THE AEROBIC REMOVAL OF MANGANESE FROM MINE DRAINAGE BY AN ALGAL MIXTURE CONTAINING *CLADOPHORA*[1]

by

Laura A. Duggan, Thomas R. Wildeman, and David M. Updegraff[2]

Abstract. Manganese is a contaminant common to most metal and coal mine drainages. Mn (II) is difficult to remove from solution without chemical treatment due to the high pH required to form insoluble manganese oxides or carbonates. Laboratory studies have indicated that an algal mixture, primarily comprised of *Cladophora* (a green alga), is effective in removing the manganese from solution and substantially raising the pH of the mine drainage through photosynthesis. Two reservoirs were constructed for a bench scale study to examine the effects of algae containing *Cladophora* on mine drainage that had passed through a constructed wetland treatment, but still contained 32 mg/L of manganese. The reservoirs were run statically for two months and then as a flow system for two months. Each reservoir initially contained 97 L of wetland effluent (pH = 5.8), 5 L of pond scum containing *Cladophora*, and one reservoir also contained 12 kg of limestone. The reservoir containing limestone reduced the manganese to concentrations of less than 0.3 mg/L and performed slightly better than the reservoir without limestone, which reduced the manganese to concentrations below 3 mg/L. The *Cladophora* grew extensively and was resistant to high manganese levels often toxic to microbes. Microscopic and phase studies have suggested that the *Cladophora* removed the manganese from solution by forming manganese oxide crusts in the algal mat. These results indicate that an algal mixture containing *Cladophora* may be used as a second stage process to remove manganese and raise the pH of water that has passed through a constructed wetland treatment. In addition, an algal pool appears to be a good candidate for a bench scale aerobic wetland.

Key Words: manganese removal, wetland, algae

Introduction

The use of microorganisms for the removal of heavy metals from acid mine drainage has become an important alternative to chemical treatment. Constructed wetlands have been proven to substantially increase the pH of the mine drainage and remove many of the heavy metals by precipitating insoluble metal complexes and precipitates (Brodie et al. 1989 and Wildeman and Laudon 1989). Manganese, a common contaminant in most acid mine drainages, is difficult to remove from solution

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[1] Paper presented at the 1992 National Meeting of the American Society for Surface Mining and Reclamation, Duluth, Minnesota, June 14-18, 1992. Publication in this proceedings does not preclude authors from publishing their manuscripts, whole or in part, in other publication outlets.

[2] Laura A. Duggan is a graduate student and Drs. Thomas R. Wildeman and David M. Updegraff are professors in the Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, Colorado 80401.

Proceedings America Society of Mining and Reclamation, 1992 pp 241-248
DOI: 10.21000/JASMR92010241

https://doi.org/10.21000/JASMR92010241

due to the high pH required to form insoluble manganese oxides, carbonates, or sulfides (Watzlaf and Casson 1990).

The Big Five Wetland in Idaho Springs, Colorado was constructed as a pilot scale anaerobic system emphasizing the microbial reduction of sulfate to sulfide and subsequent precipitation of insoluble metal sulfides (Howard et al. 1989). The pH of the mine drainage was raised from below 3 to above 6 and over 90% of the Fe, Cu, and Zn were removed (Machemer et al. 1990). However, manganese was not removed from the mine drainage due to the inability of manganese to form stable sulfide precipitates under most wetland conditions. Thus, a polishing stage treatment utilizing an alternate microbial process seemed necessary to remove the high levels of manganese.

A laboratory investigation was conducted to study the microbial oxidation of manganese as a possible removal method. Several different types of bacteria and algae were collected from local eutrophic ponds and mine drainage areas and tested with respect to manganese removal from neutralized mine drainage. A sample of pond scum (primarily green and blue-green algae) was found to substantially increase the pH of the mine drainage and remove the manganese from solution to concentrations below the detection limit (< 0.3 mg/L).

