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Biogeochemical Activities of Microorganisms in Mineral Transformations: Consequences for Metal and Nutrient Mobility

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Abstract Bacteria and fungi are fundamental biotic components of natural biogeochemical cycles for metals and metalloids, and play important roles in dissolution, precipitation, oxidation and reduction processes. Some processes catalyzed by microorganisms also have important applications in environmental biotechnology in the areas of ore leaching and bioremediation.

Key words: Bacteria, fungi, metals, metal mobilization, metal immobilization, environmental biotechnology

Fungi and bacteria from all major taxonomic groups have been found in metal-rich habitats, such as mineral ores, acidic soils, or polluted environments. Survival may depend on a number of biochemical, structural, and physiological properties that modify metal bioavailability and toxicity. Microorganisms have essential requirements for metals such as K, Na, Mg, Ca, Mn, Fe, Cu, Zn, Co, and Ni, while some metals, *e.g.* Rb, Cs, Al, Cd, Ag, Au, Hg, and Pb, and metalloids, *e.g.* As and Te, have no apparent essential function [15]. However, all metals and metalloids can be toxic in excess, which has led organisms to develop diverse mechanisms of resistance [32, 33]. Both genetically-coded mechanisms of resistance and nonspecific mechanisms, such as oxidation or reduction of the metals to produce less toxic chemical species, or sorption and precipitation on cell surfaces, can protect cells [15].

Microbial Roles in Alteration of Metal Speciation

The ability of microorganisms to affect metal speciation stems from two opposing phenomena: mobilization and immobilization, which influence the balance of metal species between soluble and insoluble phases (Table 1). Mobilization of metals can be achieved by, *e.g.* protonation, chelation, and chemical transformation. Metal immobilization can occur by precipitation or crystallization of insoluble organic or inorganic compounds, or by sorption, uptake, and intracellular sequestration. In addition, redox reactions can mobilize or immobilize metals depending on the metal species involved. The extent to which microorganisms can affect metal contaminants is dependent on the identity and chemical form of the metal and the physical and chemical nature of the contaminated site or substance. In general terms, microbial processes which solubilize metals increase bioavailability and toxicity, whereas those that immobilize them reduce bioavailability. In bioremediation, solubilization provides a route for removal from solid matrices such as soils, sediments, dumps, and industrial wastes [4, 7]. Alternatively, immobilization processes may enable metals to be transformed *in situ* into insoluble and chemically inert forms and are also particularly applicable to removing metals from mobile aqueous phases [13, 14, 16, 18, 25, 26].

Table 1. Main microbial metal transformations (adapted from Ref. 15).

$\text{Metal}_{\text{soluble}} \longleftarrow$	$\longrightarrow \text{Metal}_{\text{insoluble}}$
Chemolithotrophic leaching	Biosorption
Chemoheterotrophic leaching	Organic and inorganic precipitation
Complexation and chelation, siderophores, biomolecules	Metal-binding peptides, proteins, polysaccharides, derived products, biomolecules
Methylation	Transport and intracellular localization
Reductive mobilization	Direct and indirect reductive immobilization

METAL MOBILIZATION

Fungi and bacteria can facilitate the movement of metals into soluble forms via the dissolution of metal compounds including oxides, phosphates, sulfides, and more complex

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mineral ores, or by desorption from exchange sites on clay minerals or organic matter. A number of mechanisms may be involved in metal mobilization.

Acidification

Fungi and bacteria can acidify their environment by proton efflux via plasma membrane H^+-ATPases, maintenance of charge balance, or as a result of respiratory carbon dioxide accumulation. Acidification can lead to metal release via a number of obvious routes; *e.g.* competition between protons and the metal (in a metal-anion complex) leads to protonation of the anion, and results in the release of free metal cations. Organic acids can supply both protons and metal complexing anions (see below) [12, 19].

