Arabian Journal of Chemistry (2016) 9, 846–853

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Biosorption of chromium by using *Spirulina* sp.

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Received 12 April 2011; accepted 16 November 2013
Available online 23 November 2013

KEYWORDS

Biosorption;
Spirulina sp.;
Chromium;
Isotherm models;
Kinetic

Abstract Presence of heavy metals in the aquatic system is posing serious problems and chromium has been used in many industries and the removal of chromium ions from waste waters is significant. Biosorption is one of the economic methods that is used for the removal of heavy metals. In the present study, the biomass generated from the dried *Spirulina* sp. was used for evaluating the biosorption characteristics of chromium ions in aqueous solutions. Batch adsorption experiments were performed on these leaves and it was found that the amount of metal ions adsorbed increased with the increase in the initial metal ion concentration. In this study effect of agitation time, initial metal ion concentration, temperature, pH, and biomass dosage were studied. Maximum metal uptake was observed at pH = **5**. Maximum metal uptake (q_{max}) was 90.91 mg/g .The biosorption followed both Langmuir and Freundlich isotherm models but the Freundlich isotherm model was better than the Langmuir with R^2 = 0.997. The adsorption equilibrium was reached in about 1 h. The kinetics of biosorption followed the second-order rate. The biomass could be regenerated using 0.1 M HNO_3. FTIR Spectrums of biomasses revealed the presence of hydroxyl, amino, carboxylic and carbonyl groups. The scanning electron micrograph clearly revealed the surface texture and morphology of the biosorbent.

1. Introduction

Heavy metal pollution has posed a serious threat to the aquatic environment. At high concentrations, metals are toxic to animals and plants alike, as they could be dispersed in water and consequently in human beings through food chain biomagnifications that could cause serious health hazards. Chromium water pollution has become of considerable concern due to the fact that chromium has been widely used in metal finishing, electroplating, leather tanning, stainless steel production, textile industries, and chromate preparation (Abdel-Jawad et al., 2002). Chromium exists in the environment in either its hexavalent form (Cr) or trivalent form (Cr (III)). The metal species Cr is considered as highly toxic in that it could act as a carcinogen, mutagen, and teratogen in the biological system (Abu Al-Rub et al., 2002). It has also been noted that prolonged exposure to the metal species could cause skin allergies and cancer in human beings (N. et al., 2002). Additionally, Cr (III) should be oxidized to the state of a more carcinogenic and mutagenic Cr by some bacteria in the environment under certain conditions (Ahalya et al., 2006). Recently, the commonly used methods

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Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2013.11.008
1878-5352

applied to remove excessive chromium from aqueous solutions have included ion exchange, chemical precipitation, activated carbon adsorption, evaporation and membrane processes. However, these methods were found to be either inefficient or expensive when metal ions exist in low concentrations (< 100 mg/L) and may also be associated with the generation of secondary environmental problems from waste disposal (Ahalya et al., 2003). The list of the advantages of biosorption includes competitive performance, heavy metal selectivity, cost-effectiveness, regeneration and no sludge generation. Sources of biomass include seaweeds, microorganisms (bacteria, fungi, yeast, and molds), activated sludge and fermentation waste. Studies using Biosorbents have shown that both living and dead microbial cells are able to uptake metal ions and offer a potentially inexpensive alternative to conventional absorbents (Khoo and Ting, 2001; Knorr, 1991). However, the living cell is subject to toxic effects of the heavy metals, resulting in cell death. Moreover, living cell often requires the addition of nutrients and hence increase the BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) in the effluent. For these reasons, the use of non-living biomaterials or dead cells as metal binding compounds has been gaining advantage because toxic ions do not affect them. In addition, dead require less care and maintenance, and is cheaper (Mofa, 1995). Furthermore, dead biomass could be easily regenerated and reused. The capability of some living microorganisms to accumulate metallic elements has been observed at first from the toxicological point of view (Volesky, 1990a,b; Introduction, 1990). However, further researches have revealed that inactive/dead microbial biomass can passively bind metal ions via various physicochemical mechanisms. Research on biosorption has become an active field for the removal of metal ions or organic compounds. Biosorbent behavior for metallic ions is a function of the chemical make-up of the microbial cells of which it consists (Volesky and Holan, 1995). Mechanisms responsible for biosorption, although understood to a limited extent, may be one or a combination of ion exchange, complexation, coordination, adsorption, electrostatic interaction, chelation and micro precipitation (Veglio and Beolchini, 1997; Vijayaraghavan and Yun, 2008; Wang and Chen, 2006).

