

Evaluating the Manganese Removal Capabilities of Mushroom Compost
Under Anaerobic Conditions Using Fed-Batch Reactors

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Abstract

Abandoned mine drainage often contains heavy metals and trace metals, that when left untreated, contaminate surrounding soil and water rendering them unable to support life or maintain ecological functions, and impacts the natural environment. These metals must be removed in order to prevent environmental damage. Historically, two types of treatment systems have been proposed to treat mine drainage: passive treatment systems (PTS) or active treatment facilities. The latter of these options use chemicals harsh on the environment while the former option utilizes natural processes. Previous research done on PTS show that the removal of the trace metal manganese (Mn) in anaerobic systems can be unpredictable, necessitating further research on Mn behavior.

This study utilized 1-L fed-batch reactors to create an anaerobic environment similar to the conditions found in vertical flow bioreactors (VFBR), a common unit-process in passive treatment systems. All treatments and the control contained a 1:3 ratio of liquid and spent mushroom compost. Mushroom compost is a readily available organic substrate often used in vertical flow bioreactors (VFBR) to remove heavy and trace metals from PTS; it is for these reasons it was chosen as the substrate in this experiment. This study manipulated ionic strength, to try and understand how Mn may be retained or released in the varying conditions. Water quality was observed over the course of seven weeks and organic matter was sacrificed at the end for analysis. Organic matter analysis utilized a modified Tessier sequential extraction method to obtain the location of where manganese occurred.

Introduction

Abandoned mine drainage contains heavy metals that will destroy ecosystems if not removed.

When heavy metals are left untreated the surrounding soil and water are contaminated potentially rendering them unable to support life, maintain ecological functions, and it negatively impacts the natural environment. Treatments systems may be used to remove heavy metals in order to prevent environmental damage. In general, these treatment systems can be passive or active and the two differ in both price and methodology. In the United States there are many abandoned mine drainage sites of concern; therefore, treating each site in an effective manner is imperative. From an economic standpoint the option to treat each heavy metal site via active treatment systems (ATS) is often not an option because these require a higher investment of money due to the methods and materials implemented. ATS, although able to remove metals of concern, use harsh chemicals that add to the negative impact on the environment. As a less environmentally abrasive and potentially cost reducing alternative, passive treatment systems (PTS) use universally accessible materials like gravity, natural reduction and oxidation, and organic matter sourced from local producers (Hayton, 2020).

Vertical flow bioreactors (VFBR), a unit process in a PTS, creates an anaerobic environment. To create the same environment in a lab experiment, fed-batch reactors are a substitute that is a small-scale version of this unit process. They create the same anaerobic environment one observes in the field and is why they will be used in this experiment. In a PTS, the heavy metals being monitored for removal may include cadmium (Cd), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn), (LaBar and Nairn, 2016, 2017, 2018). Most heavy and trace metals of interest in AMD are favorably retained in substrates as sulfides, Mn does not follow this

removal technique. Previous PTS research done by LaBar and Nairn (2016, 2017, 2018), revealed that concentrations of Mn in effluent wastewater occasionally exceeded the concentrations of Mn in influent wastewater. High concentrations of Mn are a cause for concern as overexposure or continuous exposure poses health risks such as Parkinson's disease (Neculita and Rosa, 2019). High concentrations of Mn are also a cause for concern for aquatic life. Aquatic arthropods, specifically those that produce naiads or immatures that spend early development in the water, when exposed to high concentrations of Mn they will die. In fish species, over exposure to high concentrations of Mn causes the same issue. For this reason, the treatment of mine drainage is important, and in the case of Mn there are no enforced regulations of its concentration as it is a secondary water contaminant. Due to the many areas of concern in Oklahoma that produce mine drainage, along with the results of LaBar and Nairn (2016, 2017, 2018), there is a need to research how to 'naturally' remove Mn and other metals from the environment without sending the water to a treatment facility. As stated, previous research done on PTS show that the removal of the trace metal manganese (Mn) in anaerobic systems can be unpredictable, which is why further research on Mn behavior is necessary.

