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Removal of Pb (II) by Immobilized and Free Filaments of Marine *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02

Muthukannan Satheesh Kumar · Kamaraj Rajeshwari · Shani Johnson · Nooruddin Thajuddin · Muthukumaran Gunasekaran

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Abstract Pb^{2+} removal ability of the immobilized and free filaments of marine cyanobacteria *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 was studied using batch experiments. Biosorption of lead by immobilized filaments was studied as a function of pH (2, 4, 6, 8, 10), contact time (5–180 min) and initial lead concentration (1, 3, 5, 7 mg/L) and the removal efficiency of free filaments was studied by culturing in the marine medium with the initial concentration (1, 3, 5, 7 mg/L) at pH 7 and incubated for 10 days. The maximum percentage removal was observed at 25 min for immobilized *Oscillatoria* sp. NTMS01 and 30 min for immobilized *Phormidium* sp. NTMS02. At 4th and 6th day of incubation, 89% and 77% removal was observed at 1 mg/L of initial lead concentration by free filaments of *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 respectively and further the removal was decreased with increasing concentration. Chlorophyll-*a* content was decreased in a dose dependent manner. About 40 and 50% reduction of chlorophyll-*a* was observed at higher concentration in *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 respectively. The adsorption capacity of immobilized *Oscillatoria* sp. NTMS01 was found to be (Q_{max}) 217.39 which is comparatively higher than other sorbents. The Pb^{2+} removal efficiency was performed as described in terms of Langmuir and Freundlich isotherms. These organisms is found to fit better by the Langmuir isotherms.

Keywords Lead · Adsorption isotherm · Freundlich · Langmuir · Immobilized filaments · Free filaments · Bioaccumulation

M. S. Kumar · K. Rajeshwari · N. Thajuddin
Department of Microbiology, School of Life Sciences, Bharathidasan University, Palkalai perur, Tiruchirappalli 620 024, Tamilnadu, India

S. Johnson · M. Gunasekaran (✉)
Department of Biology, Fisk University, Nashville, TN 37208, USA
e-mail: mguna@fisk.edu

The discharge of heavy metals into aquatic ecosystems has become a matter of concern in India over the last few decades. These pollutants are introduced into aquatic systems significantly as a result of various anthropological activities. Lead is one of the toxic heavy metals and an important contaminant in aquatic environments. Major lead pollution is largely contributed by automobiles and battery manufacturers. Lead is even toxic at very low exposures. Short-term exposure to high levels of lead can cause vomiting, diarrhoea, convulsions, coma or even death. Anaemia is common and lead can also damage the brain and nervous system. Other symptoms are: appetite loss, abdominal pain, constipation, fatigue, sleeplessness, irritability, and headache. Long term exposure to lead can affect kidney. Lead exposure cause mental retardation (Alluri et al. 2007). The maximum allowable limit of total Pb in drinking water considered safe by the World Health Organization is 50 ppb, whereas less than 15 ppb is adopted by the United States Environmental Protection Agency (Raungsomboon et al. 2008).

Many types of biological materials including cyanobacteria (Wang et al. 1998), algae (Matheickal and Yu 1996), yeast (Volesky et al. 1993) and aquatic plants (Miranda and Ilangovan 1996) have been used for the removal of metals from wastewater. Cyanobacteria are the largest group of photosynthetic prokaryotes whose habitats vary from fresh and marine water to terrestrial environments. Studies have shown that cyanobacteira can survive and reproduce in metal-contaminated habitats. The genera *Oscillatoria*, *Phormidium*, *Plectonema* and *Schizothrix* are dominant in zinc-enriched water (Say and Whitton 1980; Whitton 1980) and the genera *Anabaena*, *Nostoc*, *Oscillatoria*, *Phormidium* and *Scytonema*, have been recovered from copper-rich soils (Whitton and Shehata 1982). Earlier, hypersaline cyanobacterium *Phormidium tenue* has been screened for degradation of anthracene and naphthalene (Kumar et al. 2009). In the present study the free filaments and immobilized filaments of two marine cyanobacteria *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 were investigated separately for the removal of Pb^{2+}. The freely suspended growing cells can create a continuous supply of unsaturated metal removing biomass (Terry and Stone 2002). In industrial or technical operations, immobilized microbial cell systems could also provide additional advantages over freely suspended cells. These advantages include ease of regeneration and reuse of the clogging in continuous flow systems (Ozdemir et al. 2005). The literature concerning the use of immobilized filaments for lead removal and adsorption isotherms for lead biosorption is scarce. The Objective of this work were novel to find out the Pb removal efficiency of immobilized and free filaments of selected organisms and to find the adsorption capacity of the selected organisms using Langmuir and Freundlich isotherms.