Further laboratory studies were conducted with pond scum collected from different local sources. Those samples which were the most effective in removing the manganese and further raising the pH of the mine drainage that had previously passed through the Big Five Wetland, were samples which contained *Cladophora*, a filamentous green alga, along with much lesser amounts of other green and blue-green algae. It appears that the manganese is removed from the water by the precipitation of manganese oxides on the algal cells. The metal precipitation is believed to be at least partially due to the increase in pH and production of oxygen during photosynthesis, as the precipitation of manganese oxides usually requires a pH between 8 and 9 (Stumm and Morgan 1981). Previous laboratory studies also found a large diurnal pH change associated with photosynthesis due to the uptake and release of carbon dioxide (Stumm and Morgan 1981 and Fuller et al. 1988).

Bench scale studies are helpful to test the effectiveness of the laboratory results and to determine their potential for larger scale studies. Anaerobic bench scale permeameters have been shown to closely model the Big Five Wetland (Bolis et al. 1991). To further examine the manganese removal process and to evaluate the potential of an aerobic pilot scale wetland, two bench scale aerobic reservoirs were constructed. The objectives of this study also included determining a manganese removal rate and testing the resistance of the *Cladophora* to high manganese concentrations and severe weather conditions.

Materials & Methods

The reservoirs were constructed from small plastic swimming pools, approximately 1.1 m in diameter. Each of the two pools initially contained 97 L of effluent from the Big Five Wetland and 5 L of pond scum comprised primarily of *Cladophora* from a local pond. The Big Five effluent contained approximately 32 mg/L of manganese and had a pH of 5.8. The only difference between the two reservoirs was that one reservoir also contained 12 kg of limestone (limestone pieces were approximately 1 cm). This reservoir will be referred to as "reservoir LS", and the reservoir which did not contain limestone will be denoted "reservoir NoLS". The reservoirs were placed outside to have full exposure to the environment.

This experiment was run for approximately four months, from August to December, incorporating a wide range of weather conditions. The reservoirs were static for the first two months of the experiment, with water being added occasionally to account for water loss due to evaporation. The weather was typically warm and sunny during this portion of the experiment. A flow system was installed during the last two months of the experiment to determine approximate loading and removal rates. This was accomplished using a peristaltic pump to monitor flow from a feed tank into the reservoirs, and an outlet tube 7 cm above the bottom of each pool. The diameter of the pools was 1 m at the height of the outlet. The outflows were collected in plastic containers which were connected to the reservoirs by plastic tubing. The weather during this portion of the experiment was typically cold and snowy, and the reservoirs froze several times. The samples were filtered and acidified after collection and then analyzed for manganese by flame atomic adsorption.

The pH of the waters were measured frequently, as the formation of manganese oxides is highly pH dependent. During the static portion of the experiment, the pH was taken at different areas in each reservoir to account for differences due to the amount of biomass present and because the amount of sunlight received at each location may affect the amount of photosynthesis. The pH values cited below are geometric averages of the several measurements. Each sample collected was a composite sample containing water from different areas within the reservoir. During the portion of the experiment when the pump was monitoring the water flow, the pH's and samples were taken from the water collected in the outflow containers.

Once during the static portion and once during the pump portion of the experiment, a high concentration manganese solution was added to the reservoirs to examine the effectiveness of the algae in removing high manganese concentrations. This 100 mg/L manganese solution was prepared using manganese sulfate and deionized water.

Results

Static Reservoirs

The initial pH of the water in each reservoir was 5.8 and contained 32 mg/L of manganese. During the first week of the experiment, the pH of each reservoir gradually rose with the pH of reservoir LS usually slightly higher than the pH of reservoir NoLS. On the sixth day of the experiment at 7:10 PM, the pH of reservoir NoLS was up to 8.6 and contained 14 mg/L of manganese, and reservoir LS had a pH of 8.8 and contained only 5.4 mg/L of manganese. However, no diurnal pH fluctuation with photosynthesis was observed up to this point. On the tenth day of the experiment, pH cycling and almost complete manganese removal were observed. On this day, the pH rose 0.3 pH units in each pool between 7:00 AM and 3:00 PM, and reservoir NoLS contained 2.5 mg/L manganese and reservoir LS only 0.5 mg/L. After the tenth day much of the water had evaporated from the reservoirs and most of the manganese had been removed. Therefore, on Day 11 at 1:30PM, 20 L of additional mine drainage effluent from the Big Five Wetland were added (pH = 6.0). This addition lowered the pH to 7.2 and 7.5 for reservoir NoLS and reservoir LS, respectively. However, on Day 12 at 3:15 PM, the pH's were back up to 8.2 and 8.5 of reservoir NoLS and reservoir LS, respectively. For the rest of the static portion of the experiment, Big Five mine drainage effluent was continuously added about twice a week and the reservoir pH's usually recovered within a day or two of the effluent addition.

