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Biosorption: An eco-friendly alternative

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ABSTRACT

Release of heavy metal without proper treatment poses a significant threat to public health because of its persistence, biomagnifications and accumulation in food chains. Non degradability and sludge production are two major constraints of metal treatment. The chemical processes are not economical and physical processes consume lot of energy. In this endeavor, microbial biomass has emerged as an option for developing economic and eco-friendly waste water treatment processes. Non living and dead microbial biomass may passively sequester metal by the process of biosorption technology. It has advantages like low operating cost and is effective in dilute solutions and generates minimum effluent. Here, the dead microbial biomass has several reactive groups available on the cell surface such as carboxyl, amine, imidazole, phosphate, sulfhydryl, sulfate and hydroxyl. The pretreatments modify the cell surface either by removing or masking the groups or exposing more metal binding sites. Immobilized biomass offers the continuous sorption-desorption system in a fixed bed reactor. Various commercial microbial biosorbents available are Alga sorbs, AMT Bioclaim and Bio-fix. The economics of these sorbents merit their commercialization, over chemical ion exchangers. Although a lot of research is done in the field of biosorption the applications made on large scale are still less.

KEYWORDS

Biosorption;
Biomass;
Biosorbents;
Pollutants.

1. INTRODUCTION

During the last few decades extensive attention has been paid to the hazards arising from contamination of the environment by heavy metals[59]. Modern industry, to a large degree, is responsible for contamination of the environment. The current pattern of industrial activity alters the natural flow of material and introduces novel chemicals into the environment[21]. The rate at which effluents are discharged into the environment especially water bodies have been on the increase as a result of urbanization. Of the variety of existing pollutants, heavy metals have received special attention, since some of them are extremely harmful to a large variety of organisms when they exceed the limit permitted by environmental legislation and/or the quantities assimilable by these organisms[47]. Most of these effluents contain toxic substances especially heavy metals. The pollutants of concern include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, silver, gold and nickel. Lead, cadmium and mercury are examples of heavy metals that have been classified as priority pollutants by U. S. Environmental protection Agency (U.S. EPA)[43]. The presence of heavy metals in the environment is of major concern because of their toxicity, bioaccumulating tendency, threat to human life and the environment[33]. Heavy metals are among the conservative pollutants that are not subject to bacterial attack or other break down or degradation process and are permanent additions to the marine environment[20]. As a result of this, their concentrations often exceed the permissible levels normally found in soil, water ways and sediments. Hence, they find their way up the food pyramid when they accumulate in the environment and in food chains they can profoundly disrupt biological processes.

The primary sources of heavy metals pollution in coastal lagoons are input from rivers, sediments and atmosphere, which can affect aquaculture profitability in certain areas[44]. The anthropogenic sources of heavy metals include wastes from the electroplating and metal finishing industries, metallurgical industries, tannery operations, chemical manufacturing, marine drainage, battery manufacturing, leather tanning industries, fertilizer industries, pigment manufacturing industries, leachates from landfills and contaminated fround water from hazardous waste sites[21]. Heavy metals are also emitted from resource recovery plants in relatively high levels on fly ash particles[56]. Due to the increasing environmental concern regarding heavy metal contamination, there has been an abundance of interest in the removal of heavy metals from contaminated waste streams. Techniques presently in existence for removal of heavy metals from wastewater are relatively expensive involving either elaborate and costly equipment or high costs of operation with ultimate disposal problems. In view of these reasons, development of a more cost effective remediation process using biological system for removal of heavy metal ions from waste water is necessary[17].

Heavy metals also enter the water supply by industrial and consumer water or even from acid rain breaking down soils and rocks and releasing heavy metals into streams, lakes and ground water. Heavy metals are widespread pollutants of great environmental concern as they are non-biodegradable and thus persistent[79]. Heavy metal pollution in the aquatic system has become a serious threat today. Metals are mobilized and carried into food web as a result of leaching from waste dumps, polluted soils and water. At every level of food chains the metals increase in concentration and are passed onto the next higher level-a phenomenon called biomagnification[61]. Heavy metals even at low

TABLE 1: Types of heavy metals and their effect on human health

Pollutants	Major sources	Effect on human Health	Permissible level
Arsenic	Pesticides, fungicides, metal smelters	Bronchitis, dermatitis	0.0 2ppm
Cadmium	Welding, electroplating, pesticide fertilizer CdNi batteries, nuclear fission plant	Kidney damage, bronchitis, gastrointestinal disorder, bonemarrow, cancer	0.06ppm
Lead	Paint, Pesticide, Smoking, automobile Emission, Mining, Burning	Liver, kidney, gastrointestinal damage, mental retardation in children	0.1ppm
Manganese	Welding, fuel addition, ferromanganese production	Inhalation or contact causes damage to central nervous system	0.26ppm
Mercury Fluorescent tubes kills fishes and fish eaters	Pesticides, batteries, paper industry,	Damage to nervous system, protoplasm poisoning	0.01ppm
Zinc	Refineries, brass manufacture, metal Plating, plumbing	Zinc fumes have corrosive effect on skin, cause damage to nervous membrane	15ppm

concentrations (TABLE 1) can cause toxicity to humans and other forms of life. The toxicity of metal ion is owing to their ability to bind with protein molecules[41] and prevent replication of DNA and subsequent cell division. To avoid health hazards it is essential to remove these toxic heavy metals from waste water before its disposal. In US, $58 million worth metals were disposed off in 1985 from the aqueous solution of electroplating industry alone. According to the U.S. EPA report, 15,000 tonnes of chromium, 19,000 tonnes of lead and 29,000 tonnes of other heavy metals were disposed in 1987. Eventually, environmental awareness is growing among consumers and industrialists and legal constraints on discharge of effluents, necessitating a need for cost-effective alternative technologies[70]. In this endeavor, microbial biomass has emerged as an option for developing economic and eco-friendly waste water treatment process[72].

2. Conventional methods for removal of metal ions

Some of the conventional techniques for removal of metals from industrial waste water include chemical precipitation, adsorption, solvent extraction, membrane separation, ion exchange, electrolytic techniques, coagulation/floatation, sedimentation, filtration, membrane process, biological process and chemical reaction[38]. Each method has its merits and limitations in application. These processes may be ineffective or expensive, especially when the heavy metal ions are in solutions containing in the order of 1-100 mg dissolved heavy metal ions/L[85]. Biological methods such as biosorption/bioaccumulation for the removal of heavy metal ions may provide an attractive alternative to physico-chemical methods[40]. Microorganisms uptake metals either actively (bioaccumulation) or passively (biosorption)[36]. Feasibility studies for large-scale applications demonstrated that, biosorptive process are more applicable than the bioaccumulative processes, because living systems (active uptake) often require the addition of nutrients and hence increase biological oxygen demand (BOD) or chemical oxygen demand (COD) in the effluent. In addition, maintenance of healthy microbial population is difficult due to metal toxicity and other unsuitable environmental factors. In addition, potential for desorptive metal recovery is restricted since metal may be intracellulary bound, metabolic products may be form complexes with metals to retain them in solution and mathematical modeling of a non-defined system is difficult[16].

