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Application of response surface methodology for the biosorption of copper using *Rhizopus arrhizus*

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Abstract

Response surface methodology was used to study the cumulative effect of the various parameters namely, initial copper ion concentration, pH, temperature, biomass loading and to optimize the process conditions for the maximum removal of copper. For obtaining the mutual interaction between the variables and optimizing these variables, a 2^4 full factorial central composite design using response surface methodology was employed. The analysis of variance (ANOVA) of the quadratic model demonstrates that the model was highly significant. The model was statistically tested and verified by experimentation. A maximum copper removal of 98% was obtained using the biosorption kinetics of copper under optimum conditions.

Keywords: Biosorption; *Rhizopus arrhizus*; Design of experiments; Central composite design; Response surface methodology

1. Introduction

Heavy metals present in the industrial effluents remain as alarming pollutants due to their nondestructive nature, toxicity, bioaccumulation and subsequent biomagnifications [1]. Metal plating industry mainly discharges huge amounts of copper and its ingestion beyond the permissible level causes various types of acute and chronic disorder in man, such as hemochromatosis, etc. [2]. There is an increasing trend in the use of microorganisms for removal and possible recovery of metal ions from industrial wastes by biosorption. This is a potential alternative to existing technologies (chemical precipitation, reverse osmosis and solvent extraction), which have significant disadvantages, such as high chemical or energy requirements and generation of toxic sludge or other products that need disposal [3]. Biosorption is an innovative technology using inactive and dead biomasses to remove heavy metals from aqueous solutions. This biological phenomenon could be explained by considering different kinds of chemical and physical interactions among the functional groups present in the cell wall and the heavy metals in solution [4]. Fungi are well suited for this purpose since they often exhibit marked tolerance towards metals and other adverse conditions, e.g., low pH. They have high capacities of metal binding to cell walls and may also exhibit high values of intracellular accumulation [5]. The cell wall of *Rhizopus arrhizus* involves a high content of chitin and the ability of chitin to complex metal ions has been confirmed in the literature [6].

This work is primarily aimed at evaluating the effects of initial copper ion concentration, pH, temperature and biomass loading on the percentage removal of copper and statistically optimizing these variables for maximum removal of copper efficiently and economically. The application of statistical experimental design in biosorption techniques, results in higher percentage yields, less treatment time with minimum costs. Most optimization studies during the development of a process involve variation of one factor at a time, keeping all other factors constant. But the experiments conducted using the factorial designs, enable all factors to vary simultaneously. This helps in quantifying linear, square and interactive effects of the test variables. Another important advantage is that, the experimental designs could be changed progressively until a fitted model is found to describe the studied phenomenon [7,8]. Response surface methodology (RSM) is an empirical statistical technique employed for multiple regression analysis of quantitative data obtained from statistically designed experiments by solving the multivariate equations simultaneously. The graphical representation of these equations

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Nomenclature

x_i	coded value of the i^{th} variable
X_i	uncoded value of the i^{th} test variable
X_o	uncoded value of the i^{th} test variable at the center point
Y	predicted response

Greek symbols

β_i	coefficient of linear effect
β_{ii}	coefficient of square effect
β_{ij}	coefficient of interaction effect
β_o	offset term

are called as response surfaces, could be used to describe the individual and cumulative effect of the test variables on the response and to determine the mutual interaction between the test variables and their subsequent effect on the response [9,10]. In this study, 2^4 full factorial central composite design using response surface methodology was employed.

2. Experimental

R. arrhizus MTCC 2233, a filamentous fungus obtained from the institute of microbial technology, Chandigarh, was used in this study. *R. arrhizus* possesses the following general characteristics: high porosity and good wetting ability, good resistance to chemicals and favorable equilibrium and kinetics [11]. The microorganism was grown at 25 °C in an agitated liquid media containing potato extract (200 g/l) and dextrose (20 g/l). The pH of the medium was adjusted to 5.3 with dilute sulphuric acid before sterilization. The cell suspension was then separated, dried, homogenized and stored for subsequent biosorption studies.

A 1000 ppm stock solution of copper was prepared by dissolving 3.93 g of copper sulphate in double distilled water. The required concentrations of copper ions were prepared from the stock solution by dilution method.

Batch experiments were carried out in Erlenmeyer flasks by adding dried cells of *R. arrhizus* in 200 ml of aqueous copper sulphate solution. The flasks were gently agitated on a shaker with a constant shaking rate at 150 rpm for 24 h until equilibrium sorption was obtained. Samples were taken from the solution at regular time intervals for the residual metal ion concentration in the solution. The residual concentration of copper ions in the solutions was determined spectrophotometrically at 475 nm using neocuproine as the complexing agent [12].

