# Microbial Biosorption of Radionuclides in Liquid Effluent Treatment

S. MCELDOWNEY

*School of Biotechnology, Polytechnic of Central London, 115 New Cavendish Street, London W1M 8JS, UK*

Received and Accepted November 2, 1989

**Index Entries:** Microbial biosorption; radionuclide biosorption; liquid effluent treatment; waste-stream treatment.

## INTRODUCTION

The application of microbial processes to recover radionuclides from liquid effluents is a relatively new development within biotechnology. It forms part of an exciting and potentially rewarding new emphasis in biotechnology towards the resolution of environmental problems.

Waste streams containing radioactive elements originate from uranium mines and milling, from nuclear power production, and from the reprocessing of spent fuel rods. The presence of radionuclides in liquid effluents may result in the loss of an economically and strategically valuable resource, e.g., thorium, a nuclear fuel element often discharged in effluent during uranium mining. In addition, any release of radioactive elements into the environment offers a possible health hazard and pollution problem. It is likely that the nuclear industry will be increasingly pressurized for commercial and environmental reasons to limit the release of radionuclides to the environment. The aims of radionuclide recovery by both conventional and new microbial processes therefore include pollution control and the recycling of nuclear fuel or radioactive elements. It has been proposed that biotechnological processes for radionuclide recovery may also permit the economic recovery of radioactive elements, e.g., uranium, from natural waters (*1*) and aid in the recovery of uranium from low-grade ores (*2*).

 

The aim of this review is to consider the ability of fungal, yeast, and bacterial radionuclide biosorbents to contribute successful and efficient radionuclide biosorption processes to liquid effluent treatment. There will be a brief description of relevant biosorption mechanisms as understood at present, an analysis of the impact of environmental factors on biosorption, and consideration of desorption mechanisms and efficiency. It is not, however, intended to discuss process design.

## Characteristics of Radioactive Liquid Effluents

The characteristics and nuclide composition of liquid effluents depends on the source. Effluents from uranium mines and mill tailings contain several radioactive elements, including uranium, isotopes of thorium, radium and polonium. It has been estimated that approx 85% of the total radioactivity in ore is discharged in the mine tailings (*3*). Typically, reprocessing streams are complex, containing from 5 to 20 different radionuclides, e.g., uranium, transuranics such as plutonium and americium, various lanthinides, ruthenium, and caesium. The concentration and radionuclide types vary with the particular waste-stream. In addition to the radioactive isotopes, the effluents carry variable numbers, types, and concentrations of nonactive anionic, e.g., $NO_3^-$, $CL^-$, $SO_4^{2-}$, cationic, e.g., $Fe^{3+}$, $Zn^{2+}$, $Cu^{2+}$, and organic species. The pH of the waste-stream also varies with the effluent source. Uranium mine and mill tailing overflow may have a high pH (*4*), whereas reprocessing effluents range from acid pH to alkaline pH, e.g., pH 12. Characteristics of the waste-streams may change in time. For example, effluent from uranium mines will fluctuate with rainfall.

## Biotechnological Processes for Waste-Stream Treatment

At present, radioactive waste-streams are decontaminated and valuable radionuclides recovered using physicochemical techniques, e.g., ion-exchange, ultrafiltration, and flocculation. Solvent extraction and ion-exchange are particularly important techniques in the concentration of dilute waste-streams throughout the nuclear industry (*5*). These "conventional" treatment processes can be expensive and do not always achieve required radionuclide removal levels, particularly if large volumes of low concentration waste are processed.

Alternatives to conventional techniques for radioactive liquid treatment include biotechnological approaches, such as radionuclide biosorption and bioaccumulation. Biosorption has been defined as "the sequestering of metal ions by solid materials of natural origin" (*6*), often whole microbial cells. The radionuclides are adsorbed and concentrated by the

microorganisms. To be successful, a biosorption process for radionuclide removal from waste-streams should comply to the following requirements (*7,8*):

1. The microbial biomass should have a high biosorption capacity, and biosorption should be rapid, efficient, and compare favorably with conventional separation techniques;
2. Ideally, the biosorption process should be pH stable and unaffected by other waste-stream constituents;
3. Biosorption should be radionuclide selective to separate valuable radionuclides from a mixed cocktail, particularly if recovery rather than pollution control is the process aim;
4. Recovery of metal ions from the biosorbent by desorption should be rapid, metal-selective, and economic;
5. After metal elution, the biosorbent should be reusable with no significant loss in radionuclide carrying capacity;
6. The biosorbent biomass should be cheap to grow and recover.

It is important that the biotechnological processes are commerically and technologically competitive with conventional techniques. Brierly et al. (*9*) suggested that a competitive biosorbent for metal removal should be >99% efficient with a loading capacity in the order of 150 mg metal/g biomass. It has already been demonstrated that microbial biosorbents compare favorably in capacity with conventional techniques (Table 1). The economic viability of biosorption processes would be improved by efficient radionuclide recovery from the biosorbent and the capability for multiple adsorption-desorption cycles.

## CHARACTERISTICS AND MECHANISMS OF RADIONUCLIDE UPTAKE BY MICROBIAL CELLS

### General Characteristics of Radionuclide Uptake

A diverse range of microorganisms are able to accumulate radionuclides, some to large amounts (Table 2). There is considerable variation in radionuclide biosorption rate and capacity among species and even strains. Attempts have been made to rank microbial types for radionuclide uptake capacity, e.g., uranium biosorption, actinomycetes > bacteria = yeasts > fungi (*10*). However, these seem inappropriate given the differences in biosorption at species level. Moreover, biomass uptake capacity varies with radionuclide type and environmental conditions.

Microorganisms can accumulate metals by several active and passive mechanisms that may act singly or in combination. The mechanisms in-

Table 1
Comparison of the Efficiency
of Biosorbent and Conventional Adsorption Techniques in Radionuclide Sorption

| Biosorbent | Radionuclide | Multiple of biosorbent efficiency compared to ion exchange resin | Multiple of biosorbent efficiency compared to activated carbon | References |
|---|---|---|---|---|
| Biomass A[1] | radium | 13.7 | 11.5 | (4) |
| Biomass B[1] | radium | 25.9 | 21.4 | (4) |
| *Rhizopus arrhizus* | uranium | 2.5 | 3.3 | (4) |
| *Aspergillus niger* | uranium | 14.0 | - | (24) |
| *Rhizopus arrhizus* | thorium | 2.0 | 2.3 | (14) |

[1] Biomass A and B were municipal waste water activated return sludges from different treatment plants.

