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Optimization of process parameters for the removal of chromium(VI) and nickel(II) from aqueous solutions by mixed biosorbents (custard apple seeds and *Aspergillus niger*) using response surface methodology

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ABSTRACT

The mixture of custard apple seeds and *Aspergillus niger* was used as biosorbents for the removal of chromium(VI) and nickel(II) from the contaminated water. The batch biosorption process was carried out and it was influenced by the different operating parameters such as initial metal ion concentration, pH, temperature, and biomass loading. The essential process variables were optimized using response surface methodology (RSM) based on the central composite design (CCD) experiments. The ANOVA data obtained from the RSM studies were analyzed using a second-degree polynomial equation and the study of the determination of contour plots showed the interactions among the variables of the biosorption system. The optimum conditions for the removal of chromium(VI) was found to be: initial chromium(VI) concentration = 100 mg/L; pH 3.0; temperature = 36˚C, and biosorbent loading = 10 g/L. At these optimized conditions, the maximum removal of chromium(VI) was found to be of 95.7%. The optimum conditions for the removal of nickel(II) was found to be: initial nickel(II) concentration = 100 mg/L; pH 5.6; temperature = 30˚C, and biosorbent loading = 10 g/L. At these optimized conditions, the maximum removal of nickel(II) was found to be of 96.41%. The mixed biosorbents showed better adsorption properties towards the removal of chromium(VI) and nickel(II) ions from the aqueous solutions. These low-cost biosorption methods can be effectively adopted for the removal of metal ions from the industrial effluents.

Keywords: *Aspergillus niger*; Biosorption; Custard apple seeds; Heavy metals; Response surface methodology

1. Introduction

Nowadays, different heavy metal ions and complex organic molecules were discharged into the environment, because of the rapid growth of agricultural and industrial production processes. Because of these activities, the polluted wastewaters are consequently accumulated in the environment. If these pollutants were persist in the environment then these pollutants can causes serious health effects to the

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human beings due to its mutagenic and carcinogenic properties [1,2].

Chromium(VI) and nickel(II) ions are the non-biodegradable toxic heavy metals which often exist in the water habitats from industrial effluents such as textile dyeing, pigments, electroplating, leather tanning, metal mining, paint manufacturing, steel fabrication, battery manufacturing, automotive, steam electric power plants, and agricultural wastes [3,4]. Inhaling chromium(VI) can cause nose irritations and nosebleeds. Other health problems that are caused by chromium(VI) are skin rashes, respiratory problems, weakened immune systems, kidney and liver damage, alteration of genetic material, lung cancer, and death. Similarly, the nickel(II) metals can causes serious problems such as allergic sensitization, dermatitis, lung, and nervous system damage [5–7].

The commonly used techniques for removing heavy metal ions from the contaminated water are ion-exchange, chemical precipitation, reverse osmosis, evaporation, and membrane filtration processes [8]. The removal of heavy metal ions using the above-affirmed methods are not viable because of the high operational cost, incomplete metal removal, and also the generation of secondary wastes [9]. Biosorption is the most successful process of wastewater treatment technology, based on the efficiency, binding capacity of diverse biological materials, and its potential marketing benefit over other conventional wastewater treatment technologies. Because the natural biosorbent is cheaper than the traditional activated carbon and ion-exchange resins, it is environmental friendly [10–12]. The different kinds of biosorbent have been tested to remove the several heavy metals from the aqueous solution [13]. Generally, the low-cost biosorbents were derived from the natural materials, industrial solid waste, biological organisms, agricultural by-products, etc., which behaves as a good effective biosorbents for the removal of heavy metal ions from the wastewater [14,15].

The aim of the present investigation is to prepare the mixed biosorbent (*Aspergillus* niger and custard apple seeds were immobilized by calcium alginate polymer) for the effective removal of chromium(VI) and nickel(II) ions from the contaminated water. The removal of chromium(VI) and nickel(II) from the aqueous solutions by mixed biosorbent was measured as a function of initial solution pH, biosorbent loading, initial metal ion concentrations, contact time, and temperature. Biosorption based on the immobilization of fungal cell (*Aspergillus niger*) in mixture with custard apple seeds powder on calcium alginate polymeric matrices was found to increase the performance, biosorptive capacity, and also be suitable for the removal of heavy metal ions from the aqueous solution.

2. Materials and methods

2.1. Preparation of immobilized mixed biosorbent

The custard apple seeds were separated from the fruit. These materials were completely washed with tap water to remove the impurities present in it and then the excess water was removed. This wet material was dried in a hot air oven for about 80℃ till the removal of moisture contents. These dried materials were ground into a fine powder in a flour mill. Finally, the powdered materials were utilized for the synthesis of mixed biosorbent for the removal of heavy metal ions.

Aspergillus niger (MTCC-132) were obtained from the Institute of Microbial Technology, Chandigarh. The organism was subcultured by using agitated potato dextrose agar (PDA) medium from the stock. This system was observed under microscope for any probable contamination and also other morphological changes in the system.

Aspergillus niger culture was grown primarily in the filamentous form with white fluffy mycelia and finally to dark colonies with black spores. At the stationary phase of the growth, the contents of the vessels were harvested by filtering through nylon mesh. The recovered biomass was washed roughly with tap water, used as suspended cells for the subsequent use.

The sorbents used were immobilized biosorbent beads. The beads were prepared by using sodium alginate (3% w/v). An identified amount of biosorbents (custard apple seeds and *Aspergillus niger*) was assorted with sodium alginate. The mixtures were persistently stirred under humid condition for the alginate gets dissolved. The beads were formed by dripping the polymer solution from a height of approximately 20 cm into an access of stirred 0.2 M calcium chloride solution with a syringe and a needle. The prepared beads were used as a mixed biosorbent for the removal of heavy metal ions from the aqueous solution.

2.2. Biosorption of chromium(VI) and nickel(II) by mixed bioadsorbents

Metal ion solutions of known concentration of chromium(VI) and nickel(II) ions were prepared by dissolving the measured amounts of potassium dichromate ($K_2Cr_2O_7$) and nickel sulphate ($NiSO_4{\cdot}7H_20$) salt in double-distilled water, respectively. The pH of each test solution was adjusted by using 0.1 N sodium hydroxide and 0.1 N hydrochloic acid solutions before the addition of biomass into the aqueous solution.

