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Biosorption of Cr(VI) by immobilized biomass of two indigenous strains of cyanobacteria isolated from metal contaminated soil

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Abstract

Biosorption of Cr(VI) using native strains of cyanobacteria from metal contaminated soil in the premises of textile mill has been reported in this paper. Biosorption was studied as a function of pH (1–5), contact time (5–180 min) and initial chromium ion concentration (5–20 mg/l) to find out the maximum biosorption capacity of alginate immobilized *Nostoc calcicola* HH-12 and *Chroococcus* sp. HH-11. The optimum conditions for Cr(VI) biosorption are almost same for the two strains (pH 3–4, contact time 30 min and initial chromium concentration of 20 mg/l) however, the biomass of *Chroococcus* sp. HH-11 was found to be more suitable for the development of an efficient biosorbent for the removal of Cr(VI) from wastewater, as it showed higher values of q_m and K_f, the Langmuir and Freundlich isotherm parameters. Both the isotherm models were suitable for describing the biosorption of Cr(VI) by the cyanobacterial biosorbents.

Keywords: Algae; Heavy metal; Adsorption isotherm

1. Introduction

Presence of toxic levels of heavy metals in wastewaters from various industries has become a major cause of environmental concern due to serious health impacts associated with them. Chromium(VI) is one such metal, which is a potent carcinogen reported to cause cancer in digestive tract and lungs [1]. Chromium(VI) is more toxic form of the metal due to its association with oxygen as chromate (CrO_4^{2-}) ions. It is a strong oxidizing agent and in the presence of organic matter, it is reduced to chromium(III), more rapidly so, in acidic environment. However, at high concentration, chromium(VI) may overcome the reducing capacity of environment and thus, persists as a pollutant. Chromium is a common contaminant of wastewater of tannery, textile, paint, ink, dye, aluminum and electroplating industries. Conventional techniques, such as chemical precipitation, ion exchange, activated carbon adsorption and membrane processes are not only cost intensive but also not very effective when the metal concentration is less than 100 mg/l [2,3]. Removal of heavy metals from wastewaters through adsorption, particularly biosorption, has emerged as an alternative technology in the recent years. A variety of biomaterials and microorganisms have been explored by researchers for biosorption and bioaccumulation including fungi [4], yeast [5], algae [6] and mosses [7]. Biosorption may occur actively through metabolism or passively through some physical and chemical processes. Cyanobacteria are suggested to have some added advantages over other microorganisms because of their large surface area, greater mucilage volume with high binding affinity and simple nutrient requirements [8]. However, one major problem associated with microbial biosorbents is separation and harvesting of the biomass after metal removal. Immobilization of the organism in some suitable matrix like silica gel, polyurethane or alginate has proved useful in tackling this problem. The physical entrapment of the organism inside a polymeric gel in the form of beads is one of the most widely used techniques for immobilization which not only tackles the above problem but also provides mechanical strength, rigidity and porosity characteristics to the biosorbents. Further, the metal can be recovered from the loaded beads using appropriate desorption techniques, thereby, minimizing the possibilities of environmental contamination [9,10].

In the present study, Cr(VI) biosorption by two locally isolated cyanobacteria from metal contaminated soil, *Nostoc*

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calcicola HH-12 and *Chroococcus* sp. HH-11 using their live and immobilized biomass has been reported.

In the recent past, a large number of studies have been carried out by several workers using marine algae and microalgae for metal biosorption [11]. Most of these studies have shown that percent removal of metal declines as metal concentration in the water increases [12]. It has however, been observed earlier by the authors that cyanobacterial strains, when isolated from the soil of an electroplating site show more Cr(VI) biosorption in the presence as well as absence of salts when initial metal ion concentration was high [13,14]. It was hypothesized that these strains, due to long exposure to metals in the contaminated site might have developed tolerance to metal ions. It was thus, thought worthwhile to explore metal biosorption potential of some other native strains of cyanobacteria isolated from the premises of industrial units at different metal concentrations. In the present study, two species of cyanobacteria, namely *N. calcicola* HH-12, a nitrogen fixing filamentous form and *Chroococcus* sp. HH-11, a unicellular non-heterocystous colonial form were selected, which were isolated from the soil within the premises of a textile mill in Haryana, India.

