



Uptake and transformation of metals and metalloids by microbial mats and their use in bioremediation

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SUMMARY

Constructed microbial mats, used for studies on the removal and transformation of metals and metalloids, are made by combining cyanobacteria inoculum with a sediment inoculum from a metal-contaminated site. These mats are a heterotrophic and autotrophic community dominated by cyanobacteria and held together by slimy secretions produced by various microbial groups. When contaminated water containing high concentrations of metals is passed over microbial mats immobilized on glass wool, there is rapid removal of the metals from the water. The mats are tolerant of high concentrations of toxic metals and metalloids, such as cadmium, lead, chromium, selenium and arsenic (up to 350 mg L⁻¹). This tolerance may be due to a number of mechanisms at the molecular, cellular and community levels. Management of toxic metals by the mats is related to deposition of metal compounds outside the cell surfaces as well as chemical modification of the aqueous environment surrounding the mats. The location of metal deposition is determined by factors such as redox gradients, cell surface micro-environments and secretion of extra-cellular biofloculents. Metal-binding flocculents (polyanionic polysaccharides) are produced in large quantities by the cyanobacterial component of the mat. Steep gradients of redox and oxygen exist from the surface through the laminated strata of microbes. These are produced by photosynthetic oxygen production at the surface and heterotrophic consumption in the deeper regions. Additionally, sulfur-reducing bacteria colonize the lower strata, removing and utilizing the reducing H₂S, rather than water, for photosynthesis. Thus, depending on the chemical character of the microzone of the mat, the sequestered metals or metalloids can be oxidized, reduced and precipitated as sulfides or oxides. For example precipitates of red amorphous elemental selenium were identified in mats exposed to selenate (Se-VI) and insoluble precipitates of manganese, chromium, cadmium, cobalt, and lead were found in mats exposed to soluble salts of these metals. Constructed microbial mats offer several advantages for use in the bioremediation of metal-contaminated sites. These include low cost, durability, ability to function in both fresh and salt water, tolerance to high concentrations of metals and metalloids and the unique capacity of mats to form associations with new microbial species. Thus one or several desired microbial species might be integrated into mats in order to design the community for specific bioremediation applications.

INTRODUCTION

This review includes the removal and transformation of metals and metalloids via metal precipitation, biofloculant-binding, metal removal by immobilized mat films, and effect of environmental parameters and microbial species on metal management. One pilot study demonstrating the application of mats in field-site remediation is reviewed.

Dense and layered populations of microbes, referred to as microbial mats, are common on aquatic sediments. These mats are a heterotrophic and autotrophic community dominated by cyanobacteria (blue-green algae). They are self-organized laminated structures annealed tightly together by slimy secretions from various microbial components (Fig. 1). The surface slime of the mats effectively immobilizes the ecosystem to a variety of substrates, thereby stabilizing the internal microbial structure. Below the cyanobacterial photozone is a multilayer community of purple bacteria (anoxygenic photosynthetic bacteria) and a variety of heterotrophs (Fig. 2). Steep gradients of redox and oxygen from the surface through the

laminated strata of microbes are due to photosynthetic oxygen production at the surface and heterotrophic consumption in the deeper regions [29,30,31]. The autotrophic and nitrogen fixing components provide nutrients for the mats. Thus, mats are essentially self sufficient, solar-driven ecosystems with few growth requirements [26].

Microbial mats likely play an important role in adsorbing, filtering and transforming various nutrients and contaminants, such as metals, which enter wetlands. Since mats have both oxic and anoxic zones and therefore can both oxidize and reduce metals, it seems probable that mats play an important role in the geochemical cycles of metals in wetlands.

The unique characteristics of mats described above and the availability of community mechanisms suggest an important potential for bioremediation of metal-contaminated water. Recent studies have used constructed microbial mats, composed of sediment and cyanobacteria inoculum to remove metals from contaminated waters [4,5]. The slimy secretions of these constructed microbial mats allow attachment of the mat to glass wool or nylon screens, so that contaminated water passed over mats will have maximum exposure to the biological surface areas.