Materials and Methods

Two axenic cultures of cyanobacterial strains, *Oscillatoria* sp. NTMS01, *Phormidium* sp. NTMS02 were obtained from Culture Collection Centre, Department of Microbiology, Bharathidasan University, Tiruchirappalli, India. Pure cultures were grown on MN medium.

For immobilization of cyanobacterial cells, sodium alginate (5.5%) was mixed with 50 mL of concentrated suspension of cyanobacterium and dropped through a syringe into 0.1 M $CaCl_2$. The beads, approximately 3–4 mm in diameter, were cured in the solution for about 1 h, and then washed three times with 150 mL of sterile distilled water. The cured beads were then stored in 5 mM $CaCl_2$ solution at 4°C until use. The dry weight of the prepared biomass was determined after drying the alginate beads overnight in an oven at 50°C (Katircioglu et al. 2008).

Synthetic stock solution of Pb (II) was prepared by dissolving a calculated quantity of $Pb(NO_3)_2$ (AR Grade) in double distilled water and working standards were obtained by further dilutions.

Lead removal capacities of the two biosorbents were studied in batches under varying pH (2, 4, 6, 8, 10), initial metal ion concentration (1, 3, 5, 7 mg/L), contact time (5–180 min) at 26°C. The experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL of metal ion solution and 50 immobilized cyanobacterial beads. The flasks were agitated for 3 h at 120 rotations per minute. The incubation time of biosorption was studied at definite intervals (5, 10, 20, 25, 30, 60, 120, 180 min). Biosorption studies for freely suspended filaments were performed by inoculating algal filaments in 10 mL of fresh MN medium at pH 7 supplemented with various initial metal concentrations (1, 3, 7, 9 mg/L). Chlorophyll-*a* content was observed for freely suspended filaments. The concentration of Pb (II) of the sample was determined by spectrophotometry (Optizen 3220 UV) at 520 nm using PAR (4-(2-pyridilazo) resorcinol) methods. Blanks were also run without the cyanobacteria to determine alginate adsorption.

An adsorption isotherm is characterized by certain constants, the values of which express the surface properties and affinity of the sorbent. Langmuir adsorption and Freundlich adsorption isotherms were applied to describe the relationship between the adsorbed amount of metal and its equilibrium concentration in solution separately. The Langmuir equation suggests the monolayer sorption on to a surface containing finite number of identical sites. Its linearized form is represented by the following equation (Ergene et al. 2006).

$$C_e/q_e = 1/Q_{max}b + C_e/Q_{max}$$

Where, C_e is the concentration of metal in aqueous solution (mg/L) at equilibrium, q_e is the amount of adsorbed metal per gram of adsorbent at equilibrium (mg/g), b is the Langmiur constant related to the energy of adsorption (L/mg) and Q_{max} is the adsorption capacity of the adsorbent (mg/g). Based on the linearized form of the adsorption isotherm derived from plots of C_e/q_e versus C_e, the constant Q_{max} and b values were calculated from the slope and intercept of the plot. The empirical Freundlich equation based on sorption on a heterogeneous surface is given by the following equation:

$$q_e = K_f C_e^{1/n}$$

Where, K_f and n are the Freundlich constants. K_f and n are indicators of adsorption capacity and adsorption intensity, respectively. The Freundlich isotherm has generally been considered an empirical relationship and has been widely used to fit the experimental data. The Freundlich constants K_f and n were obtained from the linearized logarithmic plot of above equation (Morell and Hering 1993).

Results and Discussion

Figure 1 shows that the maximum sorption of Pb (II) ions from aqueous solutions using immobilized filaments of

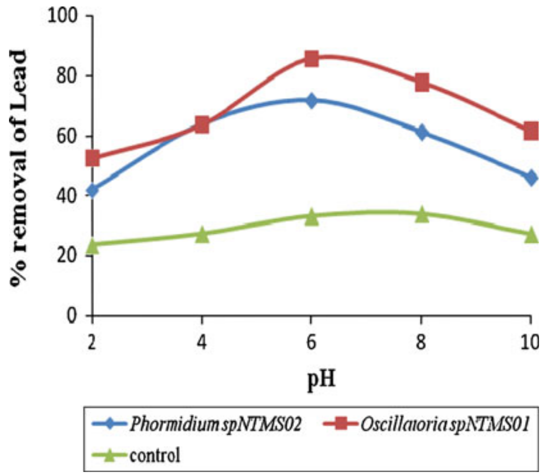

Fig. 1 Effect of pH on equilibrium Pb (II) sorption capacity of two strains of marine cyanobacteria in an immobilized form (initial Pb (II) concentration 1 mg/L, contact time 25 and 30 min for *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 respectively, tempeataure 26°C)

