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CONSTRUCTED WETLAND TREATMENT SYSTEMS FOR RISK MITIGATION OF ENERGY DERIVED WATERS

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CONSTRUCTED WETLAND TREATMENT SYSTEMS FOR RISK MITIGATION OF ENERGY DERIVED WATERS

A thesis
Presented to
The Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Wildlife and Fisheries Biology

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by
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Accepted by:
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ABSTRACT

Energy derived waters (EDWs) (e.g. petroleum refinery effluents, produced waters) can contain a variety of constituents [e.g. selenium (Se), arsenic (As), low molecular weight organics (LMWOs)]. The overall objective of this research was to provide an approach for remediation of specific constituents of concern in these waters and to measure a relationship between Se removal and abundance of Se reducing microbes. The specific objectives of this research were to: (1) evaluate removal of Se from simulated refinery effluent (SRE) using a pilot-scale constructed wetland treatment system (CWTS); (2) evaluate removal of Se, As, and LMWOs from a generic simulated fresh produced water (SFPW) using a pilot-scale CWTS; and (3) compare removal of Se from a SFPW to abundance of Se reducing microbes in a pilot-scale CWTS. Characterizations of each EDW (e.g. refinery effluent and produced water) were conducted in order to determine constituent concentrations and formulate simulated experimental EDWs for this research. An evaluation of the performance of a pilot-scale CWTS for removal of Se was conducted with pretreatment levels of 42-44 μg Se/L. Previous research indicated improved Se removal with addition of an organic carbon source; an outflow Se concentration goal of 5 μg/L was reached with this amendment. The concept of simultaneous constituent treatment of metalloids (e.g. Se, As) and LMWOs was evaluated. Pretreatment levels were approximately 50 μg Se/L, 20 μg As/L, and 25 mg LMWO/L with treatment goals of 5 μg Se/L, 5 μg As/L, and 1 mg LMWO/L, respectively. These goals were achieved for Se and LMWOs, but treatment of As was not sufficient to reach the goal of 5 μg As/L. Se removal rate coefficients and removal extents were calculated and measured, respectively, and compared to abundance of Se reducing microbes in pilot-scale CWTS sediment pore water. Relationships were calculated statistically by regression analysis and a parametric relationship was established.

DEDICATION

I dedicate this thesis to my wife, Amanda, who continuously keeps me motivated. Without her hard work and patience, this would have never been possible.

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CHAPTER ONE

INTRODUCTION

Energy derived waters (EDWs) are generated during fossil fuel extraction, energy production, and refining processes and contain a variety of elements and compounds, both inorganic and organic, which may pose risks to receiving aquatic system biota. Effluents from petroleum refining facilities and waters co-produced from oil and natural gas wells (i.e. produced waters) may contain metalloids (e.g. selenium and arsenic) as well as organic constituents [e.g. low molecular-weight aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (BTEX)] (Veil *et al*. 2004). Due to recent changes in environmental regulations implemented by the United States Environmental Protection Agency (USEPA) – National Pollutant Discharge Elimination System (NPDES), more stringent limits have been established for concentrations of constituents of concern (COCs) in water discharged from these facilities, including selenium, arsenic and organics (USEPA 2004, 2006). Initial characterizations of these specific waters are needed in order to discern ionic composition, ionic balance, COC concentrations, and potential use options. These data can be used to formulate simulated waters for experimentation as well as to develop treatment goals for further use of the treated waters.

EDWs can contain many COCs. However, metalloids [e.g. selenium (Se) and arsenic (As)] present unique challenges for treatment. Metalloids possess unique properties that can mimic both metals and non-metals, thus making treatment difficult. Further, treatment of Se and As to a designated permit level can be challenging due to speciation of these elements, the low discharge concentrations required, inconsistency and high cost of chemical treatment, disposal of sludge, and variation in aquatic parameters that control speciation and removal [e.g. dissolved oxygen concentration (DO), pH, conductivity, hardness, alkalinity, etc. (Pickett *et al*. 2006)].

Characterization data for these waters were compiled from a combination of (1) information provided from industry, federal and state agencies, (2) a search of peer-reviewed published literature, and (3) samples received at this laboratory for analysis (Chapters 2, 3, 4). The USEPA Water Quality Criteria (WQC) documents were used for comparison to constituent concentrations and use criteria in order to determine the degree of treatment needed for COCs in the specific water (USEPA 2004, 2006).

Constructed wetland treatment systems (CWTSs) offer a potential option to mitigate risks posed by untreated EDWs. Using COCs identified through initial characterization, CWTSs can be specifically designed to transfer and transform constituents in EDW to decrease concentrations of targeted COCs (Rodgers and Castle 2008). In order to investigate treatment pathways and parameters and provide proof of concept data, pilot-scale CWTSs are often used for experimentation. Pilot-scale CWTSs, while sufficiently small to enable control and manipulation of macrofeatures (e.g. hydroperiod, hydrosoil, and vegetation) and allow replication (Hawkins *et al*. 1997), are sufficiently large to accurately predict performance of full scale CWTSs in terms of COC removal rates and extents (Rodgers and Castle 2008; Dorman *et al*. 2009).

Previous research has been conducted on microbial activity and the ability of microbes to reduce metalloids (e.g. Se) to non-bioavailable species through metabolic processes (Maiers *et al*. 1988; Lortie *et al*. 1992; Tomei *et al*. 1995; Garbisu *et al*. 1996; Stolz and Oremland 1999; Ike *et al*. 2000; Zhang and Frankenberger 2005; Siddique *et al*. 2005, 2007; Zhang *et al*. 2008). However, a relationship between the abundance of Se reducing microbes and Se removal rates and extents (i.e. removal efficiency) in a pilot-scale CWTS could further scientific knowledge and understanding of the mechanisms (e.g. Se reductive pathways and fates) in CWTSs and imply a possible cost effective method of estimating Se removal performance in CWTSs.

This research investigates the composition of EDWs, potential for remediation of risks to receiving aquatic system biota through the use of CWTSs, and a possible relationship between removal of selenium and quantity of Se reducing microbes. This research had three major objectives:

1. Evaluate removal of selenium (Se) from simulated refinery effluent (SRE) using a pilot-scale constructed wetland treatment system (CWTS);
2. Evaluate removal of Se, arsenic (As), and low molecular weight organics (LMWOs) from simulated fresh produced water (SFPW) using a pilot-scale CWTS;
3. Compare Se removal rates and extents from SFPW in a pilot-scale CWTS to abundance of Se reducing microbes.

1. EVALUATE REMOVAL OF SELENIUM (SE) FROM SIMULATED REFINERY EFFLUENT (SRE) USING A PILOT-SCALE CONSTRUCTED WETLAND TREATMENT SYSTEM.

The purpose of this research was to determine the removal of Se from a simulated refinery effluent in a pilot-scale constructed wetland treatment system amended with organic carbon sources. The specific research objectives were: (1) to chemically and physically characterize an oil refinery effluent to confirm Se as a COC and to formulate simulated refinery effluent for experimentation; (2) to conduct bench-scale experiments to confirm Se removal in response to organic carbon additions; (3) to design and build pilot-scale CWTS using information from the bench-scale experiments; and (4) to measure the performance of the pilot-scale CWTS in terms of the rate and extent of Se removal and the effect of organic carbon inflow amendments.

2. EVALUATE REMOVAL OF SE, ARSENIC (AS), AND LOW MOLECULAR WEIGHT ORGANICS (LMWOS) FROM SIMULATED FRESH PRODUCED WATER (SFPW) USING A PILOT-SCALE CWTS.

The overall objective of this research was to design, build, and evaluate the performance of a pilot-scale CWTS for SFPW containing petroleum hydrocarbons and metalloids (e.g. Se and As). The specific objectives were: (1) to characterize a generic fresh produced water and determine targeted COCs based on surface water discharge limits; (2) to formulated a SFPW for experimentation; (3) to design and build a pilot-scale CWTS based on removal pathways for targeted constituents; (4) to measure performance in terms of rates and extents of removal of targeted constituents in response to aqueous amendments (e.g. organic carbon addition); and (5) to compare removal of COCs to discharge criteria.

3. COMPARE SE REMOVAL RATES AND EXTENTS FROM SFPW IN A PILOT-SCALE CWTS TO ABUNDANCE OF SE REDUCING MICROBES.

The overall objective of this research was to measure a relationship between removal of selenium from SFPW in a pilot-scale CWTS and the abundance of Se reducing microbes. The specific objectives were: (1) to measure Se removal rates and extents in response to organic carbon amendments; (2) to measure the abundance of Se reducing microbes; (3) to compare rates and extents of removal to abundance of Se reducing microbes; and (4) measure a relationship between Se removal and Se reducing microbe abundance.

SUMMARY

The goal of this research is to investigate specific constituents (e.g. Se, As, LMWOs) in EDWs and evaluate efficient and effective treatment options for mitigating risks associated with untreated EDWs in order to meet stringent discharge limits. A comprehensive understanding of the treatment possibilities and parameters involved in managing EDWs will allow not only full-scale application of these pilot-scale studies that can achieve targeted treatment goals, but enable efficient estimating of Se removal efficiency that can be combined with measurement of other explanatory parameters to aid in understanding the functional boundaries of a CWTS designed to treat Se to low levels.

This thesis is organized into chapters intended for publication (Chapters 2, 3, and 4) in peer-reviewed journals. Therefore, some of the introductory information and materials and methods are repeated. In Chapter 2, a method for increasing the rate and extent of selenium removal from a refinery effluent using a constructed wetland treatment system is evaluated. A potential strategy for renovating a fresh produced water targeting removal of selenium, arsenic, and low molecular weight organics to achieve stringent treatment goals is proposed in Chapter 3. A relationship between selenium removal in a constructed wetland treatment system and the abundance of Se reducing microbes is discerned in Chapter 4. Finally, the outcomes and potential applications for this research are summarized in Chapter 5.

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CHAPTER TWO

TREATMENT OF SELENIUM IN SIMULATED REFINERY EFFLUENT USING A PILOT-SCALE CONSTRUCTED WETLAND SYSTEM

1. INTRODUCTION

Selenium (Se) in petroleum refinery effluents, as well as other energy derived waters (EDWs), may pose risks to biota in receiving aquatic systems (Lawson and Macy 1995; Lemly 2004). Although Se is an essential micronutrient for basic cellular function (Zayed *et al*. 1998; Carlson *et al*. 2004), there is little difference between the required amount and the amount causing adverse effects (e.g. bioconcentration, toxicity) (Oremland 1994; Lemly 2004). Se can occur in petroleum refinery effluents in several oxidation states (VI, IV, 0, and -II) (Zhang *et al*. 2004) and in a variety of compounds and ionic forms such as: selenides (e.g. H_2Se, HSe^-), selenites (e.g. H_2SeO_3, $HSeO_3^-$, SeO_3^{-2}), and selenates (e.g. $HSeO_4^-$, SeO_4^{-2}) (Zhang and Moore 1996). Treatment of Se in petroleum effluents to a designated permit level [United States Environmental Protection Agency – National Pollutant Discharge Elimination System (USEPA 2004)] can be challenging due to speciation of this element, the low discharge concentrations required by permits, inconsistency and high cost of chemical treatment, disposal of sludge, and variation in parameters that control Se speciation and removal such as: dissolved oxygen concentration (DO), pH, conductivity, hardness, and alkalinity (Pickett *et al*. 2006). Because the form of Se can change in a given effluent, conventional treatment may be periodically successful but costly in terms of time, resources, and effort (Rodgers and Castle 2008). Constructed wetland treatment systems (CWTSs) are robust and may be an effective and efficient approach for consistently treating Se in complex matrices to low levels (Johnson *et al*. 2008; Rodgers and Castle 2008; Dorman *et al*. 2009).

CWTSs have been used for petroleum- and natural gas-derived waters (Johnson *et al*. 2008; Knight *et al*. 1999; Gillespie *et al*. 2000). The benefits of CWTSs can include: low cost of operation, low maintenance, effective treatment, solar energy driven, sustained effectiveness over time, tolerance of deviations in flow rate and contaminant load, and treatment of multiple constituents of concern (COCs) simultaneously and more effectively than some chemical or physical treatment processes (Bhamidimarri *et al*. 1991; Sundaravadivel and Vigneswaran 2001; Rodgers and Castle 2008; Dorman *et al*. 2009). Hybrid CWTSs that integrate other treatment methods (e.g. oil-water separators, reverse osmosis and granular activated carbon (GAC) systems) can provide effective water treatment, provided that COCs are successfully targeted through operative pathways (Murray Gulde *et al*. 2005; Johnson *et al*. 2008). CWTSs can be specifically designed based on targeted constituents in the refinery effluent and treatment pathways to transfer or transform those constituents (Hawkins *et al*. 1997; Gillespie *et al*. 1999, 2000). This research on refinery effluent provided an opportunity to evaluate the ability of an enhanced CWTS to reliably remove Se to stringent discharge limits (e.g. 5 μg Se /L).

The specific research objectives were: (1) to chemically and physically characterize the refinery effluent to confirm Se as a COC and to formulate simulated refinery effluent for experimentation; (2) to conduct bench-scale experiments to measure Se removal in response to organic carbon additions; (3) to design and build a pilot-scale

CWTS using information from the bench-scale experiments; and (4) to measure performance of a pilot-scale CWTS in terms of the rate and extent of Se removal from simulated refinery effluent and the effect of organic carbon treatments following a period of maturation and acclimation.

2. MATERIALS AND METHODS

2.1 EFFLUENT CHARACTERIZATION

Chemical and physical characterization of refinery effluent was required to determine the COCs for treatment as well as to measure the ionic composition of the water. Pretreatment effluent samples were shipped on ice in a cooler and received at the Clemson University Aquatic Toxicology Laboratory. Approximately 110 L of effluent were received and stored at 4°C (±1ºC) in preparation for analysis. This effluent was initially characterized using direct instrumentation and Standard Methods (Table 1) to measure several parameters (e.g. pH, conductivity, alkalinity, hardness, and Se concentration) (APHA 2005). Three subsamples of this effluent were analyzed by ion chromatography (IC) to determine the ion composition and ion charge balance. The effluent was examined by light microscopy to identify microorganisms associated with an observed green hue.

2.2 BENCH-SCALE EXPERIMENTS

A literature review provided information regarding an efficient pathway for Se treatment by addition of an organic carbon source to promote removal of soluble Se (selenate and selenite) from water through microbial reduction (Zawislanski *et al*. 2001; Zhang and Frankenberger 2005) in anaerobic aquatic environments (Maiers *et al*. 1988;

Siddique *et al*. 2007). A bench-scale experiment was designed to investigate responses of Se to organic carbon additions intended to increase growth or activity of heterotrophic microbes capable of dissimilatory Se reduction in an anaerobic environment. The rate and extent of removal of Se from refinery effluent was measured as a function of organic carbon or organic matter additions and the results were used to design the subsequent pilot-scale experiment.

To determine pathways and factors that could accomplish removal of Se from refinery effluent in a CWTS, bench-scale experiments were designed based on information from a literature review. In order to achieve effective removal of Se from this water, it was necessary to identify treatment pathways that are concordant with Se biogeochemistry. Methods utilizing Se volatilization have been studied (Hansen *et al*. 1998; Azaizeh *et al*. 2003; Van Huysen *et al*. 2004; Bañuelos and Lin 2005); however, displacing Se into the atmosphere is not a desired pathway as long-term effects of increasing atmospheric Se are not easily measured or monitored due to environmental variability (Zhang *et al*. 2002). Microbial activity, abundance of competitive electron acceptors, presence or absence of organic carbon, and environmental conditions (e.g. pH, alkalinity, hardness, temperature, etc.) can contribute to the rate and efficiency of Se reduction (Zhang and Frankenberger 2005). Because Se can be reduced and removed from solution by microbial pathways promoted by reduced organic carbon sources, these bench-scale experiments focused on enhancing microbial activity with four readily available carbon sources: sucrose (Dixie Crystals® Inc., Imperial Sugar®, Inc., Sugar

Land, TX), nutrient additive (AquaSmart^{TM}; Diamond V Mills$^{®}$, Cedar Rapids, IA), hay, and *Typha latifolia* Linnaeus detritus.

2.2.1 Static/Renewal Bench-Scale Experiment

Refinery effluent was used for the initial bench-scale experiment. This static renewal bench-scale experiment was conducted in an indoor laboratory environment using thirteen (13) 500 mL I-Chem$^{®}$ jars (referred to as "cells" for this initial bench-scale experiment) (treatments = 1 untreated control, 3 sucrose, 3 AquaSmart^{TM}, 3 hay, and 3 *T. latifolia* detritus). One hundred grams (100 g) of river sand (from 18-Mile Creek, Clemson, SC) were added to each cell as sediment, followed by 400 mL of refinery effluent. The untreated control received no organic carbon amendments. The sucrose treatments were 0.5 mL, 1.0 mL and 2.0 mL of 160 g/L sucrose solution to achieve concentrations of 200 mg/L, 400 mg/L, and 800 mg/L, respectively. The AquaSmart^{TM} treatments were 0.5 mL, 1.0 mL, and 2.0 mL of 160 g/L AquaSmart^{TM} solution to achieve concentrations of 200 mg/L, 400 mg/L, and 800 mg/L, respectively. The ground hay treatments and ground *T. latifolia* treatments were 2.5 g, 5.0 g, and 10.0 g of ground hay/*T. latifolia* to produce 2.5%, 5%, and 10% hay/sediment ratios (by mass), respectively. The hay and *T. latifolia* used in this experiment were dried and ground in a blender to a coarse powdery consistency. This experiment was conducted at room temperature (22ºC ±1ºC).

On the third day after initiation of the experiment (and every third day thereafter), a 100 mL sample of refinery effluent was removed from the water column of each cell with a 100 mL glass pipette and divided between two 50 mL Fisherbrand$^{®}$ centrifuge tubes. The contents of one 50 mL centrifuge tube were then acidified with trace metal grade nitric acid (Fisher Scientific®) prior to analysis by atomic absorption spectroscopy (hydride generation: Standard Method 3114 C) for total Se (APHA 2005). The water in the second 50 mL centrifuge tube was analyzed for DO and pH. After warming from 4°C to room temperature (22°C ±1ºC), 100 mL of refinery effluent was added to each cell with a 100 mL glass pipette. At each sampling, in addition to 100 mL of fresh, untreated refinery effluent, 0.125 mL, 0.25 mL and 0.5 mL of 160 g/L AquaSmart™ solution and 160 g/L sucrose solution were added, respectively, to the 200 mg/L, 400 mg/L and 800 mg/L AquaSmart™ and sucrose treatment cells. The untreated control cell, the hay treatment cells and the *T. latifolia* treatment cells received 100 mL of untreated refinery effluent.

