MANGANESE AND IRON REMOVAL FROM COAL MINE DRAINAGE BY USE OF A GREEN ALGAE-MICROBIAL MAT CONSORTIUM[1]

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Abstract: At the Tennessee Valley Authority's Fabius Coal Mine, Alabama, manganese was more effectively removed from a pond containing an algae mat consortium and limestone substrate than from ponds containing only limestone or pea gravel substrates. The algae mat resulted from the integration of a microbial mat and volunteer filamentous green algae. The microbial mat consisted of blue-green algae (predominately *Oscillatoria* spp.) and bacteria isolated from the site, cultured in the laboratory, and returned to the site. System operation ran from August 1992 through March 1993. Manganese and iron were consistently removed more efficiently in the algae mat pond (mean flow of 4.2 L/min) than through gravel-only ponds even as water temperatures dropped to less than 5° C in the winter.. Based on filtered water samples, during winter months, at 2 m from the influent point of each pond, the algae mat pond removed 2.59 $g/d/m^2$ manganese, compared with 0.80 in the limestone pond and 0.37 in the pea gravel pond. At 1 m from the influent pipe in the algae mat pond, 2.67 mg manganese and 34.25 mg iron were deposited in a gram of dried mat. In March 1993 two events likely caused the loss of much of the algae mat: (1) a 50-cm snowfall followed by a heavy runoff and (2) establishment of a snail population, as well as other possible invertebrate herbivores, which consumed the algae mat. The algae mat was reestablished in June 1993 and has persisted to date. A green algae and microbial mat consortium may be a cost- effective treatment technique for permanently removing metals from mine drainage.

Additional Key Words: acid mine drainage, blue-green algae, cyanobacteria, iron, manganese, metal removal, microbial mats, *Oscillatoria*.

Introduction

Manganese removal from acid mine drainage is a challenge due to the solubility of manganese sulfide and the alkaline conditions required to precipitate manganese as an oxide or carbonate. Therefore, it is common to find drainage with manganese above state or U.S. Environmental Protection Agency (U.S. EPA) standards (Gordon and Burr 1989). Additionally, in an oxygenated environment, ferric iron precipitates as $Fe(OH)_3$, and the consequent release of hydrogen ions will increase acidity (Caruccio and Geidel 1993). Thus the dual goal of simultaneously removing manganese and iron from mine drainage is complicated if the system does not have enough alkalinity and a high pH.

The Tennessee Valley Authority utilizes constructed wetlands technology to treat acid mine drainage. These wetlands have generally been effective in removing Mn (0.15 to 1.87 $g/d/m^2$) and Fe (0.4 to 21.3 $g/d/m^2$) (Brodie 1993). At one site within the Fabius coal mine in northeast Alabama, Mn and Fe levels are approximately 8 and 6 mg/L (0.45 μm filtered) after leaving an oxidation pond and before draining toward an extensive constructed wetland. At this point a pilot-scale field test was conducted to determine if a biological

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consortium consisting of a microbial (dominated by cyanobacteria or blue-green algae) and green algae mat would effectively remove residual manganese and iron in a very small pond surface area.

Microbial mats are natural heterotrophic and autotrophic communities dominated by cyanobacteria (blue-green algae). They are self-organized laminated structures annealed tightly together by slimy secretions from various microbial components. The surface slime of the mats effectively immobilizes the ecosystem to a variety of substrates, thereby stabilizing the efficient internal microbial structure. Since mats are both nitrogen-fixing and photosynthetic (Paerl et al. 1989), they are self-sufficient, solar-driven ecosystems with few growth requirements.

In our laboratory, we have developed microbial mats, constructed with specific microbial components, for a variety of bioremediation applications (Bender and Phillips 1993). Mats have been found to reduce selenate to elemental selenium (Bender et al. 1991), to remove Pb, Cd, Cu, Zn, Co, Cr, Fe and Mn from water (Bender 1992, Bender et al. in press), and to remove Pb from sediments (Bender et al. 1989).

