Spring 2020

Determination of iron-reducing bacterial activities in lake sediments.

Alexandra Kahn
ark114@zips.uakron.edu

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Alexandra Kahn

Honors Research Project

The Department of Geosciences and Biology, University of Akron
Akron, Ohio

May 4th, 2020

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Abstract

Algal blooms are growing rampantly in lacustrine systems due to an increase of phosphorus, a nutrient for algae. Phosphorus is being released into the environment causing overpopulation and eutrophication which damages the ecosystem. The availability of phosphorus is affected by the solubility of iron which is regulated through iron reducing and iron oxidizing bacteria. Phosphorus adheres to insoluble Fe(III), which prohibits algae from utilizing it, while phosphorus does not attach to soluble Fe(II) and therefore it remains available to algae. The purpose of this study was to determine how bacteria influence iron solubility and what are the ideal environmental conditions required for the bacteria to metabolize specific redox states of Fe(III)/Fe(II). I conducted batch experiments over a 30 day period in which the oxygen conditions varied as well as the live and sterile incubation sets. I measured the total and dissolved Fe(II) concentrations. My study revealed that iron-reducing bacteria do in fact aid in the increase of soluble Fe(II) under anoxic conditions. In oxic environments, no Fe(II) oxidation occurred. As for the total iron, all environmental conditions stayed within a small range and decreased, then increased when there was a rise in Fe(II) in anoxic conditions and decrease of Fe(II) in oxic conditions. The result indicates that total iron decreased at the beginning of bacterial metabolism of iron, and then increased when metabolism was fully active. Furthermore, the pH indicated some correlations; when iron was metabolized by iron reducing bacteria, there was a removal of H^+ ions, hence pH increased in anoxic conditions when there was an increase in Fe(II). There were issues found in this study between the live and dead batch sets; there were no variations between the two sets which could be due to improper sterilization or residual bacteria in the sterile batch sets. This work may help improve the understanding of how bacteria control

the iron solubility in lacustrine environments by identifying where iron reduction takes place. Additionally, this work may aid future studies in determining how changing iron solubility affects phosphorus levels, and if oxygenating benthic sediments can decrease the flux of limiting nutrients.

Introduction

Dangerous algal blooms are on the rise in the water systems of Ohio. The foundation of ecosystems is based on interdependent components; the limiting nutrients in the water and the various organisms. Both are essential for the normal function of the ecosystem. A stable/healthy environment is when all the organisms are interacting in balance with one another. A healthy ecosystem is capable of returning to its state of equilibrium even after a perturbation (a capacity known as resilience) and/or does not experience unexpected substantial changes in its characteristics across time (National Research Council, 2013). Limiting nutrients are essential elements for plant life to grow and survive, and their concentrations determine the amount of productivity in an ecosystem (Carlson & Simpson, 1996). Each organism needs resources to survive in its environment. If there is access to these resources and there are no outside factors to limit the population, then that organism will overpopulate to the point where the ecosystem is negatively affected (National Research Council, 2013). A large amount of accessible limiting nutrients leads to detrimental algal blooms; a drastic change to the environment in a short time poses serious long-term effects in the area (National Research Council, 2013).

An excess of limiting nutrients added in water systems is also known as nutrient pollution, which lead to unhealthy lakes. Phosphorus is one of the limiting nutrients that causes

nutrient pollution and it is involved in several essential plant functions; including energy transfer, photosynthesis, the transformation of sugars and starches, nutrient movement within the plant, and transfer of genetic characteristics (Mosaic Crop Nutrition, n.d.) Phosphorus is classified into two broad groups, organic and inorganic. Organic phosphorus is found in plant residues, manures and microbial tissues. Inorganic forms consist of apatite, complexes of iron and aluminum phosphates, and phosphorus absorbed onto clay particles (Mosaic Crop Nutrition, n.d.) Because of this fixation, phosphorus has low solubility and moves very little in the soil. The soil/rock with inorganic phosphorus can naturally erode and be recycled through plants to convert to its organic form, which are released into an ecosystem and gradual increase the concentration of phosphorus over time in a healthy environment (Rosen & White, 1999). This allows algae to grow but not at the expense of the whole ecosystem (Chaffin, 2017). However, extensive nutrient pollution has rapidly sped up this process and has caused ecosystems to become unstable (Chaffin, 2017). From improper fertilization and natural weathering, phosphorus can be released and washed into other areas through runoff or sewer systems; movement of surface water that can carry limiting nutrients that empty into water systems (Rosen & White, 1999). Fertilizer can be made from soluble/insoluble phosphorus. If fertilizer is not distributed in correct quantities and/or mixed well within the soil, or if the soil is heavily eroded, an overabundance of limiting nutrients can occur in the lake ecosystems (Rosen & White, 1999). Furthermore, erosion of soil can remove organic and inorganic phosphorus from its source and runoff can carry it into water systems and pollute it. Excess phosphorus within a body of water can allow algae populations to drastically increase and create an unstable/unhealthy ecosystem.

