Evaluation of Constructed Wetlands and Pretreatment Options For the Treatment of Flow-through Trout Farm Effluent

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(Abstract)

Horizontal subsurface flow (HSSF) constructed wetlands were evaluated for the treatment of flow-through trout farm effluent, phosphorus sorption affinity of gravel-bed media, and influence on Rhodamine WT (RWT) transport. HSSF wetlands coupled with mechanical pretreatment demonstrated significant ($p < 0.05$) removal of total ammonia-nitrogen (TAN), total phosphorus (TP), total organic carbon (TOC), total suspended solids (TSS), five-day biochemical oxygen demand (BOD_5), and turbidity. Treatment occurred predominantly within the wetland cells, with minimal removal of studied water quality parameters by means of sedimentation or microscreen filtration (80 μm mesh). HSSF wetlands removed 69% of influent TSS, 24% of influent TP, and reduced turbidity by 66%. The removal of organic matter within the wetlands, as measured by BOD_5, COD, and TOC was 62%, 50%, and 55%, respectively. After receiving effluent from a flow-through trout farm for about one year, the gravel media exhibited moderate removals of soluble phosphorus in batch and column sorption experiments. Partition coefficients (K_d) from batch sorption tests ranged from 45-90 mL/g. Low (60 mL/min) and high (165 mL/min) flow column experiments removed about 50 and 40% of influent PO_4-P, respectively. The conservative nature of RWT in subsurface media has been called into question by many authors. Tracer response curves from tests conducted in pilot-scale HSSF wetlands exhibited elongated tails and dual peaks, in addition to mean tracer retention times far exceeding the theoretical value. Laboratory column testing of RWT and the more conservative NaCl tracer supported field data, indicating that RWT was more reactive within the wetland media.

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1.1.1 Trout Farming Industry and Effluent Quality

In the United States, trout farming is the third largest sector in the aquaculture industry, selling $210.6 million (USD) worth of trout in 2007 (USDA, 2009). Between 1950 and 2008, global aquaculture output has increased three times the rate of meat production with the majority of production intended for human consumption. While global population has risen by an average of 1.6% per year between 1970 and 2008, aquaculture production of food-fish has seen an average growth rate of 8.3 % (FAO, 2010). Trout production capacity is limited by its requirement for finite locations providing high-volume, high-quality coldwater. Thus, if population trends continue to indicate growth, demand will likely follow, eventually requiring trout farmers to increase production at existing sites. Whether production demands are met by an increase in fish density or new construction to accommodate additional fish, a net increase in effluent waste is foreseeable.

Wastewater discharged from aquaculture facilities can contain metabolic excretions, uneaten fish feed, algae, parasites and pathogens, and elevated temperatures (IDEQ, 1998). This equates to an increase in conventional pollutants, as defined by the Clean Water Act, such as total suspended solids (TSS) and biochemical oxygen demand (BOD), in addition to non-conventional pollutants such as phosphorus and nitrogen (U.S. EPA, 2004). Of primary concern are pollutants that possess a direct toxicity to organisms and those that promote eutrophication in receiving waters. One such pollutant that is a product of fish excretions is ammonia. Unionized ammonia (NH_3), the principle toxic form of ammonia which can cause reductions in hatching success and growth rates on many fish species (EPA, 1986), has been found to be below regulatory limits at three trout farm facilities in the state of Virginia (Boardman et al., 1998). This is predominantly because the range of pH and temperature suitable for rearing salmonid species heavily favors ionized ammonia (ammonium ion, NH_4^+). Suspended solids can also pose a substantial threat to receiving waters. Macroinvertebrate communities downstream of trout farm effluents have been reduced due to settleable solids increasing substrate embeddedness and reducing the habitat of intolerant Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa (Selong and Helfrich, 1998). Direct biomass growth and particle accumulation on heptageniid mayflies has been also been determined to be a likely cause of toxicity (Roberts et al., 2009). An increase in suspended solids typically indicates a rise in turbidity that can interfere with sunlight penetration and hamper the photosynthesis process of aquatic plants, therefore lowering oxygen production. Numerical turbidity standards for the protection of fish and wildlife habitats do exist in select states and range from 5 nephelometric turbidity units (NTU) above natural conditions in Idaho to 25 units above natural in Alaska (in streams) and Washington (ADEC, 1978; API, 1980). Increases in organic matter in trout farm effluent have been linked with rises in BOD in receiving waters (Boaventura, et al. 1996; Pulatsu et al., 2004; Viadero et al., 2005). Oxygen depletion in receiving waters is compounded when excess nutrients are introduced, stimulating algae growth. Algae will prevent light from reaching emergent plants, limiting oxygen production, and when pervasive algal blooms decay, oxygen is typically depleted at a rapid rate by aerobic microorganisms. Nutrient concentration, quantified as total nitrogen (TN) and total phosphorus (TP), has been found to significantly ($p < 0.05$) increase between trout farm inflow and outflow (Sindilariu et al., 2009a). Phosphorus loading has been shown to vary in Nordic studies between 4.8-6.0 kg per metric ton of trout produced (Bergheim and Cripps, 1998b; Enell, 1995) while studies carried out in Turkey indicate loadings of 6.25 to 14.50 kg per ton of trout produced (Pulatsu et al., 2004). Variation is highly dependent on the particular type of feed used; however, these numbers are indicative of elevated phosphorus in receiving waters.

The United States Environmental Protection Agency (U.S. EPA) classified certain flow-through trout farms as Concentrated Aquatic Animal Production (CAAP) facilities, which are defined as point sources under the Clean Water Act (U.S. NARA, 2004). This classification requires facilities to discharge at least 30 days per year with a production output of 9,072 kg (20,000 lb) per year for cold water species or 45,359 kg (100,000 lb) per year for warm water species (U.S. NARA, 2004). In June of 2004, the EPA established a new ruling requiring best management practices and record keeping for CAAPs. These narrative requirements included the submittal of a Best Management Plan (BMP) listing how the permittee would achieve several operational goals, including minimizing feed usage, identifying harvesting and cleaning procedures that minimize solids discharge, and proper disposal of aquatic animal mortalities. According to EPA estimates, this ruling will reduce facility discharge of TSS, TN, TP, and BOD. The EPA originally proposed to establish numerical limitations on the concentration of TSS based on the production level of the facility; however, qualitative limits were deemed to better respond to regional and site-specific conditions (U.S. NARA, 2004). Though traditional wastewater treatment technologies were said to effectively remove solids, there were wide variations in TSS levels achieved through these technologies and facilities with exceptional operating controls alone, preventing the establishment of numerical limits. The EPA does however acknowledge that feed management practices may not have the "precision or consistency" that traditional wastewater treatment technologies offer (U.S. NARA, 2004). Nevertheless, the EPA sided with commenters in the final ruling, who believed that additional settling treatment structures would provide little additional benefit over feed management systems. Additionally, a lack of economically feasible pollutant control technologies, in the form of biological and advanced treatment, prevented the establishment of numerical TSS limits. Though numerical limits are absent from federal regulation, local permit writers retain the ability to impose site-specific numerical effluent limits (U.S. NARA, 2004). These limits often address impairments to water quality via waste load allocations (WLAs) resulting from the implementation of total maximum daily loads (TMDLs). NPDES permitting and TMDLs both have the potential to induce upgrades of trout farm treatment capabilities in the near future.

1.1.2 Sedimentation Basins

Sedimentation in the form of quiescent settling zones (sediment traps) is the predominant method of solids removal in flow-through trout farm facilities in the United States; however, the use of off-line settling basins and full-flow settling basins is also common practice (Hinshaw and Fornshell 2002). Quiescent settling zones are areas within a raceway, typically screened off to prevent fish access, in which solids are collected. Periodically, collected solids must be removed to ensure proper settling depth and mitigate the leaching of nutrients from settled solids. The Idaho Department of Environmental Quality (IDEQ) identifies the pumping or siphoning of solids as a common procedure with the usage of a fixed suction port being a more efficient method (IDEQ, 1998). The captured slurry is typically pumped into off-line settling basins that receive a small fraction of full flow, allowing for extended retention times. Regardless of the method, the cleaning of in-line settling basins will involve the resuspension of solids, leading to higher waste concentrations in effluents (Kendra, 1991; Hinshaw and Fornshell, 2002). The reported effectiveness of quiescent zones has varied between less than 20% solids capture (Soderberg, 2007), 75% (Colt and Tomasso, 2001), to upwards of 90% (Hinshaw and Fornshell, 2002). With design of raceway quiescent zones typically hinging on discrete particle settling velocity (modeled by Stoke's Law) being greater than the surface overflow rate, particle size becomes a crucial aspect of design.

Stoke's Law is defines as:

$$V_s = \left[\frac{8g(\rho_s - \rho)d^2}{18\mu}\right] \tag{1.1}$$

where ρ_s = mass density of a particle (kg/m^3 or lb/ft^3)

ρ = mass density of fluid (kg/m^3 or lb/ft^3)

g = acceleration due to gravity (9.81 m/sec^2 or 32.2 ft/sec^2)

d = diameter of a particle (m or ft)

μ = dynamic viscosity of water (N · sec/m^2 or lb sec/ft^2)

Discrete settling assumes negligible interparticle effects and is generally a safe assumption for effluents containing less than 500 mg/L TSS (Fornshell, 2001). Fornshell notes that this is often the case for flow-through fish farms in the western United States which typically have effluent concentrations less than 5 mg/L net (effluent concentration minus influent concentration). Particle-size studies have indicated that, even though small particles (<20 μm) outnumbered their larger counterparts by far in trout producing facilities (Maillard et al., 2005), larger particles (>60 μm) contributed more heavily towards total particle volume and mass (Cripps, 1993; Maillard et al., 2005). Maillard et al. (2005) surveyed three trout farms with fish densities ranging from 6.4-72.8 kg/m^3 and found that 54% of particles (by mass) were greater than 105 μm in size. It is important to note that Cripps (1995) determined that no individual size fraction of particles contain an especially high nutrient load. In addition to verifying that settling velocity of the desired particle size is greater than the overflow rate, the design must prevent scouring. Camp (1946) presented an equation that calculates minimum flow-through velocity required to flush a particle of specified size and weight through a settling zone.

$$V_{scour}\left(\tfrac{m}{sec}\right)=\left[\frac{8\beta g d(\rho_s-1)}{f}\right]^{1/2} \tag{1.2}$$

where β = Constant for type of scoured particles

ρ_s = Specific gravity of scoured particle

g = Acceleration due to gravity (m/sec^2)

d = Diameter (m)

f = Darcy-Weisbach friction factor

Basin geometry and flow were later incorporated into a design equation to calculate a Darcy-Weisbach friction factor (Swamee and Tyagi, 1996).

$$f = 0.223\left(\frac{\nu B}{Q}\right)^{2.5} \tag{1.3}$$

where ν = kinematic viscosity (m^2/sec)

B = channel width

Q = flow (m^3/second)

Warrer-Hansen (1982) provided guidelines that V_s not exceed 0.02-0.04 m/sec when treating trout farm effluent. Though these design parameters are helpful tools, they do not ensure absolute removal efficiencies. Short-circuiting within sedimentation basins is common and actual residence time within a basin may be significantly less than the theoretical value (Macdonald and Ernst, 1986; Marecos do Monte and Mara, 1987). Hydraulic retention time has been shown to improve with the installation of baffles prior to sedimentation (Mangelson and Watters, 1972; Pedahzur et al., 1993). Baffles at the outlet can also be implemented to prevent floating sludge within the basin (Sindilariu et al., 2007). Additionally, increasing the length to width ratio, thus enhancing plug flow conditions, can improve waste stabilization (Mangelson and Watters, 1972).

For aquaculture settling basins, length to width ratios greater than 4:1 and ideally more than 8:1 are recommended (Arceivala, 1983; Michelsen, 1991).

Retention times of 30 min or longer have been recommended by Mudrak (1981) and Henderson and Bromage (1988). Bergheim et al. (1998a) determined overflow rates of 1.0-2.7 m/h was sufficient to promote the settling of solids leaving aquaculture facilities. Investigations of the solids removal efficiency of sedimentation basins have yielded minimal treatment of low TSS concentrations with significant removal occurring only during cleaning situations in which influent TSS concentrations are elevated (Sindilariu et al., 2007, 2009a). Results from Sindilariu et al. can be corroborated by the findings of Henderson and Bromage (1988) in which desired treatment effects of sedimentation ponds begins at influent TSS concentrations greater than 6.64 mg/L. Though treatment efficiency of sedimentation basins is generally low as primary treatment under runoff conditions, Cripps and Bergheim (2000) deemed sedimentation basins appropriate for secondary de-watering or thickening.

1.1.3 Microscreen Filtration

As an alternative to sedimentation, microscreen filters intercept solid waste particles greater than a predetermined mesh size. Solids removal is largely dependent on the characteristics (i.e. particle size) of the wastewater to be treated (Cripps and Bergheim, 2000). Thus a mesh size must be carefully chosen based on particle size distribution analysis. Though greater treatment will always be possible with the minimization of the mesh size, rapid increases in head loss due to clogging will prevent large hydraulic loading rates, therefore creating a tradeoff. Frequent cleaning necessary to remove solids off the filter is carried out by flushing, pneumatic suction, mechanical vibration, or raking (Summerfelt, 1999). Summerfelt (1999) also reports that though frequent backwashing presents an operating cost, microscreen filters benefit from a small footprint, high hydraulic capacity, satisfactory pressure drop, easy installation, and economies of scale when removing solids from large flows.

The three types of screens that are predominantly used in the aquaculture industry are rotating disk, drum, and triangle filters. Rotating disk filters utilize a series of disk-shaped screens, oriented perpendicular to flow, that rotate in a cylindrical or semi-cylindrical tank. Drum filters stretch a microscreen over a cylinder and rotate; passing water from inside the drum into a trough that expels water from the treatment system or conveys it towards the next treatment step. Both drum and disk filters operate with only a portion of influent water in contact with the screen itself. When the screen becomes clogged, a float-switch activates high-pressure nozzles that can continuously clean the screen if necessary. The simplest of all screening technology is the triangle filter. A triangle filter is essentially a static screen aligned at an incline to sieve influent water and carry separated particles to a waste trough. Mäkinen and Eskelinen (1988) experimented with the triangle filter on a fish farm in central Finland with electronically activated flushing nozzles and deemed it more effective in reducing phosphorus content than a swirl concentrator.

Several studies have provided information to help guide the mesh sizing process for aquacultural effluents. For a tank-based system on a salmon smolt farm, screens with an aperture of 60-100 µm were found be most effective. In this case, reductions in BOD_5, TP, and TSS were negligible between the 30 µm and the 60-100 µm mesh size but removal was significantly better than with the 200 µm mesh. Summerfelt et al. (1994) determined that finer microscreen openings reduced TSS in the backwash by a factor of 3.5, but increased backwash frequency by a factor of 4, resulting in ten times the backwash volume. In the same evaluation, a mesh size of 80 to 110 µm was recommended by the authors. Testing of a 60 µm drum screen conducted by the Norwegian Hydrotechnical Laboratory indicated that removal efficiency of TSS (67-97%), TP (21-86%), and TN (4-89%) was proportional with effluent concentration (Cripps and Bergheim, 2000). Lower treatment efficiencies have been documented by Sindilariu et al. (2009a) when utilizing a drum filter with a 63 µm mesh size to treat trout farm effluent. With an influent TSS loading of 6.31 mg/L (±3.9), removal was found to be significant at 41%, with TP and BOD_5 also being removed appreciably at 33% and 26%, respectively (Sindilariu et al., 2009a).

1.1.4 An Introduction to Constructed Wetlands

Constructed wetlands are man-made systems that utilize high biological productivity inherent to wetland ecosystems to improve water quality. Treatment wetlands can be divided into two main categories: surface, and subsurface flow. Free water surface (FWS) wetlands contain water exposed to the atmosphere and often resemble natural marshes. Wastewater is treated by the processes of sedimentation, filtration, oxidation, reduction, adsorption, and precipitation (Kadlec and Wallace, 2008). Subsurface flow (SSF) wetlands convey flow through a soil or gravel bed and typically contain emergent vegetation. Treatment processes are generally the same for SSF and FWS wetlands. Subsurface wetlands can be broken down further into vertical flow (VF) and horizontal flow (HSSF) while surface flow wetlands are often characterized by the type of the vegetation. In a survey of over 200 FWS and SSF wetlands, the median hydraulic loading rate (HLR) of sampled HSSF wetlands was determined to be 6.79 cm/d, while the median HLR for FWS wetlands was found to be 3.05 cm/d (Kadlec, 2009). Additionally, smaller sample sizes analyzed by the United States EPA (2000) suggested design TSS loadings of 20 g/m^2d for HSSF and 5 g/m^2d for FWS wetlands to achieve the same outlet concentration of 30 mg/L. Kadlec (2009) determined these deviations in loadings were largely inflated by small sample sizes, finding no significant advantage for either system for BOD and TSS removal on an input-output concentration basis. In the same study, HSSF wetlands demonstrated lower TSS effluent concentrations for loadings greater than about 4.3 g/m^2d but lagged FWS for lower loadings. Like TSS data, the data clouds for nutrients were largely overlapping providing indistinguishable central tendencies. An operational concern which may preclude the use of FWS systems altogether, is ice formation in colder climates during the winter. Ice formation has also been found to reduce hydraulic efficiency of SSF wetlands, although this effect can be mitigated by the presence of vegetation (Muñoz et al., 2006). Colder water temperatures will lower the rate of certain removal processes such as nitrogen conversion, freezing of the surface will reduce oxygen transfer, and extensive freezing can hydraulically limit system performance (Kadlec and Wallace, 2008).

One of the most attractive aspects of constructed wetlands is the ability to treat wastewater with the minimal cost upfront and throughout the life of the wetland. Initial investments typically can include earthwork, piping and pumping to convey water, soil or gravel media, and support structures. Operating costs are often proportional to energy utilization. Brix (1999) concluded that both surface and subsurface flow wetlands use less than 0.1 $kW \cdot h/m^3$. In comparison, alternative treatment methods that can be relatively inexpensive to operate include facultative lagoons which require 0.11 kW·h/m^3 (Cambell and Ogden, 1999) when rapid infiltration is used, and 0.16 kW·h/m^3 (Crites et al., 2006) when overland flow is utilized. More expensive treatment options include trickling filters with nitrogen removal which were found to use 0.61 kW·h/m^3 (Crites et al., 2006) and the activated sludge process with nitrification, requiring 0.76 kW·h/m^3 (Cambell and Ogden, 1999).

1.1.5 Removal Processes within HSSF Constructed Wetlands

Solids removal within HSSF wetlands is primarily governed by the settling and interception of particles onto the soil, sand, or gravel media. Particulate settling will occur if the particle traveling time is greater than the settling time. Equation 1.4, as adopted from Kadlec and Wallace (2008), illustrates this scenario in HSSF wetlands using the d_{10} of the media to approximate pore size.

$$\frac{L}{v} > \frac{d_{10}}{w} \tag{1.4}$$

where L = wetland length, m

d_{10} = Particle size representing the smallest 10% of media

v = actual flow velocity, u/ε (m/s)

u = superficial velocity, L/t_{travel} (m/s)

ε = bed porosity

w = solids settling velocity (m/s)

Mechanisms of granular bed filtration are well documented in handbooks (Metcalf and Eddy Inc., 1991; Crites and Tchobanoglous, 1998) and include inertial deposition, diffusional deposition, and flow line interception. Though all mechanisms may contribute to TSS removal, it is generally accepted that the inertial deposition and diffusional deposition will dominate in fine soils, whereas interception is the primary removal mechanism in coarser media. With organic material in aquaculture wastewater being of concern, the active carbon cycle in wetlands provides many pathways for the removal of organic carbon. Plants require CO_2 for photosynthesis; methanogenesis and decomposition of surface litter release carbon to the atmosphere; and heterotrophic oxidation of carbon compounds within the wetland cell is a major contributor to carbon removal (Kadlec and Wallace, 2008).

Nitrogen exists in wetlands as organic nitrogen and inorganic forms, which include ammonium (NH_4^+), ammonia (NH_3), nitrate (NO_3^-), and nitrite (NO_2^-) (Bowden, 1987). Nitrogen transformation is a multistep process, many of which occur simultaneously. Ammonification, or mineralization, is the biological process in which organic nitrogen is transformed into ammonia through a catabolism of amino acids. Nitrification is another multistep process in which bacteria use energy from the oxidation of ammonia to form nitrite for cell synthesis. Chemolithitrophic bacteria that perform this task belong to the genera Nitrosospira, Nitrosovibrio, Nitrosolobus, Nitrosococcus and Nitrosomonas, and even Nitrosomonas europaea (Vymazal, 2007). The second step, performed by the chemolitrotrophic bacteria species Nitrobacter, is the oxidation of nitrite into nitrate. Optimal temperature for nitrification ranges from 25 to 35 °C in cultures and from 30 to 40 °C in soils (Vymazal, 2007), and minimum temperature for growth of Nitrosomonas and Nitrobacter are 5 and 4 °C, respectively (Cooper et al., 1996). Nitrogen removal in HSSF wetlands is primarily due to the denitrification process, where facultative anaerobic heterotrophs utilize oxidized forms of nitrogen in the absence of oxygen as electron acceptors in a respiratory process. Denitrification will not occur; however, at dissolved oxygen concentrations greater than 0.3-1.5 mg/L (U.S. EPA, 1993).

