Int.J.Curr.Microbiol.App.Sci (2021) *10*(07): 414-421

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 10 Number 07 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1007.045

Application of Box-Behnken Model to Study the Biosorption of Chromium Cr(VI) Ion by *Aspergillus niger* isolated from Polluted Site

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ABSTRACT

Keywords

Response surface methodology (RSM), Box-Behnken design (BBD), Heavy metal, Biosorption, Aspergillus niger

Article Info

Accepted:
15 June 2021
Available Online:
10 July 2021

The biosorption of Cr(VI) metal ion by fungal biomass isolated from polluted soil samples was optimized by using response surface methodology (RSM). The box-behnken design of RSM was used to optimize the parameters such as pH, temperature, initial metal ion concentration and contact time. The isolated strain was identified as *Aspergillus niger*. Total thirty experiments were used to evaluate the interactions between the selected independent variables. The results showed that the fungal biomass isolated from the polluted site impart an important environmental impact through biosorption of Cr(VI) metal ions from the metal solution. The maximum removal value of Cr(VI) obtained was 82.60 percent at pH 5.0 with 103.55 ppm initial metal ion concentration, 35°C temperature and contact time of 60 minutes.

Introduction

The importance of use of various heavy metals in the industrial application has resulted in an increased use (Rao *et al.*, 2014, Saleem *et al.*, 2014) in industries leading to the discarding of heavy metal laden waste material into the environment causing metal pollution (Gautam *et al.*, 2014). The hazardous nature of industrial effluents containing cadmium, chromium, copper, lead, mercury, nickel, palladium, and zinc, makes it compulsory to treat the effluent to control its toxicity (Barakat 2011). Heavy metal pollution poses a serious hazard to both aquatic and terrestrial ecosystems. As a result, scientific community places a high premium on recovering heavy metals from aqueous medium. Metal decontamination, on the other hand, is a difficult operation because all extraction techniques are neither cost-effective nor ecologically benign. To remove heavy metals in industrial effluents, various traditional technologies such as precipitation, reduction, coagulation, membrane filtration, ion exchange, and adsorption are already available (Fu and Wang 2011). These traditional methods possess various disadvantages such as high cost and ineffectiveness for low-strength wastewaters (Farooq *et al.*, 2010) and the sludge is produced (Srivastava and Majumder 2008) during use, many of these methods are less popular than adsorption (Eccles 1999).

Biosorption has evolved as an appealing and viable technology for the removal of metal species from wastewater as a result of rising environmental awareness and the drive for the development of clean remediation strategies for pollution abatement (Gautam *et al.*, 2014). In this process live or dead cells uptake the pollutants through the physico-chemical adsorption or ion exchange. Several biological materials, such as bacteria, algae and fungi, have merged as potential sorbents of heavy metal uptake from wastewater. During mid-1970s, the applicability of fungal biomass for adsorption of heavy metals was discovered and has now become an important biosorbent.

Chromium is introduced in the environment via several industrial practices like electroplating, tanning, steel industry, textile and dyeing industry, manufacturing of pigments and refractory materials etc. Chromium exists in environment in two stable forms i.e. Cr(III) and Cr(VI) and from these forms Cr(VI) is highly oxidizing in nature.

Due to its high oxidizing nature, Cr (VI) is considered as a cancer causing agent. It does not interact directly with DNA, Cr(VI) is reduced to Cr(III) intra-cellularly via reactive intermediates. Therefore it is necessary the Cr(VI) ions must be removed before the discharging of effluents. The adsorption technology is the viable method where low cost adsorbents like fungal biomass were used for uptake of Cr(VI) metal ions.

The biosorption technology is generally used under optimized condition for maximum removal of metal ion from the metal solution. The operating parameters were generally optimized by changing one variable, while keeping other variables constant. But this conventional method is tedious because it requires large number of experiments consuming lot of time. The output generated from these experiments is sometimes unreliable. So a statistical model called response surface methodology (RSM) was introduced to overcome the problems of conventional methods. RSM is basically computational statistical technique which mainly screens out the optimal operational conditions for a particular system.

In view of above, the present study was performed in batch mode for removal of Cr(VI) from aqueous solution using isolated species of *Aspergillus niger*. The optimal conditions for the removal of Cr(VI) were determined by using a Box-Behnken experimental design.

