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Optimization studies on biosorption of Cr(VI) and Cu(II) from wastewater in a packed bed bioreactor

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ABSTRACT

The objective of this present study is the optimization of process parameters for the biosorption of Cr(VI) and Cu(II) ions by *Bacillus subtilis* in a packed bed bioreactor using response surface methodology (RSM). Continuous biosorption studies were conducted under optimized bed height, initial metal ions concentration, and flow rate for the removal of Cr(VI) and Cu(II) ions using *B. subtilis*. The biosorption parameters were optimized using RSM in both batch and packed bed bioreactor studies. From the studies it is noticed that the maximum biosorption of Cr(VI) and Cu(II) by *B. subtilis* was at optimum conditions of contact time of 30 min, pH of 4.0, biomass concentration of 2 mg/mL, and temperature of 32˚C in batch biosorption studies and bed height of 20 cm, flown rate of 300 mL/h, and initial metal ion concentration of 100 mg/L in packed bed bioreactor biosorption studies. Predicted percent biosorption of the selected heavy metal ions by the design expert software is in agreement with experimental results of percent biosorption. The percent biosorption of Cr(VI) and Cu(II) in batch studies is 80 and 78.4%, respectively. The percent biosorption of Cr(VI) and Cu(II) in packed bed bioreactor studies is 91.7 and 94%, respectively.

Keywords: Packed bed bioreactor; Response surface methodology; *Bacillus subtilis*; Biosorption; Wastewater; Optimization; Breakthrough curve

1. Introduction

Chromium compounds are widely used in various industries, such as electroplating, leather tanning, mining, aluminum conversion coating, operation dyes, and pigments [1,2]. The indiscriminate discharge of chromium metal into water resources causes serious health effects to humans and environment because of its toxic nature. Cr(VI) ions are highly toxic. Inhalation of Cr(VI) ions leads to the carcinogenetic problem. Other health effects of Cr(VI) ions are skin allergy and liver and stomach problems [3]. Copper[Cu(II)] can be found in many wastewater sources, including printed circuit board manufacturing, electronics plating, wire drawing, copper polishing, paint manufacturing, and in wood preservatives and printing operations. Typical concentrations vary from several thousand mg/L from plating bath waste to less than 1 ppm from copper cleaning operations. Copper[Cu(II)] is present in the wastewater of several industries, such as metal cleaning and plating baths, refineries, paper and pulp, fertilizer, and wood

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preservatives [4]. The excessive intake of copper by man leads to severe mucosal irritation, widespread capillary damage, hepatic and renal damage, central nervous problems followed by depression, gastrointestinal irritation, and possible necrotic changes in the liver and kidney [5]. Thus, the removal of Cr(VI) and Cu(II) ions becomes mandatory. Packed bed column reactors are commonly used in both industry and research for separation of molecules, determination of relative molecular mass, identification of substances, and for purification purposes. Picanco et al. [6] reported that the efficiency of removing organic matter in fixed bed reactors is directly related to the characteristics of the support material used for immobilization of anaerobes. It is widely accepted that organic support material has a higher affinity than inorganic material [7]. In many cases, immobilized biomass reactors completely recover their performance after such deleterious occurrences [8,9]. On comparison between batch biosorption and packed bed bioreactor studies, it was noticed that the maximum biosorption yields were obtained in packed bed bioreactor at optimum conditions; so, the packed bed bioreactor would be a good choice for the removal of Cr(VI), Cu(II), Ni(II), and Cd(II) from wastewater using the isolated and identified bacterial species.

2. Materials and methods

The bacterial isolate was cultivated aerobically at 30˚C in nutrient broth by constantly agitating at 150 rpm in conical flasks. The cells were harvested by centrifugation (9,000 rpm, 10 min) from culture at early stationary phase. After rinsing in distilled water the cells were again centrifuged.

2.1. Cell immobilization

2.1.1. Immobilization with agar (A)

For the preparation of agar gel particles, the method described below was followed. Agar (100 mg) was dissolved in 4.5 mL of 0.9% (w/v) NaCl by heating at 100˚C and then cooling to 50˚C. Cell slurry (10–30 mg dry weight per 100 mL) was suspended in 0.9% (w/v) NaCl solution. 0.5 mL of the cell slurry was added to 4.5 mL of the agar solution at 50˚C and mixed. The solution was poured into Petri dishes. The solution was taken into a big syringe and beads were made. Then, the particles of gel were washed with 0.9% NaCl solution to separate them from intact cells.

2.1.2. Immobilization with polyacrylamide

Six millilitre of the prepared cell suspension (17–100 mg dry cell per mL) was rapidly mixed with a solution containing 1.9 g of acrylamide monomer, 0.1 g of N,N′-methylene-bisacrilamide, 3 mL ammonium persulfate (0.5%, w/v), and 0.2 mL TEMED (50%, w/v) on a shallow plate [10]. The solution was taken into a big syringe and beads were made, and then were rinsed with 0.9% NaCl solution to separate them from intact cells.

Immobilized biosorbents of 3 mm size were made. The size has an impact on the porosity of the bed and the time of contact in bed. As bead size decreases porosity decreases but contact time increases.

