Exploring N and P Reduction in Bioreactors

A THESIS SUBMITTED TO THE FACULTY
OF THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
NATURAL RESOURCE SCIENCE & MANAGEMENT- FOREST HYDROLOGY

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2/2015

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Acknowledgements

I would like to express my deepest appreciation to my committee chair Dr. Joe Magner for helpful comments, remarks, and encouragement through the process of this master thesis. Furthermore I would like to thank Dr. Bruce Wilson, Dr. Rodney Venterea, Dr. Gary Feyereisen, and Dr. John Nieber for their insight and help with this project. Also, I would like to thank Dr. Venterea, Dr. Feyereisen, and Dr. Kurt Spokas' labs for their generous help in data analysis and all the master and undergraduate students who provided help with the experiment. Lastly, I would like to thank the Minnesota Corn Growers Association for generously providing the funding to support this research.

Abstract

The main goal of this study was to provide information on the nitrate and phosphorus removal efficiencies of woodchip, biochar, and corn cobs. Woodchip bioreactors have proven effective in removing nitrate-nitrogen (NO_3-N) from agriculturally drained water in the Midwest USA region. Both nitrate and phosphorus can lead to hypoxia and algal blooms in the receiving surface waters and degrade the water quality.

This study explored the effectiveness of bioreactor technology in removing NO_3-N and orthophosphate, and the emission of N_2O from different media. Three types of media were examined in a pilot-scale lab experiment: deciduous mixed hardwood chips, biochar chips (created from the same type of woodchip), and corn cobs. Chemically formulated water was fed through each system using a residence time of 24 hours, and later on, 8 hours.

NO_3-N reduction occurred in all three media, although biochar showed a relatively longer lag time. An average of 9.26g N/d/m^3 NO_3-N total loading reduction was observed from the 24 hours retention time when the total input was 588.82g. An average of 0.35g orthophosphate/d/m^3 of the orthophosphate loading was reduced while the input phosphorus was 6.10g, with the biochar media providing the most reduction in outflow orthophosphate concentration. A lower reduction rate and higher nitrate output was observed from the 8 hours retention time. However, corn cobs showed the highest total nitrate removal rate of 22.40g N/d/m^3 while the total input was 1156.70g. The orthophosphate reduction rates were not significantly different among the three media

(ranging from 0.67g orthophosphate/d/m^3 to 0.86g orthophosphate/d/m^3) with a total input of 11.10g. Given the recent development of state-wide nutrient management plans to reduce nutrient concentrations in surface water, study results of these technologies will help the row-crop producer community manage nutrient export to surface water.

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Introduction

(1) Nitrogen

Nitrate export from agricultural fields has drawn a lot of environmental attention due to its significant health and environmental impact (Nitrates and Nitrites, EPA, 2007). Fertilizer application enhances overall crop production, but often adds more nutrients to the soil than what the crops actually need to successfully grow. Significant amount of nutrient is getting lost every year though the extensive drainage system in the Midwest cornbelt region. The tile drain system across the cornbelt region can increase the output nitrogen load by mineralization and left over nitrogen from previous years (Dinnes et al., 2002). Excess nitrate that ends up in groundwater, streams, lakes, and other surface water bodies substantially threaten the ecosystem health (Schipper et al., 2010). Specifically for the Midwest region, the receiving water is the Mississippi River which delivers excess nutrients to the Gulf of Mexico resulting in hypoxic conditions more frequently (Bianchi et al., 2010). Excess nitrate in drinking water causes health problems; a number of municipalities draw domestic water supply from the Mississippi River and selected tributaries (DNR).

In order to "enhance the health of aquatic life, improve public health and safety, and increase the recreational potential of" Minnesota's water resources, the Minnesota Pollution Control Agency (MPCA) proposed a nutrient management plan to guide the state-level nutrient reduction programs (The Minnesota Nutrient Reduction Strategy). Common treatment strategies being studied including cover crops, denitrifying

bioreactors, treatment wetlands, drainage water management, and saturated buffer zones have been studied and implemented to reduce nitrate load leaving row-crop land. Among which, the bioreactor is simple, efficient, and cost effective. Other strategies such as wetlands, focuses more on surface/overland flow, and they are less effective mitigating subsurface drainage. The location of the bioreactor makes it more suited for processing subsurface flow. The basic idea is an underground trench filled with woodchips. The woodchips, or other selected carbon sources, will attract denitrifying bacteria from the soil on site. The whole process can be considered as a biostimulation process. No invasive species are involved or transported to the site. Being underground also allows crops to grow on top of it, which makes it more space-efficient in an agricultural field.

Denitrification occurs naturally in the soil under anaerobic conditions with nitrate and carbon supply and appropriate temperature. The microorganisms that are responsible for the process are defined by the enzyme they contain. The genes for nitrate respiration (*nar*), nitrite respiration (*nir*), NO respiration (*nor*), and N_2O respiration (*nos*) are found in several of denitrifying bacteria. Those genes code for denitrification enzyme (Zumft, W. G., 1997). The bioreactor utilizes the natural existence of the denitrification process and encourages biostimulation process.

Nitrous oxide can be emitted from both nitrification and denitrification process. Nitrous oxide contributes 300 times more to the global warming effect than carbon dioxide (Forster et al., 2007). The Intergovernmental Panel on Climate Change (IPCC) has been producing assessment reports and greenhouse gas inventory guidelines since 1990 to

address the importance of nitrous oxide emission (Ravindranath, 2010). The 2006 IPCC Guidelines for National Greenhouse Gas Inventories Chapter 11 addresses the N_2O emission from managed soils. Studies also attempt to measure the nitrous oxide emission directly from the agricultural field. Nitrous oxide emission is potentially affected by soil management, environmental, and soil factors (Venterea et al., 2012). The reason that nitrous oxide is interesting in the bioreactor study is because the limited residence time does not ensure complete nitrate reduction to nitrogen gas. It becomes a source of nitrous oxide emission.

Schipper et al. (2010) indicated that there are various key factors that control the effectiveness of a bioreactor: temperature, nitrate concentration, carbon source, as well as residence time. Temperature is an important factor because the denitrification process is a biological process. The involvement of enzymes suggests that temperature is critical to biological reaction effectiveness. Usually, higher temperature indicates a higher biological reaction rate. However, Cameron and Schipper suggested that longer term NO_3 removal rate was less at the higher temperature due to the faster depletion of carbon source at the higher temperature (Cameron and Schipper, 2010). Using the NGAS model by Parton et al. (1996) the denitrification rate maximizes around 25 to 30 ºC and seems to plateau afterwards. In this thesis study, although the temperature was not controlled, the observed outflow nitrate concentration fluctuated with the change of temperature. This will be discussed later in the paper. The second effectiveness factor of bioreactors is nitrate concentration. It is the primary electron receiver for bacteria respiration reaction

under anaerobic conditions. Too much or too little nitrate input can both present problems. Too much nitrate-nitrogen input may be toxic to the bacteria (Glass & Silverstein, 1998); too little nitrate-nitrogen will not provide enough reactant for the bacteria respiration. Therefore, NO_3 concentration is another important factor determining the nitrate reduction rate. Alternative carbon options, being the bacteria food source, provide ecosystem diversity which helps build species diversity. Species diversity is good for increased system resilience. More labile carbon sources, like corn stalks, may support higher removal rates than wood media. However, these need to be replenished more often due to the higher depletion rate (Schipper et al., 2010). The next key factor affecting bioreactor performance is the hydraulic residence time. In many aspects of soil and water science, hydraulic residence time cannot be overlooked. Complete denitrification process is composed of 5 steps, going from nitrate (NO_3-) to nitrite (NO_2-), nitric oxide (NO), nitrous oxide (N_2O), and nitrogen gas (N_2). Hydraulic residence time is the measure of the average length of time that a soluble compound remains in a constructed bioreactor. If the residence time is short or any conditions are not met, the denitrification can yield any one of the products and cause other environmental problems.

Over the years, researchers have tested a variety of solid carbon sources (Gibert et al., 2008, Woli et al, 2010) as well as non-carbon sources like pyrite (Postma & Boesen, 1991). Woodchips are economically used in the field-scale bioreactors (Christianson et al., 2013). Studies have proven that woodchips are quite effective in removing nitrogen from the agricultural drainage. Blowes et al. used tree bark, woodchips, and leaf compost

as an organic carbon source in two 200-L bioreactors. Agricultural drainage with NO_3-N concentration of 3-6 mg/L was treated at a residence time of 1-6 days for over 1 year. They observed a significant reduction by denitrification with the effluent concentration being less than 0.02 mg/L (Blowes et al., 1994).

There is very limited information on how often they should be replaced. Robertson discovered that woodchips lose about 50% of their reactivity during first year of operation as soluble organic compounds are leached out, but relatively stable rates persist for a considerable number of years thereafter. The 7-year-old wildwood media had a mean nitrate removal rate of 9.1 $mgNL^{-1}d^{-1}$; the 2-year-old Avon media had the rate of 12.1 $mgNL^{-1}d^{-1}$; and the fresh chips had the removal rate of 15.4-23.0 $mgNL^{-1}d^{-1}$ (Robertson, 2010). Moorman et al. 2010 also identified different half-life times for wood at different depth in a bioreactor, where the wood at the shallower depth would have a lot shorter half-life. They calculated that the 90 to 100 cm depth wood had a half- life of 4.6 years; 155-170 cm depth wood had a half-life of 36.6 years.

In Minnesota, corn is the major agricultural commodity product. Labile carbon sources such as corn cobs are readily available under certain management scenarios. Studies have shown that corn cobs are quite efficient in removing nitrate. Li et al.(2012) used corn cobs as the carbon source for denitrification and observed around 97% reduction at a 23-hour residence time, 88% at 16-hour, and 59% at 8-hour retention time. They suggested that corn cobs could be effectively used by denitrifying bacteria and it is a feasible carbon

source for denitrification (Li et al., 2012). The system supported by corn cobs can be effective quickly after installation. Wang et al. (2013) reported 58% nitrate reduction on the first day with an influent nitrate concentration around 50 mg/L. After an 8-day continuous running of the reactor, the nitrate removal rate became stable at 98% at a 10-hour retention time (Wang et al., 2013). Xu et al. (2009) found that corn cobs lixivium has high concentration of Ca, K, Mg, Na, Si, and P, which are necessary for microorganism growth. Trace element like Ba and Zn were also detected. Heavy metal elements that negatively affect metabolism of microbes, such as Cu, Pb, Cr, Cd, were not detected (Xu et al, 2009). The metal ions can be beneficial to denitrification due to the fact that they are used as active center in the denitrification process (Tan and Luo, 2002).

Another carbon source that is drawing attention is biochar. It has a good potential of being used as a soil amendment to enhance crop production, but there is a lack of understanding of the mechanism. The chemical structure and elemental composition depends highly on the conditions of pyrolysis and the biomass parent material (Spokas, 2010). Thermal conversion of biomass in the absence of oxygen yields liquid, solid, and gas products. The solid residual, biochar, is more known as a soil amendment substrate to retain nitrogen and enhance production (Chan et al., 2007). Keeping low-cost and feasibility in mind, the procedure of making biochar in this particular study was made simple by caramelizing woodchips near the wood source. Cayuela et al. reported a decrease in N_2O:(N2+N20) emission when biochar was incorporated in the soil (Cayuela et al, 2013). Singh et al. (2010) did a study on the influence of biochar on N_2O emission.

They found an increase in N_2O emission during the first two wetting/drying cycles in their study; during the third cycle, N_2O emission decreased consistently from all biochar treatments (Singh et al., 2010).

(2) Phosphorus

Besides nitrate, which can cause eutrophication in waterbodies, phosphorus is another limiting nutrient (USEPA). For example, one of the algae species, *Selenastrum capricornutum*, responded to the addition of phosphate rather than nitrogen (Maloney et al., 1972). Phosphorus is an essential component of nucleic acids and intermediary metabolites process. Phosphorus enters the aquatic systems as a mixture of dissolved and particulate forms (Correll, D.L., 1998). Orthophosphate is the most stable and common form of dissolved phosphorus. In the tile drain system, the drainage water contains both orthophosphate and particulate phosphorus. The simulated ditch water in the lab contains only orthophosphate as the dissolved phase.

