

COLUMN FILTER STUDIES: PHOSPHORUS REMOVAL USING
BIOGENIC IRON OXIDES

By

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Abstract

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Phosphorus is recognized as the limiting nutrient for aquatic plant growth. When present in excess, phosphorus stimulates algal growth and the subsequent decay of organic matter consumes oxygen leading to hypoxia. Hypoxic conditions have detrimental effects on water quality and have led to increasingly stringent phosphorus limits. Among a wide variety of iron-rich substrates that have been investigated as phosphorus sorbents, biogenic iron oxides have been used as a novel substrate for both nutrient and metals removal (Rentz, Turner et al. 2009). These biogenic iron oxides perform similarly, if not better, than other natural and engineered iron-oxide substrates (Rentz, Turner et al. 2009). The average sorption (Γ_{\max}) for all samples was 15.3 ± 6.3 mg P/g solids, ranging from 6.2 – 25.4 mg P/g solids. Phosphorus sorption kinetics were rapid, removing sixty-five percent after just one hour and eighty percent after three hours. Flow-through filters represent an advanced wastewater treatment (AWT) process that is currently being investigated as a technology implemented to meet effluent phosphorus limits. This research utilized biogenic iron oxides as a filter substrate in flow-through filter columns using an upflow regime. Flow regime and flowrates were adjusted in several preliminary experiments in order to optimize the filter design. The upflow filters removed $84 \pm 16\%$ of the

phosphate compared to $53 \pm 21\%$ for the downflow filter and the low flow column removed $85 \pm 7\%$ compared to $57 \pm 11\%$ for the high flow column. When ion oxides were treated with detergents, the column filters achieved effluent concentrations of less than 0.2 mg/L for over 200 hours (greater than 90% removal). These column filter experiments showed reproducible results (standard error less than 20% for all columns) and the capability to remove phosphorus.

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1.0 INTRODUCTION

1.1 Phosphorus Pollution

Water pollution by nutrients is a historical and ever-growing concern in developed and developing countries alike. In excess, nutrients like nitrogen and phosphorus can lead to eutrophication in water bodies. Often, phosphorus is the limiting nutrient for aquatic plant growth, stimulating algal growth when present in sufficient concentrations. As algae decays, dissolved oxygen is consumed, leading to hypoxic conditions. Phosphorus remains a critical water-body pollutant, a classification which has led to more stringent treatment regulations (Washington Department of Ecology, 2004). Frequently, these effluent regulations require phosphorus concentrations that are lower than the current wastewater treatment plant (WWTP) phosphorus removal capabilities, creating a need to identify novel nutrient removal methods.

Phosphorus enters the environment through a variety of well characterized pathways (Figure 1). Point-source pollution is a quantifiable source and typically enters the environment through municipal and industrial WWTPs. Human waste, detergents, and industrial processes are the primary phosphorus sources treated at WWTPs (Wind, 2007). Non-point source (NPS) pollution is more difficult to quantify and includes agricultural and urban runoff. Agricultural industries use phosphorus in fertilizer and feed supplements which enter surface water through runoff, erosion, and drainage (Wind, 2007). There is currently not a phosphorus removal mechanism that treats both point-source and non-point source pollution.

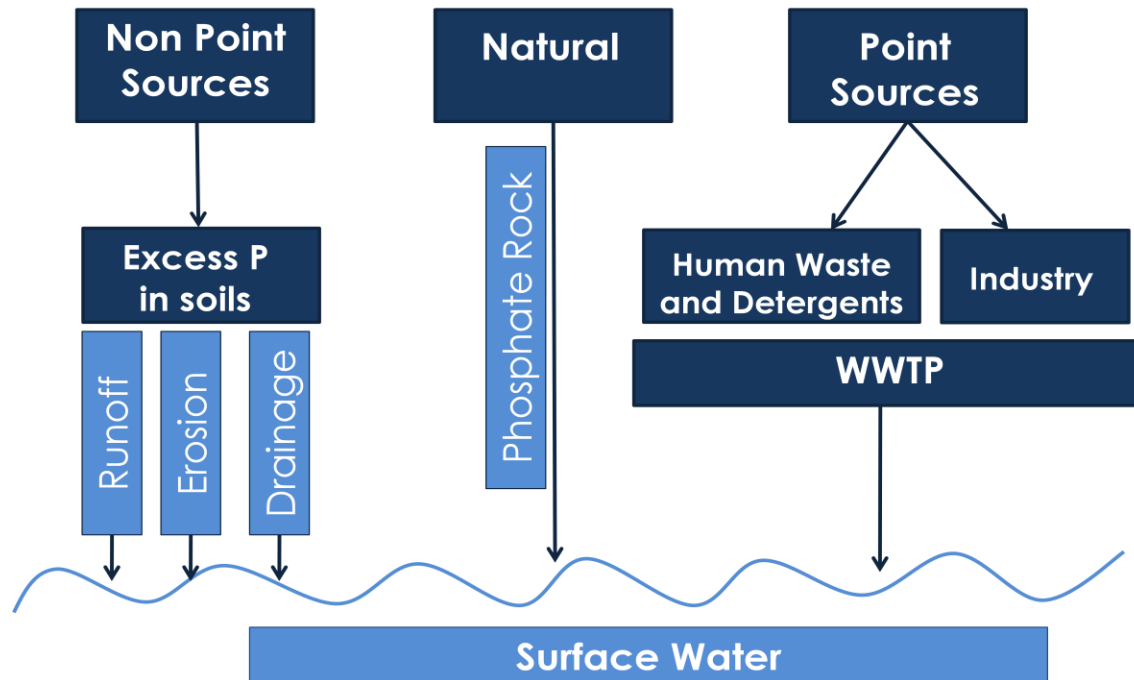


Figure 1: Phosphorus sources and their pathways to surface water (adapted from Wind, 2007)

Phosphorus Removal

Currently, phosphorus removal at WWTPs occurs in one of three ways: chemical precipitation, biological processes, or filtration (Morse, Brett et al. 1998; de-Bashan and Bashan 2004; Boujelben, Bouzid et al. 2008). Chemical precipitation involves the addition of metal salts, like ferric chloride or alum. These salts combine with phosphorus to form insoluble metal phosphates, which are subsequently removed through filtration (Morse, Brett et al. 1998; de-Bashan and Bashan 2004). Enhanced biological phosphorus removal (EBPR) utilizes bacterial phosphorus uptake during alternating anaerobic and aerobic stages (Morse, Brett et al. 1998; de-Bashan and Bashan 2004). EBPR stimulates bacteria that accumulate inorganic polyphosphate as a part of their cells (de-Bashan and Bashan 2004). Each of these processes has advantages and disadvantages. Chemical precipitation is a process with simple installation and operation while yielding high phosphorus removal. The disadvantages of chemical precipitation are the high chemical costs and significant sludge production (Morse, Brett et al. 1998). EBPR does not

require chemicals but is a more complicated system to install. While EBPR can remove 80% to greater than 90% of phosphorus, the technology is inconsistent (Morse, Brett et al. 1998; de-Bashan and Bashan 2004).

Filtration has been identified as a key mechanism for phosphorus removal. Historically, sorption-based filtration has been used as a treatment technique (Westholm 2006). It is now understood that substrates can be chosen for their ability to adsorb pollutants like phosphorus. Morse et al. (1998) identified limited sludge production and unaffected wastewater pH as potential advantages. Additional research is necessary to identify adsorbents that are capable of phosphorus removal below current EPBR effluent limits.

1.2 Iron Oxides

Iron oxides have a high capacity for P removal, and therefore have been a recent research focus. Substrates that have been evaluated include iron oxide coated crushed brick (Boujelben, Bouzid et al. 2008) as well as both naturally and synthetically coated sand (Boujelben, Bouzid et al. 2008). Recycled substrates such as iron oxide tailings (Zeng, Li et al. 2004) and iron-loaded orange waste (Biswas, Inoue et al. 2007) are also being tested. Most recently, biogenic iron oxides were identified as a substrate with phosphorus removal potential (Rentz, Turner et al. 2009).

Biogenic iron oxides have been used as a novel substrate for phosphorus removal (Rentz, Turner et al. 2009). These biogenic iron oxides perform similarly, if not better, than other natural and engineered iron-oxide substrates (Rentz, Turner et al. 2009). Previous research achieved maximum sorption normalized to total solids ranging from 10.8 ± 0.7 to 39.9 ± 3.2 mg P/g. It is understood that the biogenic iron oxides have the capability to remove P from solution. Further biogenic iron oxide research is required to determine if they are a viable substrate for flow-through filter technology.

Iron-oxidizing bacteria significantly affect iron-cycling in the natural environment where they occupy specific niches. The bacteria are microaerophilic, requiring extremely low oxygen concentrations, and they inhabit circumneutral pH environments (Neubauer, Emerson et al. 2002; Emerson and Weiss 2004; Weiss, Rentz et al. 2007). Iron-oxidizing bacteria are found at the oxic/anoxic interface, typically where anoxic sediments meet oxygenated waters or root zones (Neubauer, Emerson et al. 2002; Rentz, Kraiya et al. 2007; Weiss, Rentz et al. 2007). These bacteria survive in this niche to compete with chemical oxidation (Emerson and Moyer 1997). The most common, morphologically distinct, iron-oxidizing bacteria found in microbial iron mats are *Leptothrix ochracea* and *Gallionella ferruginea*. These bacteria generate either iron-encrusted sheaths or helical stalks, respectively (Neubauer, Emerson et al. 2002; Hashimoto, Yokoyama et al. 2007). Iron-oxidizing bacteria obtain energy through the oxidation of Fe^{2+} which is deposited as Fe^{3+} in the form of stalks or sheaths. Iron-oxidizing bacteria use oxygen as their terminal electron acceptor. The calculated available energy, at a circumneutral pH, from the oxidation of Fe^{2+} to Fe^{3+} is low ($\Delta G^{\circ} \approx 29 \text{ kJ mol of iron}^{-1}$) and the bacteria therefore grow slowly (Neubauer, Emerson et al. 2002).

Iron oxidizing bacteria generate distinctive sheaths that likely play an important role in their ability to adsorb contaminants. The diameter of rod-shaped *Leptothrix* sheaths is approximately $1\mu\text{m}$ with lengths up to $200\mu\text{m}$ (Hashimoto, Yokoyama et al. 2007). It has been theorized that the sheaths are likely brittle because of their limited length. Another defining characteristic is the nanoparticles that line the sheath wall, thereby increasing the surface area. The chemical composition, as determined by an energy dispersive X-ray spectrometer, was roughly $\text{Fe:Si:P} = 80:15:5$ with even distribution along the sheath (Hashimoto, Yokoyama et al. 2007).

1.3 Filter

Flow-through filters represent an advanced wastewater treatment process that is currently being investigated as a technology necessary to meet effluent phosphorus limits. Filter technology implemented as a tertiary treatment aims to remove point source pollution and based on pilot scale testing has been shown to be a viable option. Several design parameters influence filter effectiveness. Riahi et al. (2008) found that phosphorus sorption increased with a decreased media size and an increased surface area. The microscopic size of the biogenic iron oxide sheaths makes them an ideal filter substrate due to their increased surface area. Riahi et al. (2008) also evaluated the effect of filter media depth on turbidity and phosphorus removal. The lowest turbidity removal rates corresponded to rapid flow and low filter depth, and the highest removal corresponded to the deepest column depth with lowest flow rates. In these cases, turbidity removal rates measured between 28-55%, and phosphorus removal was between eight and 58% for the same flow and filter depth conditions (Riahi, Mammou et al.). Based on these results, column depth can be increased to improved P removal. Research completed by Haque et al. (2008) also positively correlated filter bed depth and arsenic removal. Three bed depths were tested (15, 30, and 50 cm) at the lowest flow rate (10 mL/min). Breakthrough occurred first in the column with the smallest bed depth. Increased bed depth increased Fe-LECA's sorption capacity thereby delaying breakthrough. This correlation was likely due to increased surface area in the column. Haque et al. (2008) concluded that a higher bed depth would increase sorption capacity.

Surface area is another major characteristic that affects phosphorus sorption. Studies by Riahi et al. (2008) used date palm fibers for tertiary wastewater treatment and concluded that phosphorus sorption was enhanced by smaller media diameter and increased surface area. In a similar study, sorbents included crystalline slag (CSC) and very coarse crystalline slag (CSVC)

whose surface areas were 0.50 m²/g and 0.37 m²/g, respectively (Hylander et al., 2006). Experimentally, CSC removed 0.3 mg P/g substrate compared to around 0.1 mg P/g substrate removed by CSVC. These values illustrated the relationship between surface area and pollutant removal – increasing surface area increases sorption potential.

Flowrate affect on filter efficiency varies depending upon the pollutant being removed. Riahi et al. (2008) studied the effect of flowrate on removal efficiency for several environmental parameters including turbidity, chemical oxygen demand (COD) and phosphorus. Turbidity and COD showed no change in efficiency with flow rate. Phosphorus sorption, however, was inhibited at increased flow rates. The phosphorus results were likely due to decreased contact time with filter media at higher flow rates. Ayoub et al. (2001), however, found different results. Their research examined phosphorus removal at five different flow rates (10, 20, 40, 60 and 80 mL/min) and found no correlation between removal efficiency and flow rate. The disparate findings could have been due to different influent characteristics. Ayoub et al. (2001) used distilled water with orthophosphate and Riahi et al. (2008) used treated domestic wastewater.

1.4 Experimental Objective

Our laboratory previously showed that biogenic iron oxides have a significant capacity to adsorb phosphorus. Three different field sites have been evaluated and shown to contain iron oxidizing bacteria. For this study, we expanded the experiments to apply the adsorption capacity to a filter system. The objectives of this study were: (1) to confirm significant phosphorus removal using biogenic iron oxides as an adsorbent, (2) to evaluate parameters that influence filter effectiveness, and (3) to investigate biogenic iron oxides as a filter media substrate.

2.0 EXPERIMENTAL METHODS

2.1 *Sampling and Sample Preparation*

Ten biogenic iron oxides samples were taken at three locations, Moose Creek Reservoir near Bovill, Idaho (1, 2, 3, and 4), Spring Lake near Pomeroy, Washington (1, 2, and 3), and Myron Lake near Yakima, Washington (1, 2, and 3) (Figure 3). These sites were previously identified within our lab as locations containing the iron oxide mats required for this research (Rentz, Turner et al. 2009). Samples were collected in 1 L glass jars from March 2009 to August 2009 using 50 mL sterile plastic pipettes (Figure 2). Between two and four liters were taken from each site and jars were filled to the top, minimizing atmospheric oxygen exposure. In situ parameters were analyzed including temperature, pH, ferrous iron concentration (Hach FerroVer), and dissolved oxygen concentration (YSI 55 DO meter). Samples were transported to the lab in a cooler and refrigerated at 4°C prior to analysis.



Figure 2: Site conditions at Spring Lake (WA) are shown as well as sampling conditions



Figure 3: Sampling site conditions at Moose Creek Reservoir (ID)

Biogenic iron oxides were chemically, physically, and morphologically characterized for comparison. Samples from each site were mixed in the lab to be used for batch equilibrium experiments, filter experiments, and kinetics experiments. Following a 24 hour 0.25 M oxalic acid digest, total iron was determined using the Hach FerroVer method (n=3). Total solids and organic content were determined using the gravimetric method (n=4). Samples were morphologically characterized using a Leica DMLB light microscope with SPOT software (Diagnostic Instruments Inc.). Aqueous phosphorus was analyzed using the Hach Method 8048 and pH was recorded for the settled sample.

Prior to use in batch equilibrium, filter experiments, or kinetics, samples were washed using a 0.1M sodium chloride solution to remove background phosphorus. The sample was allowed to settle for at least four hours before the supernatant was removed and replaced with a 0.1M NaCl solution. The saline wash replaced between 40% and 60% of the total sample volume. The samples were chemically and physically characterized again after the NaCl wash.