2. Materials and methods

2.1. Biomass and culture medium

In this study *Spirulina sp.* (5143) was obtained from the National Collection of Industrial Microorganisms (**NCIM**) from Pune – INDIA, which was isolated and thoroughly pure.

The *Spirulina* sp. was maintained in Spirulina Media at 28 °C using 3000 lx light intensity. After a 21 day cultivation period cells were harvested by centrifugation and were washed several times with deionised water in order to remove culture media and were kept on a filter paper to reduce the water content. The biomass was dried at 60 °C in an oven for 24 h and milled to a gritty consistency. The biomass was sieved for particle sizes smaller than 1 mm and stored in a dark bottle and kept in a dry cabinet for experiments. All of the media are sterilized by autoclaving at 121 °C for 20 min.

2.2. Preparation of synthetic sample

A stock solution of 1000 mg/L of Cr was obtained by dissolving potassium dichromate (Merck Company) in distilled water. The test solutions of various concentration ranges from 10 to 100 mg/L were prepared from the stock solution. The solution pH was adjusted using 0.1 M HNO_3 and 0.1 M NaOH at the beginning of the experiment and not controlled afterward. The conical flasks (250 mL) were shaken at 120 rpm in a temperature controlled rotatory shaker.

2.3. Analysis of chromium ions

Chromium was determined spectrophotometrically by atomic absorption spectrophotometer (UNICAM, model 929, UK).

2.4. Batch biosorption studies

Batch mode adsorption studies for individual metal compounds were carried out to investigate the effect of different parameters such as adsorbate concentration, adsorbent dose, agitation time and pH. Solution containing adsorbate and adsorbent was taken in 250 mL capacity conical flask and agitated at 120 rpm in a shaker at predetermined time intervals. The adsorbate was decanted and separated from the adsorbent using Whatman No. 41 filter paper.

2.5. Effect of contact time and initial concentration

For the determination of the rate of metal biosorption by biomasses from 100 mL (at 10, 20, 50 and 100 mg/L) on a conical 250 mL flask, the supernatant was analyzed for residual metal at different time intervals. The pH and the adsorbent dosage were kept constant at pH = 5 ± 0.01, which varied according to the adsorbent and adsorbate under consideration. Amount of biomass dosage was 0.1 ± 0.001 g for biomass (*Spirulina* sp.) and temperature was 25 ± 1 °C and agitation speed of the shaker was 120 rpm.

2.6. Effect of adsorbent dosage and initial concentration

The effect of adsorbent dosage i.e., the amount of the biomass on the adsorption of chromium was studied at different dosages ranging from 0.1 to 3 g with varied metal concentrations of 10, 20 and 50 mg/L. The equilibrium time and the pH were kept constant depending on the metal under consideration.

The pH and the adsorbent dosage was kept constant at pH = 5 ± 0.01, which varied according to the adsorbent and adsorbate under consideration. Agitation time was 120 min for biomass (*Spirulina* sp.) and temperature was 25 ± 1 °C and agitation speed of shaker was 120 rpm.

2.7. Effect of pH and initial concentration

To determine the effect of pH on the adsorption of metal solutions (100 mL) of different concentration ranges (10, 20 and 50 mg/L) in conical flasks 250 mL were adjusted to desired pH values and mixed with constant amount of adsorbent and agitated at a preset equilibrium time. The equilibrium time and adsorbent dosage varied with the metal and adsorbent under

consideration. Amount of biomass dosage was 0.1 ± 0.001 g for all three biomasses (*Spirulina* sp.) and temperature was 25 ± 1 °C and agitation speed of the shaker was 120 rpm and contact time was 120 min.

