BIOSORPTION OF HEAVY METALS BY PSEUDOMONAS BACTERIA

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ABSTRACT

Heavy metals are discharged into the environment through natural and anthropogenic activity, creating serious environment problems. Biosorption is an effective method used for removing heavy metals from environment. In the present study, spirogyra has been examined for biosorption for the removal of heavy metals from ground water. The biosorption capacity was determined from atomic absorption spectroscopy.

Key words: Biosorption, Pseudomonas, Heavy metal, AAS

1. INTRODUCTION

The presence of heavy metals in aquatic environments is known to cause severe damage to aquatic life, beside the fact that these metals kill microorganisms during biological treatment of wastewater with a consequent delay of the process of water purification. Most of the heavy metal salts are soluble in water and form aqueous solutions and consequently cannot be separated by ordinary physical means of separation.

Biosorption is one of the most effective and cost-efficient approaches for removal of heavy metal. Biosorption the functional groups, which play a dominant role in the removal of various heavy metal contaminants [Farook et al., 2010]. These biosorbents typically include algae, fungi, rice and wheat straw, hyacinth, pine bark, tea waste, starch, agricultural by-products and microbes [Pehilvan, 2009]. For ex- ample some researchers studied the effect of sawdust [Larous, 2005], rice husk [Chuah, 2005] and pomegranate peel [Elashtarkhy, 2008] for removal of copper (II) and lead (II) from aqueous solution.

Biosorption of heavy metals by microbial cells has been recognized as potential alternative to existing technologies for recovery of heavy metals from industrial waste streams. Most studies of biosorption for metal removal have involved the use of either laboratory-grown microorganism or biomass generated by the pharmacology and food processing industries or wastewater treatment units. Biological methods such as biosorption/ bioaccumulation for the removal of heavy metal ions may provide an attractive alternative to physico-chemical methods.

Biosorption is observed to be carried out with a fast surface reaction followed by slow uptake of the metal. Surface adsorption occurs in the first step there after starts the diffusion of metal ions in the cellular structures. *Acanthopora spicefora*, a red algal species, showed immense biosorption activity against a series of heavy metals, such as, Cr (VI), Cr (III), Hg (II), Cd (II) and Pb (II).Seaweeds, fungi and bacteria can also act as biosorbent for certain heavy metals. Seaweeds, due to their macroscopic nature are considered as a convenient heavy metal adsorbent. *Saccharomyces cerevisiae* executes good biosorptive activity against metals like cadmium and copper.

The initial faster rate of metal ion sorption may be attributed to the large number of available sorption sites for adsorption. For the initial blank surface, the adhering possibility is large, and therefore adsorption proceeded with a high rate. The slower adsorption rate at the end is probably due to the saturation of active sites.

The use of adsorbents of biological origin has emerged in the last decade as one of the most promising alternatives to conventional heavy metal management strategies. Because of the absence of rational method for a prior prediction of the biosorption potential of a microorganism, the only method for identifying and developing newer and efficient biosorbents is the sustained screening of microbes. (Muraleedharan et al.,1995).

The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanism. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases.

1.1 Biosorption mechanism

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The biosorption mechanism are various and are not fully understood. They may be classified according to various criteria. According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into:

a) Metabolism dependent
b) Non -metabolism dependent

According to the location where the metal removed from solution is found, biosorption can be classified as

a) Extra cellular accumulation/ precipitation
b) Cell surface sorption/ precipitation
c) Intracellular accumulation (Korrapati Narasimhulu and Parcha Sreenivasa Rao 2009)

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell's metabolism. This means that this kind of biosorption may take place only with viable cells. It is often associated with an active defence system of the microorganism, which reacts in the presence of toxic metal. During non-metabolism dependent biosorption, metal uptake is by physico-chemical interaction between the metal and the functional groups present on the microbial cell surface.

This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cells' metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of biosorption, i.e., non-metabolism dependent is relatively rapid and can be reversible (Kuyucak and Volesky, 1988). In the case of precipitation, the metal uptake may take place both in the solution and on the cell surface (Ercole *et al*., 1994).