There are different classifications for underwater biological environments that rank the overall health of an ecosystem; The trophic state index (TSI) is a classification system to rate

water bodies based on the number of biological activities they can sustain (Carlson & Simpson, 1996). To determine the TSI of a water body; concentrations of chlorophyll pigments, TSI(CHL) and total phosphorus, TSI(TP) are measured. Furthermore, a Secchi depth disk TSI(SD) is an additional method used to measure water transparency, indicating the concentration of particulate material in the water, which in turn can be used to derive the biomass (Carlson & Simpson, 1996). Concentrations of phosphorus and chlorophyll pigments within water samples and the Secchi depth are quantified through calculations, which are then categorized into an index. The categories include oligotrophic, mesoeutrophic, eutrophic, and hypereutrophic. Oligotrophic lakes with a TSI 0–40, have the least amount of biological productivity, but "good" water health (Carlson & Simpson, 1996). Mesoeutrophic lakes with a TSI 40–50, have a moderate level of biological activity and "fair" water health. Eutrophic lakes with a TSI 50–70, have a high amount of biological activity. Hypereutrophic lakes with a TSI of 70 or higher, have the highest amount of biological activity and have "poor" water health (Carlson & Simpson, 1996). Bodies of water typically gain more and more nutrients gradually over time and increase their TSI. The problem is that nutrient pollution has worsened and poses threats to other organisms within the ecosystem (Carlson & Simpson, 1996).

Algae carry out photosynthesis, which produces oxygen and biomass; however, after algae die, they are metabolized by bacteria through cellular respiration, which uses up oxygen. Cell respiration is a metabolic process that uses biochemical energy from nutrients such as glucose to produce adenosine triphosphate (ATP) as energy to sustain life. Cell respiration oxidizes and reduces substrates. Carbons in the glucose become oxidized; a process by which an atom gains bonds to more electronegative elements. Oxygen in the reaction becomes reduced; a process by which an atom gains bonds to less electronegative elements, most commonly

hydrogen. Cell respiration produces water and carbon dioxide as its products, which are released back into the system, as seen in R1 (Parker et al., 2016).

$$R1: C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + ATP$$

During oxidative phosphorylation, which is a part of cell respiration, hydrogen ions are transferred across the plasma membrane of the bacteria by a series of electron carriers. Electrons are passed from one electron carrier to the other, which use the transfer of electrons as energy to pump hydrogen ions into the periplasmic space (area between plasma membrane and cell wall of the bacteria) from the inner matrix of the bacteria to create a gradient, which will be used later to create energy. High energy electrons come from NADH and $FADH_2$, which are donated to the electron carriers; NADH and $FADH_2$ acquire the electrons from glucose through glycolysis/Krebs cycle (Parker et al., 2016). The electrons being passed by electron carriers eventually donate the electrons to dissolved oxygen, the strongest and final electron acceptor, which activate the enzyme ATP synthase. This enzyme is a complex protein that is propelled by the force of the H^+ diffusing through the enzyme down its electrochemical gradient; regenerating ATP from ADP and inorganic phosphate (Parker, et al. 2016). ATP is made up of the molecule adenosine and three phosphate groups, which is one reason why phosphorus is essential for life. Without oxygen, the electrons would have no final electron acceptor and the electron transport would stop working. Furthermore, high energy molecules NADH and $FADH_2$ would not be able to convert back into NAD and FAD to regenerate the transport of electrons (Parker et al., 2016). Therefore, when algae die and they are used as an energy resource by bacteria through cell respiration, oxygen will become depleted and halt all forms of aerobic respiration in the

ecosystem (Figure 1). The deprivation of oxygen leads to eutrophication, which causes the death of other organisms that utilize oxygen and cannot survive in conditions without it. Due to the overgrowth and consumption of dead algae; carbon dioxide becomes more prominent than oxygen and the carbon dioxide that was produced is then released into the environment. Furthermore, dead algae being decomposed at the bottom of the lake will release phosphate (Correll, 1999). Phosphorus will remain in the sediment at the bottom of the lake after decomposition, which will now be controlled by the solubility of iron.

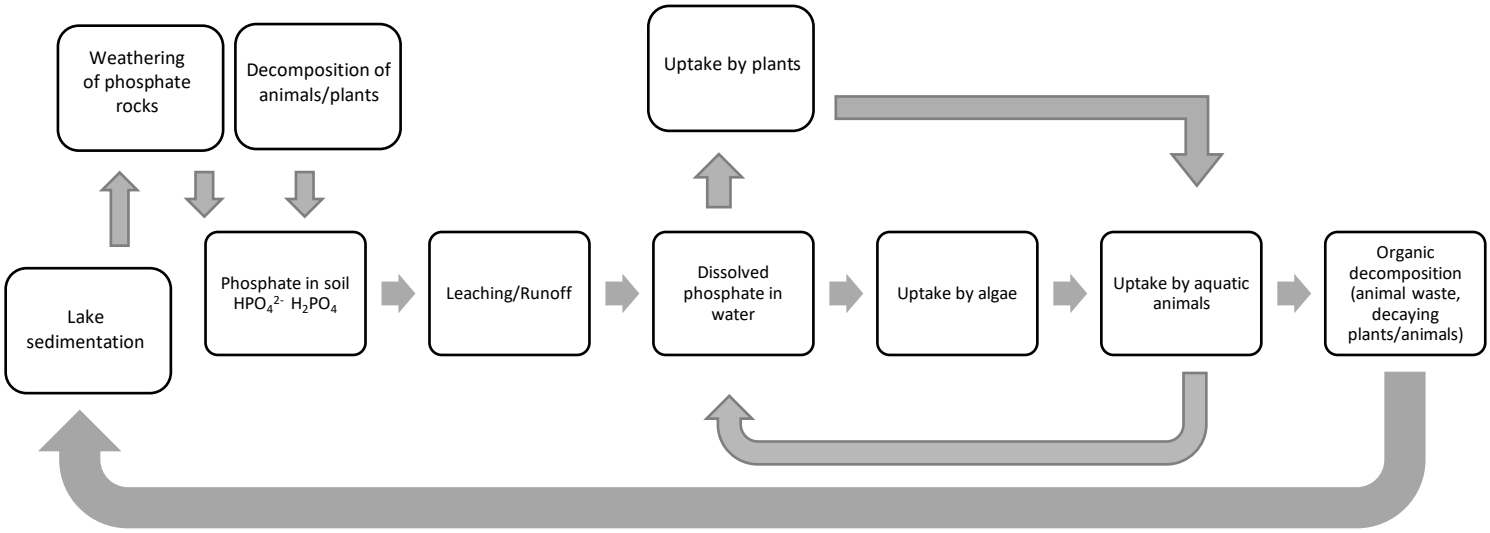

Figure 1. Diagram of phosphorus cycle, displaying how phosphorus is used by a variety of life forms (Phosphorus Cycle, 2012).