Table 2
Uranium, Thorium, and Radium Biosorption Capacities for Selected Microbial Biomass

| Metal | Microbial Type | Species | Metal uptake (mg/g cell dry wt) | Initial solution concentration ($\mu g\ mL^{-1}$) | References |
|---|---|---|---|---|---|
| U | bacteria | *Bacillus subtilis* | 80–90 | 10 | (41) |
| | | *B. subtilis* AHU 1219 | 36.33 | 10 | (10) |
| | | *Escherichia coli* AHU 1520 | 22.59 | 10 | (10) |
| | | *Pseudomonas saccharophilia* | 80–90 | 10 | (41) |
| | | *Pseudomonas aeruginosa* | 150 | 10 | (15) |
| | | *Citrobacter sp* | 9000 | 200 | (31) |
| | | *Zoogloea ramigera* | 800 | 500–2500 | (27) |
| | actinomycetes | *Actinomyces levoris* HUT 6156 | 45.47 | 10 | (10) |
| | | *Actinomyces flavoviridis* | 80–90 | 10 | (41) |
| | | *Streptomyces griseofulvus* | 143.5 | 10 | (10) |
| | yeasts | *Candida albicans* 1AM 4966 | 15.7 | 10 | (10) |
| | | *Rhodoturula glutinis* 12228 | 34.8 | 10 | (10) |
| | | *Saccharomyces cerevisiae* AHU 3818 | 33.8 | 10 | (10) |
| | | *Saccharomyces cerevisiae* | 150 | 100 | (15) |
| | fungi | *Aspergillus niger* AHU 7120 | 80.7 | 10 | (10) |
| | | *Rhizopus arrhizus* | >180 | 30 | (14) |
| | | *Penicillium digitatum* | 5.7 | 100 | (26) |
| | | *Penicillium lilacinum* | 80–90 | 10 | (41) |
| Th | fungi | *R. arrhizus* | 180 | 30 | (14) |
| Ra | fungi | *Penicillium chrysogenum* | $5 \times 10^4$[a] | 1000[b] | (4) |

[a] measured in nCi/g
[b] pCi/l

clude active transport across the cell membrane, particle entrapment by such exocellular components as flagella, adsorption, cation exchange, complexation or coordination, chelation, and microprecipitation. Essentially, active transport and particle entrapment are functions of living cells. The remainder of the mechanisms are passive physicochemical processes that may occur to living or dead cells (*11,12*) or to cellular debris (*13,14*). Pas-

sive uptake mechanisms generally occur at cell wall level and are not affected by metabolic inhibitors, such as dinitrophenol and sodium azide, corroborating the noninvolvement of microbial metabolism (*10,15*). Indeed, the metal biosorption capacity of nonliving biomass can be greater than that of equivalent living cells (*11,12*). The physicochemical processes involved in radionuclide biosorption are often rapid to reach equilibrium within minutes or even seconds (*15,16*) and are also reversible (*15,17*). No correlation has been found between metal ionic charge or effective nuclear charge of ions and adsorption, although larger ions appear to adsorb more strongly than smaller ionic radii ions (*18*).

The basis for different radionuclide accumulation capacities shown by microorganisms is partly the result of cell surface characteristics. Microbial cell walls are complex and normally charged. Certain cell wall properties, e.g., types of polar groups present and charge distribution within cell wall macromolecules, may influence the capacity and selectivity of radionuclide biosorption. Bacterial walls contain polysaccharides, proteins, lipids, and peptidoglycan macromolecules, whereas fungal and yeast walls consist predominantly of mannan polysaccharides, chitin, galactosamine, protein, and lipid. Carboxyl, hydroxyl, sulphyl, phosphyl, and amyl functional groups of the macromolecules confer the cell wall with its charge characteristics. The proportions and composition of wall macromolecular components may change with growth condition (*19*) and growth rate (*19, 20*). These fluctuations may be reflected in changes in cell surface charge characteristics (*21*) and consequently may influence radionuclide biosorption. There appears to be evidence for this type of effect, since uranium sorption capacity has been found to vary with growth phase and growth condition of batch grown cells (*6,22*). Differences in uptake between live and dead biomass (*11*) may have a similar basis.

An understanding of the basic mechanisms underlying radionuclide biosorption should aid in manipulating the process, e.g., through controlling process parameters, to achieve maximum radionuclide removal from an effluent. Although the biosorption mechanisms of only a limited number of radionuclides has been studied, differences among species and radionuclide are amply illustrated.

## Mechanisms of Uranium Biosorption

A number of different mechanisms, acting either singly or jointly, have been described in uranium biosorption. Cation exchange, complexation, and coordination appear to be particularly common. Uranium accumulation has been found to follow Freundlish adsorption isotherms and to be dependent on the physicochemistry of the cell surface (*10*).

Ion-exchange processes are among the conventional techniques frequently applied to metal recovery from waste-streams. Radionuclide sequestration by a variety of bacteria, fungi, and yeasts occurs by a similar cation exchange phenomenon (*11,23,24*). During this process,

metal cations exchange with counterions associated with cell surface anionic groups. Cation exchange uranium binding has been attributed to carboxyl groups associated with bacterial cell wall peptidoglycan, e.g., *Escherichia coli* K-12 (*25*), fungal and yeast wall proteins, glycoproteins, and chitin, e.g., *Aspergillus niger* (*24*) and *Rhizopus arrhizus* (*11*). Cell wall phosphyl groups also contribute to uranium cation exchange processes in some species. A simple stoichiometric ratio exists between uranium uptake and cell wall phosphorus content for *Streptomyces longwoodensis*. It was suggested that uranium was bound by cation exchange to phosphodiester residues in the cell wall and cytoplasm, displacing $H^+$ ions in a 1:1 ratio (*22*).

Complexation or coordination of uranium to cell wall components has been identified as an uptake mechanism for several biomass types. The anionic sites and cell surface ligands involved in uranium coordination have often not been determined. However, it has been proposed that several different active sites and ligands contributed to U(VI) ion coordination in *Penicillium digitatum* (*26*) and uranyl uptake by *R. arrhizus* (*18*). Some sites may be involved in forming primary bonds, e.g., carboxyl and phosphyl ligands, and some, which form weaker bonds with metals, may augment this complexation, e.g., hydroxyl and amyl groups (*18*). Uranium preferentially complexed with phosphyl groups at *S. cerevisiae* cell surfaces, and only on saturation of these sites did uranium complex with wall carboxyl groups (*15*). Complexation of uranyl ions to negatively charged groups associated with exocellular polysaccharide polymers has also been indicated, e.g., *Zoogloae ramigera* (*27*).

Nonstoichiometric accumulation for metal cations has been observed for several microbial/metal systems, including uranium adsorption. Biosorption of uranyl ions by *S. cerevisiae* was nonstoichiometric, apparently exceeding the anionic complexation site capacity (*15*). It has been proposed that nonstoichiometric adsorption may be the result of additional metal "crystallizing" on metal previously bound by complexation (*28*). Such a process relies on previously bound metal acting as a nucleation point and, therefore, occurs only in combination with other biosorption mechanisms.

