Removal of Heavy Metals from Aqueous Solution by Waste Biomass

A Thesis Submitted in partial fulfillment of the requirement for the award of the degree of

MASTER OF TECHNOLOGY
IN
ENVIRONMENTAL SCIENCE AND TECHNOLOGY

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Candidate's Declaration

I, hereby declare that the work presented in the dissertation entitled "Removal of heavy metals from aqueous solution by waste biomass", in partial fulfillment of the requirement for the award of the degree of Master of Technology, Department of Biotechnology and Environmental Sciences, Thapar University, Patiala, is an authentic record of my own work during the period of eleven months from July 2007 to June 2008, under the supervision of Dr. Dinesh Goyal, Associate Professor, Department of Biotechnology and Environmental Sciences, Thapar University. The thesis report has not been submitted for the award of any other degree or certificate in this or any other University.

Place: Patiala
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Certificate

This is to certify that the Thesis report entitled to "Removal of heavy metals from aqueous solution by waste biomass" submitted by Manoj Chandra Garg in the partial fulfillment of the requirement for the award of degree of the Master of Technology to the Thapar University, Patiala, is a record of student's own work carried out by his under my supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other university or Institute.

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Manoj Chandra Garg

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Chapter I

Introduction

Growing attention is being given to the potential health hazard posed by heavy metals in the environment. The major sources of heavy metal contamination are considered to be from industry such as lead from petrol based industries (Sag and Kutsal, 1996); chromium from chrome plating, petroleum refining, leather, tanning, wood preserving, textile manufacturing and pulp processing industries; zinc from paint, rubber, dye, wood preservatives, ointments and electroplating industries and nickel from galvanized, paint and powder batteries processing industries.

The health risks of heavy metal ingestion are widely ranging. Chromium causes irritation, nausea and vomiting at low level exposure, kidney, liver, circulatory and nerve tissue damage at long term exposure; zinc causes nausea and vomiting; lead causes damage to nervous, circulatory, blood forming and reproductive systems and long term exposure to nickel causes decrease in body weight, heart and liver damage.

Heavy metals, especially at trace concentration in a large volume of solution, are considerably difficult to remove by conventional techniques such as chemical precipitation of metals (changing the pH), reverse osmosis and other methods. The need for an economic and effective method for the removal of heavy metals has resulted in the development of new separation technologies.

Adsorption is one of the few alternatives available for such a situation. The use of microbial biomass for the removal of heavy metals, often referred to as the biosorption, has attracted much attention in recent times. Work has been carried out on the use of adsorbents such as activated carbon, low cost agricultural waste materials and microbial biomass.

Examples of toxic heavy metal accumulating microorganism including *Candida tropicalis* (Mattuschka et al., 1993), *Rhizopus arrhizus* (Tobin et al., 1984; Fourest and Roux, 1992), *Streptomyces noursei* (Brierley et al., 1986; Mattuschka et al., 1993) and *Penicillium* sp. (Paknikar et al., 1993; Niu et al., 1993; Townsley et al., 1986), *Fucus vesiculosus*, *Ascophyllum nodosum*, *Sargassum natans* (Holan and Volesky, 1994), *Absidia orchidis* (Volesky and Kuyucak , 1988; Holan and Volesky, 1995), *Bacillus subtilis* (Brierley et al., 1986; Brierley and Brierley, 1993; Cotoras et al., 1993), *Streptomyces longwoodensis* (Friis and Myers-Keith, 1986), *Sargassa* sp. (Davis et al., 2003) and *Saccharomyces cerevisiae* (Volesky and May-Phillips, 1995) have been reported. For biosorption purposes biomass can come from industrial wastes which should be obtained free of charge; organisms that can be obtained easily in large amounts in nature (e.g., bacteria, yeast, algae) and fast-growing organisms that are specifically cultivated or propagated (crab shells, seaweeds).

Bacteria are quite adequate for heavy metals biosorption due to their ability to adsorb metal ions, suitability for natural environments and low cost (Scott and Palmer, 1988). The surface envelopes of bacterial cells can adsorb various heavy metals by virtue of ionic bonds to their intrinsic chemical groups. The sites for metal binding are different according to bacterium species and metals.

The aim of this work was to study the biosorption of metal ions (Cr^{6+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) from single, binary and multimetal ion solutions using waste biomass, MB2 comprising of *Streptomyces* sp., a by product of pharmaceutical fermentation industry. The influence of initial concentration of heavy metals, pH and biomass quantity in the aqueous solutions on biosorption of metal ions was studied. The single, binary and multimetal biosorption data were evaluated in terms of equilibrium isotherms using the Langmuir and Freundlich adsorption isotherm model.

Chapter II

Review of Literature

1. HEAVY METAL POLLUTION

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), zinc (Zn), nickel (Ni) and lead (Pb).

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain.

Heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted.

Heavy metals can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater. Unlike organic pollutants, heavy metals do not decay and thus pose a different kind of challenge for remediation. A well documented environmental disaster associated with heavy metals is the Minamata disease cause by mercury pollution.

2. SOURCES OF HEAVY METAL POLLUTION

Heavy metal pollution can arise from many sources but most commonly arises from the purification of metals, e.g., the smelting of ores and the preparation of nuclear fuels. Electroplating is the primary source of chromium and cadmium. Some common sources of toxic heavy metals are listed below-

Metals	Sources of discharge of metals
Lead	Present in petrol-based materials and many other industrial facilities (Sag and Kutsal, 1996).
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.
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

3. THREAT FROM THE ENVIRONMENT

The greatest demand for metal sequestration today comes from the need to immobilize the metals released to the environment (or mobilized) by and partially lost through human technological activities. It has been established that dissolved metals (particularly heavy metals) escaping into the environment pose a serious health hazard (Kuyucak and Volesky, 1990). They accumulate in living tissues throughout the food chain, which has humans at its top, multiplying the danger. Some common harmful effects and health risks by some toxic heavy metals are discussed below-

Metals	Health Risks
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 effect on the living world, enters the organism by breathing, swallowing, or absorption through the skin. 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 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. Thus, it is necessary to control emissions of heavy metals into the environment.

4. THE NEED FOR NOVEL TECHNOLOGY

Conventional techniques to remove toxic metals, such as ion exchange and precipitation, lack specificity and are ineffective at low metal ion concentrations. The need for effective and economically viable technologies is driven by environmental pressures such a:

- Stricter regulations with regard to the metal discharges are being enforced, particularly in industrialized countries.
- Toxicology studies confirm the dangerous impacts of heavy metals.
- Current technologies for the removal of heavy metals from industrial effluents often create secondary problems with metal-bearing sludge.

5. CONVENTIONAL TECHNIQUES FOR HEAVY METAL REMOVAL

The conventional available “best treatment technologies” for metal bearing effluents are either not effective enough or are prohibitively expensive and inadequate considering the vast wastewater quantities.

The ultimate aim of wastewater treatment is to separate the toxic materials from the wastewater streams of various industries. The effluents obtained from such cleanup processes could then be reuse in industrial activities. The first criterion for deciding on the technology to be employed as the required water treatment is the degree of concentration of metallic species in the solution to be treated. For the solution which have a high concentration of metallic particles (hundreds or thousands of mg L^{-1}) crude metal removal technologies could be employed. However, such processes do not extract metals and when the solution concentration ranges in low hundreds of mg L^{-1} or less, more sophisticated and hence costly methods for heavy metal extraction are required but are often not employed.

Precipitation has been the crude metal removal technology most widely applied. Metal species in solution are precipitated through an increase in the pH, usually aided by the addition of certain chemicals. The process is aimed at desolubilizing the metals and increasing the precipitate particles size in the solution to bring the metal to the bottom of the settling chambers as sludge residuals. There are certain disadvantages of this type of water treatment that can be summarized as follows-

- The addition of chemicals must be accurate, making the process sensitive and often unreliable.
- The elimination of metal residuals does not always meet stricter environmental regulation.
- The precipitation process leaves behind “hazardous sludge” which need to be safely dispose of.
- Metals cannot be economically recovered.

Ion exchange can remove dissolved metals very effectively. However, since it is expensive it is not widely applied particularly for large scale clean up operations such as, for example, mining effluent treatment.

6. REMOVAL OF HEAVY METALS BY BIOSORPTION

Biosorption is the binding and concentration of heavy metals from aqueous solutions (even very dilute ones) by certain types of inactive, dead, microbial biomass (Macaskie et al., 1992). Individuals with different backgrounds, from engineering to biochemistry, can make significant contributions to the understanding of biosorption. Interdisciplinary efforts are essential to exploit this technology commercially. A chemical engineering background is particularly useful for expanding the application of this technology in large-scale process industries.

6.1. Sources of Biomass for Biosorption

Sources of biomass include:

- Seaweeds
- Microorganisms (bacteria, fungi, yeast, molds)
- Activated sludge
- Fermentation waste
- Other specially propagated biomasses

Biosorbents must be hard enough to withstand the application pressures, porous and/or "transparent" to metal ion sorbate species, and have high and fast sorption uptake even after repeated regeneration cycles (Fourest and Volesky, 1996).

Pioneering research on biosorption of heavy metals has led to the identification of a number of microbial biomass types (Benedict et al., 1981) that are extremely effective in concentrating metals. Some types of biomass are waste byproducts of large-scale industrial fermentations (*e.g.,* the mold *Rhizopus* or the bacterium *Bacillus subtilis*). Other metal-binding biomass types, such as certain abundant seaweeds (particularly brown algae, *e.g., Sargassum, Ecklonia*), can be readily harvested from the oceans. The examples of some of the natural bioresourses as adsorbents for the removal of heavy metals are as follow-

a. Wood charcoal (Deepak, 1990)
b. Moss peat (Sharma and Forster, 1993)
c. Carbon Slurry (Robinson et al., 2002)
d. Green pea skin dust (Samantaray et al.,1999)
e. Rice straw (Samanta et al., 1999)
f. Phytomass from Quercus suber (Prasad and Freitas, 2003)
g. Corncob (Nigam and Rama, 2002)
h. Bagasse (Rao et al., 2006)
i. Food industrial waste (Selvaraj et al., 1997)
j. Agricultural by product (Bishnoi et al., 2004)
k. Paper mill sludge (Ahluwalia and Goyal, 2004)

l. Rice husk, sawdust, coir pith and charcoal (Sumathia et al., 2005)
m. Microbial and plant derived biomass (Ahluwalia and Goyal, 2007)

These biomass types can accumulate in excess of 25% of their dry weight in deposited heavy metals: Pb^{2+}, Cd^{2+}, U^{6+}, Cu^{2+}, Zn^{2+}, Cr^{6+} and others. Research on biosorption is revealing that it is sometimes a complex phenomenon where the metallic species could be deposited in the solid biosorbent through various sorption processes, such as ion exchange, complexation, chelation, microprecipitation, etc.

Granulation of biomass materials into suitable cost-effective biosorbents is a crucial step for the successful application of biosorption processes.

The objectives of granulation are to:

- Establish the behavior of native biomass in a packed-bed reactor
- Establish the effectiveness of biomass granulation and reinforcement
- Determine the effect of size reduction on sorption capacity
- Determine the feasibility of biomass processing

Conventional granulation technologies are rather advanced and their adaptation will likely yield desirable biosorbent granules (Guibal et al., 1992). Because of the wide variety of biomass types, extensive experimentation will undoubtedly be required.

The need to transport raw biomass may also present some logistical problems. Microbial biomass has a high water content and is prone to decay, so drying may be required if it cannot be processed and/or granulated directly on location in the wet state.

6.1.1. Biomass Types

The assessment of the metal-binding capacity of some types of biomass has gained momentum since 1985. Indeed, some biomass types are very effective in accumulating heavy metals (Crist et al., 1993).

Availability is a major factor to be taken into account to select biomass for clean-up purposes. The economics of environmental remediation dictate that the biomass must come from nature, or even be a waste material. Seaweeds, molds, yeasts, bacteria, and 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. When choosing the biomass for metal biosorption experiments, its origin is a major factor to be considered.

Biomass can come from:

- Industrial wastes which should be obtained free of charge
- Organisms that can be obtained easily in large amounts in nature (e.g., bacteria, yeast, algae)
- Fast-growing organisms that are specifically cultivated or propagated for biosorption purposes (crab shells, seaweeds)

6.1.2. Organisms for Biosorption

There is a wide variety of microorganisms (Table 1), including bacteria, fungi, yeast, and algae, which can interact with metals and radionuclides and transform them through several mechanisms. Cost-effectiveness is the main attraction of metal biosorption. This cost-effectiveness can be maintained by using the microbial biomass directly where possible. In addition, biosorbents derived from microbial biomass through a simple process are expected to be the lowest-priced and most-economical for metal removal.

Table 1. Examples of toxic heavy metals accumulating microorganisms

Metal	Biomass Type	Biomass Class	Metal Uptake ($mg\ g^{-1}$)	Reference
Cr^{6+}	*Bacillus* biomass	Bacterium	118	Brierley et al., 1986
	Bacillus biomass	Bacterium	60	Brierley et al., 1986
	Rhizopus arrhizus	Fungus	31	Tobin et al., 1984
	Candida tropicalis	Yeast	4.6	Mattuschka et al., 1993
	Streptomyces noursei	Filamentous bacteria	1.8	Mattuschka et al., 1993
	Penicillium sp.	Fungus	0.33	Paknikar et al., 1993
Ni^{2+}	*Fucus vesiculosus*	Brown algae	40	Holan and Volesky, 1994
	Ascophyllum nodosum	Brown marine algae	30	Holan and Volesky, 1994
	Sargassum natans	Brown algae	24-44	Holan and Volesky, 1994
	Candida tropicalis	Yeast	20	Mattuschka et al., 1993
	Rhizopus arrhizus	Fungus	18	Fourest and Roux, 1992
	Absidia orchidis	Fungus	5	Volesky and Kuyucak, 1988
Pb^{2+}	*Bacillus subtilis*	Bacterium	601	Brierley et al., 1986
	Fungal biomass	Fungus	373	Brierley et al., 1986
	Absidia orchidis	Fungus	351	Holan and Volesky, 1995
	Fucus vesiculosus	Brown algae	220-370	Holan and Volesky, 1994
	Ascophyllum nodosum	Brown marine algae	270-360	Holan and Volesky, 1994
	Sargassum nodosum	Brown algae	220-270	Holan and Volesky, 1994
	Bacillus subtilis	Bacterium	189	Brierley and Brierley, 1993
	Streptomyces longwoodensis	Filamentous bacteria	100	Friis and Myers-Keith, 1986
	Streptomyces naursei	Filamentous bacteria	55	Mattuschka et al., 1993
Zn^{2+}	*Bacillus subtilis*	Bacterium	137	Brierley et al., 1986
	Fungal biomass	Bacterium	98	Brierley et al., 1986
	Sargassa sp.	Brown algae	70	Davis et al., 2003
	Saccharomyces cerevisiae	Yeast	14-40	Volesky and May-Phillips, 1995
	Candida tropicalis	Yeast	30	Mattuschka et al., 1993
	Rhizopus arhizus	Fungus	20	Fourest and Roux, 1992
	Penicillum chrysogenum	Fungus	6.5	Niu et al., 1993
	Bacillus sp.	Bacterium	3.4	Cotoras et al., 1993
	Penicillium spinulosum	Fungus	0.2	Townsley et al., 1986

Some types of industrial fermentation waste biomass are excellent metal sorbers. It is necessary to realize that some "waste" biomass is actually a commodity, not a waste. This applies particularly to the ubiquitous brewer's yeasts sold on the open market, usually as animal fodder. Activated sludge from wastewater treatment plants has not demonstrated high enough metal-sorbing capacities. Some types of seaweed biomass offer excellent metal-sorbing properties, and sometimes a local economy can benefit from turning seaweeds into a resource.

As a fallback, biomass with a high metal-sorbing capacity can be specifically grown relatively cheaply in fermenters using low-cost or even waste carbohydrate-containing growth media such as molasses or cheese whey.

6.2. Biosorption Mechanisms

The effect of any of the influencing factors can be best quantitatively estimated when the mechanism of biosorption for different ions is known and when the mathematical model is used for predicting the effect of these factors.

6.2.1. Classification of Binding Mechanisms

Various metal-binding mechanisms have been postulated to be active in biosorption (Volesky, 1990), such as:

- Chemisorption by ion exchange, complexation, coordination and/or chelation
- Physical Adsorption
- Microprecipitation

Due to the complexity of the biomaterials used, it is possible that at least some of these mechanisms are acting simultaneously to varying degrees, depending on the biosorbent and the solution environment. A systematic presentation of the relationships between different mechanisms is compiled in Fig 1. The classification of bond type (Myers, 1991) was used.