Iron also influences phosphorus and can aid its release or containment. Iron can be in a soluble or insoluble form; phosphorus can be absorbed onto insoluble iron, which prevents it from being released into the environment (Wilfert et al., 2015). When phosphorus is released due to cellular respiration of other bacteria, the phosphorus can attach to insoluble Fe(III) in the

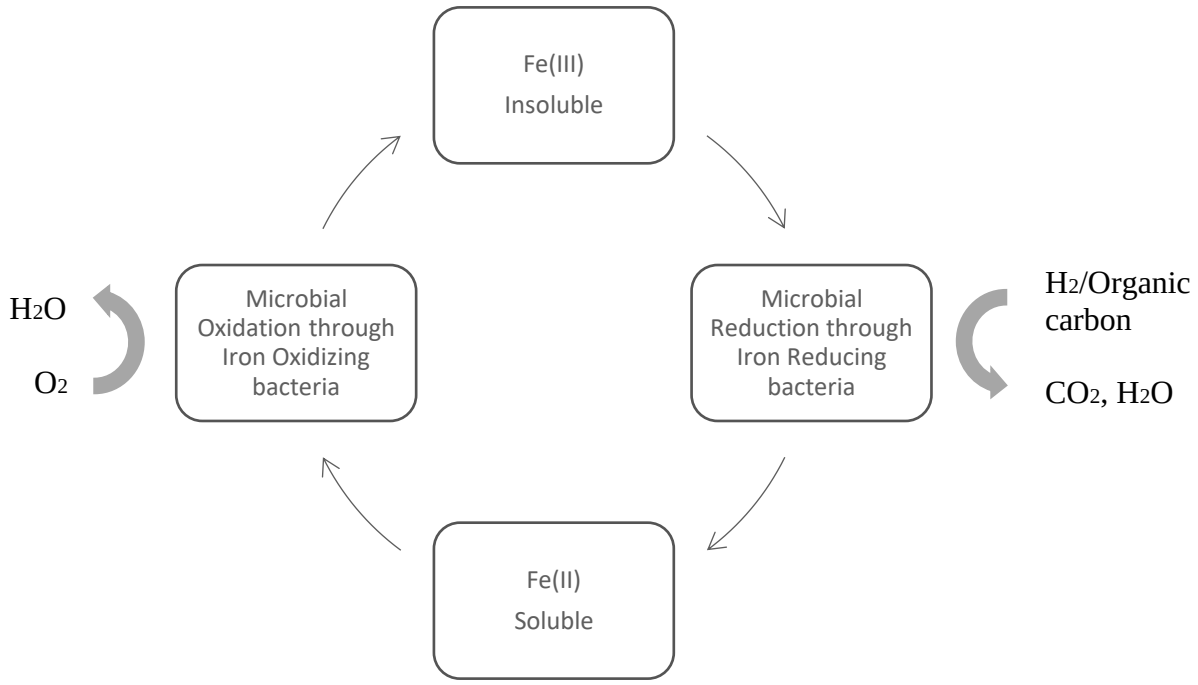

Figure 2. Diagram of microbial iron transformations in the environment (Weber et al., 2006).

sediment and can be the only substance prohibiting another algal bloom from occurring. The solubility of iron is influenced chemically by the water's oxygen levels and by microorganisms. The type of microorganisms in the environment depends on the amount of oxygen, which affects the redox state of iron. Therefore, the type of microorganisms can affect whether phosphorus is accessible in the water column based on the reactions the organisms go through. Iron oxidizing bacteria are cells found in oxic environments and take soluble Fe(II) and convert it into insoluble Fe(III). Around neutral pH, this reaction can also happen without bacterial activity. The conversion oxidizes iron by using Fe(II) as an electron donor to assimilate CO_2 into biomass (Figure 2). This reaction removes oxygen from an environment to form water (Figure 2). Phosphorus can attach to Fe(III), which is beneficial to the ecosystem because it is no longer in the water column and is inaccessible to algae. On the other hand, iron-reducing bacteria, found in anoxic conditions, are heterotrophs that conduct the opposite of iron oxidizing bacteria. They

transform iron from its insoluble Fe(III) to soluble form Fe(II) (Chaffin et al., 2019). Therefore, making phosphorus more readily available in the water column by dissolving Fe(III) (Kappler et al., 2017). When iron becomes soluble through iron reducing bacteria, the phosphorus can no longer attach to Fe(III), and therefore gets released back into the water column (Morrison et al., 2018). Any disturbances that occur can trigger another algae bloom, therefore phosphorus adhering to iron is crucial, because insoluble iron restricts the availability of phosphorus to algae. Algae are efficient at capturing phosphorus even when the nutrient is in short supply, so it is important to restrict the amount of phosphorus in the system (Chaffin et al., 2019).