Heterotrophic (Chemoorganotrophic) Leaching

Heterotrophic metabolism can also lead to leaching as a result of the efflux of protons, organic acids, and siderophores. Citrate and oxalate anions can form stable complexes with a large number of metals. Many metal citrates are highly mobile and not readily degraded and the presence of citric acid in soil may enhance metal solubility [10]. Oxalic acid can also act as a leaching agent for those metals that form soluble oxalate complexes, including Al and Fe [37]. Organic acid production is an important agent of mineral deterioration, playing a role in both biogenic chemical weathering and soil formation [12]. Heterotrophic solubilization can have consequences for other remedial treatments for contaminated soils. Pyromorphite ($Pb_5(PO_4)_3Cl$) is a stable lead mineral and can form in urban and industrially-contaminated soils. Such insolubility reduces lead bioavailability and the formation of pyromorphite has been suggested as a remediation technique for lead-contaminated land, if necessary by means of phosphate addition. However, pyromorphite can be solubilized by phosphate-solubilizing fungi, *e.g. A. niger*, and plants grown with pyromorphite as a sole phosphorus source accumulated both P and Pb [31]. During fungal transformation of pyromorphite, biogenic production of lead oxalate dihydrate can occur [29] (Fig. 1). This study emphasizes the importance of considering microbial processes in developing remediation techniques for metal-contaminated soils.

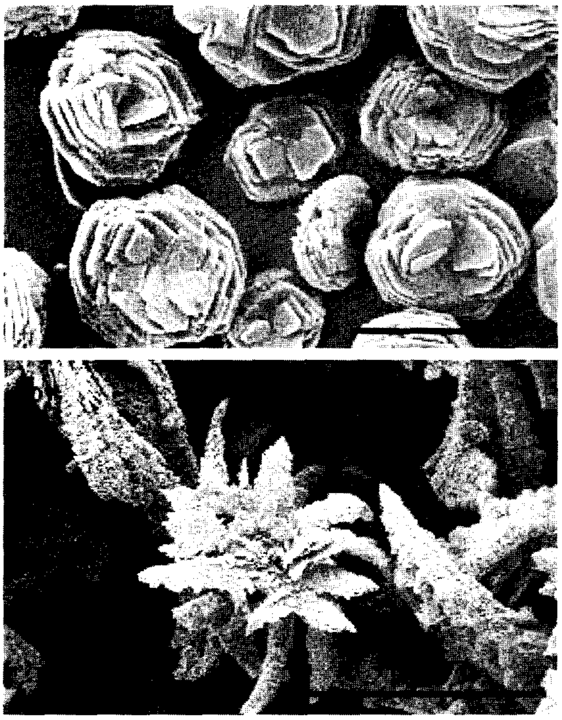

Fig. 1. (a) Calcium oxalate formed by solubilization of apatite ($Ca_5(PO_4)_3OH$) and (b) lead oxalate formed by solubilization of the lead-containing mineral pyromorphite ($Pb_5(PO_4)_3Cl$) by *Serpula himantioides*.
Bar markers=(a) 50 µm, (b) 200 µm (Moses and Gadd, unpublished data).

Autotrophic (Chemolithotrophic) Leaching

Metals can be leached from solid matrices as a result of autotrophic metabolism. Most autotrophic leaching is carried out by chemolithotrophic, acidophilic bacteria which fix carbon dioxide and obtain energy from the oxidation of ferrous iron or reduced sulfur compounds [3, 8]. These metabolic processes yield Fe(III) or H_2SO_4 as the respective end-products. The microorganisms involved in autotrotrophic leaching include sulfur-oxidizing bacteria, *e.g. Thiobacillus thiooxidans*, iron- and sulfur-oxidizing bacteria, *e.g. Thiobacillus ferrooxidans*, and iron-oxidizing bacteria, *e.g. Leptospirillum ferrooxidans*. As a result of sulfur- and iron-oxidation by these bacteria, metal sulfides are solubilized and the pH of their immediate environment is decreased, which enhances the solubilization of other metal compounds [3, 8, 48].

Complexation by Ligands

Microorganisms produce extracellular metabolites that can complex metals in solution, including polysaccharides, pigments, organic acids (see above) and siderophores [1, 2, 5, 30]. The bioavailability of a solubilized metal-ligand complex will depend ultimately on its long-term fate in the environment, including sorption onto soil components or potential utilization by biota [10].

Siderophores

Siderophores are highly specific Fe(III) ligands (formation constants often $>10^{30}$). These low-molecular weight coordination molecules are excreted by a wide variety of fungi and bacteria to aid iron assimilation [15]. Organisms may have evolved mechanisms for production of species-specific siderophores, or attachment to solid Fe minerals, *e.g.*, Fe oxides, to facilitate contact with the Fe substrate.

