serial batch sorption experiment was used to estimate the mass of clinoptilolite to be added to the acclimated pilot-scale CWTSs. Eqn. 1 was rearranged (Eqn. 2) and applied to the pilot-scale CWTSs for treating inflow with 20mg/L ammonia-N:

\[ M_c = \frac{V_w(C_o-C_{ef})}{q} \quad \text{Eqn. 2} \]

where \( M_c \) is the mass (g) of clinoptilolite required to attain a targeted outflow concentration of ammonia-N (\( C_{ef} \), 0 mg/L in this case); \( V_w \) is the volume (L) of inflow ammonia water solution to be treated; \( C_o \) is the equilibrium concentration (mg/L) of ammonia-N in the pilot-scale CWTSs determined from measuring the outflow; and \( q \) is the sorbed mass fraction of ammonia-N (mg/g) at equilibrium with \( C_o \) determined by the Freundlich isotherm.

The calculated masses of clinoptilolite (\( M_c \)) required for treating 3.5 days of ammonia solution inflow (302 L for bulrush systems and 151 L for cattail systems) were weighed on a digital scale and placed into sealed, permeable 5 cm x 5 cm bags. The
clinoptilolite bags were placed on top of the hydrosoil of one of the cattail systems and one of the bulrush systems (i.e. treated systems) near the outflow where equilibrium concentrations needed to calculate loading masses had been measured. Bags containing only sterilized sandy sediment (“control sediment”) were also placed into the two treated systems in the same locations to serve as controls for a later experiment in this investigation. The two remaining systems were used as untreated controls and did not receive any clinoptilolite treatments or sandy sediment. To detect changes in performance due to clinoptilolite addition, sampled outflow ammonia-N concentrations from the four pilot-scale CWTSs were measured daily for 10 days after addition of clinoptilolite to the treated bulrush and cattail systems. Explanatory parameters were measured just prior to clinoptilolite treatment and on the 5th day and 10th day using methods outlined in Table 3.1. Outflow ammonia-N concentrations were compared for treated and untreated bulrush systems and treated and untreated cattail systems to determine if clinoptilolite sorption enhanced ammonia removal as predicted by the optimized Freundlich sorption isotherm.

3.3.3 Clinoptilolite as a Microbial Carrier
Samples from the immersed bags from the treated systems containing either clinoptilolite or control sediment were retrieved after 50 days and added to Hach nitrification biological activity reaction tests (n-BARTs) to determine nitrification activity (3 mL sample per n-BART tube). The n-BARTs produce a visible, red color change as nitrite formed by nitrifying bacteria reacts with an added reagent after a 5-day incubation period. If active nitrifying bacteria were detected in a sample of clinoptilolite or control sediment, then that sample was interpreted as a microbial carrier under the
CWTS conditions. Nitrifying activity was compared between the clinoptilolite and control sediment for each of the two treated systems.

3.4 Results

3.4.1 Clinoptilolite Sorption Isotherm

Based on monitoring of sorption equilibration using replicate bottles containing 40 mg/L ammonia-N (Table 3.2; bottles 11, 12, 13, and 14), equilibration was achieved at day 11 as the resultant concentrations of ammonia-N for 3 replicate bottles (sampled on days 5, 7, and 11, respectively) ranged within a 95% confidence interval (Figure 3.1).

Data from the serial batch sorption experiment indicate that clinoptilolite has an affinity for ammonia (Table 3.2). Maximum values of sorption mass fractions (q) were obtained at the highest ammonia loading concentration of 80 mg/L (4.99-5.02 mg/g).

Concentrations of ammonia-N in control bottles did not change measurably during the experiment indicating that during the 11-day equilibration period, clinoptilolite did not release ammonia to the solution and ammonia did not degrade or volatilize (Table 3.2).

Linear regression of log-log normalized $C_e$ vs. q data using Excel® yielded the slope and coefficient values for generation of a Freundlich sorption isotherm $q = 0.58C_e^{0.68}$ (Figure 3.2). Optimization of the linearly derived Freundlich sorption isotherm using an Excel® based solver spreadsheet developed by Bolster and Hornberger [26] yielded alternate slope and coefficient values with an equation, $q = 0.72C_e^{0.57}$. The non-linearly derived Freundlich isotherm was selected for calculation of q because it fit the serial batch sorption data; the linearly derived Freundlich isotherm did not fit all data points due to potential log biasing (Figure 3.3).
3.4.2 Pilot-scale Constructed Wetland Application of Clinoptilolite

Values of $q$ from the pilot-scale systems on 9/16/10 (Table 3.3) were 2.55 mg/g for the treated bulrush system and 1.96 mg/g for the treated cattail system as predicted by the non-linear Freundlich isotherm (Figure 3.3). Estimated masses of clinoptilolite needed to lower the ammonia-N concentration to 0 mg/L for 3.5 days calculated using Eqn. 2 were 1,090 g for the treated bulrush system and 447 g for the treated cattail system. The actual masses loaded to the treated bulrush and cattail systems were rounded to 1,000 g and 500 g to accommodate natural variances in outflow ammonia-N concentrations observed during acclimation (Table 3.3).

During days 2-10 of the 10-day sampling period, the concentration of ammonia-N in outflow of the treated bulrush system was significantly less ($p = 8.8 \times 10^{-3}$) than that in outflow from control bulrush system (2.0 vs 5.6 mg/L, respectively, on day 10; Figure 3.4a; Table 3.4). No significant difference ($p = 0.45$) in outflow concentration of ammonia-N between the treated and untreated cattail systems was observed during the 10-day sampling period (Figure 3.4b; Table 3.4). Measurement of explanatory parameters indicated that dissolved oxygen concentration, redox, alkalinity, and hardness were lower in the cattail systems compared to the bulrush systems (Table 3.4).

3.4.3 Nitrification Activity of Clinoptilolite

Samples of clinoptilolite and sandy sediment retrieved from the treated bulrush system after 50 days contained similar, observable levels of nitrifying activity when tested using n-BARTs (Figure 3.5a) indicating that both samples were capable of serving as microbial carriers under the measured conditions (Table 3.4). Samples of clinoptilolite
and sandy sediment retrieved from the treated cattail system did not contain detectable levels of nitrification activity when tested using n-BARTs (Figure 3.5b), suggesting that neither sample was a microbial carrier under the measured conditions (Table 3.4).

3.5 Discussion

Based on the non-linear isotherm (Figure 3.3), clinoptilolite sorption capacity logarithmically increases as initial ammonia-N concentration increases to 80 mg/L. The maximum ammonia sorption capacity measured during the clinoptilolite serial batch sorption experiment was 5.03 mg ammonia-N/g clinoptilolite (Table 3.2), which is within the range of previously reported maximum ammonia sorption capacities of natural clinoptilolite (2.7-30.3 mg ammonia-N/g clinoptilolite; [21]). The optimized clinoptilolite sorption isotherm from the current investigation (Figure 3.3) predicts that as equilibrium concentrations of ammonia increase, sorption mass fractions increase logarithmically. For enhanced ammonia removal in CWTSs, clinoptilolite should be placed in areas with the highest concentration of ammonia to enhance sorption and environmental conditions most suitable to support growth of nitrifying bacteria. Therefore, clinoptilolite should be placed near the inflow of ammonia-treating CWTSs where higher equilibrium concentrations of ammonia and also higher dissolved oxygen levels are likely to occur.

In the constructed wetlands, outflow ammonia-N concentrations increased initially after clinoptilolite loading on day 1 in the bulrush systems and on days 1, 2, and 3 in the cattail systems. This increase in outflow ammonia concentration may have been the result of environmental effects, such as overcast conditions that occurred on day 1 of
the experiment. Although all four systems were located in a climate-controlled greenhouse, overcast conditions may have decreased plant photosynthesis thus decreasing oxygen supply to the systems. In this case, the delayed response of the cattail systems compared to the bulrush systems may be attributed to 4-day HRT of the cattail systems versus 2-day HRT of the bulrush systems.

Results from the loading experiments indicate that sorption by clinoptilolite added to CWTSs has the potential to enhance removal of ammonia as indicated by the significant difference ($p = 8.8 \times 10^{-3}$) in ammonia removal between the treated and untreated bulrush systems during the 10-day sampling period (Figure 3.4a). During days 2-10 of the sampling period, more ammonia was removed in the treated bulrush system than the untreated control bulrush system. Based on equilibration results from the serial batch sorption experiment, in which a majority of ammonia removal due to sorption occurred by the first sampling at day 3 (Figure 3.1), sorption capacity of the clinoptilolite was expected to be exhausted within 4-5 days. However, enhanced ammonia removal in the treated versus untreated bulrush system associated with clinoptilolite loading was observed for 9 days (from days 2-10), indicating that sorption of ammonia by clinoptilolite occurred more slowly in the treated system than in BOD bottles used during the batch sorption experiment. This may be due to limited hydraulic conductivity through the clinoptilolite in the permeable bags placed in the wetland cells.

The occurrence of nitrifying activity in clinoptilolite from the treated bulrush system suggests that clinoptilolite is capable of serving as a microbial carrier for nitrifying bacteria. Because no difference in activity between clinoptilolite and control
sediment collected from the treated bulrush system was observed, it is unclear if nitrifying bacteria have a preference for clinoptilolite over the control sediment.

Outflow concentrations of ammonia-N from the treated cattail system (2.5 mg/L on day 10) were not significantly different (p = 0.45) than outflow concentrations of ammonia-N in the untreated cattail system (3.1 mg/L on day 10) during the 10-day sampling period (Figure 3.4b), indicating that removal of ammonia due to clinoptilolite sorption did not occur. Dissolved oxygen concentration greater than 2.0 mg/L and soil redox between +100 and +350 mV are necessary for promoting nitrification [28-30]. In the treated bulrush system, both dissolved oxygen concentration and soil redox (5.3 - 5.8 mg/L and +140 to +160 mV, respectively) were within these ranges. Although dissolved oxygen concentration in the treated cattail system (2.7 - 3.2 mg/L) was within the range for nitrification, soil redox was between -20 and -42 mV, which was outside the range necessary for nitrification, suggesting that nitrification was unlikely to have occurred in the hydrosoil zone where the clinoptilolite and control sediment were placed. The lower soil redox in the cattail systems compared to the bulrush systems may be attributed to larger mass of plant litter and detritus observed in hydrosoil of the cattail system. Redox values and nitrification activity for the bulrush and cattail systems used in this study were similar to those observed in bulrush and cattail systems used during a study of ammonia removal from swine effluent using constructed wetlands [31].

The results from the treated bulrush system suggest that clinoptilolite may be used to enhance ammonia treatment in CWTSs. In future applications, care should be taken to add clinoptilolite to zones within CWTSs where equilibrium ammonia-N concentrations
are equal to or greater than those observed in the bulrush systems (~6-10 mg/L) and sediment redox values are sufficient to promote growth of nitrifying bacteria (greater than 100 mV).

