

Improving the efficiency of wood chip bioreactors for removing nitrate from drainage water

by

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## **Abstract**

Wood chip bioreactors can be installed at subsurface drainage outlets in order to decrease the concentration of nitrate ( $\text{NO}_3^-$ ) discharged to receiving waters. A laboratory column study was conducted to determine  $\text{NO}_3\text{-N}$  removal in wood chip bioreactors under saturated and unsaturated conditions. Nitrate-N was added at a concentration of 100 mg/l to columns filled with wood chips and effluent samples were collected daily for  $\text{NO}_3\text{-N}$  analysis. Once the denitrifying bacteria communities had established in the bioreactors,  $\text{NO}_3\text{-N}$  removal under saturated conditions (85.4 and 92.8%) was significantly higher ( $p < 0.0001$ ) compared to unsaturated conditions (2.8 and 21.4%). Using these results, in-field wood chip bioreactor sizing was determined for four different daily precipitation rates in southern Manitoba. The bioreactors were designed to maintain saturated conditions. The bioreactor volume was based on a three-day hydraulic retention time (HRT) to attain  $\text{NO}_3\text{-N}$  removal for the chosen precipitation values.

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## **Dedication**

I dedicate this thesis to my parents, Joanne and Jacy Whyte, for their unconditional love and support. This would have been impossible without you. Thank you.

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# **1. Introduction**

## **1.1 Overview**

Subsurface agricultural drainage, also known as tile drainage, has increased in recent years to improve drainage in poorly drained soils and to increase productivity. Although tile drainage can have a positive effect on crop production, it has the potential to deliver a significant amount of nutrients, such as nitrate, to receiving waters during high flow conditions. Excess nitrate in the environment can have an adverse impact on water quality, aquatic life, and human health. Increased nitrate concentrations in surface waters can be harmful to aquatic organisms by promoting toxic algal blooms, fish kills, and reduction in species richness (Smith and Kellman 2011). Human health is at risk with increasing nitrate levels in the environment as methemoglobinemia, also known as blue baby syndrome, can be caused by the ingestion of nitrate contaminated drinking water. Some researchers also link ingestion of nitrate contaminated water with cancer in humans (Oa et al. 2006; Powlson et al. 2008). Additional research is required to confirm the carcinogenicity of nitrate as there is conflicting evidence regarding this topic (Bryan et al. 2012; Fewtrell 2004).

Bioreactors can be installed at the edge of a field (edge-of-field bioreactors) near the tile drainage outlet to decrease the concentration of nitrate leaving the field. Edge-of-field bioreactors enhance the natural denitrification process by providing a carbon source, such as wood chips, for denitrifying bacteria. Denitrifying bacteria convert the nitrate in drainage water to nitrogen gas, which is then released back into the atmosphere. This process reduces the nitrate concentration in drainage water before reaching sensitive receptors.

Conditions for denitrification are favourable when there is an abundance of nitrate, limited oxygen (anaerobic conditions), and the presence of a degradable carbon source. Due to these conditions, the majority of studies on wood chip bioreactors have focused on maintaining anaerobic or saturated conditions in the bioreactors (Chun et al. 2009; Greenan et al. 2009). There is limited research on the ability of edge-of-field bioreactors to reduce the nitrate concentration in drainage water under unsaturated conditions. Chapter 3 of this thesis compares the effectiveness of wood chip bioreactors under saturated and unsaturated conditions.

There is limited rationale for the sizing of in-field wood chip bioreactors, however, it is assumed that sizing is generally based on the amount of land available for the bioreactor (David et al. 2016; Hartz et al. 2017). Using the results from Chapter 3, Chapter 4 explores an approach for designing denitrifying bioreactors based on precipitation data, HRT (hydraulic retention time), and porosity.

## **1.2 Scope**

Six columns were used for the laboratory-scale wood chip bioreactors in order to simulate conditions of an edge-of-field bioreactor designed to treat agricultural drainage water. Three (3) columns were completely filled with water to simulate saturated bioreactors. The other three (3) columns were filled with water then drained to simulate unsaturated bioreactors. Based on the results from the laboratory-scale study, a preliminary design is proposed for an in-field bioreactor.

### **1.3 Objectives**

The objective of this research was to compare saturated and unsaturated conditions in laboratory-scale wood chip bioreactors to determine the most favourable conditions for nitrate removal. The specific objectives were to:

1. Determine if there was a significant difference in nitrate removal between saturated and unsaturated conditions.
2. Using the most favourable condition, create a preliminary field design of a wood chip bioreactor to be implemented at the edge of an existing field containing subsurface drainage.

### **1.4 Outline of Thesis**

This thesis consists of six chapters. Chapter 1 is an overall introduction to the thesis. Chapter 2 is a literature review that details the nitrogen cycle, nitrogen in the environment, subsurface drainage systems, and wood chip bioreactors. Chapter 3 presents the laboratory study conducted which includes materials and methods and results. Chapter 4 details preliminary design calculations for a proposed field-scale bioreactor. Chapter 5 consists of a summary and conclusion of research outcomes. Chapter 6 makes recommendations for future research.

## 2. Literature Review

### 2.1 The Nitrogen Cycle

Nitrogen is essential for the existence of life because organisms require a considerable amount for the synthesis of nucleic acids and proteins (Canfield et al. 2010). Although there is an abundance of nitrogen in the environment, the majority of nitrogen is in the form of nitrogen gas ( $N_2$ ) in the atmosphere. Nitrogen gas is virtually inert and must be converted to different forms of nitrogen in order to be available for organisms. Nitrogen fixation, a process that converts nitrogen gas to ammonia ( $NH_3^+$ ), is the only biological process that allows for  $N_2$  to be accessible to organisms (Falkowski et al. 2008). In the presence of oxygen (aerobic conditions), nitrifying bacteria can oxidize ammonia to nitrate ( $NO_3^-$ ). In the absence of oxygen (anaerobic conditions), denitrifying bacteria can use nitrate as an electron acceptor to ultimately form  $N_2$ , thereby closing the nitrogen cycle (Chun et al. 2009; Falkowski et al. 2008). Nitrite ( $NO_2^-$ ) is an obligate intermediate of the denitrification process and can be produced if the environment is not favourable for complete denitrification. The nitrogen cycle describes the transformation of nitrogen within its various forms in the environment.

Another anaerobic reaction that can occur in the nitrogen cycle is known as dissimilatory nitrate reduction to ammonium (DNRA). Fermentative bacteria carry out the DNRA reaction by converting nitrate to ammonium ( $NH_4^+$ ) (Sgouridis et al. 2011). The DNRA reaction and denitrification occur under similar conditions, however, DNRA is more likely to occur when nitrate is limiting and denitrification is more likely to occur when carbon is limiting (Korom 1992; Sgouridis et al. 2011).

Nitrogen is a component of plant chlorophyll and is necessary for photosynthesis. Nitrogen is the element that most often limits plant growth due to the inaccessibility of a large fraction of

nitrogen in the atmosphere as nitrogen gas. The primary form of nitrogen for uptake in plants are the ions nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ). In order to make nitrogen easily accessible for plants, nitrogen fertilizer can be applied to the soil in various forms such as anhydrous ammonia, urea, and ammonium nitrate.

In the 20<sup>th</sup> century, the industrial process was developed to reduce  $\text{N}_2$  to  $\text{NH}_4^+$  in the form of fertilizer to boost crop yields. This discovery has had an enormous impact on the global nitrogen cycle. The use of nitrogen fertilizers has increased approximately 800% from 1960 to 2000 (Fixen and West 2002). About 50% of fertilizer use is accounted for with wheat, rice, and maize (Canfield et al. 2010). For these crops, the nitrogen use efficiency is typically below 40% (Canfield et al. 2010). Applied fertilizer that is not used by crops can leach below the root zone, be lost to surface runoff, or lost to the atmosphere. This overuse and loss of fertilizer is not only economically inefficient; it can also have an adverse impact on the environment and human health.

## **2.2 Adverse Impacts on Human Health**

The United States Environmental Protection Agency developed TEACH (Toxicity and Exposure Assessment for Children's Health) Chemical Summaries for each chemical or chemical group for use in research studies pertaining to developmental exposure and/or health effects (EPA 2007). The health effect of most concern for children, as established by the TEACH Chemical Summary for Nitrates and Nitrites (2007), is methemoglobinemia, commonly known as blue baby syndrome. Blue baby syndrome is most often seen in infants who have been fed water from nitrate contaminated wells (Greer 2005). Nitrate can be microbially reduced to nitrite ( $\text{NO}_2^-$ ) either before or after ingestion. Nitrite then oxidizes the ferrous iron in hemoglobin to the ferric form which results in the formation of methemoglobin. Methemoglobin is incapable of

binding oxygen, thus resulting in an abnormally low concentration of oxygen in the blood (Fewtrell 2004). A low concentration of oxygen in the blood can have numerous adverse effects, the most severe being coma and death (EPA 2007). Methemoglobinemia caused by nitrate contaminated drinking water is a concern for young children, especially below the age of 4 months due to a higher gastric pH in infants, greater fluid intake relative to body weight, and a higher proportion of fetal hemoglobin (Ayebo et al. 1997).

Some researchers also link ingestion of nitrate contaminated water with cancer in humans (Oa et al. 2006; Powlson et al. 2008). There is conflicting evidence regarding this topic (Bryan et al. 2012; Fewtrell 2004), however, additional research is required to confirm the carcinogenicity of nitrate and nitrite.

The Health Canada Guidelines for Canadian Drinking Water Quality state the maximum acceptable concentrations (MAC) for nitrate are 45 mg/l as nitrate and 10 mg/l as nitrate-N. The Guidelines also state the MAC for nitrite is 3 mg/l as nitrite and 1 mg/l as nitrite-N. The basis of Health Canada's MAC is the risk of methaemoglobinaemia and possible carcinogenicity under conditions that result in endogenous nitrosation (Health Canada 2017).

### **2.3 Adverse Impacts on the Environment**

Long-term environmental consequences occur with excess nutrient loading to surface waters and coastal ecosystems. The effects of excess nitrogen and phosphorus loading to receiving water can lead to eutrophication (Canfield et al. 2010; Galloway et al. 2008), harmful algal blooms, low oxygen conditions and dead zones (Diaz and Rosenberg 2008), fish kills, and loss of biodiversity (Fulweiler et al. 2012; Smith and Kellman 2011).

Eutrophication occurs when excess phosphorus and nitrogen are introduced to surface waters. The nutrient enrichment causes algae and vegetation to grow in the receiving waters. As

algae dies, this adds to the flow of organic matter to the bed of the receiving water which can fuel microbial respiration and greatly decrease the dissolved oxygen (DO) in the water (Diaz and Rosenberg 2008).

Although researchers attribute the cause of eutrophication to excess phosphorus and nitrogen in surface waters, there is less agreement among researchers on how to reverse eutrophication (Schindler et al. 2012). Researchers at Experimental Lakes Area (ELA) conducted a study over many years on the topic of nitrogen and phosphorus promoting eutrophication. Phosphorus was continuously added to a small lake in conjunction with different amounts of nitrogen over 4 decades. No nitrogen was added to the lake after 1990; however, algal blooms did not diminish. The researchers at ELA found that decreasing nitrogen inputs to lakes did not substantially reduce symptoms of eutrophication (Schindler et al. 2008). The researchers at ELA concluded that removal of nitrogen to control blue-green algae is unnecessary, and treatment should focus on the control of phosphorus (Paterson et al. 2011). There are plenty of arguments regarding whether both phosphorus and nitrogen must be decreased in order to reverse eutrophication, or whether phosphorus alone must be reduced. As more studies are completed regarding efforts to reduce eutrophication, an agreement on the best approach will hopefully be established.

