Phillips, P. & Bender, J. (1998): Bioremediation of Metals in Acid Tailings by Mixed Microbial Mats. – In: Geller, W., Klapper, H. & Salomons, W. (eds): Acidic Mining Lakes – Acid Mine Drainage, Limnology and Reclamation. – p. 347-363, 6 Abb., 5 Tab.; Berlin (Springer).

19 Bioremediation of Metals in Acid Tailings by Mixed Microbial Mats

P. Phillips and J. Bender

Clark Atlanta University, Atlanta, Georgia 30314, USA

19.1 Introduction

Mixed microbial mats were used to promote metal removal from mine drainage in Alabama and Colorado, USA. They are a microbial consortium which are highly tolerant of toxic metals and harsh environmental conditions. Under field conditions, these mats are generally modified by the voluntary invasion of local flora, especially filamentous green algae. For bioremediation work, these constructed mats take advantage of the role natural microbial mats play in filtering and transforming metals and metalloids. Constructed microbial mats are synthesized using silage with its associated bacterial flora and the blue-green algae (cyanobacteria) *Oscillatoria*.

In the laboratory, microbial mats removed an array of heavy metals and metalloids, mineralized to carbon dioxide pesticides, PCBs (polychlorinated biphenyls), solvents, oils and explosives and removed mixed waste (radionuclides and heavy metals) in a continuous flow system (Goodroad et al. 1994). In the field, they have removed manganese (Mn), zinc (Zn) and other heavy metals [silver (Ag), chromium (Cr), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni) and iron (Fe)] from mine drainage and BTEX (benzene, toluene, ethyl-benzene and xylene), a gasoline constituent, from contaminated groundwater (Goodroad et al. 1994; Bender et al. 1995). Landfill leachate is a mixture of toxic organics, metals and ammonia. In collaboration with Waste Management, Inc., a pilot project tested microbial mat reduction of ammonia and metals from sanitary landfill leachate (Goodroad et al. 1995).

Microbial mats offer several advantages for bioremediation. These include low cost, durability, the ability to function in both fresh and salt water and tolerance to high concentrations of metals, metalloids and organic contaminants, as well as mixtures of these.

19.1.1 Natural Microbial Mats

Microbial mats are natural microbial communities dominated by blue-green algae, but also containing a variety of bacteria within the laminated structure of the mat. These systems are resilient microbial communities which self-organize into stratified biofilms arranged for maximum efficiency of interspecies exchanges (Caumette 1989). Their physiological flexibility has been amply documented and includes anoxygenic and oxygenic photosynthesis, survival after desiccation and inclusion of aerobes and anaerobes within the same matrix (Shilo 1989; Stal et al. 1989). During daylight, photosynthesizing mats maintain highly aerobic conditions at the surface while retaining anaerobic zones in lower regions. Thus, the mat ecosystem has aerobic/anaerobic activities in process simultaneously. However, dark periods cause a rapid shift to anaerobic function because there is no light for photosynthesis, and, consequently, for oxygen production. Because microbial mats have evolved under hostile conditions, similar to those expected in highly contaminated environments, survival adaptations of these ecosystems are directly applicable to remediation biotechnology.

19.1.2 Constructed Microbial Mats

Mixed microbial mats can be constructed for specific metal bioremediation tasks by integrating the desired microbial components for bioremediation of metal/metalloid contamination. Among the latter, microbial mats have been found to reduce selenate to elemental selenium, remove Pb, Cd, Cu, Zn, cobalt (Co), Cr, Fe, uranium^{238} (U^{238}) and Mn from water and to remove Pb from sediments. Controlled experiments with one radionuclide, uranium (0.1 mg $U^{238}\,l^{-1}$, spiked in groundwater samples) were successful. Table 19.1 (adapted from Goodroad et al. 1994) presents a summary of metal removal from water and sediments.

Microbial mats are cultured by isolating key microorganisms from the treatment site according to Bender and Phillips (1994). They are developed by enriching a pond or bioreactor surface with ensiled vegetation together with microbial inocula. Silage provides a flora of fermentative bacteria, as well as organic acids that nutritionally support heterotrophic bacteria. Additionally, photosynthetic products of the blue-green algae will support microorganisms that are heterotrophic. The floating silage serves as a secondary structural function for floating microbial mat development. Anaerobic and microaerophilic strains colonize the anoxic zones

Table 19.1. Remediation of metals by constructed mixed microbial mats. (Adapted from Goodroad et al. 1994)

Metal	Initial concentration ($mg\ l^{-1}$)	Removal rate (mg metal m^{-2} mat h^{-1})
Free floating mats[a]	U^{238}: 0.12	3.19
Mats immobilized on glass[b] wool layered in baffled tanks	Mixture of	
	Cr: 24	10,129
	Co: 24	10,052
Mats immobilized on floaters[c]	Mixture of	
	Zn: 22	313
	Mn: 18	462
Excised mats applied to Iron[d] Mountain mine drainage sample	Mixture of	
	Cu: 284	378
	Zn: 3021	3778
	Cd: 19	356
Acid mine drainage in field[e] ponds	Mn: 3.5–7.6	1.0–4.1 g Mn m^{-2} day^{-1}

