MICROBIAL, ALGAL, AND FUNGAL STRATEGIES FOR MANGANESE OXIDATION AT A SHADE TOWNSHIP COAL MINE, SOMERSET COUNTY, PENNA.[1]

by

Eleanora I. Robbins, David L.Brant, and Paul F. Ziemkiewicz[2]

Abstract. Successful designs to eliminate Mn from mine discharge are necessary for both restoring abandoned mine lands and permitting the mining of high sulfur coal in the eastern United States. A passive in-line system that meets Mn discharge limits was built at the discharge from the former Shade Township coal mine in south central Pennsylvania. Qualitative research on monthly changes in the microbial and algal community that removes Mn is underway. Epilithic attachment of microorganisms was analyzed on artificial (glass microscope slides) and natural substrates (limestone thin sections) that were immersed in surface water for one month periods over 6 months. Organisms attached to both glass and limestone substrates. Limestone became coated with 34-86 % more Mn than did glass surfaces. Light microscopy revealed 12 different strategies are being used by bacteria, cyanobacteria, diatoms, green algae, and fungi to oxidize Mn. The dominant method used by the epilithic community to oxidize Mn is coating of holdfasts by the iron bacterium, Leptothrix discophora, and the green alga, Ulothrix sp. Other methods for Mn removal by oxidation include coating of individual cells, filaments/sheaths/ hyphae, extracellular polysaccharides, and biofilms. The unplanned community at the site is multifaceted and extremely efficient in its Mn removal ability. Community interactions or complexity may play roles in the stability of the ecosystem and the efficiency of its Mn oxidizing ability.

Introduction

Mn in the form of Mn^{2+} is highly soluble in acidic and near-neutral conditions (Hem, 1985), which means that the regulated coal-mining industry must use chemical treament to meet Mn discharge limits (Kleinmann and Watzlaf, 1988). Finding inexpensive passive treatment methods remains an important objective for government and industry (Robbins, 1998), particularly for abandoned coal mines in Pennsylvania and West Virginia. Several studies have shown that limestone is an effective and inexpensive substrate for passively removing Mn (Brant and Ziemkiewicz, 1997; Hedin et al., 1994; Sikora et al., 1996; Watzlaf, 1997).

The mechanism of manganese removal by oxidation is thought to be dominantly biological, and the details of biological removal can be elucidated at different scales. Phillips et al. (1995) analyzed the relationship from a macroscopic scale and showed that a mixed community of cyanobacteria, algae, and bacteria can be quite efficient in its Mn-removing capability. Robbins et al. (1992) used light microscope analysis on Mn-oxide growths on silicate cobbles and showed that one bacterium, Leptothrix discophora, dominated Mn-oxide precipitation. Vail et al. (1988) isolated Mn-oxidizing bacteria from a limestone remediation pond and showed that they were highly efficient when inoculated into buried limestone drains. At the angstrom scale level, many studies including Ghiorse and Ehrlich (1992) have shown the specific sites of Mn attachment to bacterial sheaths. Stone (1997) detailed 8 physiological strategies used by bacteria that interact with the Mn cycle.

The technology to isolate strains and adapt them to local conditions is quite expensive (Vail and Riley, 1995). However, the success of their studies and of Phillips et al. (1995) suggests that more studies are needed. In particular, microscopic data

---

[1] Paper presented at the 1999 National Meeting of the American Society for Surface Mining and Reclamation, Scottsdale, Arizona, August 13-19, 1999.

[2] E.I. Robbins, biogeologist, U.S. Geological Survey, Reston, VA 20192; D.L. Brandt, Research Associate, and P.F. Ziemkiewicz, Director, National Research Center for Coal and Energy, West Virginia University, Morgantown, WV 26506-6064

Proceedings America Society of Mining and Reclamation, 1999 pp 634-640
DOI: 10.21000/JASMR99010634

https://doi.org/10.21000/JASMR99010634

might reveal useful information about community interactions that may play a role in the stability of the limestone ecosystem and the efficiency of its Mn oxidizing ability.