2.2.2 Static Batch Bench-Scale Experiment

Refinery effluent was used for this bench-scale experiment in a manner similar to the previous bench-scale experiment. This bench-scale static batch reactor experiment was conducted in order to further determine factors or pathways to aid Se removal from refinery effluent and compare results to those obtained from the initial bench-scale experiment. The experimental design was similar to the previous static/renewal experiment: thirteen (13) 500 mL I-Chem® jars (referred to as “reactors” for this follow-up bench-scale experiment) with the same sediment and organic carbon additions. However, the refinery effluent was amended with additional sodium selenite (Na_2SeO_3) to increase the initial Se concentration to approximately 50 μg/L for this experiment. This experiment spanned eight days with no additional inputs of organic carbon or fresh, untreated refinery effluent. Samples of refinery effluent (10 mL) were collected from each reactor on days 2, 4, 6, and 8 and acidified with trace metal grade nitric acid (Fisher Scientific®) prior to analysis by atomic absorption spectroscopy (hydride generation: Standard Method 3114 C) for total Se (APHA 2005). The pH and DO were also measured on these days.

Defined as the percent decrease in aqueous Se concentration from experiment initiation to the experiment conclusion, the Se removal efficiency estimated from the static batch bench-scale experiment was calculated:

$$\textit{Removal efficiency}\ (\%) = \frac{[C]_o - [C]}{[C]_o} x100$$

Equation 1

where, $[C]_o$ is the initial Se concentration (μg/L) and $[C]$ is the final Se concentration at the conclusion of the experiment (μg/L). The removal rate coefficient (k) for Se was calculated using first order rate kinetics:

$$\textit{Removal rate coefficient (k)} = \frac{-\ln([C]/[C]_o)}{t}$$

Equation 2

where, $[C]_o$ is the initial Se concentration (μg/L), $[C]$ is the final Se concentration at the conclusion of the experiment (μg/L), and t is the time (days) from the experiment initiation until the experiment conclusion. The removal rate from the bench-scale experiment was used to scale the pilot-scale CWTS (i.e. to discern flow rate and hydraulic retention time).

2.3 PILOT-SCALE DESIGN AND CONSTRUCTION

Using information from the literature review and the bench-scale experiment, the pilot-scale CWTS was designed and built. While sufficiently small to facilitate control of environmental parameters (e.g. temperature, pH, flow rate, etc.) and allow replication, the pilot-scale CWTS contains the macrofeatures (e.g. hydrosoil, vegetation and hydroperiod) at sufficient scale to accurately predict performance of a full scale CWTS in terms of removal rates and extents (Rodgers and Castle 2008; Dorman *et al*. 2009). For this study, performance of the pilot-scale CWTS is defined as removal of soluble Se species from simulated refinery effluent (SRE) to less than 5 μg total Se/L. Since shipping costs for actual refinery waters would be prohibitive for a pilot-scale experiment, it was necessary to simulate the refinery effluent. As for the bench-scale experiment, removal parameters of interest in this pilot-scale experiment included the rate and extent of decline in Se concentrations after a period of maturation and acclimation as affected by organic carbon additions. Pretreatment concentrations of Se in SRE were approximately 50 μg Se/L, and the targeted mean outflow concentration was 5 μg Se/L.

The pilot-scale experiment was built and housed in a greenhouse with natural (i.e. solar) photoperiod and temperature regulation from 20 to 30°C. Twelve 378 L Rubbermaid® containers (121 cm long by 77 cm wide by 63 cm deep) were arranged in three series of four cells. The cells were connected by polyvinyl chloride (PVC) pipe fittings approximately 6 cm below the top of each Rubbermaid® container to allow gravity flow from each cell. The four cells in series provided sampling locations and prevented “short circuiting” of flow. To maintain a circumneutral pH (6.5 – 8) and increase alkalinity in the pilot-scale CWTS, 1000 g of ground oyster shells (98% $CaCO_3$

by weight) were added to each treatment cell. Osmocote® time released fertilizer (19-6-12) was added to provide essential nutrients (e.g. nitrogen, phosphorus, potassium) for the microbes and plants.

The untreated control series was designated Series C; the AquaSmart™ treatment series was designated Series A; the sucrose treatment series was designated Series S. Each cell was filled to a sediment depth of approximately 25 cm with river sand from 18-mile Creek in Clemson, SC, and subsequently planted with *T. latifolia* harvested from an aquaculture pond on the Clemson University campus. The cells were planted at a density of approximately 30 plants per cell. Three FMI®QG400 piston pumps (Fluid Metering®, Inc., Syosset, NY) were calibrated to deliver 128 mL SRE/min, to achieve a nominal 24 h hydraulic retention time (HRT) per cell, or 96 h per series. The pumps were calibrated using a 250 mL graduated cylinder and a stopwatch. The inflow concentrations of amendment solutions were 270 mg AquaSmart™ /L and 270 mg sucrose/L in the Series A inflow and Series S inflow, respectively. The AquaSmart™ and sucrose solutions were pumped from separate 19 L reservoirs which were renewed weekly (Figure 1).

2.4 PILOT-SCALE EXPERIMENT PERFORMANCE

SRE was loaded into the pilot-scale CWTS for two weeks prior to addition of organic carbon sources; sucrose and AquaSmart™ amendments were loaded into the pilot-scale CWTS inflow for one week to aid in acclimation of the system. SRE sample collection was then initiated. Samples were analyzed for total Se concentrations and general water chemistry parameters. To evaluate performance of the pilot-scale CWTS, Se concentrations were measured in the inflow and the outflow from each cell at sampling locations between each cell and at the final outflow of each series (i.e. cell 4). The performance of the pilot-scale CWTS, defined as the extent of Se removal (i.e. concentration decrease) from SRE at the outflow from the final cell (96-hr HRT), was measured by atomic absorption spectroscopy (hydride generation) using standard methods (APHA 2005). The rates of Se removal in each series were calculated. The Se removal data were analyzed for normal distribution; any significant differences in mean outflow concentrations between the untreated control and the experimental treatments were determined by one-way ANOVA and Least Significant Difference (LSD) or Tukey's test. All statistical analyses were conducted using SAS 9.1 (SAS Institute 2002).

3. RESULTS

3.1 PRETREATMENT EFFLUENT CHARACTERIZATION

The predominant cations in the pretreatment refinery effluent were sodium, calcium, magnesium and potassium; sulfate and chloride were the primary anions (Table 2). Se concentration was measured (by ion chromatography) in three pretreatment effluent subsamples by Davis & Floyd®, Inc. (Greenwood, SC) at an average concentration of 18 μg total Se/L (12-25 μg total Se/L) (Table 2). The pH of the pretreatment effluent was slightly above neutral with moderate alkalinity and hardness (Table 3). Examination by light microscopy revealed several genera of algae in the sample, including: green algae (*Ankistrodesmus* sp., *Chlorella* sp., and *Scenedesmus* sp.), diatoms (e.g. *Navicula* sp.), and Cyanobacteria (*Spirulina* sp.).

3.2 BENCH-SCALE EXPERIMENTS

3.2.1 Static/Renewal Bench-Scale Experiment

The static/renewal bench-scale experiment proceeded for 21 days in an indoor laboratory environment with a constant room temperature of 22ºC (±1ºC). For this experiment, the initial total Se concentration was 32 μg/L (Table 4). As determined by one-way ANOVA, LSD, and Tukey's test, Se removal in all treatments was significantly different from removal in the untreated control.

3.2.2 Static Batch Bench-Scale Experiment

In the second bench-scale experiment, the pretreatment concentration of Se in the refinery effluent was amended to ~50 μg/L. Se was removed in both the sucrose and the hay treatments to non-detectable levels (<1 μg/L) in 6 to 8 days (Table 5). Removal rate coefficients were greatest ($k \geq 0.367$ d^{-1}) in the sucrose and hay treatments. Based upon these bench-scale data, sucrose, AquaSmart^{TM} and *T. latifolia* were chosen for use in a pilot-scale CWTS experiment in order to evaluate effects of these carbon sources on Se removal.

3.3 PILOT-SCALE DESIGN AND CONSTRUCTION

The SRE was prepared in a 5678 L polypropylene carboy holding tank. Based on the results of analyses by ion chromatography (IC) (Table 2), the solutes used to formulate the SRE included: calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$) and sodium chloride (NaCl). Information provided by the refinery indicated that the form of Se in the site effluent was Se (IV); therefore, sodium selenite (Na_2SeO_3) was added to the SRE. The SRE was mixed for ≥24 hours with a submersible pump.

A set of working parameters from published literature was established for the pilot-scale CWTS in order to provide a suitable environment for dissimilatory Se reducing microbes. The following parameters were targeted in the pilot-scale CWTS: pH between 6.5 and 8.0, DO less than 2.0 mg/L, temperature greater than 10.0°C, and a source of organic carbon to serve as an electron donor; these conditions provided an environment for the microbial growth and activity necessary to remove Se from solution (Maiers *et al*. 1988; Pickett *et al*. 2006; Siddique *et al*. 2007; Zawislanski *et al*. 2001; Zhang and Frankenberger 2005).

During the initial phase of this experiment, a relationship between Se removal and pH became apparent. Se removal declined as pH decreased to less than 6.5. Therefore, it became important to maintain a stable circumneutral pH (6.5 – 8.0) throughout the pilot-scale CWTS. Sodium bicarbonate ($NaHCO_3$) was added to the SRE at a concentration of 5 mg/L to increase the pH to approximately 7.5 in the inflow and 1500 g of ground oyster shells (98% $CaCO_3$ by weight) were added to the treatment cells to maintain stable pH. Se removal rates and extents increased as a result of increasing and stabilizing pH in the system. Once the pH of the treatment cells stabilized, the goal of ≤ 5 μg total Se/L in the outflow water was achieved by the AquaSmart^{TM} amendment (with a 96 h HRT) [Fig. 2 (a, b, c)]

3.4 PILOT-SCALE EXPERIMENT PERFORMANCE

Se removal from SRE was achieved in a pilot-scale CWTS (Figures 2, 3, and 4) amended with an organic carbon source (e.g. AquaSmart^{TM}). The Se concentration in the pretreatment SRE ranged from 42-44 μg Se/L; the performance goal was ≤ 5 μg Se/L. During the reporting period, the pilot-scale CWTS outflow concentration, after a 96 h HRT, from the AquaSmart^{TM} treatment ranged from 3.4 to 8.5 μg Se/L with a mean outflow concentration of 5.5 μg Se/L and a mean removal efficiency of 87.1%. The pilot-scale performance goal of less than 5 μg Se/L in the pilot-scale final outflow was achieved. Both the AquaSmart^{TM} and sucrose treatment series achieved significantly greater ($\alpha = 0.05$) Se removal efficiency compared to the untreated control (Table 6).

4. DISCUSSION

The techniques used and results obtained in this study are applicable to many waters in addition to refinery effluent. Waters that contain elevated levels of Se include: effluents from coal-fired power plants equipped with flue gas desulfurization scrubbers (Mooney and Murray Gulde 2008), water produced from oil and natural gas wells (Johnson *et al*. 2008), coal mine drainage (Siddique *et al*. 2007), and agricultural drainage water (Siddique *et al*. 2005; Zhang *et al*. 2008)]. With effective treatment, these waters have the potential to be discharged to surface aquatic receiving systems or used for irrigation, livestock, groundwater recharge, and many other purposes (Rodgers and Castle 2008). Beneficial water use is especially desirable in arid regions and other areas where water is particularly scarce.

Several approaches to Se removal have been studied previously, including: phytoremediation (Zayed *et al*. 1998), biological volatilization (Hansen *et al*. 1998; Zayed *et al*. 1998), chemical treatment (Agnihotri *et al*. 1998), and bacterial/microbial reduction (Zhang and Frankenberger 2005; Zhang *et al*. 2008). Phytoremediation, biological volatilization, and chemical treatment have several disadvantages in contrast to microbial reductive processes in CWTSs. Biological volatilization can effectively remove Se from an effluent (Hansen *et al*. 1998; Zayed *et al*. 1998; Lin and Terry 2003);

however, transferring Se from water to the atmosphere does not necessarily reduce its bioavailability since the fate of airborne Se is unknown. Phytoremediation may be finite, and therefore undesirable as a long-term treatment alternative; sulfur compounds can inhibit plant uptake of their Se oxyanionic analogues (Zayed *et al*. 1998). In phytoremediation of Se, plant tissue saturation may limit achievable performance; therefore, in order to maintain an effective phytoremediation strategy, plants must be harvested up to several times per year to maintain desired performance (Cunningham and Ow 1996, deSouza *et al*. 1999). This maintenance can be expensive and does not avoid the issue of Se in harvested plant biomass. CWTSs designed to treat an inflow amended with an organic carbon source may provide a sustainable, stable, and cost effective long-term treatment alternative that can achieve rates and extents of Se removal by utilizing unimpeded pathways and consistently achieving targeted outflow COC (e.g. Se) concentrations (Hawkins *et al*. 1997; Gillespie *et al*. 1999, 2000; Knight *et al*. 1999; Sundaravadivel and Vigneswaran 2001; Rodgers and Castle 2008; Dorman *et al*. 2009). The rates of Se removal using conventional (i.e. chemical) or plant-driven technologies (e.g. biological volatilization and phytoremediation) are comparable to rates achieved in this study; however, the approach described in this study could be less costly, require less maintenance, and potentially could function indefinitely provided that an organic carbon source is available to facilitate microbial reductive pathways of Se.

With the advent of more stringent standards for discharge of Se into receiving aquatic systems, more efficient, reliable, and effective treatment alternatives are needed. CWTSs may provide cost effective and robust treatment for many of these discharges.

The benefits of CWTSs include low cost and low maintenance treatment; however, performance cannot be maintained without monitoring of functional or working parameters (e.g. pH, DO, etc.). Further research on CWTSs is being conducted currently at this laboratory for treatment of Se and other COCs [e.g. divalent metals (e.g. nickel, copper, zinc), arsenic, ammonia, oil and grease, 5-day biochemical oxygen demand (BOD_5)] found in energy related waters. The implications of this treatment strategy are far-reaching and have the potential to consistently and cost-effectively maintain an effluent below NPDES permit limits for Se discharge.

5. CONCLUSIONS

Se in a simulated refinery effluent was effectively treated in a pilot-scale CWTS. Bench-scale experiments provided data regarding rates and extents of Se removal for an effluent as affected by organic carbon amendments. The subsequent pilot-scale experiment was designed using information from the bench-scale experiments, and this experiment produced data that illustrated the feasibility of Se removal from SRE with implications regarding design of a full-scale CWTS. This approach achieved targeted Se outflow concentrations; thus, the pilot-scale data can be used, with appropriate scaling, to design and build a full-scale CWTS with organic carbon-amended inflow to achieve stringent discharge limits for Se (5 μg/L).

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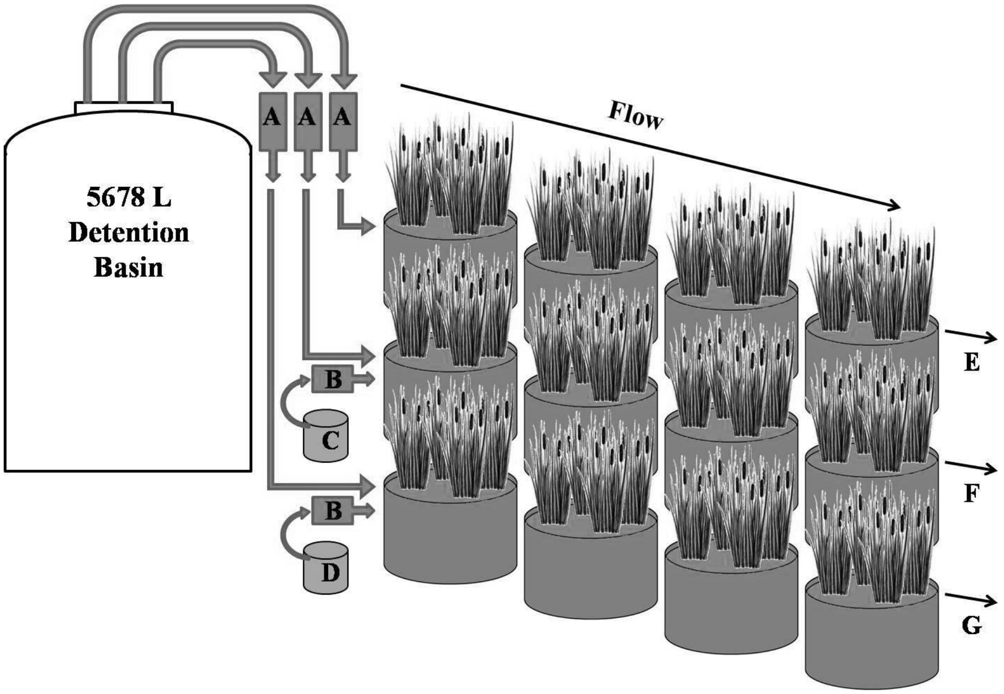