2.8. Effect of temperature

Optimum biomass concentration with optimum pH was used to monitor the temperature effect on biosorption. Experiments were carried out at different temperatures from 10 to 40 °C for each culture and kept on a rotary shaker at 120 rpm. The samples were allowed to attain equilibrium. To determine the effect of temperature on the adsorption of metal solutions (100 mL) of concentration 50 mg/L in conical flask 250 mL were adjusted to desired pH values and mixed with constant amount of adsorbent and agitated at preset equilibrium time. The equilibrium time and adsorbent dosage varied with the metal and adsorbent under consideration. Amount of biomass dosage was 0.1 ± 0.001 g for biomass (*Spirulina* sp.) and pH was 5 ± 0.001 and the agitation speed of the shaker was 120 rpm and contact time was 120 min.

2.9. Desorption studies

After adsorption, the adsorbates – loaded adsorbent were separated from the solution by centrifugation and the supernatant was drained out. The adsorbent was gently washed with water to remove any unadsorbed adsorbate. Regeneration of adsorbate from the adsorbent – laden adsorbent was carried out using the desorbing media – distilled water at pH ranges using dilute solutions of EDTA, HCl and HNO_3 (Stirred at 200 rpm for 120 min at 25 °C). Then they were agitated for the equilibrium time of the respective adsorbate. The desorbed adsorbate in the solution was separated and analyzed for the residual heavy metals.

2.10. Equilibrium isotherms

The isotherm studies were performed in the solution with the initial concentrations ranging from 10 to 100 mg/L at optimum pH values for ions (pH = 4.5 or pH = 5) .After shaking the flask containing the mixture of biomass (120 rpm, 25 °C) and ions for 120 min, the amount of residual ions in the filtrated solution was analyzed. The biosorption equilibrium uptake capacity for each sample was calculated according to mass balance on the ions expressed in this equation:

$$q_e = \frac{(C_0 - C_e)}{M} \times V$$

where V is the sample volume (L), C_0 is the initial ion concentration (mg/L), C_e is the equilibrium or final ion concentration (mg/L), M is the biomass dry weight (g), and q_e is the biomass biosorption equilibrium ions uptake capacity (mg/g). Langmuir and Freundlich isotherms, the two classical adsorption models, were used to describe the equilibrium between adsorbed ions on the biomass cell (q_e, q) and ions in the solution (C_e, q) in this study.

Langmuir isotherm model:

$$q_e = \frac{q_{\max} C_e b}{1 + C_e b}$$

Then after arrangement we have;

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} b} + \frac{C_e}{q_{\max}}.$$

These values $q_{\max}$ and b (where b, is the adsorption equilibrium constant) can be obtained from the slopes and the intercepts of the linear plots respectively, where experimental data of C_e/q_e as the function of C_e.

The empirical Freundlich equation based on sorption on a heterogeneous surface, on the other hand, is as follows: $q_e = K_f (C_e)^n$

K and 1/n: An experimental constant, K is an indication of the adsorption capacity of the adsorbent; n indicates the effect of concentration on the adsorption capacity and represents adsorption intensity. The equation can be linearized in the following logarithmic form:

$$\ln q_e = \ln k_f + \frac{1}{n} \ln C_e$$

These values n and K_f can be obtained from the slopes and the intercepts of the linear plots respectively, where experimental data of $\ln q_e$ as the function of $\ln C_e$.

2.11. Kinetic modeling

The pseudo-second-order equation is also based on the sorption capacity, which is expressed as:

$$\frac{t}{q_t} = \frac{1}{(K_2 q_e^2)} + \frac{t}{q_e}$$

where K_2 is the rate constant of pseudo-second-order sorption (g mg^{-1} min^{-1}). $K_2 qe^2$ is the initial rate constant (represented by h, mg g^{-1} min^{-1}). Plotting t/q_t versus t will give a straight line. The values of qe and K_2 can be determined from the slope and intercept of the plot, respectively.

2.12. FT-IR spectroscopy (Fourier transform infrared)

In order to determine the functional groups responsible for chromium biosorption, IR spectroscopy was used that about 0.1 g biomass was mixed with KBr for FT-IR spectra analysis (Shimadzu, Model 8400).

2.13. SEM (scanning electron microscopy)

The SEM was used to investigate the morphology of the biosorbent. We used samples with pH = 5 and C_0 = 10 mg/L. Scanning Electron Microscope (SEM, JEOL, JSM-6360A) was used for this study.

