granules and cell walls an X-ray energy dispersive microanalysis study. J. gen. Microbiol. *131* (1985) 2545–2548.
84 Penrose, W. R., Metta, D. N., Hylko, T. M., and Rinckel, L. A., The reduction of Pu V by aquatic sediments. J. envir. Radioat. *5* (1987) 169–184.
85 Perry, R. D., and Silver, S., Cadmium and manganese transport in *Staphylococcus aureus*. J. Bact. *150* (1982) 973–976.
86 Powell, P. E., Cline, G. R., Reid, C. P. P., Saniszlo, P. J., Occurrence of hydroxamate siderophore iron chelators in soils. Nature (London) *287* (1980) 833–834.
87 Premuzic, E. T., Francis, A. J., Lin, M., and Schubert, J., Induced formation of chelating agents by *Pseudomonas aeruginosa* grown in the presence of thorium and uranium. Archs envir. Contam. Toxicol. *14* (1985) 759–768.
88 Ramamoorthy, S., and Kushner, D. J., Binding of mercuric and other heavy metal ions by microbial growth media. Microb. Ecol. *2* (1975) 162–176.
89 Raspor, B., Nurnberg, H. W., Valenta, P., and Branica, M., Significance of dissolved humic substances for heavy metal speciation in natural waters. Devl Biogeochem. *1* (1984) 317–328.
90 Rayner, M. H., and Sadler, P. J., Cadmium accumulation and resistance mechanisms in bacteria, in: Metal-Microbe Interactions, SGM Special Publication 26, pp. 39–49. Eds R. K. Poole and G. M. Gadd. IRL Press, Oxford 1989.
91 Rogers, H. J., Synge, C., and Woods, V. E., Anti bacterial effect of scandium and indium complexes of enterochelin on *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. *18* (1980) 63–68.
92 Rossotti, J. C., and Rossotti, H., The Determination of Stability Constants. McGraw Hill, New York 1961.
93 Sanchez, A. L., Chemical speciation and adsorption behaviour of plutonium in natural waters. Ph. D. Dissertation, University of Washington, Seattle, Washington 1983.
94 Schlegel, H. G., General Microbiology, Cambridge University Press, Cambridge 1987.
95 Schnitzer, M., Reactions of humic substances with metals and minerals, in: Mineral Exploration: Biological Systems and Organic Matter, pp. 408–427. Eds D. Carlisle, W. L. Berry, I. R. Kaplan and J. R. Watterson. Prentice-Hall, Englewood Cliffs, N.J. 1986.
96 Schnitzer, M., and Khan, S. U., Humic Substances in the Environment. Marcel Dekker, New York 1972.
97 Schreiber, D. R., Gordon, A. S., and Millero, F. J., The toxicity of copper to the marine bacterium *Vibrio alginolyticus*. Can. J. Microbiol. *31* (1985) 83–87.
98 Shaikh, Z., The low molecular weight cadmium-, mercury-, and zinc-binding proteins (Metallothioneins): biosynthesis, metabolism and possible role in metal toxicity, in: Metallothionein, Proceedings of Metallothionein and Other Low Molecular Weight Metal-binding Proteins, pp. 331–337. Birkhäuser, Basel 1979.
99 Shiio, I., and Ujigawa, T. K., Regulation of phosphoenol pyruvate carboxylase EC-4.1.1.31 by synergistic action of aspartate and 2 oxo glutarate. Agric. Biol. Chem. *43* (1980) 2479–2486.
100 Shapiro, J., Effect of yellow organic acids on iron and other metals in water. J. Am. Water Works Ass. *56* (1964) 1062–1082.
101 Shuman, M. S., and Cromer, J. L., Copper association with aquatic fulvic and humic acids. Estimation of conditional formation constants with a titrimetric anodic stripping voltammetry procedure. Envir. Sci. Technol. *13* (1979) 543–545.
102 Sibley, T. H., Clayton, J. R., Wurtz, E. A., Sanchez, A. L., and Alberts, J. J., Effects of dissolved organic compounds on the adsorption of transuranic elements. Devl Biogeochem. *1* (1984) 289–300.
103 Snow, G. A., Mycobactins: Iron chelating growth factors from Mycobacteria. Bact. Rev. *34* (1970) 99–125.
104 Sondergard, M., and Schierup, H.-H., Release of extracellular organic carbon during a diatom bloom in Lake: Molecular weight fractionation. Freshwater Biol. *12* (1982) 313–320.
105 Sposito, G., The chemical forms of trace metals in soil, in: Applied Environmental Geochemistry, pp. 123–170. Ed. I. Thornton. Academic Press, London 1983.
106 Strandberg, G. W., Shumate, S. E., and Parrott, J. R., Microbial cells as biosorbents for heavy metals: accumulation of uranium by *Saccharomycese cerevisiae* and *Pseudomonas aeruginosa*. Appl. envir. Microbiol. *41* (1981) 237–245.
107 Summers, A. O., and Silver, S., Microbial transformations of metals. A. Rev. Microbiol. *32* (1978) 637–672.
108 Sunda, W., and Guillard, R., The relationship between cupric ion activity and the toxicity of copper to phytoplankton. J. mar. Res. *34* (1976) 511–529.
109 Thanabalasingam, P., and Pickering, W. F., The sorption of mercury-II by humic acids. Envir. Pollut. Ser. B., Chem. Phys. *9* (1985) 267–280.
110 Tynecka, Z., Gos, Z., and Zajic, J., Energy-dependent efflux of cadmium coded by a plasmid resistance determinant in *Staphylococcus aureus*. J. Bact. *147* (1981) 313–319.
111 Vangenechten, J. H. D., Chughtai, N., Bierkens, J., and Vanderborght, O., Similarity of $Am^{241}$ and $Fe^{59}$ speciation in selected freshwaters and their adsorption on Crayfish exoskeleton. J. envir. Radioact. *5* (1987) 275–286.
112 Wallace, G. T., Seibert, D. L., Holzknecht, S. M., and Thomas, W. H., The biogeochemical fate and toxicity of mercury in controlled experimental ecosystems. Estuar. Cstl. Shelf Sci. *15* (1982) 151–182.
113 Wirth, R., Taylor, D. M., and Duffield, J., Identification of transferrin as the principal neptunium binding protein in the blood serum of rats. Int. J. nucl. Med. Biol. *12* (1985) 327–330.
114 Wong, P. T. S., Chau, Y. K., and Lucon, P. L., Methylation of lead in the environment. Nature *253* (1975) 263–264.
115 Wood, J. M., and Wang, H. K., Microbial resistance to heavy metals. Envir. Sci. Technol. *17* (1983) 582–590.
116 Yamamoto, M., and Sakanoue, M., Interaction of humic acids and americium III in aqueous solution. J. Radiat. Res. *23* (1982) 261–271.

