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Cr^{6+} bioremediation efficiency of *Oscillatoria laete-virens* (Crouan & Crouan) Gomont and *Oscillatoria trichoides* Szafer: kinetics and equilibrium study

Jyothi Miranda · G. Krishnakumar · Richard Gonsalves

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Abstract Two species of cyanobacteria, *Oscillatoria laete-virens* (Crouan & Crouan) Gomont and *Oscillatoria trichoides* Szafer, were isolated from a polluted environment and studied for their Cr^{6+} removal efficiency from aqueous solutions. The parameters studied included the solution pH, contact time, initial concentration of Cr^{6+} and culture density. Living biomass is more efficient than dead biomass in Cr^{6+} removal. Removal by living biomass involves bioreduction and biosorption. Below pH 3.1, bioreduction is favored and biosorption is dominant at higher pH. The highest removal through biosorption for living biomass was achieved between pH 5 and 5.9 and for dead biomass at pH 2. The maximum removal was on the tenth day of exposure for both the species. Cr^{6+} removal increased from 0.2 to 0.4 g L^{-1} of culture biomass with a decrease with further increase in biomass. Increased Cr^{6+} concentration decreases growth of both the species over time. Both species tolerate a concentration as high as 30 mg L^{-1} Cr^{6+}. There was no evidence of bioreduction in the case of dead biomass. Living biomass of *O. laete-virens* followed both Langmuir and Freundlich models with maximum sorptive capacity (q_{max}) of 21.88 mg g^{-1}. The results of dead biomass were well fitted only to Langmuir isotherm. *O. trichoides* living biomass did not follow either of the isotherms, but removed the metal to a maximum extent of 38.7mg g^{-1}. The removal was better described by Freundlich isotherm in case of dead biomass. The pseudo-first-order model describes the kinetics better than the pseudo-second-order model in the case of living biomass. Participation of carboxylic, carbonyl, and amino groups in Cr^{6+} removal was confirmed by FTIR analysis. Both species seem to be promising biosorbents for Cr^{6+}.

Keywords Biosorption · pH dependence · Bioreduction · Langmuir isotherm · Freundlich isotherm · Kinetics · FTIR

J. Miranda (✉) · G. Krishnakumar
Department of Applied Botany, Mangalore University, Mangalagangothri 574199, India
e-mail: jyothi_ms2000@yahoo.co.in

G. Krishnakumar
e-mail: kkgmane@rediffmail.com

R. Gonsalves
Department of Chemistry, St. Aloysius College, Mangalore 575003, India
e-mail: richieag@yahoo.com

Introduction

Heavy metals as pollutants cause serious ecological problems since they are extremely toxic and tend to bioaccumulate throughout the food chain. Chromium is one such pollutant that is released from industries relating to alloys, film and photography, leather tanning, dyes, pigments, electroplating, textile, and wood preservation (Cervantes et al. 2001; Sarin and Pant 2006; Ozturk et al. 2009; Cheung and Gu 2007; Qaiser et al. 2009; Saha and Orvig 2010). Chromium is also an essential nutrient for plants (Prado et al. 2010).

Chromium exists in several oxidation states, but the most stable and common forms are Cr^{3+} and Cr^{6+} of which Cr^{6+} is more toxic (Cheung and Gu 2007; Kiran et al. 2007; Gupta and Rastogi 2008). Cr^{6+} occurs as chromate (CrO_4^{2-}) or di-chromate ($Cr_2O_7^{2-}$) ions (Sarin and Pant 2006; Ozturk et al. 2009) and Cr^{3+} in the form of oxides, hydroxides, or sulfates and exists mostly bound to organic matter in soil in the aquatic environments. Cr^{6+} is a strong oxidizing agent and in the presence of organic matter it is reduced to Cr^{3+}, a process which is fast in acidic environment. But at higher concentration, it may overcome the reducing capacity of the

environment and persist as a pollutant (Cervantes et al. 2001).

Chromate is actively transported across biological membranes in microorganisms (Dreyfuss 1964) through membrane sulfate channels (Cheung and Gu 2007). Inside the cells, Cr^{6+} is reduced to Cr^{3+} via unstable Cr^{5+} and Cr^{4+} states (Arslan et al. 1987). Cr^{6+} exists mainly in the oxyanion form and can only be trapped by the cationic components of biosorbent surfaces in an acidic pH. The cell membrane is nearly impermeable to Cr^{3+} because of its insolubility, which facilitates its precipitation and removal (Alcedo and Wetterhahn 1990). The biotransformation of Cr^{6+} to Cr^{3+} is considered as an alternative process for treating Cr^{6+} contaminated waste (Cheung and Gu 2007; Cervantes et al. 2001).

Cr^{6+} has been classified as a human carcinogen. The permissible limit for chromium in drinking water is 0.05 mg L^{-1} while for waste water it is 1 mg L^{-1} of Cr^{6+} (WHO 2004; US EPA 1998) and therefore it is essential to reduce Cr^{6+} concentration in water or waste water to acceptable limits. Physicochemical methods of toxic metal removal are energy consuming and utilize a large amount of reagents (Ozturk et al. 2009), whereas biosorption is environmentally friendly and particularly efficient with low concentration of metals. Microorganisms with high level of tolerance to chromium can be candidates for bioremediation of chromium pollution. This tolerance may be due to biosorption, diminished accumulation, precipitation, and reduction of Cr^{6+} to Cr^{3+}.

Both live and dead mass of microalgae are useful as biosorbents. Microalgae are preferred for their photosynthetic efficiency, simple nutritional requirements, ease of mass cultivation, and greater production of mucilage with high binding affinity (Khattar et al. 2007; Gupta and Rastogi 2008). Also, compared to fungi and bacteria, they have a greater capacity of metal uptake (Tüzün et al. 2005) due to the presence of functional groups like carboxyl, hydroxyl, sulfate, phosphate, and amines in their cell walls which can sequester heavy metal ions in a short period of time (Gong et al. 2005).

Cr^{6+} removal by non-viable cells of cyanobacteria has been demonstrated, using alginate-immobilized *Lyngbya putealis* (Kiran et al. 2007), *Nostoc muscorum* (Gupta and Rastogi 2008), and immobilized *Nostoc calcicola* and *Chroococcus* sp. (Anjana et al. 2007). However, only a few reports exist on the removal by viable cells such as *Nostoc linckia* (Bala et al. 2004), *Anacystis nidulans* (Khattar et al. 2007), and *Synechocystis* sp (Ozturk et al. 2009). This may be due to the low pH range required for Cr^{6+} removal. Living cells are advantageous as biosorbents because they can create a continuous supply of unsaturated metal removing biomass (Terry and Stone 2002), and some living systems can have higher metal removal efficiency than the dead cells (Rahmani and Sternberg 1999). However, the viability and metabolic activity of cells is a major limiting factor affecting the detoxification efficiency. Metal removal using cyanobacterial species isolated from polluted environments has been reported previously (Monteiro et al. 2009a, b; 2010).

To the best of our knowledge, no study has been carried out on the removal of Cr^{6+} using living biomass of *Oscillatoria* species which are very common in both fresh and polluted water habitats with acidic pH. Accordingly, the objectives of the study were to assess the Cr^{6+} removal capacity of both living and dead cells of two species of fresh water filamentous cyanobacteria, *Oscillatoria laete-virens* and *Oscillatoria trichoides*, which were isolated from an industrial polluted region in Mangalore, India. This study also tries to understand the adsorption and absorption capacities of living cells as well as to compare their biosorption capacities and compares the Cr^{6+} removal efficiency of living and dead cells, with respect to pH, contact time, initial metal ion concentration, adsorbent dose, and the impact of this metal on growth. Adsorption isotherms were applied to fit the experimental data and the kinetic models were also used to determine the sorption rates. Biomass–metal ion interactions were evaluated by FTIR analysis.

Materials and methods

Oscillatoria laete-virens and *O. trichoides* were isolated from the industrial waste waters near Mangalore, India, and identified referring to the monograph of Desikachary (1959). Pure cultures were obtained following standard isolation and culturing techniques on BG-11 medium (Stein 1973). Batch cultures were grown in sterilized optimal BG-11 medium without EDTA in 100-mL borosilicate culture flasks. Cultures were maintained at 25 ± 2°C under a 16:8 light–dark cycle and an irradiance of ~30 μmol photons m^{-2} s^{-1} provided by cool white fluorescent lamps. Filaments from the exponential growth phase were used as inoculum in the subsequent experiments. Growth was assessed by estimating the Chl-*a* content (Jeffrey and Humphrey 1975).

A stock solution of Cr^{6+} ions was prepared by dissolving $K_2Cr_2O_7$ in deionized water. Desired concentrations of Cr^{6+} were obtained by diluting the stock solution. Glassware and other materials used to handle and grow the cyanobacteria were previously rinsed with dilute nitric acid and then with double distilled water to prevent interferences with analytical assays. Experiments were carried out in triplicate.

Cyanobacterial growth at different pH and Cr^{6+} concentrations

A pH range of 2–7 was chosen for the study. Then 0.4 g L^{-1} of cells was added to BG-11 medium whose initial pH was

adjusted using 0.1 N HCl or 0.1 N NaOH. Change in the pH was recorded once in 2 days. Moreover, 0.4 g L^{-1} of biomass each was also grown in culture media having a suitable pH with 10, 20, 30, 40, and 50 mg L^{-1} of Cr^{6+} in triplicates .The cells were grown for up to 12 days. Chl-*a* content was measured at regular intervals. There was a prior adaptation to 5–10 mg L^{-1} Cr^{6+} during isolation of cyanobacterial species.

Metal removal by varying factors

The metal removal capacity of these cyanobacteria were studied by culturing 0.4 g L^{-1} of the biomass in media with initial concentration of 10, 20, 30, 40, and 50 mg L^{-1} Cr^{6+}. The extent of Cr^{6+} removal was determined as a function of pH by exposing aliquots of 0.4 g L^{-1} of living biomass to a medium containing Cr^{6+} at a pH previously adjusted between pH 2 and 7 using 0.1 N HCl or 0.1 N NaOH. Change in pH was recorded once in 2 days. To see the effect of culture density on Cr^{6+} removal, the experiments were carried out at 30 mg L^{-1} of Cr^{6+} with a culture density of 0.2, 0.4, 0.8, and 1.2 g L^{-1}.

Each of these experiments was conducted in triplicate for 12 days. Blank controls were used to ensure that the supernatant Cr^{6+} concentration initially provided was not affected by any other factor. Flasks were agitated at 100 rpm in a shaker. Reaction mixtures were separated by centrifugation at 6,000 rpm for 15 min. The supernatants were collected for chromium analysis and the pellet was washed with 0.02 M EDTA for 15 min at 100 rpm to remove Cr^{6+} ions adsorbed onto the cell surface and then centrifuged at 10,000 rpm. The pellet was acid-digested and the biosorbed intracellular Cr^{6+} estimated.

