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Response surface methodology approach for optimization of biosorption process for removal of Cr (VI), Ni (II) and Zn (II) ions by immobilized bacterial biomass sp. *Bacillus brevis*

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ABSTRACT

In this study, the Box–Behnken design matrix and response surface methodology (RSM) have been applied to design the experiments to evaluate the interactive effects of three most important operating variables: pH (2.0–6.0), temperature (25–40 °C) and initial concentration of metal ions (10.0–60.0 mg/L) on biosorption of Cr (VI), Ni (II) and Zn (II) ions with immobilized bacterial strain *Bacillus brevis*. The total 17 experiments were conducted in the present study towards the construction of a quadratic model. Independent variables have significant value 0.0001 which indicates the importance of these variables in the biosorption process. Values of “Prob > *F*” less than 0.0500 indicate that model terms are significant for the biosorption of Cr (VI), Ni (II) and Zn (II) ions. The regression equation coefficients were calculated and the data fitted to a second-order polynomial equation for removal of Cr (VI), Ni (II) and Zn (II) ions with immobilized bacterial strains *B. brevis*.

1. Introduction

The presence of toxic metals contaminated aqueous streams arising from the discharge of untreated metal containing effluent into water bodies is one of the most important environmental issues. The heavy metals such as lead, copper, cadmium, zinc and nickel are among the most common which derives from electroplating, plastics manufacturing, fertilizers, pigments, mining and metallurgical processes [1]. The imposition of strict regulations increased the demand for innovative treatment technologies to remove metals from wastewater and to attain today's toxicity-driven concentration limits. The search for new and innovative treatment technologies has focused the attention on the metal binding capacities of microorganisms such as bacteria, yeast and algae, etc. Biosorption has been recommended as cheaper and more effective technique. In biosorption, either live or dead microorganisms or their derivatives are used, which bind metal ions through the functioning of ligands or functional groups located on outer surface of cell [2,3]. Algae, bacteria, fungi and yeast have been proved to be potential metal biosorbents [4–6]. Commercial application of this microbial biomass as a biosorbent, however, has been hindered by problems associated with physical characteristics such as small particle size, low density, poor mechanical strength, rigidity and solid–liquid separation [7,8]. Immobilization of biomass within a suitable matrix can overcome these problems by offering ideal size, mechanical strength, rigidity and porous characteristics to the biological materials and provide greater opportunity for reuse and recovery [9,10]. Different techniques like flocculation, covalent binding to carriers, encapsulation in polymer gel and entrapment in polymer matrix are available for immobilization. Both living and dead biomass can be immobilized [11]. Immobilization of cells has also permitted the successful use of the reactor designs and can be used to overcome limitations such as porosity in static beds, biomass retention in expended, fluidized and attrition of the biosorbent; most importantly it allows simple separation of biomass from aqueous phase. Whereas, biosorption efficiency depends upon many factors, pH, temperature and metal ions concentration that affect the biosorption of metals ions. The variability of these factors in real wastewater makes it necessary to know how they influence biosorption performance. As a consequence of these possible multiple interactions, the comprehension of biosorption phenomena is very complex and requires a study of both the solution chemistry of metal ions depending pH, anions and/or ligands in solution and the mechanisms of metal uptake such as ion exchange, complexation, micro-precipitation, etc. The influence of parameters should be investigated to achieve the optimum experimental conditions for the biosorption of Cr (VI), Ni (II) and Zn (II) from aqueous solution. Treating each factor separately would be very time consuming; furthermore, if several factors play a role, their interactions would not be discernable even if they were dominated. Response surface

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methodology (RSM) is essentially a particular set of mathematical and statistical methods for experimental design and evaluating the effects of variables and searching optimum conditions of variables to predict targeted responses [12–14]. Its greatest applications have been in industrial research, particularly in situations where a large number of variables influencing the system feature. The RSM has already been successfully applied in other fields, i.e. food processing, biochemical engineering and adsorption processes for optimization [15–19]. Few researchers have been done work on biosorption process optimization using RSM [11,20,21]. There is no published report on optimization of biosorption process for removal of heavy metals i.e. Cr (VI), Ni (II) and Zn (II) with bacterial sp. *Bacillus brevis* using response surface methodology. It is well suited approach to the study the main and interactive effects of distinct variable and optimization of the process. Response surface methodology was applied to the Box and Behnken experimental design [18]. The Box–Behnken model was based on statistical evaluation of the following tests: root-mean-square error (RMSE), bias index and accuracy factor, and the lack-of-fit test. The Box–Behnken design minimizes the number of factor combinations and maintains good precision of the predicted response [22–24]. In this study, Box–Behnken design matrix have been used for the optimization of biosorption processes for removal of heavy metals using immobilized bacterial biomass sp. *B. brevis*. For this three most important operating variables: pH, temperature and initial concentration of metal ions were optimized and evaluated the single and interactive effects of the variables in the process for removal of heavy metals, such as Cr (VI), Ni (II) and Zn (II) ions from aqueous solution.

2. Materials and methods

2.1. Collection and analysis of soil and wastewater samples

Soil and wastewater samples were collected from Luxmi Precision Screw Ltd., Rohtak district, Haryana, India in the month of October 2006. Rohtak district is located in southeastern part of Haryana state. The climate of the study area is semi-arid with extreme temperature conditions in summer and winter. Summer spans over April to July and October have moderate temperature conditions. Average rainfall pattern is 458.5 mm. The soil samples were collected in plastic bags and stored at 4 °C. The industry mainly used chromium, nickel and zinc ions for metal plating. In the soil samples, concentration of Cr (VI), Ni (II) and Zn (II) ions was observed approximately 50 mg/L, 35 mg/L and 28 mg/L respectively. The pH of the soil samples was measured within 2 h of samples collection and found between 6.5 and 7.0. Physico-chemical parameters of electroplating wastewater samples were analyzed and results have already been published [6].