2.2. Experimental setup and procedure

A packed bed bioreactor of 50 mm internal diameter and 500 mm length made with heavy-walled borosilicate glass was used for continuous biosorption studies. The metal ion solutions of Cr(VI) and Cu(II) with initial concentrations of 50, 100, and 150 mg/L were continuously pumped downward into the column. The metal ion solution loading rate was varied from 150 to 900 mL/h. Samples were collected from the effluent to measure the residual metal ion concentrations using atomic absorption spectrophotometer.

The breakthrough curves for the biosorption of Cr(VI) and Cu(II) were measured as a function of bed height, initial metal ion concentration, and flow rate. The results were given in terms of the maximum (equilibrium) capacity of the column, $C_{i,\max}$ (mg), the amount of metal loading on the bacterium surface, $q_{i,\mathrm{eq}}$ (mg/g), and the adsorption yield (adsorbed metal percentage), $\%Y_i$. The maximum (equilibrium) capacity of the column for a given feed concentration is equal to the area under the plot of the adsorbed metal ion concentration $C_{i,\mathrm{ads}}$ (mg/L) vs. time (minutes) or the area behind the breakthrough curve. The amount of metal ion that remains in the effluent $C_{i,\mathrm{eq}}$ is the area under the breakthrough curve [11,12].

The amount of metal loading on the bacterium surface is calculated from the weight of the metal adsorbed per unit dry weight of bacterium in the column (Eq. (1)), that is the ratio of the maximum capacity of the column to the amount of biosorbent in the column, X (mg):

$$q_{i,\mathrm{eq}} = \frac{C_{i,\max}}{X} \tag{1}$$

The adsorption yield (Eq. (2)) is the ratio of the maximum capacity of the column to the amount of metal loading into the column, W_i (mg) (Eq. (3)):

$$W_i = C_i \cdot Q \cdot t \quad (2)$$

$$Y_i = \frac{C_{i,\max}}{W_i} \cdot 100 \quad (3)$$

2.3. Effect of different parameters on metal biosorption

2.3.1. Effect of bed height

Packed bed bioreactor with bed lengths of 10, 15, 20, and 25 cm was operated at a constant flow rate of 300 mL/h and inlet metal ion concentration of approximately 100 mg/L. The effect of bed height change on the equilibrium time was measured. The biosorption yields (Y_i) of both Agar-immobilized and PAA-immobilized bacterial strains for the respective heavy metals were determined at different bed heights.

2.3.2. Effect of flow rate

The metal ion solution loading rate was varied from 150 to 900 mL/h. Samples were collected from the effluent to measure the residual metal concentrations. The biosorption yields (Y_i) of both Agar-immobilized and PAA-immobilized bacterial strains of the respective heavy metals were determined at different flow rates.

2.3.3. Effect of initial concentration of metal ion

At constant flow rate and bed height, the initial metal ion concentration was changed from 50 to 150 mg/L. The biosorption capacity of the biomass and saturation values was measured with change of initial metal ion concentration. The biosorption yields (Y_i) of both Agar-immobilized and PAA-immobilized bacterial strains of the respective heavy metals were determined at different initial metal ion concentrations.

2.4. Optimization of parameters in packed bed bioreactor using response surface methodology

The relationship between the experimental variables and responses was evaluated by generating 3D response surface and contour plots. The experimental design employed in the screening of each variable consists of two levels and three independent variables. Three parameters, such as flow rate, initial metal ion concentration, and bed height, were optimized using RSM in the packed bed bioreactor. At optimum conditions results were reproduced.

2.5. Application of biosorption models on the packed bed bioreactor data

2.5.1. Redlich–Peterson isotherm model

Redlich and Peterson suggested a three parameter adsorption isotherm equation in 1959. The equation amends inaccuracies of two parameter Langmuir and Freundlich isotherm equations in some adsorption systems. Reddlich and Peterson [13] isotherm can be described as follows (Eq. (4)):

$$Q_e = \frac{K_R C_e}{1 + a_R C_e^{\beta}} \quad (4)$$

K_R (L/g), a_R (L/mg), and β (lies between 0 and 1) are empirical parameters without physical meaning.

2.5.2. Sips model

The Sips model [14] is another empirical model for representing the equilibrium adsorption data. It is a combination of the Langmuir and Freundlich isotherm type models. The Sips model takes the following form for single solute equilibrium data (Eq. (5)):

$$Q_e = \frac{K_s C_e^{1/b_s}}{1 + a_s C_e^{1/b_s}} \quad (5)$$

K_s, a_s, and b_s are the sips isotherm parameters. Unlike the other mentioned adsorption isotherm models, this model contains three parameters that can be evaluated by fitting the experimental data to this model. The Sips model can be extended to describe the multi-component adsorption equilibrium data.

2.5.3. Toth model

The Toth isotherm model [15] is another empirical equation developed to improve the fitness of the Langmuir isotherm model to the experimental data and is useful in describing heterogeneous adsorption systems. Its correlation presupposes an asymmetrical quasi-Gaussian energy distribution, with most of its sites having an adsorption energy lower than the peak (maximum) or mean value.