The Canadian Council of Ministers of the Environment (CCME) Water Quality Guidelines for the Protection of Aquatic Life outline the short-term and long-term exposure benchmark concentrations for the protection from direct toxic effects for both freshwater and marine life. The short-term benchmark concentrations are found using severe effects data (such as ability to cause death) of distinct short-term exposure periods. The short-term exposure benchmark nitrate concentration for freshwater and marine water is 550 mg/l and 1500 mg/l, respectively. The



long-term exposure benchmark concentration is the concentration below which aquatic life are intended to be protected for indefinite exposure periods. The long-term nitrate concentration for freshwater and marine water is 13 mg/l and 200 mg/l respectively. The long-term benchmark nitrite concentration for freshwater is 60 mg/l NO<sub>2</sub>-N. The short-term and long-term benchmark concentrations do not consider the indirect effects due to eutrophication.

## **2.4 Subsurface Agricultural Drainage**

In recent years, subsurface agricultural drainage (also known as tile drainage) has increased in order to maintain productivity and improve drainage in poorly drained soils. If soil is poorly drained, the soil may become water logged which will inhibit the growth of crops. A study carried out in Southern Manitoba found an increase of up to 32% yield in potato production in soils that had implemented subsurface drainage (Satchithanantham et al. 2012). Although these systems can have a positive effect on crop production, research has shown that they have the potential to deliver a significant amount of nitrate into nearby drainage ditches, streams, and rivers (Cordeiro 2014; David et al. 1997; Randall and Mulla 2001). A 6-year study completed in Illinois showed that an average of about 49% of the residual nitrate left in the soil after harvest was estimated to be leached through drain tiles (David et al. 1997). A recent study completed in Winkler, Manitoba found the average nitrate concentration draining from free drainage was 98.9 mg/l NO<sub>3</sub>-N (Cordeiro 2014).

## **2.5 Denitrifying Bioreactors**

One approach for reducing the amount of nitrate delivered to surface waters from agricultural drainage waters is edge-of-field bioreactors or denitrification walls (Greenan et al. 2009). Edge-of-field bioreactors are installed at the edge of a field containing subsurface drainage. Bioreactors provide a carbon source to the bacteria to promote the natural

denitrification process which decreases the nitrate concentration in the drainage water before reaching sensitive receptors such as streams with aquatic life or rural residential drinking water wells.

### **2.5.1 Denitrification**

Nitrate ( $\text{NO}_3^-$ ) can be used by many microbes as a respiratory electron acceptor in the absence of oxygen (anaerobic conditions). Denitrification is the process of bacteria converting nitrate to nitrogen gas. Denitrification follows the pathway:



More than 60 genera of bacteria and archaea are considered denitrifiers, as well as some eukaryotes such as fungi (Canfield et al. 2010). Matějů et al., 1992, state that the most detailed investigations into denitrification have involved a limited group of specialized bacteria. This has caused the view that denitrification can only occur under anaerobic conditions. However, in certain species, it is possible for denitrification to occur in the presence of oxygen (Lloyd 1993). Although possible in the presence of oxygen, the majority of denitrifiers are facultative anaerobic heterotrophs and therefore obtain both their energy and carbon from the oxidation of organic compounds (Rivett et al. 2008).

Concentrations of oxygen ( $\text{O}_2$ ), nitrate, and carbon (C) control the rate of heterotrophic denitrification at the microbial scale (Schipper et al. 2010). Where excess nitrate is present, the availability of a degradable carbon source to allow for denitrification is essential. Facultative anaerobic heterotrophic bacteria will use oxygen as an electron acceptor to obtain energy through the oxidation of organic compounds, until the environment becomes energetically favourable to use nitrate as an electron acceptor (Schipper et al. 2010). Therefore, conditions for denitrification

are favourable when there is an abundance of nitrate, limited oxygen, and the presence of a degradable carbon source.

### 2.5.2 How Denitrifying Bioreactors Work

Edge-of-field bioreactors enhance denitrification using a carbon source. A wide variety of materials may be used to provide the carbon source in denitrifying bioreactors such as corncobs, corn stalks, wheat and barley straw, pine and almond shells, and wood chips. In general, woody media is the preferred carbon fill due to low cost, conductivity, and longevity (Robertson 2010; Schipper et al. 2010). In this thesis, wood chips were used as the carbon source in the bioreactors and hence the name, wood chip bioreactors.

Two factors that govern nitrate removal rate in wood chip bioreactors are the rate of denitrification and the hydraulic retention time (HRT) within the bioreactor, with removal efficiency increasing at a longer HRT (Greenan et al. 2009). The HRT is determined based on pore volume and flow rate and is described by the following equation:

$$HRT = \frac{\text{Pore Volume}}{\text{Flow Rate}} = \frac{\emptyset V_{total}}{Q} \quad (2.2)$$

where  $\emptyset$  = porosity,  $V_{total}$  = total volume of bioreactor, and  $Q$  = flow rate. The total volume of the bioreactor can be measured along with the flow rate. The porosity of the bioreactor can be determined by comparing the void space to the total volume of the bioreactor using the following equation:

$$\emptyset = \frac{V_{void}}{V_{woodchips} + V_{void}} = \frac{V_{void}}{V_{total}} \quad (2.3)$$

where  $V_{void}$  = volume of void space,  $V_{woodchips}$  = volume of woodchips, and

$V_{total}$  = total volume (void space + woodchips).

Wood chip bioreactors are implemented at the edge of a field that has subsurface drainage. The wood chip bioreactor can be designed based on the size of the agricultural field serviced by subsurface drainage, the required HRT, and discharge rate. The water in the subsurface drainage is routed through the wood chip bioreactor before being discharged to a drainage ditch. Water from the subsurface drainage enters the bioreactor, nitrate is converted to  $N_2$  gas, and the treated water flows out of the bioreactor to a drainage ditch.

### **2.5.3 Previous Studies**

Edge-of-field bioreactors constructed by Blowes et al. (1994) that contained porous-medium material of coarse sand and organic carbon were successful in reducing the nitrate concentration in agricultural runoff from farm-field drainage tile. The influent nitrate concentration to the bioreactors from the farm-field drainage tile was 3-6 mg/l while the effluent nitrate concentration from the bioreactors was less than 0.02 mg/l.

A denitrification wall constructed by Schipper and Vojvodić-Vuković (2001) was successful in removing nitrate from groundwater below agricultural lands. The denitrification wall was created by filling a trench with a mixture of soil and sawdust. Over 5 years, 95% of the nitrate in the groundwater up gradient was removed. It was found that the soil/sawdust mix demonstrated declining total nitrogen concentrations, suggesting that nitrogen immobilization was not a dominating removal process and that the nitrate removal can be credited to denitrification.

As mentioned previously, the DNRA reaction occurs under similar conditions as denitrification. Greenan et al. (2006) studied the DNRA reaction in denitrifying bioreactors by completing a laboratory jar study, which assessed  $NO_3$ -N reduction in four different carbon sources (wood chips, wood chips saturated with soybean oil, dried cornstalks, and paper fibers from corrugated cardboard). All jars received a known concentration of  $NO_3$ -N and were

incubated in an anaerobic growth chamber. Water extracts from the jars were analyzed for  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$ . The DNRA reaction to  $\text{NH}_4\text{-N}$  was based on the quantity of N appearing as  $\text{NH}_4\text{-N}$ . Immobilization of  $\text{NO}_3\text{-N}$  was determined by measuring the increase in N in the solid material. It was found that less than 2.4% of the  $\text{NO}_3\text{-N}$  transformed in all treatments was due to immobilization and less than 1% of the  $\text{NO}_3\text{-N}$  transformed in all treatments was due to DNRA.

Lab-scale bioreactors constructed by Greenan et al. (2009) were created to simulate a denitrification wall designed to intercept and treat subsurface groundwater. One objective of their experiment was to determine the rate of nitrate removal at different water flow rates. It was found that nitrate removal varied with flow rates from 30% to 100% removal efficiency. Complete removal of nitrate was achieved at the lowest flow rate (2.9 ml/d) while nitrate effluent concentrations increased for increasing flow rates. It was found that increasing the flow rate decreased the HRT, which affected the ability of the microbial community to break down the nitrate.

Christianson et al. (2011) studied the impact of containing (holding) drainage water before being treated in a denitrifying bioreactor to allow for a longer, more constant HRT. Six small-scale denitrification bioreactors were built out of plywood and filled with wood chips in order to compare simulated containment prior to bioreactor treatment against letting drainage water pass directly through a bioreactor. Results showed significantly different total mass removal efficiencies with 14.0% removal efficiency for the non-containment system versus 36.9% removal efficiency for the containment system. Christianson et al. were able to conclude that more optimized nitrate removal is achieved when treating drainage at constant HRT's.

A study completed by the University of California (Hartz et al. 2017) found that, across several years of operation, denitrification in two pilot-scale bioreactors constructed on

subsurface-drained farms reduced  $\text{NO}_3\text{-N}$  concentration by an average of 8 to 10 mg/l per day of HRT in the summer and approximately 5 mg/l per day of HRT in the winter. A constant flow rate was supplied to the bioreactors to achieve an HRT of approximately 2 days. Tile drain effluent (bioreactor influent) averaged high  $\text{NO}_3\text{-N}$  concentrations, ranging between 60 and 180 mg/l at the sites. Due to the high  $\text{NO}_3\text{-N}$  concentration in the tile drain effluent, water discharged from the bioreactors had a concentration of  $\text{NO}_3\text{-N}$  that was still above the regulatory limit. If the HRT were to be increased, it is likely that there would be a greater reduction in  $\text{NO}_3\text{-N}$ . Bioreactor treatment increased nitrite ( $\text{NO}_2\text{-N}$ ) concentrations by several mg/l in the first months of operation, however, after a few months, the  $\text{NO}_2\text{-N}$  in bioreactor effluent gradually declined and remained below 0.3 mg/l thereafter. It was also found that new wood chips had to be applied at a rate of about 10% annually to maintain the chip level.

Scientific research suggests it would be beneficial to design a wood chip bioreactor to have a long and consistent HRT and the availability to replace wood chips as denitrification efficiencies decrease. Studies have been completed that ensure bioreactors remain under saturated conditions in order to maximize denitrification (Chun et al. 2009; Greenan et al. 2009). However, few studies have been completed to determine the rate of denitrification in wood chip bioreactors under unsaturated conditions.

#### **2.5.4 Adverse Environmental Effects of Denitrifying Bioreactors**

A possible detrimental effect of using denitrifying bioreactors is the production of greenhouse gases including nitrous oxide ( $\text{N}_2\text{O}$ ) and methane ( $\text{CH}_4$ ). As shown in Equation (2.1), nitrous oxide is an obligate intermediate, and some ultimately escapes into the atmosphere during the process of denitrification. A study completed in 2016 determined that less than 1% of nitrate removed from wood chip bioreactor beds was emitted as  $\text{N}_2\text{O}$  (David et al. 2016). Other studies

completed, both laboratory scale and field scale, show similar results with an  $\text{N}_2\text{O}$  production of less than 1% of total nitrate removed (Elgood et al. 2010; Greenan et al. 2009). The greenhouse gas methane has been detected in field-scale bioreactors during early operation, but disappeared after a few months (Schipper et al. 2010). It is presumed that methane concentrations should be low in bioreactors when  $\text{NO}_3^-$  concentrations are high as this will suppress the methane producing bacteria, methanogens (Schipper et al. 2010). Methanogens have to compete for the available carbon with other anaerobic bacteria, such as denitrifying bacteria. When  $\text{NO}_3^-$  concentrations are high, denitrifying bacteria will outcompete the methanogens, thus limiting the amount of methane produced (Liu et al. 2017).