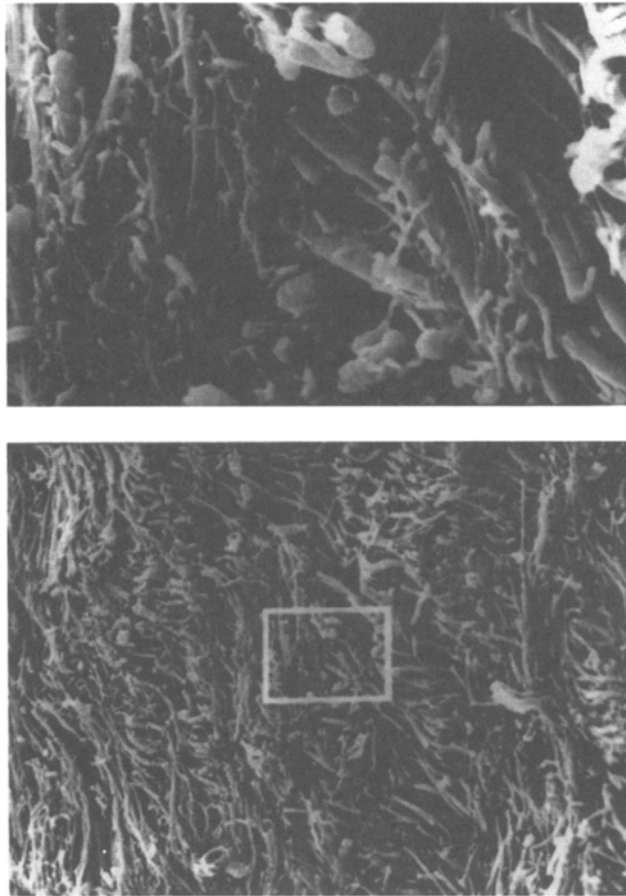


Fig. 1. Scanning electron micrograph of surface of constructed microbial mat. Note *Oscillatoria* sp. cells in the insert and the slime secretions.

Removal of metals and metalloids from contaminated water by mats

The metals and metalloids which are removed from the water by microbial mats include ions of lead, cadmium, zinc, cobalt, chromium, iron, manganese, selenium and arsenic [3–7,27,28]. The uptake can be very rapid as demonstrated in Fig. 3 showing that within a few hours most of the chromium ions, Cr(VI), and uranium ions, U(VI), were removed from laboratory-contaminated groundwater samples by mats.

A budget, prepared for many of these metal uptake studies, demonstrated that essentially all metals were removed from water and deposited in the mats and associated organic materials [4,5]. In one study the initial concentrations of zinc and manganese were 16 and 15 mg L⁻¹, respectively. When this contaminated water was passed over microbial mats attached to glass wool at a flow rate of 3.3 ml min⁻¹, mats removed 98–99% of the zinc and manganese from the water [5]. When mats were floated in tanks containing metal-contaminated water, removal required several days [3; Fig. 4].

Production of metal-binding biofloculants

The secretion of microbial biofloculants has been documented by Bar-Or and Shilo [1] and Fattom and Shilo [12]. Exposure of microbial mats to manganese and other metals

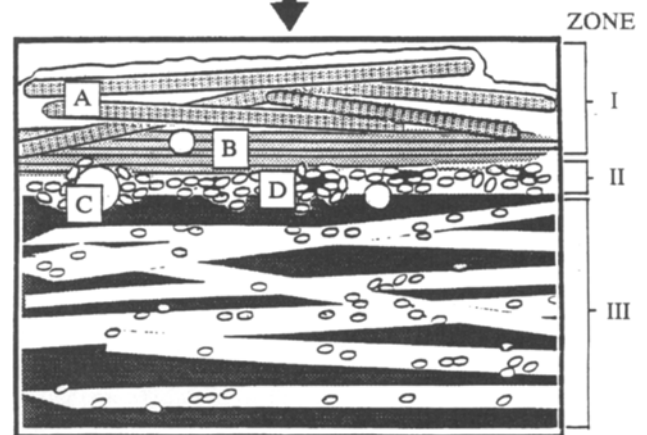
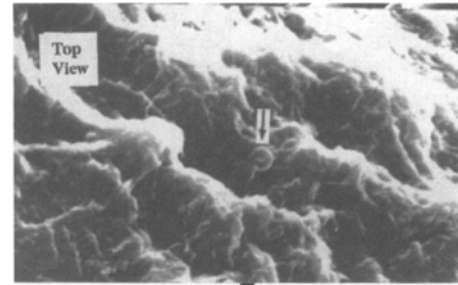


