

Biosorption of heavy metals—An overview

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During the last two decades, extensive attention has been paid on the management of environmental pollution caused by hazardous materials such as heavy metals. Decontamination of heavy metals in the soil and water around industrial plants has been a challenge for a long time. A number of methods have been developed for the removal of heavy metals from liquid wastes such as precipitation, evaporation, electroplating, ion exchange, membrane processes, etc. However, these methods have several disadvantages such as unpredictable metal ion removal, high reagent requirement, generation of toxic sludge, etc. Biosorption is a process, which represents a biotechnological innovation as well as a cost effective excellent tool for removing heavy metals from aqueous solutions. This article provides a selective overview of past achievements and present scenario of biosorption studies carried out on some promising natural biosorbents (algae, fungi, bacteria, yeast) and some waste materials which could serve as an economical means of treating effluents charged with toxic metallic ions.

Keywords: algae, fungi, bacteria, yeast, biosorption, heavy metal, biosorbent, wastewater, biomass

Introduction

In recent years, heavy metal pollution has become one of the most serious environmental problems. Presence of heavy metals even in traces is toxic and detrimental to both flora and fauna. With the rapid development of many industries (mining, surface finishing, energy and fuel producing, fertilizer, pesticide, metallurgy, iron and steel, electroplating, electrolysis, electro-osmosis, leather, photography, electric appliance manufacturing, metal surface treating) and aerospace and atomic energy installations, wastes containing metals are directly or indirectly being discharged into the environment causing serious environmental pollution and even threatening human life^{1,2}. Several methods are being used for the removal of heavy metal ions from aqueous wastes (chemical precipitation, ion exchange, electrochemical treatment, membrane technologies, adsorption on activated carbon, etc)³. Each of these methods has its own merits and demerits. Chemical precipitation and electrochemical treatments are ineffective, especially when metal ion concentration in aqueous solution is lower than 50 mg/L. Moreover, such treatments produce large amounts of sludge to be treated with great difficulties. Ion exchange, membrane technologies and activated carbon

adsorption processes are extremely expensive. Therefore, the search for new cost-effective technologies for the removal of heavy metals from wastewaters has been directed towards biosorption, which is known for the last few decades. Algae, bacteria, fungi and yeasts have proved to be potential metal biosorbents, due to metal sequestering properties and can decrease the concentration of heavy metal ions in solution⁴. In recent years, some waste materials from food industry have also shown their capability to act as biosorbents of heavy metals.

Biosorption Mechanisms

The biosorption process involves a solid phase (sorber 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 sorber for the sorbate species, the later is attracted and removed 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 sorber affinity for the sorbate determines its distribution between the solid and liquid phases.

The major advantages of biosorption over conventional treatment methods include low cost, high efficiency, minimization of chemical and biological sludge, regeneration of biosorbent and possibility of metal recovery⁵. The disadvantages of

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biosorption are (i) early saturation i.e. when metal interactive sites are occupied, metal desorption is necessary prior to further use; (ii) the potential for biological process improvement (e.g. through genetic engineering of cells) is limited because cells are not metabolizing; and (iii) there is no potential for biologically altering the metal valency state⁶.

