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The Role of Microorganisms in Biosorption of Toxic Metals and Radionuclides

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ABSTRACT

A multiplicity of physico-chemical and biological mechanisms determine the removal of toxic metals, metalloids and radionuclides from contaminated wastes. Physico-chemical mechanisms of removal, which may be encompassed by the general term "biosorption", include adsorption, ion exchange and entrapment which are features of living and dead biomass as well as derived products. In living cells, biosorption can be directly and indirectly influenced by metabolism. Metabolism-dependent mechanisms of metal removal which occur in living microorganisms include metal precipitation as sulphides, complexation by siderophores and other metabolites, sequestration by metal-binding proteins and peptides, transport and intracellular compartmentation. In addition, transformations of metal species can occur resulting in oxidation, reduction or methylation. For metalloids such as selenium, two main transformation mechanisms are the reduction of oxyanions to elemental forms, and methylation to methylated derivatives which are volatilized. Such mechanisms are important components of natural biogeochemical cycles for metals and metalloids as well as being of potential application for bioremediation.

INTRODUCTION

Pollution of the environment by toxic metals and radionuclides arises as a result of many activities, largely industrial, although sources such as agri-

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culture and sewage disposal also contribute. These pollutants are discharged or transported into the atmosphere and aquatic and terrestrial environments mainly as solutes or particulates and may reach high concentrations, especially near the site of entry. Metallic radionuclides have also entered the environment as a result of weapons-testing and accidents such as Chernobyl (Elstner *et al.*, 1987). The effects of metals on the functioning of ecosystems vary considerably and are of economic and public-health significance. Consequently environmental awareness is growing among consumers and industrialists and legal constraints on emissions are increasingly strict, leading to a need for cost-effective emission control (Gadd, 1992*a*).

Once in the environment, metals may undergo transformation, either into various mobile forms and/or immobilization in an environmental sink. Biological activity accounts for a large number of the environmental sinks for toxic metals whether derived from natural or anthropogenic sources. Essential metals, e.g. K, Ca, Mg, Cu, Zn, Fe, Co, Mn, and those with no essential biological function, e.g. Cs, Cd, Pb, Al, Sn, Hg, can be accumulated by microorganisms by non-specific physico-chemical interactions as well as specific mechanisms of sequestration or transport (Fig. 1) (Rosen & Silver, 1987; Gadd, 1988, 1992*a*,*b*, 1993*a*; Mullen *et al.*, 1989; Walker *et al.*, 1989; Beveridge, 1989*a*,*b*; Beveridge & Doyle, 1989; Silver, 1992; Misra, 1992). This article reviews the roles, both physico-chemical and biological, played by microorganisms in the removal of toxic metals, metalloids and radionuclides from contaminated wastes. The fundamental mechanisms underlying metal removal are outlined with brief discussion of their biotechnological development potential. The authors have published several other detailed accounts of this topic elsewhere (Gadd, 1988, 1990, 1992*a*,*b*,*c*, 1993*a*,*b*; Avery *et al.*, 1992; Gadd & White, 1989*a*; Tobin *et al.*, 1994).

BIOSORPTION

Although the term "biosorption" is frequently employed for the range of processes by which biomass removes metals and other substances from solution, it can also be used in a stricter sense to mean uptake by living or dead biomass via physico-chemical mechanisms such as adsorption or ion exchange although, in living biomass, metabolic processes may also influence and/or contribute to the process. In this article, we will attempt to adhere to this definition and processes which wholly rely on metabolism, or metabolically-derived products, will be dealt with in separate sections.

Biosorption by freely-suspended living or dead biomass

Because of metal toxicity, most living cell systems exploited to date have been used for decontamination of effluents containing metals at concentrations below toxic levels. These may employ a mixture of microorganisms as well as higher plants. The "meander" system used in the Homestake lead mine (Missouri) passes effluents containing Pb, Cu, Zn, Mn, Ni, Fe and Cd through channels containing cyanobacteria, algae and higher plants. Metals are removed from the water column with an efficiency >99% (Jennett & Wixson, 1983; Ehrlich & Brierley, 1990). Such complex systems clearly utilize other mechanisms, such as precipitation and entrapment of particulates in addition to biosorption, all of which concentrate the metals in the sediment in forms with greatly reduced environmental mobility and biological availability.

Fungi and yeasts have received attention in connection with metal biosorption particularly because waste fungal biomass arises as a by-product from several industrial fermentations (Tsezos & Volesky, 1981; Ehrlich & Brierley, 1990; Volesky, 1990; Tobin *et al.*, 1994), while algae (including macroalgae) can be viewed as a renewable source of metal-sorbing biomass (Gadd, 1988; Volesky, 1990). Of related interest is the removal of metal-containing particulates, e.g. zinc dust, magnetite and metal sulphides, from solution by fungal biomass including citric acid fermentation waste (*Aspergillus niger*) (Wainwright *et al.*, 1990; Singleton *et al.*, 1990). This process is independent of metabolism, but is favoured by growth, particles becoming entrapped within the hyphal mesh. The adsorption of magnetite enabled magnetic separation of loaded biomass from the suspending medium (Wainwright *et al.*, 1990).