2.3. Batch studies on biosorption of chromium(VI) and nickel(II)

Batch biosorption experiments for the individual metal ions were carried out to study the effect of diverse parameters such as initial metal concentration (100–500 mg/L), pH (2–7), temperature (27–39˚C), and biosorbent loading (5–25 g/L) for the removal of metal ions. The wastewater solution of 50 mL of known concentration of metal ions and the known amount of biosorbent dose was taken in 250 mL capacity flasks. The flasks were placed in the temperature controlled incubation shaker and the mixtures were agitated at 120 rpm for about 24 h, which is more than sufficient time for the sorption equilibrium. The samples were taken regularly at predefined time intervals and the concentrations of unadsorbed metal ions in the supernatant were measured using atomic adsorption spectrophotometer (AAS) (SL 176 Model, Elico Limited, Chennai, India).

2.4. Statistical optimization method for design of experiments

The effect of the pH, temperature, initial metal ion concentration, and biosorbent loading are calculated using central composite design (CCD).

An orthogonal 2^4 factorial central composite investigational design with eight star points ($\alpha = 2$) and seven replicates at the center point, all in duplicates, ensuing in a total of 31 experiments were used to optimize the chosen key variables for the biosorption of chromium(VI) and nickel(II) in batch adsorber.

The experiments with five diverse initial metal ion concentrations, namely, 100, 200, 300, 400, and 500 mg/L; six different pH values namely of 2, 3, 4, 5, 6, and 7; five different temperatures namely 27, 30, 33, 36, and 39˚C; and five different biomass loadings of 5, 10, 15, 20, and 25 g/L were working concurrently covering the spectrum of variables with diverse concentrations for chromium(VI) and nickel(II) in the CCD.

To understand the effects of the biosorption parameters such as pH, biosorbent loading, initial metal ion concentration, and temperature and their interactions on the biosorption process, statistically calculated experiments were used.

Tables 1 and 2 show the range and levels of the sovereign variables selected for the biosorption of chromium(VI) and nickel(II). Table 3 shows the orthogonal and real values of selected variables for chromium(VI) biosorption. Table 4 shows the orthogonal and real values of selected variables for nickel(II) biosorption. The investigational data were analyzed using CCD and optimized using response surface methodology (RSM). The second-order polynomial equation for factors takes the following form.

$$\begin{aligned} Y = \beta + \beta_1 X_1 + \beta_2 X_2 \beta_3 X_3 \beta_4 X_4 \beta_1 1 X_1^2 + \beta_2 2 X_2^2 + \beta_3 3 X_3^2 \\ + \beta_4 4 X_4^2 + \beta_1 2 X_1 X_2 + \beta_1 3 X_1 X_3 + \beta_1 4 X_1 X_4 \beta_2 3 X_2 X_3 \\ + \beta_2 4 X_2 X_4 + \beta_3 4 X_3 X_4 \end{aligned}$$

2.5. Analytical techniques

Biomass concentration was calculated as volatile-suspended solid (VSS) and analyzed according to standard methods. Chromium(VI) concentration was determined by diphenylcarbazide method using spectrophotometer at a $\lambda_{\max}$ of 540 nm. Dimethyl glyoxime method was used to determine the nickel(II) concentration at 445 nm in UV-double beam spectrophotometer using water as reference [16]. The results of the metal ions analysis were also compared with the atomic adsorption spectrophotometer (AAS) (SL 176 Model, Elico Limited, Chennai, India). Minitab 15, statistical program package was used for regression study of the data obtained and used to guesstimate the coefficient of the second-degree polynomial equations.

3. Results and Discussion

3.1. Effect of initial chromium(VI) ions concentration

The amount of biosorption for the removal of chromium(VI) highly depends on the initial metal ion concentration. Figs. 1 and 2 show the % removal and specific uptake of chromium(VI), respectively. From Fig. 1, it was observed that the % removal of chromium(VI) was decreased with an increase in initial chromium(VI) concentration. The % removal of chromium(VI) was decreased from 95.7 to 74% for the initial chromium(VI) concentration from 100 to 500 mg/L, respectively. The decrease in chromium(VI) removal with the increase in the initial concentration may be due to the reduction in biosorption sites on the biosorbent surface. Fig. 2 indicates that the specific uptake of chromium(VI) increases with increase in initial chromium(VI) concentration. The maximum biosorption capacity of 18.5 mg/g was observed at 500 mg/L of initial chromium(VI) concentration. Biosorbents offer a limited number of surface binding

Table 1
The range and levels of the independent variables selected for the biosorption of chromium(VI)

	Range and Level				
Independent variable	−2	−1	0	+1	+2
Initial metal concentration (mg/l), X_1	100	200	300	400	500
pH, X_2	2	3	4	5	6
Temperature (˚C), X_3	27	30	33	36	39
Biomass Loading (g/l), X_4	5	10	15	20	25

Table 2
The range and levels of the independent variables selected for the biosorption of nickel(II)

	Range and Level				
Independent variable	−2	−1	0	+1	+2
Initial metal concentration (mg/l), X_1	25	50	100	150	200
pH, X_2	3	4	5	6	7
Temperature (˚C), X_3	27	30	33	36	39
Biomass Loading (g/l), X_4	5	10	15	20	25

sites showed that the reduction of specific uptake at higher metal ion concentration which indicates that the accessible sites for the biosorption process were very fewer at higher initial metal ion concentrations. Furthermore, the higher initial metal ion concentration generates an important driving force to conquer all mass-transfer resistance of chromium(VI) ions between the aqueous and the solid phases [17,18].

3.2. Effect of pH

The pH of the solution is a significant parameter of the biosorption process, because its plays a major role in controlling the biosorption of heavy metals. pH changes the solubility of metals and the ionization state of the functional groups like amino groups, phosphate, and carboxyl of the cell walls of the biosorbent. Fig. 3 shows the % removal of chromium(VI) ions over a range of pH (2–6) at the temperature of 36˚C and at the biosorbent loading of 15 g/L. The maximum removal of chromium(VI) ions onto the mixed biosorbents was observed at the pH of 3.0. At lesser pH ranges, due to the high electrostatic force of desirability, the % removal of chromium(VI) is high. At higher pH values, it causes the formation of metal hydroxide complexes which decreases the concentration of chromium(VI) ions, thereby causing decrease in the equilibrium biosorption capacity [19,20]. These results shows that the optimum pH for the maximum removal of chromium(VI) was obtained at the pH of 3.