Analyzing benthic sediments and the interactions of iron and bacteria can predict the availability of phosphorus. In lake sediments there are iron reducing bacteria available as well as iron oxidizing bacteria and iron in both soluble and insoluble forms. Iron-reducing bacteria can indirectly affect phosphate release, so it is important to identify where the bacteria thrive. It is also essential to understand what conditions are required for iron to metabolize into Fe(II) and the possible role iron plays in phosphate solubility. My hypothesis: if the sediment is in live anoxic conditions, then the bacteria can release soluble iron into the water. Furthermore, by putting insoluble iron in the experiments, we can determine what factors such as bacteria and levels of oxygen are causing iron to become soluble.

Methods

The study is based on sediment taken from Silver Lake. The lake is located in a suburb of Akron, Ohio. Silver Lake is the largest lake in Summit County and is part of the Akron Metropolitan Statistical Area. From 2012 to 2014, algal blooms were becoming increasingly common, and levels of microcystin (a cyanotoxin produced from algae) were always an order of magnitude higher than the World Health Organization guideline. The water quality does not meet governmental state and federal recreational standards (Editor, 2017). The runoff that flows into Silver Lake contains an overwhelming amount of limiting nutrients. This runoff is a mixture of surface water containing organic/inorganic phosphorus from fertilizers, which mainly come from the surrounding area and have been eroded and washed away into the lake (Rosen & White, 1999)

To view the bacteria under a microscope, it is necessary to Gram stain so the bacteria become visible. The purpose of this process is to determine if cells are present in the sediments. To Gram stain, I prepared a slide smear. I transferred a drop of diluted Silver Lake sediment (with milli Q water). I air dried the culture then fixed it over a gentle flame while moving the slide circularly to avoid localized overheating. The applied heat helped the cell adhere to the glass slide, so there would not be a significant loss of the culture during Gram staining. When thoroughly dried, I added a crystal violet stain over the fixed culture. I let it stand for 20 seconds; then I poured off the dye and gently rinsed the excess stain with a stream of milli Q water. Then I added the iodine solution on the smear, enough to cover the fixed culture. I let it stand it for 20 seconds and poured off the iodine solution and rinsed the slide with a stream of milli Q water. I added a few drops of decolorizer, so the solution trickled down the slide. I rinsed it off with milli

Q water after 5 seconds. Next, I counterstained with a basic fuchsin solution for 20 seconds. I washed off the solution with milli Q water and blotted it with bibulous paper to remove the excess water. Then I looked through a microscope to analyze the biological components of the sediment.

To monitor the biological activities, I conducted batch experiments. I took sediment from the bottom of Silver Lake in Ohio, synthetic lake water (SLEW), and hydrous ferric oxide (HFO), and placed them into flasks. HFO is a suspension of $Fe(OH)_3$ particles in water. I made the SLEW by adding specific amounts of the ingredients into the solution. The following ingredients are measured in volume; $CaCO_3$ = 1 mM, Na_2SO_4 = 0.3 mM, $MgCl_2$ = 0.3 mM, KCl = 0.04mM, and $NaNO_3$ = 0.06 mM. I filled a 1000 mL volumetric flask with Milli Q water just below the 1000 mL line mark, then I mixed it with a stir bar to ensure all the particles were dissolved in the solution. I checked the pH for 30 minutes and adjusted it close to 7 pH using HCl. Afterward, I autoclaved the flask for 20 minutes.

To make the HFO, first I autoclaved 1 L of milli Q water (deionized) with an empty serum bottle. I weighed a mass of 6.76 g $FeCl_3$ $6H_2O$ and added it to a beaker and dissolved it in 250 mL of milli Q water with a stirring rod. When it dissolved, I adjusted the pH to 6.8-7 with a basic solution. Once the pH was between 6.8 and 7, I poured the solution into 6 - 50 mL falcon tubes and centrifuged them for 5 minutes at 3500 rpm. I poured off the liquid and refilled to 50 mL with milli Q water to remove NaCl. I repeated this process four times. After the last centrifugation step, I poured the remainder off and added milli Q water to 10 mL. I vortexed the mixture and poured it into a sterile serum bottle (adds up 60 mL of HFO). Using the cleanest tube, the tube that had the least amount of iron in it, I added 20 mL of milli Q water and vortexed the mixture and poured into the next cleanest tube until it had gone through each tube. Then I

poured that 20 mL into the serum bottle. I repeated this step, so now there were as many of the solids as possible in 100 mL of milli Q water in the serum bottle. I used the gassing station and attached a glass pipet and a sterile needle to two tubes in the station, putting the pipet into the serum bottle so that it reached the bottom of the solution. I inserted the needle into the headspace and bubbled the solution with N_2 gas. Then I heated the solution in a beaker of water on a hot plate for 45 minutes. When cooled enough to handle, I removed the pipet and needle quickly and replaced it with a rubber stopper and a metal cap. I then flushed N_2 gas with a needle going in and a needle going out of the bottle to make sure there was no air in the headspace. I autoclaved liquid for 15 minutes. The SLEW equaled a total of 250 mM, which is equivalent to 0.25 mol/L, so there was a total of 0.1 L from the bottle, which equals 270.3 g/mol.