On Day 20, 40 L of 100 mg/L manganese solution having a pH of 4.9 was added to each reservoir to study the tolerance and removal efficiency of the algae to high manganese concentrations. Before this addition, the water level in the reservoirs was very low (approximately 40 to 50 L). On Day 25 at 9:30 AM, reservoir NoLS had a pH of 7.6 and contained 51 mg/L of manganese, and reservoir LS had a pH of 8.1 and contained 13 mg/L of manganese. By Day 42 at 9:30 AM, reservoir NoLS had a pH of 8.8 and contained 9.7 mg/L of manganese, and reservoir LS had a pH of 9.3 and contained less than 0.3 mg/L of manganese. Thus, both reservoirs appeared to be removing the manganese from a medium pH, high manganese solution.

During the static portion of the experiment, the algal biomass had grown extensively, appeared healthy, and black precipitates could be seen in the algal mat. Therefore, a flow system was designed to simulate a possible pilot scale wetland and determine the efficiency of the reservoirs at different flow rates.

Pump Flow Reservoirs

The pump system was installed in mid-October, on Day 65, with an initial mine drainage effluent flow rate of 3.1 ml/min into each reservoir. The mine drainage effluent had a pH of 6.3 and contained approximately 23 mg/L of manganese. It took several days for the water levels in the pools to reach the outlet. The results are given in Table 1 and outflow manganese concentrations shown in Figure 1. It took a few days longer for the water to reach the outlet in reservoir NoLS, which didn't contain limestone, so the sample on Day 71 was collected from the pool rather than the collection container. No sample was collected for reservoir NoLS on Day 73. On Day 79, the mine drainage effluent in the feed tank was

Table 1. Results of the Pump Flow Experiment

RESERVOIR NoLS				RESERVOIR LS		
DAY #	FLOW (ml/min)	pH	Mn (mg/L)	FLOW (ml/min)	pH	Mn (mg/L)
71	2.1	9.1	3.2	2.5	9.2	1.0
73	3.9	9.3	----	3.9	8.7	0.7
84	2.1	8.5	32.1	2.4	8.5	14.0
86	----	8.5	25.3	----	8.8	1.4
91	3.0	8.1	13.0	1.5	8.2	0.6
94	4.5	8.2	10.1	4.5	8.5	1.2
97	4.0	8.6	4.8	4.5	8.6	2.2
99	----	8.5	3.2	----	8.4	1.4
100	----	8.3	2.3	----	8.4	0.5
103	3.7	8.5	2.3	3.9	----	1.0
120	3.1	8.1	3.8	1.8	8.1	0.6
125	3.0	8.2	5.0	3.8	8.2	0.3
127	3.0	8.2	7.2	3.7	8.3	0.7