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## Heavy metal accumulation by bacteria and other microorganisms

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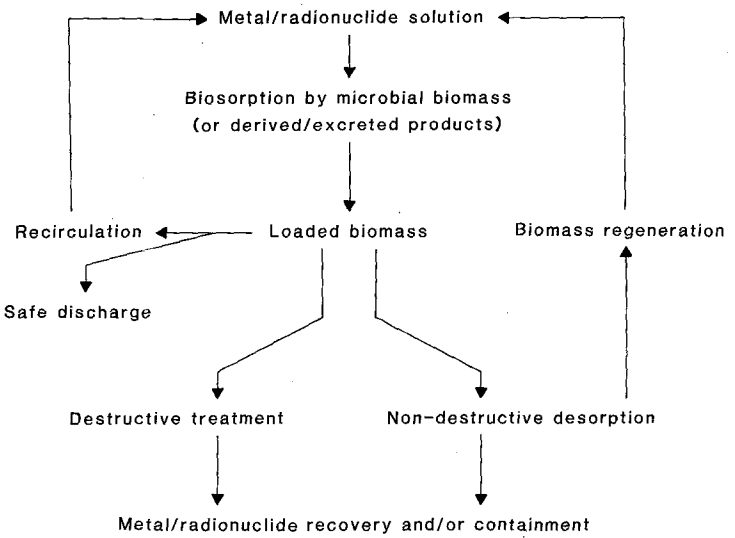
*Summary.* Bacteria, and other microorganisms, exhibit a number of metabolism-dependent and -independent processes for the uptake and accumulation of heavy metals and radionuclides. The removal of such harmful substances from effluents and waste waters by microbe-based technologies may provide an alternative or additional means of metal/radionuclide recovery for economic reasons and/or environmental protection. Both living and dead cells as well as products derived from or produced by microorganisms can be effective metal accumulators and there is evidence that some biomass-based clean-up processes are economically viable. However, many aspects of metal-microbe interactions remain unexploited in biotechnology and further development and application is necessary, particularly to the problem of radionuclide release into the environment.
*Key words.* Heavy metals; radionuclides; microorganisms; bacteria; algae; fungi; yeasts; uptake; accumulation; biosorption.




Outline scheme of metal or radionuclide removal/recovery from aqueous solutions by microbial biomass or derived products.

Bacteria (including actinomycetes), cyanobacteria, algae, fungi and yeasts are able to remove heavy metals and radionuclides from their external environment by means of mechanisms that may be physico-chemical, e.g. adsorption, or dependent on metabolic activity, e.g. transport[2,7,13–16,18,31,34]. Some physico-chemical interactions may be indirectly dependent on metabolism via the synthesis of particular cell constituents or metabolites that may act as efficient metal-chelators or the creation of a particular microenvironment in the vicinity of the cell that facilitates deposition or precipitation[22]. Thus, living or dead microbial biomass is capable of metal accumulation as well as products produced by or derived from microbial cells[14,15,18,31] (fig.).

These processes are of interest and application to a variety of industries, including those concerned with the provision of nuclear power. The removal of harmful heavy metals and radionuclides from effluents and waste waters by microbe-based technologies may provide an additional or alternative means of detoxification to existing treatments while metal recovery is possible after appropriate treatment of loaded biomass. The latter is relevant to the reclamation of valuable metals, e.g. gold, and the further containment of hazardous radionuclides. There are indications that clean-up processes based on microbial technology can be more economical than existing treatments and some are in commercial operation[8,21].

