Nitrate and Phosphate Removal from Denitrification Bioreactors Using Woodchips, Steel Chips And Agricultural Residue Media

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NITRATE AND PHOSPHATE REMOVAL FROM DENITRIFICATION BIOREACTORS USING WOODCHIPS, STEEL CHIPS AND AGRICULTURAL RESIDUE MEDIA

BY
ABDOUL AZIZ KOUANDA

A dissertation submitted in partial fulfillment of the requirements for the Doctor of Philosophy Major in Civil Engineering South Dakota State University 2021
This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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I would like to dedicate this thesis to my parents Karim and Rita. I could not have done it without your support. I am grateful for everything.
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NITRATE AND PHOSPHATE REMOVAL FROM DENITRIFICATION BIOREACTORS USING WOODCHIPS, STEEL CHIPS AND AGRICULTURAL RESIDUE MEDIA.

ABDOUL AZIZ KOUANDA

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Nitrate and phosphate present in agricultural subsurface drainage water can impact surface water quality thus impacting the environment and human health by causing eutrophication and Methemoglobinemia in young infant. Biological denitrification and phosphate adsorption filters are treatments options that can help reduce nitrate and phosphate concentration from agricultural subsurface drainage before it can be discharged in surface water.

The first objective of this dissertation was to determine the Michaelis-Menten kinetics model parameters for nitrate removal in bioreactors using the fresh, aged, and composted woodchips. The composted woodchips achieved a denitrification rate 2.4 times higher than the fresh woodchips which was in term achieved 2.9 times higher denitrification rate than the aged woodchips. Denitrification rates decreased at 5°C compared to 22°C for composted woodchips and fresh woodchips. The composted still performed better at low temperatures compared to fresh woodchips.

The second objective was to determine the impact of different pairing configurations of woodchips and steel chips on nitrate and phosphate removal capacity and how the pairing configurations affect the DOC and iron leaching from the reactors.
Three different reactors pairing configurations were evaluated in this project including woodchips followed by steel chips, steel chips followed by woodchips, and mixed woodchips/steel chips. The effect of low temperature (5°C) on phosphate removal capacity was studied. There was no statistical difference in nitrate and phosphate removed between all three reactors configurations.

The third objective was to compare the long-term nitrate removal rates of agricultural residue media and woodchips. The zero order nitrate removal constants were determined for each material at different days of the experiment. The 13 months nitrate load reduction (g/m³/d) average were as follow corn cobs > corn stover > barley straw > woodchips. Corn cobs demonstrated constantly higher nitrate removal rates than other materials throughout the 13-months experiment.

The fourth objective was to investigate the impact of woodchip pretreatments duration on nitrate removal efficiency using two types of woodchips. The different pretreatments used were solar, natural, and composted. The Solar treated woodchips exhibited higher nitrate removal throughout the 6 months experiments, followed by natural then composted and finally the raw woodchips.
1.1 Agricultural subsurface drainage

Due to the growing of the world population and the need for more food, fertilizers and subsurface drainages have been used as a method to boost agriculture to produce high crop yields. Agricultural subsurface drainage uses a system of interconnected perforated pipes below the soil surface to remove excess water from poorly drained agricultural soil. This system has given opportunities to farmers to use lands with poor natural drainage capabilities by providing a better environment for plant growth thus increasing crop production (Busman and Sands, 2002). However, agricultural subsurface drainage has been identified as a major transport mechanism for nutrients and heavy metals from agricultural fields to surface water entering the Mississippi River basin (Lavaire et al., 2017; Randall and Mulla, 2001; Rozemeijer et al., 2010; Smolders et al., 2010). The amount of precipitation controls irrigation water distribution, which controls nutrients loading into surface waters. During cycles of heavy precipitation, high nutrients loading into surface water is common (Cuadra and Vidon, 2011; Drury et al., 2009; Randall and Mulla, 2001). Discharging highly concentrated nutrients water into surface water such as lakes and rivers can cause health and environmental issues (Driel et al., 2006). The United States Environmental Protection Agency (USEPA) has set a maximum concentration level (MCL) of 10 mg/L for nitrate in drinking water. In fact, high nitrate concentration in water can cause “methaemoglobinaemia” in young infants and can also lead to the degradation of surface water quality by causing eutrophication (Almeelbi and Bezbaruah, 2012; Blowes et al., 1994; Bratieres et al., 2008; Driel et al., 2006).
Eutrophication is a condition in which excess nutrients in water sources such as lakes and rivers promote unsustainable plant growth. The death and decomposition of algae will result in a significant reduction of dissolved oxygen in the water. Due to the water's obscurity, light penetration reduces. The loss of dissolved oxygen in the water has a detrimental effect on the aquatic life causing fish death. Acid rain, air pollution, and the formation of harmful byproducts are some of the other environmental side effects of nitrogen pollution.

![Figure 1.1: Agricultural subsurface Drainage tile. (Retrieved from https://admcoalition.com/drainage-practices/controlled-drainage/)](image)

1.2 Denitrification bioreactors

Nitrate and phosphate are the main nutrients from agricultural drainage water. Several management strategies have been developed to control nutrients loss from agricultural subsurface drainage. Among them, there are crops rotation, fertilizers application rate and timing, buffers, wetlands (natural or artificial) and the use of edge of field treatments (denitrification bioreactors) (Dinnes et al., 2002; Helmers et al., 2011).
Denitrification bioreactors have been mainly used for nitrate removal. A denitrification bioreactor is an excavated channel that is filled with a carbon source, such as wood or agricultural residue media. Subsurface drainage water passes through the bioreactor, where nitrate is biologically converted to nitrogen gas. Field bioreactors come in a variety of shapes, but rectangular and trapezoidal bioreactors are the most common. The size of the bioreactor is determined by the drainage area and the amount of land available (Christianson and Helmers, 2011). Wood byproducts have been shown to provide a consistent rate of nitrate reduction, while still limiting dissolved organic leaching and nitrite formation during denitrification. It has also been shown to be a reliable source of organic carbon for denitrification (Lopez et al., 2017). Others carbon sources have been trialed over the years (Cameron and Schipper, 2010; Della Rocca et al., 2007; Feyereisen et al., 2016; Gibert et al., 2008; Saliling et al., 2007; Shao et al., 2008; Warneke et al., 2011). These alternative carbon sources include, maize cobs, wheat straw, green waste, sawdust, corn cobs, corn stover, barley straw and newspapers.

Nitrate is converted to nitrogen gas (N₂) during biological denitrification (Schipper et al., 2010). Some intermediate byproducts that can be produced during the denitrification phase include NO (Nitric oxide) and N₂O (Nitrous oxide) (Almeida et al., 1995; Wild et al., 1995). Denitrifying bacteria uses the carbon in the woodchips as a food supply (electron donor) and nitrate for respiration (electron acceptor) (Schipper et al., 2010). Most denitrifying bacteria are facultative anaerobes with the majority being heterotrophs (Korom, 1992). Pseudomonas Fluorescens and Paracoccus denitrificans and Pseudomonas denitrificans have been intensively studied as denitrifying bacteria
(Almeida et al., 1995b; Betlach and Tiedj., 1981; Blaszczyk, 1993; Kornaros et al., 1996).

Figure 1.2: Denitrification bioreactors.


1.3 Factors Affecting bioreactors performances

1.3.1 Hydraulic Retention Time

The main key design parameter of designing a bioreactor is the hydraulic retention time. The hydraulic retention time is the time that the water spends in the bioreactor or the time the water takes to travel from the inflow to the outflow. Field bioreactors inflow and outflow structures help to control the water flowrate which defines the hydraulic retention time. Typical field bioreactor HRT varies from 4 to 12 h. Studies have found that there is correlation between hydraulic retention time and the amount of nitrate removed. The longer the hydraulic retention time, more nitrate is removed.
Long hydraulic retention time in bioreactors have been linked to high dissolved organic carbon, greenhouse gas and odor. High nitrite accumulation has also been linked to long HRT (Christianson et al., 2012a; Hoover et al., 2016; Hua et al., 2016; Lepine et al., 2016). High dissolved organic carbon provokes unwanted microbial growth, odor, color, and taste issues in water. High dissolved carbon in water can contribute to a high chlorine demand during disinfection and can lead to the formation of disinfection byproducts (DBPs).

1.3.2 Temperatures

Another important factor affecting bioreactors performances is temperature. From the literature it can be found that bioreactors performance reduces when temperature becomes lower (Feyereisen et al., 2016; Healy et al., 2012; Nordström, et al., 2016). In a study by David et al. (2016), the subsurface water temperature varied between 3 to 8ºC between January and April and got back to 17ºC in July during summer. They found that there is a strong correlation between nitrate removal efficiency and temperature because nitrate removal rates increase with increasing temperature. The calculated factor of the reaction rate increases with every 10 ºC increase in temperature ($Q_{10}$) was 3.8 between 6 and 16ºC. Lower $Q_{10}$ values have been found by different studies (Cameron and shipper, 2010; Elgood et al., 2010).

1.3.3 Woodchips Type and Size

Hardwood (oak) and softwood (pine) were two main types of woodchips that have been used in the literature for denitrification bioreactors. In laboratory column and batch studies, softwood was more efficient in removing nitrate than hardwood (Cameron and
Schipper, 2010; Gibert et al., 2008). Peterson et al. (2015) found that smaller woodchip sizes performed better in removing nitrate than larger woodchips particle sizes. Small woodchips particles offer more surface area per unit mass which can give more space for biofilm growth. A small increase in nitrate removal with increasing woodchip particle size was observed by Cameron and Schipper. (2010). Larger woodchip particles will increase reactor porosity while still increasing the water retention potential, allowing bacteria more time for denitrification. The typical woodchip size used on field bioreactor is in the range of ¼” to 1“(Christianson and Helmers. 2011).

1.3.4 Wood Structure and Chemical composition

Wood is best characterized as a three-dimensional composite biopolymer composed of interconnected cellulose, hemicellulose, and lignin networks with small quantities of inorganics and extractives. Two major components are found in dried wood. The first one is lignin which is about 18% to 35% and carbohydrates which amount of 65% to 75%. Both components are polymeric materials. Other organic extractive and inorganics minerals can be found in wood which can amount 4 to 10% of the overall wood composition. In general, wood is composed of 50% carbon, 6% hydrogen, 44% oxygen and traces number of inorganics. (Rowell et al 2005)

Wood chemical composition changes depending on the part of the tree (branch, stem, and root). Other factors such as the type of wood (softwood or hardwood), climate conditions where the tree is located, and the soil conditions can influence wood chemical compositions. All these factors combined make it difficult to describe the chemical composition of wood precisely for a given tree. Generally, softwoods have a higher lignin content (26-34%), higher cellulose content (40-45%) and lower pentosan (7-14%)
compared to hardwoods that have a cellulose content between 38-49%, lignin 23-30% and pentosan 23-30%.

1.3.5 Woodchips Condition.

Wood has many interior and exterior application which requires its aesthetical and mechanical properties to be conserved over a long period of time. When wood is exposed to outdoor elements such as rainwater and ultraviolet radiation from sunlight, its physical and chemical properties are affected. This process is called weathering. This weathering process results in a change of wood color to grey, a roughening of wood texture followed by cracks on the wood surface (Williams 2005). Wood weathering is different from wood decay, but the weathering process can facilitate wood decay by microorganisms.

The weathering process is a combination of UV lights, water, oxygen, heat, and other pollutants such as SO$_2$, NO$_2$ and O$_3$ (Teaca et al., 2013). Depending on the wood type, morphology and density, wood weathering can happen fast or slow. In fact, Williams et al., 2001 found that hardwood erosion was much slower than softwood.

1.4 Phosphate filters

Recent studies have found that agricultural subsurface drainage is one of the main transports of dissolved P specially in soils that have low absorption capabilities (Sims et al. 1998). P concentrations higher than the 0.02 mg P/L threshold have been reported in agricultural subsurface drainage across the Midwest of the United States (Gentry et al. 2007; King et al. 2015). Biological denitrification is not efficient when it comes to phosphate removal. A variety of natural minerals, manufacturing byproducts, and synthetic products have been tested for their ability to adsorb P, including limestone,
olivine, zeolite, steel slag, fly ash, iron filings, granular activated carbon, Filtralite-P®, and others (Adam et al. 2007; Erickson et al. 2007; Hussain et al. 2011; Oguz 2004; Penn et al. 2011; Reddy et al. 2013). Other studies have used carbon steel byproducts to remove phosphate from water. The oxidized Fe to rust, removed P from a solution via ligand exchange, electrostatic adsorption, and the formation of Fe-P precipitates (Sellner et al., 2019; Allred and Racharaks 2014).

Figure 1.3: Two Stage Subsurface Drainage Treatment. (Hua et al., 2016).

1.5 Denitrification bioreactors current status.

Woodchip bioreactors have been used for more than 25 years to remove nitrate from subsurface drainage water (Christianson and Helmers, 2011). There are still some controversies about nitrate removal kinetics (Christianson et al., 2012). Some studies have reported that nitrate removal rate does not change with nitrate concentrations (zero order reaction) in the range of 3.1 mg/L to 50 mg/L (Gibert et al., 2008; Robertson, 2010; Schipper et al., 2010a; Driel et al., 2006). Camilo et al. (2013), Leverenz et al. (2010) and Moorman et al. (2015) found that a first order reaction better fits nitrate removal in woodchip bioreactors. Hua et al. (2016) reported a switch from zero order reaction to a
first order reaction when nitrate becomes limiting (<3 mg/L). Understanding the mechanism that governs nitrate removal rate in woodchips bioreactor is crucial for the selection of different parameters to enhance bioreactor performance and it helps in the design of field bioreactors. Robertson, (2010) explained that other factors such as dissolved organic carbon might control the reaction. The woodchips condition used in denitrification bioreactors is also one of the main factors that can explain the difference in nitrate removal rates among the literatures. Woodchip’s condition should also be a focus of research since wood storage can affect woodchips chemical and physical properties thus affecting the woodchip carbon content which also affect bioreactor performance.

1.6 Research Gasps

A summary of the literature review has identified several research gaps for nitrate removal in denitrification bioreactors as follows.

1) Most of the woodchip denitrification kinetics studies focused on the impact of operating parameters on nitrate removal. Few studies have evaluated the effect of woodchip quality on denitrification performance in bioreactors.

2) During storage, biological degradation, weathering, and UV degradation of wood could modify its chemical and physical properties of organic substrates derived from woodchips, thereby affecting the denitrification performance of bioreactors. However, little is known about the impact of woodchip storage before installation on nitrate removal in bioreactors.

3) Previous studies have used alternate carbon sources instead of woodchips for denitrification bioreactors over a short period of time. There is the lack of
research evaluating the long-term impact of different carbon sources on denitrification bioreactors.

4) There are few studies in the literature review that have evaluated the phosphate removal potential of woodchips bioreactors over a long-term period. Most of the literature do not consider woodchip bioreactor to be able to achieve phosphate removal.

1.7 Research Objectives

The objectives of this dissertation are to 1) determine the Michaelis-Menten model parameters for nitrate removal in laboratory bioreactors using the fresh, composted and aged woodchips; 2) determine the impact of different pairing configurations of woodchips and steel chips on nitrate and phosphate removal capacity; 3) investigate different carbon sources and determine their removal rates and the adverse effects of these carbon sources DOC leached in the environment over a long period of time (13 months); 4) investigate the impact of woodchip pretreatment (solar, natural, and composted) duration on nitrate removal efficiency using two different types of woodchips.

1.8 Research hypotheses

1) Denitrification in woodchip bioreactors can be operationally defined as a zero-order reaction when treating contaminated water with nitrate much higher than the \( K_m \) values. It follows a first order when treating nitrate lower than the \( K_m \) values.

2) Biological degradation, weathering, and UV degradation of wood can enhance nitrate removal rates in denitrification bioreactors.
3) Agricultural residue media specifically corn cobs can be a good replacement as a carbon source for denitrification bioreactors over a long period of time.

4) Woodchips can remove phosphate. This ability is developed overtime during bioreactor operation.

5) Denitrification bioreactors combined to phosphate filters reactors configurations does not affect the overall nitrate and phosphate removal.
Abstract

Woodchip bioreactors have emerged as a viable water management tool to reduce nitrate contamination from agricultural subsurface drainage, wastewater, and stormwater. Understanding of denitrification kinetics is critical to the design and application of field woodchip bioreactors. The denitrification process in woodchip bioreactors generally obeys a model of Michaelis-Menten type enzyme kinetics. The objective of this study was to determine Michaelis-Menten model parameters for nitrate removal in laboratory bioreactors using the fresh, composted and aged woodchips. The results showed that the maximum nitrate removal rates ($V_{\text{max}}$) were 2.09, 0.88 and 0.30 mg N/L/h, and the half saturation constants ($K_m$) were 2.60, 2.16 and 2.01 mg N/L for the composted, fresh and aged woodchip bioreactors at 22 °C. The $V_{\text{max}}$ values decreased to 0.26 and 0.05 mg N/L/h, and the $K_m$ values decreased to 1.74 and 1.19 mg N/L when the composted and fresh woodchip bioreactors were operated at 5 °C. Denitrification in woodchip bioreactors can be operationally defined as a zero-order reaction when treating contaminated water with nitrate much higher than the $K_m$ values. The nitrate removal efficiency of the bioreactors followed the order of composted woodchips > fresh woodchips > aged woodchips. The average nitrate load reduction rates were 8.81-21.0, 7.36-9.78, and 2.46-3.54 g N/m³/d for the composted, fresh, and aged woodchip bioreactors at influent nitrate concentrations of 10-50 mg N/L and 22 °C. Woodchip composting before bioreactor installation can be used as a practical strategy to enhance denitrification performance of bioreactors.
2.1 Introduction

Nitrate is an important contaminant in aquatic ecosystems worldwide. Excessive export of nitrate from anthropogenic activities can accelerate the rate and extent of eutrophication in surface waters, which can lead to significant detrimental effects on water resources and public health (Anderson et al., 2002; Dodds, et al., 2008). Therefore, managing the nitrogen cycle has been identified as one of the major challenges in various environmental systems (Galloway et al., 2003). Woodchip bioreactors have emerged as a viable management tool to reduce nitrate export from agricultural drainage, urban stormwater, and wastewaters (Schipper et al., 2010; Peterson et al., 2015; Lepine et al., 2016; Lopez-Ponnada et al., 2017). Woodchips are used in bioreactors to support the growth of denitrifying bacteria which can convert nitrate to inert nitrogen gas. Woodchips are widely available and inexpensive materials that can serve as a long-persistent organic carbon source for the denitrification process. The relatively large particle sizes and physical stability of woodchips allow high permeability such that woodchip bioreactors are well suited for nitrate removal in various sources (Christianson et al., 2010). Long-term field experiences indicate that woodchip bioreactors can provide consistent nitrate removal for 5 to 15 years with little maintenance required (Robertson et al., 2000; Schipper et al., 2010).

Nitrate removal efficiencies of woodchip bioreactors can be affected by many factors including water temperatures, influent nitrate concentrations, hydraulic retention times, and woodchip age (Addy et al., 2016). Understanding of nitrate removal kinetics is crucial for woodchip bioreactor design and application. Many field and laboratory woodchip denitrification studies showed that nitrate concentrations typically decreased
linearly along the length of reactors, suggesting zero-order nitrate removal kinetics (Schipper et al., 2005; Robertson, 2010; Warneke et al., 2011). A zero-order denitrification kinetics indicates that nitrate removal rates in woodchip bioreactors do not change with influent nitrate concentrations. Other independent parameters such as the release rate of biodegradable organic substrates may control nitrate removal rates. A number of studies also used a first-order model to describe nitrate removal in woodchip bioreactors (Chun et al., 2009; Camilo et al., 2013; Moorman et al., 2015). Influent nitrate concentrations become an important factor that limits nitrate removal rates under the first-order reaction scenario.