The use of sequential extractions is a process that can be used to analyze organic matter for concentrations of heavy and trace metals. Each study using sequential extractions has experimentally defined parameters, the most essential being number and type of substrate fractions analyzed. Sequential extractions inherently refer to the one after another process of analyzing something, in this case substrate fractions. This process utilizes chemicals that break down the substrate and release metals retained in the fraction being analyzed. The fractions being analyzed start with the portion of substrate most easily susceptible to change or breaking

down. As the sequential extractions proceed, the chemicals being used are harsher as the fraction being analyzed becomes more resistant to breaking down. The fraction most trace and heavy metals are retained in is the recalcitrant organic matter and sulfides. This fraction is more difficult to break down or alter, which is why it is preferred that metals of concern are sequestered here as they will be less likely to leach out. Mn does not follow this pattern of sequestration which is why it is important to understand whether or not Mn is retained in more labile or recalcitrant fractions of substrate. The objective of this research was to identify whether Mn is sequestered in more labile or refractory portions of the substrate to aid in understanding its susceptibility to being mobile in the system. A secondary objective of this research was to demonstrate proof of concept for using fed-batch reactors for further research on metals behavior in VFBR.

Materials and Methods

This experiment used 10 fed-batch reactors, four of which contained a high ionic strength treatment (HI), another four contained a low ionic strength treatment (LI), and two were allocated for controls. Each cubitainer was comprised of mushroom compost and water inoculated with a known Mn concentration and ionic strength. The two controls contained the same volume of mushroom compost as the two treatments and were fed deionized (DI) water. Mushroom compost is a readily available organic substrate used in VFBR to remove trace metals such as Mn from PTS; it is for these reasons it was chosen as the substrate in this experiment. The assembly of the cubitainers required the identification of dry mass equivalent for the mushroom compost. Previous research indicated a dry material to liquid ratios of 200g to 600mL. Mushroom compost was sampled directly out of the bag for dry mass analysis. Six

sample replications were taken from the compost and massed, dried to a consistent weight, and massed again. After this procedure occurred, it resulted in determining that 450g wet weight was equivalent to 200g dry weight. This amount of wet weight was added to all 10 cubitainers.

The HI and LI strength treatments were chosen to simulate water runoff originating from roads containing elevated levels of salts from winter weather road salting, as well as brine impacted in the mine drainage discharge. Ionic strength can impact the amount of competition occurring between cations. When this occurs, it affects binding and other important variables keeping Mn from being properly adsorbed or incorporated into the organic matter (Filgueiras et al., 2002). NaSO₄*H₂O was used to manipulate the ionic strength of the solution. This was used instead of NaCl, or MgCl as NaSO₄ simulates the produced brine in field or real-world scenarios. The calculations for the amount of NaSO₄*H₂O for the HI strength concentration were conducted as followed:

$$707 \frac{mg}{L} * \left(\frac{1 \text{ mmol Na}}{22.989 \text{ mg}} \right) * \left(\frac{1 \text{ mmol Na}_2\text{SO}_4}{2 \text{ mmol Na}} \right) * \left(\frac{142.014 \text{ mg}}{1 \text{ mmol Na}_2\text{SO}_4} \right) = 2,183.7 \text{ mg/L}$$

The calculations for the amount of NaSO₄*H₂O for the LI strength concentration were conducted as followed:

$$16 \frac{mg}{L} * \left(\frac{1 \text{ mmol Na}}{22.989 \text{ mg}} \right) * \left(\frac{1 \text{ mmol Na}_2\text{SO}_4}{2 \text{ mmol Na}} \right) * \left(\frac{142.014 \text{ mg}}{1 \text{ mmol Na}_2\text{SO}_4} \right) = 49.4 \text{ mg/L}$$

The calculations for the amount of MnSO₄*H₂O concentration can be obtained as followed:

$$.5 \frac{mg}{L} * \left(\frac{1 \text{ mmol}}{54.938 \text{ mg}} \right) * \left(\frac{1 \text{ mmol MnSO}_4}{1 \text{ mmol Mn}} \right) * \left(\frac{169.02 \text{ mg MnSO}_4}{1 \text{ mmol MnSO}_4} \right) = 1.538 \text{ mg/L}$$

These numbers were then multiplied by three to account for the amount of solution necessary to fill. Prior to adding these liquids to the respective cubitainers, Mn concentrations were determined. To verify the Mn concentration in the liquid was the target concentration of 0.5 mg/L, a Hach DR900 was used along with analytes discussed in method 8149: Manganese, Low Range. Once the target concentration was verified the solution was added to the cubitainers. In order to promote anaerobic conditions, there was little to no headspace in each cubitainer after liquid was added.

