Nitrate Reduction and Phosphate Uptake in Lab-Scale Denitrifying Bioreactors with Woodchip and Biochar Media

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Siobhan Powers
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Abstract

High nutrient loads in agricultural-field-adjacent waterways results in ecosystem-debilitating hypoxic zones. One potential solution for this problem is a denitrifying bioreactor, which intercepts the high nutrient (usually phosphate and nitrate from fertilizer) water from artificial drainage. This study aimed at modeling a denitrifying bioreactor in a laboratory setting, with woodchip media acting as a carbon source, and further amended by biochar in some of the experimental reactors. Water spiked with nitrate and phosphate was fed through the reactors and the concentrations were measured at both the inflows and outflows. The predicted pathway for nitrogen reduction was denitrification and the phosphate was expected to be adsorbed by the biochar. Both woodchip and biochar-mix (10% biochar, 90% woodchip by mass), had levels of nitrogen reduction above 30% with an inflow of about 5 mg Nitrate-N/L, with the biochar-mix achieving higher levels of reduction than the plain woodchip media. The phosphate uptake was no different between the woodchip and biochar-mix bioreactors across various inflow concentrations, however these influents were higher than intended for the purpose of this study. Thus, in these experiments, both woodchip and woodchip-biochar mix bioreactors decreased the nitrate concentration between inflow and outflow, but with different pathways- denitrification for woodchip and most likely adsorption for biochar-mix. Phosphate adsorption did not occur more within the biochar-mix reactors. In this study, though biochar as a bioreactor amendment was not useful in enhanced phosphate concentration reduction, its presence was indicative of higher nitrate removal so an in-situ bioreactor might benefit from subsisting of both biochar and woodchips to decrease the probability of eutrophication in adjacent waters.

Background

Agricultural Drainage Concerns

Manmade subsurface agricultural drainage allows the improvement in productivity of otherwise wet, poor quality soils throughout the world, by improving both management conditions and land properties (Skaggs and van Schelfgaarde,

1999). These practices, however, have led to water quality concerns on both local and global scales, particularly with increased nitrogen and phosphorus loads, the main sources of which are corn and soybean crops and pasture and range land uses, respectfully, (Figure 1, USGS, 2013). Although artificial drainage has been employed by farmers for over a century, in recent years high phosphorus and nitrogen loads in water have led to eutrophication and subsequent hypoxic zones. One example of great concern is in the Gulf of Mexico (Figure 2, NASA, 2007). These are sites of low dissolved oxygen concentrations, which lead to stress or death of organisms present in the ecosystems these zones affect (USGS, 2000). In 2011, the Gulf of Mexico's hypoxic zone was measured to be 6,770 square miles, roughly the size of New Jersey (USEPA, 2011).

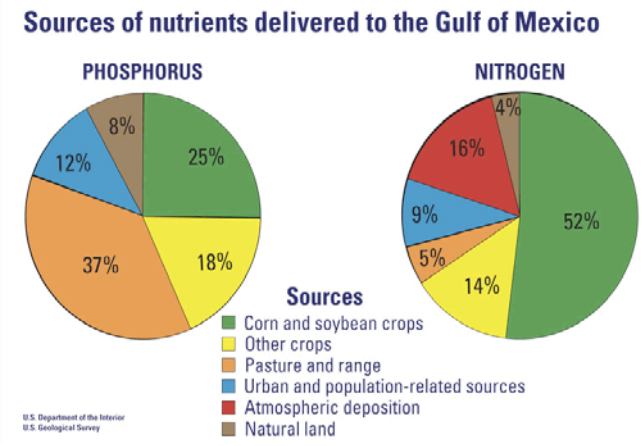


Figure 1. These pie charts, courtesy of the USGS, depict the main sources of both phosphorus and nitrogen that are delivered to the Gulf of Mexico through the hydrological network of the Mississippi Basin. (USGS,2013).

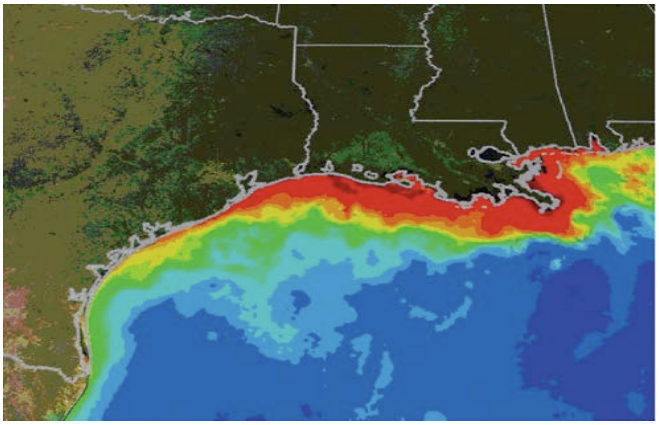


Figure 2. The figure above is a satellite image of the Gulf of Mexico taken by NASA in the summer. Red and orange represent high concentrations of phytoplankton, indicative of high nutrient concentrations of phosphate and nitrate, and river sediment (NASA, 2007).

