AN ABSTRACT OF THE THESIS OF

Jeffrey L. Hart for the degree of Master of Science in Chemical Engineering presented on March 16, 2012

Title: Evaluating the Rates of Nitrate Removal for a Nitrate Containing, Low Organic Carbon Wastewater Interacting with Carbon-containing Solid Substrates

Abstract approved:

Mark E. Dolan

The primary objective of this study was to evaluate the rates of nitrate removal for a nitrate containing, low organic carbon wastewater interacting with four different carbon-containing solid substrates (alder woodchips, corn silage, manure and woodchip biochar). Batch systems were tested for nitrate removal, and systems with a combination of three carbon substrates (75% woodchips, 12.5% silage, and 12.5% manure or woodchip biochar by mass) produced average nitrate removal rates of 571 and 275 mg-N L^{-1} D^{-1}, and systems containing the carbon substrates individually produced rates between 11.4 - 3.3 mg-N L^{-1} D^{-1}. Silage proved to be the dominant carbon substrate providing high quantities of organic carbon to fuel denitrification. With the introduction of semi-continuous flow, all systems had nitrate removal rates that converged to 13.3 – 6.4 mg-N L^{-1} D^{-1}, which is approximately two orders of magnitude smaller than the rates of the mixture systems in the batch experiment. Silage appeared to be removed from of the systems with liquid exchange potentially causing the rate decreases. Columns filled with various volume fractions of woodchips (100%, 25%, 12.5%, and 0%) produced nitrate removal rates between 30.8 – 2.4 mg-N L^{-1} D^{-1} at a 24 hour and 12 hour hydraulic residence time (HRT). Greater nitrate removal was achieved with higher HRTs and larger fractions of woodchips (the 100% woodchip system at a 24 hour HRT produced the fastest nitrate removal rate of 30.8 mg-N L^{-1} D^{-1}). When rates were normalized to the amount of woodchips in each column, higher efficiency was found in lower woodchip

fraction systems (the 12.5% woodchip column produced the highest normalized nitrate removal rate of 56 mg-N L^{-1} D^{-1} $L_{woodchips}^{-1}$). Woodchips proved to be best suited as a long term carbon substrate for nitrate removal in a system containing a nitrate concentrated, low organic carbon wastewater. However, large amounts of woodchips were necessary to achieve nitrate removal greater than 50%. A 41 acre hypothetical wetland with a 3.3 day HRT and a nitrate influent concentration of 45 mg-N L^{-1} would require 30,000 yd^3 of woodchips to achieve 68% nitrate removal based on the values obtained in the bench scale column experiment.

Evaluating the Rates of Nitrate Removal for a Nitrate Containing, Low Organic Carbon
Wastewater Interacting with Carbon-containing Solid Substrates

by
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CHAPTER 1: INTRODUCTION

1.1 Talking Water Gardens

In 2010, ground was broken on a 39 acre constructed wetland designed to treat two different wastewaters for temperature; municipal wastewater from the Albany-Millersburg Water Reclamation Facility (WRF) and process wastewater from the specialty metal manufacturer, ATI Wah Chang. Named Talked Water Gardens, the constructed wetland is located in Albany, Oregon adjacent to the Willamette River. Total maximum daily loads (TMDL) for heat, mercury, and bacteria have been placed on the Willamette River to protect natural habitat and wildlife; primarily salmon. The Albany-Millersburg Water Reclamation Facility could not meet their heat TMDL and needed a way to reduce thermal load before discharging into the Willamette River. ATI Wah Chang was required to discontinue discharge into a local creek and change to a direct Willamette River outfall. A second local industry, International Paper, had the same requirements as ATI. All three facilities joined, with CH2M-Hill, to solve individual issues with one solution, Talking Water Gardens (prior to the start of construction, International Paper shutdown its Millersburg plant leaving only Albany-Millersburg WRF and ATI Wah Chang as Talking Water Gardens inputs).

The construction of Talking Water Gardens (TWG) was a collaborative effort that effected more than the facilities involved. First, the cities of Albany and Millersburg wanted their heat treatment to be sustainable; environmentally friendly and beneficial to the community. TWG serves that purpose.

- Provides waterfowl and other wildlife habitat
- Uses zero energy except to pump water to TWG
- Provides community with outdoor recreation and educational opportunities

Second, the thermal TMDL is simplified with the combination of multiple point sources. Both Albany-Millersburg WRF and ATI have individual heat load discharge limits

depending on temperature and flow of the Willamette River and each facility. Combining the two wastewaters as one Willamette River discharge simplifies the TMDL.

1.1.1 Talking Water Garden Inputs

Albany-Millersburg Water Reclamation Facility opened in February 2009 with an average dry day capacity of 12.3 million gallons per day (MGD). The WRF was designed as an innovative secondary treatment system by CH2M-Hill and Carollo Engineers using Siemens Cannibal^{TM} technology. First, WRF wastewater (WRFWW) is pumped through a series of mechanical equipment to remove debris such as sand, grit, and large organic material. Six Vertical Loop Reactors are gravity fed WRFWW and recycled sludge from the Cannibal^{TM} process to remove BOD at a high MLSS concentration. After clarifying the bacteria-rich mixed liquor, sodium hypochlorite is used as a disinfectant before the effluent is pumped to TWG. The WRFWW effluent water characteristics are shown in Table 1.1 and the process flow diagram is depicted below (Figure 1.1).

Table 1.1: Effluent water quality parameters for ATI Wah Chang process water and Albany-Millersburg WRF wastewater. The effluents are also Talking Water Garden inputs.

Concentration (mg L^{-1})	ATI Wah Chang	Albany-Millersburg WRF
COD (ATI) or CBOD (WRF)	0-20.0	1.0-2.0
SO_4^{2-}	200-500	-
Cl^-	3000-5000	-
NO_3^- (as N)	10.0-50.0	5.0-10.0
Alkalinity (as $CaCO_3$)	130	-
TSS	-	2.0-7.0

ALBANY-MILLERSBURG WATER RECLAMATION FACILITY
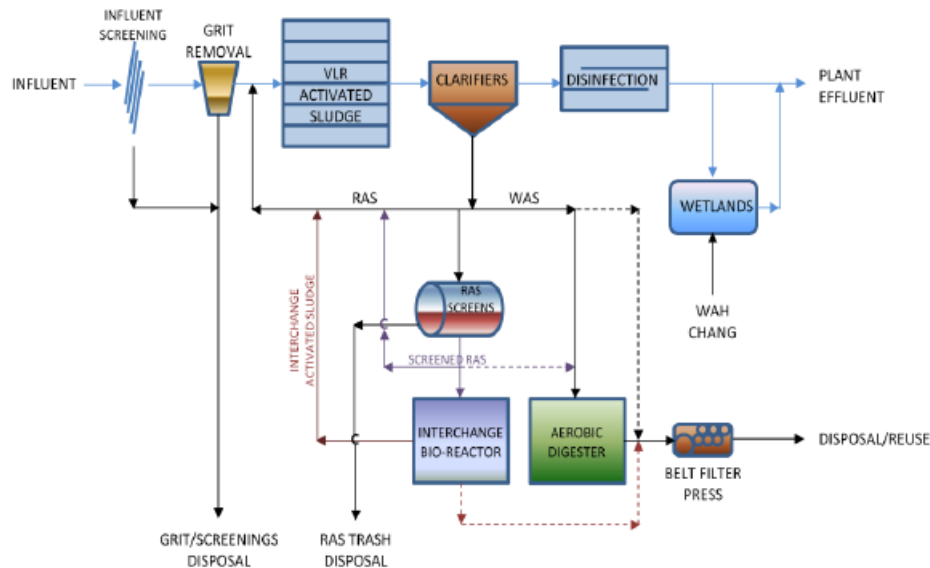
Figure 1.1: Albany-Millerburg Water Reclamation Facility Process Flow Diagram

Titanium, zirconium, niobium, hafnium, tantalum, tungsten, and vanadium are some of the specialty metals that ATI Wah Chang manufactures for a variety of applications. Many ATI manufacturing processes use water for heating, cooling, washing, metal pickling, and other purposes. The pickling process involves the use of hydrofluoric and nitric acids to remove impurities from the metals. As the acids dissociate, a low pH and nitrate containing wastewater is formed. To treat the process water, ATI applies lime settling and filter technology. Raising the pH with lime to above 8.0 provides optimal water conditions for fluoride-metal hydroxide precipitation. These metal hydroxides are collected in a clarifier and disposed of in a permit regulated landfill. The clarified water is discharged into three treatment ponds before being pumped to TWG. The treatment pond effluent characteristics are shown in Table 1.1.

1.2   Thesis Objectives

This research was conducted due to concerns of future regulation on a nitrate containing wastewater that is discharged to the Willamette River. Governmental agencies do not currently impose regulations on nitrate discharged to the Willamette; however such

regulations are likely in the near future. Excessive nitrate concentrations may lead to algal blooms and eutrophication in surface waters, which can potentially destroy habitat by depleting dissolved oxygen concentrations. On the other hand, nitrate is regulated on a federal level by limiting drinking water to less than 10 mg-N L^{-1} (EPA, 1974). Exposure to nitrites and nitrates above these concentrations can cause adverse human health effects, and can even be fatal to infants and children (McCasland, Trautmann, Porter, & Wagenet, 1985).

The fate of the nitrate containing ATI wastewater (ATIWW) through Talking Water Gardens is unknown. A separate study is being conducted to determine the hydraulic characteristics of Talking Water Gardens (TWG), which will lead to a better understanding of the nitrate fate. This study evaluates the rates of nitrate removal for the nitrate containing, low organic carbon wastewater from ATI.

The primary form of nitrogen removal within a wetland is microbial denitrification (Mayo & Bigambo, 2005). In order for denitrification to occur, organic carbon must be present in the aqueous phase. Both TWG inputs have organic carbon concentrations insufficient to stimulate denitrification. Thus, a source of organic carbon must be added to the wastewater to achieve denitrification and nitrogen removal. Literature has shown wastewater nitrogen removal in permeable reactive barriers, which are constructed with a solid substrate as porous media (Schipper, Robertson, Gold, Jaynes, & Cameron, 2010). In the case of nitrate containing, low organic carbon wastewaters, a solid substrate that provides organic carbon, such as woodchips, can stimulate denitrification and nitrogen removal (Robertson & Merkley, 2009).

The following are the objectives of this research:
- Identify cheap and locally available carbon substrates for nitrate removal evaluation of the ATI effluent
- Identify which carbon substrate or mixture of carbon substrates that are best suited for long term nitrate removal of the ATI effluent
- Quantify nitrate removal rates in conditions that best represent field conditions
- Apply nitrate removal rates to a hypothetical wetland to quantify the amounts of carbon substrate needed and cost

It is hypothesized that the addition of solid carbon substrates to a constructed wetland will produce greater nitrate removal rates than a constructed wetland without carbon substrates.

CHAPTER 2: LITERATURE REVIEW

The primary objective of this study was to evaluate the rates of nitrate removal for a nitrate containing, low organic carbon wastewater interacting with four different carbon-containing solid substrates. The following literature review is aimed to provide background on nutrient cycling in wetlands in order to elucidate the mechanisms of water treatment (nitrogen removal in particular) within a constructed wetland system.   Section 2.1 discusses the characteristics of nitrogen (and the nitrogen cycle) and its role as both an essential nutrient for life, and as a chemical of concern in the environment. Sources and implications of nitrogen pollution and conventional wastewater treatment of nitrogen are also discussed. Section 2.2 distinguishes the various types of naturally occurring wetlands and their specific roles in nutrient cycling, watershed dynamics, functionality and values. The section also discusses the causes and implications of wetland loss and degradation. Section 2.3 is concentrated on constructed wetlands, and how they are designed to take advantage of the natural processes involving wetland vegetation, soils, and microbial processes to aid the treatment of wastewater in a controlled environment. Section 2.4 focuses on the complex chemical, biological, and physical processes that occur in constructed wetlands, and their effect on water quality and characteristics. Section 2.5 introduces and describes permeable reactive barriers; a passive wastewater treatment method that utilizes a reactive porous matrix. Section 2.6 introduces and discusses biochar and its unique properties as a soil amendment.   Section 2.7 describes the potential application of permeable reactive barriers and biochar in constructed wetlands.

2.1   Nitrogen

Nitrogen is the fifth most abundant element in our solar system, and is an essential nutrient for life. It is an important component of all proteins, and necessary for the synthesis of nucleic acids. The biogeochemistry of nitrogen is largely dependent on oxidation-reduction reactions that are primarily microbially mediated (Canfield, Glaszer, & Falkowski, 2010).   The various forms of nitrogen (based on its nine oxidation states) are defined in Table 2.1.

Table 2.1: Forms of nitrogen in its nine oxidation states

Oxidation State	Species	Name
-3	NH_3, NH_4^+	Ammonia, ammonium ion
-2	N_2H_4	Hydrazine
-1	NH_2OH	Hydroxylamine
0	N_2	Nitrogen gas
+1	N_2O	Nitrous oxide
+2	NO	Nitric oxide
+3	HNO_2, NO_2^-	Nitrous acid, nitrite ion
+4	NO_2	Nitrogen dioxide
+5	HNO_3, NO_3^-	Nitric acid, nitrate ion

The mechanisms and chemistry of the various nitrogen transformations are discussed in further depth in Section 2.4.1, in order to elucidate their roles in constructed wetlands. The microbiology and chemistry of nitrification and denitrification is presented in Section 2.1.2 to provide background on how these transformations occur in conventional wastewater treatment processes. A general summary of the nitrogen cycle is discussed in the following section.

In general, plants can convert carbon dioxide and water into organic matter (photosynthesis) in the carbon cycle, which in turn provides nutrients for animals. Plants (along with associated microorganisms) also can convert inorganic nitrogen from soils into organic nitrogen in order to synthesize plant proteins. The nitrogen cycle generally involves the process of microbial break down of decaying organic nitrogen to release ammonia, which is oxidized by microbes to nitrite, then to nitrate. Denitrifying bacteria are responsible for converting nitrate to nitrogen gas. Plants fix various forms of nitrogen (mostly ammonium or nitrate; further discussed in Section 2.4) and the cycle continues. Most organisms cannot fix nitrogen, but rather uptake ammonium from the environment,

or from the reduction of nitrate to ammonium through assimilatory nitrate reduction. Some eukaryotes, such as legumes and termites, provide an additional route of nitrogen fixation via symbiotic association with nitrogen-fixing prokaryotes (Canfield, Glaszer, & Falkowski, 2010).

2.1.1   *Nitrogen Pollution*

Over the past century, the development of industrial processes, agricultural practices, and the burning of fossil fuels have disrupted the global nitrogen cycle by inducing terrestrial nitrogen fixation. Canfield et al., suggests that these anthropogenic activities double the natural terrestrial nitrogen fixation rate (2010).

Industrial processes are implemented to reduce nitrogen gas to ammonium (nitrogen fixation) in order to produce enough fertilizer to sustain agricultural demands for the human population. Synthetic nitrogen fertilizers (generally ammonium) are widely used in agricultural practices to stimulate crop growth, but typically less than 40% of the fertilizer is actually allocated to plant biomass. Nitrifying bacteria can convert the excess ammonia to highly mobile nitrate, which leaches into the groundwater and spreads into waterways (Canfield, Glaszer, & Falkowski, 2010). Nitrate (and phosphorus) is a contaminant to waterways; resulting in the production of algae blooms (eutrophication). Increased biological productivity and eutrophication restricts water use for fisheries, recreation, industry and drinking because of the large quantities of algae and aquatic weeds in the waterbody. When the algae die off and decompose, they consume dissolved oxygen; sometimes creating hypoxic zones that destroy aquatic habitat (USDA, 2003). Hypoxic zones have been reported to impact over 400 systems and a total area of more than 245,000 square kilometers around the world (Diaz & Rosenburg, 2008).

So far, nitrate is only regulated as a TMDL wastewater discharge on a watershed basis. Zollner Creek, in the Molalla-Pudding River Subbasin, Oregon, is subject to a nitrate TMDL; meaning the entities that discharge to Zollner Creek must abide by their specific nitrate loading discharge regulation. The primary sources of nitrate discharge for Zollner Creek are runoff and groundwater leaching from a landfill, septic systems, and agricultural sources (ODEQ, 2008).

Nitrate is nationally regulated by the Safe Drinking Water Act of 1974; drinking water nitrate concentrations cannot exceed 10 mg-N L^{-1} (EPA, 1974). Ingesting nitrate can lead to severe health effects in infants younger than six weeks. The most significant health effect is Methemoglobinemia, where nitrate increases the conversion of hemoglobin to methemoglobin. If the infant lacks hemoglobin there is no method of oxygen transfer throughout the body, and the infant could suffocate (McCasland, Trautmann, Porter, & Wagenet, 1985).

Ammonia production associated with intensive animal agriculture, and fertilizer production can also degrade environmental quality. Ammonia is a colorless gas with a pungent odor, which is noticeable in concentrations over 50 ppm. It is an irritant, poisonous if inhaled in great quantities, and explosive in certain environmental conditions (Phillips, 2005). Both ammonia and ammonium are regulated as discharge pollutants into water bodies; as NH_3 is known to be toxic to aquatic species at concentrations greater than 2.0 mg-N L^{-1} (Randall & Tsui, 2002) (Francis-Floyd, Watson, Petty, & Pouder, 2009).

The combustion of fossil fuels release nitrogen oxide (NO_X) and sulfur oxide (SO_x) gasses to the atmosphere, which contributes to atmospheric deposition (acid rain). In the United States, roughly one fourth of all NO_X emissions result from electric power generation that relies on the burning of fossil fuels (EPA, 2007). Acid deposition can present multiple problems to an ecosystem including acidification in water bodies, and contribution to tree damage at high elevations (EPA, 2007). NO_X gasses and their particulate matter derivatives (compounds speciated with nitrate) contribute to visibility degradation and harm human health (EPA, 2007). The EPA's Acid Rain Program was created by Congress in Title IV of the 1990 Clean Air act to reduce NO_X and SO_X emissions.

2.1.2   Nitrogen Removal in Conventional Wastewater Treatment Systems

Nitrogen removal is often required in a wastewater treatment system before the water can be discharged to water bodies, used for groundwater recharge, or reused for other applications (Metcalf & Eddy, Inc., 2003).   There are a host of biological, chemical, and physical means of accomplishing nitrogen reduction in conventional wastewater treatment systems. Examples of nitrogen removal by physical and chemical methods include air or steam stripping of ammonia, chemical oxidation, ion exchange, and any method of particulate removal for suspended organic nitrogen. Alternatively, biological systems are used more often and are generally more cost effective (Metcalf & Eddy, Inc., 2003). It is common to see separate biological, chemical, and/or physical nitrogen removal steps conjoined or added separately depending on the requirements of the system. Since this research is primarily concerned with biological nitrogen removal (BNR) processes, the scope of the following discussion will be limited to conventional biological treatment methods.

Most BNR systems are modifications of the Activated Sludge (AS) process that incorporate anoxic and/or anaerobic zones to facilitate nitrogen (and/or phosphorus) removal (Grady, Daigger, & Lim, 1999). Bacterial growth is necessary for AS systems, where microorganisms consume organic carbon by decreasing biological oxygen demand (BOD). The microorganisms consume substrates with oxidation-reduction reactions, and growth occurs by cell synthesis.   Thus, biomass in BNR systems is produced continuously as the substrate in the wastewater is utilized.

Total nitrogen (TN) is defined as the sum of organic nitrogen, ammonia, nitrite, and nitrate. The organic fraction consists of a mixture of soluble or particulate compounds, including amino acids, amino sugars, and proteins (Metcalf & Eddy, Inc., 2003). The overall goal of a BNR system is TN removal by both ammonia oxidation and nitrate reduction to nitrogen gas. Thus, the BNR system must include an aerobic zone to allow biological nitrification to take place, and an anoxic zone for biological denitrification to occur. Finally, an electron donor (usually an organic carbon source) is required for nitrogen transformations to take place. The organic carbon represents a portion of BOD and usually comes from the wastewater source or endogenous respiration

(microorganisms die off and are utilized by other microorganisms). The following processes are the individual nitrogen transformations that take place in typical BNR systems.

2.1.2.1   Nitrification

Nitrification is a two-step process in which ammonia is biologically oxidized into nitrite; and then nitrite is oxidized to nitrate. Nitrification is an essential component of sewage treatment processes due to an abundance of ammonia coming from the decomposition of organic material. The nitrification process prevents discharge of toxic levels of ammonia that may be detrimental to aquatic life in receiving waters (Prosser, 1990) (Randall & Tsui, 2002).

There are numerous species of ammonia and nitrite-oxidizing bacteria, and even ammonia oxidizing archaea in the environment. Leininger et al., suggest that archaeal ammonia oxidizers are more abundant in soils than their well known bacterial counterparts (2006). *Nitrosomonos* and *Nitrobacter* are primarily responsible for nitrification in wastewater treatment systems (i.e. activated sludge and biofilm processes) (Metcalf & Eddy, Inc., 2003), however research has also shown the occurrence of ammonia oxidizing archaea in activated sludge treatment bioreactors (Park, Wells, Bae, C, & Francis, 2006). *Nitrosomonos* and *Nitrobacter* are autotrophs that oxidize ammonia or nitrite for their energy source and fix (reduce) organic carbon from carbon dioxide for their electron acceptor to synthesize organic compounds (Prosser, 1990). Some bacteria are capable of heterotrophic nitrification, in which ammonia is oxidized to nitrite using organic energy sources. Coupled with denitrification, heterotrophic nitrification permits the complete transformation of ammonia to nitrous oxide by a single organism under aerobic growth conditions (Swiss Institute of Bioinformatics, 2012).

The following stoichiometry demonstrates the first step of nitrification, where ammonia is oxidized by *Nitrosomonos* to nitrite, shown in Equation 2.1.

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \qquad\qquad \textbf{(2.1)}$$
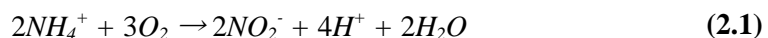
The second stage is exemplified by nitrite being oxidized by *Nitrobacter* to nitrate, shown in Equation 2.2.

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \qquad\qquad \textbf{(2.2)}$$

Based on the overall oxidation reaction (shown in Equation 2.3), the oxygen requirement is 4.57 g-O_2 g-N^{-1} with 3.43 g-O_2 g-N^{-1} used for nitrite production and 1.14 g-O_2 g-N^{-1} used for the transformation of nitrite to nitrate.[1]

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \qquad\qquad \textbf{(2.3)}$$
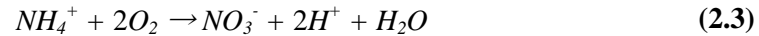
Nitrification BNR processes generally fall under attached or suspended growth treatment processes, similar to BOD removal. A common approach for suspended growth is to couple nitrification with BOD removal in a single-sludge process. Figure 2.1a shows a single-sludge process; the influent is first directed to an aeration tank for combined BOD removal and nitrification, then sent to a clarifier, and/or a sludge recycle system. Often multiple aeration basins and clarifiers are used in series to accommodate further treatment needs, as shown in Figure 2.1b, two-sludge suspended growth system. Autotrophic nitrifying bacteria grow substantially slower than heterotrophic bacteria, so hydraulic and solids residence times are generally longer for systems designed for both nitrification and BOD removal (Metcalf & Eddy, Inc., 2003).