The variations in the observed woodchip denitrification reaction orders may be explained by a model of Michaelis-Menten type enzyme kinetics (Schipper et al., 2010; Halaburka et al., 2017; Zhao et al., 2018). According to the Michaelis-Menten model, nitrate removal follows zero-order kinetics when nitrate concentrations are much higher than the half saturation constant, and switches to first-order kinetics when nitrate concentrations are much lower than the half saturation constant. Hua et al. (2016) observed that nitrate removal in a laboratory column reactor followed zero-order reaction (rate constant \( k = 1.42 \text{ mg N/L/h} \)) during the first 12 h and changed to first-order reaction \( (k = 0.49 \text{ h}^{-1}) \) after 12 h when nitrate became limiting \( (< 3.3 \text{ mg N/L}) \). Hoover et al. (2016) reported that nitrate reduction rates increased with influent nitrate concentrations, and became saturated when nitrate increased to 30-50 mg N/L. Although it has been recognized that woodchip denitrification generally obeys Michaelis-Menten kinetics, few studies have determined key parameters of this kinetic model for nitrate removal in woodchip bioreactors. Ghane et al. (2015) used a Forcheimer hydraulic model
with Michaelis-Menten kinetics to evaluate nitrate removal rates in a horizontal woodchip reactor bed. The model results showed a half saturation constant ($K_m$) of 7.2 mg N/L and a maximum removal rate ($V_{\text{max}}$) of 7.1 mg N/L/h. This $V_{\text{max}}$ value is equivalent to a load reduction rate of 144.8 g N/m$^3$/d, which is substantially higher than the nitrate reduction rates (2-22 g N/m$^3$/d) observed in field woodchip bioreactors (Schipper et al., 2010). Nordstrom and Herbert (2017) reported $V_{\text{max}}$ of 0.25-1.64 mg N/L/h and $K_m$ of 2.3-10.6 mg N/L for a series of column tests using a mixture of woodchips and activated sludge at 5-22 °C. The reported half saturation constants from both studies are somewhat higher than the expectations from several field and laboratory studies that showed nitrate limiting conditions did not occur until the concentrations were below approximately 1-3 mg N/L (Roberston, 2010; Hua et al., 2016; Halaburka et al., 2017; Zhao et al., 2018).

Most of the woodchip denitrification kinetics studies focused on the impact of operating parameters on nitrate removal. Few studies have evaluated the effect of woodchip quality on denitrification performance in bioreactors. Several studies have shown that woodchip ageing could reduce the efficiency of bioreactors over time due to the loss of available organic substrates (Robertson 2010; Halaburka et al. 2017). Woodchip quality can also be changed by natural weathering processes during woodchip storage before bioreactor installation. Biological degradation of wood by bacteria and fungi during storage could modify chemical and physical properties of organic substrates derived from woodchips, thereby affecting the denitrification performance of bioreactors. However, little is known about the impact of woodchip biodegradation before installation on nitrate removal in bioreactors. It is clear that with increased applications of woodchip
bioreactors for nitrate control, a better understanding of nitrate removal kinetics is needed in order to improve the design and operation of woodchip bioreactors. The objective of this study was to determine Michaelis-Menten kinetics model parameters for nitrate removal in bioreactors using the fresh, aged and composted woodchips. This study focused on the modelling analysis of the effect of woodchip quality and pre-treatment on nitrate removal kinetics in bioreactors. The aged woodchips were produced by operating a bioreactor for 12 months under saturated hydraulic conditions, and the composted woodchips were produced by composting woodchips with soil and moisture for three months to simulate the biological degradation of wood during storage. Laboratory column bioreactors filled with different woodchips were operated under a wide range of influent nitrate concentrations and temperatures to generate sufficient experimental data to determine nitrate removal kinetics model parameters.

2.2 Materials and Methods

2.2.1 Woodchips and Column Reactors

Woodchips made from cottonwood (Populus deltoids) were obtained from a playground woodchip distributor in Sioux Falls, South Dakota. The distributor receives woodchips produced from South Dakota and Minnesota. After collection in the spring season, woodchips larger than 6 cm were removed manually and woodchips smaller than 4 mm were removed by sieving. The woodchips were then gently washed with distilled water to remove dirt and floating fine particles, and air dried before use. The size distribution of processed woodchips was 0.4-1 cm 11%, 1-3 cm 53%, and 3-6 cm 36%, and these woodchips were referred to as the “fresh woodchips” in this study. An up-flow column reactor filled with the fresh woodchips was operated at room temperature (22 °C)
and a hydraulic retention time of 12 h for 12 months. The column reactor was fed with an artificial runoff solution (Table 2.1) that contains a nitrate concentration of 50 mg N/L. After 12 months, the reactor effluent dissolved organic carbon concentrations were below 2 mg/L. The woodchips in the bioreactor after 12-month operation were defined as the “aged woodchips”. A composting process with soil and moisture was used to treat the fresh woodchips and produce the “composted woodchips”. The composting experiments were conducted in 5-gallon plastic buckets. Approximately 2.5 kg woodchips were mixed with 1.5 kg soil in each bucket. The soil sample was collected from the South Dakota State University research farm in Volga, SD. Each bucket was capped and placed at an open space outside Crothers Engineering Hall at South Dakota State University, Brookings, SD. A volume of 500 mL of water was added to each bucket weekly to maintain moisture during the composting process. The composting experiment were conducted from June to August with daily average temperatures varied between 13.6 and 26.8 °C. Figure 2.8 shows pictures of the three woodchips used for this study.

Three clear acrylic tubes with a length of 1.2 meters and inside diameter of 8.85 cm were used to construct up-flow column reactors. Each reactor had 12 sampling ports installed along the length of the column at 10 cm intervals. Approximately 1840 g of each type of woodchips were added to each reactor, resulting in an average packing density of 0.25 g/cm³. Distilled water was added to each column to soak the woodchips for 48 h before the determination of reactor porosity. Drainable porosity was determined by draining each reactor over 1 h, and the resulting porosities were 49.9, 49.7 and 44.1%, respectively, for the fresh, aged, and composted woodchip bioreactors.

2.2.2 Reactor Hydraulic Retention Times (HRT)
After the porosity tests, distilled water was pumped to the fresh, aged and composted woodchip reactors at flow rates of 5.1, 5.1 and 4.5 mL/min, respectively. The flow travel time through each section between two sampling ports was determined based on the rising water level and recorded using a stopwatch. The resulting flow travel times of the 36 sampling sections of the three reactors were within a range of 1±0.05 h, which were very close to the theoretical flow travel time of 1 h. This indicates that the woodchips were well mixed and evenly packed in each column reactor. It was determined that theoretical HRTs based on drainable porosities were adequate to describe the flow travel times through the column reactors and were used as the reaction times in the calculation of nitrate removal rates in each sampling section.

2.2.3 Column Reactor Experimental Conditions

Variable speed peristaltic pumps (Masterflex Model L/S, Cole Parmer, Vernon Hills, IL) were used to pump an artificial runoff water from an influent tank to the column reactors during this study. The artificial runoff water was prepared based on a previously published method (Hua et al., 2016), and it was composed of various ionic constituents and trace minerals (Table S1) to ensure that microbial growth was not limited. KNO₃ was added to the artificial runoff water to achieve different influent nitrate concentrations for the column experiments. A gas diffuser was installed at the bottom of the influent tank to continuously bubble nitrogen gas to reduce the dissolved oxygen (DO) concentrations below 3.0 mg/L. This was done to minimize the potential impact of influent DO on the denitrification process.

All three column reactors were operated at an influent nitrate concentration of 50 mg N/L and an HRT of 12 h under room temperature (22 °C) for 1 month to achieve
steady-state nitrate removal conditions. It was observed that daily variations in reactor effluent nitrate were less than 10% under the steady-state conditions. After that, nitrate removal kinetics experiments were conducted by varying influent nitrate concentrations in the sequence of 50, 40, 30, 20, 10, 5 and 3 mg N/L at an HRT of 12 h. The column reactors were operated for 7 days at each concentration. The first 4 days were used as the stabilization period to ensure that steady-state removal was reached for each concentration, and the last 3 days were used as the sampling period. Two sampling events were conducted during each sampling day, and samples were taken at each sample port along the length of the column. Six replicates were obtained for each sample port at each influent concentration. In addition to the 12 h HRT experiments, the fresh and composted woodchip bioreactors were also operated at HRTs of 24 and 6 h and an influent concentration of 10 mg N/L to evaluate the impact of flow variations on reactor performance. Same sampling procedures were used during the variable HRT experiments.

After the completion of the above-mentioned experiments at 22 ºC, the fresh and composted woodchip bioreactors were moved into a cool room with a controlled temperature of 5 ºC. The two column reactors were allowed to stabilize the nitrate removal efficiency for 1 month at an influent concentration of 50 mg N/L and an HRT of 12 h in the cool room. The variation in nitrate removal during the one-month stabilization period was less than 15%. Then, nitrate removal kinetics experiments at 5 ºC were conducted by varying influent nitrate concentrations in the sequence of 50, 40, 30, 20, 10, 5 and 3 mg/L. Samples were taken based on the same procedures used during the room temperature experiments.
During each sampling event, a 40 mL sample was collected at each sampling port following a sequence of the topmost sample port to the bottom sample port. The collected samples were filtered using 0.45 µm membrane filters (MilliporeSigma, Burlington, MA) and then stored at 4º C before analysis of nitrate and nitrite. Dissolved organic carbon (DOC) concentrations were also measured for reactor effluent samples after filtration by 0.45 µm membrane filters. Weekly samples were taken from the influent tank to measure DO concentrations.

2.2.4 Analytical Methods

All solutions used in this study were prepared with ultrapure water (18 MΩ-cm) produced by a Barnstead Nanopure system (Thermo Fisher, Waltham, MA). The influent solutions were adjusted to pH 7 using sodium hydroxide or sulfuric acid solutions. The chemicals used in this study were of American Chemical Society reagent grade and were purchased from Sigma Aldrich (St Louis, MO). Nitrate and nitrite ions were measured using a DX-500 Ion Chromatography instrument with an IonPac AS-14 analytical column (Dionex, Sunnyvale, CA). The DOC concentrations were determined with a Shimadzu TOC-5000 Analyzer (Shimadzu Corp., Kyoto, Japan) according to Standard Method 5310 B (APHA et al., 2012). The pH value of each solution was measured using an Orion 290A+ advanced ISE/pH/mV/OPR meter (Thermo Fisher). DO concentrations were measured using an YSI ProOBOD probe (YSI Inc., Yellow Springs, OH).

After the bioreactor experiments, woodchips from the fresh and composted woodchip bioreactors were air dried under room temperature. Woodchip samples before and after the experiments were collected for surface characterization by a scanning electron microscope (SEM) equipment (Model Hitachi S-3400N, Santa Clara, CA).
2.2.5 Michaelis-Menten Model Parameters

Denitrifying bacteria in woodchip bioreactors remove nitrate through a respiration reaction that uses nitrate as an electron acceptor and organic carbon as an electron donor. A dual-substrate Michaelis-Menten model can be used to describe nitrate removal rates in woodchip bioreactors (Halaburka et al., 2017).

\[ V_i = V_{\text{max}} \left( \frac{C_j}{K_c + C_j} \right) \left( \frac{N_i}{K_n + N_i} \right) \]  

(1)

Where \( V_i \) is the nitrate removal rate (mg N/L/h), \( V_{\text{max}} \) is the (mg N/L/h), \( C_j \) is the concentration of DOC (mg C/L), \( K_c \) is the half saturation constant for DOC (mg C/L), \( N_i \) is the concentration of nitrate (mg N/L), and \( K_n \) is the half saturation constant for nitrate (mg N/L). Organic carbon released from woodchips is a complex mixture of heterogeneous organic compounds, and only a fraction of the DOC is biodegradable. Therefore, it is a challenge to implement this dual-substrate model to predict nitrate removal rates. During this study, each nitrate removal kinetics experiment was conducted under short-term, steady-state conditions to limit DOC variations. We assume that the release rates of biodegradable organic carbon from woodchips remain relatively stable during each kinetics experiment. Thus, a simplified, single-substrate Michaelis-Menten model can be used to describe nitrate removal rates for each kinetics experiment (Betlach and Tiedje, 1981).

\[ V_i = \frac{V_{\text{max}} S_i}{K_m + S_i} \]  

(2)

Where \( V_{\text{max}} \) is the maximum removal rate (mg N/L/h), \( K_m \) is the half saturation constant (mg N/L), and \( V_i \) is the removal rate (mg N/L/h) at a nitrate concentration of \( S_i \) (mg N/L).
The Michaelis-Menten equation can be transformed to the Lineweaver-Burk equation, which has been widely used to determine the enzyme kinetics parameters $V_{\text{max}}$ and $K_m$ using the experimental data (Lineweaver and Burk, 1934).

$$\frac{1}{V_i} = \frac{1}{V_{\text{max}}} + \frac{K_m}{V_{\text{max}}} \left(\frac{1}{S_i}\right)$$  \hfill (3)

According to the Lineweaver-Burk equation, the plot of inverse of nitrate removal rate ($1/V_i$) versus inverse of nitrate concentration ($1/S_i$) results in a straight line with the slope equal to $K_m/V_{\text{max}}$ and y-intercept equal to $1/V_{\text{max}}$. The average nitrate concentration ($C_i$) of the 6 replicates of each sampling port during the nitrate removal kinetics experiments was used as substrate concentration ($S_i$) for the Lineweaver-Burk plot. The nitrate removal rate ($V_i$) was calculated using the following equation.

$$V_i = \frac{C_i - C_{i+1}}{\Delta t}$$  \hfill (4)

Where

$V_i$ = nitrate removal rate at concentration $C_i$ (mg N/L/h)
$C_i$ = average nitrate concentration of sampling port $i$ (mg N/L)
$C_{i+1}$ = average nitrate concentrations of sampling port $i+1$ (mg N/L)
$\Delta t$ = flow travel time between sampling ports $i$ and $i+1$ (h)

For the 12 h HRT kinetics experiments, the theoretical flow travel time between 2 adjacent sampling ports was 1 h, which was used as $\Delta t$ for the calculation. Theoretically, $V_i$ is the instantaneous nitrate removal rate at concentration $C_i$ for the Michaelis-Menten model. Considering the generally slow nitrate reduction rates by woodchips, the spatial sampling time interval of 1 h was determined to be sufficient to produce nitrate removal rates that approximate the instantaneous rates. Linear regression analysis was used to determine $V_{\text{max}}$ and $K_m$ from the Lineweaver-Burk plot.
2.2.6 Denitrification Temperature Coefficient

The \( V_{\text{max}} \) values obtained at temperatures of 5 and 22 °C were used to determine the \( Q_{10} \) temperature coefficient for nitrate removal by the following equation.

\[
Q_{10} = \left( \frac{R_2}{R_1} \right)^{\frac{10^\circ\text{C}}{T_2-T_1}}
\]

(5)

Where \( R_1 \) and \( R_2 \) are the \( V_{\text{max}} \) values at temperatures \( T_1 \) (5 °C) and \( T_2 \) (22 °C), respectively.

2.2.7 Statistical Analysis

Statistical analysis was conducted using the one-way ANOVA with post-hoc Tukey HSD tests to compare the nitrate removal of different reactors. The RStudio (version 1.2) was used for the statistical tests.

2.3 Results and Discussion

2.3.1 Nitrate Removal Rates of Woodchip Column Reactors

Figure 2.1 presents the nitrate concentration profiles along the length of columns for selected influent concentrations at an HRT of 12 h and a temperature of 22 °C. All three bioreactors exhibited linear nitrate reductions with increasing flow travel time when the influent concentrations were 50 and 30 mg N/L, suggesting that the denitrification process was not largely limited by nitrate for the entire concentration range (7.2-50 mg N/L) observed in the column reactors. The average nitrate removal percentages were 19.6, 7.1 and 47.6% for the fresh, aged and composted woodchip reactors when the influent concentration was 50 mg N/L. The corresponding removal percentages increased to 32.4, 10.4, and 76.1% at an influent concentration of 30 mg N/L. The composted woodchips showed higher nitrate removal capacity than the fresh woodchips, which performed better than the aged woodchips. When the influent nitrate concentration
decreased to 10 mg N/L, the fresh and aged woodchip reactors steadily reduced nitrate to 2.6 and 7.5 mg N/L after 12 h, and no apparent nitrate limiting condition was observed. However, the composted woodchip reactor changed from linear reduction (zero-order) to non-linear reduction (first order) at approximately 3 mg N/L, likely due to its high nitrate removal rates. All three reactors demonstrated curvilinear relationships between nitrate reduction and flow travel time for an influent concentration of 3 mg N/L, indicating that nitrate limiting condition occurred in all three bioreactors.

Figure 2.1 shows the nitrate concentration profiles in the fresh and composted woodchip reactors for selected influent concentrations at an HRT of 12 h and a temperature of 5 °C. As expected, denitrification in the two reactors was substantially inhibited at this low temperature. For example, the removal percentages were only 1.1 and 5.9% for the fresh and composted woodchip reactors at an influent concentration of 50 mg N/L, and the removal percentages increased to 1.7 and 9.5% at an influent concentration of 30 mg N/L. At 5°C, the fresh woodchip reactor exhibited linear nitrate reduction for the entire concentration range of 2.5-50 mg/L shown in Figure 2.2. Curvilinear relationship between nitrate reduction and flow travel time was observed for the composted woodchip reactor at an influent concentration of 3 mg N/L. However, the degree of curve is much smaller compared to the experiments at 22 °C, suggesting reduced nitrate removal rates at 5 °C.

Table 2.2 presents the nitrate load reduction rates using the unit of g N/m³ bioreactor volume/d for the three column reactors. The nitrate load reduction rates were 8.81-21.0, 7.36-9.78, and 2.46-3.54 g N/m³/d, respectively, for the composted, fresh and aged woodchip bioreactors for influent concentrations of 10-50 mg N/L at 22 °C. These
nitrate load reductions fall well in the range (2-22 g N/m³/d) that has been observed for field bioreactors (Schipper et al., 2010). Statistical test results showed that the denitrification capacities of the three column reactors were significantly different (p<0.05) and were in the order of composted woodchips > fresh woodchips > aged woodchips. The nitrate load reduction rates of the composted woodchips were 1.20-2.15 times higher than that of the fresh woodchips, which were 2.76-3.37 times higher than that of the aged woodchips. Influent nitrate concentrations also had an impact on the nitrate load reduction rates. Variations in nitrate load reduction rates were less than 12.5% for the three column reactors when the influent concentration decreased from 50 to 30 mg N/L. When the influent concentration further declined to 10 mg N/L, all three reactors showed large decreases in nitrate load reduction rates, and the decreases were 58.0, 24.7, and 30.5% for the composted, fresh, and aged woodchips, respectively, compared to 50 mg N/L.

The nitrate load reduction rates were 2.18-2.60 and 0.53-0.55 g N/m³/d, respectively, for the composted and fresh woodchips for influent concentrations of 10-50 mg N/L at 5 °C. Compared to 22 °C, the nitrate load reduction rates decreased by 75.3-87.6% (average 84.8%) and 92.7-94.5% (average 94.1%) for composted and fresh woodchips at different nitrate concentrations. This indicates that the composted woodchip bioreactor had a better tolerance to the low temperature compared to the fresh woodchip bioreactor as evidenced by the lower reductions in denitrification performance at 5 °C.

2.3.2 Woodchip DOC Leaching and SEM Images

The results of column reactor experiments suggest that denitrification capacity of woodchips decreased after woodchip aging but increased after woodchip composting.
Reactor effluent DOC concentrations were analyzed to evaluate whether organic substrate concentrations played a role in influencing nitrate removal among the three reactors, and the results are shown in Figure 2.3. The effluent DOC concentrations of the fresh woodchip reactor varied between 7.1 and 8.9 mg/L, with an average of 8.0 mg/L during the kinetics experiments at 22 °C. The DOC concentrations were 5.5-7.4 mg/L (average 6.3 mg/L), and 1.4-1.9 mg/L (average 1.6 mg/L) for the composted and aged woodchip reactors under the same experimental conditions. Lower DOC levels in the aged woodchip reactor are expected because of the 1-year operation under saturated hydraulic conditions before the kinetics experiments. Microbial utilization and DOC leaching during the 1-year reactor operation gradually depleted available organic substrates from woodchips, thereby leading to decreased nitrate load reduction rates in the aged woodchip bioreactor. The narrow range of effluent DOC variations (< 0.5 mg/L) during the nitrate removal kinetics experiments suggest that the DOC leaching from the aged woodchips had reached a relatively steady state. Robertson (2010) compared nitrate removal of woodchips with varying age and the results showed that the nitrate removal rates of the 2-year-old media and the 7-year-old media were within 52.6-78.6% and 39.6-59.1% of the fresh woodchips. In this study, the aged woodchips exhibited nitrate load reductions that were 29.7-36.2% of that of the fresh woodchips. Therefore, the nitrate load reduction rates observed in the aged woodchip reactor could represent the long-term sustained denitrification performance of field bioreactors after several years of operation under intermittent flow conditions.

