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Optimization of Batch Biosorption of Cr(VI) and Cu(II) Ions from Wastewater using *Bacillus subtilis*

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Abstract

The objective of this present study is to optimize the process parameters for batch biosorption of Cr(VI) and Cu(II) ions by *Bacillus subtilis* using Response Surface Methodology (RSM). Batch biosorption studies were conducted under optimum pH, temperature, biomass concentration and contact time for the removal of Cr(VI) and Cu(II) ions using *Bacillus subtilis*. From the studies it is noticed that the maximum biosorption of Cr(VI) and Cu(II) was by *Bacillus subtilis* at optimum conditions of contact time of 30 minutes, pH of 4.0, biomass concentration of 2.0 mg/mL, temperature of 32°C in batch 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.

Keywords

Heavy metal ions; Response Surface Methodology; Bacillus subtilis; Biosorption; Wastewater; Optimization

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 metals into water resources causes serious health effect to human 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 the skin allergy, 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 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. Design Expert is a piece of software designed to help with the design and interpretation of multi factor experiments. The software offers a wide range of designs, including factorials, fractional factorials and composite designs. Design Expert offers computer generated D-optimal designs for cases

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where standard designs are not applicable, or where we wish to augment an existing design - for example, to fit a more flexible model.

2. Materials and Methods

2.1 Biosorption Calculation

The microorganism was isolated from NIT Warangal wastewater treatment plant and identified as *Bacillus subtilis* [6]. Biomass was harvested from the medium by centrifugation at 9000 rpm for 10 minutes. The supernatant was discarded and the cells were re-suspended in purified water for washing and again centrifuged as above to make sure that no media remain on the cell surface. The biomass was heated in a conventional hot air oven at 60°C for 24 h. This biomass was used for the biosorption experiments. Both the biomasses were added in equal amounts for biosorption experiments with mixed culture. Different concentrations of biomass (pure/ mixed cultures) were combined with 100 mL of metal ion solution in 250 mL Erlenmeyer flask. The flasks were placed on a shaker with a constant speed of 300 rpm and left to equilibrate. Samples were collected at predefined time intervals, centrifuged as above and the amount of metal in the supernatant was determined.

$$\%\text{Biosorption}=\left[\frac{\text{Initial metal ion concentration}}{\text{Final metal ion concentration}}\right]100 \qquad (1)$$

2.2 Effect of Different Parameters on Metal Biosorption

2.2 (a) Effect of pH

The metal ion sorption was monitored for pH ranging from 1.0 to 7.0. NaOH and HCL were used as pH regulators. 1 mg/mL biomass was dispersed in 100 mL of the solution containing 10 mg/L of each metal concentration. All flasks were maintained at different pH values ranging from 1.0 to 7.0 for about 12 hours. Solutions were centrifuged as above and the supernatant was analyzed for the residual concentrations of the metal ions.

2.2 (b) Effect of Biomass Concentrations

Biomass was centrifuged at 9000 rpm and different weights of the biomass ranging from 0.5 to 3.5 mg/mL were dispersed in solutions containing 10 mg/L metal ion concentration. The solutions were adjusted to the optimum pH in which maximum biosorption of the metal ion occurred. Flasks were left for equilibration. The solutions were later centrifuged at 9000 rpm and the metal ion concentrations were determined using the procedures described earlier.

2.2 (c) Effect of Temperatures

Optimum biomass concentration with optimum pH was used to monitor the temperature effect on biosorption. Experiments were carried out at different temperatures ranging from 10 to 50°C for each culture and kept on rotary shaker at 240 rpm. The samples were allowed to attain equilibrium. The sample was collected at regular intervals and was analyzed for metal ion concentration.

2.2 (d) Effect of Contact Time

The cells were dispersed in metal ion solution of 10 mg/L concentration with a working volume of 100 mL. The experiment was carried out at the optimum pH value of the system. Flasks were allowed to attain equilibrium on rotary shaker at 240 rpm and samples were collected at regular time intervals of the range from 5-35 minutes. Centrifugation at 9000 rpm was done and the supernatant was analyzed for the residual metal ion content.

2.4 Optimization of Biosorption Parameters Using Response Surface Methodology (RSM)

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing the effects of several independent variables on the response [7]. RSM has an important application in the process analysis and optimization as well as the improvement of existing design. Response surface methodology (RSM) is one of the experimental designing methods which can surmount the limitations of conventional methods collectively [8]. RSM is a combination of mathematical and statistical techniques used to determine the optimum operational conditions of the process or to determine a region that satisfies the operating specifications [9]. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions [10]. The parameters pH, Biomass concentration, Temperature, and Time were optimized for batch biosorption studies and the optimized parameters of pH and temperature were used for continuous packed bed bioreactor studies. The experiments were conducted based on central composite design (CCD). The experimental design employed in the screening of each variable consists of two levels and four independent variables.

