

Microbial Biomass: Combating Heavy Metal Pollution



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Abstract : Heavy metals are widespread pollutants of great environmental concern as they are non-degradable and thus persistent. Conventional technique for treating wastewater is not economical and it also produces large amount of toxic products. So efforts are being made to switch towards microbes for treating heavy metal contaminated wastewater. Metabolically inactive dead biomass due to its unique chemical composition sequesters metal ions and metal complexes from solution. The performance of a biosorbent can further be improved by various physical and chemical treatments. Immobilization techniques help to increase the reusability of microbial biomass for removing heavy metal ions from the wastewater. The objective of this paper is to review the available information on various biosorbents which may help in developing better biosorbents and thus can be used for heavy metal remediation.

Key words : Biosorption, heavy metals, immobilization, metal binding sites.

Introduction

Metal ions are characterized by their mobility in the liquid phase of the eco-system and by their toxicity to higher life forms even at low concentration. In addition these ions are nondegradable and thus persistent, leading to both ecological and health problems. For these reasons, the presence of heavy metal ions in the environment at concentrations above critical values is unacceptable and their removal from the wastewater is of primary importance. Recently, bioadsorbents have emerged as an eco-friendly, effective and low cost material option to physical-chemical processes involved in biosorption which are ion exchange, complexation, coordination, chelation, micro-precipitation, reverse osmosis, evaporation or adsorption. These bioadsorbents include some agricultural wastes, fungi, algae and bacteria. Studies using bioadsorbents have shown that both living and dead microbial cells are able to

adsorb metal ions and offer potential inexpensive alternative to conventional adsorbents (Al-Qodah, 2006, Congeevaram *et al.*, 2007).

Biosorption

Biosorption can be defined as sequestering of organic and inorganic species including metals, dyes and odour causing substances using live or dead biomass or their derivatives. The biosorbing biomass may be bacteria, fungi, algae, and sludge from biological wastewater treatment plants, by-products from fermentation industries, seaweeds or agricultural wastes such as rice husk, rice bran and wheat bran. In other words "Biosorption" is used to indicate a number of metabolism-independent processes (physical and chemical adsorption, electrostatic interaction, ion exchange, complexation, reduction, chelation and microprecipitation) taking place essentially on the cell surface. The biosorption capacity

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of a biomass depends on several factors. It includes type of biomass (species, age), type of sorbates, and presence of other competing ions and method of biomass preparation, along with several physicochemical factors (temperature, pH and ionic concentration) (Aksu and Balibek, 2007).

Biosorbents are prepared from the naturally abundant or waste biomass that have been killed while the biomass is pretreated by washing with acids or bases before final drying and granulation (Cabuk *et al.*, 2005). The exact mechanism by which micro-organisms take up metals is relatively unclear, but it has been demonstrated that both living and non-living fungal biomass may be utilized in biosorptive processes, as they often exhibit marked tolerance towards metals and other adverse conditions such as low pH (Yetis and Ceribasi, 2001).

Fungi as biosorbents

Several papers have been published on the role of fungi in metal biosorption. Generally fungi remove metals by adsorption, chemisorptions (ion exchange), complexation, coordination, chelation, and microprecipitation (Guibal *et al.*, 1995, Kapoor and Viraraghavan, 1997, Sarret *et al.*, 1998). There are also possible oxido-reduction taking place in the biosorbent. When metals are removed by ionic exchange, they generally replace K^+ , Mg^{2+} , Ca^{2+} and H^+ contained in biomass (Akthar *et al.*, 1995, Zhou, 1999).

Many factors affect the biosorption of metals by fungi. Metal sequestrations by fungi are influenced by the mineral and organic composition of the medium in which biomass is produced. Biomass granulometry, physiological state (living or dead), co-ions and physical parameters (temperature, pH, presence of others metals) influence heavy metal removal from polluted waters (Coulibaly *et al.*, 2003). Several fungi have been studied for the biosorption of different heavy metal ions

present in the wastewater and industrial effluent (Table 1). Biosorption characteristics of dried *Rhizopus arrhizus* for the removal of chromium (VI) ions from saline waters were examined as a function of pH, initial metal ion and salt concentration. Lower pH and increased initial chromium (VI) concentration had an increasing effect on biosorption capacity up to 250 mg L^{-1} and increasing the salinity up to 50 g L^{-1} led to a significant decrease in biosorption yield (Aksu and Balibek, 2007).