Legend

A = FMI® QG400 piston pumps

B = FMI® QG20 piston pumps

C = 35 g/L AquaSmart™ solution

D = 35 g/L sucrose solution

E = Untreated control (series C) outflow

F = AquaSmart™ treatment (series A)outflow

G = Sucrose treatment (series S) outflow

Figure 1 Schematic of pilot-scale experiment

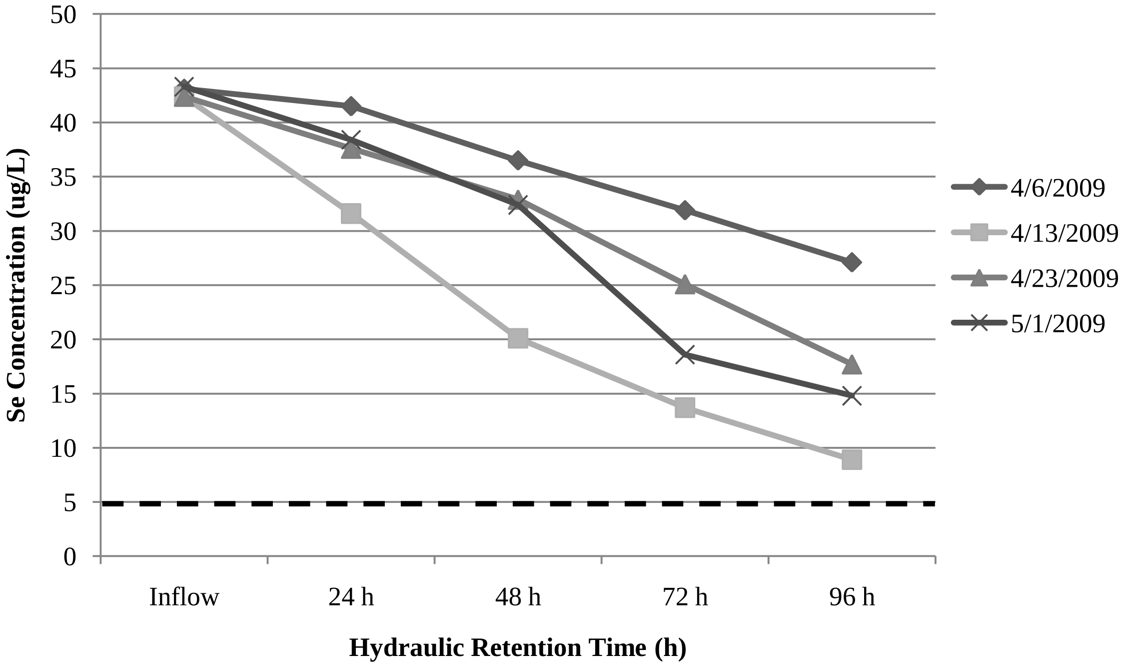

Figure 2 Removal of total selenium (μg/L) in the pilot-scale CWTS (untreated control) (dashed line = treatment goal of 5 μg Se/L)

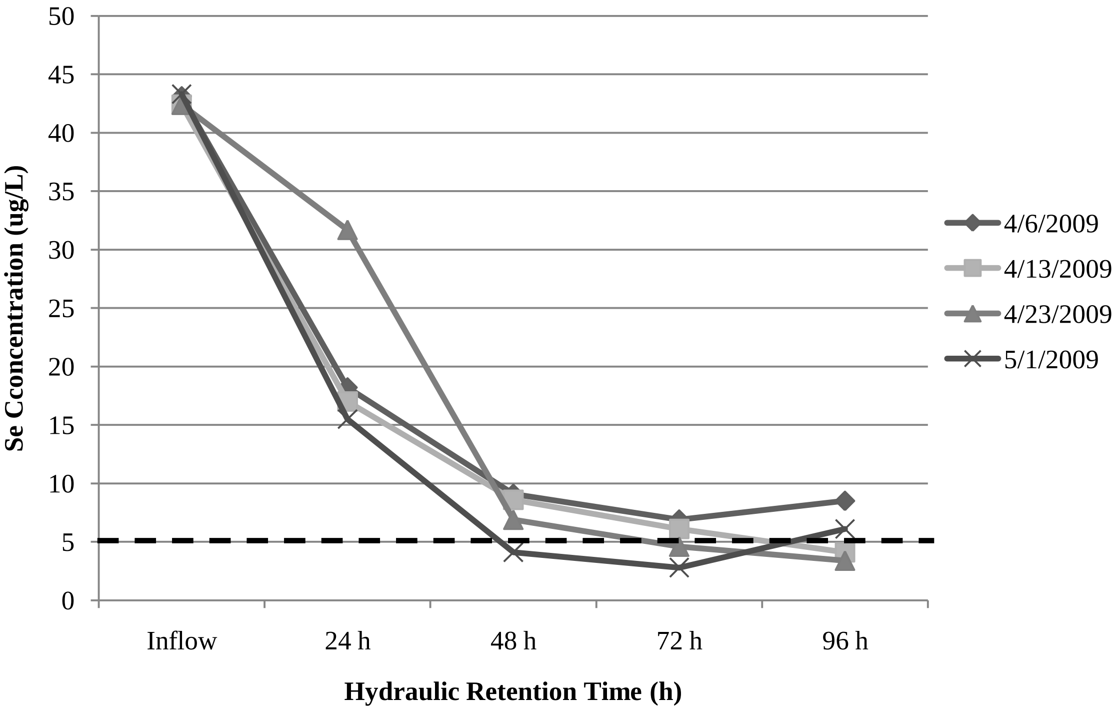

Figure 3 Removal of total selenium (μg/L) in the pilot-scale CWTS (AquaSmart^{TM}) (dashed line = treatment goal of 5 μg Se/L)

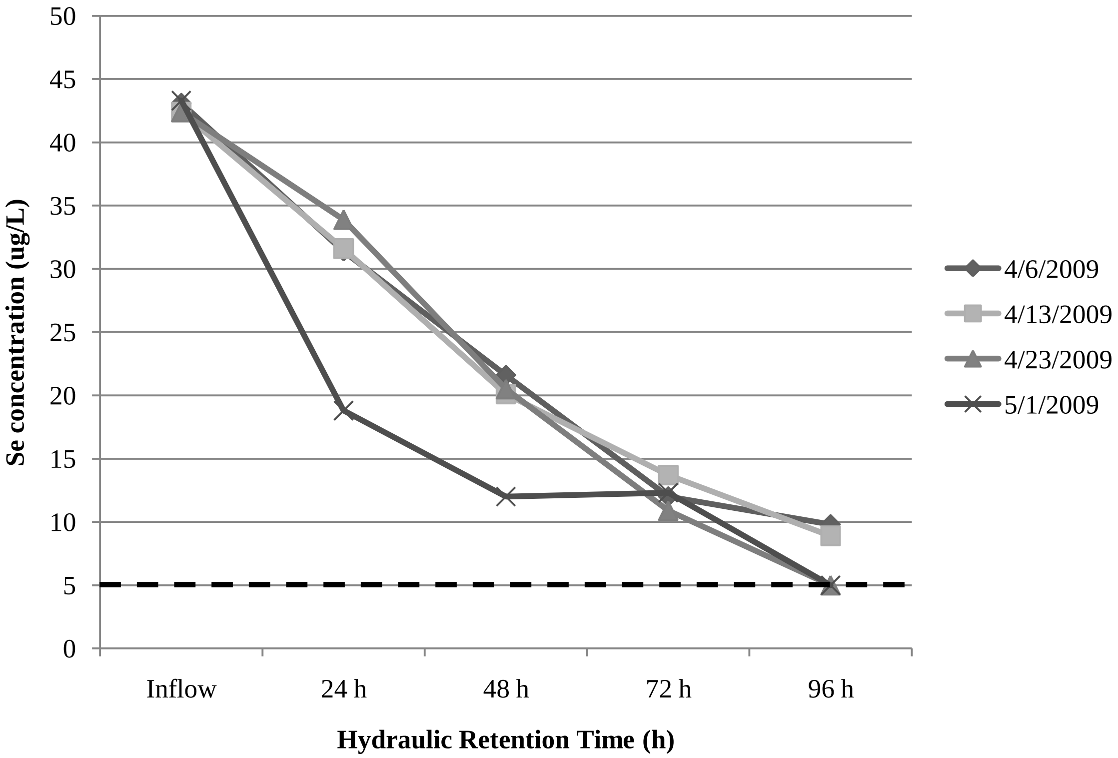

Figure 4 Removal of total selenium (μg/L) in the pilot-scale CWTS (sucrose) (dashed line = treatment goal of 5 μg Se/L)

Table 1 Analytical methods for parameters measured for refinery effluent samples

Parameter	Method	Detection limit
Se (Total)	Standard Methods*, 3500 B, 3114 C	1 μg/L
Cations	Ion Chromatography (EPA 200.7)	Varied**
Anions	Ion Chromatography (EPA 300.0)	Varied**
pH	Instrumentation, Orion® model 420A	0.01 S.U.
Dissolved oxygen	Instrumentation, YSI® 5000	0.1 mg/L
Conductivity	Instrumentation, YSI® 30	0.1 μS/cm
Alkalinity	Standard Methods*, 2320 B	2 mg/L as $CaCO_3$
Hardness	Standard Methods*, 2340 C	10 mg/L as $CaCO_3$

*(APHA 2005)

** See Table 2

Table 2 Constituents (mg/L) of refinery effluent as determined by ion chromatography[¥]

Trace metals (EPA 200.7)	Mean	Sample 1	Sample 2	Sample 3	Detection Limit
Aluminum (Total)	0.23	0.32	0.11	0.27	0.005
Antimony (Total)	-	0.005*	0.005*	0.005*	0.005
Arsenic (Total)	0.008	0.001	0.009	0.013	0.005
Barium (Total)	0.037	0.035	0.027	0.048	0.005
Beryllium (Total)	-	0.001*	0.001*	0.001*	0.001
Cadmium (Total)	-	0.002*	0.002*	0.002*	0.002
Calcium (Total)	43.1	36.7	39.7	52.6	1.0
Chromium (Total)	-	0.005*	0.041	0.009	0.005
Cobalt (Total)	-	0.020*	0.020*	0.020*	0.02
Copper (Total)	-	0.012	0.010*	0.011	0.010
Iron (Total)	0.56	0.75	0.23	0.69	0.020
Lead (Total)	-	0.002	0.002*	0.002*	0.002
Magnesium (Total)	21.6	19.3	20.8	24.6	0.05
Manganese (Total)	-	0.16	0.06	0.01*	0.01
Nickel (Total)	0.02	0.02	0.02	0.02	0.01
Potassium (Total)	19.1	17.6	18.2	21.6	1.0
Selenium (Total)	0.018	0.025	0.012	0.016	0.005
Silver (Total)	-	0.005*	0.005*	0.005*	0.005
Sodium (Total)	486	435	469	555	25.0
Thallium (Total)	-	0.002*	0.002*	0.002*	0.002
Tin (Total)	-	0.01*	0.01*	0.01*	0.01
Vanadium (Total)	-	0.02*	0.02*	0.02*	0.02
Zinc (Total)	0.04	0.04	0.03	0.04	0.01
Wet chemistry (EPA 300.0)					
Bromide (Total)	1.0	0.8	0.9	1.2	0.5
Chloride (Total)	387	350	370	440	50
Fluoride (Total)	2.0	1.9	2.1	2.0	0.20
Nitrate Nitrogen (as N)	15.6	16.6	15.6	16.6	0.5
Nitrite Nitrogen (as N)	-	0.1*	0.1*	0.1*	0.1
Sulfate	423	404	412	454	50

* Below detection limit; numbers reported are the detection limit

\- Mean incalculable

[¥] Analyses by Davis & Floyd®, Inc., Greenwood, SC 29649

Table 3 Values and concentrations for actual pretreatment refinery effluent parameters

Parameter	Value or concentration	Detection limit
pH*	7.60 SU	0.01 SU
Conductivity*	2460 μS/cm	0.1 μS/cm
Alkalinity**	78 mg/L (as $CaCO_3$)	2 mg/L (as $CaCO_3$)
Hardness**	168 mg/L (as $CaCO_3$)	10 mg/L (as $CaCO_3$)
Selenium (Total)**	16 μg/L	1 μg/L

* Measured by direct instrumentation
** Measured by Standard Methods (APHA 2005)

Table 4 DO, pH and Se concentrations for inflow, untreated control, sucrose treatment and AquaSmart^{TM} treatment at end of 21 day static/renewal bench-scale experiment

Amendment	DO (mg/L)*	pH (S.U.)*	Se (μg/L)**
Inflow	7.4	7.53	32.6
Control	6.2	7.03	3.7
Sucrose (0.2 g/L)	1.3	6.92	nd$^{¥}$
Sucrose (0.4 g/L)	1.4	5.88	nd$^{¥}$
Sucrose (0.8 g/L)	1.3	5.03	2.2
AquaSmart^{TM} (0.2 g/L)	2.1	7.15	1.4
AquaSmart^{TM} (0.4 g/L)	1.3	7.30	nd$^{¥}$
AquaSmart^{TM} (0.8 g/L)	0.6	7.54	1.1
Hay (2.5%)	0.9	6.65	nd$^{¥}$
Hay (5%)	0.8	6.62	nd$^{¥}$
Hay (10%)	0.9	5.62	1.2
T. latifolia (2.5%)	1.5	7.28	1.4
T. latifolia (5%)	1.3	7.10	1.1
T. latifolia (10%)	1.0	6.72	1.3

* Measured by direct instrumentation

**Measured by Standard Methods (APHA 2005)

$^{¥}$ nd = non-detect (<1 μg/L)

Table 5 Se concentrations (μg/L) for untreated control and organic carbon treatments for 8-day static batch reactor bench-scale experiment with an initial (Day 0) concentration of 47.3 μg Se/L

Treatment	Day 2	Day 4	Day 6	Day 8
Control	53.4	46.4	43.2	58.7
Sucrose (0.2 g/L)	20.3	12.3	5.0	nd*
Sucrose (0.4 g/L)	13.6	nd*	nd*	nd*
Sucrose (0.8 g/L)	11.3	nd*	nd*	nd*
AquaSmart^{TM} (0.2g/L)	32.9	27.6	19.0	12.2
AquaSmart^{TM} (0.4 g/L)	23.4	12.4	8.1	4.4
AquaSmart^{TM} (0.8 g/L)	19.6	9.9	4.9	3.7
Hay (2.5%)	15.7	nd*	nd*	nd*
Hay (5%)	12.4	nd*	nd*	nd*
Hay (10%)	18.4	nd*	nd*	nd*
T. latifolia (2.5%)	22.5	14.3	12.1	8.0
T. latifolia (5%)	28.1	16.5	11.0	7.8
T. latifolia (10%)	11.3	2.7	1.6	2.0

*nd = non-detect (<1 μg/L)

Table 6 Se removal efficiencies over four sampling periods after pilot-scale CWTS maturation and acclimation for the untreated control, AquaSmart^{TM} treatment, and sucrose treatment

Treatment	Range	Mean
Control (untreated)	37.1% - 79.0%	60.0%
AquaSmart^{TM}	80.3% - 92.0%	87.1%
Sucrose	79.0% - 88.5%	83.8%

CHAPTER THREE

PERFORMANCE OF A PILOT-SCALE CONSTRUCTED WETLAND TREATMENT SYSTEM FOR SELENIUM, ARSENIC, AND LOW MOLECULAR WEIGHT ORGANICS IN SIMULATED FRESH PRODUCED WATER

1. INTRODUCTION

Often generated in large quantities during petroleum extraction from geologic formations (Rice *et al*. 2000; McBeth *et al*. 2003a, 2003b; Patz *et al*. 2005), produced waters (PWs) contain both inorganic (e.g. metals, metalloids) and organic (e.g. low molecular weight hydrocarbons) constituents (Veil *et al*. 2004; Patz *et al*. 2005). Clark and Veil (2009) estimated that approximately 21 billion barrels (over 3.3 billion m^3) of PW was generated in 2007 from approximately 1 million active wells in the United States. PWs often contain elevated chloride concentrations (i.e. > 5000 mg Cl^-/L) which can limit treatment options due to the highly conservative nature of chloride. However, many PWs generated in the US would be considered fresh with chloride concentrations < 5000 mg Cl/L (Fillo and Evans 1990; Fillo et al. 1992; McBeth *et al*. 2003b; Xu *et al*. 2008). With depletion of global fresh water resources and increased consumptive water use, disposal of PW by reinjection may need to be reconsidered. Other options, such as surface water augmentation, can be pursued if constituents of PW are treated to achieve discharge criteria (USEPA 2004a). Constituents of concern (COCs) in some PWs can include selenium (Se), arsenic (As), and low molecular weight organics (LMWOs) (Hunter and Moser, 1990; Rice, 1999; Rice *et al*., 2000; Ramirez 2005; Orem *et* al. 2007; Thordsen *et al*. 2007; Singh 2010). While many PWs contain relatively low levels of these constituents (Rice *et al*. 2000), elevated levels have been found in many others

(Ramirez 2005; Orem *et al*. 2007). Removal of Se and As from aqueous media presents unique challenges since metalloids can mimic the properties of both metals and non-metals. Efficient and effective treatment systems are needed that can target these diverse constituents.

Constructed wetland treatment systems (CWTSs) have successfully treated a variety of COCs in similar complex mixtures with elevated COC concentrations to low levels (Murray Gulde *et al*. 2008; Johnson *et al*. 2008; Rodgers and Castle 2008; Spacil *et al*. in review). They have been used for treating petroleum and natural gas industrial waters (e.g. refinery effluents, produced waters) (Knight *et al*. 1999; Johnson *et al*. 2008) and can be specifically designed based on targeted constituents and treatment pathways to transfer or transform those constituents (Hawkins *et al*. 1997; Gillespie *et al*. 1999, 2000). Previous studies indicated that organic carbon amendments promote treatment of Se through microbial pathways (Maiers *et al*. 1988; Zawislanski *et al*. 2001; Ng *et al*. 2004; Zhang and Frankenberger 2005; Siddique *et al*. 2007; Spacil *et al*. in review) and that the presence of iron (Fe) promotes coprecipitation of As with Fe oxyhydroxides (Doyle and Otte 1997; Lièvremont *et al*. 2009). In addition to providing proof-of-concept data and improving potential stakeholder and regulatory confidence in performance, a pilot-scale CWTS using simulated fresh produced water (SFPW) can be effective for investigating treatment pathways and operative environmental parameters influencing ability to achieve targeted treatment goals.

The overall objective of this research was to design and build a pilot-scale CWTS for SFPW containing elevated levels of Se, As, and LMWOs and measure its performance. The specific objectives were: 1) characterize a fresh PW and determine targeted COCs relative to surface water discharge limits; 2) formulate a SFPW for experimental purposes; 3) design and build a pilot-scale CWTS for SFPW based on removal pathways for targeted constituents; and (4) measure performance in terms of targeted COC removal rates and extents in response to amendment additions.

2. MATERIALS AND METHODS

2.1 FRESH PRODUCED WATER CHARACTERIZATION AND DETERMINATION OF TARGETED COCs

Characteristics of PW were discerned by review of a variety of sources including samples received at this laboratory, presentations (Johnson *et al*. 2006), peer reviewed publications on produced waters (Johnson *et al*. 2008; Kanagy *et al*. 2008), publications by the United States Geological Survey (USGS) on coal bed methane produced water (CBMPW), as well as CBMPW water chemistry reports from the Colorado Oil and Gas Conservation Commission (COGCC), the Alabama Geological Survey (AGSA) on the Black Warrior Basin, and the Montana Groundwater Information Center (GWIC) on the northern Powder River Basin. A single database was constructed and analyzed for range and mean values for all constituents measured. This study targeted stringent treatment goals (e.g. 5 μg Se/L, 5 μg As/L, 1 mg LMWO/L) which meet or exceed present USEPA criteria for aquatic life in an effort to proactively meet lower discharge limits that may be imposed in the future (USEPA 2004).