[a] Self-buoyant microbial mat was cultured on the surface of laboratory ponds.
[b] Microbial mat, cultured on glass wool, was placed into acrylic tanks constructed with baffles to create a serpentine flow.
[c] Microbial mat was attached to glass wool balls that were floated in metal-contaminated water.
[d] Small sections of microbial mat were excised and applied to a mixed solution of Cu, Zn, Cd and Fe sample from Iron Mountain mine drainage in California, USA. pH was adjusted to 3–4 before adding microbial mat sections.
[e] A floating microbial mat (1 to 2 cm thick), composed of a filamentous green alga and blue-green algae, was developed by enriching with silage and microbial inocula (initially selected from the site) on an approximately 40-m^2 field pond. A second microbial mat formed on the limestone at the pond bottom.

within the mat. The final product is a thick, gelatinous green mat dominated by blue-green algae floating over a clear water column. Under field conditions, these mats are generally modified by the voluntary invasion of local flora, notably filamentous green algae in the field experiments discussed here. This latter assists in the long-term stability of the floating microbial mat.

Field studies, currently in progress, show that the microbial mat is easily generated in the natural environment and exhibits excellent durability in the field. Relevant research and development results from mining pilot field projects using constructed mixed microbial mats are reported here.

19.2 Acid Coal Mine Drainage Treatment

19.2.1 Background

Manganese removal from acid mine drainage represents a unique 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 US Environmental Protection Agency standards (less than 2 mg l^{-1}). Additionally, in an oxygenated environment, ferric iron precipitates as $Fe(OH)_3$, and the consequent release of hydrogen ions will increase acidity. 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 $pH > 7$.

The Tennessee Valley Authority (TVA) utilizes constructed wetlands technology to treat acid mine drainage. These wetlands have generally been effective in removing Mn (0.15–1.87 g m^{-2} day^{-1}) and Fe (0.4–21.3 g m^{-2} day^{-1}) (Brodie 1993). At one site within the abandoned Fabius coal mine fields in northeast Alabama, USA, the drainage contains total dissolved Mn and Fe at approximately 8 and 6 mg l^{-1} (0.45-µm filtered) after leaving an oxidation pond and before draining toward an extensive constructed wetland.

The research reported here, together with other TVA sponsored projects, have demonstrated the success of a comprehensive solution to this problem. The resolution of the Fe/Mn contamination in an environment of low pH, oxygen and alkalinity requires several components: an anoxic drain, an oxidation pond and a metal-tolerant microbial photosynthetic system.

19.2.2 Methods

Beginning in 1992, at a point downstream from the oxidation pond, our research group conducted a pilot-scale field test to determine if microbial mat would efficiently remove residual manganese in a 44-m^2 pond. Phillips et al. (1994, 1995) and Bender and Phillips (1995) give detailed explanations of the methodology and earlier phases of the project. The acidity in the coal mine drainage was reduced by directing the flow through an anoxic drain, a buried limestone barrier. After the limestone barrier, much of the iron precipitated in an oxidation pond of approxi-

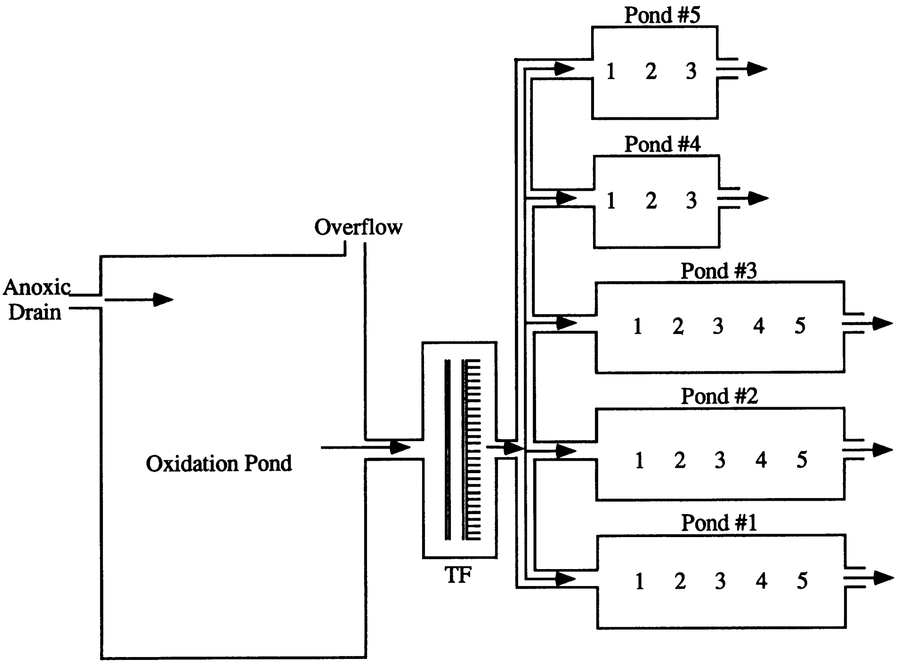

Fig. 19.1. Schematic diagram of the Tennessee Valley Authority (TVA) acid coal mine drainage remediation pilot project, Alabama, USA. Anoxic drain increases pH, and oxidation pond and trickling filter (*TF*) precipitate iron. Pond 1 was inoculated with microbial mat in 1992. Pond 2 was a limestone control pond from 1992 to 1994; then it was inoculated with microbial mat. Pond 3 was a pea gravel control pond. Pond 4 was a second limestone control pond and pond 5 was a soil substrate control pond, both operating from December 1994 to September 1995. *Numbers 1–5* refer to sampling points separated by 1 m each

