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Application of Factorial Design to Study of Heavy Metals Biosorption by Waste Biomass from Beverage Distillery

MARISTELLA A. DIAS,[1] CARLOS A. ROSA,[1] VALTER R. LINARDI,[1] ROSA A. CONTE,[2] AND HEIZIR F. DE CASTRO*,[3]

*[1]Department of Microbiology, Universidade Federal de Minas Gerais,
PO Box 486, 30270-900, Belo Horizonte-MG, Brazil;
and Departments of [2]Materials and [3]Chemical Engineering,
Faculdade de Engenharia Química de Lorena, PO Box 116, 12600-000,
Lorena-SP, Brazil, E-mail: decastro@easygold.com.br*

Abstract

A full factorial design leading to 20 sets of sorption runs was conducted to study the influence of four variables (bleaching earth and biomass concentrations, pH, and sorption time) on the iron, nickel, and chromium removal from stainless steel effluent using waste biomass from a beverage industry. Similar factor effects and interactions were found for each metal involved in this biosorption study, and the main factors were pH (positive effect) and biomass concentration (negative effect). Response surface methodology was adopted and an empirical linear polynomial model constructed on the basis of the specific uptake (mg of metal/g of biomass as dry weight) for each metal species. Under optimized process conditions (pH 4.0, biomass concentration of 2.0 g/L, absence of Celite), uptake values of 155 mg of Fe/g, 38 mg of Cr/g, and 0.4 mg of Ni/g were achieved after 3 h. This corresponded to a reduction in heavy metals concentration of approx 94% for Cr, 57% for Fe, and 25% for Ni.

Index Entries: Biosorption; waste biomass; heavy metals; experimental design; effluent detoxification.

Introduction

Removal of heavy metals by either active or inactive microorganisms is a promising technique for detoxication of highly pollutant industrial wastes *(1,2)*. The use of active biomass can be a quite effective method by employing resistant microorganisms that are able to overcome the process

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*Author to whom all correspondence and reprint requests should be addressed.

limitations including the metal toxicity to cell growth and the action of other toxic pollutants present in the effluent *(3,4)*. On the other hand, the use of inactive microorganisms can offer several advantages making this a method of choice from both economic and environmental points of view *(5–9)*. Inactive biomass is not ruled by physiologic restrictions and can be stored or used for extended periods at room temperature without the onset of putrefaction *(7,9)*. Besides having rapid and efficient metal uptake, the biomass behaves as an ion-exchange material and metals can be desorbed readily and recovered *(7)*. Moreover, if the biomass employed is a waste material (e.g., fermentation byproducts), biosorption represents a cheap alternative to conventional treatments, owing to the use of a low-cost sorbent material *(7,8)*.

Biomass samples obtained from a variety of waste streams have been reported to bind different metals, indicating that certain species might be better suited to particular metal pollutants *(1,2)*. For example, *Penicillium chrysogenum* and *Bacillus subtilis* have been shown to accumulate copper more effectively than many other microorganisms.

Interest in developing metal removal by biosorption using spent biomass has recently been indicated in the literature *(5–11)*. Suh et al. *(7)* compared the accumulation capacity of living and dead cells of *Saccharomyces cerevisiae* and *Aurebasidium pullulans* for lead. Stoll and Duncan *(8)* studied the metal sorptive properties of three types of immobilized nonviable *S. cerevisiae* biomass. Mattuschka and Straube *(9)* investigated the binding of several metals by pharmaceutical waste biomass (*Streptomyces noursei*), and Singleton and Simmons *(10)* examined the factors affecting silver biosorption by freeze-dried *S. cerevisiae* biomass produced in the brewing industry. Ferraz and Teixeira *(11)* used a flocculating brewer's yeast as biosorbent material for the removal of Cr and Pd from residual wastewater. They also determined the influence of physicochemical factors such as pH, biomass concentration, and the presence of a coion.

Our efforts in this area also concern the use of spent biomass as a biosorbent material. In a previous work, the biosorptive capacity of four types of waste biomass from beverage distilleries (*cachaça*) for different metal ions was investigated *(12)*. The metal affinities of these biomass types varied considerably, and best performance was achieved with spent biomass derived from Germana distillery, owing to its high capacity for retaining heavy metals from both synthetic and industrial effluent solutions.