Water Quality Measurements

After assembly, all ten cubitainers were placed into a dark storage container for a week to allow the systems to achieve an anaerobic environment. The suppression of light discouraged any potential algae from growing in the system. Growth of any algae in the systems could add oxygen to the system and potentially clog the substrate. At the end of the first week a dissolved oxygen (DO) probe along with an Orion Star Water Quality Meter (OSWQM) was used to confirm whether the system achieved an anaerobic environment. Once confirmed, water quality analysis occurred twice each week for ten weeks. Water quality parameters measured included: temperature, pH, conductivity, DO saturation and concentration, oxidation reduction potential (ORP), and Mn concentrations. These parameters were measured using the OSWQM. After collection of water quality data, Mn samples were extracted from each cubitainer along with a random duplicate decided at the start of each sampling event. Using a 1:10 dilution, the sample was filtered through a 0.45 micron nylon filters and added to 9 mL of DI water. A Hach DR900 was used to measure the Mn concentration of each sample. To account for the liquid lost during the Mn sampling process, two batches of liquid were made for the two different

ionic strength treatments. Each was analyzed to confirm the concentration of Mn was of the target value. The control cubitainers were topped off with DI water on the same sampling dates as the HI and LI treatments. Approximately three times during the seven weeks that water quality data was collected the cubitainers were topped off three times. The fed batch reactor portion of this experiment occurred for approximately seven weeks and the sequential extraction portion followed immediately after substrate sacrifice.

Sequential Extractions

Substrate sacrifice included emptying the liquid from the cubitainers so only the substrate remained. The remaining substrate was transferred to vacuum-sealed plastic storage bags and kept at 4° C to inhibit microbial action until sequential extraction analysis could occur. The fractions defined in this experiment are an exchangeable fraction, bound to carbonate, bound to labile organic matter and bound to refractory organic matter and sulfides. The exchangeable fraction includes a water-soluble fraction as the sacrificed substrates were not centrifuged prior to putting into the storage bags. In addition to including a water-soluble fraction, the exchangeable fraction in this experiment analyzed for metals weakly bound to the substrate making them more susceptible to release. The carbonate fraction contains ions bound to carbonates. The bound to labile organic matter fraction has particles bound to humic and fulvic acids, both of which are easily broken-down organic structures. Bound to refractory organic matter and sulfides was the last extraction in this experiment, which analyzed particles bound to organic material resistant to degradation and particles bound to sulfide.

Following a modified Tessier procedure, with a substitution of Paap et al. 1991 method for the labile fraction, 2 g of wet substrate –equivalent to 1 g dry material— was used for analysis.

When extracting each fraction, a new extraction fluid is being used (Table 1); therefore, a new matrix is created for each extraction event. Prior to each extraction, matrix effects were evaluated for each solution. The use of the chemicals that interfere with the Hach DR900 were unavoidable for this experiment. For this reason, it was necessary to understand and correct these interferences during extraction and analysis. For each extraction the blank sample was the extraction fluid being used to account for the potential reactions with the analytes used in the procedure. Using a stock solution of 50 mg/L Mn, a 10 mL sample of extraction fluid was spiked with 0.14 mL of Mn to reach a target concentration of 0.7 mg/L sample in the extraction fluid being analyzed. Once matrix corrections were accounted for the extraction process could proceed. Once agitation was completed, respective agitation times for each extraction listed in Table 1, the tubes were centrifuged at 4,700 RPM for ten minutes. Once the centrifuge process was complete, the extraction fluid was measured for Mn concentrations. In between extraction processes the substrate was rinsed with DI water and then centrifuged, this was performed twice between extraction process. This rinse procedure ensured there was no remaining extraction fluid in the substrate, or the centrifuge tube, that could interfere with the next extraction.