Biosorption by immobilized living or dead biomass

Freely-suspended microbial biomass has disadvantages that include small particle size, low mechanical strength and difficult biomass/effluent separation. Immobilized biomass particles in packed- or fluidized-bed reactors minimize these disadvantages (Macaskie & Dean, 1989; Gadd & White, 1990). Immobilized living biomass has mainly taken the form of biofilms on supports made of a range of inert materials. These have been used in a variety of bioreactor configurations including rotating biological contactors, fixed bed reactors, trickle filters, fluidized beds and air-lift bioreactors (Gadd, 1988). Reactor types can be mixed, e.g. the use of biofilm reactors in conjunction with a tandem stirred-tank reactor (Shumate & Strandberg, 1985). Living cell biofilms may provide an additional capacity for the removal of other pollutants including hydro-

carbons, pesticides and nitrates. A large-scale commercial process ($>5 \times 10^6$ gallons per day) treats effluents from gold mining and milling using rotating disc biofilm-contacting units for simultaneous degradation of cyanide, thiocyanate and ammonia as well as metal removal by biosorption (Hutchins *et al.*, 1986).

As well as biofilms, living or dead biomass of all groups has been immobilized by encapsulation or cross-linking (Brierley, 1990*a*; de Rome & Gadd, 1991; Hutchins *et al.*, 1986; Tobin *et al.*, 1994). Supports include agar, cellulose, alginates, cross-linked ethyl acrylate–ethylene glycol dimethylacrylate, polyacrylamide, silica gel and the cross-linking reagents toluene diisocyanate and glutaraldehyde. The biomass may be used in its "natural state" or modified, e.g. by alkali treatment, to improve biosorption efficiency (Hutchins *et al.*, 1986; Brierley, 1990*c*). Small-scale systems may be adequate for low volume waste containing valuable elements, e.g. Au. In order to use conventional reactor technology in larger systems, immobilized biomass particles should have similar properties to other commercial adsorbents, for example, in size (0·5–1·5 mm diameter), particle strength and chemical resistance. Diffusion into particles may present a problem, so they also require high porosity, hydrophilicity with a maximum amount of biomass and minimal amounts of binding agent (Tobin *et al.*, 1988; Tsezos *et al.*, 1989). Waste *Bacillus* sp. from industrial fermentations has been treated with alkali to enhance metal uptake, chemically cross-linked, pelleted by extrusion or milling and dried to provide an indefinite shelf life (Brierley, 1990*c*). This granulated *Bacillus* preparation is non-selective, removing Cd, Cr, Cu, Hg, Ni, Pb, U and Zn (singly or mixed) at a range of concentrations. Metal loadings are up to >10% of the dry weight, giving a removal efficiency of >99% and effluents with total metal concentrations around 10–50 ppb. The biosorbent can be used in fixed- or fluidized-bed contactors depending on the scale of process required. After loading, metals are stripped from the biomass using sulphuric acid, sodium hydroxide or complexing agents and recovered using chemical methods. The granules are regenerated by alkali treatment for re-use (Brierley *et al.*, 1989).

Immobilized particles containing *Rhizopus arrhizus* biomass of 0·7–1·3 mm diameter contained ~12–33% added polymer with improved uranium removal at lower polymer contents and lower particle diameters (Tsezos & Deutschmann, 1990). Complete uranium removal was possible from dilute uranium ore bioleaching solutions (<300 mg/l) with eluate concentrations after desorption being >5000 mg/l. The particles maintained full loading capacity (~50 mg U/g) over multiple biosorption–desorption cycles (Tsezos & Deutschmann, 1990).

Fluidized beds of alginate- and polyacrylamide-immobilized algae, e.g.

Chlorella vulgaris and *Spirulina platensis* have been used to remove a variety of metals, including Cu, Pb, Zn and Au, from mixtures and several schemes for selective recovery have been devised (Volesky, 1990; Harris & Ramelow, 1990). Alginate and polyacrylamide provide good resistance to hydrostatic pressure and mechanical degradation although it is thought that polyacrylamide is not strong enough for commercial applications (Tsezos & Deutschmann, 1990). AlgaSORB™ contains algal biomass immobilized in a silica matrix which is used in batch or column systems. Columns are slurry-packed with immobilized algal particles, 40–100-mesh size. Selective metal recovery is by treatment with appropriate reagents after which the regenerated biomass retains approximately 90% of the original metal uptake efficiency even after >18 months of regular use. AlgaSORB™ has been successfully used for the removal of Ag, Al, Au, Co, Cu, Cr, Hg, Ni, Pb, Pd, Pt, U and Zn from contaminated effluents and process streams (Bedell & Darnall, 1990). BIO-FIX is a biosorbent using biomass from a number of origins including cyanobacteria (*Spirulina*), yeast, algae and plants (*Lemna* sp. and *Sphagnum* sp.) with xanthan and guar gums, blended to give a consistent product and immobilized as beads using polysulfone. The loading of Zn^{2+} obtained is approximately four-fold that attained by an ion-exchange resin. The affinity series is Al > Cd > Zn > Mn with Ca and Mg very much lower giving good discrimination. Metal ions are eluted using hydrochloric or nitric acids and can be reused for more than 120 extraction–elution cycles (Brierley, 1990*b*).