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[1] Because of the contribution of oxygen to carbonate consumption, during cell synthesis, the oxygen requirement is slightly less than 4.57 g-O_2 g-N^{-1}. The combined process of cell synthesis and oxidation reduction occurring during nitrification result in a dissolved oxygen content of 4.3g-O_2 g-NH_4^+-N^{-1} that is fully nitrified to nitrate (Kadlec & Knight, 1996).
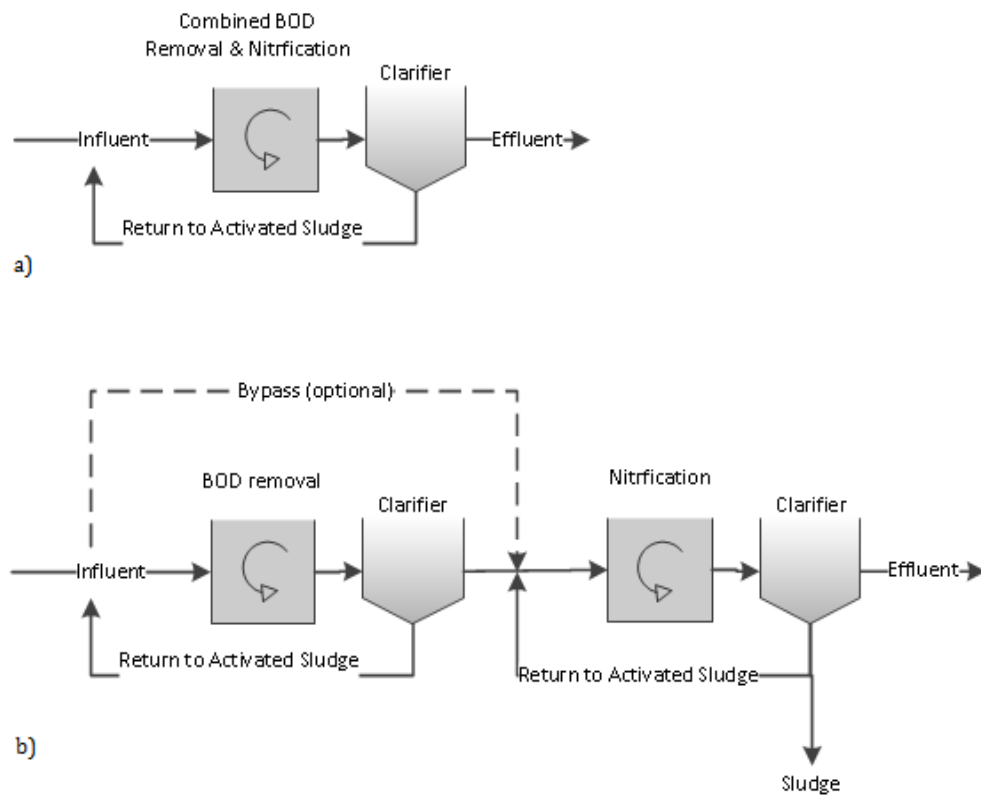
Figure 2.1: Configurations of biological nitrogen removal processes. (a) single sludge suspended growth system and (b) two-sludge suspended growth system (Adapted from Metcalf & Eddy (2003)).

2.1.2.2   Denitrification

Denitrification is one of many nitrogen transformations that take place in biological wastewater treatment (Figure 2.2).
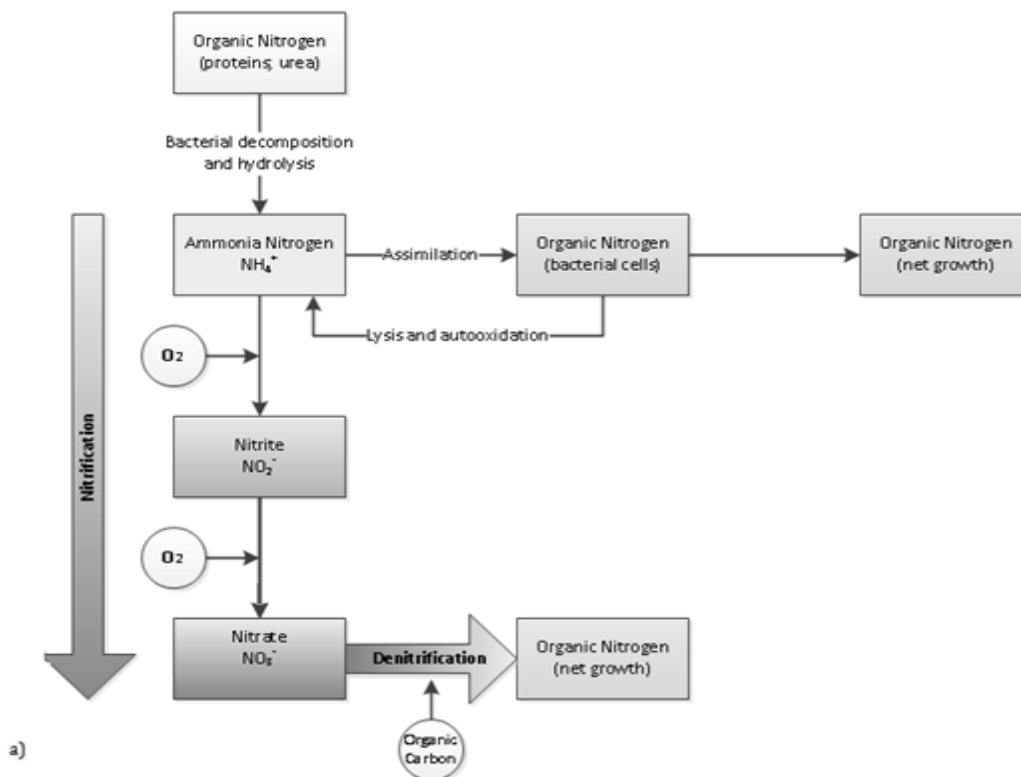
Figure 2.2: Nitrogen transformations in biological treatment processes (Adapted from Metcalf & Eddy (2003)).

The transformation of organic nitrogen into ammonia involves microbial hydrolysis. Ammonia can be transformed into nitrate by the process of nitrification when oxygen is present. Denitrification can take place when nitrate is biologically reduced to nitrite, to nitric oxide, and finally to nitrogen gas, in the absence of oxygen, as shown in Equation 2.4.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2 \tag{2.4}$$

The microbial reaction of denitrification is based on the respiratory electron transport chain where nitrate or nitrite is used as an electron acceptor in order to oxidize organic carbon into carbon dioxide and nitrogen gas.

$$CH_2O + \frac{4}{5}NO_3^- + \frac{4}{5}H^+ \rightarrow \frac{2}{5}N_2 + \frac{7}{5}H_2O + CO_2 \tag{2.5}$$
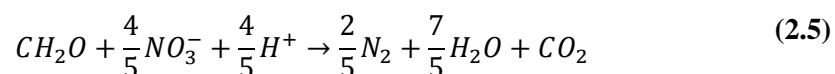
Denitrification must have a sufficient amount of organic carbon for the process to effectively remove nitrate from the system. In typical wastewater treatment systems, the organic carbon source is influent BOD, BOD produced from endogenous decay, or an added source, such as glucose or methanol (Metcalf & Eddy, Inc., 2003). Lower dissolved oxygen concentrations are also required for denitrification since oxygen can accept more electrons (redox potential), and is the favored electron acceptor for microorganisms. The half reaction for oxygen reduction has a redox potential of +820 mV, compared with +740 mV for nitrate reduction (Figure 2.3) (Wiedemeier, Rifai, Newell, & Wilson, 1999).
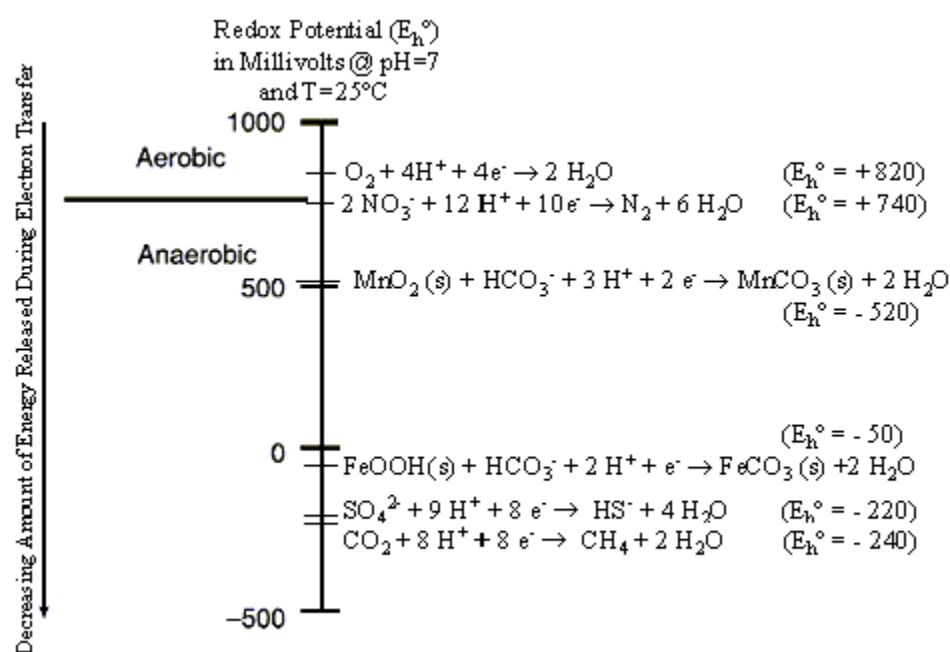
Figure 2.3: Redox potential for various half reactions (Wiedemeier, Rifai, Newell, & Wilson, 1999)

Denitrifying bacteria utilize nitrate for cell synthesis (assimilation) and as an electron acceptor in denitrification (energy).   Assimilation of nitrate occurs as a secondary source (ammonia being the primary) for incorporating nitrogen into cellular mass (Metcalf & Eddy, Inc., 2003). When assimilation occurs, nitrate is not removed from the system

unless the bacteria that consume it are removed from the system by some physical process (e.g. screening, filtering, settling, etc.).

Dissolved oxygen not only competes with nitrate as an electron acceptor, it can inhibit nitrate reduction by repressing the nitrate reductase enzyme at high concentrations. pH can also inhibit denitrification as optimal rates occur between pH 7.0 to 8.0, and pH decreases cause decreases in rates (Metcalf & Eddy, Inc., 2003). Denitrification rates increase with increased temperature until a temperature of 35˚C is obtained. For conditions below 5˚C, denitrification rates are limited (Wiesmann, Su Choi, & Dombrowski, 2007). Environmental factors that play a role in constructed wetland denitrification are discussed in Section 2.4.1.2.

A common process used for biological nitrogen removal in municipal wastewater treatment is the Modified Ludzk-Ettinger (MLE) process, shown in the flow diagram in Figure 2.4.
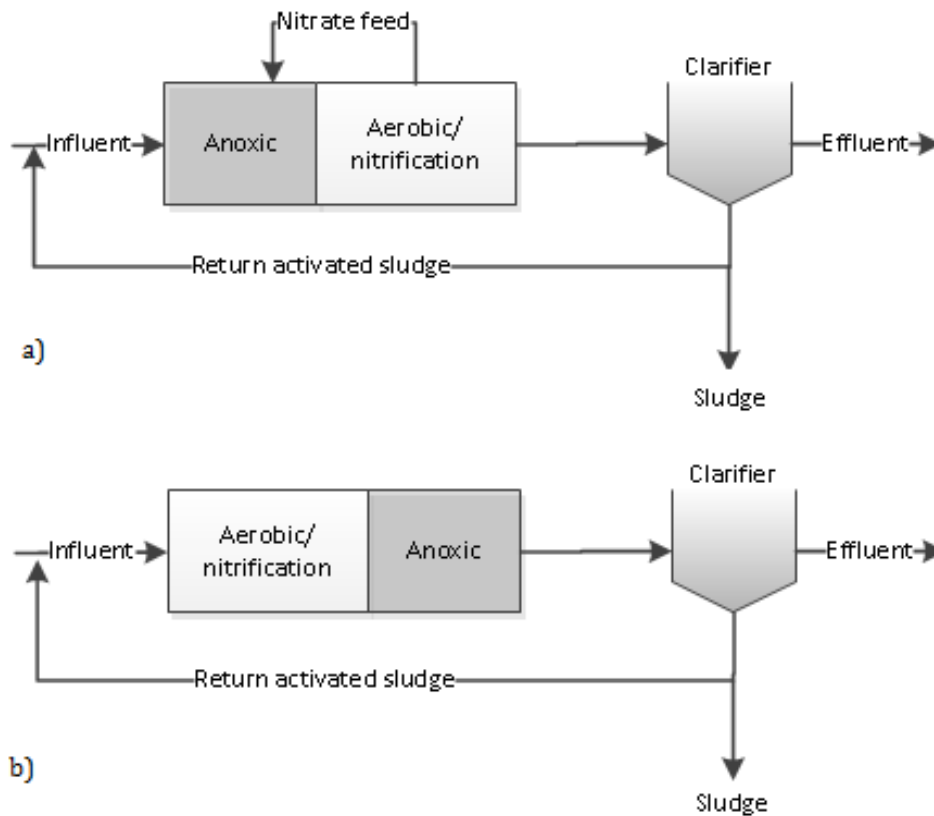
Figure 2.4: Reactors used for denitrification processes. (a) Preanoxic denitrification and (b) Postanoxic denitrification (Adopted from Metcalf & Eddy (2003)).

2.2 Wetlands

2.2.1 Overview

Wetlands are land on which water covers the soil, or is present either at or near the surface of the soil all year or for varying periods of time during the year, including the growing season. The recurrent or prolonged presence of water creates conditions that favor the growth of specially adapted plants and promotes the development of characteristic wetlands soils (EPA, 2012).

Wetlands generally include marshes, swamps, bogs, and fens. Various wetland types have distinct roles in watershed dynamics, nutrient cycling, and have different functionality relative to their position in the landscape and dominant water sources

(Turner & Gannon, 2003). Wetlands play a vital role in watershed hydrology and water quality, in addition to presenting a diverse biological habitat for vast species of plants, animals, birds, fish, insects, and microbial communities.

This thesis reports on nutrient removal by a constructed wetland system in the Pacific Northwest, United States (US), thus the background provided here will be limited to wetlands in the US. The following sections describe wetland ecosystems in the US, which have different functionalities, express different values, and are subject to different human and environmental challenges. Described are their characteristics, roles in watershed dynamics, and ecosystem status. Much research and engineering efforts have been focused on replicating these natural treatment systems, so understanding how wetlands carry out their processes in nature is fundamental to mimicking them in engineered systems. Constructed or engineered wetlands (CW) are designed to take advantage of the natural processes involving wetland vegetation, soils, and microbial processes to aid the treatment of wastewater in a controlled environment. Constructed wetlands are described later in this chapter.

2.2.2   *Functions and Values of Wetlands*

Wetland functions are the physical, chemical, and biological processes that occur in and constitute an ecosystem. Physical processes include movement of water through the wetland to other waterbodies, and organic/inorganic matter entering, leaving, or accumulating the wetland. Biological processes include the decay of organic matter and the uptake of nutrients for growth and development of wetland organisms. Chemical processes include nutrient and carbon cycling. The conversions of chemical constituents are often carried out by wetland organisms, and also occur as a function of other environmental factors throughout the wetland (Turner & Gannon, 2003).

Wetland values are distinct from wetland functions in that *values* are a result of the wetland *functions.* Wetlands are considered valuable because they improve water quality, recharge water supplies, reduce flood risks, and provide fish/wildlife habitat and biodiversity. Additionally, wetlands provide recreational opportunities, aesthetic

benefits, sites for research and education, and commercial fishery benefits (EPA, 2001).
Specific wetland functions and values are exemplified in Table 2.2. Difficulty in
quantifying the value of wetland ecosystems persists among public and private sectors,
and the organizations that work to protect wetlands. As a result, decision makers have
largely polluted, depleted, and destroyed wetland ecosystems.

Table 2.2: Examples of functions and values of wetlands. (Adopted from Turner &
Gannon (2003)).

Functions	Specific Examples of Functions	Examples of Values
Hydrology	Aquifer recharge/discharge, water storage & regulation, climate control	Water quality/quantity, flood control
Biogeochemical cycling & storage	Nutrient source/sink, nutrient transformation, sediment & organic matter sink	Water quality, erosion control
Bioproductivity & decomposition	Net primary productivity, carbon storage/release, detritus output of wetland organisms, mineralization & release of N, C, P, & S	Food chain support, water quality, recreation, commercial products
Ecosystem Processes	Habitat for species, food chain support, maintenance of biotic diversity	Recreation/Aesthetics, commercial products, water quality/quantity

2.2.3 *Wetland Loss and Degradation*

As of 2004, wetlands comprised only 108 million acres of the 220 million acres that were
documented in the 18th century for the US (Copeland, 2011). Activities resulting in
wetland loss and degradation include: agriculture; commercial and residential

development; road construction; impoundment; resource extraction; industrial siting, processes, and waste; dredge disposal; silviculture; and mosquito control (EPA, 1994b; EPA, 1993a). The primary pollutants causing degradation are sediment, nutrients, pesticides, salinity, heavy metals, weeds, low dissolved oxygen, pH, and selenium (Turner & Gannon, 2003).

To combat the destruction of wetlands, the US Congress passed the Clean Water Act that contains a specific Section (404) dedicated to protecting wetlands (EPA, 1972). The US Army Corp of Engineers teamed with the EPA to encourage wetland mitigation and restoration. A trend of wetland creation and destruction can be seen in Figure 2.5.
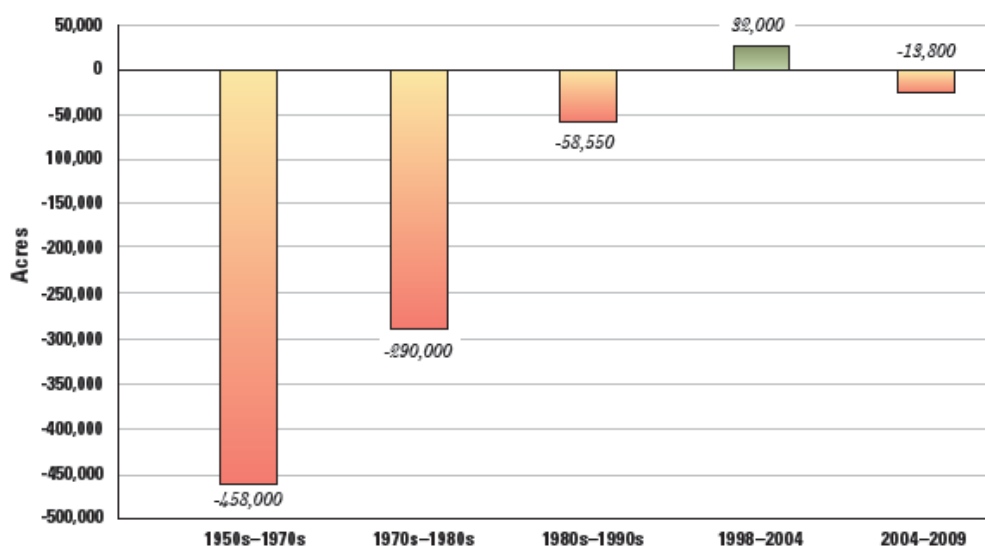
Figure 2.5: Net acres of wetland destroyed or created since 1950 in the US. Since the Clean Water Act of 1972, the combination of wetland creation and decreased wetland destruction has increased the total net acres of wetlands (USFWS).

2.2.4 Wetland Types

2.2.4.1 Overview

Wetlands are a critical entity within a watershed, and influence watershed hydrology and water quality. In 1979, a comprehensive classification system of wetlands and deepwater

habitats was developed by the U.S. Fish and Wildlife Service (Cowardin, Carter, Golet, & LaRoe, 1979). Under this system, wetlands are classified as two basic types: coastal (also known as tidal or estuarine wetlands) and inland (also known as non-tidal, freshwater, or palustrine wetlands) (Turner & Gannon, 2003). One useful way to categorize wetlands, for those interested in water quality management and watershed management, is by dominant water source. Wetlands may be precipitation-dominated, groundwater-dominated, or surface water-dominated wetlands, as specified in Table 2.3, and may have different functions as a result of their position in the landscape and their dominant water resource (Turner & Gannon, 2003).

Table 2.3: Watershed categories based on the dominant water resource (Turner & Gannon, 2003).

Ground Water	Surface Water	Precipitation
Fens	**Marshes** Tidal Freshwater Marshes Tidal Salt Marshes **Swamps** Riparian Forested Wetlands	**Marshes** Playas Vernal Pools Prairie Potholes Wet Meadows Wet Prairies **Bogs** Pocosins

2.2.4.2   Marsh Wetlands

Marshes are wetlands that are frequently or continually inundated with water, and characterized by soft-stemmed vegetation adapted to saturated soil conditions. Marshes can be freshwater or saltwater wetlands, and are typically divided into either tidal or

nontidal wetlands. Nontidal marshes are the most prevalent and widely distributed wetlands in North America, and include wet meadows, prairie potholes, vernal pools, and playa lakes. Tidal marshes (Figure 2.6) are found along protected coastlines in middle and high altitudes worldwide, and serve many important functions including buffering sea storms, slowing shoreline erosion, and absorbing excess nutrients before they reach estuaries and oceans (EPA, 2012).
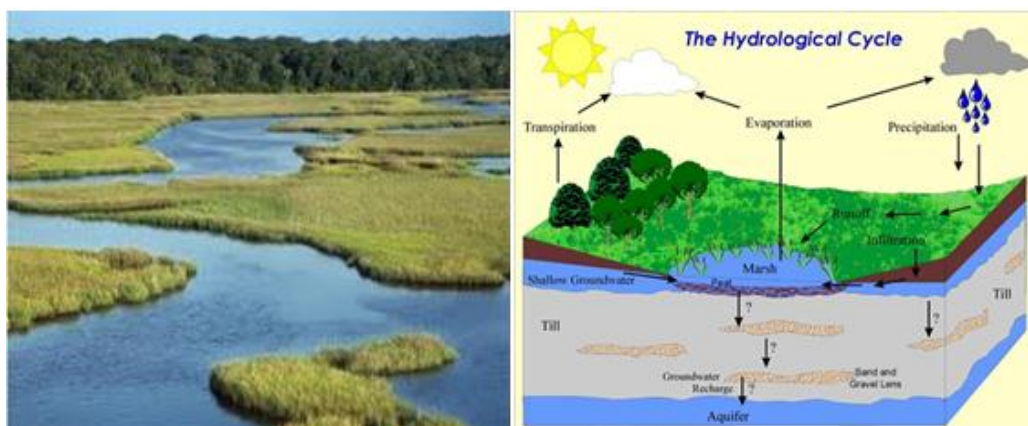
Figure 2.6: Marsh Wetlands. Left: Tidal marsh along the Edisto River, South Carolina (http://tidalmarshes1.weebly.com); Right: The hydrologic cycle adopted for a marsh area (http://www.eas.purdue.edu/geomorph/celerybog/hydrology.html).

Due to their high levels of nutrients, organics, and minerals, marshes are one of the most productive ecosystems on Earth. Marshes recharge groundwater supplies and moderate streamflow by providing water to streams, which is especially important in periods of drought. Marshes play an important role in preserving the quality of surface waters. Their presence in a watershed (Figure 2.6) helps to reduce flood damage by slowing and storing flood water. The microorganisms and marsh vegetation use excess nutrients for growth that can otherwise act to pollute surface waters (EPA, 2012).

2.2.4.3   Swamps

Swamps are dominated by woody plants and are classified by shrub or forested swamps, such as Bottomland Hardwoods and Mangrove Swamps (Figure 2.7). Both are found throughout the United States and often inundated with floodwater from nearby rivers and

streams, and are often found adjacent to one another. Swamps are characterized by saturated soils during the growing season, and standing water during certain times of the year. The highly organic soils provide a thick, black, nutrient-rich environment for the growth of water-tolerant trees. Swamps are effective in nutrient removal and flood protection; and due to their rich deposits of alluvial solids from floods, they are especially high in species diversity and productivity (EPA, 2012).

Figure 2.7: Swamp Wetlands**.** Left: Bottomland Hardwoods forests improve water quality by filtering and flushing nutrients and reducing sediment before it reaches open water (http://www.epa.gov/owow/msbasin/photopops/gulf7_pop.html). Right: Mangroves are salt-tolerant evergreen forests that are found along coastlines, lagoons, rivers or deltas in 124 tropical and subtropical countries (http://www.guardian.co.uk/environment/2008/feb/01/endangeredhabitats.conservation).

2.2.4.4   Bogs

Bogs are inundated peat deposits that have acidic waters, a floor covered by a thick carpet of sphagnum moss, and do not generally have significant influent or effluent flows. Distinctive from most other wetlands, water is received via precipitation instead of runoff, groundwater, or streams. Bogs lack the nutrients that typical wetland vegetation requires for growth, which is reinforced by acid forming peat mosses (EPA, 2012). The moss releases hydrogen ions and the peat releases organic acids – bog pH can be 3.0 – 4.0 (Mitsch & Gosselink, 1993). Bogs are formed as the moss grows over a lake or pond and slowly takes over; or as the moss carpet dries and prevents water from leaving. Many feet of acidic peat deposits accumulate over time as peat accumulation exceeds

decomposition as a result of environmental conditions. Because of the acidic, low-nutrient waters, plants and animals have made special adaptations to survive in bog environments. In the US, bogs are mostly found in the glaciated northeast and Great Lakes regions, and are called Northern Bogs (Figure 2.8). In the southeast, they are called Pocosins, which are efficient at filtering because they prevent rapid surface run-off (filters out sediments and nutrients before they enter larger water bodies). Additionally, their mucky soils act like sponges for flood control (USMC, 2002). Bogs prevent downstream flooding by absorbing precipitation, and regulate global climate by storing large amounts of carbon in peat deposits (EPA, 2012).

