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Heavy Metal Removal by Biosorption Using *Phanerochaete chrysosporium*

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Abstract

Biosorption using microbial cells as adsorbents is being seen as a cost-effective method for the removal of heavy metals from wastewaters. Biosorption studies with *Phanerochaete chrysosporium* were performed for copper (II), lead (II), and cadmium (II) to evaluate the effectiveness and to optimize the operational parameters using response surface methodology. The operational parameters chosen were initial metal ion concentration, pH, and biosorbent dosage. Using this method, the metal removal could be correlated to the operational parameters, and their values were optimized. The results showed fairly high adsorptive capacities for all the metals within the settings of the operational parameters. The removal efficiencies followed the order Pb > Cu > Cd. As a general trend, metal removal efficiency decreased as the initial metal ion concentration increased, and the results fitted the Langmuir and Freundlich isotherms well.

Index Entries: Biosorption; *Phanerochaete chrysosporium*; heavy metals; heavy metal removal; response surface methodology; adsorptive capacity.

Introduction

Heavy metals containing effluents discharged from a variety of industries have been posing a major threat to the aquatic environment worldwide. The currently practiced technologies for removal of heavy metals—precipitation, ion exchange, and reverse osmosis—appear to be inadequate and expensive. Further, they often create secondary problems associated with metal-bearing sludges and concentrates. Hence, the search is on for efficient and cost-effective alternatives. Biologic techniques, particularly biosorption, appear to be a promising solution to meet these requirements. Biosorption studies with *Zoogloea ramigera*, *Rhizopus arrhizus*, *Candida utilis*, *Saccharomyces cerevisiae*, *Citrobacter*, and *Polyporus versicolor*; waste biomass

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from pharmaceutical or food industries; and seaweed or such other naturally available resources have been reported for removal of heavy metals. A considerable amount of work has also been done on the ability of fungi to take up heavy metals *(1)*. From the standpoint of metal removal and recovery from effluents, interest has centered on the ability of filamentous fungi to take up heavy metals. The white-rot fungus *Phanerochaete chrysosporium* has attracted considerable attention because of its ability to degrade a variety of toxic organic pollutants owing to the extracellular peroxidase enzymes. However, as a filamentous fungus, it has also shown good potential for removal of toxic metals by biosorption.

The current knowledge on the influence of various process variables on biosorption is almost entirely based on a limited number of experiments treating only one factor at a time. The traditional single-factor optimization is a shotgun approach in which each parameter is considered to be insensitive to other process variables involved. In a complex case in which several factors have an important role, their possible interaction could thus not be observed even with a very large number of experiments. Being single determination, this method often does not guarantee determination of optimum condition, besides being laborious and time-consuming.

Statistical design of experiments, such as factorial design, is a powerful tool for understanding complex processes whose detailed mechanisms are unknown and for describing factor interactions in multifactor systems. In full factorial designs, experiments are carried out at all levels of every variable. This method gives the effects of all the parameters on response along with their interactive effects. Response surface methodology (RSM) *(2,3)* is an empirical statistical technique employed for multiple regression analysis of quantitative data obtained from statistically designed experiments by simultaneous solution of multivariate equations. The graphic representation of these equations, called response surfaces, can be used to describe the individual and cumulative effect of the test variables (parameters) on the response and to determine the mutual interaction between the test variables and their subsequent effect on the response.

The objective of the present work was to study the biosorption of some heavy metals using *P. chrysosporium* and to represent the adsorption kinetics in terms of suitable adsorption isotherms. In this paper, we present the results of an optimization study of some biosorption parameters using a 2^3 full factorial central composite design and RSM.

Materials and Methods

The metal salts $Pb(NO_3)_2$, $CuSO_4{\cdot}5H_2O$, $3CdCl_2{\cdot}7H_2O$, and other chemicals used were of analytical grade.

Preparation of Biosorbent

P. chrysosporium ATCC 24725 was maintained on malt agar slants. The stock culture was transferred weekly and stored in a refrigerator (4°C).

Biomass of *P. chrysosporium* was grown in dextrose, soya peptone broth (pH 5.6) at 30–32°C for 2 to 3 d. Cells in logarithmic growth phase were harvested by centrifuging at 10,000*g* for 10 min (Remi cooling centrifuge). Harvested cells were washed with distilled water and subsequently used as biosorbent.

