Evaluation of Woodchip Bioreactor Denitrification Kinetics

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EVALUATION OF WOODCHIP BIOREACTOR DENITRIFICATION KINETICS

BY

ABDOUL AZIZ KOUANDA

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EVALUATION OF WOODCHIP BIOREACTOR DENITRIFICATION KINETICS

ABDOUL AZIZ KOUANDA

This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Civil Engineering degree and is acceptable for meeting the thesis 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.
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ABSTRACT

EVALUATION OF WOODCHIP BIOREACTOR DENITRIFICATION KINETICS

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Agricultural subsurface drainage is a water management practice used to remove excess water in poorly drained soil. The use of fertilizers combined with subsurface drainage practice affects negatively surface water quality due to nutrient loss. Woodchip bioreactors have previously been used as a technology for removing nitrate from agricultural subsurface drainage. Understanding the mechanism that governs nitrate removal in woodchip bioreactors is crucial for field bioreactor design and application.

The objective of this project is to determine woodchip bioreactor denitrification kinetics parameters under different operating conditions including hydraulic retention time, temperature and influent nitrate concentrations.

Laboratory column experiments were conducted with influent nitrate concentrations varying from 50 mg N/L to 3 mg N/L at a HRT of 12 hours. The integrated Michaelis Menten equation was used to determine the denitrification kinetics parameters. The half saturation constant was found to be 2.17 mg N/L and the maximum nitrate removal rate was 0.86 mg N/L/H at 22 degree Celsius. The woodchips bioreactor achieved a denitrification rate ranging from 3.02 g NO$_3^-$ N/m$^3$/d to 9.80 g NO$_3^-$ N/m$^3$/d. At 5 degree Celsius, the bioreactor had a half saturation of 0.58 mg N/L and a maximum removal rate of 0.045 mg /L/H with denitrification rate ranging from 0.52 g N/ m$^3$/d to
0.28 g N/m³/d. At HRTs of 24 hours and 6 hours with an influent nitrate concentration of 10 mg N/L, a 98.70% and 29.4% percentage removal was achieved respectively.

Nitrite accumulation was observed in all experiments with the highest nitrite effluent concentration of 1.4 mg N/L, observed at 50 mg N/L under 12 hours HRT. The results of this study a better understanding of denitrification kinetics in woodchip bioreactors.
Chapter 1: INTRODUCTION

With the growing of population, more resources are needed, especially food and clean water. In order to avoid food scarcity, several practices are used to boost row crop agriculture to produce high crop yields. Among them, we have fertilizers and subsurface drainage. These techniques are commonly used in the Midwestern United States due to economic and climatic conditions. Agricultural subsurface drainage is a water management practices used to remove excess water in poorly drained soil. It has given more opportunities to farmers to use fields that previously were not adequate for proper usage due to their poor drainage capability, by providing an adequate environment for plant growth (Bushman and Sands, 2002). Subsurface drainage pipes can be made of fired clay, concrete and most commonly of perforated corrugated plastic. Subsurface drainage management has many agronomic and economic benefits.

From an agronomics point, subsurface drainage by removing excess water in the soil enhances water infiltration, increasing soil porosity and provides better aeration for microbial activity. It also reduces sediment loss and erosion (Kladivko et al., 2004). All those factors combined provide a better environment for plant growth resulting in more yields. Machinery works much more efficiently on drier soil than wetter soil. This reduces labor hours and saves money on machinery fuel consumption. Drier soil also provides farmers more opportunities to grow higher value crops that can be sold for a higher price. However, subsurface drainage contributes to nutrient and soluble pesticide loading from the agricultural field to surface water (Blann et al., 2009; Lavaire et al., 2017; Rahman et al., 2014). Nitrate and phosphate are the major nutrients found in
 Those nutrients can negatively impact surface water quality (Dinnes et al., 2002).

Subsurface drainage has been identified as the major transport mechanism of nitrate, phosphorus, and heavy metals from agricultural fields to surface water entering the Mississippi basin (Lavaire et al., 2017; Randall and Mulla, 2001; Rozemeijer et al., 2010; Smolders et al., 2010). Nitrate concentrations in subsurface drainage vary from 5 to 50 mg N/L depending on the season with an average nitrate concentration exceeding 10 mg N/L (Fausey et al., 1995; Greenan et al., 2006; Greenan et al., 2009; Moorman et al., 2010; Sands et al., 2008). Nitrate loading into surface waters is dependent on drainage water flow that is controlled by the amount of precipitation. High nitrate loading into surface water is often observed during periods of heavy precipitation (Cuadra and Vidon, 2011; Drury et al., 2009; Randall and Mulla, 2001). Discharging those highly concentrated waters into surface water such as lakes, rivers can cause health and environmental issues (Van Driel et al., 2006).

High nitrate concentration in drinking water has an impact on human health, especially on young infants that can get “methaemoglobinaemia” commonly called “Blue baby syndrome” (Blowes et al., 1994). The United States Environmental Protection Agency (EPA) has set a maximum concentration level (MCL) of 10 mg/l for nitrate in drinking water to limit health issues. In addition to health issues, nitrate can cause environmental issues such as eutrophication (Almeelbi and Bezbaruah, 2012; Bratieres et al., 2008; Van Driel et al., 2006). Eutrophication is the condition where water bodies such as lakes and rivers receive excess nutrient that stimulate excessive plant growth. Algae death and decomposition can cause a severe depletion of dissolved oxygen in the water. At
the same time, light penetration in the water decreases due to the obscureness of the water. The depletion of dissolved oxygen in the water has a negative impact on aquatic life causing the death of fish. Some other side effects of nutrient pollution in the environment includes acid rain, air pollution and the formation of toxic byproducts during water treatment.