Degradation of recalcitrant organic contaminants has also been observed under both dark and light conditions (Bender and Phillips 1993). The following contaminants have been degraded in water and/or soil media by constructed mats: TNT (Mondecar et al. 1993), chrysene, naphthalene, hexadecane, and phenanthrene (Phillips et al. 1993), 2,4,4' trichlorobiphenyl (PCB) (Bender 1993), trichloroethylene (TCE), and the pesticides chlordane (Bender et al. 1993), carbofuran, and paraquat. Radio-labeled experiments with mat-treated carbofuran, petroleum distillates and TCE show that these three compounds are mineralized by mats and mat products, such as biofilms and bioflocculents.

Recently, our data confirm that the mats effectively treat mixtures of organics and a heavy metal by simultaneously sequestering Zn and mineralizing TCE and chrysene. Additional research, currently in progress, shows that U^{238} can be removed from ground water samples.

This project was designed to examine the feasibility of applying mats in a field remediation pilot project to remove residual manganese and iron from acid mine drainage. The broad goals were to assess the performance of mats under environmental conditions, such as determining seasonal survival and the efficiency of the mats in removing metals under day-night conditions. Comparisons in Mn and Fe removal were made among the three ponds: algae mat pond with limestone substrate, a pond with limestone substrate, and a pond with pea gravel substrate (the latter two without mat). Specific experiments in the present study compared the effectiveness of the algae mat and limestone pond compared with controls of limestone or pea gravel alone in removing manganese and iron. Parallel laboratory experiments determined, under more ideal conditions, manganese and iron removal under simulated field conditions.

Methods

Three 40-m^2 ponds were constructed by first lining with polyvinyl chloride. Two of the ponds had limestone substrate, and one had pea gravel substrate layered to create four rises separated by five troughs. Maximum trough water depth was 30 cm. Rises nearly broke the water surface. Mine drainage flowed from an oxidation pond to a trickling filter, both designed to precipitate $Fe(OH)_3$. From the trickling filter, one pipe fed all three ponds (fig. 1). Each pond was sampled for manganese and iron at six or seven points. The ponds operated continuously from August 1992 through March 1993 and again from June 1993.

Cyanobacteria-Algae Mat Pond (CGM)

A cyanobacterial-dominated microbial mat is an entire ecosystem containing several bacteria species, but dominated by cyanobacteria, in the multilayered mat structure. In this pond, microbial mat became enmeshed with volunteer green algae, thus forming an integrated green algae and microbial mat (CGM).