The Toth model can be represented by the following equation (Eq. (6)):

$$Q_e = \frac{(K_t C_e)}{\left[(a_t + C_e)^{\frac{1}{t}}\right]} \quad (6)$$

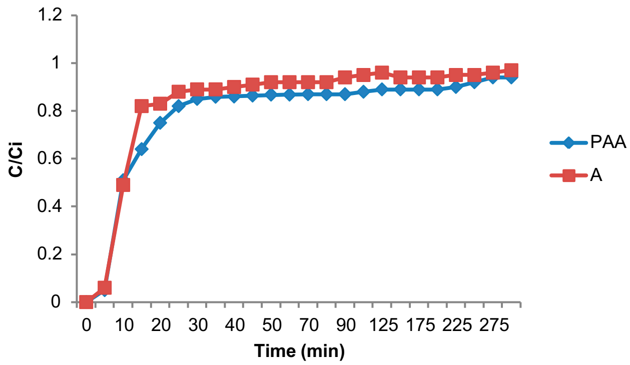

Fig. 1. Comparison of the breakthrough curves of Cr(VI) for Agar-immobilized *B. subtilis* and PAA-immobilized *B. subtilis* (determined optimum conditions; flow rate: 300 mL/h, initial metal ion concentration: 100 mg/L, $W_{\text{biosorbent}}$: 12 g of 20 cm bed height).

K_t (mg/g), a_t, and t represent the Toth isotherm constants.

These models are used to validate the results obtained from the packed bed bioreactor as these models are used when dead biomass is used. Selected models are used for energy-independent processes like biosorption.

3. Results and discussion

3.1. Effect of column operating conditions on adsorption behavior

Fixed bed column with the bed heights of 10, 15, 20, and 25 cm was operated at a flow rate in the range from 150 to 900 mL/h and initial metal ion concentration in the range from 50 to 150 mg/L. Increasing the bed height from 10 to 25 cm led to the prolongation of equilibrium time. The adsorption yields (Y_i, Eq. (3)) of both Agar (A) immobilized *Bacillus subtilis* and Polyacrylamide (PAA) immobilized *B. subtilis* were high for the bed height of 20 cm, whereas the 10 cm had lower Y_i value, especially PAA-immobilized *B. subtilis*, which may be due to a relatively small amount of adsorbents in a shorter bed. It was observed that Y_i values obtained were quite high for both Agar-immobilized *B. subtilis* and PAA-immobilized *B. subtilis* in bed height of 20 cm. The maximum value of $C_{i,\max}$ was obtained at a bed height of 20 cm and slightly decreased at 25 cm. Maximum adsorption yields (Y_i) of Cu(II) were 93.0 and 83.2%, respectively, for the Agar-immobilized *B. subtilis* and PAA-immobilized *B. subtilis*. Maximum adsorption yields (Y_i) of Cr(VI)

Table 1
Experimental data of the effect of process parameters on the percent removal of Cr(VI) and Cu(II) by *B. subtilis*

Std	Run	Bed height (cm)	Flow rate (mL/h)	IMC (mg/L)	% Cr (VI)	% Cu (II)
1	19	17.5	525	100	86.0	89.0
2	10	25.0	525	100	84.0	86.0
3	18	17.5	525	100	87.0	88.0
4	15	17.5	525	100	85.0	86.0
5	2	25.0	150	50	88.0	88.0
6	17	17.5	525	100	87.0	89.0
7	20	17.5	525	100	85.0	87.0
8	1	10.0	150	50	89.0	87.0
9	14	17.5	525	150	82.0	77.0
10	6	25.0	150	150	86.0	89.0
11	7	10.0	900	150	59.0	57.0
12	8	25.0	900	150	56.0	54.0
13	16	17.5	525	100	87.0	89.0
14	3	10.0	900	50	48.0	49.0
15	12	17.5	900	100	56.0	57.0
16	4	25.0	900	50	41.0	42.0
17	5	10.0	150	150	78.0	77.0
18	13	17.5	525	50	78.0	75.0
19	9	10.0	525	100	86.0	88.0
20	11	17.5	150	100	90.0	93.0

Table 2
Experimental design and results of percent biosorption of Cr(VI) and Cu(II) by *B. subtilis*