3.6 Conclusion

The clinoptilolite tested has an affinity for ammonia-N described by the Freundlich isotherm $q=0.72C_e^{0.57}$ for concentrations from 0.07 to 30.1 mg/L. During a 10-day sampling period, a bulrush pilot-scale CWTS containing 1,000 g clinoptilolite removed significantly more ($p = 8.8 \times 10^{-3}$) ammonia-N (mean outflow 4.5 mg/L, $\sigma = 4.1$) than a control system containing no clinoptilolite (mean outflow 8.6 mg/L, $\sigma = 2.7$). Biogeochemical conditions including soil redox (+140 to +160 mV) and dissolved oxygen (5.3 – 5.8 mg/L) were favorable for growth of nitrifying bacteria in the bulrush systems, and nitrification activity was detected using nitrifying bacteria activity reactivity tests (n-BARTs) in samples of clinoptilolite and sandy sediment retrieved from the treated bulrush system. Ammonia removal was not significantly affected ($p = 0.45$) by clinoptilolite addition to the treated cattail system, and nitrification activity was not detected in samples of clinoptilolite or control sediment retrieved from the treated cattail system. The absence of nitrification activity in samples retrieved from the treated cattail system is attributed to the low soil redox (-20 to -42 mV), which was outside the suggested range for nitrification (+100 to +350 mV). This work demonstrates that clinoptilolite can be effective for increasing ammonia removal and nitrifying activity when placed in areas within CWTSs containing equilibrium ammonia concentrations greater than or equal to those measured in outflow of the bulrush systems (~6-10 mg/L).
and having biogeochemical conditions including hydrosoil redox suitable for supporting growth of nitrifying bacteria.
3.7 Acknowledgement

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3.8 References


Figure 3.1. Equilibration Monitoring - Monitoring of ammonia-N concentration in bottles 11-14 (Table 3.2) during the serial batch sorption experiment indicated that 65.3% of the ammonia was removed by day 3 and equilibration had occurred by day 11.

Figure 3.2. Serial Batch Sorption Regression - Linear regression of transformed data \( C_e \) and \( q \) from Eqn. 1) from serial batch sorption experiment (Table 3.2). The resulting line is used to calculate the Freundlich isotherm (Figure 3.3).

Figure 3.3. Freundlich Sorption Isotherms - Linear and non-linear Freundlich sorption isotherms generated from serial batch sorption experiment (Table 3.2) showing fit of the non-linearly derived model to equilibrium concentrations. The linearly derived model did not fit the equilibrium concentrations due to potential log biasing.

Figure 3.4. Outflow Monitoring - Concentrations of ammonia-N (mg/L) in outflows during the 10-day sampling period. (A) Bulrush systems: more ammonia was removed from inflow by the treated system containing clinoptilolite (B-TRT) than from the untreated control system (B-CTL) during days 2-10. (B) Cattail systems: ammonia removal was approximately the same in both systems indicating that clinoptilolite sorption did not occur.

Figure 3.5. Nitrification Activity Tests - n-BART test kits used to detect nitrifying activity. (A) B-TRT, with two clinoptilolite samples on the left and two sandy sediment samples on the right. Active nitrifying bacteria were detected in all samples as indicated by the color change demonstrating the ability of both clinoptilolite and sandy sediment to serve as microbial carriers for nitrifying activity.
bacteria. (B) C-TRT with two clinoptilolite samples on the left and two sandy sediment samples on the right. No visible color change indicates that nitrifying bacteria activity was not present.
Figure 3.1.
Figure 3.2.

\[ y = 0.6765x - 0.234 \]
\[ R^2 = 0.9929 \]
Figure 3.3.

Linear Model
\[ q = 0.58C_e^{0.68} \]

Non-linear Model
\[ q = 0.72C_e^{0.57} \]
Figure 3.4.
Figure 3.5.
Table 3.1. Analytical methods for determining explanatory and performance parameters in the pilot scale CWTSs.

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<td>Hach n-BARTs</td>
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\(^1\) Standard Methods [32]
\(^2\) n/a – qualitative test
Table 3.2. Resultant equilibrium concentrations (C<sub>e</sub>) and calculated sorption values (q, eqn. 1) from sealed 300-mL BOD bottles containing initial ammonia-N concentration (C<sub>o</sub>) and clinoptilolite (M<sub>c</sub>).

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<td>13.9</td>
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</tr>
<tr>
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<td>3.00</td>
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</tr>
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<td>3.00</td>
<td>nd&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
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<tr>
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<td>40.0</td>
<td>39.9</td>
<td>0.00</td>
<td>nd&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Bottles 11, 12, and 13 are replicates that were sampled early to determine equilibration.

<sup>2</sup> nd: Freundlich parameters not determined for control bottles because there were no significant changes in ammonia concentration after the equilibration period.
Table 3.3. Values of performance parameters measured in outflow of treated bulrush (B-TRT), untreated control bulrush (B-CTL), treated cattail (C-TRT), and untreated control cattail (C-CTL) systems during the last month of acclimation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>8/24/10</th>
<th>9/7/10</th>
<th>9/16/10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B-TRT</td>
<td>CTL</td>
<td>C-TRT</td>
</tr>
<tr>
<td>Ammonia-N Outflow (mg/L)</td>
<td>8.7</td>
<td>8.7</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>8.3</td>
<td>8.6</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>9.2</td>
<td>9.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Nitrate-N Outflow (mg/L)</td>
<td>6.7</td>
<td>4.7</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>6.9</td>
<td>5.2</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>6.9</td>
<td>5.2</td>
<td>1.6</td>
</tr>
</tbody>
</table>
Table 3.4. Values of explanatory and performance parameters measured in outflow of treated bulrush (B-TRT), untreated control bulrush (B-CTL), treated cattail (C-TRT), and untreated control cattail (C-CTL) systems during the 10-day sampling period.

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>9/29/10</th>
<th>10/04/10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B-TRT</td>
<td>B-CTL</td>
<td>C-TRT</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>22.4</td>
<td>22.9</td>
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</tr>
<tr>
<td></td>
<td>22.3</td>
<td>22.5</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>23.1</td>
<td>23.0</td>
<td>22.5</td>
</tr>
<tr>
<td>pH (S.U.)</td>
<td>6.23</td>
<td>6.21</td>
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<td></td>
<td>6.24</td>
<td>6.32</td>
<td>6.52</td>
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<tr>
<td></td>
<td>6.10</td>
<td>6.20</td>
<td>6.34</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg/L)</td>
<td>5.5</td>
<td>5.3</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
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<td>2.7</td>
</tr>
<tr>
<td></td>
<td>5.8</td>
<td>6.0</td>
<td>2.8</td>
</tr>
<tr>
<td>Conductivity (μS/cm)</td>
<td>380</td>
<td>380</td>
<td>430</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>400</td>
<td>390</td>
</tr>
<tr>
<td></td>
<td>390</td>
<td>380</td>
<td>410</td>
</tr>
<tr>
<td>Alkalinity (mg/L as CaCO₃)</td>
<td>24</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>30</td>
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<tr>
<td></td>
<td>26</td>
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<td>32</td>
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<td>Hardness (mg/L as CaCO₃)</td>
<td>98</td>
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<td>122</td>
</tr>
<tr>
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<tr>
<td></td>
<td>98</td>
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<td>120</td>
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<tr>
<td>Soil Redox (mV)</td>
<td>140</td>
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<tr>
<td></td>
<td>142</td>
<td>84</td>
<td>-42</td>
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<tr>
<td>Ammonia-N Outflow (mg/L)</td>
<td>10.2</td>
<td>9.7</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>10.0</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>5.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Nitrate-N Outflow (mg/L)</td>
<td>6.0</td>
<td>5.4</td>
<td>2.5</td>
</tr>
<tr>
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<td>5.1</td>
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</tr>
<tr>
<td></td>
<td>4.8</td>
<td>5.7</td>
<td>2.5</td>
</tr>
</tbody>
</table>
CHAPTER 4: EFFECTS OF EVAPOTRANSPIRATION ON TREATMENT PERFORMANCE IN CONSTRUCTED WETLANDS: EXPERIMENTAL STUDIES AND MODELING

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Keywords: Constructed Wetlands, Evapotranspiration, Tracer Tests, Modeling
4.1 Abstract

Evapotranspiration (ET) can affect treatment performance in constructed wetlands by enhancing constituent transport through the hydrosoil where treatment reactions occur. Additionally, ET can decrease volumetric flow through the system thereby increasing hydraulic retention time and increasing the concentration of dissolved constituents. This research aims to determine the significance of plant transpiration on vertical transport of constituents and to assess the net effects of water loss attributed to ET on constructed wetland performance. A flowing wetland lysimeter constructed using 265-L storage containers filled with sand and *Typha latifolia* was used to record ET and determine crop coefficient during summer 2011. Results indicate that ET from the lysimeter was 2.54 times greater than calculated reference ET ($K_c = 2.54; R^2 = 0.96$). The calculated crop coefficient was used in conjunction with a first-order tank-in-series model to predict removal of both a conservative constituent ($k = 0.2 \text{ d}^{-1}$) and readily treatable constituent ($k = 1.2 \text{ d}^{-1}$) in a constructed wetland (20 cm and 40 cm water depths, 4-day nominal HRT, and 100 mg L$^{-1}$ constituent loading) operating under a range of *T. latifolia* ET (0, 10, 20, and 30 mm d$^{-1}$). The model predicts that removal efficiency of the conservative constituent decreases with increasing ET, while removal efficiency of the readily treatable constituent increases with increasing ET. In addition, eight vertical tracer tests were performed on wetland cells with either trimmed or untrimmed broadleaf *T. latifolia* to measure transport time of tracer solution from the water surface to a depth of 5 cm. Mean tracer arrival time differed significantly ($p = 1.2 \times 10^{-8}$) between the untrimmed
and trimmed cells (104 minutes versus 450 minutes, respectively) demonstrating that plant transpiration contributes significantly to vertical flow through hydrosoil.
4.2 Introduction

Water loss in constructed wetland treatment systems (CWTSs) occurs primarily through the combined effects of open water evaporation and plant transpiration, collectively termed evapotranspiration. Because CWTSs are typically constructed with a lined bottom to prevent infiltration of contaminated water to underlying soil, evapotranspiration is a key component of the water balance. Evapotranspiration is driven primarily by the transformation of energy from insolation to latent heat of vaporization of liquid water.

Numerous studies have been performed to quantify evapotranspiration from wetlands containing *Typha latifolia* (i.e. broadleaf cattails) with conflicting results attributed to differing measurement methods and lysimeter designs (Otis, 1914; Idso, 1981; Anderson and Idso, 1987; Snyder and Boyd, 1987; Idso and Anderson, 1988; Allen and Prueger, 1992; Allen et al., 1997; Towler et al., 2004). As explained by Idso and Anderson (1988), evapotranspiration can be influenced by the “oasis effect” (Shaw, 1967) resulting in elevated evapotranspiration from isolated, small stands of vegetation compared to large expanses of vegetation. Incoming latent heat from surrounding dry fetch is advectively exchanged through the periphery of isolated stands of vegetation, leading to an increase in incoming energy and corresponding increase in evapotranspiration (Idso and Anderson, 1988; Towler et al., 2004). Evapotranspiration is also dependent on regional meteorological factors including air temperature, relative humidity, solar radiation, and wind speed, as well as CWTS design features including plant species diversity and density (Allen et al., 1998).
Changes in volumetric flow attributed to evapotranspiration can alter CWTS treatment performance by removing water from the system (thus increasing hydraulic retention time) and increasing the concentration of dissolved constituents. Differences in evapotranspiration attributed to CWTS size, climatic region, and plant selection can lead to inaccurate predictions of treatment performance when using previously calculated removal rate coefficients. Because removal performance data collected from small, pilot-scale CWTS studies may be applied to designing full-scale CWTSs in different climatic regions, the extent to which differing evapotranspiration can affect treatment is of interest.