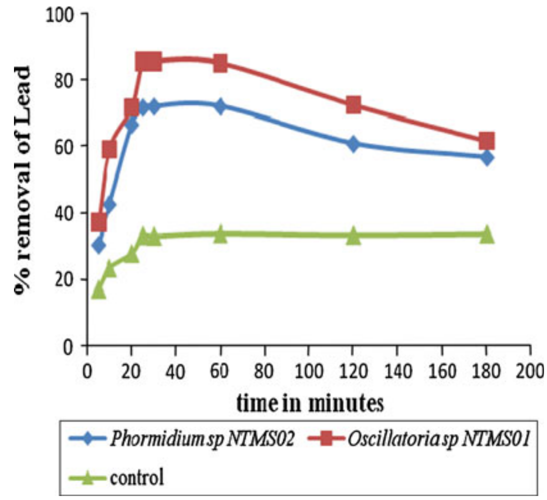

Fig. 2 Effect of contact time on equilibrium Pb (II) sorption capacity of two marine cyanobacterial strains (initial Pb (II) concentration 1 mg/L, pH 6, and temperature 26°C)

Oscillatoria sp. NTMS01 and *Phormidium* sp. NTMS02 had been achieved at pH 6. The percentage of Pb removal was increased when the pH value was increased from 2 to 6. Decreased percent removal was observed in both studied organisms, when the pH was increased from 6 to 10. It indicates, pH is the most important factor influencing the sorption efficiency. In acidic condition both adsorbent and adsorbate are positively charged and therefore interaction is that of electrostatic repulsion. Besides, higher concentration of H^+ ions present in the reaction mixture competes with the positively charged metal ions for the surface adsorption sites, resulting in decrease in the removal of metal ions (Tobin et al. 1984). The concentration of H^+ decreases, when pH increases, whereas the concentration of metal ions remain constant and therefore uptake of metal ion can be explained as an cation exchange. At alkaline pH, formation of metal oxide or hydroxide may decrease the metal removal. Adsorption of metal ion depends upon the nature of the adsorbent surface and species distribution of the metal ion. Species distribution depends on the pH of the system (Baes and Mesmer 1976; Morel 1983). The significant adsorption of metal ion was observed at pH 6. This report was found to be similar to the adsorption of Hg (II), Cd (II) and Pb (II) ions on the immobilized microalgae which showed highest values at around pH 5.0 to 6.0 (Bayramoglu et al. 2006).

The effect of contact time on biosorption of lead by immobilized filaments of *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 is shown in Fig. 2. The sorption percentage of metal increased with increase in contact time. The percent removal was started to increase initially and then attained maximum value beyond which there was no significant increase in the rate of removal and decreased slightly. The maximum percent removal was observed at 25 min for *Oscillatoria* sp. NTMS01 and 30 min for *Phormidium* sp. NTMS02. After 1 h the percent removal was decreased. This may be due to desorption of small amount of metals adsorbed by organism. Similar report observed in *Spirogyra* and *Cladophora*, has shown highest adsorption rate of lead and copper during the first 30 min of contact time (Lee and Chang 2011).

In the present study, the effect of the initial concentration of Pb (II), in the range of 1, 3, 5, 7 mg/L, on its uptake by the immobilized biomass of the two species at optimized pH and contact time was also studied and is depicted in Fig. 3. The percent removal decreased as concentration of Pb (II) increased. These two organisms *Oscillatoria* sp.

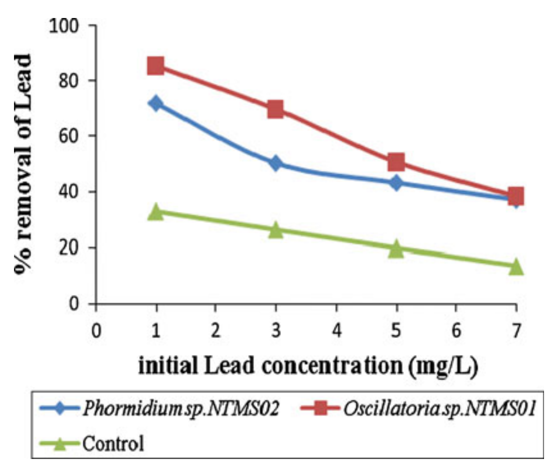

Fig. 3 Effect of initial concentration on equilibrium Pb (II) sorption capacity of two immobilized cyanobacterial strains (contact time 25 and 30 min for *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 respectively, pH 6, temperature 26°C)