*Mechanisms of microbial metal accumulation*

Although living and dead cells are capable of metal/radionuclide accumulation, there may be differences in the mechanisms involved in either case, depending on the extent of metabolic dependence. Despite many metals being essential for microbial growth at low concentrations, e.g. Cu, Zn, Mn, Co, many others have no essential biological function, e.g. Au, Ag, Cd, Pb, Sn. All these, as well as elements such as uranium and thorium, can exhibit varying degrees of toxicity towards living cells. Thus the choice of living or dead biomass is important for any envisaged process[31]. Problems of toxicity obviously do not occur with dead biomass or derived products and such materials may also be immune to other adverse conditions such as elevated temperature or nutrient limitation. Living cells have advantages in their variety of accumulation mechanisms, and the relative ease of morphological, physiological and genetical manipulation. Certain cell types or forms may be more efficient accumulators than others while resistance is a widely encountered property[15].

A wide range of uptake capacities are found in microorganisms (table). Metabolism-independent binding of heavy metals to cell walls, extracellular polysaccharides, or other materials occurs in living and dead cells and is generally rapid. Metabolism-dependent intracellular uptake or transport occurs in living cells and may be accompanied by toxic symptoms. In some cases intracellular uptake is due to increased membrane permeability arising from toxic interactions. With several metals, e.g. lead, uranium and thorium, most accumulation in microbial biomass is surface-based with little or no intracellular uptake unless by diffusion. Once inside cells, metal ions may be preferentially located within specific organelles and/or bound to proteins such as metallothionein. In growing cultures of microorganisms, metabolism-independent and -dependent phases of metal uptake can be affected by changes in medium composition and excretion of metabolites that can act as metal chelators. Thus, in a given microbial system, several mechanisms of uptake may operate simultaneously and/or sequentially[15].

Accumulation of heavy metals and radionuclides by microorganisms [a]

| Organism | Element | Uptake (% dry weight) |
|---|---|---|
| *Streptomyces* sp.[12,15] | Uranium | 2–14 |
| *Citrobacter* sp.[25,26,27] | Lead | 34–40 |
| | Cadmium | 13.5 |
| *Thiobacillus ferrooxidans*[2,22,31] | Silver | 25 |
| *Bacillus cereus*[2,22,34] | Cadmium | 3.9–8.9 |
| *Escherichia coli*[2,22,34] | Cadmium | 0.16–0.98 |
| *Zoogloea* sp.[2,15,22,31] | Cobalt | 25 |
| | Copper | 34 |
| | Nickel | 13 |
| *Chlorella vulgaris*[b19] | Gold | 10 |
| *Chlorella regularis*[b19] | Uranium | 15 |
| *Chlorella regularis*[28,29] | Uranium | 0.39 |
| | Manganese | $\leq 0.8$ |
| *Scenedesmus obliquus*[15,28] | Cadmium | 0.3 |
| *Phoma* sp.[15,28] | Silver | 2 |
| *Rhizopus arrhizus*[33,36,37] | Copper | 1.6 |
| | Cadmium | 3.0 |
| | Uranium | 19.5 |
| | Lead | 10.4 |
| | Thorium | 9.7 |
| *Saccharomyces cerevisiae*[15] | Cadmium | 0.24–3.12 |
| | Zinc | 0.45 |
| | Uranium | 10–15 |
| | Thorium | 11.6 |

[a] Data extracted from the various sources listed. Consultation of original references is recommended for full details of experimental procedures. [b] Immobilized cells.

*Metabolism-independent biosorption*

The term 'biosorption' is now frequently used to describe non-directed physico-chemical interactions between metal species and microbial biomass[31].

a) *Bacteria.* The walls of gram positive bacteria are efficient metal chelators and in *Bacillus subtilis,* the carboxyl group of the glutamic acid of peptidoglycan was the major site of metal deposition. Teichoic and teichuronic acids were important binding sites in *Bacillus licheniformis*[3]. In many bacteria, initial binding is followed by inorganic deposition of increased amounts of metal which leads to the accumulation of greater than stoichiometric amounts of metals, even to levels approaching 50 % of the dry weight.

Envelopes of gram negative bacteria consist of two distinct membrane bilayers which sandwich a thin peptidoglycan layer between them in the periplasmic space. With purified cell envelopes of *Escherichia coli* K-12, most metal deposition occurred at the polar head group regions of constituent membranes or along the peptidoglycan layer[3].

Biosorption of radionuclides, e.g. uranium, may be high in actinomycetes, e.g. *Streptomyces longwoodensis,* and maximal at acidic pH values. It is believed that phosphodiester residues are the main sites of $UO_2^{2+}$ binding[12]. Biosorption of metals and radionuclides has been demonstrated in the cyanobacterium *Synechococcus* sp. with concentration factors ranging from 0 (Cs and Np) up to $10^6$ (Sn, Hg, Pu), the sequence of accumulation efficiency being Pu, Hg, Sn $\geq$ Am > Ag > Zn > Co > Mn > Cs, Np[10]. *S. elongatus* can remove uranium from seawater, optimally at pH 5, and this was inhibited by excess $CO_3^{2-}$ and increasing cell suspension density[20]. Most uranium was located intracellularly, despite the lack of metabolic dependence, which is similar to uranium accumulation in *Pseudomonas aeruginosa*[31].

In several bacteria, metabolism-independent biosorption may be the most significant proportion of total uptake and may be affected by the previous growth history of the cells, particularly nutrient limitation which may result in changes in wall composition.

b) *Algae.* Algal cell walls and extracellular materials are good biosorptive agents. Both ionic- and covalent binding are involved in biosorption with proteins and polysaccharides playing important roles. In several species, cell walls function as a weakly acidic cation exchanger though some metals, e.g. cadmium, may be taken up as neutral complexes. This may be important under marine conditions where cadmium may exist as $CdCl_2$ and $CdCl_3^-$[34].