Additionally, because both the inflow and outflow of free water surface (FWS) CWTSs are located above the hydrosoil, a decreasing hydraulic head with depth is needed to advectively transport targeted constituents to the hydrosoil where treatment by specific redox-driven reactions occurs (Martin and Reddy, 1997; Kadlec, 1999; Martin et al., 2003; Kadlec and Wallace, 2009). Previous studies suggest that plant transpiration plays a role in establishing a vertical hydraulic gradient within wetland hydrosoil (Martin et al., 2003). The extent to which constituents are transported advectively can be estimated through a transpiration to evapotranspiration ratio based on the assumption that water lost through plant transpiration must move through the root zone (Kadlec and Wallace, 2009). However, root density and location can affect flow through the hydrosoil and water column. Therefore, the ability of plant transpiration to vertically transport constituents warrants investigation to determine if FWS CWTSs are capable of supporting treatment by redox-driven reactions in the hydrosoil.
The objectives of this paper are to (1) determine the crop coefficient for a small-stand, pilot-scale CWTS, (2) predict differences in constructed wetland treatment performance attributed to evapotranspiration-driven water-loss, and (3) measure the effects of plant transpiration on vertical flow of constituents. The completion of these objectives provides a fundamental understanding of the effects of evapotranspiration on treatment performance in FWS CWTSs containing *T. latifolia*.

4.3 Materials and Methods

4.3.1 Pilot-scale Crop Coefficients for *Typha latifolia*

*T. latifolia* evapotranspiration (*ET*_c) was monitored using a 2 m² constant-head lysimeter with dimensions similar to pilot-scale CWTSs used in many previous studies (e.g Kanagy et al., 2008; Dorman et al., 2009; Spacil et al., 2011; Horner et al., 2012). The lysimeter consisted of four 265-L troughs, each filled to a depth of 45 cm with sandy river sediment collected from nearby 18-mile Creek (Clemson, SC) and planted to field density (approximately 20 plants per trough) with *T. latifolia* collected from nearby aquaculture ponds (Figure 4.1). The four troughs were connected with 2.5-cm diameter PVC piping and arranged for gravity flow, with fixed overflow pipes installed in each trough to maintain a constant head and water-depth of 15 cm (Figure 4.2). Water was supplied to the first trough at a constant rate of 100 mL per minute by a Fluid Metering Inc. ® (FMI) pump (QG400). The lysimeter was allowed to mature for approximately 3 years before any *ET*_c data were collected. The plants were fertilized periodically to promote vigorous growth.
Volumetric outflow of the lysimeter was monitored using a RainWise® Inc. tipping bucket rain gauge placed under the outflow pipe of the last trough of the lysimeter (Figure 4.2). The rain gauge was connected to a RainWise® RainLog digital data logger with 256 kB of non-volatile memory capable of recording flow information at a resolution of 1 minute. The rain gauge was calibrated using timed intervals of a constant 100 mL per minute flow rate provided by the FMI QG400 pumps. Hourly volumetric outflow data were recorded and downloaded for three 5- to 7-day intervals in July and August after the plants had reached maturity.

\[ ETc (\text{mm h}^{-1}) = \frac{Q_{in} - Q_{out}}{SA} \]

Eqn. 1

Where \( Q_{in} \) is volumetric inflow of the lysimeter (6x10^6 mm^3 h^{-1}), \( Q_{out} \) is measured volumetric outflow of the lysimeter (mm^3 h^{-1}), and \( SA \) is measured surface area of the lysimeter (2x10^6 mm^2).

Small-stand crop coefficients for \( T. \) latifolia were determined using linear regressions of hourly \( ETc \) measured from the lysimeter and hourly reference evapotranspiration (\( ETo \)). \( ETo \) values were calculated using the FAO-56 Penman-Monteith method (Penman 1963; Allen et al., 1998) from meteorological data collected with an on-site Davis Instruments® Vantage Pro 2 weather station. The FAO-56 Penman-Monteith method (Eqn. 2) was used to calculate \( ETo \) for a reference crop with an assumed crop height of 0.12 m, fixed surface resistance of 70 s m^{-1}, and albedo of 0.23.
This method was selected because it meets the precision required for calculating crop coefficients using readily acquired meteorological data (e.g. temperature, dew point, wind speed, and solar radiation) and is commonly used in other evapotranspiration studies (e.g. Allen et al., 1998).

\[
ET_o = \frac{(0.408)\Delta(R_n)+\gamma\frac{37}{T+273}u_2(e_s-e_a)}{\Delta+\gamma(1+0.34u_2)} \quad \text{Eqn. 2}
\]

where \( ET_o \) is reference evapotranspiration (mm h\(^{-1}\)) calculated from meteorological data; \( \Delta \) is slope of the saturation vapor pressure temperature relationship (kPa °C\(^{-1}\), 1 kPa = 1x10\(^3\) pascals); \( R_n \) is measured net radiation (MJ m\(^{-2}\) h\(^{-1}\), 1 MJ = 1x10\(^6\) joules); \( \gamma \) is psychrometric constant (kPa °C\(^{-1}\)); \( T \) is measured air temperature (°C); \( u_2 \) is wind speed (m s\(^{-1}\)) measured at a height of 2 m; \( (e_s-e_a) \) represents vapor pressure deficit of the air (kPa); \( e_s \) is saturation vapor pressure of the air (kPa); \( e_a \) is vapor pressure of the air (kPa). Values for \( \Delta, \gamma, e_s, \) and \( e_a \) were calculated from meteorological data collected with the weather station (Table 4.1).

Crop coefficients \( (K_c; \text{Eqn.3}) \) can be used to predict evapotranspiration of a plant species from measured meteorological data \( (ET_o) \), and therefore were used to predict differences in evapotranspiration between large-stand (> 1 Hectare) and small-stand (e.g. pilot-scale) wetlands.

\[
K_c = \frac{ET_c-E_b}{ET_o} \quad \text{Eqn. 3}
\]

where \( K_c \) is crop coefficient (unitless); \( ET_c \) is measured \( T. \ latifolia \) evapotranspiration (mm h\(^{-1}\)); \( ET_o \) is reference evapotranspiration (mm h\(^{-1}\)); and \( E_b \) is baseline evaporation measured (mm h\(^{-1}\)).
Baseline evaporation is included in the calculation of $ET_c$ to account for incoming latent heat from the inflow (Kadlec, 2006). $K_c$ and $E_b$ were determined for the small-stand *T. latifolia* in lysimeter using a linear regression of $ET_c$ versus $ET_o$ (Eqn. 4).

$$ET_c = (ET_o \times K_c) + E_b$$  \hspace{1cm} \text{Eqn. 4}

The calculated lysimeter $K_c$ was compared with the large-stand wetland $K_c$ value (Abtew and Obeysekera, 1995) to predict differences in water loss expected between pilot-scale and full-scale CWTSs.

### 4.3.2 Analytical Evapotranspiration Performance Model

A first-order, one-dimensional, steady-state model for estimating the effects of evapotranspiration on treatment performance of a CWTS was derived from a conceptual, serial connection of continuously stirred tank reactors (CSTRs), also known as the tank-in-series (TIS) model proposed by Levenspiel (1972; example in Figure 4.3). Treatment performance was evaluated on the basis of CWTS removal extent (i.e. final outflow constituent concentration) and removal efficiency (i.e. percentage of constituent removed from inflow to outflow by the CWTS). The TIS model was selected because it can be used to recreate the hydrodynamics of FWS wetlands when the number of CSTRs connected in series (N) is calibrated to tracer test breakthrough data (Kadlec and Wallace, 2009). Other models utilizing conceptual plug-flow-reactors (PFRs) and plug-flow-reactors with dispersion (PFD) have been suggested for CWTSs, but are not ideal because they are not accurate within the mixing ranges for FWS CWTSs identified through tracer tests (Levenspiel, 1972; Kadlec and Wallace, 2009). In addition, the TIS model can be calculated as a series of mass balances using simple algebra.
The TIS model used to determine treatment performance consisted of 16 CSTRs (N = 16) of unit area connected in series. This N value was selected to model a CWTS with four wetland cells and is based on the mean N value previously determined for single FWS CWTS cells (N = 4.1; Bavor et al., 1988; Kadlec, 1994; Nolte and Associates, 1998; Wang and Jawitz, 2006; Kadlec and Wallace, 2009). The model was derived from the wetland mass balance and first-order removal kinetics (Eqn. 5).

\[ Q_{in}C_{in} - Q_{out}C_{out} = kC_{out}(A \times d) \quad \text{Eqn. 5} \]

where \( Q_{in} \) is volumetric inflow to the CSTR (m\(^3\) d\(^{-1}\)); \( C_{in} \) is constituent concentration of \( Q_{in} \) (g m\(^{-3}\)); \( Q_{out} \) is volumetric outflow of the CSTR (m\(^3\) d\(^{-1}\)); \( C_{out} \) is constituent concentration of \( Q_{out} \) (g m\(^{-3}\)); \( k \) is volumetric first-order removal coefficient (d\(^{-1}\)); \( A \) is surface area of the CSTR (m\(^2\)); and \( d \) is water depth of the CSTR (m).

Assuming a unit area for \( A \), rearrangement of Eqn. 5 yields the equation:

\[ q_{in}C_{in} - q_{out}C_{out} = kC_{out} \times d \quad \text{Eqn. 6} \]

where \( q_{in} \) is inflow hydraulic loading (m d\(^{-1}\)); and \( q_{out} \) is outflow (m d\(^{-1}\)).

Eqn. 6 rearranged for \( C_{out} \):

\[ C_{out} = \frac{q_{in}C_{in}}{q_{out} + (k \times d)} \quad \text{Eqn. 7} \]

Because \( q_{out} \) is the difference between \( q_{in} \) and water loss due to plant evapotranspiration (\( ET_c \)), Eqn. 7 can be rewritten:

\[ C_{out} = \frac{q_{in}C_{in}}{q_{in} - ET_c + (k \times d)} \quad \text{Eqn. 8} \]

Or in discrete form to allow for calculation of \( C_{out} \) for each of the 16 CSTRs (i from 1 to 16) in the TIS model:
Eqn. 9

\[ C_{(i)} = \frac{q_{(i-1)}c_{(i-1)}}{q_{(i-1)} - \frac{E_T}{16}(k \times d)} \]

where \( c_{(i)} \) is outflow constituent concentration of CSTR \( i \) (g m\(^{-3}\)); \( c_{(i-1)} \) is inflow constituent concentration of CSTR \( i \) (g m\(^{-3}\)); and \( q_{(i-1)} \) is inflow hydraulic loading (m d\(^{-1}\)).

Eqn. 9 was used to model the removal of a conservative constituent \( (k = 0.2 \text{ d}^{-1}) \) and readily treatable constituent \( (k = 1.2 \text{ d}^{-1}) \) in both shallow (20-cm water depth) and deep (40-cm water depth) FWS CWTSs operating with a 4-day nominal HRT under a range of \( ET_c \) from 0 to 30 mm d\(^{-1}\) (Table 4.2). \( ET_c \) values selected for the model were based on pilot-scale \( K_c \) measured from the lysimeter and \( K_c \) reported in previous studies for large-scale wetlands containing \( T. latifolia \) and range from baseline evapotranspiration (0 mm d\(^{-1}\)) to desert reference evapotranspiration (12 mm d\(^{-1}\); Einesr et al., 2010). Predictions from the model were compared to demonstrate the potential effects of evapotranspiration on removal efficiency of conservative and readily treatable constituents. In order to isolate and examine the effects of changes to the water balance attributed to evapotranspiration on treatment performance, removal rate coefficients used in the model were assumed to be unaffected by changes in evapotranspiration.

