Mine Water and the Environment (2018) 37:713–723
https://doi.org/10.1007/s10230-018-0523-3

TECHNICAL ARTICLE

Analysis of Biosorption Parameters, Equilibrium Isotherms and Thermodynamic Studies of Chromium (VI) Uptake by a *Nostoc* sp. Isolated from a Coal Mining Site in Meghalaya, India

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Received: 15 June 2017 / Accepted: 26 February 2018 / Published online: 1 March 2018

Abstract
A cyanobacterium isolated from a water sample collected from a coal mine in the West Khasi Hills of Meghalaya, India, and identified as *Nostoc* sp. (accession no. KX814344) using 16S rRNA analysis showed a high tolerance to chromium. It was shown to be able to grow in the presence of 15 ppm Cr, which is 30 times the highest Cr concentration recorded in the area. Cr biosorption by the cyanobacterium was optimum at pH 6.0 with 3 µg mL^{-1} biomass. The sorption showed a linear correlation with increasing metal concentration, gradually reaching saturation. An energy dispersive X-ray study verified Cr binding on the cyanobacterial biomass, and FTIR analysis revealed many negatively charged functional groups on the cell surface, which aided in metal binding. Thermodynamic studies showed the biosorption process to be energetically favorable: −0.479, −0.665, and −0.852 kJ mol^{-1} at temperatures of 293, 303, and 313K, respectively. Sorption isotherm data fit the Langmuir isotherm best, indicating the monolayer nature of the Cr sorption. The organism's maximum sorption capacity was as high as 20 mg of Cr per g of biomass. The separation factor calculated from the Langmuir isotherm was < 1, signifying favorable interaction between the cyanobacterial biomass and the Cr ions.

Keywords Cyanobacteria · SEM-EDX · FTIR · Langmuir and Freundlich isotherms.

Introduction

Meghalaya, one of the eight states of northeastern India, has large reserves of coal, lime, and uranium. Primitive rat-hole mining and subsequent roadside deposits have contaminated water bodies. The adverse effects of the mine run-off is exacerbated by the hilly terrain and the region's frequent rainfall (Bhattacharjee 2014). In these areas, most of the rural population use the contaminated water for domestic purposes, such as drinking, washing, and irrigation, with no knowledge of the harmful consequences of metal exposure (Bhanu et al. 2014; Gadd 2010). Only limited information is available on Meghalaya's mine water quality with respect to metal contamination. However, a few studies have reported high concentrations of various metal contaminants in water near the coal mining sites (Goswami et al. 2015a; Swer and Singh 2003). Among biologically relevant metals, a general sequence of relative toxicity is: Cr > V > Hg > Cd > Pb = Co > Cu > Ni > Li > Al > Zn > Fe > Mo > Mn > Na > Mg > K > Ca (Nieboer and Richardson 1980).

Chromium was present in all of the water samples collected from the coal mines in the three districts of Meghalaya (Fig. 1). The water samples from Chieruphi (Jaintia Hills) had the highest Cr concentration (0.887 ppm), followed by Shahlang in West Khasi Hills (0.415 ppm), and Cherrapunjee in East Khasi Hills (0.12 ppm). Hexavalent chromium's high solubility and rate of absorption on living surfaces, toxicity, and oxidizing and carcinogenic nature makes it a priority pollutant (Dhal et al. 2013). The World Health Organization (WHO 2003) recommends a maximum concentration limit of 0.1 mg L^{-1} of Cr(VI) for discharge into inland waters, and 0.05 mg L^{-1} in potable water. However, all water samples collected for the study exceeded these recommended limits.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10230-018-0523-3) contains supplementary material, which is available to authorized users.

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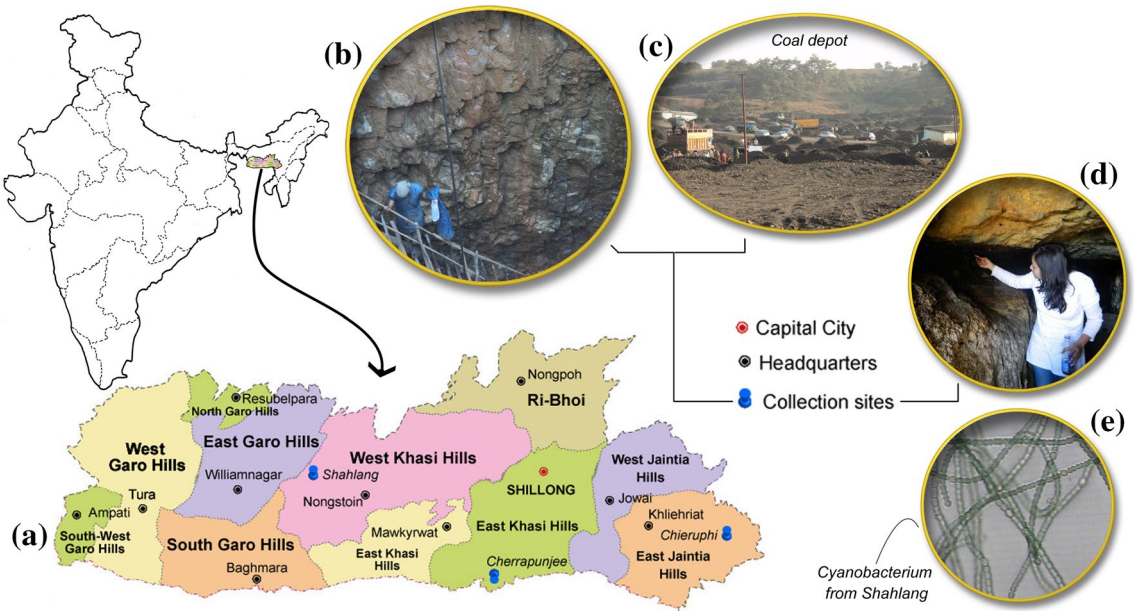