Even the waste beer yeast *Saccharomyces cerevisiae* has been exploited in biosorbing lead from battery manufacturing industrial effluent. The yeast *Saccharomyces cerevisiae* has been used to remove Cr (VI), Fe (III) (Goyal *et al.*, 2003), Pb (II) (Suh *et al.*, 1998), Cu (II) (Jianlong, 2002), Zn and Ni (Zouboulis *et al.*, 2001) from aqueous solutions. Lead uptake rose from 0.38 mg g^{-1} to 2.34 mg g^{-1} with increase in biosorbent concentration from 0.5% to 2%. Lead uptake decreased slightly when the biosorbent concentration reached 4% (2 mg g^{-1}) (Parvathi *et al.*, 2007). Apart from *Saccharomyces cerevisiae* the biosorption of lead by fungus *Aspergillus versicolor*, *Metarrhizium anisopliae* var. *anisopliae*, and *Penicillium verrucosum* were investigated. Effect of pretreatment on fungus has also been studied. Dimethyl sulfoxide, hydrogen peroxide and gluteraldehyde increased biosorption of Pb^{2+} in comparison with the *Aspergillus versicolor* live biomass. The maximum biosorption capacity of *Aspergillus versicolor* biomass subjected to dimethyl sulfoxide was 30.6 mg g^{-1} (Sari and Tuzen, 2008). *Metarrhizium anisopliae* var. *anisopliae* biomass pretreated with hydrogen peroxide, gluteraldehyde, commercial laundry detergent, dimethyl sulfoxide and formaldehyde significantly improved biosorption of Pb^{2+} in comparison with live biomass. Pretreatment with all methods of *Penicillium verrucosum* increased biosorption of Pb^{2+} in comparison with live biomass.

Table 1 : Fungal biomass used as biosorbent

Organism	Heavy metal ion	Maximum adsorption capacity (mg g ⁻¹)	Reference
<i>Aspergillus carbonarius</i>	Cu(II)	1.72	Gabriel <i>et al.</i> , 2001
<i>Penicillium chrysogenum</i>	Cu(II)	2.73	Gabriel <i>et al.</i> , 2001
<i>Aspergillus terreus</i>	Cr(VI)	96.5	Dias <i>et al.</i> , 2002
	Fe(II)	164.5	
	Ni(II)	19.6	
<i>Aspergillus flavus</i>	Pb(II)	13.46	Akar and Tunali, 2006
	Cu(II)	10.82	
<i>Rhizopus arrhizus</i>	Cu(II)	10.76	Dursun <i>et al.</i> , 2003
<i>Aspergillus niger</i>	Cu(II)	9.53	Dursun <i>et al.</i> , 2003
<i>Aspergillus niger</i>	Cu(II)	23.62	Mukhopadhyay <i>et al.</i> , 2007
<i>Phanerochaete chrysosporium</i>	Cd(II)	15.2	Li <i>et al.</i> , 2004 Li <i>et al.</i> , 2004 Yetis and Ceribasi, 2001
	Pb(II)	12.34	
	Ni(II)	55.9	
<i>Polyporus squamosus</i>	Fe(II)	153.04	Wuyep <i>et al.</i> , 2007
	Ni(II)	23.72	
	Cu(II)	31.6	
	Pb(II)	26.52	
<i>Rhizopus arrhizus</i>	Cr(VI)	31	Tobin <i>et al.</i> , 1984
	Cd(II)	30	Tobin <i>et al.</i> , 1984

For copper biosorption several wood rotting basidiomycete fungi have been studied where copper biosorption was maximum for *Oudemansiella mucida* (8.77 mg g⁻¹ dry wt), *Lepista nuda* (6.29 mg g⁻¹), *Pycnoporus cinnabarinus* (5.08 mg g⁻¹) and *Pleurotus ostreatus* (4.77 mg g⁻¹) (Gabriel *et al.*, 2001). Apart from these the mycelia of various fungi, including *Phycomyces*, *Absidia*, *Aspergillus*, and *Mucor* sp., were reported by different authors to be able to remove heavy metals from aqueous solutions.

Lot of work have been done to study the effect the pH on biosorption process. In *Aspergillus niger*, originated from citric acid fermentation industry, the maximum lead biosorption capacity obtained at pH 6 when estimated with the Langmuir model was 93

mg g⁻¹ dry biomass (Spanelova *et al.*, 2003). In case of baker yeast, copper biosorption is maximum in the range of 2-6. At higher pH copper ions precipitated due to the high concentration of OH⁻ ions in the adsorption medium (Goksungur *et al.*, 2003). Effect of temperature on biosorption experiment is also important. In baker yeast the biosorption capacity decreased from 9.8 to 9.3 mg g⁻¹ at an initial nickel (II) ion concentration of 400 mg L⁻¹, while at a lower initial concentration of 100 mg L⁻¹, it decreased from 8.2 to 4.9 mg g⁻¹, as the temperature was increased from 27°C to 60°C. The equilibrium data fit better to the Freundlich and Redlich–Peterson isotherm models compared to the Langmuir model in the concentration range studied (10–400 mg L⁻¹) (Padmavati, 2008).