When starting the batch incubations, the flasks were set up in sterile and nonsterile oxic and anoxic conditions. These conditions allowed me to determine if the bacteria in the sediment metabolized iron. I autoclaved 6 Erlenmeyer flasks, six serum bottles, and a 100 mL graduated cylinder. There were three sterile serum bottles for the live anoxic, three sterile flasks for the live oxic, three non-sterile serum bottles for the dead anoxic, and three non-sterile flasks for the dead oxic. I added 5 mL of HFO, 95 mL of SLEW, and 10 mL of sediment (~83% water). The Fe(III) concentration was 2.38 mol/L. I set up both anoxic serum bottles in the anoxic chamber and degassed them using N_2 if needed. In order to degas them, I turned on N_2 gas and put two sterile needles into the cap, connected one to the degassing tube, and let it flush for 2-3 minutes. I used a Bunsen burner while setting up the oxic samples to help keep them sterilized and made sure to cover them with foil. I autoclaved liquid the sterilized samples for 15 minutes.

Changes in the iron concentrations were measured for approximately 30 days. When pulling samples out of each batch set, I pulled 1.5 mL of sample, and from that, I put 0.1 mL into

0.4 mL of 0.5 M HCl in a microcentrifuge tube. Next, I centrifuged the HCl samples for 5 minutes at maximum speed (12000 rpm) and took them out of the microcentrifuge. I then transferred 0.4 mL of the supernatant into a fresh microcentrifuge tube to measure total Fe(II) by ferrozine assay. Then with the remaining amount from the needle, I placed 0.7 mL into an empty unacidified microcentrifuge tube and 0.7 mL into another empty microcentrifuge tube that I used for pH measurement. I centrifuged the non-acidified samples at max speed (12000 rpm) for 5 minutes and transferred 0.1 mL of the supernatant to a microcentrifuge tube with 0.4 mL of HCl to measure soluble Fe(II) by ferrozine assay.

I measured dissolved Fe(II) concentrations and the total Fe(II) concentrations using the ferrozine assay. Before the test was conducted, I had to combine four parts of dilute HCl and 1 part of the sample; this acidification was to prevent Fe(II) from oxidizing. I first switched on the spectrometer 15 minutes before taking the measurements and set the wavelength to 562nm. I added 1000 µL of ferrozine solution to all cuvettes. For the blank cuvette, only the ferrozine reagent was used. For the standards, I put specific iron concentration mixtures (20 µL) within ferrozine reagent to set up the standard curve. For all other cuvettes, I added 20 µL of HCl mixture to each cuvette and waited for 5 minutes for the color to fully develop. Then I put the blank cuvette into the spectrophotometer and pushed the zero key to adjust the measurements. After that, I measured the standards and samples in the same manner and recorded their absorbance values. If the absorbance of the samples was too intense for the spectrophotometer to measure, I diluted the solution and repeated the analysis. I plotted absorbance (y-axis) against Fe(II) (aq) concentration (in ppm iron) (x-axis) to find the concentration of Fe(II) (aq) as ppm iron in the samples. The higher the absorbance, the more iron was within the sample (Viollier, 2000).

To measure the pH of the samples I used a pH meter. These tests helped determine how much iron-reducing takes place in the lake sediment, and how much iron reduction occurs by indicating the change in H+ ions in the system. Extremely acidic environments are often associated with both ionic forms of iron (Fe(III)/Fe(II)) that are far more soluble (especially ferric iron) at a low pH than at circum-neutral pH (Johnson et al., 2012). Therefore, pH also impacts the solubility of iron, which affects if phosphorus will attach to iron or if it will be released in the water column.

Results

From my experiment, when analyzing Gram stained slides, I anticipated finding a variety of organisms that were Gram negative, because most iron reducing/oxidizing bacteria are Gram negative. From my batch experiments, I anticipated seeing high levels of soluble Fe(II) in the anoxic live incubations because it is an ideal condition for iron reducing bacteria when they reduce Fe(III). As for live oxic incubations, I expected a substantial decrease in soluble iron Fe(II) because iron oxidizing bacteria take soluble iron Fe(II) and convert it into Fe(III) in oxygen rich environments. As for total Fe(II) in anoxic/oxic live incubations, I expected to see a decrease in total Fe(II) concentrations to reflect the beginning of bacterial metabolism of iron, and then an increase for when metabolism was fully active and releasing iron back into the system. In anoxic/oxic sterile incubations, I was not anticipating any elevated levels of soluble Fe(II), as well for the total Fe(II) since sterilization should have killed off any microbe activities. I was only expecting indicators of metabolism from the live incubations; increase of soluble Fe(II) in anoxic conditions and decrease of soluble Fe(II) in oxic conditions. As for the pH, in anoxic live incubations I was expecting to see a trend between pH and soluble Fe(II); when the

pH would increase, I anticipated there would be an increase of soluble Fe(II) because during iron reduction, H^+ would be removed and joined with oxygen to form water. In oxic incubations, I expected to see no changes in H^+ because iron oxidizing bacteria do not directly affect hydrogen ion concentration. Furthermore, for the anoxic/oxic dead incubations, the pH would not change because there should not be any metabolic activities due to sterilization.