Fig. 1 **a** Larger view of Meghalaya map. Blue pin indicate collection sites; **b** a coal mine in Chiehruphi; **c** Roadside coal deposits; **d** sample collection from a mine; **e** the *Nostoc* sp. used in the study

Conventional techniques used to reduce metal concentrations in wastewater are expensive and seldom fully operative in India (Maheshwari and Gupta 2011). As an alternative to such conventional methods, various biological materials can efficiently remove some environmental contaminants (Chatterjee and Abraham 2015). Use of living organisms is often desirable as their ever-increasing biomass presents fresh surface for metal binding (Arun et al. 2017). Using microbes for metal removal is attractive because microbes have short generation times and can be highly resilient in contaminated wastewater (Deniz et al. 2011; Nongrum and Syiem 2012). To fight metal toxicity, microbes employ different mechanisms, such as biosorption, bioaccumulation, biotranformation, and/or biomineralization (Lim et al. 2005; Umrania 2006).

Cyanobacteria are a group of microbes that can remove metal ions from solutions (Diengdoh et al. 2017; Goswami et al. 2015a). Cyanobacteria occur in diverse habitual conditions, ranging from fresh and marine water to contaminated sites (Hazarika et al. 2015; Ozturk et al. 2014). Being photosynthetic and nitrogen-fixing, their nutrient requirements are simple. They grow rapidly and are generally able to endure and interact with metal ions (due to the presence of negatively charge functional groups on their cell surfaces, which serve as binding sites). These traits make them promising organisms for bioremediation studies (Arun et al. 2017; Bulgariu and Bulgariu 2013). Thus, using microbes, especially cyanobacteria, could lead to the development of eco-friendly and cost effective technology for environmental clean-up operations.

Isolation procedures carried out on the water samples from the mine sites yielded a few cyanobacterial isolates. One isolate from Shahlang, which is located 171 km from the State capital, Shillong, was chosen for Cr removal due to its rapid growth. A detailed study into its growth in the presence of Cr, factors affecting Cr uptake, surface metal binding ability, functional groups of the cell surface involved in metal binding, thermodynamics, and isotherm studies of metal removal were carried out.

Materials and Methods

Isolation, Growth and Maintenance of *Nostoc* sp.

Water samples collected from the three coal mining areas were added to BG-11_0 culture medium [macronutrients: K_2HPO_4 (40 g L^{-1}); $MgSO_4{\cdot}7H_2O$ (75 g L^{-1}); $CaCl_2{\cdot}2H_2O$ (36 g L^{-1}); citric acid (6 g L^{-1}); ferric ammonium citrate (6 g L^{-1}); Na_2CO_3 (20 g L^{-1}); EDTA (1 g L^{-1}); micronutrients: H_3BO_3 (2.86 g L^{-1}); $MnCl_2{\cdot}2H_2O$ (1.81 g L^{-1}); $ZnSO_4{\cdot}7H_2O$ (0.22 g L^{-1}); $Na_2MoO_4{\cdot}2H_2O$ (0.39 g L^{-1}); $CuSO_4{\cdot}5H_2O$ (0.079 g L^{-1}); $Co(NO_3)_2{\cdot}6H_2O$ (0.0494 g L^{-1})] (Rippka et al. 1979). After 2 weeks of incubation, visible colonies of cyanobacteria were picked and plated directly on 1.2% agar prepared in BG-11_0 medium for purification by repeated plating (Rippka 1988). Purified cultures were maintained at 30 ± 2 °C in the same medium in a culture room under continuous light with a photon rate of 50 µmol m^{-2} s^{-1}.

Identification

Among the six cyanobacteria isolated and identified by following the procedures of Desikachary (1959), the fastest growing cyanobacterial isolate was from the Shahlang sample. The organism was identified by partial sequencing of the genomic DNA, which was isolated using the Miniprep Bacterial Genomic DNA method (Ausubel et al. 1999); its quality was tested on a 1.2% agarose gel. A fragment of the 16S rRNA gene from the isolated DNA was amplified by PCR and the purified amplicon was used for sequencing. Forward and reverse DNA sequencing reactions of the PCR amplicon was carried out in Xceleris Labs, Ahmedabad, with CY106F 9 (5′-CGG ACG GGT GAG TAA CGC GTG A-3) and CY781R{equimolar mixture of CY781R(A) 5′-GAC TAC TGG GGT ATC TAA TCC CAT T-3′ and CY781R(B) 5′GAC TAC AGG GGT ATC TAA TCC CTT T-3′} primers using v3.1 cycle sequencing kit on a 3730xl genetic analyser (Applied Biosystems, USA), (Nubel et al. 1997). Related sequences were retrieved from the NCBI Genbank database using BLAST, and the phylogenetic tree was constructed using UPGMA method in MEGA 5 (Tamura et al. 2011).

Metal Treatment

Potassium dichromate ($K_2Cr_2O_7$) was used as the source of Cr(VI) for all of the experiments. A stock solution of 100 ppm was prepared in double distilled deionized water and stored in a dark bottle at 4 °C. The concentration was calculated for the metal ion, not for the whole salt. All working solutions were prepared by diluting the stock solution with double distilled deionized water.

The half lethal dose (IC_{50}) of Cr for the organism was determined by allowing the organism to grow in different concentrations (from 5 to 25 ppm in triplicates for 7 days) of Cr-supplemented BG-11_0 medium under optimal conditions of growth (pH 7.5 and 30 °C). The effect was measured in terms of chlorophyll *a* content.

The dependence of metal sorption on biomass and pH was determined by taking a week old exponentially growing culture at concentrations of 1, 2, 3, 4, 5, 10, 15, and 20 µg mL^{-1} cyanobacterial biomass in 30 mL of 10 ppm Cr supplemented medium, each adjusted to pH 6.0, 7.0, and 8.0, respectively, in triplicate. The amount of metal ions removed at the end of a 24 h incubation period was determined using an Atomic Absorption Spectrophotometer (AAS) (Graphite furnace—AAS Vario-6, Analytik Jena).