A passive in-line system that meets Mn discharge limits was built at the discharge from a former Shade Township coal mine in south central Pennsylvania, Somerset County, 7 km southwest of Windber, PA (N40° 10.574' lat., W78° 52.094' long.) (Williams et al., 1996). Following cessation of mining of the Upper Kittanning coal bed in 1982, anoxic limestone drains (ALDs) were built at seeps; these drains are removing Al and raising alkalinity. Discharge next flows through a cattail-dominated wetland that is removing Fe but lowering pH. Discharge then flows over two limestone cells that are removing Mn. The first limestone cell is composed of a bed of limestone cobbles; the bed is 0.6 to 1.2 m thick and 20 m by 20 m in width. The cell started precipitating Mn within one month of completion in 1990, and came into compliance within 15 months. In the Brant and Ziemkiewicz (1997) data set, which covered from December, 1991 to March, 1994, streamflow from the wetland averaged 19 L/min (5 gal/min). pH averaged 7.8. At the first limestone cell, influent Mn was around 25 mg/L; with the exception of the month of June, effluent Mn consistently met EPA regulations for Mn concentration <2 mg/L (Brant and Ziemkiewicz, 1997).

We designed a set of experiments to analyze seasonal changes in biological processes that are precipitating oxidized Mn on the limestone. Epilithic attachment, which is fixation to solid surfaces, was the major focus of this research. Epilithic attachment of bacteria, algae, and fungi was analyzed on artificial (glass microscope slides) and natural substrates (limestone thin sections) that were immersed for one month's duration and replaced monthly. The ultimate application of this information will be in the design of more efficient passive removal methods.

Materials and Methods

Chemistry. Water samples were analyzed quarterly using ASTM standard D858-95 for Mn content. In addition, following Morgan and Stumm (1965), orthotoluidine was used to test for the presence of oxidized Mn on slides and organic structures.

Biology. Microscopic evaluation of the dominantly aerobic microscopic community in a shallow water column was the major focus of our studies. Epilithic attachment that resulted in Mn oxidation was analyzed monthly on artificial (glass microscope slides) and natural substrates (two limestone varieties). Limestone samples from the Shade site and from the Action site nearby in Meyersdale, PA, were prepared commercially as thin sections. Macrophytes were collected periodically and analyzed microscopically to assess epiphytic attachment, which means attachment to plant surfaces.

Results and Discussion

Site Characterization

During the course of study, water levels fluctuated at the site, but limestone cobbles were usually submerged under approximately 10 cm of water. Submerged limestone cobbles are individually coated with a very densely interwoven, 1-cm-thick brown/black cyanobacterial mat that includes Oscillatoria sp. In places, the mat is continuous but sags at the junctions between individual cobbles. The green alga, Ulothrix sp., attaches to the mat using holdfasts and its long filaments float in the sag pools. These filaments do not become coated with brown/black Mn oxide. The base of the mat, where attached to limestone, is colored white from calcite crystal precipitation. When cobbles are exposed by falling water levels, the mat dehydrates into thin sheets that curl up and peel off the cobbles, thereby exposing the dark gray color of the underlying limestone.

The limestone cell, which could also be called a shallow pond, is open to full sunlight. Cattails have encroached along the pond edges and are beginning to colonize dried out patches of limestone. The mat does not grow where cattails cast shadows.

Chemical Environment

The average pH of the Shade site was 7.8 in the 6 months of observation (Table 1). The water temperature was close to that of ambient air temperature because the water column is so shallow. Specific conductance averaged around 972 µS/cm at the outflow. Dissolved Mn values for inflow and outflow show that the limestone cell efficiently

removed Mn from the water with the exception of May and July (Table 1).

Oxidized Manganese on Epiliths

The organisms at the Shade site are using a wide variety of strategies for oxidizing Mn (Table 2). These include coating of cells, filamentous sheaths, holdfasts, and excreted substances. Bacteria, cyanobacteria, fungi, and algae perform one or more of each of these processes. The character of Mn oxidation by the organisms using these strategies shifted during the experiment (Table 3). During the 6-month study, almost every organism precipitated oxidized Mn, but at varying amounts. During the spring and summer, slides were more coated than those collected during the fall.