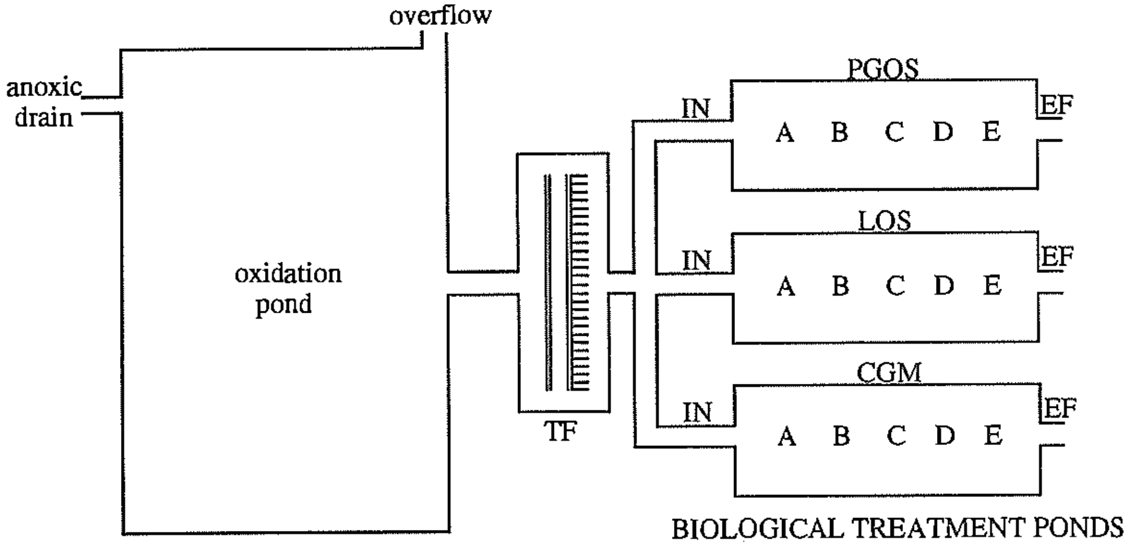

Figure 1. Oxidation pond, TF, trickling filter; IN, influent water; A-E, additional sample points for manganese and iron; CGM, cyanobacteria-algae mat pond; LOS, limestone-*Oscillatoria* pond; PGOS, pea gravel-*Oscillatoria* pond. Not drawn to scale.

Microbial strains, including *Oscillatoria* spp. and purple bacteria (likely a mixture of sulfur and nonsulfur species), were selected from the Alabama site during February 1992. Microbial mats were developed from these strains according to Bender and Phillips (1993). A microbial mat slurry (blended in water) was broadcast over the experimental pond at a rate of 1 to 1.5 L in three applications during a 4-week period. Silage, prepared from grass clippings, added organic acids and several species of ensiling bacteria to the water. Within 2 months a thick green mat covered the entire pond surface. Underneath this floating layer, the limestone itself was covered with a heavy green coating of cyanobacteria.

Limestone-*Oscillatoria* Pond (LOS)

Although no biological component was intentionally added, CGM pond cyanobacteria spread into this pond. Limestone was covered with a thin (<1-mm) film of cyanobacteria but did not develop a surface mat cover as in the CGM pond. Precipitated iron covered the entire limestone substrate of the pond including the pond, periphery beyond the effluent pipe.

Pea Gravel-*Oscillatoria* Pond (PGOS)

The pea gravel substrate in this pond also become thinly (<1 mm) covered with cyanobacteria. Additionally, there was a rapid buildup of precipitated iron at the effluent pipe by December 1992.

Flow Rate

Flow was set initially at 1 L/min and incrementally increased over a period of 2 months. Flow was adjusted on a daily basis owing to clogging by $Fe(OH)_3$. Ranges of flow rates (L/min) were 0.8 to 6.0, with the following mean values for each pond: CGM = 4.2, LOS = 3.8, PGOS = 2.8.

Mn could be removed by chemical precipitation in all ponds. By increasing the flow rate, we expected to reach a break point whereby the two ponds without the substantial biological component would no longer remove manganese as efficiently as the CGM pond. This would demonstrate the greater efficiency of Mn removal by the addition of the biological component.

Water Chemistry

Water samples were collected at the oxidation pond, the trickling filter, at each pond's influent and effluent pipes, and at five points within each pond in a horizontal profile from influent to effluent (fig. 1). If an effluent sample could not be effectively collected, the last point within the pond was considered the effluent sample.

In the field, the oxidation pond, trickling filter, and influent and effluent water was tested for pH (Orion GX series pH electrode; Orion 200 series portable pH and Eh meter), Eh (Corning platinum redox combination electrode; Orion 200 series portable pH and Eh meter), conductivity (Fisher Scientific digital conductivity meter), water temperature, and dissolved oxygen (Otterbine Sentry III dissolved oxygen meter).

For metal determination, water samples were filtered (0.45-μm cellulose acetate) and stabilized for transport with HNO_3. Oxidation pond iron was present mainly as $Fe(OH)_3$ and to a lesser extent as Fe^{+2}. Filtering removed $Fe(OH)_3$, thus only Fe^{+2} was determined. These were later analyzed for manganese and iron content by atomic absorption spectrometry (Varian, Spectra AA-20 BQ, double-beam). Reliability of manganese analysis was regularly verified (QA/QC) by comparing with a synthetic solution containing Mn and Fe (undiluted concentrations of 200 mg/L Mn and 100 mg/L Fe) and diluted with field pond water. The field pond water was a sample drawn directly from a pond.

Mn and Fe Concentration in CGM Mat

To determine the mg Mn and Fe per g algae mat, samples from the CGM pond were dried at 37° C for 24 h. Dried mats were pulverized by mortar and pestle. Triplicate 2 g samples were hydrolyzed by microwave digestion in concentrated HNO_3. Mn and Fe was determined by atomic absorption spectrometry.