SULPHIDE PRECIPITATION

Hydrogen sulphide is produced by sulphate-reducing bacteria, e.g. *Desulfovibrio* and/or *Desulfotomaculum* sp. The solubility products of most metal sulphides are extremely low and they are readily precipitated as sulphides, e.g. ZnS, CdS, CuS and FeS. Sulphate-reducing activity can occur as a result of anaerobic biomass decay in biosorption systems, forming a useful auxiliary metal-removing mechanism (Brierley, 1990*b*). A purpose-designed $9\,m^3$ stainless steel sludge-blanket reactor using sulphate-reducing bacteria has been piloted by Shell Research Ltd and Budelco B.V. (Barnes, 1990). This plant successfully removed toxic metals and sulphate from contaminated groundwater at a long-standing smelter site by precipitation as metal sulphides, yielding outflow metal concentrations below the ppb range (Barnes *et al.*, 1991). A detailed analysis of this process including mass-balance was also carried out (Barnes *et al.*, 1992). The process has since been expanded to a commercial pilot scale using an

1800 m^3 concrete reactor built by Parques B.V. (Netherlands) at a cost of 33 million guilders for the entire installation. This plant is capable of treating 7000 m^3/day and has been in operation since 30 October 1992). In another pilot-plant study carried out by the US Bureau of Mines, a 45001 reactor using spent mushroom compost as substrate and support for sulphate-reducing bacteria removed 95% of the metals and 20% of the sulphate from 19·3 m^3 of coal mine drainage waters (Dvorak *et al.*, 1992).

SIDEROPHORES

Siderophores are low molecular weight Fe(III) coordination compounds that are excreted under iron-limiting conditions by microorganisms, particularly bacteria and fungi, to enable accumulation of iron from the environment. Although virtually specific for Fe(III), siderophores and analogous compounds can complex certain other metals, e.g. Ga(III), Cr(III), Sc, In, Ni, U and Th (Premuzic *et al.*, 1985; Hausinger, 1987; Macaskie & Dean, 1990). Plutonium can also be complexed by siderophores and a potential for actinide treatment has been suggested particularly as Pu(IV) and Fe(III) [and Pu(VI) and Th(IV)] exhibit similar chemical properties (Birch & Bachofen, 1990). Cyanobacteria and algae may also produce siderophores, and analogous compounds, under iron deprivation which are capable of complexing, e.g. copper (Jardim & Pearson, 1984; Clarke *et al.*, 1987). Although most applied speculation has been directed towards Ga(III) sequestration, there seems to be relatively little progress in siderophore use for metal removal/recovery from industrial effluents.

CELL WALL COMPONENTS

Microbial exopolymers that have received most attention in relation to metal binding are those which form capsules or slime layers. Most of these are composed of polysaccharide, glycoproteins and lipopolysaccharide which may be associated with protein. Potential interactions of metals with these have been reviewed (Geesey & Jang, 1990). Generally, a correlation exists between high anionic charge and metal complexing capacity. Additionally, there may be deposition of metal in a chemically-altered form (Beveridge & Doyle, 1989).

The cell walls of bacteria also have several metal-binding components which contribute to the biosorption processes already described. The carboxyl groups of the peptidoglycan are the main binding site in Gram

positive cell walls with the phosphate groups contributing significantly in Gram negative organisms (McLean & Beveridge, 1990). Many fungi have a high chitin content in cell walls, and this polymer of *N*-acetyl glucosamine is an effective metal and radionuclide biosorbent (Macaskie & Dean, 1990; Tobin *et al.*, 1994). Actinide accumulation in intact biomass appears mainly to comprise metabolism-independent biosorption (Tobin *et al.*, 1987, 1988, 1994), the main site of uptake being the cell wall (Volesky, 1990), although permeabilization of cells with carbonates or detergents can increase uptake (Strandberg *et al.*, 1981; Tsezos & Volesky, 1982; Gadd & White, 1989*a,b,c*; Tsezos, 1983). Chitosan and other chitin derivatives also have a significant biosorptive capability (Wales & Sagar, 1990). Insoluble chitosan–glucan complexes and glucans possessing amino- or sugar acid groups from *Aspergillus niger* exhibit biosorptive properties and effect efficient removal of transition metal ions from solution (Muzzarelli *et al.*, 1986). Melanins are fungal pigments which enhance survival under environmental stress (Bell & Wheeler, 1986). Fungal melanins are located in and/or exterior to cell walls where they may appear as electron-dense deposits and granules. Granules may be released into the external medium and be termed "extracellular melanin" although this is generally of identical composition to wall-associated melanin (Bell & Wheeler, 1986). Fungal phenolic polymers and melanins contain phenolic units, peptides, carbohydrates, aliphatic hydrocarbons and fatty acids and therefore possess many potential metal-binding sites (Senesi *et al.*, 1987; Sakaguchi & Nakajima, 1987). Oxygen-containing groups in these substances, including carboxyl, phenolic and alcoholic hydroxyl, carbonyl and methoxyl groups may be particularly important in metal binding (Gadd & de Rome, 1988). A variety of heavy metals can induce or accelerate melanin production in fungi and melanized cell forms, e.g. chlamydospores, can have high metal uptake capacities for metals and organometallic compounds, e.g. tributyltin chloride (Mowll & Gadd, 1984; Senesi *et al.*, 1987; Gadd & de Rome, 1988; Gadd *et al.*, 1990).