2. MATERIALS AND METHODS

1000 ppm solutions of lead, chromium, copper, iron and manganese were prepared. It was then suitably diluted to 10 ppm solution.

Each 100 ml of mineral media for the growth of micro organism was taken in five conical flasks. 10 ppm of different metal ion solution was added incorporated in the mineral media separately and then centrifuged. The supernatant was separated.

The separated supernatant of mineral media was taken with 10 ppm solutions of Pb, Cr, Cu, Fe and Mn respectively and centrifuged at 10000 rpm, at 4 ^{o}C for 15 minutes and supernatant was separated. This culture supernatant was checked with turbidity.

Five conical flasks of 100 ml were taken for each heavy metal containing the supernatant and one ml of culture supernatant was added which contain the isolated organism of clostridium sp and *psedudomonas sp separately.* These flasks were then *separately* incubated in the rotary shaker at 120 rpm for 15 days. The growth rate of organism in each flask was studied on the 7th and 15th day using Atomic absorption spectrophotometer for the determination of heavy metals.

3. RESULTS AND DISCUSSION

The major advantages of Biodegradation,over conventional treatment methods include low cost, high efficiency, minimization of chemical and biological sludge, and regeneration of biosorbent and possibility of metal recovery. (Kratchovil *et al*. 1998).

The disadvantages of Biosorptions are (i) early saturation i.e., when metal interactive sites are occupied (ii) the potential for biological process improvement (e.g., through genetic engineering of cells) is limited because cells are not metabolizing; and (iii) there is no potential for biologically altering the metal valency state. (Ahluwalia *et al*. 2007).

The bacterial cell growth and cell division in aquatic systems is highly dependent on the availability of phosphate, substrate and mineral nutrients.

The mechanisms by which metal ions bind to cell surface include electrostatic interactions, van der waals forces, covalent bonding, redox interactions and extracellular precipitation or combination of their processes.

The negatively charged groups of the bacterial cell wall adsorb metal cations, which are then retained by mineral nucleation. The biodegradation capacities of the *psedudomonas sp* were shown in Table. The bacteria in media supplemented with metals showed higher tolerance and was able to degrade 80% of initial concentration of metals.

The ability of Pseudomonas sp. to decrease various metals concentration was examined in both media within 7 days. Maximum degradation of metals was observed in 15th day of incubation.

For *Pseudomonas sp*. ability in degrading of Pb, Cr, Cu, Fe and Mn showed that this bacterium could degrade average of 54% in 7 days and average of 76% in 15 days.

The findings prove the capacity of Pseudomonas sp. in Bioremediation of PAHs. The uptake of copper by Pseudomonas sp. was impressive and almost 50% of Pb, Cr, Cu, Fe and Mn were degrading in 7th day. This is probably due to the availability of bacterial species and their cell wall surfaces.

The metal sorption tends to reverse the negative charges on bacterial cells and numerous studies have reported that the phosphoryl residues on the outer membrane of bacterial cells are the most likely binding sites for metals for most Gram-negative bacteria.

The minimum inhibitory concentration (MIC) of metals (Pb, Cr, Cu, Fe & Mn) in the growth cycle of *Pseudomonas* sp. was observed to be highly dependent on the composition of culture media and incubation temperature.

It was found from the results of this study that metal toxicity and cell growth rate depends on the combination of media composition, substrate concentration, and incubation temperature.

The cells might compete with the components in the medium for metals binding sites. In 15th day, Pb, Cr, Cu, Fe and Mn can form more metal complexes and help the cells to grow in higher concentrations compared to that in 7th day.

The cells then provide more metal binding sites for and thus show less toxic to metals in 15th day. It was concluded that *Pseudomonas* sp biosorbed almost 75% of heavy metals in 15 days.