2.2. Source/genesis of bacterial strain

The bacterial strain *B. brevis* was isolated from electroplating industrial soil. The bacterial strain was isolated on nutrient agar medium comprising (g/L): bacteriological peptone, 5.0; sodium chloride, 8.0; beef extract, 3.0, agar, 2% and pH 7.0. Pure bacterial culture was obtained by repeat streaking on basal agar medium using standard isolation techniques. For isolation, inoculated plates were incubated at 37 °C for 72 h. The pure colony was obtained and identified from microbial type culture collection (MTCC), Institute of Microbial Technology, Chandigarh. Morphological, physiology and biochemical characteristics of the bacterial sp. *B. brevis* were shown in Table 1.

2.3. Preparation of bacterial biosorbents

For biosorption study, bacterial strain *B. brevis* was cultivated aerobically in 250 mL Erlenmeyer flask containing sterile nutrient broth on a rotary shaker 130 rpm at 37 °C. Cells were harvested at the end of exponential phase. After cultivation, the cells were centrifuged at 10,000 × *g* for 20 min and then washed twice with deionized water and finally freeze-dried. The dried biomass was powered and stored at 4 °C and used for the biosorption experiments.

2.4. Immobilization of biomass

The powdered biomass sp. *B. brevis* was immobilized by entrapment in polymer matrix of Na-alginate. 2% (w/v) slurry of sodium alginate was prepared in distilled water. After cooling, 5% (w/v) of biomass was added and stirred on magnetic stirrer. The alginate biomass slurry was introduced into 0.1 M $CaCl_2{\cdot}2H_2O$ for polymerization and bead formation using 5 mL syringe. The resultant beads were of ~4-mm diameter. The biomass entrapped beads were cured in this solution for 1 h and then washed twice with 200 mL of sterile distilled water [17,25].

2.5. Establishment of response surface methodology

The Box–Behnken design model, which is the standard RSM, was established on the basis Design Expert software (Stat Ease, 6.0 trial version) for the optimization of biosorption process. The experimental design, three independent variables, i.e. pH (2.0–6.0), temperature (20–40 °C) and initial concentration (10–60 mg/L) of ions were taken to obtained response biosorption of Cr (VI), Ni (II) and Zn (II) ions. The experimental design was applied after selection range of each variable (maximum and the minimum) as shown in Table 2. The Box–Behnken design contained a total of 17 experiments with the first 12 experiments organized in a factorial design with the experimental trials from 13 to 17 involving the replication of the central point. Repeated observations at the center point were used to estimate the experimental error employed. All the biosorption experiments were conducted in 250 mL Erlenmeyer flasks on a rotary shaker (at 125 rpm) and then the filtrate was analyzed for residual Cr (VI) concentration using a spectrophotometer

Table 1
Morphological, physiology and biochemical characteristics of the bacterial sp. *Bacillus brevis*

Morphological characteristics	Colony	Cream, round with rough surface
	Gram reaction	+
	Motility	Motile
	Cell shapes	Long rods
Physiology characteristics	UV fluorescence	–
	Growth temperature	25–45 °C
	pH	6.8–9.0
	Growth on NaCl (%)	–
Biochemical characteristics	MacConkey agar	–
	Indole	–
	Methyl red	–
	Citrate utilization	–
	Casein hydrolysis	–
	Gelatin	+
	Starch	+
	Urea	–
	Nitrate Reduction	+
	Oxidase	–
	Sorbitol	+
	Xylose	–

Table 2
The experimental domain factor and level for the Box–Behnken design

Code	Name of factor	Range and levels (coded)		
		−1	0	+1
A	pH	2.0	4.0	6.0
B	Temperature (°C)	25.0	32.5	40.0
C	Initial concentration (mg/L)	10.0	35.0	60.0

(ELICO SL, 150) at 540 nm and Zn (II) and Ni (II) concentration using atomic absorption spectrophotometer (GBC-932 Plus). The obtained response and run experiments were shown in Table 3. Biosorption efficiency was calculated using following equation:

$$\%\,\text{Efficiency} = \frac{C_0 - C_e}{C_0} \times 100 \tag{1}$$

where C_0 is the initial concentration of metal ion (mg/L) and C_e is the concentration of heavy metal ions at equilibrium (mg/L).

2.6. Statistical analysis

The quadratic equation model for predicting the optimal point was expressed according to Eq. (2). Three parameters varied, 10 coefficients has to be estimated, i.e. coefficients for the three main effects, three quadratic effects, three interactions and one constant [26]

$$Y_i = a_0 + \sum a_i X_i + \sum a_{ii} X_{ii}^2 + \sum a_{ij} X_i X_j + e \tag{2}$$

where Y_i ($i = 3$) is the predicted response, i.e. biosorption of Cr (VI), Ni (II) and Zn (II) ions with bacterial biomass, a_0 is the constant coefficient, a_i is the ith linear coefficient, a_{ii} is the ith quadratic coefficient and a_{ij} is the different interaction coefficients of the model; $X_i X_j$ are the coded independent variables related to the factors, and e is the error of model. However, in this study, the independent variables were coded as *A*, *B* and *C*. Thus, the second-order polynomial equation can be presented as follows:

$$\begin{aligned} Y\,(\%\,\text{Removal of metal ions}) = {} & a_0 + a_i A + a_i B + a_i C + a_i A^2 + a_i B^2 \\ & + a_i C^2 + a_{ij} A * B + a_{ij} A * C + a_{ij} B * C \end{aligned} \tag{3}$$

In this study, the removal of Cr (VI), Ni (II) and Zn (II) was processed for Eq. (3) including ANOVA to obtain interaction between process variables and response. The quality-of-fit of polynomial model was expressed by the coefficient of determination r^2 and statically significance was checked by *F*-test in the programme. The residual error, pure error and lack-of-fit were calculated from the repeated measurements [17]. The desirability was selected as maximum removal of heavy metal ions at optimum pH, temperature and initial concentration of metal ions. To visualize the relationship between responses and experimental levels for each of the factors, the fitted polynomial equation was expressed as surface plots.