### **3. Nitrate removal by laboratory scale wood chip bioreactors under saturated and unsaturated conditions**

#### **Abstract**

Wood chip bioreactors can be installed at subsurface drainage outlets to decrease the concentration of nitrate ( $\text{NO}_3^-$ ) discharged to receiving waters. Although subsurface drainage can increase crop production, it has the potential to deliver a significant amount of nitrate ( $\text{NO}_3^-$ ) to receiving waters during high flow events. Edge-of-field bioreactors installed at the outlet of subsurface drainage systems use a carbon source to enhance the natural denitrification process. This reduces the concentration of  $\text{NO}_3^-$  discharged to waters before reaching sensitive receptors such as drinking water wells, rivers, and lakes. A laboratory column study was conducted to determine  $\text{NO}_3\text{-N}$  reduction under saturated and unsaturated conditions in wood chip bioreactors. Columns were filled with wood chips,  $\text{NO}_3\text{-N}$  was added at a concentration of 100 ppm, and effluent concentrations were collected daily and analyzed for  $\text{NO}_3\text{-N}$  levels. It was found that after the bacteria communities had established in the bioreactors,  $\text{NO}_3\text{-N}$  reduction under saturated conditions (85.4 and 92.8%) was significantly higher ( $p < 0.0001$ ) compared to unsaturated conditions (2.8 and 21.4%).

#### **3.1 Introduction**

Subsurface agricultural drainage, also known as tile drainage, can be installed in fields to improve drainage and increase crop yields. If fields are poorly drained, the soil can become waterlogged and inhibit the growth of crops. A study carried out in Southern Manitoba found an increase of up to 32% yield in potato production in soils that had implemented subsurface drainage (Satchithanantham et al. 2012). Although tile drainage can have a positive effect on



crop production, it has the potential to discharge a significant amount of nutrients, such as nitrate, to the environment. Excess nitrate in the environment can have an adverse effect on surface waters, aquatic life, and human health.

Long-term environmental consequences occur with excess nutrient loading to surface waters and coastal ecosystems. The effects of excess nitrogen and phosphorus loading to receiving water can lead to eutrophication (Canfield et al. 2010; Galloway et al. 2008), harmful algal blooms, low oxygen conditions and dead zones (Diaz and Rosenberg 2008), fish kills, and loss of biodiversity (Fulweiler et al. 2012; Smith and Kellman 2011).

Human health is at risk with increasing nitrate levels in the environment as methemoglobinemia, also known as blue baby syndrome, can be caused by the ingestion of nitrate contaminated drinking water. Blue baby syndrome is often seen in babies who have been fed water from nitrate contaminated wells (Greer 2005). Some researchers also link ingestion of nitrate contaminated water with cancer in humans (Oa et al. 2006; Powlson et al. 2008). Additional research is required to confirm the carcinogenicity of nitrate and nitrite as there is conflicting evidence regarding this topic (Bryan et al. 2012; Fewtrell 2004).

One approach for decreasing the amount of nitrate delivered to surface waters from agricultural drainage water is edge-of-field bioreactors (Greenan et al. 2009). Edge-of-field bioreactors are installed at the edge of a field near the outlet of the subsurface drainage. Bioreactors provide a carbon source to the bacteria to promote the natural denitrification process, which decreases the nitrate concentration in the drainage water before reaching sensitive receptors such as streams with aquatic life or rural residential drinking water wells.

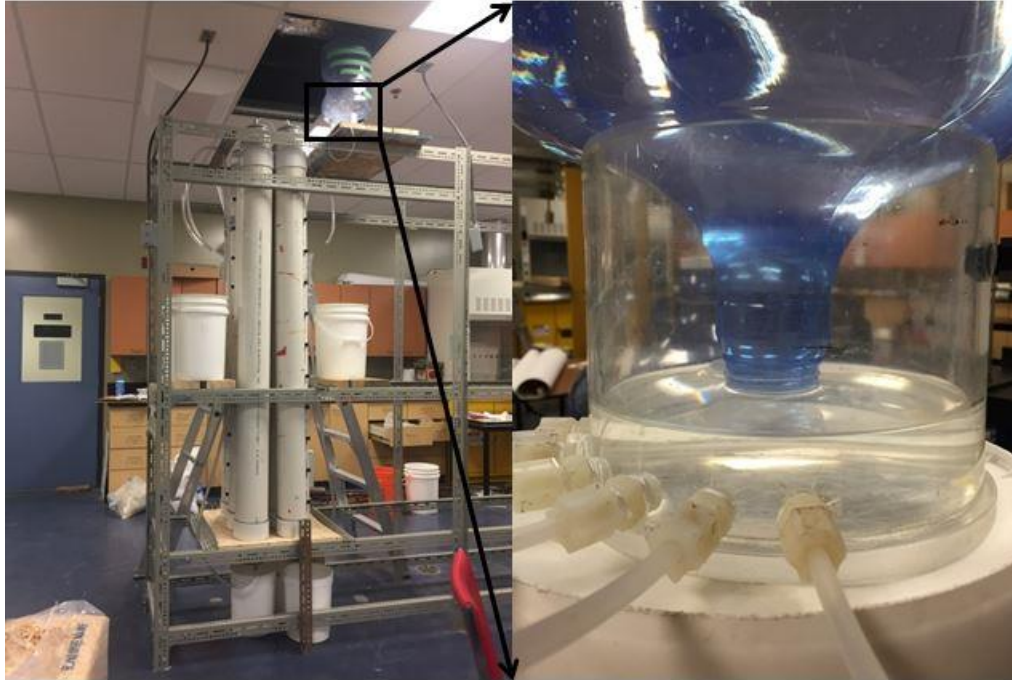
Scientific research suggests it would be beneficial to design a wood chip bioreactor to have a long and consistent HRT and the availability to replace wood chips as denitrification efficiencies decrease (Christianson et al. 2011; Hartz et al. 2017). Studies have been completed that ensure bioreactors remain under saturated conditions in order to maximize denitrification (Chun et al. 2009; Greenan et al. 2009). However, few studies have been completed to determine the rate of denitrification in wood chip bioreactors under unsaturated conditions.

## **3.2 Materials and Methods**

The laboratory column study was carried out over two time periods: once in May 2016 (Experiment A), and once in October 2016 (Experiment B). Materials and methods were the same for both experiments except where noted in the sections below. Due to the differences in the experiments, Experiment A is considered a preliminary experiment.

### **3.2.1 Materials**

Regular polyvinyl chloride (PVC) pipe columns were used to simulate the six bioreactors. The columns had an inner diameter of 15.5 cm (6 inches) and a length of 187 cm (6 feet). Three of the columns were sealed while the other three had air holes along the sides to allow for airflow. Polyethylene tubing was used to fill and drain the columns with tap water. Two carboys were used to supply water to the bioreactors while a constant head device was used to ensure a constant flow rate in each column. Potassium nitrate was used as the nitrate source. Softwood wood shavings from Spruce Products Limited (Hwy 10A North, Swan River, Manitoba) were used as the wood chip media.



**Figure 3.1. Full view of experimental set up and constant flow device.**

### **3.2.2 Filling the Columns with Wood Chips**

Wood chips were continuously poured into the top of the columns that were vertically positioned in a frame. Minimal breaks were taken during this process to prevent settling of the wood chips within the columns. All six wood chip columns were filled in the same way and on the same day.

### **3.2.3 Finding the Porosity**

A long piece of polyethylene tubing was attached to the bottom of the wood chip column. A known amount of water was poured into the polyethylene tubing using a funnel until the water was visible at the top of the wood chip column. The water was then drained out of the column and weighed. The amount of water drained out of the column was the void space ( $V_{\text{void}}$ ). The total volume ( $V_{\text{total}}$ ) was calculated using measurements of the bioreactor. Using  $V_{\text{void}}$  and the calculated  $V_{\text{total}}$ , porosity was determined with equation (2.3).

### 3.2.4 Experiment

Three columns were completely filled with water to simulate saturated conditions. The other three columns were filled with water then drained to simulate unsaturated conditions. A constant head device was constructed to provide a constant flow rate of approximately 3 – 5 ml/min for each bioreactor, except where noted in Experiment A. A measured amount of potassium nitrate ( $\text{KNO}_3$ ) was mixed with a known volume of tap water in the carboy to achieve a  $\text{KNO}_3$  concentration of 722 mg/l to attain an influent  $\text{NO}_3\text{-N}$  concentration of 100 mg/l (= ppm). The carboy was then tipped into the constant head device (Figure 3.1) to deliver the  $\text{NO}_3\text{-N}$  solution to the bioreactors. The carboy was refilled every 12-15 hours to ensure constant flow. Approximately one pore volume of  $\text{NO}_3\text{-N}$  solution was added to the bioreactors for the first few days of the experiment (5 days for Experiment A, 6 days for Experiment B). After one pore volume of  $\text{NO}_3\text{-N}$  solution was passed through the system, tap water was added using the constant head device to flush the system. For Experiment A, flushing was carried out by hooking up a garden hose to a faucet in the corner of the room and letting it flow into the constant head device continuously. For Experiment B, flushing was carried out by replacing the carboy every 12-15 hours with tap water to allow for more consistency and decrease the risk involved with running a hose constantly. Once all  $\text{NO}_3\text{-N}$  had been flushed from the system (approximately 8 - 9 days), another full pore volume of 100 ppm  $\text{NO}_3\text{-N}$  solution was added again (5 days for Experiment A, 6 days for Experiment B), and then flushed with tap water again for 9 days. The first addition of  $\text{NO}_3\text{-N}$  solution and flush is considered Round 1, the second addition of  $\text{NO}_3\text{-N}$  solution and flush is considered Round 2.

For Experiment A, water discharged from the saturated bioreactors was weighed daily to determine the amount of water that had passed through the bioreactor. For the unsaturated

bioreactors, this was calculated based on the influent flow rate (taken once daily). Averaging the influent flow rate over a full 24-hour period for the unsaturated bioreactors makes the assumption that the flow is constant for the full 24 hours. The small diameter tubes connecting the constant head device to the top of the bioreactors would sometimes fill with air bubbles and constrict the flow to the bioreactors. Averaging the influent flow rate over a 24-hour period does not account for the loss of flow caused by constrictions in the tubing. For Experiment B, water discharged from all bioreactors, both saturated and unsaturated, was weighed daily to ensure all water that had passed through the system was being accounted for. Due to the differences in flow calculations, Experiment A is considered a preliminary experiment as the calculated flow is not as accurate as the measured flow from Experiment B.

Effluent samples were collected every 24 hours throughout the course of the entire experiment. Effluent samples were filtered using 0.45 micron syringe filters, covered with Parafilm, and placed in a refrigerator until  $\text{NO}_3\text{-N}$  analysis could be completed. For Experiment A,  $\text{NO}_3\text{-N}$  concentration in the effluent samples was analyzed by a laboratory in the Civil Engineering department at the University of Manitoba and measured the  $\text{NO}_3\text{-N}$  concentration using flow injection analysis (FIA). The instrument used for FIA was the QuickChem 8500 (Lachat Instruments, Loveland, CO, USA). For Experiment B,  $\text{NO}_3\text{-N}$  concentration in the effluent samples was analyzed by an accredited laboratory, which carried out the analysis using ion chromatography. Effluent samples were tested for pH and ORP (oxidation reduction potential) daily for Experiment B using a pH tester and an ORP tester (Hanna, Woonsocket, RI, USA). Room temperature was recorded daily for Experiment B and ranged from  $23.1^\circ\text{C}$  to  $23.7^\circ\text{C}$ , with an average temperature of  $23.4^\circ\text{C}$ .

