



Biosorption technology for removal of metallic pollutants-An overview

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Abstract: The main sources of metallic pollutants to the environment are the diffuse sources such as forests and agricultural soils as well as industrial and municipal wastes, which are either discharged directly or transported in to the environment. Various conventional technologies such as chemical precipitation, solvent extraction, ion exchange, membrane separation, electrochemical treatment etc. have been employed to remove metal pollutants from aqueous solution. The exploration of new technologies involves the removal of toxic metals from wastewater with the use of biological adsorption technology. The biosorption is the selective appropriate process for removal of metal ions uptake that may involve the contribution of diffusion, adsorption, chelation, complexation, coordination, or micro-precipitation mechanisms, depending on the specific substrate (biomass). In this overview, the use of the various low cost, easily available and eco-friendly biosorbents used for removal of metallic pollutants from contaminated water and their mechanism are discussed.

Keywords: Biosorption, Metallic pollutants, Algae, Fungi, Bacteria and Agricultural by-product.

INTRODUCTION

Heavy metal pollution in the aquatic system has become a serious threat and it is of great environmental concern as these metals are non-biodegradable. Nowadays, the increasing use of the waste chemical and agricultural drainage systems represent the most hazardous chemical pollution. The most important heavy metals from the point of view of water pollution are Zn, Cu, Pb, Cd, Hg, Ni and Cr (Table 1). Some of these metals (Cu, Ni, Cr and Zn) are essential trace metals to living organisms, but become toxic at higher concentrations. Metals are mobilized and carried into food web as a result of leaching from waste dumps, polluted soil and water.

Heavy metals are groups of pollutants, which are not biodegradable and tend to accumulate in living organisms (Kobya *et al.*, 2005). Release of large quantities of heavy metals into the natural environment has resulted in a number of environmental problems (Hanif *et al.*, 2007). Their removal and recovery of heavy metals from wastewater are more important to protect public health and environment (Francesca *et al.* 2003; Guangyu and Thiruvengkatachari, 2003; Cesar and Marco, 2004 and Chen *et al.*, 2008). Conventional methods such as precipitation, oxidation/reduction, ion exchange, filtration, membranes and evaporation which are in use are extremely expensive or inefficient for metal removal from diluted solutions containing from 1 to 100 mg/L of dissolved metal. In this context, the biosorption process has been evaluated (Volesky, 1990). The biosorption is the ability of biological

materials to accumulate heavy metals from wastewater through metabolically mediated or physicochemical pathways of uptake. (Fourest *et al.*, 1992). Volesky and Schiewer (1999) have studied the uptake of contaminants via various physico-chemical mechanisms by inactive/dead biological materials that are relatively easy to operate and possess several inherent advantages, including low cost, operation over a wide range of conditions and possible reuse of biosorbents. Yun *et al.* (2001) and Yun (2004) have observed that for certain biosorbents in biosorption numerous groups, carboxyl, carbonyl, sulfonate, sulfhydryl, phosphonate, and hydroxyl groups contribute to binding contaminants.

Among the various resources in biological wastes, both dead and live biomass, exhibit particularly interesting metal-binding capacities. Many workers have used different biosorbents, including China clay (Gupta *et al.*, 1992), bentonite, steel-plant slag, fly ash (Ramakrishna and Viraraghavan, 1997), waste slurry (Yun, 2004), bagasse pith, maize cobs, sunflower, peat, orange peel, saw dust, *Corynebacterium glutamicum* (Won *et al.*, 2005 and Crini, 2006), and rice husk (Kumar and Bandyopadhyay, 2006) for the removal of contaminants from wastewater. In recent years, agricultural by-products have been widely used for metal removal from wastewater. These include peat, wood, pine bark, banana pith, soybean, and cottonseed hulls, peanut, shells, hazelnut shell, rice husk, sawdust, wool, orange peel, compost, humic substances, and leaves. The use of dead biomass eliminates the

problem of toxicity and the economics aspects of nutrient supply and culture maintenance.

The advantages of biosorption lie in both the good performance in metal removal, often comparable with their commercial competitors (ion exchangers), and cost-effectiveness, making use of raw materials for fermentation and the agricultural processes. This aspect can play an important role in improving a zero-wastes economic policy especially in case of the re-use of biomasses coming from food, pharmaceuticals, and wastewater treatments. The major advantages of biosorption over conventional treatment methods include low cost, high efficiency of metal removal from dilute solution, minimization of chemical and/or biological sludge, no additional nutrient requirement, regeneration or biosorbent and the possibility of metal recovery (Kratochvil and Volesky, 1998; Radway *et al.*, 2001, Omar, 2002 and 2008).