Several adsorption processes acting in combination is a relatively common phenomenon in uranium microbial biosorption. Two mechanisms, cation exchange and complexation, were active in accumulation by *Rhizopus oligosporus*, since uptake was reversed or inhibited by the addition of complexing ligands or by a reduction on pH ($<2$) (*6*). In an excellent analysis, Tsezos & Volesky (*29*) indicated that three mechanism were operative in uranyl ion biosorption by *R. arrhizus*. Two of the mechanisms occurred simultaneously and rapidly ($<60$ s to equilibrium). In the first process, uranyl ions coordinated with the amino nitrogen of the cell wall chitin allowing the second process, in which complexation sites acted an nucleation points for deposition of additional uranium. These two mechanisms

accounted for 66% of the total uptake capacity (0.5 mmol U/g cell dry wt). The third process was considerably slower, only reaching equilibrium after 30 min, and involving the precipitation of uranyl hydroxide within cell wall microcrystalline chitin. The three mechanisms were closely interrelated, and although the complexation mechanism only accounted for $<3\%$ of uptake capacity, the other two processes were triggered or assisted by it.

One further uranium biosorption mechanism has been elucidated. Uranium uptake by a *Citrobacter* sp. relied on insoluble metal phosphate precipitation at the cell surface (*30,31,32*). The uranium was accumulated by the activity of phosphatase enzymes released from cells and active in resting cells. The phosphatase enzyme, previously induced, cleaved organic phosphate, continuously supplied as glycerol-2-phosphate, to inorganic phosphate, which precipitated with uranium at the cell surface. It is unclear if any other microbial species have a similar uptake mechanism.

### Mechanisms of Thorium Biosorption

Not only are there distinctions in radionuclide adsorptive mechanisms with microbial species, as demonstrated for uranium (*see* Mechanisms of Uranium Biosorption), but there are also mechanistic differences in uptake by a single species with radionuclide type. For example, there were differences in thorium and uranium biosorption by *R. arrhizus* (*14,29,33*). Thorium biosorption consisted of a combination of two mechanisms (*33*) compared to the three processes of uranium sorption (*29*). The thorium biosorptive processes were rapid to saturation within 60 s, and could occur both separately or simultaneously (*33*). The first process involved coordination between thorium and the amino nitrogen of chitin. The second mechanism involved the adsorption of hydrolyzed thorium to the cell surface, a process that accounted for over 95% of thorium uptake capacity. Unlike uranium biosorption by *R. arrhizus* where three processes were interrelated, the two mechanisms of thorium sorption were unrelated (*33*).

## PROCESS CHARACTERISTICS OF MICROBIAL RADIONUCLIDE BIOSORPTION

An understanding of the equilibrium and kinetic characteristics of biosorption, of the impact of environmental factors, e.g., pH, and of biosorption selectivity, should permit an assessment, potentially quantitative, of biosorption efficiency and aid in the design of biosorption systems.

### Equilibrium and Kinetic Characteristics of Biosorption

Ideally, biosorption should be rapid and independent of radionuclide concentration. In engineering terms, rapid biosorption would permit the

use of relatively low size equipment with short residence times. Insensitivity to radionuclide concentration would safeguard against fluctuations in waste-stream concentration influencing biosorption efficiency.

The time to equilibrium for a variety of biosorption processes compares favorably with ion exchange techniques, which can take several hours to reach equilibrium. Exceptionally rapid uptake of uranium to equilibrium within seconds of exposure has been reported for several biomass types, e.g., *S. longwoodensis* (*22*) and *Pseudomonas aeruginosa* (*15*). However, other biosorbents, such as *Actinomyces levoris* and *R. arrhizus*, take minutes to reach an equilibrium plateau in uranium uptake (*10,11*). Differences in the rate of biosorption with biosorbent type are further highlighted for radium accumulation with *Penicillium chrysogenum* reaching equilibrium after 2 min whereas municipal waste activated sludge took 5 min (*34*). However, considerably slower rationuclide uptake rates have been found, biosorption reaching equilibrium only after hours of contact (*6,26*). Some of the slower rate uranium biosorption systems presented a biphasic time course. An initial rapid (s) biosorption, often involving significant (>66%) uptake, followed by a slower equilibration over a period of hours (*24,29*). Biphasic accumulation may indicate that more than one uptake mechanism was operative (*29*), or that the bulk of adsorption was occurring within the cell wall and was limited by diffusion (*24*).

The effect of initial radionuclide concentration, under batch conditions, on biosorption rate appears to vary with biomass type. Biosorption isotherms for *R. arrhizus* and other fungal species were independent of initial uranium or thorium concentration, and active biosorption occurred even at low residual equilibrium metal concentrations (*14*). However, other biosorption systems show a strong dependence on radionuclide concentration. For example, *P. chrysogenum* showed a second-order dependence for radium uptake rate on solute concentration (*34*).

There is a linear relationship between uranium adsorbed by microbial biomass and the initial and equilibrium concentration of uranium in solution (*10,26*). Often after an initial steep rise in accumulation with concentration, the increase slows (*10*) and the biomass eventually becomes saturated at high uranium concentrations (*24*). Mackaskie and Dean (*31*) have claimed that, by using immobilized cells of *Citrobacter* sp and a continuous flow system, the uranium saturation limits could be raised over those of batch systems.

It is evident that the carrying capacity of biomass can be raised by increasing cell concentration. In fact, uranium uptake at saturation has been shown to be linearly proportional to cell concentration (*22*).

## The Effect of Waste-Stream pH on Biosorption

Liquid effluent pH may have several important effects on biomass and solution characteristics, which may influence biosorption. Ionization of functional groups, e.g., carboxyl, carried on microbial surfaces is con-

trolled by solution pH. Waste-stream pH will also affect radionuclide solution chemistry. For example, uranium can exist in solution as $UO_2^{2+}$, $UO_2(OH)^+$, $(UO_2)_2(OH)_2^{2+}$, or $(UO_2)_3(OH)_5^+$ and thorium exists not only in various hydrolyzed forms, but also as colloidal particles, depending on solution pH (*14*). In addition to these effects, pH may influence the outcome of competition for cell surface adsorption sites between radionuclides and other metals.

As might be predicted, the biomass type, probably through differences in surface characteristics, and the radionuclide type induce differences in biosorption response with pH. Solution pH appears to influence both the rate of uptake and the final capacity. The pH range of maximal uranium biosorption varies with biomass type. Tsezos & Volesky (*14*) found that biosorbents could be divided into two groups, depending on the influence of pH on uptake. The first group, including *Pseudomonas fluorescens* and *P. chrysogenum,* showed biosorption independent of pH between pH2–4. The second group, e.g., *Streptomyces niveus* and industrial activated sludge, displayed lower uranium biosorption at pH2 than pH4. Both groups showed maximal adsorption capacity at pH4 to 5. It was suggested that "group 1" organisms biosorbed $UO_2^{2+}$ over all the pH range. Uranium species in solution exist in dynamic equilibrium; thus, even though $UO_2^{2+}$ represents only 9% of the species present at pH 5, its biosorption displaces the equilibrium from the hydrolyzed species toward the uranyl ion, permitting similar levels of sorption from pH2–5. "Group 2" organisms may have preferentially biosorbed hydrolyzed uranium species, or these may have enhanced uptake in some way (*14*). Lower uptake at pH 2.5–3 has also been explained in terms of competition between protons and uranium for cell surface biosorption sites (*18,22*).