2.2 *Batch Equilibrium*

Standard methods were used for the batch equilibrium experiments (Rentz, Turner et al. 2009). Batch reactors were setup using 50 mL capped tubes, phosphate stock solution, deionized water and washed sample (n=3). Equal amounts of washed sample were placed in 50 mL sterile plastic tubes. The sample volume was adjusted based on total iron concentration to achieve approximately 10 mg of iron per reactor (no greater than 15 mg). Initial reactor phosphorus concentrations ranged from 0.326 mg P/L to 19.6 mg P/L and were prepared from a 326 mg P/L stock solution. The stock solution was prepared using K_2HPO_4 and deionized water. The tubes were rotated at 20 rpm for a 24 hour period, a time previously determined to be adequate to reach equilibrium (Rentz, Turner et al. 2009). After rotation, the reactors were centrifuged at 4100 rpm for 10 minutes and the supernatant phosphate concentration was measured using the Hach Method 8048.

An additional kinetics experiment was conducted to determine appropriate residence time for the filter experiments. A biogenic iron oxide sample (1,575 mg Fe/L) that had been washed with 0.1M NaCl solution was evenly distributed to sterile plastic tubes (n=3). A 326 mg P/L phosphate stock solution was added to yield an initial concentration of 6.52 mg P/L, and DI water was added to yield a 50 mL final volume. Tubes were rotated at 20 r.p.m. at room temperature for 30 hours. Tubes were removed at pre-determined intervals 16 times in 30 hours and centrifuged at 4100 rpm for 10 minutes. Analysis was completed using the Hach 8048 method.

Non-linear curve fitting (IgorPro, Wavemetrics, Portland, OR U.S.A.) was used to fit the batch equilibrium data and to produce 95% confidence interval statistics. Pearson R^2 values (Microsoft Excel) were used to estimate the appropriateness of each fitting equation. Langmuir and Freundlich fitting equations were used to analyze this data: Langmuir (Eq. 1), Freundlich

(Eq. 2), Γ was the sorbed phosphorus concentration (mg P/g), C was the aqueous equilibrium concentration (mg P/L), Γ_{\max} was the maximum adsorbed concentration (mg P/g), b was the Langmuir equilibrium constant, K_f was the Freundlich sorption capacity, n was the Freundlich sorption intensity constant. Both equations were plotted and the associated coefficients were calculated.

$$(1) \quad \Gamma = (\Gamma_{\max} * b * C) / (1 + b * C)$$

$$(2) \quad \Gamma = K_f C^{(1/n)}$$

2.3 *Filter Setup*

After several trial experiments adjusting flow regime, flowrate, and mixing, an upflow pumped system was determined to be optimal for removing aqueous phosphate. A 1 to 4 mg/L phosphate solution was mixed in a large reservoir, placed on shelving two and a half feet above the filter. The initial filter setup had tubing from the reservoir that was split by three one-half inch connected tees to accommodate four reactor columns: one sand-only control and three experimental columns. Flow control was achieved using stopcocks at the outlet. One-half inch tubing was used to connect the reservoir to the reactor column. The reactor outlet was reduced to one-quarter inch tubing which was connected to the stopcock. Reactors were composed of one inch inside-diameter clear vinyl tubing, in eight inch lengths. Fittings on each end of the columns reduced the one inch diameter vinyl tubing to one-half inch. One 80 mesh screen was added to the base of each column to support the sand and iron oxides. Each column was filled with 40g of clean sand to support the iron oxides and then filled with 50-150 mL of iron oxide sample (Figure 5). The reservoir was used to fill four one-liter beakers with phosphate solution, one for each filter column (Figure 4). A suction line in each of the beakers was attached to 1.4 mm Tygon tubing that ran through peristaltic pump cassettes. Fittings attached the 1.4mm tubing to standard one-quarter inch vinyl tubing that fed the reactor columns. Using the average

historical sorption capacity for each site ($\text{mg PO}_4/\text{g Fe}$) as well as the flow rate and concentration of PO_4 solution being fed to the column system, a concentration of iron required in the filter was determined using Equations 3-5.

$$(3) \quad \left(\text{PO}_4 \text{ in reservoir, } \frac{\text{mg}}{\text{L}} \right) * \left(\text{flow rate, } \frac{\text{L}}{\text{d}} \right) * (\text{days to exhaustion}) = \text{total PO}_4 \text{ through system, mg PO}_4$$

$$(4) \quad \text{Fe required in filter, mg Fe} = \frac{\text{total PO}_4 \text{ through system, mg PO}_4}{\left(\text{sorption capacity of sample, } \frac{\text{mg PO}_4}{\text{mg Fe}} \right)}$$

$$(5) \quad \text{volume of sample, mL} = \frac{\text{Fe required in filter, mg Fe}}{\left(\text{postwash Fe, } \frac{\text{mg Fe}}{\text{mL}} \right)}$$

Prior to running the pump and setting up the columns, the phosphate solution was run through the pump up to the base of the sand. This ensured that the column would not drain any of the iron oxides and filtration could begin immediately. The flowrate was set on the peristaltic pump to 0.5 mL/min (0.720 L/day).

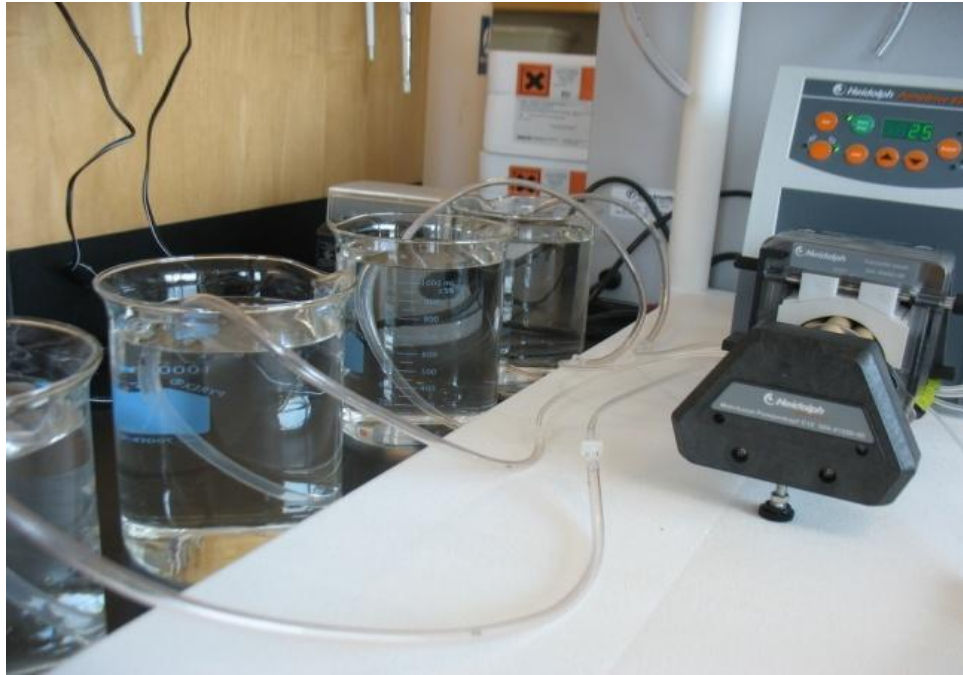


Figure 4: The 1 L jars that fed each of the filter columns and the peristaltic pump used for the final experiments



Figure 5: An example of the filter columns when filled with sand and biogenic iron oxides (3 experimental, 1 sand control)

Effluent from each filter was collected in a 1 L beaker to calculate flowrate. At each sampling event, the volume of effluent water was measured in a graduated cylinder and recorded on an Excel spreadsheet. A minimum of 6 mL was collected in addition, to be analyzed for phosphate. The volume that was collected for sampling was added to the total volume of the effluent to ensure accurate flowrate calculations. The phosphate concentration was determined using Hach Method 8048 and recorded. Each morning, a P reading was taken from each of the feed jars (called “standard”), but throughout the day, P readings were only taken from the control standard. Once per day the 1 L influent jars were refilled from the main reservoir.

After the peristaltic pump was purchased, seven filter experiments were completed. Rather than completing one experiment with three replicates, each column varied in some way. The changes that were made to each column included iron concentration, the addition of

surfactants, and mixing. Based on the previous week's results, the future columns were optimized for the highest removal, with the fewest changes to the natural sample.

Changes were made to each filter column for every new experimental setup. Moose Creek 1 consisted of downflow gravity-fed columns and varied by flow regime and iron concentration. The first filter contained 100 mL of sample (14.5 mg Fe), the second had 150 mL of sample (21.9 mg Fe), and the last filter was an upflow regime with 100 mL of sample. Moose Creek 2 varied flowrates utilizing an upflow regime for all columns each with 100 mL of sample (138 mg Fe) with a 2.2 ± 0.24 mg/L average standard solution in the reservoir. Stopcocks were adjusted to change the flowrates.

Myron Lake 1 sample was used as the initial upflow filter experiment using the pump. For this initial experiment, the filters were set-up the same ($n=3$). Each column had 40g of sand and 75 mL of sample (70 mg Fe).

Following the initial pumped experiment, several alterations to the raw sample were attempted in order to improve removal efficiency for Myron Lake 2. The sample was separated into three jars with 300 mL each. The first jar, containing raw sample only, was hand-mixed for ten minutes, the second jar was vigorously mixed with a drill and paint mixer (0-2500 RPM), not at full speed, for seven minutes, and the third jar had 100 μ L of phosphate-free consumer detergent added (0.033% of the total volume). These samples were settled overnight and 200 mL of supernatant was removed to increase the iron concentration. 75 mL of treated sample from each jar was added to the associated column (103 mg Fe). The average standard solution concentration was 2.22 ± 0.09 mg/L.

Alterations in detergent quantity were made for the Moose Creek 3 sample. One filter had no alterations to the natural sample, the second had 50 μ L of consumer detergent added to

330 mL of sample (0.015% of volume), and the third filter had 100 μ L of consumer detergent added to 330 mL of sample (0.030% of volume). Each sample was hand-mixed for two minutes and settled overnight. After settling, 200 mL of supernatant was removed to increase the iron concentration. Each column had 75 mL of sample (284 mg Fe) and 40 g of sand. Due to the high iron concentration, and the high removal efficiency, this filter was run for 15 days. The average phosphate concentration in the feed jars was 2.48 ± 0.04 mg/L.

Lab-grade detergent was tested for the Myron Lake 3 sample. The raw sample was divided between three jars with 300 mL each. The first column had no alterations to the natural sample, the second column had 100 μ L of TritonX detergent (0.033% of volume), and the third column had 100 μ L of Tween-80 detergent (0.033% of volume). The average standard phosphate solution was 2.01 ± 0.01 mg/L.

Moose Creek 4 tested the hypothesis that mixing the sample would improve filter efficiency. Two columns had 100 μ L of Tween80 added and the third column was the natural washed sample. One of the detergent-treated samples was stirred in the column every time a sample was taken (4-5 times per day). Due to the high iron concentration, only 50 mL of sample was added to each column (86 mg of Fe). The average concentration of the standard phosphate solution was 3.92 ± 0.06 mg/L.

The purpose of the Spring Lake 2 experiment was to continue testing the Tween80 detergent as well as to test the adsorption capacity of the detergent. Two columns contained iron oxides, one with no alterations to the natural sample and one with 200 μ L of Tween80 in 500 mL of sample (0.04% of volume). There were two sand-only columns, one was just sand and one was treated with 100 μ L of Tween80 in 40 g of sand for two days. The goal was to see if the

detergent was removing any of the phosphate. Each iron oxide column had 60 mL of sample (72 mg Fe) and the average phosphate standard solution was 4.00 ± 0.02 mg/L.

The Spring Lake 3 sample was treated with Tween80 for four hours before the filter was set up. One column had no alteration to the natural sample, one had 100 μ L of Tween80 in 250 mL of sample (0.04% of volume), and one had 500 μ L of Tween80 in 250 mL of sample (0.2% of volume). For this final experiment, we wanted to test the impact that a large volume of detergent would have on the sample. Each column received 65 mL of sample (56 mg Fe) and a 3.03 ± 0.04 mg/L phosphate solution was fed to the columns.

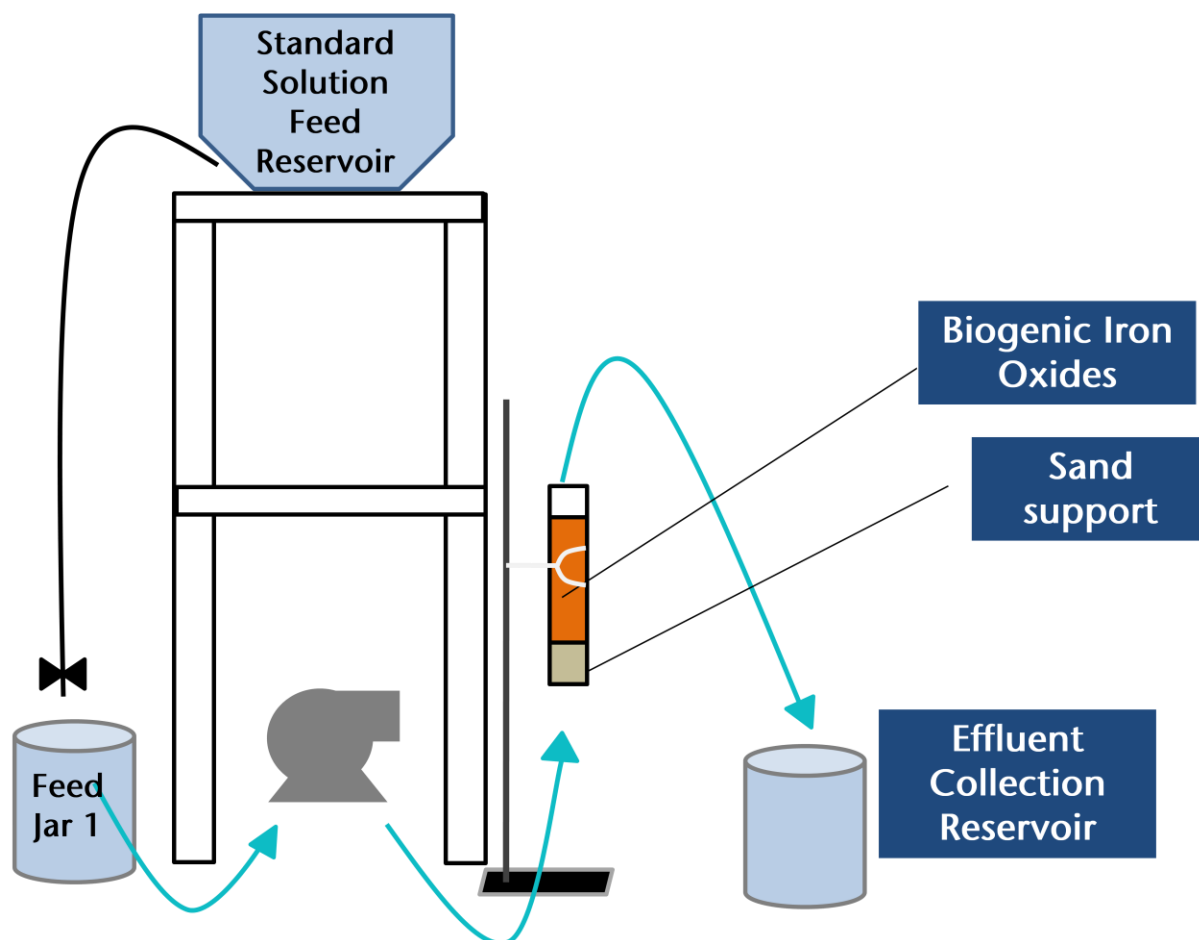


Figure 6: A schematic of the filter setup. Flow direction is indicated by the blue arrows.

