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Optimization of process variables for hexavalent chromium biosorption by psychrotrophic *Pseudomonas putida* SKG-1 isolate

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ABSTRACT

Hexavalent chromium, a priority pollutant, is well known for its carcinogenicity, mutagenicity, and teratogenicity in humans, animals, and plants. Biosorption is an attractive technique for the treatment of chromium pollution. This study was aimed to optimize the effect of various process parameters on biosorption of Cr^{6+} by *Pseudomonas putida* SKG-1 employing conventional one-factor-at-a-time and response surface methodology (RSM) approaches in a batch process. In conventional method, the maximum Cr^{6+} biosorption (97%) was achieved at 6.0 g dead cell biomass l^{-1}, pH 3.0, and 35°C during 195 min contact time with an initial Cr^{6+} concentration of 50 mg l^{-1} under shaking (150 rpm). Whereas in RSM approach, a slightly enhanced Cr^{6+} biosorption (98%) was obtained only in 180 min by *P. putida* cells (8.0 g l^{-1}). The correlation coefficient (R^2) of biosorption data to Cr^{6+} simulated by the Langmuir, Freundlich, and D–R isotherm models was 0.9953, 0.8482, and 0.8916, respectively. The best fit was obtained for Langmuir model which suggests the binding of chromium as a monolayer on the surface of the biomass.

Keywords: Pseudomonas putida; Biosorption; Chromium; Correlation coefficient; RSM

1. Introduction

The heavy metal laden industrial wastewater is a serious threat to the public life. This is due to the bioaccumulation of such metal species in the aquatic life, which is ultimately transferred to humans through various trophic levels. Besides variable levels of different heavy metals [1], several hazardous organic pollutants [2–4] are also released into the environment.

The tanning industry generally employs "chrome liquor" in the tanning process and discharges effluent into the environment that contains chrome salts more than their maximum permissible limits. In India, >50% of the total chromium effluent discharged originates from the leather, iron, and steel industries. Cr^{6+} is highly mobile and water soluble, whereas Cr^{3+} is relatively inert, chemically more stable and less bioavailable due to its negligible permeability through biomembranes [5]. Cr^{6+} is nearly 100 times more toxic and 1,000 times more mutagenic than Cr^{3+}. The intoxication by Cr^{6+} causes serious morbidity and mortality [6]. Therefore, a better approach is prevention or at least transforming the effluent pollutants to innocuous substances, before they are discharged. Tripathi and Garg [7] have earlier studied the Cr^{6+} tolerance limits

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of native *Bacillus* species isolated from the tannery effluent.

During the past few years, several conventional techniques such as chemical oxidation, coagulation, electrolysis, froth flotation, ion exchange, electrochemical/photochemical treatment, and reverse osmosis have been attempted for the removal of heavy metals and other organic/inorganic compounds from such effluents. But, due to operational limitations, high cost of treatment, and generation of toxic chemical sludge, some new techniques have been attempted for a long time. Among these techniques, the biosorption is considered as a relatively more viable option over the physiochemical treatment methods. This may be attributed to simple design, easy operation, low investment cost, high selectivity, high efficiency, short operation time, minimum sludge release, regeneration of biosorbents, possibility of metal recovery, etc. [6]. Biosorption is the binding concentration of adsorbate (s) from the aqueous solution by certain types of active or inactive/dead microbial biomass. Biosorption is metabolism-dependent when living biomass is employed, and metabolism-independent in the case of dead cells. In general, microbes can be isolated particularly from the native effluent, and the efficient, fast-growing organisms are specifically cultivated for biosorption purposes. Biosorption of various heavy metals by different bacteria has been studied by different researchers [1,8].

Biosorption is a complex phenomenon in which the metallic species is deposited in the solid biosorbent through various sorption processes such as ion exchange, complexation, chelation, microprecipitation, and oxidation/reduction. Biomass materials possess several molecular groups that are known to offer ion exchange sites such as carboxyl, phosphates, thioether, sulfate, phenol, sulfhydril, imadizole, amino, amide, and hydroxyl. [9]. The precise binding mechanisms may range from physical (electrostatic or van der Walls forces) to chemical (ionic and covalent) processes.

Two-parameter (Langmuir and Freundlich) models have been employed to describe biosorption isotherm. The reasons for their frequent and extensive use are: (i) simplicity and (ii) easy interpretability. Although most industrial applications prefer a continuous mode of operation, batch experiments are required to be performed for getting the desired fundamental information such as biosorbent efficiency, optimum experimental conditions, biosorption rate, concentration of biomass and concerned metal, possibility of biosorbent regeneration and recovery of metal ions. Hence, biosorption phenomenon is more or less like a chemical reaction, and thus, several parameters affect the process.

In view of above, the effect of various factors such as solution pH, temperature, dose of biosorbent, initial metal concentration, and contact time, have been attempted for the bioremediation of chromium from the Cr^{6+} laden solution by an efficient psychrotrophic *Pseudomonas putida* SKG-1 isolate.

2. Materials and methods

2.1. Bacterial culture

P. putida strain SKG-1 (MTCC 10510) used in the present study was previously isolated in our laboratory from dairy sludge [10]. The pure culture was preserved and maintained at 4°C on glucose yeast extract (GYE) agar slants containing (g l^{-1}): glucose 5.0, yeast extract 5.0, peptone 5.0, and agar 20.0.