Biosorption Studies

Single Sorbate Isotherm for Biosorption of Heavy Metals Using *P. chrysosporium*

To about 1.8 g of wet biomass required quantities of metal salts were added to give initial metal ion concentrations in the range of 25–300 mg/L. The flasks were shaken in an orbital shaker (150 rpm) at 32°C for 30 min. After biosorption, the biomass was separated from the treated metal solution by centrifuging at 10,000*g* for 10 min, and the supernatants were analyzed for residual metal ion concentration using an atomic absorption spectrophotometer (Varion 400 AAS model). Metal uptake by *P. chrysosporium* biomass, q (mg of metal/g of dry biomass), was calculated as follows:

$$q = V(C_{\text{initial}} - C_{\text{final}})/1000m$$

in which q is the specific metal uptake (mg of metal/g of biosorbent), V is the volume of the metal solution (mL), C_{initial} is the initial concentration of metal ion solution (mg/L), C_{final} is the final concentration of metal in the solution following biosorption (mg/L), and m is the dry weight of the biomass (g).

Optimization of Parameters Involved in Biosorption of Heavy Metals by *P. chrysosporium*

An orthogonal 2^3 factorial central composite experimental design *(4,5)* with six star points ($\alpha = 1.682$) and six replicates at the center point, all in duplicates, resulting in a total of 20 experiments, was used to optimize the chosen key variables in biosorption, in shake-flask experiments. Table 1 shows the levels of the chosen independent variables used in the experiment. The variables were coded according to the Eq. 1:

$$x_i = (X_i - X_0)/\Delta x \qquad (1)$$

in which x_i is the (dimensionless) coded value of the variable X_i, X_o is the value of X_i at the center point, and Δx is the step change. The behavior of the system was explained by the following second-degree polynomial equation:

$$Y = \beta_o + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\beta_{ij} x_i x_j \qquad (2)$$

in which Y is the predicted response, β_o is the offset term, β_i is the coefficient of linear effect, β_{ii} is the coefficient of squared effect, and β_{ij} is the coefficient of interaction effect.

The design package, Minitab (PA, USA), a statistical program package, was used for regression analysis of the data obtained and to estimate the coefficient of the regression equation. The goodness of fit of the regres-

Table 1
Experimental Range and Levels of Independent Process Variables

Independent variable	Range and level				
	−α	−1	0	+1	+α
Initial metal ion concentration (mg/L)	4.38	30	65	100	125.62
Biosorbent dose (wet wt basis) (g)	0.317	0.5	0.75	1.0	1.183
pH					
Pb	4.0	4.8	6.0	7.0	7.9
Cd	4.0	4.9	6.0	7.2	8.0
Cu	2.8	3.4	4.3	5.2	5.8

sion model obtained was given by the multiple correlation coefficient, R, and by the coefficient of determination, R^2. The statistical significance of the model was determined by the application of Fisher's F-test. The student's t-test was used to determine the significance of the partial regression coefficients and coding of the variables enabling direct comparison of them. The response surface, which is a two-dimensional graphic representation of the system's behavior, was used to determine the individual and cumulative effects of the variables and the mutual interactions between the variables on the dependent variable.

Desorption Study

After biosorption, the biomass was separated from the treated metal solution by centrifugation, suspended in 10 mL of 1 *N* HCl, and agitated for 10 min. The biomass was then separated, and the metal ion concentration in the solution was estimated.

Results and Discussion

Based on initial screening of various heavy metals for their removal by *P. chrysosporium*, Pb (II), Cu (II), and Cd (II) were chosen for this biosorption study *(6)*. The metal-binding capacity, expressed as milligrams of metal sorbed per gram of dry biomass, was analyzed by fitting data with two well-known adsorption models: Langmuir and Freundlich. Pb biosorption capacity of the organism was better than that for Cu and Cd. The effect of initial metal concentration on its removal by biosorption is shown in Fig. 1. While a marginal increase in removal efficiency was observed with increasing Pb concentration, the metal removal decreased at higher initial concentrations of Cu and Cd. Yetis et al. *(7)* found a similar trend. However, the percentage removals observed in this study were more than those reported by Yetis et al. *(7)* using *P. chrysosporium* and with other species of fungi *(8)*. The results of biosorption studies vary widely with different investigations because of the different criteria used for the selection of suitable biomass. In addition, the absence of uniform methodology often makes quantitative comparison impossible *(7)*.

