Microbial influence on metal mobility and application for bioremediation

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Abstract

Microorganisms are intimately involved in metal biogeochemistry with a variety of processes determining mobility, and therefore, bioavailability. The balance between mobilization and immobilization varies depending on the organisms involved, their environment and physicochemical conditions. Metal mobilization can arise from a variety of leaching mechanisms, complexation by metabolites and siderophores, and methylation, where this results in volatilization. Immobilization can result from sorption to biomass or exopolymers, transport and intracellular sequestration or precipitation as organic and inorganic compounds, e.g., oxalates (fungi) and sulfides. In addition, reduction of higher valency species may effect mobilization, e.g., Mn(IV) to Mn(II), or immobilization, e.g., Cr(VI) to Cr(III). In the context of bioremediation, solubilization of metal contaminants provides a means of removal from solid matrices, such as soils, sediments, dumps and other solid industrial wastes. Alternatively, immobilization processes may enable metals to be transformed in situ and are particularly applicable to removing metals from aqueous solution. This contribution will outline selected microbiological processes which are of significance in determining metal mobility and which have actual and potential application in bioremediation of metal pollution. These include autotrophic and heterotrophic leaching mechanisms, reductive precipitation, sulfate reduction and metal sulfide precipitation.
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1. Introduction

The mechanisms by which microorganisms effect changes in metal speciation and mobility are fundamental components of biogeochemical cycles for metals as well as all other elements, including carbon, nitrogen, sulfur and phosphorus, with additional implications for plant productivity and human health (Gadd, 1999, 2002). The ability of microorganisms to affect metal speciation stems from their ability to effect and/or mediate mobilization or immobilization processes that influence the balance of metal species between soluble and insoluble phases. Mobilization of metals can be achieved by, e.g., protonation, chelation, and chemical transformation while immobilization can occur by precipitation or crystallization of insoluble organic or inorganic compounds or by sorption, uptake and intracellular sequestration. Re-

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dox reactions can mobilize or immobilize metals depending on the metal species involved. As well as being an integral component of biogeochemical cycles for metals, these processes may be exploited in environmental biotechnology (Gadd, 1993a, 1996, 1999, 2000a,b, 2002; Gadd and White, 1993; White et al., 1997, 1998; Lloyd and Macaskie, 2000; Schiewer and Volesky, 2000; Lloyd and Lovley, 2001; McLean et al., 2002). Almost all metal–microbe interactions have been examined as a means for removal, recovery or detoxification of inorganic and organic metal or radionuclide pollutants (Lovley and Coates, 1997; Francis, 1998; Stephen and Macnaughton, 1999; Eccles, 1999). In such a context, solubilization may enable removal from solid matrices, such as soils, sediments, dumps and industrial wastes. Alternatively, immobilization processes may enable metals to be transformed in situ into insoluble and chemically inert forms and are also applicable to removing metals from aqueous solution. While much research is laboratory-based, there are many developments to pilot/demonstration scale with some processes apparently in successful commercial operation. It should also be noted that metal removal/transformation processes are intrinsic although less appreciated components of traditional means of water/sewage treatment as well as reed bed, lagoon and wetlands technologies (Webb et al., 1998; Stephen and Macnaughton, 1999). Molecular and genetic analysis is now furthering understanding of microbial metal metabolism, including those aspects which are of environmental and biotechnological relevance (Nies, 1999; Chen et al., 1999). The aim of this article is to outline some of the more important microbiological processes which are of significance in determining environmental metal mobility and which have actual or potential applications in bioremediation.

2. Mobilization

Microorganisms can mobilize metals through autotrophic and heterotrophic leaching, chelation by microbial metabolites and siderophores, and methylation, which can result in volatilization. Such processes can lead to dissolution of insoluble metal compounds and minerals, including oxides, phosphates, sulfides and more complex mineral ores, and desorption of metal species from exchange sites on, e.g., clay minerals or organic matter in the soil.