3. Biosorption

Biosorption can be defined as the uptake of organic and inorganic metal species, both soluble and insoluble, by physicochemical mechanisms such as adsorption. In living cells, metabolic activity may also influence this process because of changes in the physicochemical characteristics of the cellular microenvironment. Almost all biological macromolecules have some affinity for metal species with cell walls and associated materials being of the greatest significance in biosorption. As well as this, cationic species can be accumulated by cells via transport systems of varying affinity and specificity. Once inside cells, metal species may be bound, precipitated, localized within intracellular structures or organelles, or translocated to specific structures, depending on the element concerned and the organism[40].

Advantages of biosorption over conventional treatment methods

Compared to classical technologies of waste-water treatment, biosorption offers the following advantages:

- The system offers low capital investment ad low operation costs.
- The system is effective over a broad temperature and pH range and can be regenerated.
- Metals can be selectively removed.
- Minimization of chemical/biological sludge

Above all the advantages of cheap production and metal selectivity are the promising properties of microbial biomass for the development of novel industrial applications based on biosorption[84].

Advantages of using inactivated biomass

Active metabolic state of cells is not a prerequisite for biosorption since the process can occur even with inactivated/dead cells. The advantages of biosorption are listed below.

- Non living biomass is not subjected to toxicity limitation of the cells and the process is not governed by physiological constraints of microbial cells; costly nutrients for the growth and aseptic operation of cells are not required. A wider range of operating conditions such as pH, temperature and metal concentrations can be used. Waste biomass from a fermentation industry can be cheap source of biomass.
- Inactivated biomass works as an ion-exchanger. So the process is very rapid, requiring anywhere between a few minutes to few hours. Metals can be desorbed readily from the biosorbent and recovered. If the value and amount of metal recovered is significant and if the biomass is plentiful, the metal loaded biomass can be incinerated thereby eliminating further treatment[25].
- Use of inactivated biomass as adsorbent means that it can be used in established connections, theories and formulas already in routine use for adsorption system like ion exchange.

4. Process of biosorption

The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to higher affinity of the sorbent for the sorbate species the latter is attracted and bound by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of the sorbent affinity for the sorbate determines its distribution between the solid and liquid phases. While there is a preponderance of solute (sorbate) molecules (atoms) in the solution, there are none in the sorbent particle to start with. This imbalance between the two environments, amount to a driving force for the solute species. The heavy metals adsorb on the surface of biomass. Adsorption involves the inter phase accumulation or concentration of substance at a surface or inter phase. In doing so, the solid mass or particles of biomass sorbent becomes enriched in those substances of sorbate that they attracted and sequestered. A large number of microorganisms belonging to various groups, viz. bacteria, fungi, yeasts, cyanobacteria and algae have been reported to bind a variety of heavy metals to different extents[86] have presented an exhaustive list of microbes and their metal-binding capacities.

5. Biomass types, selection and sources

Indeed, some biomass types are very effective in accumulating heavy metals. Availability is a major factor to be taken into account to select biomass for clean-up purposes. The economy of environmental reme diation dictates that the biomass must come from nature or even has to be a waste material. Seaweeds, molds, yeasts, bacteria, crab shells, among other kinds of biomass, have been tested for metal biosorption with very encouraging results. Some biosorbents can bind and collect a wide range of heavy metals with no specific priority, whereas others are specific for certain types of metals[46]. The importance of any given group of biosorption of a certain metals by a certain biomass depends on factor such as: the number of sites in the biosorbent material, the accessibility of the sites, the chemical state of the site (i.e. availability) and affinity between site and metal (i.e binding strength). When choosing the biomass for metal biosorption experiments, its origin is a major factor to be taken into account. Biomass can come from (i) industrial wastes which should be obtained free of charge; (ii) organisms easily available in large amounts in nature; and (iii) organisms of quick growth, especially cultivated or propagated for biosorption purposes. Cost effectiveness is the main attraction of metal biosorption, and it should be kept that way. Not only should microbial biomass be used directly, but biosorbents derived from it in a simple process should be most low-priced for economical metal-removal process applications. If, for any reason, by-products of fermentation processes would not be available, biosorbents could be produced by using relatively unsophisticated and low-cost culture propagation techniques. Nutrients from readily available and inexpensive sources such as carbohydrate-rich industrial waste-waters, which often pose pollution/treatment problems, such as food, dairy and starch industries, might be conveniently used. On the contrary, the costs of biosorbents especially produced could be higher and affect negatively the overall economy of their application[45].

Sea weed

Sea weeds offer several advantages for biosorption because of their macroscopic structures, which offer a convenient basis for the production of biosorbent particles suitable for sorption process applications. Some sea weeds collected from the ocean have indicated impressive biosorption of metals[22]. Brown marine algae tend particularly to sequester heavy metals[75]. Aderhold et al.[1] studied the efficiency of three species of seaweed *Ecklonia maxima*, *Lessonia flavicans* and *Durvillea potatorum* at sorbing copper, nickel, zinc, lead and cadmium. They found that all three species sequestered metal ions from solution. *L.flavicans* was the poorest at removing lead ions; *D.potatorum* provided the lowest residual metal concentrations in most cases; *E.maxima* released less alginates during experimentation and showed relatively high metalion- removal ability. Ion exchange has been confirmed to be highly involved to a large degree in the metal sequestering by algal biomass[75]. Although other algal polysaccharides such as abundant carageenan have potential binding sites: red marine algae containing carageenan do not have outstanding metal-sorbing properties.

Yeasts and fungi

Other kinds of high metal-sorbing biomass such as yeast can also be considered[25]. However, the most common yeast biomass (*Saccharomyces cerevisiae*) is not usually a waste, but a commercial commodity. Some chemical compounds of yeast cells can also act as ion exchangers with rapid reversible binding of cations. Volesky et al.[88] working on cadmium biosorption by *Saccharomyces cerevisiae* demonstrated that this yeast is a reasonably potent biosorbent material for cadmium.

The majority of fungi show filamentous or hyphal growth. Fungi are easy to grow and yield large amounts of biomass. They have wide range of applications particularly in fermentation processes. The biomass of fungi and yeasts from such industries could be a ready source for metal removal processes. Cell walls of fungi present a multi-laminate architecture where up to 90% of their dry mass consists of amino or non-amino polysaccharides. The fungal cell walls can be considered as a two phase system consisting of chitin framework embedded on an amorphous polysaccharide matrix. Various metal binding groups, viz amine, imadazole, phosphate, sulphate, sulfhydryl and hydroxyl are present in the polymers[14]. The metal binding capacity depends on walls polymers as well as their alignment in the cell wall. Metal loading capacities in different fungi alter due to differences in their cell wall composition[88], working on cadmium biosorption by *Saccharomyces cerevisiae* demonstrated that this yeast is a reasonably biosorbent material for cadmium (TABLE 2a). Amongst fungi (TABLE 2b) *Penicillium chrysogenum* can extract gold from cyanide solution[18]. However, the biosorption capacity was not encouraging. Some mucoralean fungi have shown intriguing metal biosorbent properties, particularly high for uranium and thorium[10], whereby different metal deposition patterns could be clearly distinguished (**Figures** 4(A and B). Note also that a similar and conveniently available biomass of *Aspergillus* species is not very active in biosorption of metals[73].