2.2 SIMULATED FRESH PRODUCED WATER

For experimental purposes, formulation of SFPW is more economical and feasible than acquisition, shipment, and storage of large volumes of actual PW and allows greater repeatability and manipulation of specific water characteristics during a study. A SFPW was prepared in a 5678 L polypropylene carboy holding tank filled with municipal water from Clemson, SC. The SFPW, formulated using PW characterization information, was mixed with a submersible pump. Se and As were added to the SFPW as sodium selenite (Na_2SeO_3) and sodium arsenite ($NaAsO_2$), respectively. LMWOs were added as low sulfur diesel fuel to incorporate some of the same water soluble organic constituents (e.g. fluorene, naphthalene, anthracene, phenanthrene) found in PW (Orem *et al*. 2007).

2.3 PILOT-SCALE DESIGN AND CONSTRUCTION

The pilot-scale CWTS was specifically designed to incorporate pathways for treatment of Se, As, and LMWOs (e.g. diesel fuel) by microbial reduction, iron (Fe) coprecipitation, and biodegradation, respectively. These pathways were identified from a literature review targeting removal of these constituents from aqueous environments (Maiers *et al*. 1988; Hawkins *et al*. 1997; Gillespie *et al*. 2000, 1999; Zawislanski *et al*. 2001; Ng 2004; Zhang and Frankenberger 2005; Pickett *et al*. 2006; Siddique *et al*. 2007; Singh 2007). While sufficiently small to facilitate control of environmental parameters (e.g. temperature, pH, flow rate, etc.) and allow replication, the pilot-scale CWTS contains the macrofeatures (e.g. hydrosoil, vegetation and hydroperiod) at sufficient scale to accurately predict performance of a full scale CWTS in terms of removal rates and extents (Rodgers and Castle 2008; Dorman *et al*. 2009).

The pilot-scale experiment was built and housed in a greenhouse with natural (i.e. solar) photoperiod and temperature regulation from 20 to 30°C. Twelve 378 L Rubbermaid® containers (121 cm long by 77 cm wide by 63 cm deep) were arranged in three series of four cells (Figure 1). The cells were connected with polyvinyl chloride (PVC) pipe fittings approximately 6 cm below the top of each Rubbermaid® container to allow gravity flow from each cell. Each cell was filled to a sediment depth of approximately 25 cm with river sand from 18-mile Creek in Clemson, SC, and planted with *Typha latifolia* Linnaeus harvested from a Clemson University aquaculture pond. The cells were planted at a density of approximately 30 plants per cell. To maintain a circumneutral pH (6.5 – 8) and increase alkalinity, 1000 g of ground oyster shells (98% $CaCO_3$ by weight) were added to the sediments of each cell of the pilot-scale CWTS. To provide essential nutrients for the microbes and plants, 12 g of 19-6-12 Osmocote® fertilizer were added to the sediments in each cell to increase the nitrogen, phosphorus, and potassium concentrations. Three FMI® QG400 (Fluid Metering®, Inc., Syosset, NY) piston pumps were calibrated to deliver 128 mL/min of SFPW to achieve a nominal 24 hour (h) hydraulic retention time (HRT) per cell (i.e. 96 h per series). The pumps were calibrated using a 250 mL graduated cylinder and a stopwatch.

The two organic carbon amendments chosen for this study were sucrose (Dixie Crystals®, Imperial Sugar®, Inc., Sugar Land, TX) and a nutrient additive (AquaSmart™, Diamond V Mills®, Inc., Cedar Rapids, IA). The untreated control series, which was not amended, was designated Series C; the AquaSmart™ treatment series was designated Series A; the sucrose treatment series was designated Series S. After an initial stabilization period of approximately four weeks (i.e. new *T. latifolia* L. growth was observed), two additional piston pumps (FMI® QG20) were calibrated to deliver 1 mL/min of a stock solution of 35 g/L AquaSmart™ or a stock solution of 35 g/L sucrose into the inflows to treatment Series A and S, respectively. The amendment concentrations in the inflow were 270 mg/L AquaSmart™ and 270 mg/L sucrose in the series A and series S inflows, respectively. The AquaSmart™ and sucrose solutions were pumped from separate 19 L reservoirs that were renewed weekly (Fig. 1).

Samples were collected from the inflow and outflow of each cell of the pilot-scale CWTS at least once per month and analyzed for total Se, As and LMWO concentrations. Explanatory (i.e. water chemistry) parameters were measured at the time of sample collection. The following parameters were established for 1) microbial reduction of Se, 2) coprecipitation of As with Fe, and 3) biodegradation of LMWOs in the pilot-scale CWTS: pH range between 6.5 and 8.0, dissolved oxygen concentration (DO) less than 2.0 mg/L, sediment oxidation/reduction potential (redox) between -150 and -50 mV, temperature greater than 10.0°C, a source of organic carbon to serve as an electron donor for Se, and a source of reduced (elemental) Fe to serve as an As coprecipitant (Maiers *et al*. 1988; Hawkins *et al*. 1997; Gillespie *et al*. 2000, 1999; Zawislanski *et al*. 2001; Ng 2004; Zhang and Frankenberger 2005; Pickett *et al*. 2006; Siddique *et al*. 2007; Singh 2007).

2.4 PERFORMANCE OF PILOT-SCALE CWTS

SFPW samples from the pilot-scale CWTS were collected from the inflow and the outflow from each cell at sampling locations between each cell and at the final outflow of each series (i.e. cell 4) once per month for six months. To evaluate performance of the pilot-scale CWTS, Se, As, and LMWO concentrations were measured as well as general water chemistry parameters. The performance of the pilot-scale CWTS, defined as the rates and extents of Se, As, and LMWO removal from SFPW, was calculated from measurements made by: 1) hydride generation atomic absorption spectrometry using Standard Method 3114 C (for Se) (APHA 2005); 2) modified EPA method 200.9 (for As); and 3) spectrofluorometric analysis after liquid-liquid extraction with *n*-hexane (for LMWOs) modified from Zhou (2009). General water chemistry parameters were measured by direct instrumentation and standard methods (APHA 2005). Removal efficiency, defined as the mean percent decrease in COC concentration from pilot-scale CWTS inflow to final outflow, was calculated:

$$\textit{Removal efficiency}\ (\%) = \frac{[C]_o - [C]}{[C]_o} x100$$

Equation 1

where, $[C]_o$ is the initial inflow COC concentration and $[C]$ is the final outflow COC concentration. Removal rate coefficients (k) were calculated using first order rate kinetics:

$$\textit{Removal rate coefficient (k)} = \frac{-\ln([C]/[C]_o)}{t}$$

Equation 2

where, $[C]_o$ is the initial inflow COC concentration, $[C]$ is the final outflow COC concentration, and t is the time (days) from inflow to outflow. Mean removal rates were calculated for Se, As, and LMWOs.

The removal data were analyzed for normal distribution; any significant differences in mean outflow concentrations between the untreated control and the experimental treatments were determined by one-way analysis of variance (ANOVA) and Least Significant Difference (LSD) or Tukey's test. All statistical analyses were conducted using SAS 9.1 (SAS Institute 2002).

3. RESULTS

3.1 FRESH PRODUCED WATER CHARACTERIZATION

According to the characterization data, potential COCs identified for some PWs can include Se, As, and organic constituents (Table 1). Much of the fresh PW generated in the US originates from coal bed methane (CBM) wells, but depending on the location and geochemistry of the source formations, other types of PW may also be fresh. Water production rates from individual wells may vary greatly based upon drilling methods and activity, geologic location, and age of the well (Rice and Nuccio 2000; Veil *et al*. 2004; Benko and Drewes 2008). CBMPW production alone accounts for approximately 370,000 cubic meters per day (m^3/d) from the Powder River basin, approximately 42,000 m^3/d from the Uinta Basin, approximately 19,000 m^3/d from the Raton Basin, and approximately 27,000 m^3/d from the Black Warrior Basin (Rice and Nuccio 2000; Benko and Drewes 2008).

3.2 FORMULATION OF SIMULATED FRESH PRODUCED WATER

SFPW was formulated based on chemical characteristics of fresh PW (Table 1). The predominant cations in many fresh PWs are sodium, calcium, and magnesium; the predominant anions are chloride and sulfate. Based on results from the PW characterization, the solutes added to this water to formulate the SFPW included calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) at 205 mg/L, magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) at 355 mg/L, sodium chloride (NaCl) at 1230 mg/L, sodium selenite (Na_2SeO_3) at 0.109 mg/L, sodium arsenite ($NaAsO_2$) at 0.035 mg/L, and low sulfur diesel fuel at 25 mg/L. To simulate a fresh PW with elevated (i.e. greater than mean) levels of Se, As, and LMWOs, targeted concentrations of these COCs in SFPW were as follows: 50 μg/L Se, 20 μg/L As, and 25 mg/L LMWO. These concentrations of Se, As, and LMWOs fall within the range reported in Table 1 and by Orem *et al*. (2007), but were greater than the mean concentrations in the PW records used for this research.

3.3 PILOT-SCALE DESIGN AND CONSTRUCTION

SFPW was loaded into the pilot-scale CWTS during the initial stabilization period with no amendments. Sucrose and AquaSmart^{TM} amendments were then initiated to supply energy (i.e. electrons) to promote reductive processes and to acclimate the system to an increased organic carbon loading. Targeted outflow concentrations of these COCs were 5 μg/L Se, 5 μg/L As, and 1 mg/L LMWO, which are lower than USEPA standards, and allows the outflow water to be discharged to surface waters (USEPA 2004a). These targeted outflow concentrations are stringent; however, this study aims to achieve sufficient COC removal to proactively meet lower discharge permit limits that may be imposed in the future.

3.4 PILOT-SCALE EXPERIMENT PERFORMANCE

In the untreated control series, DO ranged between 7.9 and 8.9 mg O_2/L, pH between 6.4 and 7.1, redox between -209 and 112 mV, temperature between 13 and 25ºC, hardness between 126 and 138 mg/L as $CaCO_3$, and alkalinity between 22 and 34 mg/L as $CaCO_3$. In the AquaSmart^{TM} treatment, DO ranged between 0.9 and 7.7 mg O_2/L, pH between 6.4 and 7.1, redox between -158 and 68 mV, temperature between 14 and 25ºC, hardness between 126 and 138 mg/L as $CaCO_3$, and alkalinity between 34 and 78 mg/L as $CaCO_3$. For the sucrose treatment, DO ranged between 1.6 and 5.6 mg O_2/L, pH between 6.2 and 6.8, redox between -245 and -16 mV, temperature between 13 and 25ºC, hardness between 136 and 152 mg/L as $CaCO_3$, and alkalinity between 38 and 66 mg/L as $CaCO_3$.

With an inflow concentration between 42 and 76 μg Se /L, the performance goal of 5 μg Se /L in the pilot-scale CWTS outflow was consistently achieved (Figure 2) by the AquaSmart^{TM} treatment (Table 2) after a 48 h HRT. For the sucrose treatment, the performance goal of 5μg Se/L was achieved twice after a 96 h HRT and once after a 48 h HRT (Table 2). The untreated control did not achieve the targeted treatment goal at any time during the 6 month study period. With an inflow concentration of 14 to 22 μg As /L, the performance goal of 5 μg/L As was achieved once by each treatment and three times by the untreated control over the 6 month study period in the pilot-scale CWTS with no significant differences in As removal rates or extents attributed to the Fe addition (Table 3). With a targeted inflow concentration of 25 mg/L LMWO, the goal of 1 mg/L LMWO in the outflow water was achieved during every sampling period by all treatments, as well as the untreated control. This goal was accomplished in the detention basin prior to entering the pilot-scale CWTS (i.e. < 24 h HRT). The Se, As, and LMWO removal extents for each treatment series were calculated (Tables 2 and 3). Removal efficiencies for the AquaSmart^{TM} and sucrose treatments, as well as the untreated control, were calculated using *Equation 1*. The mean Se, As, and LMWO removal rate coefficients for each treatment series (i.e. 96 h HRT) were calculated using *Equation 2* (Table 4). The extents of Se and LMWO removal to meet treatment goals were achieved in the pilot-scale CWTS and the removal efficiency approached ≥99% for all LMWO treatments and the AquaSmart^{TM} Se treatment (Tables 2 and 4). The removal extent (Figure 3), efficiency, and rate coefficients for As were significantly lower ($\alpha = 0.05$) than those for Se and LMWOs (Tables 3 and 4).

4. DISCUSSION

This research demonstrated that removal of Se, As, and LMWOs from SFPW can be achieved in a specifically designed pilot-scale CWTS. The primary objective of this study was to remove Se, As, and LMWOs from SFPW with inflow concentrations of approximately 50 μg/L, 20 μg/L, and 25 mg/L, respectively. The performance goals for treatment of this SFPW were as follows: 5 μg Se/L, 5 μg As/L, and 1 mg LMWO/L. The COCs in this study (e.g. Se, As, LMWOs) were removed at varying efficiencies from aqueous phases in the pilot-scale CWTS (Table 4). Using scaling, this pilot-scale CWTS can provide information and data useful for design and construction of demonstration- or full-scale CWTSs or modification of existing CWTSs to meet stringent discharge limits (Rodgers 1994).

The techniques used in this study can be applied to many waters. Elevated levels (i.e. greater than discharge limits imposed by many NPDES permits) of Se, As, and/or LMWOs can be found in many energy derived waters [e.g. effluents from coal-fired power plants equipped with flue gas desulfurization scrubbers (Mooney and Murray Gulde 2008), petroleum refinery effluents (Lawson and Macy 1995; Hansen *et al*. 1998), coal mine drainage (Siddique *et al*. 2007), and agricultural drainage water (Lin and Terry 2003; Siddique *et al*. 2005; Zhang *et al*. 2008)]. The benefits of CWTSs can include: low cost of operation, low maintenance, effective treatment, solar energy driven, increased effectiveness over time, tolerance of deviations in flow rate and contaminant load, and treatment of multiple COCs simultaneously and more effectively than chemical or physical treatment processes (Bhamidimarri *et al*. 1991; Sundaravadivel and Vigneswaran 2001; Rodgers and Castle 2008; Dorman *et al*. 2009).

CWTSs specifically designed to treat COCs in a produced water may provide a sustainable, stable, and cost effective long-term treatment alternative that can increase or improve rates and extents of COC removal by utilizing potentially unimpeded pathways and consistently achieving targeted outflow concentrations (Hawkins *et al*. 1997; Knight *et al*. 1999; Gillespie *et al*. 1999, 2000; Sundaravadivel and Vigneswaran 2001; Rodgers and Castle 2008; Dorman *et al*. 2009). The rates of Se removal using conventional [i.e. chemical, biochemical (General Electric, Inc.)] or plant-driven technologies [e.g. biological volatilization, phytoremediation (Hansen *et al*. 1998; Zayed *et al*. 1998; de Souza *et al*. 1999; Lin and Terry 2003)] are slower than or comparable to rates found in this study; however, the technology described in this study can be less costly and could potentially function indefinitely provided that an organic carbon source is available to facilitate microbial reductive pathways of Se.

The rate and extent of As removal were less than those for Se removal. To achieve a goal of 5 μg As/L, the pilot-scale CWTS design may need revision that would allow sequential treatment of As and Se in lieu of simultaneous treatment. Since no significant differences in As treatment were apparent between the experimental treatments and untreated control (Figure 3), an As-specific design could be placed upstream of a Se-specific design which would permit sequential removal utilizing different pathways which require different conditions (e.g. redox). In the presence of Fe under oxidizing conditions (i.e. redox > +50 mV) in an aquatic system, which could precipitate Fe oxyhydroxides in the water column, As could become bound to Fe oxyhydroxides, thus decreasing aqueous As concentrations (Doyle and Otte 1997). Therefore, a series of pilot-scale CWTS cells could be designed for oxidizing conditions in the presence of Fe for treatment of As, followed by a series of reducing cells for treatment of Se through microbial reductive pathways aided by an organic carbon amendment. Due to the lack of recovery of a significant (i.e. detectable) fraction of LMWOs and a pungent diesel odor emanating from the detention basin, the LMWOs were thought to have volatilized from the detention basin prior to entering the first cell of the pilot-scale CWTS.

Several other performance parameters require consideration in a CWTS designed to treat metalloids using biogeochemical pathways promoted by specific amendments (e.g. organic carbon). Some of these parameters include: 5-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), sediment toxicity, effluent toxicity, as well as metalloid precipitation and accretion rates. Further research is needed to evaluate these parameters related to improved Se treatment by organic carbon amendment.

Efficient, reliable, and effective Se and As treatment alternatives are needed to meet stringent discharge standards for aquatic receiving systems. CWTSs may provide cost effective and robust treatment for many of these discharges. Further research on CWTSs is ongoing to seek efficient, effective, and sustainable treatment of other metalloid containing waters. The implications of this treatment for fresh PW are far-reaching and not only have the potential to prevent excess aqueous metalloid discharge into aquatic receiving systems, but also could yield a new, relatively untapped fresh water resource.

5. CONCLUSIONS

Se and LMWOs can be efficiently and effectively removed from SFPW using a pilot-scale CWTS. A literature review provided evidence regarding pathways for treatment of metalloid constituents and organic fractions in water. A pilot-scale CWTS was designed and built, and this design was successful for targeting and removing aqueous Se and LMWOs. The goal of 5 μg Se/L was achieved by the AquaSmart^{TM} treatment and the goal of 1 mg LMWO/L was reached in the detention basin (including the untreated control), the latter of which implies that a significant fraction (> 99%) of LMWOs. The goal of 5 μg As/L was not consistently achieved; however, 50 to 65% removal efficiencies were consistently achieved by each series of the pilot-scale CWTS. This approach developed the design elements of a full-scale CWTS to decrease Se and LMWO concentrations in fresh PWs with elevated Se and LMWO levels brought to the surface through extraction of petroleum resources. This pilot-scale study can be used, with scaling, to proceed to a demonstration- or full-scale CWTS to assess performance.