		% Biosorption of Cr(VI)		% Biosorption of Cu(II)	
Standard order	Run order	Actual value	Predicted value	Actual value	Predicted value
1	8	89	88.26	87.0	86.27
2	5	88	88.26	88.0	88.47
3	14	48	48.56	49.0	49.77
4	16	41	40.06	42.0	40.47
5	17	78	78.66	77.0	77.87
6	10	86	85.16	89.0	87.57
7	11	59	58.46	57.0	55.87
8	12	56	56.46	54.0	54.07
9	19	86	86.03	88.0	88.18
10	2	84	85.03	86.0	88.38
11	20	90	90.63	93.0	93.78
12	15	56	56.43	57.0	58.78
13	18	78	78.83	75.0	75.98
14	9	82	82.23	77.0	78.58
15	4	85	85.80	86.0	87.14
16	13	87	85.80	89.0	87.14
17	6	87	85.80	89.0	87.14
18	3	87	85.80	88.0	87.14
19	1	86	85.80	89.0	87.14
20	7	85	85.80	87.0	87.14

were 90.0 and 88.3%, respectively, for the A-immobilized *B. subtilis* and PAA-immobilized *B. subtilis*. The change in the inlet ionic concentration of the feed has affected the operating characteristics of the fixed bed column. When the flow rate (300 mL/h) and bed height (20 cm) were kept constant, initial metal ion concentrations were changed from 50 to 150 mg/L. The biosorption capacity of the biomass increased first with increasing the initial metal ion concentration and then reached a saturation value. These saturation values were around 100 mg/L for A- and PAA-immobilized *B. subtilis*. Maximum adsorption yields for Agar-immobilized *B. subtilis* and PAA-immobilized *B. subtilis* were determined as 88.0 and 82.8%, respectively, at their optimum initial Cu(II) concentrations. Maximum adsorption yields for Agar-immobilized *B. subtilis* and PAA-immobilized *B. subtilis* were determined as 93.5 and 86.8%, respectively, at their optimum initial Cr(VI) concentrations.

3.2. *Breakthrough curves of Cr(VI) biosorption for immobilized* B. subtilis

On comparison of the breakthrough curves (Fig. 1) of Cr(VI) for Agar-immobilized and PAA-immobilized bacterial strains at optimum conditions of flow rate of 300 mL/h, initial metal ion concentration of 100 mg/L, and bed height of 20 cm with weight of biosorbent of 12 g, it was found that the Agar-immobilized *B. subtilis* showed maximum percent biosorption and bed saturation occurred at 20 min.

3.3. *Optimization of parameters in a packed bed bioreactor using response surface methodology*

From the experimental work, it is seen that the optimum parameters for packed bed biosorption studies are bed height of 20 cm, initial metal ion concentration of 100 mg/L, and flow rate of 300 mL/h. Optimization results of Cr(VI) and Cu(II) by *B. subtilis* from the Design Expert software were obtained as bed height of 19.93 cm, initial metal ion concentration of 103.85 mg/L, and flow rate of 310.57 mL/h. The percent biosorption of Cr(VI) and Cu(II) is 91.7 and 94%, respectively. The predicted optimized parameters are in agreement with the experimental results.

3.3.1. *Optimization of operating conditions for the biosorption of Cr(VI) and Cu(II) by* B. subtilis

From the analysis of variance for the quadratic model for Cr(VI) removal at p-value of <0.0001, the model was significant and when p-value was at 0.3682 it was not significant. The coefficient of correlation was obtained as 0.9974 and standard deviation was 1.071 (Tables 1–4).

From the Analysis of variance for the quadratic model for Cu(II) removal at p-value of <0.0001, the model was significant and when p-value was at 0.1179 it was not significant. The coefficient of

Table 3
Analysis of variance for the quadratic model for Cr(VI) biosorption by *B. subtilis*

Source	Sum of squares	*df*	Mean square	*F* value	*p*-value prob > *F*	
Model	4512.33	9	501.369	437.0968	<0.0001	Significant
A-Bed height	2.5	1	2.5	2.1795	0.1706	
B-Flow rate	2924.1	1	2924.1	2549.245	<0.0001	
C-Initial metal ion con.	28.9	1	28.90	25.1951	0.0005	
AB	36.125	1	36.125	31.4939	0.0002	
AC	21.125	1	21.125	18.4168	0.0016	
BC	190.125	1	190.125	165.7519	<0.0001	
A^2	0.2045	1	0.204	0.17832	0.6818	
B^2	414.2045	1	414.204	361.1056	<0.0001	
C^2	76.4545	1	76.454	66.6534	<0.0001	
Residual	11.4704	10	1.147			
Lack of fit	6.6371	5	1.327	1.373197	0.3682	Not significant
Pure error	4.8333	5	0.966			
Cor rotal	4523.8	19				
Std. dev.	1.071002		R^2	0.9974		
Mean	76.9		Adj R^2	0.9951		
C.V. %	1.392721		Pred R^2	0.9785		
PRESS	97.0762		Adeq precision	66.7761		

Table 4
Analysis of variance for the quadratic model for Cu(II) biosorption by *B. subtilis*

Source	Sum of squares	*df*	Mean square	*F* value	*p*-value prob > *F*	
Model	4863.489	9	540.3876	163.4499	<0.0001	Significant
A-Bed height	0.1	1	0.1	0.030247	0.8654	
B-Flow rate	3062.5	1	3062.5	926.3078	<0.0001	
C-Initial metal ion con.	16.9	1	16.9	5.111707	0.0473	
AB	66.125	1	66.125	20.00069	0.0012	
AC	28.125	1	28.125	8.506909	0.0154	
BC	105.125	1	105.125	31.79693	0.0002	
A^2	3.551136	1	3.551136	1.074105	0.3244	
B^2	324.5511	1	324.5511	98.16629	<0.0001	
C^2	267.5511	1	267.5511	80.92562	<0.0001	
Residual	33.06136	10	3.306136			
Lack of fit	25.06136	5	5.012273	3.13267	0.1179	Not significant
Pure error	8	5	1.6			
Cor total	4896.55	19				
Std. dev.	1.818278		R^2	0.9932		
Mean	77.35		Adj R^2	0.9871		
C.V. %	2.350715		Pred R^2	0.9496		
PRESS	246.4416		Adeq precision	41.457		

correlation was obtained as 0.9932 and standard deviation was 1.82.

