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Response surface methodological approach for optimizing removal of Cr (VI) from aqueous solution using immobilized cyanobacterium

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Abstract

The potential use of alginate immobilized algal beads for the removal of chromium from aqueous solution has been investigated under optimized conditions in this study using a novel cyanobacterium, *Lyngbya putealis* isolated from metal contaminated soil. Batch mode experiments were performed to determine the adsorption equilibrium and kinetic behaviour of chromium in aqueous solution allowing the computation of kinetic parameters and maximum metal adsorption capacity. Influences of other parameters like initial metal ion concentration (10–100 mg/L), pH (2–6) and temperature (25–45 °C) on chromium adsorption were also examined, using Box–Behnken design. Very high regression coefficient between the variables and the response ($R^2 = 0.9984$) indicates excellent evaluation of experimental data by second-order polynomial regression model. The response surface method indicated that 50–60 mg/L initial chromium concentration, 2–3 pH and a temperature of 45 °C were optimal for biosorption of chromium by immobilized *L. putealis*, when 82% of the metal is removed from the solution.

Keywords: Immobilization; Chromium; *Lyngbya putealis*; Biosorption; Isotherms

1. Introduction

Research and development in wastewater treatment processes has gained much importance in the recent years as environmental legislations for industrial discharges have become quite stringent. Heavy metal contamination of surface waters due to industrial effluents is a major problem, which needs to be tackled due to ecotoxicological effects of the metals along with their accumulation in food chain. Some of the heavy metals are required in trace amounts by living organisms, but, beyond a threshold concentration they become toxic to plants as well as animals [1,2]. These can also cause accumulative poisoning, brain damage and even cancer in human beings [3]. Hexavalent chromium, which is more toxic than trivalent is reported to cause cancer in the digestive tract and lungs of human beings [4].

Conventional technologies like oxidation/reduction, ion exchange and membrane transfer methods available to tackle the problem of heavy metal contamination of wastewaters have a major constraint that they are not so effective and economical for effluents containing concentration of metals less than 100 mg/L [5]. Hence, there is a need to develop newer bioremediation techniques, which are cost-effective, environment-friendly and have high efficacy for metal removal from wastewaters with relatively lower metal concentration [6]. Algal biosorbents for metal removal from aqueous solution are in use for quite sometime and for this purpose both live and dead mass of algae have been tried and reported as biosorbents [7–10]. Cyanobacteria have a great potential for use as effective biosorbents, because they are easy to grow with simple nutrient requirements and unlike other microbial systems, they generally do not produce toxins. Further, cyanobacteria produce extracellular polysaccharides that have a tendency of binding metals [11]. Use of algal biomass in powder form is more in practice for biosorption purposes. However, separation of the algal powder from the wastewater after use becomes a major practical problem. Also, low strength and small particle size of the biosorbent pose hinderance in column applications [12].

Immobilization of the alga, on the other hand, not only avoids biomass–liquid separation, but also allows higher local cell

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density and retention of biomass within a definite working system that can be reused. Different techniques like flocculation, covalent bonding to carriers, encapsulation in polymer gel and entrapment in polymeric matrix, are available for immobilization. Entrapment in some polymeric matrix, usually a gel, is one of the most widely used techniques, which involve formation of beads that provides mechanical strength and rigidity to the system [13]. There are some reports on use of alginate gel for algal immobilization and heavy metal removal [14,15]. The alginate gel is a heteropolymer of L-guluronic acid and D-monouronic acid, which is available as water-soluble sodium salts [16]. Calcium alginate is formed following replacement of monovalent sodium ion with divalent calcium and ionic cross-linking among carboxylic acid groups. The polymeric network of polysaccharide molecules so formed has water entrapped in the gel [17].