Fig. 2. Schematic constructed microbial mat showing cyanobacteria attached to ensiled grass clippings. A scanning electron micrograph shows the slime on the surface. Approximate depth for zones 1, 2, and 3 are 2 mm, 1 mm, and 3.5 cm, respectively. I = Oxic zone with cyanobacteria (A) and ensiled grass (B). II = Anoxic zone with entrapped gas (C and arrow above), purple autotrophic bacteria and mixed heterotrophs such as *Pseudomonas* and sulfur-reducing bacteria (D). III = Glass wool immobilization medium with entrapped organic releases from the mat.

resulted in the production and secretion of metal-binding extracellular biofloculants from the cyanobacterial component (*Oscillatoria* sp.) [7]. The concentrations of biofloculants, which are negatively-charged polysaccharides, increased during the process of manganese removal by the mat. Analysis of these polysaccharides showed a heterogenous population of polysaccharides with respect to charge density and molecular size. The polysaccharides with the highest flocculating activity were polyanionic and had molecular weights over 200 000. The presence of negatively-charged galacturonic acid and glucuronic acids in these polysaccharides may account for the metal-binding properties of the mats. The strong binding of several metals, including copper, manganese, iron, and nickel, by bacterial exopolysaccharides is thought to be due to the presence of negatively-charged function groups, such as uronic acids, on the exopolymer [13].

Transformation and precipitation of metals and metalloids by microbial mats

The tolerance of mats to high metal concentrations (Table 1) may be due to their ability to precipitate insoluble metal salts outside the mat cells as either sulfides, oxides or hydroxides. Thick amorphous metal precipitates have been found on

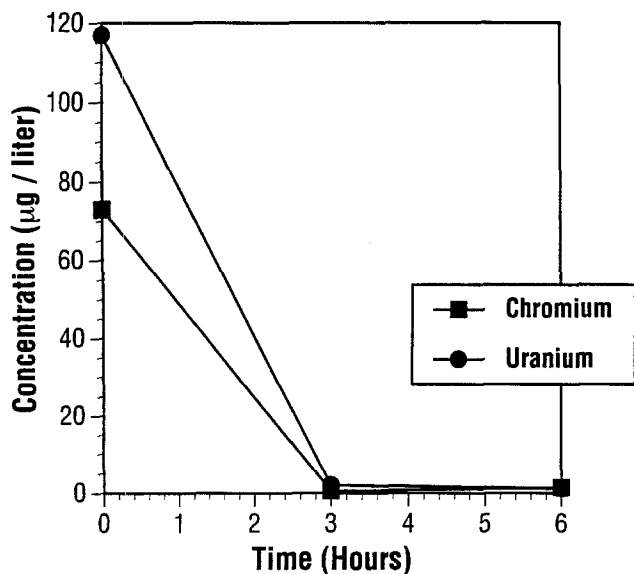


Fig. 3. Groundwater was spiked with uranium nitrate (U^{238} - VI) and potassium dichromate (Cr-VI) followed by passage of the spiked water over constructed microbial mats (J. Bender and Y. Vacharapijarn, unpublished data). Metal concentrations in the water at the various times were determined by an inductively-coupled plasma-mass spectrometer.

