Biosorption of heavy metals

Thesis

**Submitted In Partial Fulfillment of the Requirement
For The Award of Degree of
M.Sc in Biotechnology**

By

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Contents

Candidate's Declaration

I hereby declare that the work presented in the dissertation entitled, "**Biosorption of Heavy metals** " in partial fulfilment of the requirement for the award of the degree of Masters in Biotechnology, Department of Biotechnology and Environmental Sciences, Thapar Institute of Engineering and Technology, Patiala; is an authentic record of my own work during the period of five months from January 2003 to May 2003,under the supervision of Dr. Dinesh Goyal, Assistant Professor, Thapar Institute of Engineering and Technology. I have not submitted the matter embodied in this dissertation for the award of any other degree or diploma.

Place: Patiala

Date: **NAVNEET JOSHI**

This is to certify that the above statement made by the candidate is correct and true to the best of our knowledge.

Dr. Dinesh Goyal
Project Supervisor

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Head, DBTES
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Date:

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Abstract

Feasibility of five different non-conventional biosorbents of microbial and plant origin were tested for removal of priority metal ions such as Pb, Cr, Zn, Ni and Fe from synthetic effluents in single metal state and multi-metallic state. The objective was to develop inexpensive and effective metal ion adsorbents that are available in large quantity as an alternative to existing commercial adsorbents. Five biowastes such as waste carrot juice pulp, waste tea leaves, wood powder (saw dust), paper mill sludge and mycelial waste were tested for biosorption efficiency and compared with that of activated charcoal. The effect of biomass concentration, pH and metal concentration on the ability of dried biomass to remove metal from solution was investigated. Dried powder of waste carrot juice pulp can remove 74 % Zn, tea leaves 25 % lead, wood powder 36 % lead and 35 % Zinc, whereas mycelial waste could remove 74 % Pb and 46 % Fe and paper mill sludge could remove 66 % lead and 46 % Fe from 50 ppm of synthetic metal solution in 30 min. of contact time at pH 4.0 at room temperature with continuous stirring at 80 rpm. The adsorption of metal ion on paper mill sludge reached equilibrium in 30 min. and by mycelial waste in 60 min. Maximum uptake of metal ion occurred in the range of pH 4-5 by paper mill sludge and at pH 3 by mycelial waste by a biomass concentration of 2 %. For the same metal ion different adsorbents had different removal rate. A combination of biosorbents was effective in simultaneous removal of Pb, Zn and Ni from multimetal metallic solution. There is a plenty of scope for large scale application of non living biomass from fermentation industries for removal of metal ions. Paper mill sludge and mycelial waste biomass can be used in the wastewater treatment for the removal of metal ions which are available in large quantities can have tremendous usage as an alternative to existing commercial adsorbents.

Key words: Biomass, heavy metal, biosorption, bioprecipitation, and microbial removal.

Introduction

Heavy metals are on the forefront of academic and regulatory concern, since millions of gallon of water containing toxic heavy metals are generated annually from several metal processing industries and discharged in to the environment. Metals discharged into water bodies are not biodegraded but undergoes chemical or microbial transformations, creating large impact on the environment and public health (Volesky, 1993). Therefore, increasing awareness is rapidly growing over worldwide and one of the offshoots of it, is treatment and removal of heavy metals from such effluents to permissible limits before discharging into natural streams and rivers. Towards this direction, several conventional wastewater technologies were developed and are in use successfully at large scale, to reduce hazardous compounds concentration in wastewater from higher to lower level (Verma and Rahal, 1996). Application of such traditional treatments require enormous cost and continuous inputs of chemicals which becomes impractical and uneconomical and causes further environmental damages. Hence, easy, effective, economic and eco-friendly techniques are required for fine-tuning of wastewater treatment (Prakasham, *et al.,* 1999).

Sources of discharge of metals

Lead	present in petrol-based materials and many other industrial facilities (Sag and Kutsal 1997).
Chromium	industrial operations including chrome plating, petroleum refining, leather, tanning, wood preserving, textile manufacturing and pulp processing. It exists in both hexavalent and trivalent forms.
Iron	Iron and steel units, electroplating industries and galvanizing units
Zinc	widely used in industry to make paint, rubber, dye, wood preservatives, and ointments and electroplating industries.
Nickel	galvanized, paint and powder batteries processing units