Biomethylation

A range of bacteria and fungi under aerobic and anaerobic conditions can mediate methylation of Hg, As, Se, Sn, Te, and Pb [23]. Methyl groups are enzymatically transferred to the metal, and a given species may transform a number of different metal(loid)s. Methylated metal compounds formed by these processes differ in their solubility, volatility, and toxicity. Volatile methylated species, *e.g.* $(CH_3)_2Se$ and $(CH_3)_2Se_2$, can be lost to the atmosphere [11].

Redox Transformations

Microorganisms can mobilize metals, metalloids, and organometallic compounds by reduction and oxidation processes [11, 21]. For example, oxidation of metal-complexing dimethylsulfide, dimethylsulfoxide, or thiosulfate increases metal availability if metal sulfates are formed, and the solubilities of Fe and Mn increase on reduction of Fe(III) to Fe(II) and Mn(IV) to Mn(II) [25]. Most iron reduction is carried out by specialized anaerobic bacteria that use iron(III) as a terminal electron acceptor. Dissimilatory metal-reducing bacteria can use a variety of metal(loid)s with an appropriate redox couple, including Fe(III), Mn(IV), Se(IV), Cr(VI), and U(VI) [27, 36]. While Fe and Mn increase their solubility upon reduction, the solubility of other metals such as U(VI) to U(IV) and Cr(VI) to Cr(III) decreases, resulting in immobilization [28, 34] (Fig. 2). Reduction of Hg(II) to Hg(0) by bacteria and fungi results in diffusion of elemental Hg out of cells [32, 33]. Bacteria can also use redox-active organic compounds to shuttle electrons between cells and the metal being respired under anaerobic conditions [25]. Many quinoid compounds, common components of humic substances, can function as electron shuttles. These compounds move electrons from the cell membrane to the target metal, such as iron contained in ferric oxides. The shuttle molecule regenerates when the metal is reduced [25].

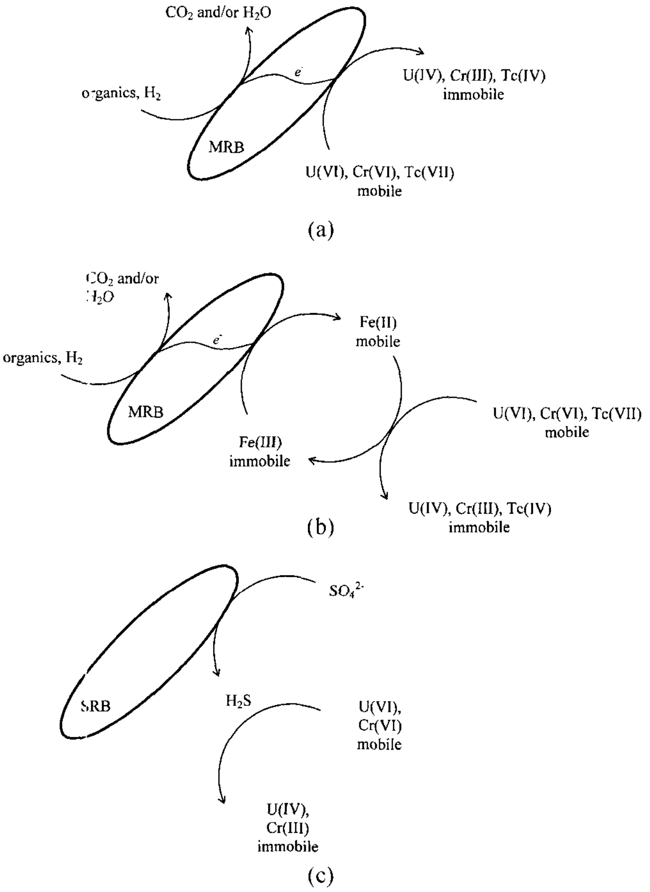

Fig. 2. Mechanisms of metal/radionuclide reduction by bacteria. (a) Direct metal/radionuclide reduction by metal-reducing bacteria (MRB). The U(IV) precipitates as the uranium oxide mineral uraninite, UO_2; Cr(III) generally as $Cr(OH)_3$; Tc(IV) as TcO_2. (b) Indirect metal/radionuclide reduction by metal-reducing bacteria (MRB) and (c) sulfate-reducing bacteria (SRB). Fe(II) and H_2S can reduce multivalent species as in (a). In the reoxidation of Fe(III), elements may be incorporated in metal oxide precipitates.