In the present investigation, a native strain of the cyanobacterium *Lyngbya putealis* HH-15 (Synonym *Phormidium putealis*; [18]) isolated from a metal contaminated industrial site was used in immobilized form to optimize its Cr (VI) removal ability from aqueous solution. There are some reports on biosorption of heavy metals like copper, iron, nickel and zinc by certain species of *Lyngbya* [19,20] but there are no reports on biosorption of metals by *L. putealis*, which grows readily and forms a good mass that can be conveniently used for biosorption studies. Rapid growth and early harvestable biomass of this cyanobacterium ensures ready availability of this system as a biosorbent, which is both cost-effective and non-toxic. Chromium (VI) which is carcinogenic in nature and present in toxic concentrations (5–100 mg/L) in effluents of several industries (electroplating, textile and fertilizer industries) needs to be brought down to permissible limits as per specified standards of WHO (0.05 mg/L) or Indian standards (0.1 mg/L). In the present investigation, kinetic parameters and maximum adsorption capacity were studied adopting a full range of response surface methodology using Box–Behnken model to analyze the effectivity of the system under different conditions. This model was adopted as an experimental design model to study the effect of interactions of combined parameters like pH, temperature (°C) and initial metal ion concentration (mg/L) of the aqueous solution while permitting reduction in the actual number of combination experiments to be performed. The experimental data was analyzed with a second order polynomial model validated by statistical analysis.

2. Materials and methods

2.1. Cyanobacterial isolation and culture

L. putealis HH-15 was isolated from metal contaminated soil collected from within the premises of an electroplating industry in Haryana, India. Pure culture of the cyanobacterium was obtained by streaking on basal agar medium at pH 8.5 using standard isolation and culturing techniques on nitrogen supplemented BG-11 medium [21]. The algal cultures were maintained at a light intensity of 3000 lx using cool fluorescent tubes at 28 ± 3 °C in a culture room. Fourteen-day-old algal cultures were harvested and the algal biomass was washed with distilled water and oven dried at 70 °C for 24 h before use.

2.2. Algal immobilization

0.1 g dry weight of the alga was suspended in 5 mL of double distilled water, mixed with 4% sodium alginate solution (w/v) and dropped into 0.5 M calcium chloride solution using a syringe to form algal beads (3.0 ± 0.1 mm diameter). The beads were kept overnight at 4 °C in $CaCl_2$ (0.5 M) for completing the process of gelation. The beads were repeatedly washed with double-distilled water and stored at 4 °C in distilled water prior to use as the biosorbent [12]. Blank alginate beads were also prepared using similar procedure, but without the alga.

2.3. Batch studies

A stock solution of the aqueous adsorbate, Cr (VI) (1000 mg/L) was prepared using potassium dichromate (AR grade) and desired concentrations of the metal were obtained by further dilutions. Batch studies were performed to determine the equilibrium time required for adsorption of Cr (VI) on the immobilized algal adsorbent. Erlenmeyer flasks containing 100 mL of the metal solution at initial pH 2 having algal beads (0.1 g algal dry weight) were shaken on an illuminated orbital shaker (Orbitek LT-IL) with fluorescent light at 120 rpm at 25 °C. Samples were withdrawn at fixed time intervals from the flasks in triplicates and analyzed spectrophotometrically for residual metal ion concentration in the aqueous solution. Adsorption of chromium by blank alginate beads was also observed by performing the experiment with beads without alga.

Adsorption isotherm studies were carried out using different initial metal ion concentrations (10–100 mg/L) with 0.1 g biosorbent concentration at pH 2 and temperature of 25 °C. Experiments were also performed by varying the temperature (25–45 °C) and pH (2–6) keeping other conditions constant to observe the effect of temperature and pH on adsorption. pH of the aqueous metal solution was adjusted using 0.1 M HCl. All the experiments were performed in triplicates and their mean values are reported here.

Amount of metal adsorbed, q_e (mg/g of dry weight of alga) was determined using the equation

$$q_e\,(\text{mg/g}) = \frac{V(C_0 - C_e)}{m} \tag{1}$$

where C_0 is initial metal concentration (mg/l), C_e the residual metal concentration (mg/L), V volume of metal solution (L) and m is the mass of dry alga (g) entrapped in the beads.