It has been reported in the literature that the presence of certain cations, such as Na^+ and Ca^{2+} ions in the native biomass may affect the extent of biosorption. Hence, pretreatment of the biomass has been carried out to get a more homogeneous biomass replacing all other ions by a single cation having a higher metal uptake capacity. It has been observed by the authors also that protonated biomass had a higher sorption capacity than native deactivated biomass (Padmavathy *et al.*, 2003, Schiewer and Volesky, 1995, Yu and Kaeswarn, 2000). Detergent, sodium hydroxide and dimethyl sulfoxide pretreatments enhanced the biosorption capacity of biomass in comparison with the heat inactivated biomass. Alkali pretreatment can increase the biosorption of heavy metals due to the fact that it may destroy autolytic enzymes causing putrefaction of biomass, remove lipids and proteins that mask binding sites and could release certain biopolymers from the cell wall that have a high affinity towards heavy metal ions (Yan and Viraraghavan, 2000). Since commercial detergents contain alkali, detergent pretreatment may be similar to an alkali pretreatment however pretreatment causes biomass losses. It is more or less compensated by the increase in biosorption potential. Pretreatment of the biomass with detergent, sodium hydroxide and dimethyl sulfoxide enhanced the biosorption capacity of *Aspergillus flavus* (Akar and Tunali, 2006). *Penicillium lilacinum* can accumulate a large quantity of uranium from solution containing uranium only, but selective uranium absorption from the mixed solution is comparatively low (Nakajima and Sakaguchi, 1986).

Bacteria as biosorbents

The use of bacterial sorbents in effluent treatment plants may be competitive compared to traditional technologies: the very small size of bacterial particles, which permits close contact between the biosorbent and the solution, and the availability of cheap raw

materials for the production of large amounts of biomass represent advantages with respect to chemical physical sorbents. *Brevibacterium* (strain PBZ) free or immobilized cells are able to remove lead, copper and cadmium from solution (Vecchio *et al.*, 1998).

Just as in case of fungi, temperature affects the biosorption process in bacteria. *Thiobacillus ferrooxidans* is an organism that has a medium-high capacity for copper adsorption ($q_{\text{max}} = 119.54 \text{ mg of Cu g}^{-1}$ of dry biomass) when pretreated with NaOH and at a temperature of 25°C . However, when the operation is carried out at 37°C , it has a greater capacity than *Bacillus subtilis*, which has been reported of having the highest copper biosorption capacity (Manriquez *et al.*, 1997).

The pH of the solution has a significant effect on the capacity for copper biosorption. This is due, principally, to the protonization that occurs at low values of pH and to the effect it has on the chemistry of the solution (Manriquez *et al.*, 1997). The pH has a strong effect on metal biosorption as optimal pH for copper and zinc biosorption by *Pseudomonas putida* is 5-6 and 7-7.5 respectively (Pardo *et al.*, 2003).

Biosorption of heavy metals by gram-positive, non-pathogenic and non-toxicogenic *Paenibacillus polymyxa* P13 which secrete EPS (exopolysaccharide) was evaluated. EPS production was associated with hyperosmotic stress by high salt (1 M NaCl), which led to a significant increase in the biosorption capacity of whole cells ($q_{\text{max}}=150 \text{ mg g}^{-1}$ of Cu) as compared to whole cells ($q_{\text{max}}=112 \text{ mg g}^{-1}$ of Cu (Acosta *et al.*, 2005).

Sometimes organic chemicals affect the biosorption process. Citrate had a stimulating effect on the bacterial sorption of chromium by *Pseudomonas aeruginosa* but had no detectable effect on *Bacillus thuringiensis* (Hassen *et al.*, 1998). Several bacteria have been studied for their role in biosorption of heavy metals (Table 2).

Table 2 : Bacterial biomass used as biosorbent

Organism	Metal ion	Maximum adsorption capacity (mg g ⁻¹)	Reference
<i>Bacillus licheniformis</i>	Cr(VI)	60.5	Zhou <i>et al.</i> , 2007
<i>Citrobacter freundii</i>	Pb(II)	59.4	Al-Garni, 2005
<i>Klebsiella pneumoniae</i>	Pb(II)	62.5	Al-Garni, 2005
<i>Thiobacillus thiooxidans</i>	Cu (II)	39.84	Liu <i>et al.</i> , 2004
<i>Staphylococcus xylosum</i>	Cr(VI)	143	Ziagova <i>et al.</i> , 2007
	Cd (II)	250	
<i>Pseudomonas sp.</i>	Cr(VI)	95	Ziagova <i>et al.</i> , 2007
	Cd (II)	8278	
<i>Bacillus circulans</i>	Cd(II)	26.5	Yilmaz and Ensari, 2005
<i>Pseudomonas aeruginosa</i>	Ni(II)	264.69	Sar <i>et al.</i> , 1999
<i>Bacillus thuringiensis</i>	Ni(II)	34.3	Ozturk, 2007
<i>Chroococcus</i>	Cr(VI)	21.36	Bala <i>et al.</i> , 2007a
<i>Enterobacter sp. J1</i>	Pb (II)	50.9	Lu <i>et al.</i> , 2006
	Cu (II)	32.5	
	Cd (II)	46.2	
<i>Ochrobactrum anthropi</i>	Cr(VI)	86.2	Ozdemir <i>et al.</i> , 2003
	Cd(II)	37.3	
	Cu(II)	32.6	
<i>Zoogloea ramigera</i>	Cu(II)	35	Sag and Kutsal, 1995
	Pb(II)	85	
	Fe(III)	67	
	Ni(II)	54	