3. Results and Discussion

3.1 Optimization of Operating Parameters in Batch Biosorption Studies using Response Surface Methodology

From the experimental work, it is seen that the optimum parameters for batch biosorption studies are pH equal to 4.0, biomass concentration of 2.0 mg/mL, temperature of 32°C and contact time of 30 minutes. Optimization results of Cr(VI) and Cu(II) by *Bacillus subtilis* from the Design Expert software were obtained as pH equal to 3.98, biomass concentration of 2.08 mg/mL, temperature of 32.07°C and contact time of 30.24 minutes. The percent biosorption of Cr(VI) and Cu(II) is 80% and 78.4% respectively. Experimental values closely agree with the values obtained from the response surface methodology, confirming that the RSM using the statistical design of experiments could be effectively used to optimize the process parameters and to study the importance of individual, cumulative and interactive effects of the test variables in biosorption.

3.2 Optimization of Operating Conditions for the Biosorption of Cr(VI) and Cu(II) by *Bacillus subtilis*

The experimental data obtained in batch biosorption of Cr(VI) and Cu(II) by *Bacillus subtilis* is used as the basis in the design of experiments using Design Expert software (shown in Table 1).

Table 1: Experimental Data of the Effect of Process Parameters on the Percent Removal of Cr(VI) and Cu(II) by *Bacillus subtilis*

Std	Run	Time (minutes)	pH	Biomass Concentration (mg/mL)	Temperature (°C)	%Cr(VI)	%Cu(II)
1	18	35	4.0	2.0	30	80.00	78.40
2	8	35	7.0	3.5	10	47.00	46.00
3	30	20	4.0	2.0	30	67.00	77.00
4	13	5	1.0	3.5	50	53.00	43.00
5	15	5	7.0	3.5	50	51.50	42.00
6	27	20	4.0	2.0	30	67.00	76.00
7	11	5	7.0	0.5	50	46.50	37.75
8	5	5	1.0	3.5	10	50.00	43.00
9	6	35	1.0	3.5	10	50.50	50.25
10	7	5	7.0	3.5	10	45.00	35.50
11	17	5	4.0	2.0	30	76.00	68.00
12	22	20	4.0	3.5	30	67.00	75.75
13	29	20	4.0	2.0	30	67.00	76.00
14	1	5	1.0	0.5	10	54.00	48.00
15	14	35	1.0	3.5	50	54.00	53.00
16	24	20	4.0	2.0	50	58.00	67.00
17	3	5	7.0	0.5	10	43.50	38.00
18	16	35	7.0	3.5	50	57.00	52.50
19	21	20	4.0	0.5	30	62.00	71.50
20	23	20	4.0	2.0	10	58.00	64.00
21	12	35	7.0	0.5	50	48.50	48.25
22	28	20	4.0	2.0	30	67.00	76.00
23	25	20	4.0	2.0	30	67.00	76.00
24	19	20	1.0	2.0	30	53.50	62.50
25	26	20	4.0	2.0	30	67.00	76.00
26	20	20	7.0	2.0	30	50.00	58.25

Std	Run	Time (minutes)	pH	Biomass Concentration (mg/mL)	Temperature (°C)	%Cr(VI)	%Cu(II)
27	10	35	1.0	0.5	50	52.00	52.50
28	4	35	7.0	0.5	10	42.00	41.75
29	2	35	1.0	0.5	10	45.50	46.00
30	9	5	1.0	0.5	50	50.00	42.00

Regression analysis was performed to fit the response functions, i.e. percentage biosorption of Cr(VI) and Cu(II). The regression models developed represent responses as functions of contact time(A), pH(B), biomass concentration(C) and temperature(D). An empirical relationship between the response and three input variables expressed by the following response surface reduced quadratic model equations 2 and 3 in coded terms for the percent biosorption of each heavy metal ion:

$$\%Cr(VI) = 67.05 + 0.22A - 1.75B + 1.72C + 1.94D + 0.81AB + 0.94AC + 1.12AD + 0.87AD + 1.06BD + 0.68CD + 9.39A^2 - 15.35B^2 - 2.61C^2 - 9.11D^2 \quad (2)$$

$$\%Cu(II) = 75.8903 + 3.8333A - 2.23611B + 0.8472C + 1.4166D + 0.5937AB + 0.9687AC + 1.375AD + 0.5937BC + 1BD + 0.5625CD - 3.6140A^2 - 15.239B^2 - 1.9890C^2 - 10.114D^2 \quad (3)$$

Positive sign in front of these terms represents synergistic effect, while negative sign represents antagonistic effect. The coefficients with one factor of contact time(A), pH(B), biomass concentration(C) and temperature(D) represent the effect of that particular factor for the preparation of biosorbent. The coefficients with two factors and others with second order terms show the interaction between the two factors and quadratic effect, respectively.