Several management strategies have been developed to control nutrient loss from agricultural fields including crop rotation, fertilizers application rate and timing, buffers, wetlands (natural or artificial) and the use of edge of field treatment (denitrification bioreactors), (Dinnes et al., 2002; Helmers et al., 2011). Denitrification bioreactors have been proven to be efficient for removing subsurface drainage nitrate, with nitrate removal efficiency ranging for 33% to 100% at nitrate removal rates ranging from 0.38 g N/m³/d to 22 g N/m³/d (Christianson et al., 2012b; Hoover et al., 2016, Schipper et al., 2010; Woli, 2010). Denitrification bioreactors are a cost effective edge of field treatment for nitrate removal because they require little maintenance and a small amount of land for the bioreactor installation (Cameron and Schipper, 2010; Christianson et al., 2009). A denitrification bioreactor is a channel, filled with a carbon source like wood products. Subsurface drainage water flows through this channel for nitrate to be removed biologically. Field bioreactors can have many different shapes but the most common on the field are rectangular and trapezoidal. The size of the bioreactor depends on the drainage area size and land availability (Christianson and Helmers, 2011). Wood byproducts have been proven to provide a steady nitrate removal rate, limit dissolved organic leaching and nitrite formation during denitrification. It has also been proven to be a durable organic carbon source for denitrification (Lopez et al., 2017).
The main mechanism for nitrate removal in woodchip bioreactors is denitrification (Robertson et al., 2000; Warneke et al., 2011a). Denitrification is an enzyme catalyzed process, so it can be modelled using the Michaelis Menten equation (Almeida et al., 1995a; Betlach and Tiedje, 1981; Dowd and Riggs, 1964; Estuardo et al., 2008; Ghane et al., 2015; Laverman et al., 2010). Michaelis Menten equation can be written as below with nitrate as a substrates. (Schnell and Maini, 2003)

\[ V_0 = \frac{V_{\text{max}} [S_0]}{K_m + [S_0]} \]

Where \( V_{\text{max}} \) is the maximum removal rate (mg N/L/h), where \( V_0 \) is the nitrate removal rate (mg N/L/h), \( K_m \) is the Michaelis Menten constant (mg N/L) that represents the nitrate concentration at which nitrate removal rate is half the maximum rate and \( S_0 \) is the inflow (initial) nitrate concentration (mg N/L). Based on the Michaelis Menten equation nitrate removal follows a zero order reaction at high nitrate concentration (\( S_0 >> K_m \)) and a first order reaction when nitrate becomes limiting (\( S_0 << K_m \)).

Two different methods have been used to determine the Michaelis Menten constants. Among them, there are the initial rate method and progress curve analysis method. Those two methods have been extensively used to determine denitrification kinetics parameters in laboratory batch studies (Balcom and Fitch, 1969; Bauer et al., 1998; Bezerra and Dias, 2007; Murray et al., 1989). Using the progress curve instead of the initial rate method considerably reduces the number of assays because a single reaction gives multiple experimental data points.

During biological denitrification, nitrate is converted to nitrogen gas (\( N_2 \)) (Schipper et al., 2010). NO (Nitric oxide), \( N_2O \) (Nitrous oxide) are some intermediate
products that can be formed during the denitrification process (Almeida et al., 1995; Hongwei et al., 2009; Wild et al., 1995). This conversion is made by denitrifying bacteria. The carbon contained in the woodchips is used as food source (electron donor) by the bacteria and nitrate for their respiration (electron acceptor) (Schipper et al., 2010). The population of denitrifiers are very large and diverse. Most of the denitrifying bacteria are facultative anaerobes with the majority being heterotrophs (Korom, 1992; Roger Knowles, 1982). Among them, Pseudomonas Fluorescens are the one that has been extensively studied (Almeida et al., 1995b; Betlach and Tiedje., 1981; Roger Knowles., 1982). Paracoccus denitrificans and Pseudomonas denitrificans have also been studied (Blaszczyk, 1993; Kornaros et al., 1996). The presence of dissolved oxygen can limit denitrification by causing an incomplete denitrification (Christianson & Helmers., 2011, Warneke et al., 2011). Gomez et al. (2002) found that dissolved oxygen inhibits organic nitrogen removal and also causes nitrite accumulation. Healy et al. (2006) found that dissolved oxygen concentrations above 3.7 mg/L inhibit nitrate removal rate in a laboratory experiment. This inhibition is caused by a competition between oxygen and nitrate as an electron acceptor.

Denitrification in woodchip bioreactors can have many side effects such as odor due to hydrogen sulfide production. Bacteria responsible for the denitrification process can also be responsible for the transformation of mercury to methyl mercury under anaerobic conditions (Shih et al., 2011). The accumulation of nitrite and also the production of greenhouse gases in woodchip bioreactor were observed (Elgood et al., 2010; Warneke et al., 2011a). Laboratory column study done by Hua et al. (2016) found nitrite accumulation higher than 1 mg/l, which is the MCL set by the EPA for drinking
water. Those greenhouse gases are highly toxic for the environment because they cause climate change and contribute to global warming. Dissolved organic carbon leaching has also been observed during the use of woodchip bioreactors (Misiti et al., 2011). Organic carbon has been linked to unwanted microbial growth, high odor, color and taste issues in the water. It also contributes to the high demand of chlorine during disinfection and formation of disinfection byproduct (DBPs) during the disinfection process (Escobar et al., 2001). Laboratory and field studies have tested the efficiency of different organic materials in removing nitrate. Among them, sawdust, wheat straw, maize cobs, rice husks and corrugated paper cardboard all succeeded in removing nitrate (Robertson, 2010; Greenan et al., 2006; Healy et al., 2015). Maize cobs had a higher removal rate compared to the other organic carbon sources which was due to more labile carbon leaching (Cameron and Schipper, 2010). Biochar is a carbon source used in combination with woodchips to enhance nitrate removal. Bock et al. (2015) combined biochar and woodchips in a pilot scale bioreactor and found that biochar enhanced nitrate removal rate for nitrate inflow concentrations above 5 mg/L to 10 mg/L, but did not affect nitrate removal rates at lower concentrations. Other parameters have also been studied in laboratory and field scale to determine their impact on woodchip bioreactor performance. Those factors includes temperature, hydraulic retention time, microbiology and woodchip type and size.