Harmful effects

Metals	Health Risks
Iron	The lack of iron in the diet may lead to iron-deficient anemia, Fatigue, Weakness, Drowsiness, Pallor (paleness), Cold extremities, Brittle nails, Loss of appetite, Constipation, Headaches, Irritability, Difficulty concentrating, Depression, Loss of libido, Tinnitus (ringing in the

	ears), Spots before eyes, Bizarre behavior, Gastrointestinal complaints, Cessation of menstruation, Jaundice
Chromium	Irritant, nausea and vomiting, carcinogen (Oxidation state of +6), Low-level exposure can irritate the skin and cause ulceration. Long-term exposure can cause kidney and liver damage, and damage too circulatory and nerve tissue.
Zinc	Nausea and vomiting. Zinc combines with other elements to form zinc compounds; common zinc compounds found at hazardous waste sites include zinc chloride, zinc oxide, zinc sulfate, zinc phosphide, zinc cyanide, and zinc sulfide.
Lead	Damage to nervous system, circulatory system, blood forming system, reproductive system, gastrointestinal tract and kidney Lead is known for its harmful affect on the living world, enters the organism by breathing, swallowing, or absorption through the skin. The greatest danger from lead comes from its tendency to accumulate in the human organism. The central nervous system is most sensitive to the effects of lead.
Nickel	Short-term overexposure to nickel is not known to cause any health problems, but long-term exposure can cause decreased body weight, heart and liver damage, and skin irritation. The EPA does not currently regulate nickel levels in drinking water.

The health risks of heavy metal ingestion are widely ranging. Some metals causes physical discomfort while others may cause life-threatening illnesses, damage to vital body system, or other damage. In many instances, the effects of heavy metals on human are not well understood or documented.

Heavy metal toxicity

Metals and their "free radicals" are highly reactive attacking other cellular structures. The ability of metals to disrupt the function of essential biological molecules, such as protein, enzyme and DNA is major cause of their toxicity. Displacement of certain metals essential for cell by a similar metal is another cause of toxicity. For example cadmium can substitute for the essential metal zinc in certain protein that require zinc for their structure and function. The alteration in protein can lead to toxic consequences. In the same way, lead can substitute for calcium in bone and in other sites where calcium is required.

Conventional Treatment Methods

- Precipitation
- Ion-Exchange Method
- Electrochemical Cells
- Reverse Osmosis
- Biological Methods

Table-1 Different technologies for the removal of heavy metals from the industrial wastewater

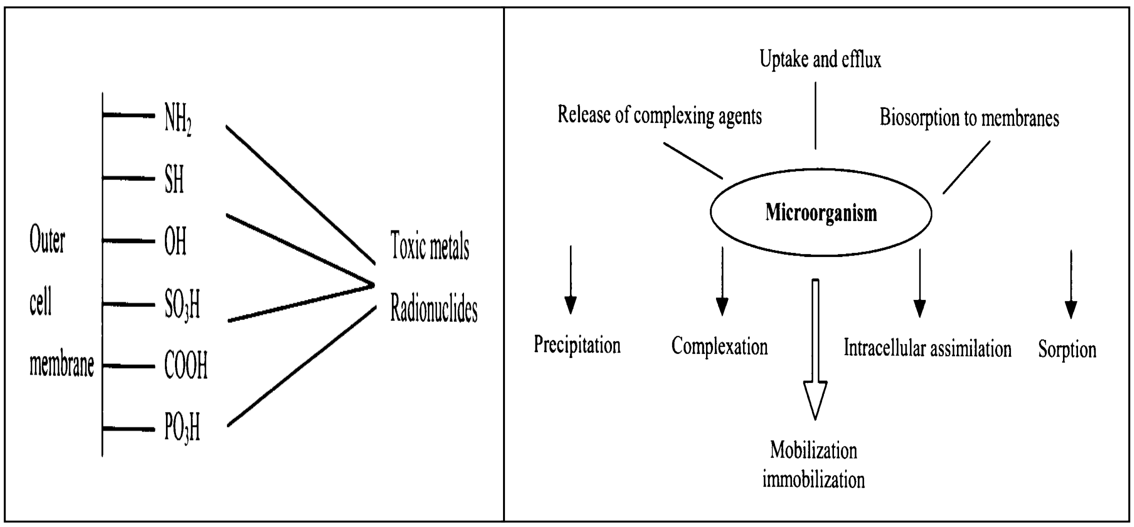
Technology	Concentration dependence	pH	Suspended solids	Effluent Concentration (mg/l)	Regeneration	Sludge generation
Biosorption	Yes	Yes	Yes	<1	Yes	No
Hydroxide Precipitation	No	No	Yes	2-5	No	Yes
Sulfide Precipitation	No	No	Yes	<1	No	Yes
Ion Exchange	Yes	Some	No	<1	Yes	Yes
Evaporation	Yes	YES	Yes	1-5	---	No
Reverse Osmosis	No	Some	No	1-5	No	No
Adsorption	Yes	Some	Yes	1-5	Yes	No

Chemical approaches are available for metal remediation, but are often expensive to apply and lack the specificity required to treat target metals against a background of competing ions. In addition, such approaches are not applicable to a cost-effective remediation of large-scale subsurface contamination *in situ*.