2. Materials and methods

2.1. Isolation of cyanobacteria and preparation of biosorbents

Cyanobacterial strains, *N. calcicola* HH-12 and *Chroococcus* sp. HH-11 were isolated from metal contaminated soil (48–70 μg/g) within the premises of a textile mill using standard plating, isolating and culturing techniques [15]. The cultures were maintained at light intensity of 3000 lux at 28 ± 3 °C. BG-11 medium was used as culture medium for both the strains using nitrogen supplement for *Chroococcus* sp. HH-11, which was non-nitrogen fixer. Composition of BG-11 medium used is: $NaNO_3$ (1.5 g/l), K_2HPO_4 (0.04 g/l), $MgSO_4$ (0.075 g/l), $CaCl_2$ (0.036 g/l), citric acid (0.006 g/l), ferric ammonium citrate (0.006 g/l), EDTA (0.001 g/l), Na_2CO_3 (0.02 g/l) and trace metal mix (1 ml) containing boric acid (2.86 g/l), manganese oxide (1.81 g/l), zinc sulphate (0.222 g/l), sodium molybdate (0.039 g/l), copper sulphate (0.079 g/l), cobalt nitrate (0.049 g/l).

Cyanobacterial cells harvested at 14th day stage were washed and dried at 70 °C for 24 h and sieved through 0.3 mm mesh. For immobilization of the cyanobacteria in alginate gel in the form of beads, powdered dry algal biomass (0.1 g) was mixed with sodium alginate (10 ml, 4% w/v) and dropped through a syringe (2.0 mm i.d.) into $CaCl_2$ (0.5 M) forming beads (diameter 1.5–2.0 mm). After keeping them overnight, the beads were rinsed with deionized water and soaked in 0.5 M HCl for 1 day and rinsed again with deionized water before use [9].

2.2. Batch mode studies

Synthetic stock solution of Cr(VI) was prepared by dissolving calculated quantity of $K_2Cr_2O_7$ (AR Grade) in double distilled water and working standards were obtained by further dilutions.

Chromium(VI) removal capacities of the two biosorbents were studied in batch mode under varying pH (1–5), initial metal ion concentration (5–20 mg/l) and contact time (5–180 min). The experiments were carried out in 250 ml Erlenmeyer flasks with algal beads of dry biomass 0.1 g / 100 ml aqueous metal solution. To study the effect of pH, buffers were used and metal solutions were maintained at desired pH level (pH 1–5) with 100 ml of 20 mg/l of Cr(VI) solution. For optimization of contact time, 100 ml of 20 mg/l chromium(VI) solutions with cyanobacterial beads were shaken on an illuminated orbital shaker (Orbitek LT-IL) at 120 rpm and at temperature of 26 °C. Ten millilitres samples were collected from the triplicate flasks at definite time intervals (5, 10, 15, 30, 90, 120, 150 and 180 min.) and were filtered through Whattman filter paper no. 40. The filtrates were analyzed for residual chromium concentration spectrophotomerically at 540 nm using 1,5-diphenyl carbazide reagent in acid solution as a complexing agent for Cr(VI) using Systronics Spectrophotometer 106 [16]. Biosorption potential of two algal species at different initial chromium ion concentration (5, 10, 15 and 20 mg/l) was studied in batch mode at optimized pH (3.0 for *N. calcicola* HH-12, 4.0 for *Chroococcus* sp. HH-11) at 26 °C by agitating the flasks for 30 min. each. For each treatment, blanks were also run without algae to account for adsorption by the alginate.

3. Result and discussion

3.1. Biosorption studies

Biosorption of Cr(VI) was studied as a function of pH, contact time and initial metal concentration.

3.1.1. Effect of contact time

Chromium(VI) adsorption by the two biosorbents as a function of time is depicted in Fig. 1.

The rate of biosorption was very high during the initial 5 min in *Chroococcus* sp. HH-11 showing more than 65% Cr(VI) removal whereas in *N. calcicola* HH-12, there was about 35% removal in first 5 min and additional 10% removal between 5 and

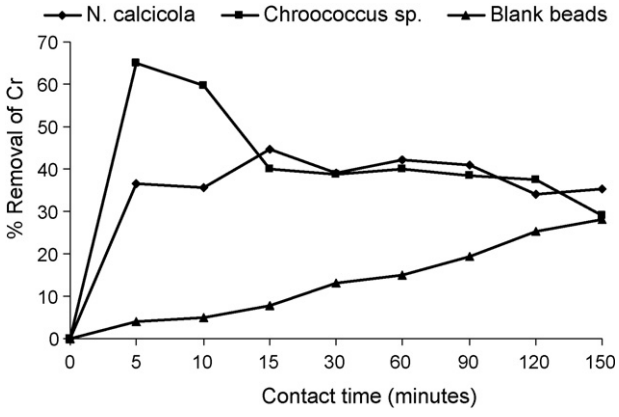

Fig. 1. Effect of agitation time on equilibrium Cr(VI) sorption capacity of two cyanobacterial strains (initial Cr concentration = 20 mg/l, pH = 2 and dry algal biomass = 0.1 g/100 ml).