mately 1 ha. The anoxic drain contributed buffer in anoxic conditions, thereby preventing iron deposit and armoring of the limestone. As the water entered the oxic region (oxidation pond) it precipitated as $Fe(OH)_3$ while maintaining neutral pH. A stream of drainage from the oxidation pond was diverted to a trickling filter system to remove additional iron. The drainage was then flowed through the pilot ponds to determine manganese removal under various treatments. The project began with three ponds (discussed below in periods 1 and 2). Two additional ponds were later added (period 3) (Fig. 19.1). All ponds were lined with 4-mm black plastic and layered to a depth of 10 cm with 2.5-cm-size limestone (ponds 1, 2 and 4), 1-cm-size pea gravel (pond 3) or soil substrate (pond 5) (Fig. 19.2). The bottom was intentionally formed into an undulating configuration to provide variable depth and to prevent vertical stratification

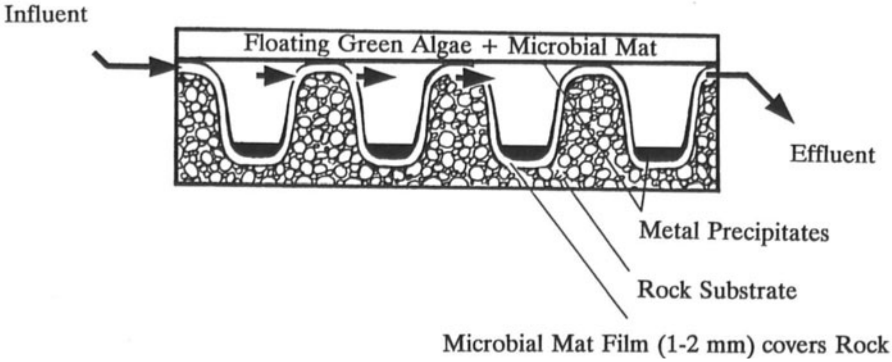

Fig. 19.2. Schematic diagram cross section of microbial mat pond at the Tennessee Valley Authority (TVA) acid mine drainage and Bureau of Mines precious metal mine drainage remediation pilot projects

and sheeting. The water depth in the troughs was 20 cm. The pilot project analysis is divided into three separate periods, which separate distinct events – either climatic or management changes.

Period 1 includes the first 4 months of the experiment from November 1992 to February 1993. Pond 1, 44 m^2 and with a limestone substrate, was inoculated with microbial mat and silage. Pond 2, 44 m^2 and with a limestone substrate, was a control pond for pond 1. Since the carbonate supplied by the limestone was expected to buffer the pond water and, in itself, be responsible for a degree of manganese precipitation, pond 3, 34 m^2 and with a pea gravel substrate, was a control pond for the pond 2 limestone substrate.

Period 2 includes the 4 months of August to November 1994. During period 2, the management strategy for the three ponds was to significantly increase flow through pond 1, inoculate pond 2 with microbial mats and silage and leave pond 3 alone. The objective in pond 1 was to determine if manganese would be released at greater than 2 mg l^{-1} through the effluent (the compliance level) at high flow rates. The objective in pond 2 was to increase the efficiency of manganese removal by direct inoculation.

Period 3 includes the 10 months from December 1994 to September 1995. Two objectives were addressed during this period. First, two small ponds were added to the pilot project. Pond 4, 17 m^2, was a new limestone substrate control to compensate for the loss of the original limestone substrate pond 2 control. Pond 5, 14 m^2, contained a soil substrate and was inoculated with microbial mat and silage. This was a control pond to measure the importance of limestone substrate. Flow rates through these

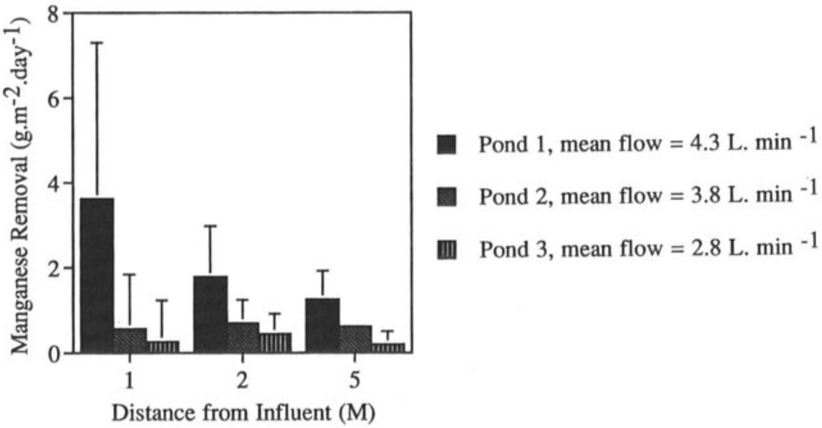

Fig. 19.3. Grams per square meter per day (g m^{-2} day^{-1}) removal of manganese in first 4 months of operation of the Tennessee Valley Authority (TVA) pilot project. The time period extended from November 1992 to February 1993

two new ponds were reduced to reflect their smaller size. Second, all management of the ponds was ended. This meant that after 1 December 1994, no additional inoculation or ensiling took place. Water quality and flow rates were monitored.