2.1. Heterotrophic (chemoorganotrophic) leaching

Microorganisms 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 or in a sorbed form, resulting in the release of free metal cations. Heterotrophic metabolism can also lead to leaching as a result of the efflux of organic acids and siderophores. Organic acids can supply both protons and metal-complexing anions (see below; Burgstaller and Schinner, 1993; Gadd, 1999; Gadd and Sayer, 2000). Citrate and oxalate anions can form stable complexes with a large number of metals. Many metal citrates are highly mobile and not readily degraded (Francis et al., 1992). Oxalic acid can also act as a leaching agent for those metals that form soluble oxalate complexes, including Al and Fe (Strasser et al., 1994). Effective leaching of a variety of wastes and low-grade minerals has been demonstrated, e.g., soils and muds, filter dust/oxides, lateritic ores, copper converter slag, fly ash and electronic waste materials (Sukla et al., 1992; Burgstaller and Schinner, 1993; Tzeferis et al., 1994; Bosshard et al., 1996; Brandl et al., 1999; Gadd, 1999). A strain of *Penicillium simplicissimum* has been used to leach Zn from insoluble ZnO contained in industrial filter dust. The production of citric acid (>100 mM) was induced by the presence of the filter dust (Schinner and Burgstaller, 1989; Franz et al., 1991, 1993). Organic acid production is also an important agent of mineral deterioration, playing a role in both biogenic chemical weathering and soil formation (Gadd, 1999). Heterotrophic solubilization can also 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. 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 during

fungal transformation of pyromorphite, biogenic production of lead oxalate dihydrate can occur (Sayer et al., 1999).

2.2. Autotrophic leaching

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 which causes the solubilization of metals because of the resulting production of Fe(III) and H_2SO_4 (Rawlings, 1997; Schippers and Sand, 1999). The microorganisms involved 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* (Ewart and Hughes, 1991; Bosecker, 1997). As a result of sulfur- and iron-oxidation, metal sulfides are solubilized concomitant with the pH of their immediate environment being decreased, therefore resulting in solubilization of other metal compounds. Such leaching of metal sulfides by *Thiobacillus* species and other acidophilic bacteria is well established for industrial scale biomining (Ewart and Hughes, 1991; Bosecker, 1997; Rawlings, 1997; Rawlings and Silver, 1995). In a bioremediation context, autotrophic production of sulfuric acid has also been used to solubilize metals from sewage sludge and soils (Sreekrishnan and Tyagi, 1994; White et al., 1998). In a two-stage process, sulfur-oxidizing bacteria were used to acidify soil and solubilize toxic metals before metal removal from the metal-contaminated liquid leachate using sulfate-reducing bacteria (White et al., 1997, 1998). Studies have also been conducted into the bioremediation of red mud, the main waste product of Al extraction from bauxite. Here, autotrophic leaching by indigenous *Thiobacillus* spp. was more efficient than heterotrophic leaching by a range of fungal strains, and decreased the toxicity of the waste product (Vachon et al., 1994). *T. ferrooxidans* has also been used to treat air pollution control residues (APCR: fly ash and used lime) which contain high amounts of toxic metals. Although growth of the thiobacilli was poor, removal of up to 95% of Cd (from APCR of ~ 270 mg Cd kg^{-1}) was achieved with ~ 69% Pb removal (from APCR of ~ 5 g Pb kg^{-1}; Mercier et al., 1999).

2.3. Siderophores

Siderophores are highly specific Fe(III) ligands (formation constants often >1030). These low molecular weight coordination molecules are excreted to aid iron assimilation (Gadd, 2001). Such assimilation may be improved by attachment to solid Fe minerals, e.g., Fe oxides, to facilitate contact with the Fe substrate. Although primarily produced as a means of obtaining iron, siderophores are also able to bind other metals such as magnesium, manganese, chromium (III), gallium (III) and radionuclides such as plutonium (IV) (Birch and Bachofen, 1990). One method for the treatment of metal-contaminated sandy soil relies 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 flocculation. This resulted in a complete decrease in the bioavailability of Cd, Zn and Pb (Diels et al., 1999).

2.4. Biomethylation

Methylation of Hg, As, Se, Sn, Te and Pb can be mediated by a range of bacteria and fungi under aerobic and anaerobic conditions. 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 $(CH_3)_2Se_2$, are often lost from the soil (Gadd, 1993a; Karlson and Frankenberger, 1993). Many environmental and soil factors, e.g., organic amendments and frequent tillage, can be optimized to increase diffusive transport through soil and enhance Se volatilization (Stork et al., 1999; Zhang and Frankenberger, 1999). Microbial methylation of selenium, resulting in volatilization, has been used successfully for in situ bioremediation of selenium-containing land and water at Kesterson Reservoir, California, reducing the selenium concentrations to acceptable levels (Thompson-Eagle and Frankenberger, 1992). Several bacterial and fungal species can methylate arsenic compounds such as arsenate [As(V), AsO_4^{3-}], arsenite [As(III), AsO_2^-] and methylarsonic acid ($CH_3H_2AsO_3$) to volatile dimethyl-[$(CH_3)_2HAs$] or trimethylarsine [$(CH_3)_3As$] (Tamaki and Frankenberger, 1992).

