Grace Duong
Biochemical Reactors : Cellulose Degradation & Sulfate Removal for Mine-Impacted Water
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Abstract

Acid mine drainage results from water infiltration into mine tailings, containing heavy metals and sulfates. Mine impacted water contains heavy metals and sulfates. Neutralization and aeration removes some iron and other metals, leaving neutral, sulfate-rich water with low concentrations of heavy metals. Sulfate-reducing bioreactors provide substrate (electron donor), resulting in an anaerobic environment for the reduction of sulfate to sulfide.

Thirty-one reactors were constructed. Daily, sulfate impacted water was run through each reactor. The influent and effluent sulfate concentrations were recorded. Every six months, the composition of the wood within the reactors was analyzed to confirm the rate of sulfate reduction. To do this, the contents of the reactor underwent a series of extractions to determine the cellulose content.

The results of the project are inconclusive. The lack of trend in removal rates and insignificant cellulose degradation suggest the bacterial communities in the bioreactors are being outcompeted or are not effective at sulfate reduction.

Introduction

Acid mine drainage results from water infiltration into mine tailings from abandoned mining operations. Precipitation events flood these areas, causing the dissolution of metal ores[1]. This results in acidic, sulfate and metal rich water. AMD often cannot be confined to the immediate area around an abandoned mine, but carry their environmental impacts downstream[2]. While high sulfate concentrations on their own are not impactful to ecology, the presence of metals poses a toxicity risk[2]. The abundant Missouri limestone acts as an anoxic limestone drain, neutralizing the mine impacted water. Following, aeration increases the dissolved oxygen of the water, converting the metal ions into less soluble forms, precipitating some of the heavy metals, predominantly iron[3]. The resulting mine impacted water is neutral with high sulfate concentrations and heavy metal deposits. One of several remediation techniques is the use of bioreactors[4]. Sulfate-reducing bioreactors provide cellulose as substrate, acting as an electron donor. In the anaerobic environment of the reactors sulfate acts as an electron acceptor, which reduces to sulfide.

The objective of the bioreactors is to reduce dissolved sulfate to sulfide. In the lab, this process is simplified by isolating the sulfate. The bioreactors reduce sulfate concentrations by creating an environment where sulfate-reducing anaerobic bacteria can thrive. These bacteria, in the absence of oxygen, utilize sulfate as their electron acceptor. In this process, the bacteria take in organic carbon to use as an electron acceptor, degrading the cellulose in the chip bark of the reactors.

The goal of this research is to determine the lifespan of bioreactors for mine-impacted water, quantifying the relationship between sulfate removal rates and cellulose degradation. The substrate, woody material, contains cellulose which bacteria consume and ferment, with the resulting acids used by sulfate reducing bacteria resulting in the reduction of dissolved sulfate to sulfide. In wetlands, this reduction causes heavy metals to co-precipitate out of solution, decreasing the concentration of metals and sulfate in the water.

Materials & Methods

Figure 1 : Bioreactor Cross-Section

Bioreactor Construction

Thirty-one bioreactors were constructed as shown in *Figure 1*. Each reactor was housed in a two-liter cylindrical plastic container, consisting of a 1 kg layer of limestone gravel, a mixture of chip bark and horse manure, and another 1 kg layer of gravel. The reactors were completely sealed with exceptions for the influent and effluent tubes. Each tube at the bottom of the reactors was attached to a pump, which simulated the inflow of sulfate impacted water. The tube at the top of each reactor was attached to a half-gallon (1.5 liter) jug collecting the effluent.