4.3.3 Vertical Tracer Tests

The effect of \( T. latifolia \) transpiration on vertical transport of dissolved constituents in bench-scale CWTSs was measured by vertical tracer tests. These tests monitored hydrosoil electrical conductivity to detect differences in dissolved tracer arrival between wetland cells containing mature plants and cells containing trimmed plants. Eight 20-L buckets were prepared as bench-scale wetland cells by filling each with approximately 20 cm of sandy sediment and approximately 8 \( T. latifolia \) plants. The
buckets were watered and fertilized regularly and stored in a climate-controlled
greenhouse for 9 months to promote plant maturation. After the maturation period, *T. latifolia* in 4 randomly selected cells were trimmed to a height of 30 cm above the
hydrosoil (corresponding to 5 cm above the waterline) immediately prior to each tracer
test to eliminate plant transpiration.

Surface water electrical conductivity data collected from each wetland cell were
used to formulate tracer solutions containing amounts of dissolved sodium chloride
needed to yield conductivity readings ten times greater than readings from the surface
water. This strength of tracer solution was selected to allow accurate resolution of tracer
arrival time.

Eight individual tracer tests (4 with untrimmed plants and 4 with trimmed plants)
were performed by placing a pair of HANNA Instruments® HI 98331 stainless steel
conductivity probes 5 cm below the hydrosoil surface of each cell. Prepared tracer
solution was then added to the cell by a FMI® QG400 pump at a rate of 200 mL min⁻¹.
After 5 minutes, a second QG400 pumping at a rate of 200 mL min⁻¹ was connected to
the system at a height of 25 cm above the hydrosoil surface to remove excess tracer
solution, maintaining a constant head. This method allowed tracer solution to be added
gently, preventing vertical flow disturbances while allowing circulation of the tracer
solution.

Hydrosoil conductivity was measured every 5 minutes until increased electrical
conductivity readings were detected for a minimum of 3 consecutive measurements at
each probe. Mean arrival times for the four untrimmed and four trimmed cells were
compared using Welch’s t-test to determine if plant transpiration significantly altered flow through hydrosoil of the wetland cells (α=0.05). If a significant difference in tracer arrival time between the untrimmed and trimmed wetland cells occurred, then it was concluded that plant evapotranspiration plays a role in transporting constituents in FWS CWTSs through the hydrosoil where specific redox-driven needed for treatment reactions can occur.

4.4 Results

4.4.1 Pilot-scale Crop Coefficients for *Typha latifolia*

A diurnal pattern of $ET_c$ was observed in the lysimeter, with lowest evapotranspiration occurring in the early morning (approximately 0.2 mm h$^{-1}$) and greatest evapotranspiration occurring in the late afternoon (approximately 2 mm h$^{-1}$; Figure 4.4). A similar diurnal pattern was observed for the calculated $ET_o$. $ET_o$ was consistently lower than $ET_c$ throughout the day and was slightly negative (approximately -0.02 mm h$^{-1}$) in the early morning when air temperature was below the dew point, indicating condensation (dew) formation.

Linear regression of $ET_c$ versus $ET_o$ for the three periods during which evapotranspiration was monitored in July and August (Figure 4.5) yielded a $K_c$ value of 2.54 ($R^2 = 0.96$) which is greater than the $K_c$ of 1.0 reported for large wetlands (Abtew and Obeysekera, 1995). Baseline evaporation ($E_b$) identified from the intercept of the linear regression was 0.27 mm h$^{-1}$. Comparison of the measured volumetric outflow from the lysimeter with theoretical volumetric outflow predicted from $ET_o$, $K_c$, $E_b$, and SA is shown as Figure 4.6.
4.4.2 Analytical Evapotranspiration Performance Model

Predictions from the TIS model (Eqn. 9) indicate changes in treatment performance associated with differing $ET_c$ from 0 to 30 mm d$^{-1}$ (Figure 4.7). Treatment performance of the conservative constituent ($k = 0.2$ d$^{-1}$) is predicted to be negatively affected by increasing $ET_c$ at water depths of both 20 cm and 40 cm (Figures 4.7A, 4.7B). As $ET_c$ increased from 0 to 30 mm d$^{-1}$, removal efficiency of the conservative tracer decreased from 54.2 to 25.4% at the 20-cm water depth and from 54.2 to 43.9% at the 40-cm water depth.

Treatment performance of the readily treatable constituent ($k = 1.2$ d$^{-1}$) is predicted to be marginally enhanced by increasing $ET_c$ at both water depths (Figures 4.7C, 4.7D). As $ET_c$ increased from 0 to 30 mm d$^{-1}$, removal efficiency of the readily tracer increased from 98.5 to 99.0% at the 20-cm water depth and from 98.5 to 99.0% at the 40-cm water depth.

4.4.3 Vertical Tracer Tests

Tracer arrival times were consistently less in the wetland cells containing untrimmed plants compared to the cells containing trimmed plants (Figure 4.8). Mean tracer arrival time from all tracer tests was 104 minutes ($\sigma = 25$ minutes) in the untrimmed cells and 450 minutes ($\sigma = 57$ minutes) in the trimmed cells. Comparison of mean arrival times in the untrimmed and trimmed cells indicates that tracer arrival times were significantly different ($p = 1.2 \times 10^{-8}$).
4.5 Discussion

Results from this study support the wetland oasis effect described by Idso and Anderson (1988) where small, isolated wetlands have greater evapotranspiration than large-stand wetlands. The calculated lysimeter crop coefficient ($K_c = 2.54$; Figure 4.5) differs from the crop coefficient measured by Abtew and Obeysekera ($K_c = 1.0$; 1995) for a large-stand $T. latifolia$. As a result, evapotranspiration from pilot-scale CWTSs is expected to be greater than full-scale CWTSs. The crop coefficient measured in this study applies to mature plants and can be used to predict evapotranspiration from small pilot-scale systems located in different climatic regions where reference evapotranspiration is known or can be measured.

Lysimeter measurements indicate that volumetric outflow decreased to approximately 20 mL min$^{-1}$ during peak evapotranspiration in the late afternoon (Figure 4.6), corresponding to an 80% decrease in volume from inflow to outflow. Over the course of each 24-hour period, the 100 mL min$^{-1}$ volumetric inflow decreased to a mean volumetric outflow of 70 mL min$^{-1}$, indicating a daily volumetric loss of 30% of inflow. A 30% decrease in water volume corresponds to a 43% increase in concentration of constituents as predicted by the law of mass conservation. However, a 30% loss of volumetric inflow is predicted to increase nominal HRT by approximately 20% based on the ratio of system volume to volumetric inflow.

Differences in CWTS treatment performance attributed to increased evapotranspiration are predicted by the model to depend on both water depth and constituent removal rate coefficients. Because increased evapotranspiration is predicted
to have a deleterious effect on removal efficiency of conservative constituents, evapotranspiration can lead to excessive salinity when CWTSs are used to treat brackish or brine waters containing high concentrations of ions such as sodium and chloride. As predicted by the model (Figure 4.7), water depth can be increased to mitigate the effects of evapotranspiration; however, removal of targeted constituents can be altered by a change in water depth due to changes in treatment conditions (Gillespie et al., 2000).

Because removal efficiency of readily treatable constituents is predicted to be only marginally enhanced by increased evapotranspiration, lengthening of HRT caused by evapotranspiration can overcome the increased concentration of constituents due to water loss. As a result, properly designed CWTSs with sufficiently high removal rate coefficients (~1.2 d\(^{-1}\)) are predicted to be resilient to changes in water loss due to evapotranspiration and can be modeled without considering changes in the water balance caused by evapotranspiration.

Results from tracer tests verify the transpiration-driven vertical flow path described by Martin et al. (2003). Therefore, it is likely that redox-driven reactions in FWS CWTS hydrosoil (e.g. dissimilatory sulfate reduction and denitrification) can contribute to removal of targeted constituents that require reducing conditions. Further research is required to determine the flux of water through hydrosoil of FWS wetlands by plant transpiration; however, maximum flux will be bound by total measured plant transpiration. Additionally, changes in flow through the hydrosoil under different evapotranspiration can contribute to changes in performance and warrants further investigation.
4.6 Conclusion

The crop coefficient for the 2 m$^2$ lysimeter was determined to be 2.54 times greater ($K_c = 2.54$) than the crop coefficient previously reported for large-stand *T. latifolia* wetlands ($K_c = 1.0$). The difference in the crop coefficient measured for the lysimeter used in this study and large-stand *T. latifolia* wetlands supports the oasis effect described in previous studies. As evapotranspiration increases from 0 to 30 mm d$^{-1}$, the TIS model predicts that removal efficiency for conservative constituents is negatively affected (from 54.2 to 25.4% at 20-cm water depth), while removal efficiency for the readily treatable constituents is marginally enhanced (from 98.5% to 99.5% at 20-cm water depth). In addition, plant transpiration was shown to significantly ($p = 1.2 \times 10^{-8}$) enhance vertical transport of constituents through FWS CWTS hydrosoil. Results from the lysimeter study and TIS model may be applied to predicting differences in evapotranspiration and performance between pilot-scale and full-scale CWTSs and among CWTSs located in different climatic regions. Results from the vertical tracer tests demonstrate the importance of plant transpiration on vertical flow of constituents in FWS CWTSs and verify that constituents can be transported vertically through the hydrosoil where redox-driven treatment reactions are known to occur.
4.7 Acknowledgement

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4.8 References


Figure 4.1. Schematic diagram of a single wetland trough of the lysimeter. Each trough was filled to a depth of 45 cm with sandy river sediment and planted to field density (approximately 20 plants per trough) with *T. latifolia*. An overflow pipe (outflow) was used to maintain a constant water depth of 15 cm.

Figure 4.2. Schematic diagram of the lysimeter showing all four wetland troughs. Water was supplied to the first trough at a rate of 100 mL min$^{-1}$ and volumetric outflow was recorded by a RainWise® tipping bucket rain gauge located at the outflow of the last trough.

Figure 4.3. Schematic diagram depicting the conceptual tank in series (TIS) model. Each wetland cell was modeled as four (N=4) continuously stirred tank reactors (CSTRs) connected in series.

Figure 4.4. Hourly plot of measured evapotranspiration ($ET_c$) and calculated reference evapotranspiration ($ET_o$). Evapotranspiration was lowest during the evening and greatest in the late afternoon.

Figure 4.5. Linear regression of $ET_c$ versus $ET_o$. The slope value of 2.54 yields the crop coefficient ($K_c$) for pilot-scale CWTSs containing *T. latifolia*.

Figure 4.6. Comparison of measured volumetric outflow from the lysimeter and volumetric outflow predicted using $ET_o$, $K_c$, and $E_b$. Mean volumetric outflow from the lysimeter during this 4-day period was 70 mL min$^{-1}$.