Cell coatings. Individual bacterial cells were completely coated, giving the impression that the bacteria became entombed in the oxide coating. This process was seen on both cocci and rods. Diatoms also became coated, but the coating appeared to be random because not all individuals were coated on the same slides.

Filament/sheath/hypha coatings. Individual bacterial filaments/sheaths, fungal hyphae, and algal filaments became lightly to strongly coated with oxidized Mn.

Holdfast coatings. In terms of total Mn precipitation, by far the dominant Mn oxidation process was by coatings on holdfasts, which are attachment structures, of the iron bacterium, Leptothrix discophora, and the green alga, Ulothrix sp. L. discophora holdfasts averaged around 5 µm in diameter, whereas those of Ulothrix sp. were 20 µm or larger. Holdfasts of the bacterium also colonized cattail stems, turning submerged plant surfaces brown/black, whereas holdfasts of the alga colonized moss leaves and stems. Holdfasts were the dominant method of Mn oxidation in the early spring (Table 3). L. discophora holdfasts are typically abundant on cobbles in swiftly moving water (Robbins et al., 1992), and were not expected in the slowly moving water of the Shade site. The Ulothrix sp. holdfasts are similar to those seen downstream from a chemical remediation site in West Virginia (Robbins et al., 1997).

Extracellular polysaccharide (EPS). EPS are substances secreted by organisms; many EPS have metal oxidizing capabilities (Fletcher and Floodgate, 1973; Puchelt et al., 1973). Bacterial holdfasts, fungal hyphae, and algal filaments and holdfasts were surrounded by brown/black substances that attach to compounds that have been excreted by the cells. EPS oxidation was most extensive during the summer months (Table 3).

Biofilms. The glass slides became coated with biofilms that turn black/brown from oxidized Mn. The biofilms were enmeshed with bacterial rods or cocci. These are typical structures formed by epilithic bacteria (Little et al., 1997).

Oxidized Manganese on Macrophytes and Cyanobacteria

Clumps. At the Shade site, only one strategy for Mn oxidation was evident among the macrophytes and cyanobacteria. Entwined filaments of cyanobacteria (Table 4) precipitate Mn as brown/black clumps.

The clumps of oxidized Mn are similar to those found by Richardson et al. (1988), on colonies of cyanobacteria in Oneida Lake, NY. At that site, microelectrodes were used to show that pH elevation during photosynthesis was the cause of Mn oxidation.

Oxidized Manganese on Limestone Thin Sections

Oxidized Mn on limestone thin sections followed the same pathways as on glass surfaces (Table 3). Coatings on cells, filaments/sheaths/ hyphae, holdfasts, EPS, and biofilms all contained oxidized Mn. The bacteria, fungi, and algae on the limestone appear to be the same species/morphotypes as on the glass surfaces (Table 3). The difference between the two substrates was in amount of oxidized Mn precipitated. Point-count transects across slides showed that between 34 and 86 % more oxidized Mn precipitated on limestone.

The two different limestone varieties being used in this experiment had different types and amounts of oxidized Mn coatings. The variety being used at Shade became more coated than the variety being used at the Action site in Meyersdale. No effort was made to learn more about the sources and chemistry of the limestone varieties, but this casual observation confirms other findings that the limestone variety is an important factor in remediation designs.

Conclusions

Oxidized Mn is being precipitated by many organisms in the water column at Shade. Bacteria, cyanobacteria, fungi, diatoms, and green algae are participating in the process, and at least 12 different strategies are being employed by these organisms to oxidize and therefore eliminate Mn from the water column. The strategies include coating on individual cells, filaments/sheaths/hyphae, holdfasts, EPS, and biofilms. While both glass and limestone surfaces are colonized by Mn oxidizing organisms, more Mn is being precipitated by the epilithic community on the limestone surfaces. Macrophytes and cyanobacteria also have strategies for precipitating Mn, but the process is related to photosynthetic pH elevation and the oxidized Mn is in the form of external clumps. Mn oxidizing bacteria also colonize macrophytes, forming coated holdfasts that color the plant tissues brown/black.