2.4. Metal analysis

Concentration of Cr (VI) ions in the synthetic solution was analyzed using a Systronics Spectrophotometer-106 at 540 nm using 1,5 diphenyl carbazide reagent in acid solution as complexing agent for Cr (VI) [22].

Table 1
Box–Behnken design matrix for three variables along with observed response

Experimental run	Variables			Response % removal of Cr (VI), Y
	Metal concentration, X_1 (mg/L)	pH, X_2	Temperature, X_3 (°C)	
1	10	2	35	31.29
2	100	2	35	37.74
3	10	6	35	21.83
4	100	6	35	25.48
5	10	4	25	24.10
6	100	4	25	27.10
7	10	4	45	30.72
8	100	4	45	35.62
9	55	2	25	70.42
10	55	6	25	59.0
11	55	2	45	81.58
12	55	6	45	63.19
13	55	4	35	60.22
14	55	4	35	60.22
15	55	4	35	60.22

2.5. *Optimization of biosorption process using RSM approach*

Response surface methodology is an approach that combines various statistical and mathematical techniques, and is useful for developing, improving and optimizing a process [23] like the adsorption process [24,25].

In the present study, Box–Behnken model for three variables (metal concentration, pH and temperature), each with two levels (the minimum and the maximum), was used as experimental design model. The model has the advantage that it permits the use of relatively few combinations of variables for determining the complex response function [26]. A total of 15 experiments are required to be performed to calculate 10 coefficients of second-order polynomial equation [27]. In the experimental design model, metal ion concentration (10–100 mg/L), pH (2–6) and temperature (25–45 °C), were taken as input variables. Percent adsorption of chromium estimated as percent removal of chromium from the solution was taken response of the system. The experimental design matrix derived from the Box–Behnken model is shown in Table 1. Percent chromium removal by the immobilized cyanobacterium in different experimental conditions based on the experimental design matrix was estimated, the results of which have also been included in the same table.

A second order polynomial model where interaction terms have been fitted to the experimental data obtained from the Box–Behnken model experiment can be stated in the form of the following equation:

$$Y = a_o + \sum a_i x_i + \sum a_{ii} x_i^2 + \sum a_{ij} x_i x_j \qquad (2)$$

where Y is the percentage of metal adsorbed, a_o offset term, a_i first-order main effect, a_{ii} second-order main effect and a_{ij} is the interaction effect.

The data were subjected to analysis of variance and the coefficient of regression (R^2) was calculated to find out the goodness of fit of the model.

3. Results and discussion

3.1. *Biosorption kinetics*

Kinetics of biosorption of Cr (VI) by immobilized algal beads was studied by varying the contact time from 5 to 180 min for two initial metal concentrations (20 and 50 mg/L), keeping other conditions constant (pH 2, temperature 25 °C) and the results are shown in Fig. 1. Metal adsorption on the beads increased with contact time till equilibrium was attained at 120 min when 60–75% adsorption occurred. Thus 120 min agitation time seemed adequate for maximum biosorption or removal of the metal by alginate immobilized algal beads. Biosorption of the metal showed a rapid exponential phase up to the first 20 min showing 40–45% removal by algal beads. Thereafter, it became gradual and during 40–50 min further metal removal of 5–15% took place. After 50 min metal biosorption tends to get slowly stabilized and 10–15% additional adsorption occurred in 70 min till equilibrium was reached at 120 min. Similar pattern of metal biosorption have been observed for other metals also. Another study [28] reported up to 65% nickel removal by immobilized alga *Chlorella vulgaris* while 50% of the metal removal was due to adsorption by blank alginate beads at 100 ppm initial metal concentration. In the present case, when we consider adsorption by the blank alginate beads (8–27%), the adsorption by the alga alone comes out to be 35–50% at different time intervals. Rangsayatorn et al. [29] have however, reported up to 10% removal of cadmium by alginate gel. Initial rapid sorption of chromium suggests the involvement of a passive process of metal binding on algal surface that is followed by a gradual and slow stage, that is likely to be associated with some active energy mediated process. Biosorption studies have been conducted by researchers using different immobilizing materials and some of them like AlgaSORB, a modified silica gel showed equilibrium in just 20 min [30]. Adsorption of cadmium by cells of *Spirulina* using alginate and silica gel beads was studied by Rangsayatorn et al. [29], who found alginate beads to have better efficiency and reusability. Blank alginate beads also show up to 25% adsorption of chromium with similar equilibrium time. The rapidity with which a major proportion of metal is adsorbed by

