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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