### **3.2.5 Post Experiment Analysis**

All columns were drained and water samples were taken from each column. Once drained, the columns were dismantled from the frame and placed horizontally on the floor of the lab. All of the wood chips were taken out of the columns, put into separate black garbage bags, and weighed. A wood chip sample of 100 grams was taken from each bag and put on an aluminum foil tray. These samples were then placed in the oven to dry for 2 days at 105 °C. The samples were weighed again after drying to find the dry mass. Another wood chip sample of 50 grams was taken from each bag. These 50 gram samples were placed into beakers with 200 ml of distilled water, covered with Parafilm, and placed in the refrigerator for 24 hours. After 24 hours, a sample of water was taken from each beaker, filtered, covered with Parafilm, and placed in a refrigerator until NO<sub>3</sub>-N analysis could be completed.

### **3.2.6 Statistical Analysis**

Analysis of variance (ANOVA) was performed on the data using the GLIMMIX procedure of the Statistical Analysis Software (SAS) version 9.4 (SAS Institute, 2014). Treatment means were compared using Fisher's Protected least significant difference (LSD) method at  $\alpha = 0.05$ . Datasets were checked for normal distribution using the PLOTS = RESIDUALPANEL option of PROC GLIMMIX; all data conformed to a normal distribution and therefore were analyzed as such. Moisture conditions (saturated and unsaturated) and Round (1 and 2) were the fixed factors in the analysis. The preliminary experiment (Experiment A) was not compared to Experiment B due to the difference in methods.

### **3.2.7 NO<sub>3</sub>-N Reduction**

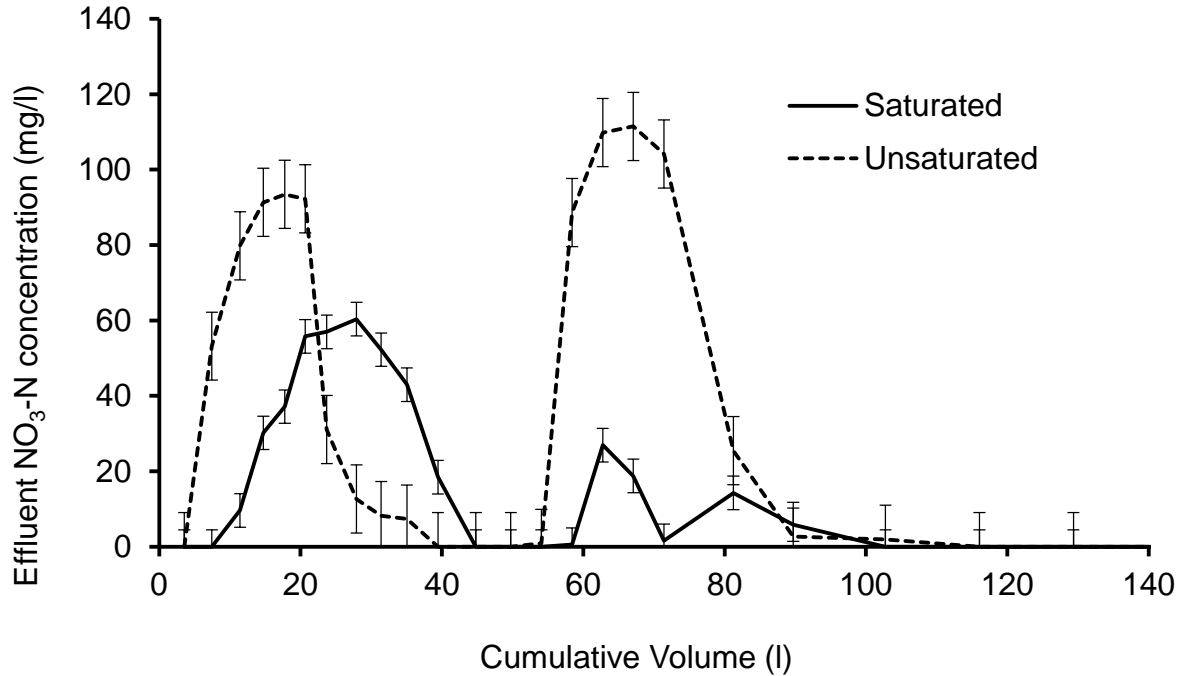
To determine the NO<sub>3</sub>-N reduction of the bioreactors, the total mass of influent NO<sub>3</sub>-N was compared with the total mass of effluent NO<sub>3</sub>-N for both Round 1 and Round 2. To find the total

mass, the amount of water passing through the bioreactors each day was multiplied by the known concentration of  $\text{NO}_3\text{-N}$  in the influent and effluent each day. This provided the total mass of  $\text{NO}_3\text{-N}$  into and out of the system.

### **3.3 Results**

#### **3.3.1 Experiment A – Preliminary Experiment**

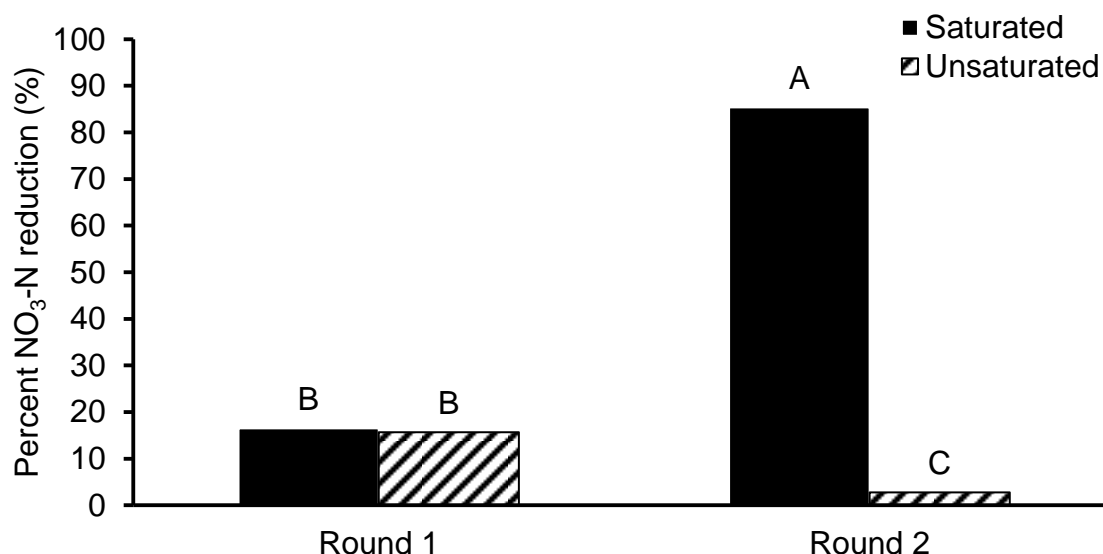
The average porosity of the saturated columns was 63%. The average porosity of the unsaturated columns was 60%. Flow rate ranged from 3 – 5 ml/min when columns were being dosed with  $\text{NO}_3\text{-N}$ , to 9 ml/min during the flush in Round 2. The flow rate was increased to 9 ml/min during this time in order to speed up the flushing. This had an effect on the  $\text{NO}_3\text{-N}$  concentration in the effluent as it decreased the HRT in the bioreactors. A flow rate of 3 ml/min is equivalent to a HRT of approximately 5 days for the bioreactors in Experiment A. A flow rate of 9 ml/min is equivalent to a HRT of approximately 1.7 days for the bioreactors in Experiment A. Figure 3.2 displays the average effluent  $\text{NO}_3\text{-N}$  concentration for the saturated and unsaturated bioreactors against the cumulative volume passing through the bioreactors. The third peak in the  $\text{NO}_3\text{-N}$  concentration for the saturated bioreactors is when the flow rate was increased.



**Figure 3.2. Experiment A effluent NO<sub>3</sub>-N concentration.**

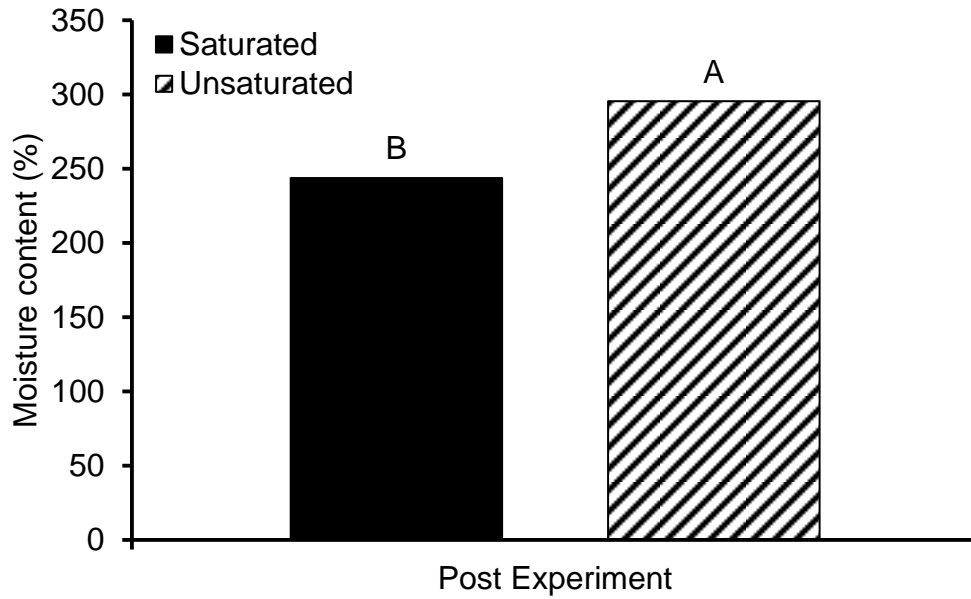
Figure 3.3 shows the average percent reduction of NO<sub>3</sub>-N in Round 1 of Experiment A was 16.5% for the saturated bioreactors and 15.7% for the unsaturated bioreactors. Figure 3.3 also shows the average percent reduction of NO<sub>3</sub>-N in Round 2 of Experiment A was 85.4% for the saturated bioreactors and 2.8% for the unsaturated bioreactors. There was not a significant difference between saturated and unsaturated conditions for Round 1 ( $p = 0.82$ ), however, there was a significant difference ( $p < 0.0001$ ) between saturated and unsaturated conditions for Round 2. There was a significant difference ( $p < 0.0001$ ) between saturated conditions in Round 1 and saturated conditions in Round 2. There was also a significant difference ( $p = 0.005$ ) between unsaturated conditions in Round 1 and unsaturated conditions in Round 2.