Currently, not many studies have reported phosphorus removal via bioreactors. Zhang et al. (2011) compared phosphorus removal effects of two plant species as well as the presence of a submerged zone with a carbon addition. Total phosphorus removal significantly increased in the treatment with the submerged zone regardless of plant presence. The average removal was more than 93% total phosphorus and more than 97% total dissolved phosphorus (Zhang et al, 2011). Different types of biochar also have different phosphorus sorption/desorption effect. Biochar formed at 600 ºC has a reduced capacity to sorb phosphorus than biochar formed at 400 ºC and 500 ºC (Morales et al.,

2013). Sarkhot et al. suggested that biochar can be used for recovering excess phosphorus. They measured 50% of the PO_4^{3-} sorption; and during desorption phase, biochar retained 60% of the sorbed PO_4^{3-} at reaction time less than 24 hours (Sarkhot et al., 2014). However, another study suggested that certain types of biochar showed little or no ability to sorb nitrate or phosphate (Yao et al, 2012), which suggests that it is important to know which type of biochar is being used when conducting a study.

Pilot-Scale Study

(1) Method

Two pilot-scale bioreactors (L=0.98m by W=0.2m by H=0.75m with 0.08m soil on top, see Figure 1) were constructed with PVC sheets in the lab to test the effectiveness of three different wood-based media. The two bioreactors simulated horizontal water flow in the field with a length to width ratio of 14:1. Each bioreactor had three chambers as shown in Figure 1. There were two baffles in each chamber that helped to guide the flow in order to minimize the dead space under horizontal flow conditions. Water was pumped into an overhead box first and flowed down though the flow meters into the bioreactor chamber by gravity. Water entered from the bottom of each chamber and was pushed upwards. One of the bioreactors had transparent sides to provide view inside the bioreactor. Both sides were covered with tarp during the experiment period to prevent light effect on microbial growth. At each outlet, a cylindrical container was placed to catch effluent for daily nitrate probe uses. Simulated ditch water was prepared in the storage tank and pumped into the overhead tank. Six flow meters were used to control the

flow rate in each chamber. Soil from a proposed field site (Clarion-Storden loam-clay loams, from Web Soil Survey) was used to cap the top. When water was running, the soil on top was always saturated, providing anaerobic reaction condition inside the bioreactor.

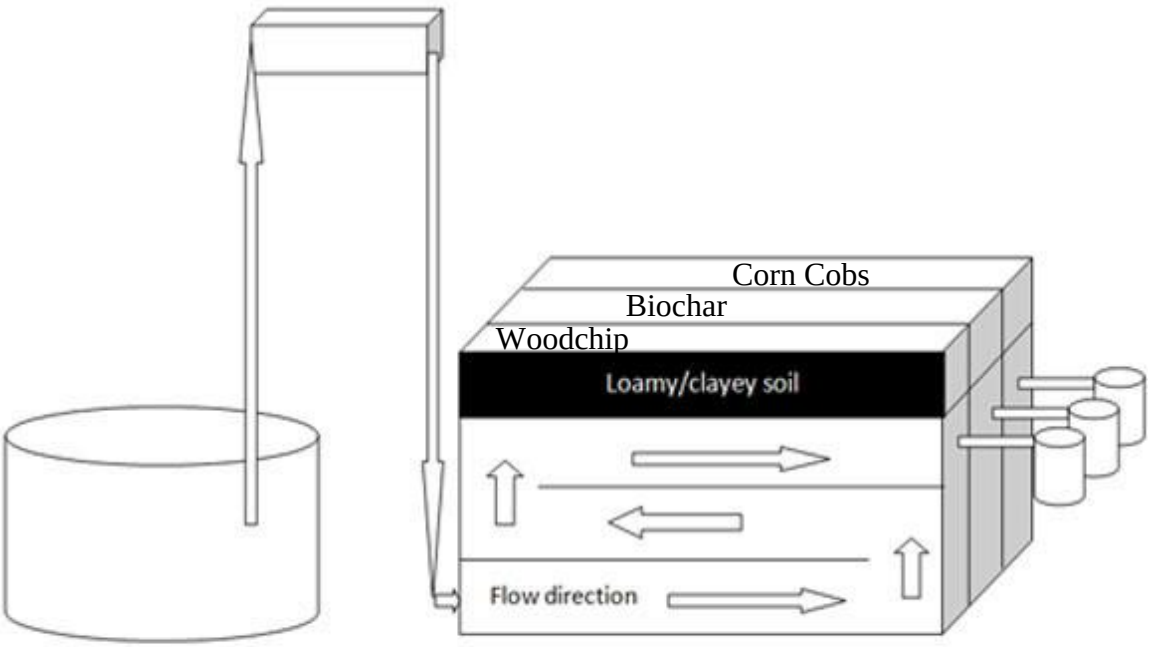

Figure 1. Schematic of the bioreactor design

Each chamber was filled with a type of media (woodchip, biochar, and corn cobs) at an average porosity of 54% with one bioreactor being the replica. All media were pre-soaked in water for 48 hours and soil was mixed in at a concentration of about 10g/L prior to use. The bioreactors were tilted towards the opening of the baffles each time to make sure all the air was pushed out.

Chemicals were mixed with tap water in a 120-gallon tank to simulate typical southern Minnesota drainage water. The water was left in the lab over night before pumped into a

325-gallon tank to ensure a room temperature (around 20ºC) starting point. The artificial drainage water was pumped into each chamber at the same rate of 50ml/min, equal to a residence time of 24 hours, for three months. The same procedure was used for 8-hour residence time. Two more tanks (120 gallon and 225 gallon) were used for water storage due to the higher flow rate (150ml/min).

1.1 Primary porosity

When woodchips were submerged in the bioreactor, only primary porosity matters in terms of hydraulic property and microbial activity. The primary porosity of each media was measured using the procedure described in Ima and Mann (2007).

Media were submerged in a jar of water for 48 hours to become completely saturated; then packed into a beaker at the same packing density as in the bioreactor. A perforated metal baffle sat slightly on top of the woodchips to prevent material floating when adding water. Water was filled right to the top of woodchip pack and the level was marked on the beaker for total volume determination. The whole system was turned upside-down onto a sieve-covered bucket to collect water. It was drained overnight to ensure all the water in between individual woodchips was drained out. The volume of the water represents the primary pore space. Primary porosity was calculated by dividing the volume of water drained out by the total volume. Each media had two replicas. Woodchip and corn cob treatment both have one chamber next to the clear side. The average

porosity was used for calculation. The porosity of woodchip was calculated to be 55%; that of biochar was 53%, and corn cobs' porosity was 57%.

1.2 Experiment preparation

Three media were used for the experiment: woodchip (mixed hardwood), biochar created from the same type of woodchip (caramelized woodchip), and corn cobs. Caramelization occurred at a proposed field site by heating the woodchips underneath burning logs. The idea was to create a high temperature and low oxygen condition for pyrolysis.

Each baffle was installed by hot glue gun in place after filling up each layer with media. After all layers were filled, loamy soil from the proposed field site (field near Elm Creek) was used to seal the top. The moisture content and organic content of the soil were pre-measured. The moisture content was measured to be 2.47%. The total organic content was 1.52% and the total nitrogen was 0.117%.

The system was then flushed with tap water for a week and drained due to the turbidity of the outflow that affected the nitrate probe reading. The chemicals were not added until the outflow cleared out.

1.3 Major nutrients

The water recipe (Table 1) was determined using measured ditch water chemistry as a reference. The sample was collected from a drain tile outlet on Elm Creek (Lenhart et al., 2009) in May of 2013. The study used a nitrate-nitrogen concentration of 21 mg/l (±1 mg/l). To simulate the groundwater characteristic in southern Minnesota, the calcium concentration was at 80 mg/l. Another nutrient of interest was phosphorus, which was maintained at 0.35 mg/l (±0.05 mg/l). Other than the nutrients mentioned above, macro nutrients were also added to more precisely simulate the ditch environment for microbial growth.

Table 1.List of nutrients added to the mixed solution

Total	mg/l	Form
NO_3^--N	20	$Ca(NO_3)_2$
PO_4^{3-}-P	0.3	KH_2PO_4
Al^{3+}	0.08	$AlCl_3$
Br^-	0.02	KBr
Ca^{2+}	80	$CaCl_2*2H_2O$, Ca(NO3)2
Cd^{2+}	0.01	CdCl2
Cr^{2+}	0.01	$CrCl_{3*}6H_2O$
Cu^{2+}	0.02	$CuSO_{4*}5H_2O$
Fe^{2+}	0.02	$FeSO_{4*}7H_2O$
Mg^{2+}	25	$MgCl_2*6H_2O$
Mn^{2+}	0.01	$MnSO_4*H_2O$
Ni^+	0.01	$NiCl_2$
Zn^{2+}	0.01	$ZnSO_4*7H_2O$
K^+	0.4	KH_2PO_4, KBr
Cl^-	170	$AlCl_3$, $CaCl_2*2H_2O$, $CdCl_2$, $CrCl_3*6H_2O$, $MgCl_2*6H_2O$, $NiCl_2$
SO_4^{2-}	0.1	MnSO4*H2O

1.4 Water tank volume calibration

A 120-gallon tank was used for water mixing and storage. The usable volume was calibrated before running the experiment. The tank was filled to maximum capacity first. Then the time it took to pump the water all out was recorded, during which, a flow rate was taken every 10 minutes by filling up an 8-L bucket. A rate versus time curve was plotted and the area underneath the curve was calculated (Figure 2). The total volume was determined by adding up the total area and the volume of the remaining water that was pumped out after the last rate measurement. The volume available was 117 gallons.

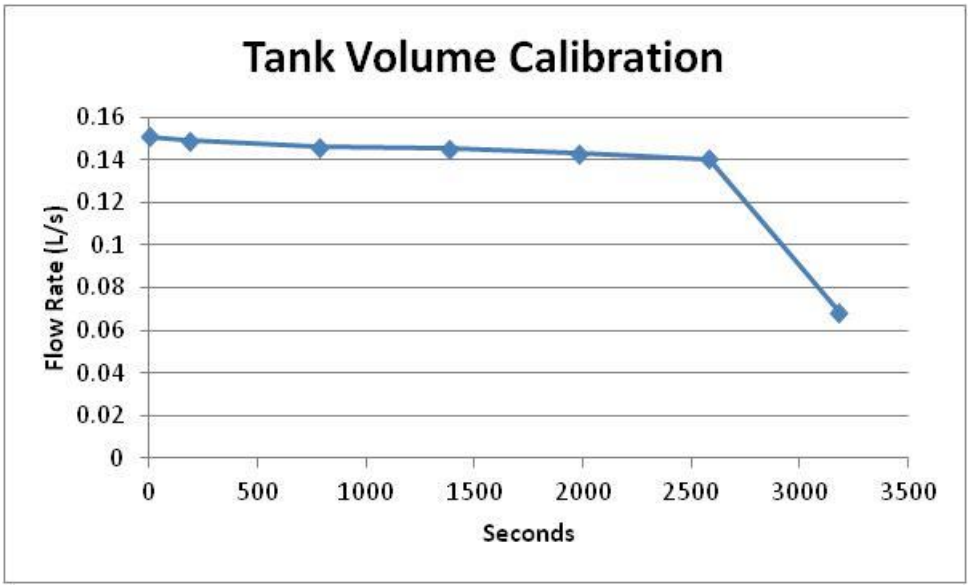

Figure 2.Rate vs. time calibration curve for the water storage tank active volume

1.5 Flow rate calibration

Six flow meters were attached to each chamber for flow control. All meters were turned to the same level to ensure same flow rate through each chamber. Prior to the start of the experiment, meters were calibrated using manually measured flow rates. After the meters

were set to the desired scale, a beaker and a timer were used to catch water at the end of each chamber for 3 minutes and ensure the flow rates per minute were the same. During the course of the experiment, flow rate was also kept track of using the same method, except that the timer was set to 1 minute.

1.6 Data collection

Nitrate concentrations going in and coming out of each chamber were measured daily; while orthophosphate from the same locations was measured every three days. Nitrate and NO_X-nitrogen in and out was measured using a HACH nitrate probe sc clear with a sc100 meter. The orthophosphate data was collected by the HACH D890 kit using standard method 79. Weekly samples were also collected from each chamber to be analyzed with Lachat instrument for nitrate and orthophosphate. At the end of the experiment period, samples of each media from the top layers were collected to be examined under scanning electron microscope (SEM) to test the hypothesis that there might be phosphorus precipitated on the media surface.