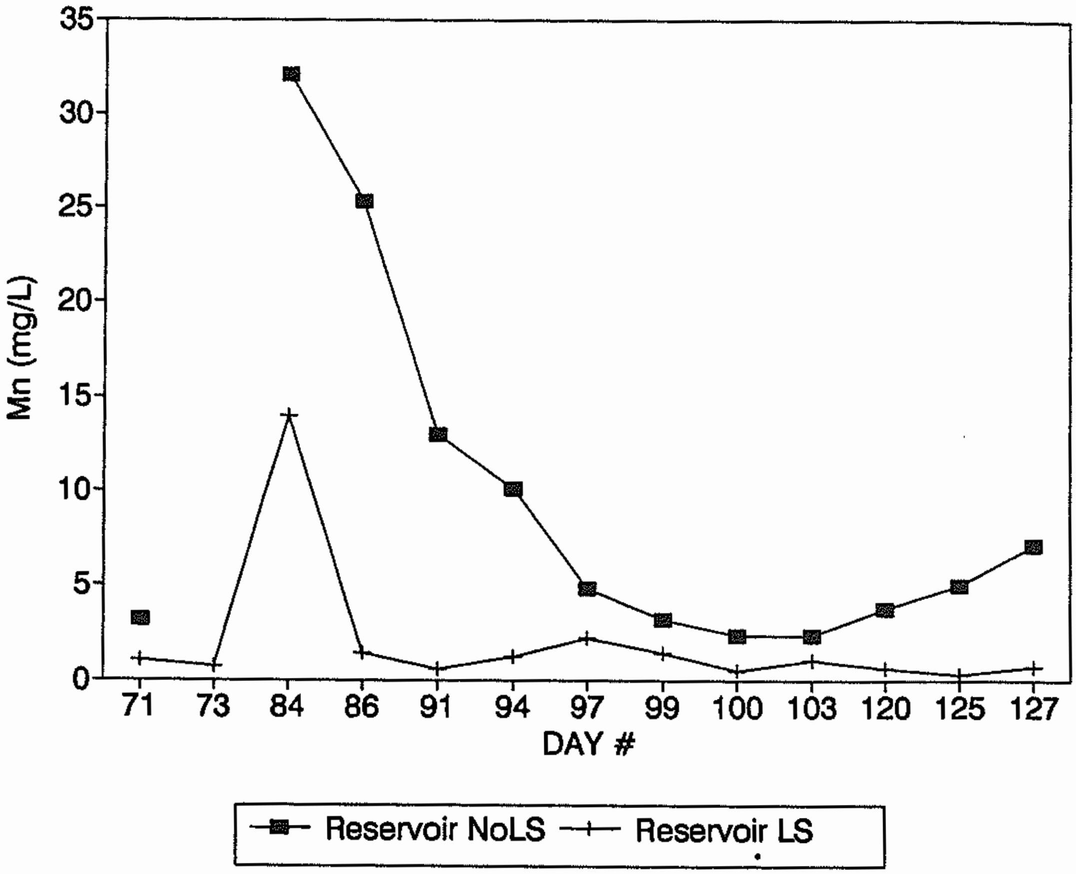

Figure 1. Effluent manganese concentrations for the pump flow experiment.

replaced with the same 100 mg/L manganese solution as was used in the static experiment. This solution was pumped at 5 ml/min until Day 80, when the flow was adjusted to 3 ml/min because the water and algae in the reservoirs were frozen solid except for a small area around the inlet. On Day 84 samples were collected and the remaining 20 L of manganese solution were mixed with 20 L of Big Five mine drainage effluent.

Manganese removal (Table 1) can be used to calculate area adjusted removal rates (Hedin 1990). The units for this rate are grams of manganese removed / day / square meter, which is abbreviated gdm. Figure 2 shows the removal rates which were determined from the following calculation (Hedin 1990):

$$\text{Mn (gdm)} = \frac{1.44 * \text{Flow (L/min)} * [\text{Mn in - Mn out}]\ (\text{mg/L})}{\text{Area } (\text{m}^2)}$$

A value of 29.3 mg/L was used for Mn inflow, which was the average (standard deviation = 3.7 mg/L) manganese concentration of the Big Five Wetland effluent over three of the months this experiment was conducted.

Discussion

The photosynthetic process involves the uptake of carbon dioxide and release of oxygen. A pH greater than 5.5 is required to maintain a supply of carbon dioxide, in the form of bicarbonate, in the water. Since most aerobic wetland treatments involve photosynthesis, this may account for why aerobic wetlands are more effective above pH 5.5 (Brodie et al. 1991). Carbon dioxide is an acid, and therefore its uptake during photosynthesis results in a pH increase. Respiration, the reverse of photosynthesis,