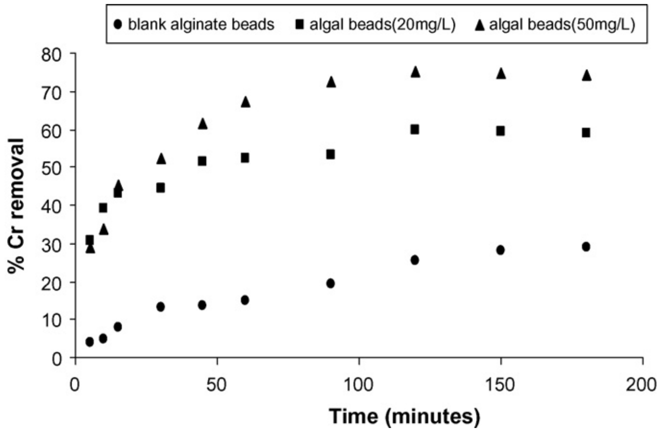

Fig. 1. Biosorption kinetics of Cr (VI) on immobilized algal beads (pH 2, temperature = 25 °C, algal dose = 0.1 g, initial Cr concentration = 20 and 50 mg/L).

Table 2
Kinetic model parameters for metal adsorption on algal beads at different initial chromium concentration

Initial metal ion concentration (mg/L)	Experimental, q_e (mg/g)	First-order model			Second-order model		
		Theoretical q_e (mg/g)	k_1 (mg/(g min))	R^2	Theoretical q_e (mg/g)	k_2 (g mg^{-1} min^{-1})	R^2
20	11.01	4.998	0.0214	0.9289	11.35	1.966	0.997
50	36.13	23.67	0.0302	0.9389	38.91	10.10	0.999

the algal beads, particularly at relatively lower Cr concentrations (20–50 mg/L) puts this biosorbent at an advantageous position for use in removal of Cr (VI) from wastewaters in an economic way and also rapid removal rate facilitates use of reactors with smaller volumes.

Assuming the biosorption capacity for chromium on the algal beads to be proportional to the number of active sites occupied on the sorbent Lagergren rate equation was applied. Rate constants for adsorption of Cr (VI) were determined using pseudo first and second order equations as shown below in the form of Eqs. (3) and (5), respectively [31,32].

$$\log(q_e - q_\mathrm{t}) = \log q_e - \frac{k_1 t}{2.303} \tag{3}$$

$$\frac{\mathrm{d}q}{\mathrm{d}t} = k_2(q_e - q_t)^2 \tag{4}$$

where k_1 is Lagergren rate constant (min^{-1}), k_2 pseudo second order constant (g mg^{-1} min^{-1}), q_e and q_t amounts of metal ion sorbed (mg g^{-1}) at equilibrium and at any time (t), respectively.