Figure 2.8: Bog Wetlands. Left: A carnivorous pitcher plant growing in a floating bed of sphagnum moss in the middle of Upper Leache's Pond, Borderland State Park, North Easton, Massachusetts. (http://losteaston.blogspot.com/2009/07/poquanticut-cedar-swamp-at-borderland.html). Right: The Venus Flytrap in a pocosin. (http://www.nhptv.org/wild/pocosins.asp).

2.2.4.5 Fens

Similar to bogs, fens are peatlands occurring mostly in the northern hemisphere, but are less acidic because they are fed by groundwater rather than precipitation. Additionally, the groundwater provides more nutrients from upslope sources through leaching and transport. However, a fen may become a bog if the peat becomes large enough to separate it from its groundwater supply, and deplete its nutrient levels (EPA, 2012).

2.3   Constructed Wetland Systems

In the 1950's, wetland applications were employed to remove various pollutants from water, and through the 70's and 80's constructed wetlands (CW) were used mostly to treat domestic or municipal sewage. Since the 1990's, CW have been used for a variety of wastewater treatments including landfill leachate, runoff (e.g. urban, highway, airport and agricultural), food processing (e.g. winery, cheese and milk production), industrial (e.g. chemicals, paper mill and oil refineries), agricultural, mine drainage or sludge dewatering effluent (Vymazal, Greenway, Tonderski, Brix, & Mander, 2006).

Constructed wetlands are designed to take advantage of the natural processes involving wetland vegetation, soils, and microbial processes to aid the treatment of wastewater in a controlled environment. The general classification is based on the type of macrophytic growth (emergent, submerged, free-floating and rooted with floating leaves). Further classification is usually based on the water flow regime (surface flow, subsurface vertical or horizontal flow). The processes responsible for the removal of specific constituents in wastewater differ in magnitude among systems. Various types of constructed wetlands may be combined (hybrid systems) in order to exploit the specific advantages of the individual systems (Vymazal, 2007). The efficiency of the CW design is also influenced by the effectiveness of various plant species, the colonization characteristics of groups of microorganisms, and how biogenic compounds and particular contaminants interact with the filter bed material (Stottmeister, 2003).

2.3.1   Constructed Wetland Configurations

Water moving through a wetland primarily experiences one of three types of flow, free water surface (FWS) flow, horizontal subsurface (HS), and vertical subsurface (VS) (Figure 2.9). FWS wetlands have numerous types of emerging or floating vegetation, areas of open water, and water that flows over soil and through vegetation.   In both horizontal and vertical subsurface flow wetlands, the vegetation is rooted in a highly porous soil matrix, usually gravel. For HS, influent water is evenly distributed vertically before making its way horizontally through the porous substrate. VS is the exact

opposite, distributing the influent water horizontally; followed by vertical percolation into a drainage pipe.
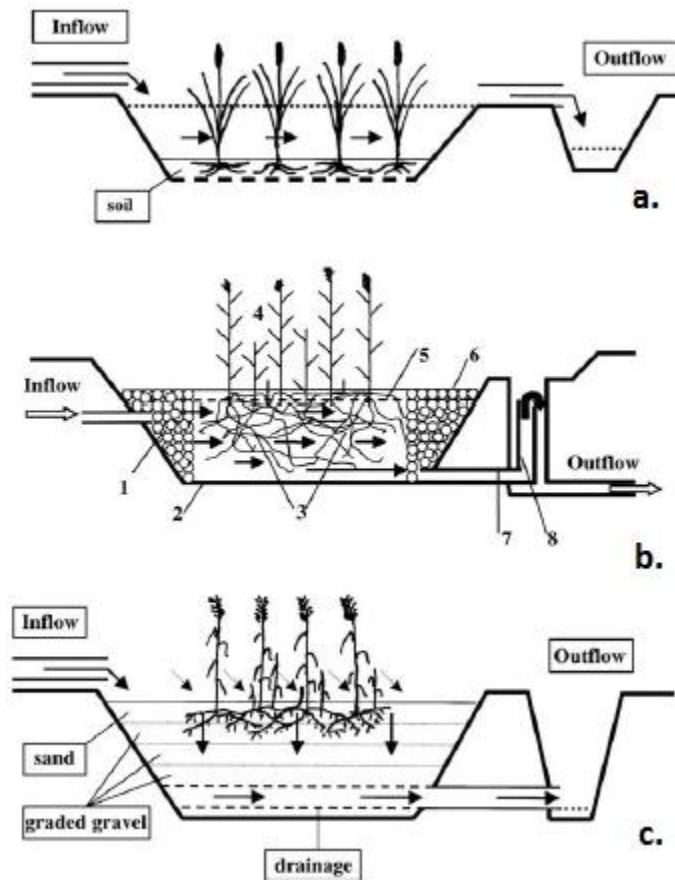
Figure 2.9: Constructed wetland configurations and flow regimes. a) Free water surface (FWS) wetland b) Horizontal subsurface (HS) wetland c) Vertical subsurface (VS) wetland. All figures include flow paths, vegetation, and other hydraulic structures (Vymazal, 2007).

Various CW configurations have been shown to remove a diverse array of constituents from polluted waters via phytoremediation and other natural mechanisms. HS wetlands provide high removal of organics and suspended solids but lower rates of nutrient removal. VS wetlands provide aerobic conditions that may aid the removal of ammonia, thus, are not ideal for the nitrate removal (Vymazal, 2007). For wastewater treatment, a combination of CW flow regimes and vegetation can be effective in reducing both BOD

and nutrients. Cheng et al., reported removal of heavy metals by aquatic plants in a CW comprising of VS, followed by a reverse-VS chamber (2002). A FWS CW was shown to be effective in the reduction of human pathogens such as total and fecal coliform by the predation of copepods (benthic wetland protozoa) (Song, Wu, Yang, Xu, & Wen, 2008).

2.4   Water Quality in Constructed Wetlands

Soil and plant interactions in a constructed wetland treatment system result in a greater variety of nutrient transformations than in conventional wastewater treatment systems. CWs offer a variety of environmental, economic and community benefits as an alternative to conventional wastewater treatment facilities. It is important to understand CW mechanisms and the environmental factors that affect these processes, in order to improve treatment efficiency.

2.4.1   Nitrogen Transformations in Constructed Wetlands

Nitrogen is mobilized and/or transformed via physical, chemical, and biological processes that are functions of environmental factors within the wetland ecosystem. Kadlec & Wallace outlined six physical processes that mobilize nitrogen in a wetland: (1) particle settling and resuspension, (2) diffusion of dissolved forms, (3) plant translocation, (4) litterfall, (5) ammonia volatilization, and (6) sorption of soluble nitrogen on solid substrates (2009).

The microbial processes that mediate chemical transformations of nitrogen into its various species include: (1) nitrification, (2) denitrification, (3) aerobic denitrification, (4) anaerobic ammonia oxidation (ANAMMOX), (4) ammonification, (5) nitrogen fixation, and (6) assimilation of nitrogen to plant biomass. The mechanisms that will ultimately remove nitrogen from wastewater in CW are ammonia volatilization, denitrification, plant uptake (with biomass harvesting), ammonia adsorption, ANAMMOX and organic nitrogen burial (Vymazal, 2007). These processes are summarized in Table 2.4, and discussed in detail in the following section.

Table 2.4: Nitrogen transformations in constructed wetlands

Process	Transformation
Nitrification	Ammonia-N $\rightarrow$ Nitrite-N $\rightarrow$ Nitrate-N
Denitrification	Nitrate-N $\rightarrow$ Nitrite-N $\rightarrow$ N_2O, Gaseous N_2
ANAMMOX	Ammonia-N, Nitrite-N $\rightarrow$ Gaseous N_2
N_2 Fixation	Gaseous N_2 $\rightarrow$ Ammonia-N (Organic-N)
Ammonia Volatilization	Ammonia-N (aq) $\rightarrow$ Ammonia-N (g)
Ammonification	Organic-N $\rightarrow$ Ammonia-N
Nitrate-ammonification	Nitrate-N $\rightarrow$ Ammonia-N
Plant/microbial uptake (assimilation)	Ammonia, Nitrite, Nitrate-N $\rightarrow$ Organic-N $\rightarrow$ Biomass
Ammonia adsorption	Ammonia-N exchange with cation from soils
Organic nitrogen burial	Organic-N gets buried and unavailable for nutrient cycling

2.4.1.1   Nitrification in Constructed Wetlands

Nitrification is a two-step process in which ammonia is biologically oxidized into nitrite; and then nitrite is oxidized to nitrate. The mechanisms and stoichiometry of nitrification have been previously discussed (Section 2.1.2.1). Wetland environments are inherently more complex than conventional wastewater treatment systems, but are influenced by common factors affecting nitrification including temperature, pH, alkalinity, inorganic carbon source, microbial population, and concentrations of ammonium and DO. However, one notable difference between conventional and natural systems is the complexity of microbial communities in soils. The previously discussed microbially mediated two-step nitrification process, which is generally used to model conventional WWTP systems (Equation (2.3)), represents the conversion of ammonium to nitrite via

between pH 6.0 – 8.0 (Paul & Clark, 1996), and denitrification slows as pH decreases; but is still significant at pH 5.0, and is negligible or absent below pH 4.0 (Vymazal, 2007). Maximum denitrification rates occur around 60 to 75˚C, but decline rapidly above this temperature ((Paul & Clark, 1996). Denitrification proceeds very slowly at temperatures below 5˚C but is still measureable (Bremner & Shaw, 1958).

A Vyzmazal review paper states that nitrogen removal rates in various constructed wetlands vary widely between 0.006 and 2.13 mg-N $L^{-1} D^{-1}$, assuming an average wetland depth of 0.48 m (2007). The 50th percentile nitrate removal rate for free water surface CWs complied by Kadlec and Wallace was 0.28 mg-N $L^{-1} D^{-1}$ or 26.5 m yr^{-1}. The horizontal subsurface CW 50th percentile rate was 0.23 mg-N $L^{-1} D^{-1}$ or 41.8 m yr^{-1} (Kadlec & Wallace, 2009)

Wastewater alone may not have sufficient carbon available to promote denitrification in treatment wetlands that receive wastewaters with high nitrate loads, and thus, an external carbon source may be required.   A growing body of research has identified possible carbon source additions to facilitate nitrogen removal in wetlands. Several studies added plant biomass to supplement carbon requirements (Gersberg, Elkins, & Goldman, 1983) (Burchell, Skaggs, Broome, & Lee, 2002). At a wastewater application rate of 16.8 cm day^{-1}, Gersburg et al. (1983) reported 95% and 60% removal of total nitrogen when supplementing methanol and biomass, respectively, compared to 25% removal without a supplemental carbon source.

Denitrification occurs in the absence of dissolved oxygen (DO), and microorganisms will consume oxygen over nitrate as the electron acceptor. However, due to low surface aeration, a subsurface wetland usually has an average DO concentration < 0.1 mg L^{-1} providing an excellent environment for denitrification. On the other hand, a FWS wetland has DO levels that vary between saturation and 0 mg L^{-1} depending on water depth, temperature, velocity, and vegetation density and type (EPA, 2000)

In addition to respiratory denitrification, various other pathways have been identified for N_2 production including aerobic denitrification, microbial denitrification using sulfur (S),

and iron (Fe) as electron donors (as opposed to carbon), acid-catalyzed destruction of NO_2^- (chemo-denitrification), and ANAMMOX (Seitzinger, et al., 2006).

Aerobic denitrification involves the microbial use of both oxygen and nitrate as oxidizing agents to reduce nitrate to N_2 or N_2O. Aerobic denitrification is often coupled to heterotrophic nitrification and carried out by a single organism (Kadlec & Wallace, 2009).

Sulfur-driven denitrification involves the biological reduction of nitrate to nitrogen gas while oxidizing elemental sulfur (S), or reduced sulfur compounds, including sulfide (S^{2-}), thiosulfate ($S_2O_3^{2-}$), and sulfite (SO_3^{2-}) (Kadlec & Wallace, 2009). *Thiobacillus denitrificans* is an autotrophic microorganism capable of this process, and the end products are usually nitrogen gas and sulfate (Batchelor & Lawrence, 1978).

$$NO_3^- + 1.1S + 0.40CO_2 + 0.76H_2O + 0.08NH_4^+ \rightarrow$$
$$0.5N_2 + 0.08C_5H_7O_2N + 1.1SO_4^{2-} + 1.2H^+ \tag{2.6}$$
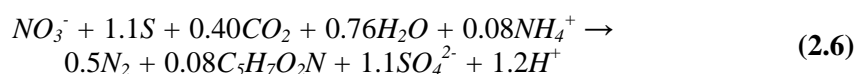
Microorganisms have been reported to also use reduced iron as an electron donor (Pauwels & Talbo, 2004).

2.4.1.3 Anaerobic Ammonia Oxidation (ANAMMOX) in Constructed Wetlands

ANAMMOX (anaerobic ammonium oxidation) is the production of nitrogen gas from the anaerobic oxidation of ammonium (Mulder, van de Graff, Robertson, & Kuenen, 1995). Nitrite is used in the ANAMMOX process as an electron acceptor. Redox balance calculations showed the following stoichiometry:

$$NH_4^+ + NO_2^- \xrightarrow[\text{eturopha}]{\substack{Planctomycetes \\ Nitrosomonas}} N_2 + 2H_2O \tag{2.7}$$
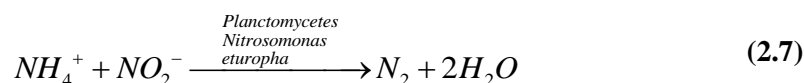
During further examination of this process, indications were obtained that nitrate could also serve as a suitable electron acceptor for the ANAMMOX process (van de Graff, Mulder, de Bruijn, Jetten, L, & Kuenen, 1995).

2.4.1.4   Ammonification in Constructed Wetlands

Ammonification is the biological transformation of organic nitrogen to ammonia. This removal process proceeds more rapidly than nitrification, which may increase ammonia concentrations along the flow path of the wetlands. The ammonification rate doubles with a $10°C$ temperature increase, with optimum temperatures ranging from $40°$ to $60°C$ (Kadlec & Knight, 1996). Ammonification is not considered a major nitrogen transformation in the wetlands system if the system is not expected to encounter this range of temperature.

2.4.1.5   Volatilization in Constructed Wetlands

Kadlec & Knight suggested that ammonia volatilization typically has limited importance in wetlands, except in specific cases where ammonia is present at concentrations greater than 20 mg-N L^{-1} (1996).

2.4.1.6   Plant Uptake (Assimilation) in Constructed Wetlands

Nitrogen assimilation occurs through a variety of biological processes that convert inorganic nitrogen (usually ammonia and nitrate) to organic compounds that serve as building blocks for cells and tissues. Although ammonia is reduced more often than nitrate, nitrate-rich waters may be an important source of plant nutrient uptake (Vymazal, 2007).

Vegetation in the wetlands can play a significant role in nitrogen removal by assimilation of nitrogen into biomass, and providing an environment in the root zone for nitrification-denitrification (NH_4 to NO_3 to N_2). Nutrients are assimilated from the sediments by emergent and rooted floating-leaved macrophytes. Plants derive most of their nitrogen from soil pore-water and a small amount from floodwater. Nitrogen removal via assimilation can be measured from plant biomass amounts and

tissue nitrogen, which vary from plant type (Vymazal, 1995). Nitrate loss in treatment wetlands can result from other mechanisms than denitrification, including assimilation of nitrate by wetland vegetation and microbes, and dissimilation of nitrate to ammonium.

The amount of plant decay in a steady state wetland will equal the amount of plant growth. Cattails can grow at an average rate of 40,000 kg ha^{-1} yr^{-1}. Plants are generally 40% carbon; therefore, 40% of the cattail growth can be considered carbon growth or carbon added to the system (Kadlec & Knight, 1996). Lin et al. planted several microcosm wetlands with various macrophytes. Harvesting results indicated that 4-11% of nitrogen removed by the planted wetland was due to vegetation uptake, and 89-96% was due to denitrification (2008).

2.4.2   Other Water Quality Factors in Constructed Wetlands

2.4.2.1   Temperature

For all bodies of water, temperature can play a vital role in the development of an ecosystem. Two major fish species, salmon and trout, need water temperatures as low as 13.0 ˚C in order to spawn (ODEQ, 2006). In response to high waterbody temperatures and the protection of ecosystems, state and federal environmental regulators have developed Total Maximum Daily Loads (TMDL) for certain river basins throughout the Pacific Northwest. One implemented treatment method to reduce waterbody temperature is the constructed wetland. Kadlec and Wallace gathered data from various constructed wetlands and concluded that wetland effluent temperature should equal the daily mean air temperature given a residence time greater than two days (2009). The constructed wetland in Tres Rios, Arizona had an average influent temperature of 30 ˚C. At a residence time greater than 2.5 days, the average effluent temperature was 5˚C lower at 25 ˚C - the daily average air temperature (2009). The following is a list of mechanisms that affect the temperature of any waterbody:

- Shading - vegetation provides shade keeping radiation from exciting water particles
- Transpiration - the vegetation reduces temperature through uptake and transpiration of water

- Conduction - when warm water makes contact with cool soil, heat is transferred to the soil.

- Convection - air flowing over the water could potentially remove heat if water temperature and vapor pressure are greater than air temperature and vapor pressure

- Evaporation – similar to convection, except evaporation is only vapor pressure dependent, not temperature dependent

- Long Wave Radiation - when the sun is down, water loses heat by radiating low energy waves

All of the above mechanisms are considered when designing a constructed wetland to achieve temperature reduction goals.

2.4.2.2 Solids

The treatment techniques for suspended solids differ in FWS and subsurface wetlands. FWS wetlands have an open water column that slows water velocity and allows for particle settling. A FWS constructed wetland in Arcata, CA, has achieved 90% suspended solids removal over a six year span. With a six day hydraulic residence time, an Arcata pilot cell showed 70% suspended solids removal at 0.75 days, and an overall suspended solids removal of 90%; indicating that a majority of removal occurred during the first day (EPA, 1999). Resuspension of the settled solids is minimal due to low water velocities (EPA, 2000). The porous substrate of subsurface wetlands acts as a filter removing particles from suspension. Vymazal compiled data for horizontal subsurface (HS) wetlands from fifteen different countries, and found the average suspended solids removal efficiency to be 83% (2001). However, high influent solids can lead to negative hydraulic effects by potentially clogging the porous substrate. After one year of operation, Robertson and Merkley observed the inlet of a HS wetland accumulated 20 cm of silt on top of the porous substrate (gravel). Removal of the silt layer doubled the hydraulic flowrate (Robertson & Merkley, 2009).

2.4.2.3   Metals

All vegetation needs specific metals, known as micronutrients (zinc, iron, manganese, copper, nickel, and molybdenum) for continual growth (Merchant, 2010). Plant uptake of metals decreases the aqueous metal concentration, consequently making plant uptake a water treatment method (metals are not completely removed from the system unless the plant is harvested). An Argentinean FWS wetland receiving wastewater from a metallurgic factory achieved removal of 95% iron, 86% chromium, and 67% nickel (Maine, Suñe, Hadad, Sánchez, & Bonetto, 2006).   However, plant uptake is not the primary metal removal mechanism. Ion exchange with soil and precipitation as solids provide the majority of metal removal. In a study examining an acid mine drainage constructed wetland, 79% of the manganese was removed where plant uptake accounted for only 2% of the removal (Mays & Edwards, 2001). Wastewater with too much metal contamination can lead to toxicity in the wetland plant species. Water hyacinth (a floating wetland species), exposed for 24 hours to cadmium concentrated waters at 1.0, 5.0, and 10.0 mg L^{-1}, exhibited physiological and genetic damage. All cadmium concentrations less than 1.0 mg L^{-1} had no effect on water hyacinth growth (Rosas, Carbajal, Gómez-Arroyo, Belmont, & Villalobos-Pietrini, 1984).

2.4.2.4   BOD

In typical municipal wastewater treatment plants, BOD is removed by solids removal or by taking advantage of the natural biological activity found in wastewaters. The primary constituent of BOD, organic carbon, is converted into carbon dioxide (CO_2) through aerobic respiration**.**

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \tag{2.8}$$
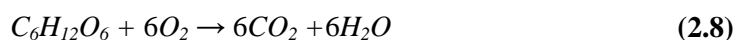
All microbial reactions require compounds willing to donate electrons, compounds accepting electrons, and microbes. The chemical reaction associated with aerobic respiration of glucose requires six moles of the electron acceptor (dissolved oxygen) for every mole of glucose (common organic carbon & electron donor). Typical municipal wastewaters have BOD concentrations high enough to deplete naturally occurring

dissolved oxygen, creating the need to add oxygen back to the wastewater to achieve BOD effluent goals (Metcalf & Eddy, Inc., 2003).

BOD removal in constructed wetlands differs from FWS to HS CWs as FWS had a 50th percentile removal rate of 33 m yr^{-1} for tertiary treatment while HS had 86 m yr^{-1} (Kadlec & Wallace, 2009).

2.4.3   Constructed Wetlands Summary

Constructed wetlands can be engineered to treat of a diverse range of wastewater types and pollutants. The incorporation of soil and plants adds a greater diversity of biological processes to passively treat wastewater over conventional systems.

2.5   Permeable Reactive Barriers

Agricultural runoff is a wastewater that usually contains high nitrate and low organic carbon concentrations. The following section will describe how agricultural runoff is produced, and how permeable reactive barriers are used to passively treat the wastewater. Fertilizer is a soil additive that contains specific nutrients (including nitrate) for crop growth. Depending on the need of the crop, different fertilizer formulas or types of fertilizers (commercial fertilizer, animal manure, wastewater, wastewater sludge, etc) are applied. Nutrients that are not consumed by the crop or mycorrihizal communities are leached into the groundwater as a non-point source discharge. In many fields across the US, drainage systems have been incorporated to increase water table depth, and turn non-point source discharge into point source. A typical drainage system encompasses perforated pipe, buried at the targeted water table depth, to transport excess irrigation/groundwater to surface waters or larger pipes in a network of drainage systems. Figure 2.10 depicts a typical drainage system.
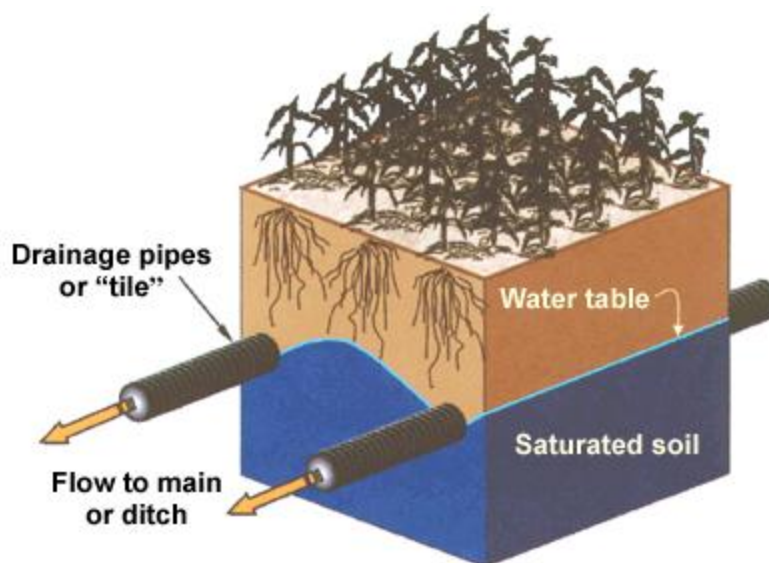
Figure 2.10: An agricultural drainage system. Used to increase water table depth and turn non-point source water discharge into point source.
http://www.extension.umn.edu/distribution/cropsystems/dc7740.html

Collected drainage water or agricultural runoff is usually a high nitrate and low organic carbon wastewater. Many experiments have been conducted to reduce the nitrate concentration of drainage system wastewaters such as wetlands (Hyberg, 2007), irrigation of cover crops, and improved fertilizer management (Saleh, Osei, Jaynes, Du, & Arnold, 2007), etc.). One passive treatment method is the permeable reactive barriers (PRB). Similar to a subsurface CW, the water passes through a porous substrate; but instead of non-reactive gravel, PRBs use a porous substrate that can provide further treatment benefits. Woodchips are a common material used for PRBs, as they have a porous and firm structure, and leach the organic carbon needed to stimulate denitrification and nitrate removal.