The influence of temperature and contact time on metal removal was studied by varying the temperature (293, 303, and 313 K) and contact time (30 min, 1, 2, and 24 h respectively). Since 3 µg mL^{-1} of biomass showed maximum uptake at pH 6.0 among all other biomass concentrations and pH, we added 3 µg mL^{-1} of biomass in flasks containing 10 ppm Cr-supplemented BG-11_0 medium adjusted to pH 6.0. After the incubation period, the amount of residual metal ions was estimated in the supernatant using AAS. This value was subtracted from the initial amount of supplemented metal ions in the test medium to arrive at the amount of metal ion removed.

The effect of increased metal concentrations on the metal uptake, expressed as q (mg of metal sorbed per g of cyanobacterial biomass) was studied by adding 3 µg mL^{-1} of biomass to each test solutions containing 1, 5, 10, 20, 30, 40, and 50 ppm Cr prepared in 30 mL BG-11_0 medium (pH 6.0). These flasks were kept under light at a photon fluence rate of 50 µmol m^{-2} s^{-1} with continuous shaking for 24 h. At the end of the incubation period, the cultures were centrifuged at 2500 rpm for 5 min; the remaining amount of Cr was measured by AAS. The percent removal of Cr was calculated using Eq. 1.

$$\%\ \text{Cr removal} = \frac{C_I - C_F}{C_I} \times 100 \quad (1)$$

where C_I is the initial Cr concentration present in the medium and C_F is the residual Cr concentration in the supernatant.

Analysis of Cr Binding to Cell Surfaces

Cr binding to the cyanobacterial cells were visualized using Scanning Electron Microscopy-Energy Dispersive X-ray (SEM-EDX; Penta FETX3 EDX, in combination with a JEOL-JSM-6360 SEM, Tokyo, Japan). The samples were pre-treated with 4% glutaraldehyde, incubated for 24 h at 4 °C for fixation and washed in 0.1 M sodium cacodylate buffer thrice. Dehydration was carried out in 30, 50, 70, 80, 90, 95, and 100% acetone for two changes at 4 °C. Samples were treated in tetra methyl silane (TMS) for 5 min before mounting on brass stubs for gold coating and were viewed under SEM.

Fourier Transformed Infra-red (FTIR) Spectroscopic Analysis

For FTIR spectroscopic analysis, 5 mL of control and Cr-treated cultures (for 24 h) were transferred onto Petriplates and dried in an oven at 40 °C. Dried samples were mixed with desiccated spectroscopic-grade potassium bromide (KBr), 1:10 (w/w) to make pellets. FTIR analysis was carried out using a Perkin Elmer, 400 FT-IR/FT-FIR spectrometer, model SP400 operating in the range of 4000–450 cm^{-1} to determine the cell surface functional groups involved in metal binding.

Biosorption Studies

Thermodynamics of Cr Sorption

The thermodynamic parameters for the bio-removal of Cr at 20 °C (293 K), 30 °C (303 K), and 40 °C (313 K) were calculated from Eq. 2 below.

$$\Delta G = -RTlnK \; Or \; \Delta G = -2.303RTlogK \tag{2}$$

where ΔG is the change in free energy content (kJ mol^{-1}), R is the molar gas constant (8.314 JK^{-1} mol^{-1}), and T is the absolute temperature (K). The equilibrium constant K can be defined as the mass action ratio between the concentration of Cr adsorbed (mg L^{-1}) on the cyanobacterial biomass and the concentration of the remaining Cr (mg L^{-1}) in the medium at equilibrium.

$$K = \frac{C_B}{C_F} \tag{3}$$

where C_B the concentration of Cr is bound on the cyanobacterial biomass and C_F is the concentration of remaining Cr (mg L^{-1}) in the medium. Alternatively, ΔG can also be calculated from the thermodynamically free energy change of the reaction:

$$\Delta G = \Delta H - T\Delta S \tag{4}$$

where ΔH is the change in enthalpy or the heat content (kJ mol^{-1}) and ΔS is the change in entropy or the measure of randomness (kJ mol^{-1} K). Equation 5, resulting from equating Eqs. 2 and 4, is known as Van't Hoff equation:

$$lnK = \frac{\Delta S}{R} - \frac{\Delta H}{R} \times \frac{1}{T} \tag{5}$$

The slope and the intercept given by $\frac{\Delta H}{R}$ and $\frac{\Delta S}{R}$ of a Van't Hoff plot between ln$K$ and $\frac{1}{T}$ can be used to calculate the ΔH and ΔS respectively (Reddy and Lee 2012).

Equilibrium Isotherm Studies

Isotherm modeling of Cr removal was studied using Langmuir and Freundlich adsorption isotherms (Freundlich 1906; Langmuir 1918), based on metal biosorption studies by other researchers (Desta 2013; Pakshirajan et al. 2013). Both of these isotherms expressed the distribution of ions between the medium and the adsorbent at equilibrium with increasing initial metal concentration in the medium. The amount of metal uptake by the cyanobacterial biomass was expressed using Eq. 6.

$$q = \frac{(C_I - C_F).V}{w} \tag{6}$$

where q = metal uptake (mg metal g^{-1} biomass), C_I = initial metal concentration (mg metal L^{-1} solution), C_F = final metal concentration (mg metal L^{-1} solution), v = volume of solution (L), and w = the biomass weight (g) of biosorbent.

The Langmuir equation assumes that: (1) there are no interactions among the adsorbed species, (2) there are a finite number of energetically uniform sites on the adsorbent and (3) the adsorbate forms a monolayer on the adsorbent surface beyond which no further adsorption takes place. The Langmuir's isotherm is mathematically expressed as

$$q_F = \frac{Q_{max}.K_L.C_F}{1 + K_L.C_F} \tag{7}$$

which can be linearized as:

$$\frac{C_F}{q_F} = \frac{1}{Q_{max}K_L} + \frac{1}{Q_{max}}C_F \tag{8}$$

where q_F = the amount of metal adsorbed per g of biosorbent at equilibrium, C_F = equilibrium concentration, Q_{max} = maximum adsorption capacity of metal ion per unit weight of the biosorbents, and K_L = equilibrium adsorption constant related to the binding affinity.