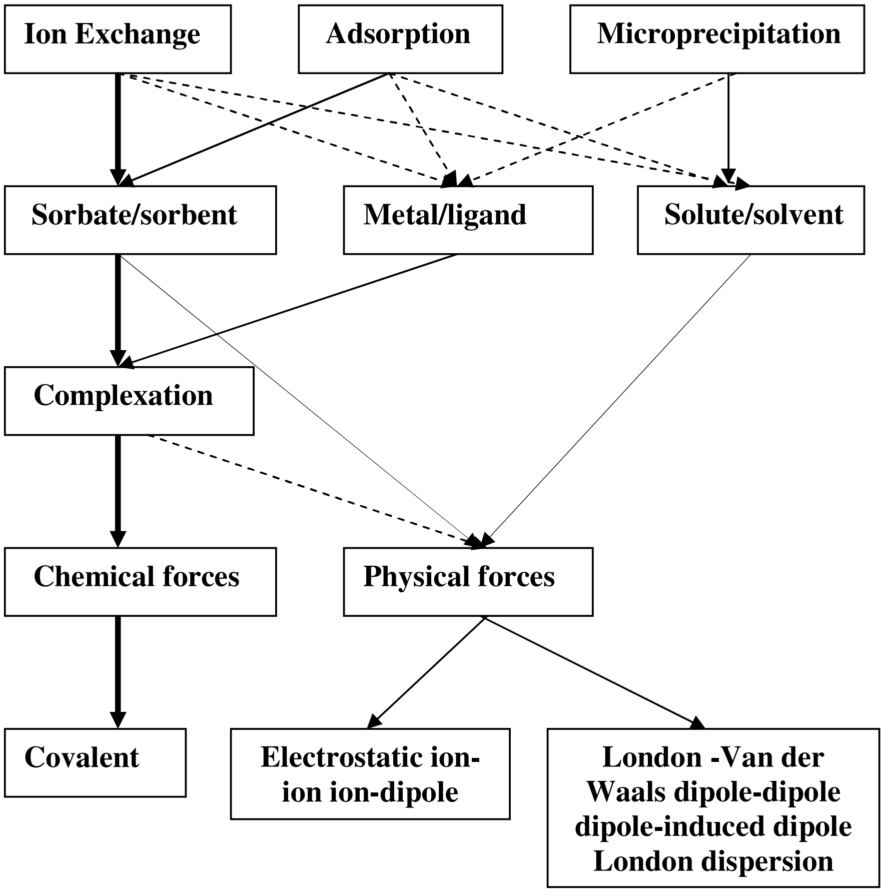


Fig 1: Metal biosorption mechanisms: bold lines, mechanism probably important in biosorption; dashed lines, biosorption binding relations of secondary importance.

❖ Ion Exchange

Ion exchange is a reversible chemical reaction wherein an ion in a solution is exchanged for a similarly charged ion attached to an immobile solid particle. These solid ion-exchange particles are either naturally occurring inorganic zeolites or synthetically produced organic resins. Synthetic organic resins are the predominant type used today because their characteristics can be tailored to specific applications. Several researchers have independently concluded that the major mechanism of heavy metal uptake by algae (Kratochvil et al., 1995), fungi (Fourest et al., 1994) and peat moss (Spinti et al., 1995) is ion exchange.

Ion exchange reactions are stoichiometric and reversible, and as such they are similar to other solution-phase reactions. For example, in the reaction

$NiSO_4 + Ca(OH)_2 \rightarrow Ni(OH)_2 + CaSO_4$

The nickel ions of the nickel sulfate ($NiSO_4$) are exchanged for the calcium ions of the calcium hydroxide $Ca(OH)_2$ molecule.

❖ Ion Exchange Versus Sorption

There has been some indication that ion exchange plays an important role in metal-ion sorption by algal biomass. Kuyucak and Volesky noted that the amount of ions (K^+, Na^{2+}, Mg^{2+}) released from the marine brown alga *A. nodosum* was much more pronounced in metal (Co) bearing than in metal free solutions. A linear correlation between Ca^{2+} released and Co^{2+} uptake was found when the biomass had been previously washed with CaCl and HCl. It was conclude that ion exchange was responsible for the sorption (Kuyucak and Volesky, 1989a). If ion exchange was responsible, an exchange ratio of 1:1 should be expected for two divalent ions such as Ca^{2+} and Co^{2+}. Since it can be expected that some sites would have been protonated due to the pretreatment with CaCl and HCl mixture and since some proton release was noticed (Kuyucak and Volesky, 1989b).

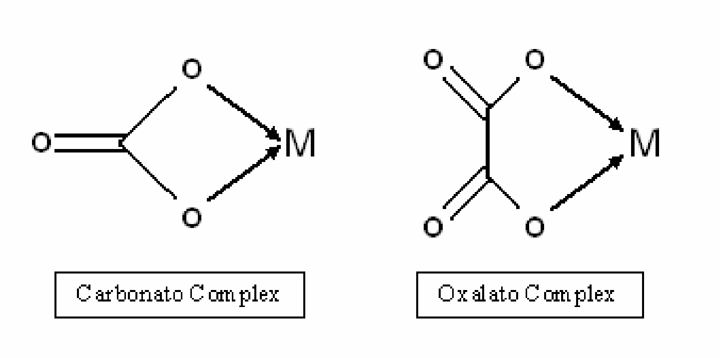
❖ Microprecipitation

Microprecipitation is the deposition of the electrically neutral material (Metal or Metal Salts) at the surface of the biomass, and does not necessarily involve a bond between the biomass and the deposited layer. Microprecipitation may, however, be facilitated by initial binding of metal ions to reactive sites of the biomass, which serve as nucleation sites for further precipitation (Mayers and Beveridge, 1989). Microprecipitation is based on interaction between the solute (dissolved solid) and solvent, and occurs when the local solubility is exceeded.

❖ Chelation

The word chelation is derived from the Greek word chele, which means claw, and is defined as the firm binding of a metal ion with an organic molecule (ligand) to form a ring structure. Some ligands are attached to a metal atom by more than one donar atom in such a manner as to form a heterocyclic ring. This type of ring has been given a specific name – chelating agent or chelator. The process of forming a chelate ring is known as chelation.

Thus, metal chelates are metal complexes where there is an organic compound bound to the metal by at least two available sites. In other words, the metal chelate is a special kind of metal complex because one can find non-chelate metal complex too. The most common metal complex occurring in aqueous solution are aquated metal ions or aquocomplexes. For most part, it is from complexes of this kind that metal chelates are formed by the replacement of water molecules. The resulting ring structure protects the mineral from entering into unwanted chemical reactions. Examples include the carbonate (CO_3^{2-}) and oxalate ($C_2O_4^{2-}$) ions:



❖ Coordination (Complex Formation)

It has been suggested that numerous chemical groups contribute to biosorption metal binding, by either whole organisms such as algae and bacteria or by molecules such as biopolymers. These include hydroxyl, carbonyl, carboxyl, sulfhydryl, thioether, sulfonate, amine, imine, amide, imidazole, phosphonate, and phosphodiester groups. The importance of any given group for biosorption of a certain metal by a certain biomass depends on such factors as the number of sites in the biosorbent material, the accessibility of the sites, the chemical state of the sites (availability), and the affinity between the site and the metal (binding strength). For covalent metal binding, even an occupied site is theoretically available; the extent to which the site can be used by a given metal depends on its binding strength and concentration compared to the metal already occupying the site.

A coordination complex is any combination of cations with molecules or anions containing free pairs of electrons. Bonding may be electrostatic, covalent or a combination of both; the metal ion is coordinately bonded to organic molecules. Examples of the formation of a coordination compound are:

$Cu^{2+} + 4H_2O \quad = \quad [Cu(H_2O)]4^{2+}$

$Cu^{2+} + 4Cl^{-} \quad = \quad [CuCl_4]^{2-}$

Where coordinate covalent bonds are formed by donation of a pair of electrons from H_2O and Cl^{-} (Lewis bases) to Cu^{2+} (Lewis acid).

In general, biosorption of toxic metals and radionuclides is based on non-enzymatic processes such as adsorption. Adsorption is due to the non-specific binding of ionic species to polysaccharides and proteins on the cell surface or outside the cell (Volesky, 1992; Mullen et al., 1992). Bacterial cell walls and envelopes, and the walls of fungi, yeasts and algae, are efficient metal biosorbents that bind charged groups. The cell walls of gram-positive bacteria bind larger quantities of toxic metals and radionuclides than the envelopes of gram-negative bacteria.

In, a Gram-negative bacterium, *Pseudomonas fluorescens* for example, the lipoplysaccharide of the outer membrane appears to be associated with platinum while gallium and uranium are bound over the whole cell envelope (Krueger et al., 1993). The homogeneous distribution of adsorbed metals in cytoplasm has also been reported for metals like copper and lead in *Pseudomonas stutzeri* (Mattuschka et al., 1994). Some metals such as lanthanide are precipitated principally in *Escherichia coli* periplasmic space (Bayer and Bayer, 1991). Periplasmic metal accumulation was reported for *Citrobacter* sp., which produced metal phosphate by a cell bound phosphatase (Jeong et al., 1997).

Heavy metal adsorption, however, appears to be stronger in Gram-positive cell walls. The metal binding sites in the cell wall of have been described *Bacillus subtilis* (Beveridge and Murray, 1976). Anionic groups such as carboxylate and phosphate groups of peptidoglycan and teichoic acids are considered the major metal binding sites (Beveridge and Murray, 1980). The heavy metal adsorption by streptomycetes, also belong to Gram-positive bacteria, has been presumed to contribute a large heavy metal binding capacity (Chergui et al., 2007).

Biomass deriving from several industrial fermentations may provide an economical source of biosorptive materials. Many species have cell walls with high concentrations of chitin, a polymer of N-acetyl-glucosamine that is an effective biosorbent.

Biosorption uses biomass raw materials that are either abundant (e.g., seaweeds) or wastes from other industrial operations (e.g., fermentation wastes) (Horikoshi et al., 1979). The metal-sorbing performance of certain types of biomass can be more or less selective for heavy metals, depending on the type of biomass, the mixture in the solution, the type of biomass preparation, and the chemical-physical environment.

It is important to note that the concentration of a specific metal in solution can be reduced either during the sorption uptake by manipulating the properties of the biosorbent or upon desorption during the regeneration cycle of the biosorbent.

6.2.1.1. Binding Forces

In general, the forces between atoms or molecules can be classified into chemical and physical ones. Chemical forces extend over very short distances (0.1 to 0.2 nm) (Myers, 1991). This type of bond is rather strong: Stumm and Morgan report an energy > 40-400 kJ mol^{-1} for chemi-sorption.

Covalent bonds are formed by merging electron clouds such that a nonionic molecule is formed. These bonds are directional (characteristics bond angle and length) and localized (Myers, 1991).

Physical forces can be subdivided into electrostatic and London van der waal forces (Myers, 1991). The energy of physico-sorption is reported as 2-20 kJ mol^{-1} and 20-40 kJ mol^{-1} (Smith et al., 1997 and Pagenkopf, 1983) respectively.

6.2.1.2. Chemical (Covalent) Versus Physical (Electrostatic) Binding

The proportion of protons among the released species varied with the metal taken up (Haug and Smidsrod, 1970) and corresponded to the binding strength: More protons were released by the stronger binding ions. It could be concluded that in those cases where no protons were involved, as for most alkaline and alkaline earth metals, electrostatic attraction was the binding mechanism (Crist et al., 1988), whereas covalent binding occurred when protons were released.

6.2.1.3. Binding Sites

Biosorption has been attributed to different types of groups such as carboxylate, carbonyl, hydroxyl, amine, amide, imidazole, phosphate, thio and thioether groups.

Which binding mechanism in any specific case depends largely on the type of biosorbent used and on the type of binding sites it contained.

6.2.1.4. Surface Charges

The relevance of electrostatic attraction in biosorption depends on the types and amount of sites present in the biomass and on whether they are ionized or occupied by proton or other ions. That, in turn, depends on the pH and pK_a value of inorganic acids and their corosponding acidic groups in biomolecules exists, as evident from Table 2.

Table 2. pK_a values of inorganic acids and acidic groups in biomolecules

Inorganic Acid	pK_a	Acidic group in Biomolecule	pK_a
Sulfuric acid (Bower and Bates, 1963)	1.9	Sulfate (Crist et al., 1992)	~1.5
Phosphoric acid (Bower and Bates, 1963)	2.1	Phosphate (Bower and Bates, 1963)	2
Benzoic acid (Bower and Bates, 1963)	4.2	Carboxyl (Buffle, 1988)	5-Mar
Acetic acid (Bower and Bates, 1963)	4.8	Carboxyl (Buffle, 1988)	5-Mar
Ammonium (Bower and Bates, 1963)	9.2	Amine (Buffle, 1988)	10-Aug
Phenol (Bower and Bates, 1963)	10	Phenolic OH (Buffle, 1988)	10

6.2.1.5. Factors Influencing the Binding Strength

There are three factors that can be determining the affinity of a metal ion.

a) Hydration effects.

A change in the orientation of the hydrated water molecules occurs in conjugation with electrostatic binding (Buffle, 1988) but in such "outer-sphere" complexes both partners retain their hydration spheres. Complete or partial dehydration takes place for "inner-sphere" complexes resulting from covalent binding (Buffle, 1988).

b) Ionic (Electrostatic) binding

If the binding groups are negatively charged, they can attract metal cations. The interaction is stronger the higher the charge density of both biosorbent and metal ion (Jain and Wagner, 1980).

c) Covalent binding

This type of bond involves sharing of the electrons. Therefore, the more similar the electro negativity of the metal ions and the coordinating atom of the ligand, the higher the covalent character of the bond (Dean, 1985).

The overall binding strength increases with increasing ionic radius and decreasing charge if binding is weak and largely due to hydration effects, decreasing hydrated radius and increasing charge if binding is intermediately strong and due to electrostatic effect and decreasing electro negativity difference if binding is strong and covalent.

6.2.2. Equilibrium Modeling

Biosorption has been studied as simplified sorption systems, usually containing one heavy metal. This is an appropriate simplification for effective experimentation. In order to evaluate feasibility and effectiveness of biosorption in wastewater treatment, it is essential to make predictions of the sorption performance (e.g., for facilitating process design). Therefore it is necessary to develop appropriate mathematical models of biosorption. Modeling the biosorption-binding equilibrium is a prerequisite for all further work involving batch kinetic studies and in particular for the continuous flow sorption-column application that represent the most effective configuration of the sorption-based process.

The amount of metal M (sorbate) bound per mass of sorbent is called the binding (uptake), q_e. The binding is not only dependent on the sorbent material but also on the equilibrium concentration [C_e] of the sorbate in the solution and on other parameter, such as, pH and equilibrium concentration of other ions in the solution. The relationship between equilibrium binding and the concentration of ions (at constant temperature) is depicted in an isotherm plot of q_e versus [C_e]. With increasing metal concentration in solution its binding increases from zero to the maximum. It is desirable for the sorbent to possess a high sorption capacity and high affinity for the sorbate species, which is reflected in a steep slope of the isotherm curve at low equilibrium concentrations.

Table 3 and 4 summarizes some of the simple sorption isotherm models that are most frequently applied. A particular model may not apply to a particular situation, and in some cases more than one model may explain the biosorption mechanism. There is no critical reason to use a more-complex model if a two-parameter model (such as the Langmuir and Freundlich isotherm models) can fit the data reasonably well.

Table 3. Frequently used single-component adsorption model (Kuyucak and Volesky, 1989a)

Isotherm	Equation	Advantages	Disadvantages
Langmuir	$q_e = \frac{q_{\max} b C_e}{1 + bC_e}$	Interpretable parameters	Not Structured; Monolayer sorption
Freundlich	$q_e = K_f C_e^{1/n}$	Simple expression	Not Structured
Combination of Langmuir and Freundlich	$q = \frac{b q_e C_e^{1/n}}{1 + b C_e^{1/n}}$	Combination of above two	Unnecessarily complicated
Radke and Prausnitz	$\frac{1}{q} = \frac{1}{aC_e} + \frac{1}{bC_e^{\beta}}$	Simple expression	Empirical; Required three parameter
Radlich Peterson	$q = \frac{aC_e^{n}}{1 + bC_e^{n}}$	Approaches at higher concentration	No significant advantages

As a matter of practicality, multi-metal biosorption models such as those in Table 4 must be used judiciously.