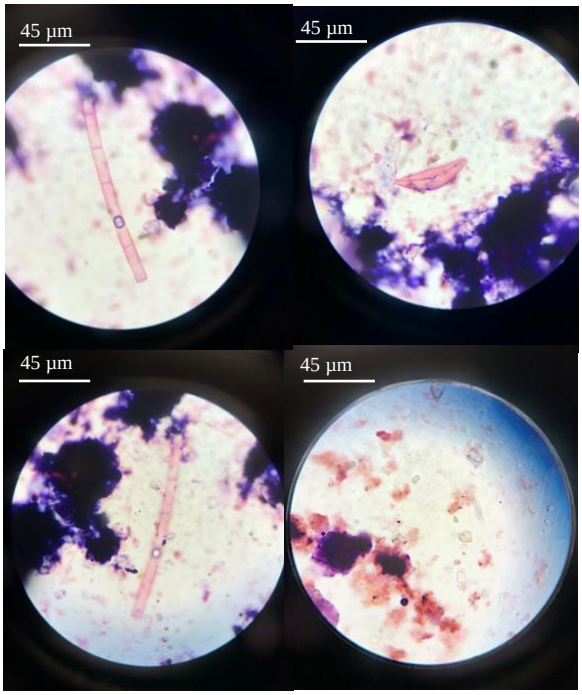

Figure 3. Microscopic images of benthic sediment from Silver Lake, OH at 1000x (total magnification). Prokaryotes are displayed, from tube like structures to rectangular gridded shapes.

In Figure 3, the sediment is diluted and made into a smear slide and Gram stained to examine the organisms within the sediment. Large purple masses are clay/silt and or iron insoluble deposits, while the pink small grains are quartz/silica material. There is a large quantity of Gram-negative cells that are visible in almost all areas throughout the smear slide. It is

probable that some of the bacteria are iron reducing bacteria or iron oxidizing bacteria; the batch sets will determine if they are active or not.

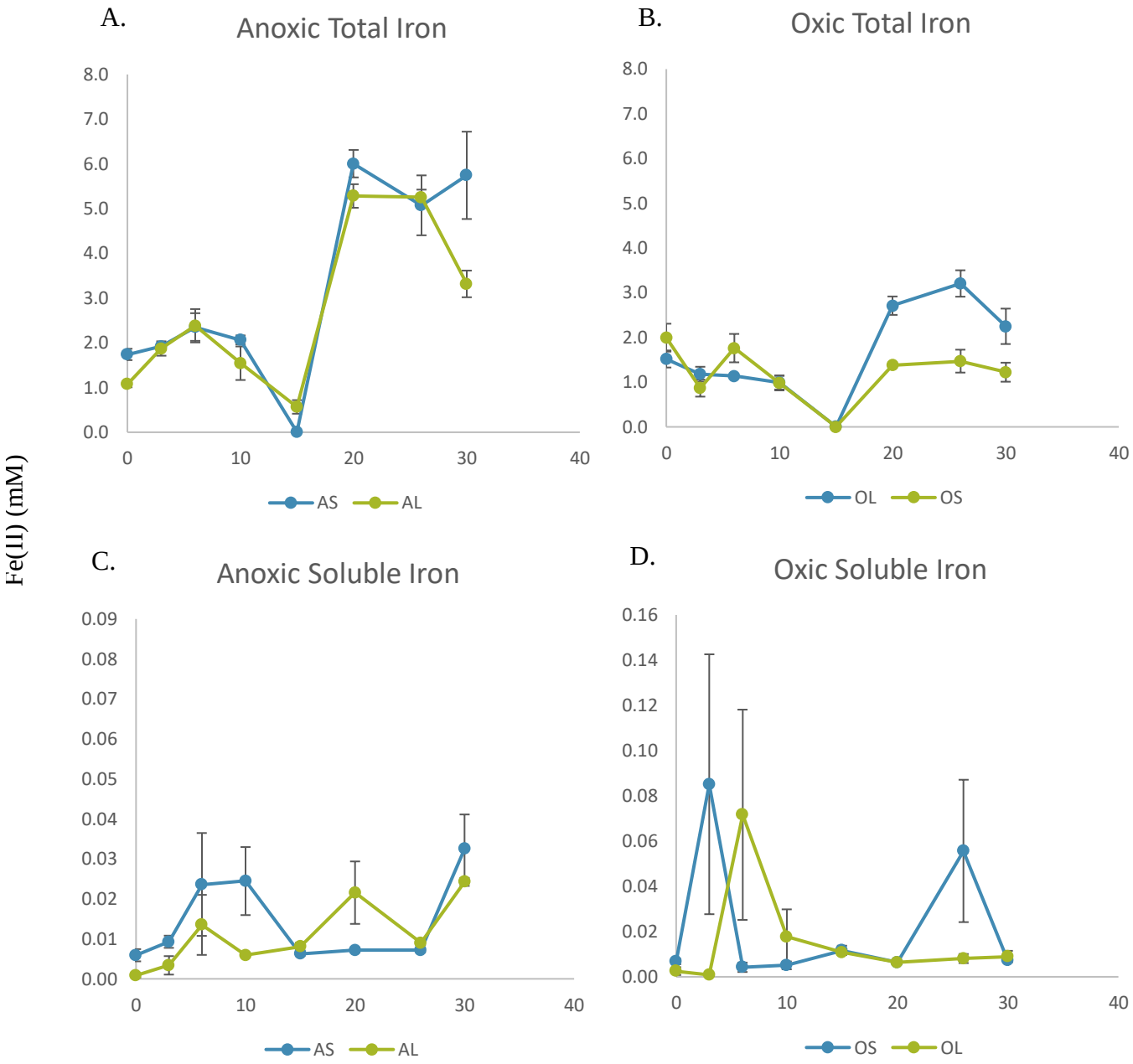

Figure 4. Soluble and total Fe(II) concentrations in anoxic and oxic incubations. AS – anoxic sterile. AL – anoxic live. OS – oxic sterile. OL – oxic live.

From these experiments, I wanted to determine what conditions iron reducing bacteria metabolize in. This information would indicate how oxygenic conditions affect iron solubility which has an important effect on phosphate levels. I hoped to learn more about the microbe reactions that occur in anoxic/oxic conditions and how iron is affected. Furthermore, I hoped to learn how long these reactions take to initiate and the relationship between total Fe(II) and soluble Fe(II).