IMMOBILIZATION

There are a number of processes whereby microorganisms immobilize metals. Although immobilization reduces the external free metal activity, it may also shift the equilibrium to release more metal into the soil solution.

Biosorption

The strong interaction of metals with cell surfaces is a result of cell wall characteristics, including component pigments and polysaccharides [16, 17, 18]. Gram-positive bacterial walls consist of a thick matrix of peptidoglycan and secondary polymers. The phosphate and carboxyl groups within the wall fabric and exposed at the surface confer pH-dependent negative charge to the cell in environments where microbes generally exist; the point of zero charge is approximately pH 2–3. This charge provides the basis for metal sorption [22]. Gram-negative cells are also negatively charged, although structurally more complex. In this case, a thin layer of peptidoglycan is located in a gel-like periplasm, sandwiched between the plasma membrane and the outer membrane. The outer membrane has characteristic surface lipopolysaccharides (LPS) containing ionizable phosphate, carboxyl, hydroxyl, and amine groups; the phosphate groups are the most metal reactive. In addition to these structures, surface capsules, sheaths, and S-layers provide additional sites for metal interactions and the heterogeneous nucleation of minerals [1, 22].

Fungal cell walls are predominantly composed of polysaccharides (80–90%) with glycoproteins and some lipids. The polysaccharide component includes microcrystalline fibrils of β-linked polysaccharides (chitin, chitosan, and β-glucans). As for bacteria, the chemical properties of the

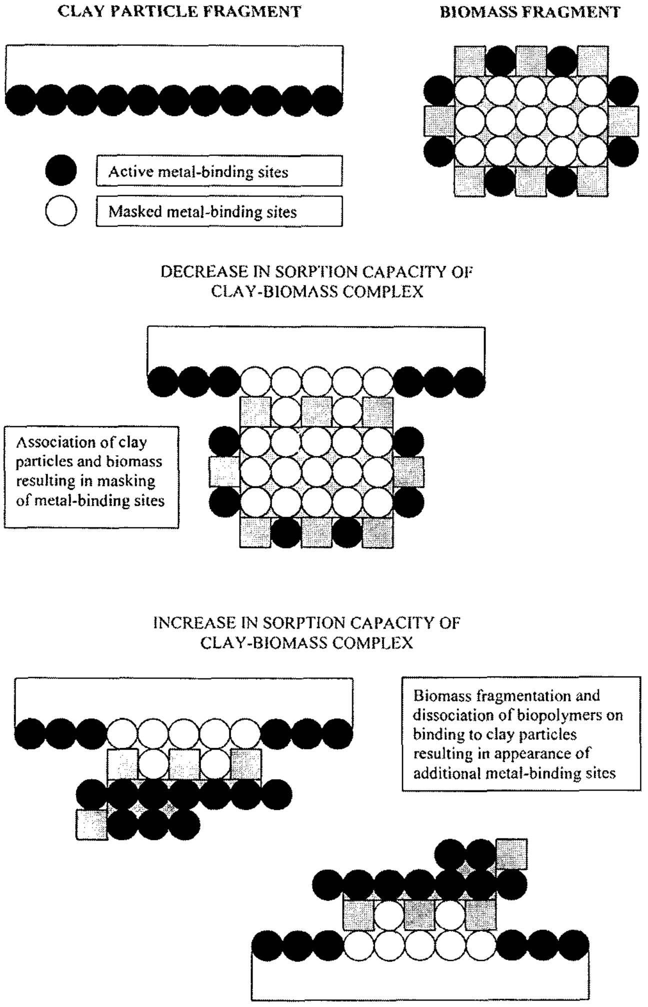

Fig. 3. Simple model of mechanisms for reduced or enhanced metal sorption abilities of fungal/clay sorbents.
(●), Available metal-binding sites on clay particles or biomass; (○), masked or blocked metal-binding sites. A decrease in sorption capacity of the clay-biomass complex may result from masking of metal-binding sites on the biomass and clay particles. An increase in sorption capacity of the clay-biomass complex may result after biomass fragmentation and/or dissociation of biopolymers after interactions with clay particles, leading to exposure of additional metal-binding sites (adapted from Ref. 9).

Table 2. Solubility products of some metal oxalates, *i.e.* the product of the concentrations (M) of the ions in a saturated solution (adapted from Ref. 19).