PRBs are typically 100-120 feet long and 10-25 feet wide, as documented in most Iowa installations and pictured in Figure 2.11. That size of PRB usually treats 30-80 acres of agricultural runoff for Iowa fields (Christianson & Helmers, 2011). Typical PRBs are installed upstream of surface waters, and downstream of the main drainage line (Figure 2.11).
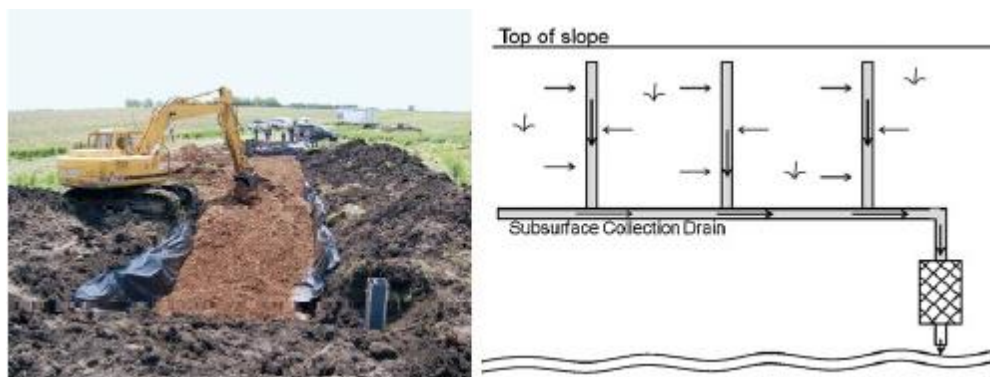
Figure 2.11: Construction of permeable reactive barriers. Left – Construction of a field installed woodchip PRB. Right – Diagram of PRB placement within a drainage system. (Christianson & Helmers, Woodchip Bioreactors for Nitrate in Agricultural Drainage, 2011) & (Schipper, Robertson, Gold, Jaynes, & Cameron, 2010)

Studies have shown that woodchip PRBs:

- Have steady nitrate removal rates - After 15 years of operation at a 10 day residence time, a nitrate removal rate of 4.6 mg-N L^{-1} D^{-1} was shown in the field (Robertson, Vogan, & Lombardo, 2008). A compilation of published woodchip PRBs, in the field, produced a nitrate removal rate range between 2-22 mg-N L^{-1} D^{-1}. The variety of rates is primarily dependent on temperature and influent nitrate concentrations (Schipper, Robertson, Gold, Jaynes, & Cameron, 2010).

- Have a long lifetime – An estimated half life of 36.6 years was calculated for the anaerobic sections of the woodchip PRB at an average hydraulic residence time of 50 days. This PRB was a 0.6 m by 1.83 m wall that was placed upstream of a drainage pipe and spanned the length of the pipe. (Moorman, Parkin, Kaspar, & Jaynes, 2010), (Jaynes, Kaspar, Moorman, & Parkin, 2008).

- Have a high hydraulic conductivity – A course woodchip PRB had a hydraulic conductivity of 3 cm s^{-1}, which was 200 times greater than the aquifer sediments from the site (Robertson, Yeung, vanDriel, & Lombardo, 2005).

- At steady state, does not leach significant amounts of excess carbon – During startup, a woodchip PRB had an average effluent BOD concentration of 100 mg L^{-1}. After one month, the effluent BOD was 11 mg L^{-1}. The PRB was a standard 55 gallon drum, had a 24 hour residence time, and removed nitrate at a rate of 4.4 mg-N L^{-1} D^{-1} (Cameron & Schipper, 2010).

- Cost between \$2.39 and \$15.17 for every kg of nitrate removed (Schipper, Robertson, Gold, Jaynes, & Cameron, 2010) (costs are based off the PRB presented in Roberston et al (2009)).
- Deplete dissolved oxygen concentrations in DO saturated water within one hour. The DO depletion experiment was conducted in a column, and suggests a residence time longer than one hour is needed to start the denitrification process (Robertson W. , 2010).

Further studies have looked into PRB substrates and amendments other than woodchips. Cameron and Schipper tested maize cob (corn) in a column, and found that it had a 6.5 times higher nitrate removal rate than woodchips (2010). However, the maize cob had a greater decline in hydraulic conductivity than the woodchips over the 24 month period. Rice husks were also examined using a 6.8 hour residence time column. A nitrate removal rate of 84.5 mg-N L^{-1} D^{-1} was produced with an influent nitrate concentration of 25 mg-N L^{-1} (Shao, Xu, Jin, & Yin, 2009). However, one study found that rapid carbon depletion produced ineffective nitrate removal rates in humus (Stewart, Carlile, & Cassel, 1979). Therefore, the use of highly labile carbon might require frequent replenishment for long term nitrate removal. Field scale and long term studies have not been conducted to verify labile carbon substrate use in field installations.

2.6   Biochar

Biochar is a unique material that has been primarily studied as a soil additive for plant growth. The nutrient provision and microbial accumulation properties suggest that biochar could also aid in the denitrification process as a carbon substrate. According to Lehmann and Joseph, biochar is a carbon rich product obtained by thermally degrading organic materials. A temperature usually ranging between 300 to 700 ˚C and an oxygen free environment must be achieved to create biochar (Lehmann & Joseph, 2009). Hydrogen, oxygen, and some carbon are volatilized, leaving behind a carbon dense solid containing many poly-aromatic hydrocarbons (Schimdt, 2000). The process of creating biochar is called pyrolysis, which is regularly used for energy generation. When biomass is degraded, the heat is used to create steam; the steam powers a turbine for electricity.

Syngas and bio-oils could also be produced in pyrolysis, and used for energy purposes. Biochar is a byproduct of the pyrolysis process.

The physical and chemical properties of biochar depend on the feedstock source and process temperature. The following properties of biochar illustrate that variability:

- Alters nutrient availability by increasing or decreasing quantities of nitrogen, phosphorus, and metals in soil.
    - Increases or decreases cation exchange capacity (CEC) of soil – Soil CEC increased 0.017 meq g-soil^{-1} with a hardwood biochar addition. A 0.002 meq g-soil^{-1} decrease in soil CEC was seen for Conifer (softwood) biochar addition (Tryon, 1948).
    - Biochar itself contains nutrients – Douglas Fir (softwood) biochar leached 0.25 mg NO_3^--N L^{-1} (DeLuca, MacKenzie, Gundale, & Holben, 2006). A 15% hardwood biochar mixture (the other 85% was soil) increased P_2O_5 soil concentrations from 16.0 to 52.7 ppm (Tryon, 1948). The micronutrient metals K, Ca, and Mg increased in soil concentration by 230.2, 2.3, and 0.9 mmol kg-soil^{-1} respectively for a 20% biochar mixture (the other 80% was soil) (Lehmann, Pereira da Silva, Steiner, Nehls, Zech, & Glaser, 2003).
    - Sorption – Total nitrogen and total phosphorous leaching was reduced by 11% and 69% respectively with a biochar addition (Laird, Fleming, Wang, Horton, & Karlen, 2010). Aluminum (a metal, not micronutrient) decreased in soil concentration by 1.9 mmol kg-soil^{-1} in a 20% biochar mixture (Lehmann, Pereira da Silva, Steiner, Nehls, Zech, & Glaser, 2003).
- Sorption of carbon – Humus biochar & crowberry biochar absorbed 26% and 42% of soil dissolved organic carbon, respectively (Pietikäinen, Kikkilä, & Fritze, 2000). Biochar is considered carbon neutral because its production creates CO_2, but it can also absorb CO_2. Out of 16 biochar variations, 14 absorbed CO_2 at rates ranging from 5.5 to 4475 ug CO_2 g-biochar^{-1} D^{-1} (Spokas & Reicosky, 2009).

- Changes soil pH – Soils with biochar showed a 0.70 pH increase over unamended soils (Tryon, 1948).
- Provides habitat for microbes
    - Biochar pore diameters can be less than 16 μm translating into high surface areas for microbial attachment (Glaser, 2007).
    - Increases in biomass – Microbial populations in humus biochar were 2.9 to 3.4 times higher than in humus alone (Pietikäinen, Kikkilä, & Fritze, 2000).
    - Stores excess microbial signaling compounds – Symbiotic relationships exist between the different soil microbes and plant roots in the rhizosphere. Signaling compounds, such as flavnoids, are used for communication between parties in symbiotic relationships; the signal can be inhibitory or stimulatory (Angelini, Castro, & Fabra, 2003). A similar product to biochar, activated carbon, has been shown to absorb and desorb signaling compounds (Akiyama, Matsuzaki, & Hayashi, 2005). Biochar could absorb signaling compounds not immediately used by the microbes, and release the compounds at a later time. During this later time, the microbes could uptake the signals as needed (Warnock, Lehmann, Kuyper, & Rillig, 2007).
    - Removes aqueous phase toxic compounds though adsorption – Aluminum has been shown to be toxic (Piña & Cervantes, 1996), however, it can be absorbed by biochar (above). The reduction of aqueous phase toxins can lead to increased microbial colonization (Vaario, Tanaka, Ide, Gill, & Suzuki, 1999).

Incorporating biochar into a woodchip PRB could provide many benefits. Any nutrients for biological growth not provided in significant concentrations by the wastewater, plants, soil, or woodchips could be leached from biochar. With greater nutrient concentrations, the probability of nutrients limiting denitrification rates decreases.

The increased microbial populations seen in biochar could increase the overall PRB microbial population. A higher denitrification rate could occur with a larger population

of microbes. However, an unpublished study by Christianson et al. discusses the influence of biochar on woodchip PRBs, and showed no significant difference in nitrate removal rates between a woodchip PRB and a biochar/woodchip PRB mix (2011). The study was conducted with four columns; 1) woodchip only, 2) 380˚C biochar mixed with woodchips at 14% by mass, 3) 550˚C biochar mixed with woodchips at 14% by mass, 4) 380˚C biochar mixed with woodchips at 7% by mass (temperatures were the pyrolysis process temperature). Five different residence times were tested, ranging from 4.1 to 14.4 hours, and the study lasted a total of 60 pore volumes. As the residence times changed, the nitrate removal rates of all four columns increased/decreased simultaneously. Overall, the study concluded that biochar had no effect on nitrate removal rates, and greater residence times increase nitrate removal rates (Christianson, Hedley, Camps, Free, & Saggar, 2011). More experiments on the effect of biochar in woodchip reactors need to be performed to determine if amendments benefit nitrate removal.

2.7 Permeable Reactive Barriers in Wetlands

Incorporating permeable reactive barriers within constructed wetlands is an idea that has been briefly explored. Leverenz et al., constructed pilot scale subsurface flow wetlands using woodchips and gravel as the porous substrate (2010). Nitrate removal rates were examined for woodchips and gravel with planted and unplanted conditions.

A 0.74 g-N m^{-2} D^{-1} nitrate removal rate was measured for the planted gravel wetland. This rate is comparable to most subsurface wetlands, like the pilot scale subsurface wetland (planted) seen in Lin et al – 1.16 g-N m^{-2} D^{-1} (2008). Schipper et al. noted that woodchip permeable reactive barriers produced nitrate removal rates between 2-22 mg-N L^{-1} D^{-1} (2010). The woodchip subsurface wetland in Leverenz et al., generated a nitrate removal rate of 8.87 mg-N L^{-1} D^{-1}, which fits within the Schipper et al. range. Having the subsurface wetland planted improved nitrate removal interdependent of porous substrate type. Plants also prevented structural woodchip settling, buffered removal rates against low air temperatures, and were a visual indicator of nitrate removal by plant health. The negative effect of plants was a decrease in woodchip wetland hydraulic conductivity to 0.15 m s^{-1} from 0.54 m s^{-1} (Leverenz, Haunschild, Hopes, Tchobanoglous, & Darby,

2010). Two year average nitrate removal rates for both the woodchip and gravel
wetlands are shown in Table 2.5.

Table 2.5: Nitrate removal rates of woodchip incorporated subsurface wetlands.
(Leverenz, Haunschild, Hopes, Tchobanoglous, & Darby, 2010).

		Nitrate Removal Rate (g-N m^{-2} D^{-1})	Nitrate Removal Rate Coefficient (D^{-1})
Woodchip Substrate	Planted	5.90	1.41
	Unplanted		1.30
Gravel Substrate	Planted	0.74	-
	Unplanted	0.00	-

CHAPTER 3: MATERIALS & METHODS

3.1   Growth of Denitrifying Culture

A denitrifying culture was established for experimental evaluation of biological nitrogen removal. The culture was enriched from soil samples collected from the top layer of sediment in the center of the Railroad wetland cell at Talking Water Gardens (TWG). In duplicate Wheaton 250 mL bottles, 20 g of soil and 250 mL of ATI wastewater (ATIWW) were emplaced to grow the culture in batch. Each bottle was amended with a 30 g L^{-1} KNO_3 solution to bring the final NO_3^- concentration to 100 mg-N L^{-1}. Three sets of the duplicates were operated to evaluate the response to glucose, methanol, and glycerin as potential carbon substrates. Carbon substrates were added to reach a final chemical oxygen demand (COD) of 1000 mg L^{-1}, and rubber septa stoppers were used to seal all bottles from the atmosphere eliminating oxygen from entering the system. A 10 minute N_2 gas purge was applied to all bottles to remove any oxygen from the closed systems, and the bottles were incubated at 20˚ C. Anions (including nitrate) and COD were measured at regular time intervals as described in Section 3.7.

The culture fed glucose was sustained throughout the entirety of the study and was used as an inoculum for subsequent experiments. Weekly COD and nitrate measurements were taken, and if the COD was below 50 mg L^{-1}, glucose would be added to reach a concentration of 1000 mg L^{-1}. If nitrate was below 25 mg-N L^{-1}, KNO_3 was added to reach a final nitrate concentration of 300 mg-N L^{-1}.

3.2   Carbon Substrate Selection

Four different carbon substrates were selected based on local availability, cost, and physical and chemical properties. Alder woodchips were obtained from a local timber company to possibly provide a slow leaching organic carbon and a firm porous structure for microbial growth. Permeable reactive barriers, for irrigation runoff, commonly use woodchips to remove nitrate (Schipper, Robertson, Gold, Jaynes, & Cameron, 2010). Two different types of biochar were also acquired from the same timber company;

manure and woodchip biochar. Alder woodchips were pyrolyzed, in the absence of O_2 gas, at 500°C to create the woodchip biochar. Anaerobically digested cow manure pellets were pyrolyzed at 350°C, and were mixed with those pyrolyzed at 600°C to make up the manure biochar. Biochar was selected because reports have shown that biochar increases microbial populations in humus, and provides nutrients necessary for microbial growth (Pietikäinen, Kikkilä, & Fritze, 2000) (Tryon, 1948). Corn silage was donated by a local farm and food processor to provide a quick leaching organic carbon for increased nitrate removal rates (Greenan, Moorman, Kaspar, Parkins, & Jaynes, 2006). Figure 3.1 depicts the carbon substrates.

Figure 3.1: Examples of carbon substrates selected for the experiment. a. Corn silage. b. woodchips. c. manure biochar. d. woodchip biochar

3.3   Batch Experiments

3.3.1   Leaching

A leaching test was conducted to evaluate the rate of COD leached from the various carbon substrates. Nine 250 mL Wheaton bottles were prepared with a total volume of 250 mL (2:1(v/v) DI water to carbon substrate ratio), and were purged with N_2 gas for 30 minutes to remove dissolved oxygen from the system. The bottles were incubated at 20°C on a 100 rpm shaker, and were measured daily for COD concentration. The experiment ended when a pseudo-steady state COD concentration was obtained. Organic

acids, anions, COD, pH, and nitrogen species were measured at the end of the experiment. All analytical techniques for the mentioned parameters are described in Section 3.7.

Leaching rate coefficients were evaluated using a first order, equilibrium based, equation. The rate coefficient at which organic carbon leaches is dependent on the difference between the current COD concentration and the equilibrium COD concentration.

$$\frac{dC}{dt} = k(C_e - C) \tag{3.1}$$

If the daily COD concentrations and time are graphed in the following form the leaching rate coefficient can be calculated as the slope of the best fit linear trend line.

$$ln\frac{C_e - C}{C_e} = kt \tag{3.2}$$

C_e – COD Equilibrium Concentration (mg L^{-1})
C – Current COD Concentration (mg L^{-1})
k – Leaching Rate Coefficient (days^{-1})
t – Time (days)

3.3.2 Carbon Substrate Adsorption

Biochar have been known to either absorb or leach carbon and nutrients such as nitrogen and phosphorus containing compounds (Tryon, 1948). Batch experiments were conducted to determine the ability of the biochar to absorb the leachate produced from the woodchips. Triplicate 250 mL Wheaton bottles sets containing a 2:1 (v/v) woodchip leachate to biochar ratio (150 mL total volume) were purged with N_2 gas for 30 minutes, and incubated on a 100 rpm shaker at 20 ˚C. Daily nitrate and COD measurements were made until a pseudo-steady state was reached. Organic acids, anions, COD, pH, and nitrogen species were analyzed at the end of the experiment. All analytical techniques for the mentioned parameters are described in Section 3.7.

Solid partitioning coefficients were calculated for the adsorption experiment. The concentration ratio of COD absorbed to the carbon substrate to COD in liquid solution is considered the solid partitioning coefficient (USEPA).

$$k_d = \frac{(COD_i - COD_f)\frac{V}{M}}{COD_f}$$
(3.3)

k_d – Solid partitioning coefficient (mL g^{-1})
COD_i – Initial COD concentration (mg L^{-1})
COD_f – Final COD concentration (mg L^{-1})
V – Liquid volume (mL)
M – Mass of carbon substrate (g)

3.3.3 *Woodchip Leachate Anoxic BOD Test*

An anoxic BOD test was conducted to determine the amount of biodegradable organic carbon leaching from woodchips. Leachate from the woodchips was placed in triplicate 250 mL Wheaton bottles, and purged with N_2 gas for 30 minutes to completely remove dissolved oxygen. A glucose-fed denitrifying culture was added to the systems at 3% of the total liquid volume, and the nitrate concentration was raised to 550 mg-N L^{-1}, by adding 30 g L^{-1} KNO_3 solution. The systems were incubated at 20 ˚C on a 100 rpm shaker. Nitrate concentrations were measured every three days until a pseudo-steady state was obtained by the method stated in Section 3.7. At pseudo-steady state, the COD concentration was measured and subtracted from the initial COD concentration. The difference in COD concentrations was assumed to be the ultimate anoxic BOD concentration or the amount of COD that was removed due to anoxic biological degradation.

$$C_u = C_i - C_s \qquad\qquad \textbf{(3.4)}$$

C_u – Ultimate Anoxic BOD Concentration (mg L^{-1})
C_i – Initial COD Concentration (mg L^{-1})
C_s – Pseudo-Steady State COD Concentration (mg L^{-1})

BOD exertion is modeled by a first order function, and depends on the amount of BOD in solution at time t (Metcalf & Eddy, Inc., 2003).

$$C_5 = C_u(1 - e^{-kt}) \qquad\qquad \textbf{(3.5)}$$

C_5 – BOD Concentration after 5 days (mg L^{-1})
C_u – Ultimate BOD Concentration (mg L^{-1})
k – First order rate coefficient (days^{-1})

3.3.4 Nitrate Removal Through Denitrification

Batch experiments were conducted to evaluate the rate of nitrate removal of the ATIWW using carbon substrates. Carbon substrates were tested individually and as mixtures with all tests conducted in triplicate sets. Two different mixtures were tested; one with manure biochar and the other with woodchip biochar. Each mixture bottle contained 75% woodchip, 12.5% silage, and 12.5% manure or woodchip biochar by mass. A 250 mL total volume comprising of an ATIWW to carbon substrate ratio of 2:1 (v/v) was obtained in every 250 mL Wheaton bottle, and KNO$_3$ solution was added to raise the NO$_3^-$ concentration to 300 mg-N L^{-1}. With rubber septa stoppers in place, a 10 minute purge with N$_2$ gas removed dissolved oxygen, and the bottles were incubated at 20˚ C. Anions (including nitrate) and COD were measured at regular time intervals using techniques mentioned in Section 3.7.

Nitrate removal rates were calculated as zero order to get an overall idea of nitrate removal capacity for each carbon substrate and mixture. Figures were created graphing nitrate concentration results versus time for each system with a best fit linear trend line. The slope of the best fit trend line was considered the zero order nitrate removal rate.

3.4   Sequential Batch Experiment Description

Sequential batch tests were conducted to evaluate nitrate removal under conditions of semi-continuous flow. Woodchips, manure biochar, and silage were combined in different fractions to provide a range of carbon substrate conditions for the tests (Table 3.1).

Table 3.1: Summary of sequential batch bottle sets material makeup. Each set is broken down by mass percentage.

	Woodchip Mass %	Manure Biochar Mass %	Silage Mass %
Set 1	87.5	12.5	0
Set 2	87.5	0	12.5
Set 3	75.0	12.5	12.5
Set 4	100	0	0

Triplicate sets using 250 mL Wheaton glass bottles were created with a total volume of 250 mL.   An ATIWW to carbon substrate ratio of 7:1 (v/v) was employed, with the ATIWW nitrate concentration either diluted or raised to 45 mg-N L^{-1} by adding a 30 g L^{-1} KNO_3 solution. Every 24 hours, the source bottle containing ATIWW was purged with N_2 gas for 15 minutes to create an anoxic exchange solution. After the 15 minute purge, the carbon substrate bottles had 100 mL of their liquid exchanged with anoxic ATIWW to create the sequencing batch process. Only 57% of the overall liquid volume was exchanged producing a 1.75 day hydraulic residence time. The average daily initial nitrate concentration varied since it was a mix of nitrate remaining from the previous incubation plus the 45 mg NO_3^- -N L^{-1} contained in the exchange solution. A five minute N_2 purge followed the liquid exchange, and the bottles were incubated at 20˚ C, on a shaker at 100 rpm. Anions (including nitrate) and COD were measured at regular time intervals using techniques mentioned in Section 3.7.

Zero order nitrate removal rates were calculated. The difference between the daily initial and final nitrate concentrations was divided by the time between liquid exchanges to achieve the nitrate removal rate for that day.

$$k = \frac{C_i - C_f}{LET}$$

<div align="right">(3.6)</div>

C_i – Initial COD Concentration (mg L^{-1}) $\qquad$ k – Nitrate Removal Rate (mg-N L^{-1} D^{-1})
C_f – Final COD Concentration (mg L^{-1}) $\qquad$ LET – Liquid Exchange Time (days)
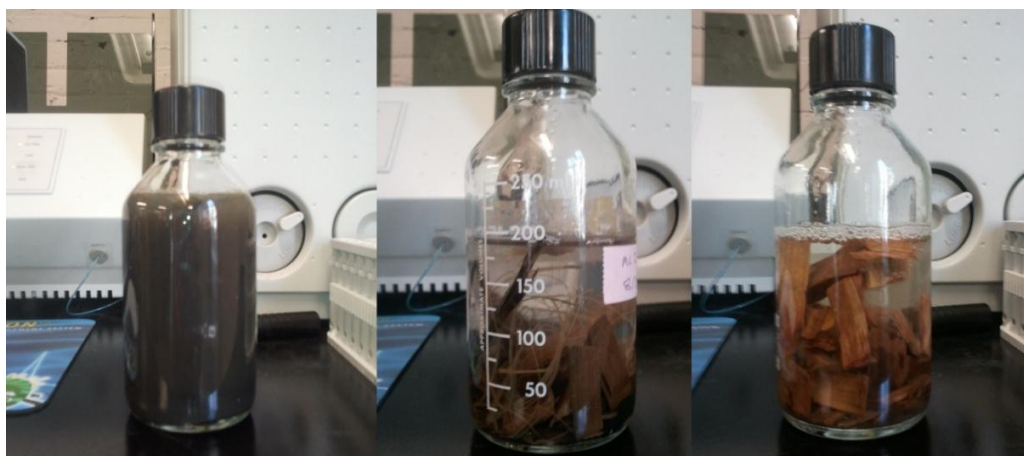
Figure 3.2: Three different experimental system conditions. From left to right – Growth of denitrifying culture (soil slurry), sequential batch 7:1 (v/v) solid to liquid ratio, batch 2:1(v/v) solid to liquid ratio.

3.5   Bench Scale Column Experiment

The purpose of the bench scale column experiment was to evaluate nitrate removal for different fractions of woodchips in continuous flow systems. Four columns, each 2 feet long with a 3 inch diameter (total column volume 2780 mL), were dry packed with the materials listed in Table 3.2.