Laboratory Comparison

Because it was not possible to keep the two field control ponds free of all biological material, controlled experiments for noting metal precipitation in the presence of a limestone substrate with and without attached microbes were performed in the laboratory.

Mn and Fe Removal. Bench-scale acrylic tanks, measuring 60- by 16.5-cm in surface area, were layered with 3.5-cm-depth limestone substrate. Three experimental tanks were inoculated with laboratory cultures of microbial mats, and three control tanks contained only limestone. The experimental tank microbial mats superficially covered the limestone.

Separate experiments examined Mn and Fe removal independently, as well as in a mixed Mn-Fe solution. The procedure was as follows. Two liters of a 10-mg/L Mn and/or Fe solution was added to a tank. The retention time in the closed tank was 2 min. The tank was elevated to 11 cm at one end. The solution was expelled in one batch. Liquid was collected, and Mn and Fe were measured by atomic absorption. Then the same solution was flowed through again. This was repeated 10 times for each of 6 tanks. Ten repeated flows with the same solution meant that the 2-L solution containing 20 mg total Mn or Fe passed through 1 m^2 (specifically 0.099 cm^2 x 10 flows = 0.99 m^2). Results are presented both graphically as a decrease in Mn and/or Fe in mg/L, and as a Mn and Re removal rate in $g/d/m^2$.

Results

Pond Characteristics

A floating mat (1 to 2 cm thick), composed of filamentous green algae mat and cyanobacteria, predominantly *Oscillatoria* spp., grew well in the CGM pond after addition of silage amd mat inocula. A secondary mat of cyanobacteria also covered the limestone at the bottom. Thus the metal-contaminated water essentially flowed between a double layer of mat. Approximately 6 weeks were required to establish a full-pond mat cover, but effective metal removal began in the early stages of mat growth.

The thin layer of cyanobacteria covering the rocks of the two ponds designed as controls consisted of an *Oscillatoria* strain resembling that of the inoculated CGM pond cyanobacteria. Small amounts of green algae produced a floating mat, but the biomass remained low compared with that of the experimental pond.

Through winter 1993, the CGM pond maintained a viable algae mat. An approximately 1-m^2 bleached area (indicating cell death) developed during this time. Beneath this bleached algae, limestone was covered with viable cyanobacteria. Iron was evident in patches over the entire surface area of the pond, yet there were also large areas of viable green algae and microbial mat over the pond surface. In contrast to the control ponds, there was no visible evidence of iron precipitates beyond the effluent pipe.

In March 1993, after a 50-cm snowfall thawed, the mat was severely damaged. This may have been due to washout or snow shading. The algae mat pond was drained and reinoculated in June. Metal deposits were left in the troughs.

Water Quality

Table 1 summarizes daytime water quality parameters from August 1992 through April 1993. Dissolved oxygen values for the single nighttime (0530 h) sample were CGM, 4.2 mg/L; LOS, 6.1; and PGOS, 6.8. The low nighttime value for the algae mat pond is likely due to high oxygen consumption by the biological component.

Table 1. Mean daytime water quality parameters per pond.

	Parameters[2]						
Pond[1]	T(sd)	DO(sd)	pH(sd)	ORP(sd)	Cond(sd)	Alk(sd)	Flow(sd)
CGM	14.1(7.6)	7.3(1.3)	7.4(0.4)	413(56.4)	648(136.7)	167(50.8)	4.2(2.4)
LOS	14.7(8.4)	8.3(1.8)	7.6(0.4)	424(73.8)	618(94.7)	168(53.2)	3.2(1.0)
PGOS	14.8(8.5)	8.2(1.9)	7.2(0.5)	410(74.4)	580(92.3)	156(49.1)	2.2(1.6)

[1] CGM=cyanobacteria-algae mat pond; LOS=limestone-*Oscillatoria* pond; PGOS=pea gravel-*Oscillatoria* pond.

