Biosorption: An attractive alternative for metal removal and recovery

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The most recent development in environmental biotechnology is the use of microbe-based sorbents for removal and recovery of strategic and precious heavy metals from industrial wastewaters. We review metal–microbe interactions, utilization of biosorbents for heavy-metal-pollution control and the mechanisms of biosorption that can help in selecting the appropriate reactor configuration for field application of this process. Studies show that this process can replace the conventional processes of heavy-metal-pollution control.

AGRICULTURAL runoff, industrial and domestic effluents and acid mine drainage have all greatly contributed to the metal loads in our natural water systems. As a result of their toxicity, environmental mobility and complex chemical forms, increasing attention is directed towards studying their removal and recovery from metal-bearing waste streams.

The capacity of micro-organisms to concentrate heavy metals has been well known. However, it is only during the last two decades that micro-organisms are being used as a potential alternative for heavy metal removal and recovery. The term 'biosorption' is defined as a process in which solids of natural origin, e.g. micro-organisms, alive or dead, or their derivatives, are employed for sequestration of heavy metals from an aqueous environment. The transfer of metal ions from aqueous to solid biosorbent phase can be due to passive, facilitated or active transport. The mechanism of uptake can be due to physical sorption, chemical complexation with microbial cell surface groups or bioaccumulation.

Metal–microbe interaction

The interaction between microbes and metals has been studied by scientists of different disciplines. While agricultural scientists are primarily interested in the microbicidal action of metals, bioinorganic chemists basically looked at the metal coordination geometry and their environment in metalloproteins and biological functions. Scientists in life sciences study the toxicological effects, its bioaccumulation and biomagnification, while the environmental scientists and engineers try to utilize this property of bioaccumulation for monitoring heavy metal pollution as well as for removal/recovery of heavy metals from metal-bearing wastewaters.

Although the oligodynamic action of some metals, particularly copper, was known since ancient civilizations, the discovery of toxic action of copper to fungi is credited to Prevost[1]. The first systematic study on the interaction of fungal spores and metal was by Wutrich[2] who reported that spores of some species took up copper more readily from copper sulphate solutions than others and the removals were so high that the metal could not be detected in the applied solution. The first quantitative study on the copper uptake by fungi was by Hecke[3] who reported that spores of *Trilletia tritici* and *Ustilago crameri* took up copper by about 1% of their weight. Metal was strongly bound as the removal of copper from the spores was achieved only with 0.5% HCl. Similar studies were also conducted by Pichler and Wobler[4], in which uptake of a number of metal ions like Ag, Cu, Ce and Hg by corn smut was evaluated. An interesting observation in these studies was that autoclaved spores took up more copper than live spores.

The phenomenon of bioaccumulation was first put to practical use for monitoring the traces of heavy metals in environment. Goodman and Roberts[5] used mosses as the indicators for aerial metal levels. Mosses, which belong to the class of Bryophyta, possess remarkable ion exchange properties, similar to many commercial ion exchangers. Standard moss bags developed by Roberts[6] can provide rapid, reliable and an inexpensive method of monitoring heavy metal pollution. Later microbes such as *Ascophyllum nodusum* were also used for biomonitoring of heavy metals[7].

Use of microbes for heavy-metal-pollution control

Although application of biosorption for pollution control has been gaining attention, the phenomenon

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was observed as early as 1940s. Ruchloft[8] observed that activated sludge efficiently removed plutonium-239 from contaminated domestic wastewater. Later, many studies on metal balance in sewage treatment plants indicated that significant quantities of various metals were removed in activated sludge process[9-12].

Various parameters that affect metal removal by activated sludge process (ASP) have been widely studied. Rudolf and Zuber[10] found that as the sludge volume index increased, the removal capacity of activated sludge for metals decreased. It was observed that of the total metal present, dissolved metals were removed to a lesser extent than other forms, resulting in a net increase in the proportion of dissolved metals to total metals in the effluent[12]. Metals with low solubility in the pH range of 7–9 (pH of sewage) were more efficiently removed. The relationship between metal concentration in sewage and metal uptake by sludge flocs was investigated by Brown *et al.*[11] who observed that for Cr, Cu, Zn, Pb and Cd within non-toxic range (2 mg/l), metal uptake was proportional to the sludge concentration. Kinetic study indicated a rapid uptake of metals in the first few minutes, followed by a slow uptake in the next few hours, reaching near total equilibrium in about two weeks[13,14]. Cells made nonviable either by sterilization at 121°C or by blending decreased their metal uptake capacity[13]. This was attributed to the alterations brought about to the cell surface during the process. In the case of chromium, its oxidation state has been found to affect the removal. While 70–90% of trivalent chromium was removed, only 20% of hexavalent chromium uptake was observed[15]. The presence of soluble-chelating agents such as fulvic acid, humic acid and ethylene diamine tetra-acetate (EDTA) greatly decreased the metal uptake by biomass as the metals have a greater affinity to these ligands as compared to activated sludge[16]. Besides mixed bacterial biomass, pure cultures of *Zooglea*, *Pseudomonas*, *Citrobacter* and many others have also been used for metal-binding studies[17-20].