Dissolved phosphorus exists in wetlands as both inorganic phosphate (orthophosphate) and dissolved organic phosphate (in combination with organic materials). Together, these species are known as soluble reactive phosphorus (SRP) which is readily taken up by plants and sorbed to wetland granular media and biomass. Sorption is dependent on the mineral properties of the aggregate with iron and aluminum oxides providing significant phosphorus retention (Rustige et al., 2003). The most crucial sorption mechanism is believed to be ligand exchange reactions, in which phosphate displaces water or hydroxyl from the surface of Al and Fe hydrous oxides (Faulkner and Richardson, 1989). Warmer water temperatures due to the endothermic nature of the sorption reaction (Jin et al., 2005) and finer aggregate sizes (Onyullo and McFarland, 2003) are also known to result in higher sorption capacity. Particulate phosphorus, measured as the difference between total phosphorus and total dissolved phosphorus, is removed by the same mechanisms responsible for the removal of TSS. Plants contain only a small fraction of the total phosphorus within a wetland, and therefore the uptake capacity of macrophytes in wetlands is limited (Brix, 1994; Vymazal, 1995).

1.1.6 Design of Constructed Wetlands

Wetland design often begins with addressing operational concerns such as treatment redundancy, cell type, and cell quantity. Regulatory authorities sometimes require wastewater treatment systems to maintain a certain performance when repairs need to be made. Therefore, multiple flow paths may become a necessary design requirement. Secondly, multiple wetland cells in series maybe desired if greater treatment efficiency or if two-stage "hybrid" wetland system is sought after to carry out multiple functions (Vymazal, 2005). Kadlec and Wallace (2008) state that while the advantage of cells in series is minimal, when an effluent quality approaches the background concentration, there are significant impacts of compartmentalization on wetland size. Designers are faced with a challenge when choosing a length to width ratio for the wetland cell which stems from contradictory design goals. The first of which is spreading the influent over a large cross sectional area to delay clogging and the second is to uniformly distribute flow. Clogging poses many problems and the EPA considers any overland flow that may result, a "failed" wetland for those treating home septic systems (U.S. EPA, 2002). Hydraulic failure is a relative term; however, and depends on regulatory requirements and the degree of flexibility the operator has with treatment performance. Wallace and Knight (2006) suggest that the cross sectional area of HSSF wetlands be configured such that the BOD loading is less than 250 $g/m^2 \cdot d$ for bed medias with a d_{10} >4 mm. It is most common; however, that coarser media be used in the inlet and outlet regions of the wetland cell. Published guidelines have indicated ranges of 25-50 mm (Wallace and Knight, 2006) and 40-80 mm (U.S. EPA, 2000) for inlet media size. To aid in maintaining uniform width distribution, the installation of an open trench preceding the wetland media has been found to retain solids while maintaining uniform width distribution (U.S. EPA, 1993; King et al., 1997; Murphy and Cooper, 2010).

A concern regarding deeper beds is that they promote vertical stratification of flow with flow paths bypassing the plant root zone (Fisher, 1990; Marsteiner et al., 1996). Kickuth's (1977) investigations on the Phragmites root systems identified that 60 cm was the maximum depth that roots would penetrate. Common reed (Phragmites spp.) is considered invasive in the United States and later research has indicated that 20-30 cm is where the majority of root profiles are most dense (Daniels and Parr, 1990; Kuusemets et al., 2002). Deeper beds reduce cross-sectional loading and have speculative benefits such as additional volume for sediment storage and can possibly provide ice formation at the top in colder climates while preserving flow at greater depths (Kadlec and Wallace, 2008). Media type is very much dependent on local availability for economic reasons, and size becomes a tradeoff between greater hydraulic conductivity and treatment. Wallace and Knight (2006) recommend main bed media sizes greater than 4 mm while the U.S. EPA (2000) has suggested greater media sizes between 20-30 mm with a 5-20 mm top layer.

Two of the most crucial aspects of hydraulic design involve verifying enough driving head is available to overcome frictional losses in the wetland bed and the ability to control water level within the wetland. The relationship between bed friction and hydraulic conductivity is dependent on whether laminar or turbulent conditions persist within the wetland. Equation 1.5 as adapted from Kadlec and Wallace (2008) relates superficial velocity, hydraulic conductivity, and a turbulence factor.

$$-\frac{dH}{dx} = \frac{1}{k}u + \omega \cdot u^2 \qquad (1.5)$$

where H = elevation of water surface (m)

x = distance from inlet, m

u = superficial velocity, m/s (L/t_{travel})

k = hydraulic conductivity, m/d

Often, the transitional region will dominate gravel-based wetlands yielding significant contributions from the turbulent term, ω. The Ergun equation (Ergun, 1952) provides an estimation of the turbulence factor and hydraulic conductivity based on the assumption that the media is composed of uniformly packed spheres.

$$-\frac{dH}{dx}=\left(\frac{150(1-\varepsilon)^2\mu}{\rho g\varepsilon^3 D^2}\right)u+\left(\frac{1.75(1-\varepsilon)\mu}{g\varepsilon^3 D}\right)u^2 \tag{1.6}$$

where H = elevation of water surface. m

x = distance from inlet, m

u = superficial velocity, m/s (L/t_{travel})

k = hydraulic conductivity, m/d

ρ = density of water, kg/m^3

μ = viscosity of water, kg/m/d

ε = bed porosity

g = gravitational constant, m/d^2

Hydraulic conductivities for crushed angular media have been found to be about three times lower than spheres (Idelchik, 1986). Inlet design is also an important aspect of hydraulic design. In the United States, subsurface infiltration chambers have become a promising development because of their ability to maximize the cross-sectional area to which solids are loaded (Cambell and Ogden, 1999; Wallace and Knight, 2006).

1.1.7 Review of Constructed Wetlands Use in Aquaculture Treatment

Several researchers have analyzed treatment capabilities of constructed wetlands when receiving effluent from various aquaculture facilities. Sindilariu et al. (2008) proposed general guidelines for trout production facilities in particular. The study revealed that at low to medium production (0.35 – 0.70 kg$(m^3s^{-1})^{-1}$year^{-1}, sedimentation appears sufficient. For increased production (0.70 – 1.15 kg$(m^3s^{-1})^{-1}$year^{-1} , micro-screens become feasible and application of biological treatment is encouraged. For example, the use of SSF wetland in combination with pre-sedimentation for TSS removal was recommended by the author. TSS removal within HSSF wetlands treating trout farm effluent is generally good to excellent with removal efficiencies reported between 95.9-97.3% (Schulz et al., 2003), 67.0% (Sindilariu et al., 2007), and 59.4% (Sindilariu et al., 2009a). Average inflow concentrations for these studies were 14.15, 7.55, and 4.88 mg/L, respectively. A survey of Czech HSSF wetlands yielded an average of 84.3 percent removal with an average influent concentration of 64.8 mg/L (Vymazal, 2002).

Removal of organics is often quantified as % reduction in oxygen demand analyzed as either chemical oxygen demand (COD), or 5-day biochemical oxygen demand (BOD_5). Sindilariu et al. (2007, 2009a) have found BOD_5 removal to vary between 48.8% and 62.1% and COD removal between 39.8 and 52.2%. Schulz et al. (2003) saw slightly higher removals between 64.8% and 71.4%, with inflow COD concentrations about three times greater. Czech HSSF wetlands have historically accomplished excellent removal of BOD_5 and COD at higher influent loadings on average, with removal efficiencies of 88.0% (n = 55) and 74.9% (n = 53), respectively (Vymazal, 2002). This is influenced by their high organic influent concentrations, represented as BOD_5 and COD, of 87.2 and 211 mg/L.

Phosphorus removal is highly dependent on the type of filtration media and removal has reported to vary between 37.3 and 68.5 % when treating trout farm effluent (Sindilariu et al., 2007; Sindilariu et al., 2009a; Schulz et al., 2003). Beyond media type, hydraulic loading rates (HLRs) have an impact on most nutrient parameters. Both phosphate and TP experienced increased area retention (eq. 2.4) when decreasing hydraulic loading by about a factor of 4, although TP removal improved only marginally from 54.2 % to 58.6% (Sindilariu, 2009a).

The reduction of total nitrogen in a single wetland cell is complicated by the diverse conditions necessary to carry out both nitrification and denitrification. This is reinforced by the relatively low treatment efficiencies reported by Sindilariu (2007, 2009a) of 4.9-6.2%. Schulz (2003) found higher treatment efficiencies, upwards of about 40%, and significant improvement in removal for the largest hydraulic residence times at two feed diets. Table 2.1 (Vymazal, 2005) provides HSSF wetland performance averages for data collected worldwide from wetlands treating various wastewaters.

1.2 An Investigation into the Sorption Potential of Soluble Phosphorus and Rhodamine WT onto Gravel Media Extracted from a Mature HSSF Constructed Wetland

1.2.1 Phosphate Sorption in Constructed Wetland Granular Media

Phosphorus sorption capacity of wetland substrates is subject to tremendous variability. Laboratory sorption studies have been conducted with a variety of media intended for use in subsurface flow (SSF) constructed wetlands including limestone, sand, gravel, topsoil, and other artificial materials (Mann and Bavor, 1993; Sakadevan and Bavor, 1998; Johansson, 1999; Pant et al., 2001; Cui et al., 2009). Limestone, a relatively common sedimentary rock, received some attention for potentially high phosphorus sorption as a result of its high calcium content. Instead, relatively low sorption ranging from 0.5-20 mg/kg was determined by Johansson (1999) with the formation of calcium complexes and precipitation being the likely mechanisms of sorption. Sands can be a viable medium for SSF constructed wetlands because of their local availability and improved hydraulic conductivity over soils. Sorption maxima for sands extracted from various regions in Denmark were found to vary between 20 mg/kg for quartz sand and 129 mg/kg for sand extracted from Løgtved (Arias et al., 2001). Data from this study showed that high calcium content will favor precipitation reactions, enhancing phosphorus removal more than the presence of iron and aluminum. A study performed by Pant et al. (2001) determined sand from a glacial kame in Niagara, Ontario to have a sorption capacity of 417 mg/kg. In this study, Lockport dolomite, a manufactured sand consisting of a mixture of calcium and magnesium carbonates, was also analyzed and found to possess a sorption maximum of 303 mg/kg.

Like sand, gravel is a widely used substrate in SSF constructed wetlands for its local availability and high hydraulic conductivities. Gravel extracted from regional sites in Australia was found to have sorption maxima of 25.8 mg/kg for particle sizes of 5-10 mm, and 47.5 mg/kg for particle sizes of 3-5 mm (Mann and Bavor, 1993). Improved performance was estimated by Cui et al. (2009), finding a maximum sorption capacity of 494 mg/kg for gravel taken from areas near Guangzhou City, China. In this study, both turf and topsoil were found to have considerably greater sorption maxima, but the author noted their tendency to become clogged in SSF constructed wetlands to be limiting factors. More exotic substrates have also been investigated such as blast furnace slag, a steel-industry byproduct, with reported sorption maxima ranging from 1598 mg/kg (Cui et al., 2008) to 44,247 mg/kg (Sakadevan and Bavor, 1998). Many of these substrates, such as blast furnace slag, are not widely available, limiting their use in constructed wetlands.

1.2.2 Tracer Studies in Subsurface Flow Media

The most common method of acquiring hydraulic flow pattern data from within wetlands is through the use of tracers. Typically introduced as a pulse, tracer concentration is measured at the outlet over a period of time until the background concentration is approached. The investigator is left with a series of residence times encompassing all pathways of travel, also known as a tracer response curve. The quality of the data is highly dependent on selecting a tracer which behaves conservatively in the medium; i.e. no physiochemical or biological interactions that may hinder or hasten travel time. Additional criteria for tracer selection may include the presence of a low background concentration, low toxicity, and ease of analysis. There is a wide array of tracers available, including radioactive tracers, salt ions, and fluorescent dyes such as Rhodamine WT (RWT). Radioactive tracers have sufficient tracer properties in wetlands (Crohn et al., 2005); however, regulatory requirements and licensing procedures (NRC, 2000) for subsurface use may prevent usage. Salt tracers are generally preferred in Horizontal Subsurface Flow (HSSF) Wetlands for their observed conservative nature in subsurface media. Bromide salts have been identified as conservative due to near 100% mass recovery in a variety of studies (Garcia et al., 2004; Drizo et al., 2000, Grismer, 2001). Sodium chloride solutions have demonstrated good mass recovery and offer an inexpensive option easily monitored by electrical conductivity probes (Chazarenc et al., 2003). Salt tracers are not without complications and can be prone to density stratification in FWS and HSSF wetlands (Schmid et al., 2004; Chazarenc et al., 2003). The fluorescent tracer RWT has been used extensively in constructed wetlands with a free water surface (FWS). Though prone to photodegradation and biodegradation (Dierberg and DeBusk, 2005), both forms of degradation have been determined to be less than 10% for studies carried out in less than one week (Lin et al., 2003). Numerous studies, however, have indicated RWT sorption in both field-scale subsurface tests (Knowles, 2010; Ríos et al., 2009) and laboratory tests (Sutton et al., 2001, Shiau et al., 1993; Sabatini and Austin, 1991; Smart and

Laidlaw, 1977). Widely available and relatively inexpensive, RWT offers many qualities other tracers cannot including low background concentration and detection limits as low as 0.02 μg/L with modern equipment (Leibundgut et al., 2009).

The sorption of tracers in subsurface flow wetlands to media and attached biomass is a multi-process phenomenon encompassing adsorption, absorption, ion exchange, and chromatography. Typically, sorption losses are defined as the tracer mass consumed by the summation of all these processes (Leibundgut, 1981). Sorption can be reversible, irreversible, or a combination of the two. Irreversible sorption implies permanent loss on the sorbent whereas reversible sorption will temporarily retain tracers within the wetland and extend tracer breakthrough curves, delaying and broadening the tracer response curves (Kadlec and Wallace, 2008). Tracers that display some nonideal behavior are generally assumed by tracer hydrologists to reach instantaneous equilibrium coupled with a linear reaction isotherm (Leibundgut et al., 2009). These reaction isotherms are typically generated from standardized laboratory batch tests that produce the term K_d, or the partition coefficient. The U.S. EPA defines K_d as the "ratio of contaminant concentration associated with the solid to the contaminant concentration in the surrounding aqueous solution when the system is at equilibrium" (U.S. EPA, 1999). This implies transport of the nonideal tracer to be slower than the flow of water by the retardation factor, R_d, which can be calculated via a series of formulations derived from the general transport equation, or calculated directly from laboratory column testing.

1.2.3 Sorption Properties of Rhodamine WT

Opportunities for the sorption of RWT in the subsurface are numerous. Above a pH of 6, RWT molecules have a net negative charge allowing for electrostatic interactions with positively charged sites. Complexation of RWT's carboxylate groups and hydrophobic exclusion are two other possible means of sorption. Sorption to sediments and attached biomass is believed to be primarily responsible for RWT losses in subsurface flow wetlands. Giraldi et al. (2009) observed increased RWT losses between initial start-up and after a vertical subsurface flow (VSSF) constructed wetland had been operating for seven months. This increase was presumably because of the development of biomass over time. Sorption to vegetation in this study and in the study of Turner et al. (1991) was found to be minimal. RWT exhibits greater sorption potential onto metal oxides in comparison to sand-based solids (Vasudevan et al., 2001). The author concluded that this was likely due to the higher surface area of metal oxides coupled with their increased potential for electrostatic interactions and complexation.

Further investigation into the properties of RWT has yielded the identification of two isomers (isomer 1 (para) and isomer 2 (meta)) with unique emission spectra and sorption tendencies (Shiau et al., 1993; Sutton et al., 2001; Vasudevan et al., 2001). Structurally, these isomers differ in the positioning of the carboxylate groups with respect to the xanthenes ring (Shiau et al., 1993; Vasudevan et al., 2001). Vasudevan et al. (2001) postulated that the geometric arrangement of the carboxylate groups in the meta isomer promoted a higher potential for hydrophobic exclusion from bulk solution, increasing the extent of sorption. Additionally, the study hypothesized that the positioning of the carboxylate groups in the meta isomer allowed for a more focused region of negative charge density. Distinct sorption tendencies of each isomer result in chromatographic separation in subsurface media. Since relative concentrations of each isomer differ from calibration and each isomer has its unique emission spectra, fluorometer error in measurements can increase to about 7.8% (Sutton et al., 2001). In addition to fluorometer error, chromatographic separation of RWT isomers has the potential to complicate interpretations of tracer response curves by producing two peaks in tracer response curves. In this situation, non-conservative behavior of the meta isomer can prolong its travel creating an additional peak beyond the initial peak that results from the travel of the para isomer and non-sorbing fraction of the meta isomer. Two-step (Shiau et al., 1993) and dual peak breakthrough curves (Sutton et al., 2001), for step and pulse tracer inputs respectively, from laboratory column tests have suggested that this may be the case.

Chapter 2: Evaluation of Constructed Wetlands and Pretreatment Options for the Treatment of Flow-through Trout Farm Effluent

2.1 Introduction

Globally recognized as the fastest-growing animal-food-producing sector, aquaculture has bloomed into a 98.4 billion dollar industry (FAO, 2010). In the United States, federal regulation of wastewaters from Concentrated Aquatic Animal Production Facilities (CAAP) has halted since narrative requirements were set forth by the EPA in 2004. Though no numerical limits were established at this time, permit writers have the ability to impose site-specific, water quality-based effluent limits when appropriate. In addition to permitting requirements established by the National Pollutant Discharge Elimination System (NPDES), Total Maximum Daily Load (TMDL) studies may impact point sources from CAAP facilities, and Waste Load Allocations (WLA) may be implemented or adjusted accordingly. Treatment technologies widely implemented already, such as sedimentation basins, have been accused of not being a viable means of treating flow-through aquaculture wastes due to the high volumes of relatively low concentrated waste inherent to such facilities (Cripps and Bergheim, 2000). Therefore, it is important for operators and permit authorities alike to be aware of practicable treatment technologies, such as constructed wetlands, that can effectively manage such effluents.

Aquaculture effluents are characterized by continuous, high volume flows with elevated loadings of conventional pollutants including TSS and BOD, in addition to non-conventional pollutants such as phosphorus and nitrogen (U.S. EPA, 2004). Waste is primarily in the form of particulate solids and nutrients embedded in uneaten feed and fecal excretions, and dissolved nutrients from trout excretions. The particulate fraction of nutrients and organic carbon is variable, with about 7-32% of TN, 30-84% of TP, and 80% of organic carbon (OC) being particle-bound (Foy and Rosell 1991; Bergheim et al., 1993a,b; Cripps and Bergheim, 2000). Effluents from trout farm facilities can have a significant effect on macroinvertebrate in receiving waters. For instance, elevated TSS levels may contribute to the embeddedness of stream beds, thus reducing macroinvertebrate habitat (Selong et al., 1998). The saprobic index, a common biological indicator combining the number of species and their saprobic rates, was found to increase to a significant extent for macroinvertebrate fauna downstream of trout farms with production intensities above 9.5 g/m^3 (Sindilariu, 2009a). In another study by Roberts et al. (2009), particle accumulation was found to be a directly toxic for Ephemeroptera, Plecoptera, and Trichoptera taxa immediately downstream of trout farm effluents.

Sedimentation basins are common Best Management Practices (BMP) found in flow-through facilities as full-flow settling basins, offline settling basins, and sediment traps (Hinshaw and Fornshell, 2002). Mechanical treatment processes, including sedimentation, are primarily designed to remove the particulate fraction of pollutants, leaving soluble nutrients and organic carbon untreated. Microscreens have also been investigated for their ability to ensure removal of the particulate fraction large than its mesh size. Some success has been witnessed already with a 63 μm microscreen removing 41% of TSS from trout farm effluents (Sindilariu et al., 2009a). Numerous investigators have advocated the use of horizontal, sub-surface constructed wetlands (HSSF) for the treatment of both the particulate and soluble pollutants in aquaculture effluents (Sindilariu et al., 2007, 2009a; Schulz et al., 2003; Lin et al., 2002a,b).

Removal processes in HSSF wetlands are diverse and benefit from the natural energies supplied by the sun, soil, and organisms—large and small. Solids removal is primarily governed by the settling and interception of particles onto soil, sand, or gravel media. Resuspension mechanisms, such as wind and animal intrusion, are limited in HSSF wetlands; however, TSS accumulation can significantly reduce hydraulic conductivity and even cause flooding of the wetland bed. A diverse body of processes governs transformations of dissolved organic matter and nutrients within HSSF wetlands. Nitrogen can be affected by nitrification, denitrification, fixation, mineralization, and plant and microbial uptake; whereas phosphorus can be removed by sorption, precipitation, and plant uptake (with harvest) (Vymazal, 2007). Transformation of dissolved organic matter in treatment wetlands is largely accomplished by biodegradation, bio-uptake, sorption, volatilization, and photolysis, with volatilization and photolysis being of lesser significance in HSSF wetlands (Barber et al., 2001). Vymazal (2002) suggested that the pairing of mechanical pre-treatment with constructed wetlands delays clogging of the wetland media, while Sindilariu et al. (2009a) concluded that a combination provides effective biological treatment of TAN and BOD_5.

In the following study, the treatment performance of two practicable treatment systems receiving trout farm effluent was investigated over the course of about one year. The specific objectives of the project were to:

1. Determine the most effective pretreatment mechanism for HSSF constructed wetlands.
2. Analyze the treatment efficiency of HSSF constructed wetlands monitored over the course of a year identifying conditions promoting increased performance.
3. Characterize pulses of increased solids loading and resultant treatment during raceway cleaning and harvesting scenarios.