Metal oxalate		Temperature	Solubility product
Cadmium	CdC_2O_4	18°C	1.53×10^{-8}
Calcium	$CaC_2O_4\cdot H_2O$	18°C	1.78×10^{-9}
	$CaC_2O_4\cdot H_2O$	25°C	2.57×10^{-9}
Copper	CuC_2O_4	25°C	2.87×10^{-8}
Iron	FeC_2O_4	25°C	2.10×10^{-7}
Lead	PbC_2O_4	18°C	2.74×10^{-11}
Magnesium	$MgC_2O_4\cdot 2H_2O$	18°C	8.57×10^{-5}
Strontium	$SrC_2O_4\cdot H_2O$	18°C	5.61×10^{-8}
Zinc	$ZnC_2O_4\cdot 2H_2O$	18°C	1.35×10^{-9}

functional groups associated with fungal walls, including carboxyl, amine, hydroxyl, phosphate, and sulfhydryl groups, provide the basis for the attraction of metals to cell walls. In addition, pigments like melanin have significant metal-binding properties, preventing metal entry into melanized structures [19]. Interactions with clay minerals can result in altered metal biosorption characteristics [9] (Fig. 3).

Precipitation of Metals and Minerals

Cellular Precipitation. At sufficient concentrations, metal precipitates nucleate on or within cells, or in the immediate extracellular environment [22]. The formation of extracellular and sorbed precipitates depends on the chemical composition of the extracellular environment and is often mediated by cellular processes. Anions, such as OH^-, SO_4^{2-}, PO_4^{3-}, and CO_3^{2-}, provide counterions for mineral hydroxides, sulfate and sulfides, phosphates, and carbonates. It is possible that cellular excretion of such anions contributes to these minerals. Metal oxide and hydroxide minerals formed by abiotic reactions also attach to cells. Inside cells, metals may be effluxed, form intracellular precipitates, bind to metal-complexing molecules, and, in eukaryotes, partition into vacuoles [19].

Oxalates. Most simple metal oxalates are insoluble, except those of alkali metals, ammonium, Al(III), and Fe(III), with Mg oxalate being the most soluble and lead oxalate the least soluble [12] (Table 2). Calcium oxalate is the most common form of oxalate associated with soils and leaf litter, occurring as dihydrate (weddellite) or the more stable monohydrate (whewellite) [12]. Calcium oxalate crystals are commonly associated with free-living, pathogenic, and plant symbiotic fungi and are formed by the precipitation of solubilized calcium as the oxalate [12, 20] (Figs. 1, 3). The formation of calcium oxalate by fungi has an important influence on biological and geochemical processes in soils, acting as a reservoir for calcium in the ecosystem, and also influencing phosphate availability. Fungi can also produce other metal oxalates with a variety of different metals and metal-bearing minerals, *e.g.* Cd, Pb, Co, Cu, Mn, Sr, and Zn [12, 31, 48].

Carbonates. In many arid and semi-arid regions, calcareous soils and near-surface limestones (calcretes) are often secondarily cemented with calcite ($CaCO_3$). This phenomenon has been partly attributed to physicochemical processes; however, the abundance of calcified fungal filaments in weathered profiles of chalky limestone and quaternary calcretes indicates fungal activity [12] (Fig. 4). Mineralized carbonate precipitates are also found in association with bacterial biofilms [22] and with fungal hyphae growing in limestone and limestone-amended microcosms (Fig. 4).

Bacterial and Fungal Oxidation. The range of bacterial species with the capability to oxidize Fe and Mn is large.