From the Langmuir equation, a dimensionless equilibrium parameter known as a separation factor can also be obtained which predicts the nature of interaction of the biomass with the metal ions. The separation factor (R_L), is calculated:

$$R_L = \frac{1}{(1 + K_L \; C_I)} \tag{9}$$

A calculated value of $R_L > 1$ indicates unfavorable interaction, $R_L = 1$ shows linear interaction, $R_L < 1$ indicates favorable interaction, and $R_L = 0$ means the interaction is irreversible (Oguz 2005).

On the other hand, Freundlich's isotherm describes adsorption characteristics of a heterogeneous surface with the assumption that enthalpy of adsorption is independent of the amount adsorbed. It is mathematically expressed as

$$q_F = K_F {C_F}^{1/n} \tag{10}$$

where K_F and n are the Freundlich constants indicating sorption capacity and intensity, respectively, and can be calculated from the linearized logarithmic form of Eq. 10:

$$logq_F = logK_F + \frac{1}{n}logC_F \tag{11}$$

Results

Analysis of Water Sample

The water sample (from which the chosen cyanobacterium was isolated) was collected from a spring contaminated by mining affluent. Its analysis is provided (Table 1).

Table 1 Elemental presence in water sample collected from Shahlang

Elements [ppm]	Metal concentration (mg L^{-1})	WHO permissible (mg L^{-1}) limit 2008
Mn	2.426	0.1
Fe	19.75	0.3
Cu	0.078	0.05
Cd	0.046	0.005
Pb	0.469	0.05
Zn	0.285	5
Cr	0.415	0.05

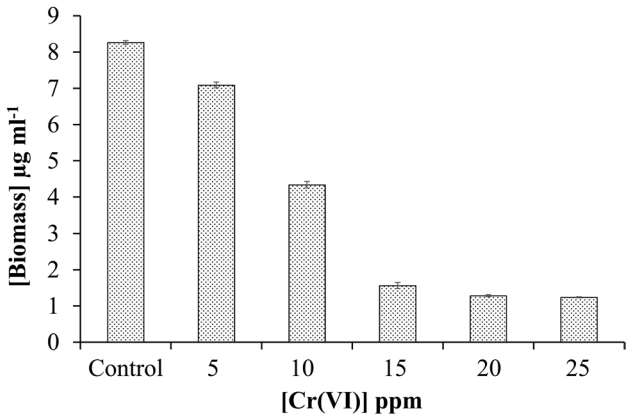

Fig. 3 IC_{50} determination for Cr on *Nostoc* sp. KX814344 after one week of incubation

Identification of Cyanobacterium

The isolated cyanobacterium was identified by comparing partial sequencing of the 16S rRNA gene with similar sequences deposited in the GenBank (NCBI) database using BLAST; based on the maximum identity score, sequences were selected, aligned, and the phylogenetic tree was constructed using the UPGMA method in MEGA 5 (Tamura et al. 2011). The NCBI accession number assigned to this *Nostoc* sp. was KX814344 (Fig. 2).

Determination of Half Lethal Dose (IC_{50})

The initial cyanobacterial biomass concentration inoculated for the study was 3 µg mL^{-1}, as this concentration of biomass yielded the greatest Cr removal. A seven day incubation period was allowed for the metal-induced changes to be noticeable. The half lethal dose was determined from Fig. 3, which shows that 15 ppm of Cr exposure for seven days resulted in a 50% reduction in cyanobacterial biomass. All further experiments were conducted keeping Cr concentration at 10 ppm since it was a sub-lethal dose, though still much higher than in the water from which the organism was isolated.

Factors Regulating Cr Uptake

Optimization of Experimental Parameters

Actual biosorption of metal ions depended on the amount of biosorbent available as well as the pH of the medium. Three other significant parameters were the temperature, concentration of metal ions available in the medium, and the contact time allowed for interaction between the metal ions and the biomass. Percent biosorption of Cr (10 ppm) with varying biosorbent concentrations at three different pH levels is shown in Fig. 4a. Biosorbent concentration determined the amount of surface area available for metal ion binding. As biomass concentration increased from 1 µg mL^{-1}, the percent of Cr sorption increased till 3 µg mL^{-1}; beyond that, there was a gradual decrease in % Cr uptake although the amount of Cr removed (in ppm) increased. The reduction seen in the higher biomass may be due to clumping of cells within the confinement of the experimental flasks, which would have reduced the effective surface area for metal binding. On the other hand, pH is the most important parameter that affected solution chemistry, modulating the activity of the functional groups on the

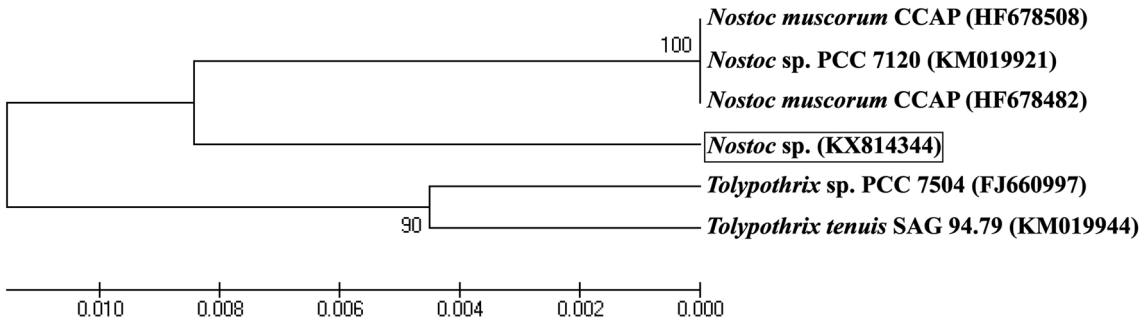

Fig. 2 Phylogenetic tree of the cyanobacterium identified as *Nostoc* sp. KX814344