3. Results and discussion

The Box–Behnken design matrix and corresponding results of RSM experiments to determine the effect of variable: pH (2.0–6.0), temperature (25–40 °C) and initial concentration of metal ions (10.0–60.0 mg/L) on response, i.e. Y_1 biosorption of Cr (VI), Y_2 biosorption of Ni (II) and Y_3 biosorption of Zn (II) with immobilized bacterial strain *B. brevis* are given in Table 3.

Table 3
The Box–Behnken design matrix for experimental design, observed and predicted responses for removal of Cr (VI), Ni (II) and Zn (II) ions with immobilized bacterial strains *B. brevis*

Response (% removal)									
Experiments run	pH (*A*)	Temperature (°C) (*B*)	Initial concentration (mg/L) (*C*)	Cr (VI) observed (Y_1)	Cr (VI) predicted (Y_1)	Ni (II) observed (Y_2)	Ni (II) predicted (Y_2)	Zn (II) observed (Y_3)	Zn (II) Predicted (Y_3)
1	2.0	25.0	35.0	70.01	70.19	22.5	22.571	30.37	30.46
2	6.0	25.0	35.0	25.3	25.29	63.035	63.0275	27.5	27.53
3	2.0	40.0	35.0	77.24	77.24	46.95	46.9575	42.25	42.22
4	6.0	40.0	35.0	38.71	38.52	75.209	75.138	35.30	35.20
5	2.0	32.5	10.0	67.75	67.66	29.65	29.582	34.67	34.65
6	6.0	32.5	10.0	26.4	26.50	52.34	52.3505	28.56	28.60
7	2.0	32.5	60.0	58.00	57.90	15.8	15.7895	29.12	29.08
8	6.0	32.5	60.0	15.35	15.43	61.59	61.658	25.17	25.19
9	4.0	25.0	10.0	33.25	33.15	25.72	25.717	48.36	48.29
10	4.0	40.0	10.0	41.8	41.87	54.47	54.5305	61.13	61.18
11	4.0	25.0	60.0	21.4	21.32	34.1	34.0395	47.02	46.97
12	4.0	40.0	60.0	32.8	32.88	41.72	41.723	53.44	53.51
13	4.0	32.5	35.0	53.4	53.48	56.53	56.542	71.5	71.54
14	4.0	32.5	35.0	53.5	53.48	56.5	56.542	71.6	71.54
15	4.0	32.5	35.0	53.4	53.48	56.6	56.542	71.6	71.54
16	4.0	32.5	35.0	53.6	53.48	56.55	56.542	71.5	71.54
17	4.0	32.5	35.0	53.5	53.48	56.53	56.542	71.5	71.54

Table 4
Analysis of variance for RSM variables fitted to quadratic model

Metal ions	Source	Sum of squares	d.f.	Mean square	*F*-value	*P*-value	Prob > *F*
Cr (VI)	Model	5211.477	9	579.053	25258.47	0.0001	Significant
	Residual	0.160476	7	0.022925			
	Lack-of-fit	0.132476	3	0.044159	6.308369	0.0536	Not significant
	Pure error	0.028	4	0.007			
	r^2	0.9996					
	r^2_{adj}	0.9999					
Ni (II)	Model	4324.402	9	480.4891	103548.9	<0.0001	Significant
	Residual	0.032482	7	0.00464			
	Lack-of-fit	0.027002	3	0.009001	6.569708	0.0503	Not significant
	Pure error	0.00548	4	0.00137			
	r^2	0.9999					
	r^2_{adj}	1.0000					
Zn (II)	Model	5331.368	9	592.3742	85346.41	<0.0001	Significant
	Residual	0.048586	7	0.006941			
	Lack-of-fit	0.036586	3	0.012195	4.065083	0.1045	Not significant
	Pure error	0.012	4	0.003			
	r^2	1.0000					
	r^2_{adj}	1.0000					

d.f. = degree of freedom.

3.1. Validation of response surface models and statistical analysis

The relationship between independent variables and response was drawn by second-order polynomial equations. The regression equation coefficients were calculated and data was fitted to a second-order polynomial equation for removal of Cr (VI), Ni (II) and Zn (II) ions with immobilized bacterial strains *B. brevis*

$$\begin{aligned}\%\,\text{Removal of Cr (VI)}\ Y_1 = {} & +53.48 - 20.91 \ast A + 5.07 \ast B - 5.21 \ast C \\ & +1.54 \ast A \ast B - 0.33 \ast A \ast C + 0.71 \ast B \ast C \\ & +4.45 \ast A^2 - 5.11 \ast B^2 - 16.05 \ast C^2 \qquad (4)\end{aligned}$$

$$\begin{aligned}\%\,\text{Removal of Ni (II)}\ Y_2 = {} & +56.54 + 17.16 \ast A + 9.12 \ast B - 1.12 \ast C \\ & -3.07 \ast A \ast B + 5.77 \ast A \ast C5.28 \ast B \ast C \\ & -1.89 \ast A^2 - 2.73 \ast B^2 - 14.81 \ast C^2 \qquad (5)\end{aligned}$$

$$\begin{aligned}\%\,\text{Removal of Zn (II)}\ Y_3 = {} & +71.54 - 2.49 \ast A + 4.86 \ast B - 2.25 \ast C \\ & -1.02 \ast A \ast B + 0.54 \ast A \ast C - 1.59 \ast B \ast C \\ & -30.40 \ast A^2 - 7.29 \ast B^2 - 11.76 \ast C^2 \qquad (6)\end{aligned}$$