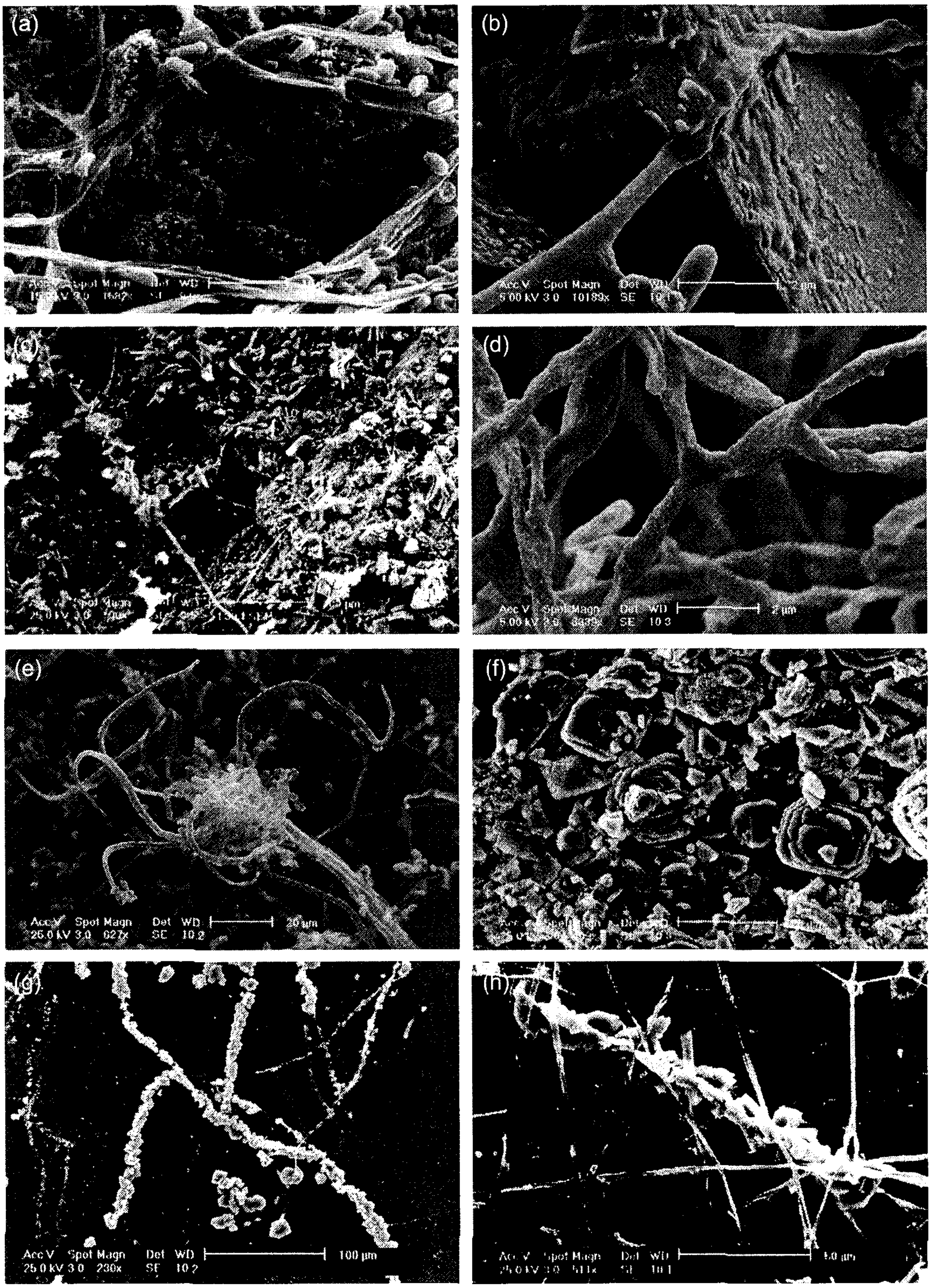

Fig. 4. Carbonate and oxalate biomineralization by fungi in natural limestone and limestone microcosms.

(a) Fungal hyphae *in situ* in limestone (bar=10 µm); (b) fungal hyphae attached to mineral surfaces *in situ* in limestone (bar=2 µm); (c) mycogenic crystalline precipitates formed on fungal hyphae *in situ* in limestone (bar=200 µm); (d) branching hyphal filaments of *Penicillium corylophilum* isolated from limestone (bar=2 µm); (e) dimorphic growth of *Chaetomium* sp. isolated from limestone (bar=20 µm); (f) mycogenic calcite ($CaCO_3$) and whewellite (calcium oxalate monohydrate) formed under colonies of a limestone isolate, *Pseudallescheria boydii*, when grown in microcosms amended with limestone (bar=50 µm); (g & h) hyphae of *Serpula himantioides* mineralized with calcite and whewellite when grown in microcosms amended with limestone (bar=100 µm and 50 µm respectively) (Burford and Gadd, unpublished data).