2.5. Redox transformations

Microorganisms can mobilize metals, metalloids and organometallic compounds by reduction and oxidation processes (Gadd, 1993a; Gharieb et al., 1999; Lovley, 2000). For example, metal solubilities increase on reduction of Fe(III) to Fe(II) and Mn(IV) to Mn(II) (Lovley and Coates, 1997; Lovley, 2000; McLean et al., 2002). 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) (Oremland et al., 1991; Stolz and Oremland, 1999). 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 (Phillips et al., 1995; Smith and Gadd, 2000). Reduction of Hg(II) to Hg(0) by bacteria and fungi results in diffusion of elemental Hg out of cells (Silver, 1996, 1998; Hobman et al., 2000). Mercuric reductase, from a recombinant *Escherichia coli* strain, has been immobilized on a chemically modified diatomaceous earth support with immobilization enhancing stability and reusability: maximal activity was 1.2 nmol Hg mg^{-1} protein s^{-1} at an initial [Hg^{2+}] of 50 μmol dm^{-3} (Chang et al., 1999). Fe(III) and Mn(IV) oxides strongly absorb metals and this may hinder metal extraction from contaminated soils. Microbial 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 important soil polymers like humic materials, or related compounds. Such compounds act as electron shuttles for, e.g., U(VI) and Cr(VI), especially if located in tight pore spaces away from microbial activity (Lovley and Coates, 1997). Bacterial Fe(III) reduction resulted in release of, e.g., Mn and Co, from goethite where 5% of the iron was substituted by these metals (Bousserrhine et al., 1999).

3. Immobilization

A number of processes lead to immobilization of metals. Although immobilization reduces the external free metal species, it may also promote solubilization in some circumstances by shifting the equilibrium to release more metal into solution.

3.1. Biosorption and intracellular accumulation

Biosorption can be defined as the microbial uptake of organic and inorganic metal species, both soluble and insoluble, by physicochemical mechanisms, such as adsorption. In living cells, metabolic activity may also influence this process because of changes in pH, E$_h$, organic and inorganic nutrients, and metabolites. Biosorption can also provide nucleation sites for the formation of stable minerals (Beveridge and Doyle, 1989; Southam, 2000; McLean et al., 2002). As well as sorption to cellular surfaces, some cationic species can be accumulated within cells via membrane transport systems of varying affinity and specificity. 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 (Gadd, 1996, 1997; White et al., 1997; Gadd and Sayer, 2000).

Peptidoglycan carboxyl groups are the main binding site for cations in Gram-positive bacterial cell walls with phosphate groups contributing significantly in Gram-negative species (Beveridge and Doyle, 1989; McLean et al., 2002). Chitin is an important structural component of fungal cell walls and this is an effective biosorbent for radionuclides, as are chitosan and other chitin derivatives (Tobin et al., 1994). In *Rhizopus arrhizus*, U biosorption involves coordination to the amine N of chitin, adsorption in the cell wall chitin structure and further precipitation of hydroxylated derivatives (Tsezos and Volesky, 1982). Fungal phenolic polymers and melanins possess many potential metal-binding sites with oxygen-containing groups including carboxyl, phenolic and alcoholic hydroxyl, carbonyl and methoxyl groups being particularly important (Gadd, 1993b).

Both freely suspended and immobilized biomass from bacterial, cyanobacterial, algal and fungal species have received attention with immobilized systems appearing to possess several advantages including higher mechanical strength, easier biomass/liquid separation and, for living cells, increased metal tolerance (Macaskie and Dean, 1989; Brierley, 1990; Macaskie,

1991; Tobin et al., 1994). 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 (Gadd, 2001; Macaskie and Dean, 1989; Gadd and White, 1990, 1993; Schiewer and Volesky, 2000).

3.2. Metal-binding peptides, proteins, polysaccharides and other biomolecules

A range of specific and nonspecific metal-binding compounds are produced by microorganisms. Nonspecific metal-binding compounds range from simple organic acids and alcohols to macromolecules, such as polysaccharides, humic and fulvic acids (Birch and Bachofen, 1990; Beech and Cheung, 1995; Bridge et al., 1999; Sayer and Gadd, 2001). Extracellular polymeric substances (EPS), a mixture of polysaccharides, mucopolysaccharides and proteins (Zinkevich et al., 1996), are produced by bacteria, algae and fungi and also bind potentially toxic metals (Beech and Cheung, 1995; White and Gadd, 1998a). Extracellular polysaccharides can also adsorb or entrap particulate matter such as precipitated metal sulfides and oxides (Flemming, 1995; Vieira and Melo, 1995). One process uses floating cyanobacterial mats to remove metals from waters, the metal-binding process being due to large polysaccharides (>200,000 Da; Bender et al., 1994).