2.2 Methods and Materials

2.2.1 Field site

The experimental setup was constructed at the end of a flow-through trout farm, that contained two species of trout: rainbow and golden rainbow (*Oncorhynchus mykiss*), and brook trout (*Salvelinus fontinalis*). Feeding intensity varies between 180 and 360 kg/day, and the feed conversion ratio is typically 1.2. Total production ranges from 36,000 – 41,000 kg/year (80,000 – 90,000 lb/year). Source water for the farm is obtained from a freshwater spring at a flowrate of 3,000 m^3/day (792,000 gallons per day) into the facility. A typical raceway is 22.9 m long, 2.1 m wide, and 0.5 m deep (75 ft x 7 ft x 1.5 ft) with a stocking density of 48-64 kg/m^3 (3-4 lb/ft^3). The upper system of the farm contains a hatchery and 18 paired concrete raceways and the lower system is comprised of eight raceways.

2.2.2 Sedimentation Basin

The sedimentation basin was constructed utilizing on wall common to an unused raceway at the tail end of the facility. A 60 mil ethylene propylene diene monomer (EPDM) liner was stretched along the walls and base of the basin to ensure a watertight seal from the raceway. The basin, shown in Figure 2.1, was 4.6 m long, 1.2 m wide, and 1.2 m deep (15 ft x 4 ft x 4ft). The sedimentation basin was originally designed for a 30 minute theoretical hydraulic residence time

(HRT); however, actual flow rates were lower than anticipated inflating the HRT. Baffles were constructed at the inlet and outlet to ensure quiescence.

2.2.3 Microscreen

Triangular style filtration was ideal due to its simplicity and cost of construction. A static microscreen with a mesh size of 80 μm was placed at an incline within plywood housing. The flow of wastewater can be controlled by two valves prior to entering a slotted distribution pipe. Figure 2.2 illustrates how treated water passed into a collection pipe below the housing and solids cascaded into a collection trough.

2.2.4 HSSF Constructed Wetlands

Wetland basins were made 6.7 m long, 2.4 m wide, and 0.9 m deep (22 ft x 8 ft x 3 ft) and constructed of plywood covered with 60 mil EPDM liner. The influent and effluent ends of each wetland featured a 1 ft wide baffle to promote even distribution of the flow through the wetland medium and some initial settling of solids. Dual valves on the pipes feeding the influent baffles allowed control of the flow rate through the wetland. Standpipes connected to the effluent baffle allowed control of the water surface elevation and were initially set to 0.86 m (34 in), but were lowered to 0.76 m (30 in) to minimize surface flow generated by inlet clogging. Lowering the effluent standpipe was also believed to help prevent freezing near the surface during the colder winter season.

The wetland basins are filled with coarse (19 – 25 mm) gravel at the influent and effluent ends to promote even distribution of flow through the wetland. The remainder of the wetland basins was filled with finer gravel (2-8 mm). Two standpipes, 0.6 m (2 ft) apart, were placed into the gravel at 2.4 m (8 ft) intervals along the length of the wetland cells to allow for the monitoring of dissolved oxygen, a critical parameter of concern for the denitrification process. Both wetlands were left unplanted; however, diverse native flora quickly emerged in the first growing season of operation. The addition of vegetation has the ability to improve treatment efficiency, as the resulting rhizosphere encourages symbiotic relationships with the microbial community that benefits pollutant removal (Naylor et al., 2003). Figures 2.3 and 2.4 illustrate the full system dimensions and orientation.

2.2.5 Sampling and Analysis

Samples were taken from five sites throughout the wetland (Figure 2.4). Table 2.2 summarizes the sampling sites. Samples were collected on fifteen days between March 2010 and March 2011. Samples collected within the raceway influent to pretreatment were collected at mid-depth using a hand pump, and emptied into either 1 L plastic bottles or 40 mL vials. All sample containers had been washed and labeled accordingly prior to collection. Sample containers were placed in a cooler with freezer packs for transportation back to the Environmental and Water Resources (EWR) lab at Virginia Tech. NO_3-N, NO_2-N, PO_4-P, and BOD_5 were analyzed within 12-48 hours of collection. Temperature and pH were monitored using a pH meter (Oakton pH 6 Acorn series) following the EPA 150.1 pH electrode method. Conductivity and dissolved oxygen were analyzed using a YSI (model 85) oxygen, conductivity, salinity, and temperature probe. Table 2.3 summarizes the parameters tested and their respective analytical methods.

Except where noted, analytical procedures were performed in accordance with Standard Methods for Examination of Water and Wastewater (Clesceri et al., 1998). Additionally, system flow rate was ascertained by noting the time required to fill a pre-measured container (68 L) with a stopwatch at the effluent standpipes of both wetland basins. On occasion, the same “bucket and stopwatch” method was used to verify flow entering the wetlands was nearly identical to that at the effluent end. Without a densely planted vegetation bed, water losses due to evapotranspiration were deemed insignificant. If necessary, control valves were adjusted to equate flow rates in each wetland cell.

BOD_5 was determined using Standard Methods 5210 B. Three dilutions were initially performed (at 150 mL, 75 mL and 60 mL of sample water) at a temperature of 20^oC for 5 days. It was eventually determined that to achieve the minimum DO depletion recommended by Standard Methods, BOD bottles needed to only be filled with the test waters. Samples for TN and TP were digested and initially submitted to the Biological Systems Engineering Department of Virginia Tech for automated analysis. Later in the project, samples were analyzed using HACH methods 10071 (TN) and 8190 (TP) on the HACH DR2800 Spectrophotometer.

2.2.6 Simulated Harvesting and Cleaning Scenarios

The simulation of raceway cleaning was performed by using a street broom to push settled solids to one section of the raceway. Normally, the accumulated solids would be siphoned out of the system; however, since the purpose was to test the performance of the treatment system under higher mass loadings, this step was omitted. The simulated cleaning was carried out in same raceway where the sedimentation basin was located, just upstream of the treatment system. Only a small portion of the raceway underwent the cleaning process as directly agitating only a small fraction of the raceway proved to be enough to disturb solids throughout its entirety. Sampling locations and methods were the same as during normal conditions with the exception of the following changes to account for the “slug” of solids entering the system. After the stirring up of solids, five samples were taken between 5 minutes and 60 minutes to better characterize the high potential loadings from raceway cleaning. A combination of theoretical retention times and tracer tests were used to create a pertinent sampling schedule. This is illustrated in Table 2.4.

In performing the simulated harvest, a screen was used to crowd the trout from one end of the raceway to the other. Trout are actually harvested from this crowded condition using a dipnet to lift the trout onto a truck. By crowding the trout in this way, if there is any sediment on the bottom of the raceway, it gets agitated very quickly and thoroughly by the trout activity. The harvesting process was simulated in the nearest stocked raceway, with three unused raceways in between the sedimentation basin and the location of harvesting. Travel time in between the simulated harvesting site and the sedimentation basin was roughly estimated by using a stop watch to track the time required for a tennis ball to travel downstream. The harvesting raceway was 23 m long and 2.1 m wide, with a water depth of 0.9 m (75 ft x 7 ft x 3 ft). This aided in creating a sampling schedule that would reflect higher loadings due to harvesting conditions. A schematic showing sampling methodology is featured in Table 2.5.

2.2.7 Calculations and statistics

If a parameter involved the collection of triplicate samples, then the results of the triplicate samples were analyzed separately and averaged, with standard deviation reported alongside. The relative treatment efficiency of both mechanical and wetland treatment systems were calculated as follows:

$$\%\Delta = (C_{in} - C_{out})/C_{in} \text{ x } 100\% \quad (2.1)$$

where C_{out} = effluent concentration

C_{in} = influent concentration

Positive treatment efficiency values indicate removal, while negative values indicate increases of the particular parameter. Hydraulic loading rates (m/day) were calculated as:

$$HLR = 86.4 \cdot Q \cdot A \quad (2.2)$$

where Q = flow rate (L/s)

A = surface area (m^2)

86.4 = conversion factor

Area loading (g/m^2day) and removals (g/m^2day) were calculated as:

$$AL = C_{in} \cdot HLR \quad (2.3)$$

$$AR = \Delta p \cdot HLR \quad (2.4)$$

where Δp = net difference between inflow and outflow concentration (mg/L)

HLR = hydraulic loading rate (m/day)

The influence of treatment on receiving waters was tested by one-way analysis of variance (ANOVA) utilizing the JMP software package (SAS, 2010). Differences between means were evaluated for significance using Tukey's HSD test ($p \leq 0.05$) for homogeneous variances (Levene test) and Dunnett's T3 test ($p \leq 0.05$) for inhomogeneous variances.

2.3 Results

2.3.1 Evaluation of Pretreatment Options

Average solids removal was similar for each pretreatment option, with the microscreen providing a 14.6% TSS reduction and the sedimentation basin removing 18.9% TSS. Average influent concentration of TSS to each treatment system was 5.4 (±3.7) mg/L. Neither hydraulic loading nor influent concentration was determined to have a significant effect on TSS removal. Removal of organic matter was minimal with the sedimentation basin providing slightly superior treatment. BOD_5 and COD were reduced on average by 4.2% and 8.7%, respectively, by microscreening, and 8.3% and 16%, respectively, within the sedimentation basin. Influent BOD_5 concentrations were 4.4 (±1.8) mg/L and COD concentrations were 12.4 (±6.7) mg/L. Though each pretreatment alternative provided some TSS removal, no statistically significant removal ($p \leq 0.05$) of any measured parameter was observed in either pretreatment mechanism.

2.3.2 Treatment Efficiency of Constructed Wetlands

The two HSSF constructed wetlands cells removed 69% of TSS when receiving low influent concentrations averaging 4.5 (±3.1) mg/L. Solids removal was accompanied by a 66% reduction in turbidity. Reduction of organic matter was similar for all three indicators: BOD_5, COD, and TOC were reduced by 62%, 50%, and 55%, respectively. Generally, lower hydraulic loading provided greater treatment of BOD_5 (Figure 2.5) and COD (Figure 2.6). HLRs below 5 m/day produced no BOD_5 removals below 40%, whereas, loadings below 4 m/day were associated with COD removals above 40%. Treatment efficiency of BOD_5 appeared to improve during the colder winter months, as illustrated in Figure 2.7.

Nitrogen transformations persisted within the wetland cells with insignificant net gain or loss of TN. Total ammonia nitrogen (TAN) was reduced by 69% while NO_3-N increased by 116%. Average influent concentrations were 0.65 (±0.2) mg/L for TAN and 0.56 (±0.1) mg/L for NO_3-N. The HSSF wetlands reduced PO_4-P and TP by 18% and 24%, respectively, with influent TP concentrations averaging 0.26 mg/L. Removal of TP was greater for higher areal loadings influent to the wetlands, as indicated by Figure 2.8. Treatment efficiencies were statistically significant ($p \leq 0.05$) for all measured parameters with the exception of NO_2-N and TN. Treatment performance of individual wetland cells is illustrated in Figure 2.9 and system performance is summarized in Table 2.6.

2.3.3 Treatment during Cleaning and Harvesting Conditions

Raceway cleaning resulted in elevated concentrations of TSS (43 mg/L), TP (0.45 mg/L), COD (22 mg/L), and turbidity (41 NTU). TSS concentrations were reduced by about 60% on average within the sedimentation basin, while the microscreen only appeared to reduce solids by 11%. The sedimentation basin reduced TP by about 28% while the microscreen had a negligible effect on phosphorus. The constructed wetlands removed 99% of TSS with influent concentrations averaging 28 mg/L and effluent concentrations at less than 1 mg/L. A 99% reduction of turbidity accompanied this reduction in solids. Wetland TP reduction averaged 43% at influent concentrations averaging 0.40 (±0.09) mg/L and effluent concentrations averaging (±0.05) mg/L. System performance was similar with each treatment train reducing 99-100% of solids and 75-76% of COD. The combination of the sedimentation basin and constructed wetland (SB-CW) reduced TP by 55%, whereas 48% removal was provided by the microscreen and constructed wetland (MS-CW).

During harvesting, influent TSS concentrations averaged 7.2 mg/L, and did not exceed 9.3 mg/L. Though influent TSS concentration during the harvesting period (Figure 2.10) indicated that the spike was likely due to a single event (i.e. harvesting), concentrations were not much higher than normal operation (Table 2.7). Table 2.8 shows the treatment efficiency of select parameters during raceway cleaning, harvesting, and normal (neither cleaning nor harvesting) operation. The microscreen and sedimentation basin demonstrated a marginal increase in TSS removal during raceway harvesting in comparison to normal operation, whereas the HSSF wetlands removed 55% of influent TSS in comparison to 69%, on average, during normal operation. Each treatment train performed similarly with COD reductions ranging from 60-62% and TSS removals ranging from 63% in the SB-CW and 71% in the MS-CW system.

2.4 Discussion

2.4.1 Treatment Performance

Superior solids removal will often dictate the most effective pretreatment of wastewater entering HSSF wetlands. Though ancillary benefits are often present, greater reduction in solids minimizes losses in hydraulic conductivity due to inlet clogging, promoting wetland efficiency and prolonging wetland life. Both pretreatment options investigated proved to be only minimally effective during normal operating conditions in this regard. The microscreen and sedimentation basin removed 15% and 19% of TSS, respectively. Low removals of organics and the particulate fractions of nitrogen and phosphorus were also documented, but all parameters were statistically insignificant ($p \leq 0.05$).TSS removals fell short of those reported by Sindilariu et al. (2009a) for their microscreen (41%) and sedimentation basin (33%) designs. Each wastewater originated from a flow-through trout farm and TSS concentrations were similar on average with the Virginia system in this study receiving 5.4 mg/L of TSS and the German system (Sindilariu et al., 2009a) receiving 5.6 mg/L. There are notable differences between the studied treatment devices. Sindilariu utilized a drum filter with a smaller mesh size of 63 μm, in comparison to the static screen used in this study with a mesh size of 80 μm. The ability to filter out a smaller particulate fraction certainly is one reason why the microscreen in this study was less efficient. More perplexing however is the performance of the sedimentation basin which had an overflow rate that was an order of magnitude lower (0.0002 vs. 0.002 m/s) than in the study carried out in Germany. A likely explanation for this poor performance is a smaller particulate fraction entering the treatment system. Three raceways just upstream of the treatment system were taken offline shortly after startup while new concrete raceways were being constructed. These raceways then performed like full-flow sedimentation basins, settling the largest particles. Unlike production units, components intended to destroy or remove particles including waterfalls, drainage channels, and maturation ponds have been found to significantly reduce particle size in flow-through fish farms (Brinker and Rösh, 2005). In the same study, waterfalls were noted for being especially effective in breaking up larger particles, reducing the TSS removal efficiency of an 80 μm drum filter by 33.5%. In addition to the obvious impairment of microscreen treatment, smaller particle size will reduce settling velocity as determined by Stokes Law, reducing the effectiveness of sedimentation. Therefore, it is plausible that the series of waterfalls and drainage channels, directly upstream of the offline raceways adjoining the treatment system, were responsible for low TSS removal in both the microscreen and sedimentation basin.

Solids reduction improved within the wetlands, removing approximately 69% of TSS. These results are comparable to the findings of Sindilariu et al. (2007, 2009a) in which treatment efficiencies of 59-68% were documented. In another study with trout farm effluent, it was found that removals were in the area of 96% at similar areal loadings (27 g/m^2d TSS) (Schulz et al., 2003). Areal loadings generally do not have an effect on the treatment efficiency of solids in HSSF wetlands (Kadlec, 2009), and this is reflected in Figure 2.11. Each of the other studies included a plant community upon startup which can both benefit solids removal by decreasing porosity with a mature root system and reduce efficiency in deeper beds by promoting flow stratification (Kadlec and Wallace, 2008). The higher removal efficiencies seen by Schulz et al. (2003) were more likely because a finer gravel (1-2 mm) was used in the wetland bed than that used in this study and HSSF wetlands created by Sindilariu et al. (2007) (4-8 mm).

The constructed wetlands significantly reduced organic matter, with treatment efficiencies of BOD_5, COD, and TOC ranging from 50-62%. Removal of BOD_5 (62%) and COD (50%) was very similar to the findings of Sindilariu et al. (2009a) where HSSF wetlands removed 62% of BOD_5 and 40% of COD with very similar influent concentrations. Treatment efficiency of COD is not greatly affected by areal loading (Schulz et al., 2003) and the data in this study indicated this was also the case. Increased hydraulic residence time (low HLR) was determined to correlate with improved removal of BOD_5 ($R^2 = 0.45$) and to a lesser extent, COD ($R^2 = 0.36$), as seen in Figures 2.5 and 2.6. This finding is in agreement with results presented by Sindilariu et al. (2009b), in which wetland cells with lower hydraulic loading rates of 3.3 m/day (largest HRT) had significantly ($p < 0.05$) greater treatment of BOD_5 than more highly loaded cells (14.5 m/day). Seasonal performance did not vary as might be expected from basic temperature dependence on biological reactions. In fact, treatment performance was slightly improved during the colder months (Figure 2.7). This can be explained by seasonal cycling of biodegradable particles within subsurface flow wetland beds. During the colder months, biodegradable particles are physically removed and accumulate due to slower degradation (Kadlec and Knight, 1996; U.S. EPA, 2000). When temperatures rise during the warmer months, rate of biodegradation of accumulated organic matter increases and is accompanied by a release of BOD.

Nitrogen removal was negligible within the HSSF wetlands, with a statistically insignificant ($p > 0.05$) average TN reduction of 3%. TAN and NO_2-N were reduced by 69% and 23%, respectively. The average inorganic fraction of nitrogen rose considerably from 75% to 92% during travel through the wetland cells, largely because of a 116% increase in NO_3-N. The decrease in organically-bound nitrogen is an indication of mineralization (ammonification). Nitrification is also evident based on transformations of nitrogen from the ammonium form to nitrate. Denitrification is highly dependent on oxygen inhibition and it was the belief that a deep wetland cell coupled with a long HRT would promote oxygen gradients in which would allow both aerobic and anoxic reactions to occur simultaneously. Standpipes within the wetland cells revealed that even though oxygen depletion was evident along the longitudinal axis, dissolved oxygen never dropped below 1.8 mg/L (Figure 2.12). Since background concentrations of TN is often between 0.5-2.5 mg/L in all types of wetlands (Kadlec and Wallace, 2008), significant removal of low influent concentrations becomes difficult. Sindilariu et al. (2007, 2009a) experienced similar results with TN treatment efficiencies ranging from -2.0% to 6.2%, whereas Schulz et al. (2003) had greater success, reporting removals of about 42% for areal loadings of 2 g/m^2day. The same study calculated TN removal between 20.6-26.2% for areal loadings (10-12 g/m^2day) closer to those witnessed in this study (9 g/m^2day).

Phosphorus removal was lower in this study than for other wetlands treating wastewater from flow-through trout farm facilities. TP removal averaged 24%, while other authors reported removals of 37-69% (Schulz et al., 2003; Sindilariu et al., 2007 and 2009a). These same studies have indicated increases in PO_4-P between the influent and effluent of treatment wetlands. While increases in TP and PO_4-P were observed on occasion in this study, this was not the norm. One reason for this is the “change over” phenomenon in which changing conditions such as a reduction in phosphorus loading can take an extended period of time to be reflected in the wetland effluent (Kadlec and Wallace, 2008). During this period, a net export in phosphorus can potentially be measured. Wetlands may take upwards of one or more years to respond to reduced loading. Pulses in phosphorus may also account for short term influxes that may not be adequately accounted for in most sampling processes. Extremely low phosphate concentrations in flow-through trout farm facilities using groundwater springs as a source are also inherently difficult to measure.

During the raceway cleaning simulation, influent concentrations increased significantly for TSS and parameters associated with higher solids loading, such as TP and COD. Treatment performance of the sedimentation basin increased substantially, but remained steady for the microscreen. TSS removals during raceway cleaning averaged 60% in the sedimentation basin which is similar to the 51% reduction found by Sindilariu et al. (2007). The microscreen only reduced TSS concentrations by 11%, which was similar to performance witnessed during normal operation. Higher influent concentrations increased the number of particle collisions, thus promoting the coalescence of particles within the sedimentation basin (Type-II settling) (Letterman, 1999). Particle characteristics of settled sludge in trout farms have been found to differ significantly from particles suspended in the effluent. Whereas 42% of particles by mass were determined to be less than 70 μm in the effluent, 92% of sludge particles were less than this diameter (Maillard et al., 2005). An 80 μm microscreen would tend to be less effective in removing this large particulate fraction and the results of this study indicate this to be true.

Phosphorus removal in the wetlands averaged 43% during raceway cleaning, as opposed to only 24% during normal loading. Greater phosphorus treatment efficiency in the wetland can be attributed to the larger particulate-bound fraction of phosphorus present during cleaning combined with effective solids removal. TSS was reduced in the wetlands by 99-100% during raceway cleaning and immediately prior to raceway cleaning during normal operation. During harvesting conditions, both pretreatment options demonstrated TSS removal exceeding performance data from normal raceway operation. Solids disturbed in stocked raceways likely do not persist long enough to be degraded to the same extent as in empty raceways. Degradation is believed to be a main cause for smaller particle sizes in trout farm sludge (Maillard et al., 2005) and is believed to explain why treatment performance during the cleaning of an empty raceway unit yielded minimal TSS reduction by the microscreen. Nevertheless, future treatment systems that are to incorporate microscreening in the treatment of trout farm facilities should use a mesh size smaller than 80 μm to accommodate small particle sizes that can persist in a long series of flow-through raceways.

2.5 Conclusion

The two treatment trains showed no significant differences in treatment for all parameters measured with the exception of raceway cleaning performance. This fact should not be surprising as the two constructed wetlands, identical upon construction, were responsible for the vast majority of treatment. When treating flow-through trout farm effluents, constructed wetlands have far more potential than mechanical pretreatment options. This is primarily due to the inability of common mechanical treatment technologies to effectively treat soluble fractions of nutrients and organic matter. Additionally, reduced particle size from the separation of treatment systems from production raceways and common raceway components such as waterfalls, can reduce the effectiveness of microscreens and sedimentation basins.