3.0 RESULTS

3.1 Site Characteristics

Sampling location site characteristics were favorable for biogenic iron oxidizing bacterial growth (Table 1). Circumneutral pH and aqueous iron were available for the growth of biogenic iron oxides. The field biofilm pH ranged from 6.6 to 8 for all sites, reinforcing the necessity for circumneutral conditions. Average aqueous iron, a measure of the overlying site water, was 4.44 ± 0.65 mg/L at Moose Creek during the early spring and 20.14 ± 11.11 mg/L in the summer. This change over time is expected because as the sample ages there is more bacterial growth, and therefore more iron production. Throughout the summer the iron is built up and causes a significant increase in aqueous iron. Aqueous iron for Myron Lake 2 and 3 were 4.0 and 1.1 mg/L, respectively and the Spring Lake samples average was 12.72 ± 7.44 mg/L. The temperature ranged from 2 to 17.5°C, indicating the wide range of temperature conditions available for biogenic iron oxide growth.

Table 1: Summary of physical and chemical site and sample characteristics with one standard deviation reported. Dissolved oxygen, pH and temperature were measured within the biofilm onsite. Aqueous iron (Fe^{2+}) was a measure of the overlying site water and total iron was the total iron concentration in the biofilm sample. Values that were not reported resulted from unavailable or broken sampling equipment.

Sample	pH	Temp (°C)	PO_4^{3-} (mg/L)	DO (mg/L)	Fe^{2+}	Tot. Fe (mg/L)	Dry Wt. (g/L)
M21	7.6	4.0	1.61	-	4.90 ± 0.5	137.1 ± 13.4	1.45 ± 0.1
M22	8.0	2.0	0.45	-	3.98 ± 2.0	746.7 ± 43.2	2.55 ± 0.2
M24	7.1	12.2	0.16	0.66	28.0 ± 3.0	1426.3 ± 52.5	3.67 ± 0.1
M25	-	14.9	0.38	1.50	12.28 ± 3.6	1957.1 ± 49.8	8.26 ± 0.2
YK10	-	-	0.17	-	-	490.0 ± 31.6	1.23 ± 0.1
YK11	-	-	0.64	-	4.01 ± 0.2	242.1 ± 13.4	0.83 ± 0.0
YK12	-	-	0.43	-	1.10 ± 0.0	519.2 ± 41.4	1.21 ± 0.0
SL25	7.9	17.5	0.85	0.62	20.88 ± 4.9	1577.9 ± 105.5	5.10 ± 0.1
SL28	-	17.8	0.27	0.50	10.85 ± 2.6	1085.0 ± 40.1	4.51 ± 0.2
SL30	6.6	16.3	3.17	0.80	6.33 ± 0.1	953.8 ± 63.1	2.37 ± 0.0

DO readings confirmed the microaerobic requirements of these iron oxidizing bacteria. Microaerobic conditions are defined by Brock Biology of Microorganisms as less than atmospheric concentrations, which is around 20%. Other literature defines microaerophilic as 7% of air saturation, which is around 0.6 mg/L (Tseng et al. 1996). Due to the timing of the purchase and the locations for sampling, only a few samples have DO readings. DO for Moose Creek 3 was 0.663 ± 0.29 mg/L, Moose Creek 4 was 1.5 mg/L, Spring Lake 1 was 0.615 ± 0.05 mg/L, Spring Lake 2 was 0.497 ± 0.13 , and Spring Lake 3 was 0.797 ± 0.33 mg/L.

Differences found between sampling sites were reflected in total iron, dry density, and organic content. Moose Creek samples specifically illustrated changes that occurred with the seasons. The first sample was taken March 11th, 2009 and the total iron concentration was low, 137.1 ± 13.4 mg/L. The dry density and organic content for this sample were 1.45 ± 0.06 g/L and 0.443 ± 0.01 g/L, respectively. The next sample was taken March 23rd, 2009 and visually, the site had changed dramatically. It was darker in color and settled on the bottom of the wetland. The total iron concentration was 746.7 ± 43.2 mg/L, the dry density was 2.55 ± 0.16 g/L and the organic content was 0.748 ± 0.08 g/L. Two samples were taken in the summer (Moose Creek 3 and 4), June 15th, 2009 and July 21st, 2009. Moose Creek 3 had an average total iron of 1426.3 ± 52.5 mg/L, dry density was 3.67 ± 0.13 g/L, and organic content was 0.90 ± 0.01 g/L. Moose Creek 4 had an average total iron of 1957.1 ± 49.8 mg/L, dry density was 8.26 ± 0.20 g/L, and organic content was 1.86 ± 0.08 mg/L. These results indicated that the sample at Moose Creek Reservoir became more concentrated with iron over time as well as having an increased dry density and organic content.

Samples from Myron Lake and Spring Lake had more consistent total iron, dry density and organic content values. The average total iron, dry density and organic content for all three

Myron Lake samples were 499.7 ± 36.5 mg/L, 3.33 ± 1.92 g/L, and 0.30 ± 0.16 g/L, respectively. The average total iron, dry density and organic content for all three Spring Lake samples were 1205.6 ± 329.1 mg/L, 3.99 ± 1.45 g/L, and 1.05 ± 0.56 g/L, respectively.

Normalizing the iron content and organic content to total solids indicated that a similar ratio of iron and organics are present at each sampling site and this range of values is necessary for the biogenic iron oxide growth. By analyzing the data this way, we are able to compare each site and understand the growth requirements of these bacteria. The iron and organic concentration ranged from 137.1 to 1957.1 mg/L and 0.183 to 1.858 g/L, respectively. Over one order of magnitude existed between all of the sampling sites. The iron/solids ratio ranged from 0.095 to 0.431 percent iron in the samples. The organic/solids ratio ranged from 0.199 to 0.312 percent organics in the samples. These results indicate that around 10-40% of the total solids are iron and around 20-30% of the total solids are organics.

All of the biogenic iron oxide samples showed a significant capacity for phosphorus adsorption. The sorption isotherms followed expected visual trends, where sorption to biogenic iron oxides tailed off at high concentrations. This suggests that the samples became saturated with respect to phosphorus. The Langmuir isotherm specifically models saturation, and gave Pearson R^2 values between 0.146 and 0.995 and the Freundlich isotherm gave Pearson R^2 values between 0.221 and 0.897. The Langmuir equation provided the best fit, visually indicating that a maximum phosphorus sorption was achieved. The fitting coefficients and maximum sorption (Γ_{\max}) values are provided in Table 2.

Pearson R^2 and Γ_{\max} values varied drastically for each site. The Moose Creek sites had the highest R^2 values, ranging from 0.940 to 0.995, but achieved the lowest Γ_{\max} , which ranged from 7.7 ± 0.01 to 16.9 ± 0.4 mg P/g solids. The opposite results were found for the Myron Lake

samples. The R^2 values ranged from 0.146 to 0.889, but the maximum sorption (Γ_{\max}) was from 19.8 ± 0.7 to 25.4 ± 2.3 mg P/g solids which was much higher than the Moose Creek samples. An inverse correlation can be seen between organic content and Γ_{\max} . The organics can block sorption sites on the iron oxides inhibiting sorption capacity. This relationship likely controls the low Γ_{\max} values seen at Moose Creek and Spring Lake. Four representative batch equilibrium experiments are illustrated in Figure 7.

Table 2: Isotherm coefficients for the Langmuir and Freundlich equations; 95% confidence interval reported

<i>Langmuir</i>				<i>Freundlich</i>			
	$\Gamma_{\max, \text{solids}}$	b	Pearson R^2		K_f, solids	n	Pearson R^2
M21	14.7 ± 1.2	1.9 ± 0.6	0.940	M21	6.6 ± 1.0	0.34 ± 0.1	0.842
M22	16.9 ± 0.8	4.2 ± 0.8	0.979	M22	8.9 ± 1.1	0.30 ± 0.1	0.878
M24	12.5 ± 0.3	3.3 ± 0.4	0.989	M24	7.9 ± 0.6	0.26 ± 0.05	0.874
M25	7.7 ± 0.1	2.4 ± 0.2	0.995	M25	4.6 ± 0.4	0.25 ± 0.05	0.897
YK10	25.4 ± 4.7	0.8 ± 0.3	0.735	YK10	10.1 ± 1.5	0.41 ± 0.1	0.623
YK11	21.7 ± 5.0	4.9 ± 4.6	0.146	YK11	16.1 ± 2.4	0.17 ± 0.1	0.221
YK12	19.8 ± 1.4	1.5 ± 0.5	0.889	YK12	10.9 ± 1.3	0.26 ± 0.1	0.758
SL28	6.2 ± 0.4	1.3 ± 0.4	0.930	SL28	3.4 ± 0.5	0.24 ± 0.1	0.748
SL30	12.7 ± 1.3	1.2 ± 0.5	0.908	SL30	6.4 ± 1.0	0.29 ± 0.1	0.771

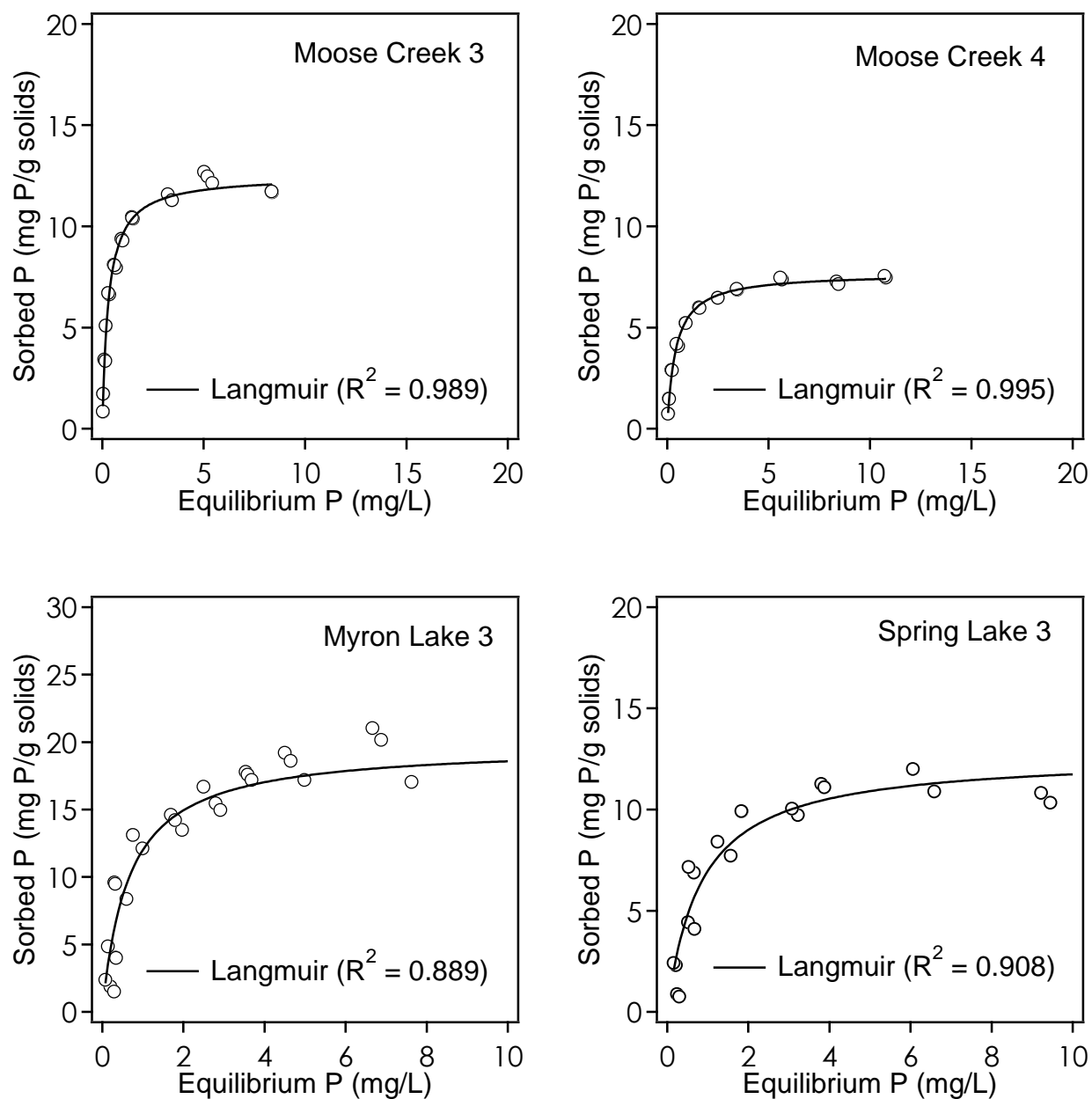


Figure 7: Representative batch equilibrium plots from each sampling site. R^2 values are reported for the Langmuir isotherm, which provided the best fit for all of the samples. Batch equilibrium plots for every experiment are provided in Appendix B.

Biogenic iron oxides proved to be efficient phosphorus adsorbents and performed well compared to other iron-rich materials (Figure 8). Phosphorus sorption is presented normalized to total solids and all replicates are shown. Iron substrates used for comparison were iron oxide coated sand, engineered materials (brick and metal-loaded orange waste), and recycled materials (tailings and two juniper fibers). The sorption range is quite significant, from less than 0.50 mg P/g solids for the sand materials to greater than 25 mg P/g solids for some of the biogenic iron oxides.

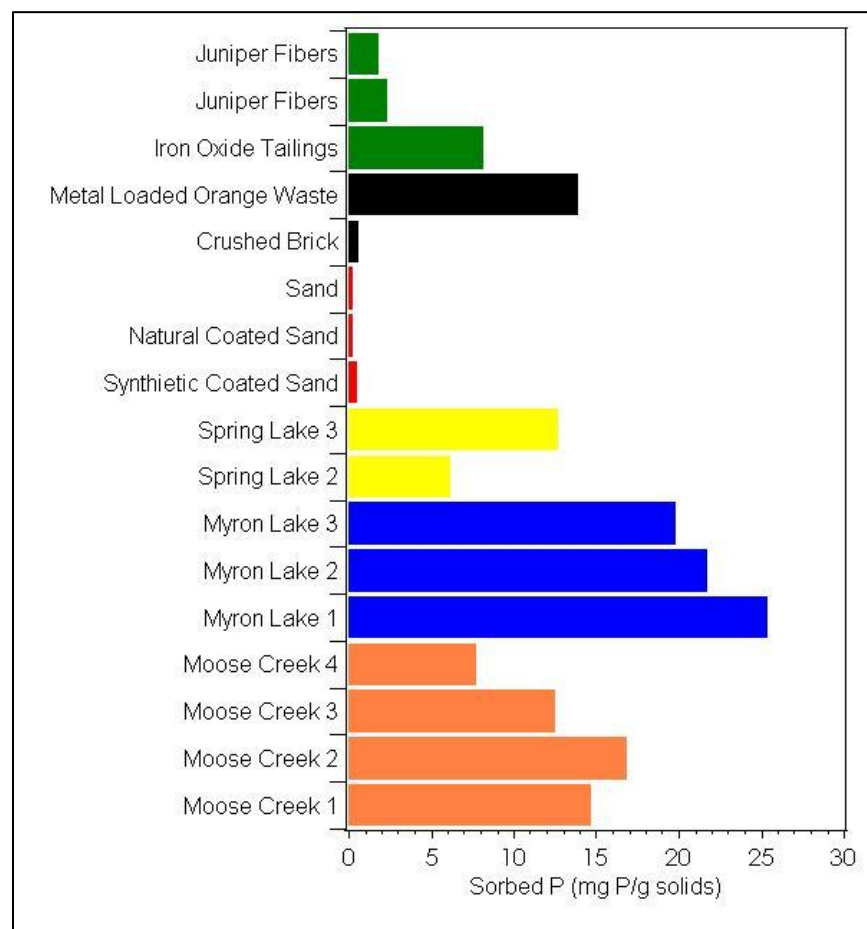


Figure 8: Maximum phosphate loadings (Langmuir Γ_{\max} , mg P/g solids) for several iron rich substrates. Sand substrate was from Boujelben et al. (2008) and Del Bubba, Arias et al. 2003. Engineered materials (brick and orange waste, respectively) were acquired from Bastin et al. (1999) and Boujelben et al. (2008). Pure iron oxides, goethite and akaganeite, were from Chitraker et al. (2006). Recycled materials were tailings from Zeng et al. (2004) and juniper fiber from Han et al. (2005).