The hydraulic retention time is one key factor in designing a bioreactor. Field woodchip bioreactors have two main components; the inflow and outflow structures that help in the regulation of the flowrate and the hydraulic retention time. The inflow structure is used to bypass water during periods of high flow. The typical HRT used on
field bioreactors is in the range of 4 to 12 h. The hydraulic retention time may decrease to less than 4 h during periods of high flow (heavy precipitation). Many studies have observed that the amount of nitrate removed is related to the hydraulic retention time. The longer the hydraulic retention time, more nitrate will be removed (Greenan et al., 2009). Longer hydraulic retention times in woodchip bioreactors have also been linked to high dissolved organic leaching, greenhouse gas production, and odor and nitrite accumulation (Christianson et al., 2012a; Hoover et al., 2016; Hua et al., 2016; Lepine et al., 2016).

Temperature is an important environmental factor that can affect denitrification efficiency. Previous studies have shown that woodchip bioreactors still remove some nitrate at low temperatures (Feyereisen et al., 2016; Healy et al., 2012; Nordström, et al., 2016). A 3 year field study done by David et al. (2016) showed a strong correlation between temperature and nitrate removal rate. The subsurface drainage water temperature during their study was in the range of 3 to 8 degree Celsius between January and April, and reached 17 degree Celsius in July. They found that Nitrate removal rate increased with increasing temperature. The calculated $Q_{10}$ (factor of the reaction rate increase with every $10^\circ$C increase in temperature) for this study was 3.8 between 6 and 16 degree Celsius. The $Q_{10}$ value for their study is higher than what have been reported by other studies (Cameron and shipper, 2010; Elgood et al., 2010). This difference may be explained by different temperature ranges and different inflow nitrate concentrations.

Different types of woodchips have been studied to assess which types perform better in removing nitrate. The two main types of woodchip used for denitrification bioreactors are hardwood (oak) and softwood (pine). Softwood has been shown to be
more efficient in removing nitrate than harwood in laboratory column and batch studies (Cameron and Schipper, 2010; Gibert et al., 2008). However, Peterson et al. (2015) reported a higher removal using hardwood. Woodchip size impact on denitrification bioreactors has also been studied by Peterson et al. (2015). They found that smaller woodchip sizes performed better in removing nitrate than larger woodchips particle sizes. This result can be explained by the fact that small woodchip particles offer more surface area per unit mass giving more space for biofilm to grow. Contrary, Cameron and Schipper. (2010) noticed a small increase in nitrate removal with increasing woodchip particle size. Larger woodchip particles can increase reactor porosity and at the same time increase the reactor water retention capacity, giving more time for bacteria to denitrification. The differences observed in different experiments can be caused by the influent nitrate concentration used, hydraulic retention time, and temperature. More research is needed to clarify the impact of particle size on denitrification efficiency of bioreactor. The typical woodchip size used on field bioreactor is in the range of ¼” to 1”(Christianson and Helmers. 2011).

Woodchip bioreactors have been used for more than 15 years to remove nitrate from subsurface drainage water (Christianson and Helmers, 2011, Long et al., 2011). There are still some controversies about nitrate removal kinetics (Christianson et al., 2012). Some studies have reported that nitrate removal rate doesn’t 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; Van Driel et al., 2006a). Robertson, (2010) explained that other factors such as dissolved organic carbon might control the reaction. 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 also it can help in the design of the bioreactor. Nitrate removal rate determination varies from study to study. Therefore, more studies are needed to elucidate nitrate removal kinetics of woodchips bioreactors.

This study was designed to determine the effect of temperature on denitrification kinetics parameters in a laboratory woodchip bioreactor using a wide range of nitrate inflow concentrations. Hydraulic retention time impacts were also studied. Three different hydraulic retention times (HRT) were studied. This study provides a better understanding of the effect of temperature on denitrification kinetics, which can help in the design of woodchip bioreactors for field applications.
Chapter 2: MATERIALS AND METHODS

2.1 Materials

2.1.1 Woodchips Characteristic

Cottonwood woodchips were collected from a supplier in Sioux Falls, SD. Several bioreactors installed in Eastern South Dakota utilize the same type of woodchips. The woodchips were covered with dirt and fine particles when collected, because they were stored outside. The woodchips were cleaned before being used. Clean buckets were filled with distilled water and the woodchips were soaked before being mixed by hand to separate fine particles from large particles. Particles that remained in suspension in the bucket were poured out. This process was repeated several times till fewer fine particles were in suspension. After cleaning, the woodchips were placed on a plastic sheet to dry. Hand screening was done after the woodchips were dried to remove big wood particles that was susceptible to cause clogging in the reactor. Three different woodchips size were chosen after the hand screening depending on the length and wideness of the particles. It was collected in three different buckets. Each bucket contained large woodchip particles (2.5 cm long by 1.5 cm wide), medium woodchips particles (1.5 cm by 1 cm wide) and small woodchips particles (1 cm long by 1 cm wide). One final bucket containing mixed woodchip particles (large, medium and small) was used to pack the reactor.