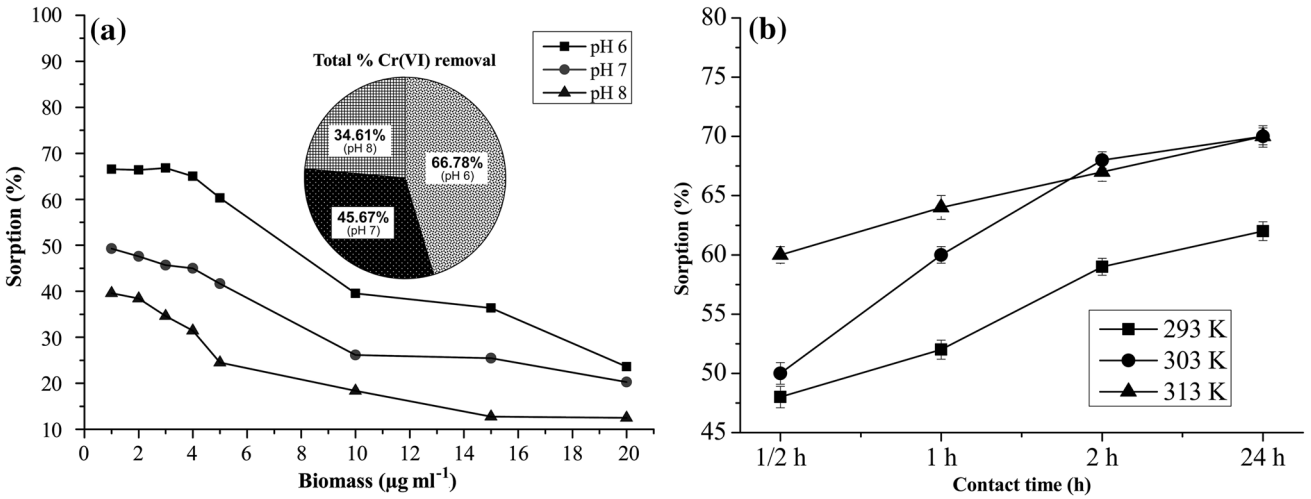

Fig. 4 **a** Effect of pH and biosorbent concentration; **b** effect of temperature and contact time on Cr sorption (%)

surface of the biomass and the competition among metal ions (Galun et al. 1987). The pH determined the number of operative, negatively-charged functional groups available for interaction with the metal ions. The three pH (i.e. pH 6.0, 7.0, and 8.0) values were chosen based on the pH range at which cyanobacteria can survive and function. As seen from Fig. 4a, Cr biosorption was best at pH 6.0. These results suggested that the sorption of Cr on the *Nostoc* sp. biomass was controlled by ionic attraction. At a pH greater than 6.0, precipitation of insoluble metal hydroxides took place, reducing the number of Cr ions in solution for binding to functional groups. In Meghalaya, many water bodies have a pH between 5 and 6. Hence, pH 6 seemed ideal for an in situ application of this *Nostoc* sp. for Cr biosorption in the region.

Also, Fig. 4b shows the effect of temperature on percent sorption of Cr ions from a 10 ppm Cr-supplemented medium as the contact time was increased from 30 min to 24 h. Readings taken after 30 min of contact time showed an increase in % sorption from 48 to 50 to 60%, as temperature increased from 293 to 303 to 313 K. When the temperature was kept constant, there was an increase in % sorption as the contact time increased. For example, at 30 °C, the % sorption increased from 50 to 70% from 30 min to 24 h. However, saturation was almost achieved by 2 h of contact time, beyond which there was little increase in uptake.

Initial Cr ion concentration also played an important role in determining the sorption capacity of the organism. Figure 5 illustrates the effect of increasing Cr concentration on the biosorption capacity of a fixed biomass (3 µg mL^{-1}) when the cells were incubated for 24 h. At lower concentrations, there was higher metal uptake, designated as q (mg g^{-1}), which reached saturation at 40 ppm. Thereafter, uptake remained constant with increasing metal

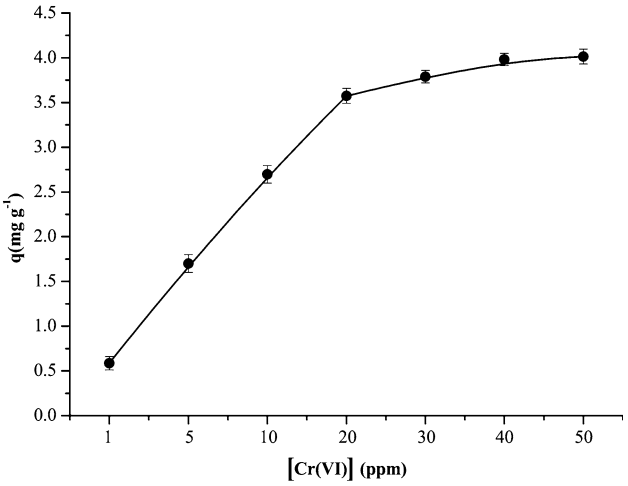

Fig. 5 Uptake *vs*. increasing Cr concentration

concentration, indicating saturation of the surface functional groups on the sorbent biomass.

Morphological Changes and Surface Metal Binding Study

The cyanobacterium showed high tolerance to Cr exposure; this was immediately visible as there were no detectible morphological changes in the treated cells after 24 h (Fig. 6a, b). Results comparing the SEM-EDX spectra of the control and 10 ppm Cr-treated cells after 24 h exposure are presented in Fig. 6c, d, respectively. The arrow in Fig. 6d indicates binding of Cr on the cell surface of the test organism.