Table 4. Frequently used multi-component adsorption models (Kuyucak and Volesky, 1989a)

Isotherm	Equation	Advantages	Disadvantages
Langmuir (Multi-component)	$q_i = \frac{b_i q_m C_m}{\sum_{n=1}^{n} b_i C_i^{1/n}}$	Constant have physical meaning; Isotherm level off at maximum saturation	Not structured; doesn't reflect the mechanism well
Combination (Langmuir and Freundlich)	$qi = \frac{a_i C^{1/n}}{\sum_{n=1}^{n} b_i C^{1/n}}$	Combination of Langmuir and Freundlich	Unnecessarily complicated

where:

C_e	Equilibrium solute concentration in the fluid
K, n	Freundlich isotherm constants
q_{max}, b	Langmuir isotherm parameters
q_m	Langmuir maximum metal uptake in mg g^{-1}
q	Metal uptake in mg g^{-1}

The sorption uptake (q_e) can be expressed in different units depending on the purpose of the exercise:

- For practical and engineering process evaluation purposes eventually concerned with process mass balances, it is customary to use weight per (dry) weight (e.g., mg of metal sorbed per gram of the (dry) sorbent material).

- Ultimately, mainly because of reactor volume considerations (e.g., a packed-bed column), the uptake may also be expressed on a per volume basis (e.g., mg L^{-1}). However, the porosity may complicate the quantitative comparison of biosorption performance.

- Only when working on the stoichiometry of the process and when studying the functional groups and metal-binding mechanisms might it be useful to express q on a molar or charge equivalent basis - again, per unit weight or volume of the sorbent (e.g., mmol g^{-1} or mequiv g^{-1}).

It is relatively easy to convert among these units; the only problem may arise with the sorbent weight-volume conversions. For scientific interpretations, the sorbent material dry-weight basis is thus preferred.

The use of "wet biomass weight" should be discouraged, unless the wet-weight-to-dry-weight conversion is well specified. Different biomass types are likely to retain different moisture contents, intracellular as well as that trapped in the interstitial space between the cells or tissue particles (e.g., seaweed particles). Different types of biomass obviously compact in a different ways. When centrifuging biomass, the g-force and time need to be specified, and even then it is difficult to make any comparisons. All this makes the "wet biomass weight" citation very approximate at best and generally undesirable.

6.2.3. Experimental Sorption Isotherms

It is relatively easy to obtain equilibrium sorption data for a single sorbate in the laboratory. A small amount of the sorbent is brought into contact with a solution containing the sorbate of interest. The conditions of the sorption system, particularly pH, must be carefully controlled at the required values over the entire period of contact until the sorption equilibrium is reached. This may take a few hours or much longer, depending on the size of the sorbent particles and the time it takes until they attain sorption equilibrium.

A simple preliminary sorption kinetics test will establish the exposure time necessary for the given sorbent particles to reach the equilibrium state. The following procedure (Mahesh, 2008) provides an example for obtaining the experimental sorption equilibrium data points for the isotherm:

- Prepare the sorbate in solution at the highest concentration of interest.
- Prepare dilutions covering the entire concentration range, from 0 (blank) to the maximum.
- Adjust the conditions, e.g., pH, ionic strength, etc.

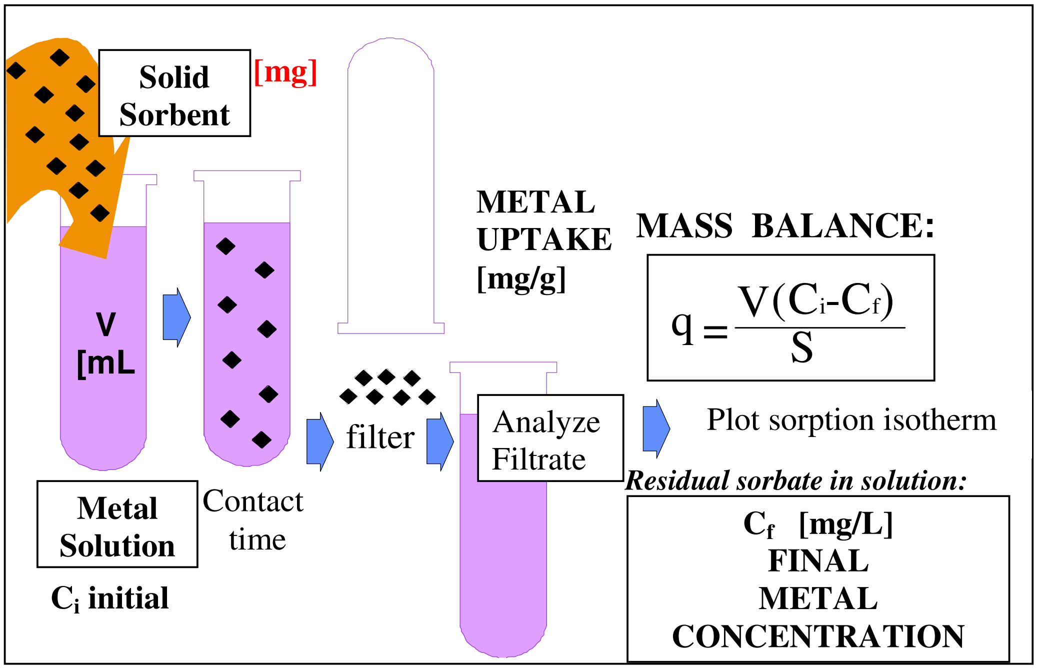


Fig 2: Experimental methodology for deriving the biosorption isotherm

- Determine the sorbate initial concentrations (C_i) in all the liquid samples.

- Distribute the samples into containers of appropriate volumes (30–150 mL of liquid) such as flasks or test tubes; prepare samples in duplicate, triplicate or as required.

- Accurately weigh each quantity of the biosorbent solids to be used in the tests and record the weights (S, mg). It may help to be able to roughly estimate the anticipated sorption uptake so that there is an easily detectable final sorbate concentration in each sample solution at equilibrium. If too much sorbent is added, there may be virtually no sorbate left in the solution, precluding a reliable analysis. Varying the initial concentration could cause the sorbent weight to fluctuate, which has to be precisely known for each sample. Metal depletion in the solution must be avoided because it renders such samples useless.

- Add the sorbent solids into each sample solution and provide rather gentle mixing over the contact period.

- Make sure the conditions (especially pH) are controlled at constant values during the contact period. Use an appropriate acid or base for this; do not dilute the sorption system by adding excessive volume.

- At the end of the contact period, separate the solids from the liquid by decantation, filtration, centrifugation, etc.

- Analyze the liquid portion to determine the residual final sorbate concentration (C_f).

- Calculate the sorbate uptake: $q = \frac{V(C_i - C_f)}{S}$. Note that q could also be determined directly by analyzing the separated solids and thus closing the material balance on the sorbate in the system. However, this usually presents analytical difficulties (digestion-liquefaction of solids, and/or very sophisticated analytical methods may be required).

- Plot the sorption isotherm q vs. (C_f) as shown in Fig. 3.

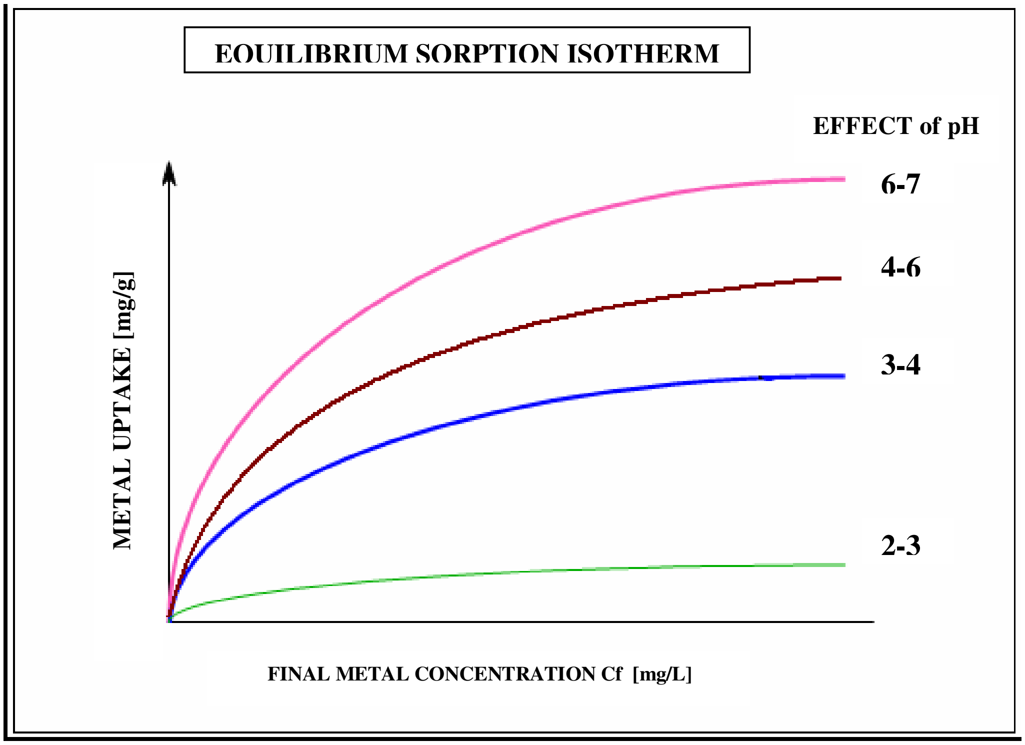


Fig 3: pH is an external factor and it has to be controlled for standard isotherm experiments – the final, equilibrium pH is the one that matters!

6.3. Factors Influencing Heavy Metal Sorption

- **Temperature Effect**

Simple physical sorption processes are generally exothermic (i.e. equilibrium constant decreases with increasing temperature). Biosorption, ion exchange among alkaline earth metals in binding to alginate was observed to be exothermic. However, when the Cu was the replacement, the reaction exhibited a positive enthalpy change. (i.e. it was endothermic-the equilibrium constant increases with temperature). The driving force in this case was attributed to a large positive entropy change, possibly caused by larger ordering effect of Cu (than Ca) on the water molecules I the hydration sphere.

Overall effect of temperature in biosorption is relatively small and perhaps slightly more pronounced in the temperature range.

- **Presence of Anions (Ligands)**

Theoretically, the presence of ligands (at level that do not cause precipitation) can lead to the following:

a. Formation of complexes that have a higher affinity to be sorbent than the free metal ions (i.e., an enhancement of sorption)
b. Formation of complexes that have lower affinity to sorbent than the free metal ions (i.e., a reduction of sorption)
c. Interaction of anions with the biomass, changing the state of the active sites such that the binding is either enhanced or reduced.

There is no indication of the third kind of interaction reported in the biosorption literature. In most cases of biosorption, however, metal bonding tends to be reduced in the presence of ligands. This means that the biomass apparently has less affinity for many metal ligand complexes than for free hydrated metal ions. This effect is, however, not very pronounced unless these anions show a strong complexing power for the metal ion of interest (e.g., EDTA, which at only 1.5 times excess caused 100% inhibition for metal binding.

Since significant concentration of strong complexing agent is rare in typical metal-bearing industrial effluents, the effect of the presence of ligands is of secondary importance.

❖ Influence of pH

It is commonly agreed that the sorption of metal cations (e.g. Cd, Cu, Zn, Pb, Ni, Mn, Al, Co) increases with increases pH (Volesky and Holan, 1995) as the metal ionic species become less stable in the solution. Only those metal ions that can occur as negatively charged complexes such as Ag, Hg or Au may show either a decrease in binding with increasing pH or may have no significant pH effect at all.

There are three ways that the solution pH can influence metal biosorption, as described in the following:

First, the state of active sites may be changed. When the binding groups are acidic, the availability of free sites depends on pH: at lower pH the active sites are protonated, therefore, competition between protons and metal ions for the sorption sites occurs (Tobin et al., 1984). At a low enough pH, virtually, all sites become protonated and complete desorption of the bound metal ions is possible, which is why acid treatment is a method for metal elution and regeneration of sorbent material. Decreasing the pH value by 2 units can in some cases result in ~90% reduction of metal binding (Ferguson and Bubela, 1974).

Second, extreme pH values, as they are employed in the regeneration (desorption) of the sorbent, may damage the structure of the biosorbent material. Microscopic observation have shown distorted cells, significant weight loss and decrease in the sorption capacity have been observed (Kuyucak and Volesky, 1989b).

Third, the speciation of the metal in the solution is pH dependent. Whereas metals in aqueous solutions occurs as hydrated cations in salvation shell when the pH is low, hydroxide may form at higher pH, especially for cations of high charge and small size. The formation of metal oxide and hydroxide complexes and precipitate is often called hydrolysis (i.e., decomposition or conversion by water) (Baes and Mesmer, 1976).

Adsorption depends not only on the attraction of the sorbate to the solid surface but also on its lyophobic behavior (Pagenkopf, 1978). This means that sorption increases with decreasing solubility. Since the solubility of many metal complexes in solution decreases with increases pH, this provides an additional possible explanation as why sorption increases with increasing pH. In the narrow range of pH, where the metal ions are hydrolyzed, sorption is especially enhanced. Further possible explanation of increasing sorption with increasing pH are that hydrolyzed species have lesser degree of hydration, that is, less energy is necessary for removal or reorientation of the hydrated water molecules upon binding (Stumm and morgan, 1970).

With further increase pH, the solubility of metal complexes decreases enough for precipitation to occur. Although precipitation may contribute to overall removal of metal from solution (and therefore desirable for metal-removal application), it renders the study of the biosorption binding more difficult. For scientific purposes it is therefore recommended to study biosorption at pH value where precipitation not occurs.

6.4. Possible Applications of Metal Biosorption

The term *biosorption* commonly refers to the passive binding of metal ions by biomass, which may even be dead. It must be distinguished from bioaccumulation, which is usually understood to be an active, metabolically mediated process occurring in living organisms.

It has been known for decades that different type of microbial biomass bind trace metal ions, achieving very high concentration factors. The biomass metal uptake phenomena has been made use of in, for example, electron microscopy, to visualize cellular components. The focus on early studies of microbial uptake has been almost exclusively on the nutritional and toxicological aspects of metal presence.

Biosorption removal of heavy metals is especially suited as a “polishing” water-treatment step because it is possible to reach drinking water quality of the treated water (initial metal concentration, e.g., 1 to 100 mg L^{-1}, final concentration < 0.01 to 0.1 mg L^{-1}), especially in the packed bed flow-through sorption column applications. To prevent unnecessarily rapid exhaustion of the sorption capacity when the metal concentration of the wastewater to be treated are high (>100 mg L^{-1}), it may be desirable to use a different pretreatment techniques, such as precipitation or electro-recovery, for removal of the bulk of the metal content. However, generation of toxic sludges during pretreatment must be considered, since these represent another type of hazard and the eventual recovery of metal from them may not be feasible.

The metal-laden biosorbent can be regenerated, incinerated, or stored in landfills. The biosorption process basically serves to reduce the waste volume. Alternatively and preferably, regeneration of the biosorbent material or multiple reuses is desirable to increase the process economy. It can be accomplished by metal desorption with, for example, acids or salt solutions. The resulting highly concentrated metal solution can be proceed by other technique such as precipitation or electro winning to remove or concentrate the metal, which could be recovered and resold. The latter process, in particular, is aimed at recuperation of the metal. The overall achievements of the biosorption (complete adsorption + desorption cycle) process is to concentrate the metal solution, possible at least a factor of 100 or more. The flow chart of the biosorption process with in-situ regeneration of the biosorbent is quite similar to the use of ion-exchange resins.

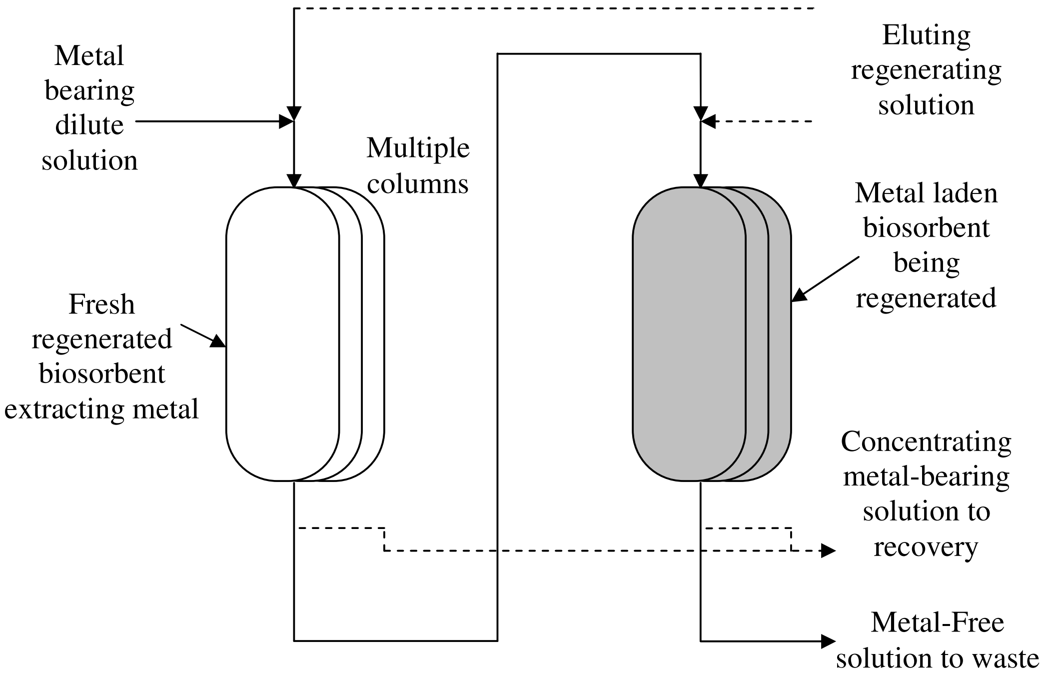


Fig 4 : Schematic flowchart of continuous flow column biosorption process

Compared to conventional wastewater "polishing" techniques such as ion-exchange, activated carbon treatment, or membrane technologies (electrodialysis, reverse osmosis), the advantage of biosorption is not only that it can be operated under broad range of conditions (pH, temperature), but especially that it may be economically attractive due to the cheap raw material that can be used as biosorbent (Kuyucak and Volesky, 1990).