Design issues remain to ensure both aerobic and anoxic conditions exist within HSSF wetlands to allow for TN removal. With aerobic conditions prevailing within the wetland (DO > 1.5 mg/L), nitrogen transformations were evident with significant TAN reduction and NO_3-N increases, but TN concentrations remained relatively constant in the effluent. Future work can refine design aspects such as wetland depth, shape, and media configuration to foster simultaneous nitrification and denitrification, and bring forth economically achievable nitrogen removal in single-stage wetlands. Modest TP reductions (24%) were witnessed over the sampling period, but can be improved in the future with incorporation of medium rich in iron and aluminum oxides and presence of vegetation designed for phosphorus uptake.

Each treatment train offered a practicable means of removing TSS, organic matter (BOD_5), and some phosphorus; contaminants that have come under scrutiny for their potential deleterious effects on receiving waters. Though the insignificance of pretreatment in this study does not support one treatment train over the other, the results reinforce HSSF constructed wetlands as a leading option for treatment of flow-through aquaculture facilities.

2.6 References

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2.7 Figures

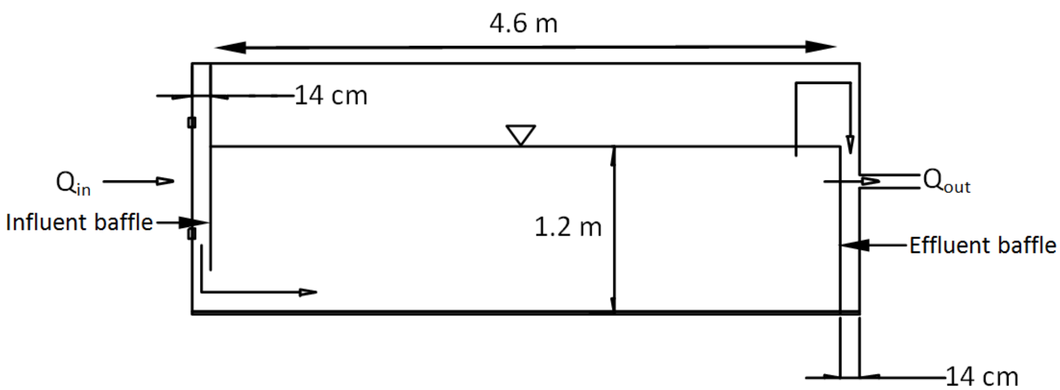

Figure 2.1: Sedimentation basin side-view

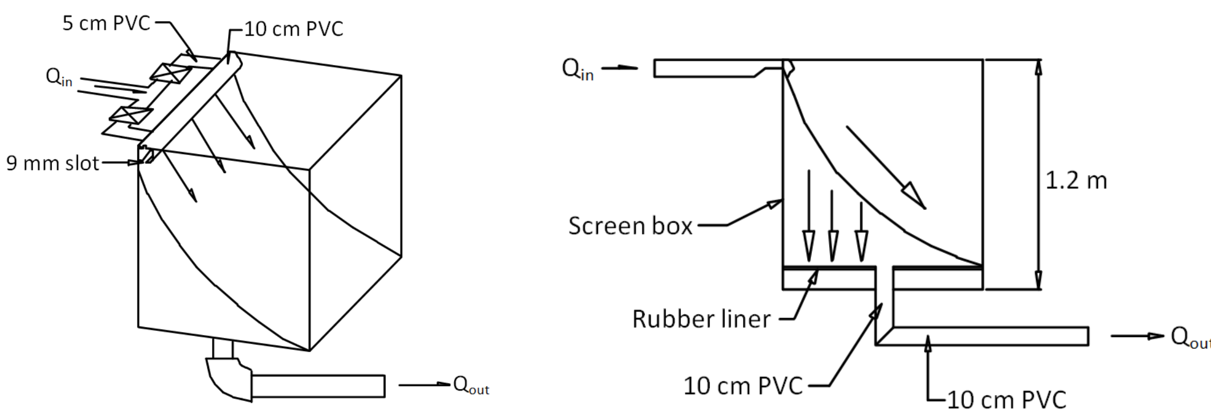

Figure 2.2: Microscreen box design, orthographic and side views

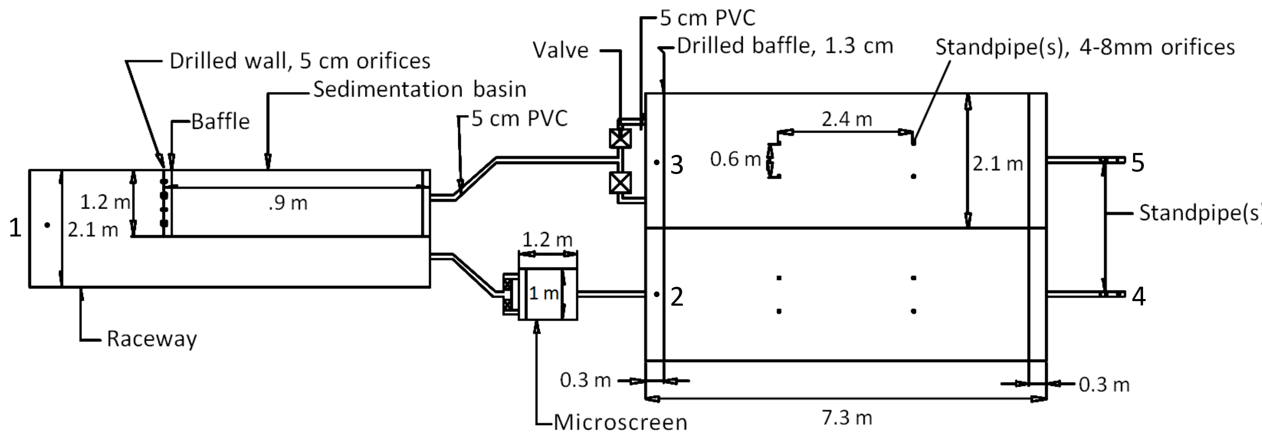

Figure 2.3: Plan view of experimental system. Numbers one through five indicate sampling locations.

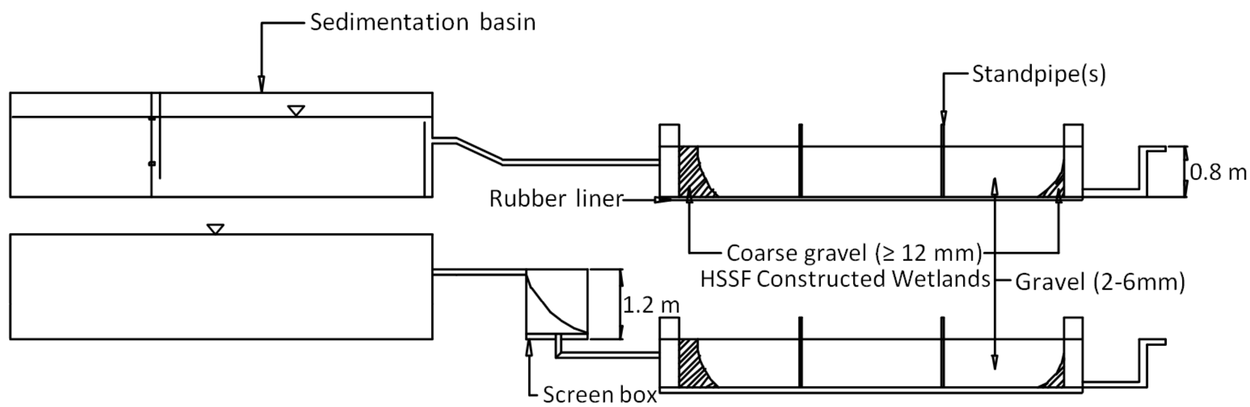

Figure 2.4: Side view of experimental system

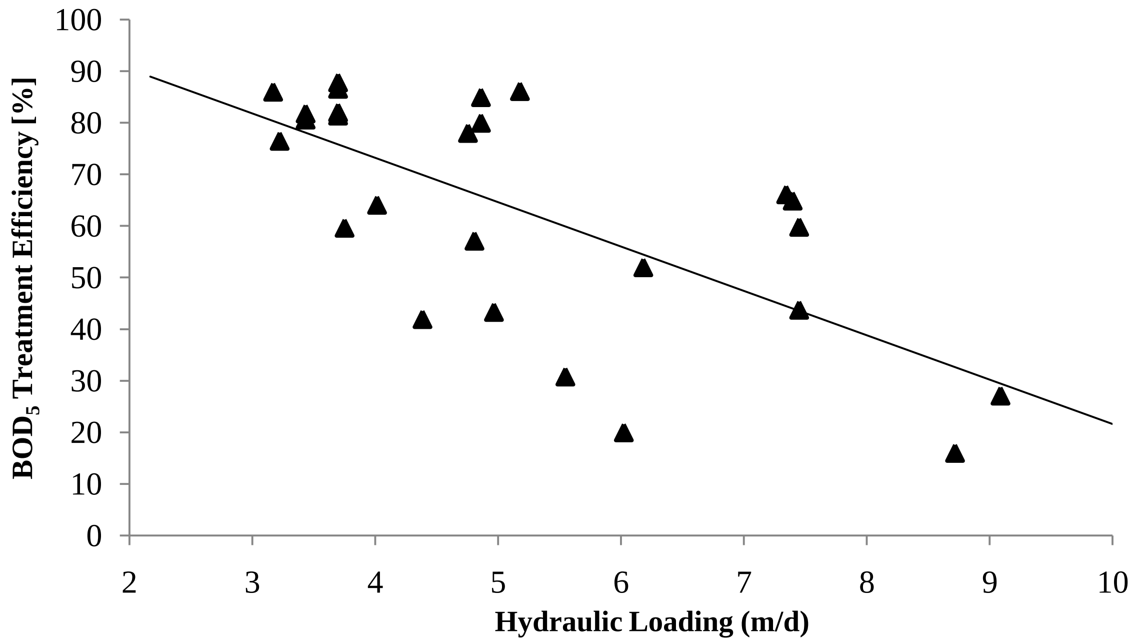

Figure 2.5: BOD_5 removal in wetland cells and hydraulic loading ($R^2 = 0.4529$)

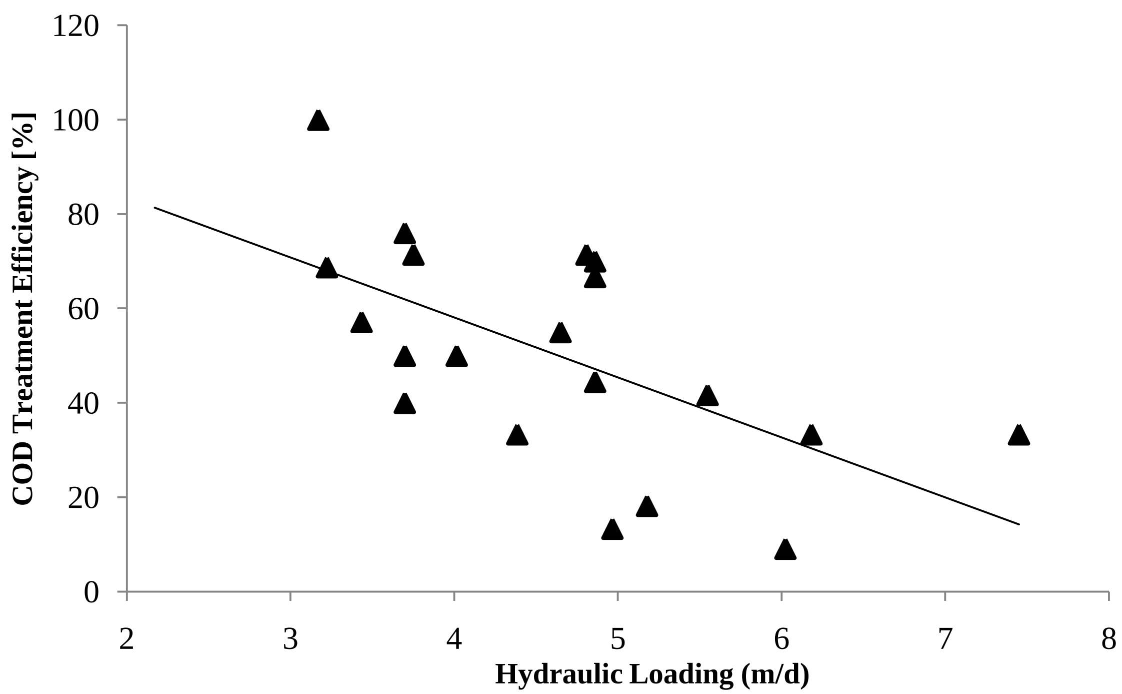

Figure 2.6: COD removal in wetland cells and hydraulic loading ($R^{2 =}$ 0.3639)

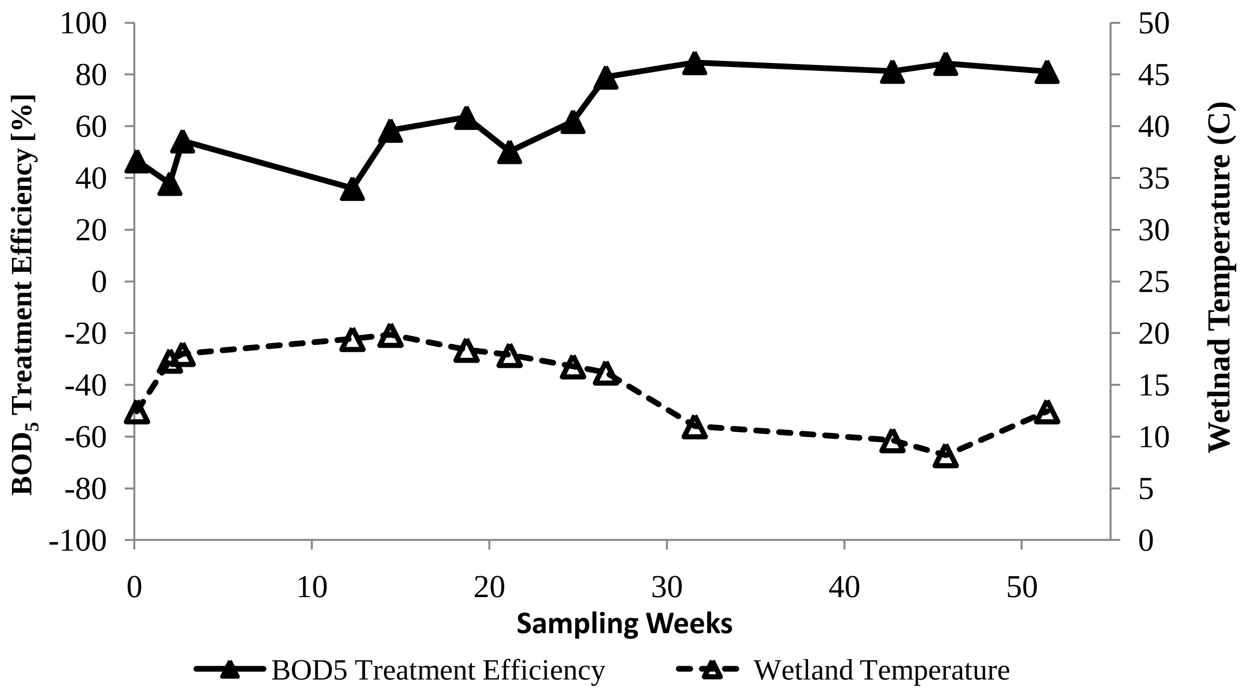

Figure 2.7: Temporal BOD_5 removal in wetland cells between March 2010 and March 2011.

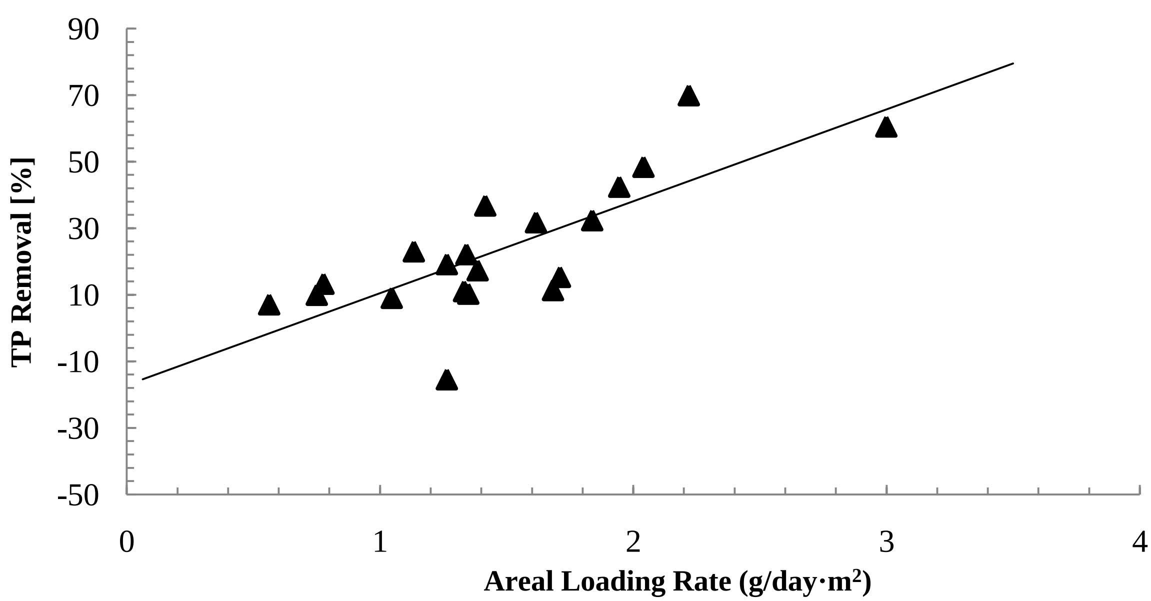

Figure 2.8: Total phosphorus (TP) removal and areal loading. ($R^2 = 0.5894$)

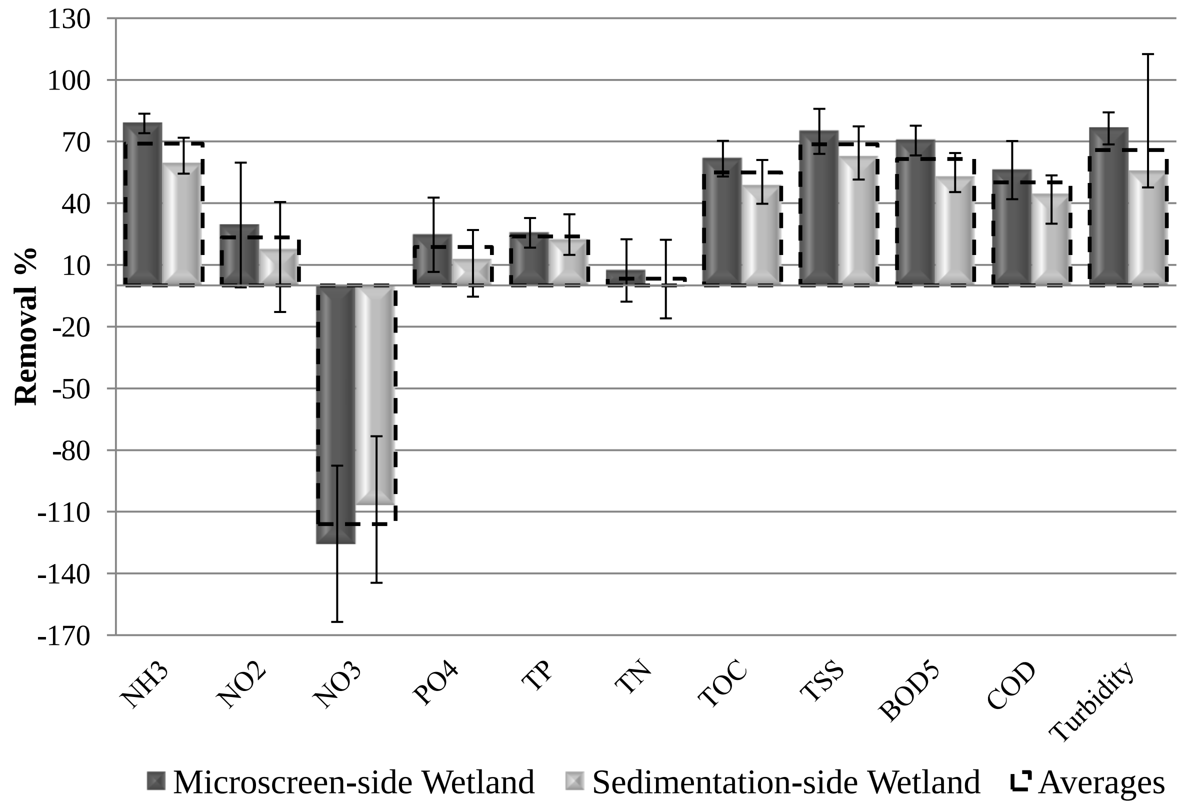

Figure 2.9: Average treatment efficiencies of HSSF constructed wetlands with standard error bars ($p < 0.1$)