Biological methods

Microbial research and need for new methods of water cleanup having led to great deal of expansion in the filed of biological methods of industrial effluent cleanup. Microbes require heavy metals as human require certain metals in their diet. There are four commonly pathways by which microbes accumulates heavy metals like a) Binding to the cell surface, b) Intracellular accumulation, c) Extra-cellular precipitation and d) Volatilization. Living biological systems are well suited for the treatment of water. The mining industry has used engineered living systems in the remediation of lagoons contaminated with heavy metal. Algae with excellent metal adsorption are deliberately grown in the lagoons. The term "biosorption" is used to describe the accumulation of metal ions from solution by material of biological origin (microbial or plant). Biosorption is a process that utilizes inexpensive dead biomass to sequester toxic heavy metals and is particularly useful for the removal of contaminants from industrial effluents.

Biosorbents are prepared from the naturally abundant and/or waste biomass of algae, fungi or bacteria. A variety of uptake mechanisms is involved including adsorption and ion exchange. It has been widely suggested that this behavior could be utilized in wastewater treatment but, to date, there has been little commercial success in this field.



Advantages of Biosorption

- Non-living cells are less sensitive to metal ion concentration (toxicity effects).
- Can be operated at ambient conditions of pH and temperature.
- Low operating cost.
- Volume of chemical or biological sludge can be minimized.
- Supply of nutrients is not required.
- Dead biomass can also be procured from industrial sources as a waste product from the fermentation processes

The need for economical, effective and safe methods for removing heavy metals from wastewater has resulted in the search for unconventional materials that may be useful in reducing the levels or accumulation of heavy metals in the environment. The newly discovered metal sequestering properties of certain types of microbial biomass of fungi, bacteria and algae offers considerable promise (Volesky, 1987) and offer an alternative to the existing methods for metal detoxification and their recovery. To date, the most successful biotechnological processes utilize biosorption and bioprecipitation, in addition to other processes such as binding by specific macromolecules. Previous studies on metal biosorption have been restricted to simple solutions of only one metal. Biomass, which is available in large quantities as a waste product, their potential utilization as a metal biosorbent, is of interest. Till now, on the large scale, microbial potential has been exploited only to a limited extent. Much wider application is possible and needs to be seriously considered. The feasibility and efficiency of a biosorption process depends not only on the properties of the biosorbents but also on the composition of the wastewater. The present investigation envisages use of dead or non-living biomass available in large quantities for removal of heavy metals from aqueous solution and to evaluate influence of co-cations on the biosorption in multimetal systems using combination of waste biomass types.

Review of literature

Microorganisms are generally the first to be affected by the discharges of heavy metals into the environment. Microbial ecosystem can drastically alter the fate of the metal entering into aquatic or soil environments. Bacteria, cyanobacteria and fungi alter the form of occurrence of metal through methylation, chelation, complexation, catalysis or adsorption affecting their bio-availability and movement in the food chain. Many types of yeast, fungi, algae, bacteria and some aquatic plants have been reported to have the capacity to concentrate metals from dilute aqueous solutions and to accumulate them inside the cell structure. (Kapoor and Viraghavan, 1995; Volesky and Holan, 1995; Modak and Natarajan, 1996). There are numerous industrial processes and related activities that result in the release of metallic species and if release of metals can be controlled before it enter in common waste streams, enormous saving in disposal costs of resulting sludge's are possible. In fact, the detoxification of sludge's can convert them from economies liability to a sellable resource.

Bioremediation is to remove, sequester or solublize the metals that are able to degrade compounds. This suggests that under the selective pressure of environmental pollution, a microbial capacity for the degradation of recalcitrant compounds exists that may be harnessed for pollutant removal by biotechnological processes. Bioremediation-using microorganisms is less intrusive, less expensive and accumulates toxics for their removal and sequester them for large-scale removal.

Adam and Holmes (1935) described the removal of Ca and Mg ions by tannin resin, black wattle bark (*Acacia mollissima*), which were treated directly so that the condensation product was fixed on the woody fibres. Strong biosorbent behaviour of certain types of microbial cells towards metallic ions is a function of the chemical makeup of the microbial cells of which it consists this aspects is particularly important when it comes to the process application, whereby new biosorbents respective "Chemicals" are capable of sequestering a relatively large amount of the metals (Volesky 1987). Some types of biosorbents could have broad range binding of the majority of heavy metals with no specific priority, while others can even be specific for certain types of metals (Volesky and Kuyvcak, 1988). Microbial biomass was used as an adsorbing agent for the removal and recovery of uranium present in industrial effluents and mine wastewater (Nakajima and Sukaguchi, 1986). A summary of microorganisms having high metal removal efficiency is presented in Table 2.