**Figure 3.3. Experiment A percent NO<sub>3</sub>-N reduction. Note: Treatments with the same letter indicate the difference between the means is not statistically significant at  $p = 0.05$ .**

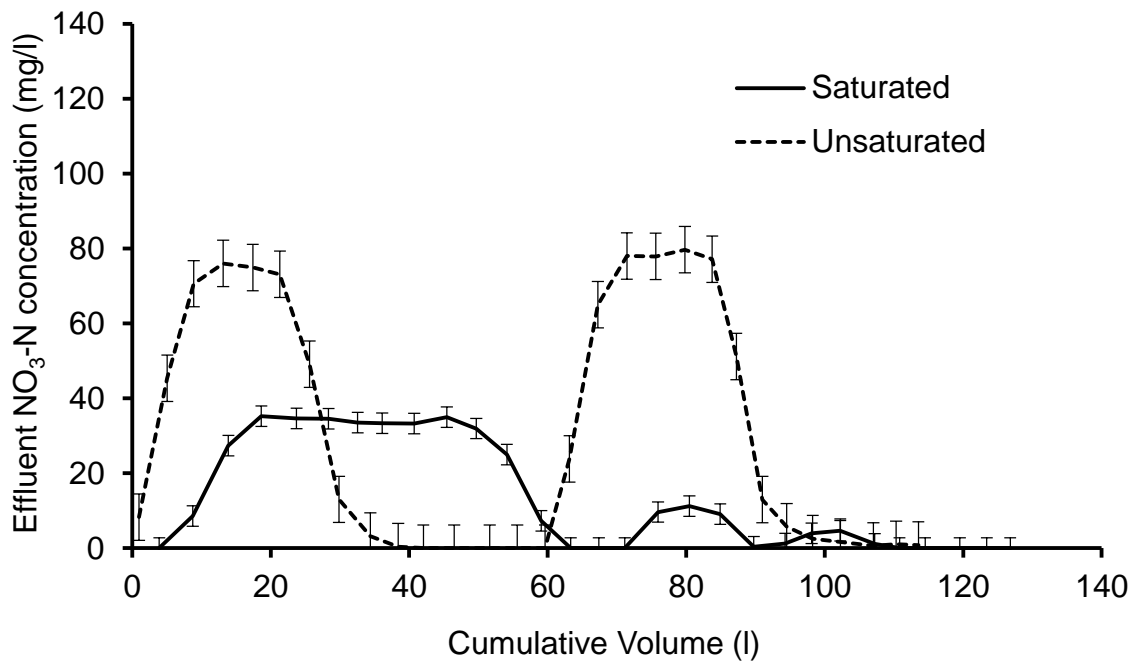
Post experiment analysis consisted of determining the moisture content of the chips, and how much residual NO<sub>3</sub>-N was in the wood chips. Figure 3.4 shows the average moisture content in the woodchips was 244% taken from the saturated bioreactors, and 295% taken from the unsaturated bioreactors. There is a significant difference ( $p < 0.0001$ ) between the moisture content in the saturated columns and the unsaturated columns. There was some residual NO<sub>3</sub>-N found in the wood chips from two of the unsaturated bioreactors at a concentration ranging from 1.7 to 1.9 mg/l, which accounts for approximately 9.7% to 13.9% of the total NO<sub>3</sub>-N removed..



**Figure 3.4. Experiment A moisture content. Note: Treatments with the same letter indicate the difference between the means is not statistically significant at  $p = 0.05$ .**

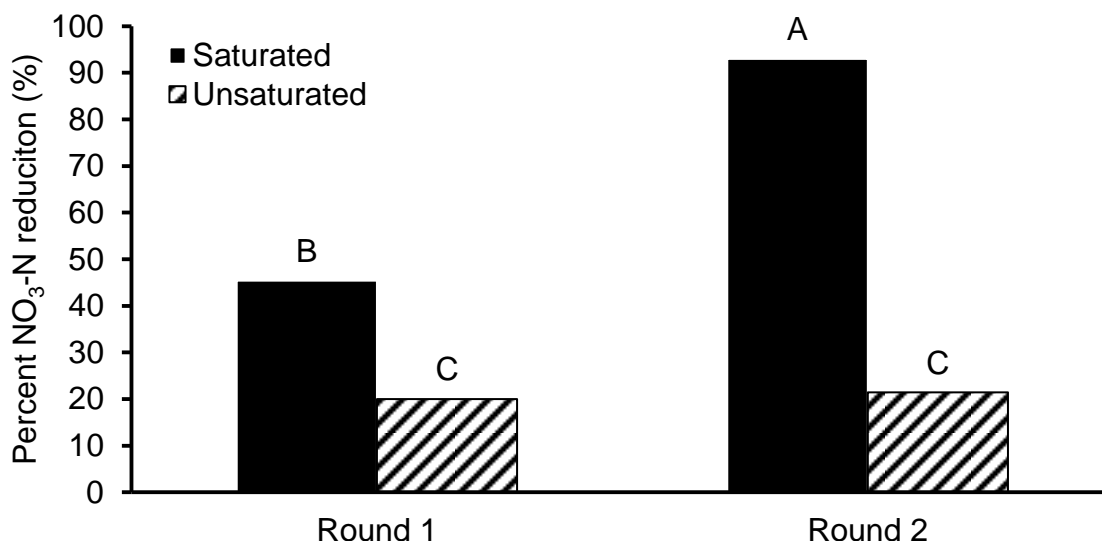
### 3.3.2 Experiment B

For Experiment B, the average porosity of the saturated columns was 67%. The average porosity of the unsaturated columns was 68%. The flow rate ranged from 3 ml/min to 5 ml/min throughout the entire experiment (equivalent to a HRT of 5.5 and 3.3 days for the bioreactors in Experiment B, respectively). Figure 3.5 displays the average effluent  $\text{NO}_3\text{-N}$  concentration for the saturated and unsaturated bioreactors against the cumulative volume passing through the bioreactors.



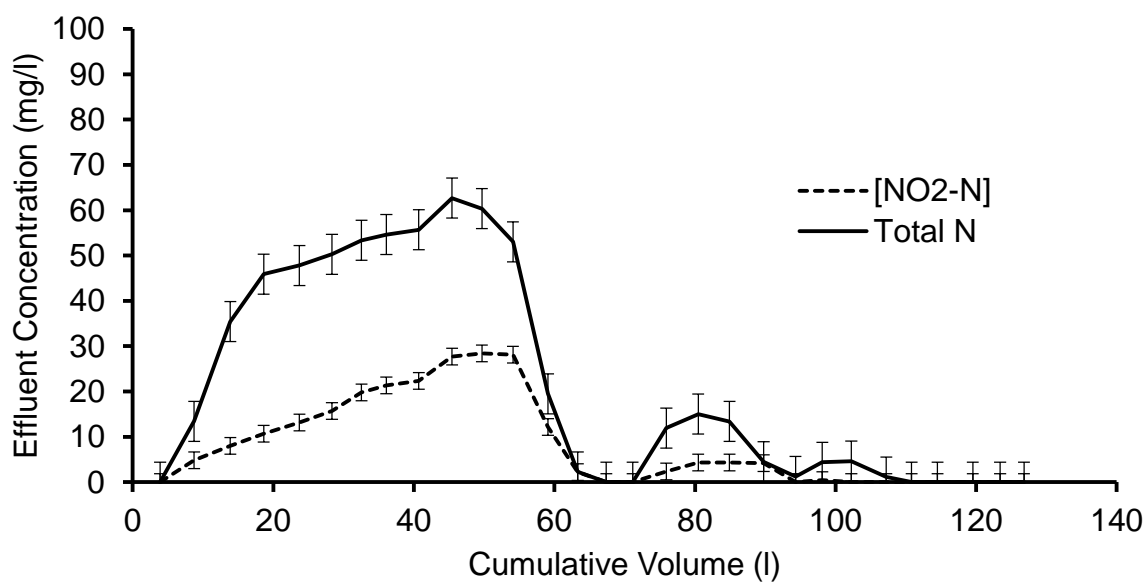
**Figure 3.5. Experiment B effluent NO<sub>3</sub>-N concentration.**

The average percent reduction of NO<sub>3</sub>-N in Round 1 of Experiment B was 45.4% for the saturated bioreactors and 20.5% for the unsaturated bioreactors. The average percent reduction of NO<sub>3</sub>-N in Round 2 of Experiment B was 92.8% for the saturated bioreactors and 21.4% for the unsaturated bioreactors. There was a significant difference between saturated and unsaturated conditions for Round 1 ( $p = 0.0001$ ), as well as for Round 2 ( $p < 0.0001$ ). There was a significant difference ( $p < 0.0001$ ) between saturated conditions in Round 1 and saturated conditions in Round 2. There was no significant difference between unsaturated conditions in Round 1 and unsaturated conditions in Round 2 ( $p = 0.80$ ).



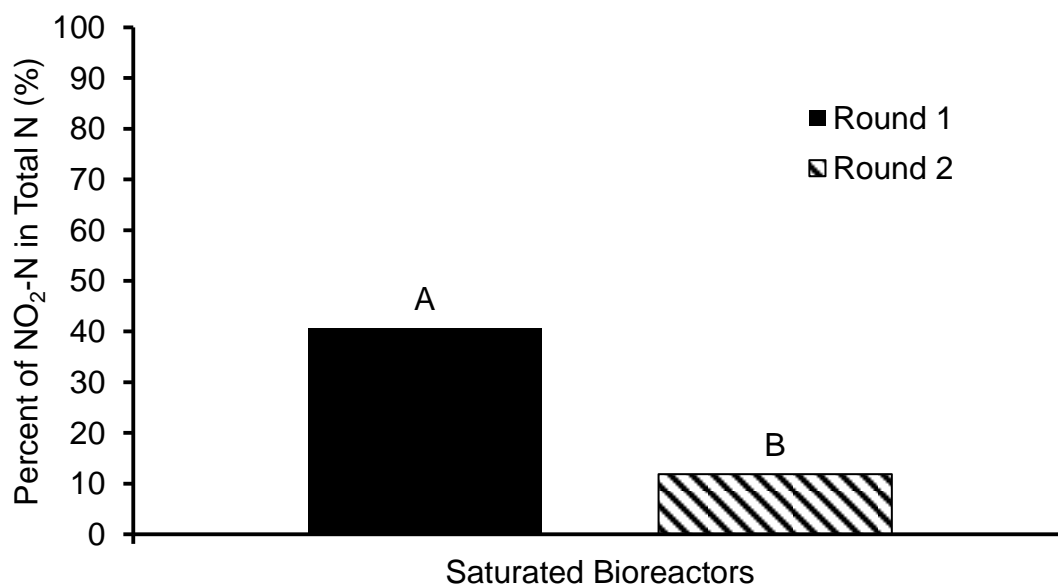
**Figure 3.6. Experiment B percent NO<sub>3</sub>-N reduction. Note: Treatments with the same letter indicate the difference between the means is not statistically significant at  $p = 0.05$ .**

Nitrite, NO<sub>2</sub>-N, was analyzed only in Experiment B. NO<sub>2</sub>-N was only produced in the saturated bioreactors. For Round 1, the average NO<sub>2</sub>-N concentration was 40.67% of the total N in the effluent. For Round 2, the average NO<sub>2</sub>-N concentration was 11.89% of the total N in the effluent. Figure 3.7 displays the total N (NO<sub>2</sub>-N + NO<sub>3</sub>-N) concentration and the NO<sub>2</sub>-N concentration against the cumulative volume. One data point in a replicate from Trial 2 was excluded from statistical analysis because it was statistically an outlier.



