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Research Article

BIOSORPTION OF CHROMIUM (VI) USING *ASPERGILLUS NIGER*

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Abstract: The response surface methodology was employed to make mathematical modeling and optimization of the concentration of chromium and temperature. A polynomial regression model equation was $Y_1 = 72.00 + 0.71\ x_1 + 7.44\ x_2 + 2.50\ x_1 x_2 - 32.06\ x_1^2 - 17.81\ x_2^2$ obtained with concentration of chromium (X_1) and temperature (X_2). The optimal concentration of chromium and temperature were found to be 126 ppm and 34°C respectively. The maximum % of chromium removal was found to be 71.05. The simulation parameter revealed that the modeled values were in good agreement with simulated ones.

Keywords: Response Surface Methodology, Optimization, Analysis Of Variance and *Aspergillus niger.*

INTRODUCTION

The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption, based on metal binding capacities of various biological materials. Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake.[1] Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents.[2]

The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases.[3]

There are several chemical groups that would attract and sequester the metals in biomass: acetamido groups of chitin, structural polysaccharides of fungi, amino and phosphate groups in nucleic acids, amido, amino, sulphydryl and carboxyl groups in proteins, hydroxyls in polysaccharide and mainly carboxyl and sulphates in polysaccharides of marine algae that belong to the divisions Phaeophyta, Rhodophyta and Chlorophyta. However, it does not necessarily mean that the presence of some functional group guarantees biosorption, perhaps due to steric, conformational or other barriers.[4] Apart from toxicological criteria, the interest in specific metals may also be based on how representative their behavior may be in terms of eventual generalization of results of studying their biosorbent uptake. The toxicity and interesting solution chemistry of elements such as chromium, arsenic and selenium make them interesting to study. Strategic and precious metals though not environmentally threatening are important from their recovery point of view.[5]

The removal of poisonous Cr (VI) from industrial wastewater by different low-cost abundant adsorbents was investigated. Wool, olive cake, sawdust, pine needles, almond shells, cactus leaves and charcoal were used at different adsorbent / metal ion ratios. The influence of pH, contact metal concentration, adsorbent nature and concentration on the selectivity and sensitivity of removal process was investigated.[6]

The aim of the present research is to get a better understanding of the relation between the variables (concentration of chromium and temperature) and determine the optimum conditions for the % of chromium removal. Response surface methodology (RSM) and Central composite design (CCD) which is an efficient statistical technique for optimization of multiple variables is applied to predict best performance conditions with minimum number of experiments.

MATERIALS AND METHODS

Experimental design and data analysis: Central Composite Design (CCD):

RSM consists of a group of empirical techniques devoted to the evaluation of relations existing

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between a cluster of controlled experimental factors and the measured responses, according to one or more selected criteria. Prior knowledge and understanding of the process variables under investigation is necessary for achieving a realistic model. The significant variables obtained by using statistical experimental design technique called the response surface methodology and central composite design was used. According to the Central composite design, the total number of treatment combinations was $2^k + 2k + n_o$, where 'k' is the number of independent variables and n_o is the number of repetition of experiments at the center point. A 2^2 factorial design with four axial points and six replicates at the center point with a total number of 13 experiments was employed for optimization of the medium components.[7] The central value (zero level) chosen for experimental design was the range of concentration of chromium (X_1) and temperature (X_2) studied in this work was between 50 to 200 and 25 to 40 respectively.

Biomass preparation:

The liquid culture was prepared by the combination of dextrose, peptone, KH_2PO_4, $MgSO_4$ and Rose Bengal. In this liquid culture transfer a organism *Aspergillus niger* and kept for 7 days. After filtered a culture by using ordinary nylon mesh, washed with distilled water and transferred to watchs glass and kept for drying for about 24 hours at 60°C. Then the dried biomass is powdered. The biomass obtained was used directly in metal uptake experiments to evaluate the biosorption capacity.

Experimental procedure:

2.83g of $K_2Cr_20_7$ crystals was weighed and dissolved to make 1 lit of solution in standard volumetric flask of different concentrations of $K_2Cr_20_7$ are prepared in 250 ml conical flasks. A detailed methodology for the optimization process is given for run number 1 (Table.1). 0.2g of biomass is added to conical flask. These solutions are kept for shaking at 60 rpm in incubator for 24 hours for different temperature given in Table 1. The solutions are taken out for the analysis.

The solution for the analysis is prepared by adding 1 ml of sample solution and 0.25 ml of H_3PO_4 is added. pH is maintained by adding 0.2 N H_2So_4 and distilled water is added to make up to 100 ml and 2 ml of diphenyl carbazide (DPC) is added to it. DPC solution is prepared by dissolving 0.25g of DPC in distilled and made up to 100 ml in standard volumetric flask. After adding DPC, the solution is kept for 10 minutes and % of absorbance is found with help of a spectrophotometer, using distilled water as reference, and the wavelength set at 540nm.[8]

RESULT AND DISCUSSION

The application of response surface methodology offers, on the basis of parameter estimate, an empirical relationship between the response variable and the test variables under consideration. Multiple regression analysis of the experimental data (using Design- Expert software) gave the following second order polynomial equation in terms of % of chromium removal result shown in Table.1.