Figure 4.7. TIS model results showing outflow concentrations of the 16 CSTRs during evapotranspiration from 0 to 30 mm d$^{-1}$ for: (A) Conservative constituent (0.2 d$^{-1}$) at a water depth of 20 cm, (B) conservative constituent at a water depth of 40 cm,
(C) readily treatable constituent (1.2 d⁻¹) at a water depth of 20 cm, (D) readily treatable constituent at a water depth of 40 cm. As evapotranspiration increased from 0 to 30 mm d⁻¹, concentrations of the conservative constituent at outflow from the final CSTR (16) increased at water depths of both 20 and 40 cm, indicating a decrease in treatment performance (A and B). Treatment performance of the readily treatable constituent was marginally enhanced as evapotranspiration increased from 0 to 30 mm d⁻¹ for both water depths as shown by the difference in outflow concentrations of the final CSTR (C and D).

Figure 4.8. Measured tracer arrival times at each of the two conductivity probes (P1 and P2) for both untrimmed and trimmed wetland cells. The arrival times were consistently lower in the untrimmed cells than the trimmed cells indicating transpiration-driven vertical transport of tracer through hydrosoil.
Figure 4.1.
Figure 4.2.
Figure 4.3.
Figure 4.4.

Evapotranspiration (mm h$^{-1}$)

- $E_{To}$
- $E_{Tc}$
Figure 4.5.

\[ y = 2.54x + 0.27 \]

\[ R^2 = 0.96 \]
Figure 4.6.
Figure 4.7.
Figure 4.8.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Units</th>
<th>Formula</th>
</tr>
</thead>
</table>
| Slope of saturation vapor pressure curve       | Δ      | kPa °C⁻¹    | \[
\frac{4098 \left[ 0.6108 \exp \left( \frac{17.27 \times T_{\text{mean}}}{T_{\text{mean}} + 237.3} \right) \right]}{(T_{\text{mean}} + 237.3)^2}
\] |
| Psychrometric constant                         | γ      | kPa °C⁻¹    | \[(6.65 \times 10^{-2}) \times P\]                                    |
| Saturation vapor pressure                      | \(e_s\) | kPa         | \[
\frac{0.6108 \exp \left( \frac{17.27 \times T_{\text{max}}}{T_{\text{max}} + 237.3} \right) - 0.6108 \exp \left( \frac{17.27 \times T_{\text{min}}}{T_{\text{min}} + 237.3} \right)}{2}
\] |
| Actual vapor pressure                          | \(e_a\) | kPa         | \[
0.6108 \exp \left( \frac{17.27 \times T_{\text{dew}}}{T_{\text{dew}} + 237.3} \right)
\] |
Table 4.2. Input parameters for scenarios modeled using a TIS receiving a hydraulic loading to maintain a nominal hydraulic retention time of 4 days and a constituent concentration loading of 100 g m\(^{-3}\).

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Evapotranspiration (mm d(^{-1}))</th>
<th>Water Depth (m)</th>
<th>Removal Rate Coefficient (1 d(^{-1}))</th>
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<td>0.2</td>
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<td>0.2</td>
</tr>
<tr>
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<td>0.2</td>
</tr>
<tr>
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<td>0.2</td>
<td>1.2</td>
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<td>0.2</td>
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<td>16</td>
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</table>
CHAPTER 5: SUMMARY AND CONCLUSION
5.1 Objectives

The impetus for this study was to determine the feasibility of using specifically designed constructed wetland treatment systems (CWTSs) to renovate produced water contaminated with ammonia. Three major objectives were completed and are presented in Chapters 2 through 4 of this dissertation: (1) design and evaluate a pilot-scale, process-based CWTS, (2) evaluate clinoptilolite for use in CWTSs, and (3) investigate the effects of evapotranspiration on CWTS treatment. These objectives were achieved through the use of pilot- and bench-scale CWTSs, laboratory experiments, and computer simulations.

5.2 Design and Evaluation of a pilot-scale, process based CWTS

The second chapter of this dissertation focuses on the design of a pilot-scale, process-based CWTS constructed to promote the biogeochemical conditions necessary for microbial transformation of ammonia to nitrogen gas. Ranges of biogeochemical conditions under which microbial nitrification and denitrification have been observed in previous studies of natural and artificial systems were identified as targeted ranges for the CWTS design. Amendments including aeration, sucrose, and crushed oyster shells were added to the CWTS to promote the targeted ranges, which were monitored during the study. Ammonia treatment performance of the CWTS was evaluated on the basis of removal extents, efficiencies, and first-order rate coefficients.

Although not all targeted conditions were met, the process system was able to treat from 20 mg/L ammonia-N to non-detectable levels (< 0.1 mg/L ammonia-N) during three of the four sampling months. The sequential design of the process system (e.g. aeration followed by organic carbon addition) allowed nitrification to precede
denitrification. In contrast, the generic system did not meet the targeted treatment goal, and ammonia removal was likely limited by the availability of alkalinity. The occurrence of nitrification and denitrification in the process system under biogeochemical conditions outside of the targeted ranges is attributed to the coexistence of an oxidizing zone in the water column and a reducing zone in the hydrosoil, and growth and attachment of bacteria to exposed, submerged surfaces. The difference in treatment performance between the process system and the generic system demonstrates the advantage of designing constructed wetlands to promote biogeochemical conditions favorable for nitrification and denitrification when targeting ammonia for treatment. This work also suggests that nitrification and denitrification operate under a wider range of conditions in constructed wetlands than in previously studied natural and engineered systems.

5.3 Evaluation of Clinoptilolite for Use in CWTSs

The third chapter of this dissertation focuses on the ability of clinoptilolite, a naturally occurring zeolite mineral, to enhance ammonia sorption and nitrification activity in CWTSs. A Freundlich ammonia sorption isotherm was determined for clinoptilolite using data collected from a serial batch sorption experiment. The isotherm was used to determine masses of clinoptilolite loaded into two pilot-scale CWTSs for increased ammonia treatment through enhanced sorption capacity. Samples of the clinoptilolite were retrieved from the CWTSs after 50 days and tested for the presence of nitrifying bacteria to determine if the clinoptilolite served as a microbial carrier.

The clinoptilolite tested has an affinity for ammonia-N described by the Freundlich isotherm $q=0.72C_e^{0.57}$ for concentrations from 0.07 to 30.1 mg/L. During a
10-day sampling period, a bulrush pilot-scale CWTS containing 1,000 g clinoptilolite removed significantly more (p = 8.8 x 10^{-3}) ammonia-N (mean outflow 4.5 mg/L, σ = 4.1) than a control system containing no clinoptilolite (mean outflow 8.6 mg/L, σ = 2.7). Biogeochemical conditions including soil redox (+140 to +160 mV) and dissolved oxygen (5.3 – 5.8 mg/L) were favorable for growth of nitrifying bacteria in the bulrush systems, and nitrification activity was detected using nitrifying bacteria activity reactivity tests (n-BARTs) in samples of clinoptilolite and sandy sediment retrieved from the treated bulrush system. Ammonia removal was not significantly affected (p = 0.45) by clinoptilolite addition to the treated cattail system, and nitrification activity was not detected in samples of clinoptilolite or control sediment retrieved from the treated cattail system. The absence of nitrification activity in samples retrieved from the treated cattail system is attributed to the low soil redox (-20 to -42 mV), which was outside the suggested range for nitrification (+100 to +350 mV). This work demonstrates that clinoptilolite can be effective for increasing ammonia removal and nitrifying activity when placed in areas within CWTSs containing equilibrium ammonia concentrations greater than or equal to those measured in outflow of the bulrush systems (~6-10 mg/L) and having biogeochemical conditions including hydrosoil redox suitable for supporting growth of nitrifying bacteria.

5.4 Investigation of the Effects of Evapotranspiration on CWTS Treatment Performance

The fourth chapter of this dissertation focuses on the effects of evapotranspiration on treatment performance in CWTSs. The process-based CWTS used in the second chapter of this dissertation was converted into a lysimeter for measuring
evapotranspiration and determining the crop coefficient for pilot-scale wetlands. The pilot-scale crop coefficient was compared with crop coefficients determined previously for large-stand wetlands (greater than 1 hectare) to predict differences in evapotranspiration between pilot-scale and full-scale CWTSs. Performance differences attributed to water loss caused by evapotranspiration were predicted using a first-order, one-dimensional tank-in-series model derived from the wetland water balance and law of mass conservation. The ability of plant transpiration to vertically transport constituents through the hydrosoil was investigated using vertical tracer tests.

The crop coefficient for the 2 m$^2$ lysimeter was determined to be 2.54 times greater ($K_c = 2.54$) than the crop coefficient previously reported for large-stand $T. latifolia$ wetlands ($K_c = 1.0$). The difference in the crop coefficient measured for the lysimeter used in this study and large-stand $T. latifolia$ wetlands supports the oasis effect described in previous studies. As evapotranspiration increases from 0 to 30 mm d$^{-1}$, the TIS model predicts that removal efficiency for conservative constituents is negatively affected (from 54.2 to 25.4% at 20-cm water depth), while removal efficiency for the readily treatable constituents is marginally enhanced (from 98.5% to 99.5% at 20-cm water depth). In addition, plant transpiration was shown to significantly ($p = 1.2 \times 10^{-8}$) enhance vertical transport of constituents through free water surface (FWS) CWTS hydrosoil. Results from the lysimeter study and TIS model may be applied to predicting differences in evapotranspiration and performance between pilot-scale and full-scale CWTSs and among CWTSs located in different climatic regions. Results from the vertical tracer tests demonstrate the importance of plant transpiration on vertical flow of
constituents in FWS CWTSs and verify that constituents can be transported vertically through the hydrosoil where redox-driven treatment reactions are known to occur.

5.5 Conclusion

Results from this study demonstrate that properly designed CWTSs are a viable treatment option for waters contaminated with ammonia. In addition, ammonia treatment performance by CWTSs can be enhanced by adding clinoptilolite to increase sorption capacity and nitrification activity. Ammonia treatment performance is not likely to change due to differing evapotranspiration water loss expected from scaling from pilot- to full-scale or building CWTSs in locations with different climates.
APPENDIX
EXPERIMENTAL PROTOCOL FOR TESTING AN AMMONIA SORPTIVE MATERIAL BY BATCH SORPTION TESTS

Alex Beebe, Jim Castle, John Rodgers, Scott Brame

1.0 INTRODUCTION

Sorptive materials may be used to remove constituents of concern from the water column during the remediation of contaminated waters. In the case of ammonia treatment using constructed wetland treatment systems (CWTSs), this transfer from the aqueous phase may enhance performance by concentrating ammonia in areas where nitrifying bacteria may be present. To determine the partitioning of dissolved ammonia in the presence of an ammonia sorptive material, a series of batch sorption experiments may be performed to plot a sorption isotherm. The resulting plot may then be used to estimate the amount of sorptive material needed when amending CWTSs.

2.0 OBJECTIVE

To determine the sorption isotherm for ammonia in the presence of a proprietary sorptive material.

3.0 MATERIALS AND METHODS

3.1 Serial Batch Sorption Experiment

The distribution between known amounts of the sorptive material and ammonia will be measured using a serial batch sorption experiment. To perform this experiment, 3g of the material will be added to 7 300mL BOD bottles along with different concentrations of an ammonium chloride solution. Table 1 shows the ammonium chloride loading concentrations to be used. These values were selected based upon the ranges of ammonium chloride to be loaded into a pilot scale CWTS and not the estimated sorption capacity of the sorptive material. As a result, a follow up experiment may need to be performed to increase data resolution. The bottles will then be sealed and placed in a dark area with a steady temperature for 7 days to allow equilibration to occur. After 7 days, the concentration of ammonia in the aqueous phase will be measured using a ion selective electrode (ISE) to determine the equilibrium concentration. Equilibration will be confirmed by sampling on the 8th day and any proceeding days as necessary.