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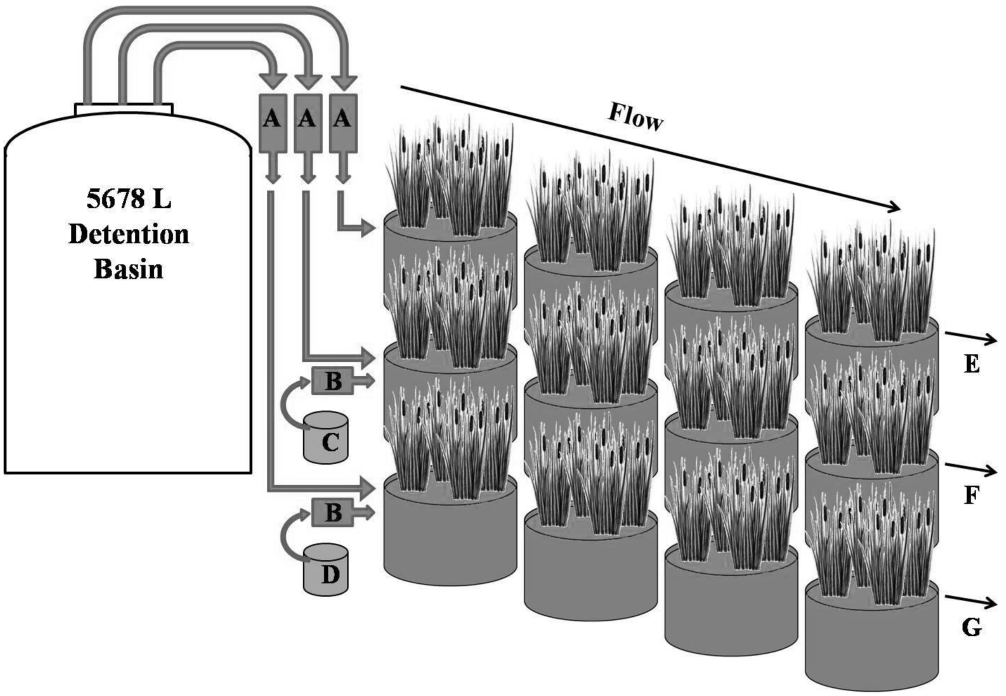

Legend

A = FMI$^{\circledR}$ QG400 piston pumps
B = FMI$^{\circledR}$ QG20 piston pumps
C = 35 g/L AquaSmart$^{\mathrm{TM}}$ solution
D = 35 g/L sucrose solution

E = Untreated control (series C) outflow
F = AquaSmart$^{\mathrm{TM}}$ treatment (series A)outflow
G = Sucrose treatment (series S) outflow

Figure 1 Schematic of pilot-scale experiment

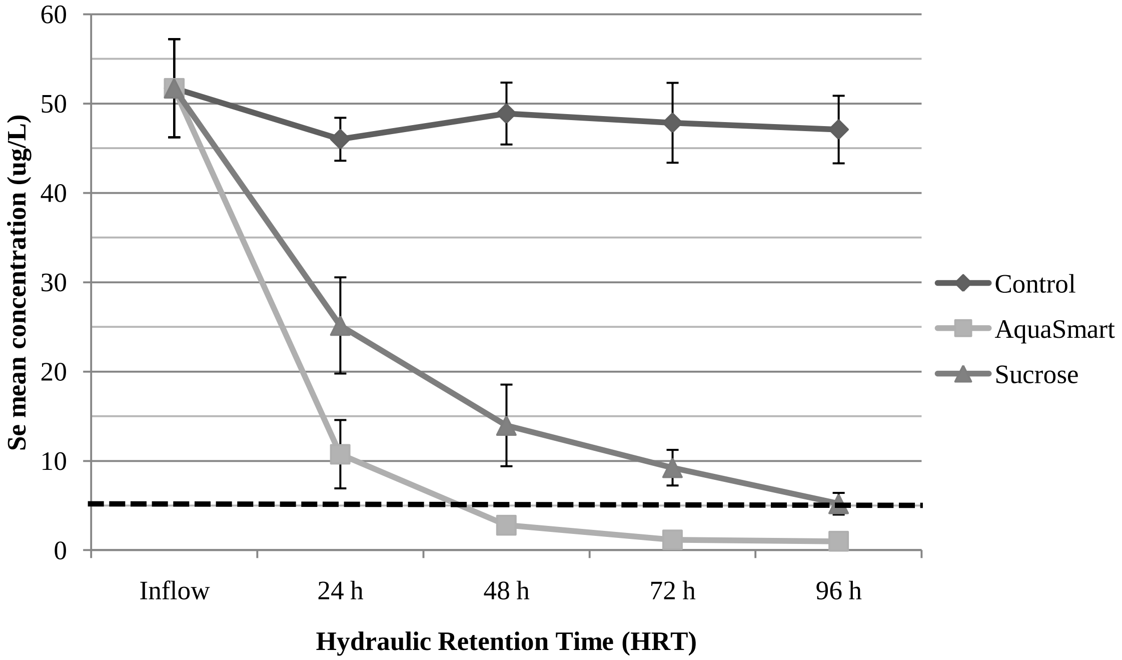

Figure 2 Comparison of mean Se removal between two organic carbon amended treatments and an untreated control at 24 hour intervals (i.e. each cell) in the pilot-scale CWTS over a six month study period (dashed line: targeted outflow concentration of 5 μg/L Se; errors bars denote std dev)

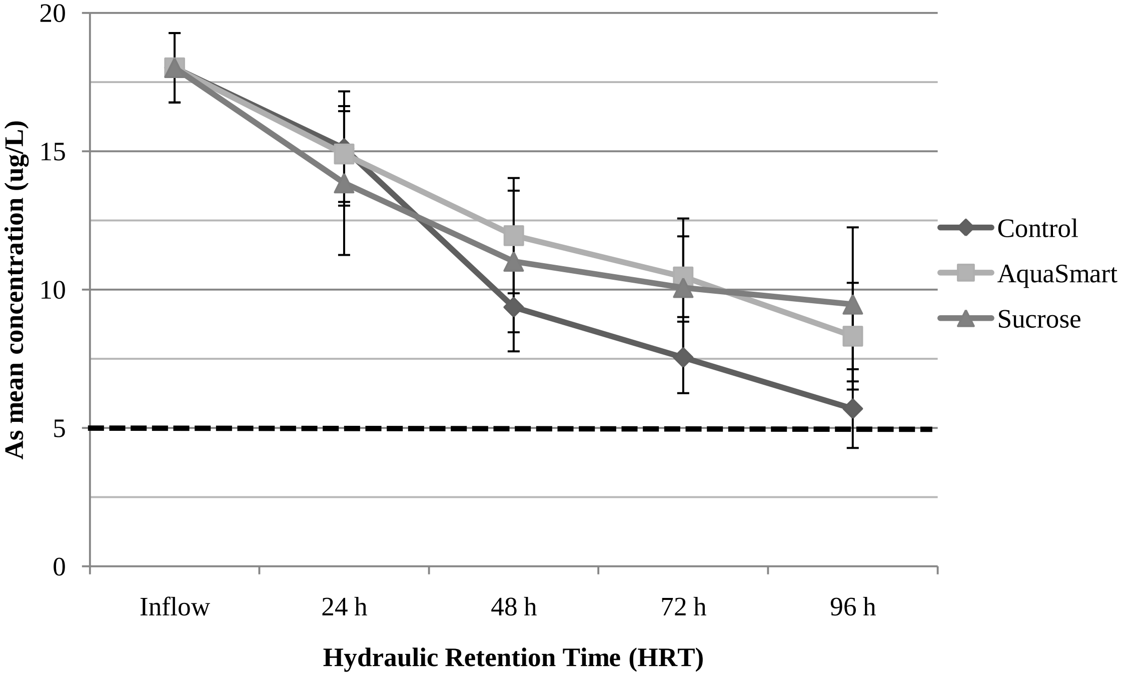

Figure 3 Comparison of mean As removal between two organic carbon amended treatments and an untreated control at 24 hour intervals (i.e. each cell) in the pilot-scale CWTS over a six month study period (dashed line: targeted outflow concentration of 5 μg/L As; error bars denote std dev)

Table 1 Summary statistics for concentrations of constituents in fresh PWs (i.e. < 5000 mg Cl^-/L)

Constituent	Units	Range	Mean	Standard Deviation	n
pH[1]	S.U.	6.56-9.87	-	-	375
Temperature[1]	ºC	1.2-41.7	18.7	6.3	299
Conductivity[1]	μS/cm	95-145000	4422	9792	324
Total Dissolved Solids[1]	mg/L	270.2-114000	5197	10400	168
Alkalinity[1]	mg/L as $CaCO_3$	54-9450	1858	1974	324
Ba[2]	mg/L	<0.01-190	7.0	21.5	41
Br[2]	mg/L	<0.002-300	37.8	66.5	41
Ca[2]	mg/L	0.8-5870	54	328	213
Cl[2, 1]	mg/L	0.7-4680	405	875	261
F[2]	mg/L	<0.05-15.22	2.5	2.3	272
Fe[2]	mg/L	<0.002-220	2.7	15.9	306
K[2]	mg/L	0.3-186	10.6	14.8	315
Mg[2]	mg/L	0.2-1830	34.6	119	356
Na[2]	mg/L	8.8-34100	1180	2430	356
Si[2]	mg/L	<0.1-49.8	10	5.5	356
Sr[2]	mg/L	0.032-565	16.3	124	356
Sodium Absorption Ratio[1]	-	5.7-32	11.7	7.3	358
Ammonia[2]	mg/L	1.05-59	3.5	8.2	375
Nitrate[6]	mg/L	<0.002-18.7	1.2	2.4	97
Phosphate[6]	mg/L	<0.050-1.5	0.2	0.4	232
Sulfate[6]	mg/L	<0.01-5590	335	656	49
Total Organic Carbon[1]	mg/L	1-100	10.7	12	174
Ag[3]	μg/L	<0.5-375	63	88	368
Al[3]	μg/L	0.2-1240	201	269	1
As[2, 3]	μg/L	0.1-614.6	14.7	75.6	51
B[3]	μg/L	1.6-2400	151	244	41
Be[3]	μg/L	nd* - <0.1	<0.1	-	1
Bi[3]	μg/L	19-32	24.5	3.4	1
Cd[3]	μg/L	<0.1-10	4.8	3.3	1
Ce[3]	μg/L	nd* - <10.0	<10.0	-	1
Co[3]	μg/L	<0.1-0.729	0.2	0.1	254
Cr[3]	μg/L	<1.0-53	11.5	12.8	205
Cs[3]	μg/L	<0.1-0.78	0.2	0.2	195
Cu[3]	μg/L	<0.2-60	8.8	8.7	246
Hg[5]	μg/L	<0.005-0.4	0.2	0.1	118
La[3]	μg/L	nd* - <10.0	<10.0	-	47
Li[3]	μg/L	0.21-6880	146	439	259
Mn[3]	μg/L	<2.0-5400	59.1	322	47
Mo[3]	μg/L	<0.5-100	28.8	25.6	105
Ni[3]	μg/L	0.304-203	19.7	30.6	259
P[3]	μg/L	<50-94	84	9.8	47

Table 1 (Cont.)

Constituent	Units	Range	Mean	Standard Deviation	n
Pb[3]	μg/L	<0.1-585	118	132	255
Rb[3]	μg/L	4.1-38.2	11.8	6.1	67
Sb[3]	μg/L	<0.1-950	575	530	47
Sc[3]	μg/L	<0.1-3	1.3	0.7	258
Se[2, 4]	μg/L	<0.1-73	11.6	20.0	358
Sn[3]	μg/L	<0.1-680	90	146	143
Th[3]	μg/L	nd* - <20.0	<20.0	-	206
Ti[3]	μg/L	<1.0-45	12.7	9.9	47
Tl[3]	μg/L	<0.1-0.34	0.3	-	237
U[3]	μg/L	<0.5-50	12.8	16.9	47
V[3]	μg/L	0.19-59	13.2	11.7	120
W[3]	μg/L	nd* - <20.0	<20.0	-	47
Y[3]	μg/L	nd *- <10.0	<10.0	-	192
Zn[3]	μg/L	0.02-590	37.0	73.4	85
Zr[3]	μg/L	0.21-131	27.7	33.2	47

*nd – non-detect

[1] Measured by Standard Methods (Rice *et al*., 2000; Johnson *et al*. 2008)

[2] Measured by ICP-AES (Rice *et al*., 2000; Johnson *et al*. 2008)

[3] Measured by ICP-MS (Rice *et al*., 2000)

[4] Measured by hydride generation atomic absorption spectroscopy (Rice *et al*., 2000)

[5] Measured by cold vapor atomic absorption spectroscopy (Rice *et al*., 2000)

[6] Measured by ion chromatography (Rice *et al*., 2000)

(Hunter and Moser, 1990; Rice, 1999; Thordsen *et al*. 2007)

Table 2 Se concentrations (μg/L) in SFPW samples collected over a 6 month study period from locations in pilot-scale CWTS illustrating the extent of Se removal at 24 h intervals and mean Se concentrations during the study period

Location	Nov	Dec	Jan	Feb	Mar	Apr	Mean
Inflow	44.6	49.7	46.5	42.7	76.0	50.8	51.7
Control (24 h)	43.4	45.1	44.9	38.3	52.7	51.6	46.0
Control (48 h)	42.8	46.3	41.9	45.7	55.2	61.4	48.9
Control (72 h)	44.3	43.7	39.1	39.7	56.7	63.6	47.8
Control (96 h)	45.7	44.6	37.9	39.7	58.2	56.4	47.1
AquaSmart^{TM} (24 h)	3.0	8.8	7.9	27.3	11.2	6.3	10.7
AquaSmart^{TM} (48 h)	1.3	2.8	2.2	4.5	1.0*	5.0	2.8
AquaSmart^{TM} (72 h)	1.0*	1.1	1.0*	1.0*	1.0*	1.9	1.2
AquaSmart^{TM} (96 h)	1.0*	1.0*	1.0*	1.0*	1.0*	1.0*	1.0*
Sucrose (24 h)	18.3	38.2	36.2	29.8	6.3	22.2	25.2
Sucrose (48 h)	15.9	24.2	26.5	3.0	2.5	11.7	14.0
Sucrose (72 h)	9.9	13.8	13.8	6.5	2.2	9.2	9.2
Sucrose (96 h)	6.1	8.1	7.3	1.0*	5.8	2.9	5.2

* non-detect, value reported as detection limit

Table 3 As concentrations (μg/L) in SFPW samples collected over a 6 month study period from locations in pilot-scale CWTS illustrating the extent of As removal at 24 h intervals and mean As concentrations during the study period

Location	Nov	Dec	Jan	Feb	Mar	Apr	Mean
Inflow	21.8	14	18.4	18.4	18.3	17.2	18.0
Control (24 h)	13.3	10.6	16.5	13.5	14.1	22.6	15.1
Control (48 h)	12.1	7.5	13.1	10.4	8.7	4.4	9.4
Control (72 h)	7.9	6.4	11.9	8.0	7.1	4.0	7.6
Control (96 h)	5.6	5	10.9	6.0	4.3	2.4	5.7
AquaSmart^{TM} (24 h)	15.2	9.5	19	15.1	17.9	12.7	14.9
AquaSmart^{TM} (48 h)	16.5	6.9	15.3	8.1	9.8	15.1	12.0
AquaSmart^{TM} (72 h)	12	7.8	15.6	8.6	8.7	10.1	10.5
AquaSmart^{TM} (96 h)	3.3	6.3	14.7	9.0	9.7	6.9	8.3
Sucrose (24 h)	19.2	8.2	16.7	18.4	7.1	13.5	13.9
Sucrose (48 h)	16.3	5.2	17.1	11.6	5.4	10.5	11.0
Sucrose (72 h)	15	5.1	17.2	8.2	5.4	9.5	10.1
Sucrose (96 h)	13.4	5.5	18.3	6.3	3.5	9.8	9.5

Table 4 Removal efficiencies (range and mean) and mean removal rate coefficients (d^{-1}) over a 6 month period for the untreated control, AquaSmart^{TM} treatment, and sucrose treatment

Removal Efficiencies	Se		As		LMWO	
	Range	Mean	Range	Mean	Range	Mean
Control (untreated)	0% - 23%	11%	41% - 77%	65%	99%	99%
AquaSmart^{TM}	99%	99%	20% - 85%	52%	99%	99%
Sucrose	84% - 92%	86%	1% - 81%	50%	99%	99%
Removal Rate Coefficients						
Control (untreated)	0.02		0.29		0.80	
AquaSmart^{TM}	0.99		0.19		0.80	
Sucrose	0.57		0.16		0.80	

CHAPTER FOUR

RELATIONSHIPS BETWEEN ABUNDANCE OF SELENIUM REDUCING MICROBES AND SELENIUM REMOVAL IN A PILOT-SCALE CONSTRUCTED WETLAND TREATMENT SYSTEM

1. INTRODUCTION

Risks due to selenium (Se) in contaminated waters may be mitigated by treatment involving Se reducing microbes (Maiers *et al*. 1988; Stolz and Oremland 1999; Zhang and Frankenberger 2005; Siddique *et al*. 2007; Zhang *et al*. 2008). Constructed wetland treatment systems (CWTSs) could support Se reducing microbes with sufficient activity and numbers to decrease concentrations or alter forms of Se in contaminated waters below risk levels. The bioavailability of Se in aquatic systems is influenced by speciation (Tomei *et al*. 1995; Garbisu *et al*. 1996; Ike *et al*. 2000; Siddique *et al*. 2007). In nature, Se can occur in several oxidation states (VI, IV, 0, and -II) (Zhang *et al*. 2004) and in a variety of compounds and ionic forms such as: selenides (e.g. H_2Se, HSe^-), selenites (e.g. H_2SeO_3, $HSeO_3^-$, SeO_3^{-2}), and selenates (e.g. $HSeO_4^-$, SeO_4^{-2}) (Zhang and Moore 1996). Selenites and selenates are highly water soluble (Maiers *et al*. 1988) and are potentially toxic to aquatic system biota at low concentrations (e.g. parts per billion) (Lemly 2004). However, elemental Se(0) is insoluble in water and biologically unavailable to aquatic organisms (Maiers *et al*. 1988; Garbisu *et al*. 1996; Ike *et al*. 2000; Siddique *et al* 2007). To mitigate risks to aquatic biota, more information is needed regarding pathways that transform bioavailable Se.