Table.1: Experimental data obtained for % of chromium removal

Run	Concentration of chromium x_1 ($\equiv X_1$)	Temperature x_2 ($\equiv X_2$)	% of chromium removal
1	-1 (50.00)	-1 (25.00)	20
2	+1 (200.00)	+1 (25.00)	15
3	-1 (50.00)	-1 (40.00)	20
4	+1 (200.00)	+1 (40.00)	25
5	-1.414 (18.93)	0 (32.50)	8
6	+1.414 (231.07)	0 (32.50)	12
7	0 (125.00)	-1.414 (21.89)	21
8	0 (125.00)	+1.414 (43.11)	56
9	0 (125.00)	0 (32.50)	72
10	0 (125.00)	0 (32.50)	72
11	0 (125.00)	0 (32.50)	72
12	0 (125.00)	0 (32.50)	72
13	0 (125.00)	0 (32.50)	72

The expression obtained in terms of coded factors is given by the equation, $Y_1 = 72.00 + 0.71\ x_1 + 7.44\ x_2 + 2.50\ x_1\ x_2 - 32.06\ x_1^2 - 17.81\ x_2^2$. Goodness of fit was examined using second order model equation with independent and dependent variables. The coefficient of determination (R^2) is 0.9744 which indicates that model response to total variability by 97.44% to various responses given. Analysis of variance (ANOVA) was used for statistical testing of the model which is part of test for adequacy and significance of the model. Models reliability was tested using Fisher's statistical test (F). The results of statistical testing using ANOVA are given in Table 2.

Table.2: Analysis of Variance (ANOVA) Table for the effect of Concentration of chromium and temperature on % of chromium removal.

Source	Sum of squares	Degrees of freedom	Mean square	F value	Probability (P) > F
Model	8937.64	5	1787.53	53.22	0.0001 significant
Error	235.13	7	33.59		

Probability with a value of less than 0.05 signifies that the model terms are significant. Table 2 indicates that the model was highly significant. The F value ($F0.005\ (5,\ 7) = 9.52$) obtained from the standard distribution table.[7] From Figure 1 it can be observed that a stationary point exists inside the range based on the shape of the contour plot. The response surface plot shown in Figure 2 for the chosen model Y1 illustrates the three dimensional relationship. CCD demonstrated that optimum of % of chromium removal. This result indicates that two variables had mutually dependent influence on the % of chromium removal can determined. The optimal concentration of chromium and temperature were found to be 126 ppm and 34°C respectively. The maximum % of chromium removal was found to be 71.05.

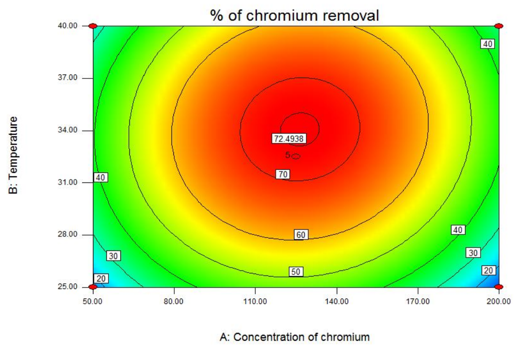

Figure.1: Isoresponse contour plots showing the effect of concentration of chromium (ppm) and temperature (°C) and their interactive effect on the % of chromium removal.

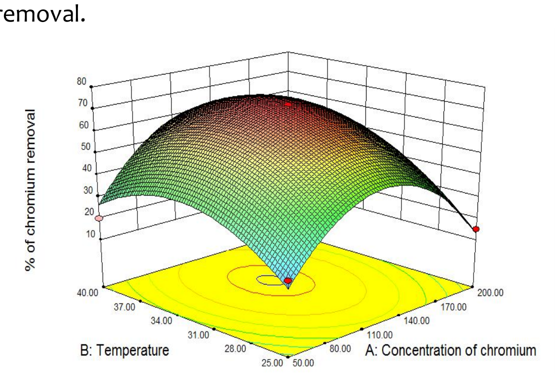

Figure.2: Response surface plot showing the effect of concentration of chromium (ppm) and temperature (°C) and their interactive effect on the % of chromium removal.

CONCLUSION

The study has demonstrated the use of a central composite design by determining the conditions leading to the optimum % of chromium removal. The optimal concentration of chromium and temperature were found to be 126 ppm and 34°C respectively. The maximum % of chromium removal was found to be 71.05. This methodology could therefore be successfully employed to any process, where an analysis of the effects and interactions of many experimental factors are referred. Response surface plots are very helpful in visualizing the main effects and interaction of its factors. Thus, smaller and less time consuming experimental designs could generally suffice the optimization of many fermentation processes.

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