Simulated Mine Water

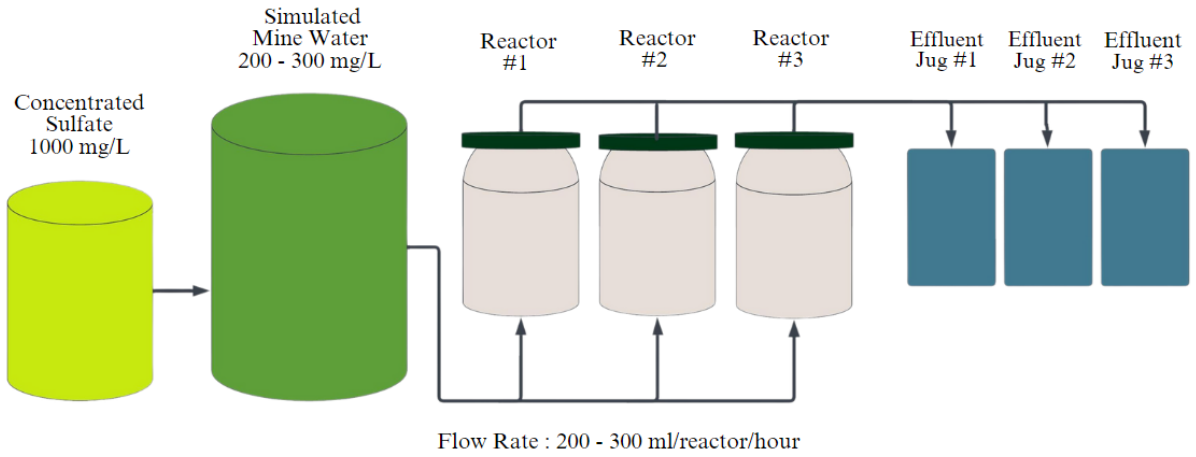

Figure 2 : Flow diagram of bioreactor system

To simulate the flow of acid mine drainage, a solution of sodium sulfate (Na_2SO_4) was pumped into each reactor on a schedule. A tank was filled with 10 gallons (37.8 liters) of water and 56 grams of sodium sulfate to create a 1000 mg/L solution of sulfate. As shown in *Figure 2* this concentrated sulfate solution was then pumped into a larger tank, diluting the solution to 200-300 mg/L of sulfate. This solution was the simulated mine impacted water. Each of the influent pumps was connected to a timer, which allowed the pumps to run for one hour every day. As the large tank was drained by the pumps to the reactors, it was refilled with tap water, until the bladder indicated the tank was full. The small tank was manually refilled when empty.

Sulfate Removal Rates

At least once a month, each reactor's flow rate and sulfate removal rate were measured and recorded. The effluent jugs were emptied the night before the flow rates were measured. The next day, the sulfate concentration of the big tank was measured, along with the sulfate concentration of each effluent basin. Effluent from each of the basins was diluted with distilled water to 10 mL and analyzed by a HACH colorimeter. After a suitable reading was obtained from the colorimeter, a vacuum was used to siphon the contents of each effluent basin into a graduated cylinder to determine the flow rate. If any of the reactors were out of range of the ideal flow rate, 300 mL to 400 mL, their pumps were tightened or loosened. Once all the readings were taken, the reactors were allowed to run for another month, unless complications arose.

Cellulose Degradation Extractions

Another benchmark of the efficiency of the reactors was the rate of degradation of the substrate within the containers. As the sulfate-reducing bacteria within the reactors grow and multiply, they consume the organic carbon from the chip bark and manure. This should result in the consumption and reduction of cellulose and the enrichment of lignin in the reactors. To track this degradation, a chosen reactor is periodically deconstructed and its components analyzed.

Following the deconstruction of Reactor 11 by a previous researcher, Reactor 12 was broken down and analyzed. After the reactor was disconnected from its influent pump and

effluent jug, it was propped up vertically and allowed to drain fully, for about one day. The reactor was moved into a large bin, where it was cut apart using a box cutter, so that the layers within the reactor remained intact. Samples were then taken from the top (near the effluent tube), the middle, and the bottom (near the pumping tube). Before grinding the wood chips into a fine powder, they were dried in the oven until the samples were dry to the touch. Once dried, they were then ground and sifted through a fine metal sieve.

The first extraction utilized a Soxhlet extraction chamber to drip an acetone water solution over the wood samples in a permeable thimble. The sample was then washed with ethanol. This isolated the extractives, leaving cellulose, hemicellulose, and lignin in the wood sample. This sample was filtered into a glass fiber filter, dried in an oven, and cooled in a desiccator. The next process, to extract the lignin, used the periodic addition of a solution of acetone and sodium chlorite into an erlenmeyer flask with the wood sample. The solution was added every hour, for six hours while submerged in a warm water bath. Then, the sample was cooled, filtered, washed, and dried. The final extraction isolated the cellulose by drawing out the hemicellulose. The sample was removed from the filter and added to a flask with sodium hydroxide and stirred in a water bath. This last sample was filtered, washed and dried[5]. The weight differences between the samples before and after each extraction were used to determine the percentage of each component.