The mechanism of metal biosorption is a complicated process. The status of biomass (living or non-living), types of biomaterials, properties of metal solution chemistry, ambient/environmental conditions such as pH, influence the mechanism of metal biosorption. The metal biosorption process by living cells is a two-step process. In the first step, metal ions are adsorbed to the surface of cells by interactions between metals and functional groups displayed on the surface of cells. All the metal ions before gaining access to the cell membrane and cell cytoplasm come across the cell wall. The cell wall consists of a variety of polysaccharides and proteins and hence offers a number of active sites capable of binding metal ions. Difference in the cell wall composition among the different groups of microorganisms, viz., algae, bacteria, cyanobacteria and fungi, cause significant differences in the type and amount of metal ion binding to them. Algal cell walls are mainly cellulosic. The potential metal binding groups in this class of microbes are carboxylates, amines, imidazoles, phosphates, sulfhydryls, sulfates and hydroxyls. Of these, amines and imidazoles are positively charged when protonated and may build negatively charged metal complexes⁷. Cell walls of bacteria are principally composed of peptidoglycans, which consist of linear chains of the disaccharide N-acetylglucosamine- β 1, 4-N-acetylmuramic acid with peptide chains. In *E. coli* K12, peptidoglycan was found to be a potent binder of the metals tested and carboxylate groups were the principal components involved in metal binding⁸. Fungal cell walls contain chitin and chitosan, which have been shown to sequester metal ions^{9,10}. The first step, passive biosorption, is metabolism independent and proceeds rapidly by any one or a combination of the following metal binding mechanisms: coordination, complexation, ion exchange, physical adsorption (e.g. electrostatic) or inorganic microprecipitation. Passive biosorption is a dynamic equilibrium of reversible adsorption-desorption. Metal ions bound on the surface can be eluted by other ions, chelating agents or acids. In the second step, due to active biosorption,

metal ions penetrate the cell membrane and enter into the cells.

Metal uptake by non-living cells is mainly in passive mode^{1,11-14}. By investigating the biosorption of Cr (VI) and Fe (III) on *Streptococcus equisimilis*, *S. cerevisiae* and *Aspergillus niger*, Goyal *et al*¹⁵ confirmed that the metal uptake by microorganisms occurs in two stages: passive uptake which takes place immediately, and active uptake which takes place slowly. The first stage is thought to be physical adsorption or ion exchange at the cell surface, reaching the adsorption equilibrium within 30-40 min. Therefore, passive mode is independent of energy, mainly through chemical functional groups of the material, comprising the cell and particularly cell wall whereas active mode is metabolism dependent and related to metal transport and deposition.

Factors Affecting Biosorption

The major factors that affect the biosorption processes are (i) initial metal ion concentration, (ii) temperature, (iii) pH, and (iv) biomass concentration in solution. Aksu *et al*¹⁶ reported that temperature does not influence the biosorption processes in the range of 20°-35°C. However, pH seems to be the most important parameter in the biosorption processes. It affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of the metallic ions^{17,18}. Biomass concentration in the solution seems to influence the specific uptake; for lower values of biomass concentration leads to interference between the binding sites. Fourest and Roux¹⁹ invalidated this hypothesis attributing the responsibility of the specific uptake decrease due to metal concentration shortage in solution. Hence, this factor needs to be taken into consideration in any application of microbial biomass as biosorbent.

Biosorption Equilibrium Models

Preliminary testing of solid-liquid absorption system is based on two types of investigations: a) equilibrium batch sorption tests and b) dynamic continuous flow sorption studies. The equilibrium of the biosorption process is often described by fitting the experimental points with models²⁰ usually used for the representation of isotherm adsorption equilibrium. The two widely accepted and linearised equilibrium adsorption isotherm models for single solute system are given by the following equations:

$$\text{Langmuir: } q = \frac{q_{\max} b C_{\text{eq}}}{1 + b C_{\text{eq}}}$$

where q is milligrams of metal accumulated per gram of the biosorbent material; C_{eq} is the metal residual concentration in solution; q_{\max} is the maximum specific uptake corresponding to the site saturation and b is the ratio of adsorption and desorption rates. This is a theoretical model for monolayer adsorption.

Another empirical model for monolayer adsorption is

$$\text{Freundlich: } q = K_{\text{F}} C_{\text{eq}}^{1/n}$$

where K_{F} and n are constants.

These models can be applied at a constant pH , and used for modeling of biosorption equilibrium in the presence of one metal.