Regression analysis was performed to fit the response functions, i.e. percentage of biosorption of Cr(VI) and Cu(II). The regression models developed represent responses as functions of bed height (a), flow rate (b), and initial concentration (c). An empirical relationship between the response and three input variables expressed by the following response surface reduced quadratic model equations (Eqs. (7) and (8)) in coded terms for the percent of biosorption of each heavy metal ion:

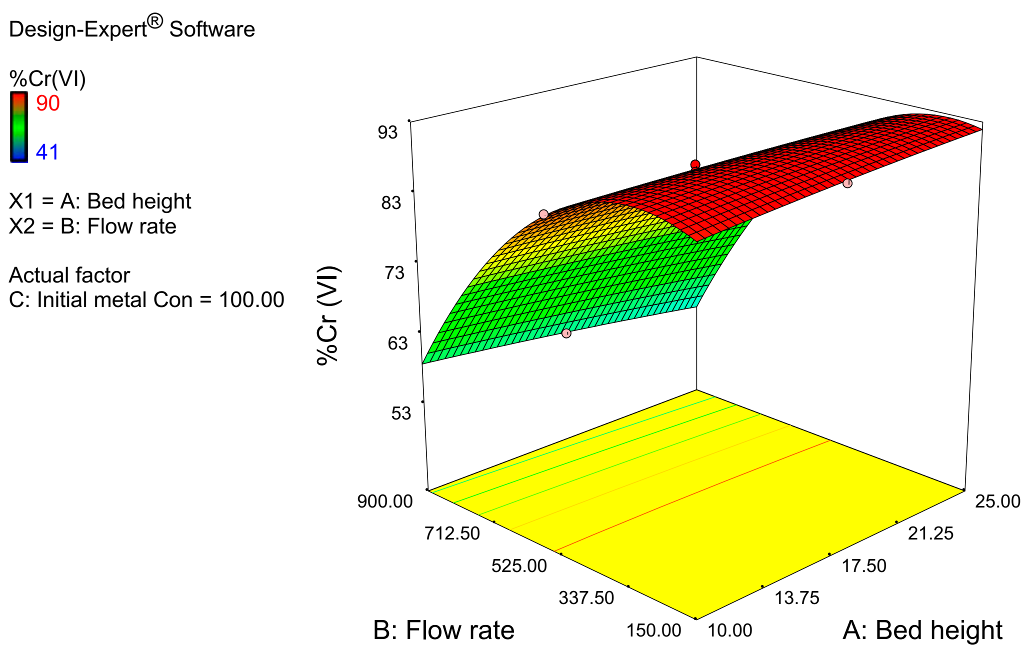

Fig. 2. Response surface 3D plot indicating the effect of interaction between bed height and flow rate on Cr(VI) removal while holding initial metal ion concentration at its design center point 100 mg/L by *B. subtilis*.

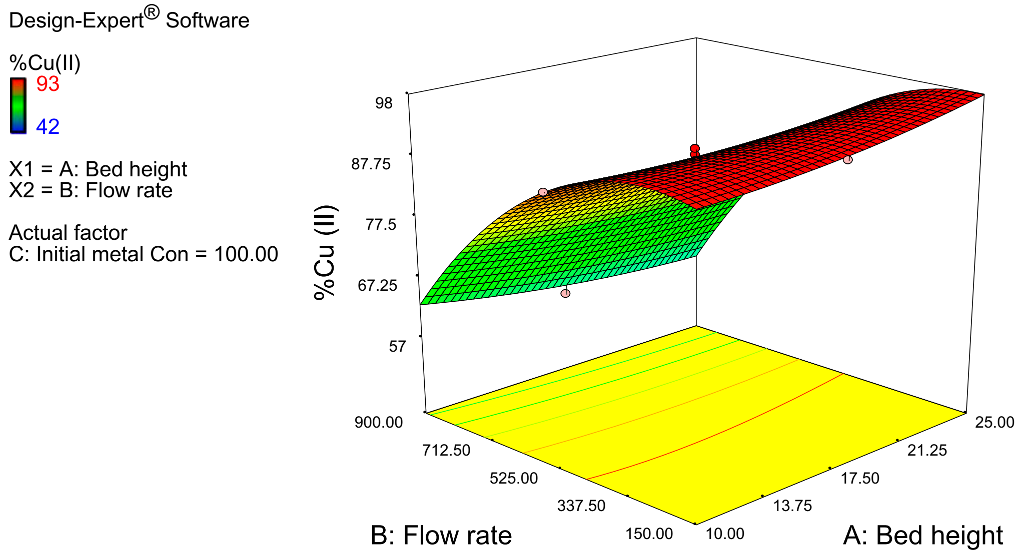

Fig. 3. Response surface 3D plot indicating the effect of interaction between flow rate and initial metal ion concentration on Cr(VI) removal while holding bed height at its design center point 17.5 cm by *B. subtilis*.