METALLIC POLLUTION IN ENVIRONMENT AND ITS EFFECTS

Contamination of heavy metals in the environment is of major concern because of their toxicity and threat to human life and the environment. The main sources of heavy metals to the environment are diffuse sources such as forest and agricultural soils as well as industrial and municipal waste, which are either discharged directly or transported in to the environment. Inputs from diffuse sources are mainly the result of old environmental sinks that after decades continue to leach pollutants into waterways via runoff. Hazardous substances from industries are emitted from all stages of the product chain - from the raw material and the production and combustion processes, from the use of products and from the handling of products as waste.

Water pollution due to heavy metals is an issue of great environmental concern. Several small and heavy industries are involved in the activities such as battery and paint manufacturing, petroleum refining, cement and ceramic production, steel production which contribute to the release of significant amounts of metals among other toxic substances into the marine environment and directly affect surface water and quality of sediments. Heavy metals have been used in many different areas for thousands of years. Heavy metal ions such as cobalt, copper, nickel, chromium, lead and zinc are detected in the waste streams from mining operations, tanneries, electronics, electroplating, batteries and petrochemical industries as well as textile mill products. Major lead pollution is through automobiles and battery manufacturers (Adesola Babarinde *et al.*, 2006). The chemically polluted water has seriously damaged the ecology of surface and ground water, which eventually impart serious consequences on agriculture due to

contamination of crops grown in a polluted area (Mufti *et al.*, 1997). Penalties for the discharge of untreated waste water pose large financial pressures on industrialists and treatment of waste water containing heavy metals is becoming most popular and financially sustainable (Jooste, 2000). Many heavy metals and their compounds have been found toxic, while some are also subjected to biomagnifications (Karvelas *et al.*, 2003).

The main source of pollution in sewage is human excreta, with smaller contribution from food preparation washing laundry, surface drainage etc. Domestic wastewater is composed mainly of proteins (40-60 percent), carbohydrates to a range of 25-50 per cent, fats and oils (1.0 per cent), urea derived from urine and a large number of trace organics, which include pesticides, surfactants, phenols, and priority pollutants. The latter category comprises nonmetals (As, Se), metals (Cd, Hg, Pb) and aromatic compounds like, benzene, ethyl benzene, lindane, tetrachloroether, trichloroether (Metchalf and Eddy, 1991). The hexavalent and trivalent chromium is often present in electroplating wastewater (Kratochvil *et al.*, 1998). Other sources of chromium pollution are leather tanning, textile, metal processing, paint and pigments, dyeing and steel fabrication. Lead is used as industrial raw material in the manufacture of storage batteries, pigments, leaded glass, fuels, photographic materials, matches and explosives (Raji and Anirudhan, 1997). The main source of pollution in sewage is human excreta, with smaller contribution from food preparation washing laundry, surface drainage etc (Preetha and Kumar, 2008). Industrial wastewater is heavily loaded with different types of inorganic and organic pollutants, which are discharged through aquatic routes either in water soluble or insoluble forms. In this regard, occurrence of heavy metals in the aquatic system has become a global phenomenon due to their carcinogenic and mutagenic nature (Javaid and Bajwa, 2008). The sad thing about the pollution of the environment with heavy metals is that they can not be biologically degraded, they can only be transformed from one oxidation state or organic complex to another (Jing *et al.*, 2007 and Lone *et al.*, 2008).

Heavy metals have a harmful effect on human physiology and other biological systems when they exceed the tolerance levels (Kobyas *et al.*, 2005). Exposure to lead can cause anaemia, diseases of the liver and kidneys, brain damage and ultimately death (Jain *et al.*, 1989). Besides, chronic exposure to these contaminants present even at low concentrations in the environment can prove to be harmful to the human health. For these reasons, heavy metals must be removed as much as possible from industrial effluents.

Acute lead poisoning in humans may cause severe damage of kidneys, liver, brain and control nervous system leading to sickness or death. The effect of

cadmium poisoning on human are very serious. They include high blood pressure, kidney damage, and destruction of red blood cells (Mustafa and Idris, 2003). The toxicity of metals most commonly involves the brain and the kidney, but other manifestations occur, and some metals, such as arsenic, are clearly capable of causing cancer. An individual with metals toxicity, even if high dose and acute, typically has very general symptoms, such as weakness or headache. This makes the diagnosis of metals toxicity in a clinical setting very difficult unless a clinician has the knowledge and training to suspect the diagnosis and is able to order the correct diagnostic test. Chronic exposure to metals at a high enough level to cause chronic toxicity effects (such as hypertension in individuals exposed to lead and renal toxicity in individuals exposed to cadmium) can also occur in individuals who have no symptoms. Agency for toxic substances and disease registry (ATSDR) (1999a) have reported ninety-nine percent of the lead that enters the adult human body and 33% that enters a child's body is excreted in about 2 weeks. Because of this, lead poisoning is a greater concern in children. Mobilized lead can be redeposited in the soft tissues of the body and can cause musculoskeletal, renal, ocular, immunological, neurological, reproductive, and developmental effects (Todd *et al.*, 1996; and ATSDR, 1999a). Various reproductive effects including decreased pregnancy rate, ovarian damage, testicular damage, testicular atrophy, cellular degeneration, and irregular estrous cycles have been shown in animal studies (ATSDR, 1999a). Copper is an essential substance to human life, but in high doses it can cause anemia, liver and kidney damage, and stomach and intestinal irritation.