2.1. Central composite design analysis and optimization by response surface methodology involved in the biosorption of copper

The effect of the biosorption parameters namely initial copper ion concentration, pH, temperature and biomass loading on percentage removal of copper was studied by statistically designed experiments and optimization by response surface methodology. An orthogonal full factorial central composite design with eight star points ($\alpha = 2$) and seven replicates at the center point, all in duplicates, resulting in a total of 31 experiments which covers the entire range of combinations of variables and were used to optimize the chosen key variables for the biosorption of copper using *R. arrhizus* in batch reactor.

Table 1
Central composite design analysis for biosorption of copper

Independent variable	Range and level				
	$-\alpha$	-1	0	$+1$	$+\alpha$
Initial copper ion concentration (ppm) (X_1)	35	45	55	65	75
pH (X_2)	2	3	4	5	6
Temperature (°C) (X_3)	30	35	40	45	50
Biomass loading (g/l) (X_4)	2.5	5	7.5	10	12.5

The experiments with five different initial copper ion concentrations namely, 35, 45, 55, 65 and 75 ppm, five different pH values of 2, 3, 4, 5 and 6, five different temperatures of 30, 35, 40, 45 and 50 °C and five different biomass loading of 2.5, 5, 7.5, 10 and 12.5 g/l were employed, coupled to each other and varied simultaneously to cover the combinations of variables in the central composite design. The levels of the chosen independent variables used in the experiments for the removal of copper are given in Table 1. The chosen independent variables used in this experiment were coded according to Eq. (1):

$$x_i = \frac{X_i - X_o}{\Delta x} \tag{1}$$

The behavior of the system is explained by the following empirical second-order polynomial model Eq. (2):

$$Y = \beta_o + \sum_{i=1}^{k}\beta_i X_i + \sum_{i=1}^{k}\beta_{ii} X_i^2 + \sum_{i=1}^{k-1}\sum_{j=2}^{k}\beta_{ij} X_i X_j \tag{2}$$

The design package Minitab 14, a statistical program package, was used for regression analysis of the data obtained and to estimate the coefficient of the regression equation. The equations were validated by the statistical tests called the ANOVA (analysis-of-variance) analysis, to determine the significance of each term in the equations fitted and to estimate the goodness of fit in each case. Response surfaces were drawn for the experimental results obtained from the effect of different variables on the percentage removal of copper in order to determine the individual and cumulative effects of these variables and the mutual interactions between them.

3. Results and discussion

The coded and uncoded values of the test variables were used to optimize the variables namely initial copper ion concentration, pH, temperature, biomass loading and the experimental results of percentage removal of copper in each case are presented in Table 2. The percentage removal of copper depends on the individual effects of combinations of test variables and the

Table 2
Full factorial central composite design matrix of orthogonal and real values along with observed responses for the removal of copper

Run no.	Independent variables								Response (% removal of copper)
	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	45	5	45	10	78.23
2	+1	−1	−1	−1	65	3	35	5	81.27
3	−1	+1	−1	+1	45	5	35	10	86.81
4	−1	−1	−1	−1	45	3	35	5	85.46
5	+1	+1	−1	+1	65	5	35	10	89.23
6	+1	+1	+1	+1	65	5	45	10	90.25
7	+1	+1	+1	−1	65	5	45	5	74.41
8	+1	+1	−1	−1	65	5	35	5	83.82
9	+1	−1	+1	+1	65	3	45	10	80.14
10	−1	−1	+1	−1	45	3	45	5	80.08
11	−1	+1	−1	−1	45	5	35	5	81.09
12	+1	−1	−1	+1	65	3	35	10	81.28
13	−1	+1	+1	−1	45	5	45	5	72.28
14	+1	−1	+1	−1	65	3	45	5	79.32
15	−1	−1	−1	+1	45	3	35	10	80.13
16	−1	−1	+1	+1	45	3	45	10	82.28
17	α	0	0	0	75	4	40	7.5	79.23
18	−α	0	0	0	35	4	40	7.5	95.09
19	0	α	0	0	55	6	40	7.5	92.23
20	0	−α	0	0	55	2	40	7.5	84.23
21	0	0	α	0	55	4	50	7.5	80.17
22	0	0	−α	0	55	4	30	7.5	99.83
23	0	0	0	α	55	4	40	12.5	81.25
24	0	0	0	−α	55	4	40	2.5	75.21
25	0	0	0	0	55	4	40	7.5	97.32
26	0	0	0	0	55	4	40	7.5	97.33
27	0	0	0	0	55	4	40	7.5	97.33
28	0	0	0	0	55	4	40	7.5	97.32
29	0	0	0	0	55	4	40	7.5	97.32
30	0	0	0	0	55	4	40	7.5	97.33
31	0	0	0	0	55	4	40	7.5	97.32