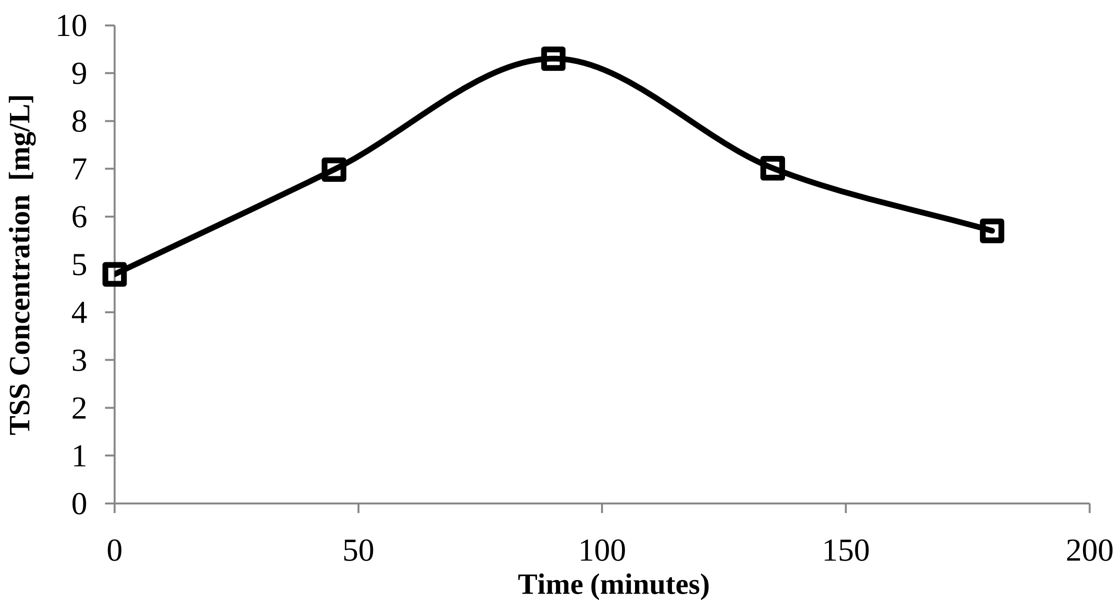

Figure 2.10: Bell curve-shape indicating spiked TSS concentrations during harvesting

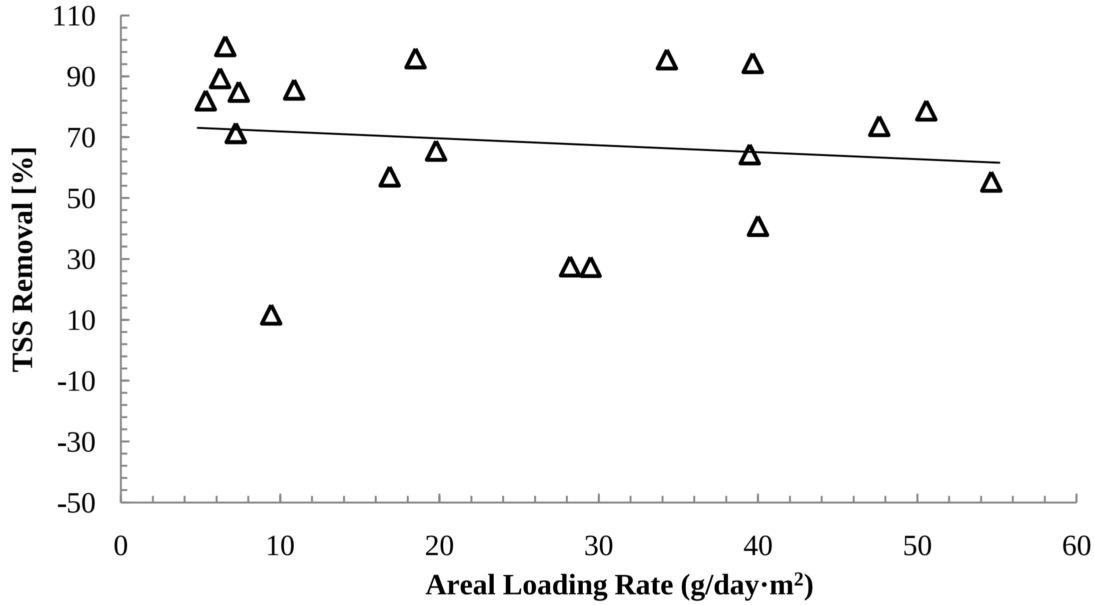

Figure 2.11: TSS removal and areal loading

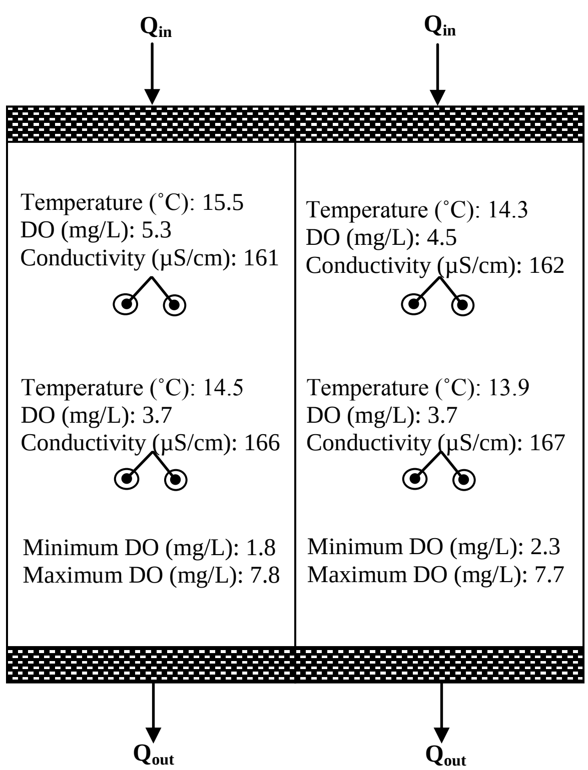

Figure 2.12: Mean values of select parameters, measured within wetland cell standpipes. (N = 8)

2.8 Tables

Table 2.1: Summary of treatment efficiencies of vegetated HSSF CWs – Adapted from Vymazal (2005)

Parameter	Inflow (mg/L)	Outflow (mg/L)	Efficiency (%)	N
BOD_5	108	16	85	164
COD	284	72	75	131
TSS	107	18.1	83	158
TP	8.74	5.15	41	149
TN	46.6	26.9	42	137
NH_4^+-N	38.9	20.1	48	151
NO_3^--N	4.38	2.87	35	79

Adapted from Vymazal (2005) Constructed wetlands with horizontal subsurface flow and hybrid systems for wastewater treatment. Ecological Engineering 25, 478-490.

Table 2.2: Location of sample collections

Site #	Description
1	Before treatment, raceway
2	After microscreen, pipe influent to wetland baffle
3	After sedimentation basin, pipe influent to wetland baffle
4	After microscreen-side wetland, standpipe effluent
5	After sedimentation-side wetland, standpipe effluent

Table 2.3: Analytical methods and equipment used to characterize water quality

Parameter	Analytical method	Equipment
NH_3-N	EPA approved Hach Methods 10023 & 8155	Hach DR-2800 Spectrophotometer
NO_3-N	DX-120 Ion Chromatograph Anion Procedure	Dionex DX-120 Ion Chromatograph
NO_2-N	DX-120 Ion Chromatograph Anion Procedure	Dionex DX-120 Ion Chromatograph
TN	Simultaneous persulfate digestion for total nitrogen and total phosphorous in water, Method No. G-172-96 Rev. 12	Seal Analytical AA3 Automated Segmented Flow Analyzer and Hach DR-2800 Spectrophotometer
TP	Simultaneous persulfate digestion for total nitrogen and total phosphorous in water, Method No. G-175-96 Rev. 13	Seal Analytical AA3 Automated Segmented Flow Analyzer and Hach DR-2800 Spectrophotometer
PO_4-P	DX-120 Ion Chromatograph Anion Procedure	Dionex DX-120 Ion Chromatograph
TOC	Sievers 800 Portable Total Organic Carbon Analyzer	Sievers 800 Portable Total Organic Carbon Analyzer
Turbidity	Standard Methods 2130 B	HF Scientific DRT-15CE Portable Turbidimeter
TSS	Standard Methods 2540 D	Filtration apparatus and drying oven
BOD_5	Standard Methods 5210 B	Constant temperature room, YSI Model 57 Oxygen Meter
COD	Reactor Digestion Method	Hach DR-2800 Spectrophotometer

Table 2.4: Sample collection times and reasoning for raceway cleaning simulation.

Site	Times of Sample Collection (minutes)	Notes
1. System Influent	0, 10, 20, 30, 40, 60	To better define "slug" input of selected parameters
2. MS Effluent	0, 20, 40, 60	RWT tracer indicated 20 minute travel-time between location of raceway cleaning and microscreen effluent sampling port
3. SB Effluent	0, 60, 120, 180	Samples taken in equal time intervals between T_0 and theoretical retention time of SB. RWT tracer injection yielded 50% mass recovery at t = 100 min.
4. MS CW Effluent	0, 130, 175, 220, 265, 310	Sampling occurred during a three hour period centered around the theoretical retention time of CW. Sampling period encapsulated peak tracer concentration from pulse input to resemble "worst-case-scenario".
5. SB CW Effluent	0, 213, 258, 303, 348, 393	Similar reasoning as Site 4 with the exception of additional travel time allowed for contaminant travel through SB.

T_0 = Culmination of raceway cleaning period

Table 2.5: Sample collection times and reasoning for raceway harvesting simulation.

Site	Times of Sample Collection (minutes)	Notes
1. System Influent	0, 10, 55, 100, 145	Ten minutes was the approximate travel time between the raceway undergoing harvesting simulation and the treatment system.
2. MS Effluent	0, 15, 60, 105	Higher flows hastened travel time (Approximately 5 minutes) between sampling location 1 and 2.
3. SB Effluent	0, 60, 105, 150	Samples taken in equal time intervals between T_0 and the theoretical retention time of SB. RWT tracer injection yielded 50% mass recovery at t = 100 min.
4. MS CW Effluent	0, 75, 120, 165	Sampling occurred during a 2.25 hour period centered around the theoretical retention time of CW.
5. SB CW Effluent	0, 120, 165, 210	Similar reasoning as Site 4 with the exception of additional travel time allowed for contaminant travel through SB.

T_0 = Culmination of raceway harvesting period

Table 2.6: Summary of Measured Parameters

Water Quality Parameter	Pretreatment Options								Secondary Treatment			
	Microscreen				Sedimentation Basin				HSSF Constructed Wetland			
	N	Inflow (mg/L) (±SD)	Outflow (mg/L) (±SD)	Removal (%)	N	Inflow (mg/L) (±SD)	Outflow (mg/L) (±SD)	Removal (%)	N	Inflow (mg/L) (±SD)	Outflow (mg/L) (±SD)	Removal (%)
TAN	13	0.69 (0.22)	0.63 (0.16)	4.5	13	0.69 (0.22)	0.66 (0.19)	1.1	26	0.65 (0.17)	0.21 (0.20)	69.0 *
NO_2-N	12	0.07 (0.03)	0.07 (0.03)	-29	12	0.07 (0.03)	0.07 (0.03)	-17	24	0.07 (0.03)	0.06 (0.04)	23.4
NO_3-N	12	0.53 (0.13)	0.57 (0.12)	-7.9	12	0.53 (0.13)	0.56 (0.13)	-6.9	24	0.56 (0.12)	1.18 (0.39)	-116 *
TN	11	1.9 (0.84)	1.6 (0.52)	13.1	11	1.9 (0.84)	1.8 (0.52)	4.6	22	1.7 (0.51)	1.6 (0.71)	3.3
PO_4-P	11	0.04 (0.01)	0.04 (0.01)	2	11	0.04 (0.01)	0.04 (0.02)	8.4	22	0.04 (0.02)	0.04 (0.01)	18.7 *
TP	10	0.26 (0.06)	0.26 (0.09)	1.2	10	0.26 (0.06)	0.26 (0.10)	1.2	20	0.26 (0.09)	0.19 (0.07)	23.9 *
BOD_5	12	4.4 (1.82)	4.3 (1.60)	4.2	12	4.4 (1.82)	4.2 (1.90)	8.3	24	4.3 (1.73)	1.7 (1.42)	61.6 *
COD	10	12.4 (6.70)	10.4 (4.80)	8.7	10	12.4 (6.70)	10.0 (5.50)	16	20	10.2 (5.06)	5.1 (3.49)	50.2 *
TOC	13	2.7 (0.60)	2.7 (0.40)	4.6	13	2.7 (0.60)	2.5 (0.40)	5.9	26	2.6 (0.40)	1.3 (0.70)	55.0 *
TSS	10	5.4 (3.70)	4.6 (3.30)	14.6	10	5.4 (3.70)	4.4 (2.90)	18.9	20	4.5 (3.05)	1.5 (1.56)	68.7 *
Turbidity (NTU)	12	3.1 (1.84)	2.9 (2.00)	7.4	12	3.1 (1.84)	2.5 (1.60)	23	24	2.7 (1.78)	0.7 (0.70)	65.9 *
pH	13	7.8 (0.42)	7.7 (0.40)	1.3	13	7.8 (0.42)	7.8 (0.30)	0.3	26	7.8 (0.40)	7.7 (0.38)	7.7
DO	13	8.4 (1.52)	8.7 (1.30)	-3.9	13	8.4 (1.52)	8.6 (1.24)	-2.1	26	8.7 (1.21)	4.2 (2.07)	51.6 *
Temperature (°C)	13	15.3 (3.71)	15.4 (3.90)	-0.6	13	15.3 (3.71)	15.3 (3.80)	1.0	26	15.4 (3.80)	14.8 (4.40)	8.4

Mean inflow and outflow concentrations of studied water quality parameters. An asterisk (*) indicates significance difference between the means. ($p \leq 0.05$)

Table 2.7: Average influent concentrations during cleaning and harvesting simulations

Event	TSS (mg/L)	Turbidity (NTU)	COD (mg/L)	TP (mg/L PO4-P)
None	5.4	3.1	12	0.26
Harvesting	7.2	3.3	17	-
Cleaning	43.1	41.0	22	0.45

Table 2.8: Treatment efficiency (% removal) during cleaning and harvesting simulations

Parameter	Microscreen			Sedimentation Basin			HSSF Wetlands		
	Harvesting	Cleaning	Avg.	Harvesting	Cleaning	Avg.	Harvesting	Cleaning	Avg.
TSS	22	11	15	29	60	19	55	99	69
Turbidity	-3	34	7	12	62	23	52	99	66
COD	34	8	9	40	32	16	38	69	50
TP	-	0	1	-	28	1	-	43	24

Chapter 3: An Investigation into the Sorption Potential of Soluble Phosphorus and Rhodamine WT onto Gravel Media Extracted from a Mature HSSF Constructed Wetland

3.1 Introduction

Constructed wetlands have emerged as a popular wastewater treatment option worldwide, harnessing the natural treatment processes in one of the most biologically active ecosystems. Horizontal subsurface flow (HSSF) constructed wetlands are particularly of interest since the lack of an exposed water surface minimizes both animal and human impact on the wetlands, and vice versa. Additionally, this does not make them prone to becoming mosquito breeding grounds, which is especially important for systems treating effluent from single-family homes or small neighborhoods. Unlike free water surface wetlands which rely largely on the sediment-water interface and suspended material for sorption sites, HSSF wetland media offer a myriad of additional potential sites. In HSSF wetlands, sorption becomes a particularly important removal mechanism for many wastewater parameters, yet can also interfere with certain tracers.

Eutrophication is of great concern in the Chesapeake Bay Watershed area where poor water quality and low populations of many species of fish and shellfish are being reported (U.S. EPA, 2010). Established in December of 2010, the Chesapeake Bay Total Maximum Daily Load stipulates reductions in major sources of nitrogen, phosphorus, and sediment for the 64,000 square mile watershed. Phosphorus is often found to be the growth-limiting nutrient in freshwater systems and annual loadings will have to be reduced by 24% within the watershed by 2025 (U.S. EPA, 2010). HSSF wetlands are an economical means of controlling effluent phosphorus loadings from agriculture, aquaculture, and other industrial sources. Energy utilization was found to be less than 0.1 $kW \cdot h/m^3$ (Brix, 1999) for subsurface flow wetlands, lower than facultative lagoons and the activated sludge process in a survey of wastewater treatment operations (Kadlec and Wallace, 2008).

Results from many studies indicate that substrate sorption is the most significant factor in the removal of phosphorus in constructed wetland systems (Mann and Bavor 1993; Sakadevan and Bavor 1998; Arias et al. 2001; Cui et al., 2008). Sorption is highly dependent on the mineral properties of the aggregate; e.g., higher calcium content favors precipitation reactions (Arias et al., 2001), iron and aluminum oxides enhance phosphorus retention (Rustige et al., 2003), and finer particles provide increased surface area, allowing for more sorption sites for phosphorus (Onyullo and McFarland, 2003). Laboratory sorption studies have been conducted with a variety of media intended for use in subsurface flow (SSF) constructed wetlands, including limestone, sand, gravel, topsoil, and other artificial materials (Mann and Bavor, 1993; Sakadevan and Bavor, 1998; Johansson, 1999; Pant et al., 2001; Cui et al., 2008). Wetland beds are predominantly composed of either gravel or sand, as these materials allow for increased hydraulic conductivities and are widely available.

Though additional sorption sites within HSSF wetlands promote enhanced phosphorus removal, water tracers such as Rhodamine WT (RWT), have exhibited increased reactivity with substrates and attached biomass in SSF wetlands, in comparison to FWS wetlands, leading to lower mass recoveries. Low mass recoveries, primarily associated with sorption, cannot always be associated with poor hydraulic data. Experiments with simultaneous injections of multiple tracers in FWS wetlands have yielded mean hydraulic residence times (HRT) between 4-10% of the conservative agents, lithium and bromide (Dierberg and DeBusk, 2005; Lin et al., 2003). Pang et al. (1998) determined that the shape and position of breakthrough curves for RWT and chloride were nearly identical in alluvial gravel aquifers. In a control dispersion study, Ríos et al. (2009) found very similar recovery and shape of residence time distribution curves for lithium and RWT tracers. In the same report RWT recovery values were in excess of 75% in HSSF wetlands with HRTs between 41 and 54 hours.

One of the main draws for tracer usage in subsurface wetlands is the identification of short-circuiting and flow stratification within a wetland cell. Short-circuiting and the development of preferential flow paths are especially of concern in colder climates, where ice formation having the potential to reduce the hydraulic efficiency of subsurface flow wetlands (Muñoz et al., 2006). Flow stratification can often be deduced visually, by the presence of multiple peaks on a tracer response curve. Multiple peaks often indicate flow traveling through strata of varying hydraulic conductivity, arriving at different times in the effluent. Studies have determined RWT to undergo chromatographic separation during experiments conducted with subsurface media (Sutton et al., 2001; Vasudevan et al., 2001), which can lead to different arrival times of two RWT isomers. This can complicate the interpretation of tracer response curves and also artificially increase theoretical residence times. Sorption of RWT to attached biomass has been considered to be the main cause of low mass recovery in subsurface flow systems (Giraldi et al., 2009; Ríos et al., 2009). Therefore, it is important to analyze the conservative nature of a tracer after a constructed wetland has been operational long enough for biomass accumulation. It has been said that some degree of nonconservative transport is inevitable due to all solutes having varying propensity to react with the subsurface (Sutton et al, 2001). Thus a case-by-case evaluation of the conservative nature of a tracer in subsurface media is important in the determination of its utility. The objectives of this study were to:

1. Characterize the sorption of phosphorus in gravel media utilized in a HSSF constructed wetland that had been operational for a period of about 1 year.
2. Investigate the utility of Rhodamine WT as a conservative tracer in gravel media, used in a HSSF constructed wetland, by conducting laboratory batch and column experiments.

3.2 Methods and Materials

3.2.1 Site Description

A pilot scale study was undertaken to evaluate the use of HSSF constructed wetlands for the treatment of effluent from a flow-through trout farm in southwest Virginia. Wetland basins were made 6.7 m long, 2.4 m wide, and 0.9 m deep (22 ft x 8 ft x 3 ft) and constructed of plywood covered with 60 mil EPDM liner. The wetland basins were filled with coarse (19 – 25 mm) gravel at the influent and effluent ends to promote even distribution of flow through the wetland. The remainder of the wetland basins was filled with finer gravel (2-6 mm), with a porosity of 0.44 and a bulk density of 1.5 g/cm^3. Both wetlands were left unplanted; however, diverse native flora quickly emerged in the first growing season of operation. The wetlands were subject to continuous loading from the trout facility, each receiving a flow of 1 L/s, on average.

Influent phosphorus concentrations averaged 0.26 mg/L as TP, and 0.04 mg/L as ortho-PO_4-P (OP).

3.2.2 Wetland bed media sampling and physical analysis

Wetland gravel was extracted from the center of the beds, 15 months after the start of operations. Following extraction, media was transported to Virginia Tech, and stored in a 6 °C refrigerator until employed in sorption experiments. Clean-bed gravel was used to determine porosity and bulk density. Porosity was analyzed using the water evaporation method. Fifty mL of saturated sample was weighed, dried at 110C, and then weighed. The pore-water volume was then calculated using the following formula:

$$V_{pw} = \frac{M_s - M_d}{\rho_w} \qquad (3.1)$$

where M_s = mass of saturated sample (g)

M_d = mass of dried sample (g)

ρ_w = density of water (1 g/ml)

Porosity was then calculated by dividing the pore volume by total sample volume. Bulk density was calculated from the following relationship with porosity (n) and media density.

$$n = \left(1 - \frac{\rho_{bulk}}{\rho_{media}}\right) \qquad (3.2)$$

where ρ_{bulk} = bulk density of media (g/cc)

ρ_{media} = density of media (2.65 g/cc)

3.2.3 Chemical analysis

Phosphorus (orthophosphate) was analyzed using the Ascorbic Acid method (equivalent to USEPA method 365.2 and Standard Method 4500-P-E). Samples were analyzed on a HACH DR2800 spectrophotometer and diluted with nanopure water when concentrations exceeded the upper limit of the test.