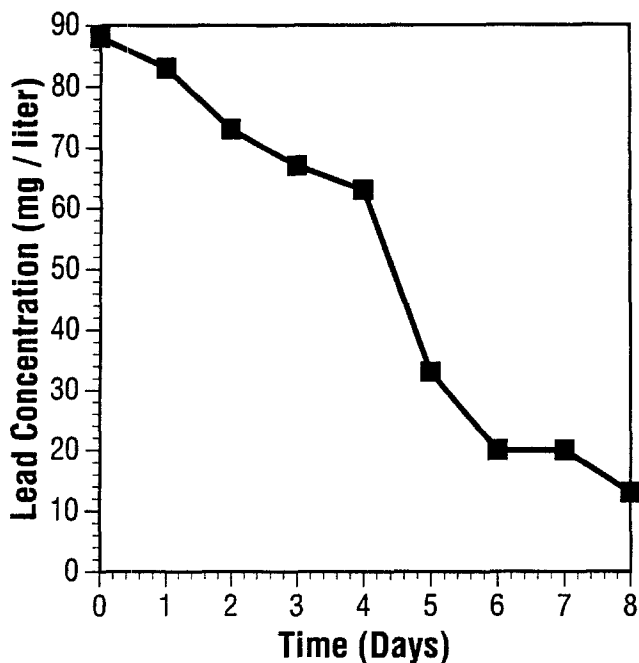


Fig. 4. Lead nitrate was added to 3 L of water in a clear plastic tank to give an initial lead concentration of 90 mg L^{-1} . Constructed microbial mats were floated on top of the tank and analysis of water for lead at the different times was by atomic absorption. Data taken from [3].

the surfaces of mats exposed to lead, zinc, and cadmium ions [3,5; Fig. 5]. The precipitation of these metals in mats is likely to be controlled by a complex of mechanisms including the zonation of oxygen and redox, pH management (to levels

TABLE 1

Tolerance of constructed microbial mats to metals and metalloids¹

Metal or metalloid ion	Tolerance ² (mg L^{-1})
Arsenic	100
Lead	350
Mixture of lead, cadmium, zinc, manganese, chromium, cadmium and selenium ³	700

¹Taken from Bender (unpublished data).

²Tolerance was the concentration above which there was no growth and blanching occurred.

³The concentration of each metal was 100 mg L^{-1} and total metal ion was 700 mg L^{-1}

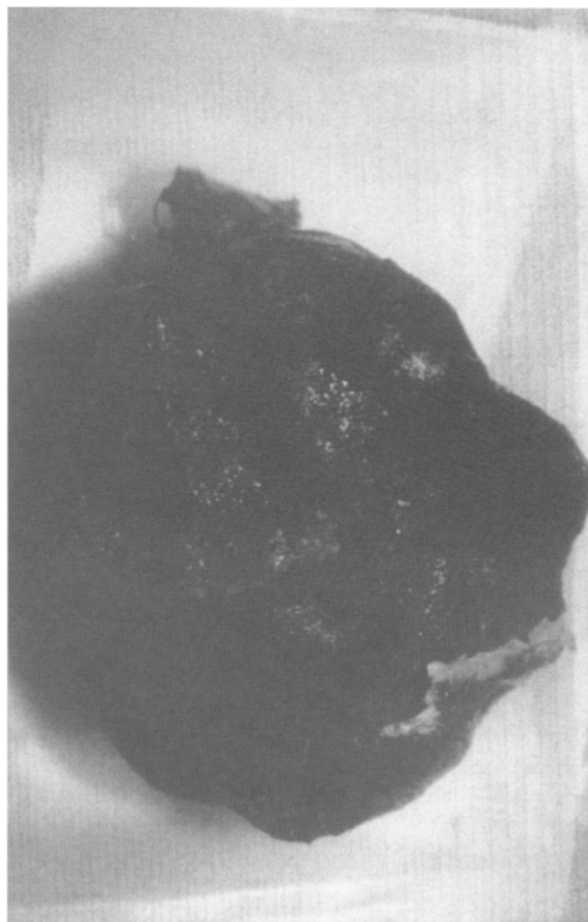


Fig. 5. This photograph shows a constructed microbial mat after exposure to a cadmium concentration of 20 mg L^{-1} . The black surfaces on the mat are precipitates of a cadmium salt. Notice the crack on the upper surface of the metal precipitate which shows that the metal deposit is on the outer mat surface, approximately 1 mm thick.

greater than 7), and the availability of anionic pools such as sulfide and hydroxide.