2.1.2 Soil

A soil sample was collected from the SDSU research farm in Volga, SD. A shovel was used to dig a hole of few inches before the soil sample was collected. A clean plastic bucket was used to collect the soil sample. The sample was stored at room temperature in
South Dakota State University Environment Engineering laboratory before cleaning. The soil particles were cleaned using distilled water to remove fine particles such as corn sticks, leaves and other plants residue. The remaining soil particles were mixed with distilled water and stored for 24h to allow particles to settle at the bottom. The supernatant was used to inoculate the reactor for 7 days at a 12 hour hydraulic retention time (5.2 ml/minute).

2.2 Column reactor

2.2.1 Reactor set up

A clear acrylic tube with a length of 1.2 meters and inside diameter of 8.85 cm was used as an up flow reactor. The reactor has a volume of 7378 cm$^3$. The reactor was packed with 1842 g of woodchips. The resulting porosity was 49.87 %. The porosity was calculated by using the ratio between the volumes of water drained from the reactor and the total reactor volume. Glass balls were used at the bottom of the reactor before packing with woodchips and also at the top of the reactor to block some wood particles from floating. Twelve samples ports were used along the length of the column with an interval of 10 cm. Each sampling port corresponds to a hydraulic retention time of 1h based on the 12 hours hydraulic retention time design. The soil supernatant previously prepared was pumped and recycled into the reactor for 7 days at a 12 hour HRT. This process was used to inoculate the woodchips with soil bacteria. The reactor was covered with aluminum foil to prevent light from entering the reactor so it can better simulate field conditions.
2.2.2 Synthetic Subsurface Drainage Water

A 55 liter tank was used to provide the simulated subsurface drainage water to the reactor. The targeted inflow concentrations were 3 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L. Nitrate stock solution was prepared using 99% purity KNO₃. Phosphorus stock solution was also made using 98% purity NaH₂PO₄·H₂O. Chloride stock solution was prepared using 0.5 mg/L as Na⁺ from NaCl, 1 mg/L as Ca⁺ from CaCl₂·2H₂O, 0.5mg/L as Mg²⁺ from MgCl₂·6H₂O, 0.5 mg/L as K⁺ from KCl with a total of 4.43 mg/L as Cl⁻ and 4.17 mg/L as SO₄²⁻ from NaSO₄. Additional macronutrients stock solution was prepared using CoCl₂·6h20 (Co), MnCl₂·4h20 (Mn), ZnCl₂ (Zn), FeCl₃·6h20 (Fe), NaMoO₄·2H₂O (Mo), NiCl₂·6H₂O (Ni), CuCl₂·H₂O (Cu), H₃BO₃ (B), NaSeO₄ (Se). All chemicals were weighted and prepared using 1 liter volume flasks with nano pure water at a conductivity of 18.1 MΩ. The different nutrients concentrations were chosen to reflect subsurface drainage water conditions (Fausey et al., 1995; Rozemeijer et al., 2010). The simulated subsurface drainage water was adjusted at pH 7 with addition of NaOH. All chemicals used were from Sigma Aldrich.

A nitrogen gas sparging unit was installed and sparging heads were inserted into the influent tank. Nitrogen gas was blown continuously into the influent water tank for the experiment. The tank was also moved into a cooler room for low temperature experiment. One sample was taken directly from the influent tank outlet for dissolved oxygen (DO) measurement. A BOD probe was used for measuring DO concentrations. DO samples were taken for each experiment condition. The average dissolved oxygen (DO) concentration achieved was 2.57 mg/l.
2.2.3 Flow Rate Control

A Masterflex l/s variable speed peristaltic pump coupled with Tygon l/s 16 ID diameter tubing was used to achieve various HRTs. The pumps were calibrated according to manufacturer’s instructions. Three different HRTs were used to determine their impact on nitrate removal. The different HRTs used in this study are 6, 12 and 24 hours. The influent nitrate concentration was maintained at 10 mg/L for the HRT test. Each HRT was run for a period of 5 days before samples were collected for 2 days.

2.3 Temperature impact

2.3.1 Room Temperature

The reactor was placed in the Water and Environmental Engineering Research Center laboratory located on the campus of South Dakota State University. The average temperature in the laboratory was about 21.5°C. This temperature is within the range of temperatures observed for subsurface drainage water during summer periods in the Midwest. Temperatures were measured over a period of 3 years by David et al. (2016) for a field bioreactor. The highest temperature observed were 17°C. Temperatures above 20°C were observed in a field bioreactor in Northern Iowa (Hoover et al., 2016) and also in a pilot bioreactor in Central Iowa (Christianson et al., 2012).

2.3.2 Low Temperature

The woodchip reactor was moved into a cooler room where the temperature was maintained at 5°C. The bioreactor, influent tank, pump and effluent bucket were placed in the cooler room. The temperature was maintained at 5°C to simulate subsurface drainage water condition that can be observed during the early spring in Midwest. The
effluent tank water temperature was regularly checked to make sure that the water leaving the reactor reaches 5º C. Temperatures as low as 3º C have been observed for field bioreactors (David et al., 2016). Nitrogen gas was also blown into the influent tank during the low temperature experiment to reduce DO levels. The low temperature experiment was done to assess the impact of temperature on nitrate removal and also to determine the kinetic parameters of nitrate removal at low temperatures.