The composted woodchips exhibited higher nitrate load reduction rates than the fresh woodchips despite the lower DOC levels found in the reactor effluents. Therefore,
the quantity of organic carbon in the reactors cannot explain the observed differences in nitrate removal between the two column reactors. It is likely that the composting process before the bioreactor experiments modified the quality of wood materials such that more biodegradable organic compounds were produced to promote the denitrification reactions. This demonstrates the importance of woodchip quality on nitrate removal in bioreactors. Other researchers have used external carbon sources and drying-rewetting cycles to improve the carbon availability for nitrate removal in woodchip reactors (Roser et al., 2018; Maxwell et al. 2019). The DOC levels in the fresh and composted woodchip reactors declined to 1.2-1.5 mg/L (average 1.3 mg/L) and 0.7-1.0 mg/L (average 0.8 mg/L), respectively, during the kinetics experiments at 5 °C. The average reactor effluent DOC at 5 °C decreased by 83.6-86.6% for the two reactors compared to 22°C. It is clear that low temperatures could inhibit both microbial denitrification rates and woodchip DOC leaching rates, which resulted in overall lower nitrate removal in the column reactors.

Figure 2.4 presents SEM images of fresh and composted woodchips before and after the bioreactor experiments. The surface of the fresh woodchip (Image A) showed fresh open cell walls by tangential cut. After the bioreactor experiments, the surface of the aged woodchip clearly exhibited deterioration and degradation by water and bacteria (Image B). Water attack and biodegradation caused erosion of cell walls into adjacent areas. Localized cavitation on the wood surface is also evident from the SEM image. Images C and D in Figure 2.4 present the surface morphological information of the composted woodchips before and after the bioreactor experiments. It is evident that fine soil particles were attached to the woodchip surface during the composting process and
partially covered the wood surface, which makes the cell wall structure illegible. Erosion, cracks, and cavitation were observed on the composted woodchip surface after the experiments. Some of the attached soil particles were not easily detached from the wood surface even after the bioreactor experiments.

Wood is primarily composed of cellulose, hemicellulose, and lignin. Cellulose is a glucose polymer, hemicellulose is a heteropolysaccharide polymer, and lignin is a complex cross-linked polymer with amorphous, aromatic, and heterogeneous structures (Andlar et al., 2018). Microbial utilization of woodchip organic substrates requires the production of soluble organic carbon by enzymatic hydrolysis first. Under anaerobic conditions, cellulase enzymes are used by bacteria to break down the crystalline cellulose into smaller soluble oligosaccharides, which can be metabolized by bacterial community in biofilm (Lynd et al., 2002). Compared to cellulose, lignin is generally resistant to bacterial biodegradation and therefore it provides a protective barrier to enzymatic decomposition of cellulose in wood. The soluble organic hydrolysis rate typically decreases over time because fewer cellulose sites are available for cellulase enzyme adsorption. Therefore, the DOC release from woodchip bioreactors generally follows the power law exponential decline over time (Abusallout and Hua, 2017). Wood-degrading fungi are the primary microorganisms responsible for lignincellulose degradation in nature (Andlar et al., 2018). During the woodchip composting process in this study, it was observed that fungi were widely distributed on the woodchip surface (Figure 2.9). The modification and removal of lignin by fungi will increase the enzyme access to hemicellulose and cellulose, which is likely one of the key factors that led to increased denitrification potential of the composted woodchips. Under aerobic conditions, fungi are
also effective at depolymerizing hemicellulose and cellulose to produce monomers and other smaller units, which can also help improve the biodegradability of organic substrates. Moreover, fungi and bacteria degradation of woodchips during composting would also cause surface erosions with depressions, channels, and honeycomb patterns, which can create more surface areas for biofilm growth. These factors together may have contributed to the increased woodchip denitrification efficiency after composting.

2.3.3 Nitrate Removal Michaelis-Menten Kinetics Parameters

Figure 2.5 uses the fresh woodchip bioreactor as an example to illustrate the application of Lineweaver-Burk plot to process the nitrate removal experimental data. The high linear regression coefficient ($R^2$) suggests that the denitrification process in the bioreactor can be sufficiently described by the Michaelis-Menten model. After the model parameters $V_{max}$ and $K_m$ were obtained from the slope ($K_m/V_{max}$) and intercept ($1/V_{max}$) of the Lineweaver-Burk plot, the developed Michaelis-Menten model was then used to predict the nitrate removal rates of the bioreactor at different nitrate concentrations. Figure 2.6 presents a comparison of nitrate removal rates obtained from the model prediction and the experimental data. It is evident that the model results closely tracked the experimental results.

Table 2.3 summarizes the Michaelis-Menten model parameters for the three woodchip bioreactors. The model $V_{max}$ values at 22°C were 2.09, 0.88, and 0.30 mg N/L/h for the composted, fresh, and aged woodchips, respectively. The maximum removal rate of the composted woodchips was 2.4 times higher than that of the fresh woodchips, which was again 2.9 times higher than that of the aged woodchips. These results quantitatively agree with the analysis of nitrate load reductions of the three
bioreactors shown in Table 2.2. Despite the large differences in the maximum removal rates, the three bioreactors exhibited similar half saturation constants. The $K_m$ values at 22 °C were 2.60, 2.16 and 2.01 mg N/L for the composted, fresh and aged woodchips, respectively. It seems that woodchips that had high maximum nitrate removal rates also exhibited relatively high half saturation constants. Both $V_{max}$ and $K_m$ values declined under the cold temperature of 5 °C. The $V_{max}$ values decreased to 0.26 and 0.05 mg N/L/h for the composted and fresh woodchips, which correspond to 87.6 and 94.3% reductions compared to 22 °C. These results confirm that low temperature of 5 °C substantially inhibited biological denitrification activities in the woodchip bioreactors and that the composted woodchips showed better resistance to the cold temperature. The better performance of the composted woodchip bioreactor at 5 °C may be attributed to lignin-cellulose fungi degradation, production of biodegradable organic substrates, and surface modification during the composting process. The cold temperature of 5 °C also decreased $K_m$ values to 1.74 and 1.19 mg/L, respectively, for composted and fresh woodchips. The model coefficients ($R^2$) of different bioreactors varied between 0.970 and 0.993, suggesting that the nitrate removal data obtained from this study fit the Michaelis-Menten model well.

The Michaelis-Menten $K_m$ values obtained at 22 °C predict that the nitrate removal by the three woodchip bioreactors follows a zero-order reaction when the nitrate concentration is much higher than 2.01-2.60 mg N/L. A switch from a zero-order reaction to a mixed order reaction occurs when the nitrate concentration is similar to 2.01-2.60 mg N/L. The denitrification reaction becomes a first-order reaction when the nitrate concentration is much lower than 2.01-2.60 mg N/L. These model results can be used to
analyze nitrate removal kinetics in woodchip bioreactors for various applications. Nitrate concentrations in agricultural subsurface drainage and some wastewater sources such as aquaculture wastewater are typically higher than 10 mg N/L (Fausey et al., 1995; Lepine et al., 2016). Therefore, nitrate removal in woodchip bioreactors for subsurface drainage and wastewater treatment can be operationally defined as a zero-order reaction. Nitrate is not a major limiting factor under such conditions. It is possible that cellulose enzymatic and abiotic hydrolysis and subsequent metabolism of oligosaccharides are the limiting factors controlling the denitrification rate when nitrate is present at relatively high concentrations. For urban stormwater with relatively low nitrate concentrations (e.g., <10 mg N/L), mixed-order and first-order reactions may be observed during woodchip denitrification. The \( K_m \) values of the bioreactors reduced to 1.19-1.74 mg/L when the temperature decreased to 5 °C, suggesting that the reduced biological activities at 5 °C decreased the half saturation constant for nitrate removal. At such a low temperature, nitrate removal in most woodchip bioreactors would follow a zero-order reaction with reduced rate constants.

The composted and fresh woodchip bioreactors showed \( Q_{10} \) values of 3.4 and 5.4, respectively. These two values translate to 3.4-fold and 5.4-fold increases in nitrate removal rates for every 10°C increase in temperature. The fresh woodchips exhibited a higher temperature dependence in nitrate removal than the composted woodchips. Similar \( Q_{10} \) values in the range of 4.4-4.7 have been reported for column experiments under controlled laboratory conditions (Schmidt and Clark, 2013; Halaburka et al, 2019). However, lower \( Q_{10} \) values (1.4-1.7) have also been reported in field woodchip bioreactors (Cameron and Schipper, 2010). The \( Q_{10} \) variability for woodchip
denitrification may be attributed to differences in treatment systems, field and laboratory experimental conditions, and temperature ranges.

2.3.4  HRT Impacts on Nitrate Removal and Nitrite Accumulation

Figure 2.7 presents the impacts of HRT on the denitrification performance of the fresh and composted woodchip bioreactors. For an influent concentration of 10 mg N/L, the average removal percentages were 98.7, 73.7, and 35.7%, respectively, for 24, 12, and 6 h HRTs by the fresh woodchip bioreactor. The nitrate load reduction rates by the fresh woodchip bioreactor were 4.93, 7.36 and 7.14 g N/m$^3$/d for 24, 12 and 6 h HRTs. Similar nitrate load reductions were achieved by 12 and 6 h HRTs. The relatively low nitrate load reduction at a 24 h HRT can be attributed to the switch of a zero-order reaction to a first-order reaction along the length of the reactor. Unlike the fresh woodchip bioreactor, near complete removal of nitrate was observed for the composted woodchip bioreactor under all three HRTs. Therefore, an HRT of 6 h is adequate for the composted woodchips to completely remove nitrate for an influent concentration of 10 mg N/L. This further demonstrates that the composted woodchips are much more efficient denitrifying media than the fresh woodchips.

Denitrification is a microbial process that involves the reduction of nitrate through the intermediate nitrite to nitrogen gas. Nitrite concentration profiles along the length of the reactors were analyzed to evaluate the nitrite accumulation in the fresh and composted woodchip bioreactors. For both reactors operated at 24 h HRT, nitrite gradually increased with increasing reaction time and reached a peak concentration when nitrate reduced to a certain level, and after that nitrite gradually declined. Similar nitrite profile was also observed for the composted woodchip bioreactor operated at 12 and 6 h
HRTs, and the final effluent nitrite was 0 for all three HRT conditions. Peak nitrite concentrations in the composted woodchip bioreactor were 0.53, 0.66 and 0.72 mg N/L when nitrate reduced to 4.86, 5.66, and 3.32 mg N/L for the three HRTs. These peak nitrite concentrations accounted for 10.3-15.2% of the reduced nitrate. For the fresh woodchip bioreactor operated at 24 h HRT, nitrite reached a peak concentration of 0.68 mg N/L when nitrate reduced to 2.18 mg N/L. This peak nitrite concentration amounted to 8.70% of the reduced nitrate. When the HRT decreased to 12 and 6 h, nitrite gradually increased with increasing flow travel time and no nitrite decline phase was observed. The final effluent nitrite concentration of the fresh woodchip bioreactor was 0.22, 1.17, and 0.65 mg N/L for 24, 12, and 6 h HRTs.

The nitrate and nitrite concentration profiles reflect the difference between the nitrate and nitrite reduction rates at different substrate concentrations. Nitrite accumulation occurs when the nitrate reduction rate is higher than the nitrite reduction rate and reaches a peak when the nitrate reduction rate is equal to the nitrite reduction rate. After that, the nitrite reduction rate overtakes the nitrate reduction rate, leading to the nitrite declining phase. The results in Figure 2.7 indicate that nitrate would need to decrease to below approximately 2.2 mg N/L before nitrite could decline for the fresh woodchip bioreactor. For the composted woodchip bioreactor nitrate would need to decrease to below approximately 3.3-5.7 mg N/L to level off nitrite accumulation and reduce nitrite in the reactor effluent. These results suggest that nitrite reduction in the bioreactors occurred only after nitrate become limiting in the bioreactors.

Nitrite reduction rates (\( r_{\text{nitrite reduction}} \)) can be calculated from the difference between nitrate reduction rates (\( r_{\text{nitrate reduction}} \)) and nitrite accumulation rates (\( r_{\text{nitrite accumulation}} \)) using
the following equation: \( r_{\text{nitrite reduction}} = r_{\text{nitrate reduction}} - r_{\text{nitrite accumulation}} \). The nitrate and nitrite concentration profiles of the bioreactors operated at 12 h HRT were used to determine the nitrite reduction rates. The linear regression was used to model nitrate and nitrite data in the time range of 0-6 h for the fresh woodchip reactor and 0-2 h for the composted woodchip reactor to obtain the reaction rates. The resulting \( r_{\text{nitrate reduction}} \) and \( r_{\text{nitrite accumulation}} \) values were 0.57 and 0.10 mg N/L/h for the fresh woodchips, and 1.41 and 0.28 mg N/L/h for the composted woodchips \((R^2 = 0.981-1)\). The calculated nitrite reduction rate \( (r_{\text{nitrite reduction}}) \) was 0.47 and 1.13 mg N/L/h, respectively, for the fresh and composted woodchip bioreactors. The nitrite reduction rates were 82.5% and 80.1% of the respective nitrate reduction rates for the two bioreactors. The comparable nitrate and nitrite reduction rates obtained from this study support the observation that nitrite accumulation is generally not high in field bioreactors. However, the fresh woodchip bioreactor produced an effluent with nitrite exceeding US Environmental Protection Agency drinking water standard (1 mg N/L) at an HRT of 12 h. Therefore, nitrite should be evaluated during woodchip bioreactor design and operation to avoid high nitrite levels in reactor effluents.

2.3.5 Implications on Woodchip Denitrification Applications

Woodchip bioreactors have been employed to remove nitrate in various sources for more than two decades (Blowes et al., 1994). However, there are still uncertainties about kinetic parameters \( V_{\text{max}} \) and \( K_m \) of Michaelis-Menten model for nitrate removal in woodchip bioreactors. The results of this study suggest that the use of a zero-order reaction model is adequate to design woodchip bioreactors for treating agricultural subsurface drainage and wastewaters that contain nitrate much higher than the \( K_m \) values.
Based on the nitrate removal rates observed in this study and several other studies (Robertson, 2010; Hua et al., 2016; Nordstrom and Herbert, 2017), we propose the following nitrate removal rates as general guidelines to facilitate the design and evaluation of woodchip bioreactors. For new woodchip bioreactors, nitrate removal rates of 1-1.5 mg N/L/h and 0.1-0.3 mg N/L/h can be used for reactor design under warm (22 °C) and cold (5 °C) seasons. These rates are equivalent to nitrate load reduction rates of 16.8-25.2 g N/m³/d at 22°C and 1.68-5.04 g N/m³/d at 5 °C when a typical field woodchip bioreactor porosity of 0.7 is used. For woodchip bioreactors that have been used for several years in the field, a nitrate removal rate of 0.3 mg N/L/h can be used for reactor analysis.

The results of this study showed that microbial degradation of woodchips during the composting process led to more than doubled nitrate removal rates compared to fresh woodchips. Therefore, microbial degradation of woodchips before bioreactor installation could be a practical and low-cost management tool to enhance the denitrification performance of woodchip bioreactors. This pre-installation management strategy can be realized through the storage of woodchips under natural conditions. Natural rain or artificial water spray, and certain soil amendment could create the required moister content and microorganism community for the wood degradation. Therefore, we recommend that woodchips should be stored in an outdoor environment for several weeks to months before the bioreactor installation. Certainly, long-term laboratory and field studies are needed to evaluate the longevity of the effect of woodchip composting on nitrate removal. This study indicates that the quality of DOC leached from woodchips can have a significant impact on nitrate removal rates. More studies are needed to determine
the biodegradability of woodchip DOC, and the release rates of biodegradable organic carbon from woodchips to better understand nitrate removal kinetics in bioreactors.

2.4 Conclusions

This study was conducted to determine the nitrate removal kinetics parameters in denitrification bioreactors using the fresh, aged and composted woodchips. The results showed that denitrification efficiency followed the order of composted woodchips > fresh woodchips > aged woodchips. The average nitrate load reduction rates were 8.81-21.0, 7.36-9.78, and 2.46-3.54 g N/m³/d for the composted, fresh, and aged woodchips at influent nitrate concentrations of 10-50 mg N/L, an HRT of 12 h, and 22 °C. The composted woodchips exhibited denitrification capacities that were 1.20-2.15 times higher than that of the fresh woodchips at 22 °C. The nitrate load reduction rates decreased to 2.18-2.60, and 0.53-0.55 g N/m³/d for the composted and fresh woodchip bioreactors operated at 5 °C. Woodchip composting before bioreactor installation can be used as a practical management tool to improve denitrification efficiency.

The Michaelis-Menten model adequately described the nitrate removal process in woodchip bioreactors (R² = 0.970-0.993). The \( V_{\text{max}} \) values were 2.09, 0.88 and 0.30 mg N/L/h, and the \( K_m \) values were 2.60, 2.16 and 2.01 mg N/L for the composted, fresh and aged woodchips at 22 °C. The \( V_{\text{max}} \) values decreased to 0.26 and 0.05 mg N/L/h, and the \( K_m \) values decreased to 1.74 and 1.19 mg N/L when the composted and fresh woodchip bioreactors were operated at 5 °C.

The nitrite reduction rates were 0.47 and 1.13 mg N/L/h for the fresh and composted woodchips, which were 82.5% and 80.1% of the respective nitrate reduction rates for an influent nitrate of 10 mg N/L and 22 °C. The fresh woodchip bioreactor
produced an effluent with nitrite exceeding drinking water standard (1 mg N/L) at an influent nitrate of 10 mg N/L and an HRT of 12 h. Nitrite should be evaluated during woodchip bioreactor operation to avoid high nitrite levels in reactor effluents.

Table 2.1: Chemical Composition of the Artificial Runoff Water

<table>
<thead>
<tr>
<th>Chemical Species</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca$^{2+}$</td>
<td>5 mg/L</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>2.5 mg/L</td>
</tr>
<tr>
<td>K$^+$</td>
<td>2.5 mg/L</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>1 mg/L</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>15.5 mg/L</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>16.7 mg/L</td>
</tr>
<tr>
<td>PO$_4^{3-}$</td>
<td>1 mg P/L</td>
</tr>
<tr>
<td>Co$^{3+}$</td>
<td>6.2 µg/L</td>
</tr>
<tr>
<td>Fe$^{3+}$</td>
<td>52 µg/L</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>13.9 µg/L</td>
</tr>
<tr>
<td>Mo$^{7+}$</td>
<td>2.2 µg/L</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>6.2 µg/L</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>2.6 µg/L</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>12 µg/L</td>
</tr>
<tr>
<td>BO$_3^{3-}$</td>
<td>23.4 µg/L</td>
</tr>
<tr>
<td>SeO$_4^{2-}$</td>
<td>18.9 µg/L</td>
</tr>
</tbody>
</table>
Table 2.2: Nitrate load reduction rates in woodchip bioreactors

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Influent Nitrate (mg N/L)</th>
<th>Nitrate Load Reduction Rates (g N/m³/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Composted Woodchips</td>
</tr>
<tr>
<td>22</td>
<td>50</td>
<td>21.00 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>20.13 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>20.12 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>17.38 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8.81 ± 0.13</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>2.60 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>2.56 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2.52 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.40 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.18 ± 0.15</td>
</tr>
</tbody>
</table>

*Experimental conditions: HRT=12 h. N/A: not available.
Table 2.3: Summary of Michaelis-Menten model parameters for nitrate removal in woodchip bioreactors

<table>
<thead>
<tr>
<th>Woodchip Type</th>
<th>Temperature (°C)</th>
<th>HRT (h)</th>
<th>$K_m$ (mg/L)</th>
<th>$V_{max}$ (mg N/L/h)</th>
<th>Model $R^2$</th>
<th>$Q_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composted Woodchips</td>
<td>22</td>
<td>12</td>
<td>2.60</td>
<td>2.09</td>
<td>0.990</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>12</td>
<td>1.74</td>
<td>0.26</td>
<td>0.970</td>
<td></td>
</tr>
<tr>
<td>Fresh Woodchips</td>
<td>22</td>
<td>12</td>
<td>2.16</td>
<td>0.88</td>
<td>0.988</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>12</td>
<td>1.19</td>
<td>0.05</td>
<td>0.993</td>
<td></td>
</tr>
<tr>
<td>Aged Woodchips</td>
<td>22</td>
<td>12</td>
<td>2.01</td>
<td>0.30</td>
<td>0.981</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Figure 2.1: Nitrate concentration profiles in woodchip bioreactors during nitrate removal kinetics experiments at 22 °C. (Experimental conditions: HRT = 12 h)
Figure 2.2: Nitrate concentration profiles in woodchip bioreactors during nitrate removal kinetics experiments at 5 °C. (Experimental conditions: HRT = 12 h.)
Figure 2.3: DOC levels in bioreactor effluents during nitrate removal kinetics experiments. (Experimental conditions: HRT = 12 h.)
Figure 2.4: Scanning electron microscope images of woodchips. (A: fresh woodchip before experiment; B: fresh woodchip after experiment; C: composted woodchip before experiment; D: composted woodchip after experiment.)
Figure 2.5: Lineweaver-Burk plot of nitrate removal rates at different concentrations in fresh woodchip bioreactor. (Experimental conditions: HRT = 12 h; temperature = 22 °C. C_i: nitrate concentration (mg N/L); V_i: nitrate removal rate (mg N/L/h).)
Figure 2.6: Comparison of experimental nitrate removal rates in fresh woodchip bioreactor with Michaelis-Menten model predictions. (Experimental conditions: HRT = 12 h; temperature = 22 °C.)
Figure 2.7: Nitrate and nitrite concentration profiles in woodchip bioreactors at different HRTs. (Experimental conditions: influent nitrate = 10 mg N/L; HRT = 24, 12, and 6 h; Temperature = 22 °C.)
Figure 2.8: Pictures of fresh woodchips (A), composted woodchips (B), and aged woodchips (C).
Figure 2.9: Fungi on Composted Woodchips.
CHAPTER THREE
Nitrate and Phosphate Removal by Woodchip Bioreactors and Steel Chip Filters Using Different Media Configurations

Abstract

Nitrogen and phosphorus are two primary nutrients that can promote eutrophication in aquatic ecosystems. Dual-nutrient removal technologies are needed to reduce nutrient inputs and protect natural water resources. The objective of this study was to evaluate the impact of three different media configurations on nitrate and phosphate removal of dual-media reactors using woodchips and steel chips. Laboratory column reactors were constructed based on three configurations: woodchips/steel chips, steel chips/woodchips, and mixture of the two media. The volumetric ratio of woodchips and steel chips media in each reactor was 11:1. The results showed that all three reactors effectively removed nitrate and phosphate and no significant differences in nutrient removal was observed among different media configurations. Steel chips, when placed downstream of woodchips reduced reactor organic carbon leaching, whereas woodchips, when placed downstream of steel chips reduced reactor iron leaching. The mixed media reactor was able to effectively control both organic carbon and iron leaching. The nitrate removal extents by the woodchip bioreactor decreased by 70.4% while the phosphate removal extents by the steel chip filter only decreased by 18.2% when decreasing hydraulic retention times by 4 times. The calculated temperature coefficients ($Q_{10}$) were 3.01 for nitrate removal by the woodchips and 1.08 for phosphate removal by the steel chips under stable operating conditions. Phosphate removal by the steel chip filter was
much less affected by flow and temperature variations than nitrate removal by the woodchip bioreactor.