Biosorption by fungi as an alternative treatment option for wastewater containing heavy metal has been reviewed by Kapoor and Viraghavan (1995) and Modak and Natarajan (1996). Any fungi can tolerate high concentration of potentially toxic metals and with other microbes; this may be correlated with decreased intracellular uptake or impermeability. A close relation between toxicity and intracellular uptake has been shown for Cu^{2+}, Cd^{2+}, Co^{2+} and Zn^{2+} in yeast *Saccharomyces cerevisiae* (Gadd, 1986; white and Gadd; 1986).

Table 2. Removal of heavy metal and removal efficiency of different microorganism

Organism Used	Metals removed	System	Reported efficiency and application
Pseudomonas flourescens	Pb, Zn	Immobilized on PVC & Packed in columns	Used in Hungarian chemical Company
Pseudomonas aeruginosa	Uranium, Plutonium	Immobilized in plasma treated Polyprppylene	75-80 % removal
Citrobacter sp.	Cd, Pb, Cu, U	Immobilized on PAG	80-90 % removal
Bacillus subitilis Saccharomyces sp.	Pb, Zn, Cu, Ni, Cd, Hg, Ag, Au, Pd	Fixed Reactor	AMT-Bioclaim TM 98 % removal
Streptomyces sp. Viridochromogenes sp.	Uranium	Trpped in silica gel matrix	80-100 % removal
Rhizopus arrhizus	Cr, Fe, Cu, U	------	50-605removal
Dead algae Barkley, 1991	Hg	Sorption column	95uptake Alga SORBTM being used
Sargassum natans	Pb, Cd, Cr	-----	3 times more efficient than ion exchange resin
S.flutians	Cu		80-90% removal
A. niger, *P. chrysoginum*	Ag, Zn		---
A. oryzae	Cd	Immobilized on reticulated foam	95 % removal

Table. 3 Metal removal by different conventional and non-conventional biosorbents

S. No.	Adsorbents	Metals	References
1	*Lemna minor*	Pb	Rahimani, *et al*.,1999
2	*Amaranthus spinosus, Solanun nigrum*	Cu	Chen, *et al*., 1996
3	Chicken feathers	Au, Pt	Suyama, *et al.,* 1996
4	Canola meal	Cr	Al-asheh, *et al.,* 1996
5	Hyacinth roots	Cr	Low, *et al.,* 1997
6	Fly ash	Cr, Pb, Cd	Bhargava, *et al.,* 1989
7	Fly ash	Cr, Pb, Mn, Fe	Sharma, *et al.,* 1990
8	Agriculture residue	Cr, Cd	Orhan and Byakungar, 1993
9	Saw dust	Cr, Pb, Cd	Campanella, *et al.,* 1986
10	Coconut fibre	Cr	Tan, *et al.,* 1993
11	*Sargassum natans*	Cr, Pb, Co, Cd	Volesky, 1995

Waste mycelia from industrial fermentation plants (*A. niger, P. chrysogenum and C. paspali*) were used to as a biosorbent for removal of Zn ions from aqueous environments, both batch wise as well as in column mode. Under optimised conditions *A.niger* and *C.paspali* were found superior to *P.chrysogenum* (Luef, *et al.,* 1991). Removal of lead ions from aqueous solution by non-living biomass of *Penicillium chrysogenum* was studied and observed that Pb^{2+} was strongly affected by the pH in the range of 4-5. Uptake of pb^{2+} was 116 mg/g dry biomass, which was higher than that of activated carbon and some other microorganisms (Niu, *et al., 1993*). Chromium biosorption by non-living biomass of *Chlorella vulgaris, Clodophora crispate, Zoogloea ramigera, Rhizopus arrhizus* and *Saccharomyces cerevisiae* was studied and observed that optimum initial pH (1.0-2.0) of the metal ion solution affected the metal uptake capacity of the biomass for all the microorganism. Maximum adsorption rates of metal ions to microbial biomass were obtained at temperature in the range of 25-35^{0}C. The adsorption rates increased with increasing the metal concentration of *Chlorella vulgaris, Clodophora crispate, Zoogloea ramigera, Rhizopus arrhizus* and *Saccharomyces cerevisiae* up to 200, 200, 75, 125 and 100 mg/l respectively (Nourbakhsh *et al.,* 1994). Dead cells of *Saccharomyces cerevisiae* removed 40% more uranium or zinc than the corresponding live cultures.

Biosorption of Uranium by *Saccharomyces cerevisiae* was a rapid process reaching 60% of the final uptake value within the 15 min contact. The deposition differing from that of other heavy metals more associated with the cell wall, uranium was deposited as fine needle-like crystal both on inside and outside the *Saccharomyces cerevisiae* cells (Volesky, *et al.,* 1995). For the removal Hg and Cd several brown seaweeds were tested for their ability to remove metal ions from aqueous solution by biosorption and 90-95% removal level was found from industrial wastewater (Wilson and Edween, 1995). *Lemna minor,* duckweed has been studied to remove the soluble lead from water. Result showed that viable biomass removed 85-90% of lead while non-viable removes the 60-75% of lead (Rahmani, *et. al.,* 1995).