$$\%\mathrm{Cr}(VI) = 85.7363 - 0.2A - 17.1B + 1.7C - 2.125AB + 1.625AC - 4.375BC - 0.091A^2 - 12.091B^2 - 5.091C^2 \quad (7)$$

$$\%\mathrm{Cu}(II) = 87.11 + 0.3A - 17.3B + 1.1C - 2.625AB + 1.625AC + 3.375BC + 1.227A^2 - 10.773B^2 - 9.773C^2 \quad (8)$$

Figs. 2 and 3 show the response surface 3D plot for the effect of interaction between bed height and flow rate on Cr(VI) and Cu(II) removal, while the initial metal ion concentration term was at the middle point of the designed range (100 mg/L). It is noticed in these figures that the removal efficiencies increased with increase in bed height. The removal increased up to the bed height of 20 cm and then decreased slightly. The flow rate would significantly affect the

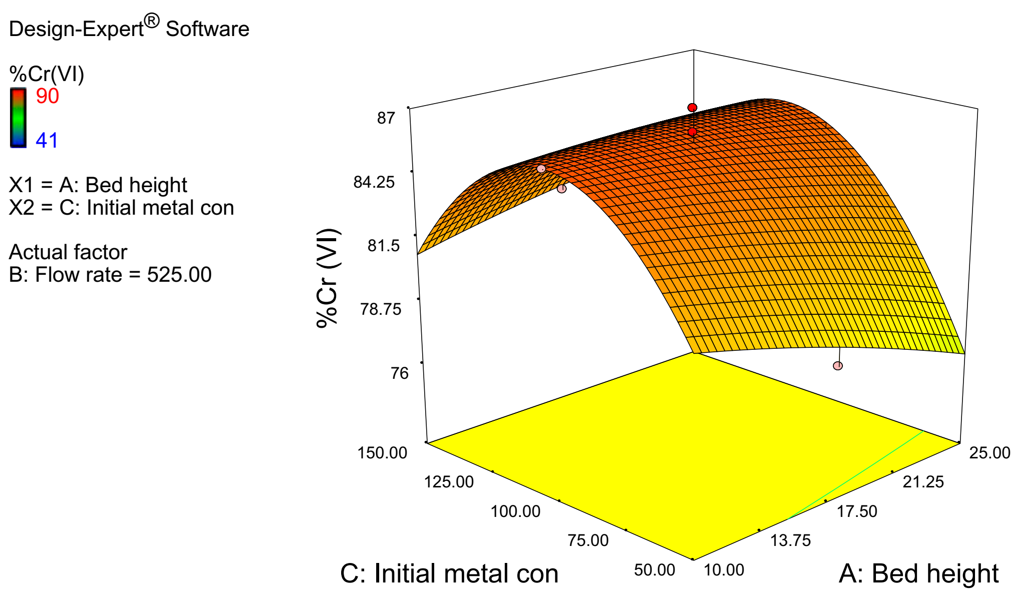

Fig. 4. Response surface 3D plot indicating the effect of interaction between bed height and initial metal ion concentration on Cu(II) removal while holding flow rate at its design center point 525 mL/h by *B. subtilis*.