2.2. Biosorbent preparation

The bacterial inoculum was prepared in above-mentioned GYE broth (pH 8.0). The sterilized medium (250 ml) was inoculated with a loopful of *P. putida* culture and incubated at 30°C in an incubator shaker (150 rpm) for 24 h. The cells were grown to late exponential phase, harvested by centrifugation at 10,000 rpm (4°C) for 30 min, and washed thrice with deionized water. The cell pellet was conditioned to desired pH (2–10) by repeated washings with 0.1 N H_2SO_4 or 0.1 N NaOH until the pH of washed water became constant. The pH-conditioned pellet was then dried in an oven at 80°C till constant weight, and finally crushed in pestle and mortar.

2.3. Preparation of working solution

The stock solution (10,000 mg l^{-1}) of Cr^{6+} was prepared in deionized water using potassium dichromate ($K_2Cr_2O_7$). All working concentrations were obtained by diluting the stock solution with deionized water. The pH was adjusted using 0.1 N H_2SO_4 or 0.1 N NaOH to desired values as per the following experimental design.

2.4. Metal biosorption

A sterilized working solution was prepared in distilled water containing 100 mg Cr^{6+} l^{-1}. Biosorption of Cr^{6+} by the culture was determined by batch equilibrium method. The dead cells were added (2.0 g l^{-1}) to 25 ml (in duplicate) of above working solution in 150 ml Erlenmeyer flasks, and incubated at 150 rpm on an incubator shaker as per the experimental conditions.

Samples were drawn periodically at 15 min intervals till equilibrium was attained, and centrifuged at 10,000 rpm (4˚C) for 10 min. The supernatant was used for analysis of the residual Cr^{6+} which was determined using 1,5-diphenyl carbazide method [11], extrapolated against the standard curve of $K_2Cr_2O_7$, and reported as per cent Cr^{6+} biosorption.

2.5. Optimization of various factors

2.5.1. pH

In order to study the effect of pH on Cr^{6+} biosorption, the pH-conditioned (pH 2.0–10.0) biosorbent was inoculated (2.0 g l^{-1}) into working solution (25 ml each in duplicate) of pH range 2.0–10.0. The pH of metal solution was also adjusted to desired corresponding value by addition of aqueous 0.1 N NaOH or 0.1 N H_2SO_4. The flasks were incubated at 30˚C in an incubator shaker (150 rpm) for 180 min. Samples were processed and analyzed for Cr^{6+} biosorption as described above.

2.5.2. Contact time

To evaluate Cr^{6+} biosorption with respect to contact time (15–210 min), the biosorbent (2.0 g l^{-1}) was inoculated in 25-ml working solution of optimized pH 3.0. The samples were drawn periodically each at 15 min interval up to 210 min, and processed for residual Cr^{6+} analysis.

2.5.3. Biosorbent concentration

The effect of biosorbent dose (at 2.0–10.0 g l^{-1}) was assessed by inoculating the cell pellet (25–125 mg) in 25-ml working solution (in duplicate) at optimized pH 3.0. The flasks were incubated for 195 min (optimized contact time) in an incubator shaker at 30˚C (150 rpm). Samples were processed and analyzed for residual Cr^{6+} as per the standard method.

2.5.4. Initial metal ion concentration

Varying concentration of Cr^{6+} (50, 100, 150, 200, and 250 mg l^{-1}) was added to working solution and inoculated with optimized biosorbent (6.0 g l^{-1}) and incubated in an incubator shaker (150 rpm) at 30˚C for 195 min. The residual Cr^{6+} was analyzed as above.

2.5.5. Temperature

Influence of temperature was assessed by inoculating the optimized biosorbent (6.0 g l^{-1}) to 25-ml working solution (containing optimized initial 50 mg Cr^{6+} l^{-1} concentration) of pH 3.0 and incubated at varied temperature (25, 28, 30, 32, 35, 38, and 40˚C) in an incubator shaker (150 rpm) for 195 min. The Cr^{6+} biosorption was analyzed as above.

2.6. Statistical optimization using Box–Behnken design

Based on the results from the experiments performed employing one-factor-at-a-time approach, three variables [initial Cr^{6+} (A), biomass (B) and contact time (C)], as the important factors, were chosen in the present study. The statistical optimization was performed using Box–Behnken design. The three-dimensional response surface plots were used for analyzing the interactive effect of each variable [12].

The effect of each variable on Cr^{6+} biosorption was studied at three different levels (−1, 0 and +1) with minimum, central, and maximum values (Table 1). Seventeen (17) experimental setups were obtained (Table 2). A second-order polynomial equation was used for the analysis of Cr^{6+} biosorption, and the data were fitted in the equation by multiple regression procedure. This resulted in an empirical model. The model equation for analysis is as under:

$$Y = \beta_o + \sum \beta_n X_n + \sum \beta_{nn} X_n^2 + \sum \beta_{nm} X_n X_m \quad (1)$$

where Y is the predicted response, β_o offset term, β_n liner coefficient, β_{nn} squared coefficient, β_{nm} interaction coefficient, X_n nth independent variable, X_n^2 squared effect, and X_nX_m interaction effects. The predicted values for Cr^{6+} biosorption were obtained by applying quadratic model (Design Expert software). Analysis of variance (ANOVA) was used to analyze the responses under different combinations as defined by the design (Table 3).