Zinc deficiency in the diet may be more detrimental to human health than too much zinc in the diet (ATSDR, 1994). Although the average daily intake of zinc in the United States is 7-16.3 mg Zn/day, the Recommended Daily Allowance (RDA) for zinc is 15 mg Zn/day for man and 12 mg Zn/day for women (ATSDR, 1994). Vomiting, diarrhea, abdominal cramping, and, in some cases, intestinal hemorrhage can occur from long-term exposure to high (i.e. >85 mg/kg/day) doses of zinc. Zinc is not a human carcinogen.

Cadmium is often a byproduct of the extraction of Pb, Zn, and Cu from their respective ores (ATSDR, 1999b). Carbonaceous shale, coal, and other fossil fuels are also a source of Cd. Anthropogenic sources of Cd in the soil and groundwater include the use of commercially available fertilizers and the disposal of sewage sludges as soil amendments (Baker *et al.*, 1979, Garcia *et al.*, 1979, Kosla, 1986, Peles *et al.*, 1998, Gallardo-Lara *et al.*, 1999). EPA has established RfDs for Cd of 0.001 mg Cd/kg/day for food and 0.0005 mg Cd/kg/day for water. These amounts represent an estimated daily oral exposure that

is likely not to cause adverse health effects (EPA, 1994).

BIOSORBENTS USED FOR WASTE WATER TREATMENT

Biosorption of heavy metals by microbial cells has been recognized as a potential alternative to existing technologies for waste streams and natural waters. Biosorbents for the removal of metals mainly come under the following categories: algae, bacteria, fungi, industrial wastes, agricultural wastes and other polysaccharide materials (Table 2). Microorganisms are known to accumulate metals by two distinct processes; (i) bioaccumulation, an energy-dependent process, and (ii) biosorption, an energy independent physical adsorption (Akthar *et al.*, 1995). In general, all types of biomaterials have shown good biosorption capacities towards all types of metal ions.

Algae: The algae have many features that make them ideal for the selective removal and concentration of heavy metals, which include high tolerance to heavy metals, ability to grow both autotrophically and heterotrophically, large surface area/volume ratios, phototaxy, phytochelatin expression and potential for genetic manipulation (Suresh and Ravishankar, 2004). Algae possess high metal binding capacities, due to the presence of polysaccharides, proteins or lipid on the cell wall surface containing functional groups such as amino, hydroxyl, carboxyl, and sulphate, which could act as binding sites for metals (Sheng *et al.*, 2004; Patricia *et al.*, 2006; Vitor *et al.*, 2007; Deng *et al.*, 2007; Olga *et al.*, 2008). The use of algae in biosorption is low as compared to those using other biomass (mainly fungi and bacteria), and the number is still lower for multimetallic systems (Romera *et al.*, 2006). Many studies have shown that the factors influencing biosorption by non-viable algae include heavy metal species (Fourest and Volesky, 1997), pH (Yu and Kaewsarn, 1999), competing ions (Lau *et al.*, 1999) and the types of algae (Radway *et al.*, 2001). Omar (2008) carried out a study on biosorption of copper, nickel and manganese using non-living biomass of marine alga, *Ulva lactuca*. The results of this study indicated that the biomass of *Ulva lactuca* is suitable for the development of an efficient metal removal system for the removal of Cu²⁺, Ni²⁺ and Mn²⁺ from the aqueous solution. Biosorption of these metals on the algal biomass showed a higher affinity for Cu²⁺ compared to Ni²⁺ and Mn²⁺. Al-fawwaz and Wan Maznah (2008) studied that biosorption of copper using live and dead green microalgae isolated from Penang rivers, Malaysia. The copper biosorptive capacity of green microalgae (*Chlorella* sp., and *Scenedesmus* sp.) has been studied to find the differences in copper uptake using live and dead microalgae in aqueous solutions. The study indicated that using dead *Chlorella* and *Scenedesmus* sp. was better than using

Table 1. Water quality standards by the Indian standards for drinking IS 100500-1991, World Health Organization (WHO) and National Agency for Food and Drugs Administration and Control (NAFDAC), Nigeria (Source: Duruibe *et al.*, 2007).