Table 3.2: Bench Scale Column Experiment – Column materials, porosities, and flowrates to achieve a 24 hour residence time

Column	Porosity	Flowrate (mL/min)
100% Gravel	0.25	0.50
100% Woodchips	0.60	1.15
25% Woodchips & 75% Gravel	0.45	0.85
12.5% Woodchips & 87.5% Gravel	0.40	0.75

Gravel, from Knife River Corporation (Corvallis, OR), was used as a filler for the mixture columns.   The purpose of the filler was to keep consistent hydraulic properties in all columns while using various fractions of the carbon substrate. Both gravel and woodchips were sized to ¼" particle length, and 30 g of TWG soil was intermittently mixed into all columns during loading as an inoculum. Soil was collected in the same manner as in Section 3.1. A reservoir was filled with deionized (DI) water, and kept at a constant nitrate concentration of 45 mg-N L^{-1} to be used as influent to the columns; each of which have an approximate hydraulic residence time of 24 hours. A 12 hour hydraulic residence time (HRT) was tested after the 24 hour HRT period by doubling all flowrates. Samples were also taken daily, and measured for anions (including nitrate) and COD. The experimental setup is shown below in Figure 3.3.

Both zero order and first order nitrate removal rates were calculated to compare rates within this study and systems seen in other reports. Zero order rates were used in the same manner as the sequential batch experiment (Equation 3.6) except the HRT was used instead of LET. First order nitrate removal rate coefficients are dependent on nitrate concentrations in the bulk solution. The rate coefficient was calculated by taking the natural log of the ratio between influent and effluent nitrate concentrations divided by hydraulic residence time.

$$k = ln(\frac{C_i}{C_f})HRT^{-1}$$

(3.7)

C_i – Influent COD Concentration (mg L^{-1})
C_f – Effluent COD Concentration (mg L^{-1})
k – Nitrate Removal Rate Coefficient (days^{-1})
HRT – Hydraulic Residence Time (days)

Figure 3.3: Bench Scale Columns – Each of the four columns has a pump supplying influent solution containing 45 mg-N L^{-1} from the common reservoir.

3.6   Pilot Scale Column Experiment

The duration of the batch, sequential batch, and bench scale column experiments were short term (50, 30, and 25 days respectively). A column experiment was conducted in a woodchip filled PVC pipe to determine long term nitrate removal rates. A 3.5 foot long, 10 inch diameter PVC pipe was dry packed with 75.6 lbs of woodchips and 33.4 L of water. With a total empty column volume of 53.0 L, the column porosity was 63%. The column was setup at the final ATI treatment pond (final location before the ATIWW is pumped to TWG), and placed in an upflow position at a 50˚ angle. Using a peristaltic pump, ATIWW was taken from the pond and supplied to the column at 24 mL min^{-1} (0.97 day residence time). In summer months, column tubing was replaced every two weeks to prevent algae plugs. Samples were taken from the pond and PVC reactor effluent, every 2-4 weeks, and measured for anions (including nitrate) and COD.

3.7   Analytical Techniques

For all experiments NO_3^-, NO_2^-, Cl^-, and SO_4^{2-} concentrations were determined with a Dionex DX-500 ion chromatograph (Sunnyvale, CA) equipped with an electrical conductivity detector and a Dionex AS14 column. Eluent was a 3.5 mM sodium carbonate and 1 mM sodium bicarbonate solution. Liquid samples were extracted, centrifuged for 2 minutes at 14,000 RPM, and diluted 26 times with ultrapure water before analysis.

Organic acids concentrations were monitored using high performance liquid chromatography (HPLC) on a Dionex DX500 HPLC (Sunnyvale, CA) as described by Azizian et al., (2008). The HPLC consists of a Dionex AD 20 Absorbance Detector, a Dionex GP50 Gradient Pump, a Dionex AS 40 Autosampler, and an Alltech Prevail Organic Acid 5u 4mm column with a 25 mM KH_2PO_4 and 38.4 mM H_3PO_4 solution as eluent.

Hach Method 10173 was used to measure COD (Hach, 2009). Depending on the sample concentration, different ranges of COD vials were used. COD medium range (0-1500 mg

L^{-1}) was used in the batch systems, where the leached COD was not removed from the system. For all other samples, the COD low range COD (0-150 mg L^{-1}) was used.

For 5 day BOD (BOD_5) measurements, Method 5210 was used from Standard Methods of Water and Wastewater (20th Ed.). Sample dilutions were estimated based on the COD concentration. Corvallis Wastewater Reclamation Plant activated sludge (RAS) was use as the seed source, which was collected an hour before the experiment. Hach BOD nutrient buffer pillow packets were used for preparation of dilution water. A Hach DO meter (Sens Ion 156) was used for the dissolved oxygen measurements.

Colorimetric measurements for ammonia were achieved with a Microplate reader consisting of PerkinElmer 1420 Multilabel Counter and a UV-Vis spectrophotometer. An ammonia test kit and measurement method from Aquarium Pharmaceuticals Inc. (API) was used for the NH_4^+ measurements. The API test kit consisted of two proprietary liquid solutions that provided a colorimetric change in the sample solution. After waiting five minutes for complete color change, the samples are placed in the above spectrophotometer at 700nm wavelength. A standard curve was developed for every measurement; it consisted of a known ammonia concentration versus an absorbance graph.

An Inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Varian, Liberty 150) was used to determine Ca^{2+}, Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Na^+, and Zn^{2+} concentrations of ATIWW and the leachate solutions. The ICP-AES procedure followed Method 3120B of Standard Methods for Water and Wastewater (19th Ed.)

3.8   T-Test

Unpaired T-tests were performed to evaluate the significance between the nitrate removal rates of the sequential batch and bench scale column experiments. To perform the T-test, a t-value was calculated as seen below (Lowry).

$$t = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{s_1}{n_1} + \frac{s_2}{n_2}}}$$

(3.8)

$\overline{x_1}$ - Mean for data set 1 $\overline{x_2}$ - Mean for data set 1
s_1 – Variance for data set 1 s_2 – Variance for data set 2
n_1 – Size of data set 1 n_2 – Size of data set 2
t – T-test value

The t-value is compared to critical values listed on a t-distribution table, and if the t-value is greater the two data sets are significantly different. Selection of the critical values on the t-distribution table was based off the degrees of freedom (combined number of data points for both data sets minus two) and a probability of exceeding the critical value of (p=) 0.05 (NIST). For the sequential batch experiment, the degrees of freedom were 24 and the critical value was 1.71. For the bench scale column experiment, the degrees of freedom were 22 and the critical value was 1.72.

CHAPTER 4: RESULTS & DISCUSSION

The primary objective of this study was to evaluate the rates of nitrate removal for a nitrate containing, low organic carbon wastewater interacting with four different carbon-containing solid substrates. Talking Water Gardens (TWG) is a 37 acre constructed wetland, located in Albany, OR, that receives wastewater from Albany-Millersburg Water Reclamation Facility and ATI Wah Chang. The primary mechanism for nitrate removal in constructed wetlands is denitrification; where the growth of microbes transform nitrate into nitrogen gas as a part of their metabolism (Mayo & Bigambo, 2005). Growth requirements of denitrifying bacteria include an anoxic environment and organic carbon as a source of energy. A simplified chemical equation for the growth of denitrifying bacteria using glucose as a carbon source is shown below.

$$C_6H_{12}O_6 + 3.37NO_3^- + 3.37H^+ \rightarrow \qquad\qquad \textbf{(4.1)}$$
$$0.311C_5H_7O_2N + 1.53N_2 + 6.55H_2O + 4.445CO_2$$
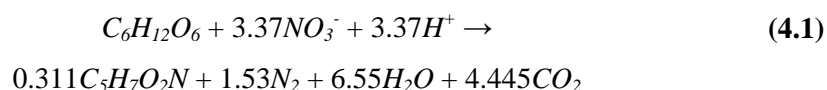
If an appropriate amount of organic carbon is not present in the nitrate containing wastewater the reaction can fail due to carbon limitations. Permeable reactive barriers (PRBs) were developed to address such carbon-limited situations. Similar to subsurface flow wetlands, PRBs are constructed with a solid media in the porous matrix of the barrier. However, PRBs utilize a carbon-containing solid material instead of inert gravel seen in most subsurface wetlands. Ideally, the carbon substrate slowly releases organic carbon over time, providing the energy needed for denitrification. Woodchips have shown slow organic carbon release, a long lifetime, high hydraulic conductivities, consistent nitrate removal rates, and are non-toxic to the denitrifying communities and environment (Schipper, Robertson, Gold, Jaynes, & Cameron, 2010). Other carbon substrates have been tested in PRBs including corn stalks, for their large amounts of labile carbon (Greenan, Moorman, Kaspar, Parkins, & Jaynes, 2006), and biochar, which is known for a wide variety of beneficial uses (Christianson, Hedley, Camps, Free, & Saggar, 2011).

The influent water to TWG (ATIWW) contains $5 - 45$ mg NO_3^--N L^{-1} but very little organic carbon. Therefore, this study was conducted to identify a carbon substrate or substrates that could be used to improve nitrate removal in TWG. The carbon substrates considered were alder woodchips, silage (fermented corn stalks), anaerobically digested cow manure biochar (a mix of 350°C and 600°C), and alder woodchip biochar (500°C) (further discussion of carbon substrate selection can be seen in 3.2).

4.1   Growth of Denitrifying Culture

A denitrifying culture was cultivated for use as inoculum in biological nitrate removal experiments. Soil from TWG was collected as the source of the denitrifying culture. TWG soil, ATIWW, and an organic carbon source were placed in sealed bottles to comprise the batch systems. The batch systems were sampled at regular time intervals, and tested for nitrate concentrations with the disappearance of nitrate indicating active denitrification.

Three different substrates were used as the organic carbon source; glucose, methanol, and glycerin. With complex organic carbon in a system (such as woodchips), bacteria and fungus must first breakdown the complex organics into simple organics before utilizing them for energy (hydrolysis). Cellulose and hemicellulose make up 70% of wood with the majority of the other 30% coming from lignins (Sjostrom, 1993). When cellulose breaks down, its final form is glucose (Garcia-Kirchner & Huitron, 1996). Thus, glucose was chosen as a simple sugar for denitrifying bacterial growth since woodchips will be studied as a carbon substrate. Methanol and glycerin were also chosen to evaluate the range of organic carbon sources.

All three systems showed nitrate removal, indicating the presence of robust denitrifying cultures in the TWG soil. The systems with glucose and methanol as carbon substrates produced the highest average nitrate removal rate (5.6 mg-NO_3^--N L^{-1} D^{-1}). Performing at a slightly lower nitrate removal rate, the culture utilizing glycerin eliminated 5.0 mg-NO_3^--N L^{-1} D^{-1} (Figure 6.1). The culture grown on glucose was chosen to be

maintained and used as the inoculum culture for future experiments due to it compatibility with woodchips as a carbon source.

4.2   Batch Experiment

Batch experiments were conducted to identify which carbon substrates or mixtures provided the highest nitrate removal rates, and to quantify the amount of COD, BOD_5, acetate, and nitrate leached or absorbed by the carbon substrates. As the simplest experimental system, batch systems do not have liquid exchange, and are expected to accumulate COD as it is leached from the carbon substrates. This leached amount was experimentally quantified, and the accumulation of COD was expected to provide a maximum capacity for denitrification.

4.2.1   Carbon Substrate Leaching and Adsorption

4.2.1.1   Leaching

The carbon substrates were selected because of the physical and chemical properties seen in other reports; woodchips provide steady and long term nitrate removal (Schipper, Robertson, Gold, Jaynes, & Cameron, 2010), biochar provides benefits for plant and microbial growth in soil (Tryon, 1948), and silage provides a highly labile organic carbon source (Greenan, Moorman, Kaspar, Parkins, & Jaynes, 2006). The leaching experiment was conducted to quantify the amount of COD, BOD, and nutrients leached from the carbon substrates. To measure the leaching properties, carbon substrates and anaerobic DI water were placed in batch bottles at a liquid to solid ratio of 2:1 (v/v). The anaerobic DI water was used to limit microbial activity, and the liquid to solid ratio was required to fully submerge the carbon substrate. COD concentrations were measured until a pseudo-steady state concentration became apparent (Figure 4.1). Once the pseudo-steady state was reached, the liquid was decanted, filtered, and measured for anions, organic acids, COD, BOD_5, and pH (Table 4.1).
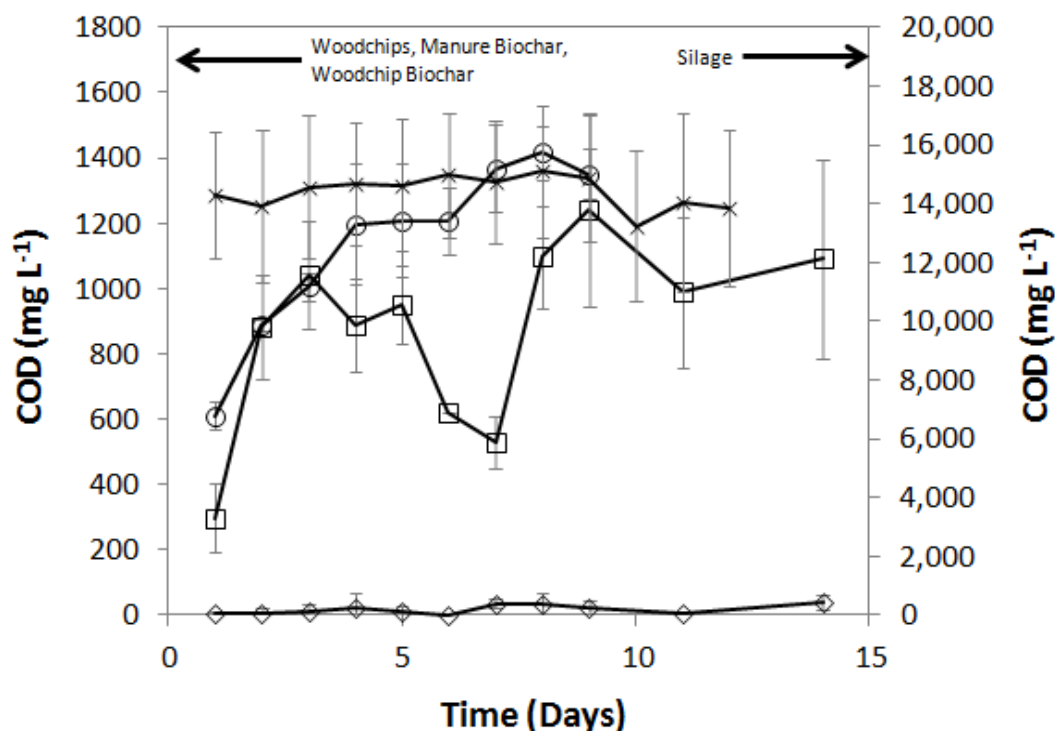
Figure 4.1: Individual carbon substrate COD leaching. Each bottle had a liquid to solid ratio of 2:1 (v/v). X Silage – right axis; ○ Woodchips, □ Manure Biochar, ◊ Woodchip Biochar – left axis.

Woodchips provided a pseudo-steady state COD concentration of 1375 ± 100 mg L^{-1}, and a BOD_5 concentration that comprises approximately 36% of the COD present (500 ± 20 mg L^{-1}). Organic acids analysis revealed an acetate concentration of 115 ± 30 mg COD L^{-1}, which was 23% of the BOD_5. Woodchips were the only carbon substrate to leach nitrate (5.0 ± 1.5 mg-N L^{-1}), and reduced the DI water pH from 6.8 to 5.8 ± 0.2; which is somewhat low for optimal denitrification (Knowles, 1982).

Manure biochar provided an average COD concentration of 1100 ± 250 mg L^{-1}, with a BOD_5 concentration of 120 ± 80 mg L^{-1} (11% of the COD present) and an acetate concentration at 20 ± 20 mg COD L^{-1}. Manure biochar increased the pH of DI water from 6.8 to 7.7 ± 0.2 presumably due to the presence of ash and other products of pyrolysis.

Woodchip biochar produced the lowest COD concentration (17.0 ± 18.5 mg L^{-1}) and no BOD$_5$. The large difference in manure biochar and woodchip biochar COD concentrations is most likely due to the different parent materials and the temperatures of pyrolysis. Lower temperature pyrolysis is expected to produce more labile carbon than higher temperature pyrolysis (Calvelo Pereira, et al., 2011). The pseudo-steady state pH of woodchip biochar was 7.9 ± 0.1.

Silage produced COD concentrations of $14,500 \pm 2400$ mg L^{-1}. BOD$_5$ concentrations exceeded the capacity of the test resulting in concentrations greater than 9000 mg L^{-1}. Organic acid analysis revealed acetate concentrations of 2385 ± 85 mg-COD L^{-1} (16.6% of the silage COD), lactate concentrations of 3460 ± 580 mg-COD L^{-1} (24.0% of COD), and three other organic acids that could not be identified. The three unidentified organic acids were estimated to comprise 20% of the silage COD based off HPLC areas and assuming a similar molar mass to acetate. Silage leachate had a pH of 4.2 ± 0.1, which is far less than optimal (7.0 - 8.0) suggesting pH inhibition could have occurred in the nitrate removal experiment (Knowles, 1982).

Table 4.1: Carbon substrate leaching results at apparent COD equilibrium with ± indicating a 95% confidence interval. All systems had a 2:1 (v/v) liquid to solid ratio.

Carbon Substrates	COD (mg L^{-1})	BOD$_5$ (mg L^{-1})	Acetate (mg-COD L^{-1})	BOD$_5$/COD	NO$_3^-$ (mg-N L^{-1})	Cl$^-$ (mg L^{-1})	SO$_4^{2-}$ (mg L^{-1})	pH
Woodchip	1375 ± 100	500 ± 20	115 ± 30	0.36	5.0 ± 1.5	12 ± 1	0	5.8 ± 0.2
Manure Biochar	1100 ± 250	120 ± 80	20 ± 20	0.11	0	625 ± 50	245 ± 2.5	7.7 ± 0.2
Woodchip Biochar	17.0 ± 18.5	0	0	0	0	0	0	7.9 ± 0.1
Silage	14,500 ± 2400	< 9000*	2385 ± 85	0.62	0	960 ± 40	85 ± 9.0	4.2 ± 0.1

*Note: Day 5 dissolved oxygen was completely depleted

4.2.1.2   Leaching Rate

The carbon substrate leaching experiment showed woodchip biochar produced the lowest COD leaching rate (Figure 4.1). Manure biochar leached COD at a high rate initially, but exhibited a strange COD drop from Day 3 to 7 before coming to an apparent equilibrium. Silage leached COD at a rate so fast it appeared to be at equilibrium within one day. Out of all the carbon substrates, woodchips was the only substrate to leach COD with approximate first order behavior (Figure 4.2). Analysis of the data resulted in a pseudo-first order rate coefficient of 0.47 days^{-1} with an r^2 value of 0.79.
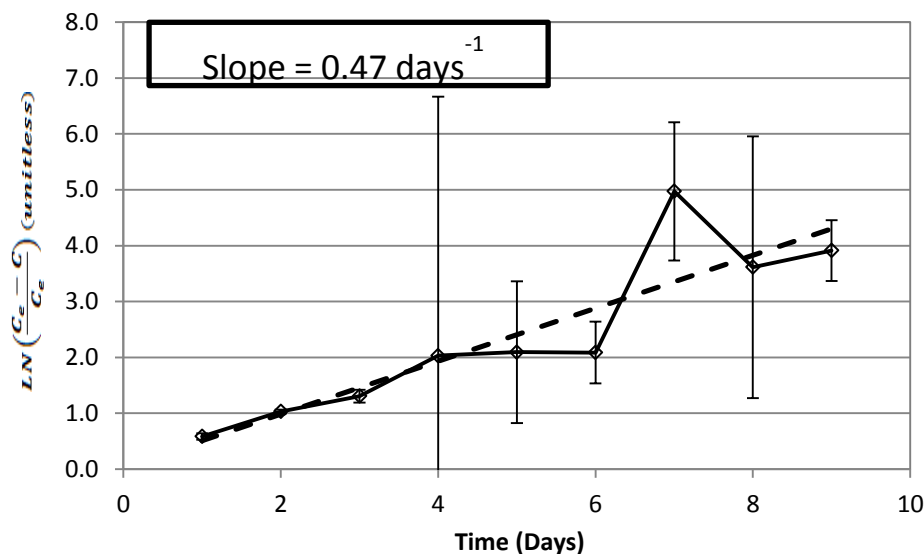
Figure 4.2: COD leaching rate coefficient for woodchips. Each bottle had a liquid to solid ratio of 2:1 (v/v). Error bars represent a 95% confidence interval**.**

4.2.1.3   Anoxic BOD Test

An anoxic BOD test was performed on the woodchip leachate as another measurement of biodegradable carbon. Denitrifying culture and nitrate were added to the woodchip leachate to induce denitrification, and quantify the COD utilized. Once a constant nitrate concentration was

established, a final COD concentration was measured and compared to the initial COD concentration.

A total of 205 mg NO_3^--N L^{-1} was removed during the experiment, along with 640 mg COD L^{-1} resulting in a COD to N ratio of 3.1 mg COD per mg NO_3^--N (data not shown). The 640 mg COD L^{-1} that was utilized or the degradable COD comprised approximately 62% of the total COD in the woodchip leachate. That 62% can also be considered the ultimate BOD. The woodchip leachate BOD_5 was measured at 490 mg L^{-1} (36% of the COD) corresponding to a BOD first order rate coefficient of 0.29 days^{-1} (3.5), which is in the range that is typically found for readily degradable carbon (Metcalf & Eddy, Inc., 2003).