3. Results

Results on the effect of pH of Cr at different initial metal ion concentrations by *Spirulina* sp. are shown in the Fig. 1. Maximum percentage of biosorption obtained at the initial concentration of 10 mg/L at the time of 120 min at pH = 5 for Cr was 82.67% and metal ion uptake capacity was 8.26 mg/g and when initial concentration of Cr increased to 50 mg/L,

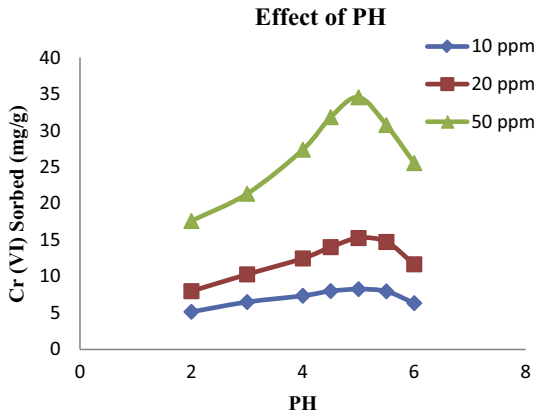


Figure 1 Effect of pH on biosorption of Cr by *Spirulina* sp. (biomass dose = 0.1 g, initial Cr ion concentration = 10, 20, 50 mg/L, temperature = 25 °C; agitation speed = 120 rpm; contact time = 120 min).

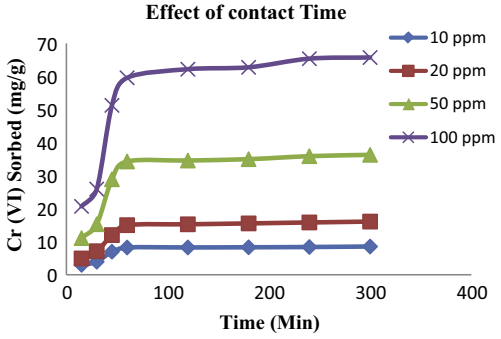


Figure 2 Effect of contact time on biosorption of Cr by *Spirulina* sp. (biomass dose = 0.1 g, pH = 5, initial Cr ion concentration = 10, 20 50 and 100 mg/L; temperature = 25 °C; agitation speed = 120 rpm).

percentage of biosortion of Cr was 63.44% and uptake capacity was 34.56 mg/g for *Spirulina* sp. With increase of the initial concentration percentage of biosorption decreased and metal ion uptake capacity increased.

Results on the contact time of chromium at different initial metal ion concentrations by *Spirulina* sp. are shown in the Fig. 2. In the initial concentration of 10 mg/L at the time of 60 min percentage of removal of Cr was 82.52% and the metal ion uptake capacity was 8.25 mg/g and when the initial concentration of Cr increased to 100 mg/L, percentage of removal of Cr was 59.57% and uptake capacity was 59.57 mg/g for *Spirulina* sp. The time taken for Cr adsorption by *Spirulina* sp. was dependent on initial metal ion concentration and increased with increase in concentration of Cr. With increase of the initial concentration percentage of biosorption decreased and metal ion uptake capacity increased.

Results on the effect of biomass dosage of Cr at different initial metal ion concentrations by *Spirulina* sp. are shown in the Fig. 3. Percentage of biosorption obtained at the initial concentration of 10 mg/L at the time of 120 min at pH = 5 and 0.1 ± 0.001 g of biomass for Cr was 82.67% and metal ion uptake capacity 8.26 mg/g and when the initial concentration of

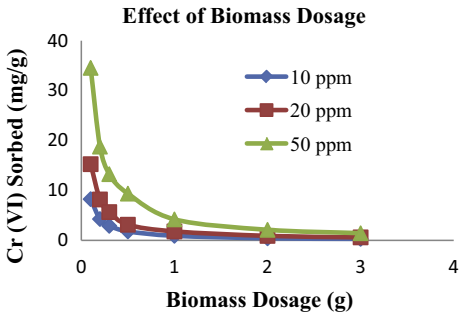


Figure 3 Effect of biomass dosage on biosorption of Cr by *Spirulina* sp. (pH = 5, initial Cr ion concentration = 10, 20 and 50 mg/L; temperature = 25 °C; agitation speed = 120 rpm; contact time = 120 min).

Cr increased to 50 mg/L, percentage of biosortion of Cr was 69.12% and uptake capacity was 34.56 mg/g for *Spirulina* sp.