3.3. Effect of temperature

Effect of temperature is an important physiochemical process parameter because the mechanism of metal ion biosorption is the temperature dependent. Batch biosorption studies were completed at diverse temperatures of 27, 30, 33, 36, and 39˚C for chromium(VI) ions at different time intervals to study the biosorption of chromium(VI) ions onto mixed biosorbents. Fig. 4 shows that the maximum % removal was observed at 36˚C at the pH of 5.6, metal ion concentration of 100 mg/L, and the biosorbent loading of 10 g/L. The results explored that the % removal of chromium(VI) was increased from 82.39 to 93.16% as the temperature increased from 27 to 36˚C. It was conditional that the biosorption of chromium(VI) ions onto mixed biosorbents is an endothermic process, because of the increment of active sites in the biosorbent or decrease of the layer thickness that surrounds the biosorbent. This statement additionally confirmed that the 30–36˚C temperature is fitted for biosorption experiments. However, a further increase in the temperature could affect the biosorption process; it notably indicates that the biosorption is an exothermic process which means biosorption capacity is inversely proportional to the temperature, and this might be due to the weakening

Table 3
The orthogonal and real values of selected variables for chromium(VI) biosorption

S. No.	Orthogonal values				Real values			
	X_1	X_2	X_3	X_4	X_1	X_2	X_3	X_4
1	−1	+1	+1	+1	200	5	33	25
2	+1	−1	−1	−1	400	3	27	15
3	−1	+1	−1	+1	200	5	27	25
4	−1	−1	−1	−1	200	3	27	15
5	+1	+1	−1	+1	400	5	27	25
6	+1	+1	+1	+1	400	5	33	25
7	+1	+1	+1	−1	400	5	33	15
8	+1	+1	−1	−1	400	5	27	15
9	+1	−1	+1	+1	400	3	33	25
10	−1	−1	+1	−1	200	3	33	15
11	−1	+1	−1	−1	200	5	27	15
12	+1	−1	−1	+1	400	3	27	25
13	−1	+1	+1	−1	200	5	33	15
14	+1	−1	+1	−1	400	3	33	15
15	−1	−1	−1	+1	200	3	27	25
16	−1	−1	+1	+1	200	3	33	25
17	+2	0	0	0	500	4	30	20
18	−2	0	0	0	100	4	30	20
19	0	2	0	0	300	6	30	20
20	0	−2	0	0	100	3	36	10
21	0	0	2	0	300	4	36	20
22	0	0	−2	0	300	4	27	20
23	0	0		2	300	4	30	30
24	0	0	0	−2	300	4	30	10
25	0	0	0	0	300	4	30	20
26	0	0	0	0	300	4	30	20
27	0	0	0	0	300	4	30	20
28	0	0	0	0	300	4	30	20
29	0	0	0	0	300	4	30	20
30	0	0	0	0	300	4	30	20
31	0	0	0	0	300	4	30	20

Table 4
The orthogonal and real values of selected variables for nickel(II) biosorption

S. No.	Orthogonal values				Real values			
	X_1	X_2	X_3	X_4	X_1	X_2	X_3	X_4
1	−1	+1	+1	+1	50	6	33	25
2	+1	−1	−1	−1	150	4	27	15
3	−1	+1	−1	+1	50	6	27	25
4	−1	−1	−1	−1	50	4	27	15
5	+1	+1	−1	+1	150	6	27	25
6	+1	+1	+1	+1	150	6	33	25
7	+1	+1	+1	−1	150	6	33	15
8	+1	+1	−1	−1	150	6	27	15
9	+1	−1	+1	+1	150	4	33	25
10	−1	−1	+1	−1	50	4	33	15
11	−1	+1	−1	−1	50	6	27	15
12	+1	−1	−1	+1	150	4	27	25
13	−1	+1	+1	−1	50	6	33	15
14	+1	−1	+1	−1	150	4	33	15
15	−1	−1	−1	+1	50	4	27	25
16	−1	−1	+1	+1	50	4	33	25
17	+2	0	0	0	200	5	30	20
18	−2	0	0	0	25	5	30	20
19	0	2	0	0	100	7	30	20
20	0	−2	0	0	100	3	30	20
21	0	0	2	0	100	5	36	20
22	0	0	−2	0	100	5	27	20
23	0	0	0	2	100	5	30	10
24	0	0	0	−2	100	5	30	10
25	0	0	0	0	100	5	30	20
26	0	0	0	0	100	5	30	20
27	0	0	0	0	100	5	30	20
28	0	0	0	0	100	5	30	20
29	0	0	0	0	100	5	30	20
30	0	0	0	0	100	5	30	20
31	0	0	0	0	100	5	30	20

of biosorptive forces between the metal ions and the active sites on the biosorbent surface [19].

3.4. Effect of biosorbent loading

Biosorbent loading is the main process parameters to resolve the capacity of biosorbent for a given amount of the biosorbent at the operating conditions. Fig. 5 shows the effect of the mixed biosorbent loading on the biosorption of chromium(VI) at different time intervals. The mixed biosorbent loading varied from 5 to 25 g/L to optimize the biosorbent loading to get the maximum removal of chromium(VI) ions. The biosorption ability of *Aspergillus niger* has been ascribed mainly to the cell wall. The cell walls are loaded with glycoproteins such as chitin, glycans, chitosan, mannans, phosphormannans, and polysaccharides. Various metal binding

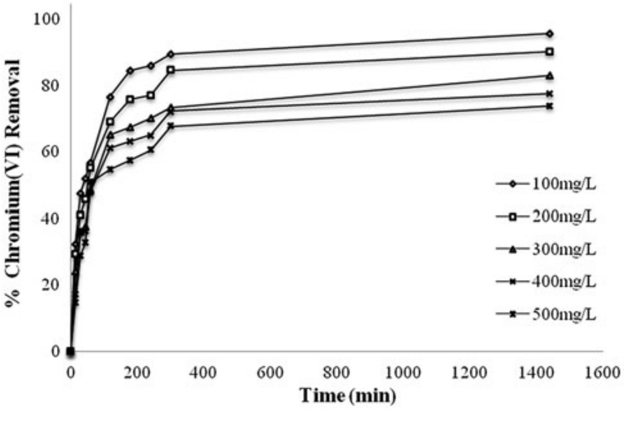

Fig. 1. Effect of initial chromium concentration on removal of chromium(VI).