Phosphorus sorption kinetics were rapid, removing sixty-five percent after just one hour and eighty percent after three hours (Figure 9). 24 hours, however, were required to reach equilibrium. For batch equilibrium studies, 24 hours is a practical timeframe, but for any engineered application, including filtration, 24 hours is unreasonable. Eighty percent removal in three hours was significant because the contact time for each of the columns was between one and three hours. Three hours is a practical time period for the design of engineered applications. The Simple Elovich model provided the best fit for the kinetics data with an R^2 of 0.988, but other kinetic models provided acceptable fits (Table 3).

Table 3: Coefficients for common kinetic models; 95% confidence intervals are reported.

Kinetic Model	Coefficients		R^2
Power Function	a	1.528 ± 0.071	0.965
$S_T/S_E = b*t^a$	b	0.151 ± 0.008	
Simple Elovich	a	1.013 ± 0.038	0.988
$S_T/S_E = a + b*\ln(t)$	b	0.469 ± 0.008	
Parabolic Function	a	-0.006 ± 0.0004	0.926
$S_T/S_E = a*t + b*t^{1/2}$	b	0.347 ± 0.015	
2nd order kinetics	a	0.019 ± 0.003	0.838
$S_T/S_E = t/((1/(a*b^2))+t/b)$	b	3.950 ± 0.090	
1st order kinetics	a	-2.750 ± 0.089	0.960
$S_T/S_E = a*\exp(-b*t)$	b	0.006 ± 0.0005	

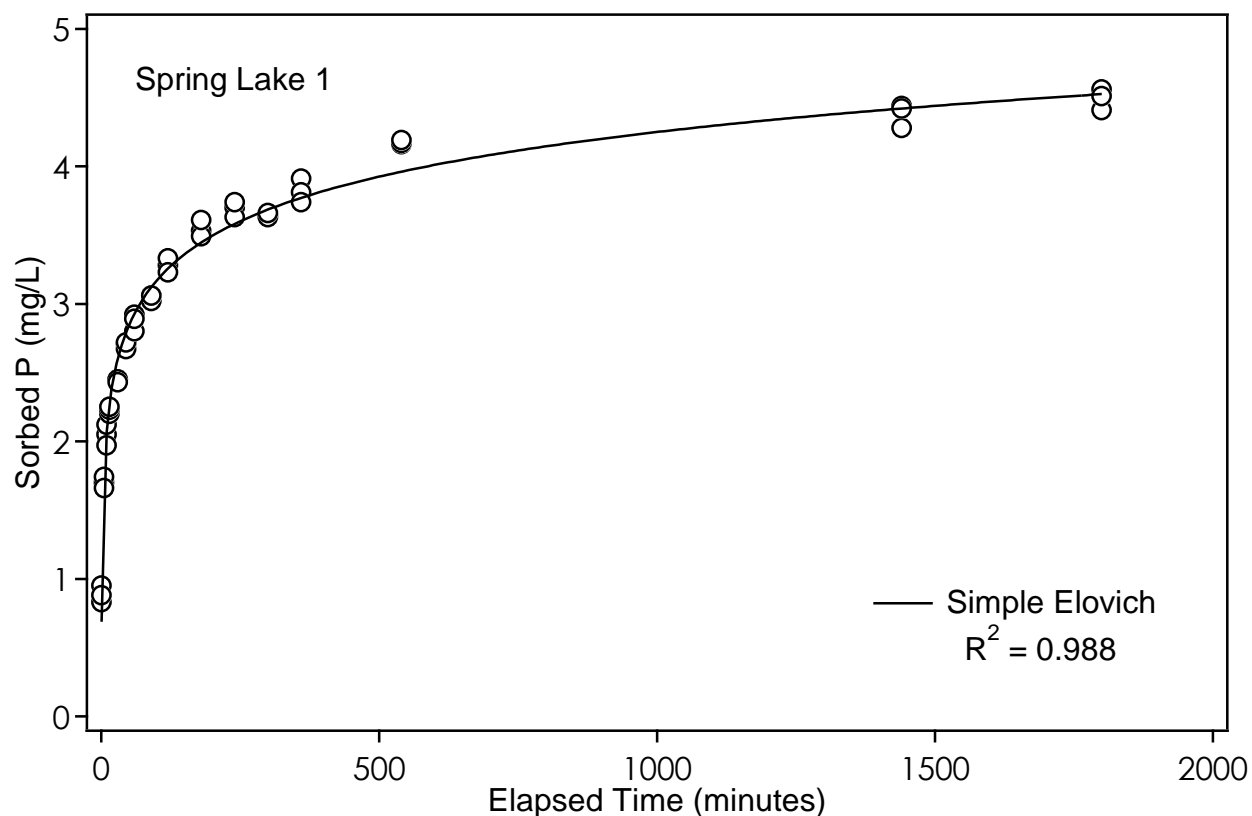


Figure 9: Phosphorus sorption kinetics for biogenic iron oxides (Spring Lake 1) over 30 hours (n=3 at each time point).

3.2 Preliminary Filter Results

These biogenic iron oxide characterizations were used to develop a column filter for phosphorus removal. Initially, a simple downflow gravity system was constructed and evaluated for phosphorus removal using biogenic iron oxides as a filter substrate. After running this experiment for one week (Moose Creek 1), it was observed that the system remained dynamic and no steady state was achieved during the monitored period. The iron oxides settled on top of the sand decreasing porosity thereby decreasing flow through the filter (Figure 11). Due to the clogging in the filter, the stopcocks were opened at every sampling event to increase the flowrate, therefore decreasing the flowrate accuracy. The lack of control over the flowrate required changes to the experiment.

Results from preliminary filter experiments determined the need for an upflow regime. A 1 mg/L standard solution fed three experimental columns that differed by flow regime and iron concentration. The downflow filter (14.5 mg Fe) had an average effluent concentration of 0.49 ± 0.23 mg/L, with concentrations ranging from 0.11 to 0.90 mg/L. The average percent removal was $53 \pm 21\%$. The other downflow filter (21.9 mg Fe) had an average effluent concentration of 0.71 ± 0.25 mg/L, with concentrations ranging from 0.08 to 0.94 mg/L. The average percent removal was $31 \pm 22\%$. The upflow filter (14.5 mg Fe) had an average effluent concentration of 0.16 ± 0.18 mg/L, with concentrations ranging from 0.0 to 0.58 mg/L. The average percent removal was $84 \pm 16\%$. The upflow regime performed dramatically better than the two downflow filters (Figure 10). This improvement was likely due to increased contact time between the suspended iron oxides and the phosphate solution.

In an attempt to keep iron oxides suspended and stabilize the flowrate (Moose Creek 2), the filter system was redesigned to allow for upflow conditions. The upflow system exhibited some settling, but the iron oxides remained largely suspended when compared to the downflow system. Screens were placed at the top of the reactor columns to keep the iron oxides within the filter. It was determined, however, that the screens led to clogging at the outflow of the filters, so they were removed. Maintaining a constant flowrate became an issue over time (Figure 11), and the stopcocks still were adjusted at every sampling event. Iron oxides would clog the screens containing them within the filter causing head losses and reducing flow.

Utilizing all upflow filters and varying the flowrates confirmed the hypothesis that low-flow conditions would improve removal efficiencies. The second filter setup had upflow columns, each with 100 mL of sample (138 mg Fe) and a 2 mg/L standard solution in the reservoir. The intent was to have a different flowrate in each column, by varying the position of

the stopcock. Due to the changing flows and clogging, however, achieving this consistently was unattainable. Over all three filters, the effluent concentrations ranged from 0.08 to 1.21 mg/L, with an average concentration of 0.69 ± 0.35 mg/L. The average percent removal for all three filters was $68 \pm 17\%$. Analyzed independent from one another, the low-flow filter performed the best ($85 \pm 7\%$ removal, compared to $64 \pm 18\%$ and $57 \pm 11\%$ for the medium and high flow filters, respectively). This improved removal at low flowrates (average 0.66 ± 0.56 L/d for the low-flow filter) is likely due to increased contact time. The filter results for the upflow filters with varying flow rates (Moose Creek 2) are represented graphically in Figure 10. The relationship between flowrate and effluent concentration is represented in Figure 12. For Moose Creek 2, the best removal rates were achieved below 1 L/d. Because of this, a flowrate less than 1 L/d was used for the pumped experiments.

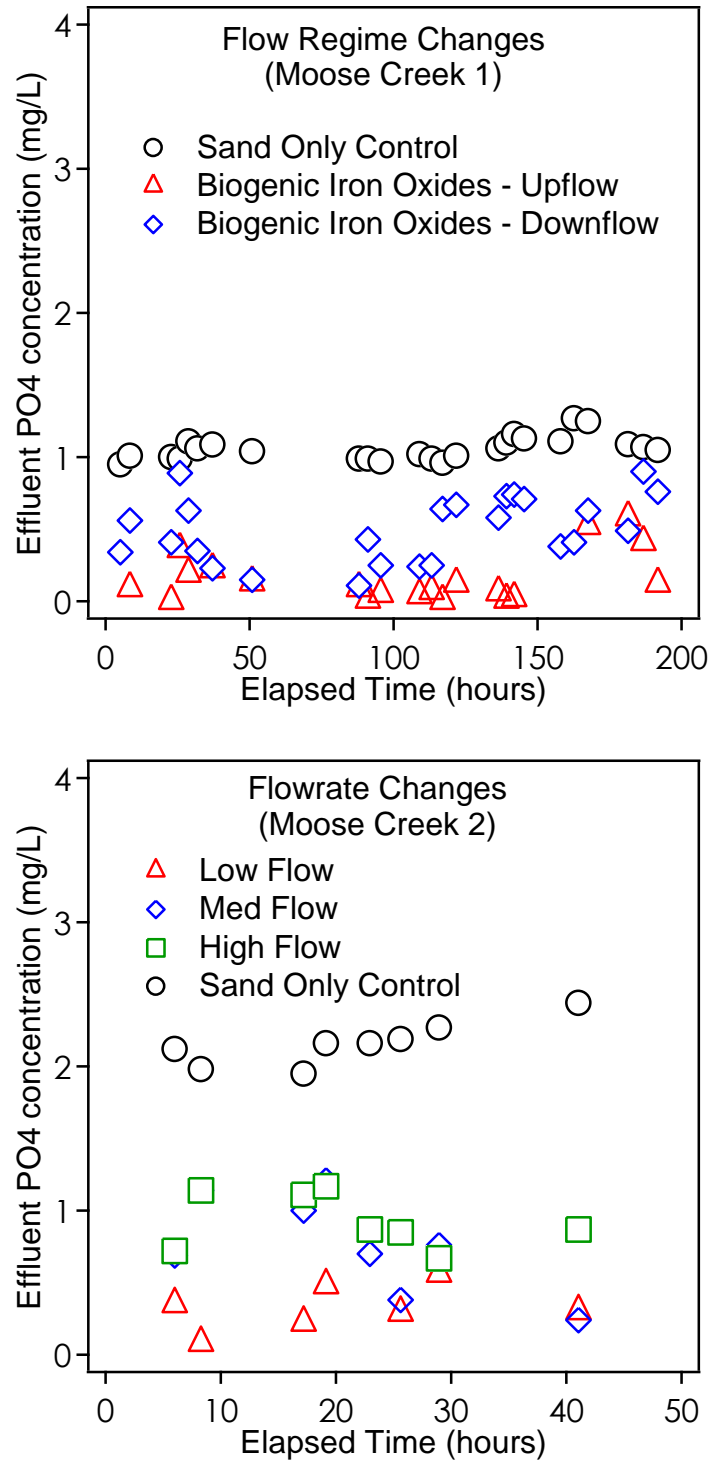


Figure 10: Preliminary filter results including flow regime changes (downflow vs upflow) and flowrate changes (low, medium, and high flow). The improved efficiency using an upflow filter is shown in the first plot. Decreased effluent concentrations were found under the low flow conditions for the second plot.

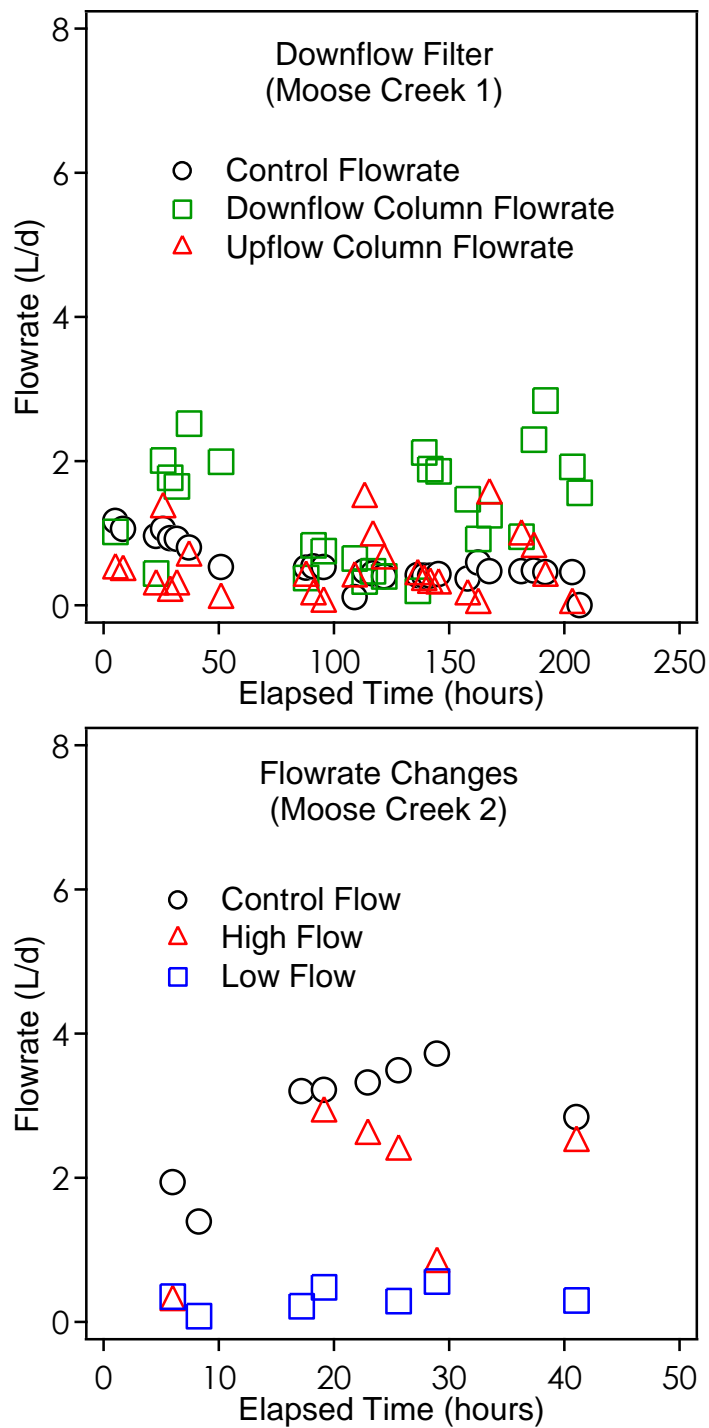


Figure 11: Highly variable flowrates observed within filters containing sample from Moose Creek 1 and Moose Creek 2. The control column contained no biogenic iron oxides and therefore was able to achieve a relatively stable flowrate. The peaks observed in flowrate occurred when the stopcock at the outlet was opened to increase flow.