Recently, some biosorbents have emerged as an eco-friendly, effective and low cost material options^{13,21,22}. These biosorbents include some agricultural wastes, fungi, algae, bacteria and yeast^{5,23}. Studies using biosorbents have shown that both living and dead microbial cells are able to uptake metal ions and offer potential inexpensive alternative to conventional absorbents^{24,25}. However, living cells are subject to toxic effect of the heavy metals, resulting in cell death. Moreover, living cells often require the addition of nutrients and hence increase the BOD and COD in the effluent. For these reasons, the use of non-living biomaterials or dead cells as metal binding compounds has been gaining advantage because toxic ions do not affect them. In addition, dead cells require less care and maintenance, and are cheaper²⁶. Furthermore, dead biomass could be easily regenerated and reused.

Algae as Biosorbent

Use of algal biomass as a biosorbent is emerging as an attractive, economical and effective proposition because of certain added advantages of algae over others^{27,28}. Algae have low nutrient requirements, being autotrophic they produce a large biomass, and unlike other biomass and microbes, such as bacteria and fungi, they generally do not produce toxic substances. Binding of metal ions on algal surface depends on different conditions like ionic charge of metal ion, algal species and chemical composition of the metal ion solution²⁹⁻³¹.

The uptake of Pb by dried biomass of a green alga, *Chlorella vulgaris* was investigated in a single-staged

batch reactor in the concentration range of 25-200 mg/L³². Sorption phenomenon at different pH values and temperatures was expressed by the Freundlich adsorption isotherm. Increased Pb uptake values at higher pH values and temperature were observed. Holan and Volesky²⁷ reported the biosorption of Pb and Ni by biomass of marine algae. The multi metal sorption system was investigated by De Carvalho *et al*³³ with brown marine alga, *Ascophyllum nodosum*. Using two metal systems comprising either (Cu + Zn), (Cu + Cd), or (Zn + Cd), they found that each of the metals inhibited the sorption of others. Biosorption of Cr (VI) from aqueous solution by green algae *Spirogyra* had also been reported³⁰. The brown seaweed, *Sargassum* sp. (Chromophyta) was used as a biosorbent for Cu ions³⁴. The influence of different experimental parameters such as initial pH , shaking rate, sorption time, temperature, equilibrium conditions and initial concentration of Cu ions on Cu uptake was evaluated.

Biosorption of Cr (III) by *Sargassum* sp. was studied by Cossich *et al*³⁵. The results showed that pH has an important effect on Cr biosorption capacity. The biosorbent size did not affect the Cr biosorption rate and capacity. The removal of Cr (VI) by *Eclonia* biomass, the brown seaweed, was examined in a binary aqueous containing Ni³⁶. The removal rate was unaffected by the presence of Ni (II). Kiran *et al*³⁷ reported biosorption of Cr (VI) by native isolate of an unexplored algal strain, *Lyngbya putealis* (HH-15) in batch system under varying range of pH (2.0-10.0). Maximum metal removal (94.8 %) took place at pH 3.0 with initial Cr concentration of 50 mg/L, which got reduced (90.1%) in the presence of 0.2% salts.

Fungi as Biosorbent

Biomaterials like fungi have been proved efficient and economical for the removal of toxic metals from dilute aqueous solutions by biosorption because fungal biomass offers the advantage of having a high percentage of cell wall material, which shows excellent metal binding properties³⁸. Moreover, large quantity of fungal biomass is available from the antibiotic- and food-industries. Ultimately, the biosorption results not only in metal removal but also provides an eco-friendly environment.

Heavy metal uptake by *Aureobasidium pullanans* and *Cladosporium resinae* was studied. There was a distinct two-phase uptake of heavy metals, the first consisting of metabolism independent cell wall uptake followed by an energy dependent cellular uptake. The

latter was affected by temperature, absence of energy source and metabolic inhibitors³⁹. Removal of Cd by nine species of fungi had been investigated by Huang *et al* in both batch and continuous reactors⁴⁰. Biomass grown in the laboratory was harvested and stored in freeze dried conditions. The uptake was found to be controlled by adsorption and not by surface precipitation. Both fresh and freeze dried biomass also took up heavy metals such as Cu, Pb, Zn and Co.