Other researchers have found a large dependence on pH for uranium biosorption capacity, i.e., similar to "Group 2" organisms above, although the pH range of maximum uptake varies with biosorbent, e.g., pH 3.5–5.0, *R. arrhizus* (*22*); *Penicillium* sp. (*2*); and *S. longwoodensis* (*11*); pH 5.6–6, *A. niger* (*24*) and; *A. levoris* (*10*). The rate of uranium uptake may also be pH dependent. For example, biosorption rate for *S. cerevisiae* increased as the pH was raised from 2.5 to 5.5, although maximum capacity only occurred between pH 3 and pH 4 (*15*).

Thorium biosorption by *R. arrhizus* was also found to be pH dependent with higher adsorption at pH 4 and pH 5 than pH 2. Since hydrolyzed thorium species, e.g., $Th(OH)^{2+}$, are dominant at pH 4 to pH 5, it was considered likely that these were preferentially adsorbed (*14,33*). Similarly, radium biosorption is affected by solution pH. Two biomass types, *P. chrysogenum* and waste-water biological treatment plant biomass, showed maximum uptake capacity at pH 7–10, with reduced uptake at pH 4 and none at pH 2 (*4*). It was thought that increased biosorption was related to a decrease in radium solubility with increasing pH.

Insensitivity to solution pH in a biosorption system would confer certain process advantages. Some biomass types do show relative pH indepen-

dence in radionuclide uptake. "Group 1" organisms described by Tsezos & Volesky (*14*) display uranium uptake with a degree of pH independence, and other biosorbants have also been found to be relatively unaffected by pH for uranium sorption (*6,17,26*) and for radium biosorption (*34*).

It is not only radionuclide solution chemistry that determines pH effects on biosorption, but also the type and ionization of cell surface groups and the mechanism of radionuclide bioaccumulation. The influence of pH may not only vary with biosorbent type, but also with preharvest growth conditions, since these will influence the nature of the microbial cell surface (*see* General Characteristics of Radionuclide Uptake). It may be possible to modulate pH effects on biosorption by carefully selecting biomass type and growth conditions.

## The Effect of Temperature on Biosorption

Temperature influences both the rate and capacity of living and dead biomass radionuclide adsorption. An increase in temperature from 4°C to 35°C increased uranium biosorption capacity of several viable and heat-inactivated biomass types by 75% and 55%, respectively (*2*). Such an increase in equilibrium biosorption capacity with temperature (4–50°C) seems relatively common for both bacterial and fungal biosorbents with respect to uranium (*14,17,26*), thorium (*14*), and other metal cations (*35*). Nakajima et al. (*17*) described this increase in terms of an endothermic (13 kJ/mol) uranium adsorption process for *Streptomyces viridochromogenes*. On occasion, over low temperature ranges, e.g., 0–30°C, uranium uptake has been found to be relatively independent of temperature, e.g., *A. levoris* (*10*).

Biosorption rate appears to be temperature dependent, increasing with increased temperature (*15,26*). In column studies on immobilized *R. arrhizus* biomass adsorption of metal cations, e.g., $Cu^{2+}$ and $Fe^{3+}$, temperature affected both the kinetics and capacity of accumulation. Increasing temperatures (20–45°C) slowed the decline in uptake with column use and raised sorption capacity (*36*).

Biosorption as a dominantly physicochemical process will inevitably be affected by temperature. It may be possible to utilize temperature to improve process characteristics, as shown by Lewis & Kiff (*36*). However, the benefits in rate and capacity of biosorption must be great enough to outweigh any additional process costs involved in temperature control.

## The Effect of Waste-Stream Contaminants on Biosorption

Liquid effluents may contain a variety of active species and nonactive anionic and cationic components (*see* Introduction). These inorganic and organic ligands may influence biosorption through several mechanisms. They may compete for cell surface active sites reducing radionuclide up-

take (*37*) or may complex with the radionuclides competing with the cell suface for the metal (*38*). Alternatively, contaminants may increase uptake capacity. It is possible that ligands may bind to the biosorbent surface and subsequently increase uptake (*6*), or radionuclide–ligand complexes may sorb more readily to the biomass than the free metal (*39*). Contaminant effects on radionuclide biosorption may be further complicated by pH, since this alters radionuclide and other waste-stream components solution chemistry.

A degree of metal selectivity for recovery of specific radionuclides from effluent and a comparative tolerance to contaminant effects is clearly desirable in biosorption processes. The impact of waste-stream contaminants on biosorption efficiency may be large, and it is essential that the role of such components should be extensively studied.

Anionic ligands have been found to leave radionuclide biosorption unaltered or cause uptake inhibition. Anionic inhibition of radionuclide uptake has been explained in simple competition terms (*6*) and appears to be related to the stability of the ligand–radionuclide complexes (*6,40*). The order of stability of uranyl complexes is $EDTA^{2-} > CH_3CO_2^- > C_2O_4^{2-} > SCN^- > SO_4^{2-} > NO_3^- \geq Cl^-$, and with the exception of thiocyanate and oxylate, large biosorption inhibition was caused by anions to the left of nitrate for *R. oligosporus* (*6*). Similarly, uranyl uptake by *R. arrhizus* was appreciably inhibited by $EDTA^{2-}$ and sulfate, but not by glutamate, carbonate, or chloride (*40*). The inhibitory effect of anions varies with metal, e.g., $Pb^{2+}$ and $UO_2^{2+}$, anion concentration, and anion type (Table 3) (*40*). It is not unusual for uranium biosorption to be unchanged by the presence of some anionic components (Table 3) (*24,40*). The importance of ligand concentration in inhibition is demonstrated by the influence of bicarbonate on *A. levoris* and *S. viridrochromogenes* uranium biosorption (*10*). Below 0.3 m*M*, no uptake inhibition occurred, whereas 3 m*M* $NaHCO_3$ induced total uptake inhibition. The formation of stable uranium–carbonate complexes, e.g., $UO_2$, $(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$ pH 8, has been suggested as an explanation for carbonate ion inhibition of uranium biosorption for several biomass types (*1,10*).

Rarely anionic ligands have enhanced uranyl ion uptake. Uranium biosorption by *R. oligosporus* was increased by thiocyanate and oxalate; however, the effective pH of enhancement was different for the two ligands, a higher pH for thiocyanate and a lower pH for oxalate (*6*). Microbial surfaces present positively charged groups to the liquid phase to which anionic ligands, e.g., thiocyanate, adsorb. However, at low pH, thiocyanate is neutralized and therefore would not bind to the appropriate cell surface sites decreasing uranyl ion uptake. On the other hand, oxalate–uranium complexes are less likely to be closed ringed at low pH, permitting bridging with the cell surface (*6*). This underlines the influence of pH on adsorption interactions through solution chemistry, but it is important to realize that changes in cell surface group ionization induced by pH may also influence the outcome of these interactions.