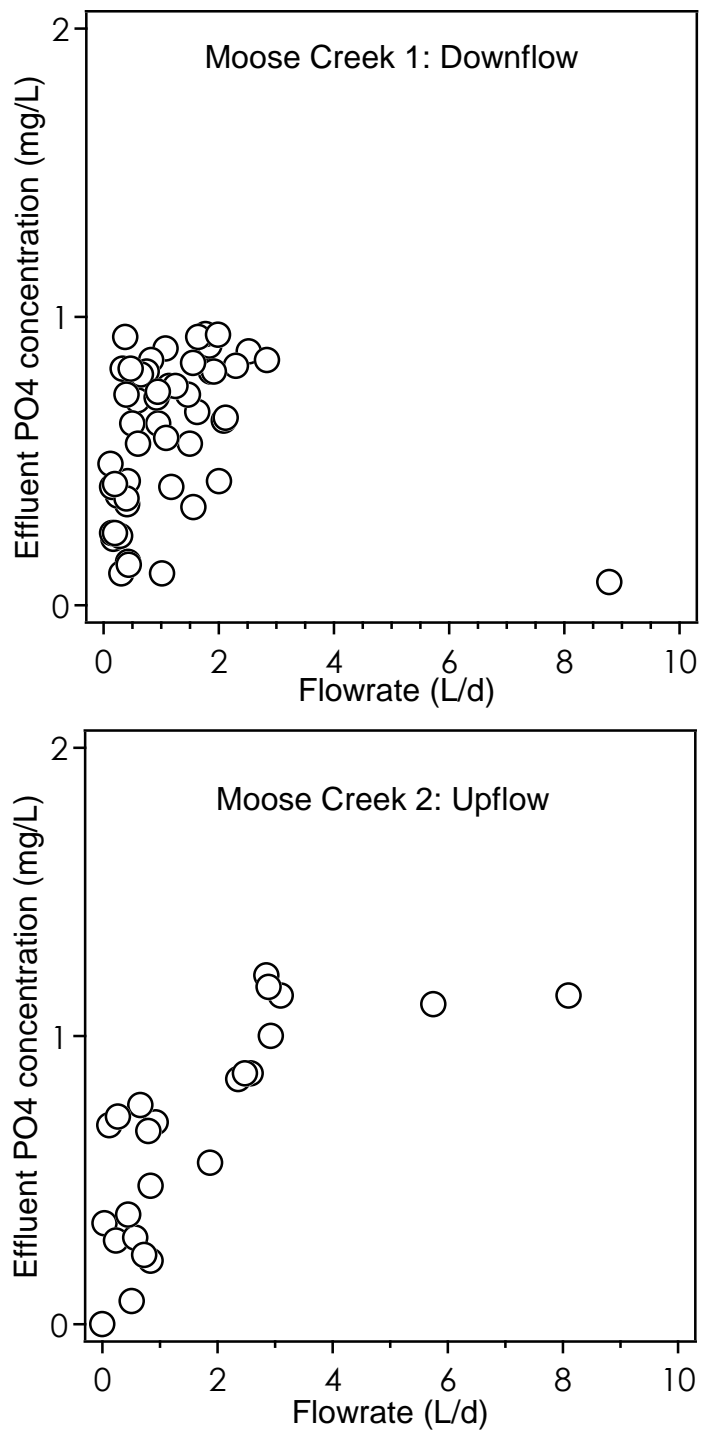


Figure 12: Flowrate versus effluent concentrations are graphed to determine the most appropriate flowrate for the pumped filter system. The second experiment (upflow filter) achieved removal lower than 0.75mg/L PO₄ for flowrates less than 1 L/d.

3.3 *Final Filter Results*

The final filter setup incorporated results from the two preliminary filter experiments and was initially tested with three column replicates. All three columns for each experiment had an upflow regime. Each column contained the same volume of sand and sample. Most importantly, flow was controlled using a peristaltic pump. The flow rate was set at 0.5 mL/min (0.720 L/d, 0.190 gpd), a value that was determined based on flowrate versus effluent concentration data (Figure 12).

Reproducible results were obtained using the upflow filter configuration with a peristaltic pump. The effluent concentrations for all three columns ranged from 0.44 to 2.23 mg/L with a standard solution of 2.55 mg/L. The average percent removal for all three columns was $45\% \pm 18\%$. The entire data set is represented in Figure 13. Additionally, with the exception of one outlier, the standard error was always less than 20%. The first pumped experiment, however, exhibited preferential flows that inhibited significant phosphorus removal.

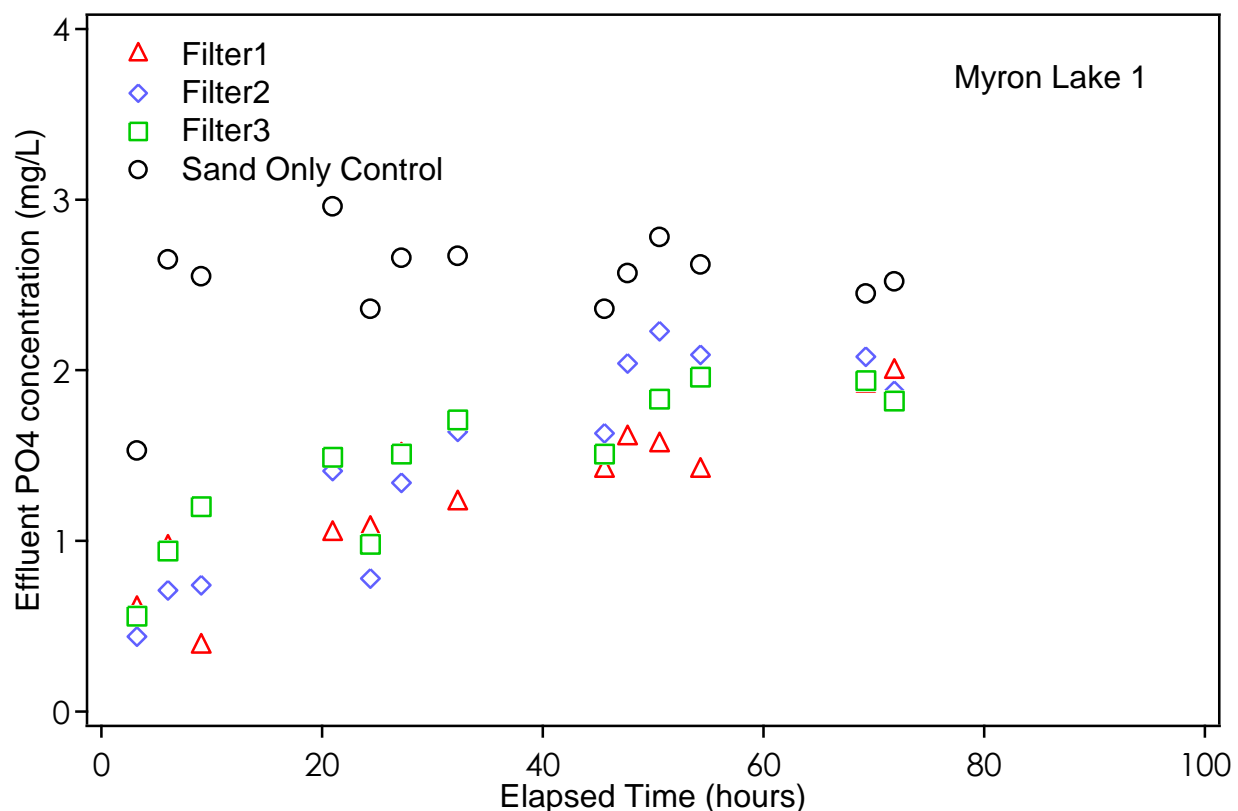


Figure 13: Effluent phosphate concentrations for all three filter replicates showing the reproducibility of the pumped system.

This sample was not well separated and therefore led to preferential flows through the iron oxides. Pathways were visually evident through the iron oxides and it is likely that this reduced removal rates. The preferential flows inhibited contact with the iron oxides. At the end of the week, a dye test was completed. Blue food coloring was added to the feed water for the filter columns. This water was run through the columns for two hours in order to see the flow pathways in each column. Preferential flow was evident in each of the columns (Figure 14).

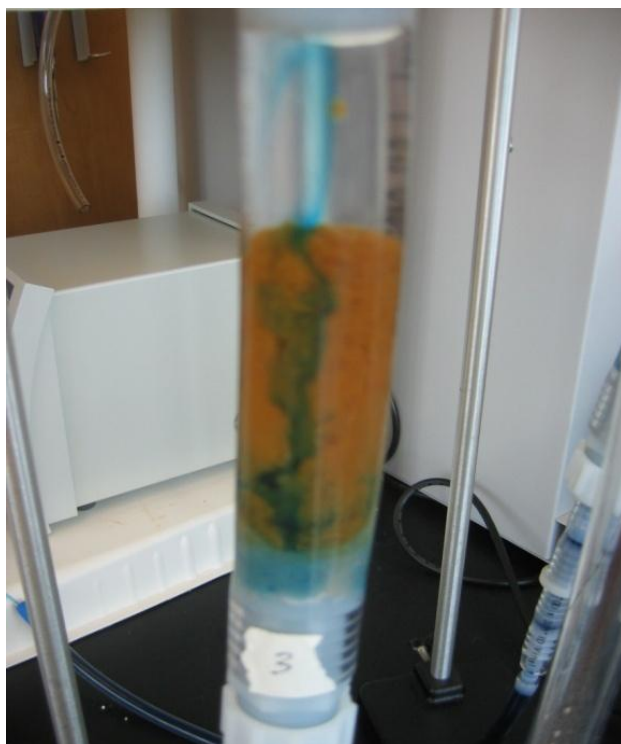


Figure 14: Preferential flows observed during an upflow pump-controlled filter experiment indicated by blue dye flowing through the iron oxides. Pathways similar to this were evident in each of the three replicates for the first pumped filter experiment.

Due to the physical nature of the sample and the preferential flows, sample disruption was necessary. The raw samples were gelatinous in nature and microscopically, the interconnection of the sheaths can be seen (Figure 15). These linkages prevent the flow from going through the sample and decrease removal. In order to improve contact time and removal efficiency the samples were disrupted with incrementally increased levels of disturbance. The first sample was hand-mixed, similar to previous samples, the second was violently mixed with a drill and paint mixer, and the last was chemically disrupted using a consumer detergent.

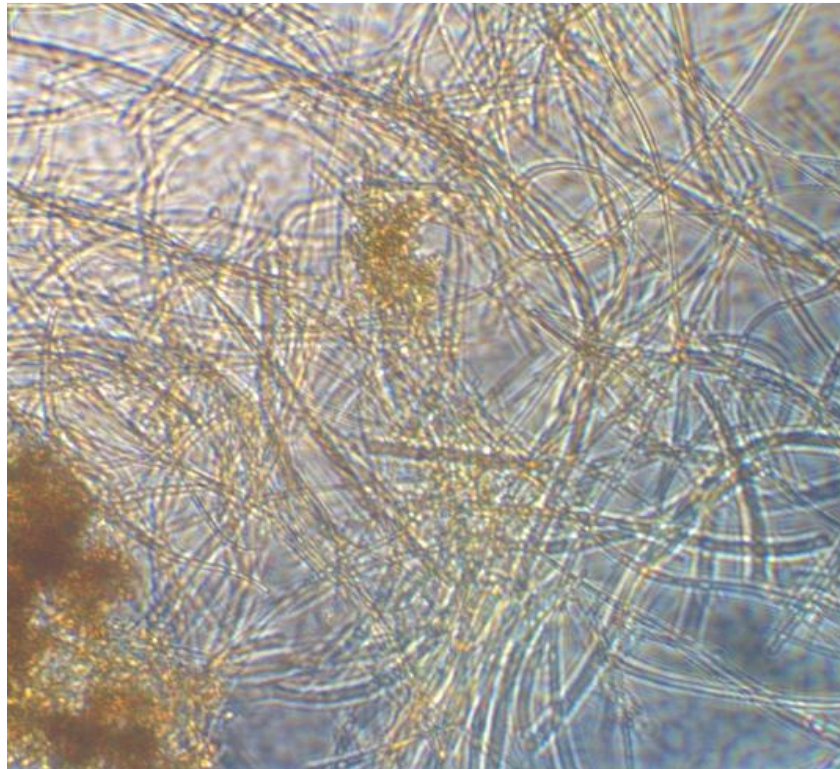


Figure 15: A 400X microscopic image of the biogenic iron oxides highlighting the interconnections between sheaths. These interconnections led to preferential flows and reduced removal efficiencies.

Filtration efficiency improvements were observed when samples were disrupted suggesting samples were inhibited by preferential flows. Myron Lake 2 tested the theory that the addition of a surfactant would decrease the surface tension and remove preferential flows.

Column 1 (hand-mixed), Column 2 (drill-mixed) and Column 3 (detergent) removed $55\% \pm 17\%$, $42\% \pm 17\%$, and $78\% \pm 7\%$ of the phosphate, respectively (Figure 16). The detergent-treated column achieved greater than 70% removal for the majority of the three day experiment period. The hand-mixed sample results are similar to those seen from the previous week and are as expected. The drill-mixed sample performed the worst, likely due to the extreme physical disturbance of the sample. Finally, the detergent amended sample performed significantly better than the others. This is because the detergent works to break up the surface tension without altering the iron oxides. A second dye test completed for this experiment confirmed that detergent addition in small volumes to the iron oxides eliminated preferential flows (Figure 17).

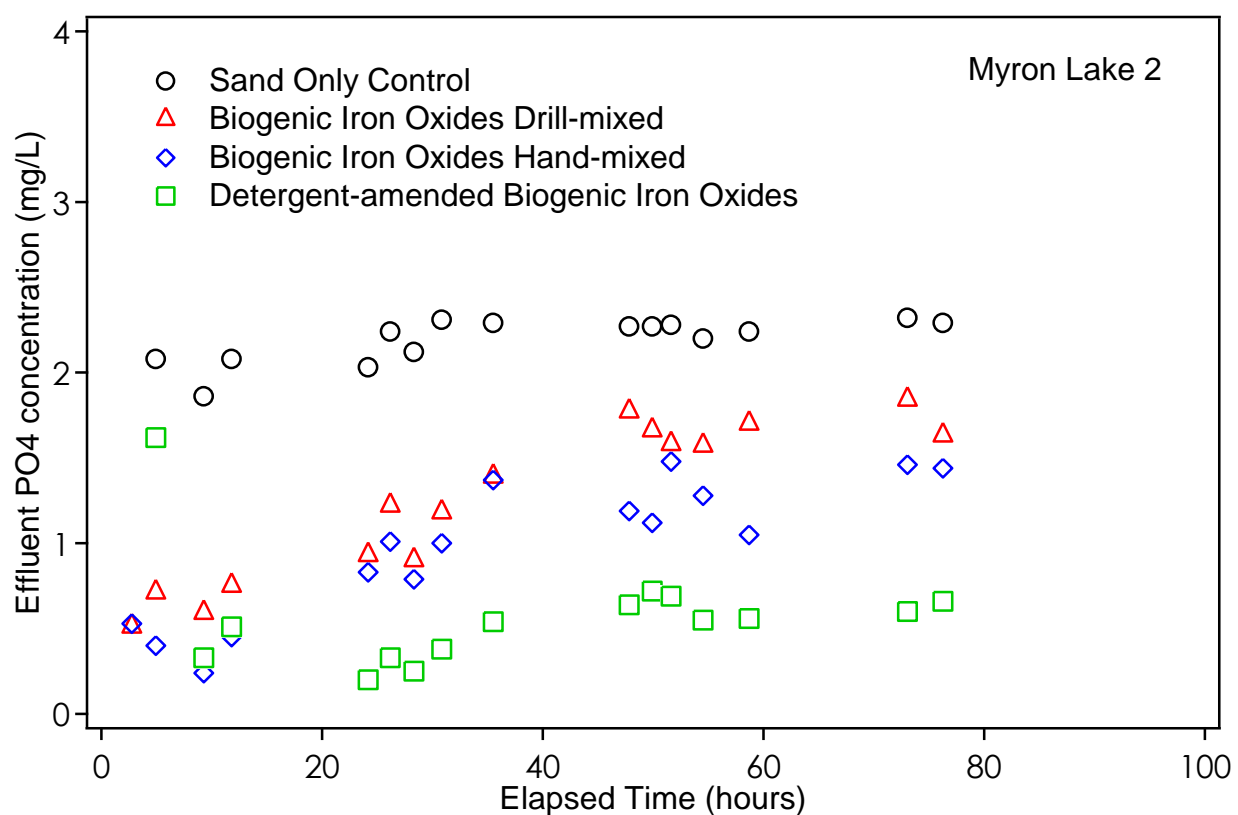


Figure 16: The detergent amended (0.033% of total volume) iron oxides (Myron Lake 2) performed significantly better than either of the well-mixed samples.