According to Howarth and Marino (2006), there is a consensus among researchers that in the United States "nitrogen represents the largest pollution problem in the nation's coastal waters". Goolsby and others (1999) acknowledge that an increasing supply of nitrogen, particularly nitrate, to the Mississippi basin is the principal cause of the growth of the hypoxic zone in the Gulf of Mexico, and, further show that commercial fertilizer is becoming an increasingly dominant source of nitrogen, with its specific annual nitrogen load growing six-fold since the 1950's (Goolsby et al., 1999). In addition to nitrate, phosphorus has been acknowledged as a limiting factor for phytoplankton in water bodies (Sylvan et al., 2006). Though total annual loads of nitrogen to the Gulf of Mexico decreased by 21% between 2001 to 2005, phosphorus increased by 12%, with the hypoxic zone's area over five times higher than the EPA's goal, specified in the 2001 Action Plan. Phosphorus is acknowledged as the leading contributor to eutrophication in many locations, including, on a large scale, the surface waters of Minnesota and, in an area more local to this study, Lake Oneida, NY, where algae growth led to the lake being labeled as an

impaired water body in 1998 under the Clean Water Act, until the recognition of reduced phosphorus loads allowed the proposal of this label to be lifted in 2008 (USEPA, 2008).

In 2008, the EPA Science Advisory Board outlined a plan to improve water quality, specifically in the Mississippi River Basin, and reduce total nitrogen as well as total phosphorus loads to the Gulf via the Mississippi River by 45% each, relative to the average loads from 1980-1996 (USEPA, 2007). On a national scale, a common standard is such that nitrate concentrations in water bodies should not exceed the drinking water standard of 10 mg NO_3^--N/L with standards for phosphorus varying by state and water body but usually less than 1 ppm (USEPA, 2012). The EPA's recommendations to avoid eutrophication are specific to certain ecoregions and for the region that includes Ithaca, NY, these values are 0.54 mg total nitrogen(TN)/L and 33.0 µg total phosphorus(TP)/L (USEPA, 2002). Even with improved in-field management strategies, drainage waters can still have nitrate concentrations above the drinking water standard depending on precipitation timing and soil organic matter mineralization (Randall and Goss, 2001). Phosphate concentrations just slightly higher than average can lead to dramatic changes within a body of water due to its status as a limiting factor (Mainstone and Parr, 2002). Thus, looking beyond in-field management strategies, a favorable method for decreasing nitrate and phosphate leaching from artificially drained fields would be one that intercepts water from the drainage before it reaches adjacent water bodies.

Denitrifying Bioreactors

A denitrifying bioreactor has the capability of treating nitrate leaching from agricultural fields before it reaches adjacent streams, thus limiting the amount of nitrate in the water and decreasing the presence of the previously mentioned hypoxic

zones. These bioreactors decrease nitrate via the denitrification process, which also occurs in soil, although less so where artificially drained because of rapid transport and a decrease in time of drainage water retention (Kellman, 2005). Denitrification, the reduction of NO_3^- to N_2, has several requirements to proceed, described further in the following paragraphs. The chemical equation for the denitrification process for the purpose of this study is displayed below in Equation 1.

$$5C + 4NO_3^- + 2H_2O \rightarrow 2N_2 + 4HCO_3^- + CO_2 \qquad Equation\ 1$$

This mechanism requires electron acceptors in the form of nitrogen oxides (e.g. NO_3^-, NO_2^-, NO, N_2O), denitrifying bacteria, an electron donor (carbon source), and suitable dissolved oxygen (DO) conditions. The limiting DO level varies among denitrifying organisms, but DO concentrations as low as 0.2 mg/L have been found to inhibit denitrification (Korom, 1992). Bacteria use oxygen to oxidize the available carbon in saturated conditions, however, when oxygen concentrations become limiting, facultative anaerobes utilize NO_3^- as electron acceptors in their respiration electron transport chain. Denitrifying bacteria are a multifarious group, made up of mostly facultative anaerobes, and with most denitrifiers being heterotrophic (Korom, 1992). Denitrification produces dinitrogen gas, carbon dioxide, and biocarbonate (Eq. 1). The bicarbonate is indicative of an increase in alkalinity and an increase in solution pH (Metcalf and Eddy, 2003). Because of molecular triple bonds, N_2 is stable, however, under certain conditions (specifically, low pH, low temperature, high DO, and low carbon to nitrogen ratio) nitrous oxide, a greenhouse gas 310 times more impactful than carbon dioxide per unit weight over a 100-year period, can be produced (Chapin III et al., 2002; USEPA, 2010).