3.1 Introduction

Nitrogen and phosphorus are two essential nutrients that support plant and algal growth in natural systems. However, an overabundance of nutrients in surface waters can result in eutrophication, a leading cause of impairment of many freshwater and coastal marine ecosystems (Smith and Schindler, 2009). Excessive inputs of nutrients to water bodies by human activities have greatly increased the rate of eutrophication and the frequency of harmful algal blooms. It has been widely recognized that reduction of anthropogenic nutrient inputs is necessary in order to reduce eutrophication in surface waters and protect drinking water supplies (Schindler, 2006). Many aquatic ecosystems require the implementation of dual-nutrient reduction strategies to control eutrophication (Conley et al, 2009). Therefore, treatment technologies that can remove both nitrogen and phosphorus from different sources are needed to meet the challenge of the dual-nutrient reduction goal.

Denitrifying bioreactors is a water quality management tool developed to reduce nitrate inputs to surface waters (Blowes et al., 1994). Solid organic carbon media are used in bioreactors to provide habitats and organic carbon sources for denitrifying bacteria to convert nitrate to nitrogen gas through a series of microbial reduction processes. Woodchips are the most commonly used organic medium in denitrifying bioreactors, and woodchip bioreactors have been increasingly applied to control nitrate exports from agricultural drainage (Christianson and Schipper, 2016). Field experiences suggest that woodchip bioreactors can achieve average nitrate load reduction rates of 2-22 mg N/m³
of media/d depending on water quality and woodchip conditions (Schipper et al., 2010). In addition to the application in agricultural settings, woodchip bioreactors have also demonstrated great potentials in nitrate removal from urban stormwater, onsite wastewater systems, and aquaculture wastewater (Peterson et al., 2015; Lepine et al., 2016; Lopez-Ponnada et al., 2017).

Phosphate removal from non-point sources is generally achieved by the filtration technology using phosphate absorption materials. Many materials have been evaluated for phosphate filtration, such as natural minerals, industrial byproducts, and commercial products (Drizo et al., 2002; Grace et al., 2015; Lalley et al., 2016; Penn et al., 2016; Sellner et al., 2019). Filtration materials that showed high phosphate adsorption potentials typically have high contents of iron, aluminum and calcium, which can remove dissolved phosphate through electrostatic attraction, precipitation, and ligand exchange (Cucarella and Renman, 2009). Pairing woodchip bioreactors and phosphate filters has been investigated to achieve simultaneous nitrate and phosphate removal. Selection of filter media that can be used in conjunction with woodchips depends on material cost, hydraulic property, physical stability, and phosphate removal capacity and longevity. Several studies evaluated the addition of phosphate sorption materials (e.g., biochar and drinking water treatment residuals) to woodchip bioreactors for phosphate removal (Gottschall et al., 2016; Povilaitis et al., 2020). Others have developed two-stage treatment systems that place phosphate filters (e.g., drinking water treatment residuals, activated alumina, expanded shale aggregates, acid mine drainage residuals, steel slag, and recycled steel byproducts) either downstream or upstream of woodchip bioreactors (Zoski et al., 2013; Goodwin et al., 2015; Christianson et al., 2017; Husk et al., 2018;
Abdi et al., 2020). The results of these studies suggest that pairing woodchip bioreactors with phosphate filters improved phosphate removal efficiency while maintaining effective nitrate removal.

Recycled steel byproducts including steel wool, chips and turnings, are waste materials generated during steel machining, cutting, and grinding processes. These cost-effective and readily available iron-based materials have shown high phosphate capturing capability from aqueous solutions (Goodwin et al., 2015; Hua et al., 2016; Sellner et al., 2019a). Hua et al. (2016) demonstrated that a woodchip column reactor followed by a steel chip column reactor successfully removed nitrate and phosphate in simulated subsurface drainage. Goodwin et al. (2015) evaluated the impact of different media arrangements of woodchips and steel turnings on nutrient removal, and showed that the two-stage treatment system achieved better nitrate removal when the steel turning filter was placed upstream of the woodchip bioreactor whereas a better phosphate removal was achieved by placing the steel turning filter downstream of the woodchip bioreactor. However, Christianson et al. (2017) showed that different placement configurations had limited impact on nitrate and phosphate removal when pairing woodchip bioreactors with iron-based acid mine drainage residual filters under normal hydraulic retention times.

Further studies are needed to elucidate the synergistic effects between woodchips and steel byproducts on nutrient removal. Woodchip bioreactors rely on biological denitrification processes for nitrate removal while steel byproducts remove phosphate through physical and chemical reactions. The responses of these two media to the changes in operation conditions such as flow rates and temperatures should also be
carefully evaluated to provide sound recommendations on field applications of the dual-media treatment system.

The use of woodchips and steel byproducts may lead to some negative environmental impacts, such as organic carbon and metal leaching from the reactor. However, little is known about the impact of media configurations on organic carbon and metal leaching of the dual-media treatment systems. The objective of this study was to evaluate the impact of different media configurations on nutrient removal, organic carbon leaching and iron leaching from the paired woodchips and steel chips reactors. In addition, the impact of flow and temperature variations on nitrate and phosphate removal by the dual-media treatment system was also investigated.

3.2 Materials and Methods

3.2.1 Woodchips and Steel Chips

Woodchips made from cottonwood (Populus deltoids) were collected from a playground woodchip distributor in Sioux Falls, South Dakota. Fine woodchip particles smaller than 4 mm were removed by sieving, and particles larger than 6 cm were removed manually. The woodchips were then gently washed with distilled water, and air dried before use. Steel chips generated from processing carbon steel were collected from a machine shop in Sioux Falls. The steel chips were washed with phosphate-free soap to remove any oil on the surface and were air dried. During the drying process, the steel chips oxidized, forming a layer of rust on the surface. After drying, the steel chips were sieved to collect particles in the size range of 1-2 mm. Figure 3.7 shows pictures of the processed woodchips and steel chips. Table 3.1 presents the physical and chemical properties of the two experimental materials. Steel chips were slightly acidic (pH = 6.3),
whereas woodchips were close to neutral pH condition (pH = 6.8). Woodchips exhibited a hydraulic conductivity of 2.95 cm/s and steel chips showed a hydraulic conductivity of 1.02 cm/s at the packing densities used for the experiments. The difference in the hydraulic properties of the two materials may be attributed to different particle shapes and sizes.

3.2.2 Column Reactors

Clear acrylic tubes with a length of 1.2 meters and inside diameter of 8.85 cm were used to construct up-flow column reactors. Each reactor had 12 sampling ports installed along the length of the column at 10 cm intervals. Figure 3.1 shows the schematic of three column reactors. These reactors were constructed based on three media configurations: (1) a woodchip bioreactor (1.1 m height) followed by a steel chip filter (0.1 m height); (2) a steel chip filter (0.1 m height) followed by a woodchip bioreactor (1.1 m height); and (3) a fully mixed reactor with woodchips and steel chips. These column reactors were referred to as “wood/steel”, “steel/wood”, and “mixed” reactors, respectively, in this study. Approximately 1596 g of woodchips and 880 g of steel chips were used to pack each column, resulting in a weight ratio of 1.8:1. The volume ratio of the woodchip section to the steel chip section in the two-stage reactors was 11:1. Drainable porosity was determined by draining each reactor section over 1 h, and the resulting porosities were 49.0, and 72.0%, respectively, for the woodchip and steel chip sections.

3.2.3 Experimental Conditions

Variable speed peristaltic pumps (Masterflex Model L/S, Cole Parmer, Vernon Hills, IL) were used to pump an artificial runoff water from an influent tank to the
column reactors during this study. The artificial runoff water was prepared based on a previously published method (Hua et al., 2016), and it was composed of various ionic constituents and trace minerals (Table S1) to ensure that microbial growth was not limited. KNO₃ and NaH₂PO₄·H₂O were added to the artificial runoff water to achieve influent nitrate and phosphate concentrations of 30 mg N/L and 10 mg P/L for the column experiments.

All three column reactors were operated at an influent pumping rate of 5.0 ml/min under room temperature (22 °C) for 100 days. The resulting hydraulic retention times (HRT) of the woodchip and the steel chips sections were 11.0 and 1.48 h based on drainable porosities, and the overall HRT of each column was 12.5 h at this flow rate. Daily samples were collected from sample ports for the woodchip effluent, the steel chip effluent, and the mixed reactor effluent during the 100 d experiment. Water samples were also taken from all sample ports along the length of each column every two weeks to monitor the concentration profiles of each parameter. After the 100 d experiments at 12.5 h HRT, the wood/steel reactor was operated at different flow rates of 5.0, 10.0 and 20.0 ml/min for 7 days each. Daily samples were collected from sample ports of the woodchip and steel chip effluents during the flow variation experiments. In addition to the room temperature experiments, a second wood/steel reactor was packed based on the same packing densities and placed in a cool room with a controlled temperature of 5 °C. The wood/steel reactor was operated at a total HRT of 12.5 h for 100 d and daily samples were collected from sample ports of the woodchip and steel chip effluents to investigate the reactor performance under the cold temperature.
During each sampling event, a 40 mL sample was collected at each sampling port. The collected samples were filtered using 0.45 µm membrane filters (MilliporeSigma, Burlington, MA) and then stored at 4º C before analysis of nitrate, phosphate, dissolved organic carbon (DOC), and dissolved iron.

3.2.4 Analytical Methods

All solutions used in this study were prepared with ultrapure water (18 MΩ-cm) produced by a Barnstead Nanopure system (Thermo Fisher, Waltham, MA). The influent solutions were adjusted to pH 7 using sodium hydroxide or sulfuric acid solutions. The chemicals used in this study were of American Chemical Society reagent grade and were purchased from Sigma Aldrich (St. Louis, MO). Nitrate and phosphate concentrations were measured using a DX-500 Ion Chromatography instrument with an IonPac AS-14 analytical column (Dionex, Sunnyvale, CA). The DOC concentrations were determined with a Shimadzu TOC-5000 Analyzer (Shimadzu Corp., Kyoto, Japan) according to Standard Method 5310 B (APHA et al., 2012). The pH value of each solution was measured using an Orion 290A+ advanced ISE/pH/mV/OPR meter (Thermo Fisher). The analysis of dissolved iron was carried out using a UV-visible spectrophotometer (DR 4000, HACH, Loveland, Colorado) based on a colorimetric method (HACH Method 8008).

3.2.5 Nitrate and Phosphate Removal Temperature Coefficients

The nitrate and phosphate removal rates obtained at temperatures of 5 and 22 ºC were used to determine the Q₁₀ temperature coefficients for nitrate removal by woodchips and phosphate removal by steel chips using the following equation.
\[ Q_{10} = \left( \frac{R_2}{R_1} \right)^{\frac{10^\circ C}{T_2-T_1}} \]

Where \( R_1 \) and \( R_2 \) are the removal rates at temperatures \( T_1 \) (5 \(^\circ\)C) and \( T_2 \) (22 \(^\circ\)C), respectively.

3.2.6 Statistical Analysis

All statistical analyses were conducted using RStudio (version 1.2). A one-way ANOVA test followed by a Duncan test were used to determine whether there were significant differences among the three media configurations on reactor performance. All statistical analyses used a 95% confidence interval.

3.3 Results and Discussion

3.3.1 Nitrate and Phosphate Removal by Column Reactors

Figure 3.1 presents the effluent nitrate and phosphate concentrations from the three reactors during the 100 d operation at a total HRT of 12.5 h and a temperature of 22 \(^\circ\)C. All three reactors effectively reduced influent nitrate (30 mg N/L) and phosphate (10 mg P/L) during the experiments. The wood/steel, steel/wood, and mixed reactors achieved nitrate removal percentages of 66.9, 71.3 and 64.4%, respectively, after the first day of operation. This indicates that denitrifying bacterial community and nitrate removal capacity quickly established in the woodchip bioreactors. The nitrate removal efficiencies gradually increased to 72.0, 73.7 and 71.2% for the three reactors at day 30. The column reactors showed moderate declines in nitrate removal between days 30 and 50, but maintained relatively stable nitrate removal performance after day 50. The nitrate removal percentages were 59.8, 60.3, and 61.7% for the three reactor at day 100. Overall, the three reactors demonstrated similar nitrate removal efficiencies and the average removal percentages were 65.1, 66.1 and 66.5% for the wood/steel, steel/wood, and
mixed reactors during the 100 days of operation at an influent concentration of 30 mg N/L. There was no significant difference (p>0.05) in nitrate removal among the three reactor configurations during this experiment.

Phosphate concentrations in the reactor effluents showed higher variations than nitrate. Near complete phosphate removal (> 99%) was observed for all three reactors during the first 7 days of operation. Phosphate breakthrough was observed for the three reactors after 7 days and the effluent phosphate increased rapidly between 7 and 20 days. The phosphate removal decreased to 77.5, 78.1, and 78.5% for the wood/steel, steel/wood, and mixed reactors at day 20. However, the increase in effluent phosphate concentrations leveled off after 20 days, and all three reactors showed relatively stable phosphate removal between days 20 and 100 (69.3-79.4%). This demonstrates that steel chip filters were able to maintain effective phosphate removal for a long duration despite the early breakthrough observed between 10 and 20 days. The phosphate breakthrough curves of the three reactors behaved differently from the conventional S-shaped adsorption breakthrough curves, where the breakthrough line would gradually increase until exhaustion. Sellner et al. (2019) also observed that phosphate adsorption onto steel chip filters was characterized by an initial fast breakthrough followed by a stable removal for a long duration during the column experiments using an empty bed contact time of 3 min. These results indicate that steel chip filtration is an effective phosphate removal technology that could be used for long-term field applications. The average phosphate removal percentages were 75.4, 79.4 and 77.5% for the wood/steel, steel/wood, and mixed reactors during the 100 days of operation for an influent phosphate concentration of 10 mg P/L. The steel/wood reactor was slightly more effective at phosphate removal
than the wood/steel reactor. However, the difference in phosphate removal among the three reactor configurations was not statistically significant (p>0.05).

Table 3.2 compares the nitrate and phosphate removal by woodchip and steel chips sections in the wood/steel and steel/wood reactors under relatively stable operating conditions (days 50-100). The average nitrate removal extents by the woodchip sections were 16.9 and 17.2 mg N/L (56.3-57.3% of the influent nitrate) for the wood/steel and steel/wood reactors. The corresponding nitrate load reduction rates based on the woodchip reactor volume were 18.1 and 18.4 g N/m$^3$/d. These nitrate volumetric load reduction rates were within a typical range obtained by relatively fresh woodchips under non-nitrate limiting conditions at similar temperatures in the literature (Hoover et al., 2016; Hua et al., 2016; Christianson et al., 2017). These results suggest that the steel chip filter placed upstream of the woodchip bioreactor had little impact on the nitrate removal performance of the woodchips. Similarly, placement of the woodchip section upstream of the steel chip section did not substantially affect the phosphate adsorption capacity of the steel chips. The average phosphate removal extents by the steel chip sections were 5.73 and 5.60 mg P/L (57.3-56.0% of the influent phosphate) for the wood/steel and steel/wood reactors. The phosphate loading reduction rates based on the steel chip filter volume were 66.9 and 65.4 g P/m$^3$/d for the two reactors.

Goodwin et al. (2015) evaluated nitrate and phosphate removal by steel turnings and woodchips using different media arrangements. The results showed that the nitrate removal by the woodchip section downstream of steel turnings (45-85%) were greater than that of the woodchip section upstream of the steel turnings (25-65%) for HRTs of 3 to 6 h. These results imply that iron released from steel turnings may have stimulated the
denitrification processes of downstream woodchips. Iron is an essential trace metal element to support microorganism growth, and it is also required by many proteins and enzymes for a variety of metabolic processes including biological denitrification (Jiang et al., 2020). It has also been reported that some microorganisms such as nitrate-reducing iron (II)-oxidizing bacteria are capable of coupling nitrate reduction and iron (II) oxidation through enzymatic pathways (Schaedler et al., 2018). Studies have shown that increasing iron concentrations improved denitrification in constructed wetlands and marine systems (Labbe et al., 2003; Song et al., 2016). Nitrate reduction using iron (II) as electron donor is less energetically favorable than heterotrophic nitrate reduction using organic carbon as electron donor. Schaedler et al. (2018) showed that DOC had a large impact on the microbial mediated coupling of nitrate reduction and iron oxidation. The highest rate of nitrate-dependent Fe (II) oxidation was observed at the low DOC concentration of 3.2 mg/L in a freshwater sediment, and the rate declined with increasing DOC concentrations. No Fe (II) oxidation was observed when the DOC increased to 21.8 mg/L. These results indicate that nitrate reduction using organic carbon as electron donor is favored by nitrate reducing bacteria, and Fe (II) can be used as electron donor under carbon-limiting conditions. Therefore, the impact of steel byproducts on woodchip denitrification would depend on the development of nitrate-reducing iron (II)-oxidizing bacteria and organic substrate concentrations. The results of the column experiments in this study showed that steel chips did not have a significant impact on nitrate removal of downstream woodchips, suggesting that the woodchips used in the experiments supplied sufficient organic substrates for denitrifying bacteria and denitrification coupled with Fe (II) oxidation was limited in the woodchip section. Goodwin et al. (2015) used aged
woodchips (three years of field operation) in their experiments and organic carbon limiting conditions may have occurred in the reactors, which promoted the nitrate-dependent Fe (II) oxidation process.