X_1 initial copper ion concentration (ppm); X_2 pH; X_3 temperature (°C); X_4 biomass loading (g/l).

results show a significant variation for each combination. Multiple regression analysis of the experimental data was obtained from the following regression equation for the biosorption of copper:

$$Y = 97.324 - 0.765X_1 + 0.923X_2 - 2.976X_3 + 1.779X_4 - 3.254X_1^2 - 2.986X_2^2 - 2.544X_3^2 - 5.486X_4^2 + 1.577X_1X_2 + 0.571X_1X_3 + 0.846X_1X_4 - 1.216X_2X_3 + 2.201X_2X_4 + 1.188X_3X_4 \quad (3)$$

in which Y is the response variable, percentage removal of copper and X_1, X_2, X_3 and X_4 are the coded values of the independent variables, initial copper ion concentration, pH, temperature and biomass loading, respectively.

Multiple regression coefficient (R) was estimated from the second-degree polynomial Eq. (3). The value of $R = 0.9236$ which is closer to one indicates that the correlation is best suited for predicting the performance of the biosorption system and the predicted values were found to be very closer to the experimental results.

The results obtained from the CCD namely the student t distribution, the p values and the parameter estimates are given in Table 3. The coefficient for the initial concentration of copper (X_1) indicates that the percentage removal of copper was more at lower copper ion concentration. Similar observations have previously been reported [13]. A higher ratio of the surface binding sites on the biosorbent to the total metal ions could be obtained with a low metal ion concentration and with fixed biomass loading which may result in total biosorption of metal and complete removal of metal ions [14]. The effect of temperature was found to be highly significant ($p = 0.004$) on percentage removal of copper. The percentage removal of copper by *R. arrhizus* was higher at low temperature of 37 °C and decreases with further increase in temperature. The results agree with the results of Sag and Kutsal [15]. Cell walls were permanently damaged at high temperatures and for this reason a reduction in copper removal was observed. The coefficients of the quadratic terms of all the parameter were also highly significant. The coefficients of the interactive effects between pH and temperature ($p = 0.061$) appear to be highly significant. The precipitation of copper ions was more pronounced when pH exceeds 5.0 and due to the increase in the concentration of OH^-

Table 3
Significance of regression coefficients for the removal of copper using Minitab 14 software

Model term	Parameter estimate (coefficients)	t	P
Constant	97.324	58.869	0.000
X_1	−0.765	−0.857	0.404
X_2	0.923	1.034	0.316
X_3	−2.976	−3.333	0.004
X_4	1.779	1.993	0.064
X_1X_1	−3.254	−3.978	0.001
X_2X_2	−2.986	−3.651	0.002
X_3X_3	−2.544	−3.110	0.007
X_4X_4	−5.486	−6.707	0.000
X_1X_2	1.577	1.443	0.168
X_1X_3	0.571	0.522	0.609
X_1X_4	0.846	0.774	0.450
X_2X_3	−1.216	−1.112	0.282
X_2X_4	2.201	2.013	0.061
X_3X_4	1.188	1.086	0.294

X_1, X_2, X_3, X_4 = linear effects; $X_1^2, X_2^2, X_3^2, X_4^2$ = square effects; $X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4, X_3X_4$ = interaction effects.

ions in the biosorption medium, biosorption studies could not be performed as reported by Sag and Kutsal [16]. The optimum pH for the maximum removal of copper was found to be 4.14 which agree with the results obtained by Sag and Kutsal [16]. The results of analysis of variance for the models used for the removal of copper are given in Table 4. The ANOVA demonstrates that the quadratic model was highly significant, as is evident from the calculated F value (6.62) and a very low probability value (p model > F = 0.0001). It was observed from Table 4, the coefficient for the linear and square effects were highly significant (p = 0.0001) when compared with interactive effects.