Statistical testing of the model was performed with *F*-test to obtain the mathematical relationship between response and process variables. In order to ensure a good model, the test for significance of regression model was performed and applying the analysis of variance (ANOVA) for biosorption of Cr (VI), Ni (II) and Zn (II) ions with immobilized bacterial biomass. Table 4 showed the results of ANOVA for removal of Cr (VI), Ni (II) and Zn (II) ions. Values of Prob > *F* less than 0.0500 indicate model terms are significant for biosorption of Cr (VI), Ni (II) and Zn (II) ions. The non-significant lack-of-fit (more than 0.05) showed that quardratic model is valid for present study. Non-significant lack-of-fit is good for data fitness in the model. The predicted r^2 0.9996 and adjusted r^2 of 0.9999 for Cr (VI), predicted r^2 0.9999 and adjusted r^2 1.0000 for Ni (II) and predicted r^2 1.0000 and adjusted r^2 1.0000 for Zn (II) is reasonable agreement with the value of r^2, which is closer to 1.0, indicates the better fitness of model in the experimental data. High value of parameter estimate for variables A, B, C, AB, AC, BC, A^2, B^2, C^2 showing a high level of significance indicates the importance of these variables in the biosorption process. The variables A (pH) and C (initial concentration of metal ions) have negative relationship in biosorption of Cr (VI) and Zn (II) ions, whereas, in case of Ni (II) ions variable A (pH) shows the positive relationship and C (initial concentration of metal ions) shows the negative relationship. Other variable B (temperature) had a significant ($P < 0.05$) positive effect on biosorption of Cr (VI), Ni (II) and Zn (II) ions.

3.2. Optimization of variables for removal of Cr (VI), Ni (II) and Zn (II) ions

On the basis of quadratic polynomial equation of response surface methodology (Eqs. (4)–(6)), the effect of independent variables: pH, temperature and initial concentration of ions on the biosorption of Cr (VI), Ni (II) and Zn (II) were analyzed.

First, pH was a most important factor ($P > 0.0001$) and had a negative effect on biosorption of Cr (VI) and Zn (II) ions (Eqs. (4) and (6)) and linear positive effect on biosorption of Ni (II) ions (Eq. (5)) from aqueous solution by immobilized bacterial biomass. It means the biosorption of Cr (VI) and Zn ions were decreased and Ni (II) ions increased when pH increased.

Second, temperature ($P > 0.0001$) significantly affects the biosorption of Cr (VI), Ni (II) and Zn (II) ions. Independent variable temperature had a positive effect on biosorption of Cr (VI), Ni (II) and Zn (II) ions (Eqs. (4)–(6)). The increase in biosorption of Cr (VI), Ni (II) and Zn (II) ions with increase in temperature is due to either the higher affinity of sites for metal or an increases in binding sites on the biomass.

Third, initial concentration effect ($P > 0.0001$) was significant also play an important role in biosorption of Cr (VI), Ni (II) and Zn (II) ions. However, initial metal ions concentration showed negative effect on biosorption of Cr (VI), Ni (II) and Zn (II) ions (Eqs. (4)–(6)). The percent removal was decreased as the initial concentration of metal ions increased because at low initial concentration more binding sites are available for complexation of ions and concentration increased the number of ions competing for the available binding sites [27].

The interactive effect of two independent variables with another variable being at fixed level on the biosorption of Cr (VI), Ni (II) and Zn (II) ions with immobilized bacterial biomass were shown in 3D surface plots (Figs. 1–3 (A–C)). Fig. 1A–C shows the interactive effect of two variables pH (A) 2.0–6.0 and temperature (B) 25–40 °C on biosorption of Cr (VI), Ni (II) and Zn (II) ions. In Fig. 1A, the removal

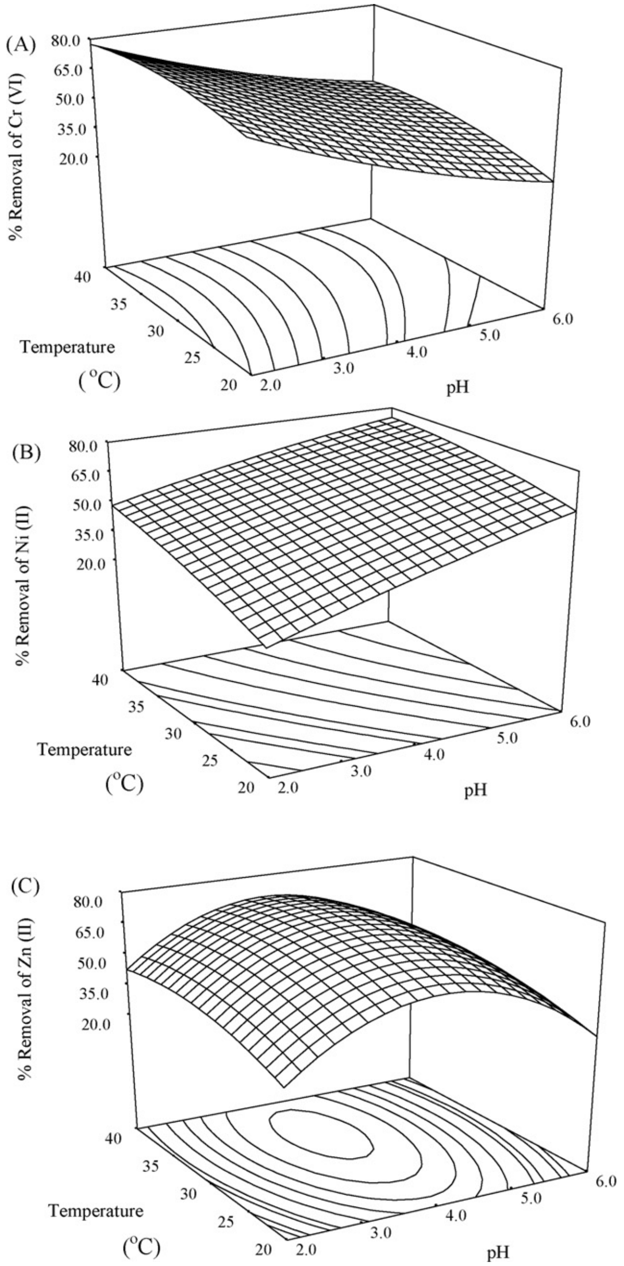

Fig. 1. 3D-surface plot showing the interactive of pH and temperature (°C) on: (A) % removal of Cr (VI), (B) % removal of Ni (II), and (C) % removal of Zn (II) by immobilized bacterial biomass sp. *Bacillus brevis*.