Extensive screening of micro-organisms for metal uptake has been carried out by Nakajima and coworkers[21-24]. They reported that the ability of microbes to accumulate uranium was in the order, actinomycetes > bacteria > yeast > fungi[21]. Although uranium uptake was affected by environmental conditions such as pH and the presence of carbonate ions, it remained unaffected in the presence of metabolic inhibitors, indicating that major accumulation of uranium is by physicochemical means at the surface[22]. Further, the release of uranium by washing the organisms with EDTA indicated the binding of metal to cell surface ligands. Similar results were earlier obtained for Cu interaction with *Chlorella regularis*[24].

Many of these studies were carried out with living organisms. Use of these for metal removal and recovery is not generally feasible due to certain inherent disadvantages. Wastewaters contain high concentrations of toxic metals and widely fluctuating pH conditions, which are not conducive for the growth and maintenance of the active microbial population. Constant energy source in the form of organic substrates has to be provided for the sustenance of life. Generally acid or alkali regenerants have to be used for metal recovery from these biosorbents which kill the organisms. These factors led to attention being focused on the use of dead microbial mass as biosorbents.

Tsezos and Volesky[25] carried out a systematic study on the uptake of uranium and thorium by dead fungal mycelia. They reported that the capacity of *Rhizopus arrhizus* (180 mg of U/g of the sorbent) was 20 times higher than those of commercially available ion exchange resins. Sorbents prepared from the same biomass have been reported to be capable of scavenging various other metal ions[26].

Muzzarelli and coworkers[27] reported that different fungal waste biomass types originating from food and pharmaceutical industries can be used for heavy metal removal after strong alkali treatment at high temperature. This treatment removes the lipid content, deproteinises and deacetylates the acetyl aminosugar moieties, leaving the alkali-resistant polysaccharides as residue.

Most of the biosorption studies reported thus far pertain to the use of microbes either grown in the laboratory or obtained as a byproduct from industrial fermentation or biological waste treatment process. The potential of macrofungi which grow widely in most tropical and temperate forests for metal uptake is being investigated in our laboratory[28-33]. Among a host of fungi that were screened, *Ganoderma lucidum*, a wood-rotting fungi, had the maximum copper-binding capacity[28]. Pretreatment with strong alkali as reported by Muzzarelli *et al.*[27] improved the engineering property of the material without significantly enhancing the metal-binding capacity[29]. Kinetic studies indicated a rapid uptake of the metal with 90% of the sorption taking place within 10 min[28,30]. Sorption capacity and potential were evaluated under a range of experimental conditions[30,31]. The presence of strong complexing agents like cyanide and EDTA decreased metal removal significantly whereas pyrophosphate, oxalate and tartrate did not have any appreciable effect[30,32,33]. Bound metal could be recovered[33] almost stoichiometrically with EDTA as well as 0.1 N HCl. Acid-regenerated sorbent could be reused for many cycles without any loss in metal-binding capacity[32].

Sharma and Venkobachar[34] reported the excellent copper-binding ability of dried anaerobic sludge generated from an upflow anaerobic sludge blanket reactor (UASB) treating the combined domestic and industrial wastewaters of Kanpur city. Gould and

Genetelli[35] examined the competition between metal ions for binding sites on anaerobic sludge using different test metals. Their results indicated the order of binding affinity as copper > cadmium ≥ zinc > nickel.

Mechanism of metal uptake by biosorbents

An understanding of the mechanisms by which organisms accumulate metals is important to the development of microbial processes for the concentration, removal and recovery of metals from aqueous solutions. For example, a knowledge of the chemical or physiological reactions during metal uptake might enable the specification and control of process parameters to increase the rate, quantity and specificity of metal accumulation. A wide variety of microbes can bind metals. However, there are large differences in the responses of microbial species to metal. A brief description of the various plausible mechanisms operating in the microbial metal uptake is given below.

The mechanisms by which micro-organisms remove metals from solution are (i) extracellular accumulation/precipitation, (ii) cell-surface sorption or complexation and (iii) intracellular accumulation[36]. Among these, process (ii) can occur whether the organism is living or dead, process (i) may be facilitated by microbial viability, whereas process (iii) requires microbial activity.