Algae as biosorbents

A number of algal species have been reported for biosorption. The brown alga *Sargassum* has been shown to possess the required mechanical properties, chemical affinity and sorption capacity (Fourest and Volesky, 1997, Davis *et al.*, 2000; Davis *et al.*, 2003) to bind metals such as Pb, Cu, Hg and Cd in an effective, reversible and cost-effective manner (Davis *et al.*, 2004).

The amount of the adsorbed metals at maximum weights differed from alga to alga, presumably owing to difference in their chemical composition. The red alga investigated (*Laurencia obtusa*) showed a completely different behavior in its biosorption of heavy metals compared with the brown algae. This is due to the different structures of

the cell wall polysaccharides (Hamdy, 2000). Some algae show remarkable capability to adsorb metal ions from aqueous solution, and this has opened up the possibility of their use in treatment of metalliferous wastewaters (Mehta and Gaur, 2005). Algae have often displayed better metal ion removal efficiency and economic feasibility than commercial ion-exchangers. For example, *Sargassum natans* and *Ascophyllum nodosum* outperformed ion exchange resins in sequestering Au²⁺ and Co²⁺ respectively from solution. Large-sized algae are better suited for the biosorption purpose than unicellular algae. Seaweeds can efficiently remove heavy metal ions and radionuclides from aqueous solutions (Kuyucak and Volesky, 1988, Singh *et al.*, 2007).

The cell walls of brown algae generally contain three components: cellulose, the

structural support; alginic acid, a polymer of mannuronic and guluronic acids and the corresponding salts of sodium, potassium, magnesium and calcium; and sulphated polysaccharides. As a consequence, carboxyl and sulphate are the predominant active groups in this alga. Red algae also contain cellulose, but their interest in connection with biosorption lies in the presence of sulphated polysaccharides made of galactanes (agar and carragenates). Green algae are mainly cellulose, and a high percentage of the cell wall are proteins bonded to polysaccharides to form glycoproteins. These compounds contain several functional groups (amino, carboxyl, sulphate, hydroxyl etc) which could play an important role in the biosorption process (Romera *et al.*, 2007).

Many workers have also investigated the performance of fresh water green algae *Chaetophora elegans*, *Scenedesmus*, *Chlorella*, *Spirogyra*, *Chlamydomonas globas*, *Zygnema* species, etc. for the removal various heavy metal ions (Ni, Cu, Pb, Zn, Cd, and Al) from wastewater. The biosorption characteristics of cadmium and lead ions were determined with brown algae, *Macrocystis pyrifera*, *Kjellmaniella crassifolia* and

Undaria pinnatifida. Several algae have been studied for their role in biosorption of heavy metals (Table 3).

Immobilization

From the laboratory trials reported it can be concluded that microbial biomass has potential to be used at industrial scale for biosorption. In order to maintain efficiency of microbial biomass to adsorb metal during continuous industrial process, it is imperative to immobilize microbes using a suitable matrix as free biomass is highly susceptible to disintegration.

Biosorption by algal biomass has been used as an alternative for the remediation of wastewaters containing toxic metals. However, the fragile nature of algal biomass is not suitable for robust wastewater treatment operations (Chu *et al.*, 1997). This has led to the interest in the use of entrapped biomass as immobilized preparations. Several immobilization media, such as alginates, carrageenans and polyacrylamide gel have been used for this purpose (Robinson, 1998).

