

Heavy Metal Removal by Microorganisms: Use of Immobilized Cells

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Due to the cumulatively toxic and environmentally detrimental effects of toxic metals, as well as the increasing value of the traditionally important gold and platinum, a number of studies of metal accumulation from the viewpoint of metal recovery and removal from solution are under way. Conventional methods for removing metals from ore processing solutions include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, membrane technology, and evaporation recovery. However these processes may be ineffective or expensive, especially when the metals are present in low concentrations (e.g. 1 mg/L-100 mg/L).

Some microorganisms, including actinomycetes, cyanobacteria, and other bacteria, algae, fungi, and yeasts, can accumulate and retain relatively high quantities of heavy metals and radionuclides from their external environments (1-4). Both living and dead cells can be used for biosorptive metal/radionuclide removal from solution. Thus microorganisms and products excreted by or derived from microbial cells (2) may provide an alternative or adjunct to conventional techniques of metal removal and recovery.

Recent approaches have separated the microbial growth and metal removal process to manipulate production of metal-adsorptive capacity of bacteria and metal removal process. If pre-grown cells are immobilized and used for metal removal, mathematical modeling can be applied to predict immobilized cell reactor behavior under specific process

conditions. Waste and microbial adsorbent could be separated from the treated flow in one step. Once treated, the metal waste is concentrated in a small volume of sorbed form for easy metal disposal or recovery.

Mechanisms of microbial metal accumulation

For the removal of heavy metals variety of mechanisms exist in microorganisms. These range from physico-chemical interactions such as adsorption to cell walls and other constituents, to mechanisms dependent on metabolism such as transport, internal compartmentation and extracellular precipitation by excreted metabolites (3). Although the mechanisms and capacities may vary widely in different microorganisms, microbially-mediated metal removal processes fall into two types i.e. biosorption and bioaccumulation. Biosorption and bioaccumulation have often been combined into the single heading of metal uptake or metal removal because the predominant mechanism in effect is not known. These two phases of uptake may not be seen in all microorganisms, and for some elements, e.g. lead, uranium, and thorium, most accumulation in living or dead cells appears to involve surface phenomena with little or no intracellular uptake unless by diffusion (3).

Biosorption is a process which is responsible for binding and accumulation of positively charged metal ions to functional groups on the cell envelope or within extracellular layers and secretions even though the cells are no longer metabolically

active or dead. This is thus a passive metal sequestering capacity of the cells caused by a number of different mechanisms (adsorption, complexation, coordination, chelation of metals, ion exchange, and inorganic microprecipitation). This is generally rapid and depends on a number of external factors (temperature, pH, redox potential and the presence of complexing anions) as well as on the type of metal, its ionic form in solution, and on the type of a particular active binding site of the cells responsible for sequestering the metal. The decoupling of the growth and propagation of the biomass from its subsequent function as a metal-sorbing material is one major advantage of biosorption. This allows the independent culture of microbial biomass in a separate fermentation process and use in a separate biosorptive process. Following metal desorption the biomass can be reused.

Bioaccumulation can be defined as an active metal accumulation by living cells, and is often dependent on metabolic activity, which in turn can be significantly affected by the presence of the metallic ions. In some cases intracellular uptake is a result of permeation and diffusion due to increased membrane permeability especially if toxicity is manifest (3). Bioaccumulation, which has traditionally been more studied by researchers for its toxicological importance, is less desirable than biosorption for metal removal because it requires that the cells should be actively metabolizing under physiologically permissive conditions.

Extracellular binding of metals

Many studies have shown or implied that metal accumulation occurs at the cell surface or within the cell wall matrix. This surface accumulation has generally been assumed to result from complexation reactions between metal ions and charged receptor cell wall components. Other extracellular metal binding mechanisms include precipitation followed by deposition at the cell surfaces, and complexation with extracellular polymers.