[2] T=temperature, Celsius; DO=dissolved oxygen, mg/L; ORP=oxidation-reduction potential (platinum combination Ag-AgCl electrode), mV; Cond=conductivity, µmho/cm; Alk=alkalinity, mg/L equivalent $CaCO_3$; Flow=L/min; sd=standard deviation.

Metals Removal

Manganese removal rates remained high as the flow rate increased in the CGM pond (fig. 2). This is especially significant because figure 2 data came from the winter months when water temperatures were between 4° and 6° C. In December 1992, the flow rate was 4.5 to 6 L/min in LOS and PGOS ponds, which was likely the reason for low Mn and Fe removal in those ponds. The CGM pond continued high-rate removal of Mn until 5 to 8 M from the inflow, while LOS and PGOS showed much lower removal rates, even though Mn concentration in the water remained high (table 2). At lower metal loading, the slower removal rates of LOS and PGOS were sufficient to remove most of the Mn before the effluent point. However, at flow rates of >4.5

L/min, the mechanisms of Mn removal present in these two ponds were saturated, and only the CGM pond continued effective removal. Ferrous iron removal patterns were similar but not as dramatic as for manganese since $Fe(OH)_3$ was removed in the 0.45-μm water filter during sample preparation.

Another way to distinguish the superior CGM pond metal removal efficiency was to determine differences between daytime and nighttime removal. Figure 3 shows the metal removal profiles in all ponds at a flow rate of 5 L/min, during light and dark periods. Mn was effectively removed beginning at 1 to 2 m from the inflow in the CGM pond. These data represent a time period after 4 months of continuous flow.