Extracellular polymers

Removal of metals by extracellular polysaccharides has been extensively studied. The literature on metal uptake by activated sludge and the role of extracellular polysaccharides produced by bacteria have been reviewed by Brown and Lester[9]. Physical entrapment of precipitated metals in the polymer matrix and the complexation of soluble species by charged constituents of polymers have been suggested to be important in metal removal. Although microbial polymers consist mainly of neutral polysaccharides, they also contain compounds such as uronic acid, hexoseamines and organically bound phosphates, which complex soluble metal ions. Since polysaccharides excreted by different micro-organisms differ in their composition, metal-binding properties also differ with microbial species. Microbial growth conditions also significantly influence the composition of polysaccharides affecting the metal removal. Once polymers are produced, metal removal by this mechanism is probably a passive phenomenon not requiring the participation of live organisms. However, there are some reports to indicate that active synthesis of these polymers is induced in the presence of toxic metals. Lawson *et al.*[37] as well as Cheng *et al.*[13] isolated polymers from ASP and studied the complexation capacities of these with metals such as copper, cadmium and nickel. Metal removal by this mechanism appears feasible only with a low concentration of heavy metal present in domestic wastewater to be treated by biological processes.

Cell-surface accumulation

It is generally assumed that surface accumulation is the result of complexation/ion exchange reactions between metal ions and the charged receptive constituents of cell walls. The composition of the cell wall constituents of prokaryotes and eukaryotes differs widely and contain active groups such as phosphodiester (constituent of teichoic acid), carboxyl (glycosides) and amine (amino and peptido glycosides and proteins) on the polymers.

Polikarpov[38] reported that radionuclides present in sea water are accumulated by marine microorganisms through adsorption. His experiments also indicated that viability of the microbial cell was not required for this sorption. Studies by Mathews *et al.*[39] and Beveridge and Murray[40] provided strong evidence for the involvement of carboxyl groups of glutamic acids of peptidoglycans present in the cell walls of gram-positive *B. subtilis* as primary sites of metal complexation. However in *B. licheniformis*, the prime site of metal deposition seems to be phosphate-containing teichoic acids, indicating the differences in mechanism even in related species[41].

The role of phosphomannans and carboxyl groups of cell wall protein of yeasts in metal binding has been studied in detail. Strandberg *et al.*[42] found that treatment of *S. cerevisiae* cells with formaldehyde increased the rate of uranium uptake, probably by decreasing the repulsive force of positively charged amino groups of surface proteins.

Many filamentous fungi (molds) exhibit very high metal uptake capacity. *Rhizopus arrhizus* could take up almost one fourth of its dry weight uranium from pure metal solutions buffered at pH 4. Experimental studies using techniques such as electron microscopy and energy-dispersive X-ray analysis have indicated that bound metal is accumulated at the cell wall[43]. The formation of coordination complex between metallic species and chitin nitrogen (or oxygen) was suggested as the initial step in metal binding. However, later studies indicated the involvement of cell components other than chitin in metal binding[44]. Horikoshi and coworkers[45] reported that two species of actinomycetes exhibited very high affinity for uranium. This binding was not affected by the presence of metabolic inhibitors or scalding, indicating that it is a surface phenomenon. Detailed investigations were carried out to delineate the mechanism of copper (II) sorption to *G. lucidum*[29]. Selective elution of cell wall components clearly

established that neither proteins nor chitins contribute significantly to metal sorption. ESR spectroscopic studies indicated the presence of a strong copper coordinating environment unaffected by the harsh treatments imparted[29].

Since biosorption is a physical/chemical reaction between positively charged metal ions and anionic groups of cell surface, it is to be expected that metal uptake is strongly influenced by the experimental conditions such as pH which affects the speciation of metal and reactive groups. Further, it is possible that the receptive sorbent groups can be metal-specific. For instance, *Chlorella vulgaris* has an unusually high affinity[45] for Au^{3+}, Ag^{+}, and Hg^{2+}. An approach to decipher this specificity is the determination of conditional stability constants (K) for the binding of metals to sorbent. Studies[13] with ASP indicate that $\log K$ values for copper, nickel and cadmium seem to lie in the range of 4 to 7. Jose[33] experimentally determined the stability conditional constants for *G. lucidum* both for native form and alkali-treated derivative and reported $\log K$ values in the range of 4–5 for these biosorbents. These studies also give an insight into the mechanism of biosorption by identifying the probable groups responsible for metal binding and useful in predicting metal removal by biosorbents in the presence of soluble ligands, which are generally present in industrial wastewater.