Biosorptive capacity of different biosorbent including dried mycelium of some species of fungi, baggase, rice rusk and fermented baggase by selected fungal species or natural micro flora was examined to remove cyanide from industrial effluent. The biomass of *Rhizopus sexualis* and the fermented baggase by *Rhizopus sexualis* or *Aspergillus terreus* showed higher sorption capacity than activated charcoal. The biomass of *Rhizopus sexualis* and *Mortierella ramanniana* exhibited higher CN sorptive capacity than ascomycetes e.g. *Aspergillus terreus* and *Penicillium capsulatum* (Azab, *et al.,* 1995). Maximal removal of Ni from electroplating industries occurred by 2.5 gm of biomass of *Saccharomyces cerevisae* with in 5 hr. Ni uptake capacity from aqueous solution was also studied in filamentous fungi such as *Rhizopus sp., Penicillium sp.* and *Aspergillus sp*. The metal uptake was highest by *Rhizopus sp. (*Gill *et al.,* 1966).

The yeast biomass *Saccharomyces cerevisiae* which is a by-product from brewery industry, was used for purifications of water polluted by uranium ions, which had an efficiency to absorb U 2.4 mMol µg/dry biomass (Omar, *et al.,* 1996). Dead biomass of actinomycetes, which is the waste product from industrial fermentation, was mixed with wastewater as a free bacterial suspension and biosorption occurred. Cadimum cations bound to negative charged sites on bacterial cell wall and could be desorbed (Butter, *et al.,* 1996). Cd (II) biosorption to non-living biomass of *Rhizopus arrhizus* and *Schizomeris leiblenii* was studied in batch reactor. Maximum adsorption rates of Cd (II) ions to microbial biomass were at 30^{O}C and at the optimum pH 5.0 for both microorganisms. The adsorption rates increased with increasing Cd (II) concentration for *Rhizopus arrhizus* and *Schizomeris leiblenii* up to 100 -150 mg/l respectively. The adsorption for *Rhizopus arrhizus* were higher than that of *Schizomeris leiblenii* **(**Ozer, *et al.,* 1997). Dry cells of *Rhizopus arrhizus* has been used for the removal of Iron (II), Pb (II) and Cd (II) ions from the industrial wastewater. Higher adsorption rates and adsorption capacities were obtained at initial metal concentration up to 100 mg/l in batch reactor. High concentration of heavy metal ions may be purified by using multistage batch reactor in series (Ozer, *et al.,* 1997).

Biosorption studies were also carried out with the white-rot fungi *Polyporous versicolor* and *Phanaerochaete chrysoporium* for Cu (II), Cr (III), Cd (II), Ni (II) and Pb(II) under same operating conditions. Result showed that both were effective in removing Pb (II) from aqueous solutions with maximum biosorption capacity of 57.5 and 110 mg Pb (II)/g dry biomass (Yetis, *et. al.,* 1998). *Mucor meihi,* a fermentation industrial waste was found to be effective biosorbent for the removal of hexavalent chromium from industrial tanning effluents. Sorption levels of 1.15 and 0.7 mmol/g were observed at pH 4 and 2 respectively. In comparative studies with the ion–exchange resins, *Mucor* biomass demonstrated chromium biosorption levels that correspond closely to those of commercial strongly acidic exchange resins, while the pH behaviour mirrored that of the weakly acidic resins in solutions. However, the chromium elution characteristic from the *Mucor* biomass was similar to those of both the weakly and strongly acid resins (Tobin and Roux, 1998).

Waste biomass from the pharmaceutical fermentation industry, i.e. non living *Rhizopus nigricans* has been used for adsorption of lead over a range of metal ions concentration, adsorption time, pH and co-ions. The process of uptake obeys the Langmuir and Freundlich isotherms. Comparison of uptake between NaOH-treated and untreated biomass shows that the adsorption takes place in the chitin structure of the cell wall (Zhang, *et al.,* 1998). The biosorption of Cu (II), Ni (II) and Cr (VI) from aqueous solution on dried algae (*Chlorella vulgaris, Scenedesmus obliquus* and *synechocystis sp*.) were tested under laboratory conditions as a function of pH, initial metal ion and biomass concentration. Experiment results showed that influence of the algae concentrations. Experiments results also showed the influence of the alga concentration on the metal uptake of all the species. Both Freundlich and Langmuir adsorption models were found to be suitable for describing the short term biosorption of Cu (II), Ni (II) and Cr (VI) by all algae species (Donmez, *et al.,* 1999).