19.2.3 Results

During period 1, in the inoculated pond 1, a floating mat (1 to 2 cm thick), composed of microbial mat and a voluntary filamentous green alga, developed. A second microbial mat formed over the rock substrate at the pond bottom (Fig. 19.2). Pond 1 had the greatest manganese removal rate across its length (from influent pipe to 5-m distance). At 1 m, manganese removal was 3.61 g m^{-2} day^{-1}, diminishing to 1.25 g m^{-2} day^{-1} at 5 m (Fig. 19.3). Both control ponds were significantly less efficient. It is important to note that the mean flow rate was greatest in pond 1 (11% greater than pond 2 and 35% greater than pond 3).

During the course of operating the pilot project, microbial mats voluntarily established over the substrate of ponds 2 and 3, similar to the microbial mat substrate cover of pond 1 (Fig. 19.2). Repeated attempts to maintain a biologically nearly sterile rock substrate environment by chlorination of the ponds failed to eliminate the microbial mat cover. Therefore, both ponds 2 and 3 showed increased efficiency of manganese removal as the ponds aged.

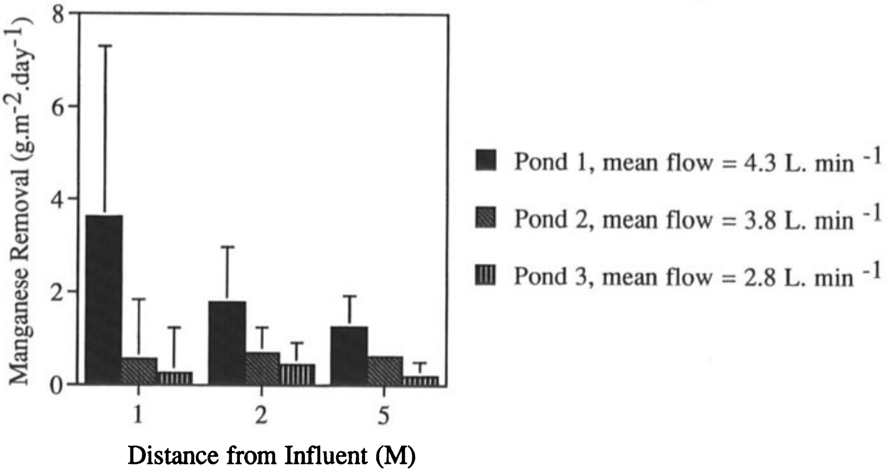

Fig. 19.4. Grams per square meter per day (g m^{-2} day^{-1}) removal of manganese after more renovation (heavy inoculation and ensiling to improve manganese removal efficiency) occurred in ponds 1 and 2. These data are the mean of 4 months from August to November 1994

During period 2, pond 2 operated as a second biological treatment pond. Average pond 1 flow rate was more than twice that of ponds 2 and 3 (Fig. 19.4) and after 2 years of continuous operation, manganese deposits had accumulated to high levels in the sediments of pond 1. The pond 1 high flow and accumulated manganese in the sediments likely resulted in an advancing front of peak manganese removal toward the effluent pipe. For example, manganese was removed at a low rate at 1 m (1.1 g m^{-2} day^{-1}), released at 2 m (−0.3 g m^{-2} day^{-1}), and then removed at a high rate (4.1 g m^{-2} day^{-1}) at 5 m. At flow rates of 6.8 and 5.9 l min^{-1}, respectively, ponds 2 and 3 showed the greatest manganese removal. The relative importance of the effect of the two factors high flow and manganese accumulation in sediments on manganese removal efficiency in pond 1 cannot be determined from this study.

During period 3, the two small control ponds 4 and 5 were operating. The only management included flow rate adjustment and occasional chlorination to maintain low levels of involuntary flora in ponds 3 and 4. The establishment of microbial mat and a green alga was maintained at a low level in pond 4 due to its newness. Pond 3 always had a microbial mat over the substrate.