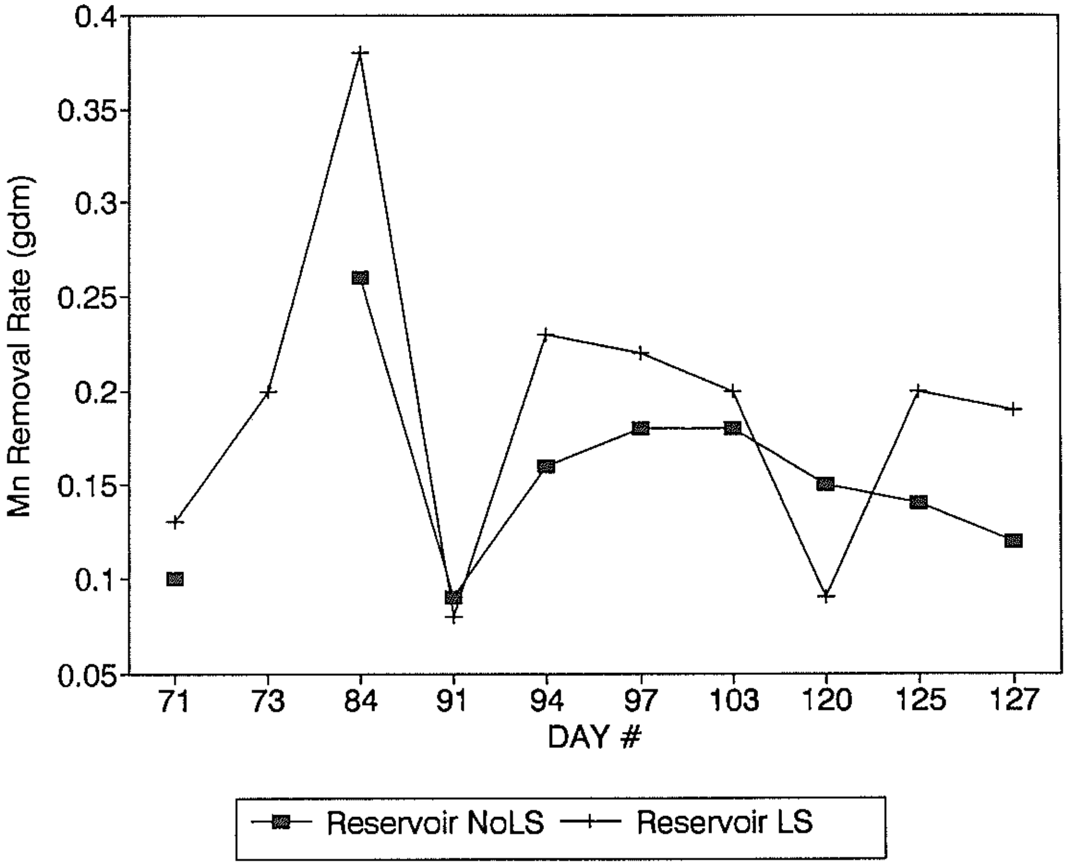

Figure 2. Area adjusted removal rates for the pump flow experiment.

occurs at night and involves the uptake of oxygen and release of carbon dioxide resulting in a lower pH. This uptake and release of carbon dioxide is the cause of the diurnal pH fluctuations observed in the laboratory and reservoir experiments.

The formation of manganese oxides, specifically MnO_2, is a desirable manganese removal method due to the extreme insolubility and large sorption capacities of manganese oxides. The reaction appears to be autocatalytic, which greatly increases the manganese removal rate from solution once some MnO_2 or Mn_3O_4 is formed (Stumm and Morgan 1981). The formation of rhodochrosite, $MnCO_3$, is also a possible manganese precipitate that could form as it is slightly less soluble than calcite.

The success of the previous laboratory studies and the static portion of this bench scale study indicated that algae containing *Cladophora* is effective in removing manganese from the effluent under warm, sunny, static conditions. However, a large scale wetland treatment system is more dynamic and subject to more environmental stresses. Thus, the pump flow portion of this experiment is probably a more accurate indicator of the potential for *Cladophora* to be used in a larger scale wetland treatment.