Nitrous oxide data collection used the same idea from USDA-ARS GRACEnet Project Protocols Chapter 3: Chamber-Based Trace Gas Flux Measurements (Parkin, T. B. and Venterea, R. T., 2010). A PVC sheet of half an inch thick was used to cover the chamber top with weather stripping around it. Gas was extracted from the septa-covered hole on

the PVC sheet. Twelve ml of gas sample was extracted from each chamber every twenty minutes for a total of three times every week. First sample was collect right after the PVC sheet was placed. The samples were analyzed with gas chromotograph in the lab for nitrous oxide.

Other than nitrate, phosphorus, and nitrous oxide, influent temperature was measured everyday using a soil/water thermometer; pH of the inflow and outflow was taken periodically to observe reaction consistency.

1.7 Statistical analysis

Two-way ANOVA and post hoc Tukey's test were run using R statistics software. Since there was more than one observation from each combination (residence time and medium) and they were not pseudoreplications, a two-way ANOVA at a significance level of 0.05 (analysis of variance) was chosen instead of paired-t test to analyze nitrate-N removal rate differences in group means of each combination. A post hoc Tukey's test was run using the ANOVA model to further determine the difference at a significance level of 0.1.

(2) Results

2.1 Nitrate reduction

The results were summarized in Figure 10. The experiment continued for a total of 89 days of 24-hour residence time, and 60 days of 8-hour residence time. The 24 hours residence time represented the media potential of nutrient removal while the 8 hours

residence time more closely represented the field condition. The replica treatment for each media matched the results as shown in Figures 3 and 4. Average reductions from each media were used in the remaining graphs. A few missing data points were included in the analysis by the Excel "NA" function and in the statistical software R. Over the three months period, the water temperature fluctuated between 16.07° C to 24.06° C. Temperature in the lab started dropping in the beginning of December, making the data incomparable to others. Therefore, the second period of study did not start until the month of March, and the data collected during the cold season was not used in this study. 95% of the input NO3-N stayed within the range from 19.9 mg/l to 21.9 mg/l. It is necessary to mention that on day 36 of the second research period, the nitrate probe was changed due to the malfunction of the original one.

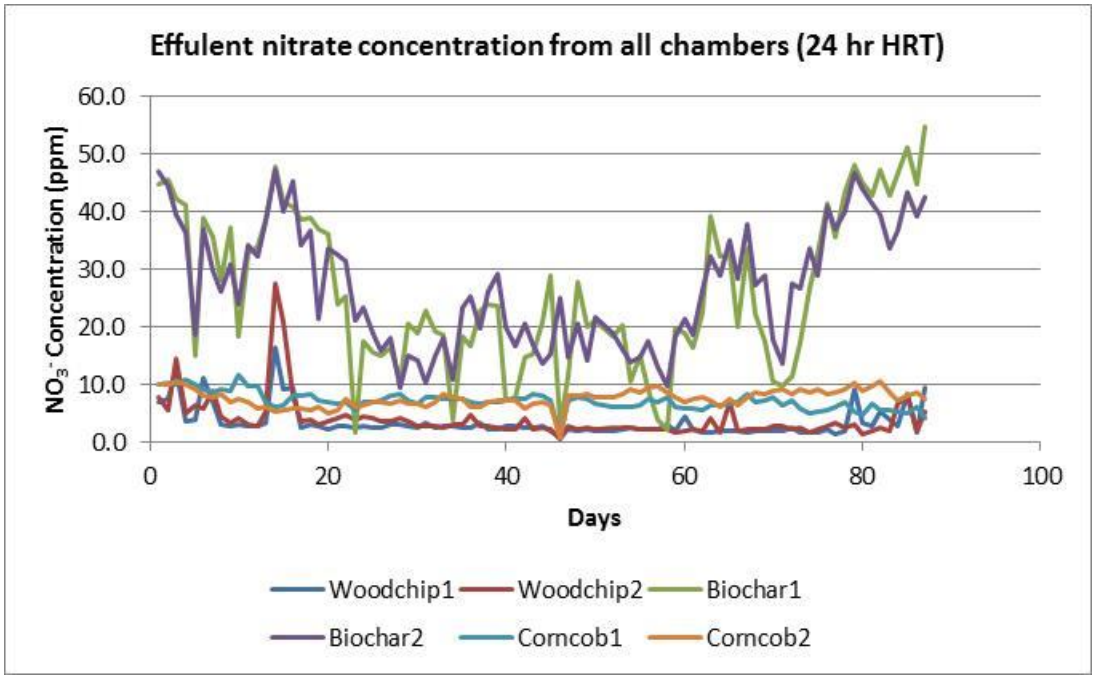

Figure 3.24-hr outflow nitrate concentrations from all chambers

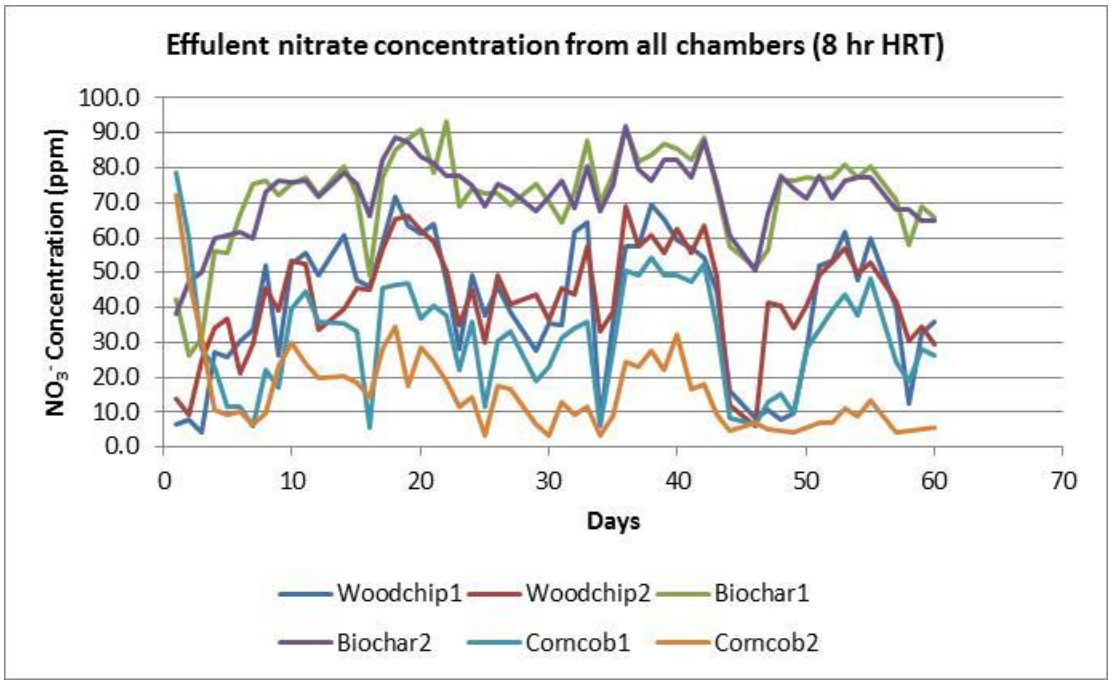

Figure 4.8-hr outflow nitrate concentrations from all chambers

The unprocessed woodchips showed the highest potential nitrate reduction rate among all three media. It had an average of 96% reduction at 24 hours residence time. It reduced the nitrate loading quickly at start compared to the other two media. The only major outflow nitrate concentration spike observed in both woodchip chambers was after a power outage on Oct. 7th (day 15) of nearly 40 minutes (Figures 5, 6, 7, and 8). At the very end of the experiment, power was cut for another 40 minutes to test the observation. The data showed an upward trend. The residence time has a great effect on the nitrate removal rate. After switching to the 8 hours residence time, the reduction rate dropped sharply to an average of 53%.