In certain cases, several mat mechanisms, at the cellular and community levels, become coupled in a complex precipi-

tation process. For example, reductive transformation of Cr(VI) to Cr(III) precedes its precipitation as Cr(OH)₃ at pH levels generated by the mat (Ibeanusi, Archibold, Gould and Bender, unpublished manuscript). Therefore, the presence of reducing mechanisms, together with the management of pH and subsequent availability of hydroxide is important to this precipitation. The mechanism of the initial Cr(VI) reduction by the mat is not known. Chromate reductase has been identified in other microbial groups which demonstrate both aerobic and anaerobic reduction of Cr(VI) [9,17,18]. However, the presence of chromate reductase has not been investigated in the microbial members that constitute the mat.

The reduction of selenate to elemental selenium, occurring in anoxic lakes and wetlands [11,21], may be mediated by a number of microbial species. In bacterial systems selenate can be reduced to selenite. Selenate in the presence of glutathione can form bis(glutathione)-Se(II) which can be reduced by glutathione reductase to elemental selenium [8,15,16,25; Fig. 6]. In the case of microbial mats this reduction of selenate (40 mg L⁻¹ NaSeO₄) is probably mediated at the community level. Gaseous dimethyl selenium, produced in the lower anoxic regions of the mat, probably rises to areas of entrapped photosynthetic oxygen on the mat surface. In these regions, elemental selenium is generated and collects as red deposits on the mat surface [4].

Another mechanism of metal transformation involves the generation of hydrogen gas by certain species within a microbial mat. It has been shown that the purple nonsulfur bacteria, *Rhodobacter sphaeroides*, grown photoheterotrophically [23], reduces tellurite, selenate and other oxyanions to their elemental forms. This process is accompanied by the production of hydrogen gas. Approximately 11.5 mmol of hydrogen were evolved per mmol of tellurium deposited. Tellurite has also been shown to be reduced to elemental tellurium by *Pseudomonas maltophilia* [8]. Since purple bacteria and *Pseudomonas* sp. are found in microbial mats, ongoing research is currently exploring metal-reduction mechanisms that may be mediated by these microbial mat components.

In some recent studies we have demonstrated that mats growing in the light take up tributyltin and transform it to hydroxybutyltins and dibutyltin (Lee, unpublished data). In the dark tributyltin metabolism was significantly lower and dibutyltin was the only identified metabolite. Previous work has shown that tributyltin was metabolized by microalgae in estu-

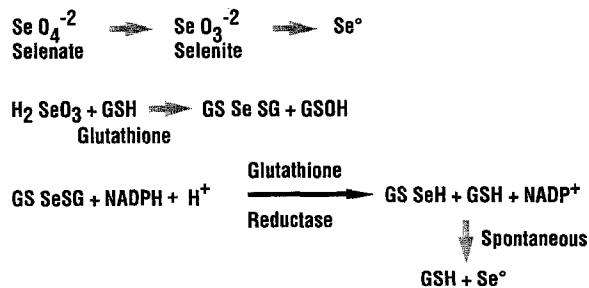


Fig. 6. The pathway used by bacteria that reduce selenate to elemental selenium. Similar pathways are presumably used by microbial mats to reduce selenate to elemental selenium.

TABLE 2

Variation in pH and zeta potential on the different parts of the surface of an individual mat¹

Sample	pH	Zeta potential (mv)
1	6.57	-69.2
2	6.84	-53.0
3	6.75	-12

¹Taken from [5].

arine waters [19]. Presumably, algal dioxygenase systems carry out the hydroxylation of organotins. For example, the freshwater chlorophyte, *A. falcatus*, metabolized trimethyllead to dimethyllead and lead (II) compounds [32]. The presence of such algal dioxygenase systems in microbial mats makes it likely that other organometallics, e.g. organoleads, would be metabolized by microbial mats.

Effect of environmental parameters and different microbial groups on metal removal and transformations by mats

Steep gradients of oxygen and redox from the surface through the laminated strata of microbes are characteristic features of microbial mats [29,30,31]. Additionally, horizontal planes of the mat are highly heterogeneous. For example Table 2 shows the variation of pH and zeta potential (a measure of surface charge density) within the same mat.

Near the surface are oxygen-producing cyanobacteria resulting in high oxygen concentrations in this zone. Below this oxygenated zone are colorless sulfur bacteria, purple sulfur bacteria and sulfate-reducing bacteria [31]. The sulfate-reducing bacteria are thought to provide sulfide as an electron donor for anoxygenic photosynthesis by purple sulfur bacteria. The activity of aerobic heterotrophic bacteria associated with mats can lead to low oxygen concentrations on the surface during dark conditions (Table 3).