Figure 1 shows the outflow manganese concentrations for reservoirs NoLS and LS. Considering that the inflow manganese concentration was 100 mg/L from Day 79 through Day 84 and varied between 28 and 65 mg/L during the rest of the experiment, both reservoirs showed excellent removal, often reducing the effluent to below 5 mg/L manganese. Reservoir LS consistently performed better than reservoir NoLS, with 85% of the samples below 2.0 mg/L manganese, the Federal monthly-average effluent limitation for coal mines (U.S. Code of Federal Regulations 1985 a & b). However, a pH difference in the two reservoirs could not account for the better performance of reservoir LS since the pH's of the two reservoirs during this portion of the experiment were very similar.

Both reservoirs recovered from the addition of the 100 mg/L (58 uM) manganese solution and continued to remove the manganese from the effluent added during the rest of the experiment. This tolerance to high manganese concentrations is important because manganese concentrations as low as 10-20 uM have been shown to greatly inhibit manganese oxidizing microbes (Nealson et al. 1988). Reservoir LS once again showed more efficient manganese removal than reservoir NoLS. It is also important to note that the reservoirs were almost frozen solid during most of the time the 100 mg/L manganese solution was being added.

The severe weather conditions present during much of the pump flow experiment had a visible effect on the health of the biomass. During the static experiment the thick algal mat was bright green and floated on the surface due to the large number of oxygen gas bubbles produced during photosynthesis. Throughout the pump flow experiment, the algae lost much of its bright green color and most of the algae sank below the water surface. Gas bubbles were still observed during the sunlight hours indicating that photosynthesis was occurring, but to a much lesser extent than during the warmer, sunnier months. At the completion of the experiment in mid-December, the reservoirs had frozen several times and the algal biomass did not appear very healthy. The fact that the reservoirs performed so well even under these adverse conditions is important, though a pilot scale system may be more efficient in a warmer climate.

Figure 2 shows the calculated area adjusted removal rates for both reservoirs. Reservoir LS had a slightly higher average removal rate of 0.19 gdm than did reservoir NoLS of 0.15 gdm. Both reservoirs showed a much higher manganese removal rate on Day 84 when the 100 mg/L manganese solution was being fed. However, the outflow manganese concentrations for both reservoirs were not below the 2.0 mg/L Federal limit (32.1 mg/L for reservoir NoLS and 14.0 mg/L for reservoir LS). Excluding the removal rates for Day 84, the average removal rates decrease to 0.14 gdm for reservoir NoLS and 0.17 gdm for reservoir LS.

There appears to be several processes that are occurring in the reservoirs to remove the manganese from solution. Low magnification microscopy indicates that black precipitates appear to be forming as crusts on the filamentous algae. SEM and XRF analysis suggest that the crusts contain calciummanganese oxides which were determined to be amorphous by XRD analysis. The photosynthetic pH increase and production of oxygen would most likely be an important factor in the formation of these manganese oxides. However, it has also been shown that nonliving algae has a high affinity for metal adsorption, indicating a surface adsorption phenomena may be occurring in addition to the formation of oxides (Darnall et al. 1989 and Jeffers et al. 1989). Thus, manganese biosorption by nonliving algae may have been an important manganese removal mechanism during the cold weather when some of the algae may have died. Though further studies need to be performed, it appears that the primary manganese removal processes are adsorption to algal cell walls, auto-oxidation due to photosynthesis, and adsorption to manganese oxides that have formed.

Summary

Both reservoirs were successful in raising the pH and removing the manganese down to below 5 mg/L during most of the experiment. Reservoir LS was more efficient in manganese removal during both the static and flow portions of the experiment, often removing the manganese to concentrations below detection limit. Photosynthesis appears to be an important process contributing to the manganese removal due to it's ability to increase pH. Though manganese is required by photosynthetic organisms in the process of oxygen evolution, only trace amounts are usually needed and high manganese concentrations are often toxic to microbes (Nealson et al. 1988). Thus, *Cladophora's* tolerance to high manganese concentrations distinguishes it from many manganese oxidizing microbes. The manganese removal method appears to be the formation of manganese oxide crusts on the filamentous algae. An aerobic wetland treatment utilizing *Cladophora* and limestone would have an estimated manganese removal rate of 0.17 gdm under adverse conditions, and possibly higher under warmer sunnier conditions.

Acknowledgement

The authors would like to acknowledge the U.S. Bureau of Mines who provided support for this research (Contract No. J021002).

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