Bacterial Fe oxidation is ubiquitous in environments with sufficient Fe^{2+} and conditions to support bacterial growth such as drainage waters and tailing piles in mined areas, pyritic and hydric soils (bogs and sediments), drain pipes and irrigation ditches, and plant rhizosphere zones. Iron-oxidizers found in acidic soil environments are acidophilic chemolithotrophs, such as *Thiobacillus ferrooxidans*, significant for its role in generating acid mine drainage [8]. Facultative chemolithotrophic microaerophiles such as *Leptothrix ochracea*, *Sphaerotilus natans*, and *Gallionella ferruginea* are common in mildly acidic to neutral environments. Fungi also oxidize metals in their environment. Desert varnish is an oxidized metal layer (patina) a few mm thick found on rocks and in soils of arid and semi-arid regions, and is believed to be of fungal and bacterial origin.

Dissimilatory Metal Reduction by Bacteria. When dissimilatory metal-reducing bacteria utilize metals such as Cr(VI) and U(VI) as electron acceptors, less soluble Cr(III) and U(IV) species result [25, 34, 36]. Once reduced, metals sorb to the cells or partition to the bulk extracellular solution, where new minerals can form. Fe^{2+} and Mn^{2+}, generated by dissimilatory reduction, can form extracellular precipitates and nodules. Fungi and other microorganisms also precipitate reduced forms of metal species, *e.g.* Ag(I) to Ag(0); selenate [Se(VI)] and selenite [Se(IV)] to Se(0); tellurite [Te(IV)] to Te(0), by a variety of mechanisms [11, 19, 21] (Fig. 5).

Table 3. Solubility products of some metal sulfides[a].

Metal	Solubility product
Aluminum	Decomposes[b]
Cadmium	3.6×10^{-29}
Calcium	Sparingly soluble
Cobalt	3.0×10^{-26}
Copper (I)	2.0×10^{-48}
Copper (II)	8.5×10^{-36}
Iron (II)	3.7×10^{-19}
Lead	3.4×10^{-24}
Magnesium	Decomposes[b]
Manganese (II)	1.4×10^{-15}
Mercury (II)	4.0×10^{-53}
Molybdenum(n)	All sulfides insoluble
Nickel	1.4×10^{-24}
Potassium	Very soluble
Selenium	Insoluble
Silver	1.6×10^{-46}
Sodium	Very soluble
Zinc	1.2×10^{-23}

[a] All solubility products were determined at 18°C.
[b] Decomposes to H_2S and the corresponding (insoluble) hydroxide (adapted from Ref. 41).

Metal Precipitation by Sulfate-Reducing and Other Bacteria. Sulfate-reducing bacteria (SRB) oxidize organic compounds or hydrogen coupled with the reduction of sulfate, producing sulfide [42, 43, 44]. The solubility products of most heavy metal sulfides are very low, (Table 3) so that even a moderate output of sulfide can remove metals to levels permitted in the environment, with metal removal being directly related to sulfide production [42, 46]. Sulfate-reducing bacteria can also create extremely reducing conditions which can chemically reduce metals such as uranium(VI) [46]. In addition, sulfate reduction partially eliminates acidity from the system as a result of the shift in equilibrium when sulfate (dissociated) is converted to sulfide (largely protonated). This can result in the further precipitation of metals as hydroxides as well as increasing the efficiency of sulfide precipitation [44]. Another form of bacterial bioprecipitation is mediated by a phosphatase enzyme, which liberates inorganic phosphate from a supplied organic