Metals	Indian standards for water quality	Max. acceptable conc. (WHO)	Max. acceptable conc. (NAFDAC)
Zinc	1.5	5 mg/l	5 mg/l
Arsenic	0.05	0.01 mg/l	0.0 mg/l,
Magnesium	-	50 mg/l	30 mg/l
Calcium	-	50 mg/l	50 mg/l
Cadmium	-	0.003 mg/l	0.0 mg/l
Lead	0.1	0.01 mg/l	0.0 mg/l
Mercury	-	0.001 mg/l	0.0 mg/l
Silver	-	0.0 mg/l	0.0 mg/l

live one and the biosorption efficiency for *Chlorella* sp. was better than *Scenedesmus* sp.

Antunes *et al.* (2003) investigated the removal of copper ions from aqueous solutions by *Sargassum* sp. that was conducted in batch conditions and found that the copper uptake capacity (1.48 mmol/g biomass) of *Sargassum* sp. proved to be an excellent biomaterial for accumulating and recovering copper from industrial solutions. Ayla *et al.* (2004) carried out a study on the equilibrium and kinetic modelling of the Biosorption of copper (II) ions on *Cladophora crispata* and concluded that the pH, the initial metal ion concentration and temperature affected the equilibrium uptake of Cu (II) ions to *C. crispata* and the optimum initial pH, the initial copper (II) ion concentration and temperature were determined as 4.5, 200 mg L⁻¹ and 25°C, respectively. The maximum biosorption capacity of algal biomass was determined as 1.03 mmol g⁻¹. A comparison of sorption capacity of *C. crispata* with those obtained in the literature for the adsorption of Cu (II) ions on some other biosorbents and adsorbents also showed that *C. crispata* was more effective for this purpose. Vieira *et al.* (2007) carried out a study on biosorption of lead by the brown seaweed *Sargassum filipendula* – batch and continuous pilot studies and results that were obtained in a continuous system showed a gradual saturation of the biomass in the reactors.

Bacteria: The metal removal ability of microorganisms including bacteria (Lo *et al.*, 1999) has been studied extensively. Microorganisms can affect heavy metal concentrations in the environment because they exhibit a strong ability for metal removal from solution through enzymatic or nonenzymatic mechanisms (Rajendran *et al.*, 2002). According to Beveridge (1989), bacteria make excellent biosorbents because of their high surface-to-volume ratios and a high content of potentially active chemisorption sites such as on teichoic acid in their cell walls. Churchill *et al.* (1995) used two Gram-negative strains *Escherichia coli* K-12 and *Pseudomonas*

aeruginosa and a Gram-positive strain *Micrococcus luteus* to demonstrate biosorption of Cu²⁺, Cr³⁺, Co²⁺ and Ni²⁺. Their sorption binding constants suggested that *E. coli* cells were the most efficient at binding copper, chromium and nickel and *M. luteus* sorbed cobalt most efficiently. The heavy metal removal capacity is higher than those conventional methods and the uptake of heavy metals can be selective (Unz and Shuttleworth, 1996). Microbial cells can also be supplied inexpensively as waste in industrial fermentation processes as well as biological wastewater treatment plants (Volesky and May-Phillips, 1995). Hu *et al.* (1996) worked with *Pseudomonas aeruginosa* strain CSU, a genetically not altered bacterial strain known to bind dissolved hexavalent uranium. *P. aeruginosa* CSU biomass was sorbing significantly more uranium than certain novel, patented biosorbents derived from algal or fungal biomass sources. A good basis for ongoing work in the field of bacterial metal biosorption has been provided by Mann (1990), which remains very attractive and exciting for the study. Most of the experiments done with metals and bacteria have really concerned metabolically mediated bioaccumulation, while the basic principle of biosorption is the use of dead biomass. Leung *et al.* (2000) studied on removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal wastewater. *Pseudomonas pseudoalcaligenes* and *Micrococcus luteus* were found to be capable of removing significant amounts of copper and lead. Kaewchai and Prasertsan (2002) made a study on biosorption of heavy metal by thermotolerant polymer-producing bacterial cells and the bioflocculant. Three strains of thermotolerant polymer-producing bacteria; *Bacillus subtilis* WD 90, *Bacillus subtilis* SM 29, and *Enterobacter agglomerans* SM 38 as well as their bioflocculants were used to investigate on the adsorption of heavy metal, nickel and cadmium. Adsorption of nickel and cadmium by the bioflocculant from the three selected strains was higher than that by the cells.