6.5. Comparison of Sorption Performance

The performance of sorbing materials needs to be evaluated and often compared. The simplest situation is when there is only one sorbate species in the system, in which case it is best to base the single-sorbate sorption performance on a complete single-sorbate sorption isotherm curve.

To fairly compare two or more sorbents, the comparison must be done under uniform conditions. These may be restricted by the environmental factors under which sorption may have to take place (pH, temperature, ionic strength, etc.), which may not necessarily be easily or widely adjustable. In particular, it is important to compare sorption performance under the same pH conditions, since isotherms can vary with pH.

The performance of the sorbent is usually gauged by its uptake (q). Sorbents can be compared based on their respective maximum uptake values (q_{max}), which can be calculated by fitting the Langmuir isotherm model to the actual experimental data (if it fits). This approach is feasible if q_{max} reaches a plateau. Some isotherms might not exhibit the asymptotic plateau represented by the Langmuir equation.

In general, one is looking for a “good” sorbent with a high sorption uptake capacity (q_{max}). Surface area in biosorption is not particularly important.

6.6. Types of Biosorption

Biosorption can be carried out as a batch process, a continuous process, or a two-stage process with continuous metal recovery.

Biomass should be defrosted and washed with deionized water. To ensure equal quality of the biomass during all experiments, different kinds of biomass should be mixed together to obtain a uniform mixture.

6.6.1. Batch Process

Batch biosorption experiments can be done in a stirred vessel with a working volume of approximately 100 mL. For example a minimal amount of concentrated solution of

$Pb(NO_3)_2$ can be added into a suspension of fungal pellets in water of various concentrations (25, 50, 100, 150 and 200 g of wet biomass per L of biomass suspension) to produce the desired initial metal concentrations of 10, 20, 50, 100 and 300 mg L^{-1} of Pb^{2+}. The decreasing metal concentration can be recorded as a function of the initial metal concentration (C_i) and the biomass loading.

6.6.2. Continuous Process

Continuous process experiments can be carried out in a glass column having an inner diameter of 5–8 cm and filled with a packed bed of biomass pellets of varying heights (20, 40 and 55 cm), set with an adjustable plug. The effluent solution of metal ions can be fed from the top of the column with the help of a pump using varying flow rates. An inert bed of glass spheres can be placed at the bottom of the column below the active biomass bed to ensure homogenous distribution of the feed. The remaining metal concentration can be measured online in the effluent at the top of the column. The breakthrough curves can be recorded as a function of the flowrate and bed height.

Measurements of metal ion concentrations in the solution can be made online with metal-detecting electrodes or ion-selective electrodes, and may be verified with an atomic absorption spectrometer.

7. RECOVERY OR DESORPTION

Regeneration of loaded biosorbent is critical to keeping costs down and to recovering the metal(s) extracted from the liquid phase. The deposited metals are washed out (desorbed) and the biosorbent is regenerated for another cycle. The desorption process should result in:

- High-concentration metal effluent
- Undiminished metal uptake upon re-use
- No physico-chemical damage to the biosorbent.

The desorption and sorbent regeneration studies might require somewhat different methodologies, beginning with screening for the most effective regenerating solution.

Because different metal ions have different affinities for the biosorbent, the uptake has some degree of metal selectivity. The selectivity of the elution-desorption operation may be different, which may serve as another means of eventually separating metals from one another if desirable.

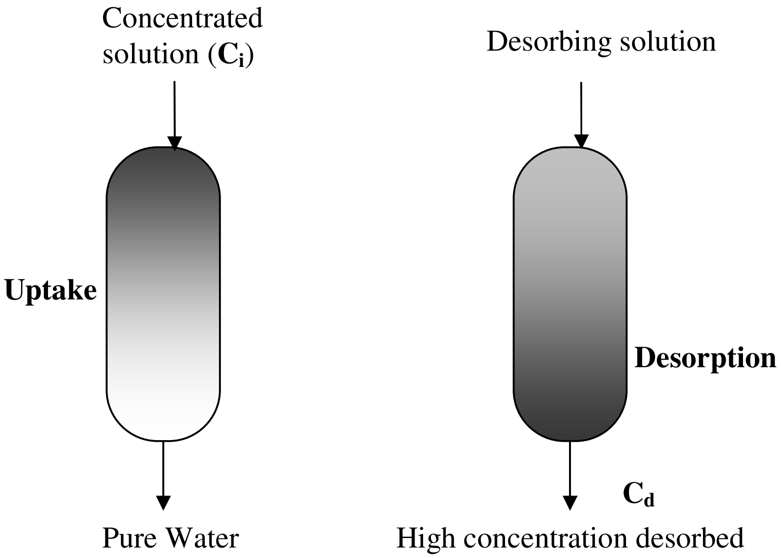


Fig 5: Concentration of the sorbate in the effluent eluate is important for its further recovery (or disposal)

The concentration ratio (CR) is used to evaluate the overall concentration effectiveness of the whole sorption-desorption process:

$$CR = \frac{Elute \quad metal \quad concentration(C_d)}{Feed \quad metal \quad concentration(C_i)}$$

Obviously, the higher the CR, the better the overall performance of the sorption process, is making the eventual recovery of the metal more feasible with higher eluate concentrations.

Considerations concerning desorption of metal laden biosorbents ‘solids’ leads to concern over another factor:

$$\frac{Solids}{Liqid}(S/L)ratio$$

S representing the amount of biosorbent (loaded with metal as high as possible, high q), while L is the amount of desorbing (eluting) liquid. Ideally, just a small amount of desorbing liquid should dislodge all the deposited metal: the S/L ratio should be as high as possible.

Recovery of the metal from these concentrated desorption solutions is carried out in a different plant by electrowinning. Following desorption of the metal(s), the column may be further pre-treated (e.g., pre-saturated with protons such as Ca, K, etc.) for optimum operation in the next metal uptake cycle. The specific type of pre-treatment used to optimize the column performance may vary.

An example of one method for prioritizing the recovery of ten metals is presented in Table 5. This may be simplistic, but it provides a useful direction by ranking metals into three general priority categories:

(1) Environmental Risk (ER)

(2) Reserve Depletion Rate (RDR)

(3) Combination of ER and RDR.

Environmental risk assessment could be based on a number of different factors, which could also be weighted. Environmental risk is assessed here only in terms of relative toxicity. Other components of environmental risk which might be of equal or even greater importance are relative mass loading of each metal into environment, principlal route of loading, environmetal mobility, species transformation of bioaccumulation, and so forth. In essence, risk assessment addresses such factors (Volesky, 1999).

Table 5: Ranking of Risks Associated with Various Metals

Relative Priority	Environmental Risk	Reserve Depletion	Combined Factors
High	Cd	Cd	Cd
	Pb	Pb	Pb
	Hg	Hg	Hg
	—	Zn	Zn
Medium	Cr	—	—
	Co	Co	Co
	Cu	Cu	Cu
	Ni	Ni	Ni
	Zn	—	—
Low	Al	—	Al
	—	Cr	Cr
	Fe	Fe	Fe

The second category is the reverse depletion rate which is used as an indication of probable future increase in the market price of the metal. The combination of the environmental risk plus the reverse depletion rate indicates that the four metals (Cd, Pb, Hg and Zn) might have the highest priority for research and development in metal recovery, by this prioritization scheme. Other metals would be ranked of medium and low priority. More sophisticated risk assessment, and better prediction of the future market value, might well change the relative priority of ranking among these example metals (Volesky, 1999).

7.1. Desorption/Ion Exchange of Metal from Biosorbent.

Due to the weak nature of the metal-binding forces, it is possible to wash the bound metal from the sorbent in a small volume of solution offering another cation (e.g., H^+, Ca^{2+}) in higher concentration. Desorption can be achieved by acids (e.g., HCl, H_2SO_4) and/or salt solution (e.g., $CaCl_2$). The effectiveness of desorption depends in this case on the binding strength of the added cation to the biosorbent (Singh and Tiwari, 1997).

7.2. Two-Stage Process with Continuous Metal Recovery

Two-stage continuous biosorption and metal recovery can be carried out. This process is similar to continuous biosorption, although the metal solution is adsorbed in two stages. After initial adsorption and filtration in stage one, the effluent is fed with fresh biosorbent into stage two, where further biosorption of the metal ion takes place. The effluent from the second stage is filtered to recover the metal ions and biosorbents. The effluent sample can be analyzed using adsorption spectroscopy (Matis et al., 2003).

8. FEASIBILITY OF BIOSORPTION

For successful application on a large scale, any operation needs to be economically viable. The feasibility of a biosorption process depends on such factors as:

- Biosorbent uptake performance
- The source of the raw biomass
- Biomass granulation and treatment
- The desorption and regeneration processes used

Often, the source of the biosorbent has a major impact on the feasibility of the operation. Biosorbents (biomass) should always be obtained from the least-expensive source, such as from the effluent of a fermenter, seaweeds from nearby bodies of water, algae, etc. The spent biosorbents can be regenerated at very low cost using water, so the material can be reused many times. Hence, considering the overall unit operations involved in biosorption, we can conclude that the process is generally economically viable (Park et al., 2006).

9. ADVANTAGES OF BIOSORPTION

Biosorption is highly competitive with the presently available technologies like ion exchange, electrodialysis, reverse osmosis, etc. Some of the key features of biosorption compared to conventional processes include (Sasek et al., 2003):

- Performance
- Heavy metal selectivity
- Cost-effectiveness
- Regenerative
- No sludge generation
- No additional nutrient requirement

Biosorption is particularly economical and competitive for environmental applications in detoxifying effluents from, for example:

- Metal plating and metal finishing operations
- Mining and ore processing operations
- Metal processing
- Battery and accumulator manufacturing operations
- Thermal power generation (coal-fired plants in particular)
- Nuclear power generation

Chapter III

Material and Methods

1. Procurement of Waste Biomass

Waste biomass, MB2 comprising of *Streptomyces* sp., generated as a by product of pharmaceutical fermentation industry involving fermentative processes, was collected from Ranbaxy, Paonta Sahib, Himachal Pradesh, India.

1.1. Preparation of Adsorbent

Dry biomass was pulverized in a blender and passed through a 2 mm sieve in order to obtain uniform particle size and washed 2-3 times in tap water at room temperature and was oven dried overnight at 80^oC for two days. Dried biomass was used for further sorption experiments.

2. Preparation of Heavy Metal Solution

Aqueous stock solution (1000 mg L^{-1}) of Cr^{6+}, Zn^{2+}, Pb^{2+} and Ni^{2+} was prepared using salts of $K_2Cr_2O_7$, $ZnSO_4$, $NiSO_4.6H_2O$ and $Pb(C_2H_3O_2)_2$ respectively. The concentration ranges varied between 5 to 50 mg L^{-1} for single metal aqueous solution. For multimetal solution (250 ml) of 5, 10, 15, 25 and 50 mg L^{-1}, 1.25, 2.5, 3.75, 6.25 and 12.5 ml of the stock solution of each Cr^{6+}, Zn^{2+}, Pb^{2+} and Ni^{2+} was added. Solution pH was adjusted with dilute 0.1 N HCl or 0.1 N NaOH.

3. Optimization of Parameters

Initial batch sorption experiments were carried out for optimization of pH (2-6), adsorbent concentration (0.2-4 g 100 mL^{-1}) and metal concentration (5-50 mg L^{-1}). All further experiments were performed with the optimized data.

4. Batch Sorption Studies

4.1. Single Batch Sorption Experiments

For all single batch sorption experiments were performed by suspending 1 g of biomass in 100 mL of metal solution and shaking on orbitek open shaker at 120 rpm for 24 hr. After constant time intervals (0.83-24 hr) the samples were filtered (Whatman filter paper No. 42) and the filtrate was analyzed by atomic absorption spectrophotometer (GBC AAS) for the residual metal content.

4.2. Multimetal Batch Sorption Experiments

Multimetal batch sorption experiments were performed in 200 mL of multimetal solution by suspending 2 g of biomass and shaking on orbitek open shaker at 120 rpm for 24 hr. After constant time intervals (0.83-24 hr) the samples were filtered (Whatman filter paper No. 42) and the filtrate was analyzed for metals as in single metal sorption experiments.

5. Determination of Total Residual Metal

Residual concentration R (%) was calculated (Zhang et al., 1998) as:

$$R(\%) = \left(\frac{(C_i - C_f)}{C_i}\right)100 \qquad (1)$$

where C_i and C_f are the initial and final metal concentrations in mg L^{-1}

6. Metal Uptake by Biomass

Specific metal uptake was calculated as follows:

$$q_e = \frac{(C_i - C_f)V}{m} \qquad (2)$$

Where C_i and C_f are the initial and final metal concentrations in mg L^{-1} respectively, initially and at a given time t, respectively, V is the volume of the metal solution in ml, m is weight of the biomass in g. V is the volume of the solution (ml) and W is the weight of biosorbent (g) taken.

7. Adsorption Isotherm

Adsorption isotherm, which are the presentation of the amount of solute adsorbed per unit of adsorbent. Two commonly used adsorptive isothermal models, Langmuir and Freundlich equations, were used to evaluate the experimental data and described as:

7.1. Langmuir Isotherms

This isotherm represents one of the first theoretical treatments of nonlinear sorption and suggested that uptake occurs on a homogeneous surface by monolayer sorption with interaction between adsorption molecules.

$$q_e = \frac{q_{\max} b C_e}{1 + b C_e} \qquad (3)$$

Where

q_{max} (mg g^{-1}) and b is Langmuir constants related to adsorption capacity and the energy of adsorption respectively.

Eq (3) is usually linerized to obtain the following form (Kinniburgh, 1986; Longhinotti et al., 1998)

$$\frac{1}{q_e} = \frac{1}{C_e}\left(\frac{1}{q_{\max} b}\right) + \left(\frac{1}{q_{\max}}\right)$$

q_{max} and b can be determined from the lineal plot of $1/q_e$ versus $1/C_e$.

7.2. Freundlich Model

This isotherm also considers monolayer sorption with a heterogenous distribution of active sites of the sorbent (Freundlich, 1907).

$$q_e = K_f C_e^{1/n} \qquad (4)$$

Where K_f stands for adsorption capacity and n for adsorption intensity.

Logarithmic from of Eq (4)

$$\log_e q_e = \log_e \left(K_f C_e^{1/n}\right)$$

$$= \log_e K_f + \log_e C_e^{1/n}$$

$$\log_e q_e = \log_e K_f + \frac{1}{n} \log_e C_e$$

Where K_f and 1/n can be determined from the linear plot of $\log_e(q_e)$ Vs $\log_e(C_e)$. Experimental values obtained for the adsorption capacity experiments were used to calculate the parameters.

Chapter IV

Result and Discussion

1. Single-Metal System

1.1 Effect of pH

Biosorption capability of waste biomass (MB2) for Cr^{6+}, Zn^{2+}, Pb^{2+} and Ni^{2+} (25 mg L^{-1}) in the single metal sorption system at different pH (2 to 6) is presented in Table 6 and Fig 6.

The maximum removal for Zn^{2+} (92.24%), Cr^{6+} (97.80%) at pH 4 was observed, whereas maximum Pb^{2+} (91.10%) and Ni^{2+} (89.32%) removal was observed at pH 2 and pH 5 respectively. At pH 4 there was maximum uptake of Cr^{6+} (2.43 mg g^{-1}) followed by Zn^{2+} (2.21 mg g^{-1}), Pb^{2+} (2.14 mg g^{-1}) and Ni^{2+} (1.6 mg g^{-1}) from 25 mg L^{-1} of metal containing solution by biomass MB2 (Fig 6 and Table 6). On increasing the pH from 4 to 5 reduction in biosorption of Zn^{2+} (2.2 to 1.8 mg g^{-1}) and Cr^{6+} (2.4 to 2.2 mg g^{-1}) was observed whereas significant increase was observed (0.9 to 2.2 mg g^{-1} for Zn^{2+} and 1.1 to 2.4 mg g^{-1} for Cr^{6+}) when the pH was shifted from 2 to 4. No significant change was observed in metal uptake for Ni^{2+} (1.7 to 1.6 mg g^{-1}) and Pb^{2+} (2.1 to 2.0 mg g^{-1}) as pH change from 2-6.