4.2.1.4   Adsorption

Since different biochars are known to exhibit different adsorption and leaching behavior (Spokas & Reicosky, 2009), an experiment was conducted to quantify the COD adsorption capacity of the biochars. Biochar was added to the woodchip leachate, in batch bottles, at a liquid to solid ratio of 2:1 (v/v), and was tested for daily COD concentrations. Initial COD concentrations averaging 1190 mg L^{-1} were measured in the woodchip leachate, and after nine days, an apparent COD equilibrium concentration was established at 565 mg L^{-1} (Figure 4.3). During this experiment, 625 mg L^{-1} of COD was apparently absorbed to the biochar, and a solid partitioning coefficient (k_d) of 3.68 mL/g was calculated (Equation (3.3). The same experiment was conducted on manure biochar; however, COD was produced instead of absorbed.
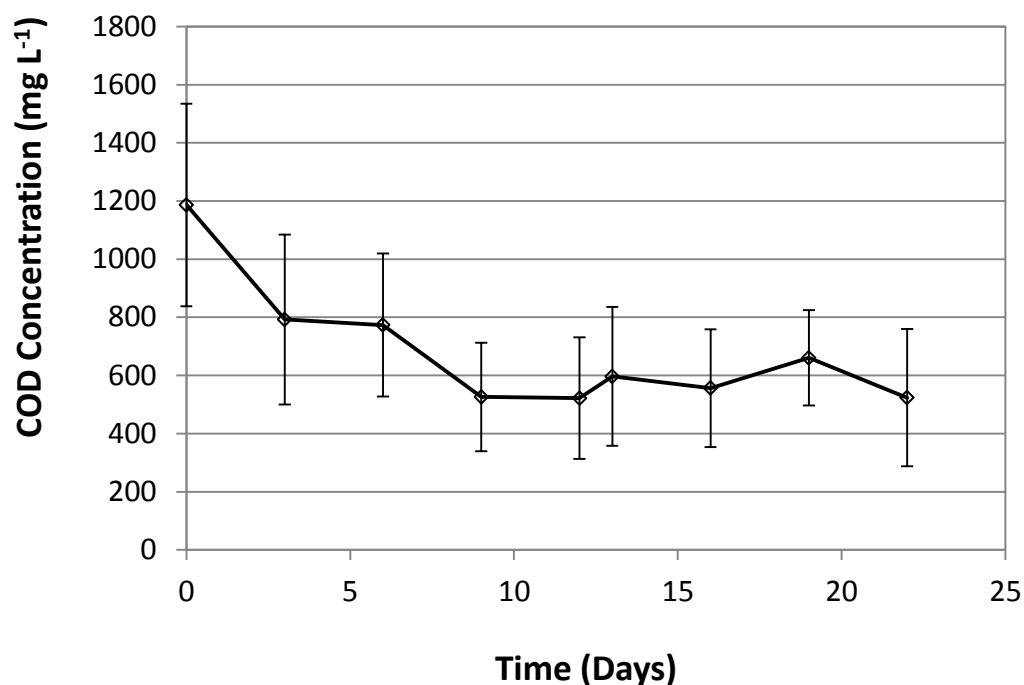
Figure 4.3: COD adsorption from woodchip biochar. Each bottle had a liquid to solid ratio of 2:1 (v/v). Error bars represent 95% confidence intervals**.**

4.2.2   *Nitrate Removal Experiment Results*

Four individual carbon substrates and two mixtures of carbon substrates were tested for maximum batch nitrate removal rates (liquid to solid ratio of 2:1 (v/v)). Nitrate removal ensued in all the systems, but the highest rates were observed in the mixture systems. The mixture systems comprised of 75% woodchip, 12.5% silage, and 12.5% woodchip or manure biochar by mass, removed around 300 mg-NO_3^- -N L^{-1} in less than two days (Figure 4.4). Woodchips provided steady nitrate removal over time eliminating 276 mg-N L^{-1} in 43 days. Both biochar bottles removed nitrate for the first 20 days, (150 mg-N L^{-1} – woodchip biochar and 233 mg-N L^{-1} – manure biochar) and then nitrate removal stopped. Silage nitrate concentrations increased for the first 20 days before being reducing from 274 mg-N L^{-1} to 0 in the following 100 days.
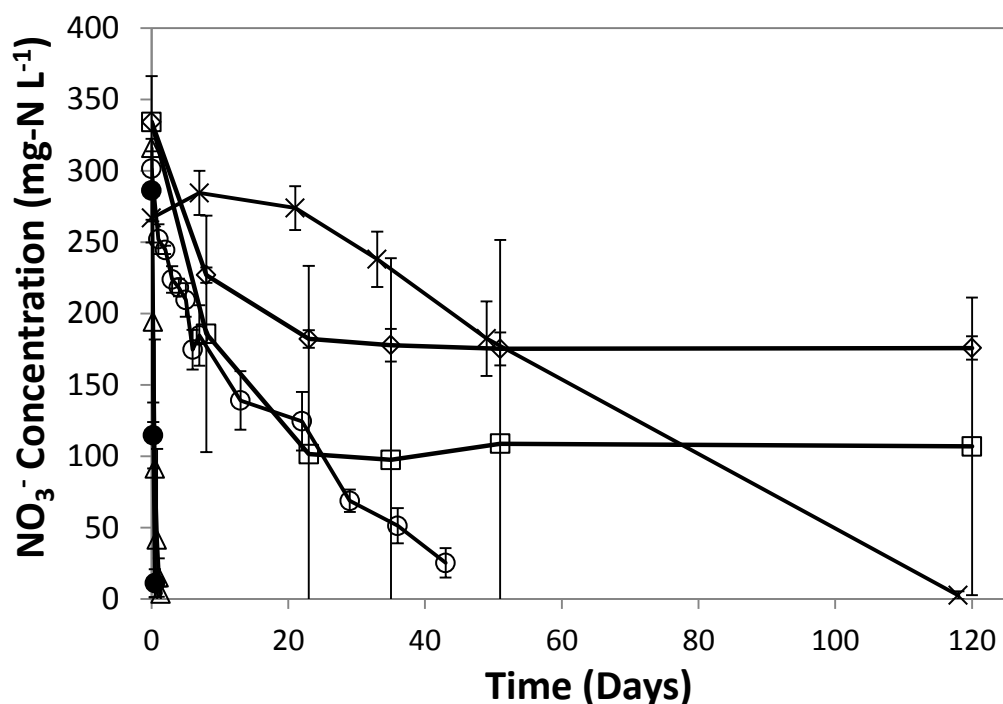
Figure 4.4: Nitrate concentrations resulting from different carbon substrates and mixtures. Each bottle had a liquid to solid ratio of 2:1 (v/v), and error bars represent 95% confidence intervals. X Silage, ◊ Woodchip Biochar, □ Manure Biochar, ○ Woodchips, Δ Mixed w/Woodchip Biochar, ● Mixed w/Manure Biochar

Initial zero order nitrate removal rates were calculated for each of the systems, and are presented in Figure 4.5 and Figure 4.6. Mixture systems had their rates calculated during the first 13 hours of the experiment, while woodchip rates were calculated from day 0 to 13. The biochars rates were calculated from day 0 to 20, and silage rates were calculated from day 20 to 50 after net nitrate removal began.
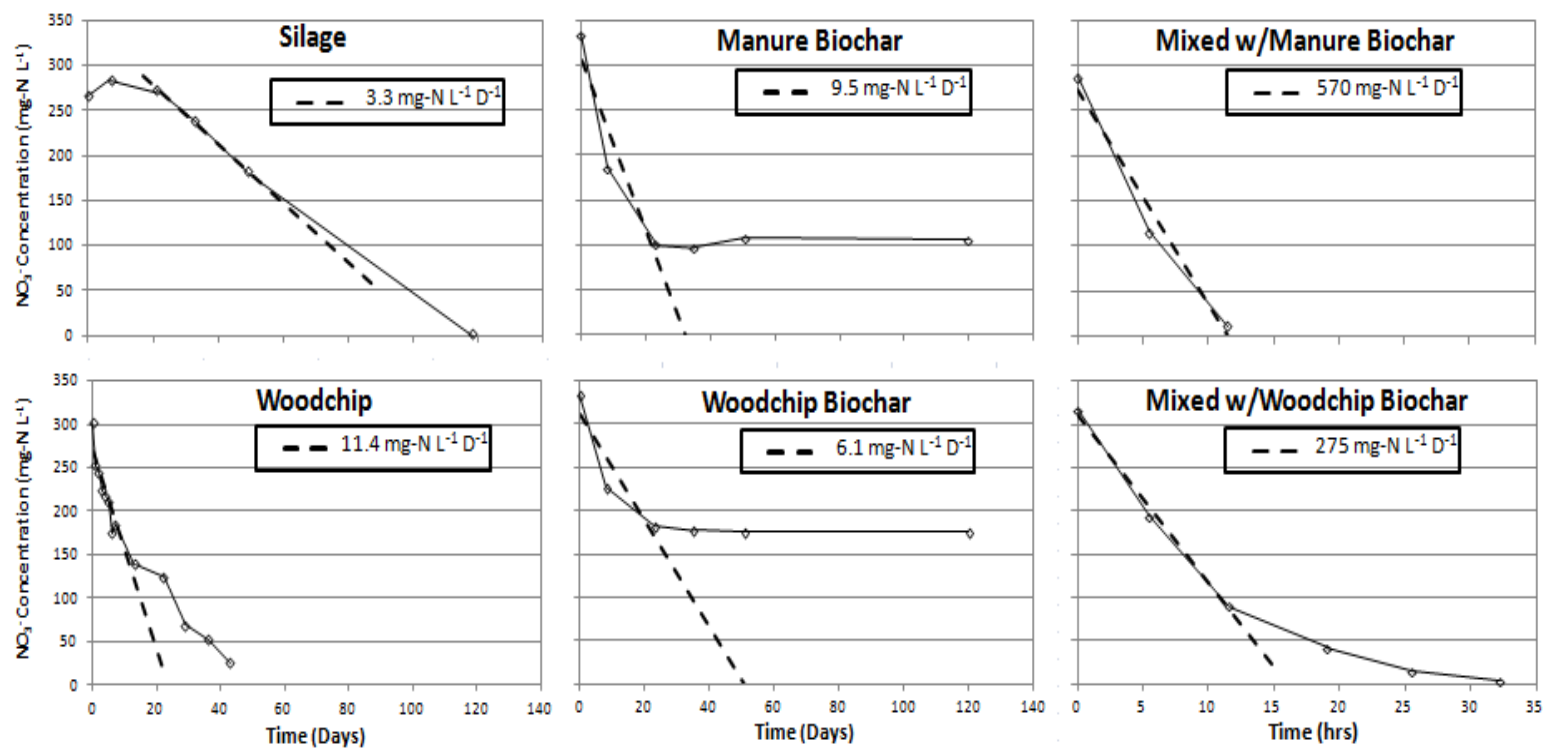
Figure 4.5: Zero order nitrate removal rates taken from days 0-20 for the biochars, day 0-13 for woodchip, day 20-50 for silage, hour 0-13 for both mixed systems. ◊ Experimental Data, - - Initial zero order nitrate removal rate
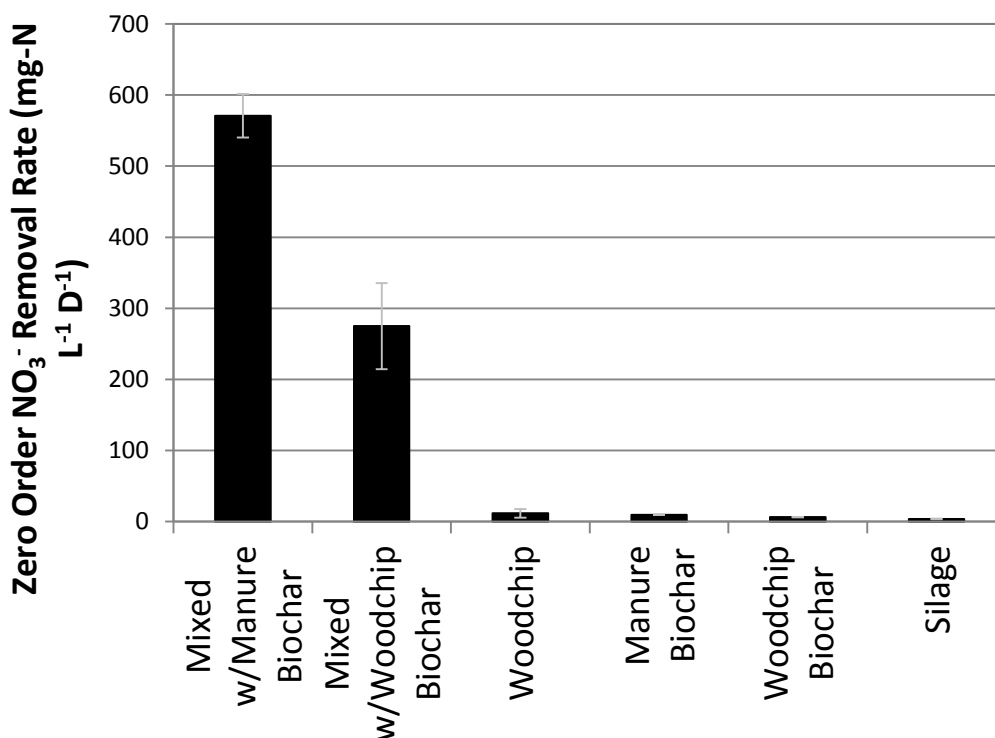
Figure 4.6: Batch zero order nitrate removal rates. Rates shown represent the largest average nitrate removal rates achieved. Error bars represent a 95% confidence interval.

Carbon substrate mixtures produced nitrate removal rates two orders of magnitude greater than any of the single carbon substrates. The mixture system with manure biochar produced the highest nitrate removal rate of 570 mg-N L^{-1} D^{-1}, which was approximately twice that of the woodchip biochar mixture (275 mg-N L^{-1} D^{-1}) (Table 4.2).

Table 4.2: Comparison of batch nitrate removal rates to literature value.

Carbon Substrate	Zero Order		COD	Liquid:Carbon Substrate Volume Ratio	Source
	mg-N L^{-1} D^{-1}	r^2	mg L^{-1}		
Mixed w/ Manure Biochar	570	0.97	3200-3680	2:1	This Study
Mixed w/ Woodchip Biochar	275	0.90	1560-2080		
Woodchip	11.4	0.88	393-797		
Manure Biochar	9.5	0.89	445-738		
Woodchip Biochar	6.1	0.84	111-205		
Silage	3.3	1.0	18,000-23,800		
Corn Stalk (unfermented silage)	62.6			3.75:1	(Greenan, Moorman, Kaspar, Parkins, & Jaynes, 2006)
Woodchip	5.8	-	-	6:1	
Woodchip	2.6	-	30-80 (TOC)	5.67:1	(Gibert, Pomierny, Rowe, & Kalin, 2008)
Glucose and Soil (Growth of Denitrifying Culture)	5.6	0.96	-	-	This Study

Woodchip was the only single carbon substrate system that produced steady nitrate reduction with average rates of 11.4 mg-N L^{-1} D^{-1}. When compared to the Greenan et al. and Gibert et al. study (Table 4.2), the nitrate removal rate from this study is greater than the others (11.4 mg-N L^{-1} D^{-1} compared to 5.8 and 2.6 mg-N L^{-1} D^{-1}). The differences in rates could be due to the different liquid to solid volume ratios. It was hypothesized that a lower ratio would have a higher nitrate removal rate as it has a higher percentage of carbon substrate. A solid to liquid volume ratio of 2:1 was experimented in this study while a ratio of 17:3 and 6:1 were employed in Gibert et al. and Greenan et al. respectively. Another factor that could explain the difference in nitrate removal rates is the woodchip type. Alder (hardwood) was used in this study, while Greenan et al. used oak (hardwood) and Gibert et al. used an unidentified hardwood. A study by F.E. Allison shows hardwoods decompose to a greater extent than softwoods (Allison, 1965). The greater decomposition in hardwoods could be beneficial for denitrification as organic carbon is one of the byproducts of the decomposition. However, all three studies used hardwood so the difference in rates could be related to hardwood tree species. The alder woodchips provided COD concentrations that did not decrease or increase over time (393-797 mg L^{-1}) even though nitrate concentrations continually decreased.

The manure biochar systems produced a nitrate removal rate of 9.5 mg-N L^{-1} D^{-1} during the first 20 days, and then remained constant. COD concentrations of manure biochar ranged from 445-738 mg L^{-1} at various points in time. The nitrate concentrations tapering off in the manure biochar system, while maintaining a COD concentration between 445-738 mg L^{-1}, could be a result of the 11% biodegradable carbon (from the BOD_5 test) being fully consumed.

Woodchip biochar produced a nitrate removal rate of 6.1 mg-N L^{-1} D^{-1}. BOD_5 was not present in the woodchip biochar suggesting any nitrate removed in the nitrate removal experiment could have been from adsorption. The lack of biodegradable carbon could also explain the 30-40% smaller nitrate removal rates than manure biochar.

Silage provided the slowest nitrate removal rate of all carbon substrates after the initial 20 day nitrate increase (3.3 mg-N L^{-1} D^{-1}). The silage used for the nitrate removal

experiment was fresh silage while the leaching experiment used silage stored in a 4°C refrigerator for four months. Age differences could account for nitrate leaching occurring in the fresh silage (nitrate removal experiment) and not detecting nitrate in the four-month-old silage (Table 4.1). It was not indicated if nitrate concentrations in the Greenan et al. paper increased in the corn stalk systems as the first nitrate measurement occurred at day 30 (Greenan, Moorman, Kaspar, Parkins, & Jaynes, 2006). The overall nitrate removal rates comparing Greenan et al. (corn stalk) and this study (silage) are 62.6 and 3.3 mg-N L^{-1} D^{-1} respectively - a 1798% difference (Table 4.2).

COD concentrations of 18,000-23,800 mg L^{-1} were measured for silage in the nitrate removal experiment. Inhibition of the denitrification culture could explain the slow nitrate removal rates with high concentrations of biodegradable carbon.   According Knowles, optimal pH for denitrification is between 7.0 and 8.0 (1982).   Silage leachate pH (4.2 ± 0.1) is far less than 7.0 suggesting pH inhibition was occurring in the nitrate removal experiment. pH was not measured during the nitrate removal experiment but could be assumed higher than 4.2 due to a less fermented product from a younger aged silage.

Presumably, the conditions within the bottles containing carbon substrate mixtures was the best suited for rapid nitrate removal. The silage provided labile organic carbon and possible nutrients; the biochar buffered the pH drop associated with woodchip and silage leaching and provided possible nutrients; and the woodchips provided the microbial habitat and slow release of organic carbon necessary for sustained denitrification.

4.2.3   *Carbon Substrates at Various Ages*

Rates of nitrate removal were measured in mixture systems to identify the effect of carbon substrate age. It has been reported in woodchip-filled PRBs that nitrate removal rates decrease by 50% after the first year, but maintain steady rates for a number of years after (Robertson W. , 2010). As noted in Table 4.2, the original mixed manure biochar systems produced a nitrate removal rate of 570 mg-N L^{-1} D^{-1}, and the mixed woodchip biochar systems were at 275 mg-N L^{-1} D^{-1}. The same systems, 45 days later, exhibited

decreased nitrate removal rates of 395 mg-N $L^{-1} D^{-1}$ (30% lower) and 135 mg-N $L^{-1} D^{-1}$ (50% lower) for the mixed manure biochar and mixed woodchip biochar systems, respectively (Table 4.3).

Table 4.3: Batch nitrate removal rates and COD concentrations at different carbon substrate ages.

Carbon Substrate Mixtures	Zero Order Nitrate Removal Rate		COD mg L^{-1}	Source
	mg-N $L^{-1} D^{-1}$	r^2		
Mixed w/ Manure Biochar	570	0.97	3200-3680	This Study (Batch)
Mixed w/ Manure Biochar + 45 Days	395	0.94	1504-2357	
Mixed w/ Woodchip Biochar	275	0.90	1560-2080	
Mixed w/ Woodchip Biochar + 45 days	135	0.86	902-1402	
Woodchip	15.4 - 23.0	0.96	7.5-22.0 (DOC)	(Robertson W. , 2010) (Continuous flow)
Woodchip + 2 years	12.1	0.94	nl	
Woodchip + 7 years	9.1	0.96	7.5-10.0 (DOC)	

nl: not listed in (Robertson W. , 2010)

The availability of readily degradable organic carbon is expected to be the primary cause for the differences in rates over time. Average COD release in the mixed manure biochar systems were 1930 mg L^{-1} after 45 days compared to 3440 mg L^{-1} initially. A similar decrease was seen in the mixed woodchip biochar systems with COD concentrations reduced 37% from an initial 1820 mg L^{-1} to 1150 mg L^{-1} after 45 days. The COD

concentration decrease over time (37-44%) was similar in proportions to the nitrate removal rate decrease (30-50%) over the same time.

In a continuous flow column study, the use of two year old and seven year old woodchips resulted in a 37% and 53% decrease in median nitrate removals rates over fresh woodchips, respectively (Robertson W. , 2010). The 30-50% nitrate removal rate decrease and 36-42% COD concentration decrease, seen in the mixture systems of this study, occurred in 45 days compared to 2 to 7 years. The addition of silage to woodchip systems could explain the similar reduction in nitrate removal rates over a shorter time period.

4.2.4   Batch Experiments Summary

- Combining carbon substrates into one batch system increased nitrate removal rates by two orders of magnitude over the carbon substrates individually.
- Woodchips provided consistent nitrate removal and COD leaching. A first order COD leaching rate coefficient of 0.47 days^{-1} was calculated during the leaching test.
- Manure biochar leached biodegradable carbon to induce nitrate removal for a short time period. It also increased pH.
- Woodchip biochar did not appear to contain biodegradable carbon. However, it absorbed COD and nitrate, and increased pH.
- Silage produced a relatively high soluble organic carbon, including organic acids which can be used for denitrification. However, the high organic carbon concentrations lead to low pH and microbial inhibition.
- Carbon substrate age was a factor in nitrate removal rates and COD leaching.

4.3   Sequential Batch Experiment

A sequential batch experiment was conducted to evaluate the nitrate removal performance of different mixtures in systems that included fluid exchange to better mimic field conditions. Every 24 hours, 100 mL of system solution was exchanged with 100 mL of ATIWW. Bottles were created with a liquid to solid ratio of 7:1 (v/v) for easy

extraction of system liquid without the interference of solids. Only 57% of the liquid was extracted every 24 hours, creating a 1.75 day hydraulic residence time. The ATIWW exchange water remained at a constant nitrate concentration of 45mg-N L^{-1}. Under these conditions, the systems were operated closer to field conditions with semi-continuous flow and lower nitrate concentrations.

Three different carbon substrate mixtures were tested and compared to woodchips alone as the carbon source (Table 4.4). All systems were measured every 24 hours for nitrate after liquid exchange.

Table 4.4: Carbon substrate mixtures used in the sequential batch tests. Values indicate the mass percentage of each carbon substrate in the system

	Woodchip Mass %	Manure Biochar Mass %	Silage Mass %
System – 1 Woodchip/Manure Biochar	87.5	12.5	0
System – 2 Woodchips/Silage	87.5	0	12.5
System – 3 All Three Substrates	75.0	12.5	12.5
System – 4 Woodchip	100	0	0

4.3.1   Sequential Batch Experiment Results

Rapid nitrate depletion occurred in systems that contained silage with complete nitrate reduction occurring during the first four days. After day 4, nitrate concentrations increased until they approximately stabilized after day 10 (Figure 4.7). The systems with silage also produced high COD concentrations early in the experiment that decreased with continual fluid exchange (Figure 4.8). The systems with woodchips alone showed similar trends to the systems with silage except nitrate concentrations were greater near the end of the experiment. Woodchip/Manure biochar supported minimal early nitrate removal rates even though it appeared to have the most COD remaining in solution at day

5. Nitrate removal in all of the systems appeared to converge after the first week of operation and exhibit very similar removal efficiencies from day 20 on.

The sequential batch experiment had COD concentrations that converged to a range of 0-100 mg L^{-1}, while the batch experiment had COD values of 393-3680 mg L^{-1} (not including the silage systems) (Table 4.2). The semi-continuous flow conditions probably caused the decrease in COD concentrations by removing any leached COD during liquid exchange.
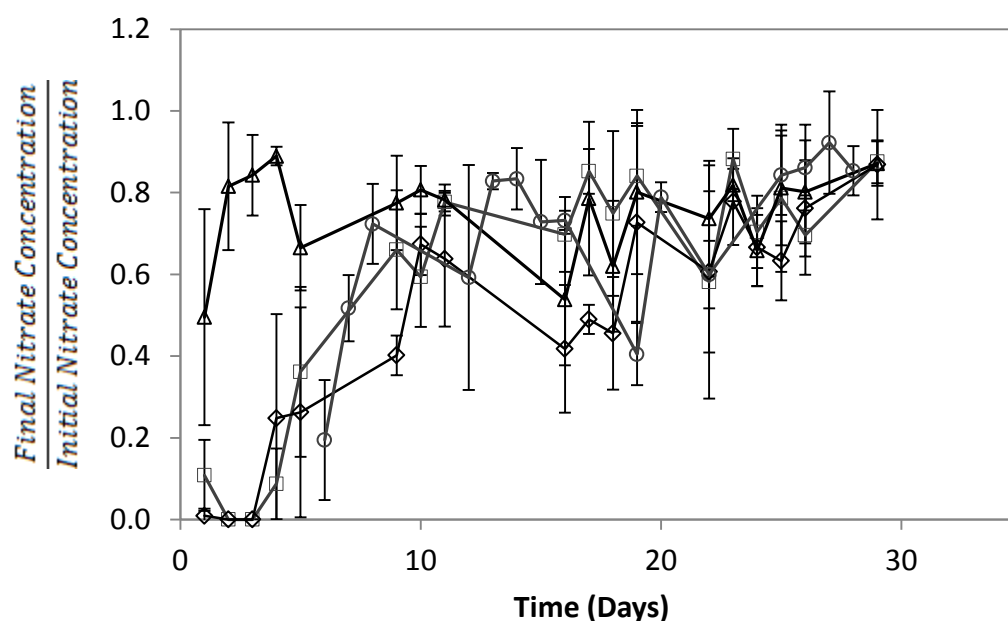
Figure 4.7: Ratio of nitrate concentrations after 24 hour incubation. Each bottle had a liquid to solid ratio of 7:1. Error bars represent a 95% confidence interval. Δ Woodchip/Manure Biochar, □ Woodchip Silage, ◊ All Three Substrates, ○ Woodchip
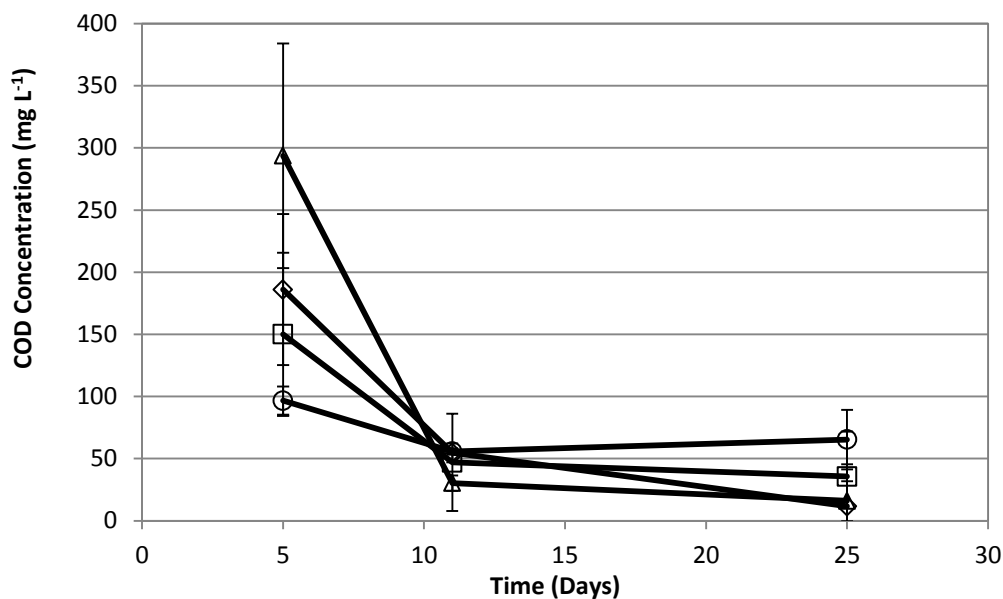
Figure 4.8: COD concentrations before liquid exchange. Each bottle had a liquid to solid ratio of 7:1. Error bars represent a 95% confidence interval. Δ Woodchip/Manure Biochar, □ Woodchip Silage, ◊ All Three Substrates, ○ Woodchip.