A linear equilibrium relationship between bound metal and the metal concentration in solution is frequently observed[23]. For *Chlorella vulgaris,* the order of binding capacities was $UO_2^{2+} \gg Cu^{2+} \gg Zn^{2+} \geq Ba^{2+}$, $Mn^{2+} > Co^{2+}$, $Cd^{2+} \geq Ni^{2+}$, $Sr^{2+}$. Other reported sequences are $Cu^{2+} > Sr^{2+} > Zn^{2+} > Mg^{2+} > Na^+$ for *Vaucheria* and $Hg^{2+} > Ag^+ > Zn^{2+} > Cd^{2+}$ for several marine algae[15]. $Au^{3+}$, $Au^+$ and gold complexes may be bound by algae, optimally at acidic pH values. $Au^{3+}$ may be reduced to $Au^+$ and then to colloidal $Au^0$ which may comprise up to 10 % of the dry weight[10].

In some algae, biosorption may be a significant proportion of total uptake, particularly where toxicity leads to non-specific intracellular accumulation. For radionuclides, such physico-chemical interactions comprise the majority of total uptake. In *Chlorella,* uranium uptake is optimal at pH 5 and reduced by $PO_4^{2-}$, $CO_3^{2-}$ and $HCO_3^-$. $UO_2^{2+}$ and $UO_2OH^+$ were the main species taken up in exchange for $H^+$[29]. Little uranium is taken up in marine conditions because of interference by $CO_3^{2-}$ which leads to the formation of $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$ complexes. Marine algae are also capable of passively accumulating Am, Po, Pb, Pu and Cf with concentration factors ranging from $5 \times 10^3$ to $4 \times 10^5$[11].

c) *Fungi and yeasts.* Because of wide variation in the chemical composition of fungal cell walls, there can be considerable variations in uptake capacities between different species[15]. In *Rhizopus arrhizus,* biosorption was related to ionic radii for $La^{3+}$, $Mn^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Ba^{2+}$, $Hg^{2+}$ $Pb^{2+}$ $UO_2^{2+}$ and $Ag^+$ but not $Cr^{3+}$, $Na^+$, $K^+$, $Rb^+$ or $Cs^+$[33]. In *R. arrhizus,* uranium biosorption comprised at least 3 processes involving rapid and simultaneous coordination of uranium to the amine nitrogen of chitin and adsorption to cell wall chitin, fol-

lowed by the slower precipitation of uranyl hydroxide [38]. Phosphate and carboxyl groups may be involved in $UO_2^{2+}$ binding to walls of yeast followed by deposition of uranium as needle-like fibrils. This can lead to large levels of accumulation with average values approaching 15 % of the dry weight [32].

Over modest ranges of temperature, e.g. 4–30 °C, fungal biosorption is relatively unaffected though uranium uptake was enhanced at 50 °C in a variety of species [15]. Low external pH generally decreases the rate and extent of metal biosorption. A reduction in solubility may accompany a rise in pH which favours biosorption. For example, at pH values $> 2$, $Th(OH)_2^{2+}$ is taken up more efficiently than $Th^{4+}$ which is the major Th species at pH values $\leq 2$ [37]. Low pH reduces uranium uptake because of competition by $H_3O^+$ for binding sites and effects on the species present. At pH $< 2.5$, $UO_2^{2+}$ is the principal cation but $>$ pH 2.5, hydrolysis products include $(UO_2)_2(OH)_2^{2+}$, $UO_2(OH)^+$ and $(UO_2)_3(OH)_5^+$ which are more readily absorbed [38]. Other anions and cations may also affect metal biosorption by e.g. precipitation or competition for binding sites [15].

As well as dissolved metal species, particulate material may also be absorbed by fungal biomass, e.g. copper, lead and zinc sulphides, zinc dust and ferric hydroxide ('ochre') [39].

*Extracellular precipitation, complexation, and crystallization*

Many bacterial extracellular polysaccharides are efficient metal binding agents and such materials are of major importance in metal removal from solution by activated sludge biomass [24].

With regard to metal crystallization on microbial surfaces it is often difficult to ascertain whether metabolism is involved in all cases. Microbes are implicated in ferromanganese nodule formation on ocean floors and a variety of bacteria, algae and fungi can become encrusted with manganic oxides [22]. Crystalline deposits of many other elements, e.g. Au and U, have been observed in a variety of microbial types [15].

Hydrogen sulphide production can lead to precipitation of metal sulphides within and on cell surfaces. Cd-grown *Klebsiella aerogenes* contained 2–4 % of the dry weight as cadmium and large numbers of electron-dense granules of CdS occurred on outer cell surfaces [1]. Such sulphide precipitation can also occur in algae, yeasts and fungi as well as metal precipitation as phosphates or oxalates [15].

Many organisms release high-affinity iron-binding molecules called siderophores, the synthesis of which may be stimulated by iron deficiency. Siderophores are also able to bind other metals, e.g. Ga, Ni, U, Th and Cu [15, 18].