When amount of biomass increased from 0.1 ± 0.001 to 3 ± 0.001 g, percentage of biosorption obtained at the initial concentration of 10 mg/L at the time of 120 min at pH = 5 for Cr was 92.57% and metal ion uptake capacity was 0.308 mg/g and when the initial concentration of Cr increased to 50 mg/L, percentage of biosortion of Cr was 86.35% and uptake capacity was 1.44 mg/g for the *Spirulina* sp. With increase in the initial concentration percentage of biosorption decreased and metal ion uptake capacity was increased. With increase in the amount of biomass observed that percentage of biosorption increased and the metal ion uptake capacity decreased.

Results on the effect of temperature of Cr at initial metal ion concentration of 50 mg/L by *Spirulina* sp. are shown in the Fig. 4. Maximum percentage of biosorption obtained at the initial concentration of 50 mg/L at the time of 120 min at pH = 5 for Cr was 72.83% and the metal ion uptake capacity was 36.41 mg/g at the temperature of 40 °C for *Spirulina* sp. The findings of *Spirulina* sp. indicate that the sorption percentage increased with increase in temperature up to 40 °C.

The equilibrium experimental results of chromium ions have been fitted in the Langmuir and Freundlich models. For biosorption of chromium using *Spirulina* sp. the coefficient of determination (R^2) of both models was mostly close to 1 as shown in Fig. 5 and Fig. 6. This indicates that both models adequately describe the experimental data of the biosorption of chromium. In the biosorption of chromium by *Spirulina*

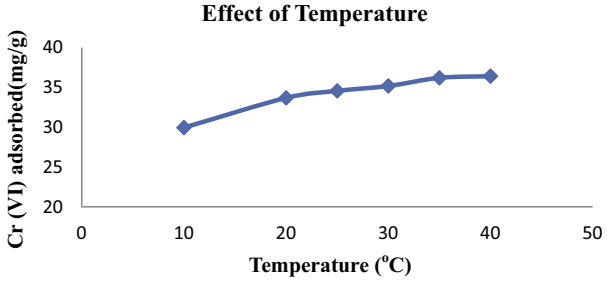


Figure 4 Effect of Temperature on biosorption of Cr by *Spirulina* sp. (biomass dose = 0.1 g, pH = 5, initial Cr ion concentration = 50 mg/L; temperature = 25 °C; agitation speed = 120 rpm; contact time = 120 min).

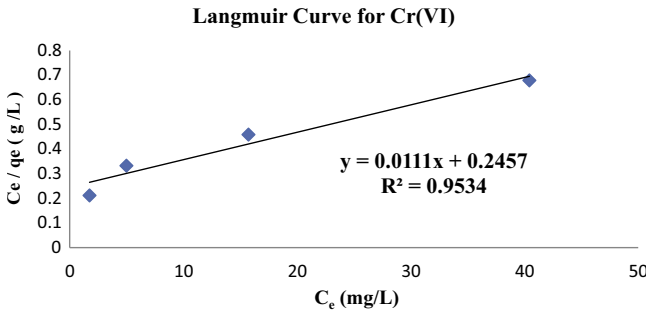


Figure 5 Langmuir adsorption isotherm for Cr by *Spirulina* sp. (q_{max} = 90.91 mg/g, b = 0.045 L/mg).

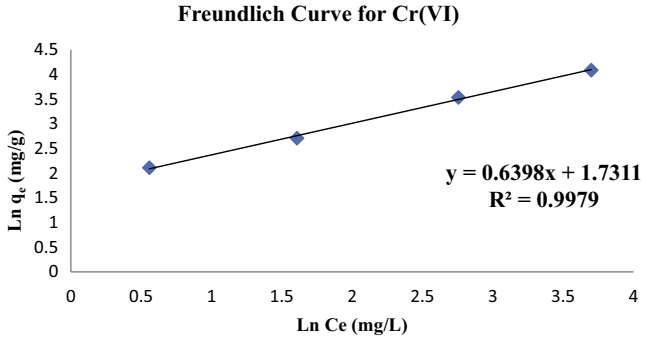


Figure 6 Freundlich adsorption isotherm for Cr by *Spirulina* sp. (n = 1.56, K = 5.65).

sp., most of the metal ions were sequestered very fast from the solutions in the first phase of contact time of 60 min and almost no increase in the level of bound metal having occurred after this time interval. Biosorption equilibrium isotherms were plotted for metal uptake q against the residual metal concentration in the solution. The q verses C_f sorption isotherm relationship was mathematically expressed by the Langmuir and Freundlich models. The higher the values of k and n; lower the value of b, the higher the affinity of the biomass. Table 1 describes summaries of linear regression data for the Langmuir and Freundlich isotherms for chromium biosorption using *Spirulina* sp. biomass (Fig. 5).