Anoxic incubations showed evidence of an increase in iron solubility; Figure 4 indicates that there is a soluble Fe(II) concentration increase after approximately day 20. As for the total iron concentration, it is evident that at day 15 there is a sharp decrease followed by an increase that stabilizes. The decrease in total iron concentrations can be due to the iron reducing bacteria starting to utilize the iron within the system and the uptake of iron is higher than output thus making iron less available in the incubation. However, once all the iron reducing bacteria started actively metabolizing Fe(III) into its soluble form Fe(II), there were an overall increase of total Fe(II) concentration as well as an increase of soluble Fe(II) which was getting released into the incubation.

In oxic conditions, the soluble Fe(II) concentration reaches a pinnacle around days 2-10 and decreases and stabilizes for the remainder of the incubation, indicating iron oxidizing bacteria were metabolizing or Fe(II) was oxidized abiotically. For total Fe(II) in oxic conditions, the iron concentrations showed transition of iron being metabolized. In Figure 4 the total Fe(II) concentrations decreased then increased around day 10. The decrease in total iron concentrations can be due to the iron oxidizing bacteria starting to uptake the soluble Fe(II) within the system. Then when there was an increase in total iron concentrations, all the iron oxidizing bacteria were starting to metabolize Fe(II) into its insoluble form Fe(III). Therefore when there was an overall

increase of total Fe(II) concentration, it is assumed there was an increase of insoluble Fe(III) in the incubation.

The oxic batch sets did not show large changes of soluble Fe(II). This shows that even with the addition of iron, iron reducing bacteria are only able to metabolize in environments without oxygen. Therefore, anoxic conditions are ideal for soluble Fe(II) to be produced. The increase in soluble iron concentrations indicates that there is a decrease in Fe(III). Knowing that the iron reducing bacteria are metabolizing Fe(III) into Fe(II) indicates these activities could release phosphorus into the solution. The phosphorus is still in the benthic zone; however, disturbances such as a storm can bring the phosphorus near the surface/photosynthetic zone and cause algae blooms to reappear. Knowing iron was added to each of these batch experiments and that phosphorus can be absorbed onto insoluble iron, it is predicted that phosphorus will be released due to the high soluble iron concentrations (Wilfert et al., 2015).

There were clear issues present between the live and dead batch sets for both anoxic and oxic iron concentrations (Figure 4). I was expecting larger indicators of metabolism from the live sets; increase of soluble Fe(II) concentrations in anoxic conditions, decrease of soluble Fe(II) concentrations in oxic conditions and a decrease then increase in total Fe(II) concentrations in both anoxic/oxic sets. I was also expecting little to no changes in any iron concentrations in the sterile incubations. There should be no microbe activity within the dead/sterile sets because they were sterilized. However, there are very little differences between live and sterile sets, concluding there must have been an error in the sterilization process. Residual bacteria may be the cause, where autoclaving materials once is not enough for the batch sets to be completely dead. Other possible errors may be flasks/serum bottles which may have not been sealed tightly, or the contamination of needles when taking samples.

Referencing back to Figure 3; it is clear there are a variety of these types of organisms that live in the sediment and from Figure 4, changing soluble Fe(II) and total Fe(II) concentrations confirms that the sediment from Silver Lake has a large biological community of bacteria that are most likely iron metabolizing bacteria.