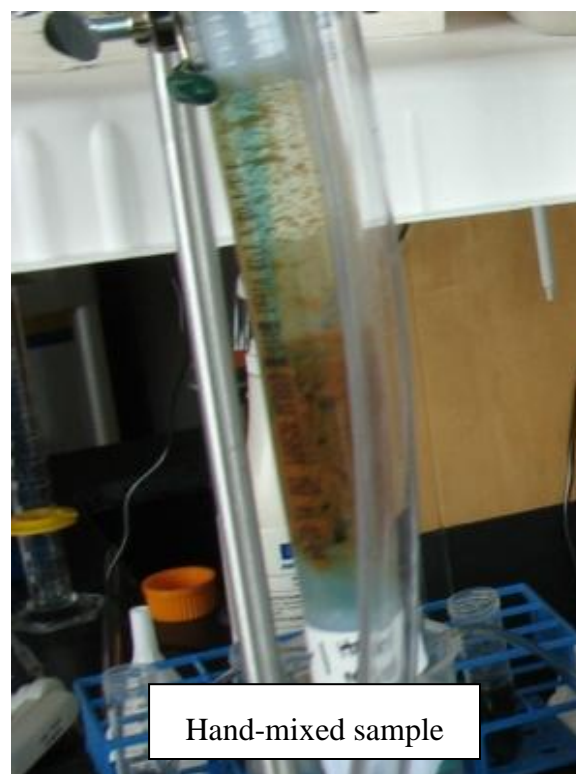
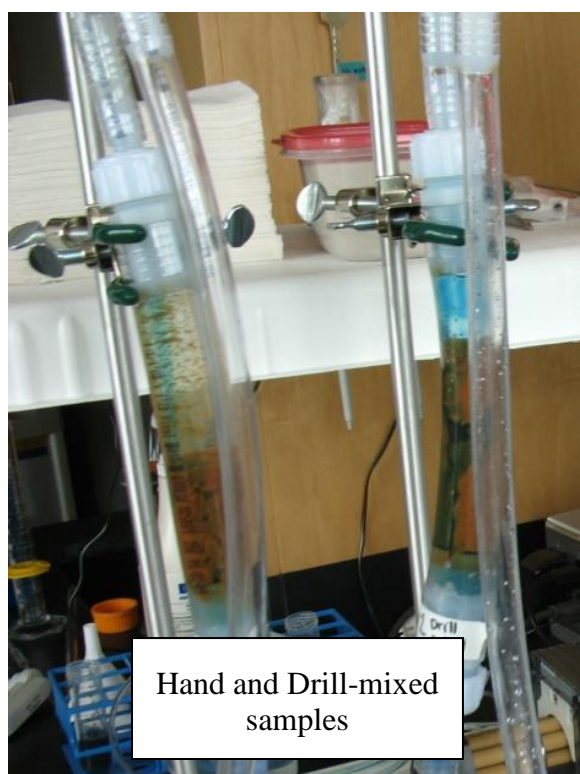
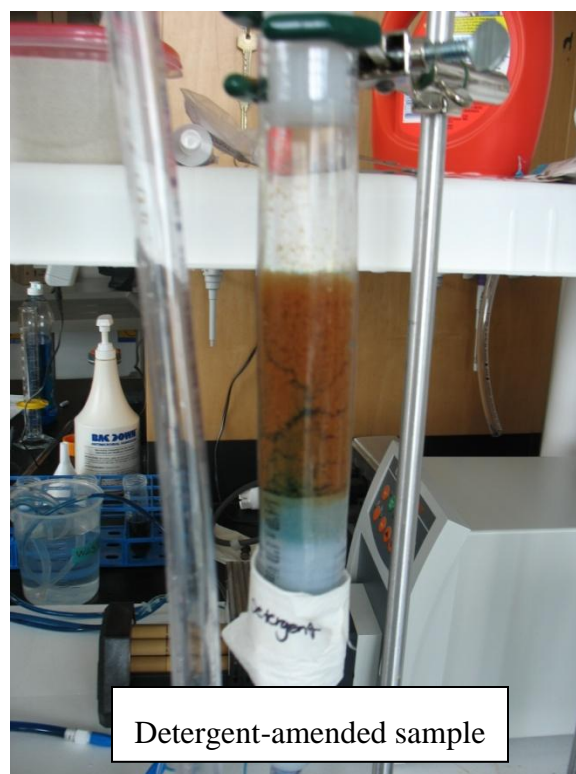
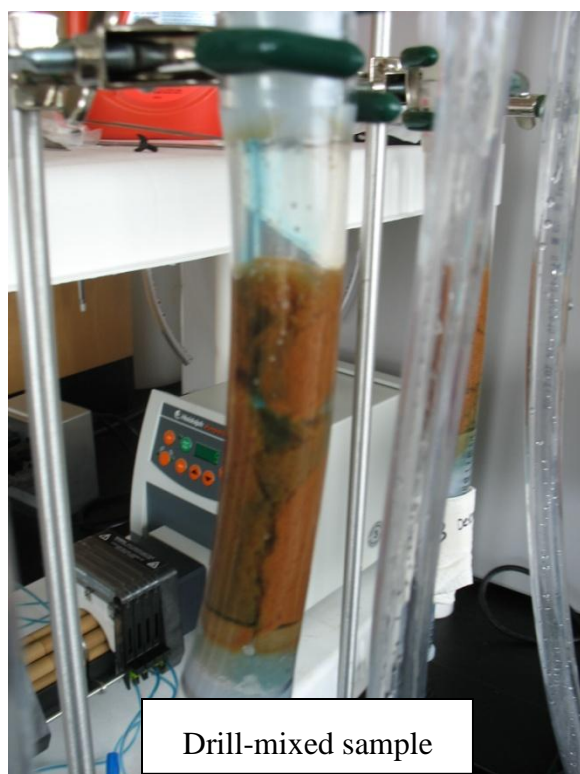


Figure 17: Photographs illustrating the preferential flows in the hand-mixed and drill mixed iron oxides. The detergent amended filter did not have the flow pathways that the other two indicated.

Extremely low phosphorus concentrations were observed when high iron concentrations and detergent were added to the filter (Figure 18). The detergent amended columns achieved $90 \pm 9\%$ and $87 \pm 14\%$, respectively, compared to the sand only control which did not remove any phosphate and the unaltered iron oxides column that removed an average of $69 \pm 14\%$. These results indicated that only a small detergent volume was required to significantly improve the phosphate removal efficiency, as the 50 μL detergent addition performed as well or better than the 100 μL detergent addition. The lowest effluent concentration achieved by the unaltered iron oxides was 0.26 mg/L, a maximum removal efficiency of 88%. The detergent amended filters realized effluent concentrations of less than 0.2 mg/L for eleven days and nine days, respectively. The removal efficiency for the detergent-added columns was greater than 91% for nine days (Figure 19).

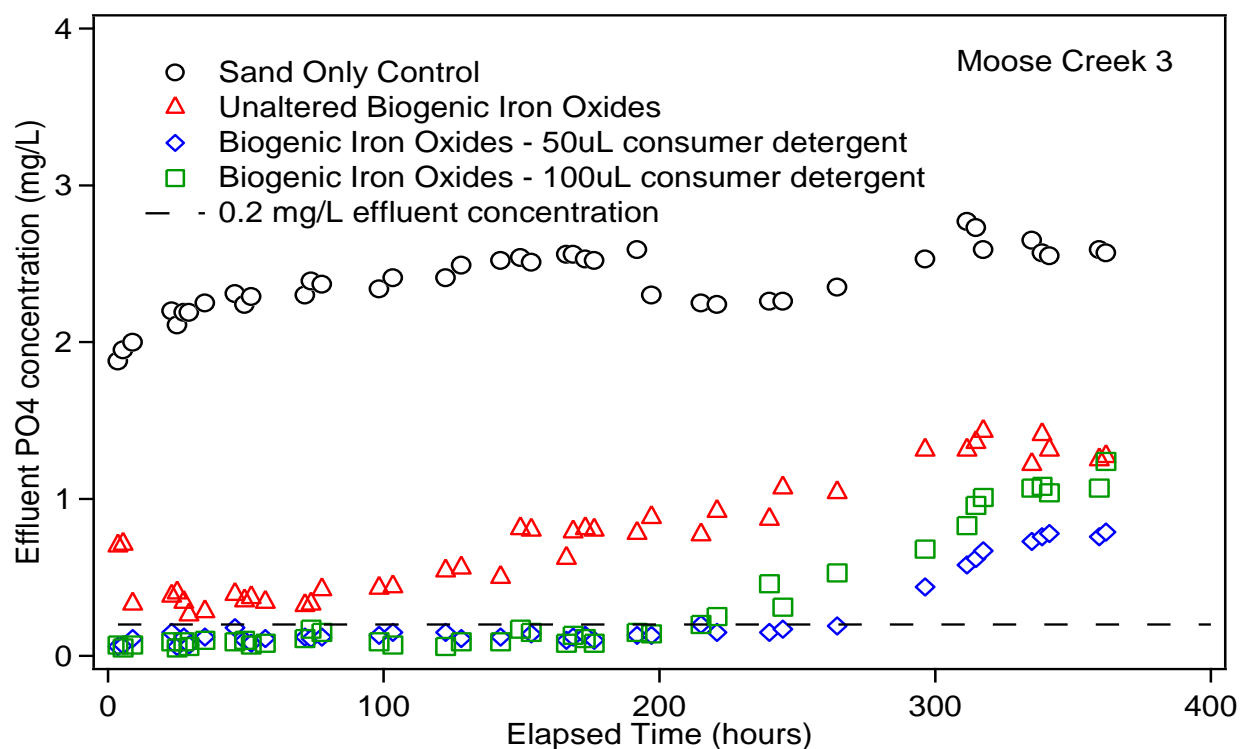


Figure 18: The detergent amended filters performed consistently better than the unaltered iron oxides. The highest performing filter had the lowest volume of detergent added. The 0.2 mg/L effluent is highlighted by the dashed line.

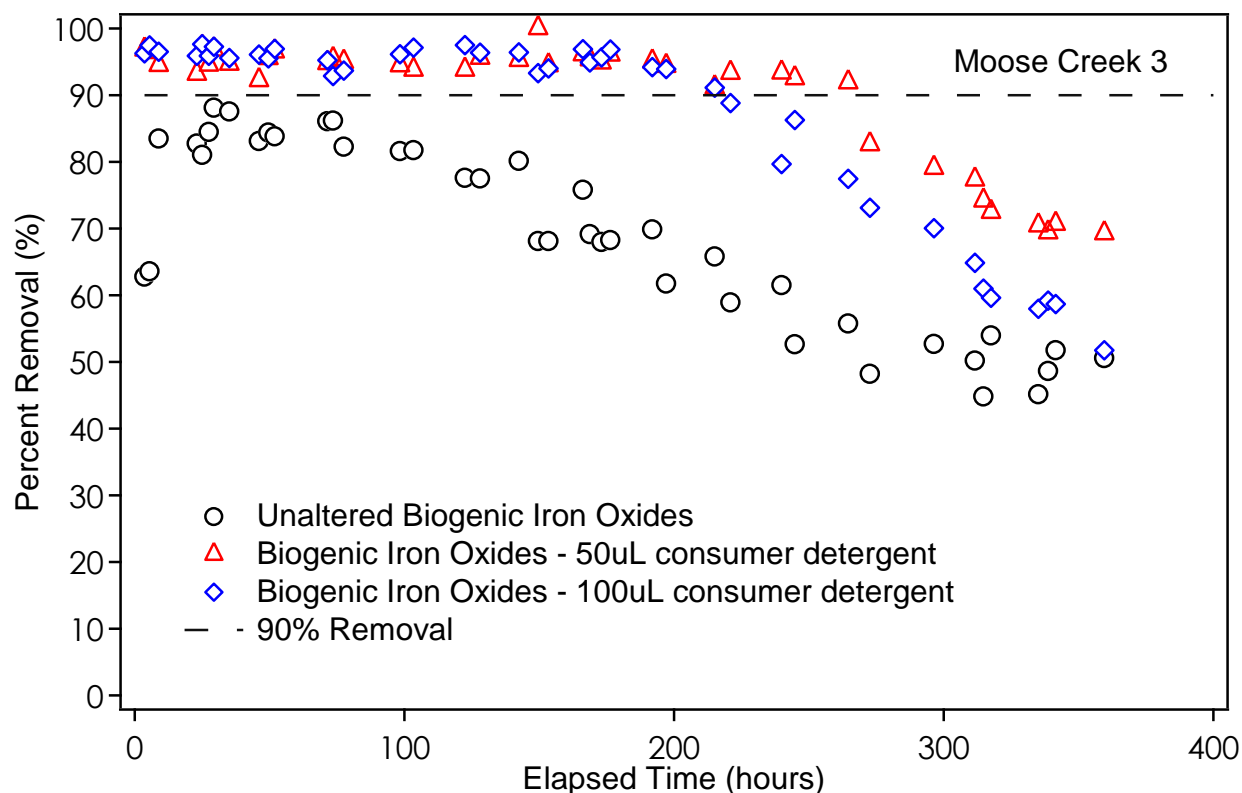


Figure 19: Percent phosphorus removal for three upflow columns (Moose Creek 3). The detergent amended columns achieved greater than 90% removal for over 200 hours compared to the natural sample, which never achieved 90% removal.

Lab-grade detergents were also an effective pre-treatment for biogenic iron oxides which was illustrated using a sample from Myron Lake. The percent removal was greater than 70% for 28 hours for both the TritonX and Tween-80 treated samples. The natural sample had 73% removal at the first reading but never achieved greater than 70% removal after that (Figure 20). The average percent removal for the natural sample was $48 \pm 13\%$. The Tween-80 worked slightly better than the TritonX as a surfactant, $58 \pm 17\%$ compared to $54 \pm 20\%$ and therefore was selected as the detergent to be used for the remaining filter experiments. The detergent amended iron oxides performed the best for all of the Myron Lake (YK10, YK11, and YK12) samples. The unaltered iron oxides for Myron Lake 10 and Myron Lake 12 removed $45 \pm 18\%$ and $48 \pm 13\%$, respectively. The detergent altered sample for Myron Lake 11 achieved the highest removal percentage. The low percent removal for the lab-grade detergent amended

samples was likely because the sample did not sit overnight. Also, the physical difference of the Myron Lake samples compared to the Moose Creek samples likely negatively affected the removal capability. The Moose Creek samples were always more settled and more individual particles could be seen. The Myron Lake samples, on the other hand, are significantly interconnected as can be seen by the photographs. Even in the water, there is a distinct structure and shape to the sample, where the Moose Creek samples were disconnected and settled.