Aspergillus niger had been found capable of removing heavy metals, Pb, Cd and Cu^{41,42}. The role played by various functional groups in the cell wall of *A. niger* was investigated. The biomass was subjected to chemical treatments to modify the functional groups, carboxyl, amino and phosphate and to study their role in biosorption of heavy metals. The effect of pretreatment on the heavy metal biosorption capacity of *Penicillium lanosa-coeruleum* was investigated by Illhan *et al*. They found that heat, NaOH and detergent pretreatments significantly improved biosorption of Pb and Cu whereas gluteraldehyde increased Ni biosorption⁴³. Kogej and Pavko reported the biosorption of Pb from aqueous solution in a batch stirred tank reactor and a continuous packed bed column using self immobilized *Rhizopus nigricans* as biosorbent⁴⁴. The effect of pretreatment on Pb biosorption capacity of fungal biomasses such as *Aspergillus versicolor*, *Metarrhizium anisopliae* var. *anisopliae* and *Penicillium verrucosum* was investigated by Cabuk *et al*⁴⁵. Fungal biomasses were subjected to physical treatments like heat and autoclaving, and chemical treatments *viz.* NaOH, formaldehyde, gluteraldehyde, acetic acid, H₂O₂, commercial laundry detergent, orthophosphoric acid and dimethyl sulfoxide. Biosorption of Pb was increased when biomass of *A. versicolor* was pretreated with dimethyl sulfoxide, H₂O₂ and gluteraldehyde. Preetha and Viruthagiri⁴⁶ investigated the absorption of Zn (II) ions by *Rhizopus arrhizus*, a filamentous fungus, in a batch reactor. Batch adsorption studies were carried out by varying biomass loading. Specific Zn (II) uptake decreased with the increase in biomass loading and these results were analysed in the light of Lagergren equation and the process followed a second order rate kinetics.

Five morphologically different fungi were isolated from leather tanning effluents in which species of *Aspergillus* and *Hirsutella* had higher potential to remove Cr⁴⁷. The potential of *Aspergillus* sp. for the

removal of Cr was evaluated in shake flask culture at different pH, temperature, inoculum size, C and N source. The maximum Cr was removed at pH 6.0 and 30° C in the presence of sodium acetate (0.2%) and yeast extract (0.1%).

Biosorption of heavy metals by macrofungi or mushroom is a known phenomenon nowadays but not much work has been done. Since mushrooms grow under natural habitats, heavy metal pollutants present in the soil or in the natural substrates are taken up by the fruiting bodies of mushrooms resulting in accumulation of metals in the mycelia and sporocarps. Size, texture and other physical characteristics are conducive for their development into absorbents without the need for immobilization or development of sophisticated reactor configuration as needed in the case of microorganisms.

Uptake of Cd, Pb, Co, Cu by mycelia and sporocarps of an edible mushroom, *Volvariella volvacea*, was reported by Purkayastha and Mitra⁴⁸. Metal (Cu II) uptake potential, mechanism and application of mushroom, *Ganoderma lucidum*, as a biosorbent had been reported⁴⁹. Uptake of heavy metal pollutants by edible mushrooms and its effect on their growth, productivity and mammalian system was also studied⁵⁰. Fruiting bodies of nine non-edible mushrooms *viz.* *Coriolopsis strumosa*, *Daedalea tenuis*, *Lentinus strigosus*, *Lenzites malaccensis*, *Phellinus xeranticus*, *Rigidoporus lineatus*, *Rigidoporus microporus*, *Trametes lactenia*, *Ganoderma lucidum* were screened for copper uptake potential⁵¹. Garcia *et al*⁵² reported the potential use of *Agaricus macrosporus* for bioextraction of heavy metals from contaminated wastes. The data indicated that *A. macrosporus* effectively extracted Cd, Hg and Cu from contaminated substrates.