Phosphate adsorption onto iron oxides can be affected by the presence of organic compounds through competitive adsorption (Weng et al., 2012). Sellner et al. (2019) showed that the presence of humic acid of 25 mg C/L decreased the amount of phosphate retained by a steel chip filter by 27.7% at an influent phosphate of 10 mg P/L and an empty bed contact time of 3 min. It would be expected that the DOC released from woodchips could compete for the available adsorption sites of steel chips and thereby reducing phosphate removal efficiency of downstream steel chip filter. However, this effect was not observed in the column experiments. The differences in phosphate removal by the steel chip sections in the wood/steel and steel/wood reactors were not statistically significant (p>0.05). It is likely that the total adsorption sites of the steel chips exceeded the demands from organic carbon and phosphate under the conditions of this study such that competitive adsorption was not apparent. Moreover, organic compounds can complex iron oxide species, which provide sorption sites for phosphate through the formation of organic matter-iron-phosphate complexes (Gerke, 2010). The formation of such ternary complexes may help the transformation and removal of free phosphate ions in the wood/steel reactor. These factors together may have resulted in similar phosphate removal performance among different media configurations. Christianson et al. (2017) also observed similar phosphate removal efficiencies by acid mine drainage residual filters when placed either upstream or downstream of woodchip bioreactors under normal woodchip HRT conditions (7.2-18 h). However, the downstream-placed phosphate filter
exhibited reduced phosphate removal compared to upstream-placed phosphate filter under conditions of overly long woodchip retention times (51 h), suggesting that highly reduced environment is detrimental to phosphate adsorption onto iron oxides.

The results of column reactor experiments in this study suggest that the three different media arrangements all achieved effective nitrate and phosphate removal, and potential synergetic effects between woodchips and steel chips did not resulted in significant differences in nutrient removal. The studies using other phosphate sorption materials including activated alumina, expanded shale aggregates, steel slag, and drinking water treatment residuals also showed that those materials improved phosphate removal while did not significantly impact nitrate removal when paired with woodchip bioreactors (Gottschall et al., 2016; Christianson et al., 2017; Husk et al., 2018; Abdi et al., 2020).

3.3.2 DOC and Iron Leaching from Column Reactors

Figure 3.3 presents the variations of dissolved organic carbon and iron concentrations in column reactor effluents during the 100 days of operation. It has been widely reported that woodchip bioreactors can release organic compounds such as tannins, lignins, and others in the reactor effluents, especially during the initial operation stage of bioreactors (Abusallout and Hua, 2017). High levels of DOC in reactor effluents were also observed during the first several days of operation in this study. The effluent DOC concentrations were 97.6, 248, and 146 mg/L for the wood/steel, steel/wood and mixed reactors after the first day. Then, the DOC declined exponentially to 8.40, 13.6 and 9.56 mg/L for the three reactors after 10 days. All three reactors exhibited moderate decreases in DOC between 10 and 50 days, and the DOC leaching from the reactors were relatively stable and the variations in DOC were less than 0.5 mg/L between 50 and 100
days. The DOC concentrations reached 1.13, 1.96 and 1.27 mg/L for the three reactors after 100 days. The average DOC concentrations in the reactor effluents during the 100 day operation were 4.72, 10.5 and 6.85 mg/L for the wood/steel, steel/wood and mixed reactors. The wood/steel reactor reduced the DOC leaching by 55.0% and 31.1% compared to the wood/steel and mixed reactors (p<0.05). This suggests that steel chips, when placed downstream of the woodchip reactor or mixed with woodchips, were able to remove DOC leached from the woodchips. These two media configurations could reduce organic loadings to the receiving water bodies.

Iron leaching may be a concern when applying steel chip filters for phosphate removal from runoff and drainage water, especially for aquatic ecosystems that are sensitive to iron contamination. Iron-enriched effluents could degrade stream benthic communities and reduce the species diversity and abundance of primary producers and aquatic invertebrates (Kotalik et al., 2019). Therefore, iron leaching should be carefully evaluated for the application of iron-based phosphate filters. As shown in Figure 3, different media configurations resulted in significantly different dissolved iron concentrations in reactor effluents (p<0.05). The first day of reactor operation led to effluent iron concentrations of 4.86, 1.08 and 3.10 mg/L, respectively, for the wood/steel, steel/wood and mixed reactors. The effluent iron concentrations rapidly decreased to 3.39, 0.23 and 1.39 mg/L at day 10 and then gradually declined to 2.63, 0.12, and 0.51 mg/L at day 100. The average effluent iron concentrations were 2.83, 0.16, and 0.78 mg/L for the wood/steel, steel/wood and mixed reactors during this experiment. The steel/wood and mixed reactors reduced iron leaching by 94.3 and 72.4% compared to the wood/steel reactor. The steel/wood reactor consistently reduced the dissolved iron
concentrations to below the United States Secondary Drinking Water Standard (0.3 mg/L) after 5 days of the reactor operation. The effluent iron concentrations in the mixed reactor effluent were close the standard between 50 and 100 days (0.50-0.60 mg/L) whereas the wood/steel reactor effluent iron concentrations were substantially higher than the standard throughout the experiment. It is clear that woodchips used in this study, when placed downstream of the steel chip filter or mixed with steel chips, effectively removed iron leached from the steel byproducts. Iron coatings on the woodchip surfaces were widely observed in woodchips collected from the steel/wood and mixed reactors after the experiments (Figure 3.7B,C), which proved the iron adsorption function of the woodchips. Previous studies have shown that wood mulches and other wood-based materials can be used to remove heavy metals such as zinc, lead and copper from stormwater runoff (Jang et al., 2005). The results of this study showed that woodchips are also an effective sorption material for iron removal from runoff and drainage water. The steel/wood configuration exhibited a better control of iron leaching than the mixed media design.

3.3.3 Nutrient, Iron and DOC Concentration Profiles in Column Reactors.

Nitrate, phosphate, iron and DOC concentration profiles along the length of the steel/wood and wood/steel columns were monitored during the stable operating conditions to elucidate the removal mechanisms in the reactors. Figure 3.4 presents the average concentration profiles obtained from three sampling events (days 50,74,88 ). Nitrate concentrations decreased linearly along the woodchip sections in both reactors, suggesting that nitrate removal by woodchips followed a zero-order reduction under the conditions of this study. The average nitrate reductions by the woodchip sections in the
wood/steel and steel/wood reactors were 16.8 mg N/L (18.0 g N/m³/d) and 17.1 mg N/L (18.2 g N/m³/d), respectively. Steel chip filters also exhibited some nitrate removal capacities and the average nitrate reductions were 1.12 mg N/L (13.0 g N/m³/d) and 1.04 mg N/L (12.2 g N/m³/d) in the wood/steel and steel/wood reactors. Steel chips can remove nitrate though adsorption onto iron oxides and chemical reduction by elemental iron. Although the nitrate load reduction rates by steel chips filters were in a similar range of woodchips, the net nitrate removal extents were much lower than the woodchips due to the smaller reactor volume. The woodchip sections were able to remove 56.0-56.9% of the influent nitrate whereas the steel chip sections only removed 3.46-3.72%. Woodchips were the primary media responsible for nitrate removal in different reactor configurations. Both steel chips and woodchips demonstrated phosphate removal capacities. The average phosphate reductions by the steel chips sections in the wood/steel and steel/wood reactors were 5.47 mg N/L (63.9 g N/m³/d) and 5.59 mg N/L (65.2 g N/m³/d), respectively. The average phosphate reductions by the woodchip sections in the wood/steel and steel/wood reactors were 1.52 mg N/L (1.62 g N/m³/d) and 1.81 mg N/L (1.93 g N/m³/d), respectively. The steel chip sections removed 54.7-55.9% of the influent phosphate, which was much higher than woodchip sections (15.2-18.1%). The media configuration had a significant impact on phosphate removal by woodchips (p<0.05). The woodchips section, when placed downstream of the steel chip section, removed 19.1% more phosphate than the upstream placed woodchip section.

The improved phosphate removal by woodchips in the steel/wood reactor can be attributed to the iron retained by the woodchips. Iron species accumulated in the woodchips can serve as additional phosphate sorption materials thereby increasing
phosphate removal capacity. The dissolved iron concentration profile along the steel/wood column supports this analysis. The steel chip section effluent in the reactor showed an average iron concentration of 2.63 mg/L. Then, the iron concentrations rapidly decreased to 1.00 mg/L after 1 h HRT in the woodchip section, and to 0.14 mg/L after 6 h HRT in the woodchip section. Only limited variations in iron concentrations (0.12-0.13 mg/L) were observed in the last 5 h HRTs, suggesting that the woodchips still had good iron removal potential after 100 days of operation. The woodchip section was able to remove an average of 95.3% of the iron released from the steel chips. The organic carbon released from woodchips exhibited linear increases in the wood/steel reactor. The DOC in the woodchip section effluent reached an average of 3.52 mg/L. The steel chip filter was able to remove an average of 66.5% of the woodchip DOC and the final reactor effluent DOC was 1.18 mg/L. These results confirm that woodchips are effective iron sorption materials and steel chips are effective DOC sorption materials, and different media configurations can have significant impacts on iron and DOC leaching.

3.3.4 Effect of HRT and Temperature on Nitrate and Phosphate Removal

Figure 3.5 presents nitrate and phosphate removal by the wood/steel reactor at three different reactor HRTs (12.5, 6.26 and 3.13 h). As shown in Figure 3.5, the nitrate and phosphate removal efficiencies of the wood/steel reactor decreased with decreasing HRTs. The average effluent nitrate concentrations were 11.8, 19.1, and 24.6 mg N/L at 12.5, 6.26, and 3.13 h HRTs. The corresponding nitrate removal percentages were 60.6, 36.2 and 17.9%, respectively. The average effluent phosphate concentrations were 2.98, 4.15, and 5.12 mg P/L at 12.5, 6.26, and 3.13 h HRTs, representing 70.2, 58.4 and 48.8% removal. The nitrate removal efficiency decreased by 70.5% when decreasing HRTs from
12.5 to 3.13 h (4 time increases in flow rates). However, the phosphate removal efficiency only reduced by 30.4%. Phosphate removal by the wood/steel reactor was much less affected by the changes in flow rates compared to nitrate removal.

Table 3.3 summarizes the nitrate and phosphate removal extents and the load reduction rates of the two media at different HRTs. The nitrate and phosphate removal extents by the woodchip section decreased by 70.4 and 76.7%, respectively, when decreasing the section HRT from 11.0 to 2.76 h. The reductions in nitrate and phosphate removal extents by woodchips were generally proportional to the increases in flow rates. Nitrate and phosphate removal by woodchips is function of HRTs when other factors are fixed. This is in agreement with concentration profiles (Figure 3.4) which showed that nitrate and phosphate decreased linearly along the column reactor. The volumetric load reduction rates by the woodchip section did not vary substantially with flow rates. The nitrate load reduction rates were 18.3, 21.7, and 21.6 g N/m$^3$/d and the phosphate load reduction rates were 1.56, 1.49 and 1.45 g P/m$^3$/d at 11.0, 5.52, and 2.76 h HRTs. Similar results were also observed for the nitrate removal by the steel chip section. The nitrate removal extent by the steel chips decreased by 72.2% when decreasing HRT from 1.48 to 0.37 h. The nitrate load reductions by steel chips varied between 12.6 and 16.1 g N/m$^3$/d at the three flow rates. The phosphate removal by the steel chips at different flow rates behaved substantially differently from others. The phosphate removal extents by the steel chips only decreased by 18.2% when the flow rate increased by 4 times. This indicates that phosphate adsorption onto steel chips was not highly affected by the flow rates for the range tested in this study. The steel chip filter was able to maintain effective phosphate removal under different flow conditions. Because of this, the phosphate load
reduction rates by the steel chips greatly increased from 64.8 to 212 g P/m$^3$/d when the HRT decreased from 1.48 to 0.37 h. Christianson et al. (2017) also showed that an upstream placed acid mine drainage residual filter improved the phosphate load reduction rate from 70 to 112 g P/m$^3$/d when the filter HRT decreased from 20 to 7.6 min for an average influent concentration of 1.39 mg P/L. Therefore, iron-based phosphate sorption materials are excellent choices the treatment of runoff and drainage water under variable flow conditions.

Figure 3.6 shows the nitrate and phosphate removal by the wood/steel reactor over 100 days at 5 °C. Woodchips showed appreciable removal of nitrate after 5 days of operation, suggesting that denitrifying bacteria requires a longer acclimation time to develop nitrate removal capacity at this low temperature. The reactor effluent nitrate decreased from 29.8 mg N/L at day 1 to 27.2 mg N/L at day 10. After that, the reactor effluent nitrate showed moderate variations from 26.2 to 27.4 mg N/L between days 10 and 100. The average nitrate concentrations removed by the woodchip section and the steel chip section during the 100 day operation were 2.55 and 0.40 mg N/L, respectively, which resulted in an average removal of 9.85% by the woodchip/steel reactor. The nitrate removal performance of the reactor at 5 °C was 84.8% lower than the nitrate removed (19.5 mg N/L) at 22 °C. Initial phosphate breakthrough was observed for the reactor. The effluent phosphate concentration steadily increased from 0.80 mg P/L at day 1 to 4.15 mg P/L at day 50, and showed moderate variations after that. The average phosphate removed by the woodchip section and the steel chip section were 0.34 and 5.99 mg P/L, respectively, which resulted in an average overall removal of 63.3% from the influent concentration. This phosphate removal by the reactor was only 16.0% lower than the
phosphate (7.54 mg P/L) removed at 22 °C. Under relatively stable operating conditions between 50 and 100 days, the average nitrate removed by the woodchip section was 2.60 mg N/L or 2.78 g N/m³/d, and the average phosphate removed by the steel chip section was 5.05 mg P/L or 58.9 g P/m³/d. The denitrification capacity of the woodchips reduced by 84.6% and the phosphate removal capacity of the steel chips reduced by 11.9% under stable conditions when reducing the temperature from 22 to 5 °C. These results showed that the activities of the denitrifying bacteria were largely suppressed at 5 °C whereas the phosphate adsorption onto steel chips was not substantially affected by temperature variations under the conditions of this study. The calculated temperature coefficients (Q_{10}) based on the removal rates under stable conditions (50-100 days) of the wood/steel reactor were 3.01 for nitrate removal by the woodchips and 1.08 for phosphate removal by the steel chips. The use of steel chips for phosphate control would be beneficial in cold regions because the adsorption process of these materials is highly resistant to temperature changes.

3.3.5 Implications on Nutrient Removal Using Woodchips and Steel Chips

The results of column experiments in this study demonstrate that the steel chip filter was able to maintain effective phosphate removal during the 100 days of operation. These steel byproducts are efficient phosphate sorption materials that can be used in conjunction with woodchips to achieve dual nutrient reduction. The phosphate removal performance of steel chips showed high resistance to the changes in flow rates and temperatures in this study. This indicates that steel chips can achieve effective phosphate removal under variable flow and temperature conditions. This quality makes steel chip filters a suitable material for phosphate control in runoff and drainage water.
All three reactor configurations evaluated in this study effectively removed nitrate and phosphate and achieved similar nutrient reduction efficiencies. Therefore, nutrient removal performance will not be a primary factor when selecting the media configuration for field applications. The selection of dual-media reactors using woodchips and steel chips would depend on other factors such as potential negative environmental impacts, easy implementation, and hydraulic properties. The results of this study showed that the steel chip filter, when placed downstream of the woodchip bioreactor, reduced organic carbon exports, whereas the woodchip bioreactor, when placed downstream of the steel chip filter, reduced effluent iron levels. The mixed reactor achieved reasonable control of both iron and organic carbon leaching during this study. The mixed media design can be easily implemented in the field using a single reactor, which is especially beneficial when upgrading the existing woodchip bioreactors to dual-media reactors. Steel chips may have negative impact on the hydraulic property of the two-stage reactors due to the smaller particle sizes and potential material agglomeration during operation. Blending steel chips with woodchips in the mixed reactor would likely mitigate the impact of steel chips on reactor hydraulics. Certainly, the advantages of the mixed media design should be further evaluated in field applications.

3.4 Conclusions

Laboratory column experiments were conducted to evaluate the impact of three different media configurations on nitrate and phosphate removal of dual-media reactors using woodchips and steel chips at a volumetric ratio of 11:1. The three column reactors based on the wood/steel, steel/wood and mixed media configurations exhibited similar nitrate and phosphate removal during the 100 days of operation. The three dual media
reactors achieved 65.1-66.5\% average removal of influent nitrate (30 mg N/L) and 75.4-79.4\% average removal of influent phosphate (10 mg P/L) at a reactor HRT of 12.5 h.

Media configurations had a significant impact on reactor effluent DOC and iron concentrations. Steel chips showed high DOC adsorption capacities and woodchips effectively removed dissolved iron. The wood/steel reactor reduced DOC leaching by 55.0\% compared to the steel/wood reactor. However, the steel/wood reactor reduced iron leaching by 94.3\% compared to the wood/steel reactor. The mixed media reactor was able to effectively control both DOC and iron leaching.

The woodchip and steel chips sections in the wood/steel reactor showed different responses to the variations in flow rates and temperatures. The nitrate removal extents by the woodchip section decreased by 70.4 \% when decreasing the section HRT from 11.0 to 2.76 h. The nitrate load reduction rates of woodchips varied between 18.3 to 21.7 g/m$^3$/d across the HRTs. The phosphate removal extents by the steel chips only decreased by 18.2\% decreasing the section HRT from 11.0 to 2.76 h. At the same time, the phosphate load reduction rates of steel chips increased substantially from 64.8 to 212 g/m$^3$/d. The calculated temperature coefficients ($Q_{10}$) were 3.01 for nitrate removal by the woodchips and 1.08 for phosphate removal by the steel chips under stable operating conditions.

Phosphate removal by the steel chip filter was much less affected by flow and temperature variations than nitrate removal by the woodchip bioreactor.
Figure 3.1: Schematic of laboratory woodchip and steel chip reactors. (Sampling ports are evenly distributed along the height of each reactor.)
Figure 3.2: Nitrate and phosphate concentrations in reactor effluents. (Experimental conditions: HRT = 12.5 h; temperature = 22 °C).
Figure 3.3: Dissolved organic carbon and dissolved iron concentrations in reactor effluents. (Experimental conditions: HRT = 12.5 h; temperature = 22 °C).
Figure 3.4: Nutrient, dissolved iron and DOC profiles of wood/steel and steel/wood reactors under stable conditions. (Vertical dash lines separate woodchip sections from steel chip sections. Experimental conditions: $\text{NO}_3^-$ = 30 mg N/L; $\text{PO}_4^{3-}$ = 10 mg P/L; woodchip HRT=11 h; steel chip HRT=1.48 h).
Figure 3.5: Effect of HRT on nitrate and phosphate removal by the wood/steel reactor. (Experimental conditions: NO$_3^-$ = 30 mg N/L; PO$_4^{3-}$ = 10 mg/L; Temperature = 22º C).
Figure 3.6: Nitrate and phosphate removal by the wood/steel reactor at 5 °C. (Experimental conditions: NO$_3^-$ = 30 mg N/L; PO$_4^{3-}$ = 10 mg/L; total HRT = 12.5 h).
Figure 3.7: Woodchips and Steel chips Surface before and after the experiment. (A: Woodchips before Steel; B: Woodchips After Steel; C: Woodchips mixed with steel; D: Steel chips before Experiment).
Table 3.1: Characteristics of woodchips and steel chips used in this study.

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>pH</th>
<th>Particle Size</th>
<th>Particle Density (g/cm³)</th>
<th>Packing Density (g/cm³)</th>
<th>Hydraulic Conductivity (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woodchips</td>
<td>Cottonwood</td>
<td>6.8</td>
<td>0.4-6 cm</td>
<td>0.62</td>
<td>0.24</td>
<td>2.95</td>
</tr>
<tr>
<td>Steel Chips</td>
<td>Carbon Steel</td>
<td>6.3</td>
<td>1-2 mm</td>
<td>5.30</td>
<td>1.43</td>
<td>1.02</td>
</tr>
</tbody>
</table>

a. Values of pH were obtained from a 1:1 by weight ratio of material and distilled water.
b. Steel chip size range was determined by standard sieve analysis. Woodchip size range was measured manually.
c. Particle densities were determined using the water displacement method.
d. Packing densities were determined by the volume occupied by the mass of each material in the column reactor.
e. Hydraulic conductivities were determined by the falling-head tests at the same column packing densities.
Table 3.2: Nitrate and phosphate average removal by woodchip and steel chip sections during days 50-100 operation (Experimental conditions: Influent nitrate = 30 mg N/L; Influent phosphate = 10 mg P/L; Temperature = 22 °C; Woodchip HRT = 11 h; Steel chip HRT = 1.48 h.)