Metal sorption performance also depends on external factors such as pH, other ions in solution (which may be in competition), organic materials in solution (such as complexing agents and cell metabolic products that can cause metal precipitation), and temperature *(1,2)*. In the present study we report the results, beyond our previous work *(12,13)*, of evaluating some of these parameters in terms of the capacity of the selected waste biomass to bind more than one metal simultaneously. The application of statistical design was used to determine the optimum operating conditions for the system. This technique is a powerful approach well suited for processes in which several variables must be considered simultaneously, a situation frequently found in industrial effluents that contain various metals ions that interfere with metal biosorption.

Materials and Methods

Sorbent Materials

Waste biomass was obtained from the stillage generated by a liquor distillery (Germana) situated in the state of Minas Gerais (Brazil). Stillage was collected and centrifuged for 20 min at 2000*g* at 25°C. The recovered biomass was washed three times with deionized water and dried at 60°C to a constant weight. Celite (341) was obtained from Aldrich (Milwaukee, WI).

Effluent and Biosorption Trials

Effluent was collected from a stainless steel company (Acesita) in the state of Minas Gerais (Brazil) and diluted with deionized water to reduce the iron (III) concentration to a level of 600 mg/L. Other metal species were also present: 2.7 mg/mL of nickel, 7.8 mg/mL of chromium (VI). Biosorption runs were carried out in 250-mL Erlenmeyer flasks containing 50 mL of industrial effluent solution. Before mixing with the waste biomass and/or Celite, the pH was adjusted to various values between 2.0 and 4.0. The flasks were shaken (150 rpm) at constant temperature (30°C) for a maximum of 6 h. Control runs were also performed without biomass to verify eventual metal precipitation. Flask contents were filtered using 0.45-μm Millipore filter membranes, and the filtrates were analyzed by flame atomic absorption spectrophotometry (Varian model AA-475) for residual metal content *(14)*.

Experimental Design

The influence of Celite concentration (X_1), biomass concentration (X_2), initial pH (X_3), and time course of biosorption (X_4) was studied using a 2^4 full factorial design. Table 1 gives the range and levels of the studied variables. The runs were performed at random. Four experiments were carried out at the center point level, for estimation of experimental error. Data processing and calculations were carried out using Statistica (version 5.0) software. The statistical significance of the regression coefficients was determined by student's t-test *(15)*, the model equation was determined by Fisher's test *(15)*, and the proportion of variance explained by the model obtained was given by the multiple coefficient of determination, R^2. The response of the process under investigation was the metal-specific uptake (q in milligrams/gram) calculated according to Eq. 1:

$$q = [(C_0 - C)/X] \qquad (1)$$

in which q (mg of metal/g of biomass) is the metal-specific uptake, C_0 (mg/L) is the initial metal concentration, C is the residual metal concentration, and X (g/L) is the biomass concentration.

Table 1
Experimental Range and Levels
of Independent Process Variables According to 2^4 Full Factorial Design

Variable	Symbol	Level –1	Level 0	Level +1
Celite (g/L)	X_1	0	2.0	4.0
Biomass (g/L)	X_2	2.0	5.0	8.0
pH	X_3	2.0	3.0	4.0
Time (h)	X_4	3.0	4.5	6.0