The fact that this unplanned community came into existence and continues to function, grow, and meet discharge limits is very significant. It suggests that local organisms which can handle elevated Mn may be universally present. It confirms the findings of Phillips et al. (1995) that a mixed community of cyanobacteria and algae is very efficient in its Mn-oxidizing capability. They showed that it was possible to manipulate different components to increase efficiency without knowledge of specific details. Our data supplements theirs by explaining some of the interacting processes that are occurring at a microscopic level.

The microscopic analysis shows that Mn which coats EPS is common and the amount increases in the warmer months; it is not presently known what factors initiate EPS excretion. Holdfasts of bacteria are efficient in the spring and holdfasts of algae are efficient in the warmer summer months. Ecological studies are particularly needed on the green alga, Ulothrix, because its Mn-oxidizing holdfasts are large. This ecological study of the former Shade coal mine is important because Mn removal met discharge limits within 15 months of construction and has operated with few failures for the past 8 years.

Acknowledgments

We would like to thank Randy Hoffman of Hoffman Mining, Inc., for access to the site and Pennsylvania Department of Environmental Protection for access to quarterly reports.

Literature Cited

Brant, D.L., and Ziemkiewicz, P.F., 1997, Passive removal of manganese from acid mine drainage: American Society for Surface Mining and Reclamation, Proceedings of 14th Annual National Meeting, p. 741-744. https://doi.org/10.21000/JASMR97010741

Fletcher, Madilyn, and Floodgate, G.D., 1973, An electron-microscopic demonstration of an acidic polysaccharide involved in the adhesion of a marine bacterium to solid surfaces: Journal of General Microbiology 74:325-334. https://doi.org/10.1099/00221287-74-2-325

Ghiorse, W.C., and Ehrlich, H.L., 1992, Microbial biomineralization of iron and manganese. in Skinner, H.C.W., and Fitzpatrick, R.W., eds., Biomineralization Processes, Iron and Manganese, Catena Supplement (Dutch Soils Jour.) 21:75-99.

Hedin, R.S., Watzlaf, G.R., and Nairn, R.W., 1994, Passive treatment of acid mine drainage with limestone: Jour. Environmental Quality 23:1338-1345. https://doi.org/10.2134/jeq1994.00472425002300060030x

Hem, J.D., 1985, Study and interpretation of the chemical characteristics of natural water: U.S. Geological Survey Water-Supply Paper 2254, 263 p.

Kleinmann, R.L.P., and Watzlaf, G.R., 1988, Should the effluent limits for Mn be modified?: Bureau of Mines Information Circular 9183, p. 305-310. https://doi.org/10.21000/JASMR88020305

Little, B.J., Wagner, P.A., and Lewandowski, Z., 1997, Spatial relationships between bacteria and mineral surfaces, in Banfield, J.F., and Nealson, K.H., eds., Geomicrobiology: Interactions Between Microbes and Minerals: Reviews in Mineralogy 35:123-159.

Morgan, J.J., and Stumm, Werner, 1965, Analytical chemistry of aqueous manganese: Journal of the American Water Works Association, v. 57, p. 107-119.

Phillips, P., Bender, J., Simms, R., Rodriguez-Eaton, and Britt, C., 1995, Manganese removal from acid coal-mine drainage by a pond containing green algae and microbial mat: Water Science Technology 31:161-170.
https://doi.org/10.1016/0273-1223(95)00503-F

Puchelt, Harald, Schock, H.H., Schroll, Erich, and Hanert, H.H., 1973, Rezente marine Eisenerze auf Santorin, Griechenland. II. Bakterogenese von Eisenhydroxid-sedimenten: Geologische Rundschau 62: 786-812.
https://doi.org/10.1007/BF01820961

Richardson, L.L., Aguilar, C., and Nealson, K.H., 1988, Manganese oxidation in pH and O_2 microenvironments produced by phyto-plankton: Limnology and Oceanography 33:352-363.
https://doi.org/10.4319/lo.1988.33.3.0352

Robbins, E.I., 1998, New roles for an old resource, Ferromanganese nodules assist mine cleanup: Geotimes 43:14-17.

Robbins, E.I., D'Agostino, J.P., Ostwald, J., Fanning, D.S., Carter, Virginia, and Van Hoven, R., 1992, Manganese nodules and microbial oxidation of manganese in the Huntley Meadows wetland, Virginia, USA: Catena Supplement (Dutch Soils Jour.) 21:179-202.