Fungi: Fungal biomass offers the advantage of having a high concentration of cell-wall material that shows excellent binding (Rajendran *et al.*, 2002). Tomko *et al.* (2006) studied on biosorption of heavy metals by dry fungi biomass. Fungal biosorbents can sorbs heavy metals such as Cu, Al and Sb from aqueous solution and the process is dependent on fungal species, biosorbent size and concentration, solution pH and ionic composition. Differences in biosorption ability found between fungi species tested are interesting. *Agaricus campester* rich the highest heavy metals sorption efficiency, except of Cu. The highest metal uptake (at about 95%) was observed in the case of Sb, except of fungus *Amanita muscaria*. In addition, biosorption by both active and inactivated microorganisms is an important process in the biogeochemical cycling of metals, radionuclides and anthropogenic organics, and should be fully studied in order to understand the source and fates of these chemicals. Wuyep *et al.* (2007) made a study on biosorption of Cr, Mn, Fe, Ni, Cu and Pb metals from petroleum refinery effluent by calcium alginate immobilized mycelia of *Polyporus squamosus*. pH played an important role in the biosorption capability of the immobilized *P. squamosus*, with the fungal biomass having maximum adsorption for cationic metal ions at pH 4 - 6. The results of this investigation could provide a basis for applying the white rot fungi for an environmentally friendly and economically feasible decontamination of pollutants and concluded that *P. squamosus*, a white rot fungus has biosorption capability, by being able to sequester substantial amounts of heavy metals from effluents. Previously, the ability of white rot fungi to degrade recalcitrant pollutants has been demonstrated. This ability is probably largely due their elaborate ligninolytic enzymes. It is difficult to say whether these heavy metals were biodegraded.

Liu *et al.* (2007) made a study on removal of hexavalent chromium by fungal biomass of *Mucor racemosus*: influencing factors and removal mechanism. The optimum pH, biomass dose, initial Cr (VI) concentration and contact time were investigated thoroughly to optimize the removal condition. The metal removal by the biomass was strongly affected by pH and the optimum pH ranged from 0.5 to 1.0. At biomass dose 6 g/l, almost all the Cr (VI) ions were removed in the optimum condition. Higher removal percentage was observed at lower initial concentrations of Cr (VI) ions, while the removal capacity of the biomass linearly depended on the initial Cr (VI) concentration. More than half of Cr (VI) ions were diminished within 1 h of contact and removal process reached a relative equilibrium in approximately 8 h. almost all of the Cr (VI) ions were removed in 24 h when initial concentrations were below 100 mg/l.

Subbaiah *et al.* (2008) studied on biosorption of Cr (VI)

from aqueous solutions using *Trametes Versicolor Polyporus* Fungi. The results of the present study demonstrated that *T. versicolor polyporous* fungi were excellent biosorbent for the removal of chromium ions. The maximum uptake of Cr (VI) ions occurred at pH 4. Increase in the amount of biosorbent increased the percent removal of the metal ions.

Agricultural by-products: Removal of heavy metals from industrial wastewater is of primary importance. Disposal of agricultural by-products such as cassava wastes from processing activities is becoming a concern in Nigeria due to its foul dour. Contamination of water by heavy metals is another serious ongoing problem because of indiscriminate discharges of wastewater containing heavy metals by small and medium-scale industries. Horsfall *et al.* (2003) made a study on removal of Cu (II) and Zn (II) ions from wastewater by cassava (*Manihot esculenta* Cranz) waste biomass.

A number of investigations have been conducted to test the low cost adsorbents for heavy metal ion removal over the last few years. Such alternatives include agricultural biowastes (Park *et al.*, 2008). Pino *et al.* (2006) studied on biosorption of heavy metals by powder of green coconut shell. Several investigations have been carried out to identify suitable and relatively cheap biosorbents that are capable of removing significant quantities of ions. Among the various resources in biological wastes, both dead and live biomass, exhibit particularly interesting metal-binding capacities. In recent years, agricultural by-products have been widely studied for metal removal from water. These include peat, wood, pine bark, banana pith, soybean, and cottonseed hulls, peanut, shells, hazelnut shell, rice husk, sawdust, wool, orange peel, compost, humic substances, and leaves (Chandra *et al.*, 2003 and Tipping, 2002). Olayinka *et al.* (2007) studied on sorption of heavy metals from electroplating effluents by low-cost adsorbents II like use of waste tea, coconut shell and coconut husk and concluded that the use of coconut husk, coconut shell and waste appears to be technically feasible, eco friendly with high efficiency in the removal of heavy metals from wastewaters. Besides, it is composed entirely of agricultural waste and this helps in the reduction of waste generation. They adsorbed comparatively like the expensive traditional activated carbon.