METAL-BINDING PROTEINS AND PEPTIDES

While virtually all biological material has a high affinity for toxic metals and radionuclides, some biomolecules function specifically to bind metals and are induced by their presence. Specific metal-binding proteins and peptides have been recorded in all microbial groups examined, although most detailed work has been carried out with yeasts (Butt & Ecker, 1987; Dameron *et al.*, 1989; Mehra & Winge, 1991).

Metallothioneins are small cysteine-rich polypeptides that can bind

essential metals, e.g. Cu and Zn, as well as non-essential metals like Cd. They mediate copper resistance in *Saccharomyces cerevisiae* (Fogel *et al.*, 1988) and also bind other metals (Mehra & Winge, 1991). Metal γ-glutamyl peptides (phytochelatins) are short peptides involved in heavy metal detoxification in algae, plants and some fungi and yeasts (Mehra & Winge, 1991; Gadd, 1993*a*). Both of these classes of metal-binding peptides may have an application for metal-removal in the future.

MICROBIAL METAL TRANSFORMATIONS

Microorganisms can transform metal and metalloid species by oxidation, reduction, methylation and dealkylation (Gadd, 1993*b*). Continuous cultures of Hg^{2+}-resistant bacteria, which can reduce Hg^{2+} to Hg^0 with mercuric reductase, volatilized Hg^0 from contaminated sewage at a rate of 2·5 mg l/h (98% removal) (Hansen *et al.*, 1984). Many bacteria, algae, fungi and yeasts can reduce Au(III) to elemental Au(0) and Ag(I) to elemental Ag(0) (Kierans *et al.*, 1991). Microbial transformations of arsenic and chromium species are also associated with a decrease in toxicity and may have relevance to wastewater treatment (Williams & Silver, 1984).

Organomercurials may be detoxified by organomercurial lyase, the resulting Hg^{2+} then being reduced to Hg^0 by mercuric reductase (Silver *et al.*, 1989; Gadd, 1990). Microbial dealkylation of organometallic compounds such as organotins can result in the formation of ionic species which could possibly be removed using a biosorptive process (Gadd, 1993*b*). Biomethylated metal derivatives, although toxic, are often volatile and may be eliminated from a system by evaporation (Trevors, 1986).

A taxonomically-diverse group of heterotrophic bacteria utilize metallic cations as terminal electron acceptors under anaerobic conditions. In the process, the metal is reduced to a lower valency state. There are several metallic elements that possess multiple valency states and which can potentially be utilized in this way by microorganisms. However, unlike sulphate-reduction, technology has not been developed to date which employs these processes, despite the microbial reduction of elements such as Fe and Mn being of great environmental significance (Lovley, 1993). Fe(III) and Mn(IV) appear to be the most commonly utilized metals as terminal electron acceptors in the biosphere and metal-reducing organisms from many habitats frequently utilize both of these metals. However, since the solubility of both Fe and Mn is increased by bacterial reduction, and neither metal poses a major toxic threat, the most potentially important dissimilatory metal reduction reactions for biotechnological treatment of toxic metal pollution utilize other metals. Molybdenum(VI) was reduced

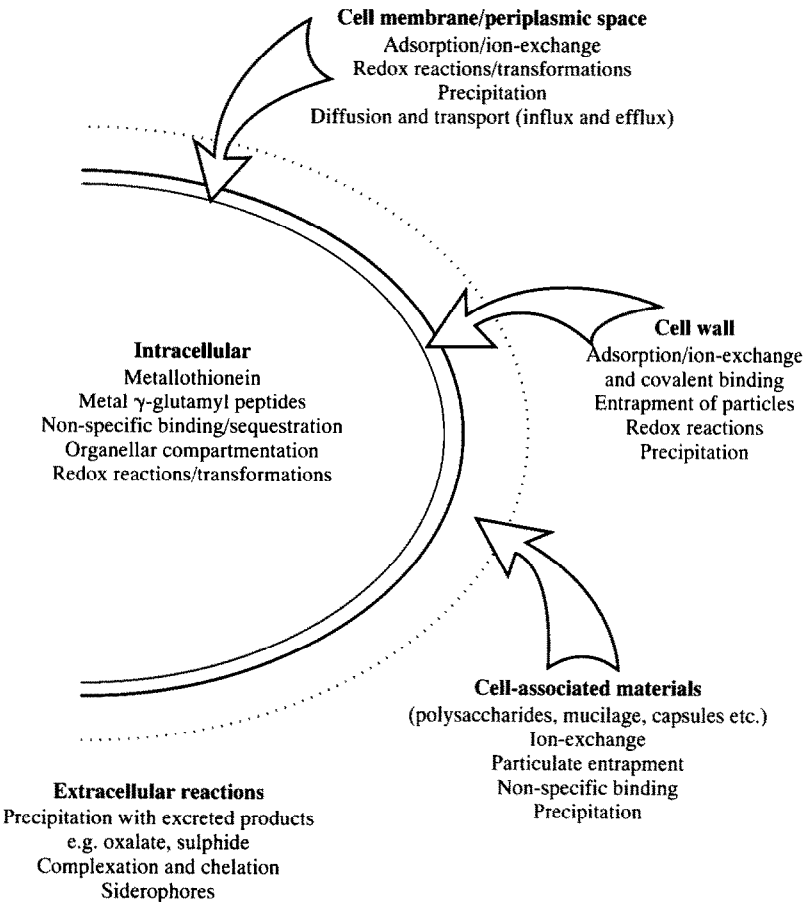


Fig. 1. Diagram representing the processes contributing to microbial uptake and detoxification of toxic metals. The localization of some of the processes, especially enzymic and redox reactions, is uncertain and postulated locations are indicated. These may vary between groups, strains and species of both prokaryotic and eukaryotic organisms (taken from Gadd & White, 1993, with permission).