Many waters with elevated (i.e. constituent concentrations > discharge criteria) levels of Se are associated with energy production (e.g. power plant flue gas desulfurization waters, refinery effluents) (Mooney and Murray Gulde 2008; Spacil *et al*. in review). A generic fresh produced water (PW) was chosen for this study because the United States generates large volumes of PW, some with low salinity (i.e. chloride concentration < 5000 mg/L) (Fillo and Evans 1990; Fillo *et al*. 1992; McBeth *et al*. 2003b; Xu *et al*. 2008). The Powder River basin alone yields approximately 370,000 cubic meters per day (m^3/d) of PW (Rice and Nuccio, 2000; Benko and Drewes, 2008). As demands on water resources intensify, remediation of PW could provide an additional freshwater source for beneficial use.

An initial fresh PW characterization from previous research (Chapter 3) and published records provided information for formulating a simulated fresh produced water (SFPW). Using simulated water for experimentation is more economical and feasible than acquisition, shipment, and storage of large volumes of actual PW and allows greater repeatability and manipulation of specific water characteristics during experimentation. The initial PW characterization indicated that Se could be a constituent of concern (COC) in these waters (Hunter and Moser 1990; Rice 1999; Rice *et al*. 2000; Ramirez 2005; Thordsen *et al*. 2007).

Constructed wetland treatment systems (CWTSs) may be a robust, effective and efficient approach for consistently treating many COCs to achieve stringent discharge limits (Murray Gulde *et al*. 2008; Johnson *et al*. 2008; Rodgers and Castle 2008). CWTSs have been used for treating a variety of constituents in energy related waters (Knight *et al*. 1999; Johnson *et al*. 2008; Spacil *et al*. in review) and can be specifically designed based on targeted constituents and treatment pathways to transfer or transform those constituents (Hawkins *et al*. 1997; Gillespie *et al*. 1999, 2000). To provide proof-of-concept data and improve potential stakeholder and regulatory confidence, a pilot-scale CWTS using SFPW could provide information regarding treatment pathways and operative environmental parameters necessary to achieve targeted treatment goals. Since Se presents unique challenges regarding removal from aqueous environments, previous studies indicated the need for organic amendments to promote treatment of aqueous selenium (Maiers *et al*. 1988; Zawislanski *et al*. 2001; Zhang and Frankenberger 2005; Siddique *et al*. 2007; Spacil *et al*. in review).

In the presence of an organic carbon amendment that can serve as an electron donor, microbes capable of dissimilatory selenium reduction can reduce Se(VI) and Se(IV) to elemental Se(0), thereby decreasing bioavailable Se in an aquatic system (Maiers *et al*. 1988; Stolz and Oremland 1999; Zhang and Frankenberger 2005; Siddique *et al*. 2007; Zhang *et al*. 2008). Data are needed that indicate a parametric relationship between microbial abundance and removal of water soluble selenium compounds. We hypothesize that Se removal efficiency in a CWTS will be proportional to the number of Se reducing microbes present in the sediment/detritus, since this is the targeted removal pathway. By understanding the relationship between Se reducing microbes and Se removal from the aqueous phase, Se treatment performance could be predicted in a CWTS.

The overall objective of this research was to evaluate the relationship between removal of selenium from SFPW and the abundance of Se reducing microbes in a pilot-scale CWTS. The specific objectives were: (1) to characterize a generic fresh PW to confirm Se as a COC based on surface water discharge limits to formulate a SFPW for experimentation, (2) to design and build a pilot-scale CWTS using information from previous studies (Spacil *et al*. in review) and peer-reviewed published literature, (3) to measure Se removal rates and extents in response to organic carbon amendments, (4) to measure Se reducing microbe abundance, and (5) to measure a statistical relationship between rates and extents of Se removal and Se reducing microbe abundance.

2. MATERIALS AND METHODS

2.1 FRESH PRODUCED WATER CHARACTERIZATION

Characteristics of PW were discerned by review of a variety of sources including samples received at this laboratory, presentations (Johnson *et al*. 2006), peer reviewed publications on produced waters (Johnson *et al*. 2008; Kanagy *et al*. 2008), publications by the United States Geological Survey (USGS) on coal bed methane produced water (CBMPW), as well as CBMPW water chemistry reports from the Colorado Oil and Gas Conservation Commission (COGCC), the Geological Survey of Alabama (GSA) on the Black Warrior Basin, and the Montana Groundwater Information Center (GWIC) on the northern Powder River Basin. A single database was constructed and analyzed for range and mean values for all constituents measured. Characteristics included general water chemistry parameters (e.g. pH, temperature, conductivity, total dissolved solids (TDS), total suspended solids (TSS), alkalinity, and hardness) as well as major cations and anions, trace metals, nitrogen species, and organic carbon (Table 1).

2.2 PILOT-SCALE DESIGN AND CONSTRUCTION

The pilot-scale CWTS was built and housed in a greenhouse with natural (i.e. solar) photoperiod and temperature regulation from 20 to 30°C. Twelve 378 L Rubbermaid® containers (121 cm long by 77 cm wide by 63 cm deep) were arranged in three series of four cells (Figure 1). The cells were connected with polyvinyl chloride (PVC) pipe fittings approximately 6 cm below the top of each Rubbermaid® container to allow gravity flow from each cell. Each cell was filled to a sediment depth of approximately 25 cm with river sand from 18-mile Creek in Clemson, SC, and planted with *Typha latifolia* Linnaeus harvested from a Clemson University aquaculture pond. The cells were planted at a density of approximately 30 plants per cell. To maintain a circumneutral pH (6.5 – 8) and increase alkalinity, 1000 g of ground oyster shells (98% $CaCO_3$ by weight) were added to the sediments of each cell of the pilot-scale CWTS. To provide essential nutrients for the microbes and plants, 12 g of 19-6-12 Osmocote® fertilizer were added to the sediments of each cell to increase the nitrogen, phosphorus, and potassium concentrations. The literature review provided evidence regarding conditions that promote Se treatment in a CWTS and the following parameters were targeted in the pilot-scale CWTS: pH range between 6.5 and 8.0, dissolved oxygen concentration (DO) less than 2.0 mg/L, temperature greater than 10.0°C, and a source of organic carbon to serve as an electron donor for Se (Maiers *et al*. 1988; Hawkins *et al*. 1997; Gillespie *et al*. 1999, 2000; Zawislanski *et al*. 2001; Ng *et al*. 2004; Zhang and Frankenberger 2005; Pickett *et al*. 2006; Siddique *et al*. 2007; Singh 2007). Three FMI® QG400 (Fluid Metering®, Inc., Syosset, NY) piston pumps were calibrated to deliver 128 mL SFPW/min, to achieve a nominal 24 hour (h) hydraulic retention time (HRT) per cell, or 96 h per series. The pumps were calibrated using a 250 mL graduated cylinder and a stopwatch.

The two organic carbon amendments chosen for this study were sucrose (Dixie Crystals®, Imperial Sugar®, Inc., Sugar Land, TX) and a nutrient additive (AquaSmart™ , Diamond V Mills, Inc.®, Cedar Rapids, IA). The untreated control series was designated Series C; the AquaSmart™ treatment series was designated Series A; the sucrose treatment series was designated Series S. After an acclimation period of 28 days, an AquaSmart™ stock solution (35 g/L) was added to the series A inflow and a sucrose stock solution (35 g/L) was added to the Series S inflow. Two FMI® QG20 pumps were calibrated to deliver 1 mL of these stock solutions per minute to the first cell of Series A and S. These additions yielded amendment concentrations in the inflow to the pilot-scale CWTS of 270 mg AquaSmart™/L and 270 mg sucrose/L in the series A and series S inflows, respectively. The AquaSmart™ and sucrose solutions were pumped from separate 19 L reservoirs that were renewed weekly (Fig. 1).

Se reducing microbes were introduced into the pilot-scale CWTS during the planting phase. When *T. latifolia* were harvested, an undetermined amount of sediment was attached to the root mass and transferred into the sediments of the pilot-scale CWTS upon planting. Since many genera of Se-reducers are also sulfate reducers [e.g. *Desulfovibrio* sp. (Tomei *et al*. 1995)] and are widespread in soils and anaerobic sediments (Tortora *et al*. 1989), Se reducing microbes were likely introduced during planting. Se reducing microbes could have also been present in the damp sediments harvested from 18-Mile Creek.

2.3 PERFORMANCE OF PILOT-SCALE CWTS

To evaluate performance of the pilot-scale CWTS, Se concentrations were measured in the pilot-scale CWTS inflow and the outflow from each cell at sampling locations between each cell and at the final outflow of each series. Water samples (e.g. inflow and outflow from each cell) were collected once per month from the pilot-scale CWTS over a period of four months. Elemental analysis of Se was conducted during each sampling period by hydride generation atomic absorption spectroscopy [Standard Method 3114C (APHA 2005)]. The performance of the pilot-scale CWTS, defined as the rate and extent of Se removal from SFPW, was calculated from these measurements. Removal efficiency, defined as the percent decrease in Se concentration from pilot-scale CWTS inflow to final outflow, was calculated:

$$\textit{Removal efficiency}\ (\%) = \frac{[C]_o - [C]}{[C]_o} x100$$

Equation 1

where, $[C]_o$ is the inflow Se concentration (μg/L) and $[C]$ is the outflow Se concentration (μg/L). The removal rate coefficient (k) for Se was calculated using first order rate kinetics:

$$\textit{Removal rate coefficient (k)} = \frac{-\ln([C]/[C]_o)}{t}$$

Equation 2

where, $[C]_o$ is the inflow Se concentration (μg/L), $[C]$ is the outflow Se concentration (μg/L), and t is the time (days) from inflow to outflow. The Se removal extent, defined as a concentration change from inflow to outflow, was calculated:

$$\textit{Removal Extent} = [C]_o - [C]$$

Equation 3

where, $[C]_o$ is the inflow Se concentration (μg/L) and $[C]$ is the outflow Se concentration (μg/L).

The Se removal data were analyzed for normal distribution. Significant differences in outflow concentrations between the untreated control and the experimental treatments were determined by one-way ANOVA and Least Significant Difference (LSD) or Tukey's test. All statistical analyses were conducted using SAS 9.1 (SAS Institute 2002).

2.4 MEASURE ABUNDANCE OF SE REDUCING MICROBES

From the two treatment series and the untreated control series, sediment samples were collected at the sediment/water interface from each cell of the pilot-scale CWTS. The samples were transported to the laboratory and immediately analyzed. In order to quantify the abundance of Se reducing microbes, a Se- and AquaSmart^{TM}-medium, modified from Zhang *et al*. (2008) by using AquaSmart^{TM} as an energy source, was prepared and poured into sterile 47 mm diameter petri plates. The respective concentrations of Se and AquaSmart^{TM} in the medium were 100 μg Se/L and 200 mg AquaSmart^{TM}/L. Using sterile technique, aliquots of each sample, with replication (n=3), were dispersed in 50 mL sterile (i.e. autoclaved at 121ºC for 15 minutes) water in a Nalgene$^{®}$ vacuum funnel and filtered through a 47 mm 0.45 μm gridded membrane filter (Pall Corporation$^{®}$, Port Washington, NY). Each filter was placed on the medium and incubated in a GasPak$^{®}$ anaerobic vessel for 72 hours at room temperature (i.e. 22ºC

±2ºC). The petri plates were scanned using an Epson® scanner. The image was expanded to full-page size and printed onto letter-size paper; Se reducing microbe colony forming units (CFUs) were then visually identified based upon the red precipitate indicative of elemental Se and counted with a Fisherbrand® digital counter pen. The counted CFUs were subsequently washed from the filters, acidified to pH < 2 with trace-metal grade nitric acid, and analyzed to confirm the presence of Se.

2.5 COMPARISON AND STATISTICS

Calculations of Se removal rates, extents and Se reducing microbial abundance were compared and contrasted to discern any relationships. These data were analyzed using Microsoft® Excel® 2007 and SAS 9.1 (SAS Institute 2002). Hypothesis tests were conducted using linear regression to test for a relationship between the dependent variable (Se removal rate coefficient) and the independent variable (Se reducing microbial abundance) (H_o: slope $\neq 0$).

3. RESULTS

3.1 FRESH PRODUCED WATER SIMULATION

Based on results from the PW characterization (Chapter 3, Table 1), the solutes added to this water to formulate the SFPW included calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), sodium chloride (NaCl), and sodium selenite (Na_2SeO_3). The SFPW was mixed with a submersible pump.

3.2 PILOT-SCALE DESIGN AND CONSTRUCTION

SFPW was loaded into the pilot-scale CWTS during the initial four week stabilization period with no amendments. Sucrose and AquaSmart™ amendments were then initiated to supply energy (i.e. electrons) to promote reductive processes and to acclimate the system to an increased organic carbon loading.

3.3 PERFORMANCE OF PILOT-SCALE CWTS

With an inflow concentration of 46 to 76 μg Se/L, the performance goal of 5 μg Se/L was achieved consistently by the AquaSmart^{TM} treatment (Tables 2, 3, 4, and 5). Both the untreated control series and sucrose treatment series achieved some Se removal, but did not consistently meet the performance goal. The Se removal rate coefficients and removal extents for each series were calculated using *Equations 2* and *3*, respectively. Both the sucrose and AquaSmart^{TM} series achieved significantly higher Se removal rate coefficients (e.g. 0.57 d^{-1} and 1.0 d^{-1}, respectively, versus 0.030 d^{-1} in the untreated control) and significantly higher Se removal extents (e.g. 49.7 μg Se/L and 54.6 μg Se/L, respectively, versus 9.9 μg Se/L in the untreated control) ($\alpha = 0.05$) (Tables 1, 2, 3, 4, and 5).

3.4 MEASURE ABUNDANCE OF SE REDUCING MICROBES

Sediment samples were collected at the sediment/water interface from the pilot-scale CWTS with synoptic SFPW samples to relate Se removal at that time to a measure of Se reducing microbes in the sediment pore water. A preliminary experimental trial was conducted to discern the volume of sediment pore water required to yield countable cultures; several different volumes and dilutions of sediment pore water were filtered and incubated with both organic carbon amendments (i.e. sucrose and AquaSmart^{TM}) to serve as energy sources in the medium. From these trials, 0.00312 mL of sediment pore water, dispersed in 50 mL sterile water and filtered through a 0.45 μm gridded membrane filter, yielded a countable number of Se reducing microbe CFUs when using AquaSmart^{TM}-specific medium at a concentration of 200 mg AquaSmart^{TM}/L. The number of CFUs counted per 3.12 mL was extrapolated to the number of CFUs per mL (Table 3).

3.5 COMPARISON AND STATISTICS

Mean Se removal extents were compared to the mean number of Se reducing CFUs in the sediment pore water of each cell in the pilot-scale CWTS. Linear regression analysis was used to determine this correlation. Mean Se removal rate coefficients calculated for each cell (Table 1) were also compared to mean counts of Se reducing CFUs (Table 3) per cell through the four month study period using linear regression. The correlation between mean Se removal extents (i.e. decrease in concentration) per cell and the CFUs counted per cell was weak ($R^2 = 0.42$) (Figure 2). However, the correlation between mean Se removal rate coefficients per cell and the CFUs counted per cell was much stronger ($R^2 = 0.86$) (Figure 3).

Several other correlations were tested as well, including: 1) Se removal extents and Se reducing microbes in the untreated control series (Figure 4), 2) Se removal extents and Se reducing microbes in the AquaSmart^{TM} treatment series (Figure 5), 3) Se removal extents and Se reducing microbes in the sucrose treatment series (Figure 6), 4) Se removal rate coefficients and Se reducing microbes in the untreated control series (Figure 7), 5) Se removal rate coefficients and Se reducing microbes in the AquaSmart^{TM} treatment series (Figure 8), and 6) Se removal rate coefficients and Se reducing microbes in the sucrose treatment series (Figure 9). Series comparisons were analyzed to determine if correlation coefficients varied with amendment type (e.g. AquaSmart^{TM} or sucrose). In regression analysis of Se removal extents and Se reducing microbes (Figures 4, 5, and 6), the correlation coefficients (R^2) for the untreated control, the AquaSmart^{TM} treatment, and the sucrose treatment were 0.03, 0.47, and $9E^{-6}$, respectively. In regression analysis of Se removal rate coefficients and Se reducing microbe CFUs (Figures 7, 8, and 9), the correlation coefficients (R^2) for the untreated control, the AquaSmart^{TM} treatment, and the sucrose treatment were 0.56, 0.97, and 0.12, respectively.

4. DISCUSSION

Microbial reduction is an attractive treatment alternative for waters containing elevated levels of Se (Zhang *et al*. 2008). With an organic carbon source that can provide energy and electrons that enable dissimilatory Se reduction (Zhang *et al*. 2004, 2008), the pilot-scale CWTS used in this study removed soluble Se species from SFPW to low levels (i.e. $\leq 5\ \mu$g/L). Several studies aimed at determining the capacity of different species of bacteria (i.e. microbes) to reduce Se to elemental and/or organic states have been conducted (Maiers *et al*. 1988; Lortie *et al*. 1992; Lawson and Macy 1995; Tomei *et al*. 1995; Garbisu *et al*. 1996; Hansen *et al*. 1998; Siddique *et al*. 2005; Zhang and Frankenberger Jr. 2005; Siddique *et al*. 2007; Takata *et al*. 2008).

The primary objective of this research was to expand on previous microbial research by comparing a measure of Se reducing microbes to an evaluation of performance (i.e. Se removal rate coefficients and removal extents) of a pilot-scale CWTS. A mean Se removal rate coefficient (k) was calculated (*Equation 2*) for each series (96 h HRT), as well as for each cell (e.g. untreated control, AquaSmart^{TM} treatment, sucrose treatment) (Table 1). Mean extents of Se removal were calculated

(*Equation 3*) as concentration decrease from inflow to outflow of individual cells and series (Table 1). With a mean inflow concentration of 55.8 μg Se/L for the four month sampling period, mean removal efficiencies (*Equation 1*) for the untreated control, AquaSmart^{TM}, and sucrose treatments, were 10.8, 98.1, and 88.7%, respectively.

In a previous study, Lortie *et al*. (1992) evaluated a specific bacterial isolate, *Pseudomonas stutzeri*, to evaluate Se removal [i.e. reduction from Se(VI) and Se(IV) to Se(0)] in a laboratory environment. Using tryptic soy broth as a growth medium, removal of Se (0) ranged from approximately 80 to 90% within a matter of hours (Lortie *et al*. 1992). In comparison, according to Quinn *et al*. (2000), microbial volatilization efficiencies in Kesterson, CA field plots ranged from 11 to 51% and were relatively slow (i.e. 2 – 4 week HRT) compared to other biological and physical remediation strategies. In a study in a California full-scale CWTS, Se removal efficiencies were approximately 77% based solely on the highest measured volatilization rates (e.g. 330 μg Se/m^2 per day) (Hansen *et al* 1998). However, Hansen *et al*. (1998) hypothesize that volatilization actually accounts for 10 to 30% of the daily removal of Se.