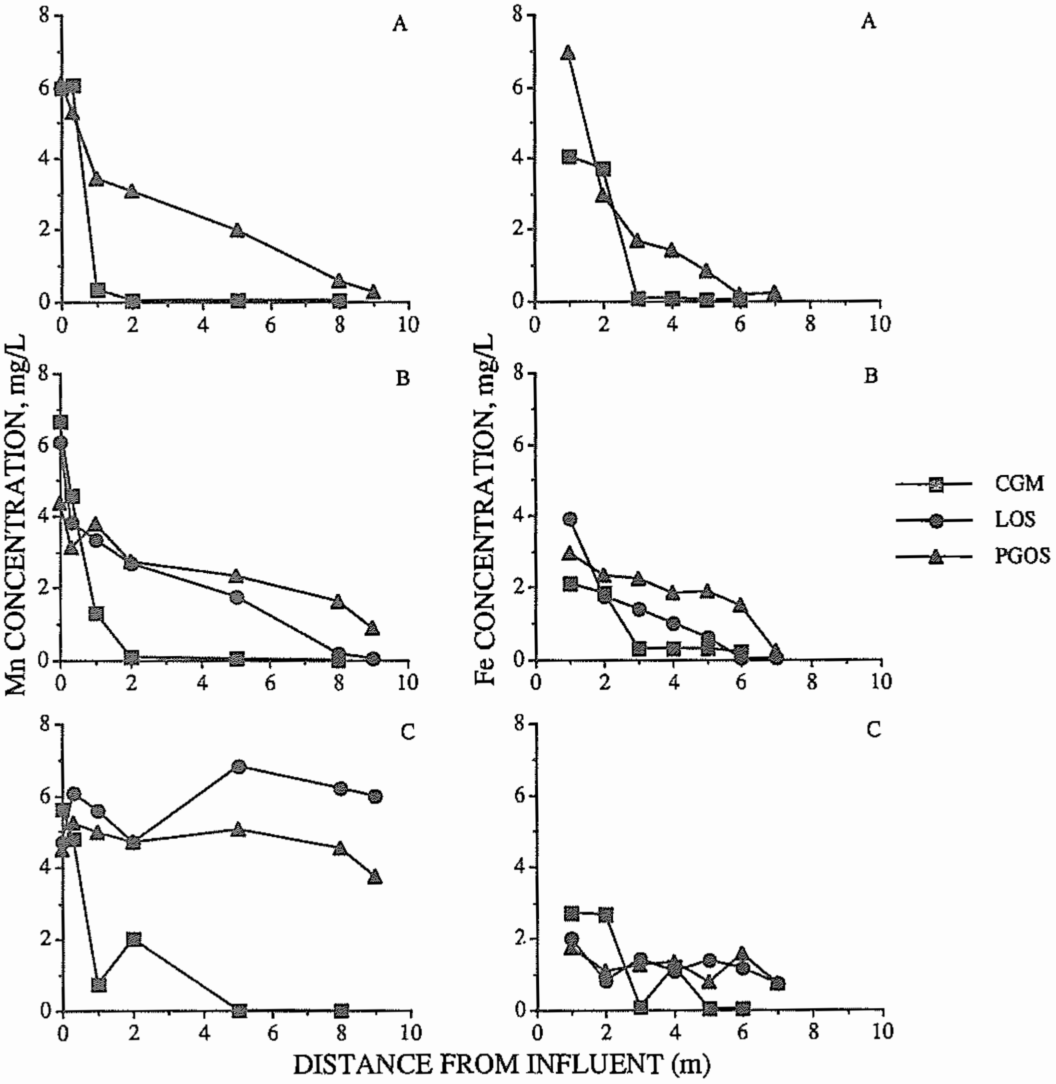

Figure 2. Comparison of Mn and Fe removal at varying rates: *A*, 1.5 L/min; *B*, 3 L/min; *C*, 4.5 to 6 L/min. CGM, cyanobacteria-algae mat pond; LOS, limestone-*Oscillatoria* pond; PGOS, pea gravel-*Oscillatoria* pond.

LOS and PGOS ponds with cyanobacteria covering only their rock substrate did demonstrate metal removal, although not as efficiently. Similar conclusions are difficult to reach with Fe because nighttime mg/L values were very low at the influent pipe (0.11 and 0.09) in the LOS and PGOS ponds. CGM pond filtered Fe enters at 4.39 mg/L and drops to 0.48 mg/L at 1 m.