Non-living waste biomass from *Aspergillus niger* along with wheat bran was used as a biosorbent for the removal of Zn and Cu from aqueous solution. The binding capacity of the biomass for Cu was observed to be higher than that of Zn. the metal uptake was found to be a function of the initial metal concentration, the biomass loading and pH. The metal uptake of Cu by the biomass decreased in the presence of Co- ion. The uptake of Cu by the biomass decreased in the presence of the Zn and vice versa. The decreased in the metal uptake was depends on the concentration of metal ions in two compounds in aqueous solution (Modak, *et al.,* 1996).

Dried, nonliving, granulated biomass of *Streptoverticillium cinnamoneum* was used for the recovery of Pb and Zn from the solution. The optimum pH of Zn and Pb was 3.5-4.5 and 5.0-6.0 respectively. The maximum loading capacity of *S. cinnamoneum* biomass was 57.7 mg/g for Pb and 21.3 mg/g for the Zn with boiling water pre-treatment. The loaded metals could be desorbed effectively with dilute HCl, nitric acid and 0.1 M EDTA. Treatment with 0.1 M Na carbonate permitted reuse of desorbed biomass although the loading capacity in subsequent cycles decreased by 14-37% (Puranik, *et al.,* 1997).

Non-living free and immobilized biomass of *Rhizopus arrhizus* was used to study the biosorption of Cr (VI). Chromium removal rate was slightly more in free biomass conditions over immobilized state. Stirred tank reactor studies indicated maximum chromium biosorption at 100 rpm and at 1:10 biomass –liquid ratio. Fluidised bed reactor is more efficient in chromium removal over stirred tank reactor. Immobilization of biomaterial has little effect on the chromium biosorption by *Rhizopus arrhizus* (Prakasham *et al*., 1999).

Wheat stem and babul bark, a raw material was used as agro waste carbons to removal the nickel metal from the effluent of the electroplating industry. Electroplating wastewater showed 2%-10% lower removal as compared to the synthetic solutions as the similar conditions. Almost 100% removal of Ni (II) was observed using wheat stem activated carbon at a pH value of 4.0 in adsorption period of 4.0 hrs at 36±2^{O}C, carbon doses of 16.0hr/l when the initial nickel concentration was 25 g/l (Verma and Shukla, 2000).

Keeping all these points in view, our study is directly related to check the bioremoval efficiency of different biosorbents (dead biomass) from synthetic metal solution and to make an efficient biosorbent (mixed) which can remove the toxic metals from mixed metal solution which are inherently present in industrial effluent.

Materials and Methods

Preparation of adsorbent

The waste tea leave and waste carrot juice pulp were collected from the Institutes cafeteria and juice corner. Wood powder was obtained as saw dust of *Eucalyptus* tree, and paper mill sludge and mycelial waste were collected from Ballarpur Industries Ltd., Yamunanagar, and Ranbaxy Pharmaceutical Ltd., Ponta Sahib (HP) respectively. Activated charcoal was obtained from SD fine chemicals Ltd., Mumbai. Waste biomass collected from different source was dried at 70°C for overnight, powderized using pestle and mortar and preserved in polythene bags for further adsorption experiments.

Chemicals and Reagents

The stock solutions containing the 1000 µg/ml concentration of Pb^{+2}, Cr^{+6}, Fe^{+3}, Zn^{+2} and Ni^{+2} were prepared by dissolving the Lead nitrate, Potassium dichromate, Ferrous sulphate, Zinc sulphate and Nickel nitrate in the distilled water and were used in the experiment. The salts of these chemicals were procured from SD fine chemical ltd., Bombay.

Batch equilibrium studies

It was done in the Erlenmeyer flask with a working volume of 100-200 ml. Dried biomass was added at different concentrations in Erlenmeyer flasks and metal concentrations (lead, chromium, nickel, iron and zinc) were kept near to 50 $\mu g\ ml^{-1}$ separately for each metal at pH 4.0 using dilute HNO_3 and were agitated at 80 rpm at room temperature. Five ml samples were drawn at different times (0, 5, 10, 15, 30 and 60 min), acidified using concentrated HCl, filtered using whatman filter paper no. 1 and metal content in the supernatant were analysed by AAS. Similarly, time course biosorption experiments were carried out at varying pH, biomass concentration (2,4, 6 and 8 g of 100 ml of metal solution) and with varying metal ion concentration (5, 10, 20, 50 and 100 ppm). Experiments with mixed metal solution (lead, nickel and zinc) were also carried out using mixed biomass of mycelial waste and paper mill sludge in the ratio of 4:1.