2.4 Denitrification Kinetics

Nitrate concentrations ranging from 1 mg N/L to 50 mg N/L were used during this experiment. This wide range of concentrations was used because of the need to cover the typical nitrate concentration range observed in agricultural tile drainage and also to assess the kinetics of nitrate removal at different nitrate concentrations. For each concentration, a 12 hour HRT was used. At least 84 data points were collected during each experiment. For each data point a removal rate associated with a specific nitrate concentration was calculated. This removal rate is the instantaneous removal rate at this specific concentration, which was calculated by nitrate reduction through next sample port divided by travel time. Each data point was fitted to the Michaelis Menten rate equation. The different parameters obtained from the Michaelis Menten equation were the $V_{\text{max}}$ that is the maximum removal rate (mg N/L/h), $K_m$ is the Michaelis Menten constant (mg N/L) which defines the nitrate concentration at which nitrate removal rate is half of the maximum rate (Ghane et al., 2015). The same process was repeated to determine the kinetics parameters at low temperature.
2.6 Sample collection and analysis method

Samples were taken at each sample port along the length of the column (total of 12 samples for each sampling event). Six (6) samples events were done for each concentration and the average was used. A 40 ml sample was collected at each sampling port and filtered using a 0.45 um filter. Samples were stored at 4°C before being analyzed for NO$_3^-$ -N (mg N/L) and NO$_2^-$ -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. Another set of samples was taken for each concentration to measure the dissolved organic carbon (DOC) along the length of the column. The DOC samples were filtered using a 0.45 um filter and each sample was acidified using a HCl solution. Samples were acidified to prevent any potential microbial utilization of carbon during the storage time. DOC was analyzed using a Total organic carbon analyzer model CSH from Shimadzu.
Chapter 3: RESULTS and DISCUSSION

3.1: Nitrate Removal Rate and Kinetics.

Influent nitrate concentration effect on nitrate removal rate was studied at room temperature (22º C) and low temperature (5º C). The reactor was loaded with 50 mg N/L for a period of 5 weeks to stabilize the bioreactor. During the first 3 weeks of operation, the bioreactor achieved a mean removal rate of 12.02 g N/m³/d with an effluent concentration of 37.60 mg N/L. Effluent nitrite started increasing after the first week of operation with the highest observed nitrite concentration of 0.8 mg N/L. After the fourth week of operation the nitrate removal rate dropped to an average of 9.68 g N/m³/d with an effluent concentration of 40.50 mg N/L. Mean nitrite concentration increased to 1.3 mg N/L. High denitrification rate was expected during the first few weeks of operation due to high initial dissolved organic carbon leaching. Cameron et al. (2010) observed a decrease in nitrate removal rate over a 23 month period using hardwood in barrels. The initial removal rate they observed was 7.4 ±1.1 g N/m³/d between the first month and the 10 month of operation and 4.4 ± 1 g N/m³/d between 10 months and 23 months of operation. Figure 3 presents the results of the bioreactor operated under different nitrate concentrations (50 mg N/L to 3 mg N/L) at a fixed HRT of 12 hours at 22 degree Celsius. Nitrate removal rate was less variable at higher nitrate concentrations but started changing at lower concentrations. From Figure 3 it can be seen that nitrate removal rate remained constant for concentrations ranging from 50 mg N/L to 20 mg N/L, with approximatively 10 ± 0.3 mg N/L removed and a denitrification rate of 9.80 ± 0.5 g N/m³/d. The denitrification rates at influent concentrations of 10 mg N/L; 5 mg N/L and 3 mg N/L were 7.43 g NO₃⁻ N/m³/d, 4.97 g NO₃⁻ N/m³/d, 3.02 g NO₃⁻ N/m³/d respectively.
which were significantly lower than the values observed at influent concentrations ranging from 20 mg N/L to 50 mg N/L. The results of our study fall within the range of what has been reported by Christianson et al. (2011) who calculated nitrate removal rates of rectangular, channel and trapezoidal bioreactors, with respectively 5.6, 3.8 and 4.1 g N/m³/d removal rates based on their volumes. Based on the nitrate removal rate it can be concluded that denitrification rate increases with increasing nitrate influent concentrations. However, the denitrification rate reached a plateau when the influent nitrate concentration was higher than 20 mg/L based on the results of this study. The same trend has also been observed by Hoover et al. (2016) who reported similar denitrification rates between 30 mg N/L and 50 mg N/L. Carbon availability and biomass population can be the limiting factors for denitrification at higher nitrate concentrations because nitrate concentration can exceed biomass capacity, which may explain why no variation in denitrification rate was observed at higher nitrate concentrations (Moorman et al., 2010). Healy et al. (2006) used woodchips combined to sand filters and found that when loaded with nitrate concentration of 60 mg N/L and 200 mg N/L, nitrate removal rates varied between 2.2 ± 0.1 g N/m³/d and 3.3 ± 0.1 g N/m³/d respectively. Zero order and first order reaction models were fitted to the experimental data to determine the reaction order. By comparing the different coefficient of determination (R²), it was determined that zero order reaction fits most of the concentrations ranging from 50 mg N/L to 5 mg N/L and first order reaction fits the concentrations below 3 mg N/L. Table 2 shows the results of the different coefficients of determination. Robertson (2010) reported a zero order reaction for nitrate concentration ranging from 3.1 mg N/L to 49 mg N/L, in a laboratory study. Hua et al., (2016) found that nitrate removal rate changed from zero
order reaction to first order reaction when nitrate becomes limiting. From Figure 3, it can also be seen that nitrate removal rate substantially changed with changing concentrations so neither the zero order nor the first order reaction can adequately describe nitrate removal in a woodchip bioreactor. The Michaelis Menten equation has been used to determine the reaction order. To determine the different kinetics parameters (Vmax and Km) nitrate removal rate was calculated at each nitrate concentration. The inverse of nitrate removal rate versus inverse of nitrate concentration were fitted to the inverse of the Michaelis Menten equation (Lineweaver Burk equation). The following equation represents the Lineweaver Burk equation:

\[
\frac{1}{V_o} = \frac{1}{V_{\text{max}}} + \frac{K_m}{V_{\text{max}} [C_i]}
\]

This plot gave us a straight line with slope corresponding to Km/Vmax and intercept of 1/Vmax.