In some cases the amount of metal accumulated by the organism is several per cent of the dry weight of biomass. It will be difficult to conceive sufficient reactive sites on the cell wall, to account for such metal binding, if complexation is the only mechanism. Beveridge[46] observed greater than stiochiometric amounts of gold and other metals being accumulated by *B. subtilis* and suggested that this is a two-step process. Initially metal reacts with carboxyl groups of peptidoglycans. The bound metal then acts as a nucleation site for depositing additional molecules. Tsezos and Volesky[43] proposed that uranium binding to *R. arrhizus* essentially involves three processes (a) initial stiochiometric reaction of uranium with amine nitrogen of chitin (b) adsorption of uranium onto the cell wall chitin structure and (c) precipitation of uranium hydroxide within the cell wall matrix.

Presence of certain enzymes in the cell membrane of micro-organisms can also lead to the precipitation of heavy metals. Lead and cadmium removal exhibited by *Citrobacter* species is associated with the presence of cell-bound alkaline phosphatase, which liberates inorganic phosphate from organic phosphates. This in turn combines with the dissolved metals to form insoluble phosphates[47,48]. *Citrobacter* cells immobilized in a gel under appropriate conditions exhibited for a long period more than 90% removal of cadmium present in the influent[49]. Similar removal of heavy metals was observed with organisms possessing a sulphur-reducing enzyme in cell membrane, which leads to the precipitation of metal sulphides[20]. Continuous synthesis of enzymes on which these reactions depend probably needs active metabolism. Further, since removals are dependent on the *in situ* activity of enzymes, optimal environmental conditions conducive to these activities should be maintained.

Metal uptake

Intracellular accumulation of many metals has been observed in bacteria, fungi and algae. Although the transport mechanisms for essential elements such as sodium, potassium and calcium have been studied extensively, very little is known about the systems that regulate the intracellular concentrations of other metals. It has been inferred in several instances that the accumulation of nonessential metal results from the lack of specificity of a normal metal-transport system. Although metals such as Ag, As, Hg, Zn, Pb and Cd are generally toxic, certain microorganisms show resistance to them. Although the specific mechanisms by which these metals are taken up by cells are essentially unknown, their uptake seems to be genetically controlled by plasmid-linked genes. Chakrabarty[49] reported that the capability of mercury accumulation was transferable via a plasmid from a resistant strain to a normally sensitive strain of pseudomonas. Intracellular deposition of metals by non-metabolically mediated processes has also been observed. Heldwein[50] reported that lead accumulated intracellularly in *S. cerevisiae* by diffusion, while in the same organism, Cd and Co uptake were energy-dependent. Although there are many cellular constituents capable of forming complexes with intracellularly accumulated metals, the nature of these is largely unknown. Sulphur-containing metal-binding proteins such as metallothionein have been largely implicated in metal binding.

Field application of biosorption

The preceding discussion shows that many microbial species exhibit metal-binding properties. However, not all of them may be suitable for field application in the treatment of metal-rich wastewaters. To evaluate the suitability of a biosorbent, it is necessary to determine the maximum sorption capacity, kinetics of sorption, recovery of the bound metal and the regeneration of biosorbent and physical state of the sorbent. It is better to select the material which can fulfil many of the following criteria: (i) the active biosorbent should be produced at a low cost and should be reusable; (ii) particle size, shape and mechanical properties should be suitable for use in a continuous-flow system in completely mixed, packed or fluidized-bed-reactor con-

figurations; (iii) uptake and release of the metal should be efficient and rapid; (iv) separation of the biosorbent from solution should be cheap, efficient and rapid; (v) it is desirable that the sorbent is metal-selective; (vi) regeneration of metals from the sorbent should preferably be metal-selective and economically feasible, and the sorbent should be in a physical state that can be used.

Despite the accumulation of vast literature on microbiological metal uptake, there appears to be no large scale plant in operation for removal and recovery of metals. However, a few instances[51-54] where this process has been contemplated in the field, are briefly discussed here. Lead mine effluents were treated using tailing ponds and artificial stream-meander systems, to achieve biological removal of metals prior to their discharge to the receiving body. Filamentous algae such as *Cladophora* growing in them were responsible for reducing the number and severity of heavy metal release episodes[51]. Spisak[52] employed an algae-based arsenic process for treating the aqueous effluent produced in refining copper.

