

## MINIREVIEW

# Microbial Metal Transformations

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There is considerable interest in how microbiological processes can affect the behaviour of metal contaminants in natural and engineered environments and their potential for bioremediation. 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 their bioavailability and potential toxicity, whereas those that immobilize them reduce bioavailability. The balance between mobilization and immobilization varies depending on the metal, the organisms, their environment and physico-chemical conditions.

**Key words:** toxic metals, metal mobilization, metal immobilization

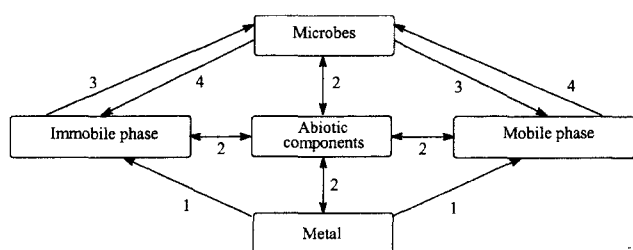
Microorganisms can mobilize metals and radionuclides through autotrophic and heterotrophic leaching, chelation by microbial metabolites and siderophores, and methylation, which can result in volatilization. Conversely, immobilization can result from sorption to cell components or exopolymers, transport into cells and intracellular sequestration or precipitation as insoluble organic and inorganic compounds, e.g. oxalates (27, 46), sulphides or phosphates (60, 61, 68,). In bioremediation, solubilization provides a route for removal from solid matrices such as soils, sed-

iments, dumps and industrial waste. 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.

### Metal Mobilization

#### 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 sulphur compounds. These metabolic processes yield Fe(III) or H<sub>2</sub>SO<sub>4</sub> as the respective end-products. The microorganisms involved in autotrophic leaching include sulphur-oxidizing bacteria, e.g. *Thiobacillus thiooxidans*, iron- and sulphur-oxidizing bacteria, e.g. *Thiobacillus ferrooxidans* and iron-oxidizing bacteria, e.g. *Leptospirillum ferrooxidans* (7, 15). As a result of sulphur- and iron-oxidation by these bacteria, metal sulphides are solubilized and the pH of their immediate environment is decreased, which enhances the solubilization of other metal compounds. The autotrophic leaching of metal sulphides by *Thiobacillus* species and other acidophilic bacteria is well established for use in industrial scale biomining processes (44). In a bioremediation context, production of sulphuric acid by *Thiobacillus* species has been used to solubilize metals from sewage sludge, thus enabling sep-



**Fig. 1.** Simple model of microbial roles in the environmental mobility of metals. Metal entry into the environment [1] is shown as well as the importance of abiotic environmental components in affecting metal speciation and microbial populations [2]. The major influence of microbes in effecting transformations between soluble and insoluble phases is emphasised [3, 4] (adapted from 19).

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aration from the sludge which can then be used as a fertiliser (5, 52). Autotrophic leaching has been used to remediate other metal-contaminated solid materials including soil (69) and red mud, the main waste product of Al extraction from bauxite (58).

#### ***Heterotrophic (chemoorganotrophic) leaching***

Heterotrophic metabolism can also lead to leaching as a result of the efflux of protons, organic acids and siderophores (9, 20, 23, 46). Organic acids provide both protons and a metal-chelating anion to complex the metal cation. Citrate and oxalate anions can form stable complexes with a large number of metals. Uranium forms very stable 1:1 and 1:2 uranium-citrate complexes with stability constants that are much higher than those of uranyl acetate, uranyl lactate, U-EDTA and uranyl ascorbate complexes (6). Many metal citrates are highly mobile and not readily degraded and the presence of citric acid in soil may enhance contaminant metal solubility for a significant time (16). Oxalic acid can also act as a leaching agent for those metals that form soluble oxalate complexes, including Al and Fe (54). Many fungi are able to leach metals from industrial waste and by-products, low grade ores (9) and metal-bearing minerals (13, 23, 47, 57). Heterotrophic solubilization can have consequences for other remedial treatments for contaminated soils. Pyromorphite ( $\text{Pb}_5(\text{PO}_4)_3\text{Cl}$ ) 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. *Aspergillus niger*, and plants grown with pyromorphite as a sole phosphorus source accumulate both P and Pb (47). During fungal transformation of pyromorphite, biogenic production of lead oxalate dihydrate was observed for the first time (47). This study emphasises the importance of considering microbial processes in developing remediation techniques for metal-contaminated soils. Another method for treatment of metal-contaminated sandy soil relied on siderophore-mediated metal solubilization by *Alcaligenes eutrophus*. Solubilized metals were adsorbed to the biomass and/or precipitated, with biomass separated from a soil slurry by a flocculation process. This resulted in a complete decrease in Cd, Zn and Pb bioavailability (12). Related to heterotrophic solubilization is fungal translocation of, e.g. Cs, Zn and Cd, which can lead to concentration in specific regions of the mycelium and/or in fruiting bodies. Whether the concentration factors observed *in vitro* can be reproduced in the field and whether such amounts can contribute to soil bioremediation remains uncertain (28).