Cell surface binding may constitute a resistance

mechanism as well as a mechanism of metal uptake, compartmentalizing toxic elements to regions of the cell where their toxicity can not be manifested. Extracellular binding is more important than intracellular uptake for the recovery and removal of metals, especially when they are likely to be encountered at toxic levels.

Metal uptake experiments are generally performed by incubating microorganisms in an aqueous solution of a soluble metal salt. Therefore the chemistry of the metals (metal speciation) may be important and quite complex, depending on the solution environment.

Bacteria

The microbial cell wall composition differs considerably between Gram negative and Gram positive bacteria, yeasts, filamentous fungi, and algae. The wall composition is not only species dependent but is also subject to the influence of environmental growth conditions (5). Baldry and Dean (6) suggested that nutrient limitation had probably affected wall composition and biosorption, since for both *Pseudomonas fluorescens* and *Bacillus subtilis* subsp. niger, copper uptake increased with the limiting nutrient in the order $C < Mg < N < K$.

A study of the binding of metal ions to purified cell envelopes of *E. coli* K12 showed that the primary sites of metal adsorption were the polar head groups of phospholipids, the available anionic sites of lipopolysaccharides and the acidic groups of exported polypeptides (7). For *E. coli*, an affinity sequence of $Zn^{2+} = Cd^{2+} > Mn^{2+} = Co^{2+} = Ni^{2+} > Mg^{2+} = Ca^{2+}$ was indicated (8).

When the precipitation of cadmium salts at the surface of *Klebsiella aerogenes* was examined using electron microscopy, dense granular deposits of cadmium sulfide were found in the extracellular matrix (9). In glucose-limited chemostats, cadmium (3.3% w/w of the bacterial dry weight) was accumulated primarily as the sulfide, while sulfate-limitation produced accumulation of 5.8% (w/w) cadmium mainly as a phosphate precipitate. Phosphate limitation sensitized *Klebsiella aerogenes* cells to 0.6mM cadmium, but when the conce-

centration of cadmium in the growth medium was reduced to 0.2 mM, electron-dense granules of cadmium sulfide were observed (10). Two mechanisms of cadmium detoxification were subsequently demonstrated in this organism (11). In addition to sulfide formation, increased accumulation of inorganic phosphate has been postulated as a mechanism of resistance to, and biosorption of cadmium. Cells grown in the absence of cadmium contained polyphosphate but this was undetectable when cadmium was present.

In *Thiobacillus ferrooxidans*, uranium removal was dependent on the external concentration of uranium. Previous growth in the presence of 1 mM Fe^{2+} , Fe^{3+} , Ni^{2+} , Co^{2+} , Zn^{2+} , or Cd^{2+} did not affect uptake, and most of the uranium was associated with the cell wall and membrane fractions (12).

The walls of Gram positive bacteria are also efficient metal binding sites. Marquis *et al.* (13) showed that the binding of metals was in the order $\text{La}^{3+} > \text{Cd}^{2+} > \text{Sr}^{2+} > \text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+ > \text{Na}^+$ in *Streptococcus mutans*, *Micrococcus lysodeikticus*, and *Bacillus subtilis*. The primary site of metal deposition was identified in isolated walls of *Bacillus subtilis* as the carboxyl groups of glutamate in the peptidoglycan layer (14,15). Teichoic and teichuronic acids were major metal binding sites in walls of *Bacillus licheniformis* (16). Various metals competed for binding sites on *Bacillus subtilis* walls, so it was suggested that the metals were complexed by identical sites (15). In two strains of *Bacillus subtilis*, differing in Cd^{2+} sensitivity, the distribution of Cd^{2+} was similar; about 86~90% of the Cd^{2+} taken up was associated with the cell wall, 3~4% with the membrane fraction, and 6~7% in the soluble fraction (17). *Streptomyces longwoodensis* accumulated uranium maximally at pH 4.6, with a simple stoichiometric relationship between uranium uptake and phosphorus content; here phosphodiester residues were proposed to be the main sites of UO_2^{2+} binding (18).