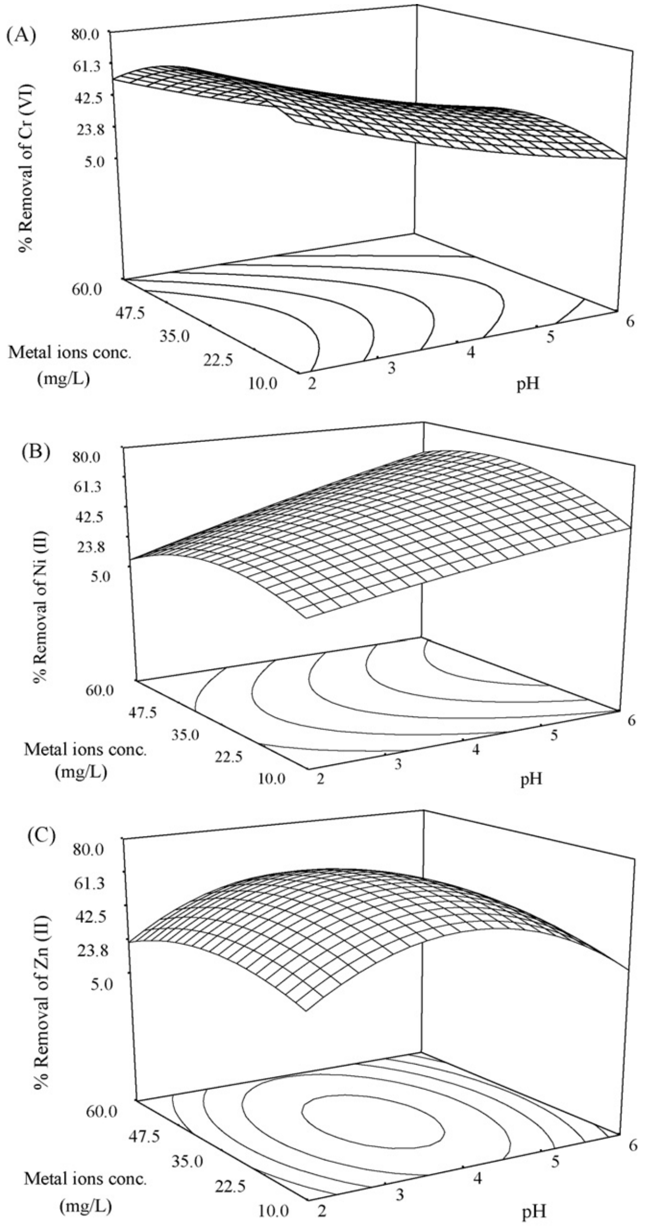

Fig. 2. 3D-surface plot showing the interactive of pH and metal ions concentration (mg/L) on: (A) % removal of Cr (VI), (B) % removal of Ni (II), and (C) % removal of Zn (II) by immobilized bacterial biomass sp. *B. brevis*.

of Cr (VI) was decreased with increase of pH from 2.0 to 6.0 and increased with increase of temperature from 25 °C to 40 °C. It was also observed that removal of Cr (VI) ions from solution is high at higher temperature 40 °C than at lower temperature 25 °C, so decreasing trend of Cr (VI) removal is slow at higher temperature 40 °C than at lower temperature 25 °C with increase in pH of the solution. Maximum removal of Cr (VI) was observed 77.24% at pH 2.0 and temperature 40 °C. High adsorption capacity observed at pH 2.0 may be attributed due to electrostatic interactions between adsorbent and adsorbate. At lower pH ($pH > 2.0$) values, $Cr_3O_{10}{}^{2-}$ and $Cr_4O_{13}{}^{2-}$ species are formed [28]. Thus decrease in solution pH causes the formation of more polymerized chromium oxide species. As pH increased, the number of negatively charged sites increased and the number of positively charged sites decreased. A negatively charged surface site on adsorbent does not favor the adsorption of anions due to electrostatic repulsion [29]. Removal of Ni (II) ions was increased with increase of pH as well as temperature of the solution. Maximum removal of Ni (II) ions was observed 75.21% at pH 6.0 and temperature 40 °C (Fig. 1B). It is observed that adsorption of Ni (II) is high at pH 6.0. At low pH, recoveries were found to be low due to the binding of H^+ ions and at higher pH, $OH-$ ions compete with Ni (II) ions for active sites on the surface of adsorbent [30]. The medium pH affects solubility of metals and ionization state of functional groups like carboxylate, phosphate and amino groups of cell wall. The carboxylate and phosphate groups carry negative charges that allow the components of cell wall to be potent scavengers of cations. When pH increased, adsorbent surface were more negatively charged with subsequent attraction of metallic ions with positive charge and biosorption process was favored until a maximum was reached. In Fig. 1C, removal of Zn (II) ions was also increased with increased pH 2.0–4.0 and tem-