Figure 4A presents the Lineweaver plot, it can be observed that 95% of our variations are explained by linear relationship. Table 2 shows a half saturation point of 2.17 mg N/L and a maximum removal rate of 0.86 mg N/L/h that were determined at 22 degree Celsius. These parameters have been determined for a stable reactor that has been run for a period of 1 month till nitrate removal rate become constant before sampling events begun. Woodchips bioreactor have shown high removal rate during the first few months of operation, this is due to the high leaching of dissolved organic carbon (Ghane et al., 2015, Jaynes et al., 2008; Robertson, 2010). The determined kinetics parameters are valid for predicting long term nitrate removal.
The half saturation constant of 2.17 mg N/L is the nitrate concentration at which nitrate removal rate is half (0.43 mg N/L/H) of the maximum removal rate. Our results fall within the range of what others have reported. Nordström and Herbert (2017) and Garcia-Ruiz et al. (1998) reported half saturation constants ranging from 0.2 to 10.6 mg N/L for woodchip bioreactor and intact sediment respectively. Our results are lower than what has been observed by Laverman et al. (2010) and Ghane et al. (2015) who reported half saturation constants ranging from 7.2 mg N/L to 30.7 mg N/L. This variation may be explained by different experimental conditions.

Figure 4B presents a comparison between predicted removal rates from Michaelis Menten kinetics and experimental data. From this figure, it can be seen that at high nitrate concentration (Ci > 2.17 mg N/L), the reaction follows a zero order reaction with a maximum removal rate of 0.86 mg N/L/h. At low nitrate concentration when (Ci < 2.17 mg N/L), an apparent first order reaction can be observed. Table 2 shows the results of the calculation of first order and zero order constants.

The switch from a zero order reaction to a first order reaction is an important aspect when designing field bioreactors because extremely long HRTs from oversizing a bioreactor could lead to several unintended consequences such as H2S production and methylmercury formation (Nordström., 2016). Hua et al., (2016) observed sulfate reduction when nitrate concentration was less than 1 mg N/L.

3.2 Nitrate Removal Rate and Kinetics: Low Temperature Impact

The reactor was moved to a temperature controlled chamber where the temperature was maintained at 5 degree Celsius. Nitrogen gas was sparged into the
influent tank to limit the effect of dissolved oxygen on denitrification. The dissolved oxygen level was maintained below 3 mg /L for the duration of the low temperature experiment. Nitrate removal rate was evaluated at different nitrate influent concentrations ranging from 50 mg N/L to 0.5 mg N/L and the HRT was maintained at 12 hours for each concentration (Figure 6). Nitrate removal rate at 50 mg N/L decreased by almost 96 % compared to the removal rate observed for 50 mg N/L at 22 degree Celsius. As observed at 22 degree Celsius, nitrate removal rate was also less variable at higher nitrate concentrations at 5º C. For nitrate influent concentrations ranging from 50 mg N/L to 20 mg N/L, nitrate removal rate was 0.55 g N/m$^3$/d ± 0.04 g N/m$^3$/d. When influent nitrate concentration decreased, the nitrate removal rate decreased with a removal rate of 0.49 g N/m$^3$/d, 0.41 g N/m$^3$/d and 0.2 g N/m$^3$/d at 10 mg N/L, 2 mg N/L and 0.5 mg N/L respectively. Christianson et al. (2012a) reported nitrate removal rates varying from 0.38 to 7.76 g N/m$^3$/d for four field bioreactors in Iowa where water temperature could decrease as low as 3 degree Celsius during the month of March. Dissolved organic carbon leaching dropped by more than half when the reactor moved from the room temperature (22 degree Celsius) to the 5º C chamber. The dissolved organic carbon leached during the low temperature experiment was 1.1 ± 0.4 mg/L compared to 5 ± 0.5 mg/L before moving the reactor into the temperature controlled chamber. The difference in nitrate removal rates observed at low temperature compare to the room temperature could be explained by low dissolved organic carbon leaching observed at low temperature. The reduced microbial community activity at low temperature could also be an important factor limiting denitrification (Rittmann and McCarty, 2001). Nitrite
accumulation was also observed at 5° C. However, the concentrations of effluent nitrite were less than those observed at 22° C.

The Lineweaver Burk equation was used to determine the kinetics parameters. As shown on Figure 7A, 96 % of our variation is explained by a linear relationship. A half saturation constant of 0.58 mg N/L and a maximum removal rate of 0.045 mg N/L/h were determined. Figure 7B presents the Michaelis Menten plot of the variation of nitrate removal rate at different concentrations. From this figure, it can be seen that nitrate removal rate remained stable for nitrate concentration above 20 mg N/L and the maximum removal rate was 0.045 mg N/L/h. At low nitrate concentrations (Ci< 0.58 mg N/L), an apparent first order reaction can be observed. It can be concluded that low temperature has a negative impact on denitrification but woodchip bioreactor is still a viable option for removing nitrate from agricultural drainage water at low temperatures.

3.3 HRT impact on nitrate removal rate

Figure 5A, 5B and 5C presents nitrate removal at different HRTs of 24 hours, 12 hours and 6 hours respectively. The influent nitrate concentration was 10 mg/l. Each HRT was ran for 7 days with two sampling event every day for the last 3 days.