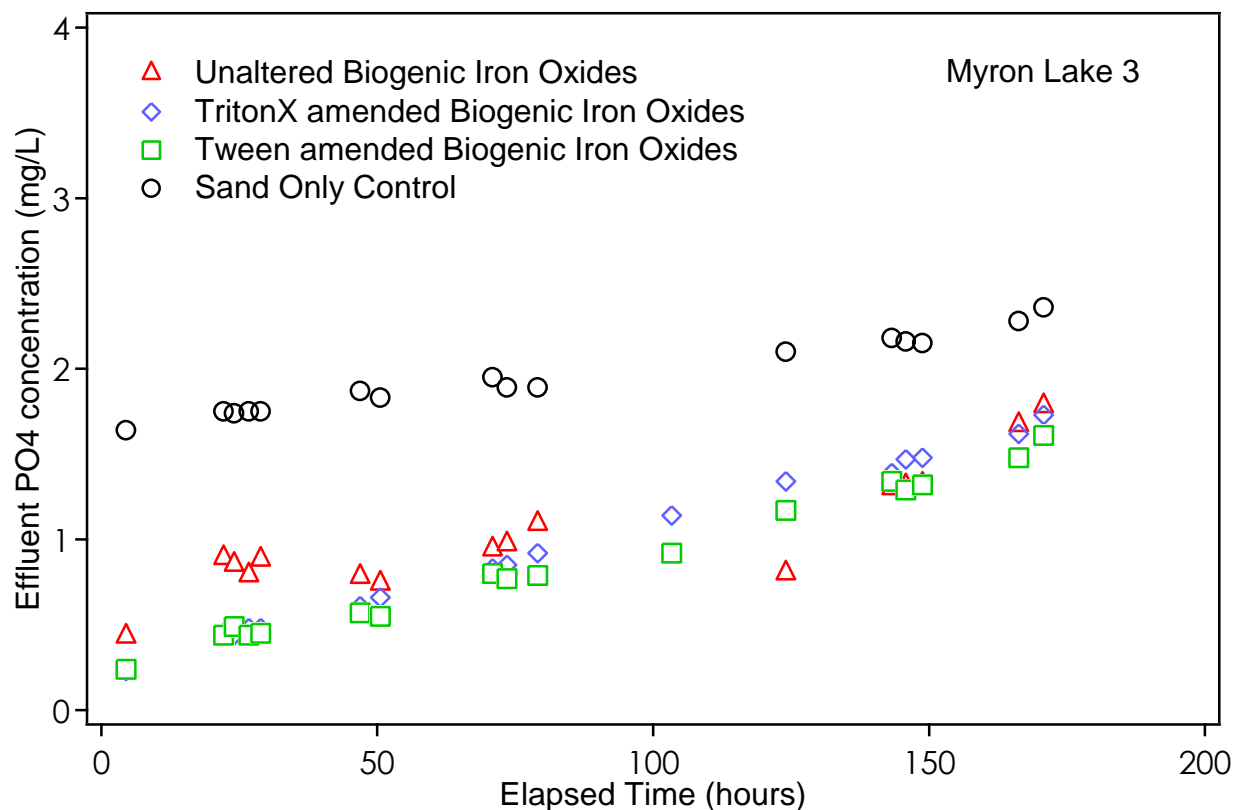


Figure 20: Upflow, pumped filter columns treated with commercial-grade detergents for a Myron Lake sample.

Frequently mixing the iron oxides within the column in an effort to increase contact time and improve filter efficiency did not work. The stirred column achieved greater than 80% removal for 28 hours, the detergent-treated column (not stirred) achieved greater than 90% removal for 50 hours, and the natural sample achieved greater than 75% removal for 28 hours

(Figure 21). This experiment confirms that the addition of a surfactant improves the removal efficiency. Stirring the iron oxides, however, likely released some of the adsorbed phosphate therefore increasing the effluent concentration.

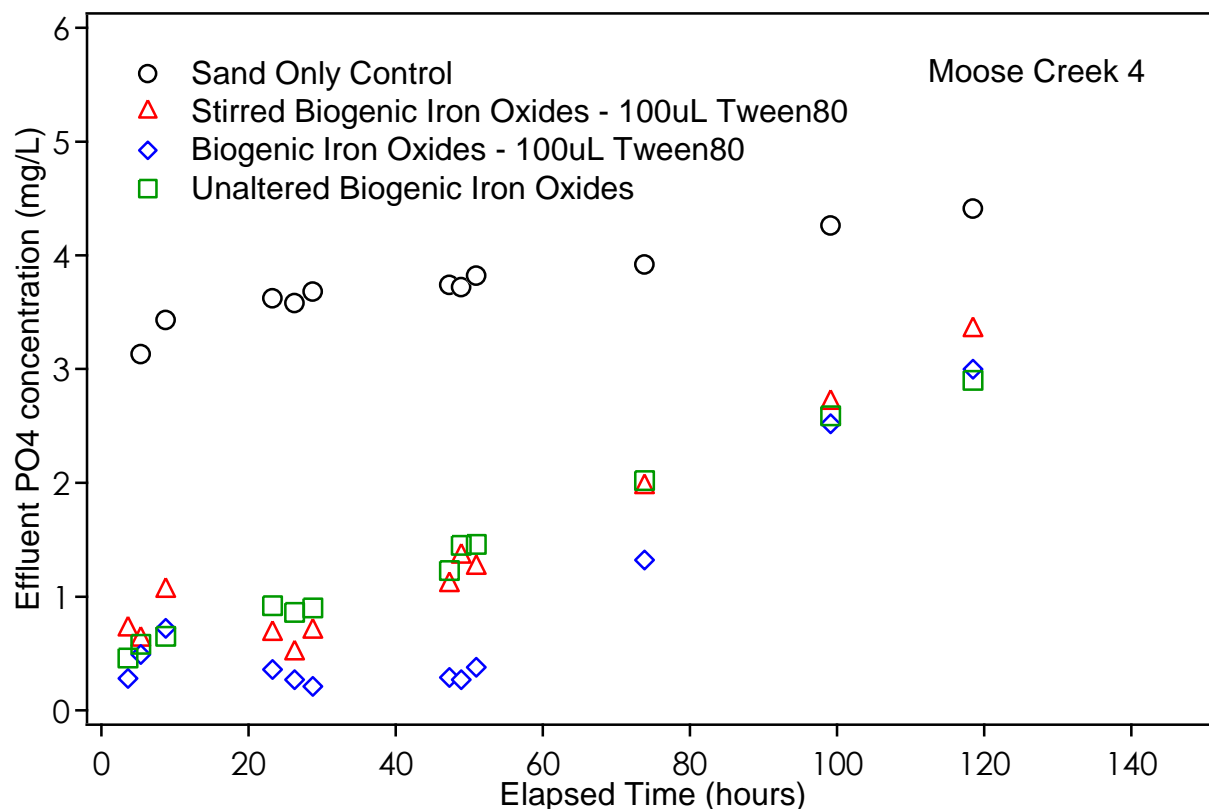


Figure 21: The detergent amended column for Moose Creek 4 performed significantly better than the unaltered biogenic iron oxides or the mixed column, which showed similar removal efficiencies.

The Tween80 detergent did not adsorb any phosphate when it was tested for sorption capacity (Figure 22). Visually, this sample was slimy and had one of the highest organic content and lowest iron/solids ratios of the samples we collected. This sample also had the lowest Γ_{\max} of all the samples (6.20 mg P/g solids). It was observed that the Tween80 did not adsorb any of the phosphate and therefore was not interfering with the iron oxide experiments except to break up the iron oxides. Due to the low Γ_{\max} for this sample, the filter results were the lowest of the

series. The unaltered iron oxide filter had an average removal efficiency of $53 \pm 12\%$ compared to $56 \pm 11\%$ for the detergent-amended sample.

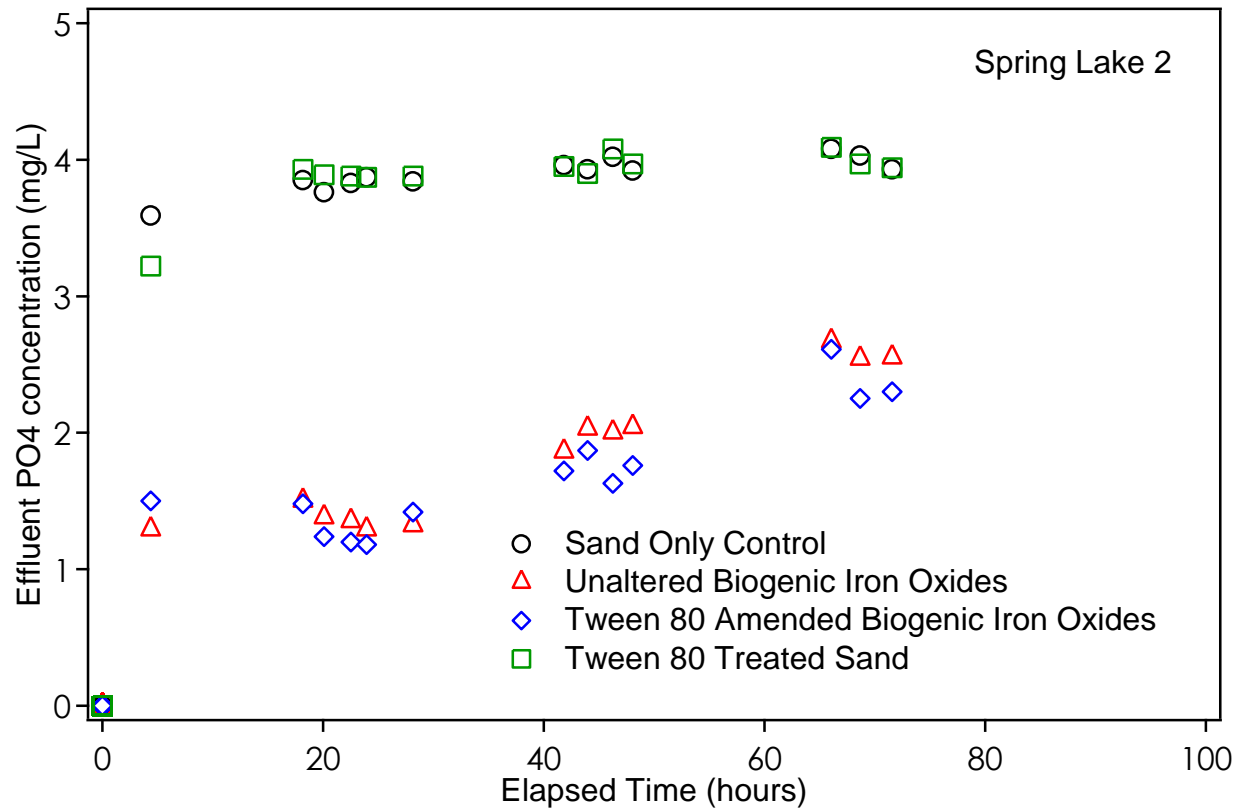


Figure 22: The detergent amended filter performed slightly better than the unaltered biogenic iron oxides. The detergent amended sand, however, did not remove any phosphate indicating that the iron oxides are doing all of the adsorption.

4.0 CONCLUSION

Column filter studies confirmed the applicability of biogenic iron oxides as a filter media for phosphorus removal. Batch equilibrium experiments for nine samples confirmed data from previous studies, and led to the following conclusions:

- Adsorption isotherms followed the Langmuir model, indicating iron oxides became saturated with phosphorus.
- Maximum adsorption using biogenic iron oxides, normalized to total solids, was consistent with previous research in our lab (Rentz et al., 2009).
- Maximum adsorption using this media was higher than values reported for other iron rich or iron oxide containing substrates

Filter design and construction was based on ease of use and bench-scale set-up simplicity. Filter design was based on simple filters developed by Leupin et. al (2005) for pilot scale removal of aqueous phosphorus using practical, functional and local materials. While pumped systems, compared to gravity-fed, increase complexity and the risk of technical problems, in the case of these filter experiments the use of a peristaltic pump dramatically improved the ease of use, reproducibility, removal efficiency.

Using bacteria within filters also increased the column design complexity. While these experiments did not require bacterial cultures, as did those described by Galera et al. (2007), the samples had to be collected on-site for every column experiment. Also, these bacteria have not been successfully grown in the laboratory, which creates a challenge for scaling up this project.

The column experiments discussed here confirmed that biogenic iron oxides will work as a filter media substrate. High removal percentages and low effluent concentrations were

achieved under well controlled conditions. The use of a pump and other manipulations suggest that operator knowledge is key to streamlining filter function.

5.0 APPLICATIONS AND RECOMMENDATIONS

Biogenic iron oxide filters may be an option for point source phosphorous removal at WWTFs. In order for this application to succeed, it must first be discovered how to grow iron oxidizing bacteria in the laboratory in order to generate them in sufficient quantity. Once generated in the laboratory, further research will need to be carried out to confirm that these bacteria can also be cultured in a WWTF setting. If the iron oxidizing bacteria could grow and self-regenerate, they would have a significant advantage over chemical iron oxides that cannot be regenerated within a filter.

Phosphorus recovery is at the forefront of research and further investigation into this application is needed. Based on previous desorption studies, adsorbed phosphorus can be recovered and reused, but the iron oxides are likely to be lost. Another possibility for phosphorus recovery is applying the used iron oxides, with adsorbed phosphate, as fertilizer, but again, the iron oxides will be spent. These two applications imply the importance of the ability to grow these bacteria in the laboratory.

Finally, constructed treatment wetlands offer a natural filtration option for phosphorus removal. If the growth of iron oxidizing bacteria could be engineered in a natural treatment system, phosphorus removal could be achieved. Future research must include attempting the growth of these bacteria in a laboratory setting and testing the filter function using phosphorus containing wastewater effluent. Other research will include a surface area analysis to compare the surface area with maximum sorption as well as compare the natural biogenic iron oxides to engineered substrates.

6.0 APPENDIX A – STANDARD OPERATING PROCEDURES

Field Sampling – Iron Oxides

Washington State University
Center for Multiphase Environmental Research (CMER)

c/o Jeremy A. Rentz
Sloan Hall
Pullman, WA, 99164

Created by: Haley Falconer, Graduate Research Assistant
Date: September 2, 2009

Adapted from: Antoine Cordray, Graduate Research Assistant
Date: May 08, 2008

Supplies:

These supplies should be in the lab backpack or the cooler.

- Copy of SOP
- FOX record sheets (one per sample)
- Field notebook
- Labeling tape and marker
- Pen/pencil
- Automatic pipette; 5 mL and 50 mL pipettes
- 1-L jars for storing sample
- Thermometer
- pH meter
- 15 mL Falcon tubes and FerroVer pillows (three per sample)
- Scissors
- Cooler and ice packs
- Waders
- Sunscreen and bug spray

Method:

The following must be recorded on the FOX sheet for each sample:

- Sample name and number (Yakima 1, YK1), date and time
- Site description (location, weather)
- Names of those on the sampling trip

Label each container in the following manner:

- Location prefix and number (YK1)
- Date
- Sampler's initials

Field Sampling Procedures:

Samples should be collected from at a site that has the most fresh iron oxides. When sampling more than one site in the same area, you should begin downstream and work upstream. This is to ensure that downstream samples are not affected by previous sample disturbances. One liter of sample is required for each batch equilibrium experiment (i.e. 1 for batch, 1 for pH, etc).