The flow rates were similar in the first three ponds (Fig. 19.5). The data show that manganese removal in pond 1 is still best at 5-m distance from the influent pipe. Pond 2 manganese removal is best nearer the influent

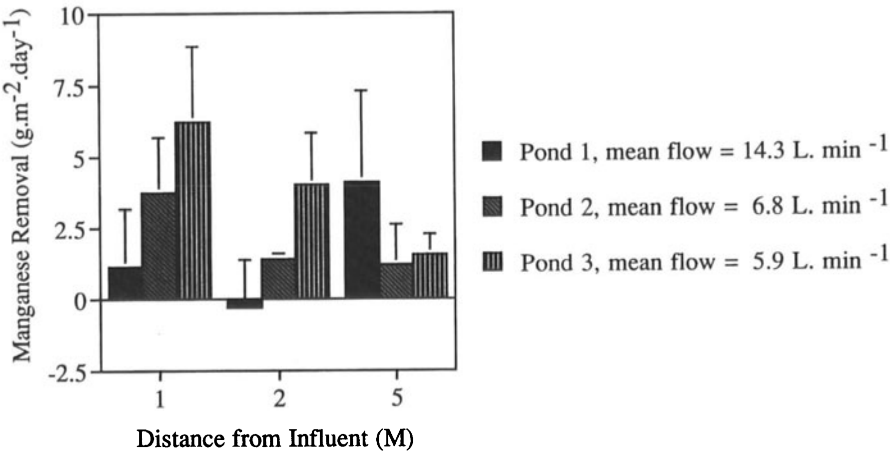

Fig. 19.5. Grams per square meter per day (g m^{-2} day^{-1}) removal of manganese after all active management was suspended (other than repeated attempts to rid pond 3 of microbial mats by chlorination; this was not successful). The intention was to observe ability of system to sustain itself. Pond 4 had a limestone substrate to compensate for loss of pond 2 as a limestone substrate control. Pond 5 had a soil substrate to compensate for the rock substrate in all other ponds. These data are the mean of 10 months from December 1994 to September 1995

pipe. This may likely be due to it being a biologically less mature pond (inoculation with microbial mat occurring in 1994 versus 1992 in pond 1). Superior removal of Mn from pond 2 versus pond 1 was significant ($p < 0.05$). Pond 3 microbial mat was not eliminated by chlorination and this may explain the similar rate (grams per meter squared per day) of manganese removal between this pond and ponds 1 and 2 ($p > 0.05$). Ponds 4 and 5 (limestone and soil substrate controls, respectively) did not appear to remove as much manganese as the first three ponds. Nevertheless, an analysis of variance revealed no significant difference between pond 1 and ponds 4 or 5 ($p > 0.05$). The difference between ponds 2 and 4 was significant ($p < 0.05$) and between 2 and 5 highly significant ($p < 0.01$). The difference between the original control ponds 3 and 4 was not significant ($p > 0.05$), but pond 3 performed significantly ($p < 0.05$) better than pond 5. Of the two newest ponds, the limestone substrate pond 4 removed a greater amount of Mn across its length compared with pond 5, but this difference was not statistically significantly ($p > 0.05$). Water quality parameters for the five ponds are shown in Table 19.2. The anoxic drain system raised pH to neutral before entering this pilot project system.

Table 19.2. Water quality parameters for the Tennessee Valley Authority (TVA) Fabius, Alabama, USA, pilot project, 1992–1995

Pond No.	Temperature, °C (range)	Dissolved oxygen, mg l^{-1} (range)	pH (range)	Oxidation-reduction potential, mV (range)	Conductivity, µΩ cm^{-1} (range)	Alkalinity, mg l^{-1} (range)	Manganese at influent, mg l^{-1} (range)	Manganese at final pond sampling point, mg l^{-1} (range)
1	17.2±8.0 (4.0–36)	6.6±1.7 (2.8–10.5)	7.2±0.4 (6.4–8.3)	425±69.8 (324–599)	559±128.1 (304–892)	146±59.4 (60–264)	5.0±1.6 (1.2–7.6)	1.2±1.6 (0.005–6.5)
2	17.1±8.0 (3.0–33)	7.1±1.8 (3.0–11.3)	7.3±0.4 (6.5–8.2)	432±75.6 (328–565)	542±122.4 (301–760)	148±60.0 (72–281)	4.5±2.2 (0.2–8.3)	1.3±2.0 (0.02–8.0)
3	16.9±8.3 (3.0–35.5)	7.2±2.0 (2.8–11.0)	7.2±0.3 (6.8–8.0)	425±67.0 (336–566)	526±106.2 (306–705)	139±63.6 (41–264)	5.0±2.0 (0.9–10.3)	2.0±2.0 (–0.007–7.4)
4	16.4±9.8 (3.5–35.0)	7.9±2.1 (5.0–11.0)	7.2±0.4 (6.4–7.6)	388±51.2 (331–488)	538±51.2 (493–618)	128±41.0 (67–190)	5.2±2.2 (0.07–7.9)	1.2±2.2 (0.006–6.9)
	17.1±10.2 (2.0–38)	8.0±2.2 (7.0–12.0)	7.1±0.3 (6.7–7.6)	396±70.5 (311–567)	319±92.2 (641–505)	116±52.5 (43–194)	5.8±1.5 (3.4–8.3)	3.3±3.6 (0.05–8.6)

A comparison of data from pond 1 results with the data shown in Figs. 19.3–19.5 reveals that as manganese fills the sediment zone of the pond, there is an advancing front of peak manganese removal. This would indicate that eventually an individual pond will be saturated with manganese, its presence will interfere with the biologically mediated mechanisms of further manganese removal, and, therefore, manganese concentrations at the effluent pipe will exceed levels permitted by the US Environmental Protection Agency. The pond will then need to be closed and this will be a permanent manganese deposit.