These equations reveal how the individual variables (linear and quadratic) or double interaction affected Cr(VI) and Cu(II) biosorption from wastewater on *Bacillus subtilis* biomass. Negative coefficient values indicate that individual or double interaction factors negatively affect percent biosorption (i.e. removal percentage decreases), whereas positive coefficient values mean that factors increase Cr(VI) and Cu(II) removal in the tested range. For instance, among all linear factors, pH had a negative effect, while contact time, biomass concentration and temperature had a positive effect on Cr(VI) and Cu(II) biosorption (Eq. 2 and 3). The most important individual effect was the temperature ($P < 0.0001$) for Cr(VI) biosorption and time ($P < 0.0001$) for Cu(II) biosorption.

In addition, the most important double effect on Cr(VI) biosorption was the interaction between time and temperature, which was significant ($P = 0.0068$). The second most important effect is the interaction between temperature and pH ($P = 0.0096$). The most important double effect on Cu(II) biosorption was the interaction between time and temperature, which was significant ($P = 0.0018$). The second most important effect is the interaction between temperature and pH ($P = 0.0149$) shown in Table 2.

A high value of the adjusted determination coefficient (Adjusted $R^2 = 0.9792$ for Cr(VI) and 0.9902 for Cu(II)) was estimated. This result means that 97% of the total variation on Cr(VI) biosorption and 99% of the total variation on Cu(II) data (Table 3 and Table 4) can be described by the selected model. The adequate precision ratio of 35.76[Cr(VI)] and 39.12[Cu(II)], for the quadratic model, indicates an appropriated signal to noise ratio. Because the adjusted determination coefficient and adequate precision ratio exceeded 70% and 4.0, respectively, the quadratic model can be used to explore the design space and to find the optimal conditions of this process. A contour plot is a graphic representation of the relationships among three numeric variables in two dimensions. Two variables are for X and Y axes, and a third variable Z is for contour levels. The contour levels are plotted as curves; the area between curves can be color coded to indicate interpolated values. That is, given a value for *z*, lines are drawn for connecting the (x, y) coordinates where that *z* value occurs. The contour plot is an alternative to a 3-D surface plot.

Experiments were conducted at established optimum conditions and results were reproduced. The results were in agreement with the predicted results (shown in table 5).

Table 2: Experimental Design and Results of Percent Biosorption of Cr(VI) and Cu(II) by *Bacillus subtilis*

		%Biosorption of Cr(VI)		%Biosorption of Cu(II)	
Standard Order	**Run Order**	**Actual Value**	**Predicted Value**	**Actual Value**	**Predicted Value**
1	14	54.0	52.60	48.00	46.05
2	29	45.5	47.63	46.00	48.11
3	17	43.5	43.60	38.00	37.20
4	28	42.0	41.88	41.75	41.64
5	8	50.0	51.04	43.00	43.49
6	9	50.5	49.82	50.25	49.43
7	10	45.0	45.54	35.50	37.02
8	2	47.0	47.57	46.00	45.33
9	30	50.0	50.74	42.00	43.01
10	27	52.0	50.26	52.50	50.57
11	7	46.5	45.99	37.75	38.16
12	21	48.5	48.76	48.25	48.09
13	4	53.0	51.93	43.00	42.70
14	15	54.0	55.21	53.00	54.14
15	5	51.5	50.68	42.00	40.23
16	18	57.0	57.21	52.50	54.04
17	11	76.0	77.34	68.00	69.34
18	1	80.0	78.12	78.40	77.27
19	24	53.5	53.23	62.50	62.71
20	26	50.0	49.73	58.25	58.24
21	19	62.0	62.51	71.50	72.88
22	12	67.0	65.95	75.75	74.58
23	20	58.0	55.79	64.00	64.19
24	16	58.0	59.68	67.00	67.02
25	23	67.0	67.26	76.00	76.05
26	25	67.0	67.26	76.00	76.05
27	6	67.0	67.26	76.00	76.05
28	22	67.0	67.26	76.00	76.05
29	13	67.0	67.26	76.00	76.05
30	3	67.0	67.26	77.00	76.05

Table 3: Analysis of Variance for the Quadratic Model for Cr(VI) Biosorption by *Bacillus subtilis*