- **Field pH:**
 - Place field pH meter in water and allow it to equilibrate.
 - Record the value on the FOX sheet
 - Repeat for each location
- **Field Temperature:**
 - Place thermometer near collection site and allow it to equilibrate.
- **Field DO:**
 - Place the DO probe in the iron oxide biofilm and allow to equilibrate
 - Record the value on the FOX sheet
 - Repeat in a nearby area to get at least three DO readings
- **Field aqueous Fe concentration (n=3):**
 - For each sample site, pipette 5mL of sample into a 15mL Falcon tube
 - Add 1 FerroVer powder pillow to each tube and shake (Hach #8008)
 - Cap tube for transportation (analysis performed in lab); transport on ice
- **Iron Sample Collection**
 - Start downstream and work upstream to disturb biofilms as little as possible
 - Use a 50mL pipette to withdraw iron sample
 - Do not pipette sediments; try not to disturb iron oxide biofilm
 - Transfer sample to 1L bottle until bottle is full; allow sample to settle
 - Place the samples on ice for transport back to the lab

Iron Oxides Characterization

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Date: September 02, 2009

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Date: May 08, 2008

Supplies:

- 12 x 50mL Falcon tubes
- 13 x 15mL Falcon tubes
- 1 x 1L jars
- 2 PhosVer (reactive phosphate) tubes for Hach method 8048
- 5mL, 10mL, 25mL and 50mL Pipettes
- 0-20 μ L, 0-200 μ L, 0-1000 μ L tips for micropipettes
- DNA microtubes
- Slides and cover glass for microscope study
- 700mL of 0.1M saline wash per sample (700mL DI water + 4.09 g NaCl + autoclave)
- 1mL of 10 μ g/L Acridine Orange solution (2 μ L of AO solution in 20mL DI water)
- 350 mL of 0.25M Oxalic Acid solution(Add 22.06g of Oxalic acid powder in 1L DI water)
- 20 mL of 1M NaHCO₃ solution (20.985g of powder NaHCO₃ in 250 mL DI water)
- 250 mL of 0.1g/L Cu (Add .2683g of CuCl₂*2H₂O powder to 250mL DI water)

Method:

Saline Wash

- Washing should be performed on a field sample in order to remove uncertainties associated with the composition of the *in situ* water.
 - Allow sample to settle for a several hours
 - Remove supernatant by pipetting without removing any solids
 - Save 2-50 mL tubes of supernatant for TOC analysis
 - Fill sample container back up with 100mM NaCl solution to 1L total volume
 - Ensure that the same volume of saline wash is added to each sample
 - Record the saline wash volume on the FOX sheet
 - Transfer 100 mL (2-50 mL tubes) of washed, mixed sample for post-wash analysis

The following procedures must be completed for a raw, unwashed sample and for the sample after the saline wash is complete. There will be two tubes for both pre- and post-wash analysis (Pre1, Pre2 and Post1, Post2). The methods below are different for tube 1 and tube 2.

Tube 1: Mixed Sample

- **pH**
 - Check pH meter using buffer solutions (pH= 4.0, 7.0, and 10.0)
 - Use pH meter to analyze; record on FOX Sheet
- **Total Fe (n=3)**
 - Add 1mL of well-mixed sample and 49mL of 0.25 oxalic acid to each of three 50mL tubes
 - Allow the digest to sit overnight
 - Add 5mL of well-mixed oxalic acid digest to 15mL tube; *slowly* add 2mL of NaHCO₃; read pH (between 3-5)
 - Dilute samples: add 1mL pH digest and 4mL DI water; add FerroVer pillows to the tubes and shake well; wait two minutes before analysis
 - Select the FerroVer program from Favorite Programs menu on the spectrophotometer; “Zero” the machine using a cuvette with DI water
 - Pipet the orange iron solution into a square cuvette, clean with a Kimwipe, and push read
 - If the concentration is out of range, the solution needs to be diluted again (total volume must be 5mL)
- Account for dilution factors in determining Total Iron concentration.
 - x2.5 for difference between pre-programmed vial size and used cuvettes.
 - x50 for initial oxalic acid digest
 - x1.4 for pH adjustment
 - xD for final 5mL dilution with FerroVer pillow
- **Dry Density**
 - Label and weigh aluminum evaporating dishes (R=pre; S=post; n=4)
 - Transfer 10mL of mixed sample to each dish
 - Evaporate water in oven (110°C) overnight
 - Place samples in dessicator to cool for 20 minutes
 - Weigh evaporating dishes with dried solids
 - Dry density = (final dried weight - initial weight) x 100mL
 - Record results on FOX sheet
- **Organic Content (n=4):**
 - Use the dried samples from the dry density characterization
 - Re-weigh dried samples

- Place samples in muffle furnace (440°C).
- Allow samples to heat for 24 hours
- Place samples in dessicator to cool for 20 minutes
- Weigh samples to determine inorganic weight
- Record results on FOX sheet

Tube 2: Settled sample

- **Aqueous Phosphate Concentration (n=1):**
 - Withdraw 5mL of supernatant from the settled sample solution.
 - Add to PhosVer vial with a PhosVer powder pillow. Wait two minutes.
 - Analyze phosphate concentration using Hach method 8048
 - Select PhosVer program on spectrophotometer
 - Record value on FOx Sheet for Pre and Post PO₄
- **Photographs of Iron-oxides:**
 - Add 50 to 100µL of the specimen to a clean glass slide
 - Add a drop of Acridine Orange (10 to 20µg/mL) to the specimen drop
 - Mix to make it spread homogeneously using the pipette tip extremity
 - Add a cover glass. Let one side of the cover glass touch the slide first. The solution is going to spread on this side. Then, let the cover slide drop to avoid air bubbles.
 - Turn on microscope (Arclamp power supply, LEP Ltd), camera (RT power supply, SPOT), polarized light and computer
 - Place glass slide on the microscope
 - Rotate numerated disk and select the position 3 (fluorescence)
 - Localize an appropriate view using 10x and 40x objectives
 - Add a drop of immersion oil on the cover slide and select the 100x objective
 - Increase or decrease light intensity to allow more or less fluorescence
 - Find appropriate settings to produce a desired image
 - Set the vision screw either on “camera” (image just on camera) or on “50/50” (image for user and camera) to allow camera to get the image
 - Open the software “SPOT advanced”. The shortcut is on the desktop
 - On the bottom right corner, select the mode “ACAO”
 - Click on the button “get image” on the right side of the screen or push F9
 - The settings might change during the capture of the picture. Make sure that the clarity of the image stays the same during the capture

Phosphate Batch Equilibrium

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Supplies:

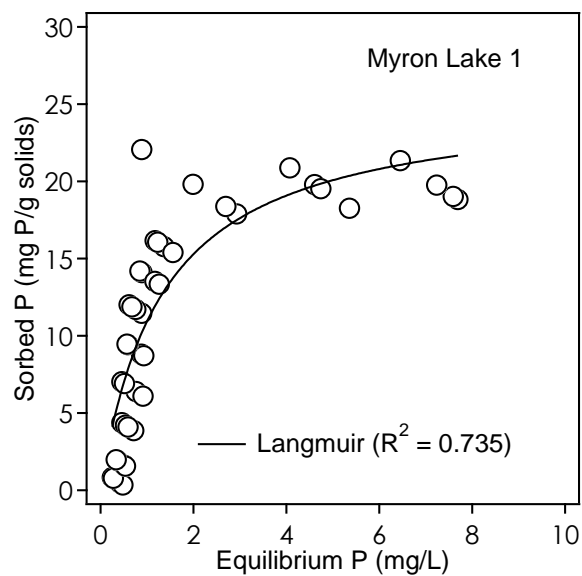
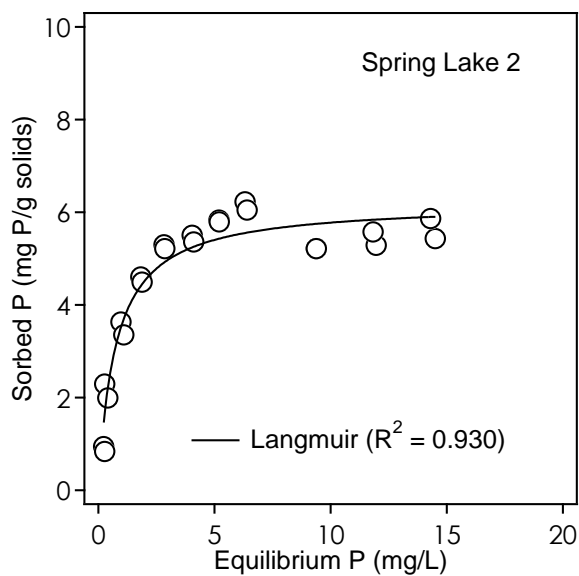
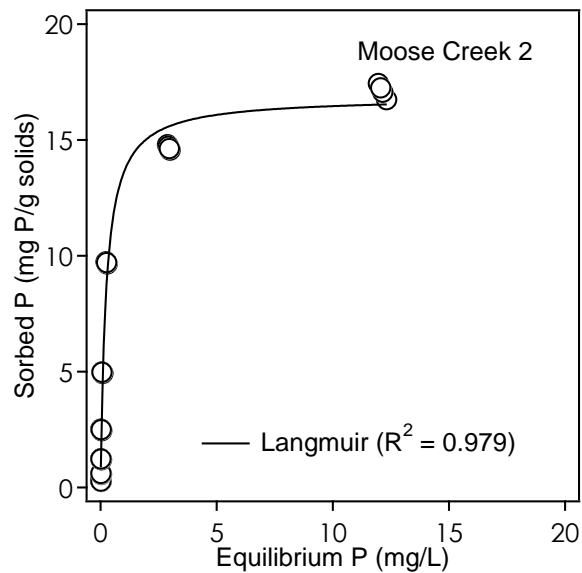
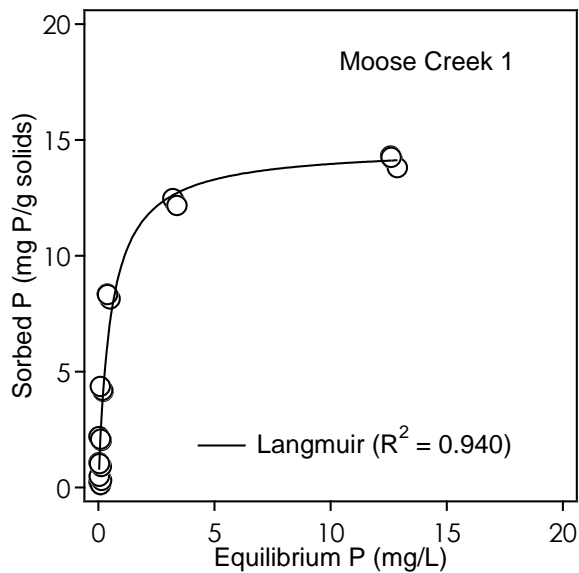
These supplies should be in the lab backpack or the cooler.

- 33 x 50mL Falcon tubes
- 33 PhosVer (reactive phosphate) Hach Method 8048
- 5mL, 10mL, 25mL and 50mL Pipettes
- 0-20 μ L, 0-200 μ L, 0-1000 μ L tips for micropipettes
- 250 mL of 1g/L PO₄ solution (Add 0.4584g of K₂HPO₄ powder to 250mL DI water)

Method:

- o Add equal volumes of washed sample (less than 15 mg of iron per reactor) to 33 x 50 mL Falcon tubes (11 concentrations, n=3)
- o Add the amount of DI water and 1g/L Cu solution given by the FOX sheet (the total volume after Cu and sample have been added should be 50mL)
- o Place reactor tubes on the inversion rack for a period of 24 hours on setting #3. Make sure that the air conditioner is turned on.
- o Centrifuge reactor tubes at 4100 rpm for 10 minutes
- o Test phosphate content using Hach method 8048

7.0 APPENDIX B – SUPPLEMENTARY DATA



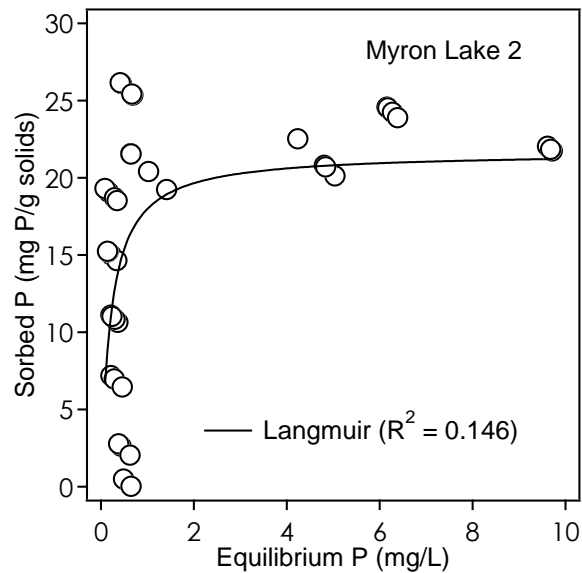


Figure B1: Representative batch equilibrium plots for the remaining sampling site associated with each filter experiment. R^2 values are reported for the Langmuir isotherm, which provided the best fit for all of the samples.

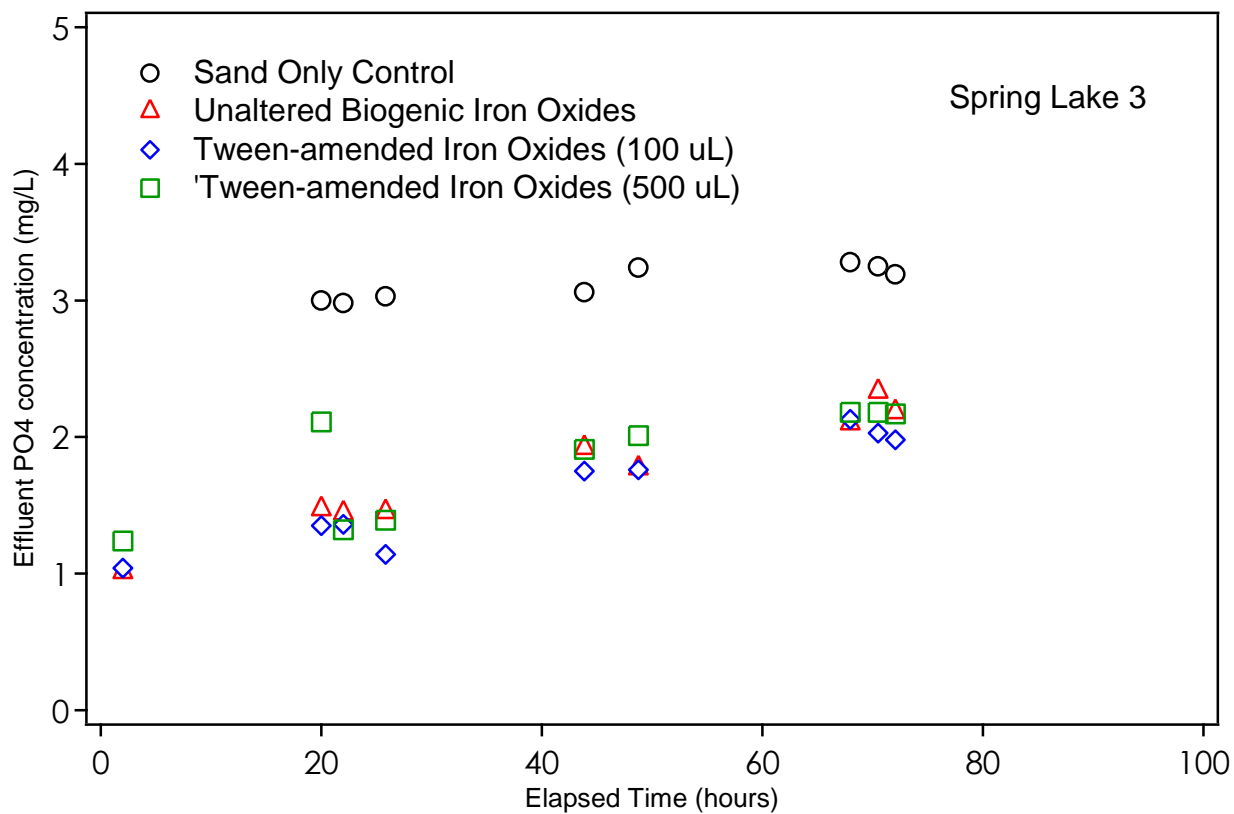


Figure B2: Results indicating that a small volume of detergent is required to achieve improved results when compared to the unaltered sample.

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