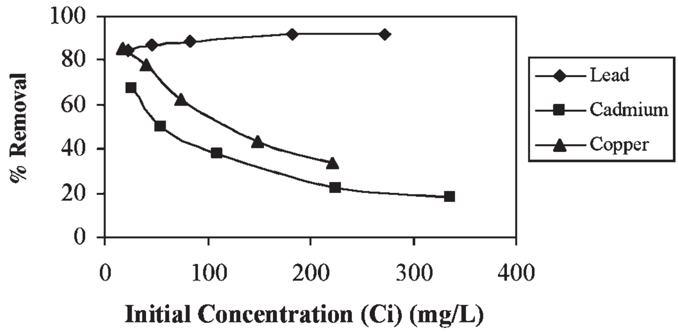

Fig. 1. Effect of initial metal concentration on heavy metal removal efficiency by *P. chrysosporium*.

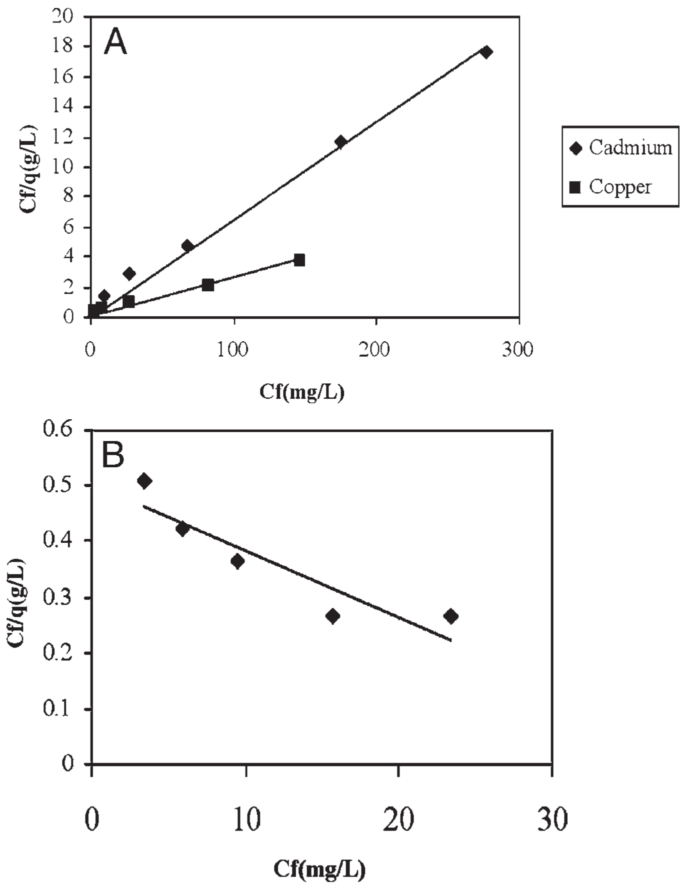

Fig. 2. **(A)** Langmuir isotherms for cadmium and copper with *P. chrysosporium*; **(B)** Langmuir isotherm for lead with *P. chrysosporium*.

Figures 2A,B and 3 show that the metal uptake ability of these three metals fitted the Langmuir and Freundlich isotherms well. The sorption parameters as determined from the two models are given in Table 2. The

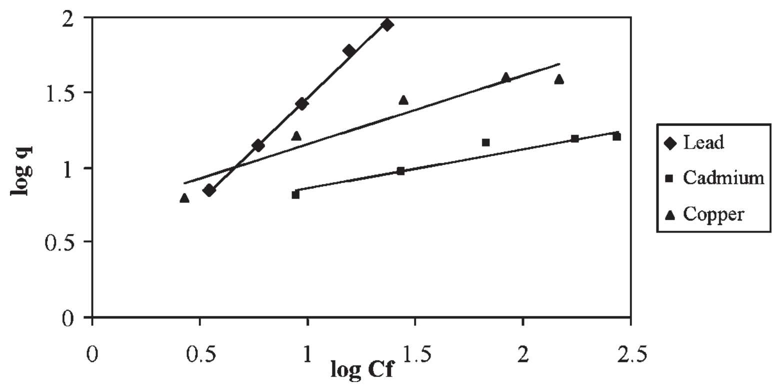

Fig. 3. Freundlich isotherms for various heavy metals with *P. chrysosporium*.