#### ***Reductive mobilization***

Fe(III) and Mn(IV) oxides absorb metals strongly and this may hinder metal extraction from contaminated soils. Micro-

bial reduction of Fe(III) and Mn(IV) may be one way for releasing such metals and this process may be enhanced with the addition of humic materials, or related compounds. Such compounds may also act as electron shuttles for, e.g. U(VI) and Cr(VI), converting them to less soluble forms, especially if located in tight pore spaces where microorganisms cannot enter (34). The solubility of certain radionuclides can also be increased by reduction and this may favour their removal from matrices such as soils. For example, iron-reducing bacterial strains solubilized 40% of the Pu present in contaminated soils within 6-7 days through reduction of Pu(IV) to the more soluble Pu(III) (45) and both iron- and sulphate-reducing bacteria were able to solubilize Ra from uranium mine tailings, although solubilization occurred largely by disruption of reducible host minerals (31). The mechanism of bacterial  $\text{Hg}^{2+}$  resistance is enzymic reduction of  $\text{Hg}^{2+}$  to non-toxic volatile  $\text{Hg}^0$  by mercuric reductase.  $\text{Hg}^{2+}$  may also arise from the action of organomercurial lyase on organomercurials (49, 50). Since  $\text{Hg}^0$  is volatile, this could provide one means of mercury removal (2, 8).

#### ***Methylation of metalloids***

Microbial methylation of metalloids to yield volatile derivatives, e.g. dimethylselenide or trimethylarsine, can be effected by a variety of bacteria, algae and fungi (18, 29). Microbial methylation of selenium, resulting in volatilization, has also been used for *in situ* bioremediation of selenium-containing land and water at Kesterson Reservoir, California (56).

#### ***Metal Immobilization***

##### ***Biosorption***

Biosorption is the uptake of organic and inorganic metal species, both soluble and insoluble, by physico-chemical mechanisms such as adsorption. In living cells, metabolic activity may also influence this process. Almost all biological macromolecules have some affinity for metal species with cell walls and associated materials being of the greatest significance. Biosorption can also provide nucleation sites for the formation of stable minerals (3). As well as sorption to cellular surfaces, cationic species can be accumulated within cells via transport systems of varying affinity and specificity (19). Once inside cells, metal species may be bound, precipitated, localised within intracellular structures or organelles, or translocated to specific structures depending on the element concerned and the organism (19, 66). Freely-suspended and immobilized microbial biomass has received attention with immobilized systems possessing advantages which include higher mechanical strength and easier biomass/liquid separation (36). Immobilized living biomass has mainly taken the form of bacterial biofilms on inert supports and is used in a variety of bioreactor configurations including rotating

biological contactors, fixed bed reactors, trickle filters, fluidized beds and air-lift bioreactors (17, 24, 25, 36). A range of specific and non-specific metal-binding compounds are also produced by microorganisms. Non-specific metal-binding compounds range from simple organic acids and alcohols to macromolecules such as polysaccharides, humic and fulvic acids (4). The metal-binding abilities of siderophores, metallothioneins, phytochelatins and other biomolecules also have potential for bioremediation. However, the earlier commercial promise and development of biosorption appears to have largely ceased and there is no adoption of biosorption as a commercially viable treatment method to date. The lack of commercial development is somewhat perplexing although the lack of specificity and lower robustness of biomass-based systems compared to ion exchange resins is often cited as a reason (14).

#### **Metal precipitation by metal-reducing bacteria**

Where reduction of a metal to a lower redox state occurs, mobility and toxicity may be reduced, offering potential bioremediation applications. Such processes may also accompany other indirect reductive metal precipitation mechanisms, e.g. in sulphate-reducing bacterial systems where reduction of Cr(VI) can be a result of indirect reduction by Fe<sup>2+</sup> and sulphide. A diverse range of metal-reducing bacteria can use oxidized species of metallic elements, e.g. Fe(III), Cr(VI) or Mn(IV) as terminal electron acceptors (34). For example, a strain of *Shewanella (Alteromonas) putrefaciens* which reduces Fe(III) and Mn(IV) also reduces U(VI) to U(IV), forming a black precipitate of U(IV) carbonate (35). Bacterial uranium reduction has also been combined with chemical extraction to produce a potential process for soil bioremediation (43). *Desulfovibrio desulphuricans* can reduce Pd(II) to cell-bound Pd(0) with hydrogen-dependent reduction being O<sub>2</sub>-insensitive, providing a means of aerobic Pd recovery (32).