Specific, low molecular weight (6000–10,000 Da) metal-binding proteins, termed metallothioneins, are produced by animals, plants and microorganisms in response to the presence of toxic metals (Howe et al., 1997). Other metal-binding proteins, phytochelatins and related peptides, contain glutamic acid and cysteine at the amino-terminal position, and have been identified in plants, algae and several microorganisms (Rauser, 1995). Eukaryotic metallothioneins and other metal-binding peptides have been expressed in *E. coli* as fusions to membrane or membrane-associated proteins such as LamB, an outer membrane protein. Such in vivo expression of metallothioneins provides a means of designing biomass with specific metal-binding properties (Valls et al., 1998; Pazirandeh et al., 1998; Chen et al., 1999).

3.3. Metal precipitation by metal- and sulfate-reducing bacteria

Where reduction of a metal to a lower redox state occurs, mobility and toxicity may be reduced offering bioremediation applications (Lovley, 2001; Finneran et al., 2002). Such processes may also accompany other indirect reductive metal precipitation mechanisms, e.g., in sulfate-reducing bacterial systems, where reduction of Cr(VI) can be a result of indirect reduction by Fe2+ and the produced sulfide. Aerobic or anaerobic reduction of Cr(VI) to Cr(III) is widespread in microorganisms (Smith and Gadd, 2000; McLean and Beveridge, 2001) and both ex situ reactor systems and in situ treatment approaches have been documented (Lovley and Coates, 1997; Wang, 2000). U(VI) can be reduced to U(IV) by certain Fe(III)-dissimilatory microorganisms and this reduction in solubility can be the basis of U removal from contaminated waters and leachates (Lovley and Coates, 1997; Lovley, 2001; Finneran et al., 2002). Sulfur and sulfate-reducing bacteria are geochemically important in reductive precipitation of toxic metals, e.g., U(VI) and Cr(VI), a process mediated by multiheme cytochrome *c* proteins (Aubert et al., 1998). *Desulfovibrio desulfuricans* can couple the oxidation of a range of electron donors to reduction of Tc(VII), which is precipitated as an insoluble oxide at cell peripheries. Resting cells, immobilized in a flow-through membrane bioreactor accumulated substantial quantities of Tc when supplied with formate as electron donor, suggesting potential of this organism for treatment of Tc-contaminated wastewater (Lloyd et al., 1999a,b). *D. desulfuricans* can also reduce Pd(II) to cell-bound Pd(0) with hydrogen-dependent reduction being O_2-insensitive thus providing a means of Pd recovery under oxic conditions (Lloyd and Macaskie, 1998). Some sulfate-reducing bacteria, like *Desulfotomaculum reducens*, share physiological properties of both sulfate- and metal-reducing groups of bacteria, and can grow with Cr(VI), Mn(IV), Fe(III) and U(IV) as sole electron acceptors (Tebo and Obraztsova, 1998).

Se(VI) reduction to elemental insoluble Se(0) has been employed to remediate contaminated waters and soils. In situ removal of SeO_4^{2-}, by reduction to Se^o, by sediment bacteria in agricultural drainage regions of Nevada has also been described (Oremland et al., 1990, 1991). Flooding of exposed sediments at Kes-

terson Reservoir with water (to create anoxic conditions) resulted in reduction and immobilization of large quantities of selenium that was present in the sediment (Long et al., 1990). Some bacteria can use such reduction to support growth, making this a natural process for in situ applications. Although 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 enough energy for growth and metabolism. Their reduction can be coupled to a variety of 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 (Stolz and Oremland, 1999; Oremland and Stolz, 2000). 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 (Gharieb et al., 1999).