Bacteria

A great deal of heterogenecity exit among different bacterial species in relation to:

- Number of surface binding sites
- Binding strength for different ions
- Binding mechanisms

Gram positive bacteria exhibit enhanced metal binding capacity than gram negative bacteria[48]. Gram positive cell walls and surfaces have a negative charge density owing to the peptidoglycan network, a macromolecule consisting of strands of alternating gluosamine and muramic acid residues, which are often N-acetylated. Carboxylate groups at the carboxyl terminus of individual strands provide bulk of anionic character to the cell wall. The phosphodiesters of teichoic acid and the carboxyl groups of teichuronic acid contribute to the ion exchange capacity of cell walls. In comparison to the ion-exchange process, the bacteria possess maximum binding capacity attributed to nucleation reaction[31]. The major anionic character in gram negative cell walls is due to the phosphate in outer and inner membranes and their peptidoglycan. Various bacterial species (TABLE 2c) are known to adsorb metals like copper, cadmium, chromium, nickel etc.

TABLE 2(a) : Various yeast species used for metal biosorption

Yeast	Metal adsorbed	Reference
Saccharomyces cerevisiae	Cd,Ur	Volesky et al. (1993)
Saccharomycescerevisiae, Kluyveromyces fragilis	Cd	Bashar et al. (2003)
Saccharomyces cerevisiae	Methyl mercury and Hg(II)	Madrid et al. (1995)

TABLE 2(b): Some fungal species used in metal biosorption

Fungi	Metal adsorbed	Reference
Phanerochaete chrysosporium	Ni(II),Pb(II)	Haluk and Ulki (2001)
Aspergillus niger	Cd	Barros et al. (2003)
Aspergillus fumigatus	Ur(VI)	Bhainsa and D'Souza (1999)
Aspergillus terreus	Cu	Ruchi et al. (2003)
Penicillium chrysogenum	Au	Niu and Volesky (1999)

TABLE 2(C): Bacterial species exploited in metal biosorption

Fungi	Metal adsorbed	Reference
1. *Bacillus polymyxa*	Cu	Philip and Venkobachr (2001)
2. *Bacillus coagulens*	Cr(VI)	Srinath et al. (2003)
3. *Eschereria coli*	Hg	Weon et al. (2003)
4. *Eschereria coli*	Cu,Cr,Ni	Churchill et al. (1995)
5. *Pseudomonas* species	Cr(VI),Cu(II), Cd(II),Ni(II)	Muraleedharan et al. (1991)

6. Effect of pre-treatment on the biosorption of heavy metals

Metal affinity to the biomass can be manipulated by pretreating the biomass with alkalies, acids, detergents and heat, which may increase the amount of the metal sorbed. The bioadsorption capacity of autoclaved

Mucor rouxii decreased as compared to the live fungus, attributed to the loss of intracellular uptake[92]. Whistler and Daniel[90] reported that the heat treatment could cause a loss of amino-functional groups on the fungal surface through the non-enzymic browning reaction. Aminofunctional groups in the polysaccharides contribute to the binding of heavy metals[50]. However, Galun et al.[27] reported that *Pencillium* biomass pretreatment at 100°C for 5 minutes increased the biadsorption of lead, cadmium, nickel and zinc and the increase was attributed to the exposure of latent binding sites after pre-treatment. In the case of alkali pretreatment, bioadsorption capacity of *Mucor rouxii* biomass was significantly enhanced in comparison with autoclaving. Acid pretreatment of *Mucor rouxii* significantly decreased the bioadsorption of heavy metals[92]. However, Huang and Huang[35] reported that acid pretreatment can strongly enhance the adsorption capacity of *Aspergillus oryzae* mycelia. In case of *A.oryzae*, live biomass after acid pre-treatment was directly used in bioadsorption of heavy metals instead of being autoc1aved and dried. The difference in results after a specific pretreatment may be attributed to the different strains of fungi used and whether the biomass was live or stead when it is used in biosorption of metal ions.

7. Biosorption mechanisms

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. They may be classified according to various criteria.

According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into:

- Metabolism dependent
- Non-Metabolism dependent.

According to location where the metal removed from solution is found, biosorption can be classified as

- Extra cellular accumulation/ precipitation.
- Cell surface sorption/ precipitation and
- Intracellular accumulation

Transport of the metal across the cell membrane yields intracellular accumulation which is dependent on the cell's metabolism i.e., it takes place only with viable cells. It is often associated with an active defense system of the microorganism, which reacts in the presence of toxic metal. During non-metabolism dependent biosorption, metal uptake is by physic-chemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption which are not dependent on the cell's metabolism. Cell walls of microbial biomass mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of biosorption, i.e., non-metabolism dependent is relatively rapid and can be reversible[46].

Transport across cell membrane

The metal transport systems may become confused by the presence of heavy metal ions of the same charge and ionic radius with essential ions. This kind of mechanism is not associated with metabolic activity. Basically, biosorption by living organisms comprises of two steps: first, a metabolism independent binding where the metals are bound to the cell walls and second, metabolism dependent intracellular uptake, whereby metal ions are transported across the cell membrane[25].

Physical adsorption

It takes place with the help of Vanderwaals forces. Electrostatic interaction has been demonstrated to be responsible for copper biosorption by bacterium *Zooglea ramigera* and alga *Chorella vulgaris*[2].

Ion exchange

Cell walls of microorganisms contain polysaccharides and bivalent metal ions exchange with the counter ions of the polysaccharides. For example, the alginates of marine algae occur as salts of potassium, sodium, calcium and magnesium. These ions can exchange with counter ions such as cobalt, copper, cadmium and zinc resulting in biosorptive uptake of heavy metals[46]. The biosorption of copper by fungi *Ganoderma lucidium* and *Aspergillus niger* was also up taken by ion exchange mechanism.

Complexation

The metal removal from solutions may take place by complex formation on the cell surface after the interaction between the metal and the active groups. Asku et al.[2] hypothesized that biosorption of copper by *Chorella vulgaris* and *Zooglea ramigera* takes place through both adsorption and formation of co-ordinate bonds between metals and amino and carboxyl groups of cell walls.

Precipitation

It may be either dependent on the cellular metabolism or independent of it. In the former case it is often associated with active defense systems of microorganisms. They react in the presence of toxic metal producing compounds which favor the precipitation process. In the case of precipitation not dependent on the cellular metabolism, it may be a consequence of the chemical interaction between the metal and the cell surface. The various biosorption mechanisms mentioned above can take place simultaneously.

Use of recombinant bacteria for metal removal

Recombinant bacteria are being investigated for removing specific metals from contaminated water as metal removal by adsorbents from water and waste water is strongly influenced by physico-chemical parameters such as ionic strength, pH and concentration of competing organic and inorganic compounds. For example, a genetically engineered *E.coli* which expressed Hg^{2+} transport system and metallothionin (a metal binding protein) was able to selectively accumulate 8μ moles of Hg^{2+}/gm cell dry weight.