to molybdenum blue by a strain of *Enterobacter cloacae* which was isolated from a molybdate-polluted aquatic environment (Ghani *et al.*, 1993). Another strain of this organism, also isolated from a polluted habitat, was able to reduce Cr(VI) to Cr(III) under similar conditions (Wang *et al.*, 1989). The fact that these strains were isolated from polluted waters may indicate that natural waters do not contain sufficient chromate or molybdate to support similar organisms and that this type of metabolism is an adaptation specifically exploiting environmental pollution by these oxyanions. Dissimilatory Cr(VI) reduction was also carried out by a strain of *Escherichia coli*, which was unusual in that it apparently reducd Cr(VI) as

a terminal electron acceptor under anaerobic conditions and also under aerobic conditions, albeit at a slower rate (Shen & Wang, 1993). A strain of *Shewanella* (*Alteromonas*) *putrefaciens* which reduced Fe(III) and Mn(IV) also reduced U(VI) to U(IV) (Lovley *et al.*, 1991). Due to the low solubility of U(IV), the reaction was accompanied by the formation of a black precipitate of U(IV) carbonate. When the organisms were contained by dialysis tubing, the precipitate was associated with the organisms, indicating that it was the result of an enzymatic reaction rather than reduction by reduced products (Gorby & Lovley, 1992). U(VI) was also reduced by the sulphate-reducing bacterium *Desulfovibrio desulfuricans* utilizing a mechanism that involved the electron transport chain although the organism could not utilize U(VI) as an electron acceptor for growth (Lovley & Phillips, 1992).

MICROBIAL METALLOID TRANSFORMATIONS

Two major transformation processes have been described for metalloids:

(i) reduction of metalloid oxyanions to elemental forms, e.g. SeO_4^{2-} and SeO_3^{2-} to Se^0;
(ii) methylation of metalloids, metalloid oxyanions or organometalloids to methyl derivatives, e.g. AsO_4^{3-}, AsO_2^- and methylarsonic acid to $(CH_3)_3As$ (trimethylarsine).

Transformation processes have geochemical significance, since they may modify the mobility and toxicity of metalloids, as well as being of biotechnological potential in bioremediation (Lovley, 1993; Karlson & Frankenberger, 1993; Gadd, 1993*a*,*b*). Reduction of SeO_4^{2-} and SeO_3^{2-} (both toxic) to elemental selenium (Se^0) results in immobilization and detoxification (Oremland *et al.*, 1990). Methylation of arsenic and selenium compounds results in volatilization (Tamaki & Frankenberger, 1992; Karlson & Frankenberger, 1993).

Microbial reduction of metalloid oxyanions

Microbially-mediated reduction of selenate [Se(VI)] and selenite [Se(IV)] to elemental selenium is well known and numerous bacterial species are known to carry out this process (Lovley, 1993). Maiers *et al.* (1988) described reduction of SeO_4^{2-} to elemental selenium, resulting in a red precipitate, by microbial populations isolated from water, sediment and soil from the selenium-rich Kesterson Reservoir in California. Microbial reduction of 100 mg Se/l (1·3 mmol Se/l) was

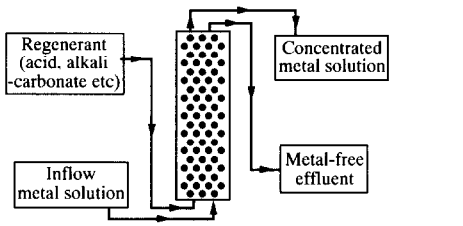
effected within 1 week of incubation. Up to 75 mg Se/l (0·9 mmol Se/l) was reduced to Se^0. *Wolinella succinogenes*, adapted to grow in SeO_4^{2-} and SeO_3^{2-} at concentrations up to 10 mmol/l, was able to reduce these oxyanions to Se^0, but only during the stationary phase of growth (Tomei *et al.*, 1992). Bacterial cells treated with SeO_4^{2-} or SeO_3^{2-} contained electron dense granules in the cytoplasm which were verified by energy dispersive X-ray analysis to consist of Se^0. Similar electron dense bodies, also consisting of Se^0, were found both inside and outside cells of *Pseudomonas maltophila* O-2, a strain isolated from a toxic waste site, following incubation in medium containing 40 mmol/l SeO_3^{2-} (Blake *et al.*, 1993), although it was thought that the presence of extracellular crystals was due to lysis of cells. Reduction of SeO_3^{2-} was inhibited by the addition of buthionine sulfoximine (BSO), a well-known inhibitor of reduced glutathione (GSH) biosynthesis, suggesting that GSH was involved in the reduction process. Steinberg and Oremland (1990) measured bacterial reduction of $^{75}SeO_4^{2-}$ to $^{75}Se^0$ in numerous sediment types. Potential rates of SeO_4^{2-} reduction ranged from 0·07 to 22 μmol SeO_4^{2-} reduced/l/h. NO_3^-, NO_2^-, MoO_4^{2-} and WO_4^{2-} inhibited SeO_4^{2-} reduction in some sediments, while SO_4^{2-} partially inhibited reducing activity in freshwater samples. Certain organisms are known to use SeO_4^{2-} as an electron acceptor to support growth. Oremland *et al.* (1989) reported oxidation of acetate, with concomitant reduction of SeO_4^{2-} to Se^0, by bacteria isolated from anoxic sediments. SeO_4^{2-} reduction was inhibited by WO_4^{2-} and CrO_4^{2-} but not by MoO_4^{2-}. The authors proposed the following mechanism for SeO_4^{2-} reduction (1):