*Biosorption by derived microbial products*

Compounds from fungal biomass have received most attention, probably because waste fungal biomass can arise in quantity from several industrial fermentations. Potential biosorptive agents in fungal walls include mannans, glucans, chitin, chitosan and melanin. Phosphorylated derivatives of chitin and chitosan were more efficient biosorptive agents than non-phosphorylated chitin and chitosan, orders of biosorption efficiency being $UO_2^{2+} \gg Cu^{2+} > Cd^{2+} > Mn^{2+} > Zn^{2+} > Mg^{2+} > Co^{2+} > Ni^{2+} > Ca^{2+}$ and $UO_2^{2+} \gg Cu^{2+} > Zn^{2+} > Mn^{2+} > Co^{2+} > Ni^{2+} > Mg^{2+} > Ca^{2+}$ respectively [30]. Fungal phenolic polymers and melanins contain phenolic units, peptides, carbohydrates, aliphatic hydrocarbons and fatty acids. Melanins in particular are good biosorptive agents whether in purified form or associated with wall material [17, 18].

*Metabolism-dependent intracellular accumulation*

Metabolism-dependent uptake of metal ions is usually a slower process than biosorption although greater amounts of metal may be accumulated by this means in some organisms, e.g. yeasts. Transport of metal ions into microbial cells is inhibited by low temperatures, metabolic inhibitors and the absence of an energy source. Rates of uptake are influenced by the metabolic state of cells and the composition of the external medium. Transport systems encountered in microorganisms are of varying specificity and both essential and non-essential elements may be taken up. Most mechanisms of metal transport appear to rely on the electrochemical proton gradient ($\Delta\tilde{\mu}_{H^+}$) across the cell membrane which has a chemical component, the pH gradient, ($\Delta$pH) and an electrical component, the membrane potential ($\Delta\Psi$), each of which can drive transport of ionized solutes across membranes. The membrane potential appears to be responsible for electrophoretic mono- and divalent cation transport in fungi although other gradients, e.g. $K^+$, may also be involved [5, 15, 18]. It should be stressed that in several organisms, notably filamentous fungi, transport may not be as significant a component of total uptake as general biosorption. In addition, in some cases, intracellular uptake may occur by diffusion, particularly where toxic effects lead to changes in membrane permeability [15].

Metal resistance may often be associated with decreased uptake and/or impermeability. In addition, those external factors which reduce uptake often result in reduced toxicity [13, 15]. In contrast, there is an example of a $Mn^{2+}$-resistant strain of *S. cerevisiae* which accumulates considerably more $Mn^{2+}$ than the sensitive parental strain, probably by more efficient internal sequestration [4].

*Intracellular localization and deposition*

After uptake into cells, metals may be compartmentalized and/or converted to more innocuous forms by binding or precipitation. Dense intracellular deposits of uranium were observed in *Pseudomonas aeruginosa* [32], while other electron-dense bodies, including polyphosphate, have been associated with intracellular metal accumula-

tion in several bacteria, algae and fungi[15]. In eukaryotic microbes, e.g. yeasts, a majority of intracellular $Co^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Mg^{2+}$ and $K^+$ is located in the vacuole where there may be binding to low molecular weight polyphosphates[18].

Another common microbial response is the synthesis of intracellular metal-binding proteins and these have been recorded in bacteria, cyanobacteria, algae, fungi and yeasts. Metallothioneins, and other metal-binding proteins, may have relevance to metal recovery since they can bind valuable metals, e.g. Au and Ag, as well as those of low value, e.g. Cu, Zn and Cd. Future work may lead to the engineering of different metal-binding proteins with specific affinities for different metals[9].

*Metal/radionuclide recovery from loaded biomass*

Metabolism-independent biosorption is often reversible by simple non-destructive physical/chemical treatments whereas intracellular accumulation may be irreversible and necessitate destructive recovery, e.g. incineration or dissolution in acids or alkalis. Technological applications of microbial metal removal systems may depend on the ease of metal recovery either for reclamation or for biomass regeneration. If cheap, waste biomass is used to reclaim valuable metals, then destructive recovery may be economically feasible. However, most attention to date has focussed on non-destructive desorption from loaded biomass. It is therefore obvious that the choice between living or dead cell systems is important because of the implications for metal recovery[14,15].

Dilute mineral acids can be effective desorption agents but high concentrations may damage the biomass. Manipulation of the pH of desorbing solutions may be a good method for selective removal of metal ions. Some metal ions, e.g. Cu and Zn, were desorbed by lowering the eluant pH to 2 yet Au, Ag and Hg were strongly bound at this pH value. $Au^{3+}$ was selectively eluted using mercaptoethanol[19].

Carbonates and/or bicarbonates appear to have potential for non-destructive recovery. The solid:liquid ratios that can be used for bicarbonate elution can exceed 120:1 (mg:ml) for a 1M $NaHCO_3$ solution with $\simeq$ 100 % uranium recovery[35]. The biosorption capacity of the biomass may be increased after bicarbonate treatment[15].

*Biotechnology of microbial metal/radionuclide removal*

a) *Living cell systems.* These have received application in the decontamination of sewage and waste waters where metal concentrations may be below toxic levels. Tailings ponds and artificial stream meanders containing photosynthetic microbes have been used to treat mining effluents while algal blooms, that can be encouraged by nutrients in sewage effluent, can also serve as biosorptive agents[6]. Where such algae die in the presence of toxic metal concentrations, decomposition of settled biomass can lead to the production of $H_2S$ by sulphate-reducing bacteria and metal precipitation as sulphides[7]. Activated sludge microbial biomass is also of applied potential, particularly where there is extensive production of extracellular polymers[24].

b) *Immobilized cell systems.* For rigorous industrial application, microbial biomass has a number of disadvantages which include small particle size, low mechanical strength and low density, which can make biomass/effluent separation difficult[36]. For use in packed-bed or fluidized-bed reactors, pelleted biomass appears of greater potential. Benefits of immobilization include easy separation of cells and effluent, high biomass loadings, minimal clogging and better capability for re-use. Particle size can be controlled and high flow rates achieved in a variety of reactor configurations.