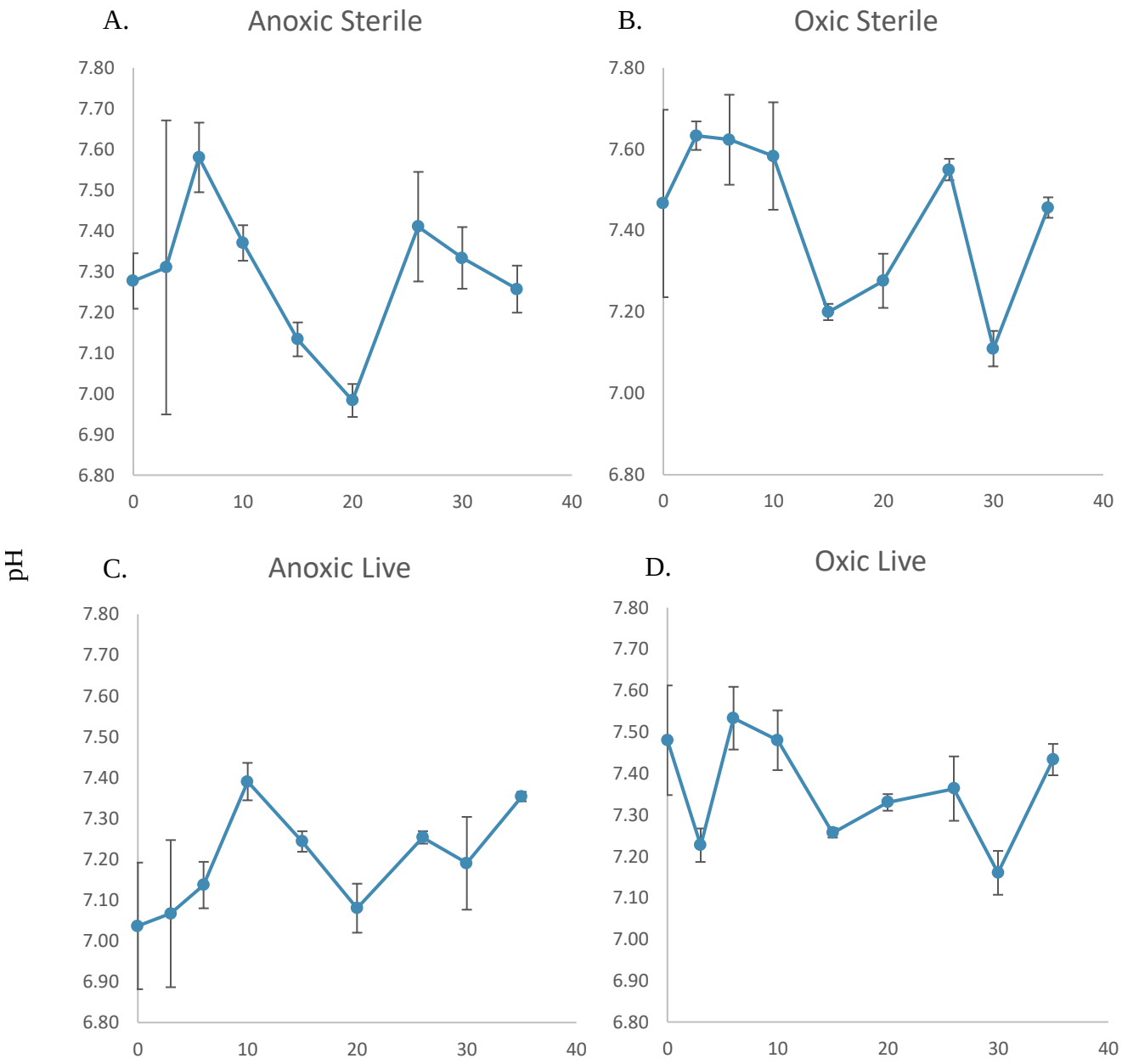

Figure 5. pH of sediment incubations over 35 days in anoxic/oxic conditions. Errors bars were produced through standard deviation.

The mean pH for anoxic incubations concluded some indicators of metabolizing iron. When iron reducing bacteria metabolize, H^+ are utilized and oxygen is added to produce water. This process decreases the availability of hydrogen ions, therefore the pH increases. This is shown in Figures 4 and 5, when Fe(II) concentration increases, the pH increases. However, there was no other evidence of correlations of pH to the remainder of soluble Fe(II) concentrations including anoxic/oxic live/dead batch sets. In oxic incubations, there were no changes in H^+ because iron oxidizing bacteria do not directly affect hydrogen ion concentration. The anoxic/oxic dead incubations were also inconclusive for pH, because there should not be any metabolic activities due to sterilization.

Discussion

The purpose of this study was to determine how bacteria from eutrophic lake sediments influence iron solubility. During the study, there were some limitations that arose in my experiments; such as the sterile batch incubations. The sterile incubations indicated there was microbe activity; however, there should not have been any because the incubations were sterilized. Nevertheless, anoxic live incubations clearly showed activities by iron-reducing bacteria converting iron into its soluble form. However, with insoluble Fe(III) being converted into Fe(II); the question arises as to how phosphorus was being affected over time, considering there was less iron for it to adhere to. This could be a possible inquiry for future studies; if there was an increase of soluble iron in anoxic conditions due to iron-reducing bacteria, does the decrease in insoluble Fe(III) affect phosphorus concentrations within the sediment/water column (Wilfert et al., 2015). Furthermore, does introducing oxygen to an anoxic environment inhibit the

release of phosphates? Quantifying phosphorus concentrations and identifying its effectors as well as inhibitors that affect its concentration levels are extremely important. In fact, introduction of oxygen to benthic sediments to control phosphate release has already taken place in Silver Lake. In 2014, a lake-wide aeration system was installed to oxygenate the benthic sediments. By having oxygen in the water column, it keeps the phosphorus in the sediment by inhibiting iron reducing bacteria from converting Fe(III) into Fe(II). Studies performed by EnviroScience show that water clarity has improved and that the amount of blue-green algae and algal toxins have decreased since the installation of the aeration system (Editor , 2017). Quantifying phosphorus concentrations and identifying one of its main effectors for altering its concentration is extremely important. This work has improved the understanding of how bacteria are regulators of nutrients in lacustrine environments and based on oxygen levels can affect the future of algal blooms. Nutrient pollution and its causations can lead to multiple possibilities to improve ecosystems and provide insight into determining how bacteria indirectly influence phosphorus concentrations.

Conclusion

Dangerous algal blooms are a prominent and growing concern for the environment. Knowing anoxic environments alter the solubility of iron within a system, it is critical to understand the possible effects on limiting nutrients. In order to eliminate enormous algal blooms, it is a necessary to know where they occur and why. In oxic conditions there was little to no soluble iron being produced, so transforming these oxygen deprived conditions into oxygen rich ones may be a solution to stop the release of phosphates into the water column. Limiting nutrients are the leading cause of these large algal blooms, and controlling these limiting

nutrients are essential for controlling algae. Comprehending how bacterial activities such as iron reducing bacteria function in anoxic/oxic conditions and its indirect effect on algal blooms is important when creating an approach to tackle this issue and further understand it.

Acknowledgements

I would like to thank the University of Akron for funding this research. I would also like to thank Dr. John M. Senko, Dr. Chelsea Monty-Bromer, Dr. Caleb W. Holyoke, John F. Beltz, Dr. John Peck, Melissa Mulford, Alexandra Simler, Anwar Sadek, Prasanna Chinthala, Clarity Gunn and Joshua Davis.

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