Linear form of Eq. (4) is as follows:

$$\frac{t}{q} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e t} \tag{5}$$

The linear plot of t/q versus t shows better fitness of data at both concentrations as compared to the plot $\log(q_e - q_t)$ versus t indicating relatively better fitness of sorption data to pseudo second order model. The values of k_1, k_2 and q_e calculated from the slope and intercept of plots are given in Table 2. The theoretical values of q_e obtained from the graph (not shown here) have been compared with experimental values of q_e in Table 2 and good agreement between calculated value of q_e from second order kinetic model and experimental value of q_e was observed. Very high value of R^2 (0.99), further proves the validity of this model to explain biosorption of Cr (VI) by algal beads at both the concentrations. Value of q_e calculated from the first order kinetic model by plotting $\log(q_e - q_t)$ versus t were however, not equal or approaching the experimental value of q_e, thus diminishing the validity of this model for the present system.

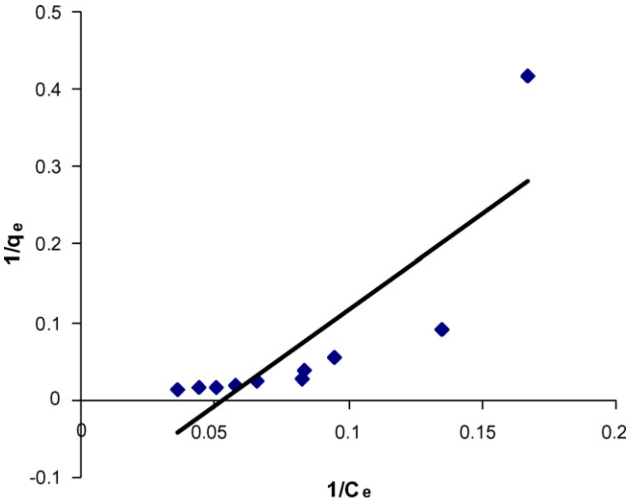

Fig. 2. Langmuir isotherm for the adsorption of chromium on algal beads (pH 2, temperature = 25 °C, algal dose = 0.1 g, initial chromium concentration = 10–100 mg/L).

3.2. Adsorption isotherms

Figs. 2 and 3 show the adsorption isotherm for chromium removal by *L. putealis*. Maximum biosorption capacity was found to be 69 mg/g at 25 °C. Metal biosorption may be influenced by several factors which may be physical or chemical in nature. Metal biosorption data is widely subjected to isotherm models to understand the mechanism of adsorption. Here to understand the biosorption mechanism and surface characteristics of the immobilized alga, the mathematical models developed by Langmuir and Freundlich have been applied to the data.

Langmuir isotherm, which assumes that there is finite number of binding sites distributed homogeneously over the surface of

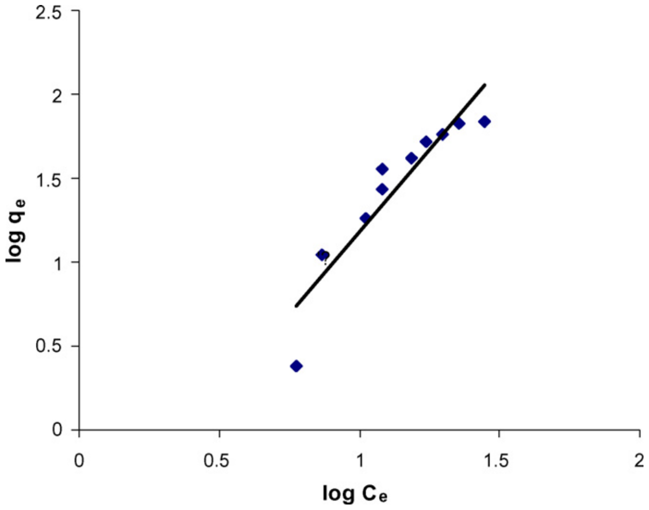

Fig. 3. Freundlich isotherm for the adsorption of chromium on algal beads (pH 2, temperature = 25 °C, algal dose = 0.1 g, initial chromium concentration = 10–100 mg/L).