#### **Reduction of metalloid oxyanions**

Se(VI) reduction to elemental insoluble Se(0) has been employed to remediate contaminated waters and soils. Some bacteria can use such reduction to support growth, making this a natural process for *in situ* applications. Though reduction of oxyanions of As and Se can occur by different mechanisms, the most environmentally significant process is dissimilatory reduction. Oxyanions of arsenic and selenium can be used in microbial anaerobic respiration as terminal electron acceptors, providing energy for growth and metabolism (11, 38, 42). Their reduction can be coupled to organic substrates, e.g. lactate, acetate and aromatics, with the bacteria found in a range of habitats and not confined to any specific genus. These organisms, and perhaps even the enzymes themselves, may have applications for bioremediation of selenium- and arsenic-contaminated environments (53). Oremland *et al.* (40,

41) utilised these metabolic processes for the *in situ* removal of SeO<sub>4</sub><sup>2-</sup> from agricultural drainage regions of Nevada. Exposed reservoir sediments were flooded to create anoxic conditions, in which the natural bacterial population reduced and immobilized large quantities of the selenium that was present in the sediments (33). The incidental ability of a variety of microorganisms from all major groups to reduce Se(VI) and Te(VI) by additional, often uncharacterised, mechanisms offers additional scope for bioreactor-based approaches (26, 30).

#### **Metal precipitation by sulphate-reducing and other bacteria**

Sulphate-reducing bacteria (SRB) oxidize organic compounds or hydrogen coupled with the reduction of sulfate, producing sulphide (59, 60). The solubility products of most heavy metal sulphides are very low, in the range of 4.65×10<sup>-14</sup> (Mn) to 6.44×10<sup>-53</sup> (Hg) so that even a moderate output of sulphide can remove metals to levels permitted in the environment (10, 55) with metal removal being directly related to sulphide production (60). Sulphate-reducing bacteria can also create extremely reducing conditions which can chemically reduce metals such as uranium(VI) (51). In addition, sulphate reduction partially eliminates acidity from the system as a result of the shift in equilibrium when sulphate (dissociated) is converted to sulphide (largely protonated) (60). This can result in the further precipitation of metals such as copper or aluminium as hydroxides as well as increasing the efficiency of sulphide precipitation. The sulphide produced from sulphate reduction plays a major role in metal sulphide immobilization in sediments but has also been applied to bioremediation of metals in water and leachates. Large-scale bioreactors have been developed using bacterial sulphate-reduction for treating metal-contaminated water. A process integrating bacterial sulphate-reduction with bioleaching by sulphur-oxidizing bacteria has also been developed to remove contaminating toxic metals from soils. Sulphur- and iron-oxidizing bacteria liberated metals from soils in the form of an acid sulphate solution that enabled almost all the metals to be removed by bacterial sulphate reduction (60, 61, 62, 66, 67). SRB biofilm reactors may offer a means of process intensification and entrap or precipitate metals, e.g. Cu and Cd, at the biofilm surface (63, 65). Regarding other organisms, the thiosulphate reductase gene from *Salmonella typhimurium* has been expressed in *Escherichia coli*. This resulted in sulphide production from inorganic thiosulphate which precipitated metals as metal-sulphide complexes (1). A Cd-resistant *Klebsiella planticola* also precipitated significant amounts of cadmium sulphide when grown in thiosulphate-containing medium (48). As an alternative to anaerobic sulphate reduction, a novel aerobic sulphate reduction pathway has been engineered for sulphide production. The assimilatory SO<sub>4</sub><sup>2-</sup>-reduction pathway was redirected to cysteine production

which was converted to  $S^{2-}$  by cysteine desulphhydrase, leading to CdS precipitation on bacterial cell surfaces (59). Another form of bacterial bioprecipitation is mediated by a phosphatase enzyme, which liberates inorganic phosphate from a supplied organic phosphate donor molecule, e.g. glycerol 2-phosphate. Metals/radionuclides are precipitated as phosphates on the biomass (36, 37).

### Conclusions

Microorganisms play important roles in the environmental fate of toxic metals and metalloids with physico-chemical 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. Although the biotechnological potential of most of these processes has only been explored at the laboratory scale, some mechanisms, notably bioleaching, biosorption and precipitation, have been employed at a commercial scale. Of these, autotrophic leaching is an established major process in mineral extraction but has also been applied to the treatment of contaminated land. There have been several attempts to commercialise biosorption using microbial biomass but success has been limited, primarily due to competition with commercially-produced ion exchange media. As a process for immobilizing metals, precipitation of metals as sulphides has achieved large-scale application, and this holds out promise of further commercial development. Exploitation of other biological processes will undoubtedly depend on a number of scientific, economic and political factors, including the availability of a market.

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