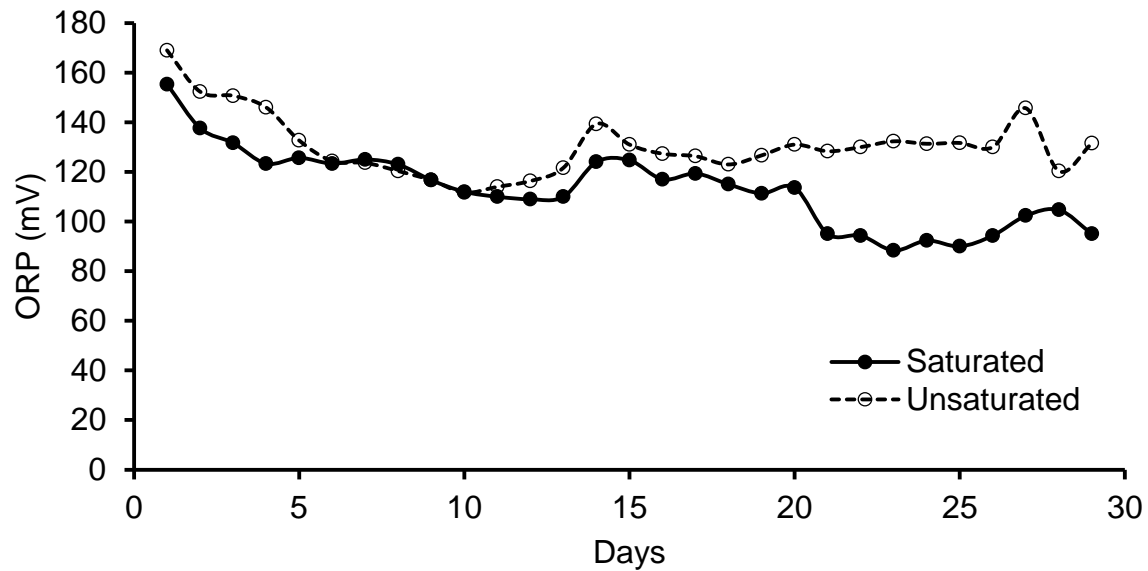
**Figure 3.7. Effluent  $\text{NO}_2\text{-N}$  and total N concentration.**

It was found that the  $\text{NO}_2\text{-N}$  concentration was significantly lower in Round 2 than in Round 1 ( $p = 0.03$ ).

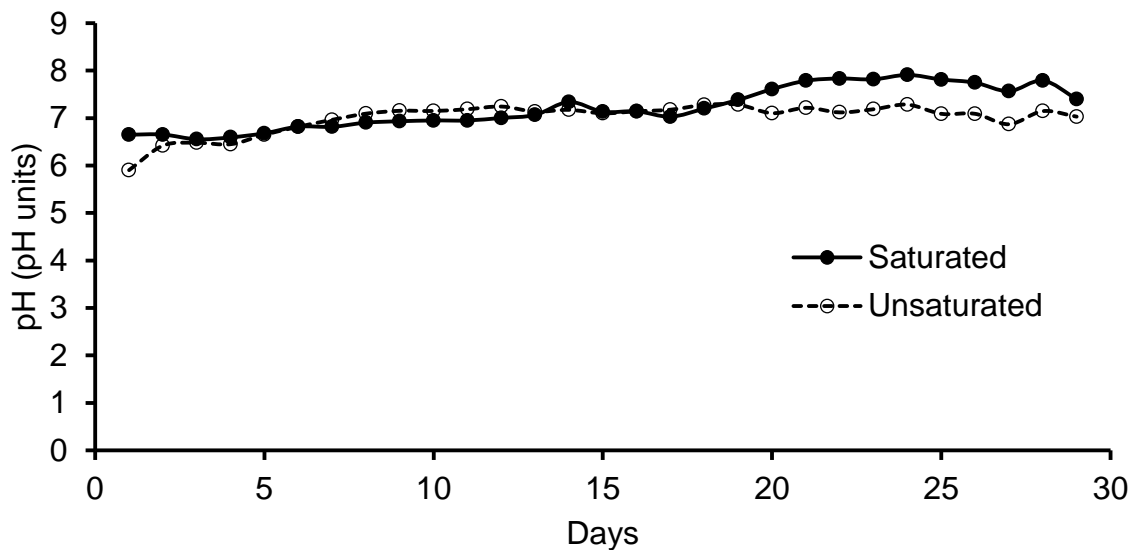


**Figure 3.8. Percent of  $\text{NO}_2\text{-N}$  in total N. Note: Treatments with the same letter indicate the difference between the means is not statistically significant at  $p = 0.05$ .**

The ORP and pH were also only analyzed in Experiment B. The average daily ORP reading for the saturated and unsaturated bioreactors are presented in Figure 3.9. The average daily pH reading for the saturated and unsaturated bioreactors are presented in Figure 3.10.

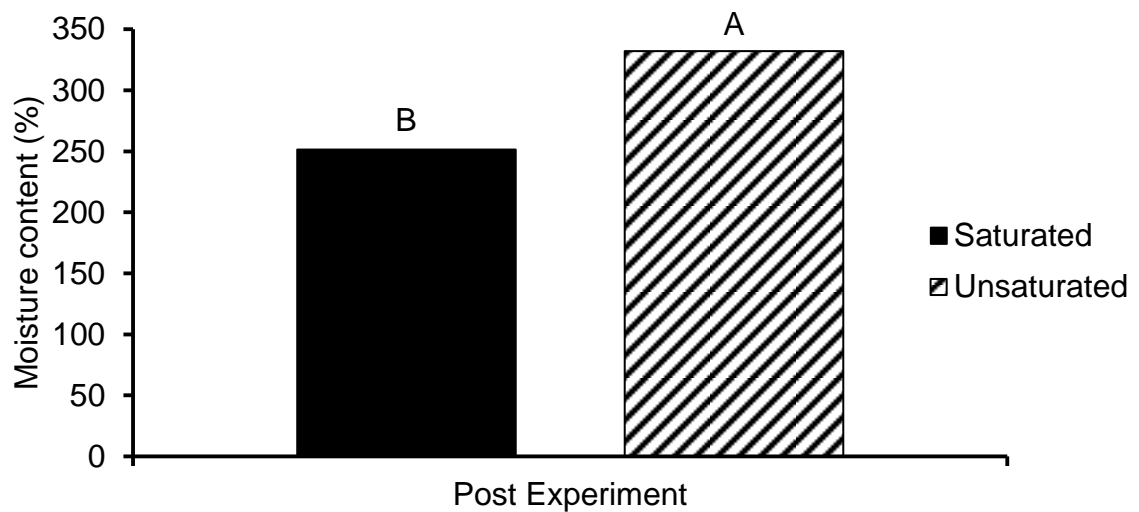


**Figure 3.9. Daily ORP readings.**



**Figure 3.10. Daily pH readings.**

Post experiment analysis consisted of determining the moisture content of the chips, and how much residual  $\text{NO}_3\text{-N}$  was in the wood chips. Figure 3.11 shows the average moisture content in the woodchips was 251% taken from the saturated bioreactors, and 332% taken from the unsaturated bioreactors. There was some residual  $\text{NO}_3\text{-N}$  found in the wood chips from the unsaturated bioreactors at a concentration ranging from 1.25 to 2.01 mg/l, which accounts for approximately 2.4% to 6.6% of the total  $\text{NO}_3\text{-N}$  removed.



**Figure 3.11. Experiment B moisture content. Note: Treatments with the same letter indicate the difference between the means is not statistically significant at  $p = 0.05$ .**

### 3.4 Discussion

In both Experiment A and Experiment B,  $\text{NO}_3\text{-N}$  reduction under saturated conditions (85.4 and 92.8%) was significantly higher ( $p < 0.0001$ ) than  $\text{NO}_3\text{-N}$  reduction under unsaturated conditions (2.8 and 21.4%). These results are for Round 2 only, as it is assumed that the communities of denitrifying bacteria were not fully established in Round 1.

### **3.4.1 Preferential Flow**

It is possible that under unsaturated conditions, the  $\text{NO}_3\text{-N}$  solution was able to find a preferential path to flow through the bioreactor. This would explain why  $\text{NO}_3\text{-N}$  was detectable early on in the unsaturated effluent. As seen in Figure 3.2 and Figure 3.5, effluent  $\text{NO}_3\text{-N}$  concentration in the unsaturated columns appeared shortly after the experiment had started, and at a higher concentration as compared to the saturated columns. If the  $\text{NO}_3\text{-N}$  solution followed a preferential path in the unsaturated bioreactors, it would have passed through the bioreactors at a much faster rate than in the saturated bioreactors. A faster flow through the bioreactor results in a decreased HRT. With a lower HRT, there is less opportunity for denitrification to occur in the unsaturated bioreactors.

### **3.4.2 Hydraulic retention time (HRT)**

It is likely that there was a difference in HRT between the unsaturated and saturated bioreactors due to preferential flow. The longer HRT in the saturated bioreactors allowed for a greater length of time for the denitrification process. Having a longer HRT in the saturated bioreactors resulted in a greater overall reduction of nitrate in the saturated bioreactors than in the unsaturated bioreactors.

### **3.4.3 Anaerobic versus Aerobic Conditions**

Another factor that could have played a role in the saturated bioreactors having greater  $\text{NO}_3\text{-N}$  reduction compared to the unsaturated bioreactors is the anaerobic conditions present in the saturated bioreactors. Denitrifying bacteria are mostly facultative anaerobic heterotrophs and utilize nitrate as an electron acceptor when there is a limited supply of oxygen. Under aerobic conditions, oxygen is present and bacteria may continue to respire oxygen instead of nitrate which would result in less reduction of nitrate. Although ORP values indicate low oxygen



conditions in both saturated and unsaturated bioreactors, there is less oxygen available in the saturated bioreactors than in the unsaturated bioreactors.

#### **3.4.4 ORP and pH**

The ORP (oxidation reduction potential) and pH were measured only in Experiment B. It was found that the daily ORP reading was lower in the saturated bioreactors than in the unsaturated bioreactors. As ORP decreases, denitrification can occur as it is less likely for oxygen to be used as an electron acceptor (ITRC 2002).

The pH remained relatively constant throughout Experiment B with the pH of the saturated bioreactors being slightly higher than the unsaturated bioreactors. The pH values observed in this experiment fall within the range preferred by heterotrophic denitrifiers, with the preferred range being generally between 5.5 to 8.0 (Rivett et al. 2008).

#### **3.4.5 NO<sub>2</sub>-N Production**

Production of NO<sub>2</sub>-N (nitrite) was analyzed in Experiment B and occurred only in the saturated bioreactors. The NO<sub>2</sub>-N concentration was significantly higher ( $p = 0.03$ ) in Round 1 than in Round 2. Previous studies have also found NO<sub>2</sub>-N early on during the start-up of a wood chip bioreactor. A study completed at the University of California found that in the initial months of operation, bioreactor treatment increased nitrite (NO<sub>2</sub>-N) concentration by several mg/l, however, after a few months, the NO<sub>2</sub>-N in bioreactor effluent gradually declined and remained below 0.3 mg/l thereafter (Hartz et al. 2017). It is possible that there was no NO<sub>2</sub>-N found in the unsaturated bioreactors because very little denitrification was occurring in those columns.

### **3.4.6 Moisture Content**

For both Experiment A and Experiment B, the average moisture content in the woodchips taken from the saturated bioreactors was significantly less than the moisture content in the wood chips taken from the unsaturated bioreactors. It is possible that the chips in the unsaturated bioreactors had room to expand, thus increasing the pore sizes in the chips. This would allow the chips to have more space to absorb water and increase the moisture content of the chips.

## **3.5 Conclusion**

The  $\text{NO}_3\text{-N}$  reduction in wood chip bioreactors under saturated and unsaturated conditions was investigated using a laboratory column study. It was found that after the bacteria communities had established in the bioreactors,  $\text{NO}_3\text{-N}$  reduction under saturated conditions (85.4 and 92.8%) was significantly higher ( $p < 0.0001$ ) than  $\text{NO}_3\text{-N}$  reduction under unsaturated conditions (2.8 and 21.4%). The difference between saturated and unsaturated conditions may be due to preferential flow, hydraulic retention time (HRT), and anaerobic versus aerobic conditions. Based on these findings, wood chip bioreactors under saturated conditions may be successful at removing  $\text{NO}_3\text{-N}$  from subsurface drainage water. This would decrease the adverse environmental impacts of discharging excess  $\text{NO}_3\text{-N}$  to the environment.