Algae

The relative capacity of metal uptake, and metal

deposition sites vary between different algal species. In *Chlorella vulgaris*, most of the metal uptake occurred on the cell wall (19). Biosorption of metals can be affected by the presence of other ions. In *Chlorella regularis* Cd^{2+} uptake was retarded by Ca^{2+} , Mg^{2+} , Na^+ , Mn^{2+} , Co^{2+} , and Ni^{2+} , but not by K^+ (20) while Cd^{2+} interfered with Mn^{2+} binding (21). However uranium uptake by *Chlorella regularis* was unaffected by Na^+ , K^+ , NH_4^+ , Ca^{2+} , Mg^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , NO_3^- or SO_4^{2-} , but was affected by PO_4^{3-} , CO_3^{2-} , and HCO_3^- (21). The biosorption of uranium from sea water by algae was low, in contrast to fresh water or decarboxylated sea water. This was thought to be due to interference by CO_3^{2-} which results in the formation of $\text{UO}_2(\text{CO}_3)_2^{2-}$ and $\text{UO}_2(\text{CO}_3)_3^{4-}$ complexes which are not taken up by cells (3).

Yeasts and fungi

Copper binding sites in walls, membranes, and cytoplasm of *Saccharomyces cerevisiae* were formed by an amide and an amine. Their configuration depended on the pH. In acidic conditions copper was bound by the oxygen of the amide, while in basic conditions NHCO became deprotonated and the metal was bound to the negatively charged N (22). Rothstein and Hayes (23) suggested phosphate and carboxyl groups as an initial binding site of UO_2^{2+} to *S. cerevisiae* walls. Cell bound uranium comprised 10%-15% of the dry weight, but only about 32% of the cells had measurable quantities of uranium associated with them (24). Because simple adsorption to phosphate and carboxyl groups could not explain such a large quantity of uranium, it was suggested that uranium crystallized on already bound uranium molecules (24). The treatment of *S. cerevisiae* cells with formaldehyde increased the rate of uranium uptake, presumably by decreasing the repulsive force of the positively charged amino groups of surface proteins (24).

Adsorption of metals to *Rhizopus arrhizus* was related to the atomic radius 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+} or the alkali metal cations, Na^+ , K^+ , Rb^+ ,

and Cs^+ which were not adsorbed (25). Studies using electron microscopy, energy dispersive X-ray analysis and IR spectroscopy showed that uranium was complexed within the chitin cell-wall matrix of *Rhizopus arrhizus* (26). This uranium adsorption involved at least three processes, including uranium coordination with the amine nitrogen of chitin, the adsorption of additional uranium in the cell-wall chitin structure, and the precipitation of uranyl hydroxide within the cell-wall matrix at a slower rate (26).

Intracellular uptake of metals

Microorganisms have evolved many highly specific, active transport systems which permit selective uptake of metals from their environment. This metabolism-dependent intracellular uptake of metal ions is usually a less important process for biotechnological application than biosorption. For microorganisms possessing extracellular polysaccharide, slime, or mucilage high biosorptive capacities may mask low rates of intracellular uptake. Metabolism-dependent metal bioaccumulation is inhibited by low temperatures, metabolic inhibitors, uncouplers, and the absence of an energy source (3).

Weinberg (27) reviewed magnesium, calcium, manganese, iron and other metal transport in microorganisms. In *E. coli* magnesium is accumulated via two uptake mechanisms. One is a constitutive, low-affinity system which also transports manganese, cobalt, nickel and zinc. The other is an inducible, high-affinity, specific system for magnesium.

Cadmium was accumulated in *E. coli* via an energy-dependent, temperature sensitive Cd^{2+} transport system which was competitively inhibited by cobalt (28). When *E. coli* was grown in the presence of a low concentration of Cd^{2+} , synthesis of a 39 KDa cytoplasmic protein was induced (29).