8. Factors influencing biosorption

The investigation of the efficacy of the metal uptake by the microbial biomass is essential for the industrial application of biosorption, as it gives information about the equilibrium of the process which is necessary for the design of the equipment[7].

Type of biomass

Owing to differences in the organisms, there are differences in biosorption capacities of different species, cells of different ages, and between different cell forms of the same organism[26] growth and cultures in different media or media supplementations shows differences in morphological form and chemical composition of the microorganisms[77]. Also, growth of microbial culture is associated with change in metabolic rates, cellular composition and cell wall structure. These factors change in different stages of growth and consequently affect the nature and number of metal binding sites. The uptake of uranium was 2.6 times more in 12hrs grown culture of *Saccharomyces cerevisiae* than the older biomass. Stationary phase *C.Cladosporioides* removed more gold than younger biomass[65].

Metal chemistry

In *Rhizopus arrhizus* adsorption was related to the ionic radius of La^{3+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ba^{2+}, Pb^{2+} and Ag^{+}. Metal binding is related to covalent index of metal[10].

Temperature

In contrast to metabolism-dependent metal uptake, biosorption is relatively unaffected by changes in temperature. Biosorption of copper by *S.cerevisiae* was not affected significantly over the temperature range of 4-45°C. However, high temperatures may cause permanent damage to microbial cells decreasing metal uptake[60].

Hydrogen ion concentration

Hydrogen ion concentration seems to be the most important parameter in the biosorption process. It affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of metallic ions[27]. At low pH (<2.0) there is minimum or negligible metal uptake. The metal uptake increases as the pH increases from 3.0-5.0. At optimum pH value, metal sorption is highest and it decreases with further increase in pH. At very acidic condition, the proton concentration in solution is high. Metal ions have to compete with H^{+} ions for surface binding sites[24]. At low pH wall ligands associated with H_3O^{+} restrain the access of metal ions due to repulsive forces. The increase in metal binding with increase in pH could be due to less ionic competition. Also, increase in pH would expose more negatively charged ligands with subsequent increase in attraction for positively charged metal ions[32]. The pH (4.0 - 8.0) is optimal for metal uptake for almost all types of biomass[8].

Concentration of biomass

At a given equilibrium concentration, the biomass adsorbs more metal ions at low cell densities than at high densities[54]. Reduction in specific metal uptake at increased biomass loading is attributable to the interaction between binding sites of higher ions. At lower biomass concentration, increase in specific metal uptake was due to the increase in metal to biosorbent ratio[23].

Initial metal concentration

The amount of metal adsorbed by the biomass increases with the concentration of metals. Copper removal efficiency of *Rhizopus arrhizus* was higher at low initial metal concentration. Thus, at a given concentration of biomass, the metal uptake increases with increase in initial metal concentration.

Competing cations

The cations compete for binding sites just as the competition by H^+ and H_3O^+ ions, because the metal binding functional groups such as COO^-, CO, OH^-, SH, etc are non specific for binding the cations[14]. An increase is reported in CO^{2+} uptake in pressure of K^+[46] and that of Pb^{2+} in pressure of U^{2+} (Niu et al., 1993) and Ca^{2+} uptake in pressure of Hg^{2+}[26]. However, Mg^{2+}, Mn^{2+}, CO^{2+} and Zn^{2+} reduced Cu^{2+} binding to *Penicillium spinulosum*[82]. Cations (K^+, Na^+ etc) reduce biosorption only when present in high concentrations.

Complexing anions

In many industrial effluents variety of anions (sulphate, chloride, phosphate) are also present in addition to the metal ions[81]. Such anions may reduce the metal binding to cell surface attributed to the formation of complexes between metal cations and the anionic ligands present in solution[49]. Following three types of interactions exist between metal ions, complexing ligands and adsorbents.

- Metal anion complexes are formed that are non-adsorbing or weakly adsorbing resulting in decrease in metal adsorption.
- Biosorbent anion interaction occur that enhance or reduce metal binding
- Metal anion complexes are formed that are more strongly adsorbed than the free metal resulting in enhanced metal uptake.

Inhibitory effect of SO_4^{2-}, PO_4^{2-}, CO_3^{2-}, NO^{3-} is reported on cobalt uptake by AMT™ metal removing agent, loading of cobalt was unaffected by SO_4^{2-}, Cl^-, NO^{3-} in concentrations nine times higher than that of cobalt.

9. Bisorption by immobilized cells

Microbial biomass consists of small particles with low density, poor mechanical strength and little rigidity. Immobilized/pelletized biomass is of greater advantage for use in packed-bed or fluidized bed-reactors, since high flow rates can be achieved, clogging is minimized, particle size can be controlled and high biomass loadings are possible. The immobilized biosorbent granules must have a high surface area, porosity, mechanical strength and water retention capacity. The immobilization method must not affect the metal binding sites of biosorbent and transfer metal ions from solution to biomass surface[62] developed a method for the preparation of matrix from fungal biomass biosorbent beads of *Cladosporium cladosporioides* prepared by the method had increased capacity of gold and silver uptake. In addition to high mechanical strength, acid/alkali/temperature stability and high porosity, the beads were easily biodegraded in soil after their useful life indicating eco-friendly nature of the process[65]. Immobilized biomass showed almost 30% less uranium uptake as compared to native biomass. For better shelf-life, the immobilized biomass has the advantage of easy and convenient usage compared to free biomass, which is easily biodegradable[42]. Various applications are available for biomass immobilization. The principal techniques available for application of biosorption are based on adsorption on inert supports, on entrapment in polymeric matrix, on covalent bonds in vector compounds or on cell cross - linking.

Adsorption on inert supports

Support materials are introduced prior to sterilization and inoculation with starter culture and are left inside the continuous culture for a period of time, after which a film of microorganisms is apparent on the support surfaces. This technique has been used for the immobilization of *Rhizopus arrhizus* fungal biomass in reticulated foam biomass support particles. Activated carbon[76] was used as a support for *Enterobacter aerogens* bio-film. A work on immobilization of Rhizopus *nigricans* on polyurethane foam cubes and coconut fibers was reported[80].

Entrapment in polymeric matrices

The polymers (TABLE 3) used are calcium alginate[29], polyacrylamide[91], polysulfone[80] and polyethylenimine[12]. Those obtained from immobilization in polysulfone and polyethylenimine are the strongest.

Covalent bonds to vector compounds

The most common vector compounds (carrier) are silica gel. The material obtained is in the form of gel

TABLE 3: Immobilization matrices used for the study of metal adsorption

Immobilization matrix	Biomass types	Metal adsorbed	References
Calcium alginate	*Chlorella vulgaris* *Spirulina platensis* *Chlorella salina* *Rhizopus arrhizus*	Au, Cu, Fe, Zn Co, Mn	Muraleedharan et al. (1991) Beveridge and Fyfe (1985)
Polyacrylamide gel	*Citrobacter*, *Rhizopus arrhizus*	Ur, Cd, Pb, Cu, Co, Cd	Macaskie and Dean (1989)
Silica	Algasorb	Cu, Ni, Ur, Pb	Beveridge and Fyfe (1985)
Polyurethane	*Pseudomonas Aeruginosa*	Ur	Hu and Reeves (1997)
Polysulfone	*Phorimidium laminosum*	Pb, Cd, Zn	Blanco et al. (1999) Puranik and Paknikar(1999)

particles. This technique mainly used for algal immobilization.