Analysis of heavy metals

The concentration of lead, Iron, Nickel and zinc in the bio-sorption medium was determined by Atomic absorption spectrophotometer using air-acetylene flame (*GBC 932AA, Australia*). The solid reference material was procured from Aldrich chemical company Inc. USA and the preparation of these stock solutions (1000 mg/l) was done as per *GBC* catalogue. The hexavalent chromium was estimated by the acidic reaction with 1,5 diphenylcarbazide. The absorbance of the resulting red-violet sample was measured at 540 nm using a spectrophotometer (*Labomed inc., USA).* The analytical method for chromium is detailed in standard method (Clesceri *et.al.,* 1998).

Specific metal uptake *(q)* by biomass (mg g^{-1}) was calculated as follows (Zhang *et al*., 1998):

$q = V \times (C_i - C_f) / 1000\ W$

Where V is the volume of the solution in the contact batch flask, C_i is the initial concentration of lead in the solution (µg ml^{-1}), C_f is the final concentration of lead in the solution and W is the mass of adsorbent (g).

Similarly biosorption efficiency R (%) can be calculated as,

$R = C_i - C_f / C_i \times 100\%$

Ash Analysis

Ash content of the samples was carried out as per IS 10158 (1982).

Results and Discussion

Feasibility of five different non-conventional biosorbents of microbial and plant origin were tested for removal of priority metal ions such as Pb, Cr, Zn, Ni and Fe from synthetic effluents in single metal state and multi-metallic state. The objective was to develop inexpensive and effective metal ion adsorbents that are available in large quantity as an alternative to existing commercial adsorbents. Five biowastes such as waste carrot juice pulp, waste tea leaves, wood powder (saw dust), paper mill sludge and mycelial waste were tested for biosorption efficiency and compared with that of activated charcoal.

Time course metal removal by different adsorbents from synthetic metal solution

Time course metal removal by different adsorbents from synthetic metal solution in batch mode was studied. Table 1gives the time course biosorption efficiency of different adsorbents at varying metal ion concentration. For the same metal different adsorbents had different removal rate. The adsorption of metal ions reached equilibrium in 30-60 minutes of contact time at pH 4.0, and temperature of 28 ± 2^0C by 2 g of powderized biomass in 100 ml of synthetic metal solution and with continuous stirring at 80 rpm. Biosorption efficiency for Cr, Pb, Fe, Ni, and Zn in 30 minutes contact time was found to be 31 %, 2.8 %, 37%, 28 % and 74 % respectively by carrot powder (Table 1). By using waste tea leaves the metal removal efficiency of Cr, Pb, Fe, Ni and Zn were 23%, 25%, 10%, 12%, and 21 % respectively. With paper mill sludge, the removal efficiencies of Pb, Fe, Ni, and Zn were 66%, 46%, 27% and 33 % respectively. Similarly mycelial waste exhibited metal removal efficiencies of 27 %, 74%, 46%, 23 %, and 28 % for Cr, Pb, Fe, Ni, and Zn respectively. Activated charcoal showed removal efficiencies for Cr, Pb, Fe, Ni, and Zn to the extent of 15%, 70%, 77%, 82%, and 36% respectively. On the whole carrot powder can remove 74% Zn, tea leaves 25% lead, wood powder 36% lead, and 35% Zinc whereas mycelial waste could remove 74 % Pb and 46 % Fe and paper mill sludge could remove 66 % lead and 46% Fe from 50 ppm metal solution in 30 minutes of contact time. These experiments showed that the removal rate occurs quickly, reaching equilibrium with in the first 30-60 minutes of initial contact time. These results indicated that paper mill sludge and mycelial waste which are available in large quantities can remove effectively Pb, Fe and Zn from 50 ppm metal solution and Pb, Fe, Zn and Ni from 100 ppm of metal solution in 30 minutes of contact time (Table 1). Table 2 gives the order of metal removal 50 and 100 ppm single metal containing solution. Time course adsorption showed particular trend with waste tea leaves and paper mill sludge, where there was increase in the metal removal with increase in contact duration. However other adsorbents showed maximum removal in either 30-60 min or up to 2 hr. Residual metal concentration decreased with time where as metal uptake showed an increasing trend, which followed Langmuir and Freundlich adsorption isotherms (Fig.   ).

Paper mill sludge and mycelial waste were selected due to their large scale availability for further studies and used individually as well as in combination to optimize pH and biomass concentration at room temperature.