Table 3
Effect of Anion and Cation Components
on Metal Uptake by *Rhizopus arrhizus* Biomass

(a) Percentage inhibition of uptake and corresponding anion-metal molar ratios.

| | Lead ($Pb^{2+}$) | | Uranium ($UO_2^{2+}$) | |
|---|---|---|---|---|
| Anion | % inhibition | Molar Ratio (anion:metal) | % inhibition | Molar Ratio |
| EDTA | 100 | equimolar | 39.0 | 5 |
| Glutamate | 0 | 12 | 0 | 12 |
| Sulphate | ppt(1) | | 15 | 10 |
| Phosphate | ppt | | ppt | |
| Carbonate | 0 | 12 | 0 | 12 |
| Chloride | 26.0 | 12 | 0 | 12 |

(1) ppt represents precipitate (From Tobin et al. (1987) (*40*))

(b) Percentage inhibition of uptake and corresponding molar ratio in cation direct competition studies.

| Primary cation | Competing cation | % change in uptake | Corresponding molar ratio |
|---|---|---|---|
| $Ag^+$ | $UO_2^{2+}$ | 88 | 1 |
| $Ag^+$ | $Cd^{2+}$ | 57 | 5 |

(From Tobin et al. (1988) (*37*))

(c) Percentage inhibition of uptake and corresponding molar ratios in exchange cation competition studies.

| Primary cation | Competing cation | % change in uptake | Corresponding molar ratio |
|---|---|---|---|
| $Ag^+$ | $UO_2^{2+}$ | 100 | 1 |

(From Tobin et al. (1988) (*37*))

Several organic compounds have been found to influence uranyl ion biosorption. The monocarboxylic acids, e.g., cysteine, and substituted monocarboxylic acids, e.g., *N*-methyl-glycine, did not interfere with uranyl ion uptake by *S. cerevisiae*. However, organic acids, e.g., acetic acid, and dicarboxylic amino acids, e.g., glutamic acid, did inhibit biosorption (*15*). The basis of the interference was related to the molecular configuration of the organic ligands, which affected complexation with uranyl ions. It was proposed that the former two types of ligands did not influence uptake, since their amino group positive charge inhibited $UO_2^{2+}$ binding to the proximal carboxyl group. In contrast, dicarboxyl amino acids and the corresponding organic acids were able to complex with $UO_2^{2+}$ through the distant carboxyl group inhibiting uranium biosorption through competition for $UO_2^{2+}$ (*15*).

The impact of metal cations on radionuclide accumulation has been investigated by several workers to assess the selectivity of biosorbents and to determine the significance of cation interference with biosorption. Biosorbents have often shown little selectivity for uranyl ions, the presence of other metal cations substantially reducing uptake (*2,37,41*). The extent of sorption inhibition varies with metal type. For example, $Fe^{3+}$, $Co^{2+}$, $Cu^{2+}$, $Ni^{2+}$, and $Zn^{2+}$ reduced uranyl biosorption by *S. levoris* by 64, 31, 25, 24, and 26%, respectively (*2*). Large inhibition of uranium uptake by iron has also been found for other biomass types (*16,42*) and may represent a significant problem, since $Fe^{3+}$ is a major contaminating metal in some liquid effluents. A preferential biosorption series of $Fe^{3+} > UO_2^{2+} > Cu^{2+} > Zn^{2+}$ was shown for *A. niger* (*24*), but no reciprocal inhibition of $Fe^{3+}$ biosorption by $UO_2^{2+}$ has been indicated (*42*). One mechanistic suggestion for $Fe^{3+}$ interference with $UO_2^{2+}$ uptake is that hydrolyzed $Fe^{3+}$ preferentially binds to cell wall active sites inhibiting uranyl ion binding (*16*).

Direct competition for a single binding site may not explain all metal–metal–biomass interactions. In a series of direct competition and exchange competition studies, it was found that some metal cations, e.g., $Cd^{2+}$ and $UO_2^{2+}$, bound to the same surface site. Uptake was reversible with one metal displacing the other (*37*). However, for other metal–metal systems, there was a multiplicity of active sites preferentially binding different metal cations. For example, below saturation concentrations, $UO_2^{2+}$ preferentially bound to certain cell wall sites on *R. arrhizus* biomass leaving other sites available for $Ag^+$ ions. As $Ag^+$ concentration increased, $UO_2^{2+}$ cations were displaced from less favorable active sites, but at $UO_2^{2+}$ saturation, all sites were occupied by uranyl ions and none by $Ag^+$ (*37*). The concentration of contaminating metal cations clearly is a major determinant in the outcome of uptake interference (Table 3b and c).

Effluent water hardness may be an important factor in biosorption efficiency, since both $Ca^{2+}$ and $Mg^{2+}$ may interfere with metal cation adsorption (*36*). Both the initial rate of biosorption and final equilibrium capacity for uranium uptake by *S. cerevisiae* were altered by the presence of $Ca^{2+}$. In addition, after 2 h of contact, $Ca^{2+}$ displaced $UO_2^{2+}$ from biomass active sites (*15*).

Waste-streams may contain significant quantities of monovalent metals. However, although a wide range of divalent and trivalent cations do interfere with uranium uptake, monovalent cations, e.g., $K^+$ and $Na^+$, apparently do not (*15,24,37*).

Some radionuclide biosorption systems show selective uptake and a tolerance to metal cation interference. For example, uranyl ions were preferentially adsorbed by immobilized streptomyces cells (*41*). The rate of radium biosorption was not affected by the presence of contaminating divalent cations (*34*), nor was thorium biosorption to *R. arrhizus* biomass much changed by $Fe^{2+}$ or $Zn^{2+}$ (*33*).

Hancock (*7*) suggests the Gram-positive bacteria may be selective in metal biosorption depending on strain and cell wall composition. It may

be the case that radionuclide selectivity may be enhanced by controlling cell wall composition and characteristics through preharvest growth conditions. Clearly, biosorption selectivity would be an advantage for a process requiring recovery and recycling of a particular waste-stream component. However, for environmental pollution control, this may not be essential, provided adequate biosorption of all the appropriate radionuclides occurred. Nonselectivity may even be an advantage in this case, permitting adsorptive removal of more effluent components. It should be remembered that the interfering action of waste-stream contaminants affects not only biosorption processes, but also conventional treatments.

## RADIONUCLIDE ELUTION AND BIOSORBENT REUSE

If biosorptive processes are to offer a competitive and practical alternative to existing technologies, it must be possible to desorb radionuclides and reuse the regenerated biosorbent efficiently for further effluent treatment. The ability to elute metals from a biosorbent has several advantages:

1. The recovery and reuse of rare or valuable radionuclides
2. The production of a highly concentrated eluate and
3. The absence of the necessity to dispose of large quantities of biodegradable and radioactive biomass.

It is desirable that an elution treatment does not have a deleterious effect on the biosorbent, so that multiple adsorption–desorption cycles are possible. In addition, only low volumes of eluant should be necessary for efficient desorption to ensure a concentrated elution solution. The criteria for eluant selection are mechanistic and economic. Good eluants are likely to form highly soluble salts or soluble complexes with the radionuclide (*43*). In economic terms, eluants should be easily obtained chemicals commonly used by industry.