4.3.2   *Nitrate Removal Rates*

The nitrate removal rates for the systems with silage started high, decreased with time, and tapered off (Figure 4.9). The decrease was most likely due to of silage being removed from the system via the 100 mL liquid exchange and utilization for denitrification. The same could be said about the soluble COD concentrations.   The average nitrate removal rates shown in Table 4.5 were calculated from day 10 on, after system behavior began to converge.

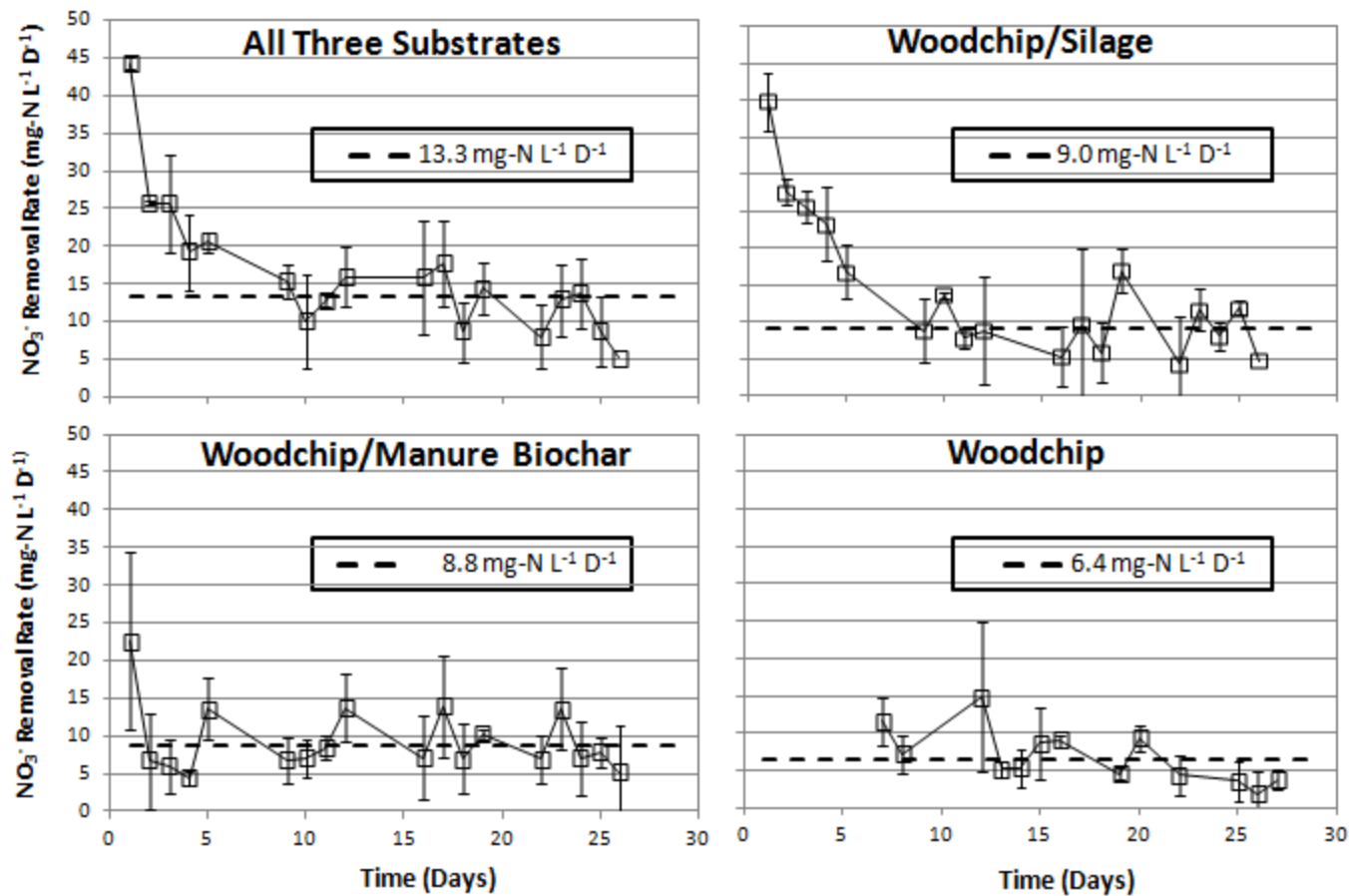**Figure 4.9:** Sequential batch nitrate removal rates vs. time. Each bottle had a liquid to solid ratio of 7:1. Error bars represent a 95% confidence interval. □ Nitrate Removal Rate, - - Average Nitrate Removal Rate from Day 10-30

Table 4.5: Average zero and first order nitrate removal rate coefficients calculated from days 10-30 data. Each bottle had a liquid to solid ratio of 7:1 (v/v). ± values represent a 95% confidence interval.

	Average Zero Order Rates (mg-N L^{-1} D^{-1})	Average First Order Rate Coefficients (Days^{-1})
All Three Substrates	13.3 ± 4.1	0.54 ± 0.19
Woodchip/Silage	9.0 ± 3.9	0.31 ± 0.16
Woodchip/Manure Biochar	8.8 ± 3.9	0.30 ± 0.15
Woodchip	6.4 ± 2.9	0.27 ± 0.13

All systems appeared to converge to average nitrate removal rates that were within 2:1 of each other, which is far smaller than the 215:1 difference seen in batch systems. Woodchips alone produced similar rates to the batch test (6.4 mg-N L^{-1} D^{-1} – sequential batch, 11.4 mg-N L^{-1} D^{-1} - batch). However, All Three Substrates systems produced rates that were 98% percent different than in batch systems (13.3 mg-N L^{-1} D^{-1} – sequential batch, 575 mg-N L^{-1} D^{-1} – batch); largely due to silage and soluble carbon washing out of the systems with the introduction of semi-continuous flow. Differences in liquid to solid ratios may also have influenced the final rates (7:1 – sequential batch, 2:1 – batch).

A T-test was performed to identify whether the average rates were statistically different from one another. According to the T-test for this data set, if the T-test value was greater than 1.71 (95% confidence limit) the two data sets were significantly different (Table 4.6). For zero order rates, only the comparison between Woodchip/Silage and Woodchip/Manure Biochar had average rates that were considered significantly the same; indicating that woodchips alone do not perform as well as woodchips mixed with other carbon substrates, and that a mixture of three carbon substrates outperformed a mixture with two carbon substrates. However, applying the T-test to the first order analysis indicated that adding a single carbon substrate to woodchips did not improve nitrate removal over woodchips alone, but if a third carbon substrate was added to the system there would be a significant improvement in nitrate removal.

Table 4.6: Sequential batch nitrate removal rates T-test values**.** If the T-test value is greater than 1.71 the two data sets are significantly different at a 95% confidence interval. Bolded T-test values identify comparisons that are significantly different.

	All Three Substrates		Woodchip/Manure Biochar		Woodchip/Silage	
	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order
All Three Substrates	-	-	-	-	-	-
Woodchip/Manure Biochar	**2.92**	**2.71**	-	-	-	-
Woodchip/Silage	**2.12**	**3.09**	0.62	0.47	-	-
Woodchip	**4.87**	**3.80**	**1.87**	1.14	**2.38**	0.64

Although the T-test analysis indicated some significant differences in nitrate removal rates, the differences are quite small in magnitude. Additionally, if the nitrate removal rates are compared from day 20 on, no significant differences in rate coefficients are observed in first order analysis. The effects of manure biochar addition to the systems are unknown other than as a potential pH buffer. In the batch experiment, manure biochar removed nitrate for 25 days then stopped, indicating that the addition of manure biochar in mixture systems may not have a significant long term effect.

A study by Christianson et al., investigated nitrate removal in column systems with different woodchip and woodchip biochar mixtures (2011). Four different columns were investigated: woodchip only, woodchip (86% by mass) mixed with biochar charred at 380˚C, woodchip (93% by mass) mixed with biochar charred at 380˚C, and woodchip (86% by mass) mixed with biochar charred at 550˚C. The influent nitrate concentration was 20 mg-N L^{-1} and five different hydraulic residence times were tested (14.1, 4.1, 13.4, 5.2, and 14.4 hours). Like the first order analysis for this study, there were no significant differences in nitrate removal performance between the woodchips alone and the woodchip/biochar mixtures.

4.3.3   *Sequential Batch Experiment Summary*

- Nitrate removal rates for all mixture systems and woodchips alone tended to converge with semi-continuous flow, and showed little or no significant differences.
- COD concentration decreases had an apparent effect on nitrate removal rate decreases.
- Silage was removed from the system via liquid exchange, and the COD concentrations and nitrate removal rates decreased rapidly during the first 10 days.
- Manure biochar additions did not produce initial nitrate removal rates as high has silage additions.

4.4   Bench Scale Column Experiment

The results from the sequential batch experiment indicated the mixture systems did not provide a significant difference in nitrate removal rates from woodchips alone. Therefore, a bench scale column experiment was conducted to determine nitrate removal rates of different fractions of woodchips in continuous flow systems.

Four 3 inch diameter, 2 foot long columns were packed with woodchip/gravel mixtures. The gravel was a filler to create approximately the same hydraulic residence times (HRTs) and flow characteristics in each column while testing different woodchip volume fractions for nitrate removal. Two different HRTs were tested with 2 weeks of operation at a 24 hour HRT followed by two weeks at a 12 hour HRT.   A common reservoir fed all four columns in an upward flow direction and contained an average influent dissolved oxygen concentration of 6.8 mg L^{-1}; meaning the organic carbon leached from the woodchips would be first utilized for aerobic microbial processes, and dissolved oxygen would be depleted before denitrification could take place. Column mixtures included a 100% woodchip column, 25% woodchip/75% gravel column, 12.5% woodchip/87.5%

gravel column, and a 100% gravel column (0% woodchip) (Figure 3.3 and Table 3.2).
Daily nitrate and COD concentrations were measured.

4.4.1   Bench Scale Column Experiment Results

The first 12 days of the experiment were conducted at hydraulic residence times that
varied between 18 and 30 hours due to pumping problems. After Day 12, flows were
stabilized and a hydraulic residence time (HRT) of 24 hours was achieved with an
average influent nitrate concentration of 47.7 ± 0.8 mg-N L^{-1} (Figure 4.10). The columns
were switched to a 12 hour HRT at day 28 to evaluate the effect that nitrate loading had
on nitrate removal rates. Effluent COD concentrations were also measured during the
experiment (Figure 4.11).
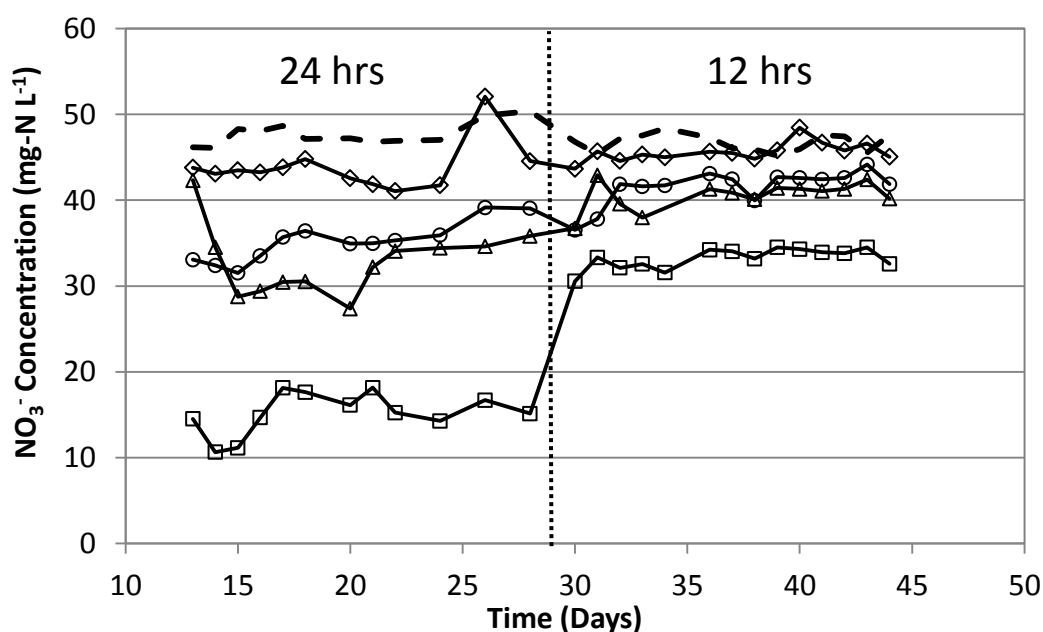
Figure 4.10: Influent and effluent nitrate concentrations for bench scale column
experiment. Two different hydraulic residence times are presented; 24 hours and 12
hours. - - Average Influent Concentration; Effluent Concentrations - ◊ 0% Woodchip, ○
12.5% Woodchip, Δ 25% Woodchip, □ 100% Woodchip
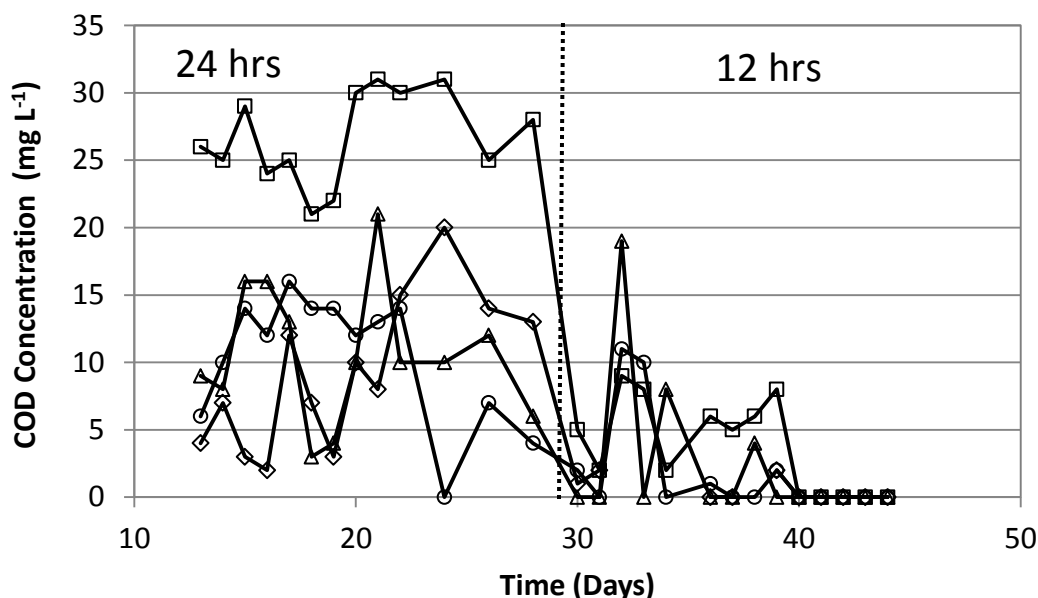
different hydraulic residence times are presented; 24 hours and 12 hours. □ 100% Woodchip, Δ 25% Woodchip, ○ 12.5% Woodchip, ◊ 0% Woodchip

The column containing 100% woodchips reduced the most nitrate during operation at a 24 hour residence time and had the highest effluent COD concentration (26.7 ± 1.8 mg L^{-1}). As the volume fraction of woodchips decreased, the amount of nitrate removed was also reduced, but not proportional to the woodchip volume percentage. The 25% woodchip column had effluent nitrate concentrations that were 54% greater than the 100% woodchip column, and the 12.5% woodchip column had concentrations 56% greater than the 100% woodchip column. Interestingly, the column containing only gravel and soil inoculum also exhibited some nitrate removal.

On Day 28, the hydraulic residence times were cut in half (12 hours). The 100% woodchip column produced the largest increase in effluent nitrate concentrations at 54% (33.2 ± 0.63 mg-N L^{-1}). The other columns did not produce effluent nitrate concentrations that increased to the extent of the 100% woodchip column (25% woodchip (WC) column had a 19% effluent nitrate concentration increase, 12.5% WC column – a 15% concentration increase, 0% WC column – a 4% concentration increase). Effluent nitrate concentrations were not proportional to woodchip volume fractions in the 12 hour

HRT. Effluent COD concentrations reduced dramatically for the 100% woodchip column after the operational HRT change (79%). All columns have effluent COD concentrations that eventually converge to 0 mg L^{-1} at day 40, which could have been caused by analytical error. COD measurements from day 40 to 44 were not retested.

4.4.2   Bench Scale Column Experiment Nitrate Removal Rates

A 30.8 ± 1.4 mg-N L^{-1} D^{-1} nitrate removal rate was observed in the 100% woodchip column when operated with a 24 hour hydraulic residence time (HRT) (Figure 4.12 and Table 4.7). This is fives time the rate observed in the sequential batch experiment (6.4 mg-N L^{-1} D^{-1}) although the retention time was longer in the sequential batch test (1.75 days). This may be largely due to the significantly different liquid to solid ratios of the two different systems (7:1 – sequential batch, 1.5:1 – column).

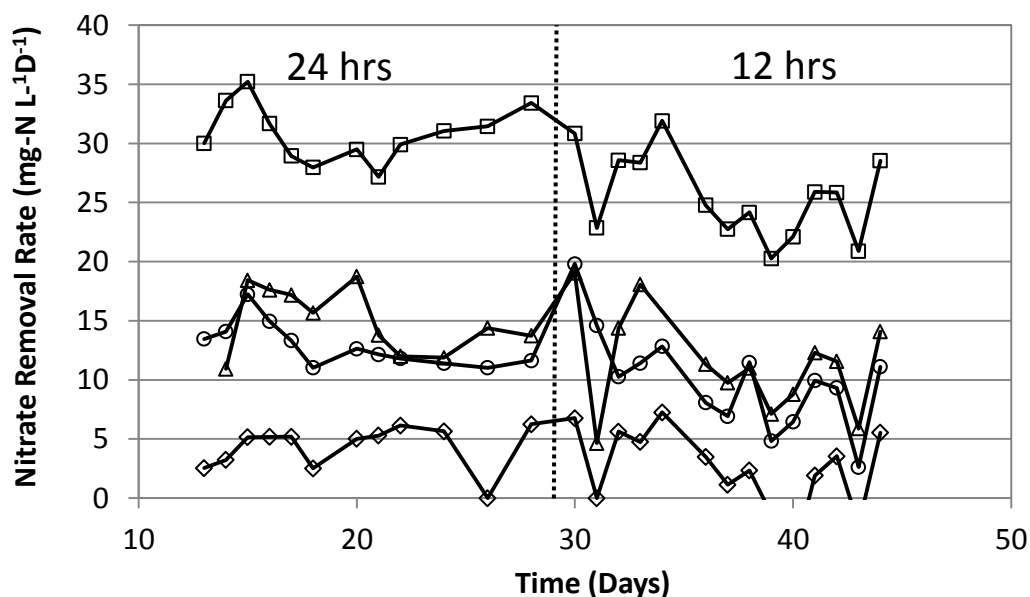**Figure 4.12**: Zero order nitrate removal rates. Two different hydraulic residence times are presented; 24 hours and 12 hours. □ 100% Woodchip, Δ 25% Woodchip, ○ 12.5% Woodchip, ◊ 0% Woodchip

Table 4.7: Zero and first order nitrate removal rates for a 24 and 12 hour hydraulic residence time. ± indicates a 95% confidence interval.

	Zero Order Nitrate Removal Rates (mg-N L^{-1} D^{-1})		First Order Nitrate Removal Rate Coefficients (Days^{-1})	
Volume Woodchip Percentage	24 hrs HRT	12 hrs HRT	k_{24}	k_{12}
100%	30.8 ± 1.4	25.6 ± 1.6	1.16 ± 0.09	0.65 ± 0.05
25%	14.9 ± 1.6	11.4 ± 2.4	0.38 ± 0.07	0.29 ± 0.06
12.5%	12.9 ± 1.0	10.0 ± 2.4	0.31 ± 0.03	0.23 ± 0.06
0%	4.3 ± 1.1	2.4 ± 2.1	0.09 ± 0.03	0.05 ± 0.04

The 25% and 12.5% woodchip columns produced similar nitrate removal rates of 14.9 ± 1.6 mg-N $L^{-1} D^{-1}$ and 12.9 ± 1.0 mg-N $L^{-1} D^{-1}$ at a 24 hour hydraulic residence time. The removal rates were only 52% and 58% lower than the 100% woodchip column even though they contained 75% and 87.5% fewer woodchips. Nitrate removal observed in the 0% woodchip column was 4.3 ± 1.1 mg-N $L^{-1} D^{-1}$ although no exogenous source of carbon was added other than the soil inoculum.

Zero order analysis of the column results indicated that the 50% reduction in HRT resulted in a uniform 20% reduction in absolute nitrate removal rates for the woodchip columns. Nitrate removal did not follow a zero order model as the rates between the two hydraulic residence times are not equivalent. The reduction in first order rate coefficients were 25-44% indicating the process cannot be adequately modeled considering first order nitrate removal.

4.4.3   *Normalized Nitrate Removal Rates*

When normalized to the volume of woodchips present in each column, greater nitrate removal efficiencies were produced at lower woodchip fractions (Table 4.8). However, this efficiency was produced at the cost of lower overall nitrate removal rates since greater rates are observed in the 100% woodchip systems.

Table 4.8: Zero and first order nitrate removal rates per liter of woodchip for a 24 and 12 hour hydraulic residence time.

Volume Woodchip Percentage	Zero Order Nitrate Removal Rates (mg-N L^{-1} D^{-1} $L_{woodchip}^{-1}$)		First Order Nitrate Removal Rate Coefficients (Days^{-1} $L_{woodchip}^{-1}$)	
	24 hrs HRT	12hrs HRT	k_{24}	k_{12}
100%	17	14	0.62	0.35
25%	32	24	0.82	0.62
12.5%	56	43	1.32	0.99
0%	4	2	0.09	0.05

4.4.4 Comparisons to Literature

Similar column conditions were employed in an experiment by Gibert et al. where woodchip columns were filled to a 50% volume ratio with sand, HRTs were 1.7 to 6.6 days, and the influent nitrate concentration was 50 mg-N L^{-1} (2008). The nitrate removals rates observed were 16.7 mg-N L^{-1} D^{-1} and 24.8 mg-N L^{-1} D^{-1}, which is in range of the rates seen in this study.