Figure 1 Bacteriological analysis for the removal of heavy metal showing gas production during the study

Table 1 Absorption capacity for Pseudomonas sp. onto metal contaminated sites

S.NO	Organism	Day	Percentage of Biosorption by organism				
			Lead (Pb)	Chromium (Cr)	Copper (Cu)	Iron (Fe)	Manganese (Mn)
1	Pseudomonas sp.	7th Day	59	52	54	55	49
		15th Day	81	73	72	78	77

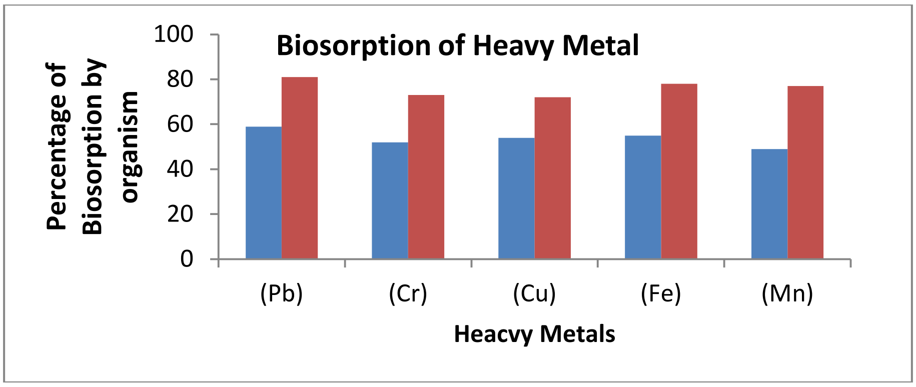

Figure 2 Biosorption of heavy metal by bacteria

4. CONCLUSION

The study was performed to understand the effect of pseudomonas sp for the removal of heavy metals in groundwater. Pseudomonas proved to be a potential bio sorbent for the removal of heavy metals in ground water. The experiment result showed that these bacteria could degrade average of 81% of heavy metals to be of low cost with high efficiency.

REFERENCES

1) Ahluwalia and Goyal, "Microbial and plant derived biomass for removal of heavy metals from waste water", Bioresour Technology, vol 98, 2007, pp2243 – 2257.
2) Aiking *et al.*,"Inorganic phosphate accumulation and cadmium detoxification in *Klebsiellaaerogenes* NCTC418 growing in continuous culture", *Appl. Environ. Microbial.* vol47, 1984, pp 374-377.
3) Brierley," Biotechnology", Blackwell, Oxford, 1985, pp163–212.
4) Chuah *et al.,* "Rice husk as a potentially low-cost biosorbent for heavy metal and dye removal an overview", Desalination, vol175, 2005, pp 305–316.
5) Farooq, "Biosorption of heavy metal ions using wheat based biosor- bents—a review of the recent literature", Bioresource Technology, vol 101, 2010, p p 5043–5053.

6) Kratchovil and Volesky, "Advances in biosorption of heavy metals, Trends Biotechnology", vol 16, 1998, pp 291-300.
7) Kuyucak and Volesky, "Accumulation of cobalt by marine algae", *Biotechnology, Bioeng, vol* 33(7), 1989, pp809– 814.
8) Korrapati Narasimhulu and Parcha sreenivasa rao, "ARPN Journal of engineering and applied science", vol 4, 2009, no 7.
9) Larous, "Experimental study of the removal of copper from aqueous solu- tions by adsorption using sawdust", Desalination, vol 185, 2009, pp 483–490.
10) Pehlivan, "Utilization of barley straws as bio sorbents for $Cu^{2+}$and Pb^{2+}ions". J Hazard Mater. vol164, 2009, pp 982-986.
11) Raras, "Biological and biotechnological waste management in material processing's", *Mineral Metals Mater, Soc,* vol 47, 1995, pp 56–63.
12) Silver, "Bacterial resistances to toxic metal ions—A review". *Gene*. vol 179, 1996, pp 9–19.
13) El-Ashtoukhyet *et al*., "Removal of lead (II) and copper (II) from aqueous solution using pomegranate peel as a new adsorbent", Desalination, vol 223, 2008, pp 162–173.