Table 6: Removal and metal uptake, q (mg g^{-1}) in single metal sorption system at different pH (Room temperature, Agitation rate = 120 rpm, C_i= 25mg L^{-1}).

Time (hr)	Zn^{2+}		Pb^{2+}		Cr^{6+}		Ni^{2+}	
	q	% Removal	q	% Removal	Q	% Removal	q	% Removal
pH 2								
0	0	0	0	0	0.6	0	1.2	0
0.083	0.05	2.28	2.1	77.02	0.7	23.74	1.7	58.01
0.25	0.11	5.15	2.2	80.87	0.9	27.19	1.9	80.18
0.5	0.10	4.43	2.3	81.47	1.1	37.58	1.9	89.66
1	0.35	15.38	2.3	82.94	0.8	46.10	1.9	89.66
2	0.65	28.49	2.3	84.62	1.0	33.06	1.8	90.38
4	0.77	33.79	2.3	85.28	1.0	40.57	1.8	88.70
6	0.84	36.59	2.3	86.08	1.1	41.76	1.9	88.12
8	0.64	27.84	2.4	86.82	1.2	44.01	1.7	89.18
24	0.97	42.33	2.4	91.10	0.6	47.11	1.2	82.20
pH 3								
0	0	0	0	0	0.6	0	1.6	0
0.083	1.82	74.64	2	75.05	0.1	25.58	1.8	77.48
0.25	2.18	89.24	1.9	74.16	0.3	47.58	1.9	86.39
0.5	2.26	92.38	1.9	73.40	1.0	13.64	1.9	90.24
1	2.29	93.73	2.1	78.63	1.0	41.79	1.9	90.33
2	2.30	94.10	2.1	81.65	0.6	39.11	1.9	88.99
4	2.27	92.90	2.1	80.68	0.6	23.99	1.7	88.99
6	2.22	91.04	2.2	84.69	0.9	23.36	1.8	83.79
8	2.05	83.89	2.2	85.17	1.0	36.00	1.7	88.36
24	1.43	55.85	2.2	85.14	0.6	41.27	1.6	82.11
pH 4								
0	0	0	0	0	2.1	0	1.2	0
0.083	1.05	43.80	1.7	68.33	2.3	86.12	1.6	60.47
0.25	1.84	76.87	1.7	70.41	2.3	90.58	1.7	79.36
0.5	2.09	87.19	1.6	67.12	2.3	90.56	1.8	87.47
1	2.17	90.52	1.9	77.13	2.4	93.70	1.8	90.88
2	2.15	89.48	1.9	78.98	2.4	94.97	1.8	90.78
4	2.19	91.20	2	74.94	2.3	94.57	1.8	89.53
6	2.13	88.85	1.8	75.14	2.4	93.94	1.6	88.68
8	2.21	91.93	1.8	84.96	2.4	96.48	1.7	78.26
24	2.21	92.24	2.1	87.17	2.1	97.80	1.2	84.47
pH 5								
0	0	0	0	0	2.1	0	1.1	0
0.083	0.96	46.18	1.6	70.54	2.2	85.58	1.5	57.89
0.25	1.54	71.49	1.6	70.09	2.2	88.35	1.7	77.41
0.5	1.97	90.35	1.5	66.12	2.2	87.99	1.8	86.10
1	1.91	87.72	1.8	67.93	2.2	87.13	1.8	89.58
2	1.93	88.29	1.5	77.64	2.2	89.13	1.8	89.88
4	1.81	83.25	1.8	75.36	2.2	88.38	1.7	89.32
6	1.76	80.83	1.7	72.09	2.3	89.48	1.7	86.46
8	1.88	86.33	1.6	85.21	2.2	90.35	1.6	82.42
24	1.88	86.46	1.9	90.78	2.1	90.21	1.1	89.32

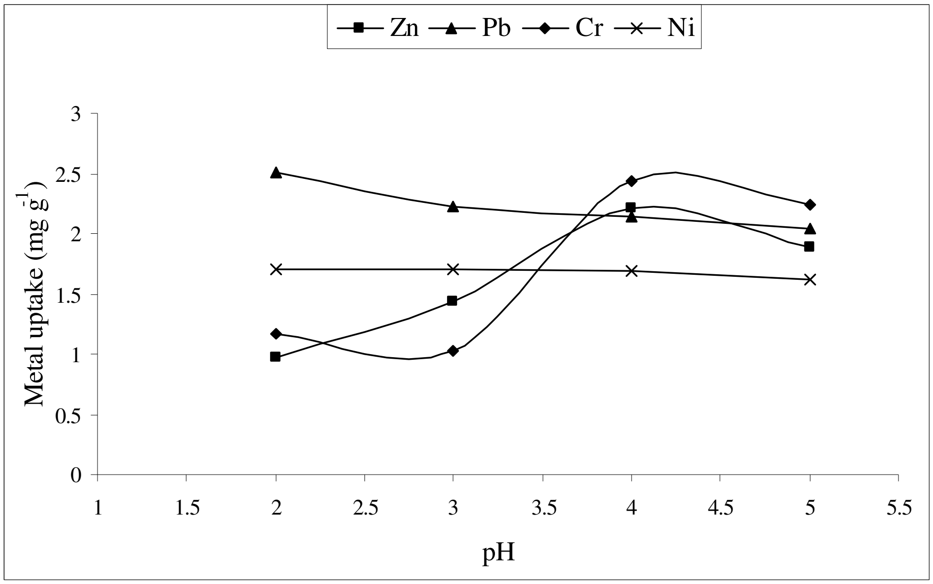


Fig 6: The effect of pH on the uptake of Zn^{2+}, Pb^{2+}, Cr^{6+} and Ni^{2+} by Waste biomass (MB2) in single metal sorption system (Room temperature, Agitation rate = 120 rpm, C_i= 25mg L^{-1}).

The adsorption of metal ions depends on solution pH, which influences electrostatic binding of ions to corresponding metal groups. At low pH (2-4) values the inactivated cell surface becomes more positively charged, reducing the attraction between metal ions and functional groups on the cell wall. In contrast, when the pH increases (4-5), the cell surface is more negatively charged and the process of retention is favoured (Volesky and Holan, 1995; Wong et al., 1993; Gardea-Torresdey et al., 1990; Sadowski, 2001). The effect of pH higher than 7, could not be studied due to the existence of insoluble hydroxide of metal, which might precipitate at the cell surface (Hahne and Kroontje, 1973). The pH of the metal solution plays a crucial role in passive microbial biosorption (Pradhan and Levine, 1995). As the pH is shifted, the equilibrium will also shift. The optimum initial pH for biosorption of Zn^{2+}, Pb^{2+}, $Cr^{6+}$and Ni^{2+} on to waste biomass (MB2) was observed at pH 4. Thus, all further experiments were performed at pH 4. Earlier studies have indicated that *Streptomyces* sp. was suitable as a biosorbent for the biosorption of Ag^{2+}, Cr^{6+}, Cu^{2+} (Mattuschka et al., 1993), Pb^{2+}, & U^{4+} (Friis and Myers-Keith, 1986).

1.2. Effect of Biomass Concentration

The effect of initial biomass quantity on equilibrium metal uptake of all metals solutions by waste biomass (MB2) are shown in Fig 7.

For the study of saturation of the sorption sites, concentration of waste biomass (MB2) was varied from 0.2 to 4 g per 100 ml and brought in contact with metal solutions of concentration 25 mg L^{-1}. The rate of increase of metal removal was not proportionate to the increase in waste biomass (MB2) as shown in Fig 7.

At 1% (1 g 100 mL^{-1}) biomass concentration there was maximum uptake of Pb^{2+} (6.4 mg g^{-1}) followed by Ni^{2+} (4.2 mg g^{-1}), Cr^{6+} (3.9 mg g^{-1}) and Zn^{2+} (3.7 mg g^{-1}) from 25 mg L^{-1} of metal containing solution by waste biomass MB2 (Fig 7).

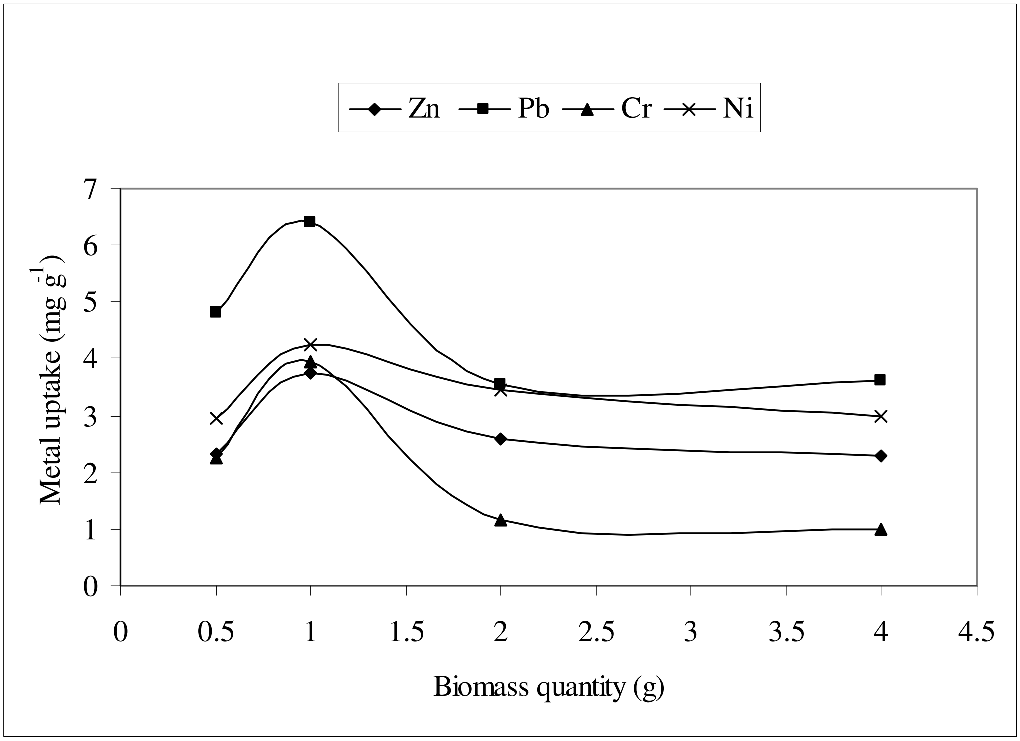


Fig 7: The effect of biomass concentration (g) on the uptake of Zn^{2+}, Pb^{2+}, Cr^{6+} and Ni^{2+} by waste biomass, MB2 (Room temperature; Agitation rate = 120 rpm, C_i=25 mg L^{-1})

This could be attributed to interference between binding sites at higher concentrations. Higher metal sorption at 1 g of waste biomass (MB2) could be due to the higher metal to biosorbent ratio, which decreases as the biomass quantity is increased (Puranik and Paknikar, 1999). There is more than one variable affecting the biosorption process, such as temperature, pH, agitation rate, metal and biosorbent concentration (Chen and Yiacaumi, 1997; Gadd, 1990; Veglio and Beolchini, 1997). As reported earlier some of these factors (biomass concentration) have greater influence on metal removal by this process (Veglio and Beolchini, 1997; Volesky and Holan 1995; Yetis et al., 2000).

1.3. Effect of Metal Concentration

Equilibrium batch adsorption experiments were conducted keeping the concentration of biosorbent constant (1 g) at different concentrations of metal ions. The amount adsorbed at different time intervals was determined. Table 7 a, b shows that, although different heavy metals were adsorbed to various extent by the waste biomass (MB2), the order of absorption at all concentration was: Cr^{6+}>Pb^{2+}>Zn^{2+}>Ni^{2+}.

The percentage removal of heavy metal ions at different metal concentrations are shown in Table 7 a, b. At 25 mg L^{-1} metal concentration there was maximum removal of Pb^{2+} (92.53%) followed by Zn^{2+} (90.76%), and Ni^{2+} (86.49%) whereas maximum remval of Cr^{6+} (97.06%) was observed at 5 mg L^{-1}. This appears to be due to the increase in the number of ions competing for the available binding sites in the biomass and also due to the lack of binding sites for the complexation of Cr^{6+} at higher concentration levels. At lower concentrations, all metal ions present in the solution would interact with the binding sites and thus facilitated 100% adsorption. At higher concentrations, more Cr^{6+} are left unabsorbed in solution due to the saturation of binding sites.

Table 7 a: The effect of metal concentration on the removal and uptake in single metal sorption system (Room temperature, Agitation rate = 120 rpm, pH = 4).

Time (hr)	Zn^{2+}		Pb^{2+}		Cr^{6+}		Ni^{2+}	
	q	% Removal	q	% Removal	q	% removal	q	% removal
5 mg L^{-1}								
0	0	0	0	0	0	0	0	0
0.083	0.4	76.89	0.1	47.46	0.18	61.87	0.4	68.67
0.25	0.4	68.40	0.1	49.15	0.20	68.23	0.3	52.80
0.5	0.4	72.78	0.2	52.34	0.22	74.58	0.3	64.41
1	0.5	82.19	0.2	70.29	0.25	84.07	0.4	73.31
2	0.4	76.77	0.2	76.3	0.27	91.15	0.4	79.88
4	0.5	83.11	0.2	79.33	0.27	91.45	0.4	81.62
6	0.5	80.29	0.2	75.10	0.28	94.95	0.4	82.01
8	0.4	77.66	0.2	70.75	0.24	81.09	0.4	81.89
10	0.5	86.94	0.2	70.86	0.26	89.26	0.4	80.23
24	0.5	86.86	0.2	73.06	0.29	97.06	0.4	79.50
10 mg L^{-1}								
0	0	0	0	0	0	0	0	0
0.083	0.9	76.39	0.4	52.68	0.47	65.67	0.6	65.34
0.25	1	80.78	0.4	56.07	0.53	73.81	0.7	68.20
0.5	1	84.61	0.6	71.03	0.57	79.55	0.7	75.43
1	1	87.56	0.6	75.03	0.6	84.84	0.8	79.41
2	1	87.61	0.6	77.64	0.62	86.32	0.8	81.86
4	1	84.47	0.6	77.35	0.64	89.67	0.8	83.28
6	1	83.59	0.6	80.77	0.66	92.65	0.8	84.91
8	1	87.95	0.6	81.38	0.66	93.10	0.8	83.59
10	1	86.89	0.6	81.37	0.66	93.15	0.8	82.53
24	1	86.54	0.7	87.35	0.67	94.06	0.8	81.04
15 mg L^{-1}								
0	0	0	0	0	0	0	0	0
0.083	1.1	64.72	1.3	69.97	0.88	70.07	1	66.85
0.25	1.3	76.75	1.3	69.85	1	79.82	1	72.10
0.5	1.4	80.61	1.3	69.29	1.02	81.27	1.2	80.46
1	1.4	78.82	1.3	69.33	1.03	82.29	1.2	84.25
2	1.4	82.48	1.4	71.29	1.05	84.40	1.3	86.26
4	1.4	82.09	1.4	75.87	1.12	89.58	1.2	85.43
6	1.4	83.68	1.6	84.43	1.13	90.43	1.2	85.43
8	1.3	77.73	1.6	86.16	1.14	91.31	1.2	85.29
10	1.4	78.59	1.7	89.72	1.15	92.93	1.2	83.89
24	1.6	79.09	1.8	90.63	1.16	94.20	1.2	79.56

Table 7 b: The effect of metal concentration on the removal and uptake in single metal sorption system (Room temperature, Agitation rate = 120 rpm, pH = 4).