At the HRT of 6 hours (Figure 5C), a 29.4 % nitrate removal was observed and the average effluent concentration was 7.06 mg N/l. Our results are within the lower range observed by Christianson et al. (2011) that reported a removal percentage ranging from 30% to 70 % at an HRT of 6 hours and an influent nitrate concentration of 10 mg/l. Their study was done on field woodchip bioreactors using different reactor geometry. The nitrate removal rate at 6 hour HRT was 5.83 g NO₃⁻ N/m³/d for the laboratory
bioreactor. Christianson et al. (2012b) also reported nitrate removal rates, for 4 field reactors, ranging from 0.38 to 7.76 g NO$_3^-$ N/m$^3$/d. A one year old laboratory column study done by Camilo et al. (2013) at a 4 hour HRT reported a nitrate removal rate of 8.4 g N/m$^3$/d, 20.5 g N/m$^3$/d, 23.4 g N/m$^3$/d in columns filled with pine bark, mixture of straw/pine bark mulch and straw respectively. The difference in nitrate removal rate observed between this study and Camilo et al. (2013) may be due to the different types of organic substrates used. Cameron and Schipper. (2010) and Gibert et al. (2008) reported that softwood performs better in removing nitrate than hardwood.

As HRT increases, nitrate removal efficiency should increase too since NO$_3^-$N removal is linearly correlated to HRTs (Christianson et al., 2011; Christianson et al., 2017). As shown in Figure 5B, increasing the HRT to 12 hours at the same 10 mg/l nitrate concentration results in an average effluent nitrate concentration of 2.63 mg N/l. The calculated percentage removal was 73.7% and the removal rate was 7.43 g N/m$^3$/d.

Figure 5C shows the results at the 24 hours HRT. The nitrate percentage removal was 98.70 % with effluent average concentration of 0.129 mg N/l. The removal rate was 4.82 g N/m$^3$/d. Most of the nitrate was removed during the first 18 hours of retention with 90% of nitrate removed. Almost 50 % of the 10 mg N/L removed within the first 6 hours.

A study done by Healy et al. (2015) on different wood carbon sources at different HRTs ranging from 4 to 22 d, reported that most of the nitrate was removed within 50 to 75% of a 0.8 m height reactor, suggesting that in some cases HRT can be reduced without significant impact on reactor performance. A 24 hour HRT is sufficient to completely remove an initial nitrate concentration of 10 mg N/L based on the results of this study at 22º C.
Based on our results, longer HRT did not affect the removal rate (g N/m³/d) 24h > 12h > 6h. The nitrate removal efficiency increased from 29.4 % to 98.70 % when increasing the HRT from 6 to 24 hours. This same trend has been observed by Hoover et al. 2016. Greenan et al. (2010) explained that nitrate removal decreases as flowrate increases, which could be due to the transport of dissolved oxygen at higher flowrates. This may not be a factor in this study since the dissolved oxygen level was maintained below 3 mg/L.

For all testing conditions, nitrite accumulated as the nitrate concentration decreased. Nitrite accumulation was also observed during woodchip bioreactor denitrification by Gibert et al. (2008) and Greenan et al. (2009). During the first week of operation, effluent nitrite concentration was almost zero. It gradually increased over time with the highest nitrite concentration observed at 50 mg N/L at a 12 hour HRT and an effluent nitrite concentration of 1.45 mg N/L. Approximately, 15 % of nitrate removed was converted to nitrite. Nitrite gradually increased along the length of the column as the nitrate concentration decreased. A straw laboratory column experiment done by Camilo et al. (2013) reported effluent nitrite concentration of 3.0 ± 3 mg N/L after 181 days of operation.

Under different nitrate concentrations, nitrite accumulation was similar at nitrate concentrations ranging from 50 mg N/L to 20 mg N/L with effluent nitrite concentrations of 1.15 ± 0.3 mg N/L under a 12 hour HRT. Nitrite reduction was not observed for this range of concentrations. When loaded with 5 mg N/L at a HRT of 12 hours a nitrite peak of 0.44 mg N/l was observed at 75 % of the reactor length. After that, nitrite reduction begun when nitrate was below 1 mg N/L. The reactor effluent nitrite concentration was
0.26 mg N/L. At nitrate concentration of 3 mg N/L and a 12 hour HRT, nitrite reduction was also observed with a peak nitrite of 0.24 mg N/L at around 25 % of the reactor length. Nitrite reduction occurred after 50 % of the nitrate was removed. The reactor effluent nitrite concentration was 0 mg N/L at 83 % of the column length because complete denitrification was already achieved.

For 10 mg N/L at a 12 hour HRT (Figure 5B), nitrite accumulated linearly from 0 to 1.2 mg N/L along the height of the reactor. Under a 10 mg N/L nitrate and a HRT of 6 hours, effluent nitrite was approximately half of what was observed at the 12 hour HRT. Approximately 16 % and 20 % of nitrate removed was converted to nitrite at 12 and 6 hour HRTs respectively.

A different scenario occurred at 10 mg N/L nitrate under a 24 hour HRT (Figure 5A). Nitrite linearly increased from 0 mg N/L to 0.71 mg N/L at the reactor sample port number eight that corresponds to a 16 hour HRT (60 % of the column length). At the same time, 84 % of nitrate was removed. Nitrite concentration decreased when the HRT was further increased to 24 hours. The effluent nitrite concentration at a 24 hour HRT was 0.22 mg N/L. Approximately 7 % of the nitrate reduced was converted into nitrite. Hua et al. (2016) also reported that the accumulated nitrite was about 9.6% -18.7% of the reduced nitrate for nitrate concentration of 20 mg N/L and 50 mg N/L at HRTs varying from 6 hours to 24 hours. From those results it can be seen that higher nitrate concentrations produced the higher effluent nitrite concentrations. Sufficient HRT is required to reduce nitrite to a low concentrations.