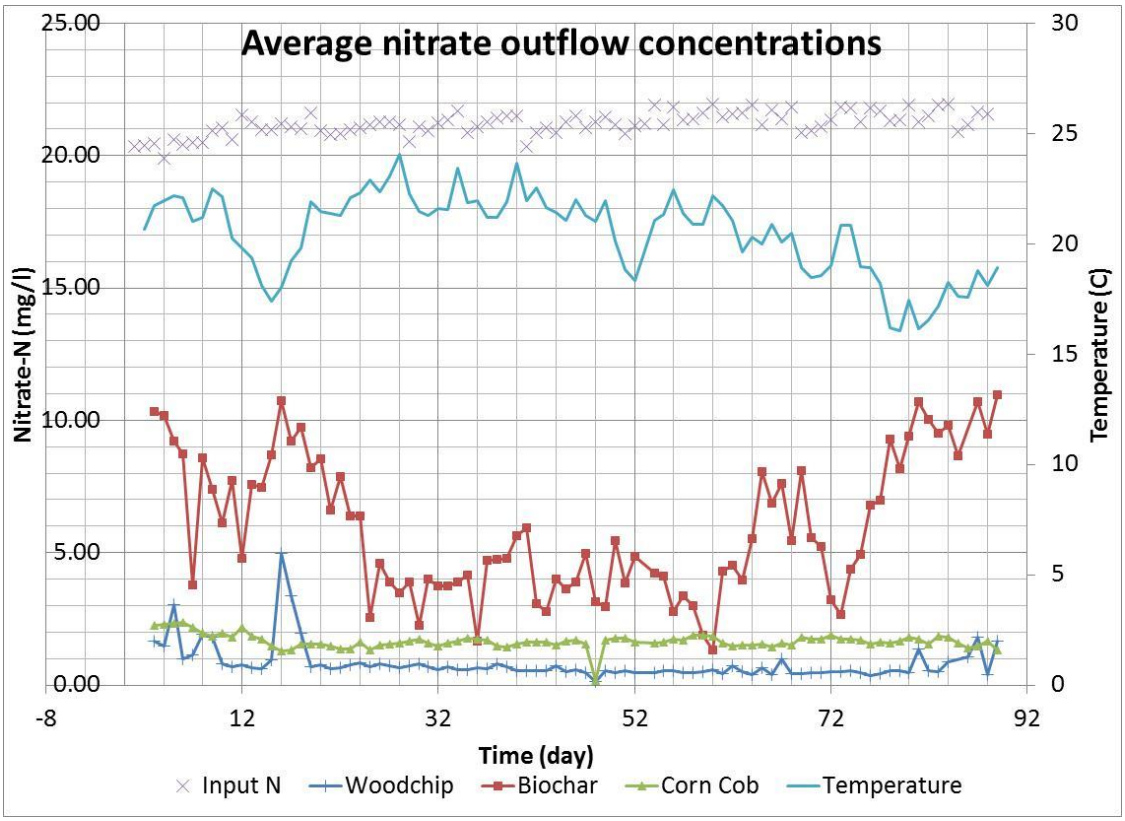

Figure 5.24-hr average outflow nitrate concentrations curves

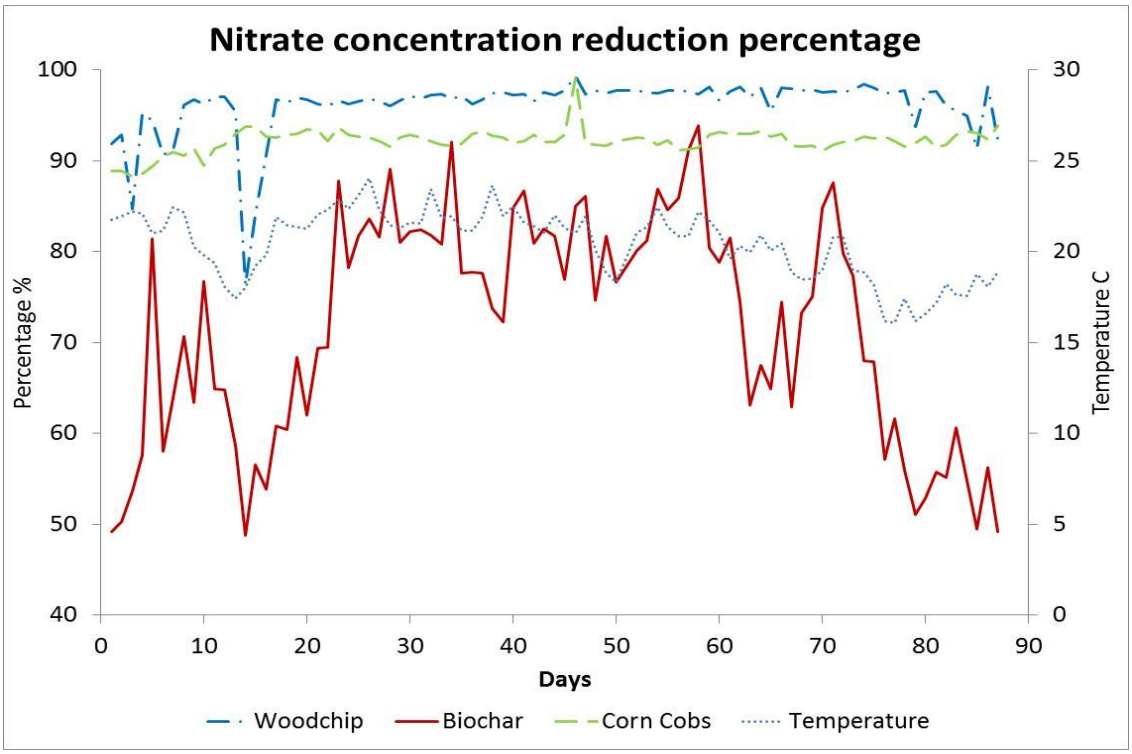

Figure 6.24-hr average nitrate concentration reduction percentage

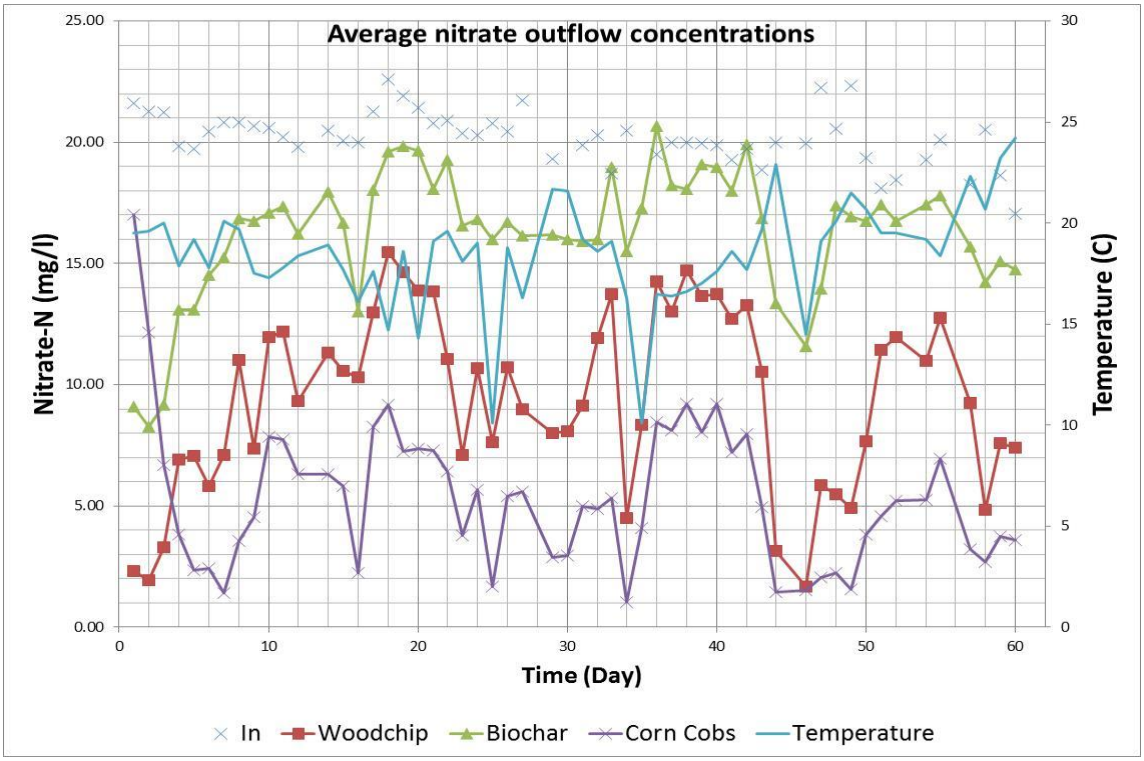

Figure 7.8-hr average outflow nitrate concentrations curves

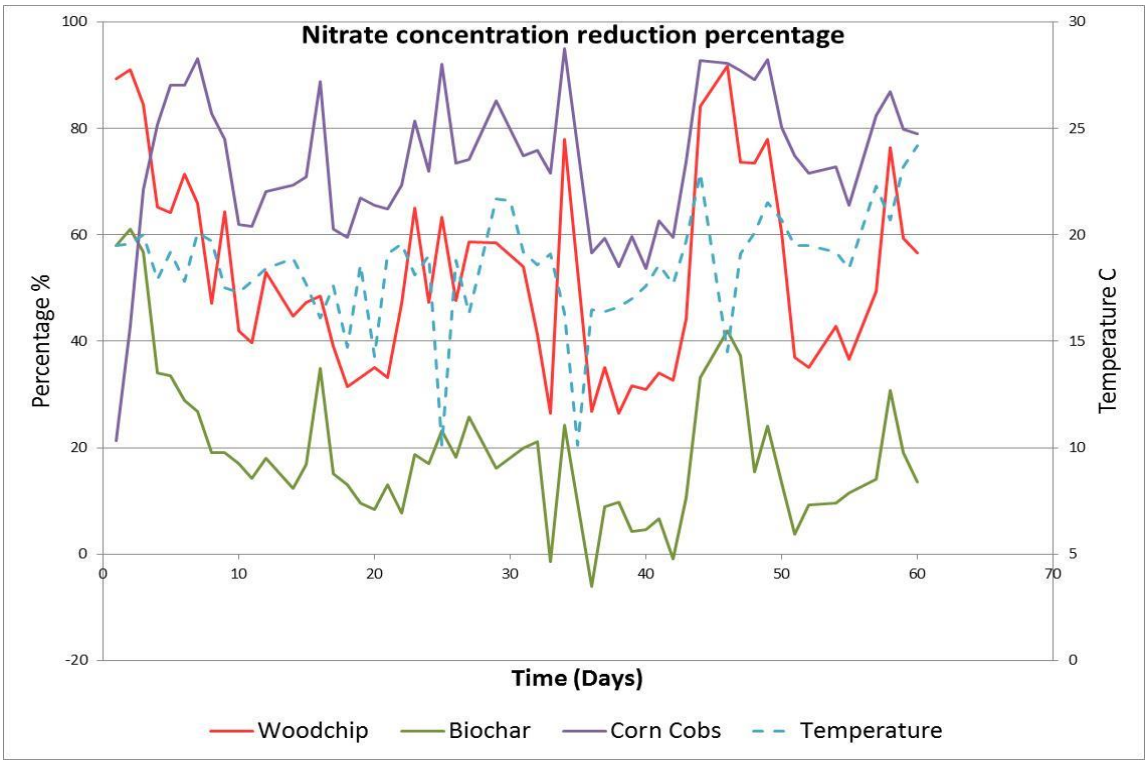

Figure 8.8-hr average nitrate concentration reduction percentage

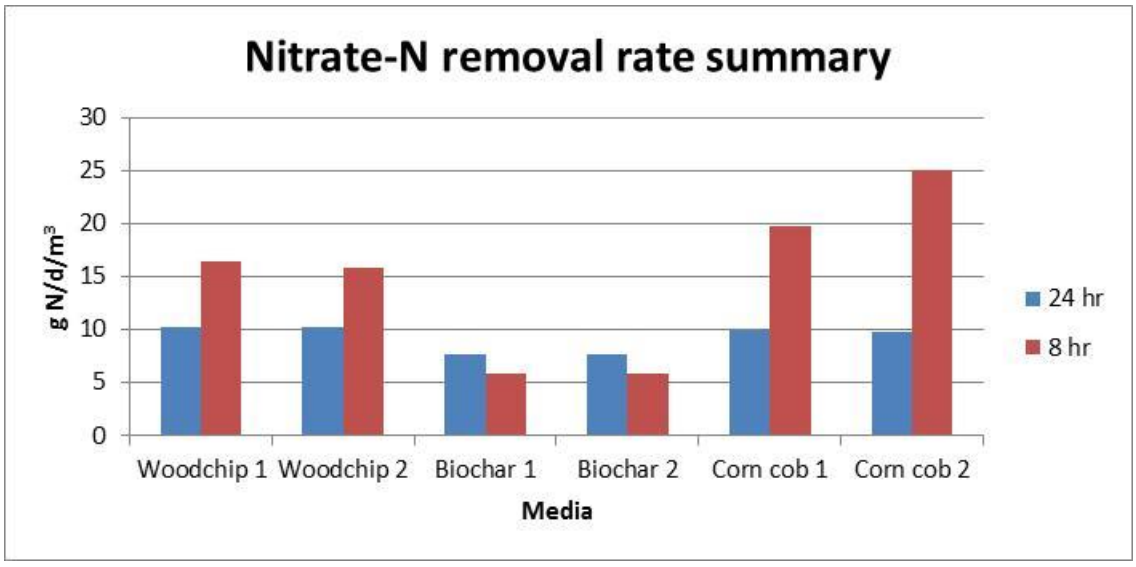

Figure 9.Summary of nitrate removal rate from all combinations

During the power outage, both biochar chambers had an increased outflow nitrate concentration like the woodchip chambers. Apart from the power disruption, the biochar N removal was less consistent than the other media. When comparing the biochar outflow nitrogen concentration curve from the 24 hours HRT with the temperature fluctuation, it appeared to be more closely related to temperature than the other two media. Therefore, a correlation chart was made using R (Table 2). Woodchip denitrification had almost no correlation with temperature. Corn cobs denitrification had a moderate correlation and biochar denitrification showed a correlation of -0.5 with temperature. Within the recorded initial water temperature range, temperature was critical in the amount of reduction by biochar. There was not enough evidence to confirm the correlation outside of the temperature and residence time range. The average nitrate reduction from biochar was 72%. After switching to the 8 hours residence time, the average removal rate was only 19%, indicating that this type of biochar is not suited for nitrogen removal at a short residence time.

Table 2.Correlation between temperature and outflow nitrate concentrations from the biochar chamber at 24-hr residence time

	T	Woodchip1	Woodchip2	Biochar1	Biochar2	Corncob1	Corncob2
T	1						
Woodchip1	-0.09	1					
Woodchip2	-0.05	0.79	1				
Biochar1	-0.51	0.53	0.38	1			
Biochar2	-0.55	0.52	0.41	0.86	1		
Corncob1	0.32	0.15	0.14	0.04	-0.05	1	
Corncob2	-0.25	0.09	-0.12	0.18	0.13	0.23	1

Corn cobs denitrification was the most stable observed over the study period. The percentage reduction over time displayed a slope of 0.012 with only one outlier. The average was 41.02 g N/d/m^3 reduction at 24 hours and 65.63 g N/d/m^3 at 8 hours which was the highest among all three media. The removal percentage ranged from 71% to 99% in the first experiment period and from 19% to 83% in the second period. Corn cobs denitrification rate curves also did not spike during the power outage. The current data suggests that corn cob media denitrification is the most resilient to environment changes including temperature and residence time.

Lachat results were attached in Appendix II. The result was compared to the probe readings and statistical significance was determined using student-t test (Tables 3 and 4). Alpha was set to be 0.05. Paired-t test assuming equal variances in Data Analysis in Excel were used for the statistical analysis. The two-tail P values from both tests suggested that there was no statistical significance between Lachat results and probe readings. Both P values are greater than 0.05, indicating that there was no significant difference between Lachat results and probe readings.