This research utilized SFPW with elevated levels of Se (i.e. $\geq$ 40-70 μg Se/L); however, these results from this study could be implemented to facilitate treatment of other waters containing elevated levels of Se [e.g. effluents from coal-fired power plants equipped with flue gas desulfurization scrubbers (Mooney and Murray Gulde 2008), petroleum refinery effluents (Spacil *et al*. in review), coal mine drainage (Siddique *et al*. 2007), and agricultural drainage water (Siddique *et al*. 2005; Zhang *et al*. 2008).

With the advent of stringent standards for discharge of Se into receiving aquatic systems, efficient, reliable, and effective treatment alternatives and monitoring techniques are needed. CWTSs can provide cost effective and robust treatment for many of these discharges and monitoring of Se reducing microbe abundance may be an effective means for estimating Se treatment in a CWTS. The benefits of CWTSs can include: low cost of operation, low maintenance, effective treatment, solar energy driven, increased effectiveness over time, tolerance of deviations in flow rate and contaminant load, and treatment of multiple COCs simultaneously and more effectively than chemical or physical treatment processes (Bhamidimarri *et al*. 1991; Sundaravadivel and Vigneswaran 2001; Rodgers and Castle 2008; Dorman *et al*. 2009).

Several performance parameters may require consideration in a CWTS designed to treat Se using biogeochemical pathways promoted by organic carbon amendments. Some of these parameters include: 5-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), sediment toxicity, effluent toxicity, temperature, seasonal variability, as well as Se precipitation and accretion rates and stability of precipitated elemental Se. Further research is needed to evaluate parameters related to Se treatment enhanced by organic carbon amendments. Investigations into CWTSs are ongoing at this laboratory to seek efficient, effective, and sustainable treatment of other Se containing waters. The implications of this treatment are far-reaching and include the potential to decrease aqueous Se discharge into aquatic receiving systems.

5. CONCLUSIONS

Se can be efficiently and effectively removed from SFPW using a pilot-scale CWTS. A literature review provided evidence regarding pathways for treatment of Se and implied that a relationship may exist between Se removal and abundance of Se reducing microbes. A pilot-scale CWTS was designed and built using information from literature. This design was successful for targeting and removing aqueous Se. Se removal rates were calculated and compared to a measure of Se reducing microbes (i.e. CFUs). Mean Se removal rate coefficients were positively correlated with mean abundance of Se reducing microbes (i.e. CFUs) ($R^2 = 0.855$). This research can assist in predicting the performance of a CWTS designed to treat Se through microbial reductive pathways by measuring explanatory parameters, using organic carbon amendments, and assessing Se reducing microbe abundance. These parameters may be useful for monitoring performance of a demonstration- or full-scale CWTS.

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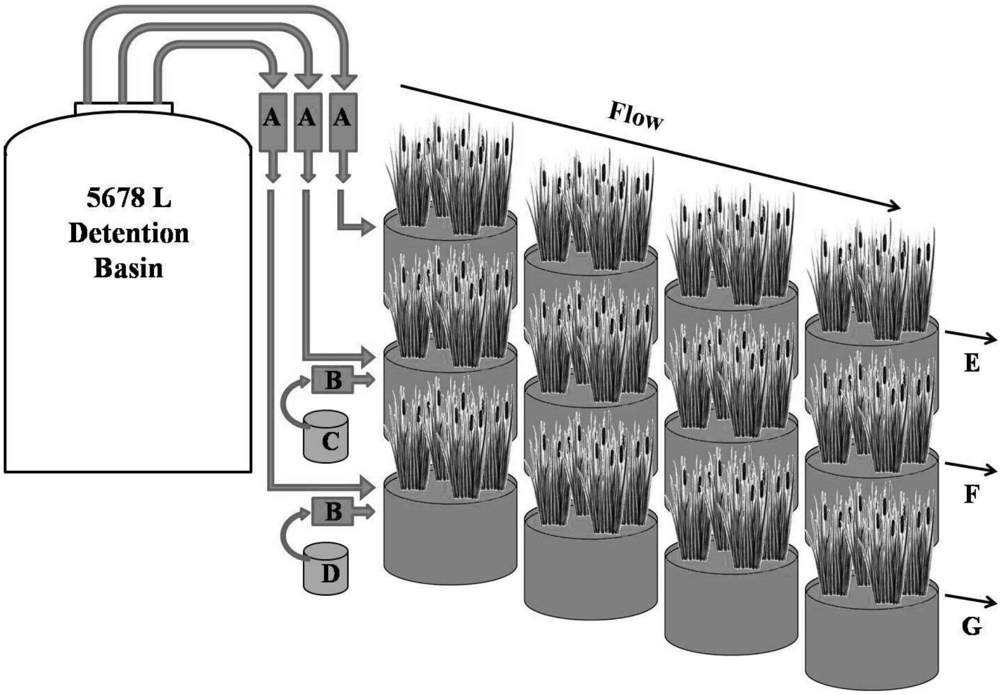

Legend

A = FMI® QG400 piston pumps

B = FMI® QG20 piston pumps

C = 35 g/L AquaSmart™ solution

D = 35 g/L sucrose solution

E = Untreated control (series C) outflow

F = AquaSmart™ treatment (series A)outflow

G = Sucrose treatment (series S) outflow

Figure 1 Schematic of pilot-scale experiment

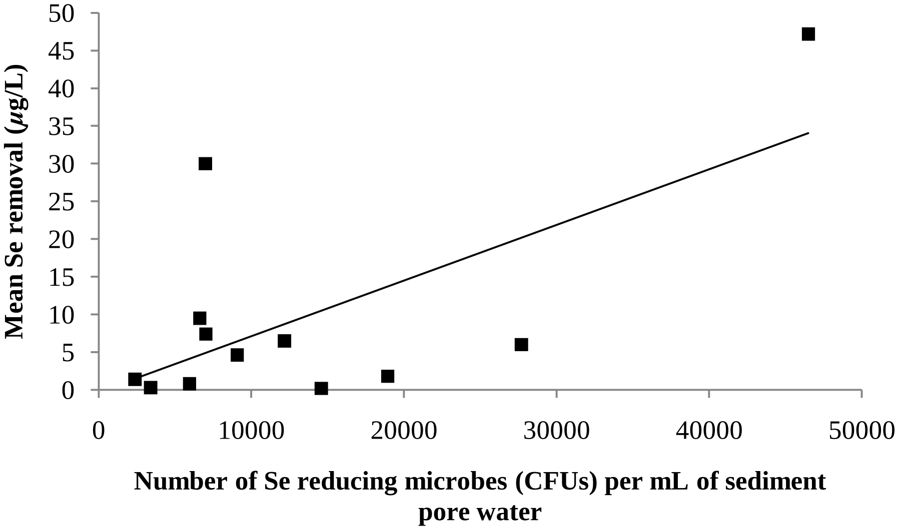

Figure 2 Comparison of mean Se removal extents (i.e. concentration decrease) to the mean abundance of associated Se reducing microbes (CFUs) counted in cultures prepared from sediment pore water in pilot-scale CWTS cells over a period of four months ($R^2 = 0.42$; $y = 7E^{-4}x – 0.2843$)

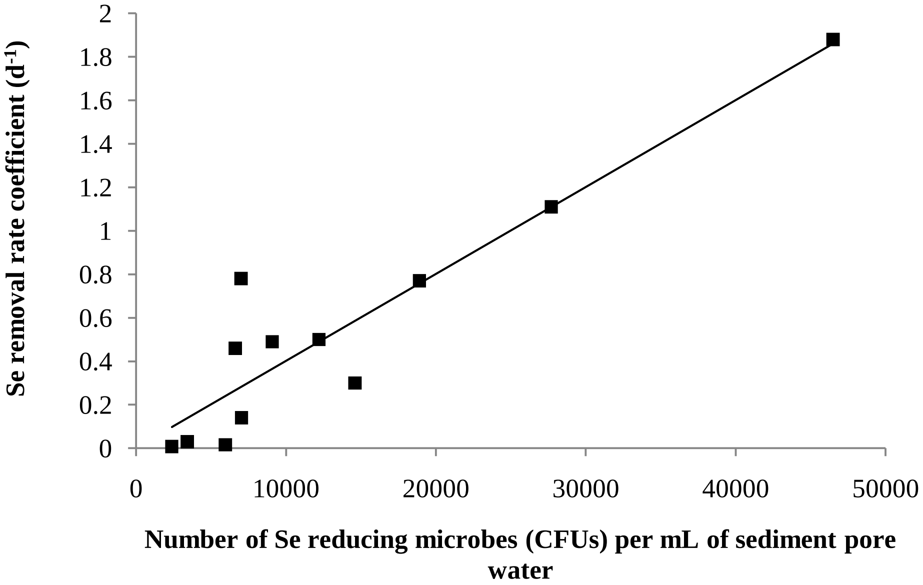

Figure 3 Comparison of mean Se removal rate coefficients to the mean abundance of associated Se reducing microbes (CFUs) counted in cultures prepared from sediment pore water in pilot-scale CWTS cells over a period of four months
(R^2 = 0.86; y = $4E^{-5}$x + 0.003)

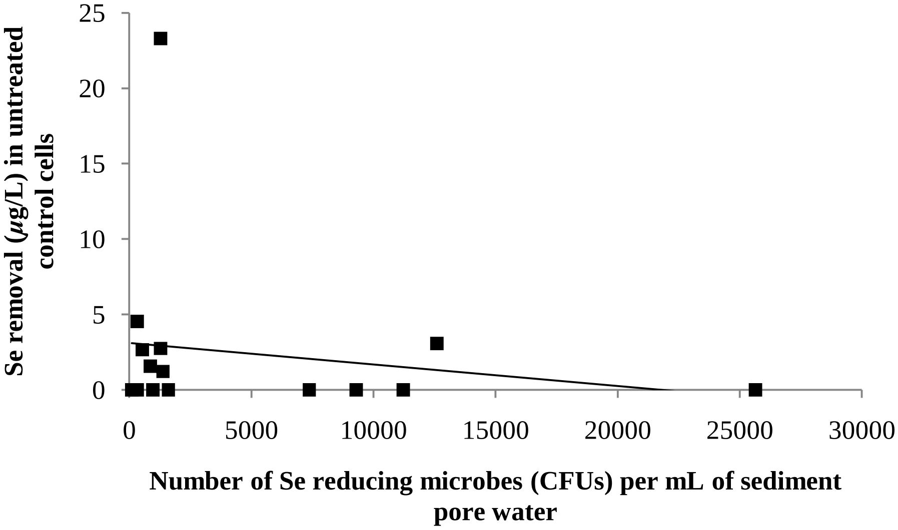

Figure 4 Comparison of Se removal extents (i.e. concentration decrease) to the abundance of associated Se reducing microbes (CFUs) counted in cultures prepared from sediment pore water in untreated control cells over a period of four months ($R^2 = 0.03$; $y = 1E^{-4}x + 3.1134$)

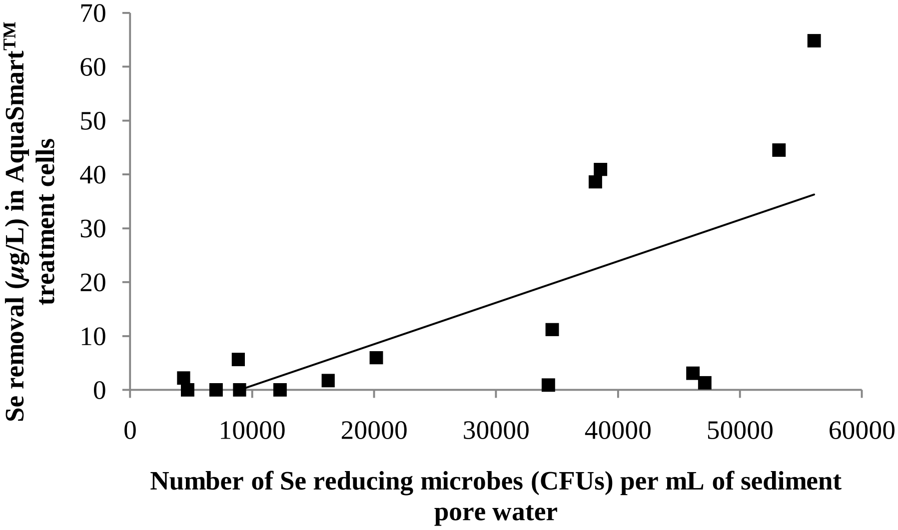

Figure 5 Comparison of Se removal extents (i.e. concentration decrease) to the abundance of associated Se reducing microbes (CFUs) counted in cultures prepared from sediment pore water in AquaSmart^{TM} treatment cells over a period of four months ($R^2 = 0.47$; $y = 8E^{-4}x + 6.9261$)

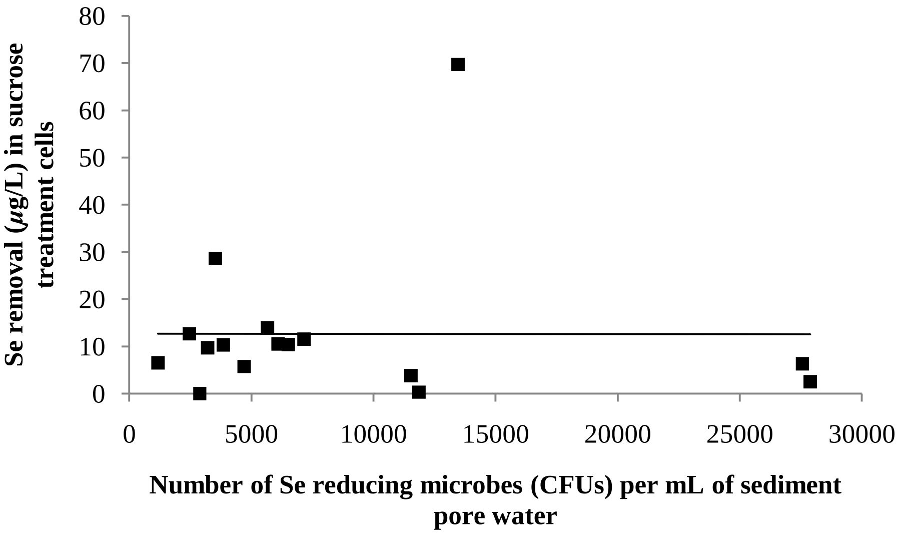

Figure 6 Comparison of Se removal extents (i.e. concentration decrease) to the abundance of associated Se reducing microbes (CFUs) counted in cultures prepared from sediment pore water in sucrose treatment cells over a period of four months ($R^2 = 9E^{-6}$; $y = -6E^{-6}x + 12.708$)

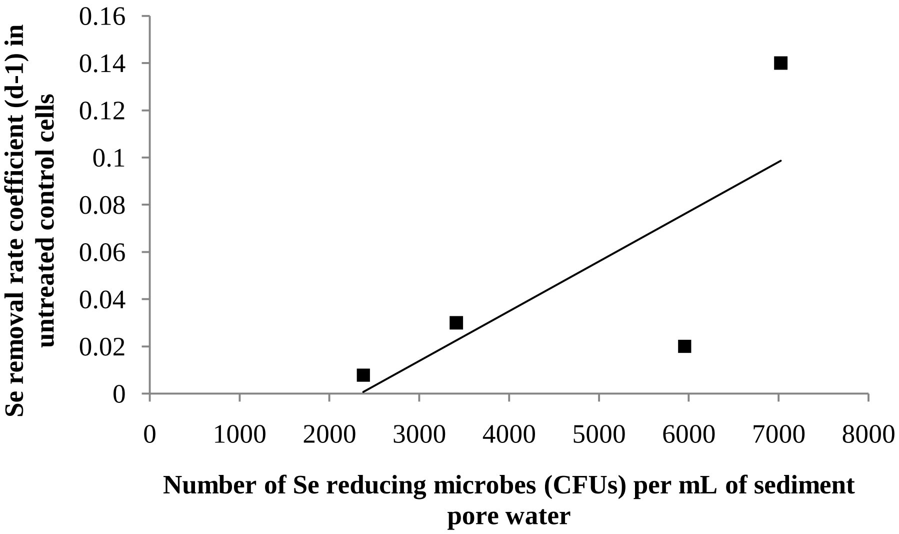

Figure 7 Comparison of mean Se removal rate coefficients per cell to the mean abundance of associated Se reducing microbes (CFUs) counted in cultures prepared from sediment pore water in untreated control cells over a period of four months ($R^2 = 0.56$; $y = 2E^{-5}x – 0.0494$)

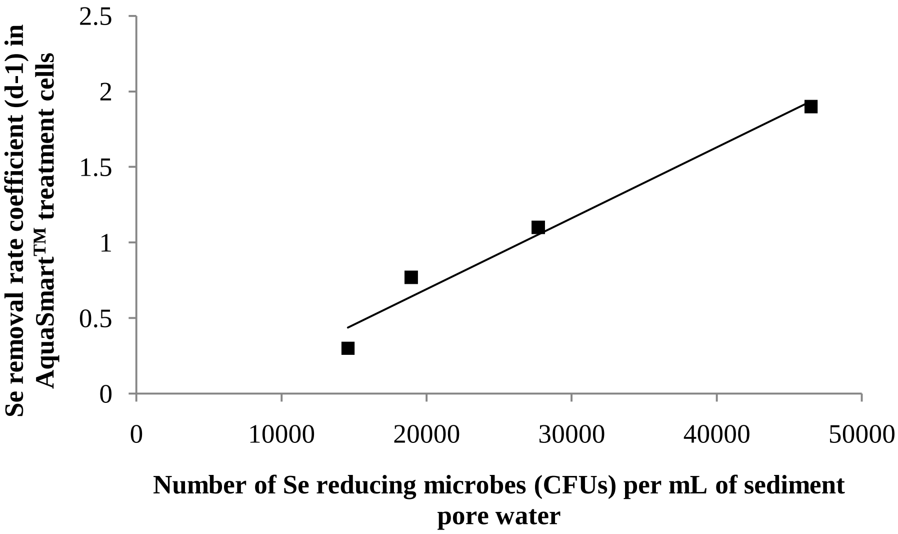

Figure 8 Comparison of mean Se removal rate coefficients per cell to the mean abundance of associated Se reducing microbes (CFUs) counted in cultures prepared from sediment pore water in AquaSmart^{TM} treatment cells over a period of four months ($R^2 = 0.97$; $y = 5E^{-5}x + 0.2471$)