Table 1
Experimental range and the levels of three independent variables employed in RSM in terms of actual and coded factors

	Levels		
Variables	–1	0	+1
Cr^{6+} concentration (mg l^{-1})	50	75	100
Biomass (g l^{-1})	4	6	8
Contact time (min)	160	180	200

Table 2
Experimental designs used in RSM studies by using three independent variables showing observed and predicted values of per cent Cr^{6+} removal

	Factor 1	Factor 2	Factor 3	Dye discoloration (%)	
Std.	Initial Cr^{6+} conc. (mg l^{-1})	Biomass (g l^{-1})	Contact time (min)	Actual response	Predicted response
1	50.0	4.0	180.0	91	91.37
2	100.0	4.0	180.0	77	76.88
3	50.0	8.0	180.0	98	98.13
4	100.0	8.0	180.0	82	81.62
5	50.0	6.0	160.0	81	80.88
6	100.0	6.0	160.0	90	89.63
7	50.0	6.0	200.0	74	74.13
8	100.0	6.0	200.0	80	79.75
9	75.0	4.0	160.0	86	86.00
10	75.0	8.0	160.0	89	89.00
11	75.0	4.0	200.0	94	94.25
12	75.0	8.0	200.0	88	88.20
13	75.0	6.0	180.0	89	88.20
14	75.0	6.0	180.0	88	88.20
15	75.0	6.0	180.0	89	88.20
16	75.0	6.0	180.0	88	88.20
17	75.0	6.0	180.0	88	88.20

Table 3
ANOVA for response surface quadratic model for Cr^{6+} biosorption

Source	Sum of squares	df	Mean square	*F*-value	*p*-value
Model	1,012.92	9	112.55	508.28	<0.0001
A-initial Cr^{6+}	480.50	1	480.50	2,170.00	<0.0001
B-biomass	66.13	1	66.13	298.63	<0.0001
C-contact time	153.13	1	153.13	691.53	<0.0001
AB	1.00	1	1.00	4.52	0.0712
AC	0.000	1	0.000	0.000	1.0000
BC	0.25	1	0.25	1.13	0.3233
A^2	126.21	1	126.21	569.99	<0.0001
B^2	76.95	1	76.95	347.52	<0.0001
C^2	114.95	1	114.95	519.13	<0.0001
Residual	1.55	7	0.22		
Lack of fit	0.75	3	0.25	1.25	0.4028
Pure error	0.80	4	0.20		
Cor total	1,014.47	16			
Standard deviation	0.47		R-squared		0.9985
Mean	85.18		Adjusted R-squared		0.9965
Coefficient of variation (C.V.%)	0.55		Predicted R-squared		0.9869
PRESS	13.25		Adequate precision		90.744

2.7. Isotherm study

2.7.1. Langmuir isotherm model

$$\frac{1}{q_e} = \frac{1}{q_o} + \frac{1}{bq_oC_e} \quad (2)$$

where q_e is the amount of adsorbate adsorbed (mol g^{-1}), C_e is the equilibrium molar concentration of the dye (mol l^{-1}), q_o is the maximum adsorption capacity over unit mass of the adsorbent (mol g^{-1}), and b is the energy of adsorption (l mol^{-1}).

2.7.2. Freundlich isotherm model

$$\log q_e = \log K_F + (1/n) \log C_e \quad (3)$$

Here, C_e and q_e are same as described above, while K_F and n are the Freundlich constants related to the adsorption capacity and adsorption intensity of the adsorbate–adsorbent system, respectively.

2.7.3. Dubinin–Radushkevich (D–R) isotherm model

$$\ln C_{ads} = \ln X_m - \beta\epsilon^2 \quad (4)$$

where C_{ads} is the amount of dye adsorbed per unit weight of adsorbent (mol g^{-1}), X_m is the maximum adsorption capacity (mol g^{-1}), β is the activity coefficient ($mol^2\ J^{-2}$) related to mean adsorption energy, and ϵ is the Polanyi potential, which is given as:

$$\epsilon = RT \ln\left(1 + \frac{1}{C_e}\right) \quad (5)$$

where R is the universal gas constant (J mol^{-1} K^{-1}), T is the temperature (K), and C_e is the concentration at equilibrium (mol l^{-1}). Polanyi sorption potential (ϵ) is the work required to remove a molecule to infinity from its location in the sorption space, independent of temperature. This model assumes the heterogeneity of sorption energies within this space. The slopes of straight lines of graphs between ln C_{ads} against ϵ^2 give activity coefficient and intercept yields adsorption capacity. The applicability of the isotherm is related with determination of the nature of the adsorption process and mean sorption energy is the decisive factor for distinguishing between chemical and physical adsorption, which is given as:

$$E = \frac{1}{\sqrt{-2\beta}} \quad (6)$$

It has been postulated that in any adsorbate–adsorbent system, when mean sorption energy "E" estimated by above expression is less than 8 kJ mol^{-1}, physiosorption dominates the sorption mechanism, whereas if "E" is between 8 and 16 kJ mol^{-1}, chemisorption is the governing factor of the process. Thus, the calculated values of E play a significant role in deciding the operative nature of the ongoing adsorption.

3. Results and discussion

3.1. Effect of pH

Hexavalent chromium removal was studied in the solutions of pH ranging 2.0–10.0 at an initial Cr^{6+} concentration of 100 mg l^{-1}. Fig. 1 reveals maximum 71% Cr^{6+} removal at pH 3.0 by *P. putida* biomass. Any increase or decrease in initial pH from optimum reduced the extent of Cr^{6+} removal. Relatively higher adsorption at low pH can be explained by the type of Cr species present and the adsorbent surface characteristics. As such, the bacterial cell walls are negatively charged under acidic pH conditions, and the cell wall chemical functional groups display a high affinity for metal ions in solution [13]. The surface of adsorbent becomes highly protonated, and favors the uptake of Cr^{6+} in anionic form under acidic conditions. As the pH increases, the net positive charge on the surface of sorbent decreases due to reduced protonation, which ultimately leads to reduced sorption capacity. Furthermore, there is competition between hydroxyl and chromate ions for binding, as the former being dominant species at higher pH values.