Table 3
Langmuir and Freundlich adsorption constants for Cr (VI) biosorption by immobilized algal beads

	Parameter value
Langmuir parameters	
Q_o (mg g^{-1})	7.72
b (L mg^{-1})	0.053
R^2	0.6932
Freundlich parameters	
K_f (mg g^{-1})	6.42
$1/n$	1.984
R^2	0.8569

the adsorbent, can be represented as

$$\frac{1}{q_e} = \frac{1}{Q_o} + \frac{1}{Q_o b C_e} \tag{6}$$

where C_e is equilibrium concentration (mg/L), q_e amount of Cr (VI) adsorbed at equilibrium (mg/g), Q_o (mg/g) and b (L mg^{-1}) are Langmuir constants showing the saturated monolayer adsorption capacity and sorption equilibrium constant, respectively [33].

Freundlich isotherm, which is widely used in environmental engineering practice, was also applied to study the adsorption behaviour. This model assumes heterogeneous surface of the adsorbent and linearized form of the model is as follows:

$$\log_{10}(q_e) = \log_{10} K_f + \frac{1}{n}(\log_{10} C_e) \tag{7}$$

where K_f is Freundlich constant indicating adsorbent capacity (mg/g dry weight) and n is Freundlich exponent known as adsorbent intensity [34].

Out of the two investigated models Freundlich fitted better to the present data as the R^2 value obtained here was higher (0.8569) as compared to that for Langmuir isotherm (0.6932). It is also supported by the high value of K_f and $1/n$, i.e. 6.4 mg/g and 1.984, respectively (Table 3). This suggests that the present biosorbent has heterogenous surface characteristics with several possible functional groups responsible for sorption of the metal ions.

3.3. RSM approach for adsorption optimization

The Box–Behnken model was used to statistically design the experiments to evaluate the interactive effects of process parameters for optimizing adsorption of Cr (VI) onto the immobilized algal beads. The empirical relationship between the response and various input variables in the RSM approach obtained from the Box–Behnken model are shown in Table 1. A perusal of Table 1 shows distinct response pattern in terms of percent chromium removal under different combinations of initial metal concentration, pH and temperature.

Statistical significance of the variables and their interactions at various levels of probability are depicted in Table 4 based on Student's t-distribution test and analysis of variance fitted to second order polynomial equation. Smaller P values (<0.05) represent the statistical significance of parameter effect [35]. Very high value of parameter estimate, for the variables X_1X_1 and high value for X_2 and X_3X_3 showing a high level of significance indicate the importance of these variables in the biosorption process. The first order effect of pH (X_2) and second order main effect of initial metal concentration (X_1^2) and temperature (X_3^2) were highly significant. The variables X_2 (pH) had negative relationship with adsorption, while other variables like X_1 (initial metal concentration) and X_3 (temperature) and second order effects, X_2^2 and X_3^2 had a significant ($P < 0.05$) positive effect on adsorption process. However, second order main effect of X_1^2 (initial metal concentration) is negative indicating optimal adsorption at a particular concentration (55 mg/L in present case) followed by decline at higher concentration.

Table 4
Estimated parameters of Box–Behnken model and their statistical significance

Variables	Parameter estimate	Probability level ($P > t$ for H_o)
Intercept	60.22	0.0001^{***}
X_1	2.25	0.0049^{***}
X_2	−6.44	0.0001^{***}
X_3	3.81	0.0005^{***}
X_1X_1	−35.15	0.0001^{***}
X_2X_2	4.01	0.0021^{***}
X_3X_3	4.31	0.0015^{***}
X_1X_2	−0.70	0.3394^{NS}
X_1X_3	0.48	0.5058^{NS}
X_2X_3	−1.74	0.0466^{*}

***Significance ($P < 0.001$), $^{*}P < 0.05$ and NS = not significant.

The parameters were then fitted into second order polynomial equation as follows:

$$Y = 60.22+2.25X_1-6.44X_2 + 3.81X_3 - 35.15X_1^2 + 4.01X_2^2 + 4.31X_3^2 - 0.7X_1X_2 + 0.48X_1X_3 - 1.74X_2X_3 \tag{8}$$