Time (hr)	Zn^{2+}		Pb^{2+}		Cr^{6+}		Ni^{2+}	
	q	% Removal	q	% Removal	q	% removal	q	% removal
25 mg L^{-1}								
0	0	0	0	0	0	0	0	0
0.083	1.8	66.62	1.3	69.97	1.12	52.13	1.3	56.80
0.25	2.1	76.88	1.3	69.85	1.33	62.00	1.6	69.45
0.5	2.2	80.45	1.3	69.29	1.50	70.01	1.9	80.32
1	2.2	83.40	1.3	69.33	1.76	82.00	2	84.02
2	2.3	84.74	1.4	71.29	1.9	88.74	2	86.20
4	2.3	86.95	1.4	75.87	1.97	92.12	2	85.47
6	2.4	87.69	1.6	84.43	1.96	91.66	2	85.64
8	2.4	88.13	1.6	86.16	2.01	93.96	2	86.07
10	2.4	89.89	1.7	89.72	2.02	93.56	2	86.02
24	2.4	90.77	1.8	92.53	2.04	92.93	2	86.49
50 mg L^{-1}								
0	0	0	0	0	0	0	0	0
0.083	3.5	67.58	3.6	57.69	2.02	37.36	2.2	39.54
0.25	3.8	74.66	4.2	77.22	3.38	62.55	3.6	67.15
0.5	4.3	84.57	4.2	76.57	3.64	67.45	4.3	79.96
1	4.5	88.24	4.2	78.19	3.88	71.81	4.7	86.18
2	4.7	90.55	4.3	80.47	4.57	84.55	4.8	88.00
4	4.7	90.71	4.6	90.27	4.75	88.05	4.8	87.54
6	4.7	91.23	4.7	94.94	4.87	90.12	4.7	86.62
8	4.7	91.85	4.7	93.80	4.98	91.98	4.7	86.62
10	4.6	90.42	4.6	90.98	4.99	92.06	4.6	86.12
24	4.6	88.24	4.7	92.51	5.06	93.66	4.5	83.28

Of these, Cr^{6+} and Pb^{2+} were highly adsorptive showing the adsorption of more than 2.36 mg g^{-1} of biomass, so that almost all metal ions dissolved initially in the reaction mixtures were retained in the biomass (Table 8). As we increased the metal concentration (5-50 mg L^{-1}), the specific metal uptake was also increased for Zn^{2+} (0.4 to 4.4 mg g^{-1}), Cr^{6+} (0.3 to 5.0 mg g^{-1}), Ni^{2+} (0.4 to 4.5 mg g^{-1}) and Pb^{2+} (0.2 to 4.6 mg g^{-1}) at pH 4 (Fig 3).

Table 8: Equilibrium metal uptake for Zn^{2+}, Ni^{2+}, Cr^{6+} and Pb^{2+} solutions in single metal sorption system (Room temperature; Agitation rate = 120 rpm, pH = 4)

Conc. (mg L^{-1})	q_{max} (mg g^{-1})			
	q_{zn}	q_{ni}	q_{cr}	q_{pb}
5	0.48	0.40	0.29	0.21
10	1.02	0.80	0.67	0.67
15	1.56	1.20	1.16	1.05
25	2.36	2.40	2.04	1.75
50	4.56	4.50	5.06	4.65

Time taken to attain equilibrium for Cr^{6+}, Zn^{2+}, Pb^{2+} and Ni^{2+} metal solution were 2 hr as illustrated in Fig 8. The present results show that sorption of the test metals increased initially with their increasing concentrations in solution (Fig 8). This could be related to the higher probability of collision between metal ion and biosorbents during the initial phase of sorption (Do¨nmez et al. 1999).

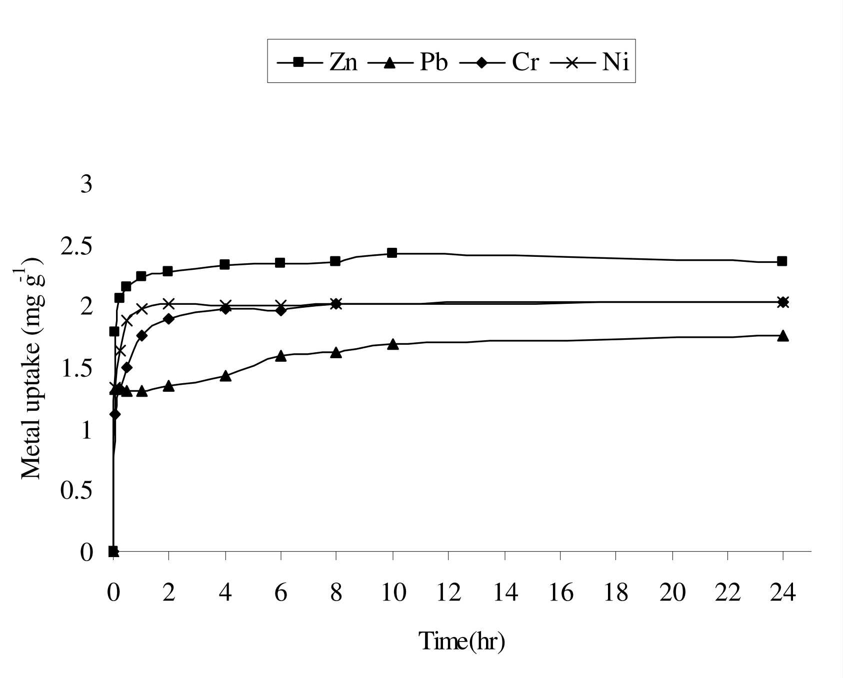


Fig 8: Equilibrium metal uptake by waste biomass (MB2) in single metal sorption system (Room temperature, Agitation rate = 120 rpm, C_i= 25mg L^{-1}).

2. Binary Metal Sorption System

Biosorption of Zn^{2+} and Pb^{2+} from binary metals solution was investigated at varying metal concentrations and contact time.

2.1. Effect of Metal Ion Concentration

The effect of the initial Zn^{2+} and Pb^{2+} concentration on the equilibrium metal uptake (mg g^{-1}) by waste biomass (MB2) in binary metal biosorption system is shown in Table 9 a, b.

Table 9 a: The effect of metal concentration on the removal (%) and metal uptake in binary metal sorption system (Room temperature, Agitation rate = 120 rpm, pH = 4).

Time (hr)	Zn^{2+}		Pb^{2+}	
	q	% Removal	q	% Removal
5 mg L^{-1}				
0	0	0	0	0
0.083	0.3	58.43	0.2	45.31
0.25	0.3	60.67	0.2	53.35
0.5	0.3	73.03	0.3	64.73
1	0.4	77.53	0.3	71.09
2	0.4	83.71	0.3	73.44
4	0.4	85.96	0.4	79.13
6	0.4	86.52	0.4	81.70
8	0.4	88.20	0.4	83.26
24	0.4	92.13	0.4	98.44
10 mg L^{-1}				
0	0	0	0	0
0.083	0.7	64.29	0.5	45.14
0.25	0.7	66.19	0.7	62.09
0.5	0.8	75.24	0.9	72.49
1	0.8	79.52	0.9	78.78
2	0.9	83.81	1	83.53
4	0.9	86.67	1	85.28
6	0.9	86.19	1	86.65
8	0.9	86.67	1.1	89.43
24	1	95.24	1.1	96.58

Table 9 b: The effect of metal concentration on the removal (%) and metal uptake in binary metal sorption system (Room temperature, Agitation rate = 120 rpm, pH = 4).

Time (hr)	Zn^{2+}		Pb^{2+}	
	q	% Removal	q	% Removal
		$15\ mg\ L^{-1}$		
0	0	0	0	0
0.083	0.9	60.07	0.6	41.03
0.25	1.1	70.96	0.8	55.45
0.5	1.2	81.85	1	67.47
1	1.3	85.81	1.1	75.73
2	1.3	86.8	1.2	81.72
4	1.4	88.78	1.3	84.63
6	1.4	88.78	1.3	85.61
8	1.4	90.76	1.3	87.68
24	1.4	94.75	1.4	95.90
		$25\ mg\ L^{-1}$		
0	0	0	0	0
0.083	1.7	60.44	0.9	40.76
0.25	2.2	76.44	1	47.91
0.5	2.4	85.33	1.4	64.51
1	2.5	89.33	1.6	75.35
2	2.5	89.78	1.7	80.69
4	2.5	90.22	1.8	83.89
6	2.6	92.00	1.8	86.45
8	2.6	92.44	1.9	87.49
24	2.7	97.05	2	94.97
		$50\ mg\ L^{-1}$		
0	0	0	0	0
0.083	2.9	49.14	3.2	74.12
0.25	4.5	77.59	3.3	77.31
0.5	5	85.34	3.6	84.73
1	5.2	88.79	3.9	89.71
2	5.2	90.09	3.9	90.93
4	5.2	90.09	4	93.5
6	5.3	90.95	4.1	94.08
8	5.5	93.97	4.1	94.51
24	5.7	97.83	4.2	97.27

The metal uptake increased with an increase of the initial Zn^{2+} and Pb^{2+} concentration up to 50 mg L^{-1} by waste biomass (MB2) for binary metal biosorption system as shown in Table 9 a, b.

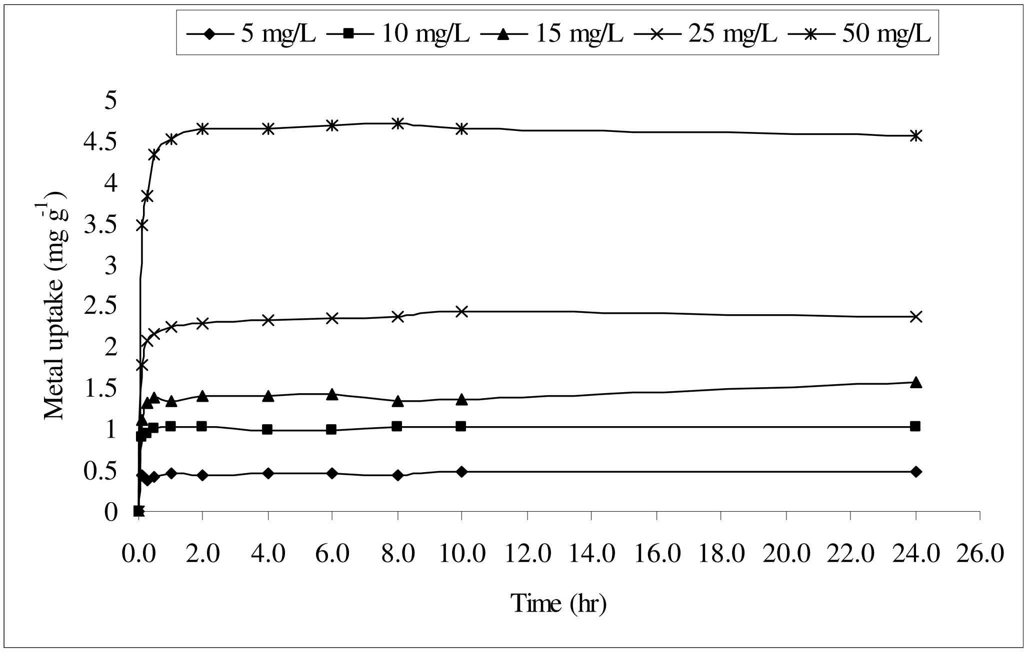


Fig 9: Effect of Zn^{2+} conc. on equilibrium metal uptake in single metal sorption system by waste biomass, MB2 (Room temperature; Agitation rate = 120 rpm, pH=4).

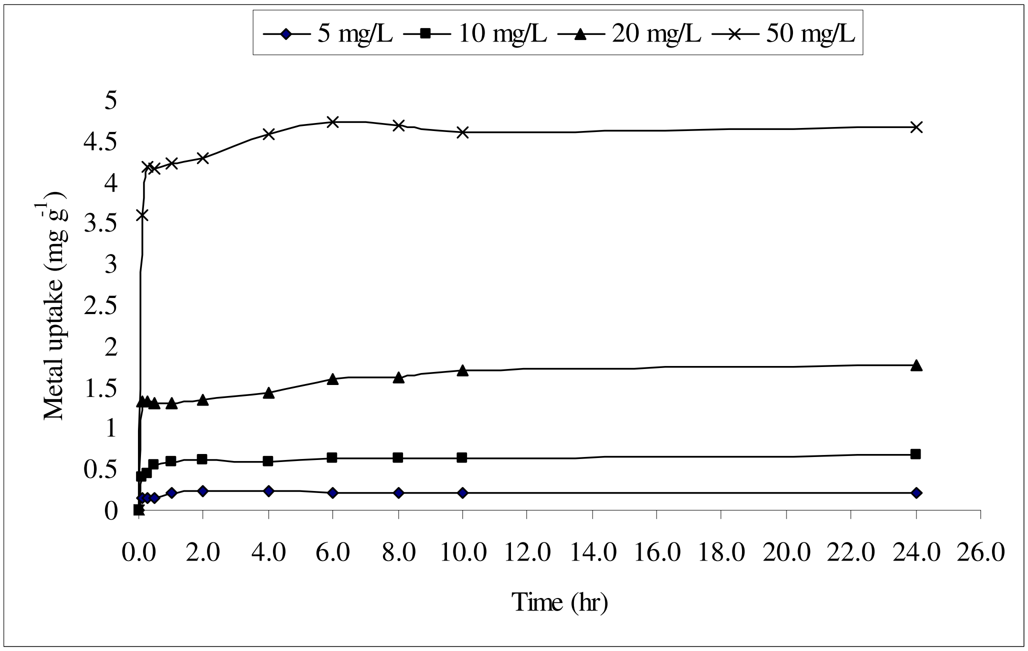


Fig 10: Effect of Pb^{2+} conc. on equilibrium metal uptake in single metal sorption system by waste biomass, MB2 (Room temperature; Agitation rate = 120 rpm, pH=4).

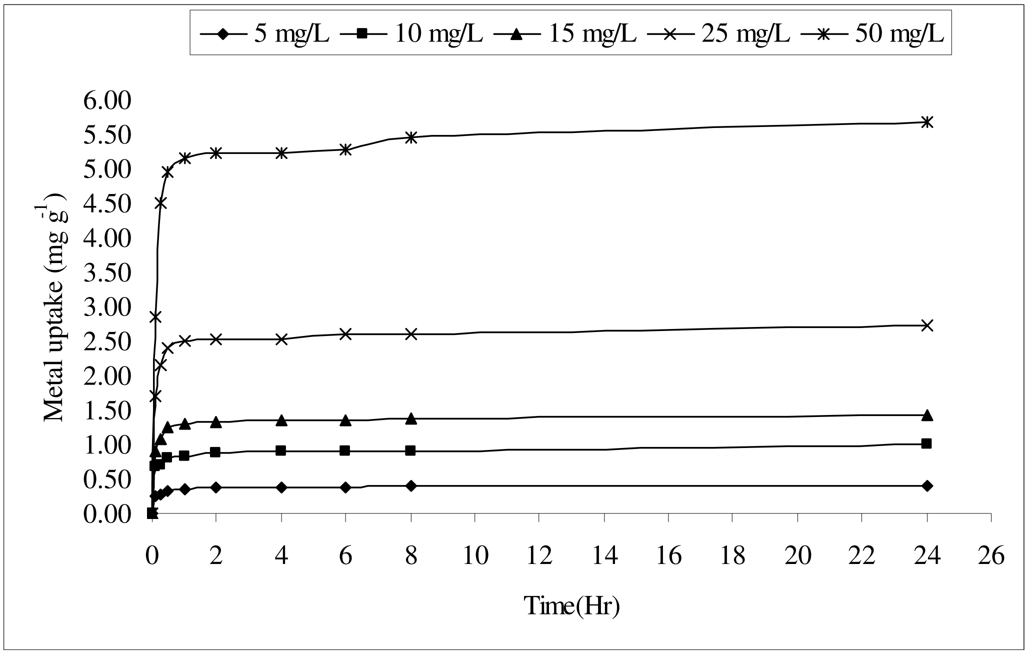


Fig 11: Effect of Zn^{2+} concentration on equilibrium metal uptake in binary metal sorption system by waste biomass, MB2 (Room temperature; Agitation rate = 120 rpm, pH=4)

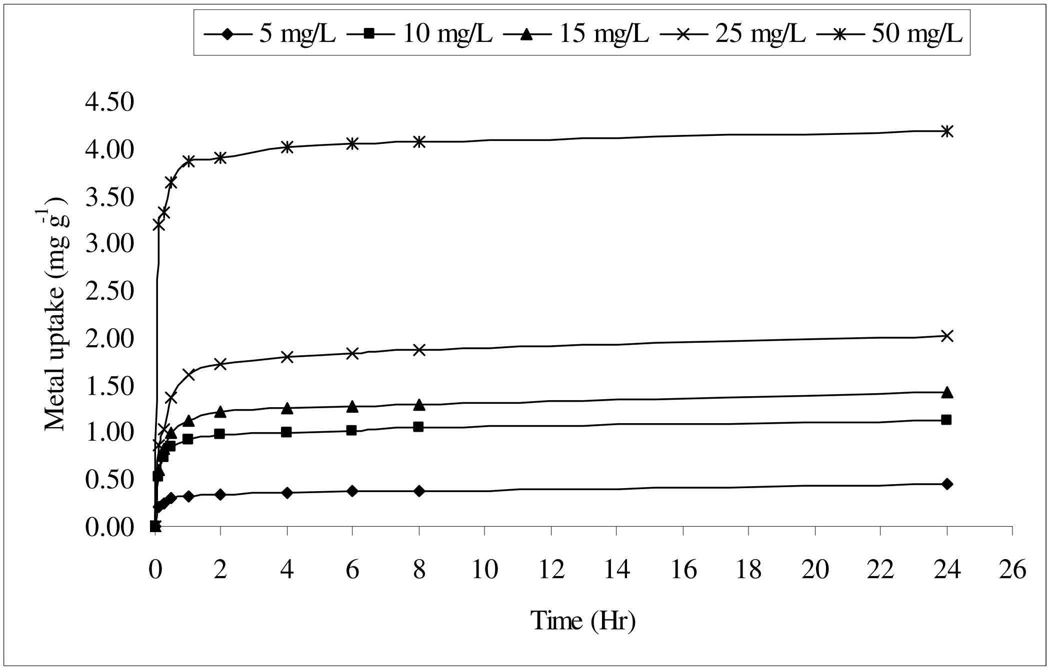


Fig 12: Effect of Pb^{2+} concentration on equilibrium metal uptake in binary metal sorption system by waste biomass, MB2 (Room temperature; Agitation rate = 120 rpm, pH=4).

