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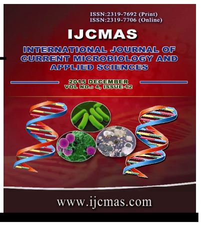

Original Research Article

Removal of heavy metals by Biosorption using Cyanobacteria isolated from Freshwater pond

R. Sivakami[1]*, M. Mahalakshmi[1] and G. Premkishore[2]

[1]Department of Zoology, Arignar Anna Govt. Arts College, Musiri – 621 211, Tamil Nadu, India
[2]Alan Feldman Public School, Kazhakkuttom, Trivandrum - 695582, Kerala, India
**Corresponding author*

ABSTRACT

Keywords

Biosorbants, *Oscillatoria limosa*, Biomass, Removal of Heavy Metals

With rapid industrialization throughout the world wastes containing metals are directly or indirectly discharged into the environment leading to serious threat to organisms including man. Of late, attention has shifted to the use of biosorbants of biological origin especially microbes as both living and dead cells are able to uptake metal ions. Hence the present study was aimed at assessing the biosorption of three metals (Cd, Hg and Pb) using the cells of *Oscillatoria limosa*. Results indicate that the order of uptake of Cd, Hg and Pb was found to be the order of 82, 78 and 72% respectively. The study also indicated that the metal uptake appeared to be a concentration independent phenomenon where an increase in metal concentration resulted in an increased uptake of metal.

Introduction

With rapid industrialization throughout the world, wastes containing metals are being directly or indirectly discharged to the environment leading to serious environmental problems posing serious threat to organisms including man. Eventhough several methods are used for removing heavy metal ions from aqueous wastes, each method has its own merits and demerits. Experiments using chemical precipitation and electrochemical treatments appear to be ineffective especially when the metal ion concentration in aqueous solution is lower than 50 mg/l while ion exchange membrane technologies and activated carbon adsorption processes are expensive (Das *et al*., 2008). This has resulted in increased research focusing on new cost effective technologies for removal of heavy metals from waste waters. Biosorption is a new technology which involves removal of heavy metals from an aqueous solution by passive binding to non-living biomass (Davis *et al*., 2003). This technique has now gathered attention as it has several advantageous over other processes like reusability of biomaterial, low operating cost, short operation time and absence of secondary compounds (Spinti *et al*., 1995; Nirmalkumar and Oommen, 2012).

Of late, the attention has shifted to the use of biosorbants of biological origin especially to non-living dry algal biomass and other microorganisms for metal removal. Cyanobacteria are cosmopolitan microorganisms which play an important role in many ecosystems. The influence of cyanobacteria on the physical, chemical and biological properties of the soil and the soil-water interface are of immense significance (Nayak and Prasanna, 2007; Prasanna and Nayak, 2007). In addition, algae have many features which make them ideal candidates for removal of heavy metals which includes high tolerance to heavy metals, ability to grow autotrophically and heterotrophically, large surface area/volume ratios, phytochelatin expression and potential for genetic manipulation (Suresh and Ravishankar, 2004; Nirmal Kumar and Oommen, 2012).

Studies using biosorbants have shown that both living and dead microbial cells are able to uptake metal ions (Knorr, 1991; Khoo and Ting, 2001; Das *et al*., 2008). However, the living cells are subject to the toxic effect of heavy metals and require more attention while non-living biomaterials or dead cells as metal binding compounds do not affect them; besides requiring less care, are cheaper and the dead biomass can be easily regenerated and reused (Mofa, 1995; Das *et al*., 2008). Under these circumstances, the present study was aimed at assessing the biosorption of heavy metals (cadmium, mercury and lead) using the living/dead cells of *Oscillatoria limosa*.

Materials and Methods

The filamentous cyanoabacteria *Oscillatoria limosa* was obtained from the Naganathar Temple Pond. The cyanobacteria was washed twice with tap water and then with double distilled water thoroughly to eliminate adhering foreign particles like sand and debris. The collected cyanobacterial samples were transferred to conical flasks with BG 11 medium (Rippka *et al*., 1979). The washed biomass was first air dried for 24 hours followed by using an oven at 80° C till constant weight was recorded. The dried biomass was then ground and sieved through a sieve (2 mm) and stored in polyethylene bottles.

Batch equilibrium experiments were performed at room temperature in 250 ml Erlenmeyer glass flasks containing aqueous solution of Cd, Hg and Pb of known concentrations (20, 40, 60 and 80 mg^{-1}) prepared using analytical grade cadmium nitrate ($Cd (NO_3)_2$), mercuric chloride ($HgCl_2$) and lead nitrate ($Pb(NO_3)_2$). An accurately weighed 250 mg portion of biomass was added to each flask and the mixtures were agitated on a rotary shaker at 180 rpm for different time intervals (30, 60, 90 and 120 min). Controls for each concentration without the addition of heavy metals were also maintained. After the respective agitation period, the solutions were separated from the biomass by filteration and subjected to further analysis. All the biosorption experiments were conducted in triplicates to substantiate the results. The data shown are the mean values of these replicate determinations. Heavy metal content in all the filtrates was quantified using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer Optima-3300 RL) at Bangalore. The amount of metal sorbed at equilibrium (mg g^{-1}) which represents the heavy metal uptake was calculated from the difference in metal concentration in the aqueous phase before and after absorption according to Shaik *et al*. (2006).