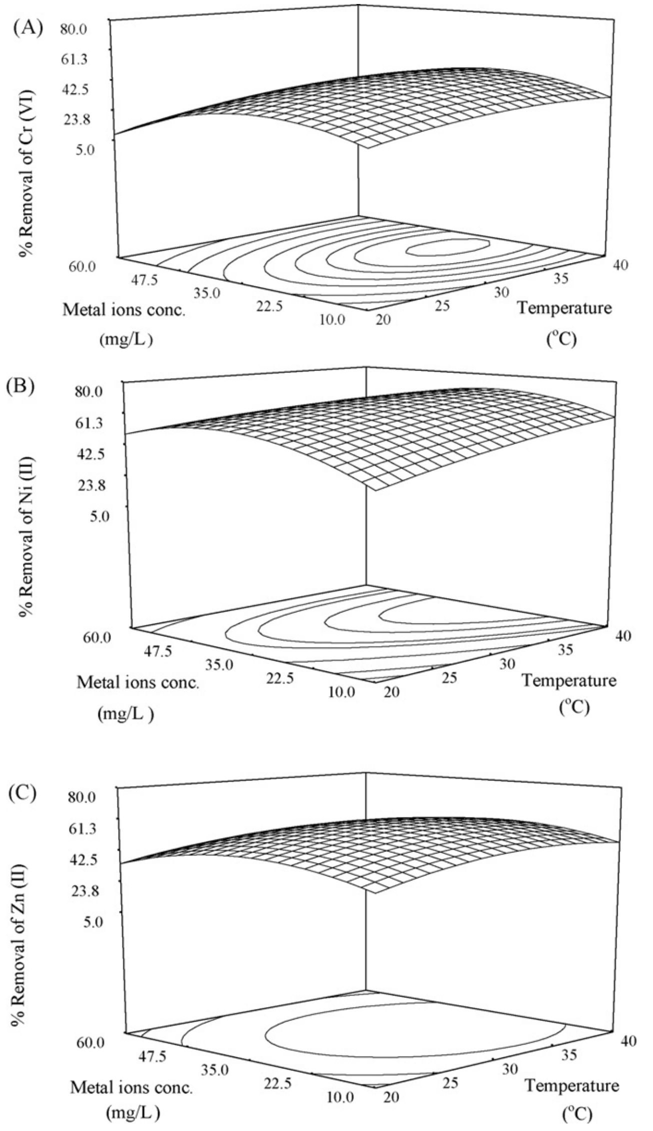

Fig. 3. 3D-surface plot showing the interactive of temperature and metal ions concentration (mg/L) on: (A) % removal of Cr (VI), (B) % removal of Ni (II), and (C) % removal of Zn (II) by immobilized bacterial biomass sp. *B. brevis*.

perature 25–32.5 °C and after that removal of Zn (II) ions decreased with increased pH and temperature. In this study, these zinc cations at pH 4.0 was found to be interact more strongly with negatively charged binding sites of adsorbent due to ionic attraction. Decrease in biosorption at higher pH is due to formation of soluble hydroxyl complexes of the metal would also compete with the active sites, and as a consequence, the retention would decrease again [31]. At pH less than 6.0, metals are in their free ionic form and as such sharp increase in metal uptake. The effect of pH on adsorption of zinc has been investigated by various investigators using a variety of different adsorbent types [32–34], and optimum adsorption capacity of zinc has been reported at below 6.0. The increase in biosorption of Cr (VI) and Ni (II) ions with increases temperature indicates an endothermic process. Whereas, in case of Zn (II) ions, maximum removal was observed at temperature 32.5 °C, after that removal of Zn (II) ions decreased. The initial increase in biosorption of Zn (II) ions indicates an endothermic process and after that when decreased in biosorption of Zn (II) ions with increased temperature indicates due to exothermic process occurred. The biosorption of Cr (VI) and NI (II) ions increased with increased temperature, but not for Zn (II) ions, this may also be due to interaction between biosorbent and Cr (VI) or Ni (II) is different from that biosorbent and Zn (II) ions. Maximum removal of Zn (II) was observed 71.6% at pH 4.0 and temperature 32.5 °C. A decrease in the biosorption of Zn (II) ions with the rise in temperature may be due to either damage of active binding sites in biomass or increasing tendency to desorb metal ions from interface to the solution [35].

Fig. 2A–C showed interactive effect of pH and initial concentration on biosorption of Cr (VI), Ni (II) and Zn (II) ions. Fig. 2A showed that removal of Cr (VI) ions was decreased with increases of pH from 2.0 to 6.0 and increased with initial increases of concentration of Cr (VI) ions up to 35 mg/L, after that removal of Cr (VI) ions decreased with increases metal ions concentration. Maximum removal was observed at 77.24% at pH 2.0 and concentration of Cr (VI) ions at 35.0 mg/L. Fig. 2B showed the removal of Ni (II) ions was increased with increases of pH from 2.0 to 6.0 and concentration from 10 mg/L to 60 mg/L of Ni (II) ions, after reached the equilibrium the removal of Ni (II) ions, no much decrease with increases of Ni (II) ions concentration. It was observed that these two independent variables pH and concentration with another variable temperature being at fixed level shows positive interactive effect in biosorption of Ni (II) ions with increases pH and Ni (II) ions concentration of the solution. Fig. 2C shows removal of Zn (II) ions was increased with increases of pH, maximum removal was at pH 4.0 and after that removal of Zn (II) ions decreased. The second variables concentration of Ni (II) ions was increased from 10 to 60 mg/L, removal of Zn (II) ions increased initially with increases concentration of Zn (II) ions. After reached equilibrium, removal of Zn (II) ions decreased with increases of concentration of Zn (II) ions. The biosorption of Cr (VI), Ni (II) and Zn (II) metal ions increased first with increases of the initial metal ions concentration and reached a saturated value as shown in Fig. 2A–C. This may be due to saturation of sorption sites and increase in number of ions competing for available binding sites in biomass for comlexation of metal ions. Difference in biosorptions of Cr (VI), Ni (II) and Zn (II) ions with bacterial biomass may be due to the properties of metal sorbets (e.g. atomic weight, ionic size or reduction potential of metal) and the properties of biosorbent (e.g. structure, functional groups and polysaccharides). These functional groups such as amino, carboxylic, sulphydryl and phosphate groups differ in their affinity and specificity for metal binding [36]. The observed enhancement of metal uptake could be due to an increase in electrostatic interactions (relative to covalent interactions), which involved sites of progressively lower affinity for metal ions [37]. It was observed that these two independent variables pH and concentration with another variable temperature being at fixed level also shows positive interactive effect (Eq. (6)) in biosorption of Cr (VI), Ni (II) and Zn (II) ions.