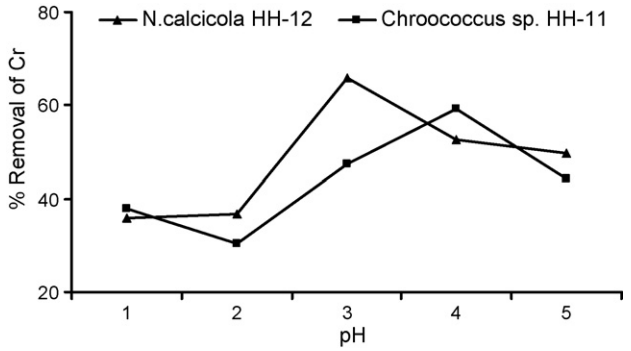

Fig. 2. Effect of pH on equilibrium Cr(VI) sorption capacity of two strains of cyanobacteria in immobilized form (initial Cr concentration = 20 mg/l, contact time = 30 min and dry algal biomass = 0.1 g/100 ml).

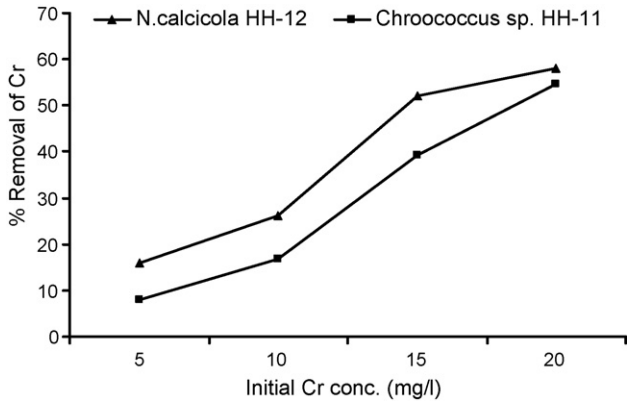

Fig. 3. Effect of initial chromium concentration on equilibrium Cr(VI) sorption capacity of two immobilized cyanobacterial strains (contact time = 30 min, pH = 3 for *N. calcicola* and pH = 4 for *Chroococcus* sp.).

15 min. In *Chroococcus* HH-11, there was a decline in percent Cr removal indicating some desorption after 5 min and equilibrium was attained after 15 min. In *N. calcicola* HH-12, again a small decline occurred after 15 min followed by attainment of equilibrium. Similar experiment was conducted with blank beads (beads without algae) and 30% removal was observed in 120–150 min.

3.1.2. Effect of pH

The pH of aqueous metal solution (20 mg/l) was found to distinctly influence biosorption of Cr(VI) by the two species (Fig. 2). Percent adsorption of chromium(VI) from the solution increased as there was a rise in pH upto 3–4, whereas further increase in pH had a negative effect. Maximum metal removal took place at pH 3.0 in case of *N. calcicola* HH-12 whereas for *Chroococcus* sp. HH-11 it was at pH 4.0 (Fig. 2). Upto 70% removal of Cr(VI) was achieved by using *N. calcicola* HH-12, while 60% removal took place using *Chroococcus* sp. HH-11. Maximum metal adsorption by microalgal surface at acidic pH (3–4) may be attributed to a net positive surface charge under this pH and protonation of certain functional groups facilitating binding of the negatively charged chromate ions existing as $HCrO_4^-$ or $Cr_2O_7^{2-}$ [12,17].