Cr^{6+} removal by dead cells

Biomass harvested from an exponential growth phase was dried at 60°C for 24 h. The amount of 0.4 g L^{-1} dry weight of dead biomass was then transferred to a metal solution with the required concentration of Cr^{6+}. The Cr^{6+} removal capacity of dead cells was assessed against pH, equilibrium time, initial metal ion concentration, and biomass density. To determine Cr^{6+} removal as a function of pH, the aliquots of 0.4g L^{-1} of biomass was added to a metal solution previously adjusted to pH 2 to 7 and containing an initial Cr^{6+} concentration of 10 mg L^{-1}. To determine the contact time required for maximum adsorption, 0.4 g L^{-1} biomass was suspended in 10 mg L^{-1} metal solution. To check the effect of initial metal ion concentration on removal of Cr^{6+}, the biomass was suspended in Cr^{6+} solution having a concentration of 10, 20, 30, 40, and 50 mg L^{-1}. The system was kept on a rotary shaker at 100 rpm and the samples were removed at 0, 15, 30, 60, 90, 120, and 150 min and the supernatant assayed for residual Cr^{6+} concentration.

Analysis of metal ions

Cr^{6+} in the supernatant was measured spectrophotometrically at 540 nm with diphenyl carbazide as the complexing agent (Clesceri et al. 1998). The concentration range studied yields a linear plot indicating adherence to the Beer–Lambert's law. A new calibration curve was prepared for each analysis. To measure the chromium incorporated into the cells, the pellets were subjected to FA-AAS (flame atomized atomic absorption spectrophotometer) in a GBC 932 plus AVANTA spectrophotometer with a lamp current of 6 mA at wavelength 359.3 nm having an optimum working range 0.1–20 mg L^{-1}.

The total Cr^{6+} removed by the cyanobacterial cells was calculated as

$$q = \frac{(C_i - C_f)v}{m} \quad (1)$$

where q=metal removed (mg g^{-1}), C_i=initial Cr^{6+}concentration in the supernatant (mg L^{-1}), C_f=final Cr^{6+}concentration in the supernatant (mg L^{-1}), v=volume of medium (L), and m= amount of biomass taken (g).

The amount of Cr^{6+} adsorbed onto the cell surface was calculated as the difference between the total Cr^{6+} removed and the amount incorporated into the cells. The amount of reduced Cr^{6+} is the difference between final total chromium and final Cr^{6+} in the supernatant (Aoyama and Tsuda 2001; Park et al. 2006).

Adsorption isotherms

The sorption data obtained for chromium uptake at 25±2°C were plotted using Langmuir equation (Langmuir 1918) (Eq. 2) and Freundlich equation (Freundlich 1907) (Eq. 3) given below.

$$\frac{1}{q_{eq}} = \frac{1}{q_{max}} + \frac{1}{b\, q_{max}\, C_{eq}} \quad (2)$$

Where q_{eq} is the amount of metal adsorbed per unit weight of adsorbent at equilibrium (mg g^{-1}), q_{max} is the maximum metal uptake per unit mass of adsorbent (mg g^{-1}), b is the Langmuir constant (L mg^{-1}) related to energy of sorption which quantitatively reflects the affinity between the sorbent and sorbate, and C_{eq} is the equilibrium concentration of adsorbate (mg L^{-1}).

$$\log q_{eq} = \log K_f + 1/n \log C_{eq} \quad (3)$$

Where K_f (mg g^{-1}) is the biosorptive uptake and n is the biosorption equilibrium constant indicative of the general shape of the isotherm.

The Langmuir equation suggests the monolayer sorption onto a surface containing definite number of identical sites.

Freundlich equation is based on sorption on a heterogeneous surface. Parameters of both models were calculated from the intercepts and slopes of $1/q_e$ versus $1/C_e$ and log q_e versus log C_e plots, respectively.

Kinetic studies

Pseudo-first-order and pseudo-second-order kinetic models are used in this study (Ho and Mckay 1998). The pseudo-first-order model is based on sorbent capacity and considers that the rate of adsorption is proportional to the number of unoccupied sites. The pseudo-second-order equation is based on adsorption capacity of solid phase (Aksu and Tunc 2005). The pseudo-first-order model is given by the derivative expression:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (4)$$

On integration, Eq. (4) and using the conditions q_t=0 at t=0, the linear form obtained is given as

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303} \quad (5)$$

Where k_1 is the rate constant of pseudo-first-order adsorption (min^{-1}), q_t is the amount of Cr^{6+} adsorbed by the sorbent at time t (mg g^{-1}), and q_e is the amount adsorbed at equilibrium (mg g^{-1}). A plot of $\log(q_e - q_t)$ against t is linear and k_1 can be determined from the slope.

The pseudo-second-order equation (Ho and Mckay 1998) is given by the derivative expression:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (6)$$

On integration, Eq. (6) and using condition q_t=0 at t=0 the linear form is obtained as

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (7)$$

Where k_2 is the rate constant of pseudo-second-order adsorption (g mg^{-1} min^{-1}). A plot of t/q_t as a function of t is linear and the value of k_2 is determined from the slope and intercept.

FTIR analysis

Biomass in the solid phase was subjected to IR analysis to assess the chemical groups present on the cell wall and analysis of control was compared with treated cultures. Infrared spectra were obtained with the help of FTIR spectrophotometer (IR Prestige-21, Shimadzu). Translucent sample disks were prepared by encapsulating 0.1 g of finely sized biomass in 1 g of KBr.

Statistical analysis

All experiments were performed in triplicate and the results were expressed as means±SD. SPSS software was used for statistical analysis. A two-way ANOVA was used to compare the significant differences and multiple comparison was performed by Bonferroni test. The relationship between total metal removed, adsorbed, and absorbed was obtained by Karl Pearson's correlation coefficient.

Results

Cyanobacterial growth

Growth of *Oscillatoria* sp. was assessed for the pH range of 2–7. At pH 2, death of algal cells was seen on the second day and at pH 3 chlorophyll-*a* content decreased with prolonged culture time (Fig. 1a, b). pH 4 showed delayed growth (growth rate=0.004 mg L^{-1} h^{-1}). In the pH range 5–7, there was an increase in growth with increase in pH. Change in pH that occurred during the course of the experiment is shown in Table 1. This change in pH was significant (p<0.05) in the range 5–7. At the end of 12 days of cultivation, initial pH of 4, 5, 6, and 7 increased to about pH 5.91±0.01, 6.33±0.014, 7.55±0.04, and 8.75±0.05 for *O. laete-virens* and pH 5.9±0.1, 6.26±0.05, 7.56±0.03, and 8.74±0.05 for *O. trichoides*.

Increased Cr^{6+} concentration showed a significant decrease (p<0.01) in growth in both the species over time (Fig. 2a, b). A two-way ANOVA indicates a significant change in cell growth in response to Cr^{6+} concentration and incubation time for each organism. The F values associated with biomass vs. time vs. initial Cr^{6+} concentration vs. their interactions were 436.13 (p<0.001), 123.65 (p<0.001), and 17.17 (p<0.001) for *O. laete-virens*. The values for *O. trichoides* were 504.40 (p<0.001), 287.77 (p<0.001), and 22.02 (p<0.001). Further, Bonferroni test suggests *O. trichoides* to be significantly more tolerant to Cr^{6+} ions (p<0.05) in all days of growth than *O. laete-virens* except on the fourth, sixth, and 12th days at 50 mg L^{-1}.

Effect of initial concentration on metal removal

Cr^{6+} removal is both by adsorption onto the cell surface and intracellular uptake. The total amount of Cr^{6+} removed, adsorbed, and incorporated into the cells as a function of exposure time and initial Cr^{6+} concentration is shown in Table 2. The initial concentration of Cr^{6+} remarkably influenced the equilibrium metal uptake (Fig. 3). When C_i was increased from 10 to 30 mg L^{-1}, the metal removal capacity increased from to 5.4±0.4 to 13.7±1.7 mg g^{-1} for *O. laete-virens*, and from 6.19±0.36 to

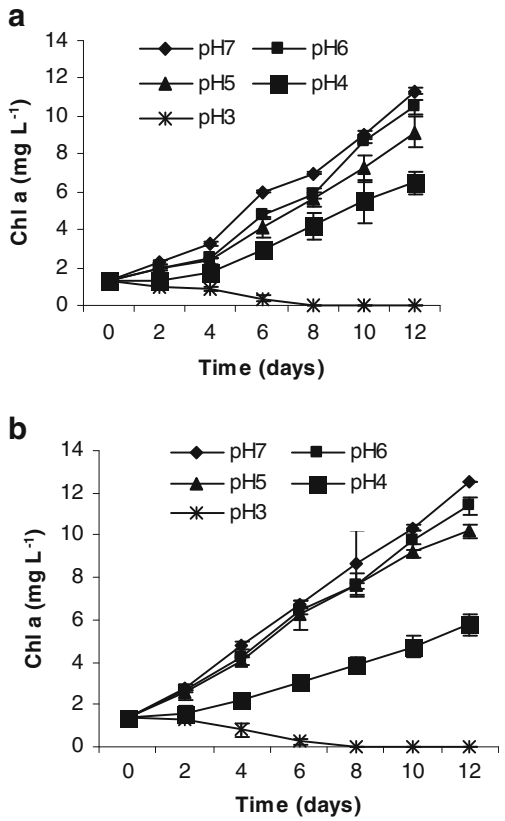

Fig. 1 Effect of pH on the growth of **a** *O. laete-virens* and **b** *O. trichoides*. Results are expressed as means; *error bars* represent standard deviation (n=3)