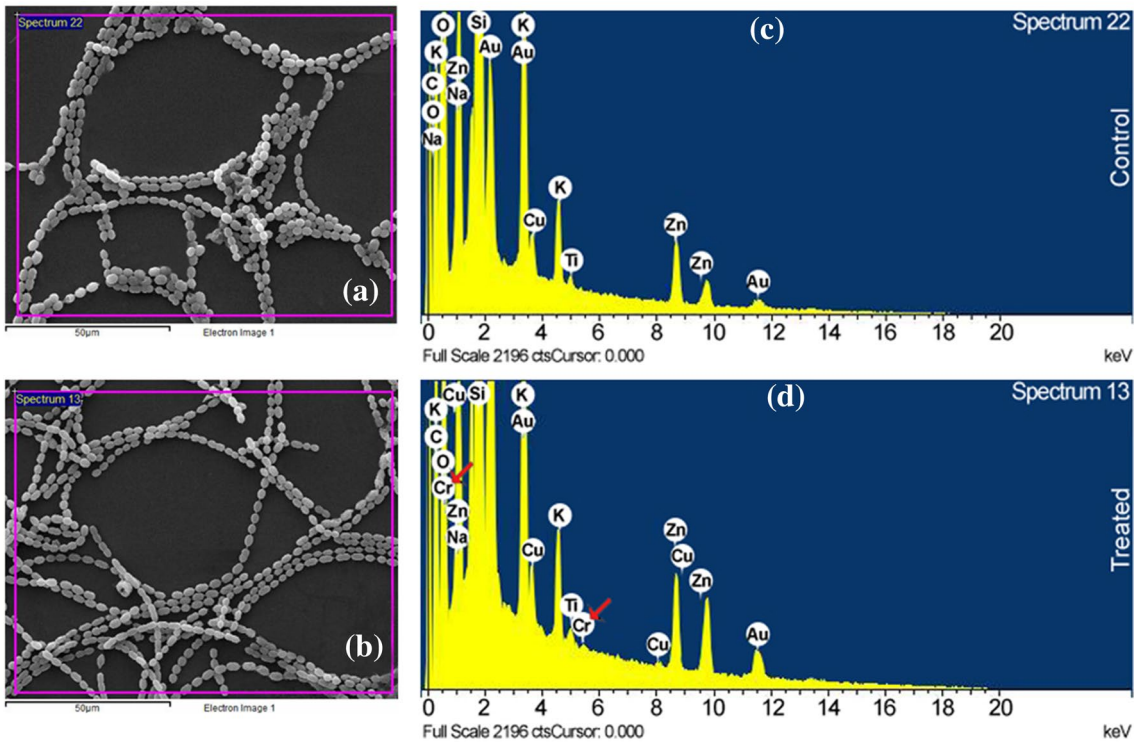

Fig. 6 SEM-EDX analysis of Cr binding on *Nostoc* sp. KX814344

FTIR Fingerprints

A comparative FTIR analysis showed changes in the peak patterns of the control and Cr-treated cells, inferring binding of metal ions. Table 2 provides the functional groups that showed alteration in the peaks (Supplementary Fig. 1). A very broad peak region between 3100 cm^{-1}→ 3600 cm^{-1} indicates the presence of exchangeable protons, typically from alcohol, amine, amide, or carboxylic acids groups. A shift towards higher frequency in the region of 3461 cm^{-1}→ 3488 cm^{-1} was indicative of involvement of O-H groups. A shift in the region of 2910 cm^{-1}→ 2934 cm^{-1} indicated the contribution of alkyl stretch. Involvement of a carbonyl group was evident from the vibrational shift from 1640 cm^{-1}→1661 cm^{-1}. A shift in frequency from 1049 cm^{-1}→1068 cm^{-1} specified C-O group involvement and a shift from 554 cm^{-1}→568 cm^{-1} specified participation of phosphate groups in metal binding.

Equilibrium Isotherm Studies

Experimental data obtained for Cr sorption were fitted into Langmuir and Freundlich isotherm models to analyze sorption performance. The temperature for the isotherm study was maintained at 30 °C as the organism's optimum growth was recorded at this temperature. The various Langmuir and Freundlich isotherm parameters are listed in Table 3. The Langmuir isotherm had the best fit, with a R^2 value of 0.9991, compared to the Freundlich R^2 value of 0.9637 (Fig. 7a, b). The organism's maximum sorption capacity, Q_{max}, was calculated to be 20 mg of Cr taken up per g of *Nostoc* sp. biomass. The calculated value obtained for n (1.63 g L^{-1}) in our study indicated favorable sorption for Cr by this cyanobacterial biomass as

Table 2 Comparative table showing shift in peaks as seen in the FTIR spectra

Observed peaks in		Absorption location wavenumber (cm^{-1})	Corresponding functional group
Control (cm^{-1})	Treated (cm^{-1})		
3461	3488	3550–3200	O–H (alcohol/phenol stretch)
2910	2934	2950–2850	C–H alkyl stretch
1640	1661	1690–1630	C=O (amide stretch)
1049	1068	1050–1645	C–O
554	568	527–715	Phosphate moieties

Table 3 Langmuir and Freundlich adsorption isotherm parameters of Cr-treated *Nostoc* sp.KX814344

Langmuir Isotherm										Freundlich Isotherm		
Q_{max}(mg g^{-1})	K_L (L mg^{-1})	R^2	$R_L$values for the studied concentrations							n(g L^{-1})	K_F(mg g^{-1})	R^2
			1 ppm	2 ppm	3 ppm	4 ppm	5 ppm	10 ppm	15 ppm			
20	0.216	0.9991	0.82	0.69	0.60	0.54	0.48	0.31	0.23	1.63	4.4	0.9637