Adding solid carbon increases the amount of carbon available for denitrifiers and also encourages aerobic respiration to reduce DO, thus allowing denitrification to proceed more efficiently (Schipper et al., 2005). This solid carbon source has been found to be effective in the form of fine and coarse wood media (Christianson, 2011), but biochar has been found to lead to a similarly significant, if not as established, nitrate removal in bioreactors (Christianson et al., 2011).

Bioreactors with amendments besides wood-based media (i.e., iron slag) have resulted in considerable phosphate removal from treated septic tank effluent and streams via simple sorption to the media (Baker et al. 1998, Robertson 2000, McDowell et al, 2008, Chen et al., 2011). Wood-based bioreactors have had little success thus far in phosphorus removal rates (Robertson et al., 2005; Jaynes et al., 2008; Schipper et al., 2010).

In practical applications, a bioreactor is a buried trench filled with carbon media that intercepts drainage from a site, most commonly an agricultural plot, but allows excess flows to bypass in high flow events. They are passive systems to ensure cost-effectiveness and few operation and maintenance concerns. This study aims at evaluating the reduction of nitrate-N between inflow and outflow of a lab-scale denitrifying bioreactor to predict how an in-field version may perform over a variety of inflow NO_3^--N concentrations, and how the nitrate- N reduction varies between bioreactors with just woodchip media and those with biochar combined with woochips at a 1:9 ratio of biochar to woodchips by mass. Also, this study aims at determining a difference between reduction in total phosphorus between inflow and outflow of a wood-based bioreactor versus one mixed with biochar.

Figure 3. The images above are various depictions of a woodchip denitrifying bioreactor from the initial development stage (top left) to the finished project in the bottom right corner (South Dakota Corn, 2013).

Methods

Lab-Scale Bioreactor Design Characteristics

Six lidded 12.5- gallon plastic containers were used to hold the carbon source and act as the denitrifying bioreactors. They were each 23 cm (9 in) deep, with the bottom of the containers slightly smaller than the top in terms of area, with dimensions approximately equal to 66 cm by 46 cm by 23 cm (26 in by 18 in by 9 in), for a total volume of about 60 L. Three of the bioreactors (LW1, LW2, and LW3) were filled solely with 8.00 kg of woodchips, occupying the trays to about 5 cm (2 in) of the top. The other three trays were filled with 7.2 kg of woodchips and 0.80 kg of biochar (LB1, LB2, and LB3), mixed evenly throughout the reactors, also filled to about 2 in of the top. The lids were then sealed to the trays to facilitate anaerobic conditions within the bioreactors. The inflow and outflow (inflows are black spigots in white

lidded tubs in Figure 4) were about 4 cm (1 ½) in. off the bottom edge on the smaller sides of the standing bioreactors, sized to tightly fit 3/8 in-diameter tubing.

Figure 4. The image above shows the lab scale denitrifying bioreactors connected to the inflow feed tubes, which had their pump rates controlled by the blue pumps.

The inflow was controlled via two Masterflex™ peristaltic pumps, each with 3 heads to feed the six bioreactors, at a rate of about 100 mL/min to maintain a hydraulic retention time (Eq. 2) of about 6 hours, which is comparable to the residence time of flow from agricultural drainage in a field-scale bioreactor. The volume of water in the bioreactor was estimated by multiplying the total volume by an approximated woodchip porosity of 0.6.

$$Hydraulic\ Retention\ Time = \frac{Bioreactor\ Volume}{Flow\ Rate\ (\frac{Volume}{Time})} \qquad Equation\ 2$$

Sampling Procedure

The bioreactors each underwent four identical runs, each run characterized by a different concentration of NO_3^--N and total phosphorus (TP) (in the form of potassium nitrate and potassium phosphate in tap water) in the inflow. All of the inflows were conducted at loads comparable to those seen in drainage outflow, both below and above the EPA's recommended standards. Run 1 was carried out with an influent concentration of 5 mg NO_3^--N /L and 0.05 mg TP/L, Run 2 with 10 mg NO_3^--N /L and 0.1 mg TP/L, Run 3 with 15 mg NO_3^--N /L and 0.2 mg TP/L, and Run 4 with 20 mg NO_3^--N /L and 0.5 mg TP/L. LW1 and LB1 were fed by the same supply, for this study called I1, as were LW2 and LB2 (supplied by I2) and then LW3 and LB3 (supplied by I3). Samples of the influent were taken at the beginning of each run, and at the 12-hour mark, when the influents had to be replenished with the same concentrations as the initial feed for the run. It was assumed that with two bed volumes run through, the bioreactors would reach a steady state in terms of denitrification process, with percent reduction in nitrate-N and total phosphorus maintaining relatively constant after this amount of time and also reaching its maximum reduction potential. Therefore, from each reactor, outflow samples, each of about 100 mL, were taken after 12 hours, or two full residence times, after the beginning of each run and then once each hour for six hours until the overall run-time reached 18 hours. At this point, the bioreactors were flushed with tap water from an inflow supply labeled I4, with outflow samples taken every hour for six hours, or one full retention time, before the next run was started through the now "clean" reactors. The trace chemical used was potassium as it was present from both potassium nitrate and potassium phosphate in the initial concentrations that characterized each run.