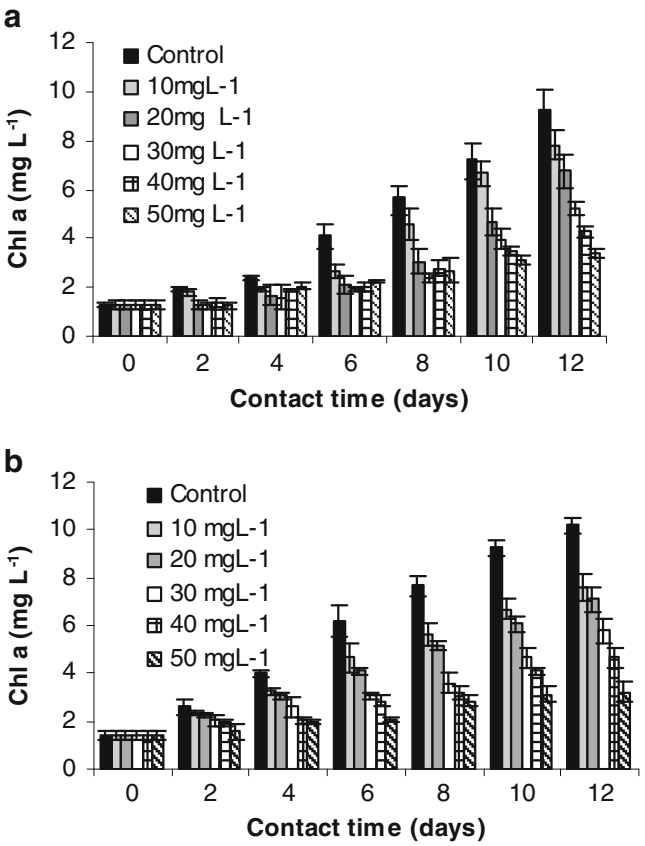

Fig. 2 **a**, **b** Growth of *O. laete-virens* and *O. trichoides* at various initial Cr^{6+} concentrations. Results are expressed as means; *error bars* represent standard deviation (n=3)

38.7±3.8 mg g^{-1} for *O. trichoides*. Further increase in C_i does not show much effect on the removal. Figure 3 shows almost similar values for q at higher C_i. The highest percent removal (20%) of Cr^{6+} was at the initial concentration of 10 mg L^{-1} for *O. laete-virens* and at 30 mg L^{-1} (51.6%) for *O. trichoides*. Increased Cr^{6+} concentrations increase Cr^{6+} absorption for both the

Table 1 Variation in pH during the growth of *Oscillatoria laete-virens* and *Oscillatoria trichoides* at different pH values

pH	Time (days)					
	2	4	6	8	10	12
Oscillatoria laete-virens						
2	2.01±0.01	2.16±0.05	2.28±0.02	2.43±0.04	2.44±0.05	2.4±0.01
3	3.08±0.06	3.2±0.01	3.29±0.01	3.3±0.01	3.30±0.01	3.29±0.01
4	4.11±0.01	4.23±0.02	4.49±0.01	4.71±0.03	5.25±0.04	5.91±0.01
5	5.13±0.03	5.31±0.02	5.59±0.01	5.85±0.05	6.18±0.07	6.33±0.014
6	6.2±0.01	6.38±0.02	6.80±0.02	7.22±0.06	7.42±0.02	7.55±0.04
7	7.18±0.04	7.5±0.05	8.2±0.03	8.40±0.02	8.52±0.01	8.75±0.05
Oscillatoria trichoides						
2	2.03±0.05	2.24±0.49	2.31±0.01	2.5±0.01	2.49±0.01	2.49±0.005
3	3.12±0.02	3.23±0.26	3.30±0.01	3.32±0.01	3.31±0.01	3.23±0.11
4	4.12±0.02	4.24±0.02	4.51±0.01	4.75±0.02	5.27±0.06	5.9±0.1
5	5.13±0.03	5.34±0.05	5.6±0.01	5.90±0.04	6.15±0.04	6.26±0.05
6	6.23±0.02	6.44±0.04	6.83±0.02	7.25±0.01	7.46±0.02	7.56±0.03
7	7.23±0.03	7.56±0.02	8.17±0.03	8.47±0.05	8.55±0.01	8.74±0.05

Results are expressed as means± standard deviation (n=3)

Table 2 Total amounts of Cr^{6+} removed, adsorbed, and accumulated by *Oscillatoria laete-virens* and *Oscillatoria trichoides* at various initial concentrations of Cr^{6+}

Concentration of Cr^{6+}	Biosorption (mg g^{-1})	Days					
		2	4	6	8	10	12
Oscillatoria laete-virens							
10 mg L^{-1}	Removal	1.8±0.1	2.99±0.2	3.63±0.2	3.53±0.37	5.4±0.4	5.2±0.1
	Adsorbed	1.59±0.01	2.70±0.15	3.23±0.09	3.8±0.36	4.58±0.46	3.97±0.16
	Accumulated	0.28±0.08	0.28±0.05	0.40±0.11	0.66±0.008	0.81±0.06	1.29±0.05
20 mg L^{-1}	Removal	1.92±0.6	3.54±0.7	4.63±0.5	5.46±0.47	6.94±0.53	6.26±0.4
	Adsorbed	1.9±0.6	3.3±0.6	4±0.3	5.1±5.1	6.54±0.3	5.57±0.3
	Accumulated	0±0	0.2±0.1	0.3±0.1	0.36±0.1	0.4±0.3	0.69±0.39
30 mg L^{-1}	Removal	4.2±0.3	5.9±0.7	8.9±3.1	12.7±1.5	13.7±1.7	13.33±1.1
	Adsorbed	4±0.3	5.5±0.7	8.46±3.27	12.14±1.6	13±1.8	12.39±1.14
	Accumulated	0.2±0.05	0.3±0.09	0.43±0.14	0.58±0.22	0.72±0.23	0.93±0.05
40 mg L^{-1}	Removal	5±1.05	6.31±0.29	8.39±0.44	12.4±1.2	13.4±0.54	13.3±0.73
	Adsorbed	4.59±1.14	5.84±0.34	7.76±0.5	11.67±1.31	12.55±0.57	12.27±0.9
	Accumulated	0.4±0.1	0.47±0.05	0.6±0.15	0.7±0.15	0.85±0.05	1.03 ±0.37
50 mg L^{-1}	Removal	6.65±0.3	6.44±0.49	8.11±0.61	10.74±1.06	12.46±0.42	12.7±0.72
	Adsorbed	6.22±0.17	5.94±0.56	7.51±0.51	10.1±1.18	11.54±0.45	11.46±0.97
	Accumulated	0.43±0.1	0.5±0.07	0.6±0.15	0.63±0.11	0.91±0.06	1.24±0.26
Oscillatoria trichoides							
10 mg L^{-1}	Removal	1.37±0.64	2.3±0.5	3.33±0.7	3.33±0.7	6.19±0.36	5.9±1.12
	Adsorbed	1.17±0.65	2.11±0.45	1.72±0.8	1.11±0.62	2.62±0.41	3.27±0.67
	Accumulated	0.19±0.05	0.22±0.06	1.61±0.1	2.23±0.4	3.56±0.2	2.63±0.55
20 mg L^{-1}	Removal	8.4±1.3	9.68±0.6	11.89±0.6	13.42±0.8	17.5±3.3	16.7±4.1
	Adsorbed	6.59±1.3	7.48±0.3	8.82±0.3	9.78±0.3	13.53±2.7	13.83±1.05
	Accumulated	1.86±0.4	2.2±0.3	3.06±0.3	3.43±0.3	3.96±0.8	2.86±0.3
30 mg L^{-1}	Removal	12.16±0.65	19.33±1.02	23.6±3.14	34.06±3.3	38.75±3.8	36.43±1.95
	Adsorbed	9.52±0.41	15.36±0.95	18.93±3.77	23±2.6	27.43±3.3	25.93±2.06
	Accumulated	2.64±0.38	3.96±0.2	5.3±1.75	11.06±1.8	11.32±1.2	10.5±0.81
40 mg L^{-1}	Removal	12±0.79	18.86±0.98	23.3±1.3	30.13±1.8	30.16±6.87	29.93±7.05
	Adsorbed	9.6±1.1	15.43±1	18.4±1.7	19.2±2.9	18.46±6	19.43±6.2
	Accumulated	2.4±0.3	3.43±0.05	4.9±1.12	10.93±1.2	11.7±0.8	10.5±0.9
50 mg L^{-1}	Removal	11.63±0.9	18.66±0.8	23.53±0.85	27.93±0.95	26.7±4.7	24.63±0.7
	Adsorbed	9.3±1.12	15.2±0.87	18.73±1.88	19.08±1.8	22.85±4.5	21.6±0.7
	Accumulated	2.33±0.25	3.46±0.1	4.8±1.05	8.85±0.7	3.9±0.3	3.03±0.15

Results are expressed as means±standard deviation (n=3)

species (Table 2). Total Cr^{6+} removal showed an initial slow uptake with maximum removal for both the species on the tenth day of exposure, irrespective of the initial concentration.

The two-way ANOVA shows a highly significant change in Cr^{6+} removal in response to incubation time and concentration for each organism. The F values for metal removal vs. time vs. initial concentration vs. their interactions for *O. laete-virens* were 126.23 (p<0.001), 183.41 (p<0.001), and 5.79 (p<0.001), respectively, and for *O. trichoides* were 71.51 (p<0.001), 236.44 (p<0.001), and 5.90 (p<0.001). Further, Bonferroni test for concentrations of 10–50 mg L^{-1} of Cr^{6+} indicate higher Cr^{6+} removal by *O. trichoides* (p<0.05) compared to *O. laete-virens* at all incubation times except on the second, fourth, sixth, and eighth days at 10 mg L^{-1}.

The Pearson correlation coefficient for the total amount of Cr^{6+} removed from the solution and the amount adsorbed and accumulated intracellularly shows a strong positive correlation (p<0.01) for both species. Therefore, adsorption is proportional to total removal. A significant correlation (p<0.01) in both species was also found between the total Cr^{6+} removed and bioaccumulated.

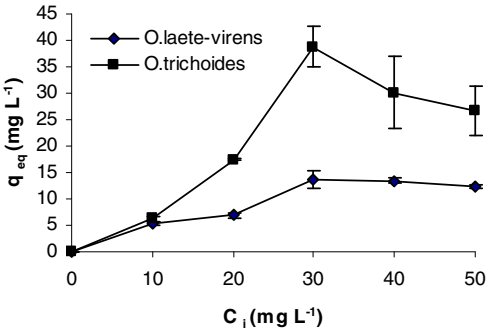

Fig. 3 Effect of initial concentration on the removal of Cr^{6+} at equilibrium by living cells

Effect of pH on metal removal

Cyanobacterial presence in the medium affects the pH with an increase in initial pH during cultivation (Table 3). The shift was marginal in the pH range of 2–3 but prominent at other pH values. pH remains unaltered in the absence of cyanobacterial cells (Table 3). The amount of bioremoval of Cr^{6+} as a function of pH is shown in Table 4. The maximum removal of Cr^{6+} by *O. laete-virens* was 12.6, 13.7, 10.7, and 5.5 mg g^{-1} at an initial pH of 4, 5, 6, and 7, respectively, on the tenth day of exposure at 30 mg L^{-1} of initial concentration. The corresponding values were 28.75, 38.75, 25.36, and 6.18 mg g^{-1} for *O. trichoides*. The cells of both the species did not survive beyond the second day (48 h) at pH 2, and the removal of Cr^{6+} was 71.23 mg g^{-1} for *O. laete-virens* and 70.33 mg g^{-1} for *O. trichoides*. The growth decreased with culture time at pH 3 and the Cr^{6+} removal was 57.23 mg g^{-1} for *O. laete-virens*, and 50.16 mg g^{-1} for *O. trichoides* within 72 h. The maximum removal was at pH 2 followed by pH 3 due to the reduction of Cr^{6+} to Cr^{3+}. This bioreduction was more pronounced in the acidic pH range and stopped beyond pH 5. Therefore, the experiments were restricted to pH 4–7. The Cr^{6+} removal by cyanobacteria decreased with increasing pH from 5 to 7. The highest removal was observed at an initial pH 5 (Fig. 4).