3.2 Sorption Isotherm

The data retrieved from the serial batch experiment may then be used to plot a sorption isotherm. This is done by plotting the equilibrium ammonia solid mass fraction ($Q_e$) versus the equilibrium ammonia aqueous concentration ($C_e$). $Q_e$ is determined using the following equation:
Q_e = V_w(C_o-C_e)/M_s

where $V_w$ is equal to the volume of solution added to the BOD bottle, $C_o$ is equal to the initial concentration of ammonia added to the bottle, and $M_s$ is the mass of sorptive material added.

The resulting isotherm may then be used to perform future experiments involving ammonia portioning mass balances. If the isotherm shows little difference between equilibrium concentrations of ammonia in the 6 bottles, additional experiments may be performed using less mass of sorptive material.

Table 1 – Ammonia loading concentrations

<table>
<thead>
<tr>
<th>Bottle Number</th>
<th>Ammonia Concentration (mg/L)</th>
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</thead>
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</table>
EXPERIMENTAL PROTOCOL FOR TESTING AN AMMONIA SORPTIVE MATERIAL IN A PILOT SCALE CONSTRUCTED WETLAND TREATMENT SYSTEM

Alex Beebe, Jim Castle, John Rodgers

1.0 INTRODUCTION

Sorptive materials may be used to remove constituents of concern from the aqueous phase during renovation of contaminated waters. In the case of ammonia treatment using constructed wetland treatment systems (CWTSs), this transfer from the aqueous phase may alter performance by concentrating ammonia in areas where nitrifying bacteria may be present. Using data from a previous batch sorption experiment to determine loading amounts, the ability of a sorptive material to recruit and enhance nitrifying bacteria populations in an ammonia-treating pilot-scale CWTS will be examined by loading the material into a pair of ammonia-treating pilot-scale CWTSs and monitoring their performance in comparison to a pair of unaltered (control) pilot-scale CWTSs. In addition, the microbial nitrifying activity of the two pairs of systems will be compared using n-BART tests.

2.0 OBJECTIVE

To determine the cumulative effects of sorption and microbial nitrification when an ammonia sorptive material is added to an ammonia-treating pilot CWTS.

3.0 MATERIALS AND METHODS

3.1 Acclimation

Two pairs of CWTSs, with each CWTS consisting of a single 70-gal Rubbermaid® trough, or microcosm, will be constructed in the greenhouse. One pair will be planted with bulrush and the other will be planted with cattails (approximately 20 plants per microcosm). These four systems will each be connected to individual Fluid Metering Inc. (FMI) metering pumps to achieve a 96h hydraulic retention time of an influent ammonia solution containing 20mg/L ammonia-N. These systems will be monitored every two weeks to determine acclimation progress. Monitoring will include explanatory parameters including pH, dissolved oxygen, conductivity, alkalinity, hardness, and redox in addition to ammonia concentration. Acclimation will be achieved when differences in performance (ammonia removal) for each system are no longer significant (\( \alpha = 0.1 \)).

3.2 Sorptive material addition
Using data from a previous batch sorption experiment, the amount of sorptive material to be added to each experimental CWTS to achieve a removal goal may be calculated using a mass balance equation:

\[ B(q_e) = V(C_o - C_e) \]

where \( B \) is the mass of sorptive material to be added (g), \( q_e \) is the equilibrium mass fraction of the sorbed ammonia (mg/g), \( V \) is the volume of water to be treated (L), \( C_o \) is the initial concentration of ammonia in the system (mg/L), and \( C_e \) is the targeted treatment concentration (mg/L).

Using the initial ammonia concentration determined during the acclimation period, an average target effluent may be predicted using the mass balance. For the purposes of this experiment, the targeted equilibrium concentration will be 3mg/L and the volume of ammonia solution to be treated will be 360L (10 days supply of ammonia solution).

The sorptive material will be added to one system from each pair of systems. The other will remain unaltered to be used as a control for monitoring any changes that may be due to factors unrelated to the sorptive material addition.

### 3.3 Performance monitoring

Once the sorptive material is added, ammonia removal will be recorded every other day for 10 days. In addition, generic parameters will be measured every 5 days to detect changes that may influence performance or changes that relate to the addition of the sorptive material.

### 3.4 Performance comparison

The average effluent concentration of the system over the course of the 10 day treatment period will be calculated and compared with the predicted average effluent concentration. If the ammonia removal is greater than predicted, nitrifying bacteria may be colonizing the sorptive material and enhancing treatment. If the ammonia removal is lower than predicted, competitive sorption by other cations may be inhibiting the sorption of ammonia.

### 3.5 Microbial activity

50 days after introduction to the CWTSs, sorptive material and background material (hydrosol) collected from the CWTSs will be tested using n-BART kits to detect nitrifying bacteria activity. The nitrifying bacteria activity will be compared between the hydrosol and the sorptive material to determine if preferential colonization is occurring.
EXPERIMENTAL PROTOCOL FOR MEASURING PLANT TRANSPIRATION DRIVEN VERTICAL TRANSPORT OF DISSOLVED CONSTITUENTS USING A VERTICAL CONDUCTIVITY TRACER TEST

Alex Beebe, Jim Castle, John Rodgers, Scott Brame

1.0 INTRODUCTION

Transpiration can play a role in establishing a vertical hydraulic gradient within constructed wetland treatment system (CWTS) hydrosoil. A vertical hydraulic gradient can be a crucial component in initiating an advective flow path required for targeted constituents to reach the hydrosoil where certain redox reactions can occur (Martin et al., 2003). Transport of target constituents through aerobic and anaerobic zones of the hydrosoil by vertical flow driven by plant transpiration can play a substantial role in treatment pathways such as nitrification and denitrification (Brix and Schierup, 1989; Weisner et al., 1994; Martin and Reddy, 1997; Martin et al., 2003). Measuring the extent to which plant transpiration affects vertical flow will improve current conceptual models of CWTS flow regimes, allowing future design considerations to include pathways that exist in the hydrosoil.

2.0 OBJECTIVE

To compare vertical migration of a dissolved tracer between bench scale wetlands with and without cattails.

3.0 MATERIALS AND METHODS

3.1 Bench scale bucket preparation

The effects of cattail transpiration on vertical transport of dissolved constituents in CWTSs will be measured by performing vertical tracer tests using soil conductivity monitoring as a method to detect tracer arrival in both planted and unplanted bench scale CWTS buckets. To perform this experiment, six five-gallon buckets will each be filled with approximately 2 gallons of fluvial river sediment collected from 18-mile creek located near Clemson, SC and planted with approximately 5 cattails each. The buckets will be kept saturated with water and fertilized periodically to allow for maturation (period of approximately 6 months). After maturation is complete, surface water conductivity of each bucket will be measured using a calibrated field conductivity probe.

3.2 Tracer formulation

The conductivity data collected from each bucket will be used to formulate tracer solutions containing amounts of dissolved sodium chloride needed to yield conductivity readings ten times greater than the surface water from each bucket. This strength of tracer
solution will allow for an accurate resolution of tracer arrival time. If an accurate resolution of tracer arrival time cannot be measured, the strength of the tracer solution may be adjusted.

3.3 Preparing buckets for tracer testing

To prepare for the tracer tests, three of the six buckets will be trimmed of cattail foliage using a pair of garden shears. The remaining plant material (stems) must be left above the surface of the water to prevent osmotic circulation of water. The root systems must be left intact so that there are no unnatural differences in preferential flowpaths between the planted and trimmed buckets. Also, all surface water will be drained prior to the tracer test by inverting the buckets to prevent dilution of the tracer solution.

A pair of stainless steel tipped soil conductivity probes will be placed into each bucket prior to the tracer test with a vertical spacing of 2 inches with the upper electrode placed 2 inches below the surface of the hydrosoil. Initial conductivity readings will be made, and a stopwatch will be prepared for continuous measurements during the tracer test.

3.4 Tracer test

To begin a tracer test, prepared tracer solution will be added first by connecting a high flow-rate FMI pump (200mL/min) to the bucket. After 10 minutes, a second FMI pump (200mL/min) will be connected to the bucket to remove tracer solution from the bucket, allowing for recirculation of tracer solution. This method allows tracer solution to be added gently, preventing vertical flow disturbances.

Measurements of soil conductivity will be made every 5 minutes until elevated conductivity levels are detected for a minimum of 3 consecutive measurements in both conductivity probes. If an accurate resolution of tracer arrival time cannot be measured, the conductivity measurement interval may be adjusted. If the tracer is not detected within one hour, the measurement interval may be increased to 10 minutes.

3.5 Identification of flow-path alteration

If the mean arrival times of the 3 planted buckets are determined to be significantly lower than the 3 trimmed buckets by using a t-test with α=0.05, then cattail transpiration plays a role in enhancing vertical transport of constituents. In addition, a statistic comparison of the advective data using a t-test with α=0.05 will be made between the planted and trimmed buckets by comparing the mean difference in arrival times between the two conductivity probes for each set of buckets to determine if plant transpiration affects constituent velocity within the subsurface.

References Cited


METHOD FOR MEASURING GENERAL WATER QUALITY PARAMETERS: pH, DISSOLVED OXYGEN, CONDUCTIVITY, TEMPERATURE, ALKALINITY, AND HARDNESS

Brenda M. Johnson, Laura E. Ober, John H. Rodgers, Jr.

1.0 OBJECTIVE

The purpose of this protocol is to measure various general water quality parameters. Parameters such as pH, dissolved oxygen (DO), conductivity, temperature, alkalinity, and hardness are fundamental water quality parameters and are necessary for all water chemistry related studies.

2.0 HEALTH AND SAFETY

Proper lab attire, including scrubs, lab coat, gloves and safety glasses must be worn at all times.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with the equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 Required and Recommended Materials

4.1 Reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milli-Q water</td>
<td>all tests</td>
</tr>
<tr>
<td>pH buffers (4, 7, &amp; 10)</td>
<td>pH, alkalinity</td>
</tr>
<tr>
<td>0.02 N standard sulfuric acid solution (H2SO4)</td>
<td>alkalinity</td>
</tr>
<tr>
<td>Eriochrome Black T indicator</td>
<td>hardness</td>
</tr>
<tr>
<td>Standard EDTA titrant (0.01M, 0.02N)</td>
<td>hardness</td>
</tr>
<tr>
<td>Buffer solution (Reference Standard Methods 2340C)</td>
<td>hardness</td>
</tr>
</tbody>
</table>

4.2 Supplies

<table>
<thead>
<tr>
<th>Supply</th>
<th>Test:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graduated cylinder</td>
<td>alkalinity, hardness</td>
</tr>
<tr>
<td>100-mL beakers</td>
<td>all tests</td>
</tr>
<tr>
<td>Magnetic stir bar</td>
<td>alkalinity, hardness</td>
</tr>
</tbody>
</table>
50-mL buret and stand  
alkalinity, hardness

4.3 Equipment

Orion-model 420A pH Meter  
YSI 500 Dissolved Oxygen Meter  
YSI 30 Salinity, Conductivity, and Temperature Meter  
Magnetic stir plate

5.0 PROCEDURE

5.1 pH

2. Rinse probe with milli-Q water to remove any prior contaminant.  
3. Remove the small blue rubber stopper from the probe.  
4. Submerge the tip of the probe in the sample and gently stir the sample with the probe or use a magnetic stir-bar.  
5. When the pH meter beeps, record reading.  
6. Rinse probe with milli-Q water and return to holder.