Table 3.Student t-test for 24-hr nitrate concentration data between Lachat result and probe reading

	Variable 1	Variable 2
Mean	17.91682	22.80791
Variance	815.6203	989.903
Observations	56	56
Pooled Variance	902.7616	
Hypothesized Mean Difference	0	
df	110	
t Stat	0.861387	
P(T<=t) one-tail	0.195449	
t Critical one-tail	1.658824	
P(T<=t) two-tail	0.390899	
t Critical two-tail	1.981765	

Table 4.Student t-test for 8-hr nitrate concentration data between Lachat result and probe reading

	Variable 1	Variable 2
Mean	50.53193	54.89773
Variance	933.4794	774.6475
Observations	49	49
Pooled Variance	854.0635	
Hypothesized Mean Difference	0	
df	96	
t Stat	0.73944	
P(T<=t) one-tail	0.230722	
t Critical one-tail	1.660881	
P(T<=t) two-tail	0.461444	
t Critical two-tail	1.984984	

2.2 Phosphorus reduction

Orthophosphate data were collected every 3 days from day 8 to day 89. A HACH colorimeter designed for orthophosphate measurement was used to measure phosphorus levels. On average, the woodchips reduced orthophosphate concentration by 70% daily,

biochar by 80% and corn cobs by 65%. Biochar showed the most promising reduction potential. The outflow orthophosphate-P concentration from both biochar chambers after day 30 was below the other 4 chambers (Figures 10, 11, 12, and 13). Orthophosphate reduction did not have significant correlation with temperature during this experiment. The 8-hour orthophosphate reduction rates were almost the same across all three media. For real world application when the residence time is relatively short, nitrate removal rate is a more important factor for decision making than phosphorus reduction rate.

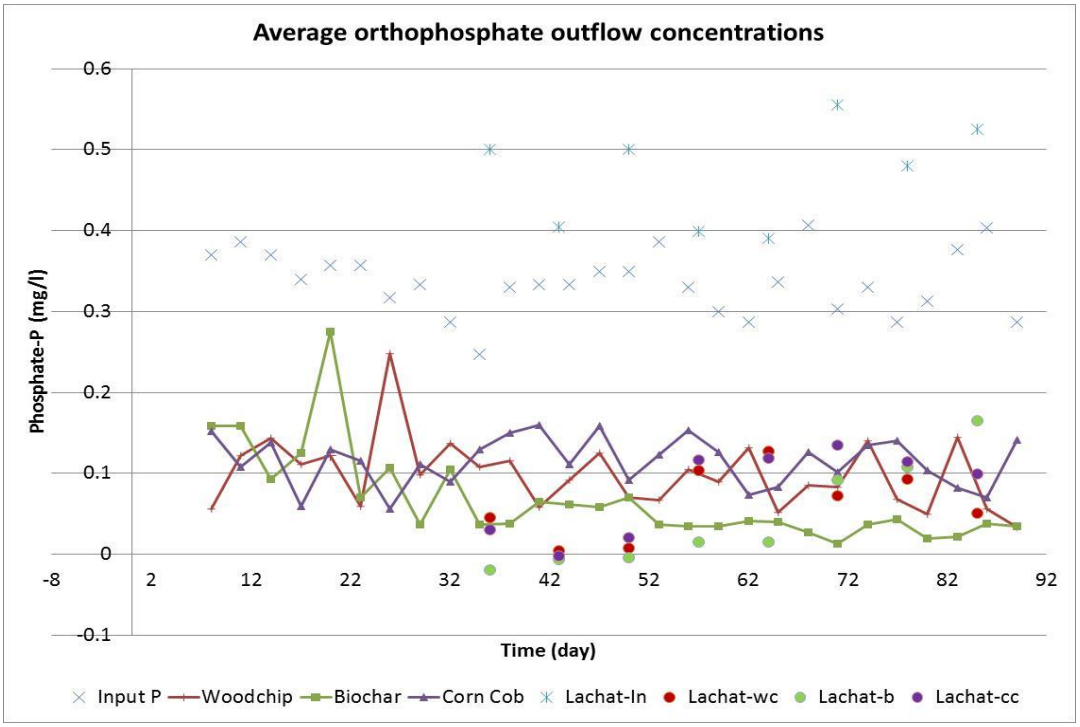

Figure 10.24-hr average outflow orthophosphate concentrations with Lachat results

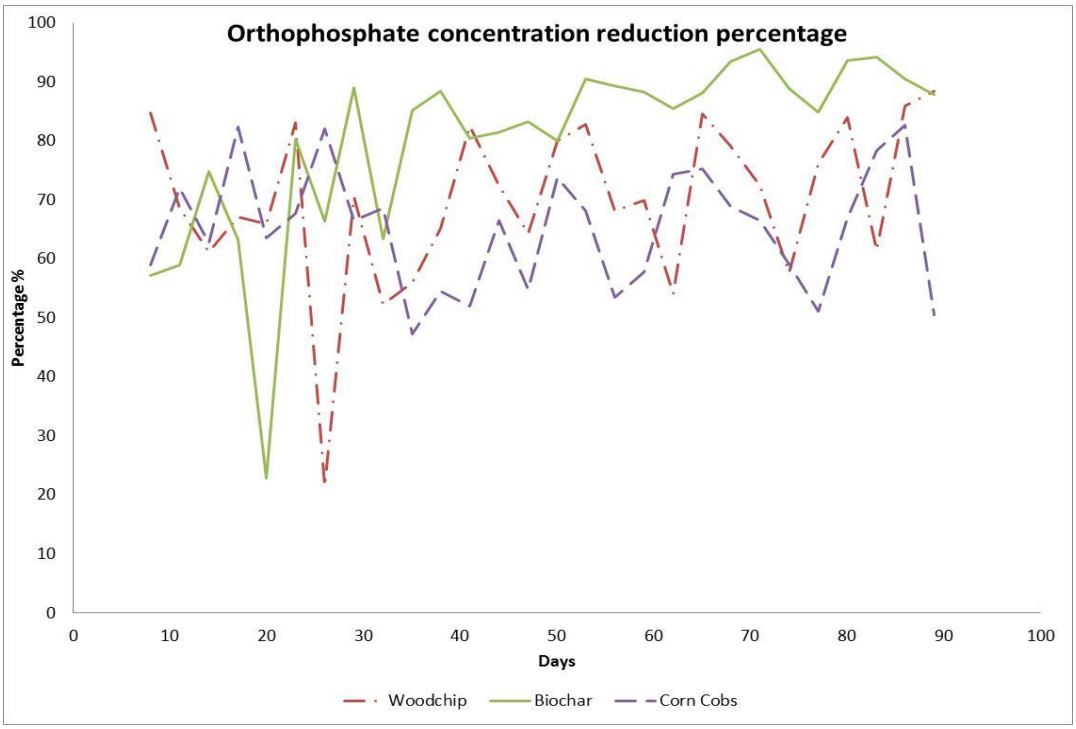

Figure 11.24-hr average orthophosphate concentration reduction percentage

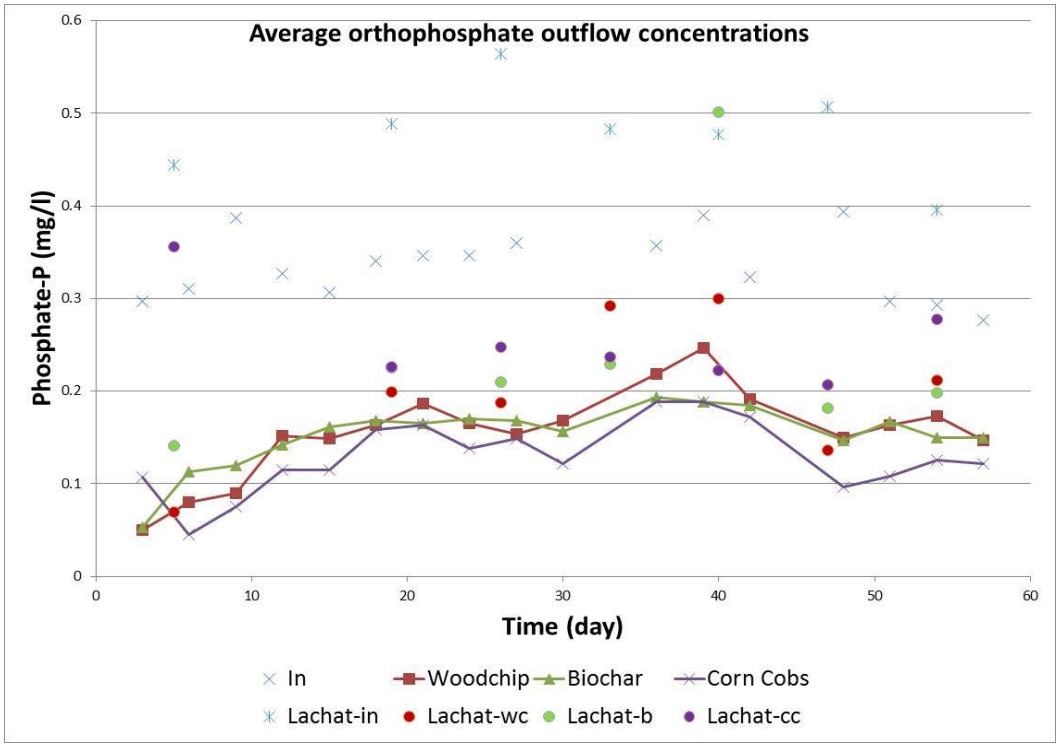

Figure 12.8-hr outflow orthophosphate concentrations with Lachat results

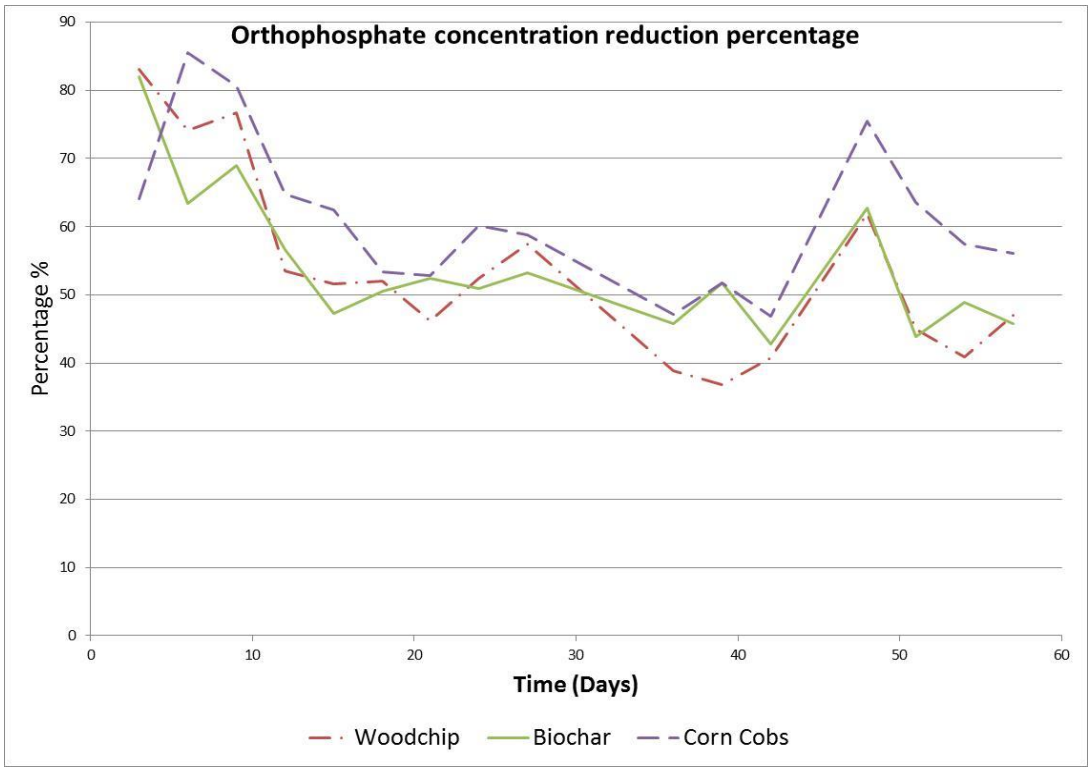

Figure 13.8-hr average orthophosphate concentration reduction percentage

The Latchat instrument was also used to analyze the weekly orthophosphate concentrations from the seven water samples. The result was directly plotted onto the reduction curve (Figure 10 & 12) since the data were not collected on a daily time step. No student-t test was used for orthophosphate data. There were some differences between Lachat data and lab readings, but the general trend stayed the same.

