

Sequestration of chromium by exopolysaccharides of *Nostoc* and *Gloeocapsa* from dilute aqueous solutions

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Abstract

This article reports the chromium removal potential of exopolysaccharides (EPS) of two indigenously isolated cyanobacterial strains, *Gloeocapsa calcarea* and *Nostoc punctiforme*. The biosorption was studied by varying pH from 2 to 6 and initial chromium concentration from 5 to 20 mg/L to find out the optimized conditions for maximum chromium removal by EPS. Two equilibrium models, Langmuir and Freundlich, were used to explain these results. The Freundlich model was found to be better applicable to the experimental data as compared to Langmuir as inferred from high value of coefficient of determination whereas the optimal conditions were found to be same for the two (pH 2 and initial chromium concentration 20 mg/L). EPS production by the two strains was also studied which was found to be higher for *Gloeocapsa*. On the basis of experimental results and model parameters, it can be inferred that the EPS extracted from *Nostoc* has comparatively high biosorption capacity and can be utilized for the removal of chromium from dilute aqueous solution. Adsorption of chromium on EPS was further confirmed by surface morphology observed in scanning electron micrographs.

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1. Introduction

Biological surfaces have been known to influence bioavailability as well as toxicity of various metals to organisms depending upon the presence of different functional groups onto them. Most of the bacteria and cyanobacteria produce extracellular polysaccharides (EPS) with a variety of ligands playing an important role in trace metal transfer through water column to sediments in aquatic systems [1]. In a recent study the authors have established superior metal adsorptive capacity of EPS of *Lyngbya putealis* as compared to its dry or immobilized biomass [2] suggesting it to be a better biosorbent for metal removal. The promising nature of EPS of *Anabaena spiroides* for heavy metal removal from dilute solutions and optimization of the process have also been suggested [3]. Exudates from several species of cyanobacteria are reported to act as strong complexing agents for some metals [4] and there is a need to compare their potential using suitable adsorption models. Modeling is important for pre-

diction and comparison of biosorption capacity of these sorbing materials.

There are some studies reporting the Cu(II) and Pb(II) adsorption by the exopolysaccharides (EPS) of cyanobacterial strains like *Nostoc* and *Cyanospira* [5] and *Gloeocapsa gelatinosa* [6] respectively. While Cr(VI) is known to be a toxic metal with carcinogenic nature, there are no reports on its removal by using cyanobacterial exopolysaccharides.

The present study was carried out to compare Cr(VI) sequestration potential of EPS produced by two cyanobacterial species, namely, *Nostoc punctiforme*, a filamentous heterocystous form and *G. calcarea*, a unicellular non-heterocystous form. Both the species isolated indigenously from metal contaminated sites possess thick mucilaginous sheath.

The objective of the present investigation was to quantify EPS production by these indigenously isolated species in response to moderately low chromium concentrations in aqueous medium as well as to study Cr sequestration by EPS as influenced by pH (1–6) and initial metal concentration (5–20 mg/L), applying isotherm models to the equilibrium data. Since earlier studies have established facilitated metal removal under acidic pH [2], hence only acidic range of pH was selected and initial chromium

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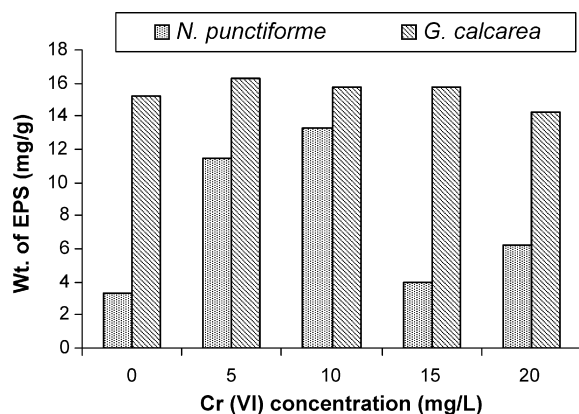


Fig. 1. Production of EPS (mg/g biomass) in response to varying concentration of chromium(VI) by two cyanobacterial species.

concentration was kept low keeping in view the fact that most of the conventional methods of metal removal are of limited use for dilute solutions. Therefore, it is worthwhile to test the applicability of the present biosorbent for metal removal from its dilute aqueous solution.