Cross-linking

The addition of cross-linker leads to the formation of stable cellular aggregates. This technique was found useful for the immobilization of algae. The most common cross-linkers are: formaldehyde, glutaric dialdehyde, divinyl sulfone.

10. Desorption and recovery of metals

If the biosorption process was to be used as an alternative to the waste water treatment scheme, the regeneration of the biosorbent may be crucially important for keeping the process costs down and in opening the possibility of recovering the metals extracted from the liquid phase. For this purpose it is desirable to desorb the sorbed metals and to regenerate the biosorbent material for another cycle of application. The desorption process should.

- Yield the metals in a concentrated form.
- Restore the biosorbent to close to the original solution for effective reuse with undiminished metal uptake and
- No physical changes or damages to the biomass.

Dilute solutions of mineral acids like HCl, H_2SO_4, CH_3COOH, and HNO_3 can be used for metal desorption from the biomass. Recovery of metals from industrials effluents is desirable when the metals are costly and rare. Although the concentration of metals in effluents may not be very high (<50mg/L), the total amount of metal may be quite significant considering the large volumes of effluent generated daily. The printed circuit board manufacturing industry discharges 1-2mg gold/L in the effluent. About 5,00,000 liters of effluent is generated every day amounting to a daily loss of 500mg of gold. Recovery of metals may also be desirable for the regeneration of the biomass for its further cycles of biosorption[83]. For an effective and viable biosorption technology, metal elution methods should be highly efficient, economical and should not cause damage to biomass. Metal ions show marked pH dependence in binding to biomass can be stripped easily by altering the pH[28], whereas metal ions showing little or no pH dependence can be desorbed by the addition of specific ligands that have higher affinity for the metal ions. Effective desportion depends on high affinity of the desorbing agent for metal ions. Dilute mineral acids (HCl, H_2SO_4, HNO_3) have been used for the removal of metals from biomass. Organic acids (citric, acetic and lactic) and complexing agents (EDTA, thiosulphate, etc) can be used for metal elution without affecting the biosorbent[53]. EDTA was effective in desorbing uranium from *Saccharomyces cerevesiae* and *Pencillium digitatum*. Various other desorbing agents include SO_4 and sodium bicarbonate. However, pressure of anions might affect desorption from *Rhizopus arrhizus*[83]. Uses of Na_2CO_3 for desorption of lead and zinc from *Streptoverticillium cinnamoneum* increases biosorption of the metal in subsequent cycles[69].

11. Metal biosorption technologies

Several micro metal removal technologies, which have been commercially employed, are as follows,

AMT bioclaim™ process

The advanced mineral technologies Inc., Gloden Co., USA, developed a waste water treatment process with Bacillus species pretreatment with caustic solution. The culture is immobilized in beads using polyethlenimize and glutaraldehyde or other appropriate builders[11]. The biomass beads (moisture 60%, specific gravity, 1.3 have a greater physical integrity than cation exchange resin, IRC 78, and are tolerant to organic chemicals and pH changes. Metals loaded on beads are eluted using sulphuric acid, NaOH and EDTA. Chemical modifications of the beads removed the anions AsO_4^{2-}, SeO_4^{2-}, CrO_4^{2-}.

Alga sorb™ process

Bio-recovery systems Inc., USA, developed a proprietary algae based material, Alga SORB ™ which comprises several types of living and non living algae[11]. The algal cultures are immobilized in silica gel in the form of beads. Desportion of metals was carried out using 0.5M H_2SO_4 to produce a concentrated solution of metal (10g/L). Alga SORB ™ material are particularly useful to remove heavy metals from water containing high load of Ca^{2+}, Mg^{2+}, Na^+ and K^+[3].

Bio-fix™ process

Bio-fix™ is a biosorption process that utilizes biomass immobilized in polysulfide developed at the US Bureau of Mines, U.S.A[37]. It consists of the thermally killed biomass of Sphagnum peat moss, algae, yeast, bacteria and/or aquatic flora having <150μmeter. Bio-Fix beads were prepared by admixing ground biomass with a solution of polysulfide-dimethyl formamide mixture. The beads are effective in treating waste water metal concentration, 0.01-15mg/L). They can be reused for metal biosorption for more than 120 extraction elution cycles with no reduction in efficiency[37]. The beads are suitable for practical applications in stirred tank reaction fixed beds and fluidized bed columns.

P.O.L sorb

P.O.L. sorb can be used as a cleansing agent. It can absorb 8-12 times its own weight and is able to remove or neutralize 95% to 100% of contaminants present in water without any specialized training and won't complicate the problem further by being hazardous to handle or difficult to dispose off. Its unique cellular structure allows P.O.L. Sorb to absorb dyes and other heavy metal compounds. Because of its chemical composition, P.O.L. Sorb can stabilize or neutralize these elements. With its ability to absorb through its porous exterior it can encapsulate, surround and lock liquids and soluble solids into its gelatinous interior, thus virtually eliminating any chance of leaching when disposed off in landfill sites. The spent peat can also be burnt without any danger to the atmosphere. The spent peat can continued to be used for horticultural purposes with excellent results. There is no danger of anything leaching out of the peat and contaminating ground waters. The cost of P.O.L. Sorb as a natural resource is minimal. The technology is priced much lower than filtration processes now in place. P.O.L Sorb has secured raw peat resources available for all current and anticipated uses. Tests show that after just two passes of effluent through a mat of peat and water, the concentration of common transition metals was lowered to well below acceptable environmental limits for these toxic substances. Even though this peat would now be considered "polluted", it is completely safe to handle or store, and disposal presents no problem. The federal government of Canada and affected provincial governments have also approved landfill as an acceptable disposal method for used peat.

Aqua sorb®

It is a solid, granular cross-linked sodium polyacrylate Advanced superabsorbent polymer that rapidly absorbs and retains large volumes of aqueous solutions, converting them into a semisolid-gel state. The absorptive properties of AQUA Sorb® are ideally suited for the absorption and solidification of industrial waste streams containing inks, heavy metals and other general contaminants. It is a remarkable, yet economical tool for spill management, containment, cleanup and disposal. It has several advantages like:

- It is non-toxic, non-hazardous; does not produce heat or off-gases.
- Meets and exceed EPA, OSHA and ANSI guidelines for absorbent material performances.
- Passes the Paint Filter Liquid Test (Method 9095). Non-Biodegradable polymer (Per 40 CFR 264.314 (e) (1) (ii)).
- Expands by less than 1% when hydrated.
- SEG certified incinerable material with heat value of 5560 BTU/lbs.
- Strong ion exchange capability allows for heavy metals to be bound and waters to pass TCLP.
- Absorbs over 250 times its weight in water.
- Freeze-Thaw Tested - will not release liquids after freezing and heating to 160° F

- Produces over 5,000 BTU's per pound when incinerated
- Solidifies most aqueous solutions in less than 2 minutes-does not require mixing.