Materials and Methods

Preparation of biosorbent

The filamentous fungus tolerant to chromium (VI) was isolated from the selected polluted sites of Bhopal, Madhya Pradesh. The chromium tolerant fungal species isolated was identified as *Aspergillus niger* by studying its morphological and microscopic characteristics. The *A. niger* species was allowed to grow on potato dextrose broth at 30±2 °C temperature with incubation period of seven days. After incubation period the fungal isolate was collected by filtration through Whatman filter paper. The obtained live biomass was washed several times with deionized water to remove the media components. Further the biomass was deactivated by autoclaving the fungal biomass in Erlenmeyer flask at 1.5 atm pressure and 121°C temperature for 5 minutes (Schiewer and Volesky, 1995). The obtained biomass was then centrifuged after washing with deionized water. The pellet was collected and dried at 45 - 50°C for 24 hours and then the dried biomass was powdered with mortar and pestle. The collected dried and sieved biomass was further stored in desiccators and used for assessing the biosorption capacity of the fungal biomass (Hajahmadi *et al.,* 2015).

Optimization of parameters with Box-Behnken design

Biosorption experiments were conducted in batch mode using Cr(VI) metal ion solution. The stock solution of Cr(VI) metal ion (1000 mg/L) was prepared by dissolving potassium dichromate ($K_2Cr_2O_7$) in double distilled water. For obtaining further desired concentrations (50mg/L to 500 mg/L), the stock solution was diluted with double distilled water. The biosorption studies were carried out using 150 ml working metal solution in 250 ml Erlenmeyer flasks. Different parameters viz., pH, temperature, initial metal ion concentration and contact time were adjusted in the metal solution to assess the optimum biosorption conditions. The response surface methodology (RSM) was used to design array of experiments to identify the optimum condition for metal ion biosorption. The biosorbent dosage and agitation speed was kept constant to 1.0 g/L and 125 rpm for all the biosorption experiments.

In this study, Box-Behnken design matrix was used to obtain the accurate prediction for operating parameters and to minimize the number of experiments without disturbing the model preciseness. Total thirty experiments were conducted for Cr(VI) metal ion according to Box-Behnken design. The effect of operating parameters viz., pH, temperature, initial metal ion concentration and contact time were systemically analyzed under biosorption studies.

In box-behnken design, combination of parameters were taken for process variables with varied pH values ranging from 5 to 9, temperature ranging from 30 to 40°C, Cr(VI) ion concentration ranging from 50 to 200 mg/L and contact time of 30 to 90 minutes. Table 1 depicts different levels and ranges of chosen independent process parameters. The generated data was analyzed by using design-expert statistical software package.

After experimentation, the biosorbent and metal solutions were centrifuged at 7000 rpm for 10 minutes and the collected supernatants were analyzed to determine the residual metal ion concentration. This will provide the uptake of metal ions from solution by the fungal biosorbent.

Result and Discussion

Optimization of biosorption

The experiments were statistically analyzed and the effect of process parameters on chromium (Cr(VI)) removal (%) was statistically evaluated by using Box-Behnken design. The results obtained from the experiments conducted as per the Box-Behnken design to study the effects of four independent process variables are presented in Table 2.

The adequacy of the model for removal of Cr(VI) was determined by the sequential model sum of squares, lack of fit tests and model summary statistics. Comparison between various statistical parameters, includes the coefficient of determination (R2), the adjusted R2 and the F-value from ANOVA, was used to identify the best fitting mathematical models of the responses. The observed regression coefficient was best for quadratic model showed by the model summary statistics. ANOVA analysis confirmed that the model adequately explained the relationship between the process variables and the response. The ANOVA results are tabulated and shown in table 3.

The relationship between various process variables and responses were determined where A, B, C and D denotes pH, initial metal ion concentration, contact time and operating temperature respectively. Values of "Prob > F" less than 0.0500 indicate model terms are significant. The analysis of variance (ANOVA) for Cr(VI) metal ion removal indicated that the main effects of pH, initial metal ion concentration, contact time, temperature, pH × initial metal ion concentration, pH^2, initial metal ion $concentration^2$ were significant model terms. Values greater than 0.1000 in analysis of variance indicates that the model terms are not significant. Multiple regression analysis of experimental data is resulted in the following equation for biosorption of Cr(VI) ions.

Biosorption Cr=

$$= +39.18 - 31.57 * A + 3.03 * B + 1.79 * C + 0.90 * D + 4.28 * AB + 0.97 * D + 4.28 * AB + 0.97 * AC + 0.97 * AD + 0.30 * BC - 0.54 * BD + 0.10 * CD + 11.41 * A^2 + 0.77 * B^2 + 1.44 * C^2 + 0.055 * D^2$$

The "Lack of Fit F-value" of 1.93 implies that it is not significant relative to the pure error. There is a 24.29% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good. The adjusted R2 is equal to 0.9959 of the quadratic model in equation 1 is in reasonable agreement with the predicted R2 value i.e. 0.9898. The Model F-value of 508.78 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. From the ANOVA results it was confirmed that the quadratic model was satisfactorily adjusted to the experimental data.