Langmuir and Freundlich constants k were obtained from the linear equations of both models. As indicated in the Table 1, the coefficients of determination (R^2) of both models are close to 1. In the Table 1 the values of K_f, n, q_{max} and b were given.

3.1. Kinetic modeling

Fig. 7 shows the experimental break through curves for the effects of contact time on a bound rate of Cr. It can be

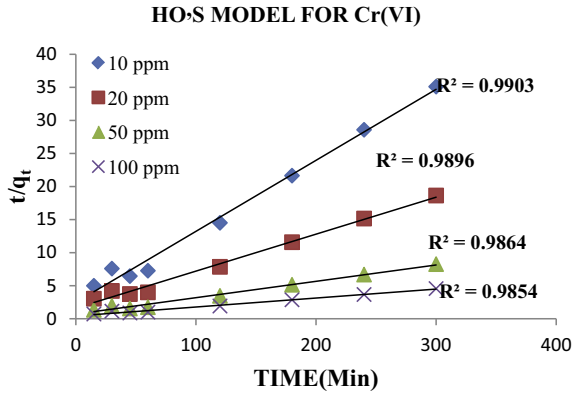


Figure 7 Plot of HOS Model (Pseudo second order rate) for Cr by *Spirulina* sp.

observed that the adsorption of chromium ions quickly increased at the beginning of biosorption, but after 15 min, the adsorption slowed down. The result indicated that the maximum adsorbed amount of the chromium ions was achieved within 60 min, and then followed by a longer equilibrium period. After this equilibrium period, the amount of adsorbed ions did not significantly change with the adsorption time. Therefore, for the following experiments, the contact time was maintained for 60 min to ensure that equilibrium was fully achieved.

The results showed that the pseudo-second-order model fitted the simulation curve much better than the pseudo-first-order model for Cr. The results of pseudo-second-order model are shown in the Table 2.The coefficient of determination (R^2) and K_2 HOS model for the different metal ion concentration under study has been established as: 10 ppm > 20 ppm > 50 ppm > 100 ppm.

With an increase the initial concentration coefficient of determination (R^2) and K_2 decreased.

3.2. Equilibrium parameter R_L

The essential characteristics of a Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or *equilibrium* parameter R_L, which is defined by

Table 2 Parameter of kinetic model for chromium of *Spirulina* sp.

Concentration of Cr	Equation	R^2	K_2
Cr, 10 ppm	$y = 0.107x + 2.477$	0.990	4.62×10^{-3}
Cr, 20 ppm	$y = 0.055x + 1.612$	0.989	1.87×10^{-3}
Cr, 50 ppm	$y = 0.024x + 0.712$	0.986	8.09×10^{-4}
Cr, 100 ppm	$y = 0.013x + 0.424$	0.985	3.98×10^{-4}

Table 1 Parameters of isotherm models for chromium.

Biomass	Langmuir parameters			Freundlich parameters		
	q_{max} (mg/g)	b (L/mg)	R^2	K_f	n	R^2
Spirulina sp.	90.91	0.045	0.953	5.65	1.56	0.997

Table 3 Type of isotherm for various R_L.

R_L	$R_L > 1$	$R_L = 1$	$0 < R_L < 1$	$R_L = 0$
Type of isotherm	Un favorable	Linear	Favorable	Irreversible

Table 4 The desorption efficiency of a different desorbent.

Desorbent	EDTA (0.1 M)	HCl (0.1 M)	HNO_3 (0.1 M)
% Desorption of Cr	72.23 ± 3.15	89.57 ± 3.39	95.04 ± 3.10

$$R_L = 1/1 + bC_o$$

where C_o is the initial adsorbate concentration (mg/L) and b is the Langmuir constant (L/mg). The parameter indicates the shape of the isotherm as follows (Table 3).