Results and Discussion

The biosorption of various metals by *O. limosa* are presented in Tables 1-3. With regard to cadmium uptake (Table 1), the lowest uptake took place at 20 mg/l in 30 m and the highest at 40 mg/l at 120 m. The uptake of cadmium at concentrations above 40 mg/l were found to decrease. However, in general, there was an increase in uptake of cadmium with increase in contact time for all the different concentrations. With regard to mercury (Table 2), the lowest uptake took place again at 20 mg/l in 30 m while the highest at 60 mg/l with a contact time of 120 m. Unlike cadmium, the uptake of mercury by *O. limosa* was found to increase with increase in concentration till 60 mg/l followed by a decline. As far as the uptake of lead by *O. limosa* was concerned (Table 3), here also the lowest uptake took place at 20 mg/l in 30 m while the maximum at 60 mg/l with a contact period of 120 m. Thus the uptake of lead by *O. limosa* was found to increase with increase in concentration and contact period till 60 mg/l followed by a decline.

Table.1 Cd Uptake (mg g^{-1}) by *Oscillatoria Limosa*

Concentration	30 min	60 min	90 min	120 min
20 ppm	2.3	2.4	2.6	2.7
40 ppm	9.4	10.2	10.5	10.8
60 ppm	8.0	8.4	8.6	8.7
80 ppm	7.4	7.8	8.0	8.2

Table.2 Mercury Uptake (mg g^{-1}) by *Oscillatoria Limosa*

Concentration	30 min	60 min	90 min	120 min
20 ppm	5.2	5.4	5.5	5.6
40 ppm	12.0	13.0	13.2	13.4
60 ppm	14.2	14.6	15.0	15.6
80 ppm	16.0	16.8	17.0	17.2

Table.3 Lead Uptake (mg g^{-1}) by *Oscillatoria lLimosa*

Concentration	30 min	60 min	90 min	120 min
20 ppm	4.6	4.7	4.9	4.8
40 ppm	7.6	7.8	8.0	8.1
60 ppm	15.6	15.8	16.2	16.4
80 ppm	11.8	11.9	12.4	12.6

Literature reveals that the sorption and removal of heavy metals by algal sorption largely depends on the initial concentration of metals in solution. According to Kant and Gaur (2001) metal sorption initially increases with increase in metal concentration and then becomes saturated after a certain concentration of metal. This statement appears to be true in the present study also. In the case of cadmium uptake, concentrations above 40 mg/l was found to result in the decrease of Cd sorption by *O. limosa*. However, with regard to mercury and lead, the concentration was 60 mg/l above which there was a decline.

From the present study, for all the metals analysed, the least metal uptake was noticed at 20 mg/l. However, the highest metal uptake was found to be different in different metals. The decrease in metal uptake after attaining maximum levels can be attributed to the increase in the number of ions competing for the available binding sites in the biomass and also due to lack of binding sites for the complexation of these ions at higher concentration levels as reported by Nirmal Kumar and Oommen (2012). Further, at higher concentrations, more ions are left unabsorbed in solution due to saturation of binding sites (Ahalya *et al*., 2005).

Literature reveals that the use of algal biomass is emerging as attractive, economical and effective proposition because of certain added advantages of algae over others (Holan and Volesky, 1994; Singh *et al*., 2001). The uptake of lead by *Chlorella vulgaris* was investigated by Aksu and Kutzal (1991) who reported increased uptake levels with increase in pH and temperature. De Carvalho *et al*. (1995) reported that biosorption using two metals (Cu + Zn; Cu + Cd or Zn + Cd) resulted in the inhibition of metal sorption by the other using *Ascophyllum nodosum*.

Among the photoautotrophs, cyanobacteria are relatively more tolerant to heavy metals (Fore and Trevors, 1995). Verma and Singh (1990) while studying the uptake of Cu using *Nostoc calcicola* reported that the metal uptake was dependent on various nutritional factors as well as the tolerance to intracellular detoxification mechanism. Studies on the uptake of various metals by Greene *et al.* (1987) and Ahuja *et al*. (1999) suggested the presence of an ion exchange mechanism.

In the present study, the order of metal uptake was Hg > Pb > Cd. Literature reveals that algal cells have revealed considerable potential in the removal of heavy metals from aqueous situations. Thus, while Singh *et al*. (2007) observed 97% removal of Pb by *Pithophora oregonia*, Abbas *et al*. (2006) achieved 90% adsorption of Hg by *Spirogyra* and Mendoza-Cozati *et al.* (2006) recorded 80% removal of lead using *Euglena gracilis*. Recently, Nirmalkumar and Oommen (2012) recorded the removal of 89% of Hg and 83% of Pb using *S. hyalina*. In the present study, the uptake of Cd, Hg and Pb was found to be of the order of 82, 78 and 72% respectively. A comparison of these levels with those of the other workers reveals similar observation.

As to the mechanism involved in this process, literature reveals that in cynobacterium two phases are involved - a passive rapid phase in which the ions bind to the cell wall followed by a slower and metabolically dependent uptake in Cytosol (Yee *et al*., 2004) while Lee *et al*. (2002) suggested that the carboxyl group in algal biomass was responsible for the binding to various ions. Nevertheless, Yee *et al.* (2004) reported that many microorganisms synthesize intracellular metal binding proteins called as metallothioneins which are rich in cystine residue and bind to metal ions. Another mechanism involved the partitioning of metal ion between exopolymer sheath and cell wall.

The present study indicates that the metal uptake appeared to be a concentration independent phenomena where an increase in metal concentration resulted in an increased uptake of metal. Similar results were also obtained by Ahuja *et al*. (1999) and Nirmalkumar and Oommen (2012). Thus, the result is typical for biobsorption of metals involving no energy mediated reactions where the metal removed from the solution is due to purely physico-chemical interactions between the biomass and metals in solution.

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