Results

As aforementioned, each reactor was emptied so its flow rate and sulfate removal rate could be measured and recorded. By subtracting the effluent sulfate concentration from the influent sulfate concentration, removal rate values were calculated and summarized in the scatter plot, *Figure 3,* below, showing values from January 2022 to March 2023.

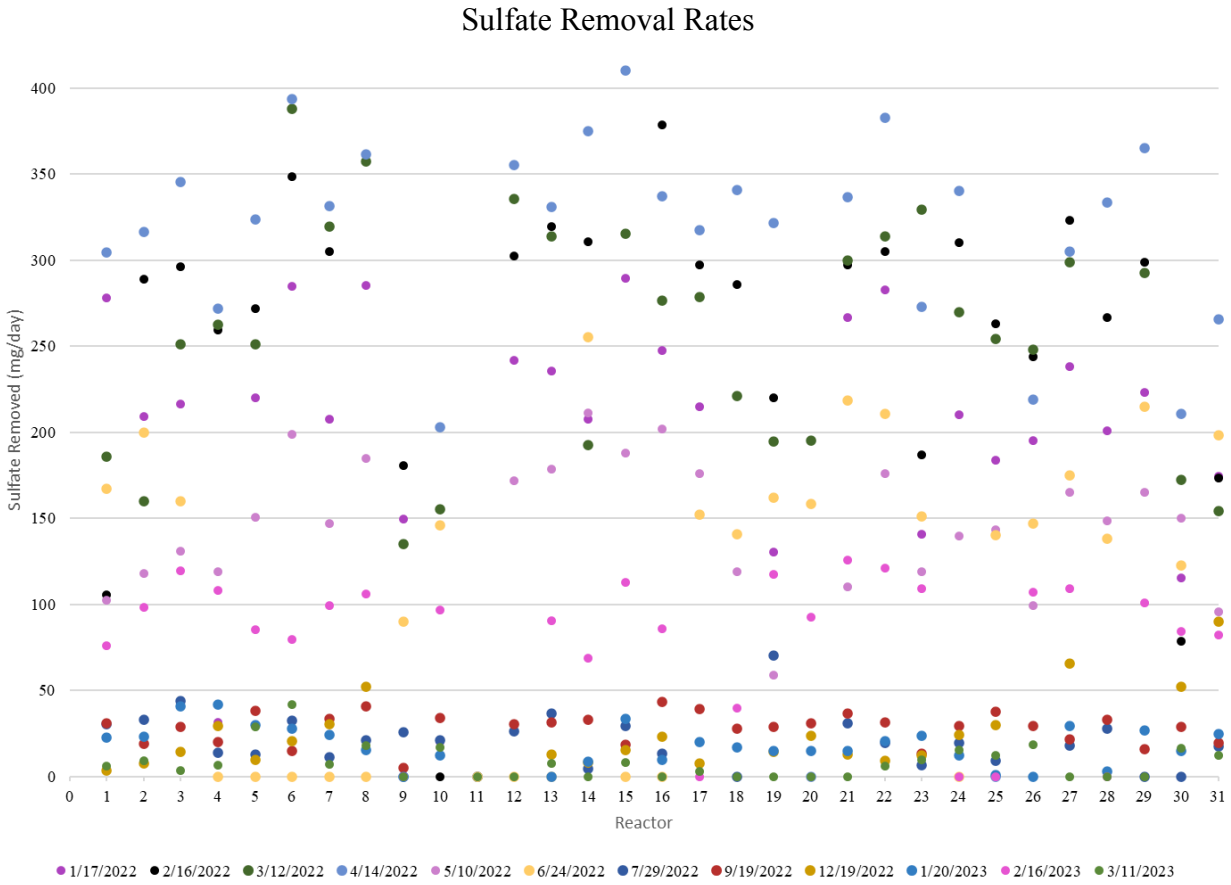

Figure 3 : Sulfate Removal Rates of 31 Bioreactors

Of the 31 reactors, reactors 19, 20, 21, and 22 were observed to have the most consistent flow rates. To calculate their sulfate removal rates per size of reactor, their flow rates and sulfate removal were recorded. Their sulfate removal rates were calculated, collected, and summarized in *Figure 4*.