The R_L values at different initial adsorbate concentrations indicate favorable adsorption for all the adsorbents and adsorbates studied.

3.3. Desorption studies

Desorption and regeneration studies of the adsorbates showed that regeneration and recovery of the adsorbates are possible. Chemisorption/ion exchange was the main mechanism by which the adsorbates (metals) were attached to the adsorbents. Physical adsorption played a minimal role in the process .The result of desorption studies of Cr in a batch system showed that HNO_3 (0.1 M) was more efficient in Cr desorption, which removed 95% chromium ions (Table 4).

4. FTIR Spectroscopy of *Spirulina* sp.

Spectrums of *Spirulina* sp. present in the Fig. 8 revealed the presence of hydroxyl, amino, carboxylic and carbonyl groups. The presence of OH group along with carbonyl group confirmed the presence of carboxylic acid groups in the biosorbent. The presence of NH group and OH group along with carbonyl group might be attributed to the presence of amino acid groups in the biosorbent. The stretching vibration of OH, NH and C═O groups shifted to a certain extent in addition, which indicated that these three groups are possibly involved in the biosorption. Assignment of bands to functional group on the surface of *Spirulina* sp. before and after

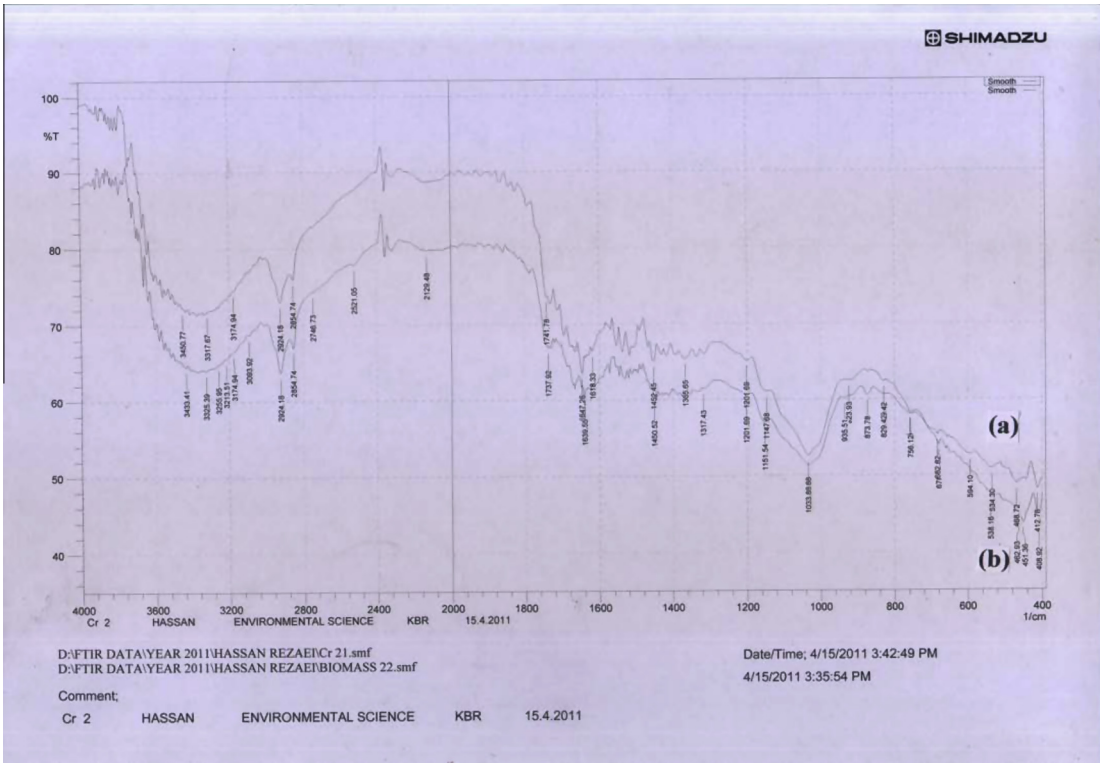
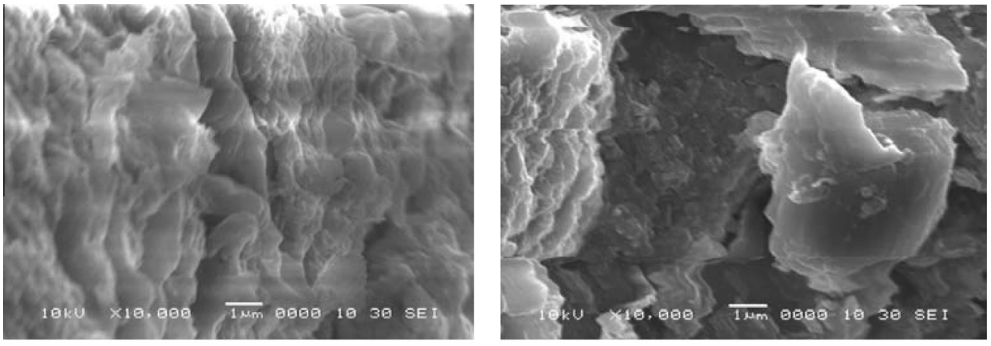