Testing Procedure

Part of each water sample was immediately tested for temperature, pH, and conductivity using a pH/ion/conductivity meter. Then, the samples were filtered and refrigerated for less than 48 hours for NO_2/NO_3^--N analysis. These concentrations were found through ion chromatography. Total phosphorus levels were found via inductively coupled plasma atomic emission spectroscopy (ICP-AES), which can determine elements at low concentrations by determining the wavelengths emitted by the sample and their intensities.

Results and Discussion

Nitrogen

Though the procedure was carried out as designed, several mechanical problems limited the nitrogen results to the 5 ppm initial concentration. Thus, the reactors' performance across different inflow concentrations could not be assessed. Because of measurement error (most likely due to an underestimation of the amount of water in the inflows' supplies), the inflow concentrations to the reactors actually averaged around 6 mg NO_3-N/L. I1 had an initial inflow concentration of 6.16 mg NO_3-N/L at time 0, and then 6.17 mg NO_3-N/L after a new inflow had to be supplied after 12 hours. I2 supplied a concentration of 5.90 mg NO_3-N/L for the first twelve hours, then 5.89 mg mg NO_3-N/L for next six hours of the run. I3 provided an inflow with 5.78 mg NO_3-N/L for twelve hours, and then 5.79 mg NO_3-N/L. Across the triplicate bioreactors, there were dissimilarities between measured values, however, the overall steady-value relationship was similar, with both woodchip and biochar-mix varieties reaching steady states. Within the woodchip bioreactors, an average nitrate removal of 32.1% was observed, while in the biochar-mix bioreactors, a noticeably higher average nitrate removal of 42.7% was measured. At every hourly measurement, the

biochar outperformed the woodchip bioreactors in % nitrate removal, as evident in the following figure.

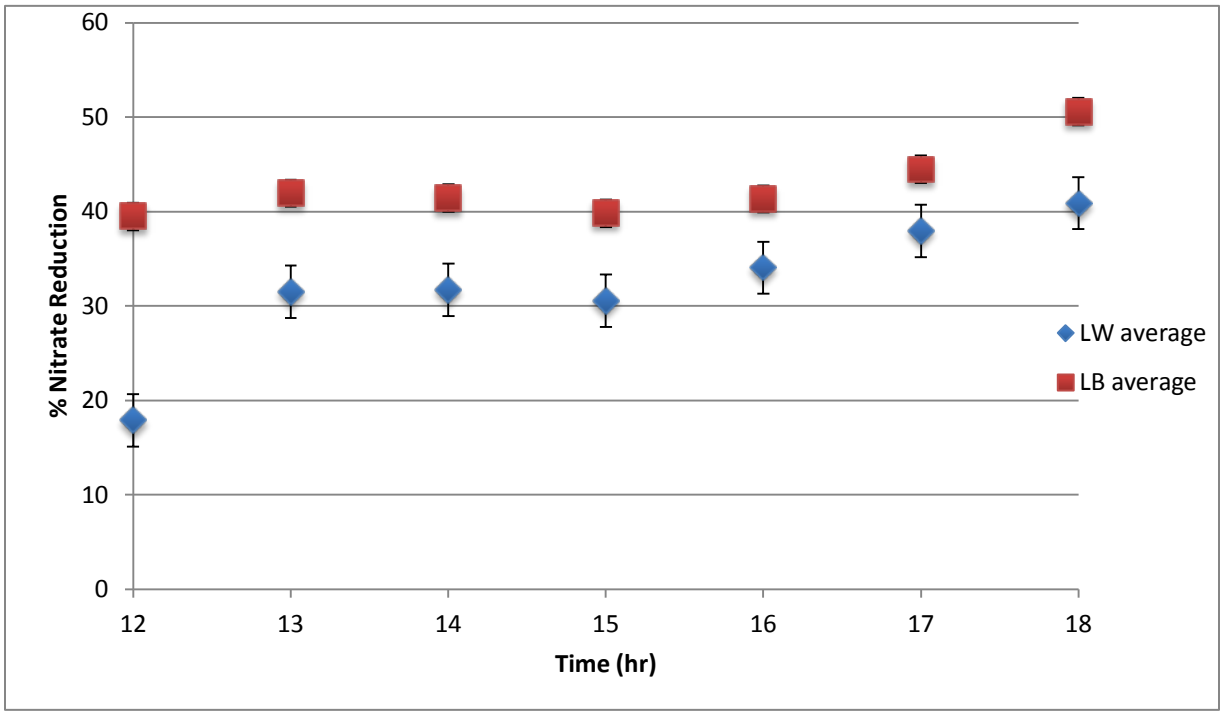
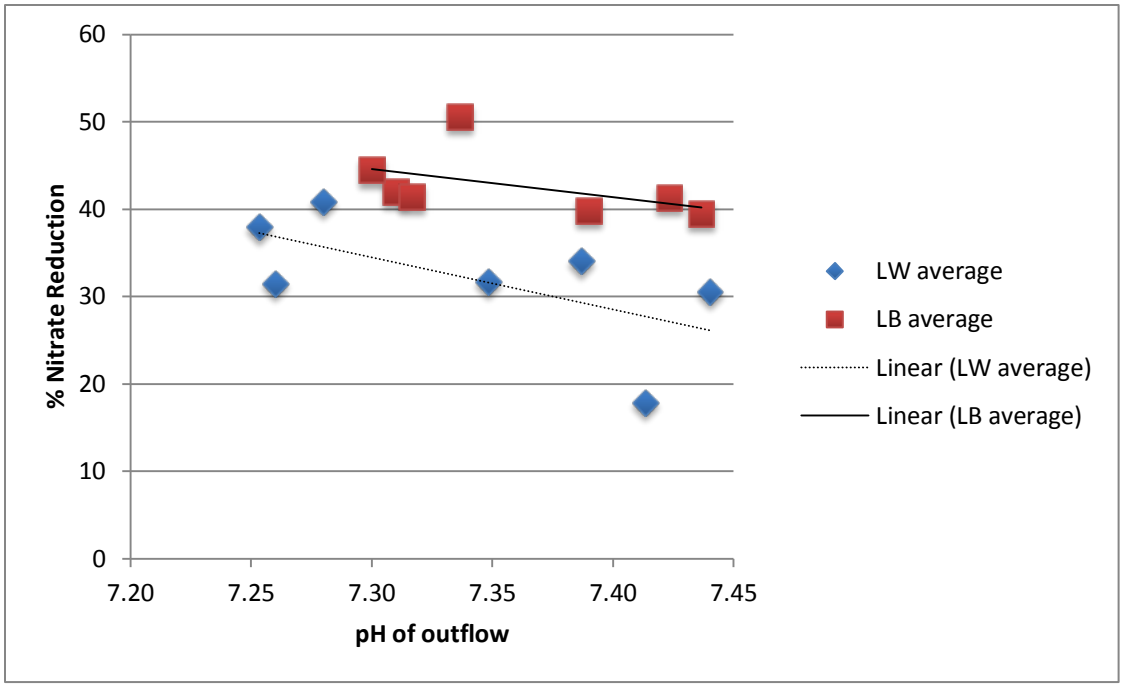