Table 1: Experimentally Defined Fractions and Extraction Chemicals

FRACTION	CHEMICAL	pH ADJUSTMENTS	AGITATION
Exchangeable	16 mL of 1M MgCl ₂ (pH 7)	NA	1 hour
Bound to carbonate	16 mL of 1M NaOA _c	Corrected to 5 using HOA _c	1 hour
Bound to labile organic matter	30 mL of 0.1M Na ₄ P ₂ O ₇ (pH 10)	NA	18 hours
Bound to refractory organic matter and sulfides	<ol style="list-style-type: none"> 1. 6 mL of .02M HNO₃ + 10 mL of 30% H₂O₂ 2. 6 mL of 30% H₂O₂ 3. 10 mL of 3.2 M NH₄OA_c 	NA	6 hours

While performing matrix corrections, the exchangeable extraction fluid, MgCl₂, caused interferences with the Hach DR900 method 8149 reagents. Matrix impacts were able to be overcome by analyzing a 1:2 dilution of the matrix and samples. The carbonate fraction extraction fluid created no matrix interferences, nor did the extraction fluid for the labile fraction. The extraction fluid for the bound to refractory organic matter and sulfides fraction created a matrix interference that prevented the titration color change from occurring. For this reason, there was no analysis of the bound to refractory or organic matter and sulfides fraction. During the sequential analysis process the fine particles in the mushroom compost created a visible pigment in all the extraction fluids. The pigment became more concentrated as the extraction process progressed and required corrections before the Hach DR900 could be used. Prior to analysis with the Hach DR900, the pigment interference required there to be a dilution factor of 1:10 for both the carbonate and labile fraction analysis. One replication of all

treatments and control were analyzed, two replications were analyzed for the original substrate.

Results

P-values were determined using a two-sample t-test assuming either unequal or equal variance, with a 95% confidence interval ($\alpha = 0.05$) for Tables 2 and 3. Using a 95% confidence interval there was no statistical difference between the amount of Mn retained in any fraction in the HI strength or LI strength treatments. These values are depicted in Table 2. The control and raw original substrate showed no statistical difference in Mn retained in all three analyzed fractions as well.

Table 2: Mean Value (mg of Mn/kg of dry substrate) \pm STDEV

Fraction	Treatment		p-value ¹
	High ionic	Low ionic	
Exchangeable	0.014 \pm .0035	0.0092 \pm .0001	.07
Carbonate	0.053 \pm .011	0.045 \pm .0043	0.26
Labile	0.15 \pm .02	0.18 \pm .043	0.23

¹ P-value obtained using a t-test ($\alpha = .05$)

Table 3: Mean Value (mg of Mn/kg of dry substrate) \pm STDEV (Cont.)

Fraction	Treatment		p-value ¹
	Control	Original	
Exchangeable	0.008 \pm .002	0.006 \pm .003	0.54
Carbonate	0.04 \pm .003	0.053 \pm .008	0.17
Labile	0.16 \pm .034	0.19 \pm .003	0.44

¹ P-value obtained using a t-test ($\alpha = .05$)

The exchangeable fraction in the LI strength treatments retained less than 4% of the total Mn extracted. The labile fraction appeared to retain the most Mn with an average of 76% of the total Mn extracted. For the HI strength treatments, the exchangeable fraction appeared to retain the least amount of Mn accumulating less than 4% of the total Mn extracted. The labile

fraction retained more than 69% of the total Mn extracted, appearing to retain the highest amount of Mn out of all the fractions in the HI strength treatments. These values are depicted in *Figure 1*. For the control treatments, the exchangeable fraction contained less than 4% of the total Mn extracted. The labile fraction appeared to contain 77% out of the total Mn extracted. For the original untouched compost, the exchangeable fraction appeared to contain the least amount of Mn with less than 3% of the total Mn extracted. The labile fraction retained more than 6% of the total Mn extracted, appearing to retain the highest amount of Mn out of all the fractions. These values are depicted in *Figure 1*.