Transformation processes of nitrogen are relatively well understood; what is less clear is where the sequestered orthophosphate went. A surface ion analysis by a Scanning Electron Microscope (SEM) for each media type pre- and post-experiment were performed to try to detect major ions residing on the surface (Appendix I). Media sampling was highly biased due to the structure of the bioreactor. The media samples

were only taken from the top layer of the chambers. There is not enough evidence indicating that phosphorus was precipitated on the top layer of woodchip, biochar, or corncobs. What is unknown at this time is if phosphorus could be either used by microbes or it could be precipitated on different areas. There is a chance that phosphorus could be deposited on the bottom of the chambers.

In both N and P reductions, biochar took longer to reach a desired reduction equilibrium; suggesting that microbes took a longer time to establish colonies on biochar. At the same time, a lower reduction rate, while still meeting the water standard, could possibly be interpreted as slower depletion of the carbon source. If this assumption is true, then this particular type of biochar could be used to increase the life span of the bioreactor.

2.3 Nitrous oxide emission

Nitrous oxide is one of the products from the denitrification process. It can be generated when denitrification does not reach completion. The results are shown in Figures 14 and 15. Biochar emitted a significant amount of nitrous oxide compared to woodchips and corncobs. This type of biochar may not have an effect on nitrous oxide reduction, or possibly, the experiment did not run long enough. In Singh et al.'s (2010) paper, they indicated that there was an increase in N_2O emission during the first two wetting/drying cycles and during the third cycle N_2O emission decreased consistently from all biochar treatments. In Figures 14 and 15, the N_2O emission curves started going down at the end of the experiment period. If the experiment continued for longer period of time, a

reduction might occur. However, with the current data, biochar does not seem to be a viable media to be used in bioreactor for the sole purpose of denitrification.

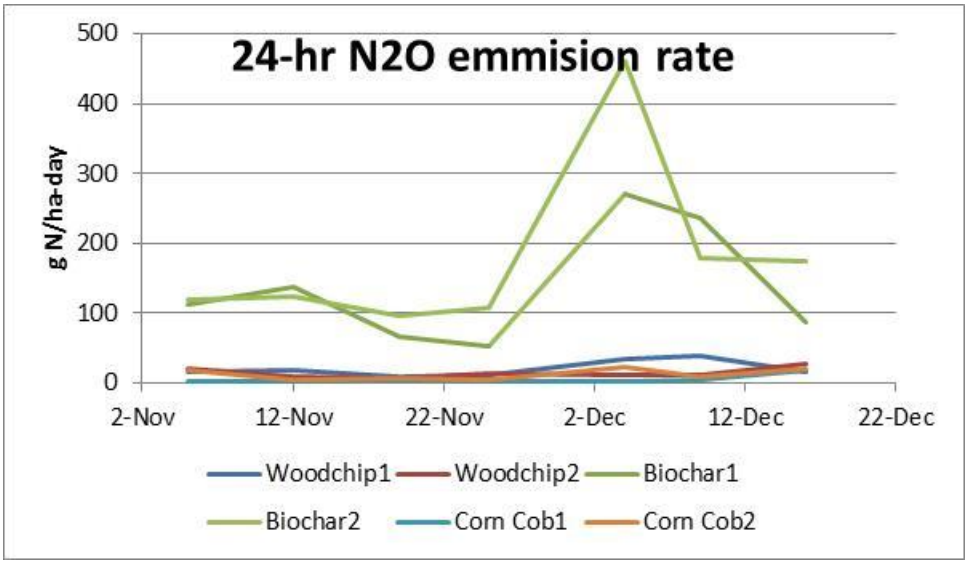

Figure 14.24-hour nitrous oxide emission from all 6 chambers

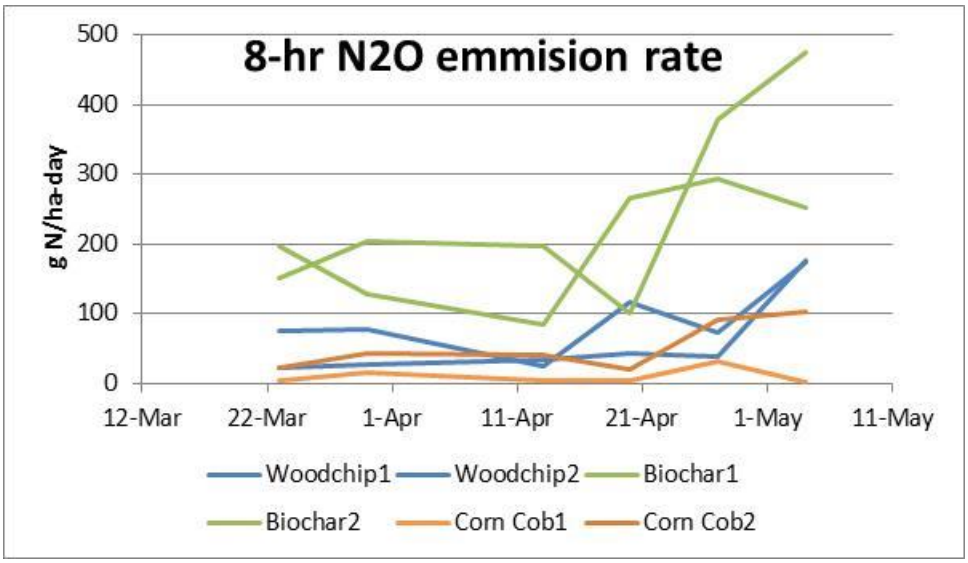

Figure 15.8-hour nitrous oxide emission from all 6 chambers

2.4 Statistical analysis

The ANOVA test results are shown in Table 5 and 6. The residual plots are shown in Figure 16 and 16. The Tukey's test results are plotted in Figure 18, 19, 20, and 21.

The p-values from the two-way ANOVA were compared to alpha which was 0.05. The only p-value that does not reject the null hypothesis assuming equal population mean is the media comparison for orthophosphate reduction rates. That means there was no significant difference in orthophosphate reduction rates among media. The residual plots do not support linear models when corn cob data points are included and not considered to be outliers. Further study of the residual plots will be needed to elucidate understanding. The post hoc Tukey's test for nitrate-N removal rate suggested difference among all media while the most difference is between corn cobs and biochar. And for orthophosphate reduction, most difference is observed between woodchips and corn cobs.

Table 5.Two way ANOVA test for nitrate-N removal rate

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
HRT	1	92.58	92.58	37.27	0.000881	***
Media	2	183.61	91.80	36.95	0.000423	***
HRT:Media	2	103.33	51.66	20.80	0.002004	**
Residuals	6	14.91	2.48			

Signif. codes:	0	'***'	0.001	'**'	0.01	'*' 0.05 '.' 0.1 ' ' 1

Table 6.Two way ANOVA test for orthophosphate reduction rate

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
HRT	1	0.4302	0.4302	262.981	3.5e-06	***
Media	2	0.0035	0.0017	1.055	0.4049	
HRT:Media	2	0.0147	0.0074	4.495	0.0641	.
Residuals	6	0.0098	0.0016			