Strandberg *et al.* (24) observed that uranium uptake in *Pseudomonas aeruginosa* was rapid and intracellular, and that the metal was highly locali-

zed inside the cell. Their hypothesis was that the metal became bound to a protein, analogous to a metallothionein.

In *Bacillus subtilis* (30) and *Staphylococcus aureus* (31) Cd^{2+} was transported via a Mn^{2+} transport system. The Cd^{2+} resistant strains of *B. subtilis* (30) showed less Cd^{2+} uptake than Cd^{2+} sensitive strains. An energy-dependent Cd^{2+} efflux system was found in Cd^{2+} resistant strains of *S. aureus*, which may prevent high internal Cd^{2+} concentration (31) and may be encoded by a plasmid (32).

Factors affecting metal removal

Although it is usual to define the metal removal capacity by cells in terms of mg (metal)/g (biomass), this is misleading and it would be advisable to present in terms of moles of metal, especially for high atomic weight elements.

Since biosorption process is a kind of ion-exchange, the products excreted by or derived from microbial cells, e.g. metal binding proteins and siderophores, biopigments, capsular and exopolymetric material, and cell wall polymers can be used for metal removal (2). However, the efficiency is not always the same and in some cases whole-cell biomass may be necessary for maximal uptake e.g. extracted chitin alone was less efficient as a uranium adsorbent (33).

Adsorption of cationic metal to an anionic bacterial substances is pH-dependent. Since various metals e.g. Cu, Fe, Mn, and Al form insoluble oxides or hydroxides at neutral or alkaline pH, their free ions are not available for binding. At acidic pH, metal binding is often limited by the increasing tendency of H^+ to compete for anionic bacterial binding sites (34). This dependency on pH varies with the affinity of the particular metal and the nature of the cell adsorption sites. For example, the pH optimum for different metals with a single strain (*Citrobacter* sp. versus Cd and Sr; *Rhizopus arrhizus* versus Ra, Th and U) are different possibly due to different speciation characters of each metals (Table 1). Similarly the pH opti-

Table 1. pH optima for metal removal by microorganisms

Metal	Organism	Optimum pH	Reference
Cd	<i>Citrobacter sp.</i>	6.5~8	Macaskie and Dean, 1984
Sr	<i>Citrobacter sp.</i>	above 9	Macaskie and Dean, 1985
Ra	<i>Rhizopus arrhizus</i>	7~10	Tsezos and Keller, 1983
Th	<i>Rhizopus arrhizus</i>	4~5	Tsezos and Volesky, 1982a
U	<i>Rhizopus arrhizus</i>	4~5	Tsezos and Volesky, 1982b
U	<i>Saccharomyces cerevisiae</i>	3~4	Strandberg <i>et al.</i> , 1981
U	<i>Aspergillus niger</i>	5.8	Yakubu and Dudeney, 1986
Zn	<i>Aspergillus niger</i>	increase as pH increased	Luef <i>et al.</i> , 1991
Cu	<i>Zoogloea ramigera</i>	above 5	Norberg and Rydin, 1984

Table 2. Effect of other cations on metal removal

Metal	Organism	Inhibiting metal	Reference
Cd (surface biosorption)	<i>Aureobasidium pullulans</i>	Hg, Co, Mg, Mn, Cu	Mowill and Gadd, 1984
Cd (influx)	<i>Aureobasidium pullulans</i>	Ca	Mowill and Gadd, 1984
	<i>Saccharomyces cerevisiae</i>	Ca	Kessels <i>et al.</i> , 1985
U (surface adsorption)	<i>Saccharomyces cerevisiae</i>	Ca	Strandberg <i>et al.</i> , 1981
U (surface adsorption)	<i>Rhizopus arrhizus</i>	Zn, Cu, Fe(II)	Tsezos, 1983
U (surface adsorption)	<i>Streptomyces levoris</i>	Co, Cu, Ni, Zn, Fe(III)	McGready and Lakshmanan, 1986