The hydrogen ion concentration is one of the most important physical parameters that influences the biosorption process [14]. The pH dependence of metal adsorption can largely be related to type as well as the ionic state of the functional group(s) on the biosorbent, and the type of metal species present in the solution. Chromium^{6+} is present as dichromate ($Cr_2O_7^{2-}$) in acidic environment (pH range 3.0–6.0) and as chromate ($Cr_2O_4^-$) in alkaline environment at above pH 8.0 [6].

3.2. Effect of contact time

Contact time of biomass with metal ions is relevant for the economic viability of a process to be employed for industrial purposes. Biosorption is a rapid phenomenon, where equilibrium is attained within few hours under optimum conditions. In the present study, the effect of contact time on biosorption of Cr^{6+} was performed at optimized pH 3.0 during 15–210 min incubation, and the results are presented in Fig. 2. The process attained equilibrium at 195 min, followed by almost constancy up to 210 min contact time and achieved maximum 73% Cr^{6+} removal.

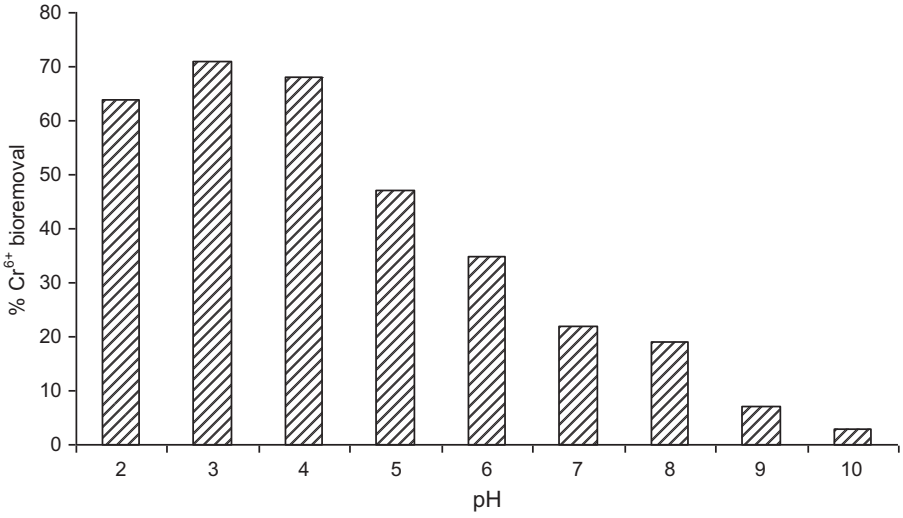

Fig. 1. Effect of pH (2.0–10.0) on Cr^{6+} biosorption by *P. putida* SKG-1 with 2 g biosorbent l^{-1}, initial Cr^{6+} 100 mg l^{-1}, 30°C and 150 rpm for 180 min.

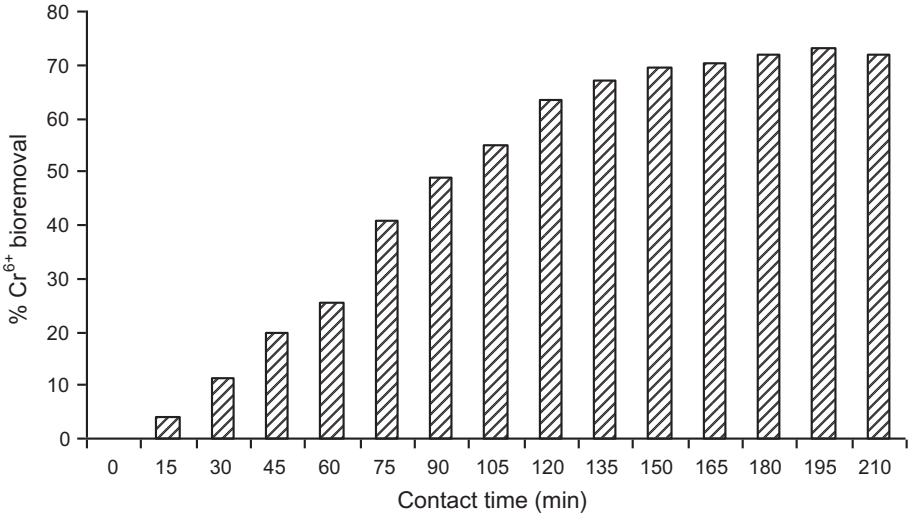

Fig. 2. Effect of contact time (15–210 min) on Cr^{6+} biosorption by *P. putida* SKG-1 at optimized pH 3.0 with 2 g biosorbent l^{-1}, initial Cr^{6+} 100 mg l^{-1}, 30°C and 150 rpm.

3.3. Effect of biomass dose and initial metal ion concentration

The initial Cr^{6+} and biosorbent concentrations are considered important factors for effective biosorption process. In order to work out the optimum biosorbent dose, varying biosorbent concentrations in the range of 2.0–10.0 g l^{-1} were taken in the presence of initial 100 mg Cr^{6+} l^{-1}, and the results are depicted in Fig. 3. The optimum biosorbent dose was 6.0 g l^{-1} for maximum (79.5%) Cr^{6+} adsorption. At optimum level, sufficient binding sites were available on the biomass for electrostatic interaction with the Cr species; whereas, at higher than optimum biosorbent concentration, the metal uptake decreased. This was attributed to excessive free extra binding sites still available on biosorbent which might have decreased the electrostatic interaction between metal and binding sites of the biosorbent [15].