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2836.855	14	202.6325	98.62	< 0.0001	significant
A-Time	2.7222	1	2.7222	1.32	0.2677	
B-pH	55.125	1	55.125	26.82	0.0001	
C-Biomass Concentration	53.3888	1	53.3888	25.98	0.0001	
D-Temp	68.0555	1	68.0555	33.12	< 0.0001	
Residual	30.82018	15	2.054678			
Lack of Fit	30.82018	10	3.082018			
Pure Error	0	5	0			
Cor Total	2867.675	29				
Std. Dev.	1.4334		R^2	0.9892		
Mean	56.55		Adj R^2	0.9792		
C.V. %	2.53		Pred R^2	0.9331		
PRESS	191.65		Adeq Precision	35.76		

Table 4: Analysis of Variance for the Quadratic Model for Cu(II) Biosorption by *Bacillus subtilis*

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	6260.117	14	447.1512	211.2651	< 0.0001	significant
A-Time	283.22	1	283.22	133.8127	< 0.0001	
B-pH	90.00347	1	90.00347	42.52386	< 0.0001	
C-Biomass Concentration	12.92014	1	12.92014	6.104366	0.0260	
D-Temp	36.125	1	36.125	17.06794	0.0009	
Residual	31.74811	15	2.116541			
Lack of Fit	30.91478	10	3.091478	18.54887	0.0024	significant
Pure Error	0.833333	5	0.166667			
Cor Total	6291.865	29				
Std. Dev.	1.45		R-Squared	0.9949		
Mean	57.3966		Adj R-Squared	0.9902		
C.V. %	2.53		Pred R-Squared	0.9626		
PRESS	234.84		Adeq Precision	39.12		

Table 5: Optimized Values Established by Design Expert for the Biosorption of Cr(VI) and Cu(II) by *Bacillus subtilis*

S.No	Time (minutes)	pH	Biomass Concentration (mg/mL)	Temperature (°C)	% Cr(VI)	% Cu(II)	Desirability	
1	34.5	3.89	2.71	33.05	76.99	76.86	0.99	
2	34.48	3.93	2.74	33.39	76.99	76.85	0.99	
3	30.24	3.98	2.08	32.07	77.00	76.44	0.99	Selected
4	34.87	3.62	3.26	33.49	77.00	76.38	0.99	
5	35.00	3.56	3.36	32.20	76.89	76.17	0.98	
6	34.09	4.22	2.41	34.29	76.21	76.59	0.98	
7	5.00	3.74	2.09	30.38	76.37	68.55	0.88	
8	5.00	3.74	2.08	30.42	76.37	68.55	0.88	
9	5.00	3.7	2.13	30.25	76.37	68.54	0.88	
10	5.00	3.73	2.04	30.72	76.36	68.55	0.88	
11	5.00	3.83	2.07	30.10	76.35	68.54	0.88	
12	5.00	3.76	1.48	29.28	75.73	68.38	0.87	

Adinarayana and Ellaiah [11] have reported that the 3D response surface plots as a function of two factors, with all of the other factors fixed, were helpful in understanding both the main effects and the interaction effects of these two factors. In addition to the 3D response surfaces, their corresponding contour plots facilitated the straightforward examination of the effects of the experimental variables on the response [12].

As shown in Figures 1-3, 3D response surface plots for the measured responses were formed based on the model equation (Eq. (2)) in order to gain a better understanding of the interaction effect of these flotation variables. The relationship between the dependent and independent variables was also further elucidated through the construction of these contour plots. One variable was held at constant at the center level for each plot. Therefore, a total of three response 3D plots and three corresponding contour plots were produced for the responses.

Figure 1: Response Surface Contour Plot Showing Interactive Effect of Time and pH on the Removal of Cr(VI) at Constant Biomass Concentration of 2.0 mg/mL and Temperature of 30°C

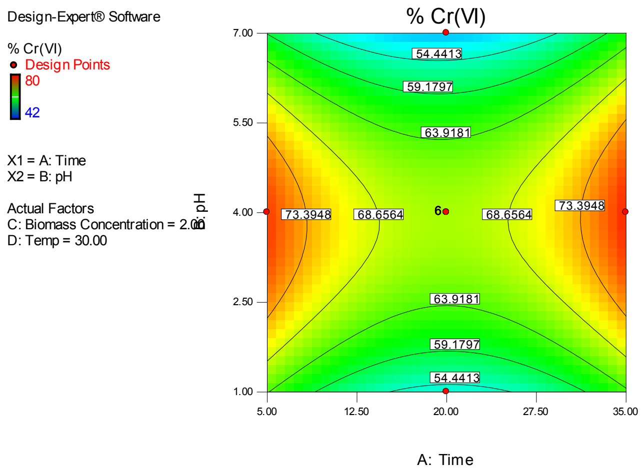


Figure 2: Response Surface Contour Plot Showing Interactive Effect of Time and Biomass Concentration on the Removal of Cr(VI) at Constant pH of 4.0 and Temperature of 30°C