12. Newer applications of biosorption

Waste CdTe photovoltaic modules contain toxic metals such as silver, cadmium and tellurium. If disposed in landfill sites, leaching of the heavy metals may potentially have severe environmental impacts. It is also important to recover and recycle expensive metals like silver and tellurium from the waste photovoltaic cells[63]. Scraping from waste photovoltaic cells were dissolved in nitric acid and diluted to get desired metal concentrations. After adjusting pH of the solution, it was passed through a biosorption column consisting of dead granulated biomass of *C.cladosporioides* for selective removal of silver. Next, the solution was conditioned for cadmium biosorption and passed through a similar column containing cadmium biosorption beads. The treated solution served as a feed for the bioreactor containing tellurium reducing bacterial culture i.e., *P.mendocina*. When the columns were operated under optimized conditions, the adsorptions efficiency obtained exceeded 90% for both silver and cadmium. The columns were saturated after adsorbing 50mg of Ag/gm biosorbent and 30mg of Cd/gm biosorbent i.e., after passing approximately 1 liter of Ag containing solution and 3 liters of Cd containing solution. The Ag containing solution and 3 liters of Cd and Te was not adsorbed by any of the two types of biosorbent beads. The metals were concentrated using eluting agents and recovered in desired form. Bacterial reduction of Te, proceeded with >99% efficiency and reduced elemental Te, could be used for recycling chemical analysis of the treated water has low metal contents (typically 1.0 mg/L). The waste was, therefore recycled and used as diluents in the process. The possible application of the process could be in the form of a modular system consisting of biosorbent columns for recovery of Ag and Cd and a simple bioreactor for Te reduction. The system could be operated with ease on site, thus, obviating the costs involved in transportation of the scrap.

Another area, important from public health point of view, is the contamination of food and food products by heavy metal ions. The estimated annual turnover of herbal based medicinal preparations in India is to the time of 4-5 crore rupees, it is being affected due to heavy metal accumulation like chromium, cadmium, lead, silver, mercury and arsenic in plants. Thus making the medicines unacceptable to statuary bodies abroad. Lead and cadmium from juices of carrot, grape and orange, and extracts of *Nordostachys jatamansi* (Jatamansi herb) and Vitis vinifera (raisin) were removed by biosorption using *C.cladosporiodes*[66]. A packed bed reactor for continuous removal of metals from carrot juice was set up. Twenty bed volumes of carrot juice were passed through the column and levels of lead and cadmium in the column effluent were <0.05 and <0.15 respectively. In a novel attempt of detoxifying metal-CN containing waste waters[64], used fungal biomass for biosorption removal of copper and Ni-CN complexes from effluents. Although bacterial species efficiently biodegrade the toxic cyano-complexes, biosorption is a non-destructive method that allows reuse of the adsorbed metal after desorption and concentration.

13. Biosorption already in use

Successful removal of heavy metals especially 'lead' from mine mill waste water by algal growth was achieved by construction of a shallow meandering stream system in Missouri New lead Beet, in which the algae developed[15]. Bioremediation of phenol, ammonia, nickel, hexavalent chromium and iron from untreated steel plant effluent of Visakhapatnam city, India was carried out using different bacteria. Live *Bacillus* species could remove 8% phenol, 100% ammonia, 92.5% nickel, 88% hexavalent chromium and 73.1% iron (II), from industrial effluent.

Biosorption technique showed 100% and 97% of chromium removal by *Staphylococcus aureus* and *Bacillus* species (BS2) respectively[19], two processes using algae on inorganic matrix which are commercially applied. These processes are by Biorecovery Systems Inc., Mexico and BB Sorbex, Canada. Kelp contaminated with oil was removed and buried on the island. A survey showed the Robben Island coastline to be mostly clean of oil on 5 July. Researchers at Miyazaki College have succeeded in recovering precious metals like gold and palladium present in industrial/mining waste water. Microorganisms can act as adsorbents to remove metals even in ppm amounts. Precious metals thus trapped by adsorption can be easily recovered further by using thiourea as an intermediary. The recovery rate is 98.6%. *Pseudomonas, Micrococcus luteus, Streptomyces phaceromogens* are capable of acting as biosorbents. Both the live and dead microorganisms can be used. Biomass discharged from amino acid production plants is used. One gram of bacteria can recover as much as 180mg of gold. Recovery occurs in 5-10 min. Recovery rate can be further improved by improved conditions. Waste water from surface treatment and electronic-recovery plants can be treated with microorganisms to recover precious metals. Reactor Systems employing granulated *Bacillus* are used in the AMT Bioclaim process (Advanced Mineral Technologies Inc. now Vista Tech Partnership Ltd., Salt Lake City, Utah). A fixed bed reactor containing 20Kg adsorbent is used for small flows of <15 lit/min, whereas larger fluidized or pulsed bed system containing 80-90Kg biomass is used for larger flows of >35 lit/min. Loaded dense granules sink to the bed bottom, enabling the addition of fresh biosorbent granules. Metals are removed from the biomass using H_2SO_4, NaOH or complexing agents and are recovered using electro winning. Regeneration of granules may be achieved by alkali treatment. Apart from the above, biotechnology firms such as Advanced BioTech, Visalia, California, market naturally occurring microorganisms packaged in a dry, dormant state. BioTech's hydrocarbon-digesting enzymes, for instance, are sold in one-half pound (227gram) or 2.5 pound (1.1kilogram) containers, including specially formulated biochemical nutrients - a concoction well suited to remediate benzene, amines, phenols, cresols, naphthalene, alcohols, petroleum hydrocarbons, and pesticides from refinery and petrochemical waste sites.

14. Disadvantages of biosorption

Early saturation of biomass limits high metal uptake. In the case of waste biomass, there is no biological control over characteristics of biosorbent, because production of the biosorbent occurs. Application of living algal cells has some disadvantages. Copper significantly damages the surface of living cells, which results in partial loss of cell-binding abilities and release of accumulated copper back into solution. The binding capacity of living cells is significantly lower than that of dead cells. There is also a possibility of de-sorption and reuse of biomass in case of biosorption[39].

CONCLUSION

Biosorption provide alternatives or supplementary to conventional physicochemical treatment method for contaminated effluents and waste waters. In developing countries, the rush for rapid industrial development coupled with lack of awareness about metal toxicity has become a serious concern to environmentalists. Therefore, there is an urgent need for developing an economical and eco-friendly technology. The metal biosorption process provides a promising alternative method for economical recovery of metals to prevent loss of metals through effluents. Biosorption is being demonstrated as a useful alternative to conventional systems for the removal of toxic metals from industrial effluents. The development of the biosorption processes requires further investigation in the direction of modeling, regeneration of biosorbent material and testing immobilized raw biomasses with industrial effluents. Due to the extensive research and significant economic benefits of biosorption, some new biosorbent materials are poised for commercial exploitation. Numerous approaches have been used to understand the process but the complex interaction between metals and microorganisms are difficult to resolve. Biosorption process is not linked to metal removal and recovery from effluents. The use of biosorption may be extended to newer applications such as recovering and reusing various polluting compounds using biosorption, such attempts would definitely give rise to a new concept of pollution management that would enable non destructive recovery of pollutants or their reuse.