Natural polymers such as cellulose derivatives, alginate, chitosan and chitin have been mostly used as the matrix for the

Table 3 : Algal biomass used as biosorbent

Organism	Metal ion	Maximum adsorption capacity (mg g ⁻¹)	Reference
<i>Spirogyra neglecta</i>	Cd(II)	27.95	Singh <i>et al.</i> , 2007
	Pb(II)	90.19	
<i>Pithophora oedogonia</i>	Cu(II)	23.08	Singh <i>et al.</i> , 2007
	Pb(II)	71.13	
<i>Cladophora calliceiema</i>	Pb(II)	40.5	Singh <i>et al.</i> , 2007
<i>Ulva lactuca</i>	Pb(II)	34.7	Sari and Tuzen, 2008
	Cd(II)	29.2	
<i>Fucus spiralis</i>	Cd(II)	114.9	Romera <i>et al.</i> , 2007
	Ni(II)	50	
<i>Chondrus crispus</i>	Pb(II)	204.1	Romera <i>et al.</i> , 2007
<i>Lyngbya putealis</i>	Cr(VI)	113.6	Bala <i>et al.</i> , 2007b

immobilization of microbial cells via entrapment. Immobilization of fungal cells in these polymer supports could also enhance fungal cell performance and adsorptive capacity of the biosorbent system for the heavy metal ions. The choice of immobilization matrix is a key factor in environmental application of immobilized biomass. The polymeric matrix determines the mechanical strength and chemical resistance of the final biosorbent particle, which is to be utilized for successive biosorption–desorption process. The most extensively investigated biopolymer in bioremediation studies is CMC (carboxymethylcellulose) (Arica and Bayramoglu, 2005).

Immobilization efficiency is strongly affected by the matrix porosity. Immobilization efficiency varied from 19.6 to 92.5% and the highest values were attained with the lowest matrix porosity independent of the dispersion medium used. This indicates that the selected matrix optimizes both the surface area, which restrains biomass, and the porosity that affects the transport of metal ions and nutrient throughout the supporting structure (Dias *et al.*, 2002).

The free and immobilized *Lentinus sajor-caju* has been successfully used as the biosorbing agent for removal of Cr (VI) ions from aqueous medium. The biosorption of Cr (VI) ions on the both biosorbents depends on the experimental conditions particularly medium pH and the concentration of Cr (VI) ions in the medium (Arica and Bayramoglu, 2005).

In the biomass of brown alga *Sargassum*, alginate was found to be the main component responsible for cadmium biosorption. It was reported that alginate was present in a gel form in the cell wall, which appears very porous and easily permeable to small ionic species (Goksungur *et al.*, 2003). The amount of Cr (VI) ions adsorbed on the immobilized *Polyporus squamosus* beads was 42.8 ± 1.52

mg g⁻¹ (non-treated) and 34.4 ± 1.52 mg g⁻¹ (treated). Immobilized Ca alginate also possess significant advantage over non-immobilized alginate, especially when Ca²⁺, Mg²⁺, and K⁺ were present as ion-exchange resins (Wuyep *et al.*, 2007).

Due to its ability to form stable structures, cross-linked alginate has been used for the immobilization of biological material for various purposes, including the removal of heavy metal from wastewater. The mechanism of divalent metal sorption on alginate beads is dominated by ion exchange involving mainly the carboxyl groups present in the alginate molecule, with hydroxyl groups playing a secondary role. Due to the differences in conformation of polyglucuronic and polymannuronic blocks, all carboxyl groups are not readily available, resulting in differences in the sorption capacity, with respect to different metals. Metals with low bonding strength (e.g. Cd²⁺) are mainly bound by polymannuronic chain carboxyls and are greatly influenced by changes in pH, while metals with higher bonding strength such as Pb²⁺, show greater affinity, they are not affected by cation exchange with protons in the solution and are adsorbed homogeneously by all available carboxyl groups. Equilibrium sorption experiments showed that the sorption of Cu²⁺, Cd²⁺ and Pb²⁺ by alginate beads from *Laminaria digitata* were greater than commercial alginate beads and other sorbents (Papageorgiou *et al.*, 2006).

Immobilized yeasts (IY) and immobilized caustic-treated yeast (ICY) could be reactivated and reused in a manner similar to ion-exchange resins. Yeast biomass immobilized in alginate reduced the quantity of heavy metal binding to the biomass, as compared to biosorption by native yeast, by about 10-25%. Caustic-treated yeast immobilized in alginate enhanced the quantity of metal biosorption. Heavy metal biosorption on the immobilized caustic-treated yeast was temperature independent at low initial metal

concentration. On the other hand, the initial pH of the heavy metal solution affected the metal-removal efficiency significantly owing to cation competition with the H⁺ ion in drastic pH regions (Lu and Wilkins, 1996). Immobilized live stationary-phase *Zoogloea ramigera* in calcium alginate improved the sorption of Cu²⁺ in flow-through columns over that attained by alginate alone (Unz and Shuttleworth, 1996). It was revealed that specific uptake of Pb (II) ion by both biomass-immobilized beads and calcium-alginate beads without biomass gradually increased from 50 to 500 mg L⁻¹ metal ion solution. In the case of immobilized beads containing biomass values of q_e (metal uptake at equilibrium) were 5.2 mg g⁻¹ and 49.75 mg g⁻¹ respectively at 50 mg L⁻¹ and 500 mg L⁻¹. When only calcium alginate beads were used for the experiment, values were 2.45 mg g⁻¹ and 22.5 mg g⁻¹ respectively at 50 mg L⁻¹ and 500 mg L⁻¹ initial metal ion solution (Paul *et al.*, 2006).