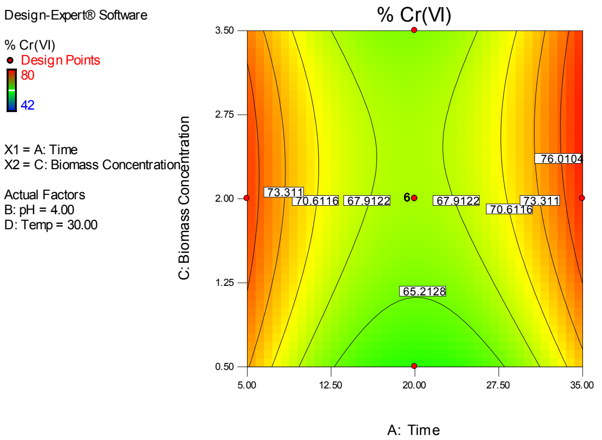


Figure 3: Response Surface Contour Plot Showing Interactive Effect of Time and Temperature on the Removal of Cr(VI) at Constant pH of 4.0 and Biomass Concentration 2.0 mg/mL

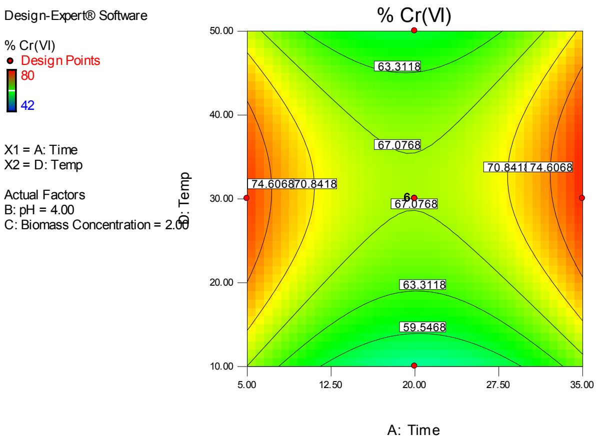


Figure 4: Response Surface 3D Plot Indicating the Effect of Interaction between pH and Biomass Concentration on Cr(VI) Removal while Holding Time at its Design Center Point of 20 minutes and Temperature at 30^{0}C by *Bacillus subtilis*

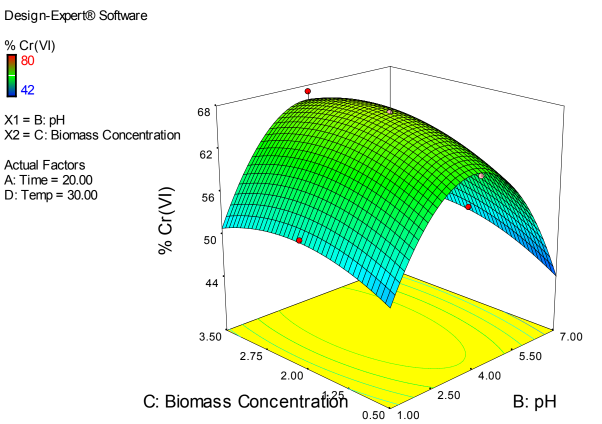


Figure 4 shows the 3D response surface relationship between pH and biomass concentration (BMC) on the Cr(VI) percent biosorption at the center level of the time 20 minutes and temperature 30^{0}C by *Bacillus subtilis*. The percent biosorption of Cr(VI) increased as the pH increased up to 4.0, then gradually decreased. The percent biosorption of Cr(VI) was best at around 2 mg/mL of biomass concentration. The 3D response surface relationship between pH and temperature, biomass concentration and temperature is also shown in Figures 5 and 6. The same trend was observed in all these combination of variables.

Figure 5: Response Surface 3D Plot Indicating the Effect of Interaction between pH and Temperature on Cr(VI) Removal while Holding Time at its Design Center Point of 20 minutes and Biomass Concentration at 2.0 mg/mL by *Bacillus subtilis*