When the initial Zn^{2+} and Pb^{2+} concentration was increased from 5 to 50 mg L^{-1} for waste biomass (MB2), the adsorption capacities of waste biomass (MB2) increased from 0.48 to 4.56 mg g^{-1} and 0.21 mg g^{-1} to 4.65 mg g^{-1} respectively for single metal biosorption system and from 0.44 to 4.18 mg g^{-1} and 0.41 mg g^{-1} to 5.67 mg g^{-1} respectively for binary metal biosorption system (Fig 9-12). The increase of adsorption capacities of the waste biomass (MB2) with the increase of Zn^{2+} concentration may be due to the higher probability of collisions between the ions and biosorbents. The adsorption yield obtained from experimental data at different initial Zn^{2+} and Pb^{2+} concentration are also presented in Table 10 for both single and binary metal biosorption systems.

Table 10: Comparison in Equilibrium metal uptake for single and binary aqueous solution by waste biomass (MB2)

Concentration (mg L^{-1})	q_{max} (mg g^{-1}) for single metal biosorption system		q_{max} (mg g^{-1}) for binary metal biosorption system		
	q_{pb}	q_{zn}	q_{pb}	q_{zn}	total q (q_{pb}+q_{zn})
5	0.21	0.50	0.41	0.44	0.85
10	0.67	1.05	1.00	1.13	2.13
15	1.75	1.60	1.43	1.42	2.85
25	2.58	2.40	2.72	2.02	4.74
50	4.65	4.60	5.67	4.18	9.85

It is interesting to note that the equilibrium uptakes were higher for Zn^{2+} biosorption by waste biomass (MB2), than those for Pb^{2+} biosorption at low concentrations (5 to 10 mg L^{-1}) that subsequently lowered at higher concentrations (15 to 50 mg L^{-1}) for both single and binary metal biosorption system (Table 10). Therefore, it seems that, Pb^{2+} occupied its binding sites, at higher metal concentration (15 to 50 mg L^{-1}), where adsorption of Zn^{2+} took place relatively poor (Rho and Kim, 2002) in binary metal sorption system.

3. Multimetal Biosorption System

The metal adsorbing activities were also determined for Cr^{6+}, Zn^{2+}, Pb^{2+} and Ni^{2+} using waste biomass (MB2) in multimetal sorption system.

3.1. Effect of pH

Initial investigation of biosorption capability of waste biomass (MB2) for Cr^{6+}, Zn^{2+}, Pb^{2+} and Ni^{2+} (25 mg L^{-1}) in the multimetal sorption system at different pH (2 to 6) is presented in Table 11 a, b and Fig 13.

Table 11 a: Removal (%) and metal uptake (mg g^{-1}) in multimetal sorption system at different pH (Room temperature, Agitation rate = 120 rpm, C_i= 25 mg L^{-1}).

Time (hr)	Zn^{2+}		Pb^{2+}		Cr^{6+}		Ni^{2+}	
	q	% Removal	q	% Removal	q	% Removal	q	% Removal
pH 2								
0	0	0	0	0	0	0	0	0
0.083	0.16	23.32	0.16	96.20	0.59	31.70	1.47	68.08
0.25	0.32	46.65	0.16	96.60	1.19	64.10	1.8	83.73
0.5	0.45	65.84	0.16	96.40	1.43	76.90	1.88	87.14
1	0.48	70.34	0.16	96.40	1.43	76.90	1.88	87.07
2	0.51	74.85	0.16	96.50	1.43	76.90	1.87	86.99
4	0.54	79.36	0.16	96.50	1.43	76.90	1.87	86.91
6	0.59	86.77	0.16	95.50	1.5	80.70	1.87	86.99
8	0.61	89.97	0.16	98.30	1.57	84.40	1.86	86.43
24	0.56	82.41	0.16	95.70	1.86	89.56	1.83	84.90
pH 3								
0	0.04	0	0	0	0	0	0	0
0.083	1.34	67.97	0.38	100	1.4	50.00	1.46	68.99
0.25	1.69	86.61	0.38	100	2.23	79.60	1.76	83.05
0.5	1.79	92.07	0.38	100	2.42	86.40	1.83	86.66
1	1.81	92.83	0.38	100	2.43	86.80	1.84	86.93
2	1.82	93.64	0.38	100	2.44	87.10	1.84	87.20
4	1.84	94.54	0.38	100	2.4	87.50	1.85	87.47
6	1.85	94.97	0.38	100	2.55	91.10	1.83	86.72
8	1.86	95.41	0.38	100	2.56	91.40	1.82	86.26
24	1.87	95.85	0.37	98.8	2.70	96.40	1.78	84.05

Table 11 b: Removal (%) and metal uptake (mg g^{-1}) in multimetal sorption system at different pH (Room temperature, Agitation rate = 120 rpm, C_i= 25 mg L^{-1}).

Time (hr)	Zn^{2+}		Pb^{2+}		Cr^{6+}		Ni^{2+}	
	q	% Removal	q	% Removal	q	% Removal	q	% Removal
pH 4								
0	0	0	0	0	0	0	0	0
0.083	1.25	70.59	0.48	99.20	0.96	39.50	1.36	66.76
0.25	1.56	88.11	0.45	92.60	1.56	64.00	1.66	81.85
0.5	1.66	93.63	0.45	94.30	1.76	72.20	1.75	86.15
1	1.68	94.70	0.46	96.00	1.75	71.70	1.70	86.59
2	1.70	95.77	0.47	97.07	1.7	71.40	1.77	87.02
4	1.71	96.84	0.48	99.05	1.73	71.00	1.78	87.46
6	1.72	97.07	0.48	100	1.79	73.40	1.77	87.05
8	1.71	96.67	0.48	100	1.89	77.50	1.75	86.03
24	1.52	85.85	0.47	97.80	2.25	92.00	1.73	85.35
pH 5								
0	0	0	0	0	0	0	0	0
0.083	1.22	75.07	0.39	99.20	0.50	30.10	1.16	67.02
0.25	1.42	87.75	0.39	99.30	1.27	69.10	1.39	80.58
0.5	1.5	92.43	0.39	99.20	1.42	77.20	1.47	85.02
1	1.5	92.67	0.39	98.10	1.43	77.60	1.46	84.7
2	1.51	92.89	0.38	97.10	1.43	77.90	1.46	84.39
4	1.51	93.16	0.38	96.10	1.44	78.30	1.40	84.07
6	1.51	93.30	0.39	98.90	1.43	77.80	1.46	84.86
8	1.51	92.98	0.39	99.40	1.43	80.50	1.45	83.85
24	1.52	93.60	0.37	93.30	1.42	77.20	1.33	77.05
pH 6								
0	0	0	0	0	0	0	0	0
0.083	0.73	69.72	0.51	99.40	1.01	45.00	0.87	63.58
0.25	0.85	80.70	0.51	99.70	1.55	68.60	1.03	74.88
0.5	0.89	84.57	0.50	97.10	1.71	75.70	1.08	78.55
1	0.87	82.49	0.50	97.80	1.71	75.70	1.05	76.48
2	0.85	80.51	0.51	98.50	1.71	75.70	1.02	74.41
4	0.83	78.52	0.5	99.30	1.71	75.70	0.99	72.34
6	0.88	83.53	0.51	99.70	1.79	79.20	1.02	74.29
8	0.83	78.80	0.5	97.20	1.74	77.00	0.96	70.00
24	0.73	69.44	0.49	95.20	1.75	77.40	0.94	68.75

It was found that the waste biomass (MB2) possessed maximum sorption capacity for Cr^{6+}, Zn^{2+}, Pb^{2+} and Ni^{2+} at pH values between 3 and 4 (Fig 13).

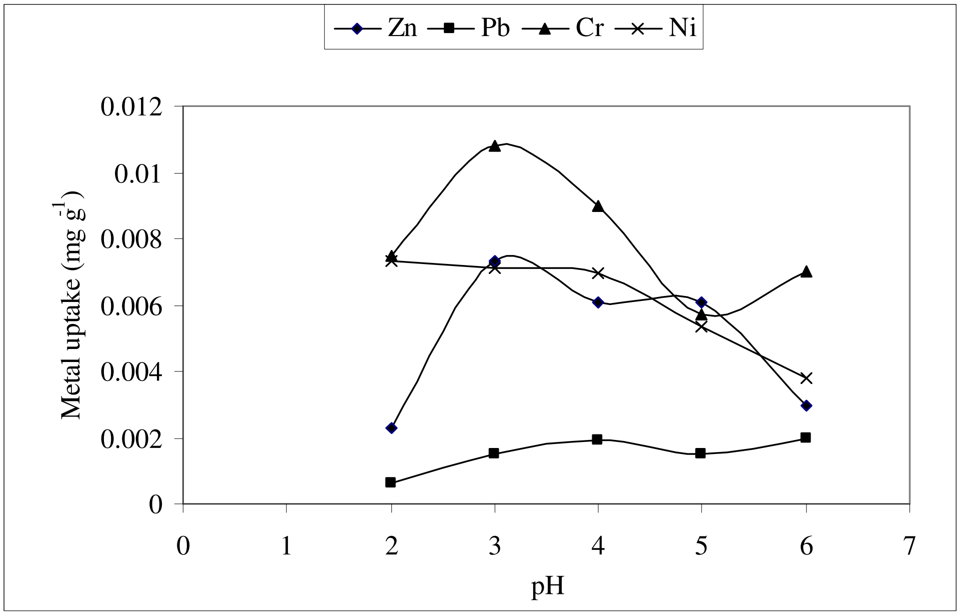


Fig 13: The effect of initial pH on the equilibrium metal uptake by waste biomass (MB2) in multimetal sorption system (Room temperature, Agitation rate = 120 rpm, C_i= 25 mg L^{-1})

The effect of pH on the biosorption of heavy metal ions by microorganisms was investigated and Pb^{2+}, Ni^{2+}, and Cu^{2+} were demonstrated to be best adsorbed on the biomass at pH 4–5 (Aksu et al., 1992; Sag and Kutsal, 1995; Sag and Kutsal, 1997). At pH between 3 and 4 there was maximum metal uptake of Cr^{6+} (2.70 mg g^{-1}) followed by Zn^{2+} (1.8 mg g^{-1}), Ni^{2+} (1.77 mg g^{-1}) and Pb^{2+} (0.47 mg g^{-1}) from 25 mg L^{-1} of metal containing solution by waste biomass (MB2). At pH below 3, uptakes of Cr^{6+}, Zn^{2+} were negligible, probably due to the cation competition effects with hydronium ion (H_3O^+). Furthermore, at pH 6 biosorption of these cationic ion metals decreased probably because of chemical precipitation. On the contrary, insignificant uptake of Pb^{2+} and Ni^{2+} by waste biomass (MB2) has been found in the whole investigated pH range, though increased removal of these ions by complexation on bacteria, algae, fungi and yeast at pH 2 was reported (Pillichshammer et al., 1995; Donmez et al., 1999; Crawford and Crawford, 1996). At pH 3 there was maximum removal of Pb^{2+} (98.80%) followed by Cr^{6+} (96.40%) and Zn^{2+} (95.85%) whereas maximum removal of Ni^{2+} (85.35%) was taken at pH 4. Since, metal ions were best adsorbed on the biomass at pH 4, studies on the simultaneous biosorption of a mixture of Pb^{2+}, Ni^{2+}, Zn^{2+} and Cr^{6+} were carried out at pH 4.

3.2. Effect of Metal Concentration

We carried out the adsorption test in reaction mixtures where biomass was exposed to the heavy metal ion mixture. In multimetal reaction, we did not see the sequential binding order found in the single metal reactions (Table 12 a, b).

Table 12 a: The effect of metal concentration on the removal (%) and metal uptake in multimetal sorption system (Room temperature, Agitation rate = 120 rpm, pH = 4).

Time (hr)	Zn^{2+}		Pb^{2+}		Cr^{6+}		Ni^{2+}	
	q	% Removal	q	% Removal	q	% removal	q	% removal
5 mg L^{-1}								
0	0	0	0	0	0	0	0	0
0.083	0.2	39.86	0.1	48.42	0.4	9.60	0.5	96.47
0.25	0.4	79.73	0.2	97.22	0	6.90	0.4	75.34
0.5	0.4	86.19	0.2	94.57	0	4.51	0.4	82.30
1	0.4	85.45	0.2	95.21	0	7.79	0.4	83.09
2	0.4	84.72	0.2	95.84	0	11.07	0.4	83.86
4	0.4	83.96	0.2	96.48	0.1	14.35	0.4	84.64
6	0.4	87.52	0.2	94.95	0.2	41.55	0.4	84.24
8	0.3	77.72	0.2	89.57	0.1	29.93	0.4	84.13
24	0.4	87.08	0.2	96.97	0.4	30.56	0.4	89.29
10 mg L^{-1}								
0	0	0	0	0	0	0	0	0
0.083	0.6	75.28	0.8	97.95	0.7	67.93	0.6	69.58
0.25	0.6	80.98	0.8	96.17	0.8	73.37	0.7	78.3
0.5	0.7	86.69	0.8	96.06	0.8	80.07	0.7	84.65
1	0.7	86.27	0.8	96.57	0.8	80.88	0.7	85.00
2	0.7	85.85	0.8	97.07	0.8	81.69	0.7	85.34
4	0.7	87.95	0.8	97.58	0.8	82.5	0.7	85.69
6	0.7	88.59	0.8	97.46	0.9	84.45	0.7	86.07
8	0.7	91.76	0.8	93.84	0.9	84.84	0.7	85.83
24	0.7	91.12	0.8	93.79	1.0	86.32	0.7	83.88

Table 12 b: The effect of metal concentration on the removal (%) and metal uptake in multimetal sorption system (Room temperature, Agitation rate = 120 rpm, pH = 4).

Time (hr)	Zn^{2+}		Pb^{2+}		Cr^{6+}		Ni^{2+}	
	q	% Removal	q	% Removal	q	% removal	q	% removal
15 mg L^{-1}								
0	0	0	0	0	0	0	0	0
0.083	0.9	67.42	0.3	98.01	0.9	52.03	0.9	74.17
0.25	1	78.78	0.3	98.64	1.4	81.05	1	81.64
0.5	1.1	81.81	0.3	97.78	1.4	82.23	1	86.14
1	1	78.78	0.3	97.36	1.4	81.99	1	85.96
2	1.1	80.3	0.3	96.95	1.4	81.76	1	85.78
4	1	78.78	0.3	96.53	1.4	81.52	1	85.60
6	1.1	80.30	0.3	93.49	1.4	81.05	1	85.08
8	1.3	95.22	0.3	96.69	1.4	85.07	1	84.90
24	1.3	95.15	0.3	93.16	1.6	90.29	1	82.98
25 mg L^{-1}								
0	0	0	0	0	0	0	0	0.04
0.083	1.4	67.70	0.6	98.8	0.9	38.92	1.6	71.72
0.25	1.8	85.95	0.6	99.26	1.1	46.89	1.8	83.83
0.5	1.9	90.75	0.6	99.12	1.2	50.44	1.9	87.88
1	1.9	91.91	0.6	98.16	1.3	54.09	1.9	87.69
2	1.9	92.97	0.6	97.19	1.3	57.74	1.9	87.55
4	1.9	94.04	0.6	96.23	1.4	61.38	1.9	87.41
6	2	94.43	0.6	98.95	1.5	62.35	1.9	86.83
8	2	94.72	0.6	97.31	1.6	67.40	1.9	86.69
24	2	95.30	0.5	93.35	2.2	92.56	1.8	84.79
50 mg L^{-1}								
0	0	0	0	0	0	0	0	0.04
0.083	2.8	63.42	1.8	98.13	1	31.92	0.4	18.01
0.25	3.7	84.89	1.8	99.09	1.1	37.26	1.6	66.31
0.5	4.1	92.00	1.8	99.38	1.3	41.93	1.8	75.45
1	4.1	93.13	1.8	99.43	1.3	44.27	1.8	76.57
2	4.2	94.38	1.8	99.49	1.4	46.61	1.8	77.69
4	4.2	95.78	1.8	99.54	1.5	48.95	1.9	78.81
6	4.3	96.69	1.8	99.59	1.7	56.05	1.9	78.95
8	4.3	97.03	1.8	99.73	1.8	61.61	1.8	77.27
24	4.2	94.42	1.8	96.41	2.6	86.96	1.8	77.67

The preference of metal sorbed was $Cr^{6+}>Pb^{2+}>Zn^{2+}>Ni^{2+}$ in single metal sorption system whereas in multimetal sorption system it was $Zn^{2+}>Cr^{6+}>Ni^{2+}>Pb^{2+}$ (Table 13).