2. Materials and methods

2.1. Cyanobacterial isolation

Cyanobacterial strains, *G. calcarea* HH-19 and *N. punctiforme* HH-17 were isolated from a metal contaminated site near a textile industry using standard plating, isolation and culturing techniques in BG-11 culture medium [7]. The cultures were maintained under controlled conditions with a light intensity of 3000 lx (with 24 h illumination) using cool fluorescent tubes at 28 ± 3 °C temperature. Nitrogen supplement was given to the medium for the non-diazotrophic *Gloeocapsa*.

2.2. Exopolysaccharide extraction and estimation

For extraction of the cyanobacterial EPS, 100 ml of each cyanobacterial culture was subjected to centrifugation (3000 rpm) and after separating the settled biomass, cell-free culture containing the EPS was taken. It was concentrated ten fold by evaporation at 40 °C for isopropanol precipitation. The precipitates so obtained were washed with isopropanol two–three times to remove any contaminants, dried at 37 °C and hydrolyzed with acid (2 M HCl) at 100 °C for 2 h. The hydrolysate was analyzed for glucose [8].

In order to examine any effect of Cr(VI) on EPS production, the two cyanobacterial cultures were spiked with 5, 10,

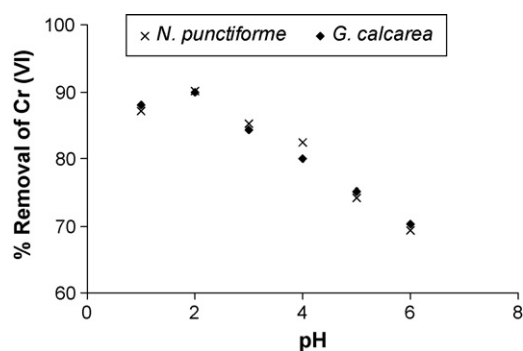


Fig. 2. Effect of pH on % removal of Cr(VI) from aqueous solution by EPS of cyanobacterial strains *G. calcarea* and *N. punctiforme*.

15, and 20 mg/L Cr(VI) using potassium dichromate and EPS production was estimated at the peak log phase (15 d).

2.3. Metal biosorption studies

Various metal concentrations were prepared from stock solution of 1000 mg/L Cr(VI) using AR grade potassium dichromate. Experiments were conducted in 250 ml Erlenmeyer flasks to determine the optimal pH and initial chromium concentration at which metal sequestration by EPS is maximum. Batch studies were performed to determine the sorption equilibrium for Cr(VI) onto the cyanobacterial EPS serving as biosorbent. Cell-free culture (100 ml) containing EPS (0.05 g/100 ml for *Gloeocapsa* and 0.02 g/100 ml for *Nostoc*) was taken in triplicate for both species at 20 ppm initial Cr(VI) concentration and at varying pH from 1 to 6 using 0.1 N HCl. The flasks were shaken for 2 h at 120 oscillations per minute at 25 °C. In another experiment, initial chromium concentration was optimized by varying the concentration (5, 10, 15 and 20 mg/L) at optimal pH 2 and constant temperature of 25 °C in triplicates following the same procedure.

Concentration of Cr(VI) ions in aqueous medium was analyzed in acid solution using Spectrophotometer-106 (Systronics) at 540 nm, using 1,5-diphenyl carbazide reagent as a complexing agent [9].

Surface morphology of the dried EPS sample before and after exposure to Cr(VI) was studied by scanning electron microscope (Philips PSEM 515).