The diffusion of the metals from the bulk solution to active sites of biosorbents predominantly occurs by passive transport mechanisms and various functional groups such as carboxyl, hydroxyl, amino and phosphate existing on the cell wall of biosorbents which can bind the heavy metals. Tree leaves from agricultural operations have generally little or no economic value (Veglio and Beolchini, 1997 and Volesky, 2001). Cost is an important parameter for comparing the sorbent materials (Bailey *et*

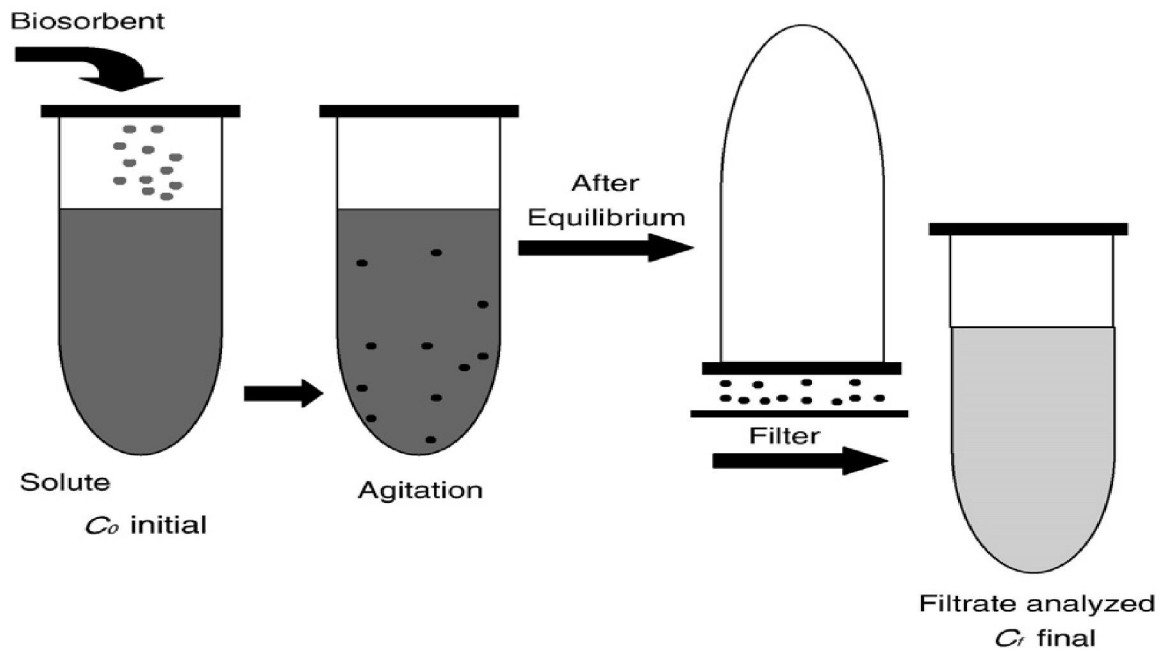


Fig. 1. Schematic diagram of batch biosorption equilibrium experimental procedure (Source: Vijayaraghavan and Yeoung- Sang, 2008).

et al., 1999).

Adsorption has emerged as a cost-effective and efficient alternative for removing heavy metals from low strength wastewaters (Manju and Anirudhan, 1997, Raji and Anirudhan, 1997, Warhusta *et al.*, 1997). Rice husk are an agricultural waste produced in excess of 100 million tons as a by-product of the rice milling industry of which 96% is generated in developing countries. The utilization of this source of biomass would solve some disposal problem as well as access to cheaper materials for adsorption in water pollutants control system (Williams and Nugranad, 2000).

BIOSORPTION MECHANISM

The mechanism of metal biosorption is a complicated process. It is rather a complex process affected by several factors viz. the status of biomass (living or non living), types of biomaterials, properties of metal solution chemistry, ambient/environmental conditions such as pH, influence the mechanism of metal biosorption (Fig. 1.). Most investigations demonstrated that biosorption mechanism for metals is very complicated, and involved in many processes, such as oxidation-reduction (Hosea *et al.*, 1986 and Schiewer, 1999), ion exchange (Kuyucak and Volesky, 1989 and Kapoor and Viraraghavan, 1997), surface complexation (Guibal *et al.*, 1995 and Figueira *et al.*, 1999), co-precipitation (Strandberg *et al.*, 1981; Tsezos and Volesky, 1982, Churchill *et al.*, 1995; Wase and Forster; 1997, Brown *et al.*, 2000, Schneider, 2001; Volesky ;2001 and Pino *et al.*, 2006) adsorption induced by static electricity or enzyme (Ashkenazy *et al.*, 1997) etc.. Additionally, many techniques have been used for the investigation in biosorption mechanism of metals (Sarret