Table 2
Freundlich and Langmuir Adsorption Constants Obtained for Biosorption of Heavy Metals by *P. chrysosporium*

	Freundlich			Langmuir		
	K (l/g)	*N*	*R*	q_{max} (mg/g)	*b* (L/mg)	*R*
Pb	1.21	0.725	0.9983	89	0.02	0.9132
Cu	4.93	2.18	0.9615	43	0.07	0.9984
Cd	3.83	3.734	0.9567	17	0.06	0.9994

Langmuir model, which reflects the two important characteristics of the sorption system, q_{max} and *b*, indicated the following order of removal of *P. chrysosporium* for the metals studied: Pb > Cu > Cd.

Pb adsorptive capacity of *P. chrysosporium* was the highest among the three metals studied; however, the value of 90 mg/g was slightly less compared with those obtained by using other species of fungi. For example, with *Absidia orchidis*, Holan and Volesky *(8)* attained 351 mg of Pb(II)/g of dry biomass. With another fungus, *Rhizopus nigricans*, they attained 166 mg of Pb(II)/g of dry biomass. Brierley et al. *(9)* were able to obtain adsorptive capacities of 601 and 373 mg/g with *Bacillus subtilis* and fungal biomass, respectively, by processing the biomass to improve biosorptive capacity.

For Cu, highest adsorptive capacity of 152 mg/g was reported with pretreated *B. subtilis (9–11)*. In the present study, the uptake capacity of Cu for *P. chrysosporium* obtained was 43 mg/g. This value was much higher than those reported for other filamentous fungi, which ranged between 0.4 and 18 mg/g. Gadd et al. *(1)* obtained 10 and 18 mg/g adsorptive capacities for Cu with *Rhizopus arrhizus* and *Cladosporium resinae*, respectively.

The adsorptive capacity value of 17 mg/g for Cd with *P. chrysosporium* was in agreement with that obtained by Yetis et al. *(7)*. Uptake capacities of 19 and 0.4 mg/g for Cd have been reported by Holan and Volesky *(8)* and Townsley et al. *(12)* using *R. nigricans* and *Penicillium spinulosum*, respectively.

Table 3
Full Factorial Central Composite Design Matrix of Three Variables Along with Observed Responses (% removal)

Run no.	X_1 (initial metal ion conc., ppm)	X_2 (biosorbent dose, g)	X_3 (pH)	Response (% removal) Pb	Cu	Cd
1	–1	–1	–1	83.84	41.85	26.94
2	+1	–1	–1	91.49	9.75	54.08
3	–1	+1	–1	79.98	38.64	31.69
4	+1	+1	–1	87.42	28.02	11.2
5	–1	–1	+1	82.49	69.78	23.78
6	+1	–1	+1	90.04	14.56	14.8
7	–1	+1	+1	81.62	43.45	31.37
8	+1	+1	+1	89.61	45.88	16.32
9	–α	0	0	61.53	70.74	33.72
10	+α	0	0	90.88	38.46	14.42
11	0	–α	0	91.23	50.82	25.05
12	0	+α	0	90.2	83.52	32.45
13	0	0	–α	91.16	65.38	26.00
14	0	0	+α	89.5	73.35	26.57
15	0	0	0	90.02	71.08	28.1
16	0	0	0	90.92	71.98	27.84
17	0	0	0	90.59	70.98	28.04
18	0	0	0	90.82	71.58	29.14
19	0	0	0	89.92	70.94	27.84
20	0	0	0	90.83	71.93	28.2