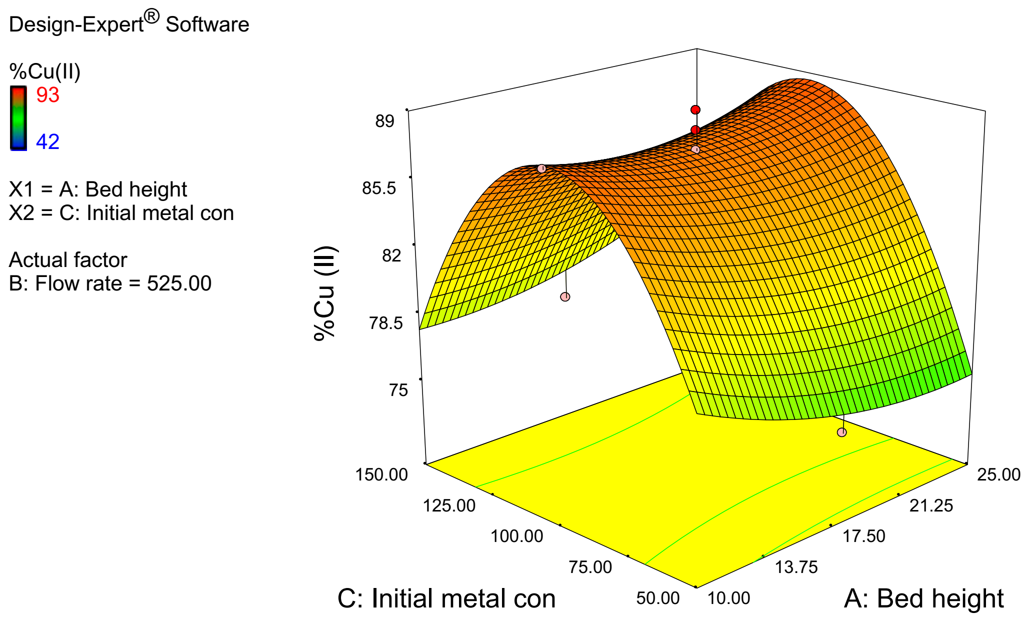

Fig. 5. Response surface 3D plot indicating the effect of interaction between bed height and initial metal ion concentration on Cr(VI) removal while holding flow rate at its design center point 525 mL/h by *B. subtilis*.

Cr(VI) and Cu(II) removal up to 300 mL/h which is predicted in optimum and after this point the removal decreased with increase in bed height.

Figs. 4 and 5 show the response surface 3D plot for the effect of interaction between bed height and initial metal ion concentration on Cr(VI) and Cu(II) removal while the flow rate term was at the middle point of the designed range (525 mL/h). It is noticed in these figures that the removal efficiencies increased with increase in bed height. The removal increased up to the bed height of 20 cm and then decreased slightly. The initial metal ion concentration would significantly affect the Cr(VI) and Cu(II) removal up to 100 mg/L which is predicted in optimum and after

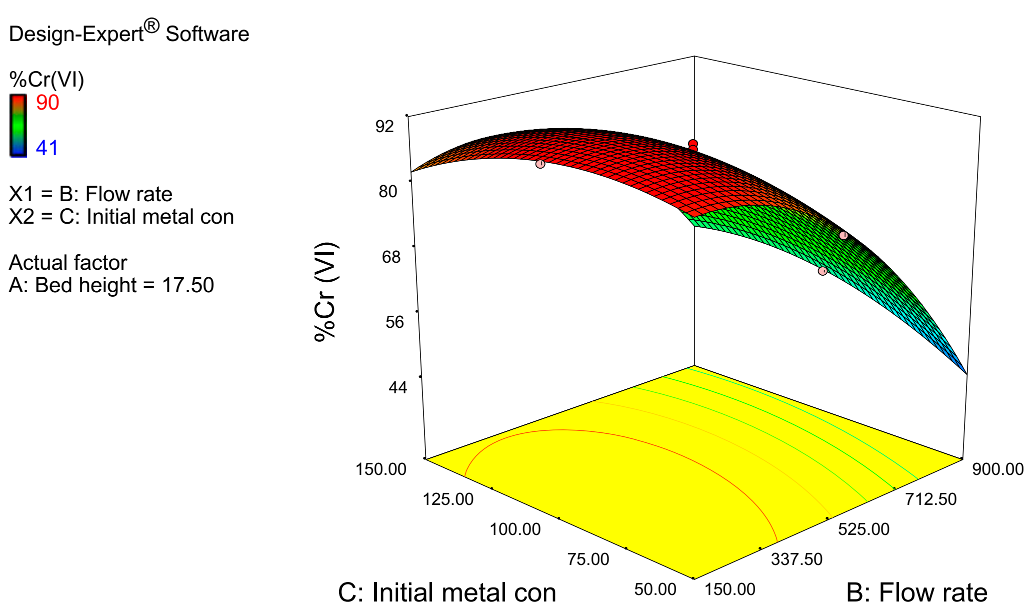

Fig. 6. Response surface 3D plot indicating the effect of interaction between bed height and flow rate on Cu(II) removal while holding initial metal ion concentration at its design center point 100 mg/L by *B. subtilis*.