et al., 1999 and Gardea-Torrnsday *et al.*, 2004), such as infra-red spectrum (IR), nuclear magnetic resonance (NMR), transmission electron microscope (TEM), electron energy loss spectrometer (EELS), electron dispersive spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), extended X-ray absorption fine structure (EXAFS), time-resolved laser induced fluorescence spectroscopy (TRLFS), even particle induced X-ray emission (PIXE) analysis (Liao *et al.*, 2008). The metal biosorption mechanism by living cells is a two step process. In the first step, metal ions are adsorbed to the surface of cells by interactions between metals and functional group displayed on the surface of cells. All the metal ions before gaining access the cell wall. The cell wall consists of a variety of polysaccharide and protins and hence offers a number of active sites capable of binding metal ions. Difference in the cell wall composition among the different group of microorganism, viz. algae bactria, cynobacteria and fungi cause significant difference in the type amount of metal ion binding to them. Algal cell walls are mainly cellulosic. The potential metal binding groups in this class of microbes are carboxylates, amines, imizadoles are positively charged when protonated and may build negatively charged metal complexes. The first step, passive biosorption, is metabolism independent and proceeds rapidly by any one or a combination of the following metal binding mechanism coordination, complexation, ion exchange or inorganic microprecipitation. In the second step, due to active biosorption, metal ions penetrate the cell membrane and enter into the cells. Actually, there are many modes of non-active metal uptake by (microbial) biomass. Any

Table 2. Summary of metal removal by different biosorbents.

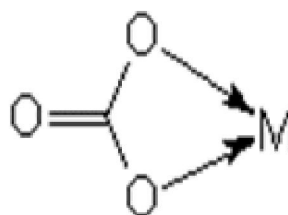
Metals	Biosorbents			References
	Algae	Bacteria, Fungi and Yeast	Agricultural product	
Cu	<i>Chlorella</i> sp., <i>Scenedesmus</i> sp.	<i>Pseudomonas putida</i> , <i>Streptomyces, coelicolor</i> , <i>Sphaerotilus natans</i>	-	Al-fawwaz and Wan Maznah (2008), Uslu and Tanyol, 2006, Öztürk <i>et al.</i> , 2004, Beolchini <i>et al.</i> , 2006 Rezaee <i>et al.</i> , 2006, Green-Ruiz, 2006
Hg	<i>Spirogyra species</i>	<i>Bacillus</i> sp.	-	Loukidou <i>et al.</i> , 2004, Yilmaz and Ensari, 2005, Pino <i>et al.</i> , 2006, Ziagova <i>et al.</i> , 2007
Cd	-	<i>Aeromonas caviae</i> , <i>Bacillus</i> <i>circulans</i> and <i>Pseudomonas</i> sp.	Green Coconut Shell	Hameed, 2006, Uslu and Tanyol, 2006, Choi and Yun, 2004
Pb	<i>Chlorella vulgaris</i>	<i>Pseudomonas putida</i> , <i>Corynebacterium glutamicum</i>	-	Omar, 2008, Öztürk, 2007, Selatnia <i>et al.</i> , 2004, Olayinka <i>et al.</i> , 2007
Ni	<i>Ulva lactuca</i>	<i>Bacillus thuringiensis</i> , <i>Streptomyces rimosus</i>	Waste tea, Coconut Shell and coconut Husk	Incharoensakdi and Kitjaharn, 2002, Apiratikul <i>et al.</i> , 2004
Zn	<i>Caulerpa lentillifera</i>	<i>Aphanothece halophytica</i>	-	Popuri <i>et al.</i> , 2007, Loukidou <i>et al.</i> , 2004, Ziagova <i>et al.</i> , 2007, Olayinka <i>et al.</i> , 2007
Cr⁺⁶	<i>Sphaeroplea</i>	<i>Aeromonas caviae</i> , <i>Pseudomonas</i> sp.	Waste tea, Coconut Shell and coconut Husk	

one or a combination of them can be functional in immobilizing metallic species on biosorbents. A number of anionic ligands participate: phosphoryl, carbonyl, sulfhydryl and hydroxyl groups can all be active to various degrees in binding the metals Volesky (1987). Biosorption can be a solution to clean the environment which is contaminated by heavy metals.