Figure 5. Nitrate reduction over time in woodchip and woodchip-biochar (10% biochar, 90% woodchip) mix reactors is depicted above. The blue diamonds represent the % nitrate reduction in lab-scale woodchip bioreactors (LW) for the specific run time, while the red squares represent the same for the lab-scale biochar-mix (LB). The black lines, barely visible in the biochar-mix values, represent the standard error for each measurement. Though both (LB and LW) measure somewhat steady and significant reductions over time, the biochar-mix reactors outperform their woodchip counterparts across every measurement and are statistically different as shown by their standard errors not intersecting.

If the main pathway for nitrate loss was denitrification by microbes, as previously mentioned (Equation 1), then the outflow would be more acidic than the inflow due to a heightened level of carbonic acid. A moderate relationship can be seen between outflow pH and nitrate reduction in the woodchip bioreactors, but not in the

biochar bioreactors, which shows that denitrification is probably not the main pathway for nitrate reduction in those reactors (Figure 6). Nitrite was usually higher in the biochar reactors than the woodchip reactors, indicative of less denitrification in the biochar-mix, however this relationship was not always observed (Figure 10 in the Appendix). The method of comparing pH to % reduction may not be very indicative of denitrification in biochar because of how basic the substance tends to be.

Figure 6. The relationship between pH and % nitrate reduction at a particular time for both the lab-scale



woodchip bioreactors as well as the 10% biochar, 90% woodchip mixed reactorsis shown above. The woodchip reactors (blue diamonds with dotted trendline) seem to have a lower outflow pH associated with higher nitrate reductions, while the biochar-mix bioreactors (red squares and normal trendline) do not display this relationship and seem to have a relatively constant nitrate reduction across pH's. Variation among woodchip nitrate reduction was shown previously in the error in Figure 5.