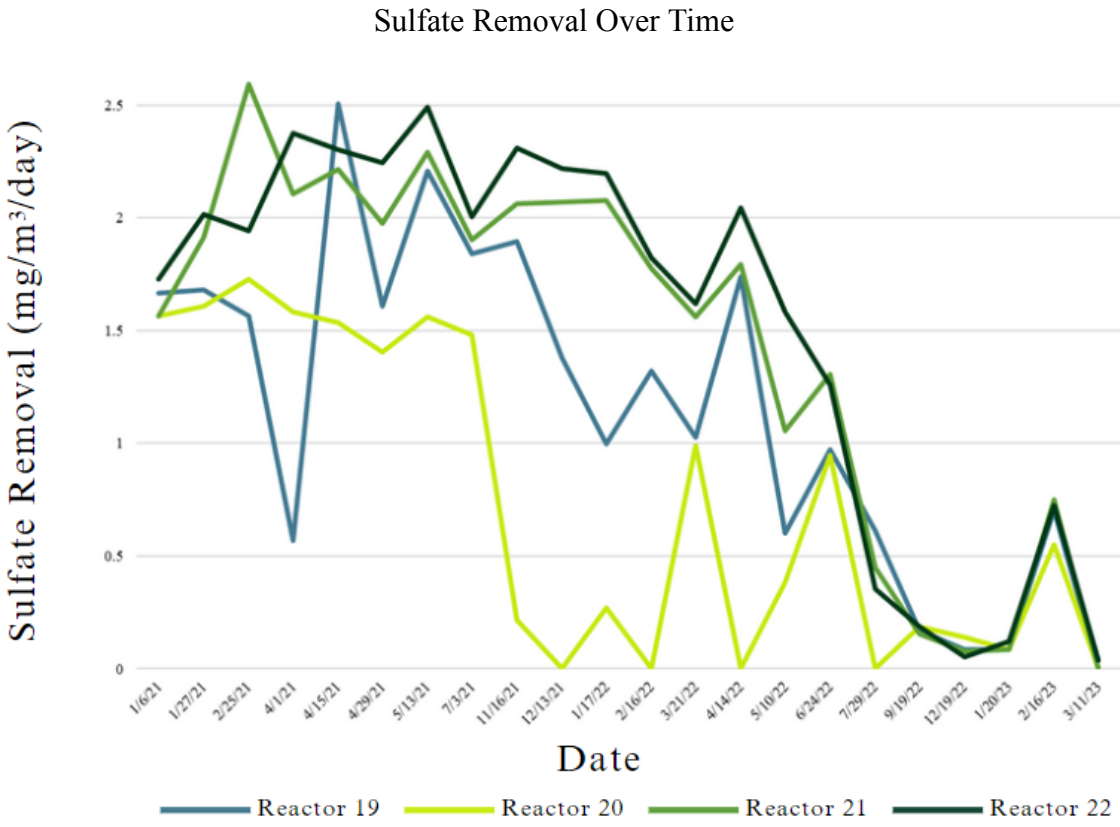

Figure 3 : Sulfate Removal Rates of Selected Bioreactors

As cellulose is another indicator of the reactors' abilities to reduce sulfate to sulfide, two reactors thus far have been disconnected and deconstructed so that the chip-bark substrate can be analyzed for its lignocellulose composition in order to determine the cellulose degradation over time. Raw chip bark had previously been analyzed. The composition of raw chip bark, along with that of reactors 11 and 12 have been analyzed and the composition of the substrate is summarized in *Table 1* below.