To test the significance of fit of the second order polynomial equation for our experimental data, ANOVA was conducted, the results of which are shown in Table 5. The second order polynomial model taken as a factor (source of variation) gave a highly significant F-value ($F = 340.76$, degree of freedom = 9, $P < 0.0001$) and a low value of standard deviation (2.89) between the measured and modeled results which shows that the equation adequately represents actual relationship between the response (Cr removal) and significant variables (initial metal concentration, pH and temperature). High value of R^2 (0.9984) indicates

Table 5
One way ANOVA for RSM parameters fitted to second-order polynomial equation

Sources of variation	Sum of squares	Degree of freedom	Mean square	F-value	Probability $> F$
Model	5392.41	9	599.16	340.76	0.0001
Error	8.79	5	1.76		
Cor total	5401.20	14			
Root MSE[a]	1.33				
Dep mean[a]	45.92				

[a] Root MSE (a) = square root of mean square error, Dep mean (b) = dependent mean (overall mean of the response) and C.V. (a/b) = 2.89, R^2 = 0.9984.

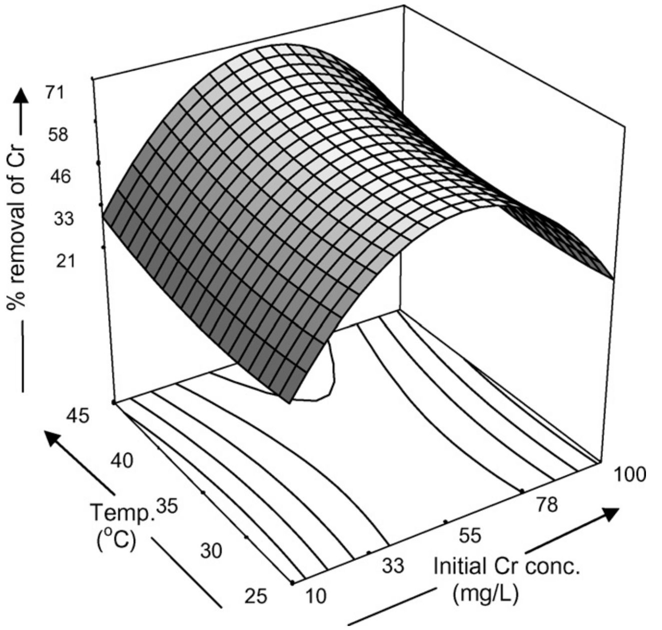

Fig. 4. Response surface plot for metal removal as a function of temperature and initial chromium concentration.

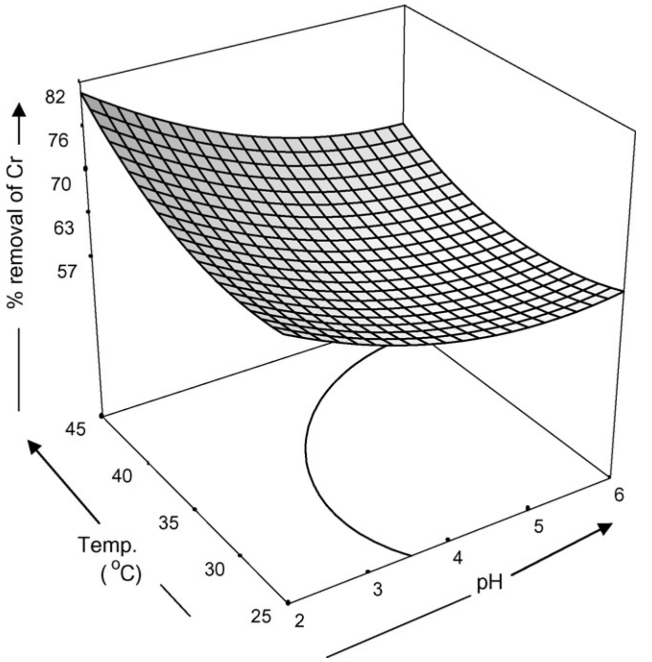

Fig. 5. Response surface plot for metal removal as a function of temperature and pH.