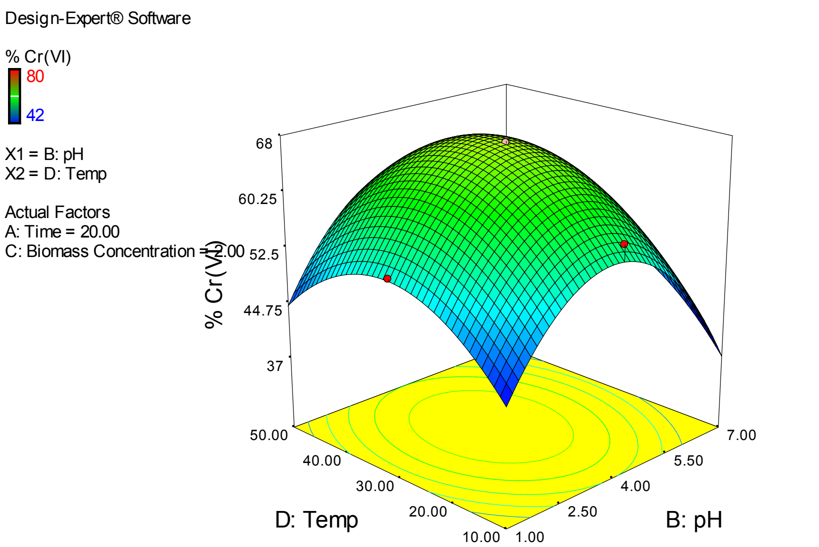
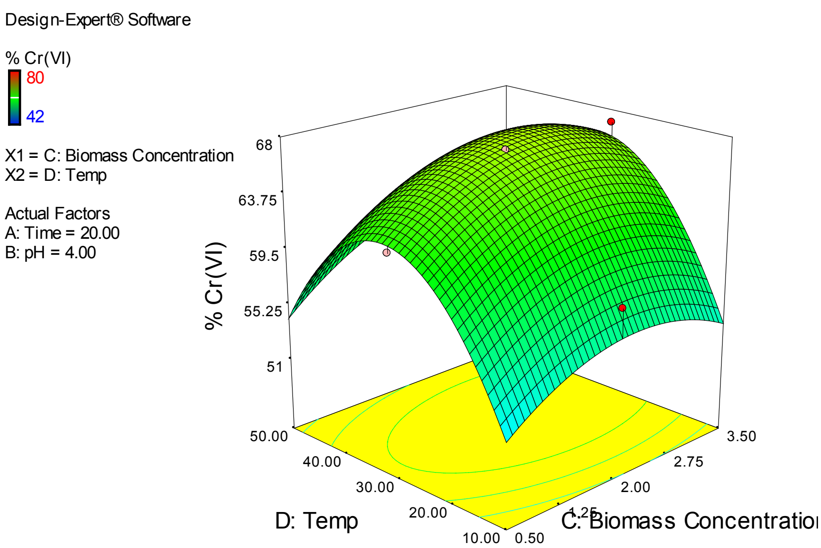


Figure 6: Response Surface 3D Plot Indicating the Effect of Interaction between Biomass Concentration and Temperature on Cr(VI) Removal while Holding Time at its Design Center Point of 20 minutes and pH at 4.0 by *Bacillus subtilis*



The sensitive response to the pH relates likely to the nature of the cellular surface. The bacterial cell wall contains several functional groups including carboxyl, phosphate, amine and hydroxyl groups. The amine group present in the bacterial biomass surface has its pKa value at around 2 to 4. This reflects that the biomass amines are partially protonated at pH 4.0 and as the pH increases, its positive charge will decrease. As the pH increased more than 4.0 the physical adsorption of Cr(VI) onto the biomass surface may be promoted, leading to enhanced sorption performance. That is possibly why there was an optimum at around pH 4.0.

Figure 7: Response Surface Contour Plot showing Interactive Effect of Time and pH on the Removal of Cu(II) at Constant Biomass Concentration of 2.0 mg/mL and Temperature of 30°C

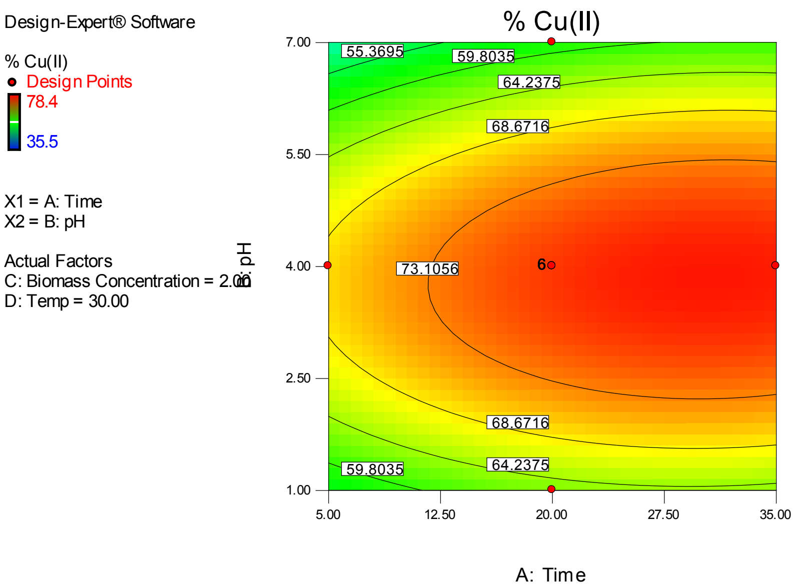


Figure 8: Response Surface Contour Plot Showing Interactive Effect of Time and Biomass Concentration on the Removal of Cu(II) at Constant pH of 4.0 and Temperature of 30°C