Within microzones of the mats, metals can be reduced, oxidized and precipitated depending on the chemical environment, which is controlled by the activities of the different microbial groups. For example, oxidized manganese (Mn-IV) forms precipitates, such as MnO₂, at high E_h and pH, while in low pH and E_h microzones manganese tends toward soluble manganese (II) [24].

TABLE 3

Oxygen concentrations and pH of mat surfaces under dark and light conditions¹

Condition	pH	Oxygen (mg L ⁻¹)
Dark	5.9-6.2	6.0-7.8
Light	7.8-7.9	24.1-25.1

¹Taken from [6].

Use of constructed microbial mats in the bioremediation of metal-contaminated sites

Mats tolerant of high metal concentrations have been isolated from contaminated sites. It seems likely that heterotrophic bacteria in these metal-tolerant mats play a role in transforming and precipitating metals. Certain strains of *Pseudomonas*, species of which are common in microbial mats, have been shown to transform a number of toxic metal ions, including mercury, chromium, selenium, lead, gold, cadmium, telluride and silver [8].

For bioremediation work, constructed microbial mats take advantage of the role natural mats play at the sites in filtering and transforming metals and metalloids. Microbial mats offer several advantages for bioremediation. These include low cost, durability, ability to function in both fresh and salt water, tolerance to high concentrations of metals and metalloids, and ability to incorporate microbial strains able to improve the uptake and transforming ability of the mats.

Field pond treatment of acid coal mine drainage by microbial mats demonstrated manganese removal of $2.6 \text{ g m}^{-2} \text{ day}^{-1}$ [2,27]. This was five times the removal rate in standard constructed wetlands treatment. Drainage containing $4\text{--}9 \text{ mg L}^{-1}$ Mn, passed under floating mats, decreased to 0.05 mg L^{-1} . A crystalline manganese deposit collected at the bottom of the pond (water characteristics: pH = 7–8, alkalinity = 167 mg L^{-1} , conductivity = $648 \mu\text{ohm cm}^{-1}$, ORP = 413 Mv). Preliminary data from X-ray analysis shows the crystals to be manganese calcite [27; J. Neal, personal communication).

Figure 3 shows that chromium and uranium, spiked in actual groundwater samples, were rapidly removed by the mats. Additionally, this experiment demonstrated the ability of mats to sequester metals presented at microgram levels. Brierley and Brierley have discussed the removal of metals from uranium mine wastewater by algae [10]. Lovely et al. found a dissimilatory Fe(III)-reducing microorganism which reduced soluble uranium (VI) to insoluble uranium (IV) and suggested that activity of such bacteria could explain the deposition of uranium which takes place in aquatic sediments [20]. We would speculate that microbial mats could similarly carry out reduction of uranium (VI).

By incorporating microbes cultured from contaminated sites into mats, it is possible to construct mats for specific remediation tasks. For example, bacteria strains able to degrade trinitrotoluene (TNT) were integrated into a mat and this mat was able to rapidly degrade TNT [22]. A *Pseudomonas* strain isolated from a toxic waste site was able to transform and precipitate a variety of toxic metal cations and oxyanions [8]. The suggestion was made by the authors that this *Pseudomonas* strain might be used to remove toxic metal wastes from contaminated sites. This *Pseudomonas* strain might be integrated into the mat and, thereby, protected, nutritionally supported and stabilized at the site of contamination by the mat system. The presence of the *Pseudomonas* strain may then amplify the metal removal processes.

A final role of mats in bioremediation could be the use of processed mats or their biofloculents in filters, assuming the metal-binding properties of the mat remain after processing.

An adsorptive filter containing immobilized, processed algae has been used to remove metals from wastewater [14].

Thus, we believe that sufficient studies have been carried out to suggest that constructed microbial mats can be used to remove metals and metalloids efficiently and cost effectively from contaminated waters. Further engineering work will be necessary to design equipment that will provide a large surface mat area and will allow rapid flow of contaminated water over microbial mats.

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