<table>
<thead>
<tr>
<th>Nutrient Removal</th>
<th>Nitrate Removal by the Woodchip Section</th>
<th>Phosphate Removal by the Steel Chip Section</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wood/Steel Reactor</td>
<td>Steel/Wood Reactor</td>
</tr>
<tr>
<td>Removal Extent (mg/L)</td>
<td>16.9 ± 0.4</td>
<td>17.2 ± 0.3</td>
</tr>
<tr>
<td>Load Reduction Rate (g/m³/d)</td>
<td>18.1 ± 0.4</td>
<td>18.4 ± 0.3</td>
</tr>
</tbody>
</table>
Table 3.3: Impact of HRTs on nitrate and phosphate removal by the wood/steel reactor. (Experimental conditions: Influent nitrate = 30 mg N/L; Influent phosphate = 10 mg P/L; Temperature = 22 °C.)

<table>
<thead>
<tr>
<th>Total HRT (h)</th>
<th>Woodchip Section</th>
<th>Steel Chip Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT (h)</td>
<td>NO$_3^-$-N Removal</td>
<td>PO$_4^{3-}$-P Removal</td>
</tr>
<tr>
<td>mg/L</td>
<td>g/m$^3$/d</td>
<td>mg/L</td>
</tr>
<tr>
<td>3.13</td>
<td>5.06</td>
<td>21.6</td>
</tr>
<tr>
<td>6.26</td>
<td>10.2</td>
<td>21.7</td>
</tr>
<tr>
<td>12.5</td>
<td>17.1</td>
<td>18.3</td>
</tr>
</tbody>
</table>
CHAPTER FOUR
LONG TERM NITRATE AND PHOSPHATE REMOVAL USING AGRICULTURAL RESIDUE MEDIA AND WOODCHIPS

Abstract

Denitrification bioreactors have been used as an effective method for removing nitrate from agricultural subsurface drainage. Woodchips have been primarily used as the primary carbon source for denitrification bioreactors. There are new concerns about optimizing nitrate removal in denitrification bioreactors. Agricultural residue medias have shown greater nitrate removal than wood due the more labile carbon content. The objective of this study was to investigate different ranges of carbon sources and determine their removal rates and the adverse effects of these carbon sources DOC leached in the environment. In this study, Corn Cobs, Corn Stover, Barley straw and woodchips were used as carbon sources for the denitrification bioreactors.

The laboratory study was run for a period of 13 months continuously. Four denitrification reactors filled with corn cobs, corn stover, barley straw and woodchips were used to treat simulated agricultural drainage water with 100 mg N/L of nitrate and 1 mg P/L of phosphate at a fixed hydraulic retention time of 12 hours for 13 months. The 13 months nitrate load reduction average were as follow corn cobs > corn stover > barley straw > woodchip. The 13 months nitrate removal rates average achieved by each material were 57.01 g N/m³/d, 21.94 g N/m³/d, 19.67 g N/m³/d and 9.10 g N/m³/d respectively for corn cobs, corn stover, barley straw and woodchips. The phosphate removal rates follow the same trend as the nitrate removal rates with corn cobs > corn stover > barley straw > woodchips. The average phosphate removal rates achieved by
each material were 0.35 g P/m$^3$/d, 0.16 g P/m$^3$/d, 0.14 g P/m$^3$/d and 0.05 g P/m$^3$/d respectively for corn cobs, corn stover, barley straw and woodchips.

Dissolved organic carbon leached was observed in the effluent of each reactors with the DOC concentration after 13 months of operation being 1.51 mg/L, 0.57 mg/L, 0.26 mg/L and 0.84 mg/L, respectively, for corn cobs, corn stover, barley straw and woodchips. The SUVA values after 13 months of experiment was 4.93 L/mg-m, 6.48 L/mg-m, 6.52 L/mg-m and 6.87 L/mg-m, respectively for corn cobs, woodchips, corn stover and barley straw.

Corn cobs demonstrated constantly higher nitrate removal rates than other materials throughout the 13-month experiments. The results of this study suggest that corn cobs could be potentially used as an alternative organic carbon source for long-term use in denitrification bioreactors.

4.4 Introduction

The increase of intensive agriculture over the few years has been one of the main factors that contributed to some important environmental issues leading to the deterioration of surface water quality and eutrophication (Withers et al., 2014). The main nutrients leached from this heavy agriculture are nitrate and phosphate that come from the use of fertilizers. Nitrate contamination can be avoided by monitoring and treating the contaminated water.

For a very long time, different types of technologies have been used to remove nitrate. Among them, reverse osmosis, ion exchange, chemical reduction, and biological denitrification (Della Rocca, Belgiorno, & Meriç, 2007; Mohsenipour, Shahid, &
Ebrahimi, 2014). Biological denitrification was the most cost effective and environmentally friendly compared to the other methods already available. During the biological denitrification, a carbon source is necessary as the electron donor to successfully reduce nitrate to nitrogen gas with nitrite being formed as an intermediate byproduct. Woodchips have been intensively used as the carbon source for biological denitrification due to its practicality and cost effectiveness (Aalto et al., 2020; Feyereisen et al., 2020; Hellman et al., 2021; Jang et al., 2019).

Over the years, it has been found that woodchips ability to remove nitrate decreases over time (Christianson et al., 2020; Mala., 2020; Robertson., 2010). New concerns regarding optimizing nitrate removal and mitigating the release of unintended contaminants have arose. For this reason, many different carbon sources were trialed over the years as a media for biological bioreactors (Cameron and Schipper, 2010; Della Rocca et al., 2007; Feyereisen et al., 2016; Gibert et al., 2008; Saliling et al., 2007; Shao et al., 2008; Warneke et al., 2011). Among these alternative carbon sources, maize cobs, wheat straw, green waste, sawdust, corn cobs, corn stover, barley straw and newspapers. These different media have consistently shown higher nitrate removal rates compared to woodchips (Greenan et al., 2006; Cameron and Schipper, 2010; Warneke et al., 2011a; Krause Camilo et al., 2013; Xu et al., 2009).

According to the current literature, High nutrients leaching levels have been correlated with agricultural residue media during bioreactor start up (Gibert et al., 2008; Healy et al., 2012; Krause Camilo et al., 2013). It was also found that the carbon in these media degrade quickly which can lead to a decrease in nitrate removal rates and the frequent replacement of the media which can be a tedious work (Soares and Abeliovich,
1998; Aslan and Türkman, 2005; Greenan et al., 2006; Saliling et al., 2007, Christianson et al., 2020). Although agricultural residue media have been used in biological reactors, their long-term nitrate removal rates were not evaluated since most of the experiment were just for a few months duration.

This study was meant to compare the long-term nitrate removal rates of agricultural residue media and woodchips. The zero order nitrate removal constants were determined for each material at different days of the experiment.

4.5 Materials and methods

4.5.1 Media characteristics and Collection

Corn cobs, corn stover and barley straw were collected at the South Dakota State University Southeast Research Farm in Beresford, SD. The woodchips were collected from Hardscapes outlet in Sioux Falls, SD. The corn cobs, corn stover and barley were cut each to the length of 3 cm and were gently washed using distilled water to remove any dirt and fine particles. They were put on a plastic sheet to dry overnight. The woodchips also were gently washed using distilled water, dried on a plastic sheet and then hand screening was done to remove wood particles bigger than 6 cm. The final woodchips size distribution used for the experiment was 0.4-1 cm 11%, 1-3 cm 53%, and 3-6 cm 36%.

4.5.2 Column Reactor set up and Experimental conditions

Four clear acrylic tubes with a length of 1.2 meters and inside diameter of 8.85 cm were used to construct up flow column reactors (Figure 4.2). Each reactor had 12 sampling ports installed along the length of the column at 10 cm intervals. Each reactor was packed with approximately 1301.74 g, 515.52 g, 473.64 g and 1470.68 g of Corn
cobs, corn stover, barley straw and woodchips, respectively. The resulting porosity for each reactor was 64.51%, 47.16%, 43.64% and 41.33% respectively for barley straw, corn cobs, corn stover and woodchips. Each reactor was fed with artificial subsurface drainage water containing micronutrients as described in Hua et al., 2016. The particles sizes of each materials are listed in table 4.1.

The experiment was divided into 3 main categories. The first phase was nutrients and DOC leaching using distilled water that lasted for a period of 1 week. The second phase was the long-term nitrate and phosphate removal. All four column reactors were operated at an influent nitrate concentration of 100 mg N/L and Phosphate concentration of 1 mg P/L at a fixed HRT of 12 h for 13 months under laboratory room temperature (22°C). The simulated agricultural subsurface drainage water contained also various ionic constituents and traces of minerals. The recipe was described in Hua et al., 2016. A Masterflex l/s variable speed peristaltic pump coupled with a multichannel head using Tygon l/s 16 ID diameter tubing was used to achieve the same HRT across all four reactors.

The third phase was the determination of the zero order nitrate removal constants at different operation days. The zero-order constant were determined at day 1, day 180 and day 390 of operation. The denitrification kinetics experiments were conducted using a nitrate concentration of 100 mg N/L at an HRT of 12 h at a temperature of 22°C. Two sampling events were conducted during each sampling day, and samples were taken at each sample port along the length of the column. Six replicates were obtained from each sample port.

4.5.3 Phosphate desorption experiments
The phosphate desorption experiment was performed after the bioreactor 13 months experiment to evaluate the phosphate recovery potential of each media. Samples from the corn cobs, corn stover, barley straw and woodchips reactors were collected and air dried under room temperature. A 1 L volumetric flask was used as a batch reactor. For each material, 30.08g, 20.09, 10.10g, 30.08g of corn cobs, corn stover, barley straw and woodchips were used respectively for the batch desorption test. The desorption solutions used for this study included 0.005 M NaCl and 0.5 M NaOH. The samples were placed on a Thermo Scientific shaker model MAXQ 4000 at 125 rpm. Daily samples were taken to measure phosphate concentration. The sampling was stopped when phosphate leached could not be detected.

4.5.4 Analytical Methods

All solutions used in this study were prepared with ultrapure water (18 MΩ-cm) produced by a Barnstead Nanopure system (Thermo Fisher Scientific Inc., Waltham, MA). The influent solutions were adjusted to pH 7 using sodium hydroxide or sulfuric acid solutions. The chemicals used in this study were of American Chemical Society reagent grade and were purchased from Sigma Aldrich (St Luis, MO) and Fisher Scientific. Nitrate, nitrite, and phosphate ions were measured using a Dionex Integrion RFIC Ion Chromatography instrument with a Dionex Ion-PAC AG 11-HC (4x50 mm) Guard Column and an AS 11-HC (4x250 mm) analytical column. The DOC concentrations were determined with a Shimadzu TOC-5000 Analyzer (Shimadzu Corp., Kyoto, Japan) according to Standard Method 5310 B (APHA et al., 2012). The pH value of each solution was measured using an Orion 290A+ advanced ISE/pH/mV/OPR meter (Thermo Fisher Scientific Inc, Waltham, MA). DO concentrations were measured using
an YSI probe (YSI Inc., Yellow Springs, Ohio). The UV absorbance at 254 nm (UV254) was measured by a Hach DR4000U spectrophotometer (Hach, Loveland, CO). The DOC and UV254 samples were filtered through a 0.45 μm glass fiber membranes (Whatman, Clifton, NJ) prior to analysis. SUVA was calculated from UV254 (cm-1) divided by the DOC (mg/L) an then multiplying by 100 cm/M.

4.5.5 Statistical Analysis

All statistical analysis were conducted using Rstudio version 1.2. For all four reactors configurations, the difference between nitrate removal, phosphate removal, and DOC leached were examined with a one-way anova test followed by a Duncan test to differentiate which reactors configurations parameters were significantly different from each other.

4.6 Results and Discussion

4.6.1 Initial Nutrients and DOC leaching from different reactors using distilled water.

Figure 4.3 presents the nitrate, phosphate and DOC leached from each material using distilled water at a 12-hour HRT. All four reactors released nitrate, phosphate, and DOC for seven days. The averaged seven days nitrate trend in the effluent was as follows (barley straw > corn stover > corn cobs > woodchip). The total cumulative amount of nitrate exported during the seven days for each material was 47.13 mg N/L, 43.26 mg N/L, 13.97 mg N/L and 3.241 mg N/L respectively for barley straw, corn stover, corn cobs, and woodchips. The averaged phosphate leached trend for seven days was as follows (barley straw > corn stover > corn cobs > woodchip). The total cumulative amount of phosphate leached from each material was 6.89 mg P/L, 5.889 mg P/L, 3.17 mg P/L and 1.58 mg P/L respectively for barley straw, corn stover, corn cobs and woodchips. The
concentration of nutrients leached from woodchips is considerably lower than other agricultural residue media because wood consists mostly of organic molecules, such as lignin and polysaccharides, which lack nitrogen. Each material achieved high DOC leaching during the first seven days of operation. The average seven days DOC leached was 124.38 mg/L, 40.34 mg/L, 39.19 mg/L and 8.66 mg/L respectively for corn cobs, corn stover, woodchips and barley straw.

Previous studies have also associated agricultural residue with high levels of nutrients and DOC leaching (Cameron and shipper et al., 2010; Gibert et al., 2008; Healy et al., 2012; Krause Camilo et al., 2013). To mitigate the potential effect of those nutrients leaching into surface water, different techniques have been proposed during bioreactor start up. Among them, starting up bioreactor in low temperatures, using shorter HRT or pre flushing. The pre flushing technique was the one used in our study by using distilled water to flush all nutrients and high DOC before starting the nitrate removal experiment.

4.6.2 Long Term Nitrate and Phosphate removal

Figure 4.4 shows nitrate and phosphate removal for all four reactors during the 13 months period. The experiment was performed at room temperature (22 °C) and fixed HRT of 12 hours with an influent nitrate and phosphate concentration of 100 mg N/L and 1 mg P/L respectively. All four reactors successfully removed nitrate and phosphate. The average 13 months nitrate removal percentage was 60.44%, 25.14%, 15.25% and 11.01% respectively for corn cobs, corn stover, barley straw and woodchip. The average nitrate load reduction was 57.01 g N/m³/d, 21.94 g N/m³/d, 19.67 g N/m³/d and 9.10 g N/m³/d respectively for corn cobs, corn stover, barley straw and woodchip (table 4.2). The
moderately high removal of nitrate by barley straw faded to one of the poorest performances by the end of the 13 months experiment. Corn stover follows the same trend as barley straw by losing its high nitrate load reduction by the end of the 13 months of operation. The same observations were made by previous studies (Feyereisen et al., 2016; Soares and Abeliovich, 1998; Aslan and Türkman, 2005; Greenan et al., 2006).

The agricultural residues media nitrate load reductions are within the range of what was observed by other studies that used agricultural residue media as a carbon source. In fact, in denitrifying woodchip bioreactors, NO$_3$ removal rates typically vary between 2 and 22 g N/m$^3$/d, while NO$_3$ removal rates have been observed to be as high as 73 g N/m$^3$/d (Hassanpour et al., 2017; Schipper et al., 2010). Feyereisen et al., 2016 and Cameron and Schipper (2010) found that corn cobs achieved a nitrate load reduction between 34.9 and 34.6 g N/m$^3$/d at 15°C and 14°C respectively. The overall performance of woodchips was also still within the range of what was previously reported for woodchips (2 to 22 g N/m$^3$/d) by Christianson et al., 2012 and Schipper et al., 2010. At 13 months of operation, corn cobs, corn stover, woodchips and barley achieved, respectively, a nitrate removal percentage of 47.15%, 6.22%, 5.21% and 1.08%.

A statistical analysis (anova-test) was performed on the 13 months nitrate removal to further understand if there are differences in nitrate removal among the four different materials. The results show that there is a significant difference in nitrate removal between those four materials with a F value of 178.7 and P-value <2.2e-16<0.05. To further validate or refute this results a Duncan and Tukey’s HSD (honestly significant difference) tests were performed and found that there is no statistical difference in nitrate removal between woodchips and barley straw. Which means that overtime, barley straw
is not a viable option to replace wood chips. The statistical difference was between corn cobs, corn stover and woodchips or barley straw.

Phosphate removal was observed for all four reactors with the averaged 13 months phosphate removal percentage of 36.74 %, 17.58%, 10.91% and 5.36% respectively for corn cobs, corn stover, barley straw and woodchips (CC>CS>BS>WC). The phosphate load reduction varies from 0.35 g P/m³/d to 0.05 g P/m³/d for all four reactors (table 4.2). Corn cobs and barley straw achieved almost 100% phosphate removal during the first 30 days of operation. A breakthrough phosphate removal was observed after 100 days of operation for corn cobs, corn stover and barley straw. At day 390, corn cobs were still able to remove 15% of the influent phosphate. After 100 days of operation, barley straw lost its ability to remove phosphate and started leaching phosphate. The highest percentage of phosphate leached was 7% of the influent phosphate. From day 150 to 390, barley straw was still leaching phosphate with an effluent phosphate concentration being 2% higher than the influent phosphate concentration. After 200 days of operation, corn stover removed only 5% of the influent phosphate concentration. Woodchip ability to remove phosphate developed overtime. After 60 days woodchip phosphate removal gradually increases with its peak removal occurring at day 170 with a removal percentage of 23%. The same observation was made by Hua et al., 2016 that found woodchips were able to remove phosphate after 50 days of operation with an average of 60% of phosphate removed based on the 1 mg P/L influent phosphate concentration. Woodchips started losing it ability to remove phosphate after 200 days of operation with less than 5 % phosphate removal percentage. The phosphate removal percentage of woodchips will remain stable from day 200 to 390.
The 13 months anova test shows that there is a statistical difference between the phosphate removal achieved by all four materials. The F value is 32.88 and P value 2.2e-16 < 0.05. The Duncan and Tukey’s HSD (honestly significant difference) show that corn cobs, corn stover, woodchip and barley phosphate removals are statistically different from each other but there is no statistical difference in phosphate removal between woodchip and barley straw. Which means replacing woodchip by barley straw for phosphate removal is not a viable option on the long term.

4.6.3 Nitrite Accumulation

Nitrite accumulation was observed for all four materials. figure 4.5 shows nitrite accumulation during the 390 days operation. The highest nitrite effluent concentration achieved by each media was 3.9 mg N/L, 1.94 mg N/L, 0.32 mg N/L, 0.11 mg N/L respectively for barley straw, woodchips, corn stover and corn cobs. Barley straw had the highest nitrite effluent concentration observed which happened at the beginning of the experiment and lasted for about 100 days this high nitrite concentration in the effluent is also linked to the high nitrate removal observed during the same period of operation. About 6% of nitrate removed is converted to nitrite. After 100 days, the nitrite concentration started decreasing, at the same time barley straw ability to remove nitrate also started decreasing. Woodchip’s nitrite effluent concentration gradually increased overtime and peaked at day 314 and started decreasing. The decrease in nitrite effluent concentration in the woodchip’s reactor is also linked to the decrease in woodchip ability to remove nitrate. Corn cobs and corn stover had lower nitrite concentration in their effluent since the beginning of the experiment. This can be explained by an almost
complete denitrification happening in the reactor which was not the case in the other reactors.

4.6.4 DOC leaching Characteristics

Submerged organic materials degradation starts with a leaching process, followed by a hydrolysis phase which is characterized by the breakdown of the released macromolecules into simpler compounds (Ramírez-Godínez et al., 2015). Dissolved organic carbon played an important role in the denitrification process. DOC leaching from biological bioreactors have been observed by many studies (Gosch, Liu, & Lennartz, 2020). In fact, the 13 months average DOC leached was 5.42 mg/L, 2.71 mg/L, 2.78 mg/L and 1.18 mg/L respectively for corn cobs, corn stover, woodchips and barley straw. Figure 4.6 presents the DOC values during the long-term DOC leaching experiments. DOC characteristics vary with time. The initial DOC leached by each reactor was 10.01 mg/L, 5.19 mg/L, 2.4 mg/L and 4.4 mg/L, respectively, for corn cobs, corn stover, barley straw and woodchips. The 13 months average SUVA values for all four reactors were 4.31, 5.86, 6.34, 6.69 L/mg-m, respectively for corn cobs, corn stover, woodchips and barley straw. Corn cobs has the lower average SUVA value which is attributed to a lower humic substances concentration which correlate to a higher nitrate removal rate achieved compared to the other materials. Barley straw had the higher average SUVA value which also correlated to the poor performance of barley straw when it comes to nitrate removal. All four materials had lower SUVA values at the beginning of the experiment which led to a higher nitrate removal rate. Overtime the SUVA started increasing which means more humic substance was present in the DOC making more difficult for bacteria to degrade. The increase in the SUVA value was also linked to the
reduce in nitrate removal percentage achieved by each material. Mopper and Schultz 1993; Wetzel et al. 1995; Moran and Zepp 1997 found that the biodegradation of dissolved organic matter can lead to a loss of labile and low molecular weight aliphatic material. The low molecular weight materials such as carbohydrates and organic acids are easily biodegradable by microorganisms which explain the high denitrification rates achieved by different material during the first few months of operation. As time goes by, the biodegradation process can produce high molecular weight aromatic material such as fulvic and humic acid by heterotrophs bacteria. (Repeta et al. 2002; Stepanauskas et al. 2005) Which can be link to the high SUVA values overtime. These high molecular weight materials are difficult to degrade by bacteria which can lead to a decrease in the denitrification rates overtime.