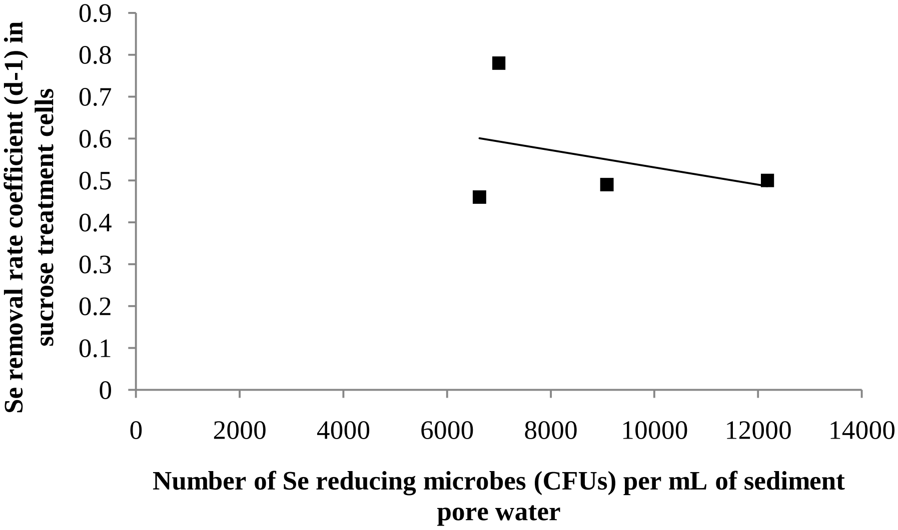

Figure 9 Comparison of mean Se removal rate coefficients per cell to the mean abundance of associated Se reducing microbes (CFUs) counted in cultures prepared from sediment pore water in sucrose treatment cells over a period of four months ($R^2 = 0.12$; $y = -2E^{-5}x + 0.7377$)

Table 1 Mean Se removal rate coefficients and removal extents for each cell (HRT=24 h) and for each series (HRT=96 h) during 4 month study period

Series	Cell	Mean removal rate coefficient per cell (*k*) (d^{-1})	Mean removal extent per cell (μg Se/L)	Mean removal rate coefficient for series (*k*) (d^{-1})	Mean removal extent for series (μg Se/L)
Untreated control	C1	0.14	7.4	0.030	9.9
	C2	0.02	0.8		
	C3	0.0078	1.4		
	C4	0.03	0.3		
AquaSmart^{TM} treatment	A1	1.9	47	1.0*	54.6*
	A2	1.1*	5.8*		
	A3	0.77*	1.5*		
	A4	0.3*	0.3*		
Sucrose treatment	S1	0.78	30	0.57	49.7
	S2	0.46	9.5		
	S3	0.50	6.5		
	S4	0.49	3.7		

* calculated with non-detectable Se levels reported as detection limit

Table 2 Se concentrations and explanatory parameters for January sampling period

Sample	Se (μg/L)	DO (mg/L)	Conductivity (μS/cm)	pH (S.U.)	Redox (mV)	Temp (ºC)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Inflow	49.7	8.4	2612	7.26	n/a[¥]	13.2	42	140
Control 1	45.1	8.2	2630	7.06	-208.6	15.1	26	138
Control 2	46.3	8.1	2643	6.86	-188.2	15.9	26	136
Control 3	43.7	8.2	2666	6.88	+9.22	16.5	24	130
Control 4	44.6	8.2	2701	6.80	-125.2	16.9	28	132
AquaSmart^{TM} 1	8.8	1.2	2642	6.49	-135.2	14.9	40	142
AquaSmart^{TM} 2	2.8	2.3	2665	6.64	-84.6	15.7	38	142
AquaSmart^{TM} 3	1.1	5.7	2684	6.94	-51.3	16.7	76	152
AquaSmart^{TM} 4	1.0*	7.4	2713	7.10	+46.5	16.5	74	154
Sucrose 1	38.2	4.3	2680	6.34	-241.2	15.2	42	140
Sucrose 2	24.2	2.2	2695	6.54	-101.3	15.8	48	140
Sucrose 3	13.8	4.5	2721	6.72	-91.2	16.3	50	138
Sucrose 4	8.1	5.4	2732	6.74	-49.2	16.9	66	152

* nd – Se concentration reported as detection limit

[¥] not applicable

Table 3 Se concentrations and explanatory parameters for February sampling period

Sample	Se (μg/L)	DO (mg/L)	Conductivity (μS/cm)	pH (S.U.)	Redox (mV)	Temp (ºC)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Inflow	46.5	8.5	1727	7.15	n/a[¥]	11.5	40	136
Control 1	44.9	8.3	1865	6.89	-194.9	13.0	28	132
Control 2	41.9	8.0	2086	6.71	-133.0	14.8	24	132
Control 3	39.1	8.2	2145	6.59	+100.4	15.0	26	128
Control 4	37.9	8.1	2128	6.62	+111.6	14.6	26	128
AquaSmart^{TM} 1	7.9	1.1	1849	6.43	-112.2	13.5	42	140
AquaSmart^{TM} 2	2.2	2.1	1908	6.54	-46.2	14.7	36	140
AquaSmart^{TM} 3	1.0*	5.2	2055	6.87	-36.3	13.7	74	148
AquaSmart^{TM} 4	1.0*	7.7	2020	6.90	+68.4	13.5	76	152
Sucrose 1	36.2	3.9	1805	6.21	-221.7	13.0	40	138
Sucrose 2	26.5	2.0	1867	6.43	-63.5	13.7	46	136
Sucrose 3	13.8	4.3	2055	6.75	-46.3	14.2	48	140
Sucrose 4	7.3	5.1	2020	6.73	-16.3	13.6	58	148

* nd – Se concentration reported as detection limit

[¥] not applicable

Table 4 Se concentrations and explanatory parameters for March sampling period

Sample	Se (μg/L)	DO (mg/L)	Conductivity (μS/cm)	pH (S.U.)	Redox (mV)	Temp (ºC)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Inflow	76.0	8.2	2210	7.52	n/a[¥]	15.9	44	146
Control 1	52.7	8.0	2264	6.89	-171.2	16.2	32	132
Control 2	55.2	8.0	2300	6.74	-119.4	16.7	32	136
Control 3	56.7	7.8	2323	6.64	+52.3	17.0	32	134
Control 4	58.2	7.9	2384	6.41	+65.3	17.5	30	128
AquaSmart™ 1	11.2	1.1	2225	6.58	-154.8	16.3	46	152
AquaSmart™ 2	1.0*	1.7	2284	6.69	-71.6	16.9	36	148
AquaSmart™ 3	1.0*	5.2	2317	6.87	-35.2	17.4	68	154
AquaSmart™ 4	1.0*	7.5	2345	6.96	+54.3	17.6	76	156
Sucrose 1	6.3	1.9	2231	6.25	-234.5	16.5	40	142
Sucrose 2	2.5	2.0	2256	6.49	-64.2	16.7	46	144
Sucrose 3	2.2	4.0	2289	6.54	-42.1	17.0	42	138
Sucrose 4	5.8	4.3	2321	6.59	-34.8	17.5	54	146

* nd – Se concentration reported as detection limit

[¥] not applicable

Table 5 Se concentrations and explanatory parameters for April sampling period

Sample	Se (μg/L)	DO (mg/L)	Conductivity (μS/cm)	pH (S.U.)	Redox (mV)	Temp (ºC)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Inflow	50.8	8.6	2525	7.40	n/a[¥]	16.2	42	144
Control 1	51.6	8.4	2528	6.91	-170.3	16.7	34	134
Control 2	61.4	8.9	2500	6.93	-120.4	16.9	32	134
Control 3	63.6	8.4	2523	6.96	+51.8	17.5	34	136
Control 4	56.4	7.8	2489	6.92	+65.4	16.7	32	130
AquaSmart^{TM} 1	6.3	3.5	2345	6.60	-157.5	16.9	48	152
AquaSmart^{TM} 2	5.0	4.8	2360	6.82	-72.6	17.4	38	150
AquaSmart^{TM} 3	1.9	6.1	2230	6.82	-38.7	17.7	70	156
AquaSmart^{TM} 4	1.0*	6.8	2290	6.79	+52.6	18.0	78	158
Sucrose 1	22.2	5.6	2330	6.32	-245.1	17.0	42	144
Sucrose 2	11.7	2.4	2280	6.51	-68.2	17.3	48	146
Sucrose 3	9.2	5.0	2260	6.68	-45.6	17.2	44	140
Sucrose 4	2.9	4.9	2265	6.71	-35.6	17.8	56	148

* nd – Se concentration reported as detection limit

[¥] not applicable

Table 6 Mean number of Se reducing microbial colonies (CFUs) estimated per mL of sediment pore water

Series	Cell	Jan	Feb	Mar	Apr	Mean	Std. Dev.
Untreated control	C1	320	856	25600	1280	7030	12400
	C2	1600	12600	9300	321	5950	5940
	C3	535	1280	7370	321	2380	3360
	C4	961	138	11200	96	3410	5230
AquaSmart$^{\text{TM}}$ treatment	A1	38600	38100	53200	56100	46500	9490
	A2	20200	8870	47100	34600	27700	16700
	A3	16200	4380	46200	8970	18900	18800
	A4	12300	4700	34300	7050	14600	13500
Sucrose treatment	S1	716	3850	3530	13500	7000	4610
	S2	5660	3210	6090	11500	6620	3510
	S3	6520	2460	27900	11900	12200	11160
	S4	4700	1180	27600	2890	9080	12400

CHAPTER FIVE

SUMMARY AND CONCLUSIONS

1. INTRODUCTION

Energy derived waters (EDWs) are diverse and can contain a variety of elements and compounds, both inorganic and organic, which may pose risks to receiving aquatic system biota. The overall objective of this research was to provide an approach for mitigation of risks posed by aquatic constituents of concern as well as determine relationships between Se reducing microbes and Se removal in a pilot-scale constructed wetland treatment system (CWTS).

This research addressed questions concerning treatment of elevated metalloid (e.g. Se, As) and organic constituent levels in simulated refinery effluent (SRE) and simulated fresh produced water (SFPW) and sought to relate Se reducing microbe abundance to Se removal rates and/or extents. This research had three primary objectives: (1) evaluate removal of Se from SRE using a pilot-scale CWTS; (2) evaluate removal of Se, As, and LMWOs from SFPW using a pilot-scale CWTS, and; (3) compare removal of Se from SFPW in a pilot-scale CWTS to quantity of Se reducing microbes.

This research was initiated to contribute possible remediation approaches to treat identified problems in EDWs and provide a potential microbial relationship useful for monitoring Se removal capacity.

1.1 TREATMENT OF SELENIUM IN SIMULATED REFINERY EFFLUENT USING A PILOT-SCALE CONSTRUCTED WETLAND SYSTEM

The purpose of this research was to evaluate performance of a pilot-scale CWTS and provide data useful in illustrating the feasibility of organic carbon amendments for Se in petroleum refinery effluent. Specific objectives of this research were to: (1) chemically and physically characterize a specific petroleum refinery effluent for simulation and confirmation of Se as a constituent of concern; (2) design and conduct bench-scale experiments to measure Se removal in response to organic carbon additions; (3) design and build a pilot-scale CWTS using information from the bench-scale experiments, and; (4) measure the performance of the pilot-scale CWTS in terms of rate and extent of Se removal in response to organic carbon additions following a period of maturation and acclimation.

The treatment effectiveness and performance for each experimental treatment (i.e. organic carbon amendment) were evaluated based upon a decrease in concentration of Se from inflow (i.e. 42-44 μg Se/L) to outflow (i.e. target of $\leq 5\mu$g Se/L) in the pilot-scale CWTS. Two organic carbon amendments (e.g. sucrose and the nutrient additive AquaSmart^{TM}) were evaluated in this study. Se removal efficiencies and rate coefficients for the sucrose treatment ranged from 79.0 to 88.5% and 0.37 to 0.54 d^{-1}, respectively. In the AquaSmart^{TM} treatment, Se removal efficiencies and rate coefficients ranged from 80.3 to 92.0% and 0.41 to 0.63 d^{-1}, respectively. The untreated control had significantly lower efficiencies and rate coefficients ($\alpha = 0.05$).

This study provided proof-of concept that constituents of concern (COCs) (e.g. Se) can be sufficiently removed from the water column and meet discharge criteria. This study illustrates that properly designed CWTSs are a viable option for mitigating the risks of refinery effluent to receiving system biota. The performance results from these pilot-scale CWTS studies can be used to design full scale systems to treat problematic Se-containing effluents.

1.2 PERFORMANCE OF A PILOT-SCALE CONSTRUCTED WETLAND TREATMENT SYSTEM FOR SELENIUM, ARSENIC, AND LOW MOLECULAR WEIGHT ORGANICS IN A FRESH SIMULATED PRODUCED WATER

The purpose of this research was to evaluate performance of a pilot-scale CWTS and provide data useful in illustrating the feasibility of this treatment approach for fresh produced water (PW) amended with an organic carbon source. The specific objectives of this study were: 1) to characterize a fresh PW and determine targeted COCs based on surface water discharge limits; 2) to formulate a simulated fresh produced water (SFPW) for experimental purposes; 3) to design and build a pilot-scale CWTS for SFPW based on removal pathways for targeted constituents; and (4) to measure treatment performance in terms of targeted COC removal rates and extents in response to specific amendments (e.g. organic carbon).

The treatment effectiveness and performance for each experimental treatment (i.e. organic carbon amendment) were evaluated based upon a decrease in: (1) concentration of Se from inflow (i.e. 42-76 μg Se/L) to outflow (i.e. target of $\leq 5\mu$g Se/L); (2) concentration of As from inflow (i.e. 14-22 μg As/L) to outflow (i.e. target of $\leq 5\mu$gAs/L) and; (3) concentration of LMWO from inflow (i.e. 25 mg LMWO/L) to outflow (i.e. target of $\leq$ 1 mg LMWO/L) in the pilot-scale CWTS. Two organic carbon amendments (e.g. sucrose and the nutrient additive AquaSmart^{TM}) were evaluated in this study. Mean Se removal efficiencies and rate coefficients for the sucrose treatment were 86% and 0.57 d^{-1}, respectively. In the AquaSmart^{TM} treatment, Se removal efficiencies and rate coefficients were 99% and 0.99 d^{-1}, respectively. The untreated control had significantly lower efficiencies and rate coefficients ($\alpha = 0.05$). Mean As removal efficiencies and rate coefficients for the sucrose treatment were 50% and 0.16 d^{-1}, respectively. In the AquaSmart^{TM} treatment, As removal efficiencies and rate coefficients were 52% and 0.19 d^{-1}, respectively. The untreated control did not have significantly different efficiencies and/or rate coefficients ($\alpha = 0.05$). In this pilot-scale CWTS preceded by a detention basin, LMWOs were removed prior to entry of the SFPW into the first cell.

This study provided proof-of concept data that show that constituents of concern (COCs) (e.g. Se) can be removed from the water column aided by an amended inflow. This study further illustrated that properly designed CWTSs are a viable option for mitigating the risks posed by Se and LMWOs in fresh PW to receiving system biota. More research is needed to determine efficient and effective treatment strategies for remediation of As in water using CWTSs. The performance results from these pilot-scale CWTS studies can be used to design demonstration- or full-scale systems to treat problematic Se- and/or LMWO-containing effluents.

1.3 RELATIONSHIPS BETWEEN ABUNDANCE OF SELENIUM REDUCING MICROBES AND SELENIUM REMOVAL IN A PILOT-SCALE CONSTRUCTED WETLAND TREATMENT SYSTEM

The purpose of this study was to measure a parametric relationship between removal of aqueous Se from a specific water (e.g. SFPW) to the abundance of Se reducing microbes in a pilot-scale CWTS. The specific objectives of this research were: (1) to characterize a fresh PW to confirm Se as a COC based on surface water discharge limits, (2) to formulate a SFPW for experimentation, (3) to design and build a pilot-scale CWTS using information from previous studies (Chapter 2) and peer-reviewed published literature, (4) to measure Se removal rates and extents in response to organic carbon amendments after a period of maturation and acclimation, (5) to measure Se reducing microbe abundance, and (6) to compare rates and extents of removal to measures of Se reducing microbe abundance to evaluate a potential relationship.

Se removal rate coefficients and Se removal extents were calculated for each treatment as well as the untreated control. Sediment samples were collected from each pilot-scale CWTS cell that coincided with water sampling events in order to relate Se removal to abundance of Se reducing microbes at those times. Inflow concentrations of Se ranged from 46-76 μg Se/L. Mean Se removal efficiencies and Se removal rate coefficients were 18% and 0.03 d^{-1} for the untreated control, 98% and 1.01 d^{-1} for the AquaSmart^{TM} treatment, and 89% and 0.56 d^{-1} for the sucrose treatment, respectively. Se reducing microbe counts were conducted by filtering sediment pore water with a gridded 0.45 μm membrane filter placed on a Se-specific medium and anaerobically cultured in a GasPak® chamber for 72 hours. Se removal rate coefficients and removal extents were compared to Se reducing microbe counts using linear regression. These relationships were evaluated at the series-level as well at the individual cell-level.

This study provided proof-of-concept data that demonstrate that Se removal rates may be estimated by conducting cultures of Se reducing microbes and counts using sediment pore water from a CWTS designed to treat Se. More research is needed to repeat and/or expand upon this topic in order to further the understanding of this relationship.

2. CONCLUSIONS

Overall, this research assessed treatment performance of pilot-scale CWTSs for metalloid and organic constituents, as well as investigated potential relationships between Se removal and quantity of Se reducing microbes. These studies were designed to expand our knowledge and understanding of treatment mechanisms and pathways present in CWTSs.

Chapter 2 of this thesis, which contains performance data on a pilot-scale CWTS regarding Se removal aided by organic carbon amendments, will be submitted to *Water, Air & Soil Pollution* for publication. Chapter 3 assesses performance of a pilot-scale CWTS for treatment of Se, As, and LMWOs aided by amended (e.g. sucrose, AquaSmart^{TM}, zero-valent iron) inflow and will be submitted to *Environmental Geosciences* for publication. Chapter 4, which illustrates relationships between Se removal rate coefficients and Se removal extents and Se reducing microbial assemblages in a pilot-scale CWTS, will be submitted for publication to *Water Research*.