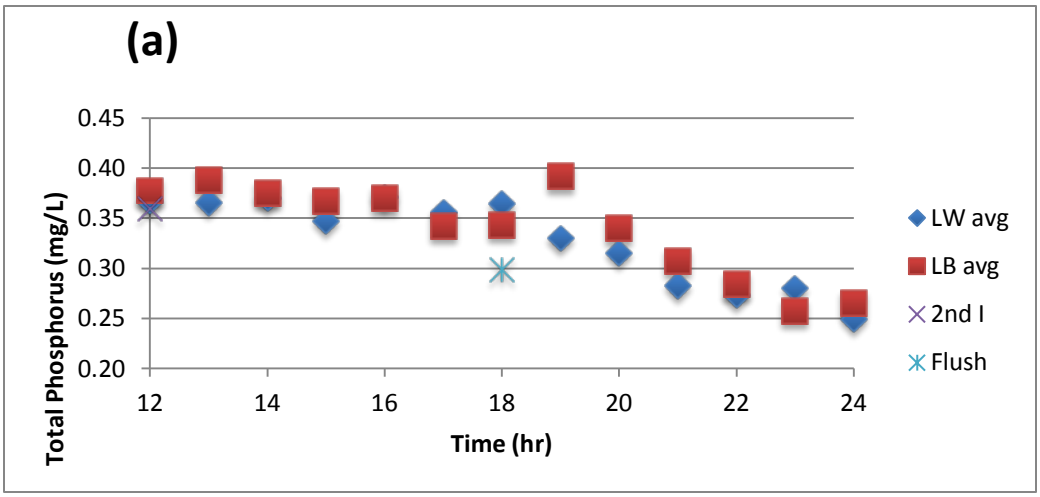
Phosphorus

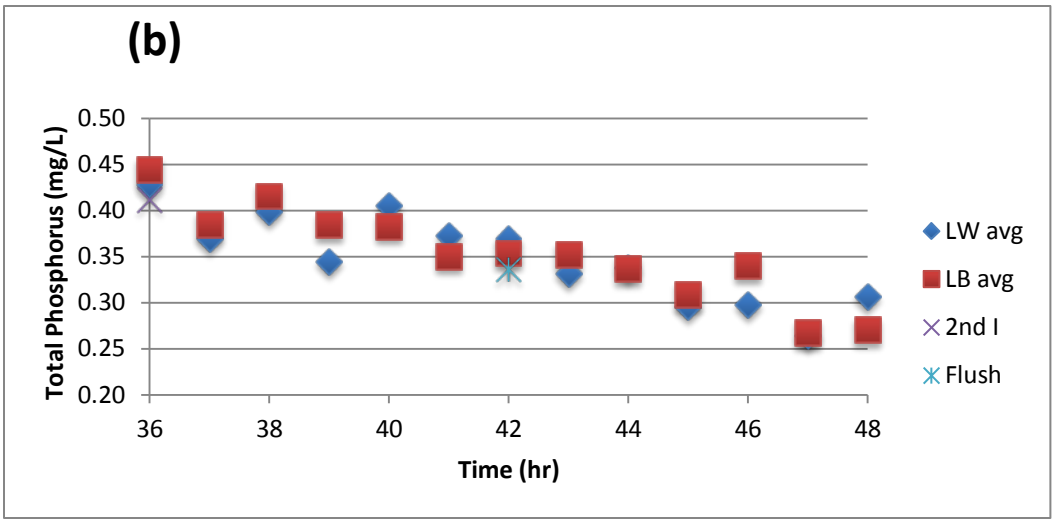
The phosphorus results were more successful in terms adhering to our experiment design. Phosphorus levels were higher than expected, as there was some phosphorus already present in the tap water used in the inflows as well as the flushes. Thus, due to this oversight as well as an over-measurement previously mentioned within the nitrogen results, the actual inflow concentration measurements are displayed in Table 1.

Table 1. Actual inflow concentrations as related to their intended counterparts are displayed for each run time. Every 12-hour actual inflow concentration measurement is marked by a decrease with respect to the actual inflow concentration at the initial run time.For Run 2 this corresponds to a low I2 measurement, but for the others an over-dilution at this time.

	Intended Inflow Concentration (mg TP/L)	Actual Inflow Concentration (mg TP/L)
Run 1 initial	0.05	0.41
Run 1 hour 12	0.05	0.36
Run 1 flush	0.00	0.30
Run 2 initial	0.10	0.48
Run 2 hour 12	0.10	0.41
Run 2 flush	0.00	0.34
Run 3 initial	0.20	0.84
Run 3 hour 12	0.20	0.69
Run 3 flush	0.00	0.30
Run 4 initial	0.50	0.57
Run 4 hour 12	0.50	1.05

The outflows approached a steady state in the case of the first run (Figure 7a), considering the inflows were not as varied as with the other runs, especially runs 3 and 4. There was no difference in outflow total phosphorus concentration between the woodchip and biochar-mix reactors although the biochar reactors were expected to facilitate phosphate sorption. There were lower levels of total phosphorus in the outflows than the influents, but the actual sorption is difficult to quantify with the measured differences between the inflow concentrations of phosphorus across each run.





Figures 7a and 7b. Total phosphorus in outflow of woodchip (blue diamonds) and biochar-mix (red squares) bioreactors for Runs 1 (a) and 2(b), at the intended lowest influent concentrations. A steady state has been achieved before hour 12 of the run, which is maintained until hour 18, and then the concentration drops when the "flush" begins. 2nd I (purple X) is the inflow concentration at the 2nd batch of mix in hour 12 of the run and the flush (blue I interlaced with an X) at hour 18 of each run is the concentration of the inflow during the tap water flush.