5.2 Dissolved Oxygen (DO)/Temperature

1. Calibrate the YSI 500 Dissolved Oxygen Meter.  
2. Rinse probe with milli-Q water to remove any prior contaminant.  
3. Completely submerge the tip of the probe in the sample and turn on the mixer.  
   Note: If sample contains live organisms, do not use the mixer. Instead, gently stir the sample with the probe.  
4. When the DO meter beeps, record DO in mg/L (a “*” should also appear by the mg/L and the % symbol). Also record the Temperature to a tenth of a degree (i.e. 20.1°C).  
5. Rinse probe with milli-Q water and return to holder.

5.3 Conductivity

1. Turn on the YSI 30 Salinity, Conductivity, and Temperature Meter.  
2. Rinse probe with milli-Q water to remove any prior contaminant.  
3. Submerge the probe in the sample and gently stir the sample with the probe.  
4. When the conductivity reading has stabilized the conductivity. Conductivity will record in _S/cm (mS/cm) and temperature in degrees Celsius.
5. Rinse probe with milli-Q water and return to holder.
6. When finished turn off the meter.

5.4 Alkalinity

1. Using a graduated cylinder, measure 50mL of sample water and pour it into a 100mL beaker with a magnetic stir-bar.
2. Place sample beaker on magnetic stir-plate. Turn on stir-plate to begin mixing sample.
3. Calibrate pH meter. Place probe in the appropriate stand, with the tip completely submerged in the sample water. (Make sure the stir-bar does not hit the pH probe).
4. Record the initial level of titrant (0.02 N H2SO4) in the buret (fill buret as necessary).
5. Slowly drip titrant into the sample, allowing time for the pH meter to stabilize.
6. Titrate to pH 4.5.
7. Record the volume (mL) of titrant used to reach the pH endpoint (pH=4.5).
8. Calculate: Total Alkalinity (mg/L as CaCO3) = vol. titrant (mL) x 20
9. Turn off stir-plate and discard sample.

5.5 Hardness

1. Using a graduated cylinder, measure 50mL of sample water and pour it into a 100mL beaker with a magnetic stir-bar. (Dilutions can be made to conserve EDTA titrant, be sure to calculate dilutions into the final equation.)
2. Add 2–5 mL of buffer solution (to give the sample a pH of 10.0–10.1).
3. Add 2–4 drops of Eriochrome Black T Indicator. Sample should turn gold (deep yellow).
4. Place sample beaker on magnetic stir-plate. Turn on plate to mix sample.
5. Record the level of titrant (EDTA) in the buret (fill buret as necessary).
6. Slowly drip titrant into the sample, allowing time for the color change to stabilize.
7. Titrate until the gold turns to a bright yellow (very similar to pH buffer 7).
8. Record the volume of titrant (mL) used to reach the color change.
9. Calculate: Hardness (mg/L CaCO3) = volume titrant(mL) x 20
10. Turn off stir-plate and discard sample.

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.
METHOD FOR MEASURING OXIDATION-REDUCTION POTENTIAL OF HYDROSOIL IN A CWTS

Sarah E. Sundberg, Derek Eggert, J. Chris Arrington, John H. Rodgers, Jr., amended by Jennifer E. Horner

1.0 OBJECTIVE

Oxidation and reduction (redox) reactions mediate the behavior of many chemical constituents in wastewaters. The reactivities and nobilities of important elements in biological systems, as well as those of a number of other metallic elements, depend strongly on redox conditions. Like pH, Eh (redox) represents an intensity factor; it does not characterize the capacity of the system for oxidation or reduction. Measurements are made by potentiometric determination of electron activity (or intensity) with an inert indicator electrode and a suitable reference electrode. Electrodes made of platinum are most commonly used for Eh measurements. This protocol describes the method used to measure redox in the hydrosoil of a constructed wetland treatment system.

2.0 HEALTH AND SAFETY

Proper lab attire, including lab coat, gloves, and safety glasses must be worn at all times.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with the equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies

Potassium ferrocyanide, $K_4Fe(CN)_6 \cdot 3H_2O$
Potassium ferricyanide, $K_3Fe(CN)_6$
Potassium chloride, KCl

4.2 Equipment

pH or millivolt meter
Reference electrode
Oxidation-reduction indicator electrode
Beakers and magnetic stirrer
5.0 PROCEDURE

Prepare ZoBell’s standard redox solution by adding 1.4080 grams potassium ferrocyanide, 1.0975 grams potassium ferricyanide, and 7.4555 grams potassium chloride to 1000 mL of Milli-Q water at 25°C. These measurements must be as accurate as possible to result in a reliable solution. When stored in dark plastic bottles in a refrigerator, this solution is stable for several months.

Follow the manufacturer’s instructions for using the pH/millivolt meter and in preparing electrodes for use. Immerse the reference electrode connected to the millivolt meter and the redox indicator electrode (platinum tip end) in the gently stirred, standard solution in a beaker. Connect the millivolt meter to the end of the indicator electrode opposite the platinum tip. Allow several minutes for electrode equilibrium then record the reading to the nearest millivolt. If the reading is within ±10 mV from the theoretical redox standard value at 25°C (+183 mV), record the reading. The indicator electrode is ready for placement in the hydrosoil. If the reading is not within ±10 mV, the indicator electrode must be re-made.

In free-water surface microcosm place the indicator electrode’s platinum tip approximately four inches deep into the sediment making certain it is not near the plant roots. Secure the electrode with cable ties. In subsurface flow microcosms the indicator electrode’s platinum tip can be installed in a PVC casing to the midpoint of hydrosoil depth. Allow the electrode to equilibrate for 24 hours prior to taking any readings. To measure redox potential of the hydrosoil place the reference electrode approximately four inches deep into the hydrosoil in the subsurface flow microcosms or submerge completely in the water of the free-water surface microcosms. Be sure that the reference electrode is not placed directly next to the plant roots (this may be hard to avoid in the subsurface flow microcosms because of the advantageous root systems of Phragmites australis). Connect the millivolt reader to the end of the indicator electrode opposite the platinum tip. Record the redox potential in mV. Repeat a second time by placing the reference electrode in another location in the hydrosoil or water. Successive readings that vary less than ±10 mV over 10 minutes are adequate for most purposes. Adjust the reading according to field corrections and electrode calibration corrections.

Example: The field redox measurement of a hydrosoil was -206mV. When the electrode was initially calibrated in the lab, the redox reading was +193mV (which is +10mV difference from the theoretical redox standard value of +183mV). The field redox measurement must be corrected for this difference by subtracting 10mV from -206mV. This gives a redox measurement of -216mV. The standard correction factor for field redox measurements for the milli volt reader is +240mV. Therefore, this correction factor is added to the redox measurement of -216mV to yield a final redox measurement of +24mV.

\[ \text{Eh}_{\text{system}} = \text{Eh}_{\text{observed}} + \text{Eh}_{\text{reference standard}} - \text{Eh}_{\text{reference observed}} + \text{Eh}_{\text{field correction}} \]
\[ E_{h_{\text{system}}} = -206 \text{mV} + 183 \text{mV} - 193 \text{mV} + 240 \text{mV} \]

### 6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.

### 7.0 REFERENCES


METHOD FOR CALCULATING WASTEWATER FLOW RATES AND ADJUSTING WATER VOLUMES IN CONSTRUCTED WETLANDS FOR WASTEWATER TREATMENT BASED ON HYDRAULIC RETENTION TIMES

Sarah E. Sundberg, Derek Eggert, J. Chris Arrington, John H. Rodgers, Jr., amended by Jennifer E. Horner

1.0 OBJECTIVE

The hydraulic retention time (HRT) is the time it takes wastewater to flow through a constructed wetland treatment system by gravity flow. Accurate HRTs are necessary to ensure that the desired contact times of wastewater with sediment are being achieved. HRT can greatly influence the chemical, physical, and biological treatment processes occurring in the system to treat constituents in the wastewater. HRT is a function of water flow rate and water volume. Prior to setting the appropriate flow rates, it is necessary to adjust water volumes in the wetland microcosms to constant and known volumes. HRTs are chosen based on land constraints, wastewater flow rates, and costs at industrial sites where the wetland system will be constructed full-scale. This method describes how to efficiently adjust water volumes in wetland cells and calculate the necessary water flow rates based on desired HRTs. Common HRTs are 24-, 36-, or 48-hrs per wetland microcosm.

2.0 HEALTH AND SAFETY

Proper lab attire, including lab coat, gloves, and safety glasses must be worn at all times.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with the equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies

- Microcosms containing hydrosoil
- 5 gallon bucket

5.0 PROCEDURE
Based on the site requirements the HRT must first be decided upon and the initial water volumes of each wetland cell must be obtained. Fill the subsurface flow microcosms (already containing gravel hydrosoil) with water from a 5 gallon bucket while recording the amount of water needed to fill the microcosm. When water flows through the outflow elbow the microcosm is full. The volume of water for the free-water surface microcosms containing hydrosoil can be measured using the same method. The volume of water needed to fill the subsurface flow microcosms should be measured periodically and the flow rate adjusted to account for root growth and maturity (decrease in void volume). The water flow rate can then be calculated:

\[
\text{FlowRate (mL/min)} = \frac{\text{Volume (mL)}}{\text{HRT (min)}}
\]

Note: in this equation, water volume is given in mL and HRT is given in minutes

6.0 QUALITY CONTROL CHECKS AND ASSURANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.
1.0 OBJECTIVE

The objective of this standard operating procedure (SOP) is to clearly outline and define the requirements of loading for OPW to insure quality assurance and quality control measures.

2.0 HEALTH AND SAFETY

Proper personnel protective equipment will be worn at all times.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with the equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies

Hose
1000 gallon detention basin
Mixing pump
1000 mL beaker

5.0 PROCEDURE

Fill the detention basin to 250 gal and turn on the submersible mixing pump. Keep the hose and mixing pump running while adding the desired concentrations (formulated from target constituent concentrations) of salts. Dissolve salts in 500mL of water before adding to the detention basin. Continue to run the mixing pump throughout the loading of the CWTS to ensure that the O&G is continually mixed in the simulated OPW.

After the detention basin is adequately mixed the pumps to the CWTS can be turned on, the calibration of the pumps must be verified. This is completed one at a time by turning...
on the pumps, and measuring the collected volume in a 200mL graduated cylinder over two minutes. If this volume is different than 292mL (for the free-water surface series) and 184mL (for the subsurface flow series) then the pumps must be adjusted accordingly to achieve the flow rate of 146mL/min and 92mL/min, respectively. After the pumps are calibrated, the pumps may be turned on to pump the simulated OPW into the CWTS. Note: If the volume of water in microcosms is measured the HRT and flow rates need to be adjusted.