REFERENCES

[1] D.Aderhold, C.J.Williams, R.G.Edyvean; Biores. Technol, **58**, 16 **(1996)**.
[2] Z.Asku, Y.Sag, T.Kutsal; 'The biosorption of Cu (II)', C.Vulgaris, Z.Ramigera; Environ.Technol, **13**, 579-586 **(1992)**.
[3] G.E.Arkipo, M.E.Kja, L.O.Ogbonnaya; Global J. Pure and Applied Sciences,**10**, 257-262 **(2004)**.
[4] L.M.Barros, G.R.Macedo, M.L.Duarte, E.P.Silva et al.; Braz J.Chem.Eng., **20(3)**, **(2003)**.
[5] H.Bashar, A.Margaritis, F.Berutti, B.Maurice; Int J.of Chem.Reactor.Eng, **1**, A47 **(2003)**.
[6] T.J.Beveridge, W.S.Fyfe; Can J.Earth.Sci., **22**, 1892-1898 **(1985)**.

[7] K.C.Bhainsa, S.F.D'Souza; Biotechnol.Techniq, **13**, 695-699 **(1999)**.

[8] K.J.Blackwell, I.Singleton, J.M.Tobin; Appl. Microbiol.Biotechnol, **43**, 579-584 **(1995)**.

[9] A.Blanco, B.Sanz, M.J.Llama, J.L.Serra; J. Biotechnol, **69**, 227-240 **(1999)**.

[10] D.Brady, J.M.Tobin; Enzyme.Microbiol.Technol, **17**, 791-796 **(1995)**.

[11] C.L.Brierley; Geomicrobiology, **J8**, 201-223 **(1990)**.

[12] C.L.Brierley, J.A.Brierley; 'Immobilization of Biomass for Industrial Application of Biosorption', In: 'Biohydrometallurgical technologies', A.E.Torma, M.L.Apel, C.L.Brierley (Eds); The Minerals, Metals and Materials Society, 35-44 **(1993)**.

[13] S.A.Churchill, J.V.Walters, P.F.Churchill; J. Environ.Eng., **121**, 706-711 **(1995)**.

[14] R.H.Crist, K.Oberholser, K.Shank, M.Nguyen; Environ.Sci.Technol, **15**, 1212-1217 **(1981)**.

[15] L.Daphne, Stone; 'Biotechnology for Treatment of Hazarding Waste', CRC press, Enivormental studies, **(1993)**.

[16] F.B.Dilek, C.F.Gokcay, U.Yetis; Water Research, **32(2)**, 303-312 **(1998)**.

[17] W.Donbebe, H.Michael; I.S.Ayabaemi; Electronic J.Biotechnol., **(2006)**.

[18] L.R.Drake, S.Lin, G.D.Rayson, P.Jackson; Environ. Sci.Technol, **59**, 110-114 **(1996)**.

[19] K.M.Elizabeth, E.G.Rani; Asian J.Chemistry, **16**, 1269-1273 **(2004)**.

[20] F.E.El-Nady, M.M.Atta; J.Environ.Sci.Health, **A31(7)**, 1529-1545 **(1996)**.

[21] M.Faisal, S.Hasnain; African J.Biotechnol., **3(11)**, 610-617 **(2004)**;

[22] M.M.F.Figueira, B.Volesky, V.S.T.Ciminelli; Water Res., **34**, 196-204 **(2000)**.

[23] E.Fourest, J.C.Roux; Appl.Microbiol.Biotechnol., **37**, 399-403 **(1996)**.

[24] G.M.Gadd; 'Accumulation of Metals by Microorganisms of Algae in Microbial Mineral Recovery, edited by H.J.Rehm; McGraw-Hill. New York, 249-276 **(1988)**.

[25] G.M.Gadd; 'Fungi and Yeasts for Metal Accumulation in Microbial Mineral Recovery', edited by H.L.Ehlrich, C.L.Brierley; Mc Graw Hill, New York, 249-276 **(1990)**.

[26] G.M.Gadd, J.L.Mowll; Exp.Mycol., **9**, 230-240 **(1985)**.

[27] M.Galun, E.Galun, B.Z.Siegel et al.; Water, Air, and Soil Pollution, **33**, 359-371 **(1987)**.

[28] B.Greene, D.W.Darnall; In 'Microbial Mineral Recovery', Editors H.L.Ehrlich, C.L.Brierley; McGraw Hill, 277-301 **(1990)**.

[29] B.Gulay, D.Adil, B.Sema, M.Y.Arica; Microchemical Journal, **7**, 63-76 **(2002)**.

[30] C.Haluk, Y.Ulki; Water SA, **27**, 15-20 **(2001)**.

[31] L.C.Hancock; 'The use of Gram-Positive Bacteria for the Removal of Metals from Aqueous Solution', edited by R.Thompson; The Royal Society of Chemistry, London, 277-302 **(1986)**.

[32] P.O.Harris, G.J.Ramelow; Environ.Sci.Technol, **24**, 220-228 **(1990)**.

[33] M.Horsfall, A.I.Spiff; Electronic Journal of Biotechnology, **8(2)**, **(2005)**.

[34] M.Z.C.Hu, M.Reeves; Biotechnol.Prog., **13**, 60-70 **(1997)**.

[35] C.Huang, C.P.Huang; Water Res., **9**, 1985-1990 **(1996)**.

[36] H.Hussein, S.Farag, H.Moawad; Arab Journal of Biotechnology, **7**, 13-22 **(2003)**.

[37] T.H.Jeffers, C.R.Ferguson, P.G.Bennett; Biohydro metallurgy-Proceedings of the International Symposium, **(1989)**.

[38] C.Jeon, J.Y.Park, Y.J.Yoo; Korean J.Chem.Eng., **18**, 955 **(2001)**.

[39] Kadukova, E.Vircikova; Environ.Int., **31**, 227-32 **(2005)**.

[40] A.Kapoor, T.Viraraghavan; Biores.Technol, **53**, 195-206 **(1995)**.

[41] R.N.Kar, B.N.Sahoo, C.B.Sukla; Pollution Research, **11**, 1-13 **(1992)**.

[42] D.Karunasagar, J.Arunachalam, K.Rashmi, J. Naveena Lavanya Latha et al.; World J.Microbiol Biotechnol., **19**, 291-295 **(2003)**.

[43] L.H.Keith, W.A.Telliard; Environ.Sci.Tech., **13**, 416-424 **(1979)**.

[44] K.K.Krishnani, V.Parmala, X.Meng; Water S.A, **30(4)**, 541-545 **(2004)**.

[45] N.Kuyucak; 'Feasibility of Biosorbents Application', In: B.Volesky (ed); 'Biosorption of Heavy Metals', CRC Press, Boca Raton, FL. 371-378 **(1990)**.

[46] N.Kuyucak, B.Volesky; Biotechnol.Bioeng., **33**, 809-814 **(1988)**.