### Radionuclide Elution by Mineral Acids

Among elution treatments so far examined, the mineral acids efficiently desorb uranium from biomass forming highly soluble uranium salts. Elution can be 100% efficient, as was found for uranium desorption from *R. arrhizus* biosorbent by 0.1*N* $H_2SO_4$ or 1*N* HCl (*44*). However, acid concentration influences desorption. At higher concentrations, the efficiency of HCl uranium elution increased while that by $H_2SO_4$ declined (Table 4) (*44*). One explanation for the reduction in $H_2SO_4$ induced desorption is that $SO_4^{2-}$ ions interacted with cell wall chitosan, changing biopolymer crystallinity and retaining biosorbed uranium within the chitin

Table 4
Efficiency of Radionuclide Elution from Biomass

| Organism | Radionuclide | Eluant | Eluant Concn. | % Elution[1] efficiency | References |
|---|---|---|---|---|---|
| *Rhizopus arrhizus* | U | $H_2SO_4$ | 0.1N | 100 | (*44*) |
| *Rhizopus arrhizus* | U | $H_2SO_4$ | 1N | 90 | (*44*) |
| *Rhizopus arrhizus* | U | HCl | 0.01N | ineffectual | (*44*) |
| *Rhizopus arrhizus* | U | HCl | 0.1N | 94 | (*44*) |
| *Rhizopus arrhizus* | U | HCl | 1N | 100 | (*44*) |
| *Saccharomyces cerevisiae* | U | $HNO_3$ | 0.1M | 59.3 | (*15*) |
| *Penicillium chrysogenum* | Ra | HCl | 0.01M | 100 | (*43*) |
| Activated sludge | Ra | HCl | 0.04M | 100 | (*43*) |
| *P. chrysogenum* | Ra | $HNO_3$ | 0.01M | 100 | (*43*) |
| Activated sludge | Ra | $HNO_3$ | 0.01M | 100 | (*43*) |
| *Saccharomyces cerevisiae* | U | EDTA | 0.1M | 72.3 | (*15*) |
| *Actinomyces levoris* | U | EDTA | 0.01M | 82–88 | (*10*) |
| *Streptomyces viridochromogenes* | U | EDTA | 0.01M | 82–88 | (*10*) |
| *Penicillium digitatum* | U | EDTA | 0.1M | 61 | (*45*) |
| *P. chrysogenum* | Ra | EDTA | 0.01M | 100 | (*43*) |
| Activated sludge | Ra | EDTA | 0.01M | 13 | (*43*) |
| *P. chrysogenum* | Ra | EDTA | 0.1M | 100 | (*43*) |
| Activated sludge | Ra | EDTA | 0.1M | 100 | (*43*) |
| *P. chrysogenum* | Ra | NTTA | 0.01M | 27 | (*43*) |
| Activated sludge | Ra | NTTA | 0.01M | 0 | (*43*) |
| *P. digitatum* | U | lactic acid | 10% v/v | 0–7 | (*45*) |
| *P. digitatum* | U | acetic acid | 10% v/v | 0–7 | (*45*) |
| *P. chrysogenum* | Ra | triammonium citrate | 0.01M | 27 | (*43*) |
| Activated sludge | Ra | triammonium citrate | 0.01M | 9 | (*43*) |
| *Citrobacter sp* | U | citrate buffer[2] | 1M | 73 | (*31*) |
| *R. arrhizus* | U | $Na_2CO_3$ | 0.1N | 100 | (*44*) |
| *R. arrhizus* | U | $NaHCO_3$ | 1N | 100 | (*44*) |
| *Saccharomyces cerevisiae* | U | $(NH_3)_2CO_3$ | 0.1M | 83.5 | (*15*) |
| *Streptomyces levoris* | U | $NaHCO_3$ | 0.1M | >90 | (*2*) |
| *Tritira* | U | $Na_2CO_3$ | 0.1M | >95 | (*2*) |

[1] % elution efficiency is calculated as % metal loading initially on biomass/loading remaining as residue on biomass after elution.
[2] the citrate buffer consisted of tri Na citrate-citric acid 2 mM.

(*44*). Ammonium sulfate may be a poor eluant for *R. arrhizus* for similar reasons (*44*). The type of mineral acid used in desorption treatment clearly influences elution efficiency. For example, nitric acid appears to have only limited elution capacity, e.g., uranyl ions from *S. cerevisiae* biosorbent 59.3% efficiency (Table 4) (*15*). Other radionuclides, e.g., radium, can also be effectively desorbed using mineral acid eluates, the efficiency again varying with acid concentration (Table 4) (*43*).

The mechanism of mineral acid radionuclide desorption is probably in part related to direct competition between $H^+$ and radionuclide ions for cell wall active sites. However, the low pH induced by mineral acids damages biosorbents (*2,43,44*). Acid desorption alters the rate of subsequent radionuclide uptake and the adsorption capacity of the biomass. The initial rate of uranium biosorption by *S. cerevisiae* was increased by 0.1*M* $HNO_3$ treatment, but the final equilibrium uptake capacity was reduced (*15*). Similarly, mineral acid treatment reduced radium biosorption capacity of *P. chrysogenum* biosorbent (*43*). After this initial reduction in adsorption capacity with mineral acid elution, there may be no further deterioration in capacity or integretory of the biosorbent during subsequent adsorption–desorption cycles (*6*).

Mineral acid eluants may be largely inappropriate for desorption processes. Any reduction in uptake capacity caused by acid eluates may be unacceptable in terms of biosorption efficiency. Furthermore, acid-induced physical damage to biomass may lower biosorbent stability during subsequent treatment cycles and limit reuse of the system.

## Radionuclide Elution by Complexing Agents

Complexing agent effectiveness in radionuclide desorption is related to biosorbent type, complexing agent type and concentration, and radionuclide type (Table 4). As with mineral acids, biomass treatment with complexing agents may influence the rate and capacity of subsequent radionuclide biosorption.

### *The Effect of Ethylenediaminetetracetic Acid (EDTA) and Nitriloacetic Acid (NTAA) Eluants*

The efficiency of chelating agents, such as EDTA, in desorption processes is concentration and pH dependent. Uranium elution from *P. digittum* biomass increased with EDTA concentration (0.01–0.1*M*) (*45*). One reason for the importance of eluate concentration in radionuclide, e.g., radium, elution appears to be pH fluctuations. During treatment of biosorbent with 0.01*M* EDTA, the pH dropped to below 7.8, and this lowered Ra–EDTA complex stability reducing extraction efficiency. However, a pH of 8.4 was maintained with 0.1*M* EDTA eluate, and Ra elution was complete (*43*). Changes in pH also influenced uranium desorption by EDTA with poor desorption occurring between pH 4–6 for *P. digitatum* and 0.1*M* EDTA (*45*).

The effect of chelating agent treatment on future biomass adsorption characteristics, i.e., rate of adsorption and biomass loading capacity, is apparently species dependent. For example, uranium desorption by 0.1*M* EDTA from *S. cerevisiae* biosorbent resulted in subsequent adsorption to occur at an increased rate, but with lower equilibrium capacity (*15*),

whereas EDTA treatment of *P. digitatum* biomass increased uranyl ion adsorption capacity, acting as an activator (*45*).