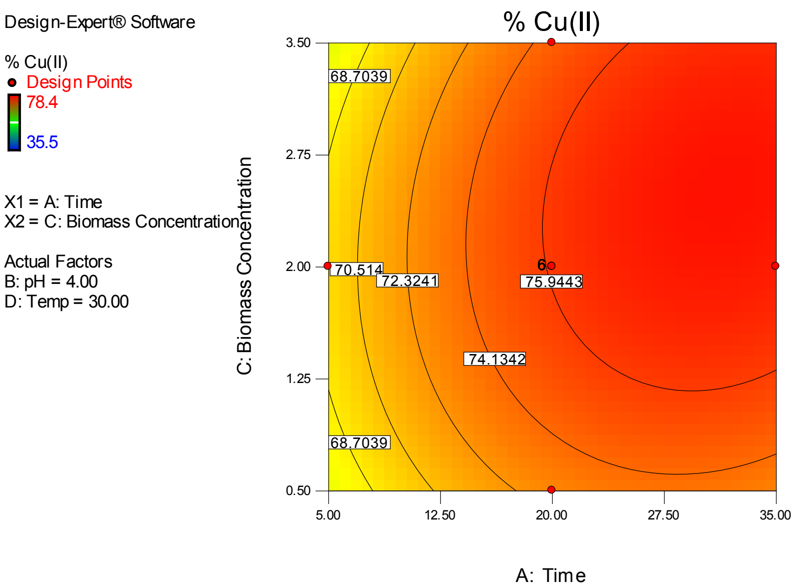


Figure 9: Response Surface Contour Plot Showing Interactive Effect of Time and Temperature on the Removal of Cu(II) at Constant pH of 4.0 and Biomass Concentration 2.0 mg/mL

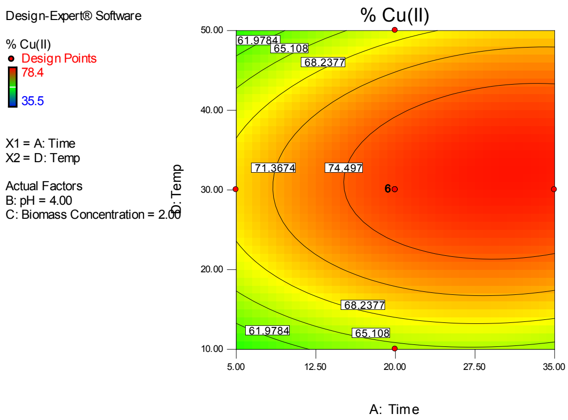


Figure 10: Response Surface 3D Plot Indicating the Effect of Interaction Between pH and Biomass Concentration on Cu(II) Removal while Holding Time at its Design Center Point of 20 minutes and Temperature at 30^{0}C by *Bacillus subtilis*

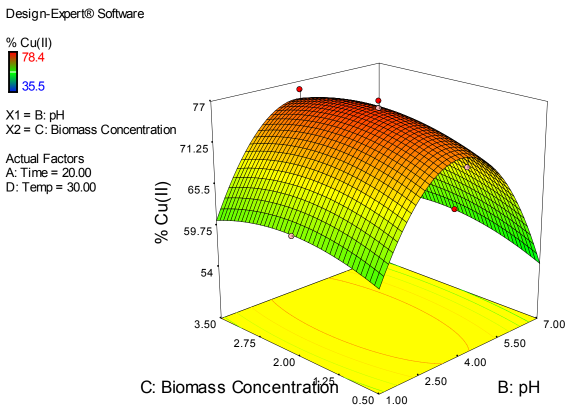


Figure 11: Response Surface 3D Plot Indicating the Effect of Interaction between pH and Temperature on Cu(II) Removal while Holding Time at its Design Center Point of 20 minutes and Biomass Concentration at 2.0 mg/mL by *Bacillus subtilis*