Rhodamine WT (2.5% active ingredient) was acquired in two different lots from Cole Parmer (EW-00298-16). A new lot of RWT tracer was acquired for laboratory batch and column studies, whereas dye purchased for previous experiments was utilized in the field. Both lots of dye presumably retained similar analytical characteristics, such as fluorescence with a maximum absorbance of 550 nm and emission of 588 nm. Chemical constituents of each RWT lot were analyzed using high performance liquid chromatography (HPLC) according to methods proposed by Sutton et al. (2001), to assess their presumed uniformity. For each lot, a 50 mg/L (active ingredient) solution was prepared and analyzed with a HP 1090 Liquid Chromatograph with a Photo Diode Array (PDA) detector. Separation was achieved using a 250 x 4.6 mm column as the immobile phase and a solution of 65% methanol and 35% 5×10^{-3} M phosphoric acid as the mobile phase. The UV spectra were used to analyze the two primary isomers of RWT (Sutton et al., 2001).

Prior to fluorometer analysis, calibration curves were made to assure instrument (Turner Digital Fluorometer Model 450) accuracy and dye integrity. NaCl was analyzed using a handheld YSI conductivity probe (YSI Model 85). Standards were made with tap water and a series of blanks were used to zero out background conductivities. A standard curve was constructed to correlate conductivity and NaCl concentration.

3.2.4 Field Tracer Study

Tracer studies were conducted on two occasions with Rhodamine WT (RWT) dye in the HSSF wetlands described in Section 3.2.1. A preliminary tracer experiment was performed to determine a satisfactory sampling scheme and tracer mass input, in the absence of a field fluorometer. An impulse of tracer was introduced by pipetting 9 mL of concentrated dye (2.5%) into the baffled quiescent settling zone, staged prior to wetland distribution. Immediately after, the settling zone was stirred for a period of 5 minutes to ensure homogenous distribution into the wetland cell. For this preliminary experiment, samples were withdrawn from the effluent of the wetland cells every 10-20 minutes over the course of about 12 hours.

A second tracer experiment was performed approximately four months later, in the same wetland cells as the preliminary tracer study. Introduction of the tracer impulse was carried out in the same way as the preliminary study, with the exception that 10 mL of RWT tracer was injected, rather than 9 mL. Samples were taken every 15 minutes during the first five hours of the study to ensure that the tracer response curve was captured in sufficient detail between the head of the curve and the peak. Sampling frequency was reduced to every 75 minutes until the final sample was taken about 30 hours into the study. Overnight, autosamplers (ISCO 3700) were utilized to attain samples. Samples were transferred to amber glass vials the next morning to prevent possible sorption to the plastic bottles that were filled by the autosampler. Following the study, samples were transported back to a laboratory at Virginia Tech and brought to room temperature (23°C) prior to analysis.

Nominal detention time in the constructed wetlands (τ_n) were calculated with equation 3.3.

$$\tau_n = \frac{nV_w}{Q} \tag{3.3}$$

where n = clean bed porosity of wetland media
V_w = wetland volume (L)
Q = flow rate (L/hr)

Tracer detention time (τ) was calculated from tracer output data using first moment analysis, illustrated in equation 3.4.

$$\tau = \frac{1}{M_e}\int_0^\infty tQC\,dt \tag{3.4}$$

where M_e = Total mass of tracer recovered in the effluent (μg)
t = time (hr)
Q = flow rate (L/hr)
C = Tracer effluent concentration (μg/L)

The upper limit of the integration was originally truncated by temporal restraints imposed by sampling complications. Enough data were collected, however, to exponentially extrapolate beyond the final sampling point. Extension of the tracer response curve was described using an exponential decay function, as suggested by Pope et al. (1994) and Wang and Jawitz (2006).

$$C(t) = C_b e^{\left(\frac{t-t_b}{a}\right)} \qquad (3.5)$$

where t_b = earliest time in regression (hr)

t = time (hr)

C_b = concentration at t_b (μg/L)

$-a^{-1}$ = slope of linear regression

Existing tail data, between hours 15.5 and 30.5, was plotted on a semi-log scale with linear regression being used to determine "a."

3.2.5 Batch sorption experiments

Batch-type phosphorus sorption experiments were conducted according to techniques outlined by the U.S. EPA (1992). Gravel samples were air-dried until constant weight and passed through a 2 mm sieve. Remaining sample was crushed, and passed through the 2 mm sieve until sufficient sample was obtained. Agitation intervals of 24, 48, and 72 hours indicated that 48 hours was sufficient equilibrium time. Soil to solution ratios of 1:40, 1:20, and 1:10 were experimented with until a 1:20 ratio was determined to be optimal. Water for batch testing was taken from the groundwater spring head directly upstream of the trout farm to ensure the mineral properties of the water were the same as on site. Five grams of sample were placed into 125 mL amber glass bottles containing solutions of 0, 0.5, 1, 1.5, 2, and 4 mg/L PO_4-P. Samples were then mixed on a rotary shaker for 48 hours at 21C (±1°C). Whatman 934-AH grade glass microfiber filters were used ensure only soluble reactive P was in the filtrate. Trials comparing these filters with 0.2 μm polycarbonate filters, indicated negligible difference between PO_4-P measurements. Phosphorus not recovered in solutions was considered to be retained by the wetland bed media.

Batch sorption tests with Rhodamine WT (RWT) were carried out in a manner similar to the phosphorus batch experiments, with a 1:10 soil:solution ratio and 48 hour equilibration time. Solutions of RWT were made at concentrations of 10, 50, and 100 μg/L. Sorption parameters were estimated using the Langmuir and Freundlich isotherms. The Langmuir expression is given in equation 3.6:

$$\frac{C_e}{q_e} = \frac{1}{q_{max} \cdot b} + \frac{C}{q_{max}} \qquad (3.6)$$

where q_{max} = sorption maxima (mg/kg)

b = binding energy coefficient

C_e = equilibrium concentration in solution (mg/L)

q_e = equilibrium sorbed concentration (mg/kg)

The maximum sorption is calculated from the slope of the resultant curve. The Freundlich equation is provided in equations 3.7 and 3.8:

$$\frac{x}{m} = KC^{1/n} = \text{equilibrium sorbed concentration (mg/g)} \qquad (3.7)$$

and

$$\log\frac{x}{m} = \log K + \frac{1}{n}\log C_e \qquad (3.8)$$

where K = Freundlich adsorption constant ($mg^{1-n}\,kg^{-1}\,L^{n}$)

n = empirical constant related to binding intensity

x = mass of adsorbate (mg)

m = mass of adsorbent (mg)

C = equilibrium solution concentration (mg/L)

Partition coefficients were calculated according to the following equation:

$$K_d = \frac{V_w (C_0 - C_i)}{M_{media} \cdot C_i} \quad (3.9)$$

where K_d = partition coefficient (mL/g)

V_w = solution volume (ml)

C_0 = initial solution concentration (mg/L)

C_i = equilibrium solution concentration (mg/L)

M_{media} = mass of gravel media (g)

3.2.6 Column sorption experiments

Unlike laboratory batch tests, media was taken in its original state (not crushed) to investigate phosphorus and RWT sorption. Columns were constructed from PVC pipe, 15 cm (6 in) in diameter, and 0.76 m (2.5 ft) long. Water was pumped from a reservoir into the bottom of the column by a Fisher variable flow peristaltic pump (Model 3389). Minimal vinyl tubing was used in order to minimize sorption sites other than the wetland substrate. Flow progressed up the column until it emerged from the media into a small (< 1 cm) free water surface (FWS), where it then cascaded into a collection reservoir. Step inputs of phosphorus and RWT were injected into empty (no media) columns to assess sorption onto column materials. The study was conducted using tap water at room temperature (22.0 ±2.0°C). Background phosphorus concentrations were zeroed out when conducting analysis. There was no interference for RWT measurements in the tap water.

Columns were constructed according to guidelines published by Relyea (1982). In order to prevent spiked solute inputs from peaking in fewer pore volumes than might be expected, it was recommended that pore-water velocity be large enough to minimize the effects of diffusion in laboratory columns. Pore-water velocity can be estimated from the following equation:

$$V_x = \frac{Q}{A \cdot n} \quad (3.10)$$

where Q = column flow rate (cm^3/s)

A = cross sectional area (cm^2)

n = media porosity

Minimum pore-water velocity was calculated using equation 3.11:

$$V_w \geq \frac{1.6 \cdot 10^{-3}}{L} \text{ cm/sec} \qquad (3.11)$$

where L = column length (cm)

Low flow phosphorus and RWT tests were performed at an average flow of 60 mL/min, corresponding to a pore-water velocity of $1.2 \cdot 10^{-2}$ cm/s. High flow experiments, only performed with phosphorus, were run at 165 mL/min.

To minimize the impact of high pore-water velocities on the effective pore volume, Relyea (1982) suggested column length be greater than, or equal to, four column diameters. The ratio of column length to width used in this experiment was 5:1 to ensure compliance with these guidelines. Lastly, to avoid velocity effects such as channeling or radial velocity gradient, it was proposed that the column diameter be 30-40 times the particle diameter of solids used to pack the column. Though sieve analysis was not performed in this study, the fraction of media less than or equal to 5 mm in size fits these specifications for a column 152 mm in diameter.

Step inputs of phosphorus were pumped continuously from a reservoir containing a KH_2PO_4 solution. Phosphorus concentrations were 2 mg/L PO_4-P for both the low and high flow experiments. Samples were filtered through Whatman 934-AH grade glass microfiber filters prior to analysis, to ensure only the soluble fraction of phosphorus in the filtrate. For a step input, retardation factors (R) can easily be calculated by setting R equal to the number of pore-volumes required to reach 50% of the influent concentration (Van Genuchten and Wierenga, 1986). This estimation, however, assumes symmetrical sigmoidal breakthrough curves and sorption equilibrium (Nkedl-Kizza et al., 1987). Retardation factors for this study were calculated according to equation 3.12, based on the conservation of mass principle (Van Genuchten and Parker, 1984; Nkedl-Kizza et al., 1987)

$$R = \int_0^{p\max} (1 - F) dp \qquad (3.12)$$

where F = normalized breakthrough curve for step input

p_{max} = pore volumes consumed when equilibrium conditions prevail

For this technique, the area above the normalized breakthrough curve was analyzed using Microcal Origin 8.5.1. The partition coefficient can be correlated to the partition coefficient from equation 3.13:

$$R_f = 1 + \frac{\rho_b K_d}{n} \qquad (3.13)$$

where ρ_b = media bulk density (g/cm^3)

K_d = partition coefficient

Tracers were pulsed into the column by temporarily switching the pump intake line into a volumetric cylinder containing 61 mL of tracer solution. The tracer solution contained 1.83 mg of RWT (active ingredient) and 1,530 mg of NaCl. The integration of RWT and NaCl was evaluated for possible interferences, and none were detected for the purposes of this study. The mean residence time for a pulse input was calculated according to the following equation (U.S. EPA, 1999).

$$t_{pulse} = \frac{\int_{t\,min}^{t\,max} t C_i dt}{\int_{t\,min}^{t\,max} C_i dt} \qquad (3.14)$$

where t_{pulse} = mean residence time for a pulse input (hr)

t_{min} = beginning of breakthrough curve (hr)

C_i = tracer concentration (mg/L)

Velocity of contaminant transport was then calculated using the calculated mean residence time and column length as the distance traveled. The retardation factor was then calculated as follows:

$$R_f = \frac{v_p}{v_c} \qquad (3.15)$$

where v_p = pore-water velocity (cm/s)

v_c = velocity of contaminant (cm/s)

The pore-water velocity, or the velocity of a non-adsorbing tracer, was estimated from equation 3.10. For tracer column experiments, the 1D transport equation was applied as follows:

$$D_L \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} = \frac{\partial C}{\partial t} \qquad (3.16)$$

where v_p = pore-water velocity (cm/s)

v_c = velocity of contaminant (cm/s)

By applying boundary conditions appropriate in column experiments, Lenda and Zuber (1970) created a normalized solution:

$$C(t) = \frac{M}{Qt_0} \frac{1}{\sqrt{4\pi P_D (t/t_0)^3}} \exp\left[- \frac{(1 - t/t_0)^2}{4P_D t/t_0} \right] \qquad (3.17)$$

where Q = column flow rate (L/hour)

M = mass of tracer injected (mg or μg)

t_0 = parameter used in the calculation of the effective porosity

P_D = Peclet number

Two unknown parameters (T_0 , P_D) were solved using breakthrough curve data and MS Excel solver to minimize the sum of squared error. This normalized transport equation was utilized to model tracer response curves for NaCl and RWT, and to calculate mass recoveries and mean retention time from first moment analysis.

3.3 Results

3.3.1 Phosphorus Batch Sorption Experiments

Sorption of phosphorus to the gravel media and attached biomass was well modeled by both the Langmuir and Freundlich isotherms. The Langmuir isotherm generated a sorption capacity of 93.2 mg/Kg and a binding strength of 1.07 L/mg. The Freundlich constant was 47.4 (mg^{1-n} kg^{-1} L^n), with an empirical constant of 1.60. Linearized versions of the Langmuir and Freundlich equations are presented in Figures 3.1 and 3.2, respectively. Correlation coefficients were 0.99 and 1 for Freundlich and Langmuir isotherms, respectively. Percentage removal of orthophosphate was inversely related to initial concentration, with removal from solution decreasing with increasing initial concentration. These data were modeled by a logarithmic function (R^2 = 0.9946) in Figure 3.3. Data for the initial solution of 0.5 mg/L PO_4-P was removed from isotherm construction due to equilibrium concentrations falling below method detection limits. Results from batch testing are summarized in Table 3.1.

3.3.2 Phosphorus Column Sorption Tests

Decreased hydraulic loading was found to have a positive impact on phosphate reductions within the column. For a flow rate of 165 mL/min (pore-water velocity of 2.1 cm/min), soluble phosphorus concentrations in the outflow reached approximately 60% of the inflow concentrations after 6.4 pore volumes. A flow rate of 60 mL/min (pore-water velocity of 0.7 cm/min) resulted in equilibrium effluent concentrations of about 50% of inflow concentrations after 6.61 pore volumes. Flow-cell experiments were modeled with exponential decay functions, illustrated in Figure 3.4. Retardation coefficients were 3.79 and 4.13, for high and low-flow experiments, respectively. Partition coefficients, calculated from equation 3.13, were 0.82 for the high flow experiment and 0.92 for the low flow experiment. Results from the phosphorus column sorption experiments are summarized in Table 3.2.

3.3.3 Separation of RWT Constituents

The separation of RWT constituents by HPLC analysis was primarily performed to determine whether isomer composition varied among the two different lots of tracer-grade RWT. A newer lot, acquired immediately prior to column and batch testing, exhibited a UV spectra very similar to that presented by Sutton et al. (2001). Three major constituents, depicted in Figure 3.5, are believed to be an inactive ingredient, isomer 1, and isomer 2. The UV spectra of the older lot used for the field study, was starkly different than that of the new lot. Only two constituents were detectable in the chromatogram (Figure 3.6), with significant absorbance recorded by a single constituent, detected in the eluent between 12 and 14 minutes. It is unknown at this time, whether RWT degradation or changes in chemical formulation was responsible for differences in UV absorption, or whether these differences are implications for dissimilar solute transport.

3.3.4 Field Tracer Tests

Preliminary tracer tests resulted in residence time distribution (RTD) curves that were asymmetrical in nature, with a sharp rise followed by an extended tail. Dual peaks were evident in the RTD (Figure 3.7) for both HSSF Wetland Cells 1 and 2, though more pronounced in Wetland Cell 2. Effluent concentrations peaked near the nominal detention time of each wetland (2.9 hours). Wetland Cell 1 peaked after 3.2 hours at a concentration of 7.3 μg/L and Cell 2 peaked after 2.8 hours at a concentration of 6.1 μg/L. A second tracer experiment on HSSF Wetland Cell 1 did not indicate any signs of this dual peak phenomenon in the RTD (Figure 3.8). Mean tracer retention time, as calculated from the method of moments, was nearly four times greater than nominal retention time at 11.2 hours. Mass recovery of the RWT tracer was 67%.

3.3.5 Laboratory Tracer Experiments

RWT exhibited a direct relationship between sorption losses and initial concentration as illustrated in Figure 3.9. Percent removals of RWT from solutions of 10 ug/L, 50 ug/L, and 100 ug/L at equilibrium were 4.0%, 9.4%, and 14.0%, respectively. Parameters from the RWT column sorption experiments are summarized in Table 3.2.

Tracer response curves from the column experiment (Figure 3.10) indicated that RWT was more reactive within the wetland media than NaCl. The NaCl tracer peaked after approximately 0.8 pore volumes had passed through the column, reaching a maximum concentration of 129 mg/L. Correspondingly, peak RWT was measured after 1.1 pore volumes at a concentration of 80.8 μg/L. Mean residence times, using first moment analysis, were 4.2 and 7.4 hr for NaCl and RWT, respectively. Tracer response curves indicated that 10% mass recovery occurred at 1.5 hours for NaCl and 1.9 hours for RWT. The difference between tracer breakthrough was more pronounced towards the tail of each curve, with 90% breakthrough at 6.6 hours for NaCl, and 16.6 hours for RWT. Using the ratio of the velocity of the conservative tracer, NaCl, to the velocity of RWT, a retardation factor of 1.4 was calculated for RWT. A Péclet number of 0.24 was calculated graphically from NaCl tracer response curve data and equation 3.17. Mass recoveries were satisfactory, with 111% recovery of NaCl, and 96% recovery of RWT tracer. Blank column tests were conducted to assess sorption onto column material. Figure 3.11 illustrates that 97% of influent orthophosphate and 94% of RWT were detected after 6.7 hours.

3.4 Discussion

3.4.1 Phosphorus Sorption Experiments

Sorption capacities of gravel substrates, determined from batch experiments using the Langmuir isotherm, vary tremendously throughout the literature. The value determined in this experiment, 93.2 mg/Kg, exceeded the maxima (25.8-47.5 mg/Kg) determined by Mann and Bavor (1993) for gravel employed in constructed wetland systems. Cui et al. (2008) determined a sorption capacity of 494 mg/Kg for gravel substrate, clearly demonstrating more sorption potential for gravel. In their experiments, initial phosphorus standards were made in 0.01 M KCl solution, to serve as a supporting electrolyte. For comparison, a 0.01 M KCl solution is equivalent to a conductivity of 1,412 μS/cm at 25°C (YSI, 1998), whereas conductivities in this study were about 200 μS/cm. The effects of supporting electrolyte solutions are also more pronounced at higher equilibrium phosphorus concentrations (Nair et al, 1984), with Cui et al. (2008) using initial phosphorus concentrations of 100-500 mg/L. Sorption maxima of gravel media in this experiment was comparable to that determined from a sampling of sands used in subsurface flow constructed reed beds in Denmark (Arias et al, 2001). In the Denmark tests, sands characterized as having high hydraulic conductivities (>200 m/day) had phosphorus sorption capacities of 49-86 mg/Kg.

Gravel is limited in its ability to sorb phosphorus, in comparison to other substrates utilized in constructed wetlands. A survey of nine substrate materials for constructed wetlands (Cui et al., 2008) revealed that gravel had the second lowest phosphorus sorption capacity.

Superior sorption appears to be related to higher concentrations of exchangeable Ca and Mg in basic wetlands, and Al and Fe in acidic wetlands. This is because under basic conditions, soluble phosphorus can react with Ca to form hydroxyapatite, which can precipitate out of solution (Ryden et al., 1977). When receiving acidic effluent, substrates high in Al and Fe content are believed to chemically adsorb phosphate ions onto the surfaces of hydrous oxides of Fe and Al by ligand exchange (Nichols, 1983; Faulkner and Richardson, 1989). Phosphate ions can further be immobilized by chemical precipitation reactions involving metallic cations such as Fe, Al, Ca, or Mg, especially at higher concentrations of either metallic cations or phosphate (Rhue and Harris, 1999).

It is important to note that a higher maximum P sorption capacity for a given substrate does not ensure lower effluent phosphorus concentrations, since the equilibrium phosphorus concentration (concentration at which no net sorption or desorption occurs) can increase with sorption maxima (Pant et al., 2001). The sorption capacity is also limited in that it represents the maximum for fast, reversible sorption, whereas constructed wetlands often process phosphorus through fast and slow sorption processes. Desorption from rapid sorption sites, induced from dilution of the solution, is predominantly from rapid sorption sites (McGechan and Lewis, 2002). McGechan and Lewis (2002) note that the extent to which slow sorption processes progress will impact the amount of sorbed phosphorus available for desorption. Therefore the impact of certain processes, including desorption, cannot be directly associated with sorption maxima. Sorption capacities do however, remain effective indicators of a substrates affinity for a sorbate, and serve as an important basis of comparison among potential wetland media.

Column sorption experiments indicated a much smaller partition coefficient for PO_4-P, in comparison to the batch sorption experiments. Batch experiments with an initial concentration of 2 mg/L PO_4-P generated a partition coefficient of 62 mL/g, whereas flow-through tests resulted in a partition coefficient of 0.8-0.9 mL/g. Phosphorus was 76% removed from solution in the aforementioned batch test, while removals were 40-50% in columns operated at low and high flows, respectively. Flow-through experiments reflect both hydrodynamic conditions and substrate composition in the field more closely than batch sorption tests. Additionally, unlike batch experiments, column tests can closer mimic field conditions by producing slow movements through the porous medium, with limited contact-time with the sediment matrix (Richardson and Vaithiyanathan, 1995). This is partially offset by the incorporation of phosphorus onto biomass and plant litter. Sorption and storage in biomass are processes subject to saturation and are not believed to contribute to long term removal (Dunne and Reddy, 2005). Soil adsorption was found to play a major factor in controlling long-term phosphorus sequestration in wetlands (Richardson and Marshall, 1986).