Polyacrylamide-immobilized *Streptomyces* removed uranium, copper and cobalt, the order of selectivity being $UO_2^{2+} \gg Cu^{2+} > Co^{2+}$, and desorption was achieved using 0.1M $Na_2CO_3$. Such preparations could be used in repeated biosorption-desorption cycles[28]. Immobilized *Citrobacter* can remove a variety of metals from solutions supplemented with glycerol-2-phosphate. The uptake mechanism involved phosphatase-mediated cleavage of glycerol-2-phosphate which released $HPO_4^{2-}$ and this precipitated metals extracellularly as insoluble phosphates, e.g. $CdHPO_4$[26,27]. The biomass could be regenerated and repeatedly used over extended time periods and a temperature range of 2–45 °C[26,27]. Polyacrylamide-entrapped *Chlorella* can remove $UO_2^{2+}$, $Au^{3+}$, $Cu^{2+}$, $Hg^{2+}$ and $Zn^{2+}$ and these cations be selectively eluted[19]. Other systems in commercial operation use various kinds of immobilized microbial biomass which can be used in multiple cycles. High metal loadings are possible, e.g. (all in mmol $g^{-1}$) Ag, 0.8; Cd, 1.9; Cu, 2.4; Pb, 2.9; Au, 2.0 and Zn, 2.1. Metal removal from low concentrations (10–100 mg $l^{-1}$) exceeds 99 % and the granules can be used in fixed-bed or fluidized-bed reactors[21].

A further means of cell immobilization involves the use of biofilms on inert matrices. A biofilm of *Citrobacter* exhibited uptake capacities similar to those of gel-immobilized cells[25].

Both living and dead biomass can be used in immobilized systems. If living cells are used, there may be possibilities for removal of other pollutants from effluents. There are examples of simultaneous denitrification and metal removal using mixed bacterial cultures grown as a film on anthracite coal particles or immobilized on polyvinyl chloride (PVC) or polypropylene webs[31]. A large-scale ($5.5 \times 10^6$ gallons effluent per day) commercial process uses rotating-disc biological-contacting units to simultaneously degrade cyanide, thiocyanate and ammonia from gold mining and milling effluents. Metals are removed by biosorption onto the microbial biofilm[21].

*Conclusions*

The future development of microbial metal/radionuclide removal/recovery systems depends on such factors as

uptake capacities, biosorbent selectivity, ease of recovery and equivalence to existing treatments in performance, economics and insensitivity to adverse operating conditions. For full competition, it has been stated that removal efficiencies should be > 99 % with loading capacities > 150 mg metal $g^{-1}$ [7,8]. However, it should be stressed that microbe-based methods may not necessarily replace existing treatments but may be used as a supplement or polishing system to existing processes that are not fully efficient. There is no doubt that many kinds of biomass, or derived products, are highly efficient with high uptake capacities. Selectivity may be a problem but this can be enhanced by strain selection or in systems where metals vary widely in their affinities for the biomass. Recovery may also be selectively controlled by appropriate choice of elution protocols.

In a general sense, it seems dead biomass, or derived products, has advantages over living material. However, many attributes of living cells remain unexploited in an industrial context, including metallothionein production, particulate metal accumulation, extracellular precipitation and complex formation, and these are all worthy of further attention since they may be of use for specific applications.

A general consensus is that for improved industrial use, immobilized or pelleted preparations should be used with recovery involving a cheap stripping agent. Some described processes are competitive in cost and operational characteristics with existing treatments [8,31,36]. However, most attention has focussed on economically-valuable elements. The situation is less clear for non-precious metals or radionuclides, although their removal is more important because of environmental considerations. With continued pollution of the biosphere with toxic heavy metals and radionuclides, it is clear that microbe-based technologies may have an important role in environmental protection.


1 Aiking, H., Stijnman, A., Van Garderen, C., van Heerikhuizen, H., and Van't Riet, J., Inorganic phosphate accumulation and cadmium detoxification in *Klebsiella aerogenes* NCTC 418 growing in continuous culture. Appl. envir. Microbiol. *47* (1984) 374–377.

2 Belliveau, B. H., Starodub, M. E., Cotter, C., and Trevors, J. T., Metal resistance and accumulation in bacteria. Biotechnol. Adv. *5* (1987) 101–127.

3 Beveridge, T. J., and Fyfe, W. S., Metal fixation by bacterial cell walls. Can. J. Earth Sci. *22* (1985) 1893–1898.

4 Bianchi, M. E., Carbone, M. L., and Lucchini, G., $Mn^{2+}$ and $Mg^{2+}$ uptake in Mn-sensitive and Mn-resistant yeast strains. Plant. Sci. Lett. *22* (1981) 345–352.