imum for one metal (e.g. uranium) uptake is organism dependent possibly due to different adsorptive sites of each organisms (Table 1). As with surface binding, pH can affect intracellular uptake. In *Aureobasidium pullulans*, both surface binding and rates of intracellular uptake of Cu and Cd were markedly decreased at pH values less than 6.5 (35). The pH can also affect the precipitation of metal ions. The copper removal by *Zoogloea ramigera* is increased as pH increased above 5 (Table 1). However only part of the copper removed is due to adsorption to bacterial biomass since the formation of copper hydroxide is initiated above pH 5 and then be trapped within polymer matrix (36).

Competition between metal ions can also reduce the metal removal efficiency (Table 2). Intracellular cadmium accumulation in *A. pullulans* is inhibited by Ca. However surface biosorption of Cd in the same organism was inhibited by Hg, Co,

Mg, Mn and Cu while Ca and Zn had no effect (35). Uranium uptake by *S. cerevisiae* was affected by Ca while *Pseudomonas aeruginosa* was not affected (24). Uranium uptake by *R. arrhizus* was inhibited by Zn, Cu, and Fe (II) (33) although thorium uptake in this organism was unaffected by Zn or Fe (II) (37).

Further complication arise in the presence of anionic species such as CN^- , $EDTA$, Cl^- , CO_3^{2-} , and HCO_3^- since chelated or complexed metals are a major problem in metal removal by microorganisms. For example, cadmium adsorption by an immobilized *Citrobacter sp.* was similarly inhibited by 5 mM CN^- and 250 mM Cl^- . At these concentrations, the molar ratio of $CN^-:Cd$ and $Cl^-:Cd$ were 2.8:1 and 140:1, respectively (38). Thus, even though the metal-removal mechanism was largely cyanide resistant, cyanide was 50 times more inhibitory than chloride under these conditions. This result could be explained in terms of

stability of $\text{Cd}(\text{CN})_3^-$ and $\text{Cd}(\text{Cl})_3^-$ formation.

Traditional biosorption process

Traditional metal removal process using microbial biosorbent relies on a solid-liquid contact process consisting of the metal sequestering and metal deposition cycle. Two distinctive types of reactor, classified according to the nature of bacterial growth in them, have been used: activated sludge process and trickling filters.

The activated sludge process depends on flocculated bacteria which are grown in suspension in aerated tank reactors. The flocculation of the microorganisms within microbially produced extracellular polymers results in coprecipitation of microbial cells and metals (39). Although metal desolubilization is basically a fortuitous process onto the extracellular polymers and cell surfaces, the microbial metabolic functions of the reactor are sensitive to metal toxicity; even the main flocculative organism, *Zoogloea ramigera*, was inhibited at 10 mg/liter of heavy metals (40). Since in a continuous treatment process the input flow composition vary unpredictably, the nature of ill-defined mixed culture of this system is somewhat unpredictable and is difficult to reproduce, and makes it questionable to apply to specific metal pollution control.

Trickling filter system relies on the growth of bacteria as biofilm on the surfaces of solid support.

The biological fluidized bed (BFB) process is a hybrid of the activated sludge process and the trickling filter process. It is an attached-growth process in which the bacteria are self-immobilized on fluidized suspended particles (41). This process provides large surface/unit volume, enables to use reduced plant size. However, with an aerobic BFB reactor, the oxygen demand per unit volume is accordingly increased such that air alone can not provide sufficient oxygen and commercial oxygen must be supplied. It is also difficult to recycle the bacterial and the metal components of sludge.