4.6.5 Nitrate and DOC zero order Kinetics constants at different days.

Figure 4.7 shows the course of nitrate removal along the length of the reactors at different dates. In all four reactors, nitrate removal decreases near linearly along the length of the reactors which means that nitrate removal follows a zero-order reaction. The nitrate removal zero order constants at day 1 were 7.90 mg/L/h, 6.40 mg/L/h, 5.48 mg/L/h and 2.56 mg/L/h respectively for Corn cobs, Corn Stover, Barley straw and Woodchips. From day 1 to day 180, a 42.9 %, 79.38%, 94.34% and 75.29% decline in nitrate removal rates were observed respectively for corn cobs, corn stover, barley straw and woodchips. From day 180 to 390, only 12.86%, 60.60%, 67.74% and 31.74% decline in nitrate removal rates were observed respectively for Corn cobs, Corn Stover, Barley straw and Woodchips. From figure 4.7, most of the nitrate removal rates decrease happened during the first 180 days and a relatively steady rate persist for the remaining
period of the experiment. Roberston et al., 2010 found that woodchips lose about 50% of its ability to remove nitrate after 1 year of operation and maintained a stable removal for the remaining years of operation. Table 4.3 summarizes the different first order constants at different dates for all four materials.

In fact, the decline in nitrate removal can also be linked to the decline in the rate of DOC release for each material at different dates. The DOC release rate follows a linear trend along the length of each reactor (figure 4.8). A zero-order kinetics was used to model amount of DOC released from each reactor. The DOC release rates trend were as follow day 1>day 180> day 390. More DOC was release during the first day of operation which contributed to the highest nitrate removal rates observed for all four materials at day1. The rates constants at day 1 were 0.87 mg/L/h ,0.43 mg/L/h,0.2 mg/L/h and 0.36 mg/L/h respectively for corn cobs, corn stover, barley straw and woodchips. At 180 days, the rate constants dropped to 0.47 mg/L/h,0.2 mg/L/h,0.07 mg/L/h and 0.19 mg/L/h respectively for corn cobs, corn stover, barley straw and woodchips. The percentage drop was between 43.37% and 60 % from day 1 to day 180. After 390 days, DOC release rate drop was between 63.15% and 75% for all four materials compared to day 180. Table 4.3 summarize DOC release rates constants at different dates for all four materials. From this observation, overtime, when DOC release decreases the different materials also achieved a lower nitrate removal rate. Figure 4.9 shows DOC color changes during the experiment. Which means that there is a strong correlation between nitrate removal and the amount of DOC released overtime. The same observation was made by previous studies that found that when the carbon source become limiting it affects denitrification rates which tend to decrease. Hassanpour et al., 2017 found that during the winter, DOC availability was a
limiting factor in the performance of the bioreactor. Since low temperature inhibited the release of DOC. Cameron and Schipper, 2010; Greenan et al., 2006 found that a labile source of carbon has a huge impact on bioreactor efficiency.

4.6.6 Batch Phosphate recovery after the experiment.

The two solutions of NaCl and NaOH were effective in stripping the phosphate off the different agricultural materials and woodchips. By using NaOH as a strong basic solution, it was anticipated that most of the adsorbed phosphate would be desorbed due to the effect of $P^H$ (Zach-Maor, Semiat, & Shemer, 2011). The 0.005 M NaCl was to simulate the ionic strength of natural water in the environment. Figure 4.10 shows the amount of phosphate leached per grams of materials during a 4-day period. After 4 days no phosphate leaching was observed. For both type of desorption solutions, the trend of the amount of phosphate released was as follow corn stover > corn cobs > barley straw > woodchips. The total amount of phosphate removed by each material after 13 months of operation were 87.861 g, 42.045 g, 37.00g and 12.793 g respectively for corn cobs, corn stover, barley and woodchips. When using the 0.5 M NaOH solution, the phosphate recovery percentage achieved was 28.40%, 45%, 9.97%, 30.46% respectively for corn cobs, corn stover, barley straw, woodchips. The 0.005 M NaCl solution achieved a phosphate recovery percentage of 8.71%, 56.64, 5.60% and 8.39 % respectively for corn cobs, corn stover, barley straw and woodchips. For both desorption solution, the phosphate release rates were relatively fast during the first day and gradually slowed for the remaining following days. P absorption and release are not only dependent on the concentration of phosphate in water, but also on environmental factors which in our study is the nature of the desorption solution used (Wang and Liang., 2015). Corn stover ability
to leach back more phosphate might be due to its physical properties. In fact, corn stover has a porous media inside it shells which might be able to release phosphate compare to other media that are hard on the outside and inside. Zach-Maor, Semiat, & Shemer, 2011 found that alkaline eluent phosphate desorption was primarily a surface reaction.

4.7 Conclusions

This study was conducted to evaluate the performance of agricultural residues as alternative organic carbon sources for denitrification bioreactors. Three agricultural residues were chosen for this study including corn cobs, corn stover and barley straw. Woodchips were also used to compare the denitrification performance with agricultural residues. Influent nitrate concentration of 100 mg N/L was maintained at a 12 h HRT at 22 °C for a period of 13 months. The results showed that denitrification efficiency followed the order of corn cobs > corn stover > barley straw>woodchips. The 13 months average nitrate load reduction rates were 57.01 g N/m³/d, 21.94 g N/m³/d, 19.67 g N/m³/d and 9.10 g N/m³/d respectively for corn cobs, corn stover, barley straw and woodchips. The corn cobs exhibited denitrification capacities that were 1.20-2.15 times higher than the woodchips at 22 °C.

The zero-order nitrate removal constant values at day 1 of operation were 7.90 mg/L/h, 6.40 mg/L/h, 5.48 mg/L/h and 2.56 mg/L/h respectively for Corn cobs, Corn Stover, Barley straw and Woodchips. After 180 days the zero-order nitrate removal constant decreased to 4.51 mg/L/h, 1.32 mg/L/h, 0.31 mg/L/h and 0.63 mg/L/h respectively for Corn cobs, Corn Stover, Barley straw and Woodchips. At 13 months of operation, corn cobs still achieved higher nitrate removal with a zero-order constant of
3.93 mg/L/h, first followed by corn stover 0.52 mg/L/h and second by woodchips 0.43 mg/L/h and last by barley straw 0.1 mg/L/h.

Although barley straw and corn stover showed high nitrate removal rates during the first three months of operation, the nitrate removal capacities of these two materials declined quickly specially for barley straw which removal capacities fell below the one achieved by woodchips. Therefore, barley straw and corn stover may not be suitable for long-term bioreactor applications.
Figures 4.1: Pictures Of The Different Carbon Sources Used To Pack The Different Reactors.
Figure 4.2: Laboratory Reactors Set Up of The Different Media. A: Without Aluminum Foil; B: With Aluminum Foil.
Figure 4.3: Leached Nitrate, Phosphate and DOC using Distilled water during the first week of operation (Experimental conditions: HRT = 12 h; temperature = 22 ºC).
Figure 4.4: Long Term Nitrate and Phosphate effluent concentration (Experimental conditions: HRT = 12 h; temperature = 22 °C).
Figure 4.5: Long Term Nitrite Accumulation. (Experimental conditions: HRT = 12 h; temperature = 22 °C)
Figure 4.6: Dissolved organic Carbon leaching and SUVA. (Experimental conditions: HRT = 12 h; temperature = 22 °C).
Figure 4.7: Nitrate Removal kinetics at different Experimental dates. (Experimental conditions: HRT = 12 h, temperature=22 °C).
Figure 4.8: DOC Release kinetics at different Experimental dates. (Experimental conditions: HRT = 12 h, temperature=22 °C).
Figure 4.9: DOC Leached Color Evolution During the Experiment.
Figure 4.10: Phosphate desorption after 1 year of experiment. (Number of days = 4 Temperature = 22º C).
Table 4.1: Characteristics of the different materials.

<table>
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<th>Material</th>
<th>Type</th>
<th>Reactor porosity (%)</th>
<th>Particle size</th>
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<td>Woodchip</td>
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<td>41.33</td>
<td>36% Large: 3-6 cm long, 0.5-2 cm wide</td>
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<td></td>
<td></td>
<td></td>
<td>52% Medium: 1-3 cm long, 0.5-1.5 cm wide</td>
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<td></td>
<td></td>
<td>12% Small: 0.4-1 cm long, 0.1-1 cm wide</td>
</tr>
<tr>
<td>Corn Cobs</td>
<td></td>
<td>47.16</td>
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<tr>
<td>Corn Stover</td>
<td></td>
<td>43.64</td>
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</tr>
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<td>Barley Straw</td>
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<td>64.51</td>
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Table 4.2: Average Nitrate load and percentage reduction rates in woodchip bioreactors. (Experimental conditions: HRT=12h; temperature = 22°C)

<table>
<thead>
<tr>
<th>Influent Nitrate and phosphate Concentration (mg/L)</th>
<th>Materials</th>
<th>Average 390 days Nitrate and Phosphate Load and Percentage Reduction Rates</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nitrate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>100 mg N/L 1 mg P/L</td>
<td>Corn Cobs</td>
<td>60.44</td>
</tr>
<tr>
<td></td>
<td>Corn Stover</td>
<td>25.14</td>
</tr>
<tr>
<td></td>
<td>Barley Straw</td>
<td>15.25</td>
</tr>
<tr>
<td></td>
<td>Woodchip</td>
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</table>
Table 4.3: Nitrate and Dissolved Organic Carbon Zero Order Kinetics Constants at nitrate concentration of 100 mg N/L.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Nitrate Removal Zero Order Constant (mg/L/h)</th>
<th>DOC Leaching Zero Order Constant (mg/L/h)</th>
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<tr>
<td></td>
<td>Day 1</td>
<td>Day 180</td>
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<tr>
<td>Corn Cobs</td>
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<td>Corn Stover</td>
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<td>Barley Straw</td>
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<tr>
<td>Woodchips</td>
<td>2.56</td>
<td>0.74</td>
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</table>
CHAPTER FIVE
SOLAR, NATURAL AND COMPOSTED WOOD PRETREATMENT DURATION IMPACT ON NITRATE REMOVAL RATES

Abstract

Denitrification bioreactors have been intensively used over the years with woodchips being the main carbon source. Many factors affect denitrification bioreactors performances. Among them temperature, HRT, woodchips types and size. However, there are still some lacks on understanding when it comes to woodchips condition impact on the bioreactor performance. The objective of this study was to investigate the impact of woodchip different pretreatments duration on nitrate removal efficiency using two type of woodchips. The different pretreatments used were solar, natural, and composted. Oak wood and cottonwood woodchips were the two types of woodchips used.

A laboratory column study was performed for 6 months continuously. A batch study was also performed for 20 days. Eight denitrification reactors columns were filled with different pretreated woodchips. The different reactors were filled with oak raw, oak solar, oak natural, oak composted, cottonwood raw, cottonwood solar, cottonwood natural and cottonwood composted. The treatments duration were 5 months. Agricultural subsurface drainage water with a nitrate concentration of 50 mg N/L and 100 mg N/L were used respectively for the column experiment and the batch test respectively. The average 6 months nitrate removal rates achieved by oak wood were 41.55 g N/m³/d, 26.44 g N/m³/d, 20.24 g N/m³/d and 16.71 g N/m³/d respectively for oak solar, oak natural, oak composted and oak raw. The cottonwood achieved nitrate removal rates of 37.35 g N/m³/d, 24.17 g N/m³/d, 22.29 g N/m³/d and 18.43 g N/m³/d respectively for cottonwood
solar, cottonwood natural, cottonwood composted and cottonwood raw. The same nitrate removal trend was observed during the batch test. Solar treated woodchips achieved higher denitrification rates compared to other treated woodchips. The 5 months solar treated woodchips achieved between 2-2.5 times better denitrification rates compared to the raw wood respectively for cottonwood and oak wood during the column experiment.

The average DOC leaching observed during the 6 months operation were 17.6 mg/L, 9.13 mg/L, 6.21 mg/L and 13.22 mg/L respectively for oak solar, oak natural, oak composted and oak raw. The average DOC effluent achieved by cottonwood solar, cottonwood natural, cottonwood composted and cottonwood raw were 16.74 mg/L, 10.77, 10.22 mg/L and 13.32 mg/L.

The solar treated woodchips demonstrated higher nitrate removal compared to the other treatments. The other treated woodchips also performed better than the raw woodchips. The results suggest that pretreatment of woodchip before using it in denitrification bioreactors can be beneficial in enhancing nitrate removal.

5.1 Introduction

Nitrate and phosphate are important nutrients that have been used to boost crop growth. The use of these nutrients is not without any consequences for the environment. Over the years, nutrients leaching from agricultural fields have contaminated surface water creating algae blooms, oxygen depletion and the reduction of biodiversity of ecosystems (Almeelbi and Bezbaruah, 2012; Bratieres et al. 2008; van Driel et al., 2006).

Biological denitrification bioreactors have been used over the years to remove nitrate from agricultural subsurface drainage field due to their cost effectiveness.
Microorganisms use organic or inorganic carbon as an electron donor and nitrate as an electron acceptor to convert nitrate to inert nitrogen gas in biological denitrification (Schipper et al., 2010). Different carbon sources have been used in biological denitrification, among them, woodchips, corn cobs, wheat straw, papers fibers, sawdust, and rice husks. Nitrate load reduction achieved by denitrification bioreactors varies between 3 g N/m³/d to 96 g N/m³/d (Greenan et al., 2006; Greenan et al., 2009; Saliling et al., 2007; van Driel et al., 2006; Robertson 2010; Cameron and Schipper, 2010; Schipper et al., 2010; Chun et al., 2009; Shao et al., 2008).

Woodchips have been intensively used as the main carbon source for denitrifying bioreactors due to their ability to remove nitrate overtime compared to others carbon sources and it also cheaper. One of the main disadvantages of using woodchips as a carbon source is that its ability to remove nitrate declines over the years and the nitrate load reduction achieved by woodchips is lower compared to other carbon source used such as corn cobs (Robertson, 2010).

Wood is mainly composed of cellulose, hemicellulose, and lignin. Carbohydrates in wood is the combination of cellulose and hemicellulose. The cellulose content of a dry wood ranges from 40 % to 50 % and the hemicellulose content ranges from 25 to 35 %. Wood physical and chemical properties change when it is exposed to outdoor elements such as the sun and rain specially for a long period of time. This process is called weathering (William 2005). The weathering process is a combination of UV lights, water, oxygen, heat, and other pollutants such as SO₂, NO₂ and O₃ (Teaca et al., 2013). Biological denitrification of wood by bacteria and fungi can happen during wood storage outdoor. The changes in wood physical and chemical properties can in fact affect the
amount and type of carbon content in the wood thus affecting nitrate removal in
denitrification bioreactors. FTIR spectroscopy has proved to be a very useful method for
investigating wood chemical composition and changes during various wood treatments,
including weathering, decay, and chemical treatment (Moore and Owen. 2001). It is a
simple, fast, and reliable procedure that requires a small amount of sample with little to
no preparation. There is still a poor understanding of the impact of wood weathering on
bioreactor performances.

This study had for objective to determine the impact of wood weathering and
pretreatments on the nitrate removal rates for two different type of woodchips. The effect
of woodchip pretreatment duration on woodchips nitrate removal efficiency was
evaluated in batch study. FTIR analysis was performed on the raw woodchips before and
after the wood solar, natural, and composting pretreatments. This was used to investigate
the impact of wood pretreatment on its physical and chemical properties which can then
be correlated to the denitrification efficiency achieved by each wood pretreated.

5.2 Materials and Methods

5.2.1 Woodchip’s pretreatment

Woodchips made from cottonwood and oak wood were obtained from a
playground woodchip distributor in Sioux Falls, South Dakota (SD). The same types of
woodchips have been used for several field bioreactors. After collection, both woodchips’
types were hand screened and woodchips larger than 6 cm were removed manually and
woodchips smaller than 4 mm were removed by sieving. The woodchips were then gently
washed with distilled water to remove dirt and floating fine particles, and air dried before
use. The size distribution of processed woodchips was 0.4-1 cm 11%, 1-3 cm 53%, and
3-6 cm 36%. These woodchips were referred as raw woodchips. Figure 5.8 shows the different woodchips used in the reactor. Three pretreatments were applied for each woodchip’s types. Among them, there are composting, Solar and natural pretreatments. A composting process with soil and moisture was used to treat the raw woodchips and produce the “composted woodchips”. The composting experiments were conducted in 5-gallon plastic buckets. Approximately 3 kg woodchips were mixed with 1.5 kg soil in each bucket. The soil sample was collected from the South Dakota State University research farm in Volga, SD. Each bucket was capped and placed at an open space outside. A 500 mL of water was added to each bucket weekly to maintain saturated moisture condition during the composting process. During the solar pretreatment, 2.5 kg of raw woodchips were put into a plastic pan wrapped with aluminum paper. This was to maximize the impact of solar treatment on the wood. Solar intensity was measured throughout the experiment period and the maximum solar irradiance recorded was 1024 w/m² and the lowest on less sunny days was 272 w/m². The plastic pans containing the woodchips were stored outside on the sun and were covered every time it rains so water does not get into the pans (figure 5.7). The last pretreatment was called natural pretreatment. During the natural pretreatment, woodchips were put into fishing nets and stored outside on the ground without any kind of control. The natural pretreatment woodchips were exposed to all the elements of nature such as sun, rain, bacteria degradation. All three pretreatments were done outside of Crothers Engineering Hall at South Dakota State University, Brookings, SD (figure 5.7). The woodchip pretreatment experiment was conducted from May to September. The average temperature during that
period was 25.8 °C. Figures 5.7 shows the pictures of the different woodchip condition during pretreatments.

5.2.2 Reactor set up and experimental conditions

Eight clear acrylic tubes with a length of 20 centimeters and inside diameter of 8.85 cm were used to construct up-flow column reactors. Each reactor had an influent and effluent sampling port where samples were collected. Distilled water was added to each column to soak the woodchips for 48 h before the determination of reactor porosity. Drainable porosity was determined by draining each reactor over 1 h, and the resulting porosities and mass of the woodchip put into each column are listed on table 5.4.

Two Variable speed peristaltic pumps (Masterflex Model L/S, Cole Parmer, Vernon Hills, IL) were used to pump an artificial runoff water from an influent tank to the column reactors during this study. To limit HRT variation between column reactors the Masterflex l/s variable speed peristaltic pump was coupled with a multichannel head using Tygon l/s 16 ID diameter tubing. The artificial runoff water was composed of various ionic constituents and trace minerals to ensure that microbial growth was not limited. The recipe is described in Hua et al., 2016. KNO₃ was added to the artificial runoff water to achieve different influent nitrate concentrations for the column experiments.

The experiment was divided into 2 main phases. The first phase was the long-term nitrate and phosphate removal. All 8 column reactors were operated at an influent nitrate concentration of 50 mg N/L and Phosphate concentration of 1 mg P/L at a fixed HRT of 12 h for 6 months under laboratory room temperature (22°C). Figure 5.9 (A) shows the column reactors set up.
The second phase of the experiment was a batch scale experiment conducted to
test nitrate removal by the different woodchips at different treatments duration. The ratio
of woodchips to water was 1:20. Each volumetric flask contained 200 ml of nutrient
solution with 10 grams of woodchips and were samples daily. The initial nitrate
concentration used was 100 mg N/L to avoid nitrate limiting condition during the batch
experiment. Woodchips collected at 2 and 5 months of pretreatment were used for the
batch test. Total of 14 batch reactors were set up. The batch reactors were covered with
aluminum foil to simulates field bioreactors condition. During the static batch test, 10
grams of woodchips was put into 200 ml of simulated agricultural subsurface drainage
water containing nitrate. Figure 5.9 (B) shows the batch reactors set up.