	Initial Chip Bark	**Reactor 11**				**Reactor 12**		
						Top	**Middle**	**Bottom**
% Extractives	5.000	13.701	5.337	9.735	12.739	7.163	14.208	4.830
% Lignin	19.380	24.588	14.831	17.277	18.248	31.461	29.434	30.451
% Hemicellulose	22.620	17.363	26.821	32.663	22.774	5.115	15.994	8.414
% Cellulose	53.00	44.348	53.011	40.325	46.239	56.261	40.365	61.703

Table 1 : Composition of Chip Bark in Reactors

Discussion

The goal of the bioreactors was to reduce the sulfate in the influent water. As shown in *Figure 2*, the removal rates of the reactors was not consistent between months, or even between reactors. The rate of removal for Reactor 14 ranged from over 300 mg/day in May to nearly zero mg/day in December. With the significant amount of noise in the data in the seven months it was recorded, it is difficult to come to any conclusions on the effectiveness of the bioreactors based on their sulfate removal rates. As shown in *Figure 3*, the bioreactors appeared to reduce sulfate until a zero sulfate influent event in March of 2022, where removal rates drop off and do not appear to recover. This may suggest the sulfate-bacteria died off or were outcompeted by other bacteria. For some months, including June of 2022, the effluent contained more sulfate than was measured in the big tank that fed influent to the bioreactors. In this case, the reactors were documented to have zero removal, which may be a poor representation of the data. In other cases, the bioreactors had zero flow, and are absent from the graph for that month. The cause of the noisy data will be discussed further in the errors section.

Another indicator of the bioreactors' effectiveness was the degradation of cellulose within the chip bark. Similarly to the noisy data of the sulfate removal rates, there was no significant decrease in the cellulose content of the chip bark. Shown in *Table 1*, the percentage of cellulose in the Reactor 12 ranged from 40 to 61, while the initial chip bark was determined to be 53% cellulose. This, along with the incomplete conclusions of the sulfate removal rates, suggests the bioreactors have not been efficiently removing sulfates from the influent water. Further degradation is likely to occur with more time.

Error

As shown in *Figure 3*, the data collected during the span showed no clear correlation, suggesting significant errors in measurement or method. One influence on the noisy data was the sulfate in the tap water that dilutes both the small and large sulfate tanks. Data on the pump cycle and sulfate concentration of each well was collected from the water marshall for the City of Rolla. As shown in *Table 2*, the sulfate concentration in the Rolla tap water ranges from 12.7 mg/L to 72.3 mg/L. While this may explain some of the noise in the data, it does not adequately bridge the gap between the negative removal rates and the sulfate concentration in the big tank.

Well #	Sulfate (mg/L)	Well #	Sulfate (mg/L)
HP 1	50.6	11	44.4
HP 2	25.9	12	42.6
4	31.4	13	16.3
5	72.3	14	12.7
7	53.8	15	41.8
8	30.1	16	25.5
9	34.1	17	25.9
10	39.0		

Table 2: Sulfate Concentrations in Rolla Wells

Another source of error was the interference of the hardness of the Rolla tap water. A sample of distilled water and tap water were run through the colorimeter with the sulfate indicator pillow. The distilled water sample gave the same result as a blank sample, 0 mg/L, while the tap water sample resulted in a sulfate concentration of 20-30 mg/L. This indicates that the water hardness introduced error into the experiment.

One procedural change that may have increased the accuracy of the data was the time between the measurement of the sulfate concentration in the tank and the sulfate concentration of the effluent tanks of each reactor. Because the ideal flow rate of the pumping system was between 300-400 mL/day and the volume of each reactor was several liters, each influent cycle did not reach the effluent containers. If the reactors operated under plug flow assumptions, the sulfate concentration of the influent should have been measured days before the effluent sulfate concentration was measured.

While ideally, the smaller sulfate tank would be refilled before it emptied into the larger tank entirely, a lack of supervision allowed the small tank to run dry on occasion. While the reactors were being fed residual sulfate from the big tank, the lower concentration of sulfate may have halted the growth of the bacteria within the reactors, or caused some of them to starve and die off. This mass death may not have been evident immediately, rather affecting later data collected.

Conclusions

This project is in the fourth year of a planned ten-year span. Thus far, no significant cellulose degradation has been observed. The cellulose mass in the wood is expected to diminish with time, leading to decreased sulfate removal as the food source is depleted. The low sulfate removal rates and lack of cellulose degradation may be an effect of the zero sulfate influent in March of 2022 where the bacteria may have died and never recovered. Another explanation for the inconclusive results is that the sulfate reducing bacteria or cellulose degrading bacteria are being outcompeted by other bacteria. These hypotheses can be explored by performing a culture test to determine the bacterial community makeup of the bioreactors. Other data that may be useful in determining the reasons for the ineffective reactors include the dissolved oxygen of the reactors and the gasses in their effluent. Quantifying the relationship between cellulose degradation and sulfate removal rates over the span of operation will lead to an improved understanding of the lifespans of bioreactors for mine impacted water.