Table 13: Comparison in equilibrium metal uptake for single and multimetal biosorption system by waste biomass (MB2).

Conc. (mg L^{-1})	q_{max} (mg g^{-1}) for single metal biosorption system				q_{max} (mg g^{-1}) for multi metal biosorption system			
	q_{Zn}	q_{Ni}	q_{Cr}	q_{Pb}	q_{Zn}	q_{Ni}	q_{Cr}	q_{Pb}
5	0.48	0.41	0.29	0.21	0.35	0.44	0.33	0.16
10	1.02	0.8	0.67	0.67	0.72	0.77	0.58	0.69
15	1.56	1.15	1.17	1.63	1.23	0.96	1.03	0.28
25	2.36	2.35	2.04	1.75	1.94	1.79	1.33	0.56
50	4.56	4.52	5.06	4.65	4.18	1.79	2.64	1.74

The sorption processes were found to be slower in multi-component than those in the single-component metal solutions (Fig 8 and 14). When the multimetal concentration was increased from 5 to 50 mg L^{-1}, the adsorption capacities of biosorbents increased for Zn^{2+}, Ni^{2+}, Cr^{6+} and Pb^{2+} from 0.48 to 4.56 mg g^{-1}, 0.41 to 4.52 mg g^{-1}, 0.29 to 5.06 mg g^{-1} and 0.21 mg g^{-1} to 4.65 mg g^{-1} respectively for single and from 0.35 to 4.18 mg g^{-1}, 0.44 to 1.79 mg g^{-1}, 0.33 to 2.64 mg g^{-1} and 0.16 to 1.74 mg g^{-1} respectively for multimetal biosorption system. Thus, compared with the single metal system, the decrease in Cr^{6+}, Pb^{2+}, $Zn^{2+}$and Ni^{2+} adsorption in multi metal sorption system were notable for all metal concentrations. At 25 mg L^{-1} concentration of metal solution there was maximum removal of Zn^{2+} (95.30%) followed by Cr^{6+} (92.56%) and Ni^{2+} (84.79%) whereas maximum removal of Pb^{2+} (96.97%) was taken at 5 mg L^{-1} concentration.

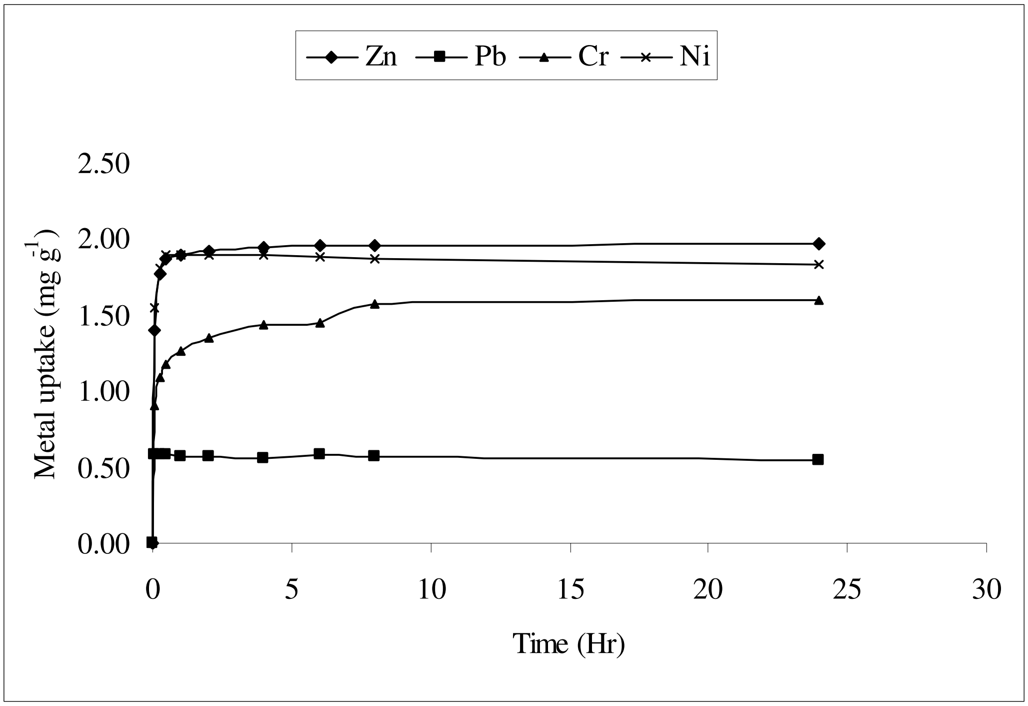


Fig 14: Equilibrium metal uptake by waste biomass (MB2) in multimetal sorption system (Room temperature, Agitation rate = 120 rpm, C_i= 25mg L^{-1}).

In multimetal experiments, we did not see the sequential binding order found in the single metal reactions. The adsorption pattern was changed in multimetal sorption system as $Zn^{2+}>Cr^{6+}>Ni^{2+}>Pb^{2+}$ in contrast to $Cr^{6+}>Pb^{2+}>Zn^{2+}>Ni^{2}$ in single metal sorption system (Fig 3-4). The Cr^{6+} adsorption decreased at all concentrations. Therefore, it seems that, Pb^{2+}, $Zn^{2+}$and Ni^{2+} compete for the binding sites with Cr^{6+}. The maximum sorption capacity was found for Zn^{2+} in multimetal sorption system. Earlier studies showed, in multimetal biosorption of Cr^{6+}, Cd^{2+} and Cu^{2+}, at higher Cr^{6+} and Cd^{2+} concentrations, the inhibitory effects of Cr^{6+} and Cd^{2+} on the biosorptive uptake of Cu^{2+} predicted by the Freundlich-type model (Sag et al., 2003).

4. Equilibrium Modeling

In order to evaluate feasibility and effectiveness of biosorption in wastewater treatment, it is essential to make predictions of the sorption performance (e.g., for facilitating process design). Therefore it is necessary to develop appropriate mathematical models of biosorption. The amount of metal M (or, in general, sorbate) bound per mass of sorbent is called the binding (or uptake), q is usually expressed in milligrams of metal sorbed per gram of the (dry) sorbent (Kratochvil and Volesky, 1998). The binding is not only dependent on the sorbent material but also on the equilibrium concentration $[C_e]$ of the sorbate in the solution and but also on other parameters, such as, pH and equilibrium concentration of other ions in the solution. The relationship between equilibrium binding and the concentration of ions (at constant temperature) is depicted in an isotherm plot of q versus $[C_e]$. With increasing metal concentration in solution its binding increases from zero to the maximum. It is desirable for the sorbent to possess a high sorption capacity and high affinity for the sorbate species, which is reflected in a steep slope of the isotherm curve at low equilibrium concentrations.

The comparison in sorption performance of waste biomass (MB2) was evaluated through determining sorption isotherm between single and binary heavy metals (Zn^{2+} and Pb^{2+}) sorption system (Table 14) and single and multi-metal sorption system (Table 15 a, b). It was observed that, in both single and binary metal sorption system, the adsorption capacity of the waste biomass (MB2) for the Zn^{2+} (1.5 and 3.28) was always higher than that for the Pb^{2+} (0.8769 and 2.4).

Table 14: Comparison in biosorption isotherm parameters for single and binary metal biosorption of Zn^{2+} and Pb^{2+} by waste biomass (MB2)

	Freundlich constant					
Metal	Single sorption system			Binary sorption system		
	K_f^*	$n^\#$	R^2	K_f^*	$n^\#$	R^2
Zn^{2+}	1.50	2.88	0.9543	3.28	0.83	0.9463
Pb^{2+}	0.87	0.36	0.9348	2.40	1.47	0.8857

* Adsorption capacity; # Adsorption intensity

This indicated strongly that there was a competitive adsorption of Zn^{2+} on the surface of the waste biomass (MB2). The total adsorption capacity for the heavy metals seemed to be lower in the single metal system than the binary metal system.

Cell envelopes of microorganisms typically exhibit negative charges (Camesano and Logan, 2000) and are therefore able to sequester cationic metals. The biosorption capacity of bacteria depends on a number of features concerning the cell architecture, chemical composition of cell wall and adjacent extracellular layers, and compounds like the extracellular polysaccharides of streptomycetes. The main functional groups contributing to the charges on bacteria are phosphate moieties, either in phosphodiester bridges as in teichoic acids or at the end of a polymer as in phospholipids, protein or peptidoglycan-associated carboxylic groups, polysaccharide-associated carboxylic groups, protonated phosphates, and peptidoglycan or protein-associated ammonium (Camesano and Logan 2000, Conn et al., 1987).

The sorption performance of waste biomass (MB2) was evaluated through determining sorption isotherm with heavy metals Zn^{2+}, Ni^{2+}, Cr^{6+} and Pb^{2+} for single and multi metal sorption system are summarized in Table 15 a, b.

Table 15 a: Comparison in Langmuir isotherm parameters for single and multimetal sorption system of by waste biomass (MB2)

Langmuir constants							
Metal	Single component sorption System			Multi component sorption system			
	q_{max}	b	R^2	q_{max}	b	K	R^2
Zn^{2+}	-2.44	-0.27	0.8595	-1.76	-0.42	-2.38	0.5973
Pb^{2+}	0.38	0.52	0.775	0.96	4.04	0.24	0.7353
Cr^{6+}	2.44	1.49	0.9535	2.12	19.06	0.05	0.6178
Ni^{2+}	-5.56	-0.07	0.9737	2.44	0.39	2.54	0.9587

Table 15 b: Comparison in Freundlich isotherm parameters for single and multimetal sorption system of by waste biomass (MB2)

Freundlich constants						
Metal	Single component sorption System			Multi component sorption system		
	K_f	n	R^2	K_f	n	R^2
Zn^{2+}	1.50	2.88	0.9543	0.71	0.73	0.778
Pb^{2+}	0.88	0.36	0.9348	0.66	1.23	0.7868
Cr^{6+}	0.60	1.27	0.9611	0.55	3.80	0.803
Ni^{2+}	2.45	0.88	0.938	1.55	1.44	0.9519

It can be observed (Table 15 a, b) that, the adsorption capacity of the waste biomass (MB2) for the Zn^{2+}, Pb^{2+}, Cr^{6+} and Ni^{2+} was always higher in single metal sorption system (1.5, 0.88, 0.60 and 2.45) than that for multi-metal sorption system (0.71, 0.66, 0.55 and 1.55). The sorption processes were found to be slower in multi-component solutions than those in the single-component metal solutions.

4.1. Multicomponent Langmuirian Models

The Langmuirian sorption behavior conventionally assumes the formation of a surface molecular monolayer whereby sorbate become bound by originally empty reaction sites. While there may even be several types of binding sites considered, most often, the 1:1 binding stoichiometry is implied. This type of model can not reflect the mechanism of the metal binding reaction and so it is insensitive to situations when ion exchange mechanism prevails as well as to the 'environmental' external factors of the sorption system, such as e.g. pH or ionic strength, that so not appear anywhere in the model equation.

The most often used basic Langmuir isotherm model can be easily modified to account for different competing ions. Multi-sites model versions can also apply for cases where a mixed ion-exchange and covalent binding phenomena are at work. The Langmuir model has also been used to analyze multicomponent biosorption equilibrium data (Chong and Volesky, 1995; Sag and Kutsal, 1996; Chong and Volesky, 1996; Sánchez et al., 1999; Figueira et al., 1997). This model resembles the competitive inhibition in enzyme kinetics studies. Since the equilibrium sorption system is considered, there are no net changes in concentrations of the biomass-bound metal complexes in time. The Langmuir isotherm based on multicomponent sorption isotherm model is represented by the following equation:

$$q(M_1) = \frac{(q_{\max} / K_1) C_f [M_1]}{1 + (1/K_1) C_f [M_1]^{K_3} + (1/K_2) C_f [M_2]^{K_4}}$$

Where q_{max}, $K_{1,}$ $K_{2,}$ K_3 and K_4 are Langmuir constants.

Maximum metal uptake (q_{max}) of waste biomass (MB2) for Zn^{2+}, Pb^{2+}, Cr^{6+} and Ni^{2+} in multimetal sorption system with multicomponent Langmuirian isotherm model is presented in Table 16.

Table 16: Maximum metal uptake (q_{max}) in multi-metal sorption system by waste biomass (MB2) with multi-component Langmuirian isotherm model.

Metal	q_{max} (mg g^{-1})
Zn^{2+}	0.0334
Pb^{2+}	0.0017
Cr^{6+}	0.0024
Ni^{2+}	0.0065

The preference of maximum metal uptake (q_{max}) was Ni^{2+}>Cr^{6+}>Pb^{2+}>Zn^{2+} in multimetal sorption system with simple Langmuir isotherm model, whereas it was Zn^{2+}>Ni^{2+}>Cr^{6+}>Pb^{2+} with multicomponent Langmuirain isotherm model by waste biomass, MB2 (Table 15 a, b and 16). However, with multicomponent Langmuirian isotherm model no significant values were obtained.

Chapter IV

Conclusion

1. The highest equilibrium uptake (q) was observed at 1 g biomass concentration for Zn^{2+} (3.7 mg g^{-1}), Cr^{6+} (3.9 mg g^{-1}), Ni^{2+} (4.2 mg g^{-1}) and Pb^{2+} (6.4 mg g^{-1}) at 25 mg L^{-1}.

2. The metal uptake (q) was increased with an increase of the initial Zn^{2+} and Pb^{2+} concentration up to 50 mg L^{-1} by waste biomass (MB2) in both single and binary metal biosorption system. The metal uptake (q) was higher for Zn^{2+} than those for Pb^{2+} at lower concentrations (5 to 10 mg L^{-1}) that subsequently lowered at higher concentrations (15 to 50 mg L^{-1}) for both single and binary metal biosorption system.

3. The adsorption capacity (K_f) of the waste biomass (MB2) for Zn^{2+} (1.5) was higher than Pb^{2+} (0.8769) in single metal sorption system whereas it was 3.28 and 2.4 respectively in binary metal sorption system.

4. The adsorption capacity (K_f) of the waste biomass (MB2) for the Zn^{2+}, Pb^{2+}, Cr^{6+} and Ni^{2+} was always lower in single (1.50, 0.88, 0.60 and 2.45) metal sorption system than that for the multimetal (0.71, 0.66, 0.55 and 1.55) sorption system.

5. Waste biomass (MB2) biomass possessed maximum sorption capacity (K_f) for Cr^{6+} and Zn^{2+} at pH ranging from 4 to 5 in single and 3 to 4 in multimetal sorption system, while insignificant metal uptake (q) of Pb^{2+} and Ni^{2+} was noticed in both single and multimetal sorption system.

6. The preference of metal sorption was $Cr^{6+}>Pb^{2+}>Zn^{2+}>Ni^{2+}$ in single metal sorption system whereas in multimetal sorption system it was $Zn^{2+}>Cr^{6+}>Ni^{2+}>Pb^{2+}$.

7. At pH between 3 and 4 there was maximum metal uptake (q) of Cr^{6+} (2.70 mg g^{-1}) followed by Zn^{2+} (1.8 mg g^{-1}), Ni^{2+} (1.77 mg g^{-1}) and Pb^{2+} (0.47 mg g^{-1}) from 25 mg L^{-1} of metal containing solution by waste biomass (MB2) possessed in multimetal sorption system whereas at pH 4 the biomass MB2 showed maximum metal uptake of Cr^{6+} (2.43 mg g^{-1}) followed by Zn^{2+} (2.21 mg g^{-1}), Pb^{2+} (2.14 mg

g^{-1}) and Ni^{2+} (1.6 mg g^{-1}) from 25 mg L^{-1} of metal containing solution in single sorption system.

8. The pattern of adsorption capacities (K_f) was found as Ni^{2+} (2.45) > Zn^{2+} (1.50) > Pb^{2+} (0.88) > Cr^{6+} (0.60) in single metal sorption system whereas it was 1.55, 0.71, 0.66 and 0.55 respectively in multimetal sorption system.
9. This study showed that waste biomass (MB2) generated in great quantities can be used for metal uptake in waste water treatment of different metal finishing industries.

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