Robbins, E.I., Maggard, R.R., and Kirk, E.J., 1997, Manganese removal by chemical and microbial oxidation and the effect on benthic macroinvertebrates as a coal mine in Wayne County, western West Virginia: Proceeding of the 18th Annual West Virginia Surface Mine Drainage Task Force Symposium, Morgantown, WV, p. 110-124.

Sikora, F.J., Brodie, G.A., and Behrends, L.L., 1996, Manganese removal in saturated gravel beds: Obtaining design criteria (abs.): Proceedings, 13th Annual Meeting American Society for Surface Mining and Reclamation, Knoxville, TN, p. 857.

Stone, A.T., 1997, reactions of extracellular organic ligands with dissolved metal ions and mineral surfaces, in Banfield, J.F., and Nealson, K.H., eds., Geomicrobiology: Interactions Between Microbes and Minerals: Reviews in Mineralogy 35:309-344.

Vail, W.J., and Riley, R.K., 1995, U.S. Patent 5,441,641, Process for removing manganese from solutions including aqueous industrial waste: U.S. Patent Office, 2 p.

Vail, W.J., Wilson, S., and Riley, R.K., 1988, Mine water and mine waste: Isolations and culture of manganese-oxidizing bacterium from a man-made cattail wetland: Bureau of Mines Information Circular 9183, p. 399.

Watzlaf, G.R., 1997, Passive treatment of acid mine drainage in down-flow limestone systems: American Society for Surface Mining and Reclamation, 14th Annual Meeting, Austin, TX, p. 611-622.
https://doi.org/10.21000/JASMR97010611

Williams, D.R., Sams, J.I., III, and Mulkerrin, M.E., 1996, Effects of coal-mine discharges on the quality of the Stonycreek River and its tributaries, Somerset and Cambria Counties, Pennsylvania: U.S. Geological Survey Water-Resources Investigations Report 96-4133, 95 p.

Table 1. Chemical characteristics of Shade Township site water samples (Abbreviations: nd, no data; ORP, oxidation-reduction potential).

Sample date	pH influent	pH effluent	Temperature influent (°C)	Temperature effluent (°C)	Specific conductance influent ($\mu S/cm^2$)	Specific conductance effluent ($\mu S/cm^2$)	ORP (mv)	Mn influent (mg/L)	Mn effluent (mg/L)
4/6/98	nd	7.8	nd	20	nd	1080	-39.1	nd	nd
4/21/98	6.3	7.6	11	13	800	620	nd	10.2	0.1
5/26/98	6.2	7.4	13	17	895	772	nd	12.9	2.9
6/26/98	6.6	7.7	12	20	837	663	nd	11.1	0.3
7/21/98	7.3	8.0	16	21	943	776	nd	12.5	3.8
8/28/98	7.7	8.2	16	26	1,200	797	nd	12.2	0.3
9/4/98	nd	7.87	nd	28.4	nd	nd	338	nd	nd
9/9/98	7.3	8.2	13	18	1,160	763	nd	14.5	0.2
9/16/98	nd	nd	nd	nd	nd	1200	196	nd	nd

Table 2. Strategies used by microorganisms to oxidize Mn (EPS=extracellular polysaccharide).

BACTERIA	CYANOBACTERIA	FUNGI	ALGAE
Coating individual cells (rods, cocci)	Lumps on entwined filaments	Coating hyphae	Coating filaments
Coating filaments/sheaths		Coating EPS	Coating diatom frustules
Coating biofilm			Coating Ulothrix sp. holdfasts
Coating Leptothrix discophora holdfasts			Coating EPS
Coating EPS			

Table 4. Macroscopic biological data and chemical characteristics at Shade Township site (Symbols: see Table 3; ls, limestone).