Optimization of biomass concentration for metal removal

Figure 15 and 16 represents the purification of a solution of 50 ppm of Ni, Zn, Pb, at various biosorbent concentrations of paper mill sludge and mycelial waste respectively. This experiment shows that Zn uptake decreases when biomass concentration rises by both the type of metal adsorbents. This reduction is attributable to metal concentration shortage in the solution. Therefore it is not useful to increase the biomass beyond 2-4 g/100 ml to purify 50 ppm zinc solution (Fourest and Roux, 1992). Using paper mill sludge there was increase in metal uptake (Zn, Ni, Pb) up to 4 g biomass, however thereafter it decreased up to 8 g biomass per 100 ml. Similarly with mycelial waste there was increase in Zn uptake upto 2 g and there after it decreased (Fig. 16) and for Ni and Pb, it increased. Higher biomass concentration were required as against reported earlier which may be due to that these wastes are directly obtained from industry which also contains several impurities and are not of pure microbial origin. Optimum concentration of 2 g / 100 ml were used in all other experiments. Reduction in biomass concentration in the suspension at a given metal concentration enhances the metal / biosorbent ratio and thus increases metal uptake per gram of biosorbent, as long as the later is not saturated. All these results can be of great interest in scale up processes to optimize industrial effluent purifications.

Time course effect of pH on biosorption efficiency

Paper mill sludge had optimum removal of Cr, Zn and Pb at pH 4.0 (Fig. 1) B. whereas by mycelial waste it was at pH 3.0 (Fig. 2) from 50 ppm metal solution. At pH 4 and 60 min. of contact time chromium, zinc and lead from 50 ppm solution were removed up to 35%, 32% and 36% respectively by paper mill sludge (2 g) as compared to pH 2, 3, 5. Mycelial waste could remove Cr, Zn and Pb by 60%, 41% and 77% respectively at pH 3 in 60 min. of contact. Residual concentration and specific metal uptake from 50 ppm metal solution indicated that almost at Ph 4.0 to 5.0 there was maximum removal and uptake. By paper mill sludge there was increase in metal uptake by increasing contact time however maximum uptake of Cr, Zn was at pH 4.0 and of Pb at pH 5.0 in 2 hr contact time (Fig. 4, 6, 8). Residual concentration decreased with increasing contact duration (Fig. 3, 5, 7). With mycelial waste residual concentration (Fig. 9, 11, 13) and metal uptake (Fig. 10, 12, 14) followed the same trend however maximum removal of Cr was at pH 2.0 – pH 3.0, Zn at pH 4.0 and Pb at pH 3.0 in 90 min. of contact time. pH control during biosorption of Zn, Ni, Pb clearly enhanced metal uptake. Several authors have previously described an optimal pH 4.0. (Tobin *et al*., 1984; Tsezos and Volesky, 1981). There was no change in pH of final solution after completion of adsorption. The results also indicated that maximum adsorption of different metal species occurs at different pH. However, from practical point of view pH of 4-5 were adequate.

Metal removal from multimetal solution by combination of adsorbents

Many of the reported biosorption studies are conducted with single metal ion species in aqueous solutions while industrial effluents invariably contain more than one metal ion (Modak and Natrajan, 1995.) It is desirable to develop general purpose biosorbents that can remove a variety of metal cations. Therefore time course metal removal from multimetallic solution (C_i for Ni =5.9 ppm; Pb = 14.2 ppm; Zn = 13.1 ppm) by combination of 2 g of paper mill sludge and mycelial waste /100ml at pH 5.0 was carried out (Fig. 17). Time course experiment clearly indicates that there is rapid adsorption of all the three metal species in the initial 15-30 min. contact time where metal removal was in the order of Pb > Zn > Ni by 93 %, 87 %, 76 % respectively. The metal uptake by non-living biomass is affected significantly in the presence of other cations in solution, depending on the chemical interaction of the other chemical species (co-ions) with the metal of interest and the biomass (Somers, 1963; Khovrychev, 1973; Tsezo, 1983; Gadd and Mowll, 1985; Tobin et al, 1984; Garnham et al.,1993). Many of the functional groups present on the cell wall and membrane are nonspecific and different cations compete for the binding sites.

Conclusions

1. On the whole dried powder of waste carrot juice pulp can remove 74 % Zn, tea leaves 25 % lead, wood powder 36 % lead, and 35 % Zinc whereas mycelial waste could remove 74 % Pb and 46 % Fe and paper mill sludge could remove 66 % lead and 46 % Fe from 50 ppm synthetic metal solution in 30 min. of contact time at pH 4.0 at room temperature with continuous stirring at 80 rpm.
2. The adsorption of metal ion on paper mill sludge reached equilibrium in 30 min. and by mycelial waste in 60 min.
3. Maximum uptake of metal ion occurred in the range of pH 4-5 by paper mill sludge and at pH 2.0-3.0 by mycelial waste.
4. For the same metal ion different adsorbents had different removal rate.
5. Paper mill sludge and mycelial waste biomass can be used in the wastewater treatment for the removal of metal ions.
6. There is a plenty of scope for large scale application of non living biomass from fermentation industries for removal of metal ions. And both the wastes paper mill sludge and mycelial wastes which are available in large quantity, appears to have tremendous potential as an alternative to existing commercial adsorbents.

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