Fig. 3A–C showed interactive effect between temperature and initial concentration on biosorption of Cr (VI), Ni (II) and Zn (II) ions. The biosorption of Cr (VI), Ni (II) and Zn (II) ions increased first with increases of initial metal ions concentration and reached a saturated value and after that removal of Cr (VI), Ni (II) and Zn (II) ions were decreased with increases of concentration as shown in Fig. 3A–C respectively. However, with increases of temperature from 25 °C to 40 °C, removal of Cr (VI) and Ni (II) ions increased with increases of concentration up to 35 mg/L and after that removal of Cr (VI) and Ni (II) ions decreased with increases of concentration of metal ions. The increase in biosorption with increases of temperature is due to either higher affinity of sites for metal or increases in binding sites on biomass. It was observed that at higher temperature 40 °C, rate of metal Cr (VI) and Ni (II) ions removal is high than low temperature. Whereas, in case of Zn (II) ions biosorption increased with increases of temperature from 25 °C to 32.5 °C up to concentration of metal ions 35 mg/L and after that removal was decreased with increases temperature and concentration of ions in solution. Maximum removal of Zn (II) was observed 71.6% at 32.5 °C at concentration 35 mg/L. A decrease in biosorption of Zn (II) ions with the rise in temperature may be due to either damage of active binding sites in biomass or increasing tendency to desorb metal ions from the interface to the solution [35].

4. Conclusions

For the biosorption of heavy metals Cr (VI), Ni (II) and Zn (II) ions response surface methodology was used to optimize the process variables, such as pH 2.0–6.0, temperature 25–40 °C and initial concentration of ions 10–60 mg/L using immobilized bacterial biomass sp. *B. brevis*. On the basis of RSM using the Box–Behnken design, found that combination of pH, temperature and concentration of metal ions have significant effect on biosorption of Cr (VI), Ni (II) and Zn (II) ions. Values of "Prob > *F*" less than 0.0500 indicate model terms have significant effect on biosorption of Cr (VI), Ni (II) and Zn (II) ion using immobilized bacterial biomass sp. *B. brevis*. Maximum removal of Cr (VI) was observed 77.24% at pH 2.0, temperature 40 °C and concentration 35 mg/L. Whereas, removal of Ni (II) ions was 75.21% at pH 6.0, temperature 40 °C and concentration 35 mg/L and 71.6% of Zn (II) ions at pH 4.0, temperature 32.5 °C and concentration 35 mg/L respectively. From the significant model and mathematical evaluation, RSM approach proved to be a very useful and accurate methodology to optimize biosorption process and need to apply at pilot scale for industrial wastewater treatment.

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References

[1] A. Selatnia, M.Z. Bakhti, A. Madani, L. Kertous, Y. Mansouri, Biosorption of Cd (II) from aqueous solution by a NaOH-treated bacterial dead *Streptomyces rimosus* biomass, Hydrometallurgy 75 (2004) 11–24.

[2] D.H. Nies, Microbial heavy metal resistance, Appl. Microbiol. Biotechnol. 51 (1999) 730–750.

[3] M.R. Bruins, S. Kapil, F.W. Oehme, Microbial resistance to metals in the environment, Ecotoxicol. Environ. Saf. 45 (2000) 198–207.

[4] J.C. Fry, G.M. Gadd, R.A. Herbert, C.W. Jones, L.A. Watson-Craik, Microbioal Control of Pollution, Cambridge University Press, 1992.

[5] B. Volesky, Biosorbent materials, Biotechnol. Bioeng. Symp. 16 (1986) 121–126.

[6] R. Kumar, N.R. Bishnoi, G.K. Bishnoi, Biosorption of chromium (VI) from aqueous solution and electroplating wastewater using fungal biomass, Chem. Eng. J. 135 (2008) 202–208.

[7] A.P. McHale, S. McHale, Microbial biosorption of metals: potential in the treatment of metal pollution, Biotechnol. Adv. 12 (1994) 647–652.

[8] B. Volesky, Sorption and Biosorption, BV. Sorbex, Inc, Montreal, St. Lambert, Quebec, Canada, 2003.

[9] E.M. Trujillo, M. Sprinti, H. Zhuang, A.K. Sengupta (Eds.), Ion Exchange Technology: Advances in Pollution Control, Technomic Publishing Company Inc., Pennsylvania, 1995.

[10] N.R. Bishnoi, R. Kumar, K. Bishnoi, Biosorption Cr (VI) with *Trichoderma viride* immobilized fungal biomass and cell free Ca-Alginate beads, J. Exp. Biol. 45 (2007) 657–664.

[11] K. Bala, A. Kaushik, C.P. Kaushik, Response surface methodological approach for optimizing removal of Cr (VI) from aqueous solution using immobilized cynobacterium, Chem. Eng. J. 126 (2007) 147–153.

[12] X.B. Gu, Z.M. Zheng, H.Q. Yu, J. Wang, F.L. Liang, R.L. Liu, Optimization of medium constituent for a novel lipopeptide production by *Bacillus subtilis* MO–O1 by a response surface method, Process. Biochem. 40 (2005) 3196–3201.

[13] S.R. Senthilkumar, B. Ashokkumar, K. Chandra Raj, P. Gunasekaran, Optimization of medium composition for alkali-stable xylanase production by *Aspergillus fischeri* Fxn 1 in solid-state fermentation using central composite rotary design, Bioresour. Technol. 96 (2005) 1380–1386.