5 Borst-Pauwels, G. W. F. H., Ion transport in yeast. Biochim. biophys. Acta *650* (1981) 88–127.

6 Brierley, J. A., and Brierley, C. L., Biological accumulation of some heavy metals – biotechnological applications, in: Biomineralization and Biological Metal Accumulation, pp. 499–509. Reidel Publishing Company, Dordrecht 1983.

7 Brierley, C. L., Kelly, D. P., Seal, K. J., and Best, D. J., Materials and biotechnology, in: Biotechnology, pp. 163–212. Blackwell Scientific Publications, Oxford 1985.

8 Brierley, J. A., Goyak, G. M., and Brierley, C. L., Considerations for commercial use of natural products for metals recovery, in: Immobilisation of Ions by Bio-sorption, pp. 105–117. Ellis Horwood, Chichester 1986.

9 Butt, T. R., and Ecker, D. J., Yeast metallothionein and applications in biotechnology. Microbiol. Rev. *51* (1987) 351–364.

10 Fisher, N. S., Accumulation of metals by marine picoplankton. Mar. Biol. *87* (1985) 137–142.

11 Fisher, N. S., Burns, K. A., Cherry, R. D., and Heyraud, M., Accumulation and cellular distribution of $^{241}Am$, $^{210}Po$ and $^{210}Pb$ in two marine algae. Mar. Ecol. *11* (1983) 233–237.

12 Friis, N., and Myers-Keith, P., Biosorption of uranium and lead by *Streptomyces longwoodensis*. Biotechnol. Bioeng. *28* (1986) 21–28.

13 Gadd, G. M., Fungal responses towards heavy metals, in: Microbes in Extreme Environments, pp. 83–110. Academic Press, London 1986.

14 Gadd, G. M., The uptake of heavy metals by fungi and yeasts: the chemistry and physiology of the process and applications for biotechnology, in: Immobilisation of Ions by Biosorption, pp. 135–147. Ellis Horwood, Chichester 1986.

15 Gadd, G. M., Accumulation of metals by microorganisms and algae, in: Biotechnology – A Comprehensive Treatise, vol. 6b, pp. 401–433. VCH Verlagsgesellschaft, Weinheim 1988.

16 Gadd, G. M., and Griffiths, A. J., Microorganisms and heavy metal toxicity. Microb. Ecol. *4* (1978) 303–317.

17 Gadd, G. M., and de Rome, L., Biosorption of copper by fungal melanin. Appl. Microbiol. Biotechnol. *29* (1988) 610–617.

18 Gadd, G. M., and White, C., Heavy metal and radionuclide accumulation and toxicity in fungi and yeasts, in: Metal-Microbe Interactions, pp. 19–38. IRL Press, Oxford 1989.

19 Greene, B., Hosea, M., McPherson, R., Henzl, M., Alexander, M. D., and Darnall, D. W., Interaction of gold (I) and gold (III) complexes with algal biomass. Envir. Sci. Technol. *20* (1986) 627–632.

20 Horikoshi, T., Nakajima, A., and Sakaguchi, T., Uptake of uranium by *Chlorella vulgaris*. Agric. biol. Chem. *43* (1979) 617–623.

21 Hutchins, S. R., Davidson, M. S., Brierley, J. A., and Brierley, C. L., Microorganisms in reclamation of metals. A. Rev. Microbiol. *40* (1986) 311–336.

22 Kelly, D. P., Norris, P. R., and Brierley, C. L., Microbiological methods for the extraction and recovery of metals, in: Microbial Technology: Current State, Future Prospects, pp. 263–308. Cambridge University Press, Cambridge 1979.

23 Khummongkol, D., Canterford, G. A., and Fryer, C., Accumulation of heavy metals in unicellular algae. Biotechnol. Bioeng. *24* (1982) 2643–2660.

24 Lester, J. N., Sterritt, R. M., Rudd, T., and Brown, M. J., Assessment of the role of bacterial extracellular polymers in controlling metal removal in biological waste water treatment, in: Microbiological Methods for Environmental Biotechnology, pp. 197–217. Academic Press, London 1984.

25 Macaskie, L. E., and Dean, A. C. R., Use of immobilised biofilm of *Citrobacter* sp. for the removal of uranium and lead from aqueous flows. Enzyme Microb. Technol. *9* (1987) 2–4.

26 Macaskie, L. E., Dean, A. C. R., Cheetham, A. K., Jakeman, R. J. B., and Skarnulis, J., Cadmium accumulation by a *Citrobacter* sp.: the chemical nature of the accumulated metal precipitate and its location on the bacterial cells. J. gen. Microbiol. *133* (1987) 539–544.

27 Macaskie, L. E., Wates, J. M., and Dean, A. C. R., Cadmium accumulation by a *Citrobacter* sp. immobilized on gel and solid supports: applicability to the treatment of liquid wastes containing heavy metal cations. Biotechnol. Bioeng. *30* (1987) 66–73.

28 Nakajima, A., and Sakaguchi, T., Selective accumulation of heavy metals by microorganisms. Appl. Microbiol. Biotechnol. *24* (1986) 59–64.

29 Nakajima, A., Horikoshi, T., and Sakaguchi, T., Ion effects on the uptake of uranium by *Chlorella vulgaris*. Agric. biol. Chem. *43* (1979) 625–629.

30 Sakaguchi, T., and Nakajima, A., Recovery of uranium by chitin phosphate and chitosan phosphate, in: Chitin and Chitosan, pp. 177–182. Japanese Society of Chitin and Chitosan, Tottori, Japan 1982.