Chelating ligands have been functionalized in several support materials, including commercially available XAD resin series. Amberlite XAD are Adsorber resins (polystyrene divinyl benzene) widely used to develop several chelating materials for preconcentration procedures due to its good physical and chemical properties such as

porosity, high surface area, durability, and purity. Amberlite XAD-4 has been often used as a solid sorbent to prepare a ligand-loaded resin. Recently, the XAD-4 resin, impregnated with many compounds as complexing agents has been used for preconcentration of heavy metals (Dogru *et al.*, 2007). Another component used in immobilization is PVA (polyvinyl alcohol). PVA offers various advantages over the conventional immobilization methods, such as low cost, high durability and chemical stability and non-toxicity to viable cells. Up to now, several methods of immobilization using PVA have been reported. Among them, PVA-boric acid method was widely used and activated sludge was immobilized. However, this method has a problem of hydration of immobilized biomass PVA-containing hydroxyl group can also adsorb heavy metals. In order to investigate the adsorption capacity of PVA alone, PVA bead without alginic acid was made. From the experiment result, it was found that PVA bead have adsorption capacity of 30 mg g⁻¹ Pb dry mass at pH 4 (Jeon *et al.*, 2002). This result shows that the lead ion was adsorbed mainly on alginic acid. List of various immobilization matrices used for remediation of heavy metals are given in Table 4.

Table 4 : Immobilization techniques used for study of metal biosorption

Organism	Metal removed	Immobilization matrix	Reference
<i>Laminaria digitata</i>	Cu, Cd, Pb	Alginate beads	Papageorgiou <i>et al.</i> , 2006
<i>Citrobacter</i>	Cd, Pb	Polyacrylamide gel	Macaskie and Dean, 1989
<i>Spirulina platensis</i>	Cu, Fe, Zn	Calcium alginate	Volesky, 1984
<i>Pseudomonas aeruginosa</i>	U	Polyurethane	Hu and Reeves, 1997
<i>Phormidium laminosum</i>	Pb, Cd	Polysulfone	Blanco <i>et al.</i> , 1999
<i>Pseudomonas fluorescens</i>	Pb	PVC	Tengerdy <i>et al.</i> , 1981
<i>Chlorella sorokiniana</i>	Pb	Luffa cylindrica	Akthar <i>et al.</i> , 2004
<i>Aspergillus oryzae</i>	Cd, Cu, Fe, Mn, Pd	Reticulated foam	Kiff and Little, 1986

Metal binding sites

Studies on the mechanism of biosorption of heavy metals on fungi show that fungal cell walls mainly consist of polysaccharides, proteins and lipids have many functional groups that are responsible for the binding of metals. It has been reported that when heavy metals become tightly bound to acid groups in the side chains of amino acids on the cell surface, salt linkages are thus broken and the proteins are dissolved from the cell wall (Kapoor and Viraraghavan, 1997, Akthar *et al.*, 1996)

Bacillus subtilis was found to possess carboxyl, phosphate, hydroxyl amino functional groups in the cell wall. *B. subtilis* is gram-positive aerobic species whose surface is uncharged below pH 2.2 but becomes increasingly negatively charged at higher pH values. Bacterial cell walls are negatively charged under pH conditions and the cell wall chemical functional groups such as carboxyl groups display a high affinity for metal ions in solution (Dogru *et al.*, 2007).

The cell surface becomes positively charged at low pH values which decrease the attraction between metal ions and the functional groups on the cell wall, whereas the cell surface becomes negatively charged at high pH values, increasing the attraction until a maximum is reached at around pH 7. For pH values higher than the optimum values, the retention decreases again due to the competition between the hydroxylated complexes of the metal and active sites of the cell (Dogru *et al.*, 2007). Another important factor of biosorption in real wastewater is the content of organic materials. They can interact with heavy metal ions by chelation and complexation reaction so that it can cause the uptake capacity to decrease (Jeon *et al.*, 2005).