[14] F.J. Cui, Y. Li, Z.H. Xu, H.Y. Xu, K. Sun, W.Y. Tao, Optimization of the medium composition for production of mycelial biomass and exo-polymer by *Grifola frondosa* GF9801 using response surface methodology, Bioresour. Technol. 97 (2006) 1209–1216.

[15] S. Oh, S. Rheem, J. Sim, S. Kim, Y. Baek, Optimizing conditions for the growth of *Lactobacillus casei* YIT 9018 in tryptone-glucose medium by using response surface methodology, Appl. Environ. Microbiol. 61 (1995) 3809–3814.

[16] S. Puri, Q.K. Beg, R. Gupta, Optimization of alkaline protease production from *Bacillus* sp. by response surface methodology, Curr. Microbiol. 44 (2001) 286–290.

[17] R.H. Myers, D.C. Montgomery, Response Surface Methodology Process and Product Optimization Using Designed Experiments, 2nd ed., John Wiley, New York, 2002.

[18] G. Box, N. Draper, Empirical Model Building and Response Surfaces, John Wiley & Sons, New York, 1987.

[19] P. Ricou, I. Lecuyer, P. Le Cloirec, Influence of pH on removal of heavy metallic cations by fly ash in aqueous solution, Water Res. 35 (2001) 965–976.

[20] M. Amini, H. Younesi, N. Bahramifar, A.Z.L. Ali, F. Ghorbani, D. Ali, M. Sharifzadeh, Application of response surface methodology for optimization of lead biosorption in an aqueous solution by *Aspergillus niger*, J. Hazard. Mater. 154 (2008) 694–702.

[21] W.B. Lu, W.C. Kao, J.J. Shi, J.S. Chang, Exploring multi-metal biosorption by indigenous metal-hyperresistant *Enterobacter* sp. J1 using experimental design methodologies, J. Hazard. Mater. 153 (2008) 372–381.

[22] T. Ross, Indices for performance evaluation of predictive models in food microbiology, J. Appl. Bacteriol. 81 (1996) 501–508.

[23] S. Samapundo, F. Devlieghere, B. De Meulenar, A.H. Geeraerd, J.F. Van Impe, J.M. Debevere, Predictive modelling of the individual and combined effect of water activity and temperature on the radial growth of *Fusarium verticilloides* and *F. proliferatum* on corn, Int. J. Food Microbiol. 105 (2005) 35–52.

[24] R. Lahlali, M.N. Serrhini, D. Friel, M.H. Jijakli, Predictive modeling of temperature and water activity (solutes) on the in vitro radial growth of *Botrytis cinerea* Pers, Int. J. Food Microbiol. 114 (2007) 1–9.

[25] M.Y. Arica, Y. Kacar, O. Genc, Entrapment of white-rot fungus *Trametes versicolor* in Ca alginate beads: preparation and biosorption kinetic analysis for cadmium removal from an aqueous solution, Bioresour. Technol. 80 (2001) 121–129.

[26] L.V.A. Reddy, W. Young-Jung, Y. Jong-Sun, R. Hwa-Won, Optimization of alkaline protease production by batch culture of *Bacillus* sp. RKY3 through Plackett–Burman and response surface methodological approaches, Bioresour. Technol. 99 (2008) 2242–2249.

[27] N.R. Bishnoi, M. Bajaj, K. Sanatomba, Biosorption of Zn (II) using *Spirogyra* species from electroplating effluent, J. Environ. Biol. 26 (2005) 661–664.

[28] S. Mor, R. Khaiwal, N.R. Bishnoi, Adsorption of chromium from aqueous solution by activated alumina and activated charcoal, Bioresour. Technol. 8 (2006) 954–957.

[29] P.N. Malik, Use of activated carbons prepared from sawdust and rice husk for adsorption of acid-dyes: a case study of Acid Yellow 36, Dyes Pigments 56 (2003) 239–249.

[30] S. Kalyani, P. Ajitha Priya, P. Srinivasa Rao, A. Krishnaiah, Adsorption of nickel on flyash in natural and acid treated forms, Indian J. Environ. Health 45 (2003) 163–168.

[31] E. Malkoc, Ni (II) removal from aqueous solution using cone biomass of *Thuja orientalis*, J. Hazard. Mater. 137 (2006) 899–908.

[32] L. Norton, K. Baskaran, T. McKenzie, Biosorption of zinc from aqueous solutions using biosolids, Adv. Environ. Res. 8 (2004) 629–635.

[33] B. Nasernejad, T. Esslam Zadeh, B. Bonakdar Pour, M. Esmaail Bygi, A. Zamani, Comparison for biosorption modeling of heavy metals (Cr(III), Cu(II), Zn(II)) adsorption from wastewater by carrot residues, Process. Biochem. 40 (2005) 1319–1322.

[34] X.C. Chen, Y.P. Wang, Q. Lin, J.Y. Shi, W.X. Wu, Y.X. Chen, Biosorption of copper(II) and zinc(II) from aqueous solution by *Pseudomonas putida* CZI, Colloid Surf. B: Biointerf. 46 (2005) 101–107.

[35] K. Saltalı, A. Sarı, M. Aydın, Removal of ammonium ion from aqueous solution by natural Turkish (Yıldızeli) zeolite for environmental quality, J. Hazard. Mater. B 141 (2007) 258–263.

[36] G. Ozdemir, N. Ceyhan, T. Ozturk, F. Akirmak, T. Cosar, Biosorption of chromium (VI), cadmium (II) and copper (II) by *Pantoea* sp. TEM18, Chem. Eng. J. 102 (2004) 249–253.

[37] A.M. Marques, X. Raca, M.D. Simon-Pujol, M.C. Fuste, F. Congregado, Uranium accumulation by *Pseudomonas* sp. Eps-5028, Appl. Microbiol. Biotechnol. 35 (1991) 406–410.