Metal sorption by many microorganisms is influenced by the pH of the solution, initial metal concentration, biomass concentration, and type of growth medium. Other parameters of lesser relevance are the age of the culture or temperature *(13)*. To understand the effects of the biosorption parameters initial metal ion concentration, biosorbent dose, and pH, and their interactions on the biosorption process, statistically designed experiments were used to perform the experiments. Table 3 gives the coded values of the combination of the variables and the experimental results. A considerable variation in the metal removal depending on the value of the parameters was observed. The experimental data were analyzed using statistical methods appropriate to the experimental design used. Multiple regression analysis of the data yielded the following regression equations for the three metals: Regression equation for Cd:

$$Y = 29.414 - 5.991X_1 + 5.495X_2 + 5.038X_3 - 11.777X_1^2 - 7.334X_2^2 - 6.559X_3^2 + 9.881X_1X_2 - 1.259X_1 \cdot X_3 - 1.259X_2 \cdot X_3 \quad (3)$$

Regression equation for Pb:

$$Y = 90.908 + 5.856X_1 - 0.803X_2 - 0.13X_3 - 5.123X_1^2 + 0.006X_2^2 - 0.13X_3^2 + 0.028X_1 \cdot X_2 + 0.055X_1 \cdot X_3 + 0.828X_2 \cdot X_3 \quad (4)$$

Regression equation for Cu:

$$Y = 72.83 - 10.968X_1 + 5.495X_2 + 5.038X_3 - 11.777X_1^2 - 7.334X_2^2 - 6.559X_3^2 + 9.881X_1 \cdot X_2 - 1.259X_1 \cdot X_3 + 1.259X_2 \cdot X_3 \quad (5)$$

in which Y in all cases is the response variable, and percentage metal removal and X_1, X_2, and X_3 are the coded values of the independent variables, initial metal ion concentration, biosorbent dose, and pH respectively.

The correlation measures for testing the goodness of fit of the regression equation are the multiple correlation coefficient, R, and the determination coefficient, R^2. The values of $R = 0.9291$ for Cd, 0.8325 for Cu, and 0.955 for Pb being close to 1 indicates a high degree of correlation between the observed and predicted values. The value of $R^2 = 0.8632$ for Cd, 0.693 for Cu, and 0.912 for Pb suggests that only about 14, 31, and 9% for Cd, Cu, and Pb, respectively, of the total variation are not explained by the model. To test the significance and adequacy of the model, statistical testing of the model in the form of analysis of variance (ANOVA) was done.

The mean squares were obtained by dividing the sum of squares of each of the two sources of variations, the model and the error variance, by the respective degrees of freedom. The Fisher's variance ratio, F value = (Sr^2/Se^2), is the ratio of the mean square owing to regression to the mean square owing to the error. It is a measure of the variation in the data about the mean. Generally, the calculated F value should be several times greater than the tabulated F value if the model is a good prediction of the experimental results and the estimated factor effects are real. Here, the ANOVA of the regression model demonstrates that the model is highly significant, as is evident from the calculated F value (=7.319 for Cd, 2.509 for Cu, and 11.54 for Pb) and a very low probability value (p model $> F = 0.0023$, 0.0841, 0.0003 for Cd, Cu, and Pb, respectively). The p values are used as a tool to check the significance of each of the coefficients, which, in turn, may indicate the patterns of the interaction among the variables. The larger the magnitude of t and smaller the value of p, the more significant is the corresponding coefficient.

It was observed that the coefficient for the linear effect of metal concentration was highly significant for all the metals ($p = 0.0001$, 0.0034, and 0.001 for Cd, Cu, and Pb, respectively) and that the coefficient for the linear effect of pH was the least significant. The biomass concentration was slightly significant only in the case of cadmium ($p = 0.0561$). The coefficient of the quadratic terms of initial metal concentration was also quite significant for all the metals ($p = 0.0167$ for Cd, 0.0216 for Cu, and 0.001 for Pb), whereas those of the other two variables were not very significant. The coefficients of the interactive effects among the variables do not appear to be very significant in comparison to the linear effects for all the metals. However, the interactive effects between initial metal concentration and biosorbent dose for Cu ($p = 0.1197$) and that between initial metal concentration and pH for Cd ($p = 0.2081$) appear to be slightly significant.