Bacteria as Biosorbent

Numerous studies have identified a number of potential bacterial species capable of accumulating metals from aqueous environment. Among the bacteria, *Bacillus* sp. has been identified as having a high potential of metal sequestration and has been used in commercial biosorbent preparation⁵³. Besides, there are reports on the biosorption of metals using *Pseudomonas* sp., *Zoogloea ramigera* and *Streptomyces* sp⁵⁴⁻⁵⁹. A study of the application of bacteria for the recovery of heavy metals from aqueous environments was carried out by Seki *et al*⁶⁰. The biosorption characteristics of Cd and Pb ions were determined with purple non-sulfur bacteria,

Rhodobacter sphaeroides, and hydrogen bacteria, *Alcaligenes eutrophus* H16. Ilhan *et al*⁶¹ reported the removal of Cr, Pb and Cu ions from industrial waste waters by *Staphylococcus saprophyticus*; the optimum pH values for Cr, Pb and Cu biosorption were found to be 2.0, 4.5 and 3.5, respectively. The biosorption properties of bacterial biomass and the effects of environmental factors (pH, metal concentration, contact time, etc) on Cr, Cd and Cu biosorption were explored by Ozdemir *et al*⁶². They reported that a species of Gram negative bacterium, *Pantoea* TEM 18, isolated from wastewater treatment of a petrochemical industry along with other microorganisms exhibited the greatest Cu tolerance. Cell walls of Gram negative bacteria are somewhat thinner than the Gram positive ones and are also not heavily cross-linked. They have an outer membrane, which is composed of an outer layer of lipopolysaccharide (LPS), phospholipids and proteins⁶³. Gourdon *et al*⁶⁴ compared the Cd²⁺ biosorption capacities of Gram positive and Gram negative bacteria. Glycoproteins present on the outer side of Gram positive bacterial cell walls were suggested to have more potential binding sites for Cd²⁺ than the phospholipids and LPS and hence are responsible for the observed difference in capacity.

Rabbani *et al*⁶⁵ reported biosorption of Cr (III) by 17 bacterial strains isolated from Ramsar warm spring, Iran. A new strain of Gram positive coccobacilli bacteria (NRC-BT-2) was found to be highly capable for the biosorption of Cr (III). In the batch experiments with various initial concentrations of Cr ions to obtain the sorption capacity and isotherms, the biosorption of Cr (III) and Cr (VI) onto the cell surface of *Pseudomonas aeruginosa* was investigated⁶⁶.

Yeast as Biosorbent

Among the promising biosorbents for heavy metal removal, which have been researched during the past decades, yeast (*Saccharomyces cerevisiae*) has received increasing attention due to its unique nature in spite of the mediocre capacity for metal uptake. *S. cerevisiae* in different forms has been studied in biosorption research. For example, living cell/dead cell³⁸, immobilized cell/free cell¹¹, wild type/mutant type, flocculent/non-flocculent cell⁶⁷, engineered and non-engineered cell, laboratory culture/waste cells from different industries⁶⁸.

Comparative results of metal biosorbent capacities between *S. cerevisiae* and other microorganisms has