Adsorption experiments at varying initial Cr^{6+} concentrations (50–250 mg l^{-1}) were performed with optimized pH 3.0 and fixed dose (6.0 g l^{-1}) of bacterial biomass. The results depicted in Fig. 4 indicate that

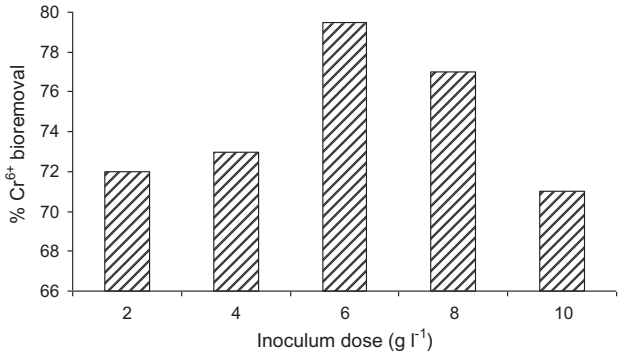

Fig. 3. Cr^{6+} biosorption at different concentrations (2.0–10.0 g l^{-1}) of biosorbent at optimized pH 3.0 and contact time 195 min with initial Cr^{6+} 100 mg l^{-1}, 30°C and 150 rpm.

Cr^{6+} removal from the solution decreased with an increase in the initial Cr^{6+} concentration. The Cr^{6+} removal ranged from 95 to 27% at initial 50–250 mg l^{-1} Cr^{6+} concentration (Fig. 3). Our findings are in accordance with Quintelas et al. [16] who reported decreased Cr^{6+} removal at higher initial concentrations, and this was possibly due to chromate (Cr_20_4) toxicity and high oxidative potential that might have inhibited the biological activity of *Arthobacter viscosus* biofilm. Further, at higher Cr^{6+} concentrations, the average distance between adsorbing species is reduced, which affects the charge distribution of its neighbors, thereby altering the ability of species to migrate to the biomass surface, resulting in reduced adsorption [17]. At lower concentrations (<250 mg Cr^{6+} l^{-1}), metal ions present in the solution possibly interacted with the binding sites, and thus facilitated more Cr^{6+} removal. Therefore, it is very essential to know the optimum ratio of biomass to metal concentration; the increase in metal uptake was evident as long as free binding sites on the biomass were available [18]. The biosorption results reveal that Cr^{6+} removal by biomass was chemically equilibrated and involved saturable mechanism (Figs. 3 and 4).

3.4. Effect of temperature

The results in Fig. 5 reveal that isolate offers a broad range of temperature from 5 to 40°C for Cr^{6+} biosorption. With an increase in the temperature from 5 to 35°C, there was concomitant marginal increase in Cr^{6+} biosorption ranging 83.0–97.0% by *P. putida* biomass at optimized initial 50 mg l^{-1} concentration. Further increase in temperature to 40°C caused slight decrease in the extent of Cr^{6+} removal from the solution. Therefore, maximum Cr^{6+} removal (97%) was achieved at optimum 35°C (Fig. 5). However, Cr^{6+} biosorption performance was good at lower temperatures also, i.e. 83.0, 84.5, 86.0, 87% at 5, 10, 15, and 20°C, respectively.

It may be inferred from the results that fairly good efficiency of Cr^{6+} removal was achieved throughout the temperature range under study. Our findings are

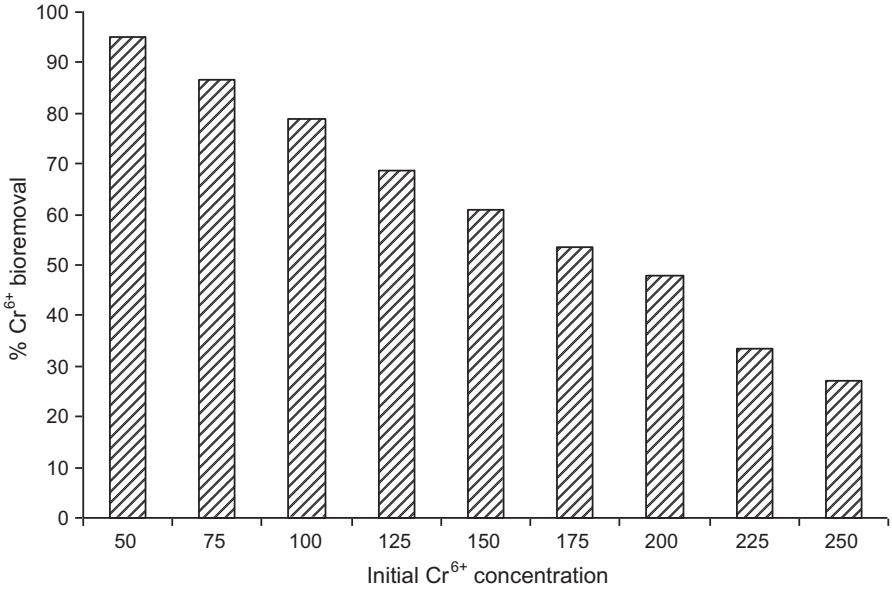

Fig. 4. Effect of initial Cr^{6+} concentrations (50–250 mg l^{-1}) on Cr^{6+} biosorption by *P. putida* SKG-1 at optimized pH 3.0, 6 g biosorbent $l^{-1}$and 195 min contact time with initial Cr^{6+} 100 mg l^{-1}, 30°C and 150 rpm.