The metal binding ability of the *Escherichia coli* cell wall has been studied in detail. The specific native groups of the outer membrane (OM) participating in metal binding are the polar head groups of phospholipids

acting mainly at the inner layer of the OM and the acidic groups of the exposed (hydrophilic) polypeptides and at the outer half of the OM LPSs (lipopolysaccharides). The LPSs provide both carboxyl and phosphoryl groups as ligands. However, only the latter group is responsible for the high affinity of divalent metal ions for LPS. The peptidoglycan (PG) layer of *E. coli* binds metal ions via the carboxyl group of the D-glutamic acid of the peptide stem and the hydroxyl groups of the glycan backbone. The two-step deposition process may increase the apparent metal binding capacity of PG and would include the stoichiometric binding of metal ion, generating a nucleation site for the subsequent precipitation of metal above the stoichiometric amounts (Kotrba *et al.*, 1999).

It has been reported that the incorporation of Cys-Gly and Cys-Cys-Gly motifs developed high affinity to Cd^{+2} and Hg^{+} (Pazirandeh *et al.*, 1998). The mechanism of biosorption is ascribed to a multitude of ligands and depends on the chemical behaviour of the particular metal ion (Weppen and Hornburg, 1995). There have been reports which indicate the importance of phosphate for efficient metal binding by cells of *B. thuringiensis*. This observation can be interpreted mainly as an effect of phosphate functional groups on medium pH as well as on cell wall composition and consequently, metal biosorption capabilities of biomass (Bontrone *et al.*, 2000, Collins and Stotzky, 1996, Lang *et al.*, 1982). The presence of the two potassium phosphates in the medium at their optimized concentrations resulted in a buffered medium with a pH of 6.9, which magnifies net negative charge on the outer surfaces of Gram-positive bacterial walls (Doyle, 1989). It is also known that teichoic acid is a major cell wall component in Gram-positive bacteria (Papachristou, 1984). The presence of ample phosphate levels during the growth of *Bacillus* cells also results in high density of the electronegative phosphate groups situated between the glycerol polymers of the teichoic acid (Amer *et al.*, 2000).

Developmental regulation of gene expression in the genus *Bacillus* involves a number of two component signal transduction systems that act by phosphate transfer. Among the most well understood two-component regulators in *B. subtilis* and *B. thuringiensis* are the PhoP-PhoR (Hulett, 1996) and KinA-Spo0A/Spo0F (Hoch, 1993, Malvar *et al.*, 1994) systems. They respond to nutrient depletion by autophosphorylation of the sensors (histidine kinases) followed by transfer of phosphate to their downstream cognates, which are transition state regulatory proteins (Amer *et al.*, 2000).

At a low pH biosorption is increased in *Bacillus licheniformis*. First as the pH decreases, the overall surface charge of cell surface of *Bacillus licheniformis* becomes positive and the surface of sorbent would also be surrounded by the hydronium ions, which could enhance the chromium (VI) interaction with binding sites of the biosorbent by greater attractive forces. As the pH increases, the overall surface charge on the cells will become negative, resulting in a decrease of anionic Cr (VI) biosorption (Zhou *et al.*, 2007).

The reason why some biomass show high affinity for a given metal and low sorption capacity, or *vice versa*, may be related to the degree of affinity of a specific biomass for each metal. Although the total amount of metal anchored on its surface will also depend on the number of active sites present and on how easily they can be accessed (Hashim and Chu, 2004, Romera *et al.*, 2007).

Engineering biosorbents

Immobilization of heavy metals into biomass or precipitation through reduction to lesser bioactive metal species, such as metal sulfide is the major mechanism employed by the microbes to counteract heavy metal toxicity. These natural mechanisms can be easily exploited to optimize biosorbents that are more efficient for heavy metal removal. In one

example, a sulfide-dependent metal removal strategy was developed by engineering the sulfate reduction pathway into *E. coli*. Under anaerobic condition the resulting strains produced significantly more sulfide and removed more than 98% of the available cadmium. Further improvement in metal precipitation was achieved by engineering effective sulfate reduction under aerobic conditions. *E. coli* expressing both serine acetyltransferase and cysteine desulfhydrase overproduced cysteine and converted it to sulfide (Wang *et al.*, 2000, Bang *et al.*, 2000). The resulting strain was effective in aerobically precipitating cadmium. This aerobic approach of metal precipitation is particularly attractive as large-scale processes could be implemented under aerobic conditions. However challenges are to incorporate these genetic modifications into a robust environmental microbe that could survive and thrive under the required operation conditions.

Similar success in engineering enhanced biosorbents has been achieved by displaying metal-binding peptides onto the cell surface. One example was reported by creating a repetitive metal-binding motif consisting of (Glu-Cys)_nGly (Bae *et al.*, 2000). These peptides emulate the structure of phytochelatins, metalchelating molecules that play a major role in metal detoxification in plants and fungi. The phytochelatin analogs were presented on the bacterial surface, enhancing Cd⁺² and Hg⁺² bioaccumulation by 12-fold and 20-fold respectively. These novel binding peptides offer the potential of improved affinity and selectivity for heavy metals.