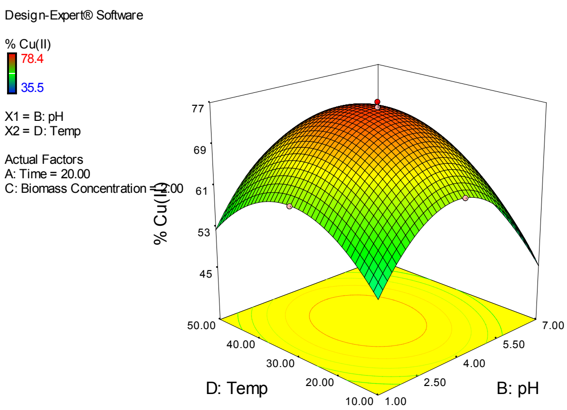
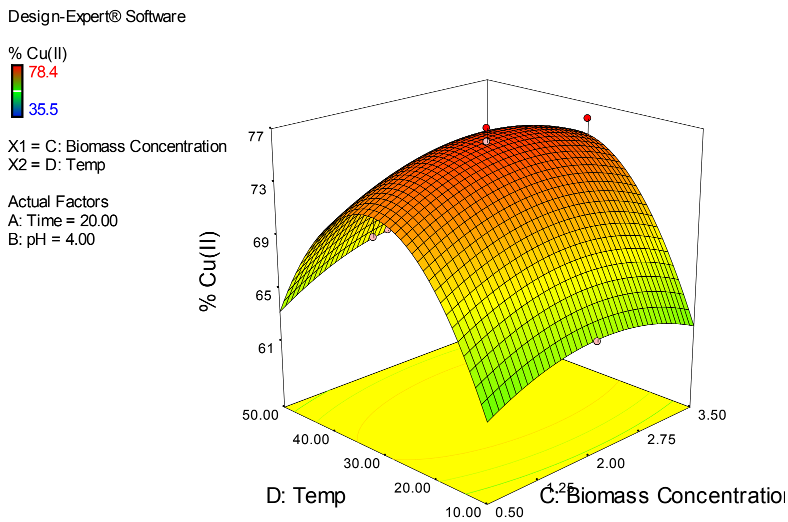


Figure 12: Response Surface 3D Plot Indicating the Effect of Interaction between Biomass Concentration and Temperature on Cu(II) Removal while Holding Time at its Design Center Point of 20 minutes and pH at 4.0 by *Bacillus subtilis*



From the Figures 7 to 12 it was observed that with the increase in pH from 4.0 to 7.0, the degree of protonation of the adsorbent functional group decreased gradually and hence removal was decreased. A close relationship between the surface basicity of the adsorbents and the anions is evident [13]. This is similar to the findings of others, where the interaction between oxygen-free Lewis basic sites and the free electrons of the anions, as well as the electrostatic interactions between the anions. The protonated sites of the adsorbent are the main adsorption mechanism [14].

Experiments were carried out at established optimum conditions of contact time of 30.24 minutes, pH of 3.98, biomass concentration of 2.08 mg/mL and temperature of 32.07°C and results were reproduced and they were in agreement with the predicted results. Earlier studies showed that the biosorption of Chromium (VI) showed that 77.6% for mixed cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis* [15].

4. Conclusions

The present study showed that the maximum biosorption of Cr(VI) and Cu(II) was by *Bacillus subtilis* at optimum conditions of contact time of 30 minutes, pH of 4.0, biomass concentration of 2.0 mg/mL, temperature of 32°C in batch biosorption studies. In batch biosorption studies the effect of pH on the selected heavy metals was studied. Using *Bacillus subtilis* the biosorption of Cr(VI) and Cu(II) was found to be 84% and 80% respectively at pH of 4.0. The maximum biosorption for Cr(VI) and Cu(II) was 80% and 80% respectively at the biomass concentration of 2.0 mg/mL. The maximum biosorption for Cr(VI) and Cu(II) was 82% and 82% respectively at the temperature of 32°C.

The equilibrium time was 30 minutes for Cr(VI) and Cu(II) at which percent biosorption was 82% and 81% respectively. From the batch biosorption experimental work, it is seen that the optimum parameters for batch biosorption studies are pH equal to 4.0, biomass concentration of 2.0 mg/mL, temperature of 32°C and contact time of 30 minutes. Optimization results of Cr(VI) and Cu(II) by *Bacillus subtilis* from the Design Expert software were obtained as pH equal to 3.98, biomass concentration of 2.08 mg/mL, temperature of 32.07°C and contact time of 30.24 minutes. The percent biosorption of Cr(VI) and Cu(II) is 80% and 78.4% respectively. Batch biosorption experiments were carried out at established optimum conditions (using Response Surface Methodology) of contact time of 30.24 minutes, pH of 3.98, biomass concentration of 2.08 mg/mL and temperature of 32.07°C and results of biosorption of Cr(VI) and Cu(II) were reproduced and they were in agreement with the predicted results. It was observed that the *Bacillus subtilis* was suitable species to remove Cr(VI) and Cu(II) from wastewater.

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