An interesting approach for simultaneous removal of metallic and nonmetallic pollutants from industrial wastewaters was proposed by Shumate *et al.*[53]. A mixed culture of bacteria, predominantly pseudomonads, was developed, which was very effective in converting nitrate to nitrogen gas. Cells formed as a byproduct of denitrification reaction, were used to remove uranium from a segregated stream of industrial process. The scheme suggested by Shumate *et al.*[53] involves the growth of microbial cells on the surface of anthracite coal. The cell-laden particles were harvested from the dentrification bioreactor and placed in a vibrating particle feeder. The biosorbent particles were continuously fed into a column in a counter current direction to the flow of uranium solution to be treated. The removal of uranium was 98% corresponding to a mean hydraulic retention time of only 8 min.

Brierly *et al.*[54] reported the details of a pilot plant scale evaluation of a patented biosorbent, AMT-Bioclaim, for wastewater treatment and metal recovery. The material is a granulated non-viable biomass, with a very high capacity for soluble metals such as Ag, Cd, Cu, Pb and Zn. The metal recovery granules accumulated gold even from gold cyanide solutions. The granules employed could be in either fixed bed canisters or fluid-bed reactor systems for treatment of wastewaters and metal recovery.

Volesky[55] suggested a wide variety of reactor configurations for heavy metal removal using active powder or granules. Continuous flow-stirred tank reactors are useful when biosorbent powder is used, whereas packed bed contactors may be more suitable with granules. Fluidized bed contactor, which operates with upflow mode, requires higher flow velocities to maintain the biosorbent particles in fluidized state.

Delineation of mechanism of metal uptake by biomass can greatly aid in selecting the most appropriate reactor configuration for field application. If the extracellular polymers play a dominant role in the process as in ASP, probably CSTR-containing heterogeneous culture can handle metal-bearing wastes along with domestic wastewater. Since live organisms are involved, the environmental conditions should be appropriate for the maintenance of their viability. However, if environmental conditions are such that the precipitation of metals is favoured, even wastewaters with high metal concentrations may be treated. Immobilization of organisms on an appropriate surface and their use in column reactor can be a method of choice, where intracellular accumulation of metals is the main mechanism of removal. This may be suitable with the cell enzyme surface-mediated metal precipitation. For example, citrobacter is immobilized in gel matrix. Although the viability of citrobacter may not be required, it is reported that live cell augments the reaction.

It is appropriate to use nonviable cells to handle wastewaters which contain metal concentrations higher than toxic limits. These can be made into granules or pellets. Since, with nonviable cells, complexation/ion exchange with the surface is the main metal-removal mechanism, upflow fixed-bed reactor may be better suited as more efficient contact can be achieved in this mode[55]. Some of the appropriate reactor configurations which depend upon the dominant metal removal mechanisms are presented in Table 1.

Future of biosorption technology

It appears from the above review that biosorption process can supplement or even substitute the conventional recovery processes. Broad screening of microbial biomass types should be undertaken urgently for the development of new materials. This approach taken up in the Environmental Engineering Laboratory at IIT Kanpur, has yielded positive results in identifying *Ganoderma lucidum*, a widely and wildly growing wood-rotting macrofungus, as a highly potential biosorbent material. The economics of biosorption process need to be evaluated after obtaining sufficient technical data on the performance of the biosorbents and their engineering suitability. Elucidation of the metal-binding mechanism is essential to manipulate the biosorbents so that its capacity can be increased and effective regeneration process developed. A knowledge of the cellular component involved in metal complexing and their biosynthesis is required. This may eventually lead to manipulation of the microbe genetics to biosynthesize more of such active ligands. Understanding the effects

Table 1. Metal-binding mechanisms and reactor configuration.

Dominant metal-binding mechanism	Status of biomass	Metal concentration	Reactor configuration	Mode of metal recovery
Extracellular polymer	Viable	Below toxic level	CSTR	Destructive acidification of biomass
Surface complexation/ ion exchange	a. Viable	Below toxic level	Fixed bed reactor with upflow mode	Elution with EDTA
	b. Non-viable	Above toxic level	Fixed bed reactor with upflow mode	Elution with dilute HCL
Intracellular accumulation	Viable	Below toxic limits	Fixed bed reactor with upflow mode	Destructive acidification of biomass
Surface physical adsorption	a. Viable	Below toxic limits	Fluidized bed reactor	Elution with water
	b. Non-viable	Above toxic limits	Fluidized bed reactor	Elusion with water

of environmental factors and nutrition requirement may enable the cultivation of a desirable type of biomass using cheap materials. However, efficient regeneration processes of the biosorbent may bring down the cost considerably.

Kinetic data on the interaction of metals and biosorbents are urgently needed for process design, scale-up and evaluating the economics. Thus the development of the biosorption process requires an interdisciplinary approach.

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