## **4. Sizing a wood chip bioreactor for field implementation**

### **Abstract**

Wood chip bioreactors can be installed at the edge of a field that has subsurface drainage to reduce the nitrate concentration in water discharging from the drainage outlet. Wood chip bioreactors enhance the natural denitrification process by providing a carbon source to denitrifying bacteria which convert nitrate to nitrogen gas. Using the results from Chapter 3, in-field wood chip bioreactor sizing has been determined for four different precipitation amounts. The four different sizes were based on precipitation values for an area in southern Manitoba. These precipitation values were 0.2, 5, 10, and 25 mm. The volume of the bioreactor was determined using the precipitation values, HRT (hydraulic retention time), and porosity. The bioreactors were designed to ensure saturated conditions remain throughout the entire bioreactor. Assuming the area serviced by the bioreactor is a quarter section of land (160 acres), the volumes of the various bioreactor sizes were found to be 598 m<sup>3</sup> for 0.2 mm of precipitation, 14,942 m<sup>3</sup> for 5 mm of precipitation, 29,884 m<sup>3</sup> for 10 mm of precipitation, and 74,711 m<sup>3</sup> for 25 mm of precipitation.

### **4.1 Introduction**

The use of tile drainage has increased in recent years in order to improve drainage in poorly drained soils and to boost crop yield. Although tile drainage can have a positive effect on crop production, it has the potential to deliver a large amount of nutrients, such as nitrate, to receiving waters. One approach for reducing the amount of nitrate delivered to surface waters from agricultural drainage waters is edge-of-field bioreactors or denitrification walls (Greenan et al. 2009). Edge-of-field bioreactors are installed at the edge of a tile drained field and enhance the

natural denitrification process by providing a carbon source to denitrifying bacteria. The denitrifying bacteria in the bioreactor convert nitrate to nitrogen gas which is then released to the atmosphere. Edge-of-field bioreactors decrease the concentration of nitrate in the drainage water before reaching sensitive receptors such as streams with aquatic life or rural residential drinking water wells.

In Chapter 3, wood chips were used as a carbon source in laboratory-scale denitrifying bioreactors. Wood chips are a common carbon source for denitrifying bioreactors due to their low cost, conductivity, and longevity (Robertson 2010; Schipper et al. 2010). There is limited rationale for the sizing of in-field wood chip bioreactors, however, it is assumed that sizing is generally based on the amount of land available for the bioreactor (David et al. 2016; Hartz et al. 2017). Chapter 4 explores an approach for designing denitrifying bioreactors based on precipitation data.

A study completed by David et al. (2016) assessed the performance of a wood chip bioreactor over the first three years of operation. The bioreactor was designed to treat a 20-ha field and was 6 by 15 by 1.3 m deep, for a total volume of 117 m<sup>3</sup>. The average monthly nitrate removal rate was 23 to 44 g NO<sub>3</sub>-N/m<sup>3</sup>/day in Year 1 and 1.2 to 11 g NO<sub>3</sub>-N/m<sup>3</sup>/day in Years 2 and 3. Highly degradable carbon in the wood chips likely caused the greater NO<sub>3</sub>-N removal rates in Year 1. Overall efficiency was low in Years 2 and 3 due to high concentrations of NO<sub>3</sub>-N in the tile drain effluent (bioreactor influent). It was determined that the bioreactor would have needed to be 9 times as large as what was originally constructed to remove 50% of the nitrate load.

A study completed by the University of California (Hartz et al. 2017) found that, across several years of operation, denitrification in two pilot-scale bioreactors (20.8 and 9.8 m<sup>3</sup>)

constructed on subsurface-drained farms reduced NO<sub>3</sub>-N concentration by an average of 8 to 10 mg/l per day of HRT in the summer and approximately 5 mg/l per day of HRT in the winter. A constant flow rate was supplied to the bioreactors to achieve an HRT of approximately 2 days. Tile drain effluent (bioreactor influent) averaged high NO<sub>3</sub>-N concentrations, ranging between 60 and 180 mg/l at the sites. Due to the high NO<sub>3</sub>-N concentration in the tile drain effluent; water discharged from the bioreactors had a concentration of NO<sub>3</sub>-N that was still above the regulatory limit. If the bioreactors were designed to hold a larger volume of drainage water and for a longer HRT, the decrease in NO<sub>3</sub>-N concentration likely would have been greater than what was observed.

## 4.2 Materials and Methods

The study site used for designing the conceptual bioreactor is based on drainage water exiting a quarter section of land in southern Manitoba (160 acres or 647,497 m<sup>2</sup>). Table 4.1 shows the thirty years of precipitation data from The Weather Network that was analyzed for the Winkler – Emerson – Morris area (The Weather Network 2017).

**Table 4.1. Thirty year average.**

No. of days with precipitation	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Above 0.2 mm	8	9	7	7	11	16	10	14	9	7	8	8
Above 5 mm	0	2	2	2	5	6	3	3	1	2	3	1
Above 10 mm	0	1	1	1	3	4	2	3	0	1	2	0
Above 25 mm	0	1	0	0	1	0	0	2	0	0	1	0

Using this precipitation data, the number of days with precipitation exceeding a certain amount (0.2, 5, 10, and 25 mm) during the growing season from May to September (153 days in

total) was found. On average, there were 60, 18, 12, and 3 days in the growing season when the precipitation was greater than 0.2, 5, 10, and 25 mm, respectively.

A porosity of 65% and a HRT of 3 days were used for the field design based on results from the laboratory column experiment in Chapter 3. For the laboratory column experiment, the flow rate varied from 3 ml/min (5 day HRT) to 5 ml/min (3 day HRT). In order to minimize the field space required for the bioreactor, the 3 day HRT was chosen for the field design. Field design calculations are based solely on precipitation and do not take into account the amount of water held in the soil profile. It is assumed that all bioreactors will be built to a depth of 2 m. The daily precipitation (mm/day) was multiplied by the size of the field (647,497 m<sup>2</sup>) to find the total quantity of water to be treated. This total volume (m<sup>3</sup>/day) was multiplied by the required HRT (3 days) then divided by the wood chip porosity in order to find the total volume required for the bioreactor. An example calculation for a precipitation of 5 mm/day is shown below.

$$3 \text{ days} = \frac{0.65 * V_{total} m^3}{\left(\frac{5 mm}{day}\right) * \left(\frac{1 m}{1000 mm}\right) * (647,497 m^2)} \quad (4.1)$$

$$3 \text{ days} = \frac{0.65 * V_{total} m^3}{3237 m^3/day} \quad (4.2)$$

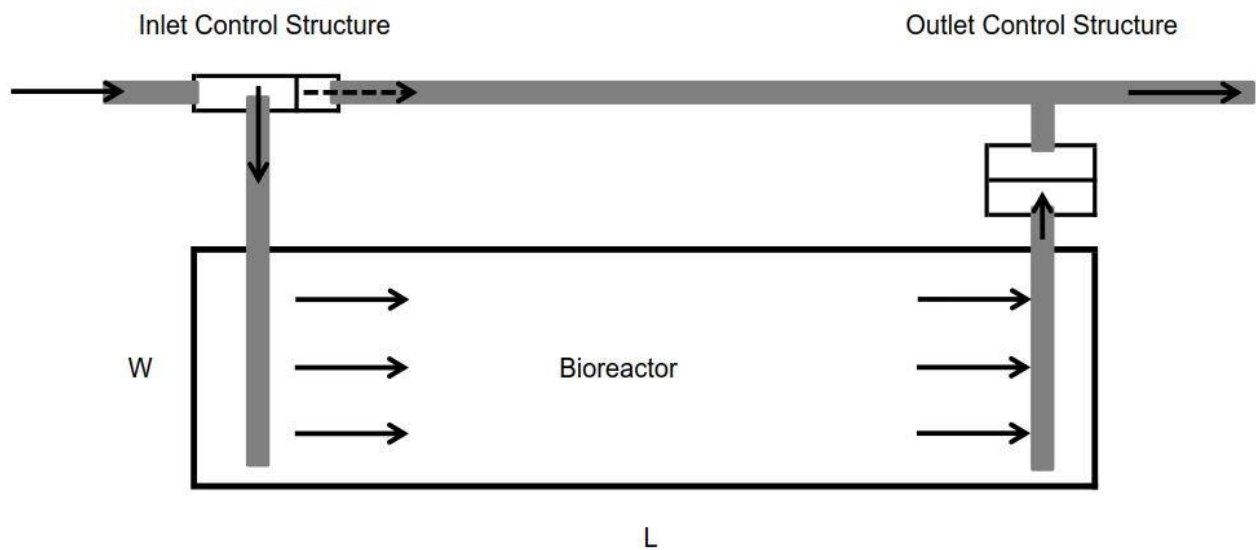
$$V_{total} = \frac{(3 \text{ days}) * (3237 \frac{m^3}{day})}{0.65} \quad (4.3)$$

$$V_{total} = 14,942 m^3 \quad (4.4)$$

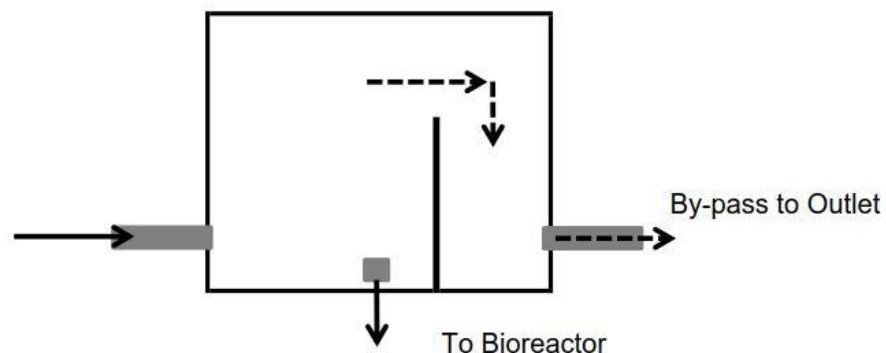
### 4.3 Results and Discussion

An example of the overall bioreactor design is presented in Figure 4.1. Water from the drainage outlet first flows into the inlet control structure (Figure 4.2). If flow is below a certain limit, all water will be directed into the bioreactor. If the flow is over a certain limit, some water

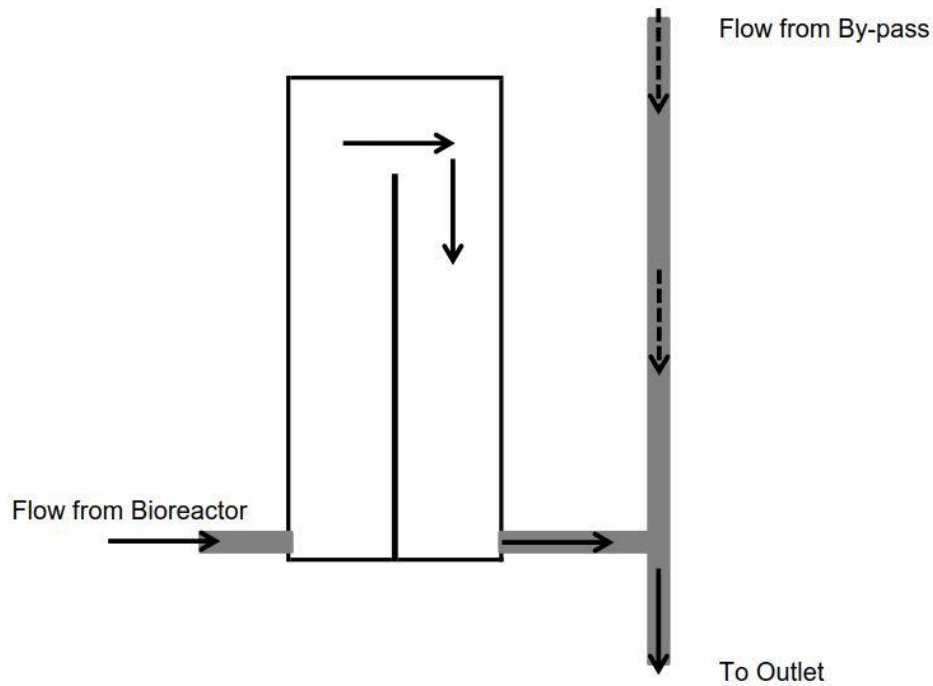
will by-pass the bioreactor and be directed straight to the ditch. After water has filtered through the bioreactor, it will be directed to an outlet control structure (Figure 4.3), which contains a 2 m high barrier wall that the water must overcome in order to reach the discharge pipe. The height of the wall is designed to the same depth of the bioreactor to ensure the bioreactor remains saturated at all times.