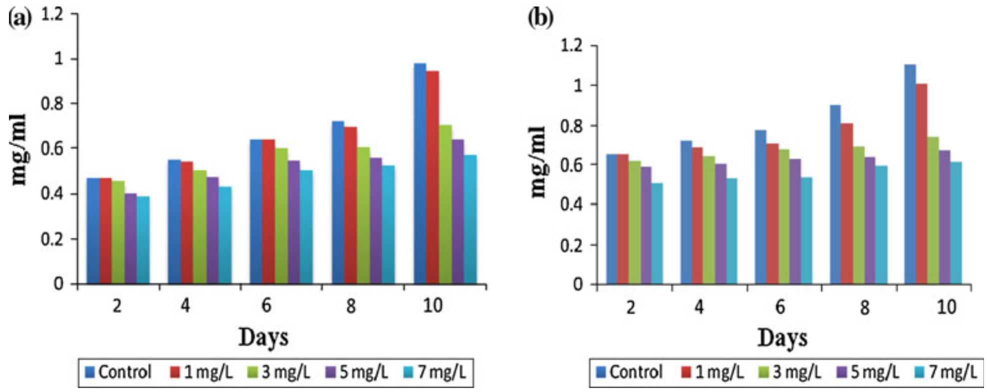

Fig. 4 Effect of lead initial concentration on Chlorophyll-*a* pigment **a** *Oscillatoria* sp. NTMS01 and **b** *Phormidium* sp. NTMS02

NTMS01 and *Phormidium* sp. NTMS02 showed maximum percent removal of lead at lower concentration and decreased percent removal with increasing concentration. Higher removal efficiency was observed at lower Pb^{2+} concentration than at high Pb^{2+} concentration. This was possibly due to the relatively large amount of binding sites for Pb^{2+} on cell surfaces at low concentration. However, at high Pb^{2+} concentration repulsive force could occur among Pb^{2+} ions and thus resulting in a lower adsorption than at low Pb^{2+} ions concentration (Raungsomboon et al. 2008). Immobilized filaments of *Oscillatoria* and *Phormidium* sp. were also found to be a good biosorbent for the removal of Chromium (VI) from aqueous solutions (Rajeshwari et al. 2011).

Effect of initial concentration on lead biosorption by freely supended filaments of *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 is depicted in the Fig. 4. At 4th and 6th day of incubation, 89% and 77% removal was observed at 1 mg/L of initial lead concentration by *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 respectively and further the removal was decreased with increasing concentration. This may be due to the less available sites in higher concentration of metal. The maximum percent removal of lead was achieved at 1 mg/L initial concentration by freely suspended filaments than immobilized filaments. Similarly *Spirogyra* and *Cladophora* filamentous macroalgae were found to be good biosorbent for the removal of lead and copper (Lee and Chang 2011).

Table 1. represent the effect of contact time on lead removal using freely suspended filaments of *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 respectively. The percent removal was increased with exposure of time upto 4th and 6th day for *Oscilatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 respectively. This may be due to the bioaccumulation of metals which is a slow process followed by adsorption. Physical adsorption on to the surface of the organism is fast but accumulation process includes transport of metal across the membrane which requires long exposure time. The *Phormidium* sp. NTMS02 requires long exposure time for its maximum removal than the *Oscillatoria* sp. NTMS01. After 4th day of incubation, the concentration of metal increased in the medium which denotes the decreased percent removal. This may be due to desorption of metal which is adsorbed on to the surface of the organism. Similar evidence could be found in cyanobacterium, *Spirulina* which shows decreased lead metal accumulation with exposure time (Arunakumara et al. 2008).

Chlorophyll biosynthesis was reported to be inhibited by both Cd^{2+} and Pb^{2+}, leading to the lowered chlorophyll contents (Pahlsson 1989). Some metals (Pb^{2+} and Cd^{2+}) at higher concentrations can destroy the chloroplasts (Lamaia et al. 2005) and thylakoid membranes (Heng et al. 2004). Chlorophyll-*a* was estimated for freely suspended filaments and is represented in Fig. 4a and b. About 40 and 50% reduction of chlorophyll was observed at higher concentration in *Oscillatoria* sp. NTMS01 and *Phormidium* sp.