Signif. codes:	0	'***'	0.001	'**'	0.01	'*' 0.05 '.' 0.1 ' ' 1

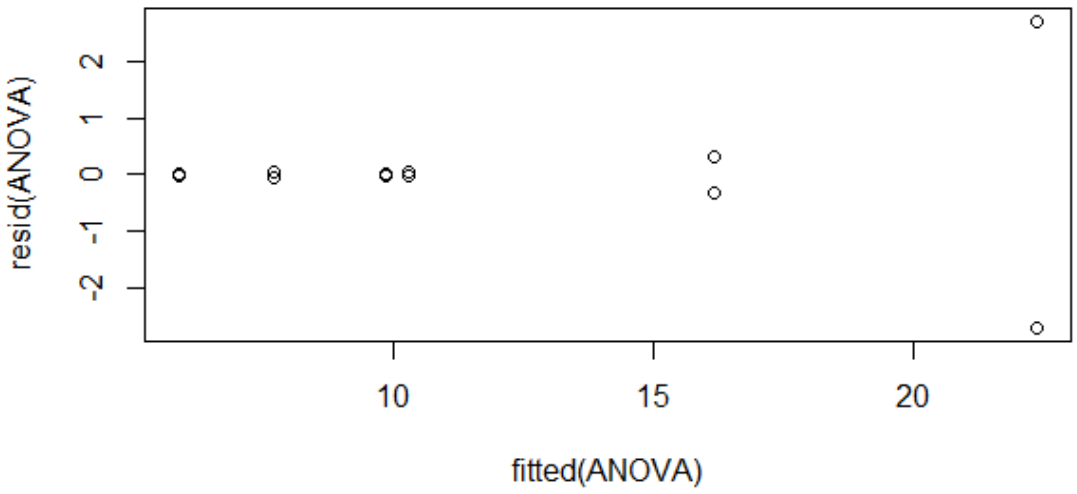

Figure 16.Residual plot for nitrate-N removal rate ANOVA model

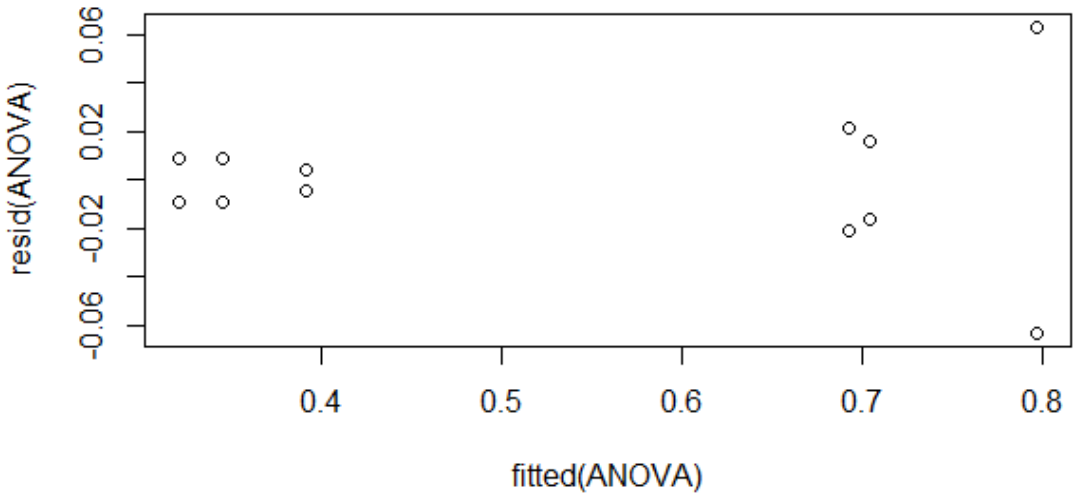

Figure 17.Residual plot for orthophosphate reduction rate ANOVA model

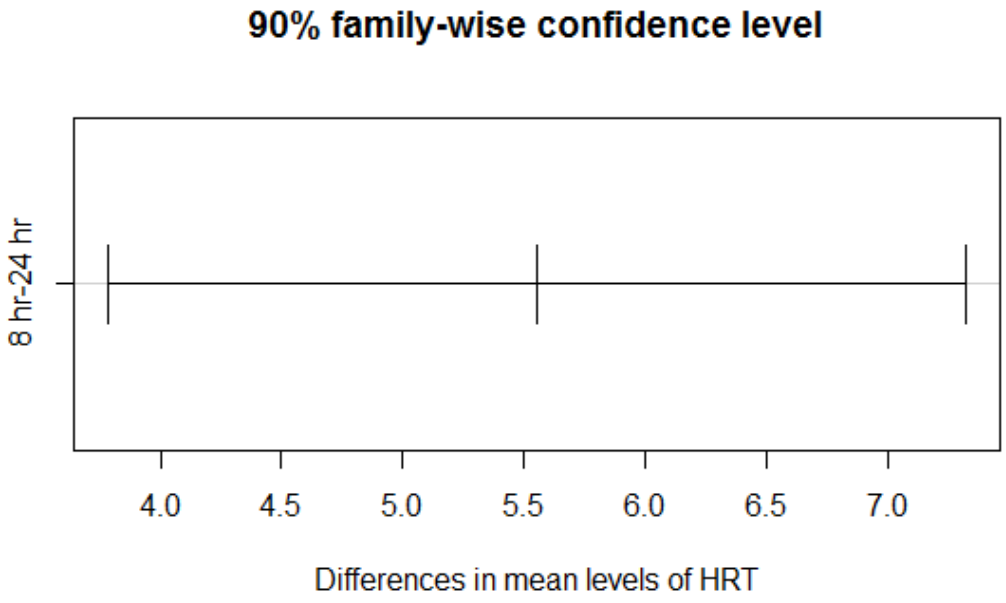

Figure 18.Tukey's test plot for nitrate-N removal rate between HRT

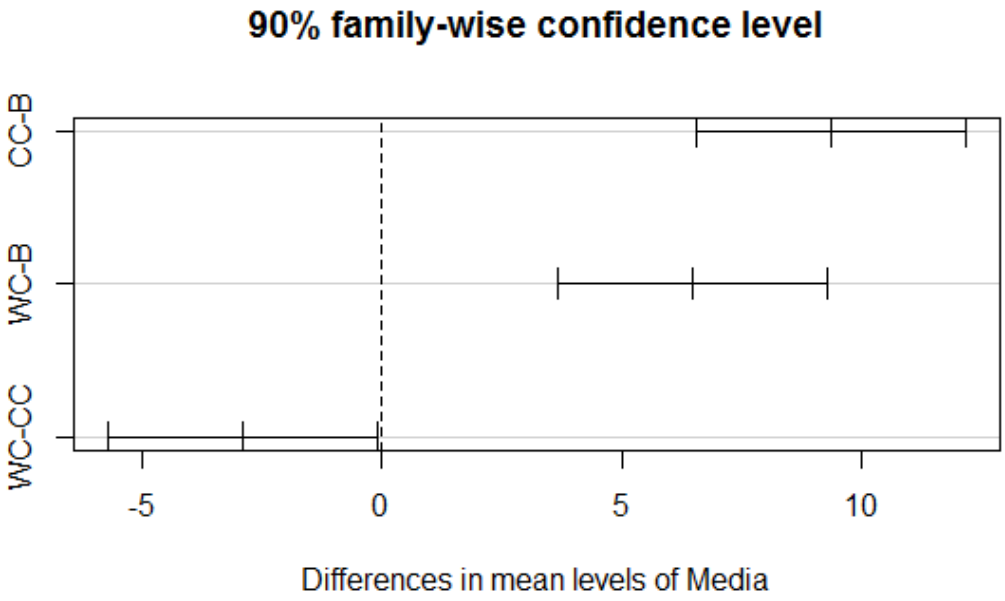

Figure 19.Tukey's test plot for nitrate-N removal rate between media

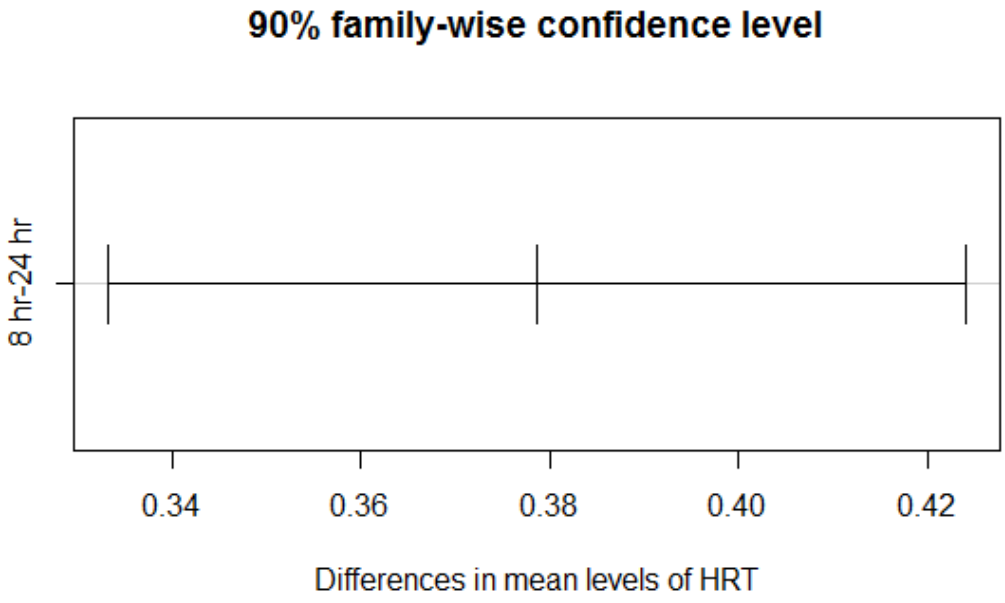

Figure 20.Tukey's test plot for orthophosphate reduction rate between HRT

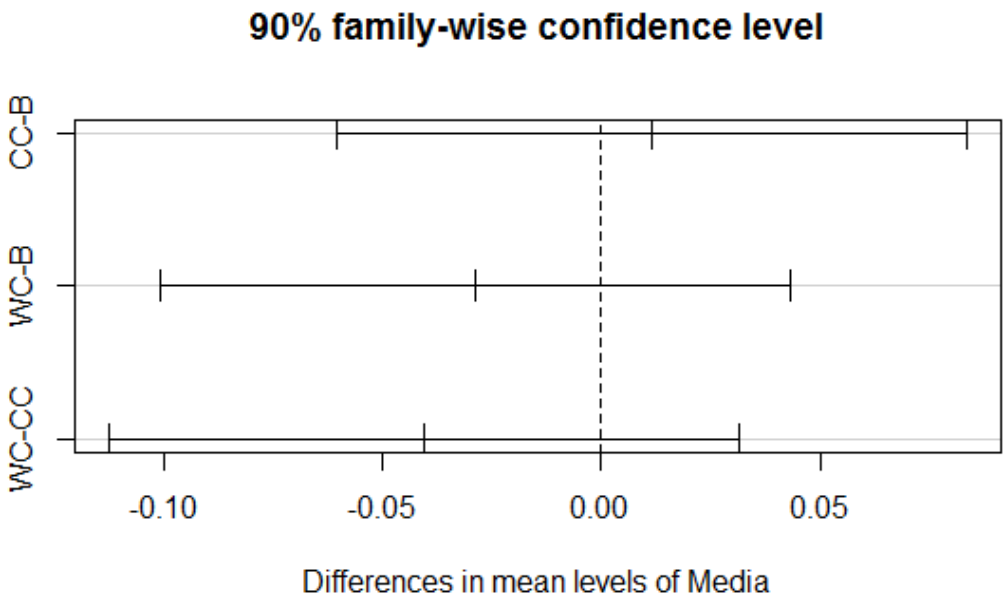

Figure 21.Tukey's test plot for orthophosphate reduction rate between media

Summary and Conclusion

A bioreactor is an effective method to manage nutrients from leaky row-crop terrain. Data measured in this study showed a strong potential for N and P reductions from all three media (woodchips, biochar, and corn cobs). In general, woodchips showed the largest denitrification potential; whereas biochar was more favorable for phosphorus removal and may add to system longevity. Corn cobs had the most stable nitrate removal percentage in spite of temperature change and unexpected power disruption, despite the inconsistency in the statistical analysis. The consistent reduction rate disregarding environmental change demonstrated better environmental resilience among all three media.

This experiment only tested each medium individually. The effectiveness of a mixed media may produce a value added synergy. During the experiment, we lacked microbial insight; thus exploring the microbiology aspect of this study could move the science toward better understanding of the hot spot and/or hot moment mechanisms occurring in the bioreactors.

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Appendix I- SEM results

Original woodchip

Element	*Net Counts*	*K-Ratio*	*Weight %*	*Weight % Error*	*Atom %*	*Atom % Error*
C	3144	0.445	42.10	+/- 0.59	53.42	+/- 0.75
N	0	0.000	0.00	---	0.00	+/- 0.00
O	1785	0.392	41.26	+/- 0.81	39.30	+/- 0.77
Na	8	0.001	0.11	+/- 0.19	0.08	+/- 0.12
Mg	11	0.002	0.16	+/- 0.19	0.10	+/- 0.12
Al	153	0.027	2.45	+/- 0.26	1.39	+/- 0.14
Si	335	0.073	6.44	+/- 0.37	3.49	+/- 0.20
P	0	0.000	0.00	---	0.00	+/- 0.00
S	0	0.000	0.00	---	0.00	+/- 0.00
S	0	0.000	---	---	---	---
Cl	0	0.000	0.00	---	0.00	+/- 0.00
Cl	35	0.000	---	---	---	---
K	10	0.016	1.57	+/- 0.78	0.61	+/- 0.31
K	0	0.000	---	---	---	---
Ca	0	0.000	0.00	---	0.00	+/- 0.00
Ca	0	0.000	---	---	---	---
Mn	0	0.000	0.00	---	0.00	+/- 0.00
Fe	94	0.045	5.91	+/- 1.70	1.61	+/- 0.46
Cu	0	0.000	0.00	---	0.00	+/- 0.00
Total			100.00		100.00	

Post experiment woodchip sample 1 & 2

Element	*Net Counts*	*K-Ratio*	*Weight %*	*Weight % Error*	*Atom %*	*Atom % Error*
C	9110	0.401	40.78	+/- 0.49	59.84	+/- 0.72
N	74	0.007	0.98	+/- 1.16	1.23	+/- 1.46
O	2813	0.192	19.21	+/- 0.49	21.16	+/- 0.54
Na	1252	0.055	5.13	+/- 0.38	3.93	+/- 0.29
Mg	190	0.009	0.81	+/- 0.17	0.58	+/- 0.13
Al	1855	0.101	8.68	+/- 0.29	5.67	+/- 0.19
Si	248	0.017	1.39	+/- 0.16	0.87	+/- 0.10
P	6	0.001	0.04	+/- 0.17	0.03	+/- 0.10
S	18	0.002	0.18	+/- 0.21	0.10	+/- 0.11
S	0	0.000	---	---	---	---
Cl	26	0.005	0.42	+/- 0.29	0.21	+/- 0.14
Cl	145	0.000	---	---	---	---
K	12	0.006	0.55	+/- 0.59	0.25	+/- 0.27
K	0	0.000	---	---	---	---
Ca	0	0.000	0.00	---	0.00	+/- 0.00
Ca	0	0.000	---	---	---	---
Mn	0	0.000	0.00	---	0.00	+/- 0.00
Fe	117	0.017	1.89	+/- 1.26	0.60	+/- 0.40
Cu	2376	0.187	19.94	+/- 0.86	5.53	+/- 0.24
Total			100.00		100.00	

Element	Net Counts	K-Ratio	Weight %	Weight % Error	Atom %	Atom % Error
C	1914	0.119	16.06	+/- 0.32	25.53	+/- 0.51
N	0	0.000	0.00	---	0.00	+/- 0.00
O	4145	0.400	39.43	+/- 0.51	47.05	+/- 0.61
Na	73	0.005	0.43	+/- 0.15	0.36	+/- 0.12
Mg	166	0.011	1.02	+/- 0.15	0.80	+/- 0.12
Al	940	0.072	6.42	+/- 0.23	4.55	+/- 0.16
Si	3017	0.288	25.03	+/- 0.61	17.02	+/- 0.42
P	0	0.000	0.00	---	0.00	+/- 0.00
S	19	0.003	0.28	+/- 0.25	0.17	+/- 0.15
S	0	0.000	---	---	---	---
Cl	25	0.006	0.60	+/- 0.36	0.32	+/- 0.19
Cl	33	0.000	---	---	---	---
K	33	0.024	2.22	+/- 0.88	1.09	+/- 0.43
K	0	0.000	---	---	---	---
Ca	11	0.018	1.64	+/- 1.79	0.78	+/- 0.85
Ca	0	0.000	---	---	---	---
Mn	0	0.000	0.00	---	0.00	+/- 0.00
Fe	261	0.055	6.84	+/- 1.15	2.34	+/- 0.39
Cu	1	0.000	0.02	+/- 0.58	0.01	+/- 0.17
Total			100.00		100.00	