The normal probability plot of studentized residuals are shown in figure 1. The data points on this plot lie reasonably close to the straight line, supporting the significance of the model which confirms that the assumptions of the analysis were satisfied.

The response surface curves for Cr (VI) metal ion removal are shown in fig. 2 which analyses the interaction between independent variables.

These three-dimensional mesh diagrams were used to the point where optimum response was pointed along with the effect of each parameter on the process of biosorption (Varsihini *et al.*, 2014).

The six mesh diagrams were obtained by varying pH and initial metal ion concentration when temperature and contact time were kept constant (Fig. 2a). Similarly the mesh diagram in figure 2b was obtained by varying pH and time while keeping temperature and initial metal ion concentration keeping constant. The 3-d surface (Fig. 2c) was obtained by varying pH and temperature when contact time and initial metal ion concentration was kept constant.

Optimization of parameters using desirability function

The desirability functions are very much important for computing optimization of desired goal for each factor and responses.

The possible goals for the present experiment are: with in range (pH), within range (initial metal ion concentration), target (time) and temperature (target) and none (for response).

Table.1 Process variables in coded and un-coded form for Cr(VI) ions biosorption using isolated *Aspergillus niger* strain

Factor	Parameters	Unit	Range and levels		
			-1	0	+1
A	pH	--	5.0	7.0	9.0
B	Initial Cr(VI) ion concentration	Mg/L	50	125	200
C	Contact time	Minutes	30	60	90
D	Temperature	°C	30	35	40

Table.2 Experimental and predicted response for Cr(VI) ions using *Aspergillus niger*

Run	A: pH	B: Initial metal ion concentration (mg/L)	C: Time (Minutes)	D: Temperature (°C)	Biosorption	
					Experimental	Predicted
1	9	125	60	30	17.32	17.20
2	9	50	60	35	12.28	12.47
3	7	50	60	30	35.19	35.52
4	7	125	60	35	37.23	39.18
5	7	50	90	35	41.3	39.83
6	5	50	60	35	83.26	84.18
7	7	125	30	30	38.56	38.09
8	7	50	60	40	38.96	38.40
9	7	125	60	35	38.97	39.18
10	5	125	90	35	84.95	84.42
11	7	125	60	35	40.24	39.18
12	9	125	60	40	19.35	20.94
13	9	125	90	35	23.56	23.21
14	7	125	30	40	40.97	39.68
15	7	125	60	35	39.65	39.18
16	7	125	60	35	39.09	39.18
17	7	200	90	35	46.15	46.50
18	5	125	30	35	83.48	82.78
19	5	125	60	40	81.09	82.14
20	9	200	60	35	27.91	27.10
21	7	125	90	30	40.05	41.46
22	7	125	90	40	42.87	43.46
23	7	200	30	35	39.93	42.33
24	7	200	60	30	43.17	42.68
25	7	200	60	40	44.77	43.39
26	9	125	30	35	18.21	17.70
27	5	200	60	35	81.76	81.68
28	5	125	60	30	82.95	82.29
29	7	50	30	35	36.28	36.86
30	7	125	60	35	39.88	39.18

Table.3 Analysis of variance for Cr(VI) metal ion.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	13116.08	14	936.86	508.78	< 0.0001	significant
A-pH	11961.24	1	11961.24	6495.74	< 0.0001	
B-Initial metal ion Concentration	110.53	1	110.53	60.03	< 0.0001	
C-Time	38.34	1	38.34	20.82	0.0004	
D-Temperature	9.67	1	9.67	5.25	0.0368	
AB	73.36	1	73.36	39.84	< 0.0001	
AC	3.76	1	3.76	2.04	0.1733	
AD	3.78	1	3.78	2.05	0.1723	
BC	0.36	1	0.36	0.20	0.6647	
BD	1.18	1	1.18	0.64	0.4364	
CD	0.042	1	0.042	0.023	0.8819	
A^2	893.24	1	893.24	485.09	< 0.0001	
B^2	4.05	1	4.05	2.20	0.1589	
C^2	14.16	1	14.16	7.69	0.0142	
D^2	0.020	1	0.020	0.011	0.9175	
Residual	27.62	15	1.84			
Lack of Fit	21.93	10	2.19	1.93	0.2429	not significant
Pure Error	5.69	5	1.14			
Cor Total	13143.70	29				

Fig.1 Normal Probability plot of studentized residuals versus normal % probability for the % removal of Cr (VI) metal ions.