In addition to peptides, metalloregulatory proteins are another group of useful metal-binding moiety with striking affinity and specificity. The highly specific nature of these proteins is the result of a cleverly designed genetic circuit that is tightly under their control. Examples are MerR and ArsR, which are regulatory proteins used for controlling the

expression of enzymes responsible for mercury and arsenic detoxification, respectively. The high affinity and selectivity of MerR toward mercury has been exploited for the construction of microbial biosorbents specific for mercury removal. Presence of surface-exposed MerR on an engineered strain enabled increased Hg²⁺ biosorption. Similarly, cells overexpressing ArsR accumulated higher levels of arsenate (Kotsal *et al.*, 2004).

However higher organisms respond to the presence of heavy metals with the production of cysteine-rich peptides such as glutathione (GSH) (Singhal *et al.*, 1997), phytochelatins (PCs), and metallothioneins (MTs) (Mehra and Winge, 1991) that bind metal ions (such as cadmium, lead, mercury, copper) and sequester them in biologically inactive forms (Hamer, 1986, Stillman *et al.*, 1992). Overexpression of MTs in bacterial cells results in enhanced metal accumulation and thus offers a promising strategy for the development of microbial-based biosorbents to remediate metal contamination (Kille *et al.*, 1991, Pazirandeh *et al.*, 1995, Romeyer *et al.*, 1990).

Recently, a gene fusion system consisting of the signal sequence and the first nine amino acids of lipoprotein (Lpp) joined to a transmembrane domain from outer membrane protein A (OmpA) has been used successfully to anchor a variety of proteins and enzymes onto the cell surface. It has been demonstrated that *E. coli* strains with EC20 (20 cysteines) displayed on the cell surface using the Lpp-OmpA fusion system accumulated a substantially higher amount of cadmium than the wild-type cells (Francisco *et al.*, 1992, Richins *et al.*, 1997).

The soil bacterium has been engineered to survive in contaminated environments for a long period of time. For these *Pseudomonas* and *Moraxella* have proved to be effective. It has been recently demonstrated that expression of organophosphorous hydrolase on the cell surface was more efficient in

Moraxella sp. than in *E. coli* using the ice nucleation protein anchor (INPNC anchor). The resulting recombinant strains accumulated more than 10-fold higher mercury than the wild-type *Moraxella* sp. For expression of INPNC-EC20 in *Moraxella* sp., plasmid pPNC20, carrying the *inpnc-ec20* fusion was constructed by inserting the INPNC-EC20 fragment into a shuttle vector pVLT33. Expression of the INPNC-EC20 fusion was under control of a *tac* promoter.

Similarly genetically engineered *E. coli* JM109, expressing cadmium transport system and metallothionein (MT) have shown increased accumulation of cadmium from the aqueous solution (Deng *et al.*, 2007). These strategies suggested the application of genetic engineering technique in the treatment of heavy metal wastewater would be better alternative to conventional methods.

Feasibility of biosorption technology

The feasibility of applying the biosorption technology into wastewater detoxification would be best assessed on a stage-wise approach. The highest priority for biosorption venture would be approximate assessment of the commercial potential and feasibility of application of the new technology based on the family of new biosorbent products. So, the preliminary stages should consist of some basic studies:

Study and assessment of competing technologies

The study of conventional process for metal removal should be done and its current costs and market share have to be summarized and assessed. The process alternatives like precipitation, reverse osmosis, ion-exchange etc can be considered for a more detailed evaluation and feasibility assessment.

Study of market size

Although it is known that environmentally-based market for metal removal from effluents

is enormous, but it is essential to know the actual figures for convincingly launching company enterprise. A quantitative review of the potential of biosorption process need to be carried out for different places where application of biosorption technology would be considered. The cost difference between the conventional and the new technology would establish the feasibility of biosorbent applications and their competitiveness in the market place.

Study and assessment of costs of new biosorbents

Approximate costs of different types of raw biomass needs to be ascertained, as well as the costs of processing the biomass into applicable biosorbent materials. Estimation of the costs of processing and treatment of raw biomass to prevent its degradation needs to be carried out for selecting representative type of biomass. All these preliminary assessment should be carried out as part of better quantitative estimation of the venture feasibility (Volesky, 2003).

Conclusions

The process of biosorption has many attractive features including the selective removal of metals over a broad range of pH and temperature, rapid kinetics of adsorption and desorption and low capital and operation cost. Biosorbent can easily be produced using inexpensive growth media or obtained as a by-product from industry. It is desirable to develop biosorbents with a wide range of metal affinities that can remove a variety of metal cations. These will be particularly useful for industrial effluents, which carry more than one type of metals. To attract more usage of biosorption, it is necessary to develop strategies so that the biosorbent can be reused. Biosorption thus, can be used for combating heavy metal pollution in aqueous system.

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