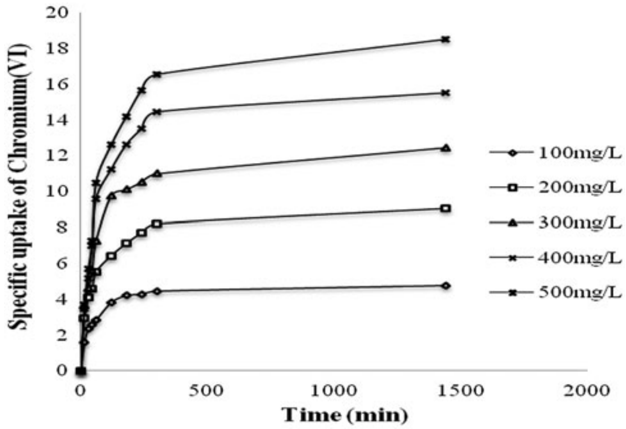

Fig. 2. Effect of initial chromium concentration on specific uptake of chromium(VI).

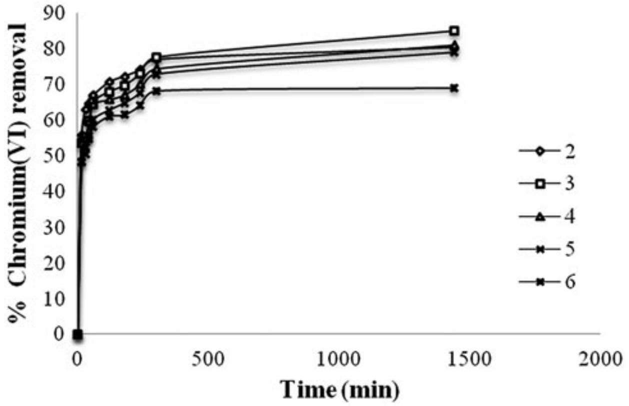

Fig. 3. Effect of pH on removal of chromium(VI).

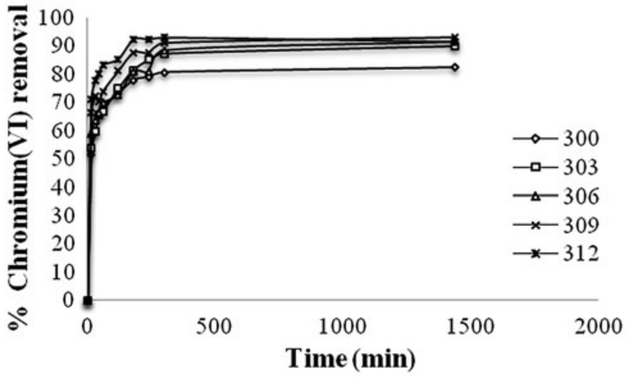

Fig. 4. Effect of temperature on removal of chromium(VI).

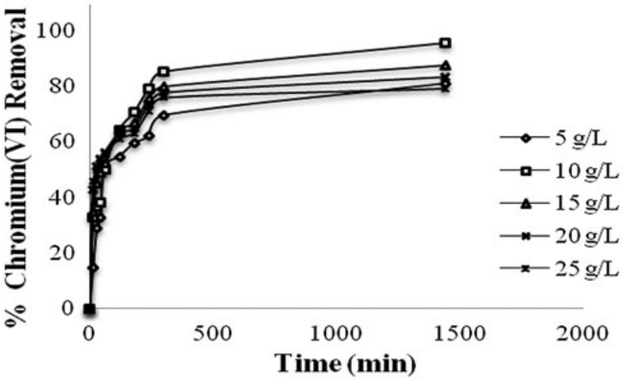

Fig. 5. Effect of biosorbent loading on removal of chromium(VI).

groups such as amine, imidazole, sulfhydryl, sulphate, hydroxylare, and phosphate present in the polymers. It has been stated that *Aspergillus niger* have a high biosorption capability for metal ions. The custard apple seed powder has the surface bearing groups of carbon and oxygen. During the biosorption process, the carbon and oxygen interact with metal ions and observe the metal ions in their surface. Hence, it is confirmed that the custard apple seeds powder has a metal biosorption potential. These two biosorbents were mixed by using immobilization technique [16]. Fig. 5 clearly shows that the primarily there is a pointed increase in the % removal of chromium(VI) ions as the mixed biosorbent loading was increased. The maximum % removal of chromium(VI) ions was 95.6% at the mixed biosorbent loading of 10 g/L. They ascribe this performance to the higher number of available biosorption sites. On the other side, the maximum biosorption potential of mixed biosorbent was decreased with increase in biosorbent loading might be due to the overlapping of available active sites leading to a decline in total surface area [21].

3.5. Effect of initial nickel(II) ions concentration

The effect of initial nickel(II) ion concentration on metal biosorption by mixed biosorbents was investigated at a pH of 5.6 for the biosorbent loading of 10 g/L and temperature 30°C. Figs. 6 and 7 indicate the effect of initial nickel(II) concentration on removal and specific uptake of nickel(II), respectively. It is observed from Fig. 6 that the % removal of nickel decreased from 96.41 to 74.78% with an increase in initial nickel(II) ions concentration might be due to the saturation of available active sites on the mixed biosorbent. Fig. 7 shows that there is an increase in the specific uptake of nickel(II) ions with the increase in initial nickel(II) ions concentration. Biosorbent offer a limited number of surface binding sites showed the reduction of specific uptake at higher metal ion

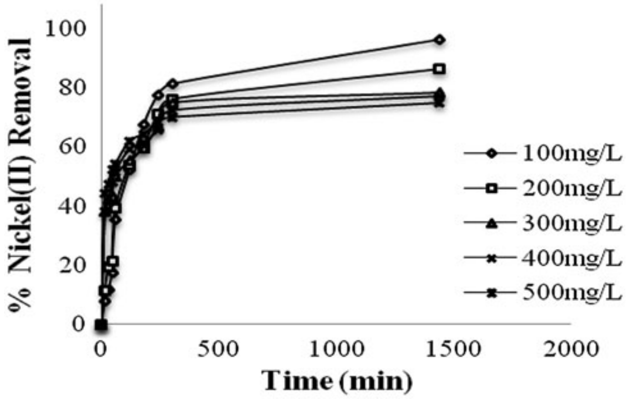

Fig. 6. Effect of initial nickel(II) concentration on removal of nickel(II).