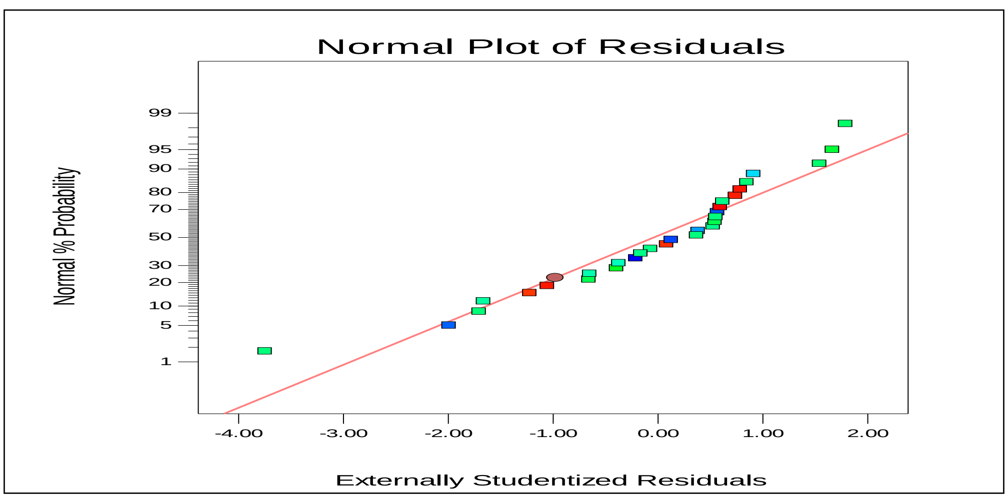
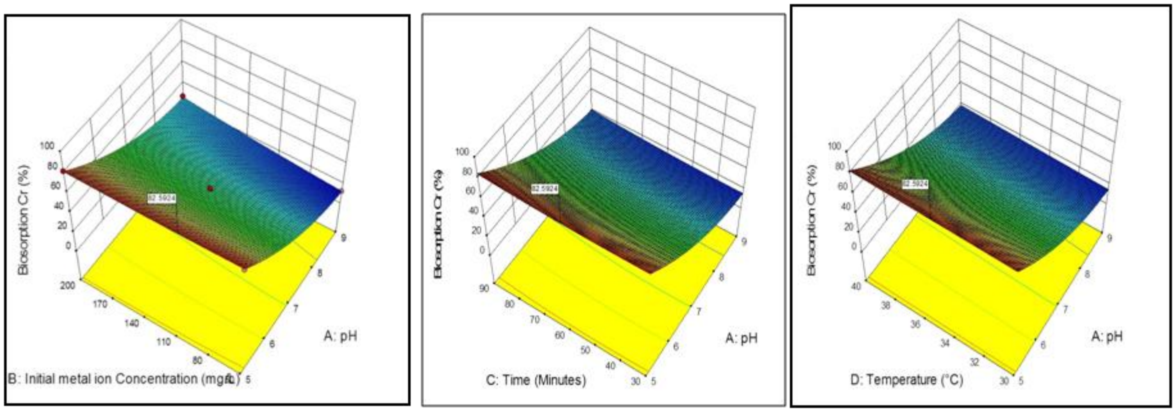


Fig.2 Three dimentional response surface plots showing the effect of different independent process variables on Cr(VI) biosorption using A. niger



(a) (b (c

The overall desirability function was determined finally by combining all the desired goals. The optimization of Cr(VI) metal ion biosorption was determined at an initial concentration of 103.55 mg/L, pH 5.0, temperature 35°C and contact time of 60 minutes.

Under these optimized conditions, maximum removal of Cr(VI) metal ion of 82.60 percent was recorded with desirability function of 0.99. This value of desirability shows that the estimated function may represent the experimental model and desired conditions. The results obtained in this study are comparable to the study of Mondal *et al.*, 2017.

The strain of *Aspergillus niger* isolated from polluted sites was further selected for biosorption studies. In this study process parameters for efficient removal of Cr(VI) metal ions were optimized using Box-Behnken design of RSM. The quadratic model suited best and validated the good agreement between the experimental values and the predicted values. The high coefficient of determination (R^2) was shown by the Analysis of variance (ANOVA) studies. The optimum conditions were selected which showed the maximum efficiency for removal of metal ions from metal solution with the help of numerical optimization.

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How to cite this article:

Shobha Shrivastava and Mayuri Gupta. 2021. Application of Box-Behnken Model to Study the Biosorption of Chromium Cr(VI) Ion by *Aspergillus niger* Isolated from Polluted Site. *Int.J.Curr.Microbiol.App.Sci.* 10(07): 414-421. doi: https://doi.org/10.20546/ijcmas.2021.1007.045