The response surface contour plots of percentage removal of copper versus the interactive effect of initial copper ion concentration, pH, temperature and biomass loading are shown in Figs. 1–3. Each contour plot represents a number of combinations of two test variables with the other variable maintained at zero levels. The maximum percentage removal of copper is indicated by the surface confined in the smallest curve (circular or elliptical) of the contour plot. The studies of the contour plots also reveal the best optimal values of the process conditions and are given below: initial copper ion concentration 50–55 ppm, pH 3.5–4.5, temperature 35–40 °C and biomass loading 8–9 g/l. The second-degree polynomial regression equation Eq. (3) was solved by the sequential quadratic programming

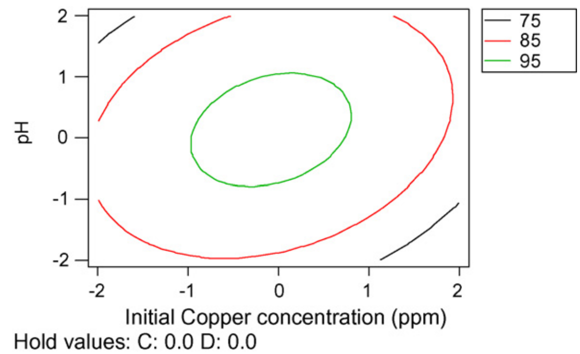

Fig. 1. Response surface contour plot showing interactive effect of initial concentration and pH on the removal of copper.

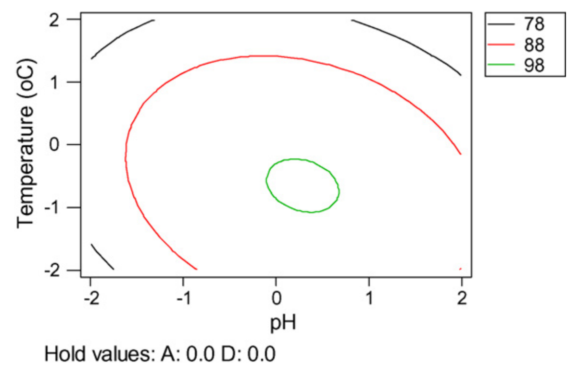

Fig. 2. Response surface contour plot showing interactive effect of pH and temperature on the removal of copper.

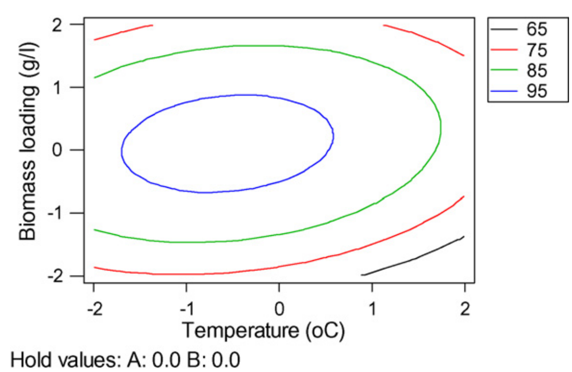

Fig. 3. Response surface contour plot showing interactive effect of temperature and biomass loading on the removal of copper.

using MATLAB 6.5. The optimum values of the test variables and the corresponding maximum percentage removal of copper (98.34%) were obtained in coded units as $X_1 = -0.11581$, $X_2 = 0.14014$, $X_3 = -0.45095$ and $X_4 = 0.26895$ and they were

Table 4
Analysis of variance (ANOVA) for the selected quadratic model for the removal of copper

Sources of variation	Sum of squares	Degrees of freedom	Mean square	F	P
Regression	1772	14	126.573	6.62	0.000
Linear	323.03	4	80.753	4.22	0.016
Square	1268.76	4	317.190	16.58	0.000
Interaction	180.25	6	30.042	1.57	0.219
Residual error	306.12	16	19.133		
Total	1985.24	30			

Table 5
Optimum values of variables obtained from regression equations for the removal of copper

Parameter	Optimum value for copper
Initial copper ion concentration (ppm)	53.84
pH	4.14
Temperature (°C)	37.75
Biomass loading (g/l)	8.17
Copper removal (%)	98.34

converted to the uncoded units for the actual values, as given in Table 5.

A maximum copper removal of 98% was obtained using the biosorption kinetics of copper under optimum conditions. This experimental value closely agrees 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.

4. Conclusion

This work has demonstrated the use of a full factorial central composite design by determining the optimum process conditions leading to the maximum percentage removal of copper from aqueous solutions. Using this experimental design and multiple regression, the parameters namely, initial metal ion concentration, pH, temperature, biomass loading were studied effectively and optimized with a lesser number of experiments. This methodology could therefore be successfully employed to study the importance of individual, cumulative and interactive effects of the test variables in biosorption and other processes.

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