Nitrite accumulation was also observed at low temperature but its extent was limited. The highest effluent nitrite concentration was 0.21 mg N/L, observed at 50 mg
N/L and a HRT of 12 hours (Figure 6A). Effluent nitrite concentration was proportional to the influent nitrate concentration. A decrease in effluent nitrite concentrations was observed when influent nitrate concentration was reduced from 50 mg N/L to 5 mg N/L. For nitrate concentration below 5 mg N/L, nitrite effluent concentration was below detection limit (Figures 6C, 6D). Nitrite reduction was not observed at low temperature.

For all temperature experiment conditions, nitrite concentration started increasing when nitrate concentration started decreasing. Also as temperature decreased, the amount of nitrite that accumulated along the length of the reactor was reduced. This decrease in nitrite concentration may be related to the low denitrification rate caused by a low bacterial activity.

### 3.4 Dissolved Organic Carbon leaching

Figure 8 presents DOC leaching from the woodchips for the 5 month period at 22 degree Celsius (room temperature) before the low temperature experiment. High DOC leaching was observed within the first week of operation. This is commonly called the flash out period (Nordström and Herbert, 2017). The DOC level was about 10 mg/L at 12 hours HRT after 6 weeks of operation. DOC leaching is also dependent of HRT. The longer the HRT the more leaching will be observed. This has been observed during the HRT impact run where the DOC leaching doubled with the increase of HRT from 12 hours to 24 hours. At 6 hour HRT, DOC leaching was half of the concentration observed at a 12 hour HRT. The DOC level dropped to about 4 mg/L approximatively 4 months after start up. The reactor was moved to the cool room for the low temperature experiment for about two months (1 month of stabilization and 1 month of data collection). DOC level dropped to below 2 mg/L for the duration of the low temperature
experiment. The reactor was brought back to normal temperature and the DOC level rose back to approximately 3 mg/L.
Chapter 4: CONCLUSIONS

Column experiment was conducted to determine denitrification kinetics parameters using a laboratory denitrification bioreactor filled with cottonwood woodchips. The experiment was done at two different temperatures. Hydraulic retention time impact was studied to determine bioreactor efficiency. From the experiment, the half saturation constant and maximum removal rate were found to be 2.17 mg N/L and 0.86 mg N/L/H respectively.

The laboratory woodchip bioreactor successfully removed nitrate with nitrate removal percentages ranging from 18.8 % to 100% over nitrate concentrations ranging from 50 mg N/L to 3 mg N/L at a fixed 12 hours HRT. This yielded nitrate removal rate of 9.48 g N m$^3$/d for nitrate concentrations ranging from 50 mg N/L to 20 mg N/L. From 10 mg N/L, 5 mg N/L, 3 mg N/L, the removal rates were 7.43 g N/m$^3$/d, 4.97 g N/m$^3$/d, 3.02 g N/m$^3$/d respectively. By changing the hydraulic retention time to 24 hours and 6 hours, the reactor removed 98.8 % and 29.5 % of the nitrate respectively with influent nitrate concentration of 10 mg N/L. The yielded removal rates were 5.85 g N/m$^3$/d at 24 hours HRT and 4.82 g N/m$^3$/d AT 6 hours HRT respectively.

Nitrite was observed for all conditions. The highest nitrate effluent concentration was observed at 50 mg N/L under a 12 hours HRT, with nitrite concentration above 1 mg N/L. During HRT changes, nitrite accumulation and reduction occurred with the highest nitrite peak observed at 24 hours HRT with influent nitrate concentration of 10 mg N/L. Nitrite accumulated during the first 15 hours before being reduced gradually with effluent nitrite concentration around 0.2 mg N/L. Under low temperature experiment (5 degree
Celsius), nitrite accumulation extent was lower than what was observed at room temperature experiment (22 degree Celsius).

During the reactor start up high Dissolved organic carbon leaching was observed. DOC leaching remained constant after the fourth month of operation at room temperature. During low temperature experiment, DOC leaching dropped by almost half of what was observed before moving the reactor into the cooler room. Based on the results of the laboratory woodchip bioreactor experiment, it can be seen that woodchip bioreactor is efficient in removing nitrate from agricultural drainage water. The results of this study brought a significant contribution in better understanding denitrification kinetics in Woodchip bioreactor.
References


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Table 2: Nitrate removal kinetics parameters (half saturation constant, maximum removal rate, zero and first order constant)

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Figure 1: Picture of the woodchips used to pack the column. (A) Woodchips collection in Sioux Falls (Hardscape Outlet) (B) woodchips before cleaning (C) woodchips after cleaning.
FIGURE 2A Picture of the reactor. (2A): room temperature (22º C)
Figure 2B Pictures of the reactor. Low temperature (5º C)
Figure 3: Nitrate removal at different concentrations. (A) 50 mg/l, (B) 20 mg/l, (C) 5 mg/l, (D) 3 mg/l. HRT = 12 HOURS (22º C)
FIGURE 4A: Inverse of nitrate removal rate versus the inverse of inflow nitrate concentration from the experimental data (22º C).
FIGURE 4B: Graph showing the simulated and the observed nitrate removal rate versus nitrate concentration (22° C).
Figure 5: Hydraulic Retention time Impact. (Experimental conditions: (A) HRT = 24 hours, (B) HRT = 12 hours, (C) HRT = 6 hours (22º C))
Figure 6: Nitrate removal at different concentrations. (A) 50 mg/l, (B) 20 mg/l, (C) 2 mg/l, (D) 0.5 mg/l. HRT = 12 HOURS (5º C)
FIGURE 7A: Inverse of nitrate removal rate versus the inverse of inflow nitrate concentration from the experimental data at 5º C.

\[ y = 11.747x + 23.395 \]

\[ R^2 = 0.9664 \]
FIGURE 7B: Graph showing the simulated and the observed nitrate removal rate versus nitrate concentration at 5 degree Celsius at 5º C.
Figure 8: Averaged monthly DOC leaching from woodchip reactor over a five months period.