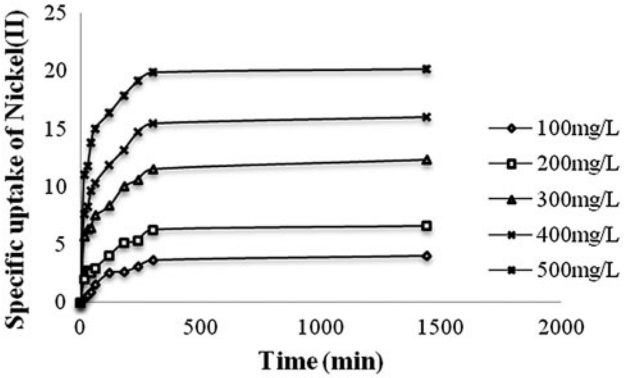

Fig. 7. Effect of initial nickel(II) concentration on specific uptake of nickel(II).

concentration which indicates that the accessible sites for the biosorption process were very fewer at higher initial metal ion concentrations. Furthermore, the higher initial metal ion concentration generates an important driving force to conquer all mass-transfer resistance of nickel(II) ions between the aqueous and the solid phases [21,22].

3.6. CCD and optimization using RSM for the biosorption of chromium(VI)

RSM is very valuable and admired tool to optimize the process parameters having equal significance and influence each other in the process. Table 5 gives the coded and uncoded values of the combination of the variables along with the experimental responses (% removal of chromium) used to optimize the parameters of pH, initial metal ion concentration, biosorbent loading, and temperature for the biosorption of chromium(VI). The removal of chromium(VI) depends on the individual effects of the grouping of test variables and the significant difference was observed for each combination [23,24].

Multiple regression coefficient R was projected from the second-degree polynomial equation. The regression value 0.90 closer to one indicates that the correlation coefficient definites the performance of the system. The investigational and predicted data were in fine accordance with each other. Multiple regression analysis of the investigational data yielded the following regression equation for the biosorption of chromium(VI).

$$\begin{aligned} Y = {} & 90.54 - 2.172X_1 + 8,424X_2 - 1.3529X_3 + 0.1188X_4 \\ & - 1.7751X_2^2.1289X_2^2 - 4.4564X_2^3 - 1.0139X_4^2 \\ & - 0.7644X_1X_2 - 0.7644X_1X_3 - +2.3481\mathrm{X}_1\mathrm{X}_4 \\ & - 0.9919X_2X_3 - 1.0594X_2X_4 - 0.3631X_3X_4 \end{aligned}$$

Y—response variable, the removal of chromium(VI), and X_1, X_2, X_3, and X_4 were the coded values of the independent variables namely pH, initial metal ion concentration, biosorbent loading, and temperature, respectively.

The data obtained from CCD namely the T distribution, the p values, and the parameters estimates are given in Table 6. The p values were used as a utensil to check the importance of each of the coefficients, which in turn, showed the patterns of the interface among the variables. Larger the magnitude of t and smaller the value of p depicted that the consequent coefficient was more significant. The effect of initial chromium(VI) ion concentration was found to be highly significant ($p = 0.060$) on % removal of chromium. It was observed from the coefficient of X_1, that the % removal of chromium was high at 100 mg/L. Further increase in initial chromium(VI) ion concentration decreased the % removal of chromium(VI). The elevated removal rates indicated a high affinity of mixed biosorbent for chromium(VI) molecules. The squared effect of temperature ($p = 0.000$) was found to be significant. The coefficient of the interaction terms of biomass loading and initial metal ion concentration was also found be highly significant.

Table 7 expose the analysis of variance (ANOVA) summary of metal for chromium(VI) biosorption. ANOVA is essential to test the consequence and capability of the model. The mean squares are obtained by dividing the total of squares of each of the two sources of variations, the model and the error variance, by the respective degrees of freedom. The fishers variance ratio, F value = (S_r^2/S_e^2), is the ratio of the mean square outstanding to regression to the mean square owing to error. It is the quantity of variation in

Table 5
Full factorial central composite design matrix of orthogonal and real values along with observed responses for the biosorption of chromium(VI)

	Orthogonal values				Real values				
S. No.	X_1	X_2	X_3	X_4	X_1	X_2	X_3	X_4	Response (percentage removal of chromium)
1	−1	+1	+1	+1	200	5	33	25	78.7
2	+1	−1	−1	−1	400	3	27	15	74.13
3	−1	+1	−1	+1	200	5	27	25	81.27
4	−1	−1	−1	−1	200	3	27	15	85.7
5	+1	+1	−1	+1	400	5	27	25	83.12
6	+1	+1	+1	+1	400	5	33	25	81.7
7	+1	+1	+1	−1	400	5	33	15	79.67
8	+1	+1	−1	−1	400	5	27	15	76.33
9	+1	−1	+1	+1	400	3	33	25	82.28
10	−1	−1	+1	−1	200	3	33	15	87.58
11	−1	+1	−1	−1	200	5	27	15	76.67
12	+1	−1	−1	+1	400	3	27	25	77.1
13	−1	+1	+1	−1	200	5	33	15	87.46
14	+1	−1	+1	−1	400	3	33	15	79.1
15	−1	−1	−1	+1	200	3	27	25	70.43
16	−1	−1	+1	+1	200	3	33	25	84.41
17	+2	0	0	0	500	4	30	20	76.73
18	−2	0	0	0	100	4	30	20	93.4
19	0	2	0	0	300	6	30	20	71.56
20	0	−2	0	0	100	3	36	10	95.74
21	0	0	2	0	300	4	36	20	73.42
22	0	0	−2	0	300	4	27	20	75.26
23	0	0	0	2	300	4	30	30	90.73
24	0	0	0	−2	300	4	30	10	85.49
25	0	0	0	0	300	4	30	20	90.54
26	0	0	0	0	300	4	30	20	90.54
27	0	0	0	0	300	4	30	20	90.54
28	0	0	0	0	300	4	30	20	90.54
29	0	0	0	0	300	4	30	20	90.54
30	0	0	0	0	300	4	30	20	90.54
31	0	0	0	0	300	4	30	20	90.54

the data about the mean. Here, the ANOVA of the regression model demonstrates that the model is extremely important, as was obvious from the calculated F value (2.77) and a very low possibility value (P model < $F = 0.027$), and it is observed that the coefficient for the squared effect is extremely important ($p = 0.003$) when match with the individual and interactive effects.