been investigated by Bakkaloglu *et al*⁶⁹. They investigated various types of microorganisms including bacteria (*S. rimosus*), yeast (*S. cerevisiae*), fungi (*P. chrysogenum*), activated sludge as well as marine algae (*F. vesiculosus* and *A. nodosum*) for biosorption of metals. They compared the removal efficiency for Zn, Cu and Ni ions at the stage of biosorption, sedimentation and desorption. The results showed that *S. cerevisiae* has a mediocre efficiency for one or multi metal biosorption systems. By comparing the index q_{max} of Langmuir equation with seven types of waste biomass for the removal of Pb ion, Kogej and Pavko⁴⁴ indicated that Pb uptake capacity by *S. cerevisiae* is in the middle, in comparison to other six biomaterials used. Vianna *et al*⁷⁰ studied the biosorption capability for Cu, Cd, and Zn, using three kinds of waste biomass from fermentation industries, that is, *Bacillus lentus*, *Aspergillus oryzae* and *S. cerevisiae*. The results showed that protonated *B. lentus* had the highest sorption capacity for Cu and Cd, followed by protonated biomass of *A. oryzae* and *S. cerevisiae*. Donmez and Aksu⁷¹ studied Cu ion bioaccumulation by adapted and growing cells of *S. cerevisiae*, *Kluyveromyces marxianus*, *Schizosaccharomyces pombe* and *Candida* sp. They found that the biosorptive capacity for Cu²⁺ decreased in the following order: *S. cerevisiae* (7.11)>*K. marxianus* (6.44)>*Candida* sp. (4.80)> *S. pombe* (1.27). However, the results indicated that *Candida* sp and *K. marxianus* were more efficient than *S. cerevisiae* and *S. pombe* for heavy metal resistance and Cu (II) bioaccumulation at higher concentrations. Compared with the excellent biosorbent fungi, *Rhizopus* for the biosorption of Pb, Cd, Cu, Zn and U, the common yeast *S. cerevisiae* is regarded as a 'mediocre' metal biosorbent⁷². Comparing with other fungi biomaterials, in spite of the mediocre metal biosorption capacity, *S. cerevisiae* is a unique biomaterial in biosorption research.

In *S. cerevisiae*, free cells appear unsuitable in practical application, largely due to solid/liquid separation problem. However, Veglio and Beolchini¹¹ pointed out that investigation on the performance of free cells for metal uptake can provide fundamental information on the equilibrium of the biosorption process, which is useful for practical application. Meanwhile, flocculating cell has been suggested for biosorption, attempting to overcome the separation problem of free cells⁷³. Brady *et al*⁷⁴ proved that the

cells of *S. cerevisiae* treated with hot alkali were capable of accumulating a wide range of heavy metal cations (Fe^{3+} , Cu^{2+} , Cr^{3+} , Hg^{2+} , Pb^{2+} , Cd^{2+} , Co^{2+} , Ag^+ , Ni^{2+} and Fe^{2+}). Some toxic metal ions studied in *S. cerevisiae* biosorption are $\text{Pb}^{75,78}$, $\text{Cu}^{69,79}$, Zn^{69} and $\text{Cd}^{68,75,80,81}$, $\text{Hg}^{82,83}$, Co^{82} , $\text{Ni}^{69,84}$ and Cr^{85} . Biosorption of Cr (VI) and As (V) onto methylated yeast biomass has been studied by Sekei *et al*⁸⁶. *S. cerevisiae* can distinguish different metal species based on their toxicity such as Se (IV) and Se (VI), Sb (III) and Hg (II). This kind of property makes *S. cerevisiae* useful not only for the bioremediation, removal and recovery of metal ions, but also for their analytical measurement^{12,87,88}. Mapolelo and Torto⁸⁹ proved that biosorption of Cd (II), Cr (III), Cr (VI), Cu (II), Pb (II) and Zn (II) by *S. cerevisiae* is dependent on optimum pH values above 5.

Waste Materials of Food and Agricultural Industry as Biosorbents

Agricultural by-products have been widely studied for metal removal from water. Peat, wood, pine bark, banana pith, soybean and cotton hulls, rice bran, saw dust, wool, orange peel have been demonstrated to remove heavy metals from wastewater^{76,90-92}. These agricultural waste biosorbents are inexhaustible, cheap and non-hazardous materials, which are specifically selective for heavy metals and can be easily disposed by incineration.

The feasibility of using bio-waste obtained from fruit juice industry for the removal of toxic heavy metals, Hg (II), Pb (II), Cd (II), Cu (II), Zn (II), and Ni (II), from wastewaters was explored by Senthilkumaar *et al*⁹³. Fruit residues and phosphated fruit residues were separately used as biosorbents in which the latter showed higher biosorption capacity. The pH of solution was identified as the most important variable influencing metal adsorption on biosorbents. Sorption of six metal ions (Pb^{2+} , Ni^{2+} , Cd^{2+} , Cr^{3+} and Zn^{2+}) from contaminated water by petiolar felt-sheath of palm was studied⁹⁴. The uptake was rapid, with more than 70% completed within 15 min. This biosorbent was found to remove all toxic metal ions efficiently with selectivity order of $\text{Pb}^{2+} > \text{Cd}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Cr}^{3+}$.