$$4\,CH_3COO^- + 3\,SeO_4^{2-} \rightarrow 3\,Se^0 + 8\,CO_2 + 4\,H_2O + 4\,H^+. \quad (1)$$

It was not clear, however, whether reduction occurred in a single step or via SeO_4^{2-}. Macy *et al.* (1989) reported that a *Pseudomonas* sp. was able to respire SeO_4^{2-} to SeO_3^{2-}, with oxidation of ^{14}C-labelled acetate to $^{14}CO_2$ by the mechanism given in equation (2):

$$CH_3COO^- + H^+ + 4\,SeO_4^{2-} \rightarrow 2\,CO_2 + 4\,SeO_3^{2-} + 2\,H_2O. \quad (2)$$

Another isolate, a strict anaerobe, was able to reduce SeO_3^{2-} to Se^0, though it was not able to reduce SeO_4^{2-}. Co-culture of these organisms resulted in reduction of SeO_4^{2-} to Se^0. More recently, a novel species, *Thauera selenatis*, has been isolated that is capable of anaerobic SeO_4^{2-} respiration to SeO_3^{2-} with concomitant reduction of NO_3^- (Macy *et al.*, 1993*a*,*b*; Macy & Lawson, 1993). SeO_3^{2-} formed from this reduction was further reduced to Se^0, though reduction of SeO_3^{2-} to Se^0 did not support growth. Mutants of *T. selenatis* lacking nitrite



Packed-bed reactor

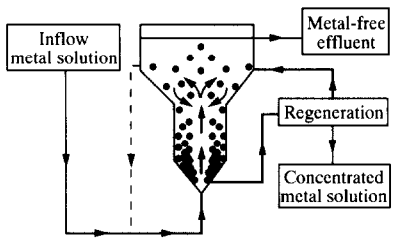
Process: biosorption

Biomass: immobilized

Regeneration: *in-situ*

Scale: up to 20kg biomass
(but modular construction is possible)

Commercial applications: AMT-Bioclaim™, Algasorb, Bio-fix



Fluidized-bed reactor

Process: biosorption,
current applications use non-living material

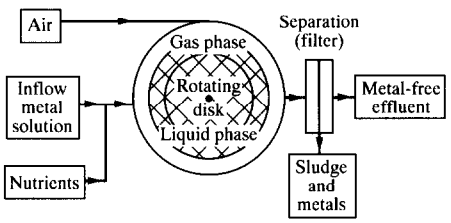
Biomass: immobilized

Regeneration: separation cycle
(can use pulsed removal)

Scale: 80–90kg biomass

Commercial application: AMT-Bioclaim™

Key: • • • Biomass particles
—► Inflow, outflows
-►- Recirculation
—► Biomass regeneration



Rotating-disk reactor

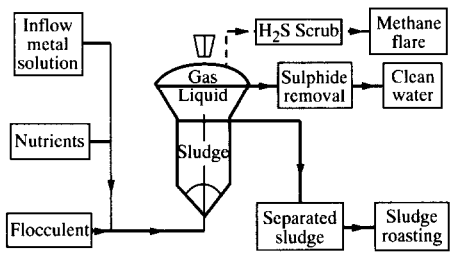
Process: biosorption, precipitation and aerobic transformations (and degradation of organic wastes)

Biomass: biofilm

Regeneration: none, metals are removed during biofilm sloughing

Scale: = 2.5 x 10^4 m^3 day^{-1}

Commercial application: Homestake mine biological treatment process



Sludge-blanket bioreactor

Process: biosorption;
sulphide precipitation (anaerobic)

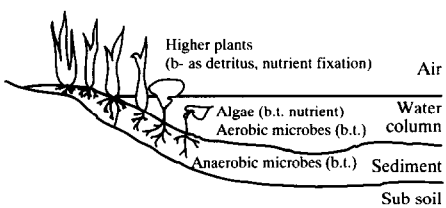
Biomass: flocculated sludge

Regeneration: none

Scale: potentially large

Commercial application: Shell-Budelco process

Key: -►- Gas
—► Liquid
—► Sludge



Artificial wetlands/Stream meanders

Process: biosorption (b), biotransformation (t), sulphide-precipitation (s)

Biomass: naturally occurring plants and microorganisms

Regeneration: none (metals are immobilized in sediment)

Scale: very large

Commercial application: Meander system Missouri lead mine

Fig. 2. Simplified diagrams of some biotechnological processes currently in use for alleviating toxic metal pollution (taken from Gadd & White, 1993, with permission).