The response surfaces plots could be used to calculate the finest range for diverse values of the test variable from the rounded or cryptic nature of the contours. The rounded nature of the curve signifies that the interactive effects between tests were not significant. Figs. 8–10 show the response surface plot for the biosorption of chromium(VI) and interactive effects pH, initial metal ion concentration, biosorbent loading, and temperature on % removal of chromium(VI). It was clear from the rounded nature of the contours that the contact between the character variables was negligible [25–27].

The second-degree polynomial regression equation was noted and the most favorable values of the test variables were obtained in coded units and then renewed into uncoded units and the consequences are given in Table 8.

Batch biosorption experiments were performed under the exceeding optimized conditions and the investigational values are given in Fig. 11. Maximum % removal of chromium(VI) (95.7%) was obtained under the finest conditions. The obtained value strongly agrees with the values obtained from the response surface analysis that the RSM using statistical design of

Table 6
Significance of regression coefficients for the biosorption of chromium(VI) using minitab 15 software

Model term	Parameter estimate (coefficients)	*T*	*P*
Constant	90.54	45.611	0.000
X_1	−2.1721	−2.026	0.060
X_2	−1.8404	−1.717	0.105
X_3	1.3529	−1.262	0.225
X_4	0.1188	0.111	0.913
X_1X_1	−1.7751	−1.807	0.090
X_2X_2	−2.1289	−2.168	0.046
X_3X_3	−4.4564	−4.537	0.000
X_4X_4	−1.0139	−1.032	0.317
X_1X_2	0.7644	0.582	0.567
X_1X_3	−0.7506	−0.572	0.575
X_1X_4	2.3481	1.788	0.093
X_2X_3	−0.9919	−0.755	0.461
X_2X_4	1.0594	0.807	0.432
X_3X_4	−0.3631	−0.277	0.786

Table 7
Analysis of variance (ANOVA) for the selected quadratic model for the biosorption of chromium(VI)

Sources of variation	Sum of squares	Degrees of freedom	Mean square	*F*	*P*
Regression	1068.65	14	76.332	2.77	0.027
Linear	238.79	4	59.697	2.16	0.120
Square	687.47	4	171.867	6.23	0.003
Interaction	142.39	6	23.732	0.86	0.544
Residual	441.33	16	27.583		
Error	0.00	6			
Total	1509.98	30			

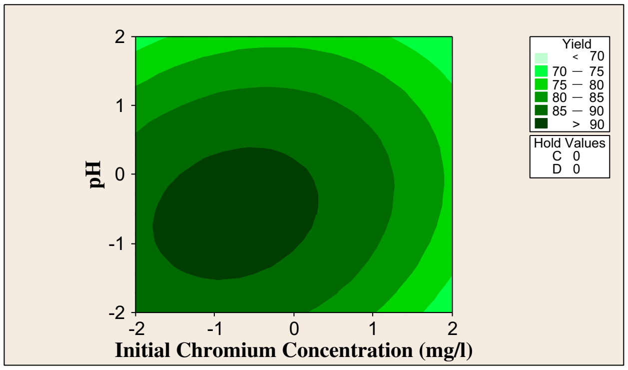

Fig. 8. Response surface contour plot showing interactive effect of initial chromium concentration and pH on the removal of chromium(VI).

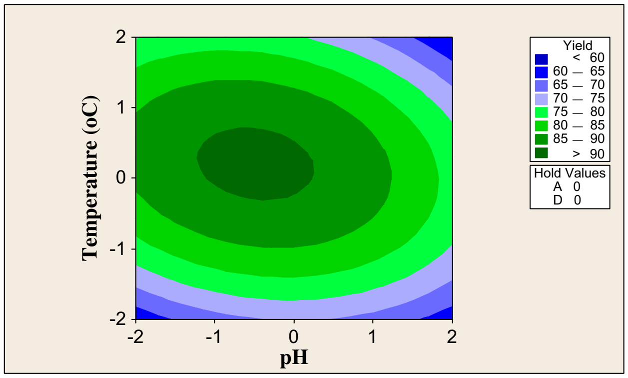

Fig. 9. Response surface contour plot showing interactive effect of pH and temperature on the removal of chromium (VI).

experiments could be successfully used to optimize the process parameters and to learn the significance of individual, collective, and interactive effects of the test variables in the biosorption of chromium(VI) [28–30]. Response surface were drained for the investigational results obtained from the effect of diverse variables on the % removal of chromium in order to conclude the individual and escalating effects of these variables.

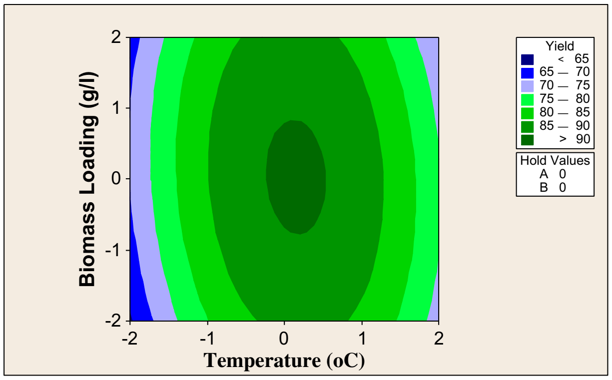

Fig. 10. Response surface contour plot showing interactive effect of temperature and biomass loading on the removal of chromium(VI).