5.2.3 Sample collection and Analysis

Weekly samples were collected for all 8 reactors starting from the first day of
operation and lasted for 6 months during the first phase of the experiment. Samples were
taken from effluent sample port. A 40 ml sample was collected at each reactor effluent
and filtered using a 0.45 um filter. During the second phase of the experiment, samples
were taken daily from the static batch test. 1 ml of sample was collected from each batch
reactor for nitrate analysis. the batch test lasted for 20 days. Samples were stored at 4º C
before being analyzed for NO₃⁻-N (mg N/L) and NO₂⁻-N (mg N/L) using a DX-500 Ion
chromatography instrument with an Ion Pac AS14 Guard Column and an AS-14
analytical column with an AS-40 auto sampler. Samples were analyzed within one week
of storage. Additional samples were collected for DOC (dissolved organic carbon) and
Absorbance measurement. The samples were also filtered using a 0.45 um filter and
analyzed using a Shimadzu TOC-5000 Analyzer (Shimadzu Corp., Kyoto, Japan) according to Standard Method 5310 B (APHA et al., 2012).

5.3 Results and discussions

5.3.1 Long Term Nitrate Removal using different pretreated woodchips

Solar, natural, and composted woodchip pretreated for a period of 5 months were used for a long-term column reactor study. The column reactors were run for a period of 6 months. Figures 5.1 shows nitrate effluent concentrations for all different woodchips. All 8 bioreactors were able to remove nitrate with nitrate influent concentration of 100 mg N/L at 12 h HRT and 22°C. The average 6 months nitrate removal rates achieved by oak wood were 41.55 g N/m$^3$/d, 26.44 g N/m$^3$/d, 20.24 g N/m$^3$/d and 16.71 g N/m$^3$/d respectively for oak solar, oak natural, oak composted and oak raw. The 6 months cottonwood achieved nitrate removal rates of 37.35 g N/m$^3$/d, 24.1 g N/m$^3$/d, 22.29 g N/m$^3$/d and 18.43 g N/m$^3$/d respectively for cottonwood solar, cottonwood natural, cottonwood composted and cottonwood raw. Reactors stable condition was achieved after around 90 days of operation. Among the 8 reactors, Oak solar and cottonwood solar achieved the highest nitrate removal rates, followed by the oak natural and cottonwood natural and then the oak composted and cottonwood composted. The raw untreated oak and cottonwood woodchip achieved the least nitrate removal rates. From this long-term experiment, it can be found that wood pretreatment enhances nitrate removal rates. For the oak wood, solar treated achieved nitrate removal rates 2.5 times higher than the raw untreated oak wood. The solar treated cottonwood achieved 2 times higher nitrate removal rates compared to the raw cottonwood. One of the main reasons why solar treatment achieved the highest nitrate removal rates is because ultraviolet radiation has
been found to be the most damaging element when wood is outdoor because of the
discoloration and deterioration it causes to the wood thus affecting wood chemical and
mechanical properties. Wood is made of polymer which are sensitive to UV radiation.
These components in the wood go through a photochemical reaction which result in
discoloration and degradation (Sharratt et al., 2009). The chromophores present in the
wood are responsible for wood discoloration since they can absorb UV light in the range
of 300 to 400 nm. In fact, wood discoloration was observed during the pretreatment
stage.

In fact, lignin and cellulose are depolymerized during solar radiation and water
leaches the degraded particle from the wood (Derbyshire and Miller.1981; Evans et al.,
2008). The wood becomes more hydrophilic due to the lignin being degraded and leached
out. Lignin contributes to the structural strength of the wood, so a decrease in lignin
during the photo degradation, results in the loss of wood strength. Wood
photodegradation by UV irradiation is provoked by the formation of free radicals (Moore
and Owen 2001; Muller et al 2003). Lignin forms aromatic free radicals (Phenoxy
radicals) that reacts with oxygen to produce carbonyl and carboxyl free radicals which
are bond to wood discoloration through unsaturated carbonyl compound called quinones
(Pandey 2005, Teaca et al., 2013). Besides an increase in the carboxyl content of wood
and the decrease of wood lignin content, the photo degradation in wood increases the
cellulose content of wood surface (George et al 2005; Wang and Lin 1991). So, by using
Solar treatment on wood, the cellulose content increases which is easily degraded by
microorganisms compared to lignin. Lignin content in wood is about 30 %, thus
pretreatment of wood is critical for the breakdown of lignin and the release of cellulose
and hemicellulose from wood. Pretreatment is going to provide more organic available carbon source which is going to provide a long-lasting denitrification (Mosier et al., 2005). Evidence of wood chemical changes during biodegradation and photo degradation can be collected using different analysis methods. FTIR spectroscopy is the most widely used wood analysis techniques. Figure 5.3 and 5.4 shows the results of the FTIR analysis. From the FTIR figures, main changes were found at 3360, 1730, 1650, 1590, 1510, 1230 and 1050 cm\(^{-1}\). The peaks at 1590 and 1510 cm\(^{-1}\) were attributed to C=C aromatic skeletal vibration of lignin, and the peak at 1230 cm\(^{-1}\) was C–O stretching vibration in lignin and hemicelluloses. These peaks decreased significantly after all different treatments compared to the raw woodchips. Peaks at 2920 cm\(^{-1}\) (alkane CH vibrations of methylene in cellulose), was characteristics for cellulose. This peaks almost remained the same for the solar treatment but decrease for composted and natural. This indicated that Solar treatment hardly affected the cellulose but contributed to the degradation of lignin. This can explain why solar treated woodchips achieved higher denitrification rates compared to the other treated woodchips. The peak at 1030 cm\(^{-1}\) for the C-O stretching was associated with cellulose and its extractives, and the peak at 3360 cm\(^{-1}\) was associated with the free –OH groups. The same observations were made by Kubovsky et al., 2020 and Teaca et al. (2013) found that during wood photo degradation and heat treatment, the absorption band for lignin incrementally decreased at 1265 and 1510 cm\(^{-1}\). The oxidation of cellulose and lignin cause the increase in the carbonyl group whereas lignin reduction was caused by light degradation (Feist and Hon 1984).
5.3.2 Long Term Phosphate Removal using different pretreated woodchips

Figure 5.2 shows phosphate effluent concentration for all 8 reactors. Woodchip’s phosphate removal capability increased overtime and reached its maximum at around 30 days of operation. The average phosphate removal rates achieved by each pretreated woodchip were 0.6 g P/m\(^3\)/d, 0.5 g P/m\(^3\)/d, 0.45 g P/m\(^3\)/d and 0.44 g P/m\(^3\)/d respectively for oak solar, oak natural, oak composted and oak raw. Cottonwood achieved phosphate removal rates of 0.88 g P/m\(^3\)/d, 0.45 g P/m\(^3\)/d, 0.38 g P/m\(^3\)/d and 0.35 g P/m\(^3\)/d respectively for cottonwood solar, cottonwood natural, cottonwood composted and cottonwood raw. All pretreated woodchips achieved higher phosphate removal rates compared to raw woodchips. Husk and al., 2018 also found that woodchips bioreactors had the ability to remove phosphate but with a limited extent. Woodchip’s ability to remove phosphate is more a physical process than a chemical process. Microbial absorb phosphate since phosphate is part of their cell composition. Table 5.1 shows phosphate removal rates for all 8 reactors.

5.3.3 Nitrate Batch Kinetics Test

Figure 5.5 shows nitrate removal by the different woodchips type at 2 months treatment and 5 months treatment. There is a clear difference in nitrate removal based on duration of the wood treatment. This trend can be observed in both oak wood and cottonwood. They all have a linear reduction which can be described as a zero-order reaction. The calculated zero order constants for the 5 months treated oak wood were 11.90 mg N/L/d, 7.77 mg N/L/day, 5.43 mg N/L/day respectively for oak solar, oak natural, oak composted. These zero order kinetics constants at 5 months treatment were greater than the one observed at 2 months (table 5.2). The same observation can be
observed for the cottonwood woodchips. At 5 months treatment, the calculated nitrate zero order constants were 12.30 mg N/L/d, 6.26 mg N/L/d and 4.91 mg N/L/d respectively for cottonwood solar, cottonwood natural, cottonwood composted. Table 5.2 summarizes the zero order nitrate removal constants for all woodchip types and treatment duration. From this short-term batch test, for all woodchips type, the treatment duration impacts the nitrate removal. The longer the treatment duration the better the nitrate removal achieved. All treated woodchips achieved higher nitrate removal compared to the raw untreated woodchips. The 5 months solar treated woodchips achieved 3.35 times higher removal compared to the raw undertreated woodchips for both oak and cottonwood.

During wood pretreatment, many parameters such as moisture, temperature, visible light, and infrared light should be taken in consideration. Allowing sufficient time for these parameters to affect the wood is crucial.

5.3.4 Dissolved Organic Carbon Leaching and SUVA

Figure 5.6 presents DOC and SUVA values during the long-term column experiment. During the reactor’s initial startup, a strong initial release of DOC was observed and followed by a gradual decline (Schipper et al., 2010). The initial DOC effluent for each reactor was 135.5 mg/L, 80.77 mg/L, 60.76 mg/L and 40.8 mg/L respectively for Oak raw, oak solar, oak natural and oak composted. The DOC released by the cottonwood reactors were 110 mg/L, 100 mg/L, 80.63 mg/L and 60.56 mg/L respectively for cottonwood raw, cottonwood solar, cottonwood composted and cottonwood natural. DOC leaching follows a linear decrease overtime and follow the trend of DOC day 1 > DOC day 75 > DOC day 150. Table 5.3 summarizes the DOC zero
order kinetics constant at day 1, 75 and 150. During the 6 months reactors run, microbial utilization and DOC leaching steadily exhausted usable organic substrates from woodchips, resulting in lower nitrate load reduction rates overtime. Hassanpour et al., 2017 found that DOC availability was a limiting factor in the performance of woodchip bioreactor. The readily soluble organic compounds in the woodchips may be represented by the DOC leached during this period (Abusallout et al., 2017). The average DOC leaching observed during the 6 months operation were 17.6 mg/L, 9.13 mg/L, 6.21 mg/L and 13.22 mg/L respectively for oak solar, oak natural, oak composted and oak raw. The average DOC effluent achieved by cottonwood solar, cottonwood natural, cottonwood composted and cottonwood raw were 16.74 mg/L, 10.77, 10.22 mg/L mg/L and 13.32 mg/L. All 8 woodchips reactors achieved medium to high SUVA values (between 1 -3) during the first 7 days. This high SUVA values were mostly due to high concentrations of humic substances emitted quickly from the wood particles. After 60 days all SUVA values fell below 0.5. The SUVA values decline follows similar trend as for the DOC. The same observation was made by Hua et al., 2015 and Abusallout et al., 2017 that found that woodchip DOC effluent contained medium to high humic content based on the SUVA values when column experiment was performed.

5.5 Conclusions

This study was conducted to determine the impact of woodchip pretreatments duration on nitrate removal rates. Three different pretreatments were used. among them, solar, natural and composted. the results showed that denitrification efficiency followed the order of solar woodchips> natural woodchips> composted woodchips> raw woodchips. The 6 months Column study operation average nitrate removal rates achieved by oak
wood were 41.55 g N/m³/d, 26.44 g N/m³/d, 20.24 g N/m³/d and 16.71 g N/m³/d respectively for oak solar, oak natural, oak composted and oak raw. The cottonwood achieved nitrate removal rates of 37.35 g N/m³/d, 24.17 g N/m³/d, 22.29 g N/m³/d and 28.43 g N/m³/d respectively for cottonwood solar, cottonwood natural, cottonwood composted and cottonwood raw. The influent nitrate concentration was 50 mg N/L at 12 h HRT and 22 °C.

The batch Kinetics test was performed to determine woodchip treatment duration impact on nitrate removal rates. The calculated zero order constants for the 5 months treated oak wood were 11.9 mg N/L/d, 7.77 mg N/L/day, 5.43 mg N/L/day respectively for oak solar, oak natural, oak composted. At 5 months treatment, the calculated nitrate zero order constants were 12.3 mg N/L/d, 6.26 mg N/L/d and 4.91 mg N/L/d respectively for cottonwood solar, cottonwood natural, cottonwood composted. Woodchips treated for 5 months exhibited nitrate removal constant between 0.85 to 137 times higher than the woodchip treated for 2 months for both oak and cottonwood. The 5 months solar treated woodchips achieved nitrate removal constant which were between 3.35 times higher than the raw untreated woodchips. These results shows that woodchip solar treatment before installation can help enhance bioreactor performance.
Figure 5.1: Nitrate effluent concentration for Oak wood and Cottonwood (Experimental conditions: HRT = 12 h; temperature = 22 °C).
Figure 5.2: Phosphate effluent concentration for Oak wood and Cottonwood (Experimental conditions: HRT = 12 h; temperature = 22 ºC).
Figure 5.3: FTIR Spectra of the Raw Oak Wood before and after Treatment.
Figure 5.4: FTIR Spectra of the Raw Cottonwood Wood before and after Treatment.
Figure 5.5: Short Term Batch Kinetics for Oak wood and Cottonwood (Experimental condition: woodchips to water ratio 1:10; temperature = 22 °C).
Figure 5.6: Dissolved Organic Carbon leaching and SUVA. (Experimental conditions: HRT = 12 h; temperature = 22 °C).
Figure 5.7: Woodchips Different treatment
Figure 5.8: Woodchips used to pack the reactors.
Figure 5.9: A (Column Reactor Set Up), B (Batch Reactors Set up)
Table 5.1: Nitrate and phosphate removal Rate for the Different Woodchips Types.  
(Experimental conditions: Influent nitrate concentration: 100 mg N/L; Influent Phosphate concentration: 1 mg/L; HRT = 12 h).

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<th>Temperature °C</th>
<th>Woodchip Type</th>
<th>Nitrate (g N/m³/d)</th>
<th>Phosphate (g P/m³/d)</th>
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</thead>
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<td>22</td>
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</tr>
<tr>
<td></td>
<td>Oak Composted</td>
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<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Oak Natural</td>
<td>26.44</td>
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<td></td>
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<td>Cottonwood Natural</td>
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<td></td>
<td>Cottonwood Solar</td>
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Table 5.2: Short Term Batch Nitrate Zero Order Kinetics Constants. (Experimental conditions: Nitrate concentration: 100 mg N/L; Wood treatment duration: 2 months and 5 months)

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Table 5.3: Dissolved Organic Carbon Zero Order Kinetics Constants at nitrate concentration of 50 mg N/L. (Experimental conditions: HRT = 12 h)

<table>
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<th>Materials</th>
<th>Day 1</th>
<th>Day 75</th>
<th>Day 150</th>
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<td>5.06</td>
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<td>0.26</td>
</tr>
<tr>
<td>Oak Natural</td>
<td>3.4</td>
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<td>Oak Solar</td>
<td>6.73</td>
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<td>Oak Raw</td>
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<td>Cottonwood Composted</td>
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<td>Cottonwood Natural</td>
<td>5.04</td>
<td>0.6</td>
<td>0.18</td>
</tr>
<tr>
<td>Cottonwood Solar</td>
<td>8.3</td>
<td>1</td>
<td>0.46</td>
</tr>
<tr>
<td>Cottonwood Raw</td>
<td>9.16</td>
<td>0.45</td>
<td>0.26</td>
</tr>
</tbody>
</table>
Table 5.4: Mass of woodchips and reactors porosities

<table>
<thead>
<tr>
<th>Woodchip reactor</th>
<th>Mass (g)</th>
<th>Porosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oak Raw</td>
<td>267.82</td>
<td>50.5</td>
</tr>
<tr>
<td>Oak Composted</td>
<td>382.45</td>
<td>44.61</td>
</tr>
<tr>
<td>Oak Natural</td>
<td>268.31</td>
<td>49.66</td>
</tr>
<tr>
<td>Oak Solar</td>
<td>267.90</td>
<td>49.66</td>
</tr>
<tr>
<td>Cottonwood Raw</td>
<td>296.49</td>
<td>47.97</td>
</tr>
<tr>
<td>Cottonwood Composted</td>
<td>399.694</td>
<td>43.77</td>
</tr>
<tr>
<td>Cottonwood Natural</td>
<td>293.86</td>
<td>43.77</td>
</tr>
<tr>
<td>Cottonwood Solar</td>
<td>296.03</td>
<td>43.77</td>
</tr>
</tbody>
</table>
6.1 Overall summary

Eutrophication is caused by excessive nutrients loading into surface water bodies. Agricultural subsurface drainage has been found to be one of the major transports of nutrient from agricultural field to surface water. Denitrification bioreactors have been widely used to treat agricultural drainage water. Woodchips has been used for many years as the carbon source. The wide application of wood is due to its chemical structure which is excellent for modification and reconfiguration. Different type of treatments has been applied to wood to change its physical and chemical properties to make it more suitable depending on which application area the wood will be used. Determining the impact of wood quality and pretreatment on nitrate removal kinetics in bioreactors is crucial since it can help with field bioreactors design.

Laboratory column experiment were conducted using different woodchips condition to determine Michaelis-Menten model parameters for nitrate removal. Composted, fresh and aged woodchips were the three different woodchips conditions used. The results show that the composted woodchips exhibited higher nitrate load reduction rates than the fresh and the aged woodchips. It also shows that nitrate removal in woodchip bioreactors for subsurface drainage and wastewater treatment can be operationally defined as a zero-order reaction and that nitrate is not a major limiting factor under such conditions. A first order reaction was observed when nitrate concentration decreases below 3 mg N/L. The quantity of organic carbon in the reactors cannot explain the observed differences in nitrate removal between the two woodchips.
column reactors. It is likely that the composting process before the bioreactor experiments modified the quality of wood materials such that more biodegradable organic compounds were produced to promote the denitrification reactions. Low temperature has been found to impact nitrate removal rates for both woodchips type. Fresh woodchips were more impacted by low temperature compared to the composted woodchips. This demonstrates the importance of woodchip quality on nitrate removal in bioreactors.

Phosphate is also one of the major nutrients leached from agricultural subsurface drainage. Phosphorus filters have been used as a method for removing phosphate from water by a process called adsorption. Phosphate filters use natural minerals, industrial byproducts, steel byproducts. Woodchip’s ability to remove phosphate is limited. Simultaneously removing nitrate and phosphate has been proposed. Laboratory dual nutrients technology combining woodchips to steel chips has been used. laboratory column experiment was performed using three different dual reactors configurations. Woodchips before steel chips, steel chips before woodchips and mixed woodchips and steel chips were the three different reactors configuration used. The results show that the reactor configuration did not substantially affected the nitrate and phosphate removal. The woodchips were able to remove iron particles leached from the steel section. The steel section was able to remove dissolved organic carbon leached from the woodchips section. The nitrate removal was more negatively impacted by low temperature compared to phosphate removal. This means that phosphate removal is a physical process compare to nitrate removal that is a biological process.
Agricultural residue media was used as an alternate carbon source for
denitrification bioreactors in a laboratory column reactor. Corn cob, corn stover, barley
was used as an alternative carbon source. The laboratory column result showed that corn
cobs achieved the highest denitrification rate compared to corn stover and barley straw.
Dissolved organic carbon leaching was one of the major downsides of using agricultural
subsurface drainage. Based on the experiment results, corn cobs can be used as an
alternative carbon source for denitrification bioreactors. One of the main advantages of
agricultural residue media is that phosphate can be leached back. This demonstrates that
agricultural residue media can be applied on the field as a nutrient source for plant after
being used in bioreactors. This can help save cost of buying new fertilizers.

Environmental conditions and wood type can affect the way wood is degraded.
From previous study it was found that wood condition affects nitrate removal in
bioreactors. Wood biodegradation, and wood weathering are the most common ways that
woodchips condition can be changed when wood is stored outside. This is because some
organisms can tolerate better a wide variation in environmental parameters while some
others cannot. Batch and laboratory woodchips column reactors were used to evaluate
nitrate removal rates based on three different woodchips conditions. Solar, natural and
composted woodchips were used in this study. The woodchips were pretreated for a
period of 2 and 5 months. This can help to determine the impact of pretreatment duration
on woodchip denitrification performances. The results showed that 5 months solar treated
woodchips achieved higher denitrification rates compared to natural and composted
woodchips. Wood photodegradation by UV irradiation increase the carboxyl content of
wood and decrease of wood lignin content, an increase in cellulose content was also
observed during the photodegradation. Based on these results, woodchips pretreatment is necessary to help improve bioreactor performances.

6.2 Recommendations for Future Work

1. More studies are needed to determine the biodegradability of woodchip DOC, and the release rates of biodegradable organic carbon from woodchips to better understand nitrate removal kinetics in bioreactors.

2. Determine the mechanisms that lead to higher denitrification potential after woodchip biodegradation and evaluate the appropriate woodchip storage conditions and duration before the bioreactor installation.

3. Pilot scale and full-scale studies must be conducted on the impact of wood pretreatment on bioreactors performance.

4. Investigating the effect of wet and dry cycle on woodchip bioreactors performance.