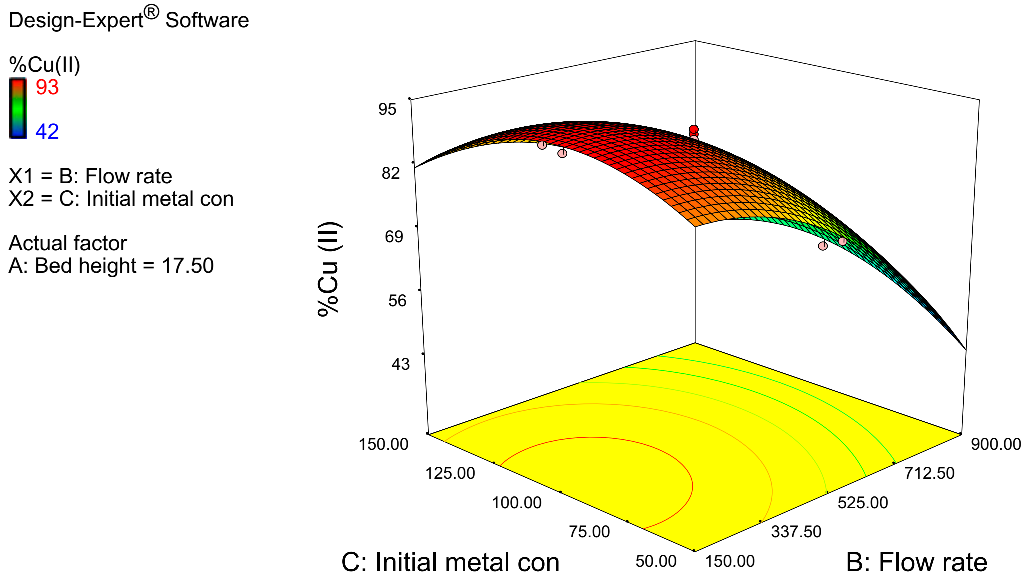

Fig. 7. Response surface 3D plot indicating the effect of interaction between flow rate and initial metal ion concentration on Cu(II) removal while holding bed height at its design center point 17.5 cm by *B. subtilis*.

this point the removal decreased with increase in bed height.

Figs. 6 and 7 show the response surface 3D plot for the effect of interaction between flow rate and initial metal ion concentration on Cr(VI) and Cu(II) removal while the bed height term was at the middle point of the designed range (20 cm). It is noticed in these figures that the removal efficiencies increased with increase in flow rate up to 300 mL/h then decreased drastically (Table 5).

Experiments were carried out at established optimum conditions of bed height of 19.93 cm, flow rate of 316.57 mL/h, and initial metal ion concentration of 103.85 mg/L; results were reproduced and they were in agreement with the predicted results.

Table 5
Optimized values established by Design Expert for the biosorption of Cr(VI) and Cu(II) by *B. subtilis*

Number	Bed height (cm)	Flow rate (mL/h)	Initial metal ion concentration (mg/L)	%Cr(VI)	%Cu(II)	Desirability	
1	11.63	230.41	91.4	90.79	93.15	1	
2	19.93	316.57	103.85	91.79	94.01	1	Selected
3	17.21	241.33	101.94	91.62	93.87	1	
4	24.96	281.57	105.3	92.91	96.95	1	
5	16.99	246.5	89.62	91.79	93.76	1	
6	22.24	185.82	76.78	91.90	94.44	1	
7	23.63	358.48	116.22	91.39	93.95	1	
8	12.74	268.17	93.54	91.09	93.19	1	
9	24.82	238.92	79.8	92.33	95.81	1	
10	12.77	235.9	86.75	91.08	93.04	1	
11	23.42	178.75	82.58	92.38	95.88	1	
12	23.05	346.97	121.92	91.13	93.13	1	
13	24.11	356.28	80.51	90.43	93.09	1	
14	15.56	156.17	87.57	90.77	93.09	1	
15	13.16	216.45	95.49	90.85	93.19	1	
16	15.1	297.25	97.06	91.34	93.21	1	
17	21.44	232.7	120.61	91.29	93.67	1	
18	23.9	305.74	122.64	91.70	94.23	1	
19	11.25	258.27	87.71	90.91	93.07	1	
20	21.69	270.46	113.12	92.04	94.68	1	

Table 6
Kinetic parameters of Redlich and Peterson model for Cr(VI) and Cu(II) biosorption in a packed bed bioreactor

	Reddlich and Peterson model			
Metal ion	K_R	a_R	β	R^2
Cr(VI)	9,155	5,023	0.5234	0.9996
Cu(II)	6,736	5,094	0.469	0.9862

3.4. Application of biosorption models on the packed bed bioreactor data

Redlich–Peterson isotherm model (Eq. (4)), Sips model (Eq. (5)), and Toth model (Eq. (6)) were used to validate and model the packed bed bioreactor biosorption studies and found that the Redlich–Peterson isotherm model fit with the experimental values well. The kinetic parameters of the Redlich and Peterson model for Cr(VI) and Cu(II) biosorption in a packed bed bioreactor are given in Table 6.

The R^2 values ranged from 0.9645 to 1.0, indicating good linearity. On comparison with fitted models, it can, therefore, be said that the high R^2 value indicates that the Redlich and Peterson model equation of linear regression analysis describes the breakthrough data under the studied conditions.

4. Conclusions

In packed bed biosorption studies, experiments were carried out at established optimum conditions of bed height of 19.93 cm, flow rate of 316.57 mL/h, and initial metal ion concentration of 103.85 mg/L; results of biosorption of Cr(VI) and Cu(II) were reproduced and they were in agreement with the predicted results. The R^2 values of Cr(VI) and Cu(II) are 0.9952 and 0.9916, respectively. The adjusted R^2 values of Cr(VI) and Cu(II) are 0.9909 and 0.9840, respectively. The predicted R^2 values of Cr(VI) and Cu(II) are 0.9374 and 0.9248, respectively. The predicted R^2 is in reasonable agreement with the adjusted R^2. Regression analysis was performed to fit the response functions, i.e. percentage biosorption of Cr(VI) and Cu(II). From the packed bed bioreactor studies, it was noticed that the maximum biosorption yields were obtained in packed bed bioreactor at optimum conditions, and that the packed bed bioreactor would be a good choice for the removal of Cr(VI) and Cu(II) from wastewater using *B. subtilis.*

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