a high dependence and correlation between the observed and the predicted values of response. The inferences obtained from the response surface methodology based on the Box–Behnken experimental design model in relation to adsorption of Cr (VI) by the algal beads with respect to each variable (X_1, X_2, X_3) are discussed here:

3.3.1. *Effect of interactive variables*

Response surface plot was used to determine percent adsorption of the metal over interactive variables temperature and initial metal concentration (Fig. 4) and the variables temperature and pH (Fig. 5).

Increasing the temperature from 25 to 45 °C facilitated the removal of Cr (VI) ions. The increase in metal uptake with increasing temperature may be due to either higher affinity of sites for metal or an increase in number of binding sited on biomass [36].

Fig. 4 also shows the effect of metal concentration on adsorption. It shows that adsorption increases with increasing metal concentration up to 50–60 mg/L and afterwards shows a slight decrease. Another study has reported decreased percent metal removal as metal concentration increases [37]. In the present study, the results of RSM approach indicates first order effect of metal concentration (X_1) as positive while second order main effect (X_1^2) as negative. Increased biosorption up to 50–60 mg/L concentration may be attributed to inherent surface characteristics of the alga, which was locally isolated from metal contaminated soils with almost similar metal concentrations. However, at higher concentration, a decline in chromium biosorption occurred. This decrease at high concentration may be due to competition among metal ions for smaller number of available binding sites and also saturation of most of the binding sites.

Fig. 5 shows the interactive effect of pH and temperature of the solution on percent adsorption of Cr (VI) onto algal beads. It shows that there is maximum adsorption at pH 2–3 and with increasing pH a decrease in biosorption is observed. This may be due to net positive charge on algal surface at low pH. Isoelectric point at pH 3.0 for algal biomass, protonation of certain functional groups and presence of hydronium ions around the binding sites, cause greater attraction of Cr (VI) to algal surface at this pH [37]. But as pH of the solution increases, algal cell wall becomes negatively charged due to functional groups, which repulse negatively charged chromate ions ($HCrO_4^-$, $Cr_2O_7^{2-}$, and $Cr_4O_{13}^{2-}$) thereby affecting Cr (VI) biosorption on algal beads.

4. Conclusion

The objective of the present study was to find out and optimize the chromium adsorption capacity of a new biosorbent *L. putealis* immobilized in alginate gel. Biosorption kinetics indicated an initial rapid phase of chromium adsorption by algal beads suggesting its use in smaller sized reactors with less contact time requirement. Higher values of K_f and R^2 from Freundlich isotherm over Q_o and R^2 for Langmuir isotherm indicate better fitness of Freundlich isotherm to the data. It may be inferred from here that the algal surface is heterogenous containing different functional groups on which adsorption takes place. The high value of K_f supports the use of this immobilized alga for Cr removal in wastewater treatment system.

On the basis of RSM approach using Box–Behnken model for experimental design and fitness of polynomial equation, optimal conditions for chromium adsorption were found to be pH 2–3, temperature 45 °C and metal concentration 50–60 mg/L when 82% chromium removal can be achieved using the alginate immobilized alga. The present strain of *L. putealis* that has been isolated from within the premises of an electroplating industry seems to be best suited for removal of higher concentration of chromium (50–60 mg/L) as it has been exposed to these levels of the metal in the soil within the industrial complex for a long time. This work suggests that the present biosorbent can be more useful for the removal of chromium from effluents discharged by electroplating industry that usually have up to 50 mg/L Cr (VI). The alga immobilized into alginate beads can be used repeatedly used due to its mechanical strength in a number of cycles. Further, chromium from wastewaters can be gradually removed using a series of reactors with the immobilized cyanobacterium till prescribed limits of chromium, are achieved.

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