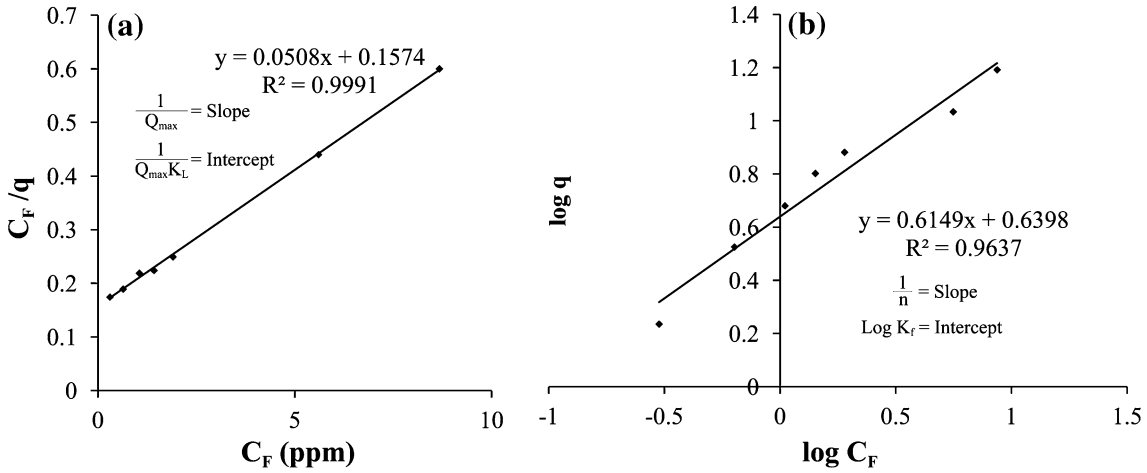

Fig. 7 **a** Langmuir isotherm plot. **b** Freundlich isotherm plot

established earlier (Ahad et al. 2017; Bern and Goldberg 2005; Lai and Lan 2015).

Van't Hoff Plot and Calculation of Cr Sorption Thermodynamic Parameters

The Van't Hoff plot drawn between $lnK$and $\frac{1}{T}$ resulted in a highly linear plot for Cr sorption by the cyanobacterium (Fig. 8). All of the ΔG values (Supplementary Table 1) calculated for the three temperatures studied produced negative values, indicating that the process of Cr biosorption was spontaneous. As seen from the graph,lnK values increased from 0.20067 to 0.27115 to 0.33411, as temperature was raised from 293K to 303K to 313K, respectively, identifying the affinity of the *Nostoc* sp. for Cr ions as a function of temperature. The intercept and the slope of the plot provided a ΔH (i.e. the change in enthalpy) of 4.98 kJ mol^{-1} and a ΔS (the entropy change) of 0.0186 kJ mol^{-1} K^{-1}. The positive ΔH and ΔS values signified the process to be endothermic; the Cr sorption indicated randomness, making the process thermodynamically favourable.

Discussion

The choice of metals for a biosorption study is determined by factors such as value, toxicity, and impact on health and environment. Our interest in Cr stemmed from the fact that it was present in many water samples collected from the coal mining areas. Many different microbes and plants have been tested for biosorption for the clean-up of degraded environments (Singh et al. 2014). Among microbes, cyanobacteria, being both photosynthetic and nitrogen-fixing, require minimal nutrient input, thereby making them cost effective for potential biotechnological applications (Cepoi et al. 2016; Mohamed 2001).

Removal of metal ions by live cells is a complex process as various metabolic activities control the complicated particulars of metal uptake and internalization. Other than that, external factors such as pH, temperature, initial metal concentration, amount of biomass available, and contact time guide metal removal by active cells. The sorption increased as the temperature was raised from 20 to 40 °C,

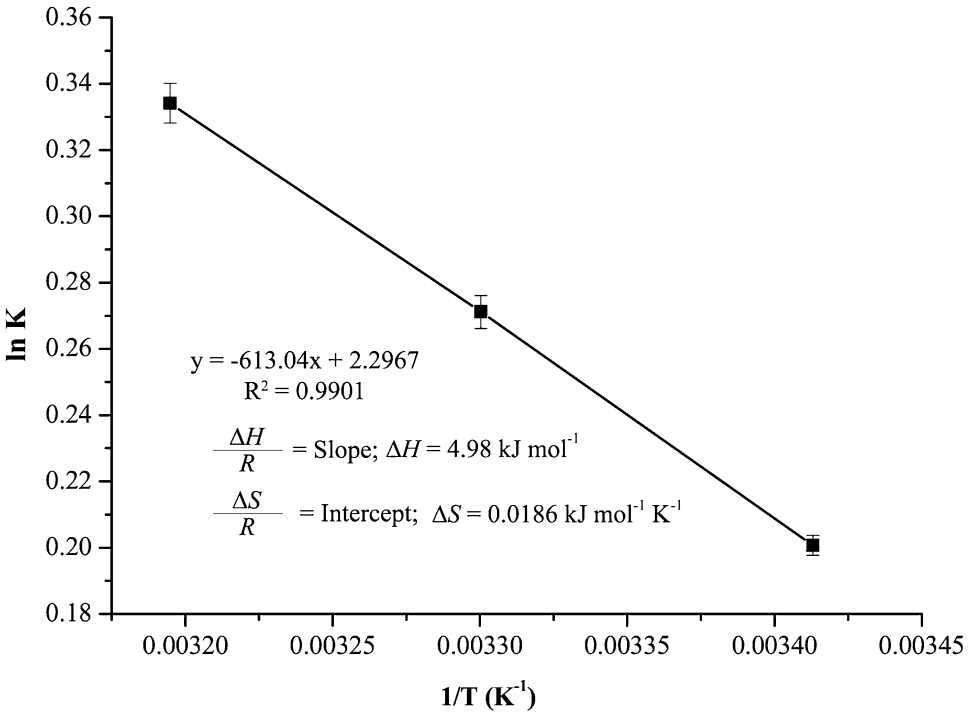

Fig. 8 The Van't Hoff plot

which suggests that increasing the kinetic energy of the sorbent-sorbate system led to more collisions, favouring binding. However, keeping in mind that live cells were the sorbent system and that these grew best at 30 °C, this temperature was maintained for all further studies.