3.4.2 Tracer Studies

Tracer response curves from studies performed in a pilot-scale HSSF constructed wetland revealed features indicative of non-conservative transport. Dual peaks were clearly visible in HSSF Wetland Cell 2 at hours 2.8 and 4.2, while all RWT tracer response curves featured elongated tails. There are multiple reasons why a tracer response curves can exhibit such elements. Chromatographic separation of RWT isomers, as suggested by other authors (Shiau et al., 1993; Sutton et al., 2001; Vasudevan et al., 2001), is believed to account for some non-conservative behavior. Sutton et al. (2001) noted that chromatographic separation of RWT's two isomers is one plausible reason for dual peaks. Isomer 1 sorbs to a lesser extent and reaches equilibrium sorption an order of magnitude faster than isomer 2. Thus, the flow of isomer 2 has the potential be retarded in subsurface media, and can thus arrive in the effluent of a HSSF wetland at a later time than isomer 1. Chromatographic separation of RWT's two isomers complicates the interpretation of multiple flow paths, as multiple flow paths will also produce distinctive peaks. For instance, clogging in the inlet region of a HSSF constructed wetland can result in surface flow until hydraulic conductivity at the surface is large enough to allow water to percolate into the wetland cell. This short-circuiting flow will arrive at the outlet much quicker than flow retarded from traveling entirely through the lower hydraulic conductivities of the subsurface. Knowles et al. (2010) documented this scenario with a tracer study and reported the subsurface flow path to arrive 8 hours later than the short-circuiting flow path. The lack of distinct multiple peaks in HSSF Wetland Cell 1 for both the first and second tracer tests indicate that wetland heterogeneity was more likely than chromatographic separation to have been the cause of multiple peaks in Wetland Cell 2. The elongation of the tracer response tail, as indicated by the presence of tracer in the effluent nearly 50 hours into the second tracer study, is believed to be attributed to a mixture of transient storage and reversible sorption. The 33% of tracer mass not recovered in the second field study was mainly attributed to sorption.

The transport of RWT within the gravel media was further confirmed to be non conservative in laboratory column testing. A retardation factor of 1.74 was calculated using NaCl as a non-reactive tracer. The retardation factor does not decipher the degree to which surface adsorption, absorption, precipitation, or other mechanisms interfere with contaminant transport. Both Giraldi et al. (2009) and Ríos et al. (2009) have indicated sorption to attached biomass as the primary reason for RWT loss in subsurface wetland media field experiments. Lin et al. (2003) determined that 10% of RWT sorption was reversible. When sorption is reversible, wetland media temporarily adsorbs a fraction of influent RWT. When influent concentrations decrease, desorption occurs, releasing RWT into the effluent. Rhodamine WT was detectible in the column effluent until 34.1 pore volumes (69.5 hours) had passed. This is quite different than the 6.6 pore volumes (13.5 hours) required for the NaCl tracer to pass through the system. Though only 10% of RWT sorption may be reversible, reversible sorption can still greatly impact the shape of the tracer response curve. This is because a very small percentage of tracer mass can be responsible for a significant extension of the tracer tail. For instance, 90% of tracer mass was recovered within the first 17.4 hours of the column experiment, leaving just 6% to be recovered until hour 69.5, when the effluent concentration reached zero. Thus, even if reversible sorption was a fraction of the 4% not recovered, it can significantly inflate mean tracer residence times.

Upon first glance of the tracer response curve for RWT, it appeared that a change in inflection at approximately hour 8 may have indicated the arrival of the less conservative isomer 2. Chromatographic separation of RWT isomers and heterogeneity of the subsurface are two potential causes for different arrival times of the tracer. Chromatographic separation is not believed to be the primary reason, as both tracers exhibited a similar change in inflection on the tail end of the tracer response curve, with the NaCl tracer exhibiting this change at hour 8, about one hour prior to RWT. The "bump" on the tracer response curve is likely due to non-homogeneous velocity profiles along the column cross-section induced by the effect of higher drag associated with the inner circumference of the PVC column. The photodegradation and biodegradation of RWT were believed to be only minor contributors to RWT losses due to relatively short retention times (Lin et al., 2003).

Though the method of moments can exaggerate the mean detention time for tracer response curves featuring elongated tails, it is obvious that RWT transport was far more reactive than NaCl within the HSSF wetland media. Therefore, RWT did not serve as a truly conservative tracer in this study, when utilizing traditional fluorometer analysis.

3.5 Conclusion

Gravel media employed in an operational HSSF constructed wetland has the potential to sorb moderate levels of soluble phosphorus. Though increased PO_4-P concentration resulted in an increase of phosphorus associated with the solid phase, the percentage removal of phosphorus from solution decreased with increasing concentration. Removals of PO_4-P from column experiments will resemble potential field removals more closely than batch experiments. Column experiments allow for the presence of hydrodynamic effects which mimic internal hydraulics within a HSSF constructed wetland to a greater degree than the 48 hour batch tests. Decreased pore-water velocity (increased retention time) was found to have a positive impact on PO_4-P removals in laboratory column tests. The sorption experiments in this study indicated that the gravel media within a HSSF constructed wetland that had received relatively constant loadings of phosphorus for approximately 15 months, still had potential to remove considerable soluble phosphorus from wastewater.

An investigation into the potential of RWT to exhibit characteristics of a conservative tracer in gravel media with attached biomass, indicated that RWT was more reactive than the salt tracer, NaCl. Mean detention times for RWT were significantly inflated due to an elongated tail of its tracer response curve. This undesirable characteristic was exacerbated by the utilization of method of moments to calculate mean detention time. Therefore, one needs to be cautious in using the results of RWT tracer studies to predict the fate of agents in the subsurface systems. Even though RWT did not behave conservatively in this study, RWT can still be used qualitatively in subsurface flow tracer studies and perhaps quantitatively on a case-by-case basis, when transport is evaluated, and compared with the behavior of known conservative tracers beforehand.

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3.7 Figures

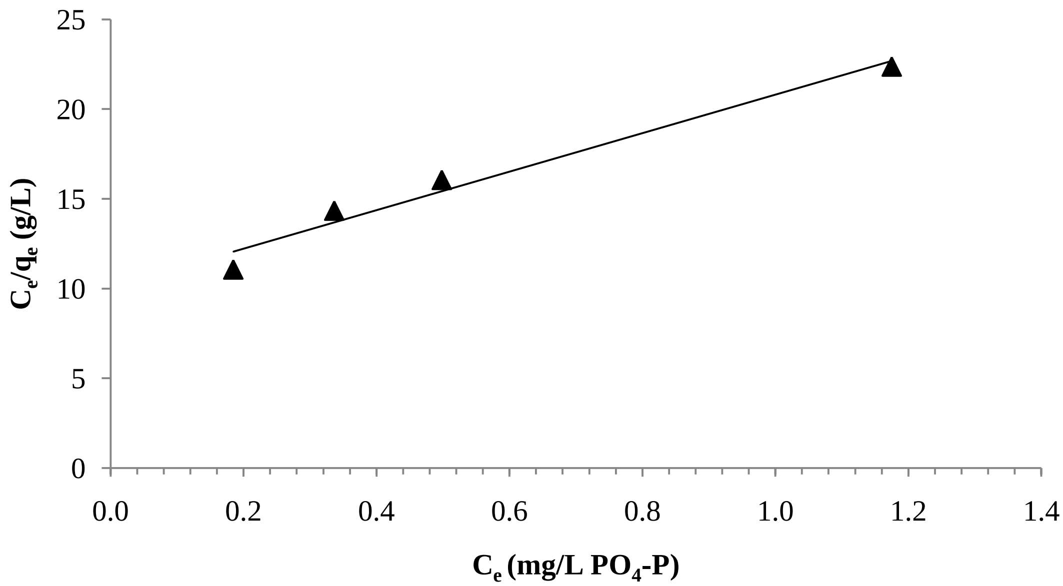


Figure 3.1: Linearized PO_4-P Langmuir isotherm. (R^2 = 0.9717)

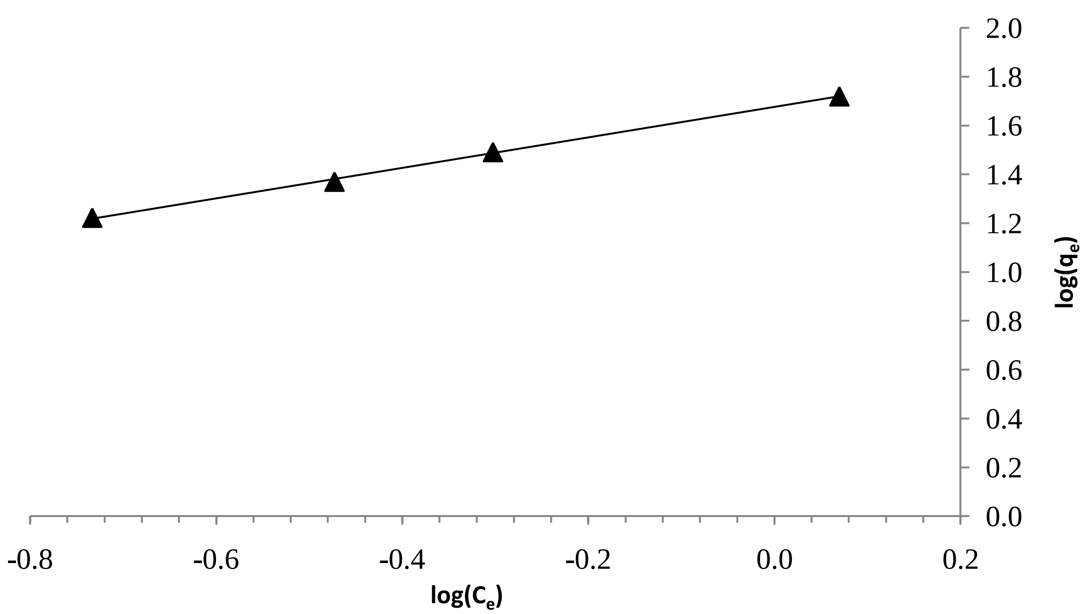


Figure 3.2: Linearized PO_4-P Freundlich isotherm. (R^2 = 0.9989)

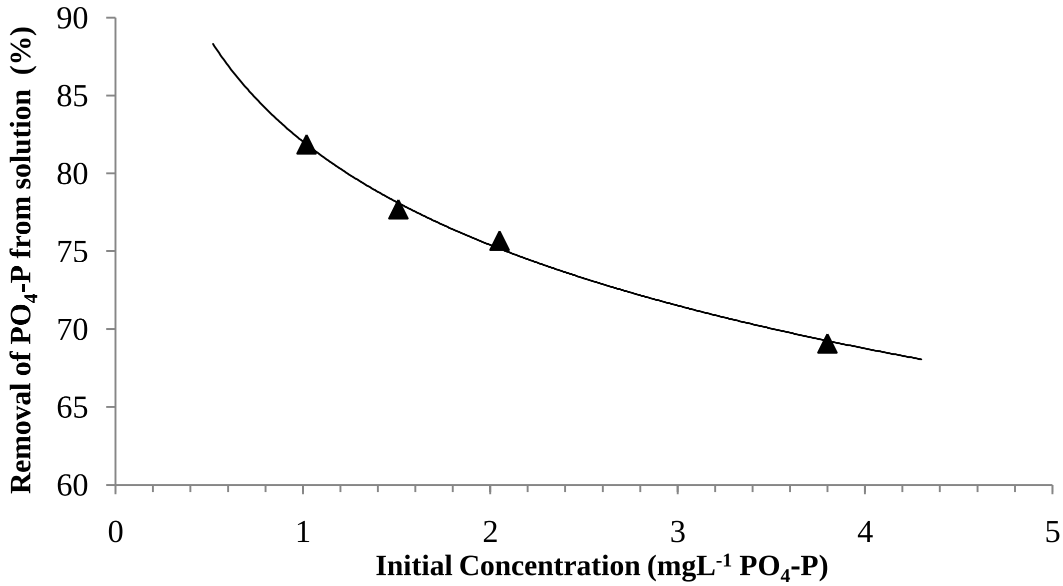


Figure 3.3: Percentage removal of PO_4-P from gravel substrate as a function of concentration with logarithmic fit ($R^2 = 0.9946$)

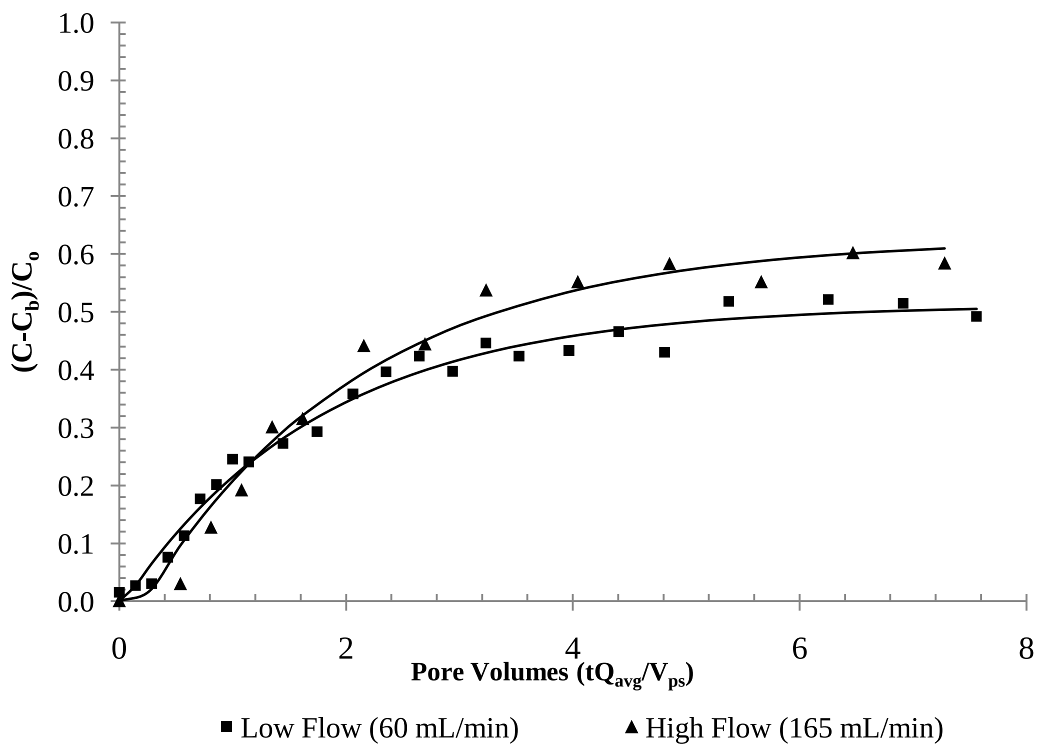
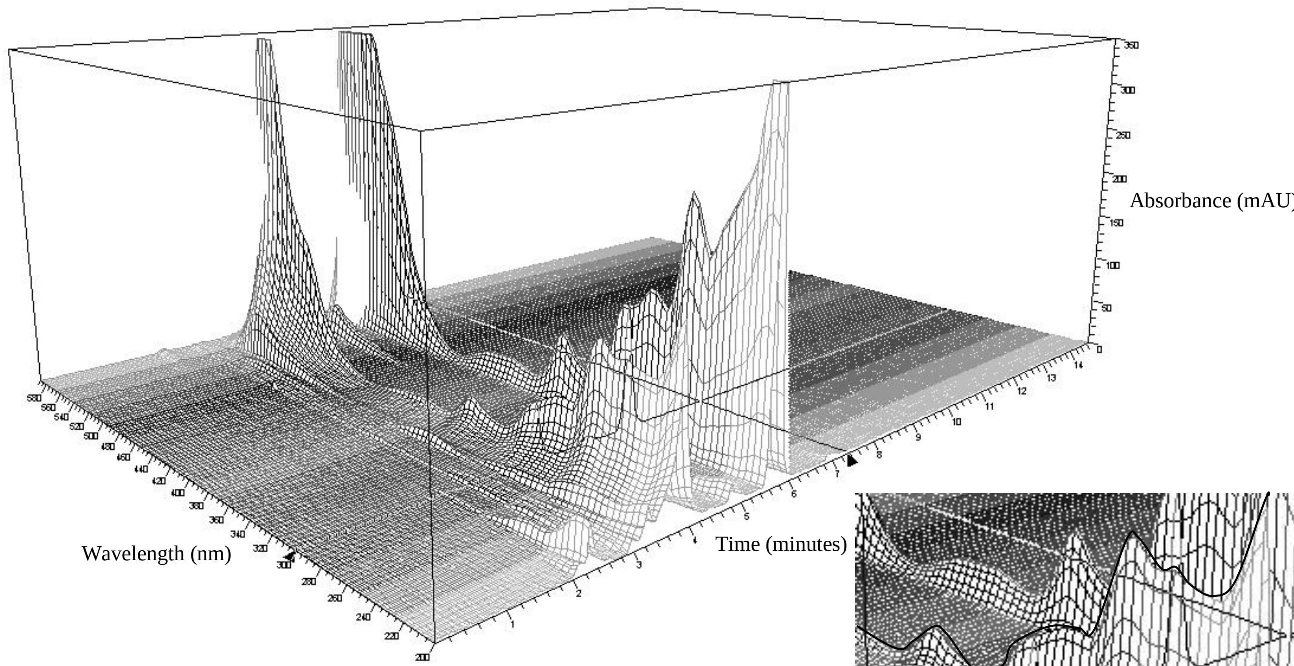


Figure 3.4: PO_4-P breakthrough curves for column experiments with exponential decay fit (high flow $R^2 = 0.9686$; low flow $R^2 = 0.9799$)



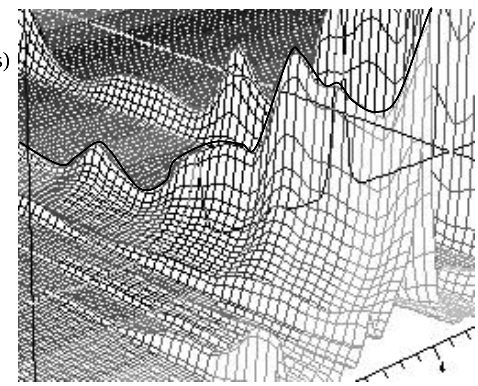


Figure 3.5: Separation of RWT constituents (new lot) by HPLC (high pressure liquid chromatography) Cutout focuses on the UV spectra of isomer 1.

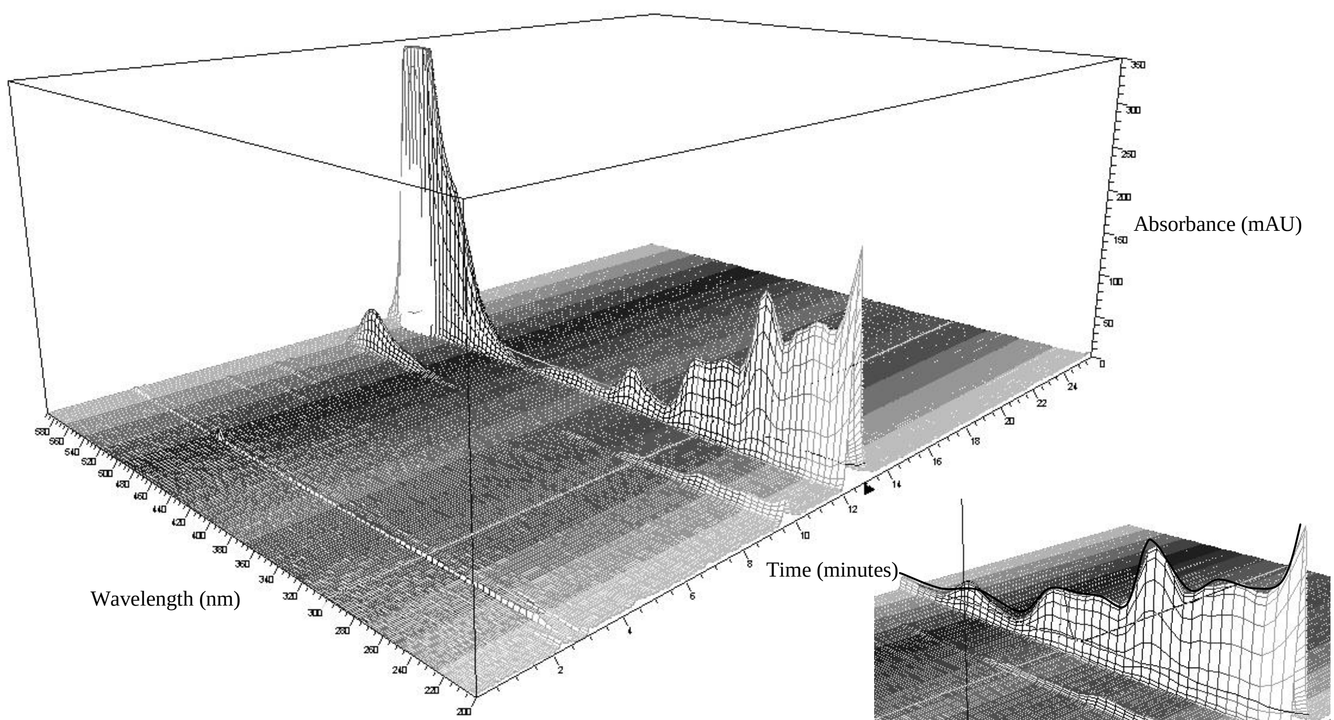


Figure 3.6: Separation of RWT constituents (old lot) by HPLC (high pressure liquid chromatography) Cutout focuses on the UV spectra of unknown constituent.