Process using defined immobilized bacteria

Microorganisms are small particles with low density and low rigidity. The use of such small particles in the CSTR (continuous stirred tank reactors) with a large volume of metal-containing solution makes the rapid and efficient separation using techniques such as filtration, sedimentation or centrifugation difficult. If biosorbent (biomass) are modified to have a particle size in the range of commercial adsorbents (0.5~1.5 mm), it would have an increased mechanical strength, high particle porosity, hydrophilicity and increased resistance to aggressive chemical environments (42). This will be substantial advantages in terms of efficiency and operation cost. These modification of biomass is usually achieved by immobilization of microorganisms. The speed and efficiency of metal uptake may vary depending on the quantity of biomass, methods of immobilization, flow rate, and concentration of input metal. Some examples of the use of immobilization for metal removal are shown in Table 3. Several methods of immobilization are in use. These includes cross-linking with a bifunctional reagent (e.g. formaldehyde), entrapment into inorganic material (e.g. silica gel, polyacrylamide) or into organic ones (e.g. synthetic resins), and biofilm formation on support material (e.g. polyester foam, sand, polyvinyl chloride). Simple adsorption to carrier surfaces relies on cell surface/carrier electrostatic interactions and is weak and pH-dependent, while chemical coupling to supports presents toxicity problems. Entrapment within gels gives a low mechanical strength in addition to diffusional limitations. Thus biofilm formation (auto immobilization) on the surface of a solid carrier has been used for large-scale metal waste treatment.

Because both nonliving (nonmetabolizing) biomass and living cells accumulate metals, either type of material can be immobilized for metal removal. Each method has its own advantages and disadvantages (Table 4). By using the immobilized nonliving biomass, there are no toxicity limitations

Table 3. Some examples of metal removal by immobilized microorganisms

Organism	Metal	Mechanism	Immobilization type	Reference
<i>Ascohyllum nodosum</i>	Cd (149 mg/g biomass)	biosorption	crosslinked with formaldehyde	Holan <i>et al.</i> , 1993
	Cd (26 mg/g biomass)	biosorption	entrapped in silica gel	Holan <i>et al.</i> , 1993
<i>Stichococcus bacillaris</i>	Pb (not stated)	adsorption (loss of 40% capacity after immobilization)	entrapped in silica gel	Mahan and Holcombe, 1991
<i>Chlorella homosphaera</i>	Cd (99.5% of 41 mg/L input)	biosorption (alginic matrix are responsible of 40% of gold uptake)	sodium alginate immobilized	Costa and Leite, 1991
	Zn (99.6% of 720 mg/L input)			
	Au (90% of 213 mg/L input)			
<i>Chlorella regularis</i>	U (159 mg/g biomass)	endothermic reaction	polyacrylamide immobilized	Nikajima <i>et al.</i> , 1982
<i>Streptomyces viridochromogenes</i>	U (312 mg/g biomass)	endothermic reaction	polyacrylamide immobilized	Nakajima <i>et al.</i> , 1982
<i>Citrobacter</i> sp.	Cd (9000 mg/g biomass)	formation of metal phosphate with HPO_4^{2-} liberated by phosphate reactiob	polyacrylamide gel immobilized	Macaskie and Dean, 1984
	Pb (99.9% of 1 mM input)		immobilized biofilm	Macaskie and Dean, 1987a
<i>Aspergillus oryzae</i>	Cd (10 mg/g biomass)	biosorption	reticulated foam immobilized biofilm	Kiff and Little, 1986
<i>Arthrobacter viscosus</i>	Cd (90 mg/g biomass)	adsorption by extracellular polysaccharide capsular coat	sand-immobilized biofilm	Scott <i>et al.</i> , 1986
<i>Pseudomonas aeruginosa</i>	Pu (95% of a 1.7 nCi PuCl ₄)	entrapment of particulate PuO ₂	polypropylene web immobilized biofilm	Tengerdy <i>et al.</i> , 1981
<i>Pseudomonas fluorescens</i>	Pb (90% of 1 mg/L input)	adsorption	polyvinyl chloride granule immobilized biofilm	Tengerdy <i>et al.</i> , 1981
	Zn (50% of 10 mg/L input)			

Table 4. Comparison of immobilization of nonliving biomass and living cells.