3. Results and discussion

Gloeocapsa produced about 60–95% more EPS as compared to that by *Nostoc* (Fig. 1). Both the cyanobacterial species showed increased production of EPS when exposed to dilute

Table 1
Cr adsorption and % removal by EPS of *Nostoc* and *Gloeocapsa* with increasing initial chromium concentration

C_0 (mg/L)	<i>Nostoc</i> (EPS) q_e (mg/g)	% Removal	<i>Gloeocapsa</i> (EPS) q_e (mg/g)	% Removal
5	23.93	95.7	9.37	93.7
10	45.58	91.15	18.23	91.15
15	68.3	91.06	27.34	91.13
20	90.05	90.05	36	90.0

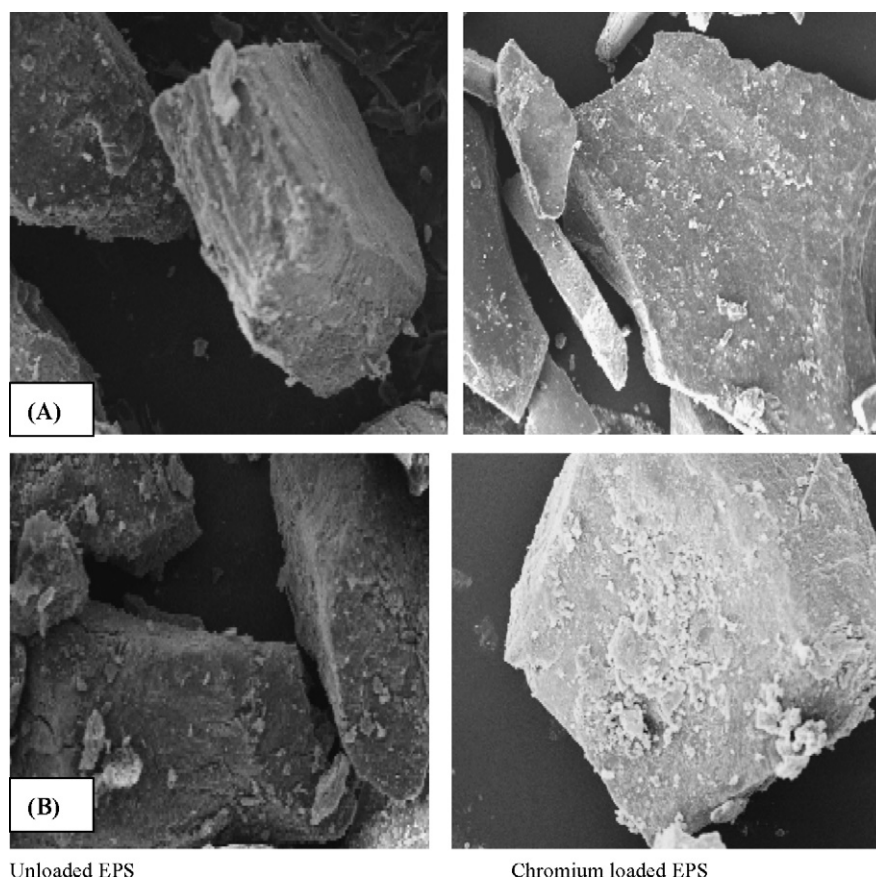


Fig. 3. Scanning electron micrographs of unloaded and chromium loaded EPS of (A) *Gloeocapsa calcarea* (B) *Nostoc punctiforme*.

metal concentration (5–10 mg/L Cr), but declined at higher concentration, particularly, in case of *Nostoc*.

3.1. Effect of pH on Cr(VI) biosorption

Removal of hexavalent chromium by EPS of the two cyanobacterial species was found to be pH-dependent and the trend was similar for both, showing maximum removal at pH 2 (Fig. 2). Decrease in adsorption capacity of extracellular polysaccharides at pH higher than 2 is explained by the fact that due to the exposure of more negatively charged functional groups, negatively charged chromate ions (HCrO_4^- , CrO_7^- , $\text{Cr}_4\text{O}_{13}^{2-}$ and $\text{Cr}_3\text{O}_{10}^{2-}$) present in solution are repelled [10,11]. A net positive charge at pH 2, due to protonation of functional groups and presence of hydronium ions around the surface, further facilitates the adsorption of negatively charged hexavalent chromate ions [2,12,13].

3.2. Effect of initial concentration of metal

Initial metal concentration of the solution is an important factor determining the metal removal efficiency of a biosorbent. The effect of initial metal concentration on percent Cr(VI) removal and adsorption capacity q_e of EPS is presented in Table 1. With increase in initial metal ion concentration from 5 to 20 mg/L, the chromium adsorption capacity (mg/g of EPS) increases con-

sistently in both the species of cyanobacteria. However, percent Cr removal somewhat declined at higher concentration. While 94–96% removal took place at C_0 of 5 mg/L, it was 90–91% at C_0 of 10–20 mg/L.