Table 8
Optimum values of variables obtained from regression equations for the biosorption of chromium(VI)

Parameter	Optimum value for biosorption of chromium
Initial metal concentration(mg/l), X_1	100
pH, X_2	3
Temperature (°C), X_3	36
Biomass loading(g/l), X_4	10
Percentage removal of chromium	95.7

Table 9
Full-factorial central composite design matrix of orthogonal and real values along with observed responses for the biosorption of nickel(II)

	Orthogonal values				Real values				
S. No.	X_1	X_2	X_3	X_4	X_1	X_2	X_3	X_4	Response (percentage removal of chromium)
1	−1	+1	+1	+1	50	6	33	25	77.54
2	+1	−1	−1	−1	150	4	27	15	66.41
3	−1	+1	−1	+1	50	6	27	25	79.23
4	−1	−1	−1	−1	50	4	27	15	63.56
5	+1	+1	−1	+1	150	6	27	25	74.63
6	+1	+1	+1	+1	150	6	33	25	74.23
7	+1	+1	+1	−1	150	6	33	15	74.88
8	+1	+1	−1	−1	150	6	27	15	79.75
9	+1	−1	+1	+1	150	4	33	25	70.16
10	−1	−1	+1	−1	50	4	33	15	68.96
11	−1	+1	−1	−1	50	6	27	15	84.25
12	+1	−1	−1	+1	150	4	27	25	66.99
13	−1	+1	+1	−1	50	6	33	15	77.19
14	+1	−1	+1	−1	150	4	33	15	69.98
15	−1	−1	−1	+1	50	4	27	25	72.16
16	−1	−1	+1	+1	50	4	33	25	80.34
17	+2	0	0	0	200	5	30	20	77.09
18	−2	0	0	0	25	5	30	20	76.73
19	0	2	0	0	100	7	30	20	77.1
20	0	−2	0	0	100	3	30	20	62.34
21	0	0	2	0	100	5	36	20	64.38
22	0	0	−2	0	100	5	27	20	69.08
23	0	0	0	2	100	5	30	10	96.41
24	0	0	0	−2	100	5	30	10	80.09
25	0	0	0	0	100	5	30	20	81.4
26	0	0	0	0	100	5	30	20	81.4
27	0	0	0	0	100	5	30	20	81.4
28	0	0	0	0	100	5	30	20	81.4
29	0	0	0	0	100	5	30	20	81.4
30	0	0	0	0	100	5	30	20	81.4
31	0	0	0	0	100	5	30	20	81.4

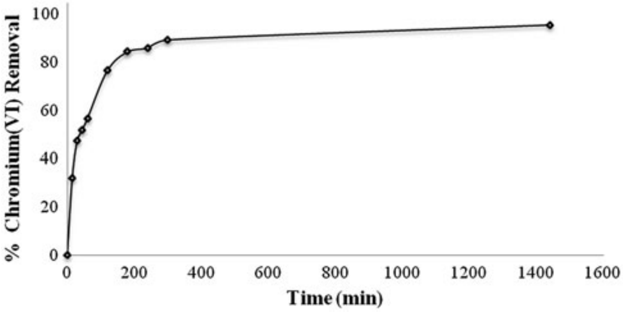

Fig. 11. Biosorption of chromium(VI) by mixed biosorbents under optimum conditions.

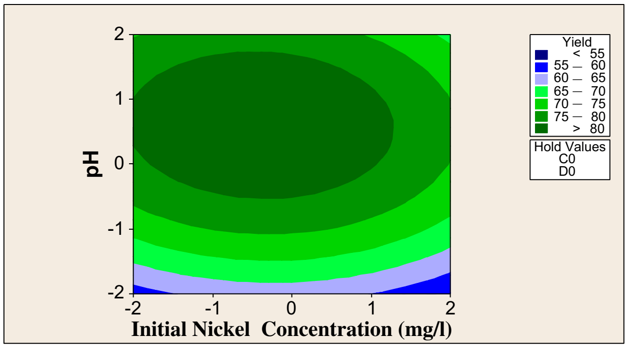

Fig. 12. Response surface contour plot showing interactive effect of initial nickel concentration and pH on the removal of nickel(II).

Table 10
Significance of regression coefficients for the biosorption of nickel(II) using minitab 15 software

Model term	Parameter estimate (coefficients)	*T*	*P*
Constant	82.81	96.298	0.000
X_1	−1.0617	−2.286	0.036
X_2	3.8608	8.314	0.000
X_3	−0.1292	−0.278	0.784
X_4	0.6975	1.502	0.153
X_1X_1	−1.4737	−3.464	0.003
X_2X_2	−3.2712	−7.689	0.000
X_3X_3	−4.0187	−9.446	0.000
X_4X_4	−0.2762	−0.649	0.525
X_1X_2	−0.2025	−0.356	0.726
X_1X_3	−0.2100	−0.369	0.717
X_1X_4	−1.2700	−2.233	0.040
X_2X_3	−2.1463	−3.774	0.002
X_2X_4	−1.9488	−3.426	0.003
X_3X_4	0.7638	1.343	0.198

Table 11
Analysis of variance (ANOVA) for the selected quadratic model for the biosorption of nickel(II)

Sources of variation	Sum of squares	Degrees of freedom	Mean square	F	P
Regression	1,288.12	14	1,288.12	17.78	0.000
Linear	396.87	4	396.87	19.17	0.000
Square	720.28	4	720.28	34.79	0.000
Interaction	170.97	6	170.97	5.51	0.003
Residual	82.81	16	82.81		
Error	21.26	6	21.26		
Total	1,370.94	30			

3.7. CCD and optimization using RSM for the biosorption of nickel(II)

Table 9 gives the coded and uncoded values of the mixture of the variables along with the observed responses (% removal of nickel) used to optimize the process parameters of pH, initial metal ion concentration, biosorbent loading, and temperature for the biosorption of nickel(II). The investigational data are analyzed using CCD and optimized with RSM [31–33].