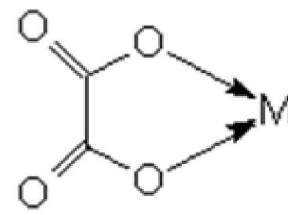
Complexation: The metal removal from solution may also take place by complex formation on the cell surface after the interaction between the metal and the active groups. It has been found to be the only mechanism responsible for calcium, magnesium, cadmium, zinc, copper and mercury accumulation by *Pseudomonas syringae*. Aksu *et al.*, (1992) hypothesized that biosorption of copper by *C. vulgaris* and *Z. ramigera* takes place through both adsorption and formation of coordination bonds between metals and amino and carboxyl groups of cell wall polysaccharides. As noted above complex formation of metal ions with organic molecules involves ligand centres in the organic species i.e. the presence of an atom or atoms having lone pair electrons to donate. Complexation may be electrostatic or covalent and the simplest case is complexation by a mono-dentate ligand such as RNH₂. Micro-organisms may also produce organic acids (e.g., citric, oxalic, gluonic, fumaric, lactic and malic acids), which may chelate toxic metals resulting in the formation of metallo-organic molecules (Ahalya *et al.*, 2003).

Chelation: It has been defined as the firm binding of a

metal ion with an organic molecule (ligand) to form a ring structure. Organic molecules containing more than one functional group with donor electron pairs can simultaneously donate these to a metal atom. This can result in the formation of a ring structure involving the metal atom. The resulting ring structure protects the mineral from entering into unwanted chemical reactions. In general, since a chelating agent may bond to a metal ion in more than one place simultaneously, chelated compounds are more stable than complexes involving monodentate ligands. Stability tends to increase with the number of chelating sites available on the ligand. Thus chelation of metals by donor ligands of biopolymers leads to the formation of stable species. Examples include the carbonate (CO₃²⁻) and oxalate (C₂O₄²⁻) ions (The Chemical Engineers' Resource Page, 2008):



Carbonato complex



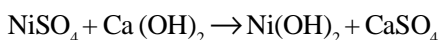
Oxalato complex

Coordination: A coordination complex is any combination of cations with molecules or anions containing free pairs of electrons. 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 (Tsezos and Volesky, 1981 and 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.

Metal atoms have preferences for specific donor atoms (“hard/hard” / “soft/soft”) and the stereochemical arrangements that play an important role in the binding with the available ligands on the microbial cell. Limited information of surface complexation models, based on the theory of surface co-ordination chemistry, is available to describe metal biosorption; however the preferences of the metal species should be considered to explain observed metal biosorption capacities and to elucidate biosorption mechanisms (Tsezos *et al.*, 1995 and Remoudaki *et al.*, 1999).

Ion Exchange: It is a process very similar to biosorption whereby the latter is known to actually function predominantly on the basis of ion exchange. Metal ions from dilute solutions are exchanged with ions held by electrostatic forces on the exchange resin. 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. According to Muraleedharan and Venkobachr (1990) ion exchange reactions are stoichiometric and reversible, and as such they are similar to other solution-phase reactions. For example, in the reaction



The nickel ions of the nickel sulfate (NiSO_4) are exchanged for the calcium ions of the calcium hydroxide $\text{Ca}(\text{OH})_2$ molecule (The Chemical Engineers’ Resource Page, 2008).

Precipitation: Precipitation may be either dependent on the cellular metabolism or independent of it. In the former case, the metal removal from solution is often associated with active defense system of the microorganisms (Ahalya *et al.*, 2003). By the addition of appropriate chemicals, followed by conventional solid-liquid removal that could be by sedimentation, flotation, filtration, in extreme cases even by more expensive centrifugation. Precipitation of metals is achieved by the addition of coagulants such as alum, lime, iron salts and other organic polymers. The large amount of sludge containing toxic compounds produced during the process is the main disadvantage.

Conclusion

The presence of heavy metals in the environment is of major concern because of their toxicity, bio-accumulating

tendency, threat to human life and the environment. However the natural biogeochemical cycles are disrupted due to various human activities like ore mining and industrial processes causing increased deposition of heavy metals in terrestrial and aquatic environment.

Biosorption of heavy metals by microbial cells has been recognized as a potential alternative to existing conventional technologies for waste streams and natural waters. Some biosorbents such as algae, bacteria, fungi and agricultural by-products are examples of biomass tested for metal biosorption with very hopeful results and very effective in accumulating heavy metals. Therefore, biosorption of heavy metals by microbial biomass is being strongly recommended as a powerful technique for the removal of heavy metals from polluted effluents. This will be beneficial not only to the industries, but also to the living organisms and the surrounding environment. The obvious advantages of biosorption process as compared to conventional techniques are their low cost involved when agricultural products and by-products are used. Agricultural products and by-products are an abundant waste materials that may be procured free of cost. The agricultural waste could be used as potential adsorbent for the removal of metals from waste water. Thus, the removal of metals from aqueous solutions using biosorption process can be a solution to clean the environment. This advance technology is not only cheaper and more effective, but also quick and easy to operate.

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