	Nonliving biomass	Living cells
Growth	independent	dependent
Pregrowth	important	less important
Nutrient	unnecessary	necessary
Metal complexation with nutrients or metabolic products	no	possible
Metal toxicity	less important	important
Choice of immobilization technique	many	few (governed by toxicity limitations or thermal inactivation)
Potential for metal recovery	good	limited (metals may be intracellularly bound)
Mathematical modeling	possible	difficult
Potential for altering metal valency state	yes	no
Sensitivity to external factors (pH, metal speciation)	sensitive	less sensitive

since cell growth and metal response are decoupled. The use of nonmetabolizing cells removes interference of metabolic end products with soluble metal speciation. In general, metal removal occurs rapidly and efficiently since metal uptake rely on the ion exchanger characteristics of cell surface polymer. However the biological use may be limited to the choice of growth conditions since pre-growth conditions are important for biomass and biosorbent production (43).

The use of living cells has a potential for a long-term continuous process in which desorption is unnecessary. Metal detoxification by degradation, oxidation, or reductive processes are also possible i.e. the potential to alter the metal speciation to suit the need is possible. However industrial waste may be so polluted as to be lethal to living microorganisms. The presence of toxic metals and surface-active agents together with the variability of pH and salt concentrations in industrial wastes make it difficult to use living system. Moreover, need for the nutrients to maintain microbial viability in waste treatment plant is impractical unless readily available cheap nutrient source are used. This nutrients addition may also result in the contamination of unwanted bacteria.

Metal desorption and biomass recycling

One critical factor in the evaluation of economic feasibility and technical applications of immobilization is the metal desorption and biomass recycling. Desorption of metal permits production of a small volume of concentrated metal when compared with the initial large volume, dilute inflow. For maximum benefit, desorption techniques should be efficient, economical, and result in minimal damage to the biomass.

Although dilute acid (0.1 M HNO₃) is effective desorption agent (44,45), nitrogenous waste is environmentally unacceptable. Organic acid (0.5 M citrate, pH 5) was used successfully to desorb Cd from metal-loaded *Citrobacter* sp. (46). Although EDTA appeared to have potential and apparently did not have adverse effects on the biomass (47), final disposal of chelated wastes could be problem.

Carbonates are also efficient and perhaps have the most commercial potential. Sodium carbonate (Na₂CO₃) is efficient, but the equilibrium elution pH is too high (pH 11~12) and may result in some damage to the biomass (48). Among several elution systems examined for U desorption from *R. arrhizus*, sodium bicarbonate (NaHCO₃) appeared the most promising, and gave the least da-

mage to the biomass, thus allowed the biomass uranium uptake capacity to remain near 90% of the original value following multiple adsorption-desorption cycles (48).

Conclusion

Two types of research on metal removal are underway. One is the continuing search for new potent biosorbent materials or finding the growth conditions of the cells to give optimal biosorbent quality. The other is to derive further knowledge of the biosorbent phenomenon as well as the scale-up parameters for the biosorption process.

Living cells, dead biomass, and derived products can be effective in the removal of metals and radionuclides, but for industrial application any biomass-related process must be economically competitive with existing technologies. Microorganisms are highly efficient at dilute metal solution and therefore may not completely replace existing technologies, but it may be needed after an existing treatment that is not completely efficient for low concentrations of metal solutions. The current trend is the use of immobilized preparations with recovery of the concentrated metal in small volume using an inexpensive desorption agent. The advantages of immobilized cell systems are enormous including high biomass concentration in reactor, easy separation of liquid and biomass etc. However other variables such as anions, cations, organic compounds, and pH should be considered when immobilized cell system is used. It is to be hoped that there will be successful use of microorganisms in the near future to give economic and environmental benefits. More basic and applied researches are required in order to make better and more efficient use of the biosorption phenomenon and its potential.

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