Nomenclature

AMD (Acid Mine Drainage)
Sodium Sulfate ($NaSO_4$)
Sulfate (SO_4^{2-})
Sulfide (S^{2-})

Acknowledgements

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As my first introduction into research, I could not be more pleased with the experience I've had in the last semesters and over the summer. Though I have had very few doubts about my degree choice and academic path, this experience has cemented my passion for environmental engineering. This project is unique, as while Kelly, my undergraduate partner in crime, and I have endless insight from our advisor, Dr. Fitch, there is no graduate student on the project with us. This has led to several complications with only one solution - to fix them with Kelly. The necessity of problem solving skills and on the spot brainstorming has developed skills that I feel could be better represented in the undergraduate curriculum.

In environmental engineering, research can be conducted to address a problem conceptually or to prove that concept in practice. The research I have aided in would fall under the category of the former. The isolation of the concept allowed me to take a deeper look at the processes that controlled the reactors. While general experimental methods were provided to me, any obstacles that arose during the duration of the project allowed me to utilize the process of experimental design to address the problems. Being forced to apply the knowledge I've gained from my classes has shown me the value of the information I've learned. With each roadbump, with the help of Kelly and Dr. Fitch, I hypothesized a reason for a failure in the system or unexpected result. After consulting my partner, advisor, and literature, I made a plan of action. After implementation of any changes needed, I collected data and analyzed the results. If these results were inconsistent with my previous hypothesis, I would repeat the process. Because of the interdisciplinary nature of environmental engineering research, adjustments and literature review are integral to an insightful project.

The biggest resources I have utilized throughout these semesters are the research and documentation of past students as well as the support from my advisor, Dr. Fitch. The abundance of literature available on the same concepts as the project I worked on greatly aided in my understanding and success in the project. While the articles were initially difficult for me to read and deconstruct into useful information, as the project progressed and I learned more of the terminology, the journals were increasingly easier to consume. Now, I am comfortable seeking out literature on the internet. I found that many of the concepts I learned in class and the textbooks that accompanied the courses aided in my understanding of the reactors. In the time that I worked on the bioreactors, I took Water and Wastewater, which expanded upon the design of reactors within a wastewater treatment plant. This deepened my understanding of the different reactor models. An important concept that I applied to the bioreactors is the plug flow model, and the assumptions that need to be made. This class also expanded upon the differences between suspended and attached growth in reactors, which helped me understand what was going on in the containers. The application of the concepts from my coursework have provided me with the background to understand the project.

My research partner, Kelly, and I have encountered numerous setbacks in the duration of our work on the project. My ability to problem solve and adjust my plan of action has developed significantly since I've started maintaining the bioreactors. Flexibility and willingness to try something new were the most important skills we applied. One facet of experimental design that

I became aware of was the importance of simplification. There were complications that seemed to require a complex solution, but would have cost more money and time. While I was not involved in securing funding for the project, I understood that overcomplications cost more than the solutions they provided were worth.

The results of the research have been generally inconclusive due to an event that occurred when I joined the project. While initially, I concluded that there were no significant results in the project, I have since learned that inconclusive results are still valid and can be used to infer next steps and errors in experimental design. For the sulfate removal rates, the amount of data was intimidating and a trend was hard to find. By selecting a few bioreactors and looking at only the data for those reactors, a trend was much easier to follow, suggesting that something occurred in the reactors around my introduction to the project and the reactors have not recovered from this. The composition of the wood within Reactor 12 was higher than the original chip bark composition found by a past researcher, which did not make sense within the scope of the project. To get a better comparison of the cellulose composition change, I instead looked at data from another reactor, which made more sense. I learned that error and variance are a significant part of doing research and learning to interpret unexpected results is vital. Because Kelly and I were the sole researchers maintaining the reactors, I had the opportunity to see the full process of a research project and am excited to conduct more research in my future.