3.1.3. Effect of initial Cr(VI) concentration

Relationship between percent removal of chromium and initial chromium(VI) concentration at optimized pH and contact time is shown in Fig. 3. Continuous increase in percent removal of chromium(VI) with increasing initial metal ion concentration was observed for both strains. These strains showed maximum percent removal of Cr(VI) at 20 mg/l. This sort of biosorption behaviour was different from that reported by other workers for various heavy metals by different algal species, where there was decline in percent metal removal at higher concentrations [5,18]. These species which were isolated from metal contaminated sites produced a large quantity of exopolysaccharides (results not shown here), seem to be responsible for adsorbing high concentration of these metals. At higher concentration, the number of ions available for competing at the binding sites of algal surfaces or mucilage layer is more, thus, increasing biosorption [19].

3.2. Adsorption isotherms

Biosorption of chromium(VI) by two cyanobacterial biosorbents was studied further for understanding the mechanism by fitting the experimental data to Langmuir and Freundlich models. The Langmuir model assumes monolayer biosorption onto a surface with a finite number of identical sites and the model is described by the following linear equation [17]:

$$\frac{C_e}{q_e} = \frac{I}{q_m C_e} + \frac{K_d}{q_m} \quad (1)$$

where C_e is the equilibrium chromium concentration (mg/l), q_e the metal adsorbed on the adsorbent (mg/g dry wt.), q_m the maximal biosorption capacity and K_d is the Langmuir constant of the system. In this model, C_e/q_e is linearly related to C_e.

Model parameters obtained for the two species showed greater q_m for *Chroococcus* HH-11 (21.36 mg/g) as compared to *N. calcicola* HH-12 (q_m = 12.23 mg/g) as shown in Table 1. However, there are chances that a molecule adsorbed onto the surface may make it more or less difficult for another molecule to get attached to a neighbouring site on the biosorbent and this might lead to a deviation from the Langmuir biosorption equation. Under such a situation, Freundlich isotherm may be

Table 1
Langmuir and Freundlich adsorption constants for Cr(VI) biosorption by two strains

Species	Langmuir parameters			Freundlich parameters		
	q_m (mg/g)	K_d (l/min)	R^2	K_f (mg/g)	n	R^2
Chroococcus sp.	21.36	0.59	0.92	210.37	0.36	0.91
N. calcicola	12.23	0.97	0.77	40.07	0.44	0.88

more suitable, which can be expressed by the linear equation in logarithmic form as:

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \quad (2)$$

where K_f is Freundlich constant indicating adsorbent capacity (mg/g dry wt.) and n is the Freundlich exponent known as adsorbent intensity [20]. This model shows that $\log q_e$ is linearly related to C_e. The Freundlich's model constants, K_f and n were calculated and the values obtained for *N. calcicola* HH-12 were, $K_f = 40.07$ and $n = 0.45$, whereas in case of *Chroococcus* sp. HH-11 $K_f = 210.37$ and $n = 0.36$ as shown in Table 1. The results obtained indicate that coefficient of regression R^2 are higher for Freundlich model in case of *N. calcicola* HH-12 whereas, it is almost similar for the two models in case of *Chroococcus* sp. HH-11 Biosorption process in *Nostoc* fits better to Freundlich isotherm indicating heterogeneity of algal surface and significant influence of one occupied site on biosorption at another site. *Nostoc* secrets a lot of exopolysaccharides with several functional groups which possibly impart it its heterogenous surface features. Biosorption capacity of extracellular polysaccharides produced by cyanobacteria is being studied further by the authors for optimizing the process. In case of *Chroococcus*, the biosorption process seemed to fit in equally well to both the models ($R^2 = 0.91$–0.92). Adsorption capacity of *Chroococcus* was found to be higher $K_f = 210.37$ mg/g and $q_m = 21.36$ mg/g as compared to that of *N. calcicola* HH-12 (Table 1) indicating it to be a better biosorbent.

4. Conclusions

The objective of the present study was to optimize chromium biosorption capacity of two immobilized strains of cyanobacteria from metal contaminated soil and also to compare their chromium adsorption using isotherms. Cr(VI) biosorption was found to be optimum at initial pH 3–4 at 30 min contact time. Both Langmuir and Freundlich isotherms were suitable for describing biosorption of Cr(VI) by these species. Higher values q_m and K_f along with higher regression coefficients in case of *Chroococcus* sp. HH-11 indicates its greater suitability for removal of chromium ions from wastewater. Further, with increasing Cr(VI) concentration in the aqueous solution (tested upto 20 mg/l) both the cyanobacterial isolates show increased percent metal removal, indicating their added advantage in bioremediation measure. Systematic studies to characterize the changes in the adapted strains at molecular level can prove to be very useful in future bioremediation programmes.

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