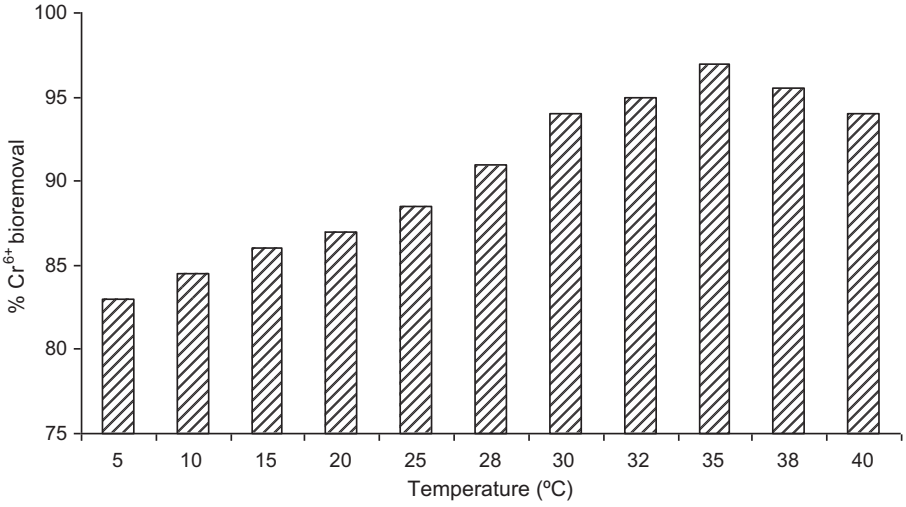

Fig. 5. Influence of temperature (5–40˚C) on Cr^{6+} biosorption by *P. putida* SKG-1 at optimized pH 3.0, 6 g biosorbent $l^{-1}$and 195 min contact time, initial Cr^{6+} 50 mg l^{-1} with 150 rpm.

in agreement with Srinath [19] who observed that the biosorption of Cr^{6+} by *Bacillus coagulans* was less affected by temperature, and had a broad optimal range of 20–40˚C for the purpose.

The biosorption mechanism is metabolism independent, and appears to be little affected by changes in certain temperature range; however, this cannot be generalized for all the biosorbents and metals. Such a situation is evident when the chemical adsorption plays a dominant role [20]. The enhancement in metal sorption with increase in temperature increase can be attributed to elevation in energy level of the system that facilitates metal attachment to the cell surface. Further increase in temperature from optimum causes decrease in sorption possibly due to distortion of some chemical sites on the cell surface available for metal adsorption [21]. The flexibility in temperature range for Cr^{6+} biosorption makes the process suitable to various geographical locations. Further, the scope of process application is broadened for various industrial sectors which are facing the problem of safe disposal of Cr^{6+} laden effluent. Our results reveal that the adsorption sites present on *P. putida* biosorbent are sufficiently stable, and therefore offer better efficiency for Cr^{6+} interaction.

3.5. Statistical response surface methodology (RSM) optimization for Cr^{6+} biosorption

This is the first report on statistical optimization for Cr^{6+} biosorption by *P. putida* SKG-1 strain. Interactive effects of the important conventionally optimized factors, viz., initial Cr^{6+} concentration, biomass and contact time were examined using Box–Behnken design. The ANOVA yielded the following regression equation in terms of Cr^{6+} biosorption (Y) as a function of initial Cr^{6+} concentration (A), biomass (B), and contact time (C):

$$Y = 88.20 - 7.75 \times A + 2.88 \times B + 4.38 \times C - 0.50 \times AB + 0.00 \times AC - 0.25 \times BC - 5.48 \times A^2 + 4.27 \times B^2 - 5.22 \times C^2 \quad (7)$$

Table 2 shows the predicted responses of Box–Behnken design on the basis of above polynomial equation. This regression equation was assessed statistically for the ANOVA, and the results are presented in Table 3. ANOVA of regression model for Cr^{6+} biosorption demonstrated the correlation coefficient (R^2) 0.9985, which means 99.85% variability in the response could be explained by this model. The R^2 value is always between 0 and 1.0. The model is stronger and predicts better response when R^2 value is closer to 1.0 [22].

The adequate precision values of 90.744 measure signal-to-noise-ratio, and a ratio >4.0 is desirable. In this case, higher ratio indicates an adequate signal, and also proves that model can be used to navigate the design space. The *F*-value of 508.28 (Table 3) implies that the model is significant. From ANOVA analysis, lower value of the coefficient of variation (C.V. 0.55% for Cr^{6+} biosorption) indicates a better precision and reliability of the experiments performed. The C.V. as the ratio of the standard error of estimated to the mean value of the observed response

(as a percentage) is a measure of reproducibility of the model. As a general rule, a model can be considered reasonably reproducible if its C.V. is not greater than 10% [23].

Response surface curves for the variation in Cr^{6+} biosorption were constructed and are depicted in Fig. 6. In each set, two variables varied within their experimental range, while third variable remained constant at zero level. Fig. 6(a) depicts Cr^{6+} biosorption with respect to biomass vs. Cr^{6+} concentration. The curve indicates that Cr^{6+} biosorption decreased with the increased levels of metal concentration ranging from 50 to 100 mg l^{-1}. Fig. 6(b) depicts Cr^{6+} biosorption with respect to contact time vs. initial Cr^{6+} concentration, and reveal that increasing metal levels were inhibitory for Cr^{6+} biosorption. Fig. 6(c) shows the maximum 98% Cr^{6+} biosorption with optimal 50 mg Cr^{6+} l^{-1} concentration and 8.0 g l^{-1} biomass dose during 180 min incubation. This accorded a run number of 3, which is considered as the optimal condition of test variables (Table 2).