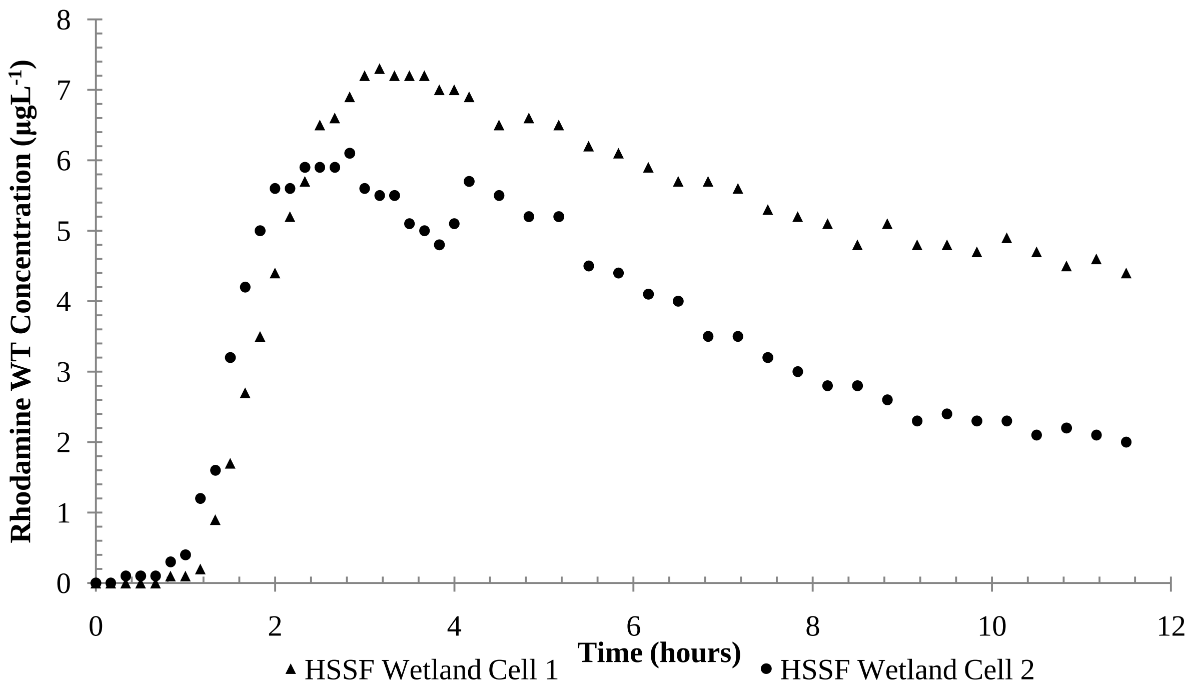


Figure 3.7: Tracer response curve from RWT impulse addition (9 mL) to HSSF Wetland Cells 1 and 2 (November, 2010). Data truncated at 11.8 hours due to temporal restraints. Mass Recovery of Cells 1 and 2 were 66% and 49%, respectively.

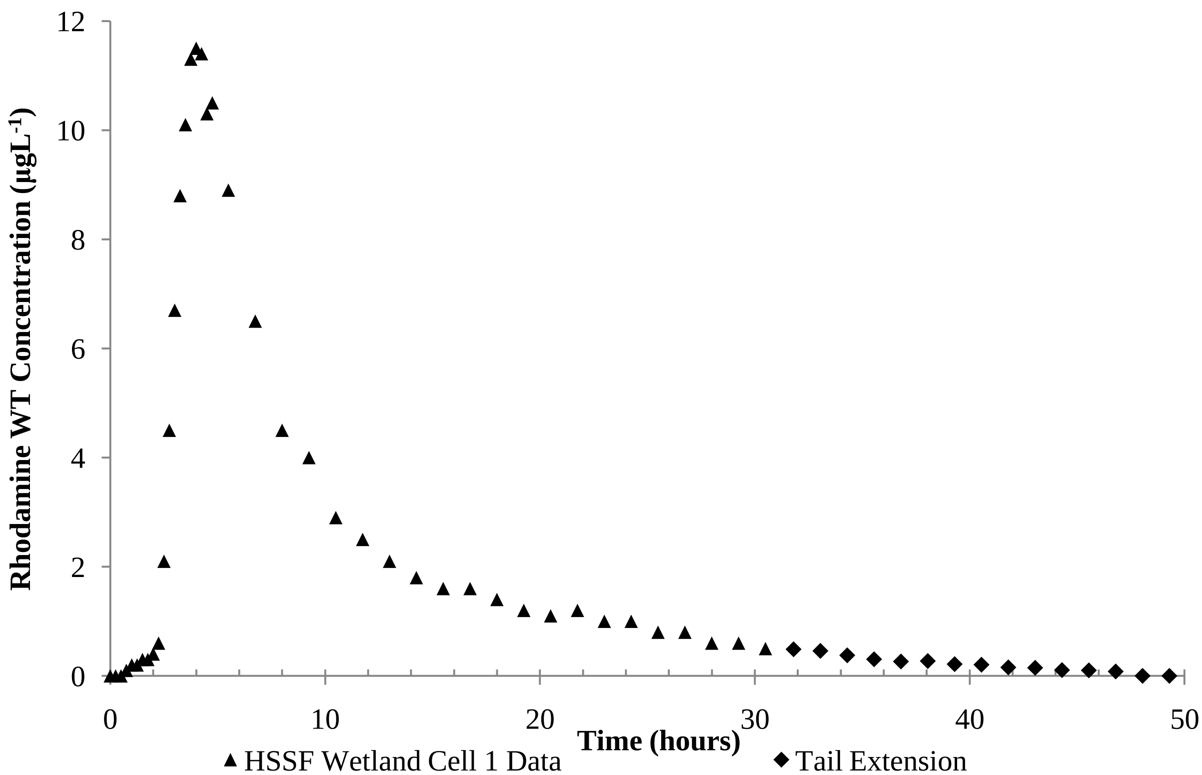


Figure 3.8: Tracer response curve from RWT impulse addition (10 mL) to HSSF Wetland Cell 1. (March, 2011) Data extrapolated from t = 30.5 hours to completion with exponential decay equation. Tracer mass recovery was 67%.

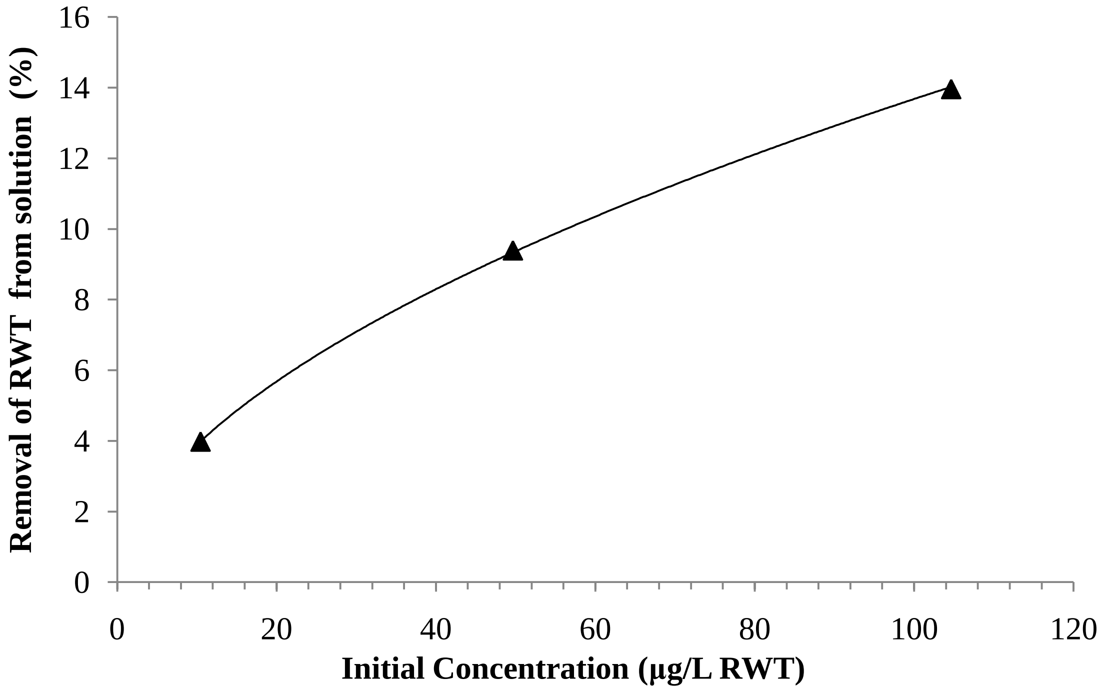


Figure 3.9: Percentage removal of RWT from gravel substrate as a function of concentration (R^2 = 0.9998)

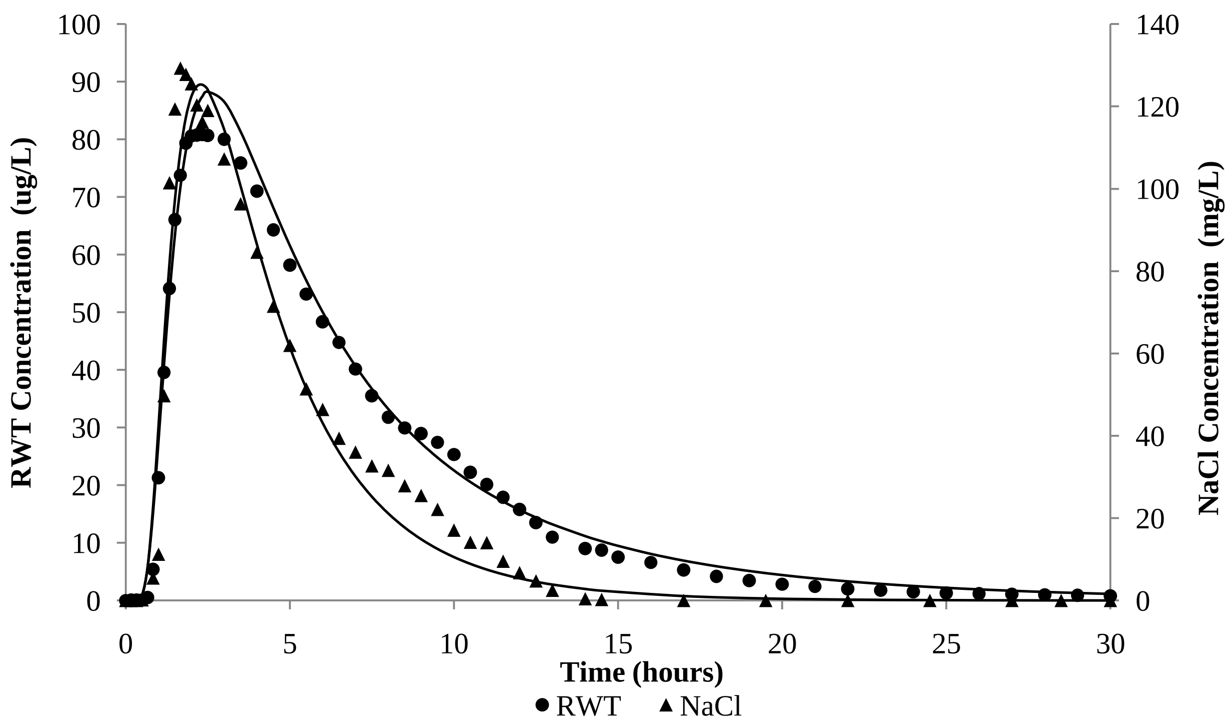


Figure 3.10: Tracer response curves for RWT and NaCl pulse injections. Curves illustrate 1-D transport equation proposed by Lenda and Zuber (1970). Mass recoveries for RWT and NaCl were 96% and 111%, respectively.

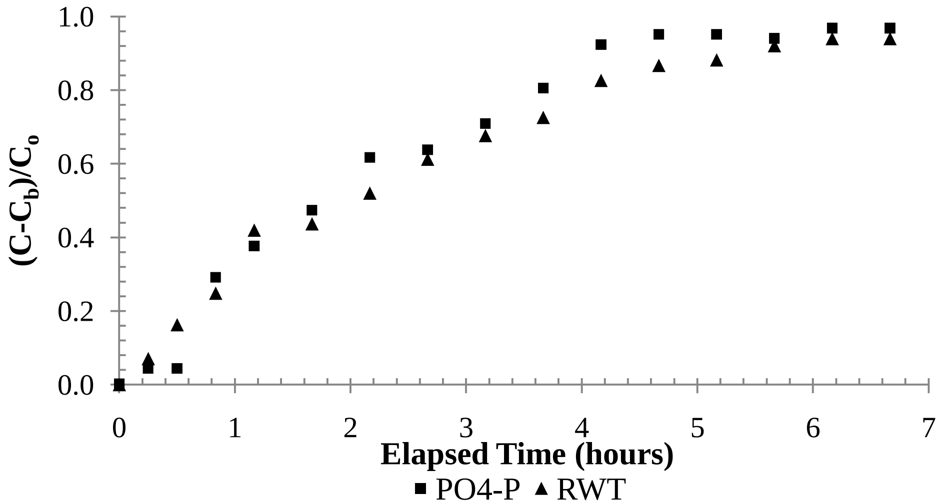


Figure 3.11: Step input of PO_4-P and RWT into blank column. Effluent concentrations reach 97% and 94% of influent after about 7 hours for PO_4-P and RWT, respectively

3.8 Tables

Table 3.1: Summary of batch sorption parameters

Parameter	C_i (mg/L PO4-P)	Soil:Solution Ratio	$V_{solution}$ (mL)	T_{eq} (hr)	Temperature (°C)	pH	C_{eq} (mg/L PO_4-P)	K_d (mL/g)	Removal (%)
PO_4	3.8					9.2	1.17	44.7	69
PO_4	2.0					9.2	0.50	62.3	76
PO_4	1.5	1:20	100	48	21 ± 1	9.1	0.34	69.7	78
PO_4	1.0					9.0	0.18	90.0	82
PO_4	0.5					8.9	0.12*	70.0	78
	(?g/L RWT)						(?g/L RWT)		
RWT	105					8.0	90.05	1.6	14
RWT	50	1:10	100	48	21 ± 1	8.0	45.00	1.0	9
RWT	10					8.0	10.05	0.4	4

An asterisk (*) indicates that equilibrium concentration fell below method detection limit (0.15 mg/L PO_4-P)
C_i = initial concentration, T_{eq} = equilibrium time, C_{eq} = equilibrium concentration, K_d = Partition Coefficient

Table 3.2: Summary of flow-through column parameters

Parameter	Input	C_i (mg/L PO4-P)	Q_{avg} (mL/min)	t_{eq} (hr)	t_{eq} (pore volumes)	Temperature (°C)	pH	C_{eq} or C_{max} (mg/L PO4-P)	R	K_d (mL/g)
PO_4 (165mL/min)	Step	2	165	4.0	6.4	21 ± 1	7.9	1.23	3.79	0.92
PO_4 (60mL/min)	Step	2	60	10.5	6.6		8.0	0.23	4.13	0.82
		(?g/L RWT)	Q_{avg}	t_b (10%)	t_b (90%) (hr)	Temperature	pH	(?g/L RWT)	R	
RWT	Pulse	N/A	50	1.9	16.0	21 ± 1	8.0	0.15	1.74	0.24
		(mg/L NaCl)	Q_{avg}	t_b (10%)	t_b (90%) (hr)	Temperature	pH	(mg/L NaCl)	R	
NaCl	Pulse	N/A	50	1.5	6.6	21 ± 1	8.0	0.00	N/A	N/A

C_i = initial concentration, Q_{avg} = average flow rate, t_{eq} = equilibrium time, t_b (%) = time in which % mass recovered, C_{eq} = equilibrium concentration, C_{max} = peak concentration, R = Retardation coefficient, K_d = Partition Coefficient

Chapter 4: Summary, Recommendations, and Future Outlook

The evaluation of HSSF constructed wetlands and pretreatment options over the course of about one year revealed the potential for effective year-round treatment of flow-through trout farm effluent. This study has also presented both design and operational limitations that will require future research to overcome. A growing body of research on the treatment performance of constructed wetlands and internal processes governing pollutant removal is a significant step towards widespread implementation, but not a full resolution to the issue of trout farm effluent treatment. Before implementation can take place, many aspects of design must be optimized to fully take advantage of the wetland ecosystem in temperate climates.

Sizing of subsurface flow wetlands to achieve a desired treatment performance is one method of design optimization. Sizing is largely accomplished by using prescriptive criteria. This method uses tools such as loading charts to help select a statistical level of performance (i.e. median) from a large data set. Wetland size is then tied to a parameter such as influent loading or detention time to scale to the desired size. Data from this study have demonstrated limited sizing criteria in the form of loading charts for select parameters. Being that many removal mechanisms are temperature dependent, it is not recommended that these charts be extrapolated beyond the trout farm utilized in this study. Data found in this study can, however, contribute to the development of prescriptive sizing criteria for flow-through trout farm wetlands by adding to a larger data set which can eventually capture system variability. Perhaps the second most important aspect of design is the type and size of material to utilize in the wetland bed.

A continual challenge for subsurface-flow wetland design is the selection of a bed media that maintains desirable hydraulic conditions while providing the best possible treatment. Generally speaking, media size is inversely proportional to treatment efficiency for a given material. Finer materials allow greater surface area for sorption sites and microbial biofilms, but will have lower hydraulic conductivities and be more prone to clogging. For wastewaters characterized by high and continuous flow rates such as flow-through aquaculture effluents, substantial hydraulic conductivities are desirable. Since materials that are economically feasible generally have to be harvested regionally, choices for trout farmers will be limited. Gravel media generally allows sufficient hydraulic conductivity; however, clogging of the inlet region produced surface flow only eight months into operation at an average hydraulic loading of 5 m/day. At the time of clogging, it was visually apparent that the cross-sectional area available for wastewater infiltration was insufficient. Orifices drilled above the bed media were distributing a large portion of flow onto the surface of the wetland, before hydraulic conductivity was sufficient to allow subsurface flow to prevail. Wastewater distribution, accomplished in this study via orifices drilled into an influent baffle and direct exposure to coarse media on the baffle floor, should be improved to avoid poor wetland operation. In order to enhance the longevity of the wetland cells at full scale, either continuous cleaning of the influent baffle or a more effective means of distributing organic loading will be necessary. For instance, the implementation of infiltration chambers in a "T" arrangement (Wallace and Knight, 2006) is one distribution system that has seen some success in reducing the organic cross-sectional loading.

Wetland tracer response curves identified the presence of dual peaks on at least one occasion, primarily in the sedimentation-side wetland cell that became more prone to clogging. As a result of clogging, varying extents of short-circuiting, as indicated by surface flow, were documented in this wetland cell. RWT proved useful to identify qualitative information such as the development of multiple flow paths, but was found to behave non-conservatively in the subsurface media. Fluorescent tracers are generally popular due to their high sensitivity and ease of analysis, low background concentrations, and low eco-toxicity. Though RWT is one of the most accessible tracers, perhaps other fluorescent tracers such as Pyranine and Uranine should be investigated alongside RWT to determine which tracer behaves most conservatively in mature HSSF wetlands.

An important lingering question is whether the treatment of parameters studied in this experiment is significant enough for trout farmers to invest in HSSF constructed wetlands. The answer is heavily dependent on three main factors. The first being whether demand for aquaculture products increases. An increase in demand will correlate with an increase in production intensity and accompanying waste loads. This could potentially elevate effluent concentrations above effluent limits, requiring more effective treatment options. Secondly, existing and future regulation might target aquaculture facilities in order to remediate bodies of water that the EPA deems "impaired." This can come in the form of NPDES permit compliance and wasteload allocations as a result of TMDLs, such as those recently implemented in the Chesapeake Bay. Finally, new research may eventually improve upon design, increasing treatment performance and viability of HSSF constructed wetlands.

Two parameters that are believed to have greater potential for removal in HSSF wetlands are nitrogen and phosphorus. Insignificant nitrogen removal demonstrated in this study was primarily due to aerobic conditions persisting throughout the wetland cells preventing substantial denitrification from occurring. Future research can focus on practicable means of either promoting aerobic and anoxic zones within a single wetland, or dimensioning a second wetland cell for denitrification. Detention times greater than 24 hours may be necessary to promote denitrification (Wang et al., 1996), therefore significant land area may be required to construct wetlands for nitrogen removal. Both capital and opportunity costs may be offset by planting a cash crop on a portion of the wetland of the wetland cell. Analyzing potential crops that have the potential to thrive in HSSF wetlands, aid in nutrient removal, and bring in an annual net profit for trout farmers may be an attractive area of future research. Phosphorus removal is another area in which future research may enhance removal. The positive effect of decreased pore-water velocity on phosphorus removal in this study may suggest rate-limited sorption. However, decreasing pore-water velocity by increasing detention times, and thus wetland size, may not be the most practical means of improving phosphorus removal. Previous research has identified substrates that have greater potential for sorption of the soluble fraction of phosphorus than gravel media, which was utilized in this study. Large volumes of wastewater, characteristic of trout farm effluent, prohibit the use of many of these substrates due to their low hydraulic conductivities accelerating the clogging process. However, future pilot scale studies may investigate other practicable substrates will greater potential for phosphorus sorption.

Like many industries, aquaculture production needs to be competitive on an international stage, requiring trout farmers to minimize production costs. Therefore, trout farm owners and operators must optimize wastewater treatment, in order to minimize expense. Though HSSF wetlands are recommended as a cost-effective means of biological treatment, future design improvements, economic climate, and environmental regulation will dictate whether flow-through trout production facilities implement HSSF constructed wetlands on a larger scale.

Appendices

Appendix A: Literature Review References

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Appendix B: Project Images

Image 1: Sedimentation basin

Image 2: Microscreen

Image 3: Orthogonal view of constructed wetlands

Image 4: Front view of constructed wetlands