Microbial mat entraps photosynthetic oxygen, generating consistently elevated dissolved oxygen levels (average 7.3 mg l^{-1}). Unlike the control ponds, the microbial mat-containing ponds consistently maintained manganese effluent levels at less than 2 mg l^{-1}. The microbial mat promotes maintenance of high pH, therefore minimizing the possibility of remobilization of manganese.

During period 1, when the pilot project system was new, day/night and winter/summer manganese removal was essentially the same. At this time, control ponds showed manganese breakthrough (manganese at greater than 2 mg l^{-1}) during night-time sampling or when mine drainage flow exceeded 4.5 l min^{-1} (Phillips et al. 1994). Although there was some binding of manganese to the microbial mat, it was primarily deposited as a precipitate at the pond bottom. Samples analysed by x-ray diffraction of floating microbial mat showed that it contained manganous carbonate. In bottom deposits, major minerals were manganese carbonate and calcite. Minor minerals were quartz, gypsum and maghemite. Trace minerals were amorphous iron oxyhydroxides and/or hydroxides.

The problem remains of the validity of comparing results in high flow (10–15 l min^{-1}) versus medium (5–9 l min^{-1}) or slow (1–4 l min^{-1}) flow periods, as the kinetics of manganese removal may be very different at different flow rates. Additionally, pond 1 (containing the heaviest microbial mat) deposited the highest quantity of metal over the duration of the project. This was clearly indicated by the nearly total filling of the sediment troughs with metal. Other ponds showed minimal deposits in their troughs.

Manganese removal rates, calculated under various treatments during this pilot study, have generated the basic information necessary for recommending sizing and flow rates needed for full-scale treatment to compliance levels for manganese removal in coal mine drainage. Each pond creates a sink of precipitated manganese oxides and carbonates, and will eventually require closure.

19.3 Precious Metal Mine Drainage

19.3.1 Background

Microbial mat is being used to treat an abandoned gold and silver mine drainage located at approximately 3100-m elevation in central Colorado, USA. The drainage, of neutral pH, contains manganese and zinc at up to 18 mg l^{-1}. These were the target contaminants for remediation. Additionally, Ag, Cd, Cr, Cu, Pb, Ni and Fe, present in micrograms per liter levels, were also concentrated by microbial mats.

19.3.2 Methods

Microbial mat was applied in two ways: (1) it was previously cultured on coconut mesh, dehydrated and shipped to the site; or (2) dry coconut mesh was applied to the pond surface and microbial mat inocula plus silage were added in the field. Under high altitude conditions, the microbial mat must be shaded to avoid ultraviolet radiation damage.

During summer 1994, microbial mat was applied to three 4×4-m treatment ponds, as well as the larger and deeper retention pond (Fig. 19.6). The pond substrate was similar to the Alabama coal mine drainage ponds. By February 1995, during the harsh winter weather, the treatment ponds had produced a thick green biomass due to the microbial mat inocula, as well as to an invasion of a filamentous green alga.

19.3.3 Results

Historical metal concentrations in the mine drainage are shown in Table 19.3. By contrast, after 2 months (between July and September 1994), the microbial mat biomass had accumulated these same metals to very high mean milligram per kilogram concentrations. In August 1995, manganese and zinc levels entering the three pilot project ponds from the retention pond were significantly reduced in the two biological treatment ponds compared with the control pond which was not inoculated (Table 19.4). In pond 1 (treatment pond), there was a 52% decrease in both manganese and zinc concentrations and in pond 2 (treatment pond), there was a 64% decrease in manganese and a 63% decrease in

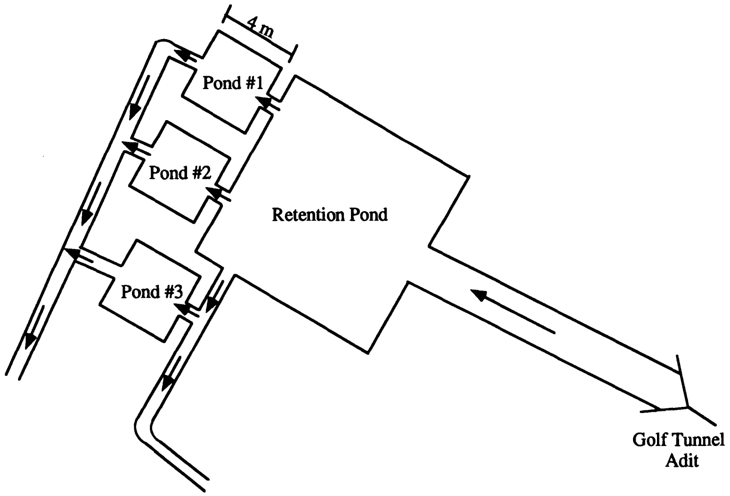

Fig. 19.6. Schematic diagram of the Golf Tunnel mine drainage remediation pilot project, Colorado, USA. The large pond is a retention pond for collecting mine drainage. The three small ponds: *pond 1* with microbial mat; *pond 2* with microbial mat; *pond 3* control, no microbial mat