Over all of the runs, there was little difference in outflow total phosphorus concentrations between the woodchip and biochar bioreactors. Some dissimilarities were noticeable in the final two runs, however, the data for these runs were noisier and the patterns were less established than in the first two runs, so inferences about the dissimilarities of the total phosphorus concentrations in the outflow between the woodchip and biochar-mix bioreactors are inconclusive.

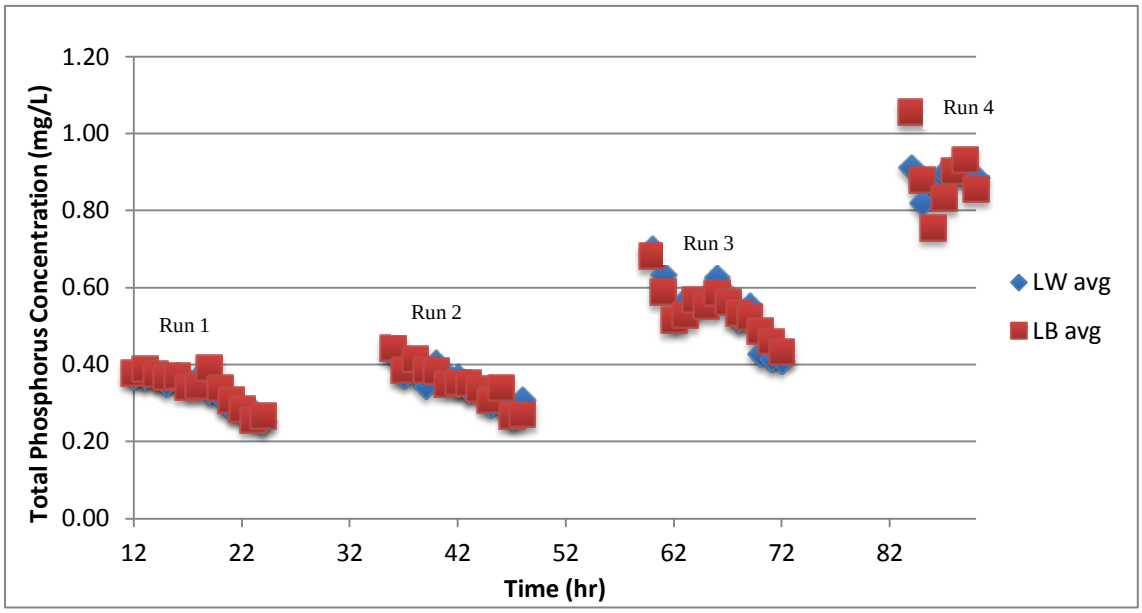


Figure 9. Outflow phosphorus concentrations from woodchip (blue diamond) and biochar-mix(red squares) bioreactors over time. Each of the four runs is characterized by their own influent concentration, which increased with each run. The spaces between data (i.e. hours 24-36) are indicative of a new run, and no measurements were taken while the bioreactor was assumed to be reaching steady state. Run 4 did not have a flush, unlike the other three.

Conclusion and Further Research

Although the nitrate reduction results were interesting, there were not enough data to conclude a difference between the woodchip and biochar-mix across several concentrations. At the low concentration of 6 mg NO_3^--N/L, the lab denitrifying bioreactors with 10% biochar by mass outperformed the bioreactors with only

woodchips. This difference in performance was possibly not due to the anaerobic denitrification process, which would have made the water more acidic, but another process. This active process would not necessarily produce more nitrite, as that did not show a particular trend across the times measured. With more data, a better relationship might be established between media type and nitrate reduction, perhaps across several influent concentrations as intended in this study, or across various retention times.

Phosphate adsorption did not take place more effectively in the biochar-amended bioreactors as expected. This could be due to the biochar composition, so in the future, biochar with varied biomass feedstocks could be used as bioreactor amendments and how sorption varies across those types of biomass can be identified. Further research should be done with deionized water because of the levels of phosphate in some tap water, as evident in this study. Lower concentrations of influent phosphate should be used as the levels discussed in this study were an order of magnitude higher than what is usually found in agricultural settings. The phosphate levels should also be measured and diluted evenly in a similar study setting as this so as to limit noise in the data. Denitrifying bioreactors have the potential to reduce nitrate loads based on this study, however, further research must go into their capabiity for uptaking phosphorus. Well-employed bioreactors with woodchips and biochar could make a more economical, sustainable impact on the agricultural industry and the environment.