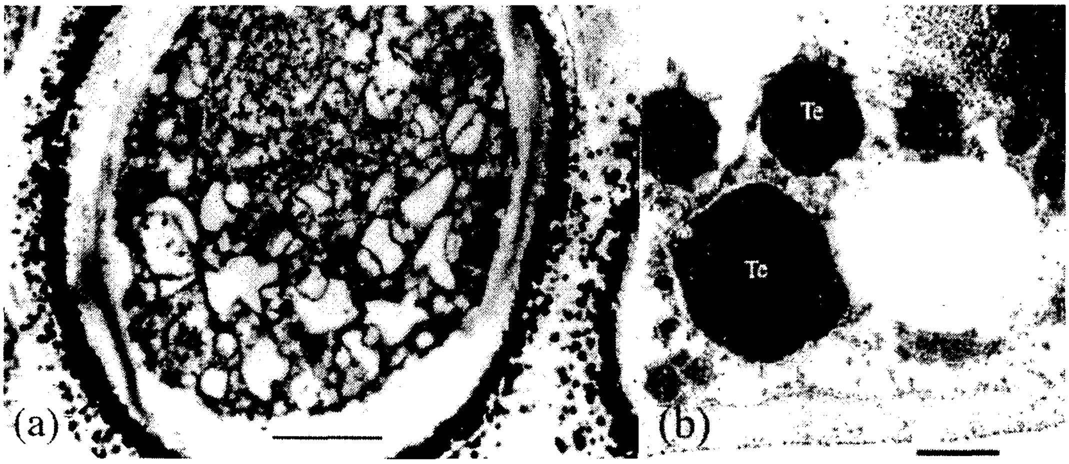

Fig. 5. Reductive precipitation of (a) elemental silver (Ag^0) around cells of *Aureobasidium pullulans* and (b) elemental tellurium (Te^0) within a *Fusarium* sp.
Bar markers=(a) 1 µm and (b) 400 nm. (Adapted from Refs. 21 and 24.)

phosphate donor molecule, *e.g.* glycerol-2-phosphate. Metals/radionuclides are precipitated as phosphates on the biomass [26, 50].

ENVIRONMENTAL BIOTECHNOLOGY

Ore Leaching

Metals in ores that are not worth recovering by smelting can be effectively leached by bacteria and fungi [3, 4, 6, 8, 29]. Important species of bacteria are Fe and S oxidizers such as *Thiobacillus ferrooxidans*, *T. thiooxidans*, and *Leptospirillum ferrooxidans*. Their activities solubilize Fe and S contained in metal sulfides, with the concomitant release of associated metals such as Cu and Zn. Large amounts of Fe^{2+} and sulfuric acid are generated in the process [8]. Industry can take advantage of the acidic leachate by recirculating it through the ore body to dissolve more metal: soluble metals are then separated by sedimentation, solvents, or electrodialysis. When uncontrolled, such as at abandoned mining sites, the same process can have disastrous environmental consequences resulting in acid mine drainage.

Extracellular ligands produced by fungi, including *Aspergillus* and *Penicillium* spp., have also been used to leach metals such as Zn, Cu, Ni, and Co from a variety of solid materials, including low-grade mineral ore, solid waste, and electronic scrap materials [4, 6, 12, 19, 30, 37, 39, 42].

Bioremediation

Some of the processes outlined above have potential for application for treating contaminated land. In a bioremediation context, production of sulfuric acid by *Thiobacillus* species has been used to solubilize metals from sewage sludge, thus enabling separation from the sludge which can then be used as a fertilizer [35]. Autotrophic leaching has been used to remediate other metal-contaminated solid materials including soil and red mud, the main waste product of Al extraction from bauxite [40]. Although some processes could be used *in situ* (*e.g.* leaching using S-oxidizing bacteria), many are probably most suitable for *ex situ* use in bioreactors, where the mobilized or immobilized metal can be separated from soil components [49]. Living or dead fungal and bacterial biomass and metabolites have been used to remove metals and metalloids from solution by biosorption or chelation [15, 26].

The increased interest in microbial activities in anaerobic, subsurface environments has opened up new possibilities for the bioremediation of metal contaminants. Metal(loid)s that form insoluble precipitates when reduced may be of particular interest for *in situ* treatment, such as Se(0), Cr(III), Tc(IV), and U(IV) [25, 36, 38]. Natural processes in subsurface soil may immobilize contaminants otherwise predicted to leach. The sulfide produced from sulfate reduction plays a major role in metal sulfide immobilization in sediments but has also been applied to bioremediation of metals in waters and leachates. Large-scale bioreactors have been developed using bacterial sulfate-reduction for treating metal-contaminated waters. A process integrating bacterial sulfate-reduction with bioleaching by sulfur-oxidizing bacteria has also been developed to remove contaminating toxic metals from soils. Sulfur- and iron-oxidizing bacteria liberated metals from soils in the form of an acid sulfate solution that enabled almost all the metals to be removed by bacterial sulfate reduction [49]. SRB biofilm reactors may offer a means of process intensification and entrap or precipitate metals, *e.g.* Cu and Cd, at the biofilm surface [45, 47].

CONCLUSIONS

Microorganisms play important roles in the environmental fate of metals, radionuclides, and metalloids, with a multiplicity of physicochemical and biological mechanisms effecting transformations between soluble and insoluble phases. Such mechanisms are important components of natural biogeochemical cycles, with some processes being of potential application to the treatment of contaminated materials.

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