Effect of culture density

Culture density affects metal removal efficiency. The amount removed increased from 0.2 to 0.4 g L^{-1}, and there was a decrease with the further increase in culture density (Fig. 5).

Adsorption by dead cells

For dead cells, pH 2 favors maximum Cr^{6+} removal (Fig. 6a, b). For an initial concentration of 10 mg L^{-1}, equilibrium was attained at 60 min with a removal of 3±0.5 mg g^{-1} by *O. laete-virens* and at 90 min with a removal of 3.33±0.28 mg g^{-1} by *O. trichoides*. The increase in initial concentration enhanced Cr^{6+} removal in both species; the highest

Table 3 Variation in pH during the growth of *Oscillatoria laete-virens* and *Oscillatoria trichoides* at 30 mg L^{-1} of Cr^{6+}

pH		Time (days)					
		2	4	6	8	10	12
Oscillatoria laete-virens							
2		2.03±0.01	2.10±0.4	2.11±0.02	2.2±0.01	2.13±0.01	2.24±0.01
3		3.12±0.02	3.15±0.26	3.22±0.01	3.22±0.01	3.22±0.01	3.22±0.11
4		4.12±0.01	4.19±0.02	4.44±0.01	4.62±0.23	5.07±0.04	5.21±0.2
5		5.11±0.02	5.23±0.02	5.42±0.02	5.72±0.04	5.82±0.02	5.89±0.03
6		6.23±0.02	6.32±0.06	6.72±0.02	7.00±0.03	7.05±0.02	7.1±0.02
7		7.23±0.02	7.42±0.02	8.07±0.02	8.15±0.01	8.22±0.02	8.3±0.03
Oscillatoria laete-virens							
5	Control	5	5	5	5	5	5
	Test	5.11±0.02	5.23±0.02	5.42±0.02	5.72±0.04	5.82±0.02	5.89±0.03
Oscillatoria trichoides							
2		2.03±0.01	2.04±0.4	2.11±0.02	2.2±0.01	2.23±0.01	2.21±0.01
3		3.12±0.02	3.13±0.26	3.20±0.01	3.22±0.01	3.21±0.01	3.23±0.11
4		4.12±0.02	4.20±0.03	4.41±0.01	5.20±0.03	5.15±0.04	5.6±0.1
5		5.12±0.02	5.35±0.02	5.72±0.02	5.93±0.04	5.91±0.02	5.92±0.03
6		6.23±0.01	6.32±0.02	6.72±0.02	7.00±0.01	7.05±0.01	7.1±0.04
7		7.23±0.02	7.42±0.01	8.07±0.03	8.15±0.02	8.22±0.01	8.3±0.04
Oscillatoria trichoides							
5	Control	5	5	5	5	5	5
	Test	5.12±0.02	5.35±0.02	5.72±0.02	5.93±0.04	5.91±0.02	5.92±0.03

Results are expressed as means ± standard deviation ($n = 3$)

Control—no cells, *Test*—with cells

Table 4 Removal of Cr^{6+} and total chromium by *Oscillatoria trichoides* and *Oscillatoria laete-virens* at different pH values

Initial solution pH	Removal (mg g^{-1})		Removal (%)		Removal (mg g^{-1})		Removal (%)	
	O. trichoides				*O. laete-virens*			
	As Cr^{6+}	As total Cr	As Cr^{6+}	As total Cr	As Cr^{6+}	As total Cr	As Cr^{6+}	As total Cr
2	70.33±0.05	10.2±0.26	93.3	13	71.23±0.25	10.3±0.5	95	13
3	50.16±0.40	10.2±0.26	66.6	13	57.23±0.25	10.3±0.5	76.6	13
4	28.75±0.05	27.23±0.25	38.3	36.6	12.6±0.1	12±0.5	16	15
5	38.7±0.07	38.7±0.07	51.6	51.6	13.7±0.1	13.7±0.1	18.3	18.3
6	25.36±0.32	25.3±0.32	33.3	33.3	10.7±0.6	10.7±0.6	13	13
7	6.18±0.07	6.18±0.07	8.3	8.3	5.5±0.5	5.5±0.5	6	6

Results are expressed as means±standard deviation (n = 3). Adsorbent 0.4 g L^{-1} at 30 mg L^{-1} initial concentration

removal for both the species was at 50 mg L^{-1}. *O. trichoides* removed 6.25±0.52 mg g^{-1} and *O. laete-virens* removed 7.83±1.04 mg g^{-1}. The equilibrium was attained at 90 min and 120 min, respectively (Table 5).

The F values for *O. laete-virens* Cr^{6+} removal vs. time vs. initial concentration vs. their interactions were 45.43 (p<0.001), 136.18 (p<0.001), and 5.05 (p<0.001), respectively, while the F values for *O. trichoides* were 90.27 (p<0.001), 54.43 (p<0.001), and 4.80 (p<0.001), respectively. The Bonferroni test showed a significantly higher metal removal by living cells than by non-living cells in all the concentrations tested. Living cells of *O. trichoides* are nearly nine times (38.7±3.8 mg g^{-1}) more efficient than non-living cells and *O. laete-virens* living cells are almost twice (13.7±1.7 mg g^{-1}) more efficient than non-living cells.

Adsorption isotherms

The experimental results were plotted using the linearized Langmuir and Freundlich adsorption isotherms over a concentration range of 10–40 mg L^{-1} (Figs. 7a, b and 8a, b). The obtained parameters are given in Table 6. The data indicate that the experimental results for *O. laete-virens* living biomass are consistent with both the isotherms with an R^2 of 0.8408 and 0.8462 with a q_{max} of 21.88 mg g^{-1}. However, the non-living biomass of *O. laete-virens* followed only the Langmuir isotherm model with an R^2 of 0.732 and a q_{max} of 7.58 mg g^{-1}. Living biomass of O. *trichoides* does not follow either of the models, but removes the metal to a maximum extent of 38.7 mg g^{-1} at equilibrium. The removal by nonliving biomass was well described by the Freundlich model with a R^2 of 0.7642. The adsorption capacity (q_{max}) in terms of monolayer was 5.67 mg g^{-1}.

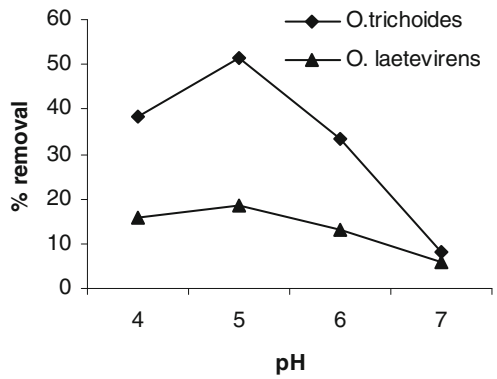

Fig. 4 Effect of pH on the removal of Cr^{6+} by living cells of two species of *Oscillatoria* at 30 mg L^{-1}

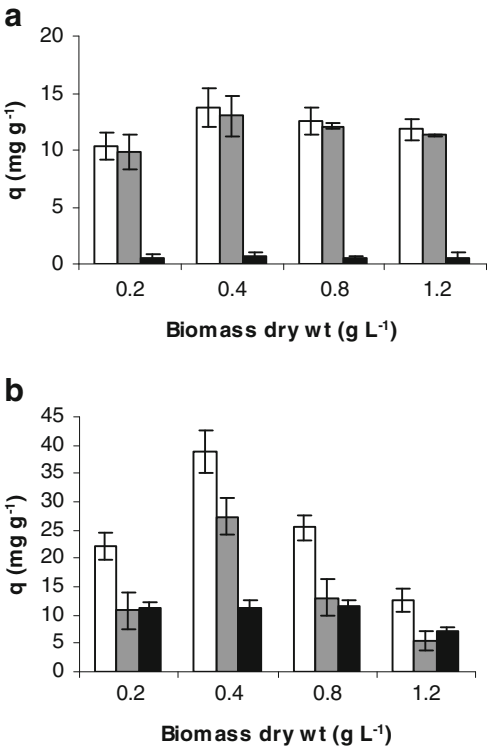

Fig. 5 Effect of culture density on the removal of Cr^{6+} by the living cells of **a** *O. laete-virens* and **b** *O. trichoides*. Results are means± standard deviation (n=3). *White bars*—removed, *gray bars*—adsorbed, *black bars*—accumulated

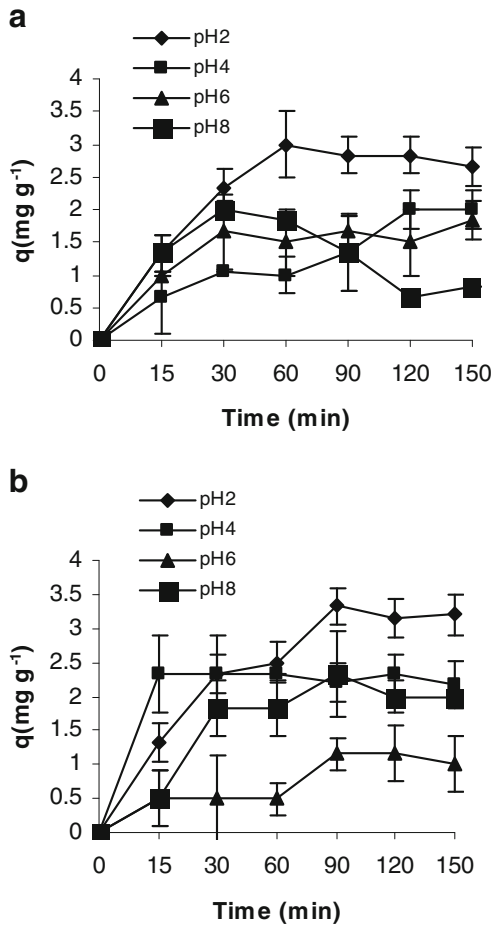

Fig. 6 Effect of pH on the removal of Cr^{6+} by the dead cells of **a** *O. laete-virens* and **b** *O. trichoides* at 10 mg L^{-1}. Results are expressed as means; *error bars* represent standard deviation (n=3)

Kinetic studies

For pseudo-first-order kinetics, the straight line plots of log ($q_e - q_t$) against t were made for both living and dead biomass (Fig. 9a, b) of both cyanobacterial species. The linear plots of t/q_t against t for pseudo-second-order model (Fig. 10a, b) were made for living and dead mass of both species. From the linear regression analysis, R^2 values were determined (Table 7a, 7b).