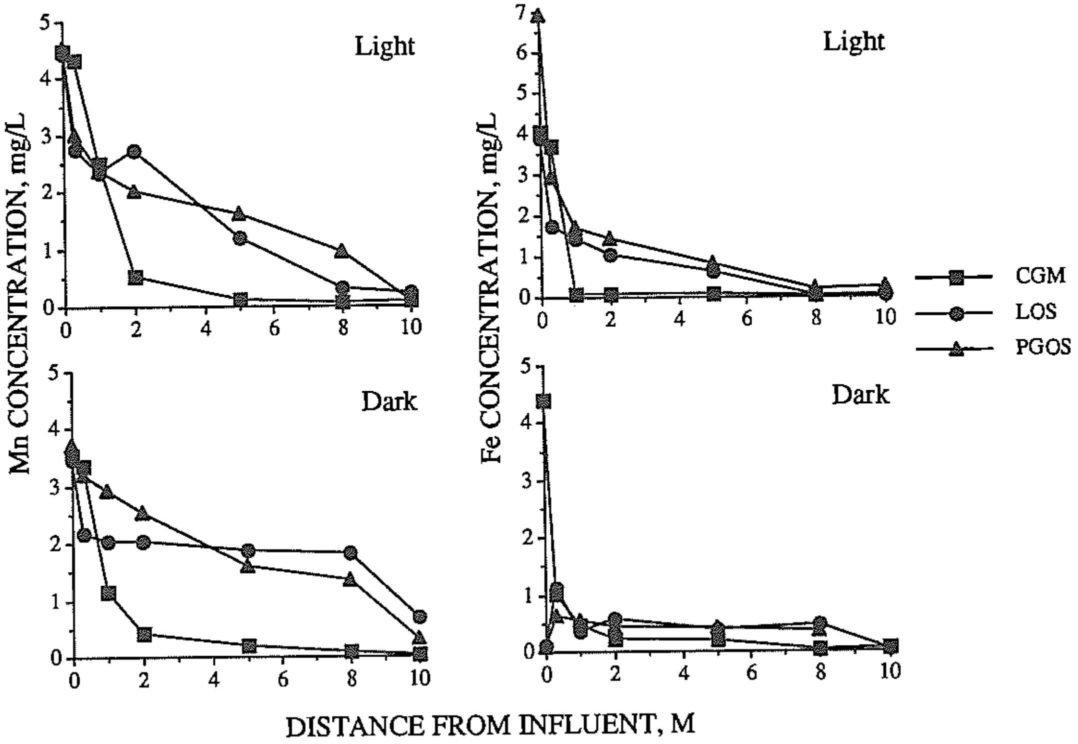

Figure 3. Day-night comparison of Mn and Fe removal among three ponds after 4 months of continuous flow. CGM, cyanobacteria-algae mat pond; LOS, limestone-*Oscillatoria* pond; PGOS, pea gravel-*Oscillatoria* pond.

Table 2. Manganese removal rates (g/day/m^2).^{1}

Pond	Flow L/min	Influent Mn	2 m	5 m	8m
CGM	4.2	4.79	2.59	3.87	.74
LOS	3.8	3.78	.80	.55	.52
PGOS	2.8	4.47	.37	.86	.58

[1] Because of the plumbing design, it was not possible to standardize the three pond flows to the same rate.

[2] CGM=cyanobacteria-algae mat pond; LOS= limestone-*Oscillatoria* pond; PGOS=pea gravel-*Oscillatoria* pond.

[3] Two, 5, and 8 m represent points C, D, and E in figure 1.

Table 3. Metal concentration (mg metal per g dried mat) from CGM pond, January 1993.[1]

Location	Mn	Fe
1 m from Influent	2.67	34.25
Pond center	.98	11.20
Point E (near effluent)	.45	.79

[1] The highest concentration of metal was present as crystalline deposits in the pond troughs. Metal speciation of these precipitates is currently in progress (J. Neil, US Geological Survey).

Manganese Removal Rate. Mn removal rates presented in table 2 for the three ponds were calculated at 2, 5, and 8 m from the influent point. Five meters cooresponds to point D and 8 m point E in figure 1.

Mn and Fe Concentration in CGM Mat. Dried, ground, and hydrolyzed mat samples from the CGM pond revealed large decrease in manganese and iron concentration from influent to effluent points (table 3). Mn concentrations declined by 83% and Fe concentrations declined by 98%.

Laboratory Comparison

Figure 4 illustrates the removal of Mn and Fe in laboratory tanks by measuring the mg/L metal decrease in the effluent solution. Overall pH was approximately 8.0 in all tanks.

Mn Removal Alone. After 10 flows of an initial 10-mg/L Mn solution, data conversion to g/d/m^2 metal removal for three experimental tanks showed Mn removed at 1.93, 1.62, and 1.48 g/d/m^2 (overall average 1.68 g/d/m^2). Three control tanks had removed Mn at 1.07, 1.34, and 0.88 g/d/m^2 (overall average 1.10 g/d/m^2).

Mn-Fe Mixed Solution Removal. In another experimental set (fig. 4; right), Mn removal may have been inhibited by the presence of a similar 10-mg/L Fe concentration.

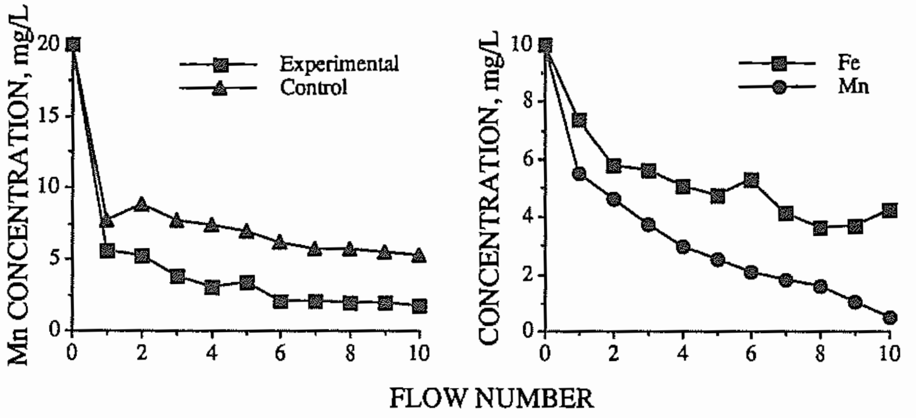

Figure 4. Left: Laboratory tank experiments in Mn removal without and with an algae mat. Right: Laboratory tank removal of Mn-Fe mixed solution.