Sample number	Sample type	Collection date	pH	Temp. (°C)	Brown clumps	Other information
SH-1	algal mat	4/6/98	7.8	20	+++	microcrustaceans
SH-2	white filaments on algal mat	4/6/98	7.8	20	+++	---
SH-3	black suspension	4/6/98	nd	nd	+++	reducing bacteria and black sulfides on microcrustacean fecal pellets
SH-4	green algae	4/6/98	7.8	20	---	---
SH-6	black scrape from ls	4/6/98	nd	nd	+++	---
SH-11	brown on catttail	4/6/98	nd	nd	---	Ulothrix colonizes moss
SH-12	brown on moss	4/6/98	nd	nd	---	brown color from brown plant hairs
SH-13	algal mat on ls	7/7/98	nd	nd	+++	brown filaments colonize mat
SH-14-1	algal mat on black limestone	9/4/98	7.87	28.4	+++	brown filaments colonize mat
SH-15	surface of mat	9/4/98	7.00	24.0	+++	brown filaments colonize mat
SH-15B	base of mat	9/4/98	7.53	26.1	---	white color from calcite crystals
SH-19	microbial mat	9/16/98	7.35	28.1	+++	brown filaments and brown hyphae in mat

Table 3. Microscopic biological data and chemical characteristics (Explanation of symbols: ---, absent, +, 1 on slide; ++, 1 every field; +++, 2-4 every field; ++++, 5 or more every field) (Abbreviations and symbols: bn, brown; dk, dark; gn, green; lt, light; med, medium; EPS, extracellular polysaccharide)

Sample number	Substrate	Submersion dates	Brown holdfasts of Leptothrix discophora	Brown cocci (co) or rods (r) (l,long; m,medium, s,short)	Brown coated bacterial filaments or chains	Iron bacteria	Colored biofilms.	Brown coated fungal hyphae	Brown holdfasts of Ulothrix	Brown coated algal filaments	Leaking brown EPS	Diatom species (brown-coated)	Brown coated animals
1-2	glass	4/6/98-5/6/98	++++	co,sr, mr	+ filaments mr chains	---	lt bn	+++	+	+	++	6 (bn)	?
3-4	glass	5/6/98-6/5/98	++++	co, sr	+++ filaments	L. discophora	lt med bn	+	+	+	+	4 (bn)	---
5	Action limestone	5/6/98-6/5/98	+++	co,sr	+ filaments	---	med bn	+	+	---	---	3 (bn)	---
6	Shade limestone	5/6/98-6/5/98	++	sr, co	---	---	---	+	+	---	+	3 (bn)	---
7-8	glass	6/5/98-7/7/98	+++	sr	+ filaments mr chains	Siderocystis	med bn	+	+	+	++++	3 (bn)	---
9	Action limestone	6/5/98-7/7/98	+	co, sr	----	---	med-dk bn	+	+	+	+++	2 (bn)	---
10	Shade limestone	6/5/98-7/7/98	+	co, sr	+ filaments	---	---	+++	+	---	+	2 (bn)	coated ostracod
11-12	glass	7/7/98-8/11/98	+	sr, co	++ filaments + mr chains	---	med bn	++	+	+	+++	2 (bn)	---
13	Action limestone	7/7/98-8/11/98	+	sr	++ filaments	Siderocystis	med bn	--	++	---	+++	2 (bn)	---
14	Shade limestone	7/7/98-8/11/98	--	sr, co	++++ filaments	---	med bn	+++	+	+	++++	2 (bn)	coated ostracod
15-16	glass	8/11/98-9/4/98	+	sr,co	+ filaments + mr chains	---	---	+	++	---	+	6	---
17	Action limestone	8/11/98-9/4/98	+	sr, co	+ filaments	---	----	+	+	---	+	1	---
18	Shade limestone	8/11/98-9/4/98	+	sr, co	+ filaments	---	med bn	--	+	---	+	1	---
19-20	glass	9/4/98-10/16/98	+++	sr,co	++ filaments + mr chains	---	med bn	+	++	+	+	1	coated micro-crustacean
25-26	glass	10/16/98-11/18/98	+	co, sr	++ filaments	L. discophora	---	---	++	---	---	6 (bn)	---
27	Action limestone	10/16/98-11/18/98	+	co, sr	---	----	---	+	+	---	---	4	---
28	Shade limestone	10/16/98-11/18/98	---	co, sr	+ filaments	---	---	---	++	---	---	---	---