FTIR analysis

The spectral studies reveal biosorbent heterogenesity shown by different characteristic peaks with the possible presence of amino, carboxylic, hydroxyl, and carbonyl groups. The IR adsorption bands and corresponding groups that are capable of interacting with metal ions are presented in Table 8 and Fig. 11a–d. It was observed that there were slight changes in the absorption peak frequencies in both species after chromium adsorption which is suggestive of metal binding process taking place on the biomass surface.

Discussion

The ability to grow at low pH is a desirable feature of cyanobacteria as waters polluted with heavy metals generally have low pH (Rai et al. 1981). pH below 4 was found to be toxic to both cyanobacteria. A reduced cation uptake (NH_4^+, Na^+, K^+, Ca^{2+}, Cu^{2+}, and Ni^{2+}) and an accelerated uptake of anions (NO_3^- and PO_4^{3-}) builds a positive charge on the membranes preventing the entry of cations and also helps to maintain a near neutral cytoplasmic pH (Rai et al. 1996). These mechanisms may be involved to protect the cells from acidity in the present study.

There is a significant increase in pH of the culture medium (p<0.05) due to CO_2 uptake for photosynthesis. This leads to a decrease in CO_2 partial pressure, when CO_2

Table 5 Total amounts of Cr^{6+} removed by dead biomass of *Oscillatoria laete-virens* and *Oscillatoria trichoides* at various initial concentrations of Cr^{6+}

Concentration of Cr^{6+}	Time (min)					
	15	30	60	90	120	150
Oscillatoria laete-virens						
10 mg L^{-1}	1.33±0.28	2.33±0.28	3±0.5	2.83±0.28	2.83±0.28	2.66±0.28
20 mg L^{-1}	1.5±0	2.83±0.28	3.33±0.28	3±0.5	3.33±0.28	3.33±0.28
30 mg L^{-1}	1.83±0.28	3.33±0.28	4.16±0.28	3.16±0.28	4±0.5	3.66±0.57
40 mg L^{-1}	2.5±1	4±0.5	4.66±0.76	6.83±0.76	6.16±0.76	5.83±0.76
50 mg L^{-1}	4.33±0.28	4.83±0.28	4.83±0.76	6.83±0.76	7.83±1.04	7.5±0.86
Oscillatoria trichoides						
10 mg L^{-1}	1.33±0.57	2.33±0.57	2.5±0	3.33±0.28	3.16±0.28	3.2±0.34
20 mg L^{-1}	1.06±0.11	2.16±0.28	2.16±0.28	3.16±0.28	3.16±0.28	3.23±0.25
30 mg L^{-1}	1.16±0.28	2.16±0.28	2.16±0.28	3.16±0.28	4.3±0.1	4.25±0.06
40 mg L^{-1}	1.16±0.28	2±0	2.33±0.28	3.66±0.28	5.06±0.30	5.03±0.16
50 mg L^{-1}	1.16±0.28	3.16±0.28	4.33±0.28	6.25±0.52	6.22±0.65	5.96±0.44

Results are expressed as means± standard deviation (n=3)

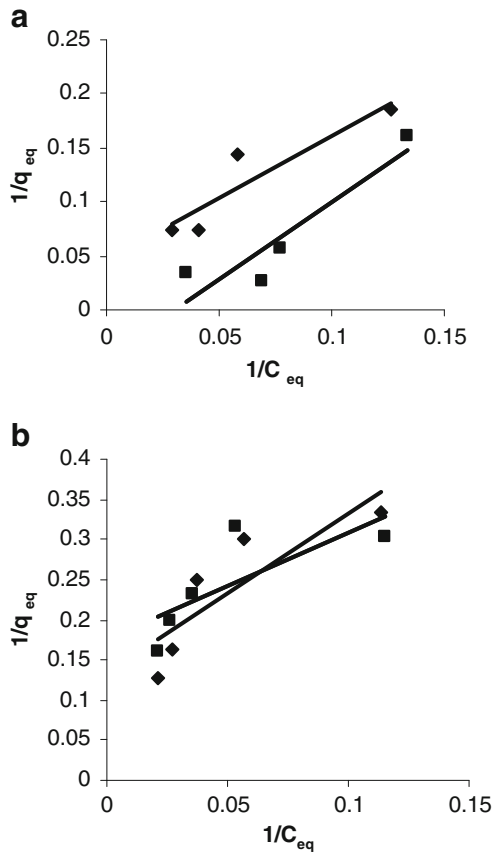

Fig. 7 Langmuir isotherm for the removal of Cr^{6+} by **a** living biomass of *O. laete-virens* $y=1.1567x + 0.0457$ $R^2=0.8408$, *O. trichoides* $y=1.4273x - 0.043$ $R^2=0.8473$ and **b** dead biomass of *O. laete-virens* $y=2.0086x + 0.1318$ $R^2=0.732$, *O. trichoides* $y=1.3214x + 0.1761$ $R^2=0.5715$. *Filled diamonds—O. laete-virens*, *filled squares—O. trichoides*

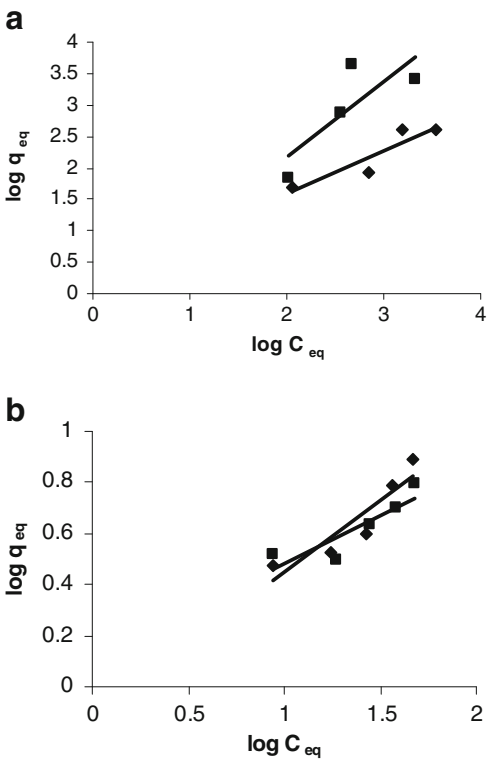

Fig. 8 Freundlich isotherm for the removal of Cr^{6+} by **a** living biomass of *O. laete-virens* $y = 0.6848x + 0.2139$ $R^2=0.8462$, *O. trichoides* $y=1.199x - 0.2362$ $R^2=0.6348$ and **b** dead biomass of *O. laete-virens* $y=0.5674x - 0.121$ $R^2=0.8388$, *O. trichoides* $y=0.3733x + 0.1127$ $R^2=0.7642$. *Filled diamonds—O. laete-virens*, *filled squares—O. trichoides*

replacement is slower than the utilization (Chen and Durbin 1994; Dubinsky and Rotem 1974).

The significant decrease ($p<0.01$) in growth shown by both the species over time in increased Cr^{6+} concentrations may be due to the oxidative stress occurring in the chloroplasts and other cell parts via a Fenton-type mechanism which has the capacity to reduce the activity of some antioxidant enzymes (Shi and Dalal 1990; Panda and Choudhary 2005). Khattar et al. (2007) have reported decreasing growth over time in *Anacystis nidulans* and Ozturk et al. (2009) in *Synechocystis* sp. Rehman et al. (2007) reported 71% growth after 192 h (8 days) of Cr^{6+} stress in *Distigma proteus*.

Both the cyanobacterial species studied by us tolerate a Cr^{6+} concentration as high as 30 mg L^{-1}, which has not so far been reported for any other algae. Exopolymeric substances (EPS) are common in many cyanobacteria. These are rich in uronic acids (De Philippis and Vincenzini 1998) which bind to metal ions conferring on these cyanobacteria an increased tolerance to metal ions. Uronic acids occurring in the EPS produced by these species (results not shown here) may bind to Cr^{6+} resulting in an increased tolerance to the metal. The ability of cyanobacteria to reduce toxic Cr^{6+} to less toxic Cr^{3+} and to accumulate this also results in an increased resistance of these algae to Cr^{6+} (Garcia et al. 2009).

Increased initial concentration increases the number of ions competing for the available binding sites in the biomass leading to saturation of functional groups at equilibrium. This also helps to overcome all mass transfer resistance of metal ions between the aqueous and solid phases. Hence, as initial concentration increases, it will increase the biosorption rate until it reaches an equilibrium state leading to saturation of functional groups (Arief et al. 2008). The greater biosorption capacity of *O. trichoides* over *O. laete-virens* could be explained by the fact that metal binding groups differ in their affinity and specificity for metal uptake (Crist et al. 1981). Concentration-dependent removal of Cr^{6+} has been reported by Dönmez and Aksu (2002) in *Dunaliella* sp. Bala et al. (2004) have reported a maximum uptake of 3.3 mg g^{-1} at 20 mg L^{-1} in *Nostoc linckia*. Energy-dependent Cr^{6+} uptake in the cyanobacterium *Anabaena doliolum* also showed concentration dependence (Rai et al. 1992).

Both the reduction and adsorption of Cr^{6+} are highly dependent on the solution pH. In most cases, Cr^{6+} is reduced to Cr^{3+} at pH 2–3 along with anionic adsorption at a lower

Table 6 Langmuir and Freundlich isotherm parameters for the removal of Cr^{6+} by *Oscillatoria laete-virens* and *Oscillatoria trichoides*

Adsorbent	Langmuir isotherm			Freundlich isotherm		
	q_{max} (mg g^{-1})	b (L mg^{-1})	R^2	K_f (mg g^{-1})	n	R^2
Oscillatoria laete-virens (living)	21.88	0.03	0.8408	1.63	1.46	0.8462
Oscillatoria laete-virens (dead)	7.58	0.06	0.732	–	–	–
Oscillatoria trichoides (living)	–	–	–	–	–	–
Oscillatoria trichoides (dead)	5.67	0.13	0.5715	1.29	2.6	0.7642

pH (Aoyama et al. 2005; Aoyama and Tsuda 2001; Park et al. 2005; 2006; Sarin and Pant 2006; Sanghi et al. 2009; Hasan et al. 2008; Cui et al. 2011; Qaiser et al. 2009). Kratochvil et al. (1998), using the Nernst equation, showed a greater redox potential of chromate at lower pH. This supports the results of living biomass obtained in this study. Also, at these pH values, the sorption of Cr^{3+} is also not favored because positively charged H^+ ions will compete with metal ions for the anions on the cell wall. In addition, there is repulsion of positively charged Cr^{3+} by positively charged ions of biomass surface at acidic pH (Tan et al. 2003).