3.3. Adsorption isotherms

In order to understand, compare and predict the biosorption process and surface characteristics of the EPS serving as biosorbent, Langmuir and Freundlich isotherms were applied to the equilibrium data.

Freundlich isotherm [14], which assumes surface of adsorbent to be heterogeneous, is expressed in its linearized form as follows:

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

where K_f and n are Freundlich constants representing adsorption capacity (mg/g of EPS) and intensity of adsorption, C_e = equilibrium constant (mg/L), q_e = amount of metal adsorption at equilibrium (mg/g).

A curve was plotted between $\log q_e$ vs. $\log C_e$ and from the intercept and slope of the curve, values of K_f and n were calculated, which are depicted in Table 2.

Langmuir Isotherm [15], which assumes homogeneous distribution of binding sites over the surface of the adsorbent, is

Table 2
Isotherm constants of Langmuir and Freundlich models for Cr(VI) biosorption onto the EPS of *Nostoc* and *Gloeocapsa*

Isotherm	Parameter	<i>Nostoc punctiforme</i>	<i>Gloeocapsa calcarea</i>
Langmuir	Q_0 (mg/g)	142.9	83.3
	b	0.71	0.36
	R^2	0.8058	0.858
Freundlich	K_f	56.2	21.4
	n	1.71	1.36
	R^2	0.9738	0.9933

expressed in its linearized form as follows:

$$\frac{C_e}{q_e} = \frac{1}{Q_0 b} + \frac{C_e}{Q_0} \quad (2)$$

where Q_0 = adsorption capacity, C_e = equilibrium concentration (mg/g), q_e = amount of metal adsorbed at equilibrium (mg/g), b = Langmuir constant

A curve was plotted between C_e/q_e vs. C_e and from the intercept and slope of the curve values of Q_0 and b were calculated, which are presented in Table 2.

The higher R^2 value i.e. 0.9738 and 0.9933 for EPS of *N. punctiforme* and *G. calcarea*, respectively, shows the better applicability of Freundlich isotherm for these biosorbents as compared to Langmuir isotherm. Between the two biosorbents, the value of Q_0 and K_f representing of adsorption capacity are found to be higher for EPS of *N. punctiforme* as compared to *G. calcarea*, indicating the former to be a better biosorbent.

3.4. Scanning electron microscopy (SEM)

The scanning electron micrographs of EPS of the two cyanobacteria reveals their porous nature along with depressions and grooves on the surface, indicating availability of a large number of binding sites for the metal ions. The binding of the metal ions on the surface of EPS is clearly visible as white encrustations over the pores in the SEM of Cr loaded EPS of both species (Fig. 3). The white encrustations are more prominent in image of *Nostoc*. Our experimental results of metal removal and adsorption isotherms also indicate EPS of *Nostoc* to have better biosorption capacity. The SEM images confirm our experimental observations.

4. Conclusions

The present work compares the effectiveness of chromium biosorption potential of EPS extracted from two natively isolated cyanobacterial strains. While the optimal conditions for chromium removal by the two biosorbents were found to be same (pH 2 and initial metal concentration 20 mg/L) yet there were large differences in quantitative and qualitative nature of

EPS of the two species. *Gloeocapsa* produced substantially greater quantities of EPS as compared to *Nostoc*, which further increased with increasing chromium concentration in the medium. But, the adsorption capacity of EPS of *N. punctiforme* in terms of metal adsorbed per unit weight of EPS was much higher ($K_f = 56.2$ and $Q_0 = 142.9$ mg/g) as compared to that of EPS of *G. calcarea* ($K_f = 21.39$ and $Q_0 = 83.3$ mg/g) as evident from observation of adsorption isotherms. This clearly indicates that functional groups present on the EPS of the two species must be quite different, and those in case of *Nostoc* have a high affinity for chromium ions, which is also indicated in SEM images, characterization of the functional groups of EPS needs to be carried out in future studies to understand the mechanism.

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