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Appendix

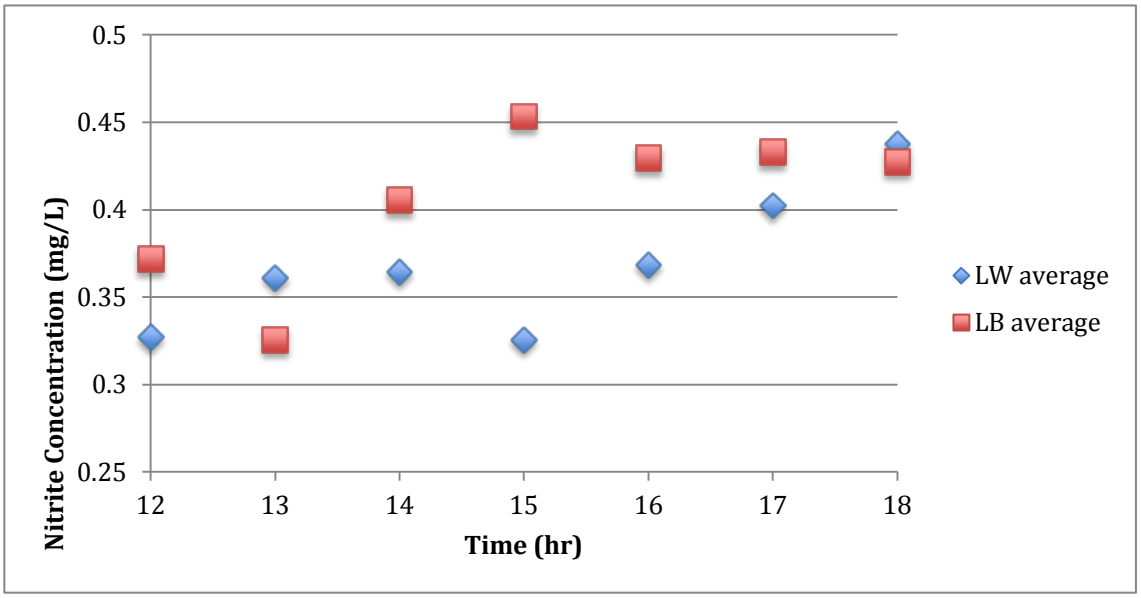

Figure 10. Comparison of outflow nitrite concentrations in woodchip (blue diamonds) and woochip-biochar mix (red squares) reactors for the first run. There was no nitrite present in the inflow. Nitrite was higher in the biochar reactors, but not always. This seems indicative that the woodchip bioreactors had more denitrification processes occurring in them, leading to the lower nitrite output.

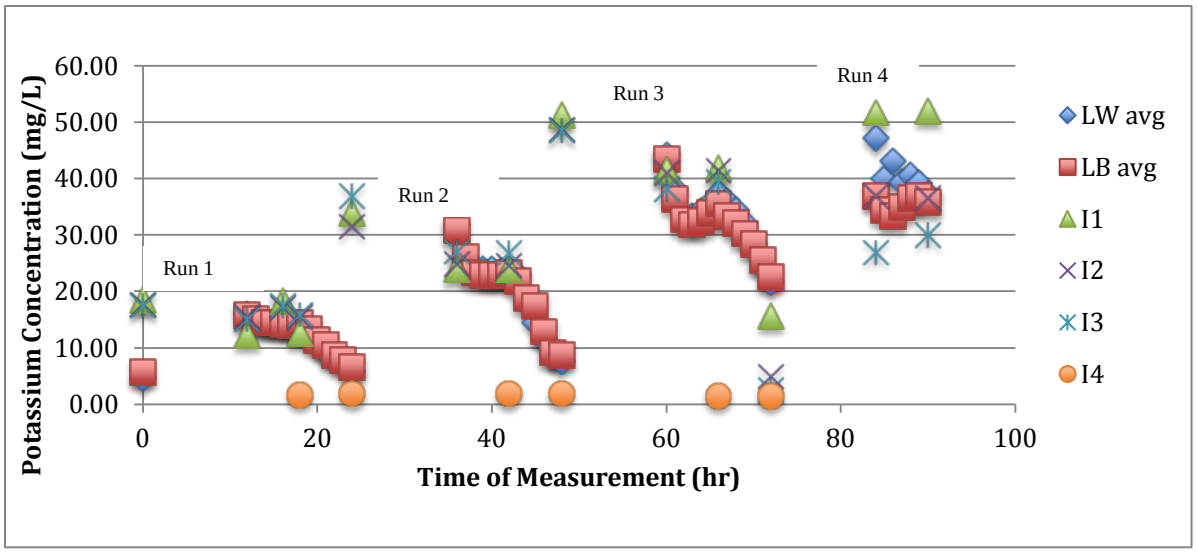

Figure 11. Potassium concentrations into and out of the woodchip(blue diamond) and biochar-mix (red square) bioreactors over time. Inflow concentrations for each supply (I1-green triangle, I2-purple X, I3-blue X interlaced with I, I4- orange circle) were taken at the beginning and near the end of each run. Potassium was used as the tracer chemical and was used to find the source of variability in phosphorus and nitrogen data.