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.
METHOD FOR SAMPLING PETROLEUM PRODUCED WATER (PW) FROM A CONSTRUCTED WETLAND TREATMENT SYSTEM (CWTS) FOR MULTIPLE CHEMICAL ANALYSES

Brenda M. Johnson, Laura E. Ober, John H. Rodgers, Jr., amended by Jennifer E. Horner

1.0 OBJECTIVE

The objective of this standard operating procedure (SOP) is to clearly outline and define the requirements of aqueous sample collection of PW to ensure quality assurance and quality control measures.

2.0 HEALTH AND SAFETY

Proper personnel protective equipment will be worn at all times.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with the equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies

- Glass bottles (1000mL) with secured seal (screw top)
- Filter paper (0.45µm) and syringe
- Centrifuge tubes (50mL)
- Trace metal grade nitric acid (HNO₃)

5.0 PROCEDURE

Simulated OPW (loading predetermined) will be introduced into the pilot-scale constructed wetland treatment systems (CWTS) starting at approximately time-0 hrs from the detention basin (1000 gallon carboy). CWTS influent should be sampled from the plastic tube delivering simulated OPW to the first microcosm in series (1-2 L of water should be collected in glass containers depending on the volume of water needed for intended analyses). If metal analysis is needed collect additional water in a 50 mL centrifuge tube.
Water can be sampled along the flow path of the CWTSs at sampling ports (breaks in PVC pipes connecting microcosms). Water should be sampled after the first microcosm (microcosm A) 24 hours after the influent to the CWTS was sampled (assuming a 24-hr HRT per microcosm). Water should be sampled after the second microcosm (microcosm B) in series 48 hours after the influent was sampled, continue for microcosms C and D. Depending on intended analyses 1-2 L of water should be collected, in addition to a 50 mL centrifuge tube. Subsurface flow and free-water surface series can be sampled in the same way.

All water samples will be immediately transported to the Ecotoxicology laboratory in Lehotsky Hall, room 228, and prepared for analyses. Soluble metal preparation for ICP-AES analysis will be conducted by filtering 50 mL of sample water with a 0.45 µm membrane filter (Millipore MF 25mm) and syringe into a 50 mL centrifuge tube acidified with 0.5 mL (1% of sample water volume) trace metal grade nitric acid (11N•HNO₃). Centrifuge tubes intended for total and dissolved metals analysis with an ICP-AES will be checked for an adequate seal and analyzed within ≤ 6 months. The remaining sample will be divided into required volumes for analysis of water quality parameters, COD, BOD, O&G, TDS, and TSS (see individual methods) or refrigerated at 4oC until analyses can be conducted.

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.
METHOD FOR BIOLOGICAL ACTIVITY REACTION TEST (BART) FOR NITRIFYING AND DENITRIFYING BACTERIA

Yun Song, amended for determining soil nitrification activity by Alex Beebe

1.0 OBJECTIVE

Nitrifying bacteria can convert ammonium to nitrate, and the N-BART tests the activity of nitrifying bacteria by testing for the production of nitrate in water. Denitrifying bacteria reduce nitrate to nitrite and some continue converting nitrite to nitrogen gas (complete denitrification). The DN-Bart tests the activity of denitrifying bacteria by testing for the production of nitrogen gas.

2.0 HEALTH AND SAFETY

Proper lab attire, lab coat, gloves, and safety glasses must be worn at all times.

3.0 PERSONAL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with the equipment and laboratory techniques and trained in this reference SOP may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

N-BART test kit (HACH)
DN-BART test kit (HACH)
Pipette 500-2500 uL

5.0 PROCEDURES

5.1 N-BART:

1. Tear the wrapper off the N-BART and take out the reaction tube. Remove the inner tube from the outer tube.

2. Using the outer tube from the BART, collect a 20 mL water sample or a 3 mL sediment sample and 17 mL of MilliQ water.

3. Tightly screw the cap back on the inner tube. Return the inner tube to the outer tube and screw the outer tube cap tightly. Do not shake or swirl the tube.

4. Label the outer tube with the sample date and origin.
5. Place the assembled BART tube on its side away from direct sunlight for five days at room temperature.

7. After five days, return the tube to a vertical position. Remove the inner tube from the outer tube and replace the white cap from the inner tube with the reactor cap from the kit. Screw the reactor cap on tightly.

8. Invert tube for three minutes to allow the reagents in the reactor cap to mix with the solution. Return tube to a vertical position and replace to outer tube.

9. After three hours, compare the observed reactions on the reaction comparison chart.

5.2 DN-BART:

1. Remove the cap from the inner BART vial and place it on a clean surface.

2. Using the outer tube from the BART collect a 20 mL water sample.

3. Fill the inner tube with sample until the level reaches the fill line.

4. Tightly screw the cap back on the inner tube. Return the inner tube to the outer tube and screw the outer cap on tightly. Do not shake or swirl the tube.

5. Label the outer cap with the sample date and origin.

6. Place the BART tube away from direct sunlight and incubate at room temperature. Measure activity on a daily basis using the standard interpretation charts.

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by Quality Assurance Unit.
METHOD FOR MEASURING AQUEOUS AMMONIA CONCENTRATION IN WATER SAMPLES

Yun Song, D. Alexander Beebe, Laura E. Ober, Brenda M. Johnson, John H. Rodgers, Jr.

1.0 OBJECTIVE

Ammonia may be present in oil-field produced water at concentrations that present a risk to receiving systems. At pH values below the 9.25, ammonia exists primarily as a soluble ion, ammonium. At pH values above 9.25, ammonia exists primarily as free ammonia which will partition to the atmosphere. Using an ammonia ISE equipped with a hydrophobic membrane, the concentration of ammonia in a buffered solution (pH of 11) may be determined.

2.0 HEALTH AND SAFETY

Proper lab attire, including scrubs, lab coat, gloves and safety glasses must be worn at all times.

3.0 PERSONAL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with the equipment and laboratory techniques and trained in this reference SOP may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

Reagents ammonia stock standard 1000ppm (as N)
Ammonia Ion Strength adjuster solution (ISA)
Orion Model 95-12 electrode
150 mL beakers stir plate stir bar

5.0 PROCEDURE

5.1 Slope Check

1. Rinse all glassware with MilliQ water.

2. Warm samples to approximately 20 °C.

3. Rinse the ammonia probe with MilliQ water, gently wipe with a Kimwipe and place in the pH 4 buffer.

4. Plug probe into meter.
5. Press “Slope” to ensure the meter is clear. If a number appears, press “reset” to clear all stored data.

6. Put mode on Mv by pressing “Mode” until the red light appears next to Mv.
7. Press “0, Cal 1”

8. In a 150 mL beaker, add 100 mL of MilliQ water and 1.0mL 1000ppm ammonia stock standard.

9. Place the beaker on the stir plate and begin stirring with a stir bar without creating a vortex.

10. Rinse the probe, gently wipe, and place in the beaker.

11. Add 2.0 mL ISA solution to the beaker and press “read”.

12. Press “Cal 1” and then “Clear” when the reading stabilized.

13. Without removing the probe, add an additional 10 mL of the ammonia stock standard and press “Read”.

14. Wait for the numbers to stabilize. The reading should display -57.00=3. *Note : If the reading deviates considerably (<60 or >-50), soak the probe in pH 4 buffer for 10 minutes, redo the slope check, and refer to the trouble shooting section of ammonia probe users’ manual.

5.2 Calibration

1. Press “Clear”.

2. Rinse and wipe the ammonia probe before placing it in ph 4 buffer.

3. Rinse three 100 mL volumetric flasks and fill with approximately 85 mL MilliQ water. Label the flasks 20 ppm, 10 ppm, 1.0 ppm, and 0.1 ppm.

4. Prepare stock solution in concentration of 10 mg/L, 10 mg/L, 1.0 mg/L and 0.1mg/L in flasks by using 1000 ppm ammonia standard solution.

5. Change the mode of the meter to “Activity”.

6. Pour the 10 ppm solution into a rinsed beaker, and put the beaker with stir bar inside on stir plate.
7. Rinse the probe, wipe, and place in the beaker.

8. Add 2.0 ISA solution to the beaker and press “Read”.

9. Press “Cal 1” when number stabilizes.


11. Put beaker containing 10 ppm dilution on the stir plate with stir bar.


13. Put beaker containing 1.0 ppm dilution on the stir plate with stir bar.


15. Press “Clear”, then “Slope”. The number should read -57.00. If the reading deviates considerably ((<60 or >-50), check dilutions, check the troubleshooting section of ammonia probe users’ manual, and recalibrate.

5.3 Measuring samples

1. Warm up samples to approximately 20°C.

2. Rinse beaker with MilliQ water and add 100 mL of samples.

3. Place beaker on stir plate and stir without creating a vortex. Place probe in beaker.

4. Add 2.0 mL ISA to the sample and press “Read”

5. Record reading after number stabilizes.

6. Press “Clear”. Remove the probe, rinse, and wipe and place in the pH 4 buffer.

7. Repeat step 2-6 for each sample.

8. When samples are completed, rinse and wipe the probe. Place the probe in the ammonia stock standard and turn off equipment.

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by Quality Assurance Unit.
METHOD FOR MEASURING AQUEOUS NITRATE CONCENTRATION IN WATER SAMPLES

Yun Song, D. Alexander Beebe

1.0 OBJECTIVE

Nitrates in water can be a potential health risk, particularly to infants who have not yet developed a tolerance to nitrate. This method uses cadmium reduction to measure the concentration of nitration.

2.0 HEALTH AND SAFETY

Proper lab attire, including scrubs, lab coat, gloves and safety glasses must be worn at all times.

3.0 PERSONAL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with the equipment and laboratory techniques and trained in this referenced SOP may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

NitraVer 5 Nitrate Reagent
Nitrate standard 100 ppm
10 mL sample vials with caps
Pipette 500-2500uL
Test Tube Cooling Rack
Clean cuvette
Spectrophotometer
100 mL volumetric flasks

5.0 PROCEDURE

4.1 Calibration curve

1. Prepare dilute nitrate solutions (30 ppm, 20 ppm, 10 ppm, 5 ppm, 1 ppm) using nitrate standard solution, MilliQ water, and 100-mL volumetric flasks.

2. Add 10 mL of each standard to a separate sample vial. Prepare a separate vial with 10 mL of MilliQ water (blank).

3. Add the contents of one NitraVer 5 Nitrate reagent powder pillow to each vial and seal the cap.
4. Start the instrument timer with one-minute reaction time.

5. Shake the vial vigorously or use a vortexer until the timer expires.

6. When the timer expires, start timer again. A five-minute reaction period will begin.

7. When the timer expires, pipette the reacted blank to a cuvette and insert in spectrophotometer.

9. Set wavelength for spectrophotometer to 500 nm and zero the instrument by pressing the “0 absorbance” button.

10. Remove the blank cuvette from the spectrophotometer, pipette a reacted standard into clean cuvette, and insert it into the spectrometer.

11. Read and record the displayed absorbance.

12. Repeat steps 10 and 11 for all standards.

13. Turn off the spectrophotometer when finished with all measurements.

14. Plot the nitrate concentrations versus spectrophotometer readings in excel and fit the plotted data with a linear trend-line. The linear trend-line is the calibration curve.

5.2 Sample analysis

1. Repeat steps 2-11 using 10 mL aliquots of each sample.

2. Use the calibration curve and spectrometer readings to calculate sample nitrate concentrations.

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by Quality Assurance Unit.