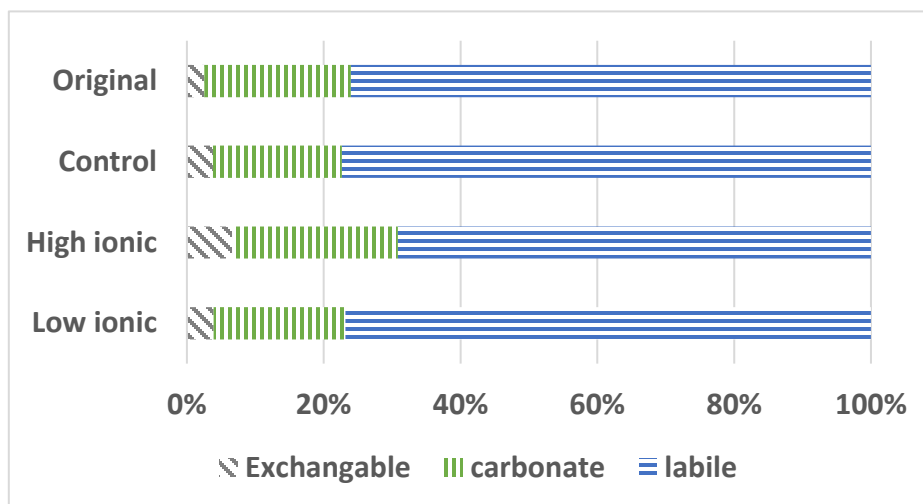


Figure 1: Percentage of Mn Extracted

Not included are the t-tests performed, which analyzed each treatment in comparison to the control and original compost data. The data was separated by fraction and analyzed for statistical significance. Using a two tailed test and equal variance, excluding the analysis performed on the LI and HI strength data for the exchangeable fraction and the LI and original data for the carbonate fraction which had unequal variances, a t-test analysis was performed.

These analyses showed that neither the HI strength nor the LI strength were statistically different from the control and the original sample data at any fraction.

Discussion

The results and analysis of this research were limited by time and resources. Seven weeks was not enough time for this experiment to run, though important results were found despite this limitation. As for the resource limitation aspect of this research resulted in inability to run extractions on the bound to organic matter and sulfide fraction. In the future this type of experiment utilizing fed-batch reactors will need more time allotted to the sequestration process and will require a change in the method measuring Mn concentration. Due to the Hach methods interfering with multiple extraction liquids, resulting in the inability to conduct one extraction, a new method of analysis for Mn concentration must be identified. This research also struggled with fine particles present in the compost, in both the extraction fluid and liquid in the fed-batch reactors, potentially interfering with the Mn concentration results. One majorly evident color interference occurred when analyzing the amount of Mn in the labile fraction, pictured in Figure 2. After dilution the dark pigment was present and had the potential to cause discrepancy in the data. This pigmentation can be mitigated in the future by flushing the compost and allowing the fine particles to be washed out.

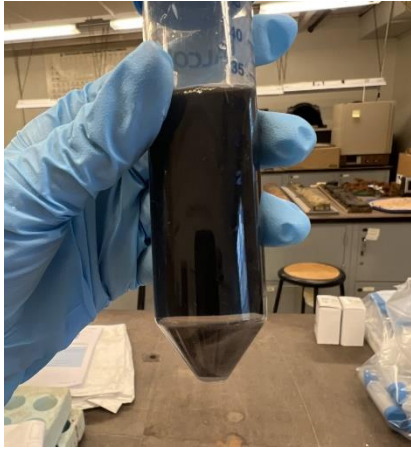


Figure 2: Labile Fraction Extraction Fluid Post Centrifuge

Results of this experiment showed that the LI and HI strength treatments did not differ in the amount of Mn sequestered in each fraction. This being said, when conducting the t-test the p-values for the HI strength compared to the original substrate and LI strength for the exchangeable fraction and the HI strength compared to the original substrate were statistically different at the 93% confidence interval. Based on the results of this experiment and at the confidence interval used, manipulation of ionic strength had no effect on the amount of Mn sequestered in each fraction. Also, the HI and LI strength treatments did not sequester any Mn as the concentrations for each fraction showed no statistical changes compared to the control and original substrate.

Comparing the results of this study to previous studies, the concentrations of Mn for the labile fraction is very high (LaBar and Nairn, 2016). Which brings up the lack of data for the bound to refractory organic matter and sulfides fraction. Due to the lack of data for this fraction, it cannot be confirmed whether or not this fraction would contain a high or low amount of Mn. Though, based on previous research Mn in this fraction is typically low (LaBar and Nairn, 2016).

Conclusion

Overall, this experiment provided evidence that the experimental set up used can be used to simulate vertical flow bioreactors in the form of fed-batch reactors, though more time is needed for future experiments. It cannot be said whether Mn is sequestered in a more labile or recalcitrant fraction of the substrate; therefore, more research needs to be conducted using this experimental set up to understand this. The methods used to analyze Mn concentrations were unsuccessful and not recommended for future testing processes.

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