Table 1 Effect of contact time on lead biosorption by free filaments of *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 at pH 7 and temperature 26°C

Organisms	Contact time (days)	1 mg/L		3 mg/L		5 mg/L		7 mg/L	
		C_e (mg/L)	% removal	C_e (mg/L)	% removal	C_e (mg/L)	% removal	C_e (mg/L)	% removal
Oscillatoria sp.NTMS01	4	0.104	89.6	1.03	65.6	2.79	44.2	4.50	37.5
Phormidium sp.NTMS02	6	0.21	79	0.77	74.3	2.01	59.8	4.85	30.7

Table 2 Langmuir and Freundlich parameters for the adsorption isotherms for Pb^{2+} ion

Organisms	Langmuir parameters			Freundlich parameters		
	Q_{max}	b	R^2	K_f	n	R^2
Immobilized *Oscillatoria* sp.NTMS01	217.39	2.519	0.9999	0.757	2.899	0.9382
Immobilized *Phormidium* sp. NTMS02	212.76	2.3500	0.9953	0.7407	2.655	0.8579
Live *Oscillatoria* sp. NTMS01	86.20	3.515	0.9947	0.5650	3.664	0.9690
Live *Phormidium* sp. NTMS02	92.592	21.618	0.963	0.6087	2.869	0.6539

NTMS02 respectively. The chlorophyll-*a* content decreased with increasing metal concentration. Similar report was found in *Synechocystis* sp. PCC 6803 (Arunakumara and Xuecheng 2009). Reduction of chlorophyll-*a* was higher in *Phormidium* sp. NTMS02 compared to *Oscillatoria* sp. NTMS01, which indicates that *Phormidium* sp. NTMS02 was more sensitive to lead contamination. Chlorophyll-*a* reduction may be due to the binding of the metal to chloroplast which deteriorates the routing functions (Heng et al. 2004).

The Freundlich and Langmuir isotherms were tested and compared. The results are presented in Table 2. The Langmuir adsorption isotherm had a higher correlation coefficient (r) than that of Freundlich isotherm. Therefore, in this study the mechanisms involved in Pb^{2+} removal by immobilized and free filaments of *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 respectively were discussed based on the Langmuir isotherm parameters. The adsorption capacity of immobilized *Oscillatoria* sp. NTMS01 was found to be (Q_{max}) 217.39 which is comparatively higher than the other sorbents. Langmuir parameters indicated a significant increase in Pb (II) uptake by immobilization of *Oscillatoria* sp. NTMS01 in sodium alginate beads (Fig. 5).

This study investigated the adsorption of Pb^{2+} by the immobilized filaments of *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 at various pH, contact time and

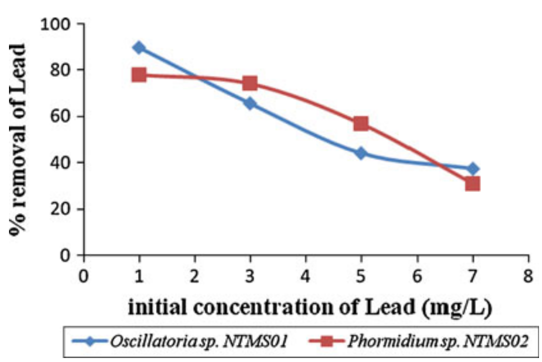

Fig. 5 Effect of initial metal concentration at temperature 26°C, pH 7, and contact time 6th and 4th days for *Phormidium* sp. NTMS02, *Oscillatoria* sp. NTMS01 respectively

Pb^{2+} concentrations. This study also investigated the adsorption of Pb^{2+} by the free filaments of *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02 at various contact time and Pb^{2+} concentration. Biosorption capacities for Pb (II) were found to be dependent on the pH of the solution and the maximum capacities were obtained at pH 6 with a contact time of 25 and 30 min for immobilized *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02. The maximum lead removal capacity of free filaments were observed at pH7, with a contact time of 4th and 6th day for *Oscillatoria* sp. NTMS01 and *Phormidium* sp. NTMS02. Based on its adsorption characteristics and capacity, this cyanobacterium was considered as the effective adsorbent for Pb^{2+}. Its adsorption characteristics followed the Langmuir adsorption model which indicates that immobilized *Oscillatoria* sp. NTMS01 was found to be an efficient sorbent compared to other sorbents. This immobilized sorbents were even found to recover the metals after sorption using appropriate method. This work will prove that cyanobacteria could be a potent environment friendly caring sorbent and a potent biofilter in heavy metal contamination.

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