The increase in the biosorption of Cr^{6+} at lower pH (anionic adsorption) suggests that these bind through electrostatic attraction to the positively charged functional groups on the biomass surface (Gupta et al. 2001) since at lower pH, the overall surface charge on the biomass is positive. This has also been reported in a variety of biosorbents (Tunali et al. 2005; Niu and Volesky 2003). At higher pH, the concentration of OH^- ions increases and overall negative charge on the biomass surface causes a hindrance to the biosorption of negatively charged ions such as $Cr_2O_7^{2-}$, CrO_4^{2-} resulting in a decrease of biosorption of Cr^{6+} (Tewari et al. 2005; Niu and Volesky 2003; Saha and Orvig 2010; Anjana et al. 2007).

In our study using living biomass, the reduction rate of Cr^{6+} increased at lower solution pH (2–3) and the contact time required for the removal of Cr^{6+} (anionic adsorption along with accumulation) increased from hours to days at pH 5. Since there was no Cr^{3+} left in the medium, reduction of Cr^{6+}

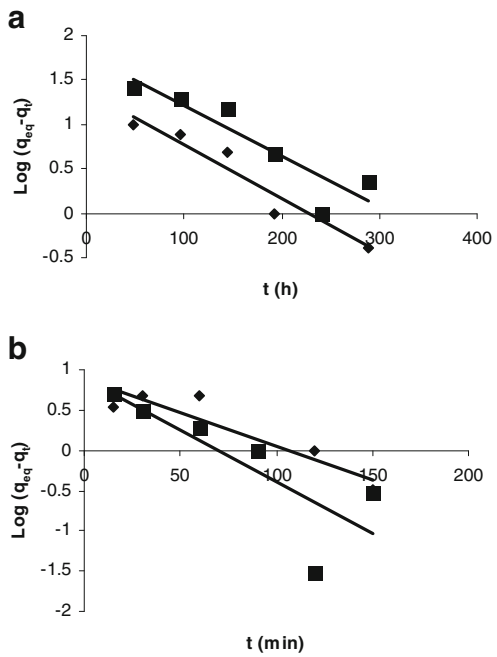

Fig. 9 The pseudo-first-order plot of kinetic study of Cr^{6+} biosorption by **a** *O. laete-virens* $y=-0.0061x + 1.3909$ $R^2=0.9359$, *O. trichoides* $y=-0.0058x + 1.7887$ $R^2=0.8267$ at a concentration of 30 mg L^{-1} and 28°C for living biomass and **b** *O. laete-virens* $y=-0.0083x + 0.8803$ $R^2=0.8366$, *O. trichoides* $y=-0.0129x + 0.8993$ $R^2=0.6716$ at a concentration of 50 mg L^{-1} and a temperature of 28°C for dead biomass. *Filled diamonds—O. laete-virens*, *filled squares—O. trichoides*

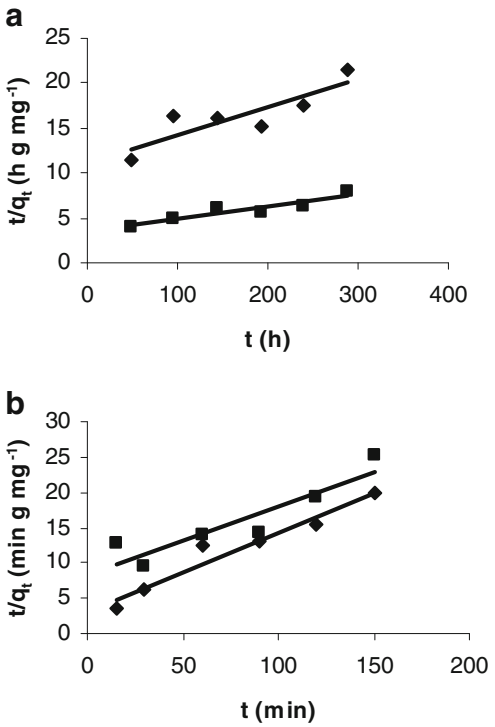

Fig. 10 The pseudo-second-order plot of kinetic study of Cr^{6+} biosorption by **a** *O. laete-virens* $y=0.0318x + 11$ $R^2=0.7443$, *O. trichoides* $y=0.0137x+3.4911$ $R^2=0.8562$ at a concentration of 30 mg L^{-1} and 28°C for living biomass and **b** *O. laete-virens* $y=0.1123x + 3.0634$ $R^2=0.945$, *O. trichoides* $y=0.0971x+8.319$ $R^2=0.8354$ at a concentration of 50 mg L^{-1} and 28°C for dead biomass. *Filled diamonds—O. laete-virens*, *filled squares—O. trichoides*

Table 7 Comparison of pseudo-first-order and second-order adsorption rate constants and the calculated and experimental q_e values

Sorbent	Pseudo-first-order kinetic model				Pseudo-second-order kinetic model		
	q_e (exp) (mg g^{-1})	k_1	q_e (cal) (mg g^{-1})	R^2	k_2	q_e (cal) (mg g^{-1})	R^2
Oscillatoria trichoides[a]	38.75	1.33×10^{-2}	61.48	0.8267	5×10^{-5}	72.99	0.8562
Oscillatoria laete-virens[a]	13.73	1.40×10^{-2}	24.60	0.9359	9×10^{-5}	31.44	0.7443
Oscillatoria trichoides[b]	6.25	2.9×10^{-2}	7.93	0.6716	1.1×10^{-3}	10.29	0.8354
Oscillatoria laete-virens[b]	7.83	1.9×10^{-2}	7.59	0.8354	4.1×10^{-3}	8.90	0.945

[a] living biomass at 30 mg L^{-1} initial Cr^{6+} concentrations, k_1 (h^{-1}) and k_2 (g mg^{-1} h^{-1})

[b] dead biomass at 50 mg L^{-1} initial Cr^{6+} concentrations, k_1 (min^{-1}) and k_2 (g mg^{-1} min^{-1})

was not observed and Cr^{6+} was removed mainly through adsorption at the cell surfaces at pH 5, as in the mechanism of anionic adsorption (Sarin and Pant 2006; Mungasavalli et al. 2007; Anjana et al. 2007). In the present study, there is a removal of Cr^{6+} at pH >3.0. But the percentage is less since at higher pH an electrostatic force of repulsion occurs. At the same time, some amount of removal could be due to the presence of other mechanism such as physical adsorption on the surface of biosorbent (Gupta et al. 2001).

pH influences both cell surface metal binding sites and metal chemistry in water. Hexavalent chromium exists in different forms in aqueous solution and the stability of these forms is pH dependent. $HCrO_4^-$ of Cr^{6+} form is more stable in aqueous solution up to pH 7. The H_2CrO_4 (chromic acid) form is stable in the low pH range, whose concentration decreases sharply with increasing pH. Similarly, the CrO_4^{2-} form is stable at higher pH range (above pH 6). At pH 2, it is the $HCrO_4^-$ form of Cr^{6+} that binds to the surfaces (Gardea-Torresdey et al. 2000; Cimino et al. 2000).

In experiments using living biomass which were conducted at pH 5, the percentage of Cr^{6+} removal is less compared to acidic pH of 2 and 3. At pH 4, since the growth was less compared to pH 5 (Fig. 1a, b), the removal capacity

Table 8 Frequencies and assignments of FTIR bands of free and Cr^{6+} bound living cells of *Oscillatoria laete-virens* and *Oscillatoria trichoides*

	Free cells	Cr^{6+} bound cells at 30 mg L^{-1}	Assignment
Oscillatoria laete-virens			
1.	3,506–3,597 cm^{-1}	3,651–3,672 cm^{-1}	Hydrogen bonded O–H stretching, N–H stretching of secondary amines
2.	2,922–2,960 cm^{-1}	2,823–2,850 cm^{-1}	C–H stretching (methyl, methylene, and methyne groups)
3.	2,370 cm^{-1}	2,370 cm^{-1}	Asymmetric stretching of –N=C=O group
4.	1,654 cm^{-1}	1,741 cm^{-1}	C=O stretching in amide groups
5.	1,570 cm^{-1}	1,593 cm^{-1}	N–H bending in –CONH–
6.	1,382 cm^{-1}	1,382 cm^{-1}	Bending of O–H group
7.	1,348 cm^{-1}	1,350 cm^{-1}	C–N stretching
8.	1,060 cm^{-1}	1,089 cm^{-1}	C–O symmetric stretching
9.	–	560–513 cm^{-1}	Out of plane N–H bending for hydrogen bonded amides
Oscillatoria trichoides			
1.	3,630–3,570 cm^{-1}	3,639–3,680 cm^{-1}	Hydrogen bonded O–H stretching, N–H stretching of secondary amines
2.	2,927–2,960 cm^{-1}	2,927–2,964 cm^{-1}	C–H stretching (methyl, methylene, and methyne groups)
3.	2,339 cm^{-1}	2,335 cm^{-1}	Asymmetric stretching of –N=C=O group
4.	1,631 cm^{-1}	1,637 cm^{-1}	C=O stretching in amide groups
5.	1,570 cm^{-1}	1,587 cm^{-1}	N–H bending in –CONH–
6.	1,440 cm^{-1}	1,458 cm^{-1}	Bending of O–H group
7.	1,348 cm^{-1}	1,348 cm^{-1}	C–N stretching
8.	1,163 cm^{-1}	1,159 cm^{-1}	C–O symmetric stretching
9.	–	528–482 cm^{-1}	Out of plane N–H bending for hydrogen bonded amides