Table 19.3. Historical Golf Tunnel, Colorado, USA, metal concentrations in mine drainage, and those same metals concentrated by microbial mat

Metal concentration in drainage ($mg\,l^{-1}$):								
Mn	Zn	Ag	Cd	Cu	Cr	Ni	Pb	Fe
3–34	6–43	<0.002	<0.1	<0.3	<0.01	<0.04	<0.04	<3
Metal concentration in microbial mat ($mg\,kg^{-1}$):								
Mn	Zn	Ag	Cd	Cu	Cr	Ni	Pb	Fe
12,050	30,300	14	122	2566	106	32	27,275	118,800

zinc concentrations. In the control pond 3, there was a 4 and 3% decrease in manganese and zinc concentrations, respectively. Table 19.5 summarizes the water quality parameters for the three ponds.

19.4 Mechanisms of Metal Removal

Metals are known to complex with a wide range of organic material, including microorganisms and their organic releases. Dunbabin and Bowmer (1992) identified four dominant binding processes that incorporate metals into organic materials: (1) cation exchange; (2) adsorption; (3)

Table 19.4. Manganese and zinc concentrations in the Golf Tunnel drainage, Colorado, USA, August 1995. Retention pond inlet is equivalent to mine tunnel drainage. Retention pond outlet is the drainage from the major holding pond. Ponds 1 and 2 are those inoculated with microbial mats. Pond 3 is a control pond. All ponds have a limestone substrate and measure 16 m^2. Water temperature was 5 °C

Station		Mn concentration ($mg\ l^{-1}$)	Zn concentration ($mg\ l^{-1}$)
Retention pond	Inlet	13.9	15.5
	Outlet	23.0	25.6
Pond 1 (treatment)	Inlet	25.6	28.6
	Outlet	12.2	13.7
Pond 2 (treatment)	Inlet	28.8	31.8
	Outlet	10.8	11.8
Pond 3 (control)	Inlet	31.8	34.8
	Outlet	30.4	33.6

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, microbial mat promotes high pH levels to prevent remobilization of metals. Additionally, high dissolved oxygen and redox levels (mediated by the biological component) favour the chemical precipitation of metal oxides and hydroxides. These oxides and hydroxides, in turn, act as reservoirs for additional metal deposit. Other metal removal mechanisms available with microbial mat include flocculation, cell sorption and autocatalysis. For example, laboratory research has shown that specific bioflocculants are released by microbial mat in response to the presence of a positively charged metal ion (Bender and Phillips 1994). These materials carry 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 bioflocculant. This provides initial protection to the microbial community contacting the toxic metal. Another example would be that at the community level, the anaerobic zones harbour sulfur-reducing bacteria, which generate hydrogen sulfide in the anoxic zones. Thus, sulfide is available for metal precipitation in the interstitial spaces of the mat. A third example is the mechanism of metal transport through the water. Scanning electron microscopy/microanalysis research, correlated with chemotaxis studies of the motile bacteria in microbial mat, suggests that these microbes become bonded to the metals and migrate to the mat by responding chemotactically to the blue-green algae and silage (Bender et al. 1989).

Table 19.5. Water quality parameters for the Bureau of Mines, Colorado, USA, pilot project, 1994–1995

Pond No.		Temperature, °C (range)	Dissolved oxygen, mg l^{-1} (range)	pH (range)	Oxidation-reduction potential, mV (range)	Alkalinity, mg l^{-1} (range)	Manganese mg l^{-1} (range)	Zinc, mg l^{-1} (range)
1	Inlet	3.0±2.0 (0.9–5.0)	10.4±2.9 (8.4–13.8)	7.3±0.4 (6.7–7.9)	359±156.6 (235–584)	48±4.6 (40–51)	12.1±7.9 (4.0–25.6)	13.2±8.5 (5.9–28.6)
	Outlet	3.5±2.7 (0.6–6.5)	9.8±2.4 (8.1–11.5)	7.3±0.5 (6.8–7.9)	394±168.4 (235–582)	47±4.8 (40–50)	10.3±4.3 (6.1–16.1)	10.9±4.2 (6.4–16.5)
2	Inlet	3.0±2.0 (1.0–5.1)	9.0±0.2 (8.8–9.1)	7.4±0.3 (7.0–7.9)	359±168.9 (215–592)	52±8.3 (40–60)	11.4±8.9 (3.8–28.8)	12.6±9.3 (5.6–31.8)
	Outlet	3.6±3.6 (0.0–9.0)	8.6±2.2 (7.2–10.3)	7.4±0.3 (7.1–7.9)	359±156.5 (235–586)	51±4.1 (45–56)	8.4±4.2 (3.3–13.4)	9.3±3.1 (5.3–13.3)
3	Inlet	3.0±1.9 (1.1–5.0)	9.0±0.4 (8.7–9.2)	7.4±0.4 (7.1–8.1)	353±161.6 (220–590)	45±8.1 (40–58)	12.9±10.3 (3.9–31.8)	14.0±10.9 (5.8–34.8)
	Outlet	3.0±2.3 (0.7–5.5)	8.0±1.3 (7.0–8.9)	7.4±0.4 (7.0–7.9)	353±155.2 (235–590)	45±7.4 (40–56)	12.6±9.7 (4.1–30.4)	14.1±10.2 (6.8–33.6)