reductase activity were unable to reduce NO_2^- and SeO_3^{2-}, while those with higher nitrate reductase activity than the wild-type strain were capable of greater levels of NO_2^- and SeO_3^{2-} reduction. The authors suggested that the periplasmic nitrite reductase was responsible for SeO_3^{2-} reduction; cytochrome C551 may be a component of this enzyme, though levels of C551 in mutant and wild-type cells were not greatly different (Demolldecker & Macy, 1993). It is generally believed that while SeO_4^{2-} may act as a terminal electron acceptor to support growth of some organisms, SeO_3^{2-} reduction does not support growth and is more likely to be a detoxification mechanism (Lovley, 1993). Reduction of elemental selenium to selenide (Se^{2-}) has been noted in cultures of *Thiobacillus ferroxidans* (Bacon & Ingledew, 1989).

Reduction of TeO_3^{2-} (also toxic) to Te^0 is also an apparent means of detoxification found in bacteria. The genetics of tellurium resistance in Gram negative bacteria has been reviewed (Walter & Taylor, 1992). Uptake of TeO_3^{2-} by resistant cells is followed by reduction to Te^0 which is deposited in or around cells, especially near the cytoplasmic membrane (Bradley *et al.*, 1988; Taylor *et al.*, 1988; Lloyd-Jones *et al.*, 1991, 1994). Blake *et al.* (1993) described TeO_3^{2-} reduction to Te^0 by *P. maltophila* O-2. This process was inhibited by BSO, as was SeO_3^{2-} reduction, indicating that GSH was involved in TeO_3^{2-} reduction. The photosynthetic bacterium *Rhodobacter sphaeroides*, grown photoheterotrophically, reduced TeO_3^{2-} at 100 μg/ml (0·4 mmol/l) to Te^0 (Moore & Kaplan, 1992, 1994). The latter was deposited intracellularly, mainly at the cytoplasmic membrane. TeO_3^{2-} is toxic to most microorganisms at 4 μmol/l (Summers & Jacoby, 1977). The concomitant evolution of H_2 gas from photoheterotrophically-grown cells was observed with reduction of both SeO_3^{2-} and TeO_3^{2-}. Significant levels of $FADH_2$-dependent TeO_3^{2-}-reducing activity were measured in the membrane fraction of *R. sphaeroides* cells (Moore & Kaplan, 1992).

In contrast to bacterial systems, fungal reduction of metalloids has received relatively little attention (see Morley *et al.*, in press). Reduction of SeO_3^{2-} to Se^0 has been observed with *Fusarium* sp. (Ramadan *et al.*, 1988; Gharieb, 1993; Gharieb *et al.*, 1995), *Mortierella* sp. (Zieve *et al.*, 1985), *Saccharomyces cerevisiae*, *Candida albicans* (Falcone & Nickerson, 1963) and *Aspergillus funiculosus* (Gharieb, 1993; Gharieb *et al.*, 1995). Both extracellular and intracellular deposition of Se^0 has been demonstrated. Gharieb *et al.* (1995) showed that numerous filamentous and unicellular fungal species were capable of SeO_3^{2-} reduction to Se^0, resulting in a red colouration of colonies. Less work has been done on TeO_3^{2-} reduction by fungi; Smith (1974) showed that *Schizosaccharomyces pombe* reduced TeO_3^{2-} to Te^0, giving black or grey colonies.

Methylation of metalloids

Microbial methylation of metalloids to yield volatile derivatives such as dimethylselenide or trimethylarsine is a well-known phenomenon (see Karlson & Frankenberger, 1993; Gadd, 1993*a*,*b*). Methyl derivatives of metalloids have a characteristic garlic-like odour. Bacterial species known to produce methyl derivatives of selenium from SeO_4^{2-} and SeO_3^{2-} include *Aeromonas* sp. (Chau *et al.*, 1976), *Bacillus* sp. (Razak *et al.*, 1990) and *Pseudomonas* sp. (Chasteen *et al.*, 1990). Dimethylselenide [$(CH_3)_2Se$] is the most common methylated product. There has been much research into selenium methylation by soil fungi (see Thompson-Eagle *et al.*, 1991; Karlson & Frankenberger, 1993). *Alternaria alternata* methylated inorganic selenium (SeO_4^{2-}, SeO_3^{2-}) more rapidly than organic forms (Se-containing amino acids, purines and pyrimidines) (Thompson-Eagle *et al.*, 1989). Both dimethylselenide and dimethyldiselenide [$(CH_3)_2Se_2$] have been detected as volatile products of fungal selenium methylation. While bacteria and fungi are thought to be important in volatilization of selenium from soils and sediments, bacteria are thought to play a more dominant role in selenium-contaminated waters (Thompson-Eagle *et al.*, 1989). The mechanism for selenium methylation appears to involve transfer of methyl groups as carbonium (CH_3^+) ions via the S-adenosyl methionine system (Gadd, 1993*b*). Much less work has been done on tellurium methylation by fungi (Karlson & Frankenberger, 1993); there is evidence of dimethyltelluride and dimethylditelluride production by *Penicillium* sp. (Huysmans & Frankenberger, 1991). However, it is not clear whether tellurium methylation is a detoxification process, since certain methyl derivatives of tellurium are no less toxic than TeO_3^{2-} (Karlson & Frankenberger, 1993). Several bacterial and fungal species have been shown to 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 [$(CH_3)_3As$] (see Tamaki & Frankenberger, 1992). Methyl derivatives of arsenic are less toxic to bacteria than organic forms. The mechanism for anaerobic biomethylation of AsO_3^- by *Methanobacterium* sp. appeared to consist of reduction to AsO_2^- and subsequent methylation to methylarsonic acid, dimethylarsenic acid and finally dimethylarsine. Vitamin B_{12} was thought to be the methyl group donor (McBride & Wolfe, 1971). Under aerobic conditions, however, fungal methylation of arsenic compounds appears to involve S-adenosylmethionine (see Gadd, 1993*b*). Huysmans & Frankenberger (1991) demonstrated production of dimethyl- and trimethylarsine from methylarsonic acid by *Penicillium* sp., though not from arsenate or arsenite.