Adsorption behaviour of Ni (II), Zn (II), Cd (II) and Cr (III) on untreated and phosphate-treated rice husk was studied by Ajmal *et al*⁹⁵. The adsorption of Ni (II) and Cd (II) was found to be greater when phosphate treated rice husk was used as an adsorbent. Sorption of Cd (II) was dependent on contact time,

concentration, temperature, adsorbent doses and pH of the solution. Basci *et al*⁹⁶ reported the adsorption capacity of wheat shell for Cu (II) from aqueous solutions. The maximum biosorption efficiency was at pH 5. An increase in Cu concentration had a negative effect on the efficiency of biosorption.

Sorption of Cr (III) ions from aqueous solutions by hen eggshells was investigated⁹⁷. Effect of process parameters [pH, temperature, and initial concentration of Cr (III) ions] was studied. The potential of papaya wood waste to treat wastewaters contaminated with heavy metals Cd (III), Cu (II) and Zn (II) was studied by Saeed *et al*⁹⁸. Sorption was most efficient at pH 5 during contact time of 60 min. Metal ion biosorption was found to increase as the ratio of metal solution to the biomass quantity decreased. The affinity of papaya wood to biosorb metals was in the order of $\text{Cu (II)} > \text{Cd (II)} > \text{Zn (II)}$.

Removal and recovery of Pb (II) from single and multi metal (Cd, Cu, Ni, and Zn) solutions by crop milling waste (black gram husk) was reported by Saeed *et al*⁹⁹. Selectivity order of the biosorbent for metals was $\text{Pb} > \text{Cd} > \text{Zn} > \text{Cu} > \text{Ni}$. Complete desorption of Pb and other metals in single and multi metal solutions was achieved with 0.1 M HCl in both shake flask and fixed bed column studies.

Biosorption characteristics of dried sugar beet pulp for removing Cu (II) from aqueous solutions revealed that at 250 mg L^{-1} initial Cu (II) concentration and pH 4, highest Cu (II) uptake capacity (28.5 mg g^{-1}) at 25°C was observed¹⁰⁰. The capacity of raw rice bran to remove Cr and Ni from aqueous solutions was investigated by Oliveira *et al*¹⁰¹. The adsorption equilibrium was modeled using the Langmuir and Freundlich isotherm and the experimental data were well fitted to the Freundlich equation. The possibility of utilization of the rice bran for sorption of Cu ions from aqueous solutions was also studied by Wang and Qin¹⁰². The experimental results were fitted to the Langmuir, Freundlich, Temkin and Redlich-Peterson isotherms to obtain the characteristic parameters of each model. Except Freundlich, the sorption data fitted well in other three isotherm models.

Hossain and Kumita¹⁰³ studied the dynamic characteristics of Cr (VI) sorption using black tea leaves as biosorbent. Batch experiments were conducted to evaluate the effects of Cr (VI). Experimental and calculated kinetics data for equilibrium were expressed by Langmuir effect on the adsorption rate.

The potential to remove Cr (VI) from aqueous solutions through biosorption using the husk of Bengal gram (*Cicer arietinum*) was investigated in batch experiments by Ahalya *et al.*¹⁰⁴. The results showed 99.9% removal of Cr from 10 mg L⁻¹ Cr solution; the biomass required at saturation was 1 g mg⁻¹. The adsorption data fitted well with the Langmuir and Freundlich isotherm models. Vijayaragavan *et al.*¹⁰⁵ investigated the biosorption of Cu (II) and Co (II) by crab shell. At optimum particle size (0.767 mm), biosorbent dosage (5 g/L) and initial pH of solution (pH 6), the biosorbent recorded 243.9 and 322.6 mg/g, uptakes of Cu and Co, respectively according to Langmuir model. Yohimbe bark and grape stalks waste were used as ligands in composite and PVC-based membranes for the development of Cr (VI) and Hg (II) selective electrodes by Fiol *et al.*¹⁰⁶.