19.5 Microbial Mat as an Ideal Bioremediation System

Microbial mat has broad potential in the treatment of mine wastes. Because microbial mat is photosynthetic and nitrogen-fixing, it is generally self-maintained and, after establishment, does not require outside supplies of nutrients. A central issue in microbial biotechnology is retention of the integrity of the biological system by maintaining the optimum populations of inoculated microbes. The mixed microbial consortium of microbial mat possesses a natural system of checks and balances and it should be less difficult to achieve this integrity in a complex microbial mat system than it is in technologies employing single species. Another distinct advantage of a mixed microbial remediation system is that specific detoxification mechanisms unique to constituent strains of microbial mat are accessible to all members of the consortium. Thus, a broader variety of cellular releases (enzymes, bioflocculants) are available within a microbial mat consortium compared with that offered by a single microorganism treatment system. Challenging the complex system of microorganisms in microbial mat will likely result in population shifts favouring the microorganisms most efficient in that particular remediation. These properties of self-maintenance, resiliency and efficiency under fluctuating environmental conditions may resolve a number of maintenance problems often associated with bioremediation technologies. These properties suggest that microbial mats have excellent potential for mine drainage remediation.

Acknowledgements. Support of the United States Environmental Protection Agency Assistance ID CR-818689, the Tennessee Valley Authority Contract TV-89721V and the United States Department of the Interior Bureau of Mines Cooperative Agreement 1432-C0240006 is gratefully acknowledged. J. Neil, United States Geological Survey, conducted the X-ray diffraction analysis of metal precipitates.

References

Bender J, Phillips P (1994) Implementation of microbial mats for bioremediation. In: JL Means, Hinchee RE (eds) Emerging technology for bioremediation of metals. Lewis, Boca Raton, pp 85–98

Bender J, Phillips P (1995) Biotreatment of mine drainage. Mining Environ Manag (UK) 3(3):25–27

Bender J, Graves B, Wright W (1995) Evaluation of the removal of BTEX from groundwater using a microbial mat. Air and Waste Management Association, 88th Annu Meeting and Exhibition, San Antonio, Texas, 18–23 June 1995, 15 pp

Bender JA, Archibold ER, Ibeanusi V, Gould JP (1989) Lead removal from contaminated water by a mixed microbial ecosystem. Water Sci Technol 21:1661–1664
Brodie G (1993) Aerobic constructed wetlands and anoxic limestone drains to treat acid drainage. Constructed wetlands workshop for electric power utilities, Tennessee Valley Authority, Chattanooga, Tennessee, 24–26 Aug 1993, 10 pp
Caumette P (1989) Ecology and general physiology of anoxygenic phototrophic bacteria in benthic environments. In: Cohen Y, Rosenberg E (eds) Microbial mats, physiological ecology of benthic microbial communities. American Society for Microbiology, Washington, DC, pp 283–304
Dunbabin J S, Bowmer KH (1992) Potential use of constructed wetlands for treatment of industrial wastewaters containing metals. Sci Total Environ 111:151–168
Goodroad L, Bender J, Phillips P, Gould J, Saha G, Rodríguez-Eaton S, Vatcharapijarn Y, Lee R, Word J (1994) Potential for bioremediation using constructed mixed microbial mats. Hazardous Materials Control Resources Institute, Washington, DC, 29 Nov–1 Dec 1994
Goodroad L, Bender J, Phillips P, Gould J, Hater G, Burrow B (1995) Use of constructed mixed microbial mats for landfill leachate treatment. 18th Int Madison Waste Conf, Department of Engineering Professional Development, University of Wisconsin, Madison, 20–21 Sept 1995, 13 p
Phillips P, Bender J, Simms R, Rodríguez-Eaton S (1994) Use of microbial mat and green algae for manganese and iron removal from coal mine drainage. 1994 Int Land reclamation and mine drainage Conf and the 3rd Int Conf on Abatement of acidic drainage, Pittsburgh, Pennsylvania, 24–29 April 1994, vol 1, pp 99–108
Phillips P, Bender J, Simms R, Rodríguez-Eaton S (1995) Manganese removal from acid coal mine drainage by a pond containing green algae and microbial mat. Water Sci Technol 31:161–170
Shilo M (1989) The unique characteristics of benthic cyanobacteria. In: Cohen Y, Rosenberg E (eds) Microbial mats, physiological ecology of benthic microbial communities. American Society for Microbiology, Washington, DC, pp 207–213
Stal LJ, Heike H, Bekker S, Villbrandt M, Krumbein WE (1989) Aerobic-anaerobic metabolism in the cyanobacterium *Oscillatoria limosa*. In: Cohen Y, Rosenberg E (eds) Microbial mats: physiological ecology of benthic microbial communities. American Society for Microbiology, Washington, DC, pp 255–276