### *The Effect of Organic Acids and Their Salts on Radionuclide Elution*

In general, the efficiency of organic acids and their salts in radionuclide elution from biosorbents is low. Lactc acid and acetic acid were no more efficient at desorbing uranium than was water showing only 0–7% efficiency, depending on the amount of biosorbed uranium (*45*). Radium eluted from two biosorbent types by triammonium citrate was in a lower concentration than in the original mock waste-stream (Table 4). Apparently, radium had a lower affinity for citrate than for either biomass type (*43*).

An exception to these low elution efficiencies was found by Mackaskie and Dean (*31*). Citrate buffer was considered to be highly effective at desorbing uranium from columns of immobilized *Citrobacter* sp cells, although 27% of the uranium remained bound after passage of 5 1 of eluate through the column. No further elution was felt worthwhile.

### *The Effect of Carbonate and Bicarbonate Eluants*

Carbonate and bicarbonate are considered good eluants for a variety of fungal and bacterial biosorbents, showing uranium elution efficiencies between 80–100% (Table 4). The high affinity of carbonate or bicarbonate for uranium shifts the biomass-liquid equilibrium in favor of the liquid phase and desorption. Desorption treatments with these eluants may affect subsequent adsorption characteristics. For example, ammonium carbonate treatment induced a faster rate of subsequent uranium biosorption by *S. cerevisiae,* but no difference in final uptake capacity (*15*).

Although carbonate, e.g, sodium carbonate, may be a very efficient eluate, it may cause cellular damage, probably because of high pH (*17,41, 44*). The damage can be considerable. The dry wt of free *Streptomyces albus* cells fell by 50% during five adsorption-desorption cycles. However, the stability of immobilized cells was much greater, showing a loss of only 2% dry wt (*41*).

Sodium bicarbonate is less harmful to biomass and is a suitable eluate for multiple adsorption-desorption cycles, with uranium biosorption capacity remaining at 90% of the original value (*44*). A further advantage of $NaHCO_3$ elution was the high solid-to-liquid ratio (S/L ratio defined as the ratio of loaded biomass [in mg] to eluant volume [in mL]) (*44*); often found possible for complete uranium desorption, e.g., 120 for *R. arrhizus* (*44*). This is crucial since uranium is extracted in highly concentrated solution. Tsezos (*44*) has developed an interesting empirical model to estimate roughly the S/L ratio for a desired uranium eluate concentration and 100% elution using bicarbonate treatments.

## CONCLUSION

There is no doubt that biosorption processes offer a promising and potentially highly efficient technology for removing radionuclides from liquid effluents. Several biosorption systems so far studied largely comply with the criteria for a competitive and successful alternative to conventional techniques (Section 1). Limitations in biosorption processes such as uptake inhibition by other waste-stream components, sensitivity to pH, nonselectivity and so on, are undoubtedly problematic but may be shared by conventional procedures. However, it may be possible to manipulate biosorbents by a variety of techniques to achieve the most favorable and economic biosorption process and overcome some of the process difficulties.

The use of relatively cheap waste-biomass from other industrial processes e.g., fermentations, polymer production, activated sludge, would improve the cost effectiveness of the biosorption process. Since there are mechanistic differences in radionuclide uptake with biomass type careful selection of biomass may help to accomplish maximum biosorption efficiency. If biomass is specifically grown for biosorption processes, careful control of growth condition is essential to ensure good biosorption process characteristics. However, it may be possible to modulate uptake by other procedures. Pretreatment of biomass by agents such as alcohol, KOH, and boiling can improve radionuclide biosorption capacity by large amounts (*12,15,26*). These pretreatments work through their denaturation or solvent effects exposing cell wall binding sites. More elaborate procedures such as genetic manipulation may also improve metal-binding characteristics (*7*).

Biosorbents consisting of dead microbial biomass offer a number of process advantages. There would be no necessity to supply nutrients and growth factors nor to control environmental factors, e.g., $O_2$ tension and pH, in order to maintain active or resting cells in appropriate conditions. Furthermore, the problem of waste-stream toxicity and its potentially detrimental effect on uptake would be eliminated. Toxic components might influence physico-chemical adsorption in two ways. First, viable microorganisms would be stressed, possibly resulting in changed cell wall characteristics, and hence, changed radionuclide uptake. Second, cell bound enzyme activity such as the *Citrobacter* sp phosphatase system (*31*) may be inhibited. Process sterility would not be an absolute requirement for dead biosorbents. However, microbial contamination should be constrained to avoid biodegradation of the biomass. Advanced Mineral Technologies have developed and operated a metal recovery biosorption technology (AMT-BIOCLAIM$^{TH}$). It was found, over a six mo period of semicontinuous operation, that the problem of microbial contamination could be controlled (*9*).

Careful choice of eluant may improve the efficiency of nuclide desorption and subsequent concentration, re-use of the biosorbent, and overall

radionuclide selectivity. It may be possible to improve metal selectivity by sequentially desorbing and collecting different sorbed radionuclides through different elution treatments.

Several types of technical systems have been proposed for biosorptive processes including immobilized cell column systems (*31,46*), stirred tank reactors (*2*), and stationary and fluidized bed systems (*35*). However, few workers have developed systems appropriate for scale-up to large effluent volumes although Brierly et al. (*9*) have run substantial pilot plant systems. One major requirement of any biosorption process is a biosorbent of mechanical stability and integrity, particularly for multiple adsorption–desorption cycles. Researchers have attempted to achieve this through biomass immobilization in gels e.g., polyacrylamide (*17*), on sand or coal columns (*46*), as biofilms on glass surfaces (*32*); through biomass granulation (*9*); or through floc formation (*27*). Gel immobilization may not confer sufficeint mechanical strength for industrial uses (*32*) and may be uneconomic. Immobilization of cells in sand or coal columns or as adsorbed biofilms may necessitate the inclusion of down-stream processing in the biosorption system to remove released radionuclide carrying microbial cells. Both monolayers and biofilms of microorganisms are able to desorb from solid surfaces (*47,48*). Formation of biomass into pellets does appear to confer an appropriate level of mechanical stability for large scale-use (*9*) while offering an additional advantage, particularly for fungal biosorbents, of higher area to biomass ratio that favors adsorption processes (*26*).

Further research is essential to determine the influence of complex mixtures of waste-stream contaminants on biosorption and methods to combat any adverse effects e.g., prior precipitation of competing cations such as $Fe^{3+}$ (*2*). The majority of workers have limited their study of biosorption processes to radionuclides common to mining and milling operations. If microbial biosorption is to have application to reprocessing waste streams, then biosorption of a broader range of nuclides singly and in mixtures must be examined.

At present, some conventional techniques are at their limits of sensitivity in removing active radionuclides from waste-streams. Biosorption systems in combination with existing processes or individually offer an exciting possibility for achieving even lower environmental discharge levels.

## REFERENCES

1. Sakaguchi, T., Horikoshi, T. & Nakajima, A. (1978), *J. Ferment. Tech.* **56,** 561–565.
2. McGready, R. G. L. & Lakshmanan, V. I. (1986), *Immobilization of Ions by Biosorption,* Eccles, H. & Hunt, S., eds. Soc. Chem. Ind. Lndn. Ellis, Horwood Ltd. Chicester. pp. 219–226.
3. Clark, D. A. (1974), EPA 660/2-74-038 Ottawa, Ontario cited in Tsezos, M. & Volesky, B. (1981), *Biotech. & Bioeng.* **23,** 583–604.