Multiple regression study of the investigational data yielded the following regression equation for the biosorption of nickel(II).

$$\begin{aligned} Y = {} & 82.81 - 1.0617X_1 + 3.8608X_2 - 0.1292X_3 + 0.6975X_4 \\ & - 1.4737X_1^2 - 3.2712X_2^2 - 4.0187X_2^3 - 0.2762X_4^2 \\ & - 0.2025X_1X_2 - 0.21X_1X_3 - 1.27X_1X_4 \\ & - 2.1463X_2X_3 - 1.9488X_2X_4 + 0.7638X_3X_4 \end{aligned}$$

In which Y is the response variable, the % removal of nickel(II), and X_1, X_2, X_3, and X_4 are the coded values of the independent variables namely pH, initial metal ion concentration, biosorbent loading, and temperature, respectively [34–36].

Multiple regression coefficient R was predictable from the second-degree polynomial equation. The value of 0.9396 showed that the correlation coefficient strongly agrees with the investigational results. The results obtained from CCD are given in Table 10. The effect of pH was originate to be extremely important ($p = 0.000$) on % removal of nickel(II). The squared effect of pH, initial metal ion concentration, biosorbent loading, and temperature was found to be extremely important. The coefficient of the interaction terms of pH, temperature, and biomass loading was also found be highly significant [37–39]. Table 11 shows the study of variance (ANOVA) summary of model for nickel(II) biosorption. Here, the ANOVA of the regression model exposed that the model was extremely important, and was obvious from the calculated F value (17.78) and a very low possibility value (P model < $F = 0.000$). It was observed that the coefficient for all the effects were exceedingly significant.

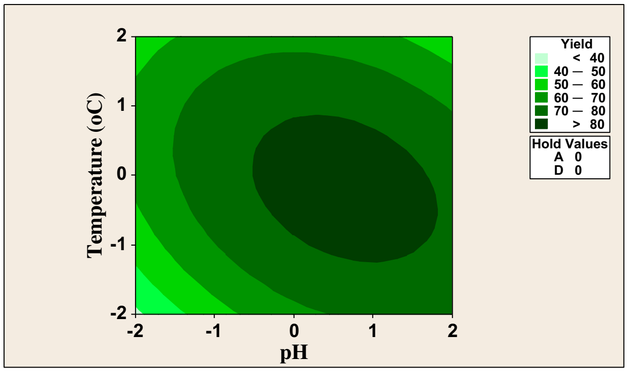

Fig. 13. Response surface contour plot showing interactive effect of pH and temperature on the removal of nickel(II).

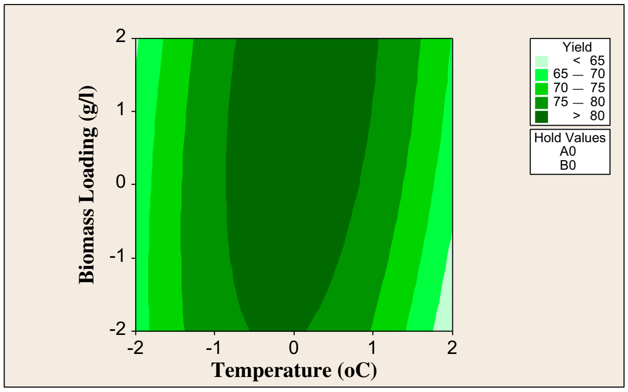

Fig. 14. Response surface contour plot showing interactive effect of temperature and biomass loading on the removal of nickel(II).

Table 12
Optimum values of variables obtained from regression equations for the biosorption of nickel(II)

Parameter	Optimum value for Biosorption of nickel
Initial metal concentration (mg/l), X_1	100
pH, X_2	5.6
Temperature (°C), X_3	30
Biomass loading (g/l), X_4	10
Percentage removal of nickel	96.41

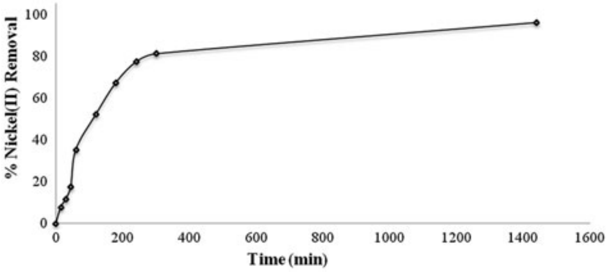

Fig. 15. Biosorption of nickel(II) by mixed biosorbents under optimum conditions.

Figs. 12–14 show the response surface plot for the biosorption of nickel(II) and interactive effects of pH, initial metal ion concentration, biosorbent loading, and temperature on % removal of nickel(II). It was noticeable from the rounded nature of the contours that the interface between the individual variables was negligible. The second-degree polynomial regression equation was noted and the results are given in Table 12.

Batch biosorption experiments are performed under the exceeding optimized conditions and the experimental values are given in Fig. 15. Maximum % removal of nickel(II) (96.41%) was obtained under finest conditions. These results were in conformity with response surface analysis results confirming that the RSM could be successfully used to optimize the process parameters. The inferences obtained from the RSM based on the investigational design model in relation to biosorption of nickel(II) by the mixed biosorbent authenticated that RSM were very capable to optimize the process parameters.

4. Conclusions

The result of this research showed that the immobilized mixed biosorbents (custard apple seeds and *Aspergillus niger*) can be used as an efficient biosorbent for the removal of chromium(VI) and nickel(II) ions from the contaminated water. The biosorption experiments were performed by several factors such as initial metal ion concentration, biosorbent loading, pH, and temperature. The maximum removal of chromium(VI) was obtained at 36°C, 10 g/L (biosorbent loading) and 100 mg/L (initial metal ion concentration). Similarly, for nickel(II), it was found to be at 30°C, 10 g/L (biosorbent loading) and 100 mg/L (initial metal ion concentration). The present study shows that the newly prepared mixed biosorbent has high potential biosorption capacity for the removal of chromium(VI) and nickel(II) ions from aqueous solutions. The experimental values were in good agreement with the simulated values from the response surface analysis and its confirming that the RSM using statistical design of experiments could be effectively used to optimize the process parameters and to study the importance of individual, increasing, and interactive effects of the text variables in the biosorption of chromium(VI) and nickel(II). The obtained results showed that the mixed biosorbent can be used as a suitable biosorbent for the removal of chromium(VI) and nickel(II) ions from the wastewater.

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