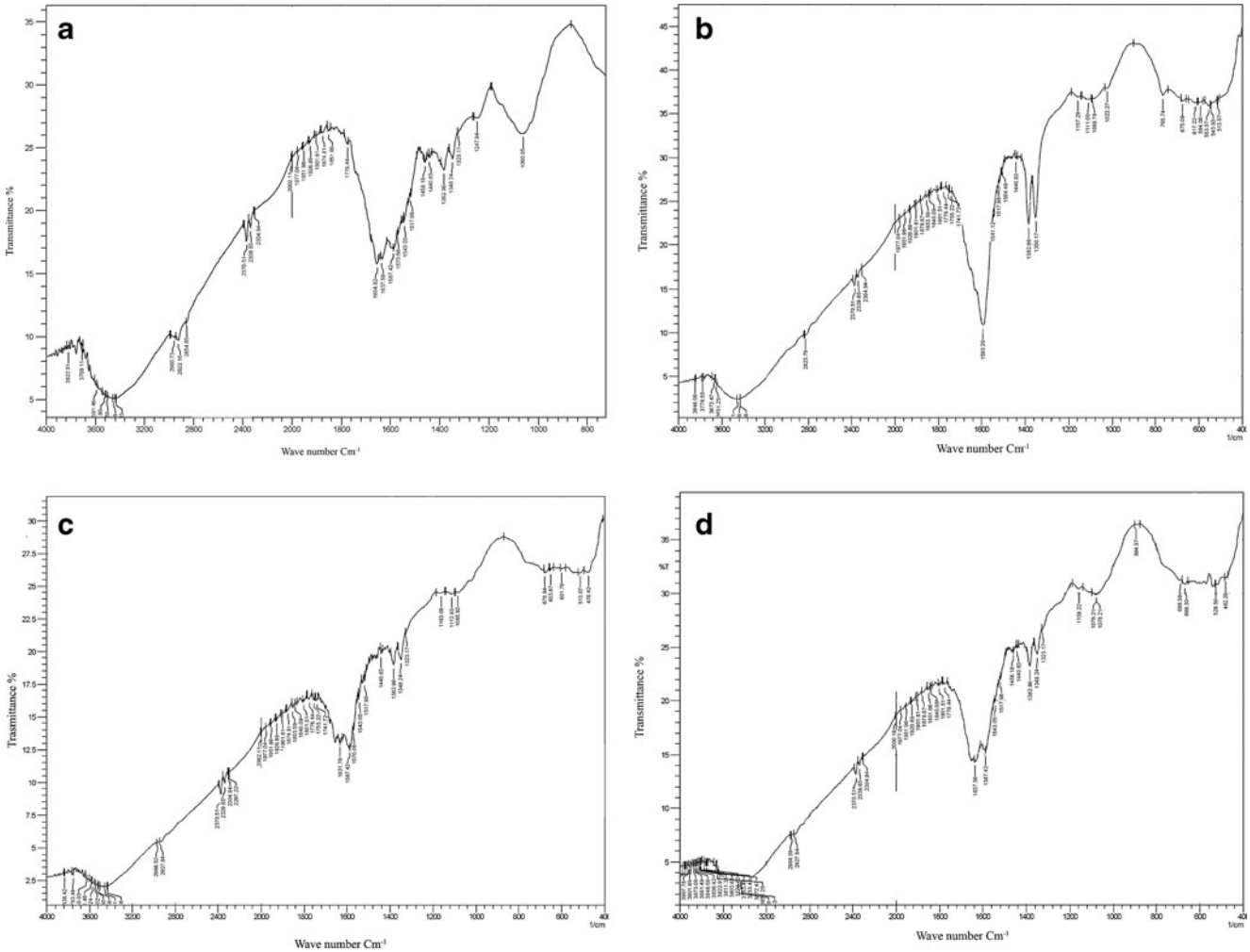

Fig. 11 FTIR analysis for the biomass of *O. laete-virens* (**a**, **b**) and *O. trichoides* (**c**, **d**)—free and Cr^{6+} bound living cells at 30 mg L^{-1} concentration

might have been affected even though this acidic pH is favorable for Cr^{6+} adsorption (anionic adsorption).

Decreased biosorption at higher culture density is due to the limited availability of metal electrostatic interactions (Khattar et al. 2007) or by the attainment of equilibrium between adsorbate and adsorbent (Rai and Kumar 1999).

Metal removal by dead cyanobacterial cells is exclusively through adsorption on to the cell wall surface (Chu and Hashim 2004). The Cr^{6+} removal by dead cells in this study seems to be by anionic adsorption and not by reduction at low pH since there was no Cr^{3+} left out in the medium. The process of adsorption reaches equilibrium after a few minutes (90–120 min) of contact in this study. Similar results were reported by Ozer et al. (1999).

The lesser amount of metal removal by dead cells over living cells may be due to the unavailability of functional groups such as carboxyl, hydroxyl, or phosphoryl present on the cell surface following heating in terms of stereochemically adequate positioning and chemical integrity (Monteiro et al. 2009a). Information on the comparative removal of Cr^{6+} by living and dead cells of cyanobacteria is limited. Cr^{6+} removal from polluted waters through dead biomass can be more economical because constrained environmental conditions for the growth and maintenance of the cells is not needed. They withstand highly toxic environments, and metals contained in them can be recovered when used in industrial applications (Aksu and Dönmez 2006).

The Freundlich and Langmuir isotherms are widely used in biosorption studies (Martins et al. 2006; Volesky 2007) to predict the adsorption capacity of a biosorbent. Langmuir model gives a good description of experimental behavior in a wide range of operating conditions (Yun et al. 2001) which helps us in evaluating q_{max}—maximum possible quantity of metal ions adsorbed per gram of adsorbent and the constant b which is related to the affinity of binding sites for the metal ions. Lower value of b, i.e., 0.03 for living biomass of *O. laete-virens* and 0.06 and 0.13 for dead biomass of *O.*

laete-virens and *O. trichoides*, is indicative of high metal removal capacity of these cyanobacteria (Langmuir 1918). Similar observations were also proposed by Kratochvil and Volesky (1998). Hence, these species seem to be good biosorbents for Cr^{6+}as they have a high q_{max} and a low b.

Living biomass of *O. trichoides* does not follow either of the models and may involve some other mechanism. Studies by Kratochvil and Volesky (1998) and Volesky and Holan (1995) suggest that these sorption isotherms do not necessarily reflect the adsorption mechanisms involved.

Even values of K_f and n (Table 6) for *O. laete-virens* living biomass and *O. trichoides* dead biomass suggest their higher biosorption capacity for Cr^{6+}. Such observations were made in many previous studies (Gokhale et al. 2008; Gabr et al. 2009). Higher value of K_f and n and the lower values of b indicate better affinity of the biomass towards metal ions (Saravanane et al. 2002; Aksu et al. 1991).

The sorption of Cr^{6+} follows relatively well with pseudo-first-order kinetic model with higher values of R^2 in living biomass of both species. Besides, the proximity of values of predicted q_e and experimental q_e shows predictive relevance of the model. The pseudo-second-order kinetic model fits better with the dead biomass of *O. laete-virens* with predicted q_e nearer to experimental q_e and a higher rate constant indicates increased uptake of adsorbate ions with higher values of R^2. For the dead biomass of *O. trichoides*, pseudo-first-order model is relevant since predicted q_e is nearer to experimental q_e, and a higher rate constant.

The FTIR results obtained indicate the presence of carboxylic, amino, and carbonyl groups on the algal cell surfaces and is also indicative of the mechanism of adsorption. The extent of band shifting also gives an indication of degree of interaction of functional groups with Cr^{6+} ions. Studies on Cr^{6+} adsorption in *Oedogonium hatei* had similar results (Gupta and Rastogi 2009). FTIR and XPS spectra suggest that carboxylate and carboxyl groups on the surface of biomass are responsible for Cr^{6+} binding and reduction, whereas amide and other groups play a minor role in the Cr^{6+} removal process (Cui et al. 2011).

In conclusion, we found that the removal of toxic hexavalent chromium from solutions was possible using these two species. Both bioreduction and biosorption contribute to the bioremoval of Cr^{6+} during the growth of the cyanobacterial species. A comparison of the bioreduction and biosorption process in living cells reveals that bioreduction contributes to a greater extent to the overall bioremoval below pH 3.1, while biosorption is more dominant beyond that pH. The highest removal through biosorption for living biomass was achieved between pH 5 and 5.9 and for dead biomass at pH 2. Of the two species, living cells of *O. trichoides* were most effective for which removal was 38.7 mg g^{-1} and reached 51.6% of the total Cr^{6+} at 30 mg L^{-1} at pH 5–5.9. But the results of this species did not follow either of the models. Most of the Cr^{6+} removal occurred through adsorption on to the cell surface. Living cells of both species outperform dead cells significantly. Kinetics of living biomass was well described by pseudo-first-order model. FTIR analysis indicated the participation of carboxylic, carbonyl, and amino groups in Cr^{6+} removal. Thus *O. laete-virens* and *O. trichoides* can be good candidates for Cr^{6+} sorption from polluted environments. However, further research is needed to establish the process with specific attention to use in the industrial effluents by immobilization of cyanobacteria and regeneration of the sorbed metal.

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References

Aksu Z, Dönmez G (2006) Binary biosorption of cadmium (II) and nickel (II) onto dried *Chlorella vulgaris*: co-ion effect on mono-component isotherm parameters. Process Biochem 41:860–868

Aksu Z, Tunc O (2005) Application of biosorption for penicillin G removal: comparison with activated carbon. Process Biochem 40:831–847

Aksu Z, Kustal T, Guin S, Haciosmanoglu N, Ghdminesad M (1991) Investigation of biosorption of Cu (II), Ni (II), and Cr (VI) ions to activated sludge bacteria. Environ Technol 12:915–921

Alcedo J, Wetterhahn KE (1990) Chromium toxicity and carcinogenesis. Int Rev Exp Path 31:85–107

Anjana K, Kaushik A, Kiran B, Nisha R (2007) Biosorption of Cr (VI) by immobilized biomass of two indigenous strains of cyanobacteria isolated from metal contaminated soil. J Hazard Mater 148:383–386

Aoyama M, Tsuda M (2001) Removal of Cr^{6+} from aqueous solutions by larch bark. Wood Sci Technol 35:425–434

Aoyama M, Sugiyama T, Doi S, Cho NS, Kim HE (2005) Removal of hexavalent chromium from dilute aqueous solution by coniferous leaves. Holzforschung 53:365–368

Arief VO, Trilestari K, Sunarso J, Indraswati N, Ismadji S (2008) Recent progress on biosorption of heavy metals from liquids using low cost biosorbents: characterization, biosorption parameters and mechanism studies. Clean 36:937–962

Arslan P, Beltrame M, Tomasi A (1987) Intracellular chromium reduction. Biochim Biophys Acta 931:10–15

Bala K, Rani N, Kaushik A (2004) Biosorption uptake of Cr(VI) by indigenous cyanobacterium *Nostoc linckia*. In: Reddy MS, Khanna S (eds) Biotechnological approaches for sustainable development. Allied Publishers, India, pp 205–212

Cervantes C, Campos-Garcia J, Devars S, Gutiérrez-Corona F, Loza-Tavera H, Torres-Guzmán JC, Moreno-Sánchez R (2001) Interactions of chromium with microorganisms and plants. FEMS Microbiol Rev 25:335–347

Chen CY, Durbin EG (1994) Effects of pH on the growth and carbon uptake of marine phytoplankton. Mar Ecol Prog Ser 109:83–94

Cheung KH, Gu JD (2007) Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: a review. Int Biodeterior Biodegrad 59:8–15

Chu KH, Hashim MA (2004) Quantitative analysis of copper biosorption by a microalga *Chlorella vulgaris*. J Environ Sci Health A Environ Sci Eng 21:139–147

Cimino G, Passerini A, Toscano G (2000) Removal of toxic cation and Cr(VI) from aqueous solution by hazelnut shell. Water Res 34:2955–62

Clesceri LS, Greenberg AE, Eaton AD (1998) Standard methods for the examination of water and waste water 20th ed. American Public Health Association. American Water Works Association and Water Environment Federation Washington, pp. 366–368.