Sulfate-reducing bacteria oxidize organic compounds or hydrogen coupled with the reduction of sulfate, producing sulfide (White and Gadd, 1996a,b, 1997). The solubility products of most heavy metal sulfides are very low, in the range of 4.65×10^{-14} (Mn) to 6.44×10^{-53} (Hg) 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 (White and Gadd, 1998a,b). Sulfate-reducing bacteria can also create extremely reducing conditions which can chemically reduce metals such as uranium(VI) (White and Gadd, 1998b). 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. Sulfate reduction can provide both in situ (Uhrie et al., 1996) and ex situ metal removal from contaminated wastes (Barnes et al., 1991, 1994; Hammack and Edenborn, 1992; Lyew et al., 1994; Christensen et al., 1996) and contribute to removal of metals and acidity in artificial and natural wetlands

(Hedin and Nairn, 1991; Perry, 1995). A process integrating bacterial sulfate reduction with bioleaching by sulfur-oxidizing bacteria has been developed to remove contaminating toxic metals from soils. In this process, sulfur- and iron-oxidizing bacteria were employed to liberate metals from soils by the breakdown of sulfide minerals and production of sulfuric acid (White and Gadd, 1996a,b, 1997; White et al., 1998). Metals are liberated in the form of an acid sulfate solution which enables both a large proportion of the acidity and almost the entirety of the metals to be removed by bacterial sulfate reduction (White and Gadd, 1997; White et al., 1998). Sulfate-reducing bacterial biofilm reactors may offer a means of process intensification and entrap or precipitate metals, e.g., Cu and Cd, at the biofilm surface (White and Gadd, 1998a, 2000). Mixed sulfate-reducing bacterial cultures were more effective than pure cultures with metal removal being enhanced by the production of exopolymers (White and Gadd, 1998a, 2000).

3.4. Bacterial and fungal oxidation

Bacterial Fe oxidation is ubiquitous in environments with sufficient Fe^{2+} and conditions to support bacterial growth, such as drainage waters and tailings piles in mined areas, pyritic and hydric soils (bogs and sediments), drain pipes and irrigation ditches, and plant rhizospheres. Iron-oxidizers found in acidic soil environments are acidophilic chemolithotrophs, such as *T. ferrooxidans*, significant for its role in generating acid mine drainage (Ewart and Hughes, 1991). 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 millimeters thick, found on rocks and in soils of arid and semiarid regions, and is believed to be of fungal and bacterial origin.

3.5. Phosphatase-mediated metal precipitation

In this process, metal or radionuclide accumulation by bacterial biomass is mediated by a phosphatase which liberates inorganic phosphate from a supplied organic phosphate donor molecule, e.g., glycerol 2-phosphate. Metal/radionuclide cations are then pre-

cipitated as phosphates on the biomass (Macaskie and Dean, 1989; Macaskie, 1991; Yong and Macaskie, 1995). Most work has been carried out with a *Citrobacter* sp., and a range of bioreactors, including those using immobilized biofilms, have been described (Macaskie et al., 1994; Tolley et al., 1995). Zirconium was mineralized by a *Citrobacter* sp. to a mixture of $Zr(HPO_4)_2$ and hydrated zirconia (ZrO_2): biomineralization of uranium as HUO_2PO_4 was repressed by zirconium in the presence of excess inorganic phosphate. Cell-bound HUO_2PO_4 facilitated Ni^{2+} removal by intercalative ion exchange into the polycrystalline lattice and also promoted Zr removal (Brasnakova and Macaskie, 1999).

3.6. Oxalates and carbonates

Calcium oxalate is the most common form of oxalate encountered in the environment, mostly occurring as the dihydrate (weddellite) or the more stable monohydrate (whewellite; Gadd, 1999). 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 (Gadd, 1999; Gharieb et al., 1998). This has an important influence on biogeochemical processes in soils, acting as a reservoir for calcium, 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, Co, Cu, Mn, Sr and Zn (White et al., 1997; Gadd, 1999; Sayer et al., 1999).

In many arid and semiarid 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 (Verrecchia and Dumont, 1996; Gadd, 1999). Mineralized carbonate precipitates are also found in association with bacterial biofilms (Glasauer et al., 2004).

4. Concluding remarks

Microorganisms play important roles in the environmental fate of toxic metals and metalloids with a multiplicity of physicochemical and biological mechanisms effecting transformations between soluble and insoluble phases. Although the biotechnological potential of most of these processes has only been explored at 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 metal extraction but has also been applied to treatment of contaminated soil. 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 sulfides has achieved large-scale application. Continued exploitation of other biological processes will undoubtedly depend on a number of scientific, economic and political factors. Finally, it should be emphasised that this area of research also provides understanding of the biogeochemistry of metal(loid) cycling in the environment and the central role of microorganisms in affecting metal mobility and transfer between different biotic and abiotic locations.

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