With RSM, the interactions of possible influencing parameters on treatment ability can be evaluated with a limited number of experiments. Whereas in conventional approach, optimization is usually carried out by varying one-factor-at-a-time, while keeping all other factors constant under specific set of conditions. The conventional approach is time-consuming, because the optimization is performed by varying one-factor-at-a-time, while keeping all other variables constant. Further, this approach is usually incapable of getting the true optimum value due to ignoring the interaction among variables. On the other hand, the interaction of best influencing variables can be evaluated with a limited number of experiments in RSM approach.

3.6. Biosorption isotherms

The batch study data were obtained from adsorption isotherm models, namely Langmuir, Freundlich, and Dubinin–Radushkevich isotherms. The process of adsorption is from one phase to the surface of another in a specific system. This leads to a thermodynamically defined distribution of that substance between two phases as soon as the system reaches at equilibrium. This distribution can be expressed in terms of adsorption isotherms, whereby the metal species is sequestered by the biosorbent (*P. putida* biomass). Langmuir and Freundlich models are widely employed in the equilibrium analysis in order to understand the sorption mechanisms. In this study, the adsorption isotherms were experimentally

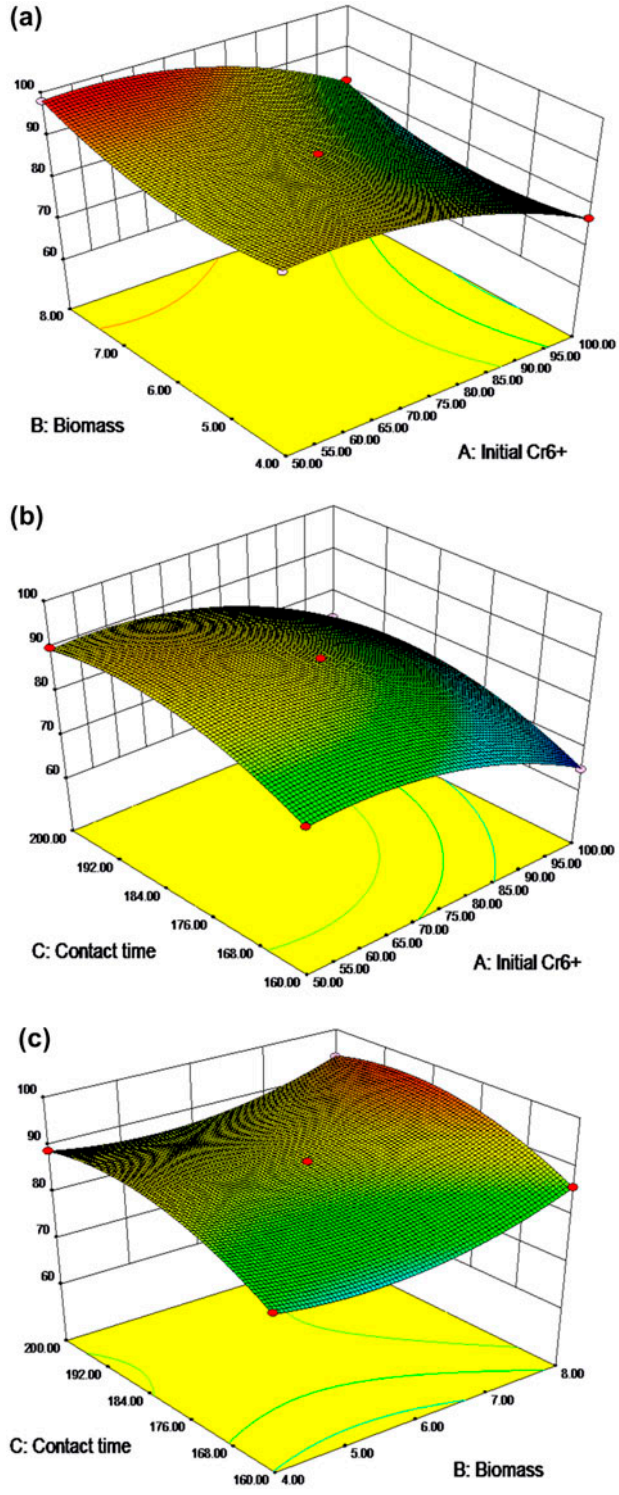

Fig. 6. Response surface curves showing interactive effects of (a) biomass and initial Cr^{6+}, (b) contact time and initial Cr^{6+}, and (c) contact time and biomass, on Cr^{6+} biosorption by *P. putida* SKG-1 biomass.