Figure 8 198 FTIR Spectrum of the *Spirulina* sp. (before (b) and after (a) biosorption of Cr.

Table 5 Assignment of bands to the functional group on the surface of *Spirulina* sp. as observed from FTIR spectroscopy.

Wave numbers (cm^{-1})		Assignment
Before	Chromium	
3350.46	3308.03	O–H stretching/N–H stretching
3217.37	3174.94	
3090.07		C–H stretching aromatic
2956.97	2955.04	C–H stretching aliphatic
2926.11	2926.11	
2856.67	2854.74	C–H stretching
	1730.21	C═O stretching vibration
1672.34	1647.26	C═O stretching vibration/N–H stretching vibration
1627.97	1622.19	
1531.53	1539.25	
1446.66	1454.38	CH_2 bonding vibration
1039.67	1043.52	C–O Stretching
777.34	773.48	C–X (X = F, Cl, Br and I)



(b) **(a)**

Figure 9 Scanning electron microscopy (SEM) micrographs of the *Chlorella pyrenoidosa* before (b) and after (a) of biosorption.

biosorption of chromium as observed from FTIR spectroscopy is summarized in the Table 5.

4.1. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) values of *Spirulina* sp. before and after biosorption of chromium are shown in the Fig. 9. The scanning electron micrograph clearly revealed the surface texture and morphology of the biosorbent at different magnifications. The SEM analysis revealed important information on surface morphology. In these micrographs structures with large surface area were evident. The results obviously show the difference between before and after loading of ions on the biomass surface.

5. Conclusions

The batch experiment conducted with the biosorption demonstrated that the biomass of *Spirulina* sp. exhibited the potential for Cr removal from aqueous solution. Optimum pH and temperature for biosorption in this study were 5 and 25 °C, respectively. The time taken for Cr adsorption by *Spirulina* sp. was dependent on the initial metal ion concentration and increased with an increase in the concentration of Cr. With increase in the initial concentration percentage of biosorption decreased and metal ion uptake capacity increased. With increase in the amount of biomass we observed that percentage of biosorption increased and metal ion uptake capacity decreased. The findings of *Spirulina* sp. indicate that the sorption percentage increased with increase in temperature up to 40 °C and there was a decrease in sorption percentage with further increase in temperature. The removal of Cr increases with increase in biosorbent. The biosorption process followed both the Langmuir and Freundlich isotherm models but the Freundlich isotherm model was better than the Langmuir with $R^2 = 0.997$. The pseudo second-order kinetics described the experimental data well. The equilibrium time was 60 min. The R_L values at different initial adsorbate concentrations indicate favorable ($0 < R_L < 1$) adsorption for all the adsorbents and adsorbates studied. HNO_3 (0.1 M) had higher efficiency of Cr desorption than EDTA (0.1 M) and HCl (0.1 M) with 95% efficiency desorption. FTIR spectrums of *Spirulina* revealed the presence of hydroxyl, amino, carboxylic and carbonyl groups. The presence of OH group along with carbonyl group confirmed the presence of carboxylic acid groups in the biosorbent. The presence of NH group and OH group along with carbonyl group might be attributed the presence of amino acid groups in the biosorbent. Scanning electron microscopy (SEM) of *Spirulina* sp. before and after biosorption of chromium clearly showed the biosorption of heavy metals by algal biomass.

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