Immobilization of Microbial Biomass for Bioreactors

In order to retain the ability of microbial biomass to absorb metals during the continuous industrial process, it is important to utilize an appropriate immobilization technique. The free cells can provide valuable information in the laboratory experimentation but are not suited for column packing in industrial applications^{107,108}. The free cells generally have low mechanical strength and small particle size and, therefore, excessive hydrostatic pressures are required to generate suitable flow rates. High pressures can cause disintegration of free biomass. These problems can be avoided by the use of immobilized cell systems^{109,110}. Immobilized biomass offers many advantages including better reusability, high biomass loading and minimal clogging in continuous flow systems^{27,64}. Table 1 summarizes some matrices used for immobilization of microbial biomass.

In accordance with the process mechanism, kinetics, uptake capacity and physical characteristics of the biosorbents, many reactor configurations have been suggested in both batch and continuous mode. The typical reactor configurations suggested are: a) batch stirred tank reactor, b) continuous flow stirred tank reactor, c) fixed packed bed contactor, d) pulsating bed contactor, e) fluidized bed contactor, and f) multiple bed contact arrangement.

Commercial Applications of Biosorption Technology

By critical analysis of various microbial masses, a few potential metal sequestering biosorbents have been commercialized. Bio-Fix biosorbent was developed using biomass from a variety of sources

Table 1—Matrices used for immobilization of microbial biomass for the metal adsorption studies

| Immobilization matrix | Type of biomass | Metal adsorbed | References |
|-----------------------|--------------------------|---|------------|
| Calcium alginate | <i>C. vulgaris</i> | Au, Cu, Fe, Zn Co, Mn | 72,116,117 |
| | <i>S. platensis</i> | | |
| | <i>C. salina</i> | | |
| | <i>R. arrhizus</i> | | |
| Polyacrylamide gel | <i>Citrobacter</i> | U, Cd, Pb, Cu, Co, Cd | 118 |
| Silica | <i>Rhizopus arrhizus</i> | Cu, Ni, U, Pb, Hg, Cd, Zn, As, Ag | 117 |
| | Algasorb | | |
| Polyurethane | <i>P. aeruginosa</i> | U | 119 |
| Polysulfone | <i>P. laminosum</i> | Pb, Cd, Zn | 120,121 |
| | <i>Citrobacter</i> | | |

like cyanobacterium (*Spirulina*), yeast, algae and plants (*Lemna* sp. and *Sphagnum* sp.)¹¹¹⁻¹¹³. The biomass was blended with xanthan and guar gums to give a consistent product and many attractive features including selective removal of metals over a broad range of pH and temperature, its rapid kinetics of adsorption and desorption and low capital and operational costs.

AlgaSorbTM, a potent algal biosorbent was developed using a fresh water alga, *Chlorella vulgaris*, to treat wastewater¹¹⁴. It efficiently removed metallic ions from dilute solutions. Another metal sorption agent, AMT-BIOCLAIMTM (MRA), was developed using *Bacillus* biomass to manufacture granulated material for wastewater treatment and metal recovery¹¹⁵. This can accumulate metal cations with efficient removal of more than 90% from dilute solutions.

Conclusions

Research over the past decade has provided a better understanding of metal biosorption by certain potential biosorbents. The group of cheap biosorbent materials based on natural and waste biomasses constitutes the basis for a new cost-effective technology that can find its largest application in the removal of metal contaminated industrial effluents. Application aspects of biosorption are being aimed at biosorption process optimization. Mathematical models are helpful in this regard in order to guide further experimental work and provide predictions of the performance of the biosorption process under different operating conditions. To attract more usage of biosorbent technology, some strategies have to be developed where further processing of biosorbent can

be done to regenerate the biomass and then convert the recovered metal into usable form.

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