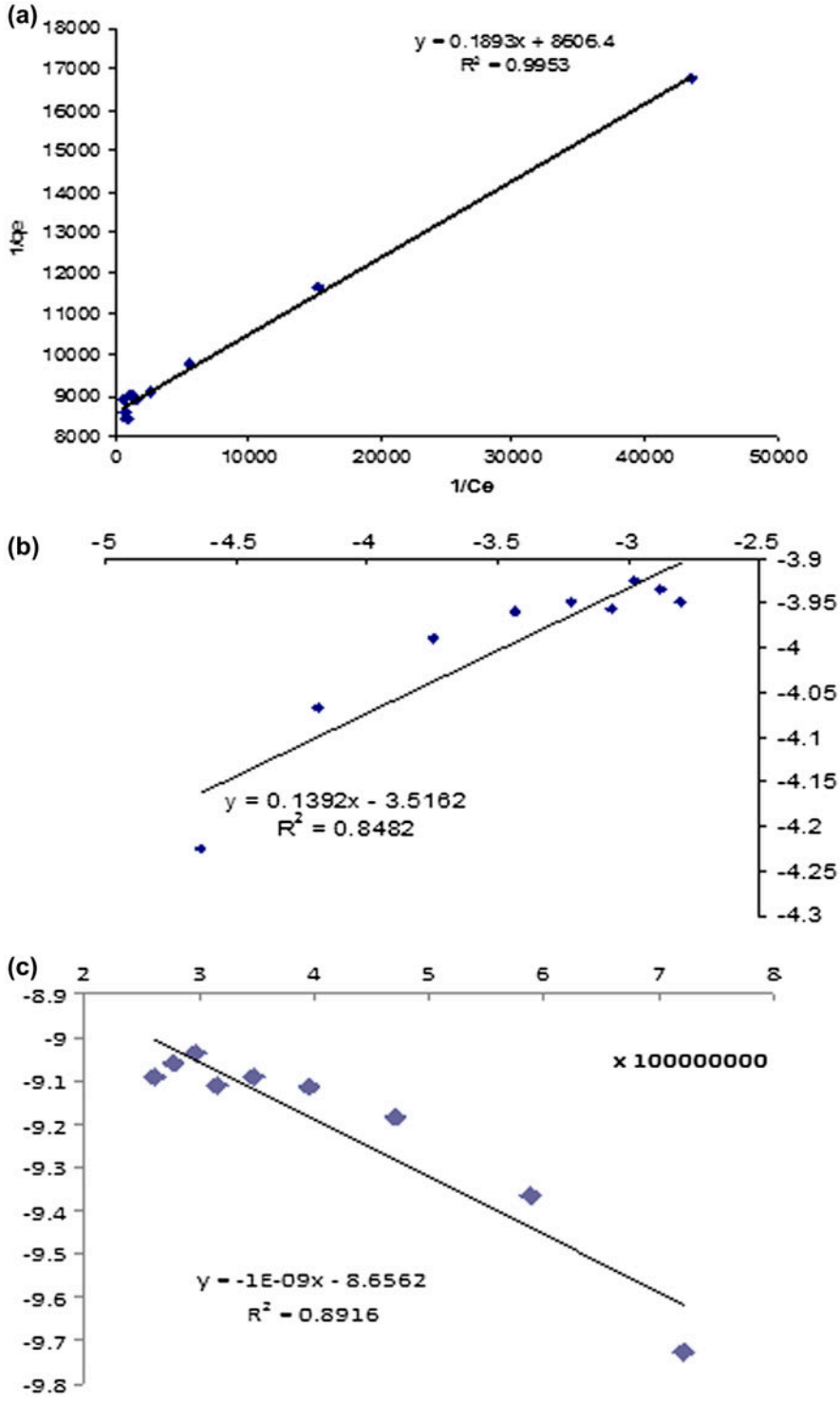

Fig. 7. Cr^{6+} biosorption models (a) Langmuir, (b) Freundlich, and (c) Dubinin–Radushkevich isotherms.

determined for the biosorbent used, and the results are depicted in Fig. 7. The above three models were fitted and constants calculated (Table 4). In Freundlich isotherm model, the adsorption characteristic is defined by both K_F and "n" values, where K_F represents the adsorption coefficient and "n" is related to the effect of metal ion concentrations. In our study, the value of "n" is 7.194245 (Table 4). A favorable

Table 4
Comparative isotherm constants of Cr^{6+} biosorption

Langmuir constants	Slope	Intercept	Q_0	b
	0.189	8.61E+03	1.09E–04	4.55E+04
Freundlich constants	Slope	Intercept	n	K_F
	0.139	–3.516	7.194245	3.05E–04
Dubinin–Radushkevich (D–R) constants	Slope	Intercept	X_m	E (kJ)
	–1.00E–09	–8.656	0.000174	2.24E+01
				$E > 16$

adsorption tends to have the constant "n" between 1 and 10. A larger value of "n" implies stronger interaction between biosorbent and heavy metal.

Table 4 further reveals that the correlation coefficient (R^2) of biosorption data to Cr^{6+} simulated by the Langmuir, Freundlich, and D–R isotherm models are 0.9953, 0.8482, and 0.8916, respectively. For *P. putida* biomass, the best fit is obtained for Langmuir model; whereas, the worst fit is noted for Freundlich and Dubinin–Radushkevich models. This suggests that the binding of chromium occurs as a monolayer. The Langmuir model suggests that the binding/sorption of Cr occurs as a monolayer on the biomass surface, and supposes that all the active sites on the sorbent surface have the same affinity with the sorbate. The Freundlich isotherm is an empirical equation, which assumes a heterogeneous biosorption system with different active sites.

4. Conclusions

This is the first report on optimization of Cr^{6+} biosorption variables using conventional one-factor-at-a-time and RSM approaches by a psychrotrophic *P. putida* SKG-1 isolate. Efficient bacterial biosorption of Cr^{6+} under wide range of environmental conditions makes the process eco-friendly and applicable to broad geological locations. Isothermal equilibrium data analyzed with Langmuir, Freundlich, and D–R isotherm models reveal that Cr^{6+} is considerably adsorbed on bacterial biomass and would be a suitable method for this metal removal from the aqueous solution.

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