**Figure 4.1. Plan view bioreactor design.**



**Figure 4.2. Elevation view inlet control structure.**



**Figure 4.3. Elevation view outlet control structure.**

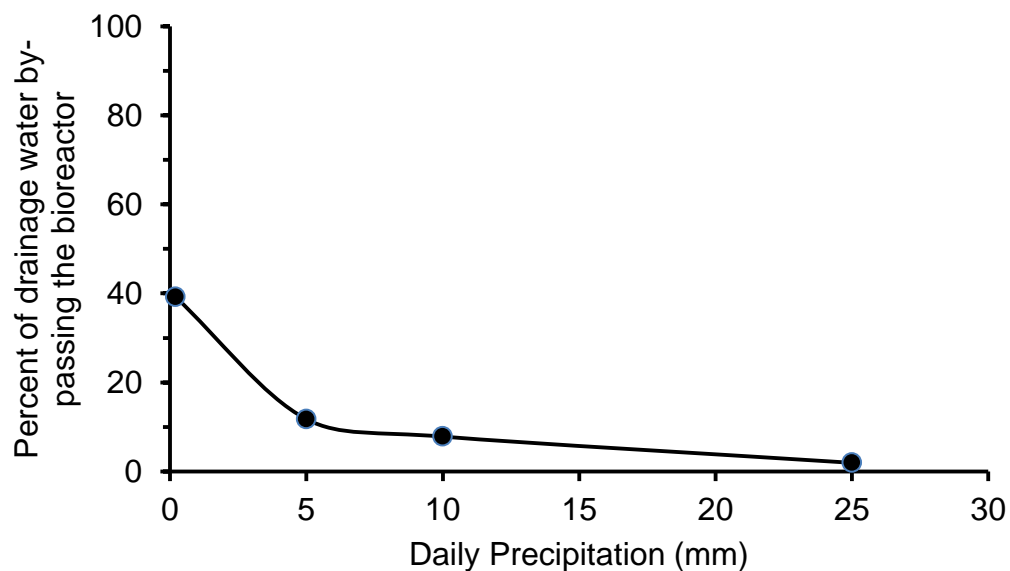
The total volume required for the bioreactor will depend on the chosen design precipitation value. The ranges in size based on the precipitation values for the Winkler – Emerson – Morris area are outlined in Table 4.2. The amount of area required will increase based on the design precipitation value. For a precipitation value of 0.2 mm/day, the area required for the bioreactor is only 0.05% of the total land area that the bioreactor is treating. This percentage increases to 1.15, 2.31, and 5.77 % for a precipitation value of 5, 10, and 25 mm, respectively.

**Table 4.2. Bioreactor size for different precipitation values.**

Precipitation (mm/day)	Volume (m <sup>3</sup> )	Area (m <sup>2</sup> )	Percent of Land Area
0.2	598	299	0.05%
5	14,942	7,471	1.15%
10	29,884	14,942	2.31%
25	74,711	37,356	5.77%

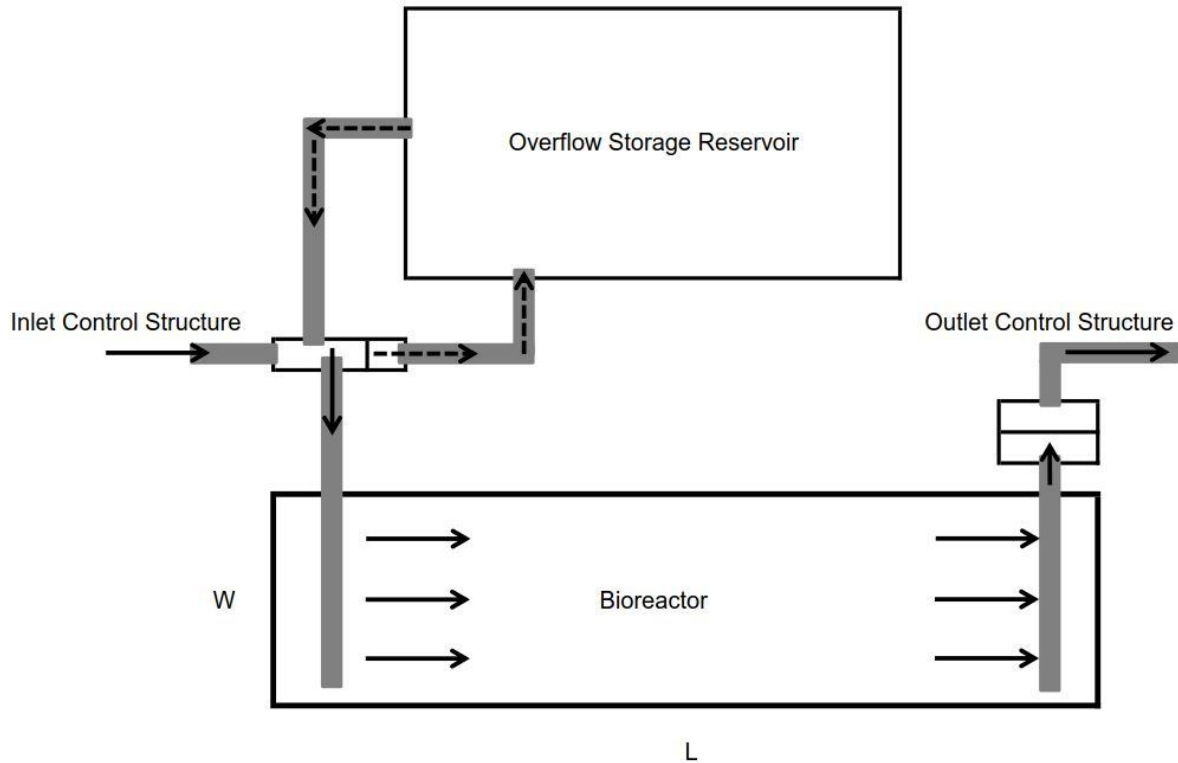


Figure 4.4 summarizes the percentage of flow that would by-pass the bioreactor based on the precipitation it is designed for. If the bioreactor is designed for a 0.2 mm rainfall, 39% of rainfall that growing season would by-pass the bioreactor. If the bioreactor is designed for a 5 mm rainfall, 12% of the rainfall that growing season would bypass the bioreactor. If the bioreactor is designed for a 10 mm or a 25 mm rainfall, the amount that would by-pass the bioreactor would be 8% and 2%, respectively.



**Figure 4.4. Percent of drainage water by-passing the bioreactor.**

If space permits, an overflow storage reservoir could be installed to collect the drainage water from the by-pass line (Figure 4.5). This collected overflow drainage water could be pumped back through the bioreactor once large flow events have passed. The size of the overflow storage reservoir will depend on the precipitation for the area as well as the maximum allowable space.



**Figure 4.5. Bioreactor with overflow storage reservoir.**

Along with ensuring the bioreactor remains saturated, another way to maximize denitrification would be to install baffles or panels in the bioreactor to force the water around the wood chips. It is possible for water to skim the surface of the bioreactor, or find a preferential pathway. Introducing baffles would decrease the chance of this happening and would ensure the drainage water remains in the bioreactor for the full designed HRT.

#### **4.4 Conclusion**

In-field wood chip bioreactor sizing was determined for four different precipitation values in southern Manitoba (0.2, 5, 10, and 25 mm). The volume of the bioreactor was determined using the precipitation values, a HRT (hydraulic retention time) of 3 days, and an average porosity of 65%. The bioreactors were designed to ensure saturated conditions remain throughout the entire

bioreactor. Assuming the area serviced by the bioreactor is a quarter section of land, the volumes of the various bioreactor sizes were found to be 598 m<sup>3</sup> for 0.2 mm of precipitation, 14,942 m<sup>3</sup> for 5 mm of precipitation, 29,884 m<sup>3</sup> for 10 mm of precipitation, and 74,711 m<sup>3</sup> for 25 mm of precipitation. Assuming a bioreactor depth of 2 m, for a precipitation value of 0.2 mm/day, the area required for the bioreactor is only 0.05% of the total land area that the bioreactor is treating. This percentage increases to 1.15, 2.31, and 5.77 % for a precipitation value of 5, 10, and 25 mm, respectively.

## 5. Overall Conclusion

A laboratory column study was completed to investigate the  $\text{NO}_3\text{-N}$  reduction in wood chip bioreactors under saturated and unsaturated conditions. It was found that after the denitrifying bacteria communities had established in the bioreactors,  $\text{NO}_3\text{-N}$  reduction under saturated conditions (85.4 and 92.8%) was significantly higher ( $p < 0.0001$ ) than  $\text{NO}_3\text{-N}$  reduction under unsaturated conditions (2.8 and 21.4%). Based on these findings, wood chip bioreactors under saturated conditions could be used to remove  $\text{NO}_3\text{-N}$  from subsurface drainage water. This would decrease the adverse environmental impacts of discharging excess  $\text{NO}_3\text{-N}$  to the environment.

Using results from the laboratory experiment, preliminary calculations were carried out for sizing an in-field bioreactor. Four different precipitation values in southern Manitoba (0.2, 5, 10, and 25 mm) were used to determine different bioreactor sizes. The required volume was determined using the precipitation values, a HRT (hydraulic retention time) of 3 days, and an average porosity of 65%. The bioreactors were designed to ensure saturated conditions remain throughout the entire bioreactor. Assuming the area serviced by the bioreactor is a quarter section of land, the volumes of the various bioreactor sizes were found to be  $598 \text{ m}^3$  for 0.2 mm of precipitation,  $14,942 \text{ m}^3$  for 5 mm of precipitation,  $29,884 \text{ m}^3$  for 10 mm of precipitation, and  $74,711 \text{ m}^3$  for 25 mm of precipitation.

## **6. Recommendations for future research**

1. A laboratory column experiment could be run with different flow rates to see if different flow rates have an impact on saturated and unsaturated conditions.
2. Different influent nitrate concentrations could be run in a laboratory column experiment to see if there is a relationship between nitrate reduction and influent nitrate concentration.
3. The laboratory column study could be run with different types of wood chips to see if there is a relationship between nitrate reduction and the type of wood chip.
4. Using the design presented in Chapter 4, a wood chip bioreactor could be implemented in a field to test the efficiency of the field design.

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