Discussion

In the CGM pond, although there was manganese-cell binding tothe floating algae mat, visual observations indicated that even more manganese was deposited as precipitaates at the pnd bottom. Fe entered the treatment pond primarily as a flocculated precipitate which becme entrapped in the filamentous algae. in general, day-night and winter-summer meal removals were essentially the same for the CGM pond. The LOS and PGOS ponds showed Mn breakthrough (Mn outflow release > U.S. EPA regulations of 2 mg/L) during nighttime sampling or when mine drainage flow exceeded 4.5 L/min. $Fe(OH)_3$ accumulated at the end of the effluent pipes in the LOS and PGOS ponds. This did not occur at the effluent of the CGM pond.

The biological component and biological processes were responsible for the addition consistent metal removal in the CGM pond. In addition to the algae mat, all exposed limestone surfaces were effectively covered by cyanobacteria. Metals are known to complex with a wide range of organic material, including microbes and their organic releases. Dunbabin and Bowmer (1992) identify four dominant binding processes that incorporate metals into organic materials: (1) cation exchange, (2) adsorption, (3) precipitation and coprecipitation, and (4) complexation or chelation. Although metals that are adsorbed, precipitated, or complexed can be released back into solution in an equilibrium response, no such fluctuations were detected in the CGM pond. Additionally, conditions of neutral pH with high dissolved oxygen and redox levels (mediated by the biological component) favor the chemical precipitation of manganese oxides and iron hydroxides. These oxides and hydroxides, in turn, act as reservoirs for additional metal deposit.

It is curious that, although LOS and PGOS do not function as well as CGM in Mn removal, they show high oxidation-reduction potential and dissolved oxygen levels. It may be important to consider the microzones present in the thick algal mat, which are absent in the LOS and PGOS ponds. While the gross water quality measurements were similar in the three ponds, the microlevel water quality conditions have shown large variability in previous laboratory research with microbial mats (Bender and Phillips 1993). Mats in the laboratory are known to entrap oxygen in their slime. If this occurred in the CGM pond, as the metal-laden water passed through the labyrinth of algae filaments, it was possibly exposed to elevated oxygen regions, which rapidly precipitated Mn. Examination of the underside of the mat showed that initial precipitation may occur in that location. As precipitation continues, sections of mat break and fall to the bottom, collecting in the troughs. These regions of concentrated manganese oxide deposits function as autocatalytic nuclei for further deposition of the Mn.

Although the conditions of high oxygen and high Eh generated in algae mat microzones as well as autocatalysis may be central to the deposit of Mn oxides, other factors may be functional as well. Other laboratory research has shown that specific bioflocculents were released by the mat in response to the presence of Mn^{+2} (Rodriguez-Eaton et al. 1993). These materials carried surface charges ranging from -58.8 to -65.7 mV. The charges changed to +1.8 in the presence of divalent metal, indicating metal binding to the bioflocculent.

The potential bioavailability of metals is favored by increases in acidity, reducing power, and salinity. Since the mat generally reduces acidity and sequesters certain anions from the water column, including chloride, the stability of the metal deposit is favored. Although anaerobic zones can be identified within the mat in laboratory experiments (Bender and Phillips 1993), the redox conditions of field water remained high. The purple sulfur bacteria, probably *Chromatium* spp.(positive identification not completed), was inoculated as part of the microbial mat group in order to maintain low-sulfide conditions. These autotrophs sequester H_2S instead of H_2O for photosynthesis, thereby decreasing the reducing power of the aqueous environment. Since *Chromatium* spp. typically colonizes in natural mats found in the environment, it can be expected to persist in the constructed field mats. Purple photosynthetic bacteria were also identified in the CGM pond.

It is likely that several mechanisms, including flocculation, cell sorption, autocatalysis, and mediation of the environmental conditions of pH, Eh, and oxygen concentration, contribute to the metal removal.

Acknowledgment

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