4. Tsezos, M. & Keller, D. M. (1983), *Biotech. Bioeng.* **25,** 201–215.
5. Carley-MacAuly, K. W. (1985), *Solvent Extraction and Ion Exchange in the Nuclear Fuel Cycle,* Logsdail, D. H. & Mills, A. L., eds., pp. 128–135.
6. Treen-Sears, M. E., Volesky, B. & Neufeld, R. J. (1984), *Biotech. Bioeng.* **26,** 1323–1329.
7. Hancock, I. C. (1986), *Trace Metal Removal from Aqueous Solution,* Thompson, R. ed., Spec. Pub. No. 61, The Royal Soc. of Chem. pp. 25–43.
8. Volesky, B. (1987), *Trends in Biotech.* **5,** 95–101.
9. Brierley, J. A., Goyak, G. M. & Brierley, C. L. (1986), *Immobilization of Ions by Bio-sorption,* Eccles, H. & Hunt, S, eds., Soc. Chem. Ind. Lndn. Ellis Horwood, Chichester, pp. 105–117.
10. Horikoshi, T., Nakajima, A., & Sakaguchi, T. (1981), *Eur. J. Applied Microbiol. Biotech.* **12,** 90–96.
11. Kuyucak, N. & Volesky, B. (1988), *Biotech. Letts.* **10,** 137–142.
12. Galun, M., Keller, P., Malki, D., Feldstein, H., Galun, E., Siegel, S., & Siegel, B. (1983), *Science* **219,** 285–286.
13. Beveridge, T. J. & Murray, R. G. E. (1980), *J. Bacteriol.* **141,** 876–887.
14. Tsezos, M. & Volesky, B. (1981), *Biotech. Bioeng.* **23,** 583–604.
15. Strandberg, G. W., Shumate II, S. E. & Parrott, J. R. (1981), *Applied Environ. Microbiol.* **41,** 237–245.
16. Tsezos, M. (1986), *Immobilization of Ions by Bio-sorption,* Eccles, H. & Hunt, S., eds., Soc. Chem. Ind. Lndn. Ellis Horwood Ltd. Chichester, pp. 201–218.
17. Nakajima, A., Horikoshi, T. & Sakaguchi, T. (1982), *Eur. J. Applied Microbiol. Biotech.* **16,** 88–91.
18. Tobin, J. M., Cooper, D. G. & Neufeld, R. J. (1984), *Appl. Environ. Microbiol.* **47,** 821–824.
19. Ellwood, D. C. & Tempest, D. W. (1972), *Adv. Microb. Physiol.* **7,** 43–47.
20. McMurrough, I. & Rose, A. M. (1967), *Biochem. J.* **105,** 189–203.
21. McEldowney, S. & Fletcher, M. (1986), *J. Gen. Microbiol.* **132,** 513–523.
22. Friis, M. & Myers-Keith, P. (1986), *Biotech. & Bioeng.* **28,** 21–28.
23. Marquis, R. E., Mayzel, K., and Cartense, E. L. (1976), *Can. J. Microbiol.* **22,** 75–98.
24. Yakubu, N. A. & Dudeney, A. W. L. (1986), *Immobilization of ions by Bio-sorption,* Eccles, H. & Hunt, S., eds., Soc. Chem. Ind. Lndn. Ellis Horwood Ltd. Chichester pp. 183–200.
25. Hoyle, B. & Beveridge, T. J. (1984), *Can. J. Microbiol.* **30,** 204–211.
26. Galun, M., Keller, P., Malki, D., Feldstein, H., Galun, E., Siegel, S. & Siegel, B. (1983), *Water, Air & Soil Pollution* **20,** 221–232.
27. Norberg, A. B. & Persson, H. (1984), *Biotech. Bioeng.* **26,** 239–246.
28. Beveridge, T. J. (1978), *Can. J. Microbiol.* **24,** 89–104.
29. Tsezos, M. & Volesky, B. (1982), *Biotech. Bioeng.* **24,** 385–401.
30. Aickin, R. M., Dean, A. C. R., Cheetham, A. K., and Skarnulis, A. J. (1979), *Microbiol. Letts* **9,** 7–15.
31. Macaskie, L. E. & Dean, A. C. R. (1985), *Biotech. Letts* **7,** 457–462.
32. Macaskie, L. E. & Dean, A. C. R. (1987), *Enzyme Microbiol. Technol.* **9,** 2–4.
33. Tsezos, M. & Volesky, B. (1982), *Biotech. Bioeng.* **24,** 955–969.
34. Tsezos, M., Baird, M. H. I. & Schemilt, L. W. (1986), *Chem. Eng. J.* **33,** B35–B41.

35. Brierley, J. A., Brierley, C. L., and Goyak, G. M. (1986), *Fundamental and Applied Biohydrometallurgy,* Lawrence, R. W., Branion, R. M. R., and Ebner, H. G., eds., Elsevier, Cambridge, pp. 291–304.
36. Lewis, D. & Kiff, R. J. (1988), *Environ. Technol. Letts.* **9,** 991–998.
37. Tobin, J. M., Cooper, D. G. & Neufeld, R. J. (1988), *Biotech. Bioeng.* **31,** 282–286.
38. Davis, J. A. & Leckie, J. O. (1978), *Enviorn. Sci. Technol.* **12,** 1309–1312.
39. Benjamin, M. M. & Leckie, J. O. (1981), *Environ. Sci. Technol.* **15,** 1050–1056.
40. Tobin, J. M., Cooper, D. G. & Neufeld, R. J. (1987), *Biotech. Bioeng.* **30,** 882–886.
41. Nakajima, A. & Sakaguchi, T. (1986), *Applied Microbiol. Biotech.* **24,** 59–64.
42. Galun, M., Keller, P., Malki, D., Feldstein, H., Galun, E., Siegel, S., & Siegel, B. (1984), *Water, Air & Soil Pollution* **21,** 411–414.
43. Tsezos, M., Baird, M. H. I. & Schemilt, L. W. (1987), *Chem. Eng. J.* **34,** B57–B64.
44. Tsezos, M. (1984), *Biotech. Bioeng.* **26,** 973–981.
45. Galun, M., Keller, P., Feldstein, H., Galun, E., Siegel, S. & Siegel, B. (1983), *Water, Air & Soil Pollution* **20,** 277–285.
46. Remacle, J. & Houba, C. (1983), *Environ. Tech. Letts.* **4,** 53–58.
47. McEldowney, S. & Fletcher, M. (1988), *Microbiol. Ecol.* **15,** 229–237.
48. Characklis, W. G. (1984), *Microbial Adhesion and Aggregation,* Marshall, K. C. ed., Report of the Dahlem Workshop on Microbial Adhesion and Aggregation, Berlin 1984, Jan. 15–20. Springer-Verlag, pp. 137–158.