[47] E.A.Laws; 'Aquatic Pollution: An Introductory Text', 2nd ed., New York, Interscience, 611 **(1993)**.

[48] M.C.Lean, T.J.Beveridge; 'Metal-binding Capacity of Bacterial Surface and their Ability to form Mineralized Aggregates in Microbial Mineral Recovery', edited by H.L.Ehlrich, C.L.Brierley; Mc Graw- Hill, New York, 185-222 **(1990)**.

[49] C.F.Lin, M.M.Benjamin; Water Res., **26**, 397-407 **(1992)**.

[50] M.Loaec, R.Olier, J.Guezennec; Water Res., **31(5)**, 1171-1179 **(1997)**.

[51] L.E.Macaskie, A.C.R.Dean; 'Biological Waste Treatment', A.Alan, R.Liss; New York, 159-201 **(1989)**.

[52] Y.Madrid, C.Cabrera, T.Perez-Corona, C.Camara; Anal.Chem., **67**, 750-754 **(1995)**.

[53] B.Mattuschka, G.Straube; J.Chem.Technol Biotechnol, **58**, 57-63 **(1993)**.

[54] J.M.Modak, K.A.Natarajan; Mineral Metal Process, **12**, 189-196 **(1995)**.

[55] T.R.Muraleedharan, L.Iyengar, C.Venkobachar; Current Sci., **61**, 379-385 **(1991)**.

[56] B.G.Neal, E.B.Lawrence, J.L.Wendt; Combust Sci. Tech., **74**, 211-214 **(1990)**.

[57] H.Niu, X.S.Xu, J.H.Wang; Biotechnol.Bioeng., **42**, 785-787 **(1993)**.

[58] H.Niu, Volesky; J.Chem.Tech.Biotechnol., **74**, 778-784 **(1999)**.

[59] J.O.Nriagu, J.M.Pacyna; Nature, **333**, 134-139 **(1989)**.

[60] J.W.Oliver, W.C.Kreye, P.H.King; J.Water.Pollut. Control.Fed., **47**, 2490 **(1975)**.

[61] K.M.Paknikar, A.V.Pethkar, P.R.Puranik; Indian Journal of Biotehnol., **2**, 426-443 **(2003)**.

[62] K.M.Paknikar, A.V.Pethkar, J.V.Vernekar;: Developing a Process for The Preparation of A Matrix from Poultry Waste and its use in the Immobilization of Biomass, (No.472/BOM/95), **(1995)**.

[63] K.M.Paknikar, J.M.Rajwade, A.V.Pethkar, D.J. Goyal, et al.; 'Environmental safety and Health issues in IC production', R.Reif et al.; (Eds.); Materials Research Society Pittsburgh, Pennysylvania, U.S.A., 133-138 **(1997)**.

[64] Y.B.Patil; Studies on Biological Detoxification of Metal-CN Containing Industrial Effective, PhD Thesis, University of Pune, Pune, **(1999)**.

[65] A.V.Pethkar, K.M.Paknikar; J.Biotechnol., **63**, 121-136 **(1998)**.

[66] A.V.Pethkar, R.P.Gaikaiwari, K.M.Paknikar; Current Sci., **80**, 1216-1219 **(2001)**.

[67] L.Philip, C.Venkobachr; Indian Journal of Environ and Pollution, **15**, 448-460 **(2001)**.

[68] P.R.Puranik, K.M.Paknikar; Biotechnol Prog., **15**, 228-237 **(1999)**.

[69] P.R.Puranik; 'Studies on Recovery of Metals from the Waste using Microbial Biomass', PhD Thesis University of Pune, Pune, India, **(1998)**.

[70] K.Rama Rao, K.Rashmi, N.J.Lavanya Latha, P.Maruthi Mohan; Indian J., **B4**, 139-143 **(2005)**.

[71] M.T.Ramchandra, D.A.Jeyakar, R.Chellaraj, T.Edison Raja et al.; IJEP, **239**, 317-320 **(2003)**.

[72] G.Rani, A.Prerna, K.Seema, R.K.Saxena, et al.; Current Science, **78**, **(2000)**.

[73] L.H.Rosa, P.F.Pimentel, M.M.Figueira, L.C.S. Mendona-Hagler, N.C.M.Gomes; 'Biosorption of Free and Complexed Cadmium Ions by Aspergillus Niger', In: R.Amils, A.Ballester (eds); Elsevier, Amsterdam, 513-519 **(1999)**.

[74] G.Ruchi, R.Saxena, G.Rani; Process Biochemistry, **39**, 1231-1235 **(2003)**.

[75] S.Schiewer, B.Volesky; 'Biosorption by Marine Algae', In: J.J.Valdes (ed); Remediation. Kluwer, Dordrecht (In press), **(2000)**.

[76] J.A.Scott, A.M.Karanjakar; Biotechnol Left., **14**, 737-740 **(1992)**.

[77] P.Simmons, I.Singleton; Appl.Microbiol.Biotechnol., **45**, 278-285 **(1996)**.

[78] T.Srinath, S.K.Garg, P.W.Ramteke; Indian Journal of Expt.Biol., **41**, 986-990 **(2003)**.

[79] G.W.Stratton; 'Review in Environmental Toxicology', E.Hodgson (Eds.); Elsevier, Amsterdam, 85-94 **(1987)**.

[80] B.R.Sudha, E.Abraham; Biores.Technol, **87**, 17-26 **(2003)**.

[81] J.M.Tobin, D.G.Cooper, R.J.Neufeld; Biotechnol. Bioeng., **30**, 882-886 **(1987)**.

[82] C.C.Tounsley, I.S.Ross; Microbios., **44**, 125-132 **(1985)**.

[83] M.Tsezos; Biotechnol.Bioeng., **26**, 973-981 **(1984)**.

[84] S.Vaclav, A.G. John, P.Baveye; 'The Utilization of Bioremediation to Reduce Soil Contamination: Problems and Solutions', Boston: Kluwer Academic Publishers, In Earth and Environmental Sciences, **19**, 1-422 **(2003)**.

[85] B.Volesky; 'Biosorption and Biosorbents, In: Biosorption of Heavy Metals', Boston, USA, CRC Press 3-5 **(1990)**.

[86] B.Volesky, Z.R.Holan; Biotechnol Program, **11**, 235-250 **(1995)**.

[87] B.Volesky, N.Kuyucak; US Patent, **4**, 769, 233 **(1988)**.

[88] B.Volesky, H.May, Z.R.Holan; Biotechnol.Bioeng., **41**, 826-829 **(1993)**.

[89] B.Weon, H.Cindy, J.K.Wu, M.Ashok et al.; Applied and Environ Microbiol., **69**, 3176-3180 **(2003)**.

[90] R.Whistler, T.R.Daniel; 'Carbonhydrates', In: Owen R.Fennema (ed.); Food Chemistry, 96-105 **(1985)**.

[91] P.K.Wong, S.C.Kwok; Biotechnol.Left., **14**, 629-634 **(1992)**.

[92] G.Yan, T.Viraraghavan; Water Research, **26**, 119-123 **(2000)**.