Original biochar

Element	Net Counts	K-Ratio	Weight %	Weight % Error	Atom %	Atom % Error
C	6560	0.552	51.73	+/- 0.51	62.27	+/- 0.62
N	0	0.000	0.00	---	0.00	+/- 0.00
O	2429	0.318	35.28	+/- 0.61	31.89	+/- 0.55
Na	33	0.003	0.28	+/- 0.13	0.17	+/- 0.08
Mg	68	0.006	0.58	+/- 0.15	0.35	+/- 0.09
Al	190	0.020	1.83	+/- 0.20	0.98	+/- 0.11
Si	541	0.070	6.26	+/- 0.44	3.22	+/- 0.23
P	24	0.004	0.36	+/- 0.22	0.17	+/- 0.10
S	0	0.000	0.00	---	0.00	+/- 0.00
S	0	0.000	---	---	---	---
Cl	0	0.000	0.00	---	0.00	+/- 0.00
Cl	103	0.000	---	---	---	---
K	0	0.000	0.00	---	0.00	+/- 0.00
K	0	0.000	---	---	---	---
Ca	0	0.000	0.00	---	0.00	+/- 0.00
Ca	0	0.000	---	---	---	---
Mn	0	0.000	0.00	---	0.00	+/- 0.00
Fe	98	0.028	3.68	+/- 1.28	0.95	+/- 0.33
Cu	0	0.000	0.00	---	0.00	+/- 0.00
Total			100.00		100.00	

Post experiment biochar sample 1 & 2

Element	*Net Counts*	*K-Ratio*	*Weight %*	*Weight % Error*	*Atom %*	*Atom % Error*
C	3325	0.174	22.16	+/- 0.43	33.50	+/- 0.64
N	0	0.000	0.00	---	0.00	+/- 0.00
O	4844	0.393	38.04	+/- 0.47	43.18	+/- 0.53
Na	67	0.004	0.33	+/- 0.11	0.26	+/- 0.09
Mg	152	0.009	0.77	+/- 0.14	0.57	+/- 0.10
Al	1155	0.075	6.56	+/- 0.22	4.41	+/- 0.15
Si	3360	0.270	23.18	+/- 0.55	14.99	+/- 0.36
P	23	0.002	0.21	+/- 0.21	0.12	+/- 0.12
S	0	0.000	0.00	---	0.00	+/- 0.00
S	0	0.000	---	---	---	---
Cl	1	0.000	0.03	+/- 0.50	0.02	+/- 0.26
Cl	49	0.000	---	---	---	---
K	15	0.009	0.82	+/- 0.71	0.38	+/- 0.33
K	0	0.000	---	---	---	---
Ca	0	0.000	0.00	---	0.00	+/- 0.00
Ca	0	0.000	---	---	---	---
Mn	0	0.000	0.00	---	0.00	+/- 0.00
Fe	367	0.065	7.91	+/- 1.03	2.57	+/- 0.34
Cu	0	0.000	0.00	---	0.00	+/- 0.00
Total			100.00		100.00	

Element	Net Counts	K-Ratio	Weight %	Weight % Error	Atom %	Atom % Error
C	1495	0.280	31.71	+/- 0.64	44.93	+/- 0.90
N	0	0.000	0.00	---	0.00	+/- 0.00
O	1215	0.354	35.04	+/- 0.84	37.27	+/- 0.89
Na	1	0.000	0.03	+/- 0.30	0.02	+/- 0.22
Mg	38	0.008	0.67	+/- 0.25	0.47	+/- 0.17
Al	255	0.059	5.09	+/- 0.38	3.21	+/- 0.24
Si	742	0.214	17.95	+/- 0.60	10.87	+/- 0.37
P	2	0.001	0.07	+/- 0.46	0.04	+/- 0.25
S	0	0.000	0.00	---	0.00	+/- 0.00
S	0	0.000	---	---	---	---
Cl	7	0.005	0.46	+/- 0.53	0.22	+/- 0.25
Cl	0	0.000	---	---	---	---
K	3	0.006	0.59	+/- 1.18	0.26	+/- 0.51
K	0	0.000	---	---	---	---
Ca	3	0.014	1.25	+/- 1.67	0.53	+/- 0.71
Ca	0	0.000	---	---	---	---
Mn	0	0.000	0.00	---	0.00	+/- 0.00
Fe	94	0.059	7.14	+/- 1.90	2.18	+/- 0.58
Cu	0	0.000	0.00	---	0.00	+/- 0.00
Total			100.00		100.00	

Original corn cob

Element	Net Counts	K-Ratio	Weight %	Weight % Error	Atom %	Atom % Error
C	7352	0.626	54.78	+/- 0.57	62.53	+/- 0.65
N	54	0.010	1.72	+/- 2.35	1.68	+/- 2.30
O	2517	0.333	40.32	+/- 0.88	34.55	+/- 0.76
Na	32	0.003	0.29	+/- 0.16	0.17	+/- 0.10
Mg	0	0.000	0.00	---	0.00	+/- 0.00
Al	12	0.001	0.13	+/- 0.15	0.07	+/- 0.08
Si	30	0.004	0.37	+/- 0.16	0.18	+/- 0.08
P	0	0.000	0.00	---	0.00	+/- 0.00
S	13	0.003	0.31	+/- 0.28	0.13	+/- 0.12
S	31	0.000	---	---	---	---
Cl	11	0.004	0.42	+/- 0.38	0.16	+/- 0.15
Cl	153	0.000	---	---	---	---
K	2	0.002	0.20	+/- 0.49	0.07	+/- 0.17
K	0	0.000	---	---	---	---
Ca	5	0.010	1.06	+/- 1.06	0.36	+/- 0.36
Ca	0	0.000	---	---	---	---
Mn	0	0.000	0.00	---	0.00	+/- 0.00
Fe	0	0.000	0.00	---	0.00	+/- 0.00
Cu	20	0.003	0.41	+/- 0.43	0.09	+/- 0.09
Total			100.00		100.00	

Post experiment biochar sample 1 & 2

Element	Net Counts	K-Ratio	Weight %	Weight % Error	Atom %	Atom % Error
C	5777	0.305	32.49	+/- 0.56	43.76	+/- 0.75
N	132	0.016	1.98	+/- 1.29	2.29	+/- 1.49
O	5010	0.411	40.67	+/- 0.64	41.12	+/- 0.65
Na	26	0.001	0.13	+/- 0.11	0.09	+/- 0.07
Mg	125	0.007	0.64	+/- 0.12	0.43	+/- 0.08
Al	692	0.045	3.95	+/- 0.18	2.37	+/- 0.11
Si	2045	0.166	14.13	+/- 0.44	8.14	+/- 0.25
P	30	0.003	0.27	+/- 0.19	0.14	+/- 0.10
S	0	0.000	0.00	---	0.00	+/- 0.00
S	44	0.000	---	---	---	---
Cl	0	0.000	0.00	---	0.00	+/- 0.00
Cl	158	0.000	---	---	---	---
K	0	0.000	0.00	---	0.00	+/- 0.00
K	0	0.000	---	---	---	---
Ca	0	0.000	0.00	---	0.00	+/- 0.00
Ca	0	0.000	---	---	---	---
Mn	0	0.000	0.00	---	0.00	+/- 0.00
Fe	257	0.046	5.74	+/- 1.03	1.66	+/- 0.30
Cu	0	0.000	0.00	---	0.00	+/- 0.00
Total			100.00		100.00	

Element	Net Counts	K-Ratio	Weight %	Weight % Error	Atom %	Atom % Error
C	9301	0.551	50.63	+/- 0.48	61.30	+/- 0.58
N	93	0.012	1.92	+/- 1.74	2.00	+/- 1.81
O	3208	0.295	33.67	+/- 0.68	30.61	+/- 0.62
Na	61	0.004	0.35	+/- 0.13	0.22	+/- 0.08
Mg	26	0.002	0.15	+/- 0.11	0.09	+/- 0.06
Al	288	0.021	1.94	+/- 0.15	1.04	+/- 0.08
Si	700	0.064	5.67	+/- 0.32	2.94	+/- 0.17
P	0	0.000	0.00	---	0.00	+/- 0.00
S	24	0.004	0.35	+/- 0.22	0.16	+/- 0.10
S	2	0.000	---	---	---	---
Cl	0	0.000	0.00	---	0.00	+/- 0.00
Cl	205	0.000	---	---	---	---
K	16	0.011	1.04	+/- 0.59	0.39	+/- 0.22
K	0	0.000	---	---	---	---
Ca	10	0.015	1.47	+/- 1.03	0.53	+/- 0.37
Ca	0	0.000	---	---	---	---
Mn	0	0.000	0.00	---	0.00	+/- 0.00
Fe	101	0.020	2.67	+/- 1.03	0.70	+/- 0.27
Cu	9	0.001	0.12	+/- 0.34	0.03	+/- 0.08
Total			100.00		100.00	

Appendix II-24 hour Lachat result compared to probe reading

Label	NO3	NO3-N	Probe
10/28 P IN	21.276	94.25268	92.5
10/28 P WOODCHIP 1	0.296	1.31128	2.8
10/28 P WOODCHIP 2	0.309	1.36887	3.1
10/28 P BIOCHAR 1	0.629	2.78647	3.8
10/28 P BIOCHAR 2	1.078	4.77554	10.8
10/28 P CORN COB 1	0.313	1.38659	7.5
10/28 P CORN COB 2	0.326	1.44418	7.8
11/4 P IN	15.957	70.68951	92.5
11/4 P WOODCHIP 1	0.309	1.36887	2.6
11/4 P WOODCHIP 2	0.417	1.84731	2.3
11/4 P BIOCHAR 1	2.338	10.35734	7.8
11/4 P BIOCHAR 2	4.075	18.05225	16.8
11/4 P CORN COB 1	0.309	1.36887	7.4
11/4 P CORN COB 2	0.311	1.37773	7.1
11/11 P IN	20.778	92.04654	95.1
11/11 P WOODCHIP 1	0.304	1.34672	2.0
11/11 P WOODCHIP 2	0.311	1.37773	2.2
11/11 P BIOCHAR 1	4.808	21.29944	27.7
11/11 P BIOCHAR 2	4.811	21.31273	20.5
11/11 P CORN COB 1	0.311	1.37773	7.8
11/11 P CORN COB 2	0.312	1.38216	7.9
11/18 P IN	17.621	78.06103	96.8
11/18 P WOODCHIP 1	0.291	1.28913	2.2
11/18 P WOODCHIP 2	0.293	1.29799	2.1
11/18 P BIOCHAR 1	1.302	5.76786	15.1
11/18 P BIOCHAR 2	3.856	17.08208	14.7
11/18 P CORN COB 1	0.295	1.30685	6.4
11/18 P CORN COB 2	0.292	1.29356	8.5
11/25 P IN	14.544	64.42992	95.8
11/25 P WOODCHIP 2	0.613	2.71559	1.7
11/25 P WOODCHIP 1	0.294	1.30242	1.8
11/25 P BIOCHAR 1	6.996	30.99228	22.6
11/25 P BIOCHAR 2	6.279	27.81597	22.4
11/25 P CORN COB 1	0.293	1.29799	5.6
11/25 P CORN COB 2	0.288	1.27584	7.8
12/2 P IN	21.441	94.98363	92.7
12/2 P WOODCHIP 1	0.287	1.27141	1.9
12/2 P WOOD CHIP 2	0.29	1.2847	2.1
12/2 P BIOCHAR 1	1.633	7.23419	17.5
12/2 P BIOCHAR 2	1.594	7.06142	28.8
12/2 P CORN COB 1	0.293	1.29799	7.2
12/2 P CORN COB 2	0.292	1.29356	8.2
12/9 P IN	13.542	59.99106	96.1
12/9 P WOODCHIP 1	0.3	1.329	2.2
12/9 P WOODCHIP 2	0.288	1.27584	2.7
12/9 BIOCHAR 1	5.234	23.18662	41.5
12/9 P BIOCHAR 2	5.213	23.09359	40.9
12/9 P CORN COB 1	0.292	1.29356	5.6
12/9 P CORN COB 2	0.291	1.28913	8.4
12/16 P IN	21.376	94.69568	97.3
12/16 P WOODCHIP 1	0.343	1.51949	
12/16 P WOODCHIP 2	0.542	2.40106	1.8
12/16 P BIOCHAR 1	11.72	51.9196	42.9
12/16 P BIOCHAR 2	8.082	35.80326	33.7
12/16 P CORN COB 1	0.304	1.34672	5.4
12/16 P CORN COB 2	0.296	1.31128	8.5