A 176 x 5 x 1.5 m PRB was constructed to treat glasshouse effluent that had a nitrate concentration ranging from 200-300 mg-N L^{-1} (Schipper, Cameron, & Warneke, 2010). The hydraulic residence time was 5.6 days assuming the same porosity (0.64) found in the 100% woodchip column on this study (Table 3.2). Over a period of two years, the nitrate removal rate ranged from 0-39 mg-N L^{-1} D^{-1}. The highest nitrate removals rates occurred earlier in the experiment, and the average rate was 5-10 mg-N L^{-1} D^{-1} thereafter. Lower nitrate removal rates were produced when the system had a low influent nitrate concentration. A smaller PRB was studied by Schipper et al. (13 x 4 x 1.6 m). A hydraulic residence time could not be calculated for this PRB because an assumption on porosity could not be made; the PRB is a 50:50 woodchip/sawdust mixture. The empty bed hydraulic residence time was 52.0 days, and the influent nitrate concentration averaged 53 mg-N L^{-1}. The average nitrate removal rate during the two year span was 1.4 mg-N L^{-1} D^{-1}, with many low rates occurring due to the low nitrate influent concentrations. Both average nitrate removal rates presented by Schipper et al. are lower

than this study, but are within the same order of magnitude (2010). A similar trend was seen in the compilation of nitrate removal rates presented in a Schipper et al. review article of 2-22 mg-N L^{-1} D^{-1} (Schipper et al.) and 10.0-30.8 mg-N L^{-1} D^{-1} in this study (2010).

First order nitrate removal rate coefficients were calculated for this study to compare rate coefficients from wetland literature. Kadlec and Wallace compiled a list of 72 free water surface wetlands (same type as Talking Water Gardens), and noted the 50th percentile of average nitrate removal rate coefficient was 0.15 $days^{-1}$, assuming an average wetland depth of 0.5 m (2009). The woodchip columns of this study produced rate coefficients between 0.23-1.16 $days^{-1}$. Subsurface flow wetlands are expected to produce higher denitrification rates as they utilize larger anoxic zones with relatively high surface area for microbial growth. The 50th percentile nitrate removal rate coefficient for horizontal subsurface wetlands (HS) was 0.23 $days^{-1}$, which is at the lower range of the column rate coefficients from this study (Kadlec & Wallace, 2009).

Having woodchips replace gravel in HS CWs was the basis of a study performed by Leverenz et al. in systems with HRTs ranging from 1.2 to 2.2 days (2010). The average nitrate removal rate coefficient that Leverenz et al. obtained was 1.30 $days^{-1}$ for the pilot scale woodchip HS CW, which is greater than the rate coefficient range seen in this study (0.23 – 1.16 $days^{-1}$). A separate HS CW was constructed with vegetation planted on top of the media, and produced a higher rate coefficient of 1.41 $days^{-1}$.

4.4.5   Bench Scale Column Experiment Conclusion

- Larger volumes of woodchips and longer hydraulic residence times tended to produce higher nitrate removal rates.
- A minimum hydraulic residence time of 24 hours and a woodchip fraction of 100% were required to produce greater than 50% nitrate removal of the 45 mg NO_3^--N L^{-1} influent.

- The lower woodchip fraction systems produced the highest nitrate removal rates per volume of woodchips. However, this efficiency was achieved at lower overall rates of nitrate removal.
- Rates obtained in this experiment were within range of rates normally seen in literature for similar systems.

4.5   Pilot Scale Column Experiment

All experiments in this study were conducted with short time durations relative to PRB lifetimes (see Section 2.5). A longer term column experiment was conducted to address questions regarding carbon substrate performance in the field (variations in age, environmental temperature, and variations in nitrate load). Only woodchips were used in the experiment as they are known to be effective in field conditions (Schipper, Robertson, Gold, Jaynes, & Cameron, 2010). A 10" diameter PVC pipe (3.5' length) was filled with woodchips and setup at the ATI effluent pond. ATIWW was continually pumped through the column at an average flowrate of 24 mL min^{-1} that resulted in a 24 hour residence time.

The column was operated for three months from late fall through winter, and samples were taken periodically to determine nitrate removal rates (Figure 4.13). Nitrate removal rates decreased as the mean daily temperature decreased and the woodchips aged. Temperatures below 5°C did not exhibit nitrate removal as expected (Bremner & Shaw, 1958).
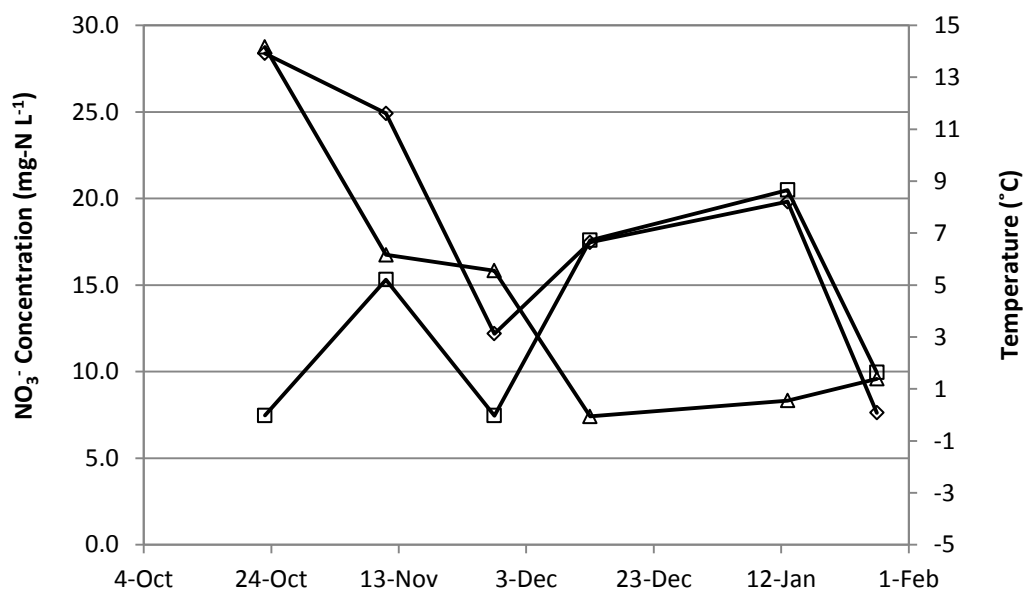
Figure 4.13: Influent and effluent nitrate concentrations in the long term woodchip column. Mean daily temperatures are also depicted. Δ Mean Daily Temperature – right axis; ◊ Influent, □ Effluent – left axis.

4.5.1 *Pilot Scale Column Conclusions*

- Initial nitrate removal rates around 20 mg-N L^{-1} D^{-1} were observed in October when the mean daily air temperature was 14˚C.
- Nitrate removal stopped as mean daily air temperatures decreased below 5˚C.
- The pilot scale column experiment is planned to continue for two more years, which can help identify the influences that temperature, reactor age, and influent nitrate concentration has on nitrate removal rates.

CHAPTER 5: CONCLUSIONS

5.1   Conclusions

The addition of carbon substrates and anoxic zones, to any type of nitrate containing and low organic carbon wastewater improves nitrate removal. In systems studied here, a higher volume of carbon substrate and longer anoxic hydraulic residence times resulted in greater nitrate removal. Both types of biochar showed limited effects on nitrate removal individually; yet, there could be potential benefits to adding biochar as part of a multi-component substrate due to its ability to buffer low pH and possibly provide nutrients. Silage provided high amounts of organic carbon for nitrate removal; however silage washes away in systems with flow deeming it ineffective in the long term. Alder woodchips provided consistent nitrate removal, and would be best suited for placement into constructed wetlands as a long term source of soluble organic carbon to fuel denitrification reactions. Out of 100, 25, and 12.5% woodchip systems by volume (with the remaining volume being gravel), the 12.5% system provided the greatest nitrate removal per volume of woodchip. However, the 100% woodchip system provided the highest rates of removal.

5.2   Further Research

Carbon substrate leaching tests were performed only in batch systems leaving the dynamics of COD leaching in flow through systems an unknown. It is still uncertain if COD leaching dynamics are best attributed to solid/liquid equilibrium, mass transfer kinetics, hydraulic residence time, nutrient and electron acceptor availability, or other unknown factors. Comparisons between different system conditions and system size scaling would be more accurate with a better understanding of the dynamics of COD leaching, readily degradable soluble carbon production, and the effects on denitrification rates in anoxic systems.

One option for woodchip placement within a free water surface (FWS) wetland is to place the woodchips in a deep anoxic zone. In this setup, there would be water flow

through the woodchips and flow over the top of the woodchips. The dynamics of nitrate removal and COD leaching in this system are unknown, and would be useful as a comparison to 100% porous media systems such as the column experiments studied here and subsurface flow wetlands. Natural stratification in FWS wetlands can induce nitrate removal in sediments and organic carbon/nitrate mass transfer and advection in the water column. The addition of woodchips to a FWS CW could increase organic carbon availability and the anoxic zone depth. To better estimate potential nitrate removal in FWS wetlands, continuous flow systems that are open to the atmosphere could serve as test models. In the lab, an aquarium tank could be used with horizontal flow over porous media.

Continuing the pilot scale column experiment, in greater detail, would provide information on environmental effects, carbon substrate age, and variations in influent water quality parameters. The time duration tested in Section 4.5 was throughout the fall and winter, a time of low nitrate removal activity. Summer nitrate removal rates are expected to increase due to increased temperature and quantifying the summer rate relative to winter would be useful information. If the test was continued for two more years comparisons between woodchip age during different yearly seasons could also be made. Influent and effluent concentrations of all nitrogen species, pH, anions, and organic carbon variations would provide detail into nitrate removal rate trends obtained over the long time period.

5.3   Carbon Substrate Application into Constructed Wetlands

The results obtained in this study is most applicable to the design of a horizontal subsurface (HS) zone for the enhancement of nitrate removal within an existing constructed wetland. The amount and cost of woodchips and gravel, and the volume of the wetland needed to convert to subsurface flow are presented in Table 5.1. In this scenario, a 41 acre wetland, with an average depth of 0.5 m (20 Mgal volume), has a hydraulic flowrate of 6 MGD (3.3 day HRT), and an influent nitrate concentration of 45 mg-N L^{-1}. The cost of woodchips used for this study is \$53.50 yd^{-3} (local source) and gravel is \$26.75 yd^{-3} (local source) with a reactor lifetime of 20 years.

Table 5.1: Cost and nitrate removal efficiency of incorporating woodchips into a constructed wetland (based off bench scale column results). System conditions – 41 acre wetland (20 Mgal), 6 MDG hydraulic residence time, 45 mg-N L^{-1} influent nitrate concentration, 20 year reactor lifetime, $53.50 yd^{-3} cost of woodchips and $26.75 yd^{-3} cost of gravel.

Woodchip System Volume Percentage	HRT (hrs)	Woodchip Volume (yd^3)	Gravel Volume (yd^3)	Acres of Reactor	**Percent Nitrate Removal**	**Total Cost[1]**	**Cost per kg-N removed**
100	24	30,000	0	12.4	**0.68**	**$1,605,000**	**$0.32**
100	12	15,000	0	6.2	**0.29**	**$802,500**	**$0.37**
25	24	7,500	22,500	12.4	**0.31**	**$1,003,125**	**$0.43**
12.5	24	4,000	26,000	12.4	**0.26**	**$909,500**	**$0.46**
25	12	4,000	11,000	6.2	**0.13**	**$508,250**	**$0.52**
12.5	12	2,000	13,000	6.2	**0.11**	**$454,750**	**$0.55**

[1]Total cost only includes cost of purchasing the porous media, not transportation, installation, and maintenance costs

The highest percentage of nitrate removal with the lowest cost is the 100% woodchip system with a 24 hour hydraulic residence time. The effluent nitrate concentration coming out of the subsurface woodchip system would be around 14 mg-N L^{-1}, and 30% of the overall wetland volume (12.4 acres) would be filled with woodchips based on a horizontal subsurface depth of 0.5 m.

There are many considerations to take into account when using this analysis. The nitrate removal rates were based off the bench scale column experiment, and lower rates are often achieved in field settings. Field settings have various flowrates, temperatures, plant and microbial growth, influent nitrate concentrations, oxygen intrusion, and mass transfer limitations that effect nitrate removal. Nitrate removal rates produced by the 100% woodchip systems of this study (30.8 mg-N L^{-1} D^{-1} for the 24 hour HRT) were greater than the rates from field PRBs in Schipper et al. (2-22 mg-N L^{-1} D^{-1}) (2010). Cost per kg-N removed was also cheaper in this study (\$0.32-0.55 kg-N^{-1}) compared to Schipper et al. (\$2.39-15.17 kg-N^{-1}) (2010).

There are at least two ways to add woodchips to a constructed wetland – as a subsurface (HS) flow system or setting the woodchips on the bottom bed of the wetland. Rates used in this analysis were those produced in bench scale column tests, and more closely reflect conditions of HS wetlands. Adding porous material to a FWS wetland for the creation of a HS flow zones, will affect the overall hydraulic residence time in the wetland, which was not taken into account during this analysis (if it was taken into account nitrate removal would decrease or more woodchips would be needed to achieve the same nitrate removal). The area required for HS zone emplacement can be minimized by creating a zone of greater depth than the average FWS depth of 0.5 m to provide the required HRT. Nitrate removal would also be less than predicted in the analysis if the woodchips were set on the bottom bed of a FWS wetland. A portion of the flow would not be traveling through the woodchips, decreasing the mass transfer of organic carbon and nitrate into the anoxic zone where the microbes are attached to the woodchips.

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CHAPTER 6: APPENDICES

6.1   Appendix A: Supplementary Figures and Tables

A denitrifying culture was cultivated for use as inoculum in the biological nitrate removal experiments. Three different substrates were used as the organic carbon source; glucose, methanol, and glycerin. All three systems produced nitrate removal, indicating the presence of robust denitrifying cultures (Figure 6.1).
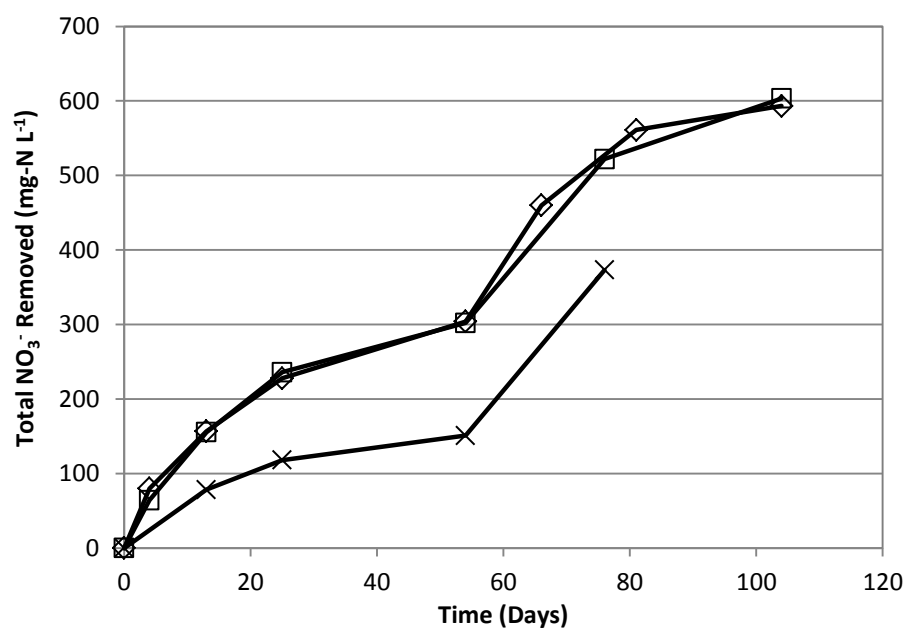

Figure 6.1: Nitrate removed by denitrifying culture. Each bottle was filled with 10 g of soil from TWG. ◊ Glucose, □ Methanol, X Glycerin

The carbon substrate leaching experiment was conducted to quantify the amount of COD, BOD, and nutrients leached from the carbon substrates. Selected metals were also analyzed during the experiment (Table 6.1).

Table 6.1: Carbon substrate leaching results at apparent COD equilibrium. All systems maintained 2:1 (v/v) liquid to solid ratio. ± indicates a 95% confidence interval.

Carbon Substrates	Batch Leaching Experiment Metal Concentrations (mg L^{-1})							
	Ca^{2+}	Cd^{2+}	Cr^{3+}	Cu^{2+}	Fe^{2+}	Mn^{2+}	Na^+	Zn^{2+}
Woodchips	55.6 ± 3.2	0.06 ± 0.02	0.03 ± 0.06	0.13 ± 0	0.43 ± 0.05	0.41 ± 0.02	15.3 ± 8.3	0.23 ± 0.05
Manure Biochar	106 ± 3.1	0.05 ± 0.02	0.05 ± 0.06	0.27 ± 0.04	0.83 ± 0.18	0.16 ± 0.02	844 ± 80	0.32 ± 0.05
Woodchip Biochar	8.30 ± 2.2	0.05 ± 0.01	0	0.10 ± 0.02	0.04 ± 0	0.04 ± 0	11.9 ± 8.8	0.08 ± 0.03
Silage	65.9 ± 0.2	0	0.03 ± 0.02	0.13 ± 0.03	123 ± 35.7	7.45 ± 0.96	16.9 ±1.7	0.97 ± 0.19

The batch adsorption experiment was conducted to quantify the COD adsorption capacity of the biochars. Biochars were employed to woodchip leachate, and were measured for COD until a pseudo-steady state was achieved. Acetate, anions, pH and selected metals were also measured to further quantify adsorption capacity, and in some cases, to quantify the parameters associated with the mixture of woodchip and biochar leachate (Table 6.2). For example, Cl^- was not absorbed with the addition of biochar, but both biochars leached more Cl^- into the woodchip leachate.

Table 6.2: Carbon substrate adsorption results at apparent COD equilibrium for COD, acetate, anions, pH, and selected metals. ± indicates a 95% confidence interval.

Carbon Substrates	COD (mg L^{-1})	Acetate (mg-COD L^{-1})	NO_3^- (mg-N L^{-1})	Cl^- (mg L^{-1})	SO_4^{2-} (mg L^{-1})	Ca^{2+} (mg L^{-1})	Cd^{2+} (mg L^{-1})	Cr^{3+} (mg L^{-1})	Cu^{2+} (mg L^{-1})	Fe^{2+} (mg L^{-1})	Mn^{2+} (mg L^{-1})	Na^+ (mg L^{-1})	Zn^{2+} (mg L^{-1})
Woodchip Leachate/ Initial Concentrations	1375 ± 100	115 ± 30	5.0 ± 1.5	12 ± 1	0	55.6 ± 3.2	0.06 ± 0.02	0.03 ± 0.1	0.13 ± 0	0.43 ± 0.05	0.41 ± 0.02	15 ± 8.3	0.23 ± 0.05
After Manure Biochar Addition	3200 ±1600	50 ± 30	0	1515 ± 50	0	180 ± 40	0.04 ± 0	0	0.21 ± 0.04	2.62 ± 0.65	0.38 ± 0.12	1625 ± 15	0.38 ± 0.13
After Woodchip Biochar Addition	650 ± 300	0	0	65 ± 25	0	45.4 ± 3.8	0.05 ± 0.01	0	0.15 ± 0.01	1.43 ± 1.59	0.23 ± 0.11	61.2 ± 46	0.23 ± 0.04

The bench scale column experiment lasted 72 days and experienced three different system conditions. The system conditions for two time periods (days 13-28 and days 61-72) were a 24 hour hydraulic residence time (HRT) and an influent nitrate concentration around 45 mg-N L^{-1}. Between days 28-44, the system conditions were a 12 hour HRT and an influent nitrate concentration around 45 mg-N L^{-1}. Between days 44-61, the HRT was 24 hours and the influent solution was DI water that was continuously purged with N_2 gas. Therefore, it was assumed that dissolved oxygen was not present and that electron acceptors (including nitrate) were absent in the influent (Figure 6.2). The objective for conducting the experiment without electron acceptors in the influent was to quantify the amount of COD leached from the woodchips before it is utilized. However, COD was not present during the experimental phase without electron acceptors in the influent (Figure 6.3).
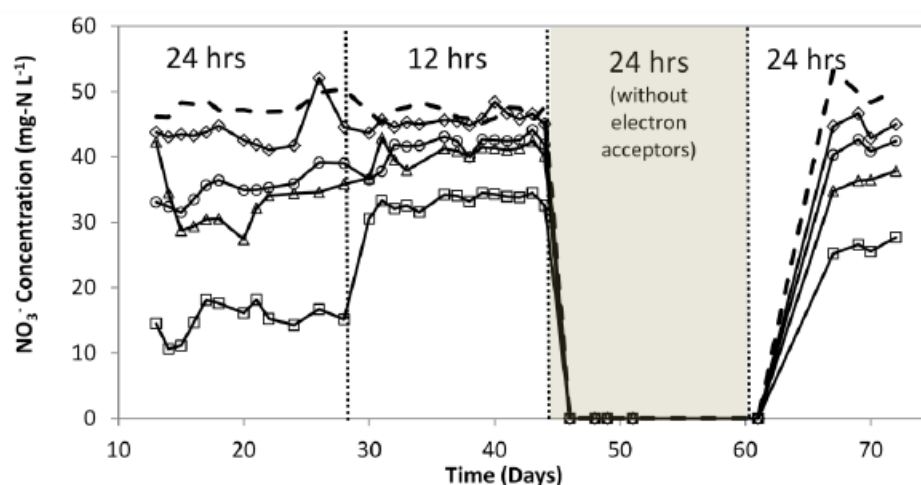

Figure 6.2: Influent and effluent nitrate concentrations for bench scale column experiment. Two different hydraulic residence times are presented; 24 hours and 12 hours, and one system condition did not have electron acceptors in the influent. - - Average Influent Concentration; Effluent Concentrations - ◊ 0% Woodchip, ○ 12.5% Woodchip, ∆ 25% Woodchip, □ 100% Woodchip
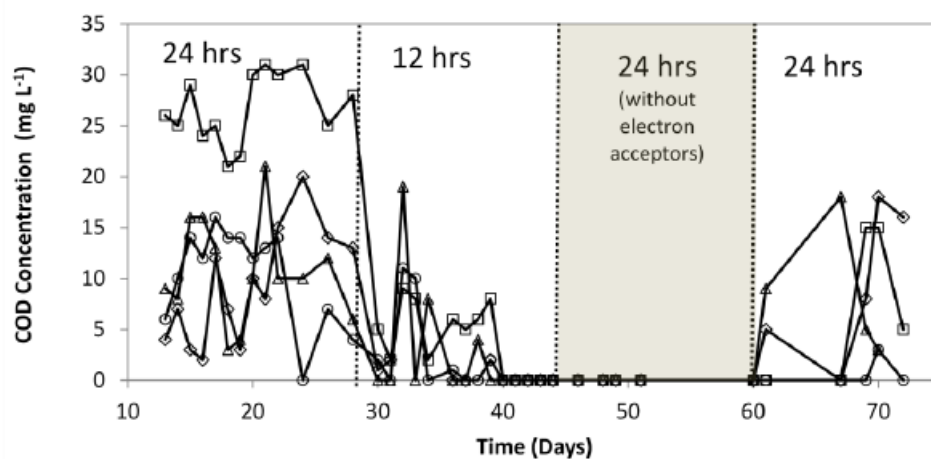
Figure 6.3: Effluent COD concentrations for bench scale column experiment. Two different hydraulic residence times are presented; 24 hours and 12 hours, and one system condition did not have electron acceptors in the influent. □ 100% Woodchip, Δ 25% Woodchip, ○ 12.5% Woodchip, ◊ 0% Woodchip

When oxygen and nitrate were added back to the influent during days 61-72, COD became present in the effluent, and nitrate removal occurred (Table 6.3). COD leaching is caused by the microbial process hydrolysis (Grady, Daigger, & Lim, 1999). Electron acceptors were not in the influent between days 44-61, and COD was not measured in the effluent indicating hydrolysis was inhibited.

Table 6.3: Zero and first order nitrate removal rates for a 24 and 12 hour hydraulic residence time. ± indicates a 95% confidence interval.

Volume Woodchip Percentage	Zero Order Nitrate Removal Rates (mg-N L^{-1} D^{-1})			First Order Nitrate Removal Rate Coefficients (Days^{-1})		
	24 hrs HRT (day 13-28)	12 hrs HRT	24 hrs HRT (day 61-72)	k_{24} (day 13-28)	k_{12}	k_{24} (day 61-72)
100%	30.8 ± 1.4	25.6 ± 1.6	22.9 ± 2.2	1.16 ± 0.09	0.65 ± 0.05	0.65 ± 0.07
25%	14.9 ± 1.6	11.4 ± 2.4	13.2 ± 2.6	0.38 ± 0.07	0.29 ± 0.06	0.33 ± 0.07
12.5%	12.9 ± 1.0	10.0 ± 2.4	8.5 ± 2.4	0.31 ± 0.03	0.23 ± 0.06	0.19 ± 0.06
0%	4.3 ± 1.1	2.4 ± 2.1	6.0 ± 2.1	0.09 ± 0.03	0.05 ± 0.04	0.12 ± 0.04