The pH influences both protonation of cell surface functional groups as well as metal chemistry (Dixit and Singh 2013). At a low pH, the high amount of H^+ ions compete with the positively charged metal ions, thereby effectively reducing binding of the metal of interest. In contrast, as pH increases, more negatively charged ligands get exposed on the sorbent surface, favouring more metal binding (Aksu 2001; Gong et al. 2005; Mehta and Gaur 2005). However, at higher pH, there could be metal hydroxylation leading to lower metal ion concentrations in solution (Goswami et al. 2015b; Reddy and Lee 2012). The present study established various parameters such as pH 6.0, temperature 30 °C, a biomass of 3 µg mL^{-1}, and 2 h of contact time to be optimum for biosorption of Cr by the cyanobacterium *Nostoc* sp. KX814344.

Although a comparative picture of Cr removal capacity by different organisms can be assembled from Table 4, almost all researchers have shown Cr uptake in acidic conditions. Even for the cyanobacterial strains, a sorption capacity between 12 and 23 mg g^{-1} was achieved only at quite an acidic pH between 3.0 and 4.0 (Anjana et al. 2007; Gupta and Rastogi 2008). However, the survivability of most such organisms would be extremely compromised at this pH, making continuous Cr removal problematic. This was a gap in the Cr-related bioremediation

Table 4 Comparative table showing reports of Cr (VI) uptake by different biosorbents

Organisms used for Cr(VI) sorption	Biosorbent type	Cr(VI) uptake capacity (mg g^{-1})	pH	References
Marine *Aspergillus niger*	Fungus	117.33	1	Khambhaty et al. (2009)
Nostoc muscorum	Cyanobacteria	22.92	3.0	Gupta and Rastogi (2008)
*Chroococcus*sp.HH-11	Cyanobacteria	21.36	4.0	Anjana et al. (2007)
Eragrostistef	Grass	21.30	6.5	Desta (2013)
Nostoc sp. KX814344	Cyanobacteria	20.0	6.0	This study
Nostoc calcicola HH- 12	Cyanobacteria	12.23	3.0	Anjana et al. (2007)
Agaricusbisporus	Mushroom	8	1	Ertugay and Bayhan (2008)
Rhizophora mangle L	Mangrove tree	5.72	4.5–5.5	Sahmoune et al. (2010)
Banana peel	Fruit	3.0	3.0	Pakshirajan et al. (2013)

research. Although bioremediation using dead biomass is a feasible option, the advantage of using live biomass to provide new cell surfaces for continuous Cr sorption would not be an alternative at such an acidic pH. Our study showed substantial growth of the organism at pH 6.0 and that a lower pH impaired survivability, so a pH of 6.0 was maintained in all of our experiments. At this pH, the live biomass of the *Nostoc* sp. evaluated in this study showed a Cr uptake of 20.0 mg g^{-1}, comparable to Cr uptake of 22.92 mg g^{-1} by dead biomass of *Nostoc muscorum* at pH 3.0 (Gupta and Rastogi 2008). This indicated that the live cells of this organism has a similar Cr removal capacity but at a much higher pH that would support its growth.

As the biomass was increased from 1 µg mL^{-1}, a maximum sorption was noticed in 3 µg mL^{-1} when the pH was maintained at 6.0, although higher biomass concentrations were also tested for a fixed metal concentration. As explained earlier, this may be due to filament overlapping and cell clumping at higher biomass concentrations reducing the effective surface area available for metal binding. There was a positive correlation between Cr sorption and initial metal concentration until the functional groups on the fixed biomass were saturated. Maximum sorption was at ≈66% in the optimum conditions fixed for this study, i.e. 30 °C, pH 6.0, and 3 µg mL^{-1} biomass at an initial concentration of 40 ppm Cr in the medium (Fig. 5), indicating a potential saturation limit.

From the isotherm study, Cr removal showed a better fit in the Langmuir isotherm model, suggesting a monolayer Cr sorption process on the cyanobacterial biomass. The separation factors calculated from the Langmuir Eq. (0.82 for 1 ppm ….. 0.23 for 15 ppm) indicated favourable interaction between the Cr ions and the sorbent. Thermodynamic analysis showed Cr sorption to be endothermic, given the increase in ln*K* values with increasing temperature. The negative ΔG values obtained from the Van't Hoff plot further confirmed the feasibility and spontaneity of the process. All of these results indicated the potential of this cyanobacterium to remediate contaminated environments by metal sorption. The fact that this cyanobacterium *Nostoc* sp. KX814344 was isolated from water near a mining site highlighted its ability to acclimatize physiologically and metabolically to environments with high metal contaminants. It also showed potential towards hyper-accumulation of Cr at pH relevant for its growth. Thus, such indigenous isolates from polluted sites could be ideal for future research in metal bioremediation technology where removal of metals such as Cr could be achieved in continuous flow bioreactors, with the added advantage of live organisms unceasingly providing newer cell surface for biosorption.

Conclusion

The cyanobacterium *Nostoc* sp. KX814344 was found growing in coal mining contaminated water and showed moderately high removal capacity for Cr. The thermodynamic study showed the process to be endothermic and energetically favorable with a negative ΔG value. The sorption pattern followed a Langmuir monolayer isotherm. The pH, temperature, biomass concentration, and contact time between the metal ion and the cyanobacterium dictated Cr uptake. Use of optimized experimental parameters maximized Cr removal, indicating the possibility of manipulation in experimental conditions for higher metal removal by live organisms. Cumulative understanding of all these results supports our assumption that *Nostoc* sp. KX814344 has the potential to be a live sorbent system for Cr removal from wastewater.

Acknowledgements The authors acknowledge the University Grants Commission, New Delhi, Government of India for financial assistance and granting the National Fellowship for Higher Education of ST students. The authors also acknowledge the Department of Chemistry for FTIR facility and SAIF, North Eastern Hill University for GF-AAS and SEM services.

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