Crist RH, Oberholser K, Shank N, Nguyen M (1981) Nature of bonding between metallic ions and algal cell walls. Environ Sci Technol 15:1212–1217

Cui H, Fu M, Yu S, Wang MK (2011) Reduction and removal of Cr (VI) from aqueous solutions using modified byproducts of beer production. J Hazard Mater 186:1625–1631

De Philippis R, Vincenzini M (1998) Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol Rev 22:151–175

Desikachary TV (1959) Cyanophyta. Indian Council of Agricultural Research, New Delhi

Dönmez G, Aksu Z (2002) Removal of chromium (VI) from saline wastewaters by *Dunaliella* species. Process Biochem 38:751–762

Dreyfuss J (1964) Characterization of a sulphate and thiosulphate transporting systems in *Salmonella typhimurium*. J Biol Chem 239:2292–2297

Dubinsky Z, Rotem J (1974) Relations between algal populations and the pH of their media. Oecologia 16:53–60

Freundlich H (1907) Ueber die Adsorption in Loesungen Z. Physic Chem 57:385–470

Gabr RM, Gad-Elrab SMF, Abskharon RNN, Hassan SHA, Shoreit AAM (2009) Biosorption of hexavalent chromium using biofilm of *E. coli* supported on granulated activated carbon. World J Microbiol Biotechnol 25:1695–1703

Garcia JD, Zavala JS, Chavez R, Cozatl D, Sanchez R (2009) Chromium uptake, retention and reduction in photosynthetic *Euglena gracilis*. Arch Microbiol 191:431–440

Gardea-Torresdey JL, Tiemann KJ, Armendariz V, Bess-Oberto L, Chinelli RR, Rios J, Parsons JG, Gamez G (2000) Characterization of Cr(VI) binding and reduction to Cr(III) by the agricultural byproducts of *Arena monida* (Oat) biomass. J Hazard Mater 80:175–88

Gokhale SV, Jyoti KK, Lele SS (2008) Kinetic and equilibrium modeling of chromium (VI) biosorption of fresh and spent *Spirulina platensis*/*Chlorella vulgaris* biomass. Bioresour Technol 99:3600–3608

Gong R, Ding Y, Liu H, Chen Q, Liu Z (2005) Lead biosorption and desorption by intact and pretreated *Spirulina maxima* biomass. Chemosphere 58:125–130

Gupta VK, Rastogi A (2008) Sorption and desorption studies of chromium (VI) from non viable cyanobacterium *Nostoc muscorum* biomass. J Hazard Mater 154:347–354

Gupta VK, Rastogi A (2009) Biosorption of hexavalent chromium by raw and acid treated green alga *Oedogonium hatei* from aqueous solutions. J Hazard Mater 163:396–402

Gupta VK, Shrivastava AK, Jain N (2001) Biosorption of Cr^{6+} from aqueous solutions by green algae *Spirogyra* species. Water Res 35:4079–4085

Hasan SH, Singh KK, Prakash O, Talat M, Ho YS (2008) Removal of Cr(VI) from aqueous solutions using agricultural waste 'maize bran'. J Hazard Mater 152:356–365

Ho YS, McKay G (1998) Sorption of dye from aqueous solution by peat. Chem Eng J 70:115–124

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls-a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanzen 167:191–194

Khattar J, Sarma T, Sharma A (2007) Optimization of chromium removal by the chromium resistant mutant of the cyanobacterium *Anacystis nidulans* in a continuous flow bio-reactor. J Chem Tech Biot 82:652–657

Kiran B, Kaushik A, Kaushik CP (2007) Response surface methodological approach for optimizing removal of Cr(VI) from aqueous solution using immobilized cyanobacterium. Chem Eng J 126:147–153

Kratochvil D, Volesky B (1998) Advances in the biosorption of heavy metals. Trends Biotechnol 16:291–300

Kratochvil D, Pimentel P, Volesky B (1998) Removal of trivalent and hexavalent chromium by seaweed biosorbent. Environ Sci Technol 32:2693–2698

Langmuir I (1918) The adsorption of gases on plane surfaces of glass, mica, and platinum. J Am Chem Soc 40:1361–1403

Martins BL, Cruz CCV, Luna AS, Henriques CA (2006) Sorption and desorption of Pb^{2+} ions by dead *Sargassum* sp. biomass. Biochem Eng J 27:310–314

Monteiro CM, Marques Ana PGC, Castro PML, Malcata FX (2009a) Characterization of *Desmodesmus pleiomorphus* isolated from a heavy metal-contaminated site: biosorption of zinc. Biodegradation 20:629–641

Monteiro CM, Castro PML, Malcata FX (2009b) Use of the microalga *Scenedesmus obliquus* to remove cadmium cations from aqueous solutions. World J Microbiol Biotechnol 25:1573–1578

Monteiro CM, Castro PML, Malcata FX (2010) Cadmium removal by two strains of *Desmodesmus pleiomorphus* cells. Water Air Soil Pollut 208:17–27

Mungasavalli DP, Viraraghavan T, Jin YC (2007) Biosorption of chromium from aqueous solutions by pretreated *Aspergillus niger*: batch and column studies. J Colloid Surface A: Physicochem Eng 301:214–223

Niu H, Volesky B (2003) Characteristics of anionic metal species biosorption with waste crab shells. Hydrometallurgy 71:209–215

OzerA OD, Dursun G, Bulak S (1999) Cadmium adsorption on *Cladophora crispata* in batch stirred reactors in series. Waste Manage 19:233–240

Ozturk S, Aslim B, Suludere Z (2009) Evaluation of chromium (VI) removal behavior by two isolates of *Synechocystis* sp. in terms of exopolysaccharide (EPS) production and monomer composition. Bioresour Technol 100:5588–5593

Panda S, Choudhary S (2005) Chromium stress in plants. Braz J Plant Physiol 17:95–102

Park D, Yun YS, Jo JH, Park JM (2005) Mechanism of hexavalent chromium removal by dead fungal biomass of *Aspergillus niger*. Water Res 39:533–540

Park D, Yun Y, Yim KH, Park JM (2006) Effect of Ni(II) on the reduction of Cr(VI) by *Ecklonia* biomass. Bioresour Technol 97:1592–1598

Prado C, Rodríguez-Montelongo L, González JA, Pagano EA, Hilal M, Prado FE (2010) Uptake of chromium by *Salvinia minima*: effect on plant growth, leaf respiration and carbohydrate metabolism. J Hazard Mater 15:546–53

Qaiser S, Saleemi A, Umar M (2009) Biosorption of Pb^{2+} and Cr^{6+} on ground nut hull: equilibrium, kinetics and thermodynamics study. Electr J Biotechnol 12:1–9

Rahmani GNH, Sternberg SPK (1999) Bioremoval of lead from water using *Lemna minor*. Bioresour Technol 70:225–230

Rai AK, Kumar S (1999) Treatment of chromium bearing wastewater by adsorption on brick kiln ash and fly ash. Ind J Environ Health 41:65–73

Rai LC, Gaur JP, Kumar HD (1981) Phycology and heavy metal pollution. Biol Rev 56:99–151

Rai LC, Dubey SK, Mallick N (1992) Influence of chromium on some physiological variables of *Anabaena doliolum*: interaction with metabolic inhibitors. Biometals 5:13–16

Rai LC, Rai PK, Mallick N (1996) Regulation of heavy metal toxicity in acid tolerant *Chlorella*: physiological and biochemical approaches. Environ Exp Bot 36:99–109

Rehman A, Shakoori FR, Shakoori AR (2007) Heavy metal resistant *Distigma proteus* (Euglenophyta) isolated from industrial effluents and its possible role in bioremediation of contaminated waste waters. World J Microbiol Biotechnol 23:753–758

Saha B, Orvig C (2010) Biosorbents for hexavalent chromium elimination from industrial and municipal effluents. Coord Chem Rev 254:2959–2972

Sanghi R, Sankararamakrishnan N, Dave B (2009) Fungal bioremediation of chromates: conformational changes of biomass during sequestration, binding, and reduction of hexavalent chromium ions. J Hazard Mater 169:1074–1080

Saravanane R, Sundararajan T, Sivamurthyreddy S (2002) Efficiency of chemically modified low cost adsorbents for the removal of heavy metals from wastewater: a comparative study. Indian J Environ Health 44:78–81

Sarin V, Pant K (2006) Removal of chromium from industrial waste by using *Eucalyptus* bark. Bioresour Technol 97:15–20

Shi X, Dalal N (1990) Evidence for a Fenton type of mechanism for the generation of OH radicals in the reduction of Cr (VI) in cellular media. Arch Biochem Biophys 281:90–95

Stein JR (1973) Handbook of phycological methods: culture methods and growth measurements. Cambridge University Press, New York

Tan LH, Chen JP, Ting YP (2003) Biosorption of chromium (VI) by marine algal biomass. Proceedings of the 15th International Biohydrometallurgy Symposium 807–816.

Terry PA, Stone W (2002) Biosorption of Cd^{+2} and Cu^{+2} contaminated water by *Scenedesmus abundans*. Chemosphere 47:249–255

Tewari N, Vasudevan P, Guha BK (2005) Study of biosorption of Cr (VI) by *Mucor hiemalis*. Biochem Eng J 23:185–192

Tunali S, Kiran I, Akar T (2005) Chromium(VI) biosorption characteristics of *Neurospora crassa* fungal biomass. Miner Eng 18:681–689

Tüzün I, Bayramoğlu G, Yalcin E, Basaran G, Celik G, Arica MY (2005) Equilibrium and kinetic studies on biosorption of Hg^{2+} Cd^{2+} and Pb^{2+} ions on to microalga *Chlamydomonas reinhardtii*. J Environ Manage 77:85–92

United States Environmental Protection Agency (US EPA) 1998 Toxicological review of hexavalent chromium. US EPA, Washington DC.

Volesky B (2007) Biosorption and me. Water Res 41:4017–4029

Volesky B, Holan ZR (1995) Biosorption of heavy metals. Biotechnol Progress 11:235–250

WHO (2004) Guidelines for drinking water quality, 3rd edn. World Health Organization, Geneva

Yun YS, Park D, Park JM, Volesky B (2001) Biosorption of trivalent chromium on the brown seaweed biomass. Environ Sci Technol 35:4353–4358