31 Shumate, S. E., and Strandberg, G. W., Accumulation of metals by microbial cells, in: Comprehensive Biotechnology, vol. 4, pp. 235–247. Pergamon Press, New York 1985.

32 Strandberg, G. W., Shumate, S. E., and Parrott, J. R., Microbial cells as biosorbents for heavy metals: accumulation of uranium by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*. Appl. envir. Microbiol. *41* (1981) 237–245.

33 Tobin, J. M., Cooper, D. G., and Neufeld, R. J., Uptake of metal ions by *Rhizopus arrhizus* biomass. Appl. envir. Microbiol. *47* (1984) 821–824.

34 Trevors, J. T., Stratton, G. W., and Gadd, G. M., Cadmium transport, resistance and toxicity in bacteria, algae and fungi. Can. J. Microbiol. *32* (1986) 447–464.
35 Tsezos, M., Recovery of uranium from biological adsorbents-desorption equilibrium. Biotechnol. Bioeng. *26* (1984) 973–981.
36 Tsezos, M., Absorption by microbial biomass as a process for removal of ions from process or waste solutions, in: Immobilization of Ions by Bio-sorption, pp. 201–218. Ellis Horwood, Chichester 1986.
37 Tsezos, M., and Volesky, B., Biosorption of uranium and thorium. Biotechnol. Bioeng. *22* (1981) 583–604.
38 Tsezos, M., and Volesky, B., The mechanism of uranium biosorption by *Rhizopus arrhizus*. Biotechnol. Bioeng. *24* (1982) 385–401.
39 Wainwright, M., and Grayston, S. J., Accumulation and oxidation of metal sulphides by fungi, in: Metal-Microbe Interactions, pp. 119–130. IRL Press, Oxford 1989.

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# Microbial dissolution and stabilization of toxic metals and radionuclides in mixed wastes

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*Summary.* Microbial activity in mixed wastes can have an appreciable effect on the dissolution or precipitation of toxic metals and radionuclides. Fundamental information on microbial dissolution and stabilization (immobilization) of toxic metals and radionuclides, in particular actinides and fission products, in nuclear wastes under various microbial process conditions, e.g., aerobic, denitrifying, iron-reducing, fermentative, sulfate-reducing, and methanogenic conditions is very limited. Microbial transformations of typical waste components such as metal oxides, metal coprecipitates, naturally occurring minerals, and metal organic complexes are reviewed. Such information can be useful in the development of 1) predictive models on the fate and long-term transport of toxic metals and radionuclides from waste disposal sites, and 2) biotechnological applications of waste treatment leading to volume reduction and stabilization as well as recovery and recycling of radionuclides and toxic metals.
*Key words.* Toxic metals; radionuclides; natural radioactive mineral deposits; metal oxides; carbonate complexes; organic complexes; coprecipitates; uranium; plutonium; low-level radioactive wastes; transuranic wastes; coal wastes.

## *Introduction*

Microorganisms, which are ubiquitous throughout nature, have long been recognized for their ability to bring about transformations of organic and inorganic compounds. Such microbial processes have not been fully exploited in the treatment and management of nuclear and fossil-energy wastes, particularly in regulating the mobility of toxic metals and radionuclides or in the biodegradation of organic constituents to innocuous products. The contaminants in wastes may be present initially as soluble forms or they may be formed after disposal by chemical or microbiological processes. The organic compounds and inorganic elements (major and minor metals and radionuclides), depending upon their chemical forms, may react with each other to varying degrees in the waste. These include organic-inorganic complex formation, precipitation reactions, coprecipitation of metals and radionuclides with Fe- and Mn-oxides, and formation of minerals. In figure 1, the interactions between the organics, inorganics, radionuclides and microbes are depicted. Because of the complexity of the system, it is often difficult to elucidate clearly the exact mechanisms involved in the transformation of mixed wastes. Nevertheless, studies with pure or model compounds under defined conditions should provide information on the mechanisms involved in the transformations of such mixed wastes. There is a paucity of information on the fate and long-term transport of toxic metals and radionuclides present in energy-related wastes disposed of in subsurface environments. Of particular concern is the lack of information on specific microbial processes and the biochemical mechanisms involved in the dissolution (mobilization) or stabilization (immobi-

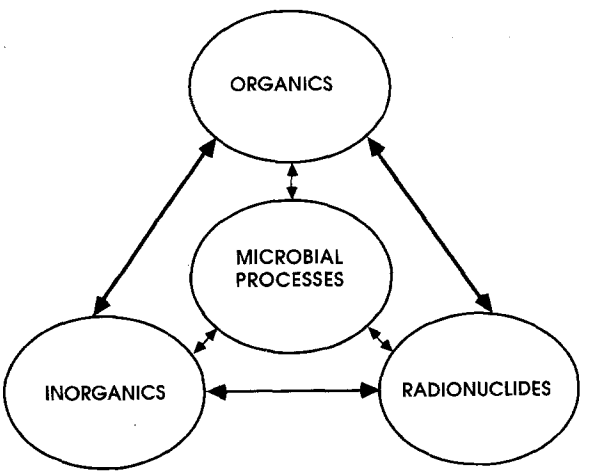


Figure 1. Interactions of organics, inorganics, radionuclides, and microbes in mixed wastes.