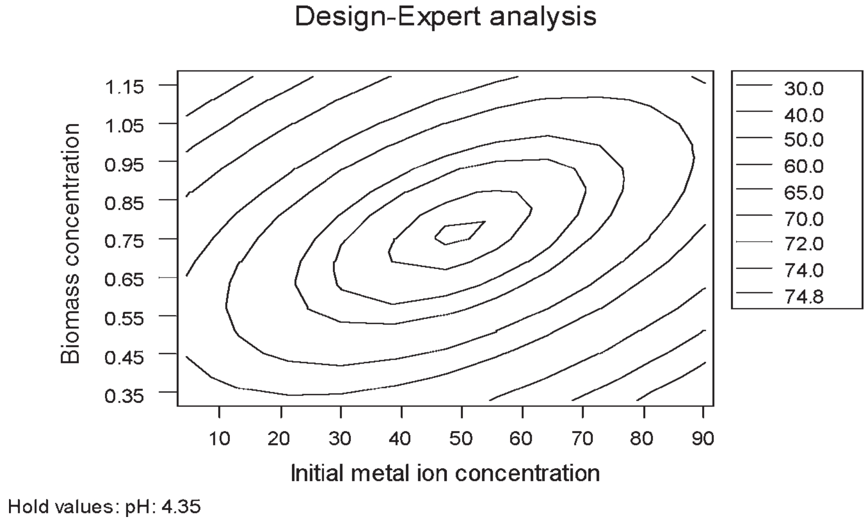

Fig. 4. Response surface contour plot of metal removal (%) showing interactive effect of initial metal ion and biomass concentration on copper removal.

The graphic representations of the regression equation called the response surfaces were obtained using the same software package. Each contour plot represents an infinite number of combinations of two test variables with the other variable maintained at 0 level. The maximum predicted yield is indicated by the surface confined in the smallest curve of the contour diagram. The shapes of the response surfaces, circular or elliptical, indicate whether the interactions among the variables are significant or not (Fig. 4). Although these interaction effects are not readily evident from the significance test of the parameter estimates, the response of the contour plot is able to reveal the same *(5)*. The response surface contours confirm the findings of the significance tests. However, the contour plot analysis showed that interaction between initial metal concentration and pH on Pb removal is also significant. It was also observed from the response surfaces that lower metal concentration and higher biosorbent dose were generally favorable for metal removal in the case of both Cu and Cd.

The optimum values of the process condition for metal removal were obtained by solving the regression Eqs. 3–5 using the multistage Monte-Carlo technique *(14)*. The optimal values of the test variables were first obtained in coded units and then converted to the uncoded units, as given in Table 4. Relatively high removals of Pb and Cu could be achieved even at fairly high concentrations of these metals. Cd removal was only moderate. These results agree closely with those obtained from the response surface analysis, confirming that the RSM using the statistical design of experiments can be effectively used to optimize the process parameters in complex biotechnologic processes. Although few studies on the effects of parameters on biosorption have been reported in the literature, no attempt has been made to optimize them using statistical optimization methods.

Table 4
Optimum Values of Variables Obtained by Solving Regression Equations

	Optimum value		
Parameter	Pb	Cu	Cd
Initial metal ion concentration (ppm)	85.24	41.27	7.43
Biosorbent dose (g)	0.35	0.75	1.18
pH	6.34	4.69	5.32
Metal removal (%)	92.4	76.5	36.6

The desorption of metals from the loaded biosorbent was studied using 1 *N* HCl as the recovery agent. Cd was more relatively easily desorbed (34.7%) than the other metals (Cu = 17% and Pb = 15.5%). The ease with which the metals were desorbed from the loaded biomass indicated that the affinity of the organism toward the metal ions seemed to follow the order Pb > Cu > Cd. This desorption study also revealed the potential for recovering the biosorbed metals for reuse.

Conclusion

The present study proves that *P. chrysosporium* could be an effective biosorbent for the removal of Pb, Cu, and Cd from wastewaters. The removal efficiency followed the order Pb > Cu > Cd. Both Langmuir and Freundlich isotherms were found to fit the experimental metal uptake rates with a high degree of correlation. The q_{max} values of the Langmuir isotherm also confirmed the metal affinities to the biosorbent. The desorption experiment indicated the potential for recovery of the removed metal ions. Statistical analysis of full factorial central composite design of the experiments revealed that the initial metal ion concentration was the most significant variable compared to the biosorbent dose and pH of the metal solution. The metal-removal efficiency was found to decrease with an increase in metal concentration. Response surface contour plots showed the effects of interaction among process variables on metal-removal efficiency. Optimization studies indicated that the statistically designed experiment using RSM could be effectively adopted for optimization of process variables.

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