Biotechnological applications of microbial metalloid transformations

Removal of selenium (as SeO_4^{2-}) from contaminated water and soil by bacterial and fungal species or populations has been demonstrated. Oremland *et al.* (1990, 1991) described the *in situ* removal of SeO_4^{2-}, by reduction to Se^0, by sediment bacteria in agricultural drainage regions of Nevada. Selenate removal from overlying water was rapid, with real removal rates estimated at up to 155 μmol/m/day, depending on the ambient selenium oxyanion concentration. Experiments using $^{75}SeO_4^{2-}$ showed that 85% of *in situ* SeO_4^{2-} removal occurred in the upper 4–8 cm of sediments, while most SeO_4^{2-} reducing activity was found at depths below 8 cm (Oremland *et al.*, 1990). Flooding of exposed sediments at Kesterson Reservoir with selenium-free water (in order to create anoxic conditions) resulted in reduction (and thereby immobilization) of large quantities of selenium that had been present in sediments (Long *et al.*, 1990). Anoxic conditions must be maintained, however, if selenium is to remain immobilized (Alemi *et al.*, 1988). Microbial methylkation of selenium, resulting in its volatization, has also been used for *in situ* bioremediation of selenium-containing land and water, particularly at Kesterson Reservoir (Thompson-Eagle & Frankenberger, 1992). Selenium-contaminated agricultural drainage water was evaporated to dryness until the sediment selenium concentration approached 100 mg Se/kg dry weight. Conditions such as carbon source, moisture, temperature and aeration were then optimized for selenium volatilization and the process continued until selenium levels in sediments declined to acceptable levels. Atmospheric levels of selenium did not exceed safety limits, and prevailing weather conditions resulted in selenium being blown towards selenium-deficient areas.

Annual costs for implementation of this process have been calculated and range from $227 to $372 per hectare ($92–$151 per acre, respectively), depending on land and water quality (Thompson-Eagle *et al.*, 1991).

The potential for *ex situ* treatment of selenium-contaminated waters has also been demonstrated. SeO_4^{2-} in uranium-mine discharge waters was microbially reduced to Se^0 after passage through a soil column (Kauffman *et al.*, 1986). Removal of SeO_4^{2-} in an algal-bacterial system has also been suggested (Gerhardt *et al.*, 1991). NO_3^-, an inhibitor of SeO_4^{2-} reduction, is removed during algal growth. The water is then treated in an anaerobic digestor. The algal biomass also provides a source of electron donors for the removal of any residual NO_3^- by denitrifying bacteria and for SeO_4^{2-} reduction, and thus precipitation, to Se^0. Macy *et al.* (1993*a*) described the simultaneous removal of SeO_4^{2-} and NO_3^- under anaerobic conditions

from selenium-contaminated drainage water by *Thauera selenatis* in recycled sludge blanket and fluidized bed reactors (1 l working volume). SeO_4^{2-} reduction by *T. selenatis* was not inhibited by NO_3^-, indeed both SeO_4^{2-} and NO_3^- were reduced concomitantly. SeO_4^{2-} levels declined from 350 to 400 μg Se/l (4–5 μmol Se/l) to 5·4 μg Se/l (0·07 μmol Se/l). The final product of the reduction process was Se^0. NO_3^- in drainage water was reduced by 98%.

POTENTIAL FOR FUTURE DEVELOPMENT

Several biotechnological approaches seem to be established as a means of combating toxic metal pollution from industrial and other sources, although none are yet in widespread use. Several design preferences are beginning to emerge within the field with the processes currently in or near to practical operation mainly utilizing biosorption and or bioprecipitation (Figs 1 and 2) as these require the least modification of biological material and can be used in those process technologies which are already well established for ion-exchange or biological treatment of organic wastes. There has also been some attention given to the possibilities of integrating biological metal removal with sewage and other waste treatment (Cowling *et al.*, 1992). A technically simpler approach using artificial wetlands with undefined biota has been found useful for cost-effective treatment of high-volume, low concentration wastes such as mine runoff (Brierley, 1990*b*). In the longer term, the use of purified biopolymers as specific metal binding agents holds out considerable potential (Ashley & Roach, 1990). The application of both genetic and protein engineering could conceivably lead to peptides or other biopolymers with enhanced metal specificity, stability and other useful properties. It is clear that some microbiological methods for the treatment of metal/radionuclide-containing wastes offer potentially efficient and cost-effective alternatives or adjuncts to existing treatment technologies.

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