Table 2
Experimental Design and Results According to 2^4 Full Factorial Design

	Variable				Response		
Run	X_1 (g/L)	X_2 (g/L)	X_3	X_4 (h)	Fe (mg/g)	Ni (mg/g)	Cr (mg/g)
1	0	2.0	2.0	3	0	0.15	1.20
2	4.0	2.0	2.0	3	0	0.05	0.31
3	0	8.0	2.0	3	10.4	0.05	1.25
4	4.0	8.0	2.0	3	5.2	0.02	0.60
5	0	2.0	4.0	3	155.5	0.40	38.05
6	4.0	2.0	4.0	3	40.5	0.11	12.40
7	0	8.0	4.0	3	43.2	0.08	9.10
8	4.0	8.0	4.0	3	25.7	0.06	6.13
9	0	2.0	2.0	6	3.2	0.10	2.80
10	4.0	2.0	2.0	6	8.3	0.05	1.85
11	0	8.0	2.0	6	0	0.02	0.45
12	4.0	8.0	2.0	6	0.2	0.02	0.25
13	0	2.0	4.0	6	153.0	0.40	37.85
14	4.0	2.0	4.0	6	38.1	0.05	12.10
15	0	8.0	4.0	6	41.7	0.08	9.27
16	4.0	8.0	4.0	6	29.0	0.05	6.16
17	2.0	5.0	3.0	4.5	36.3	0.08	7.58
18	2.0	5.0	3.0	4.5	30.4	0.08	7.64
19	2.0	5.0	3.0	4.5	19.1	0.05	4.00
20	2.0	5.0	3.0	4.5	20.4	0.05	4.00

Results

The effects of four variables (Celite and biomass concentrations, pH, and sorption time) on metal-binding affinity of the waste biomass were simultaneously investigated using a 2^4 full factorial design leading to 20 sets of experiments. The choice of variables was made based on their importance to this kind of process. The association of waste biomass (biosorbent) with inorganic sorbent (Celite) was used as a strategy to enhance the iron uptake as previously described by Dias et al. *(13)*. The design of this experiment is given in Table 2, together with the experimental results.

The results clearly showed that biomass adsorption capacity was strongly affected by initial pH, independently of the other variables. At pH 2.0 (runs 1–4 and 9–12) the biomass adsorption capacity was very low for Fe and Ni uptakes (<12 mg/g). This was likely owing to the nature of chemical interactions of each metal species and the waste biomass; at low pH, the overall surface charge on the biomass will become positive, which will inhibit the approach of positively charged metal cations. It is likely that protons then compete with metal ions for the binding sites and thereby decrease the interaction of biomass and metal ions *(7,8)*. By increasing the pH from 2.0 to 4.0 (runs 5–8 and 13–18), the biomass sorption capacity for all studied metal species reached their maximum values.

Another important factor in the biosorption performance was biomass concentration (Table 2). At a biomass concentration of 2 g/L, specific metal uptake values were maximized (runs 5 and 13). As the biomass concentration increased to the high level (8 g/L), a decrease in capacity was observed, suggesting an inverse relationship between biomass concentration and its biosorptive capacity. Similar behavior has been found for other biosorbent types, as reported by Singleton and Simmons *(10)*. Various reasons have been suggested to explain this effect, including limited availability of solute, electrostatic interactions, interference between binding sites, and reduced mixing at high biomass densities *(10)*. The time course of biosorption trials did not promote any positive effect on the parameters evaluated, showing that 3 h was sufficient to attain adsorption equilibrium. On the other hand, the addition of Celite to the biosorptive runs exerted a negative influence for all response variables. This suggests that, in the range investigated, association of sorbent materials is not necessary to attain high metal uptake.

The experimental results shown in Table 2 were used to estimate the main effects of variables and their interactions. The statistical analyses for each of the response variables evaluated—Fe, Ni, and Cr uptake—are summarized in Table 3. According to student's *t*-test results, the effects and interactions are similar for all metals involved in this biosorption. The most important variable was pH (X_3), since it presented a significant effect for Ni response (95% confidence level) and for both iron and chromium responses (99% confidence level). The effect of Celite (X_1) and biomass concentration (X_2) and their interactions with pH (X_1X_3, X_2X_3 and $X_1X_2X_3$) were also significant at the same confidence level ($p < 0.05$).

Based on the response evaluated, mathematical models were developed for each metal species. The main effects and their interactions for the responses were fitted by multiple regression analysis (Table 4) to a linear model since tests for curvature ($p > 0.05$) using center points showed that square terms were not important for the models.

By analyzing Table 4, it can be seen that polynomial models were adequate for describing the relationships among all the responses under study and the experimental factors. The regression models were highly significant ($p < 0.001$), at a 99% confidence level, and presented high deter-

Table 3
Estimated Effects, Standard Errors, and Student's *t*-Test for Fe, Ni, and Cr Uptake (mg/g) Using 2^4 Full Factorial Design

	Fe (mg/g)			Ni (mg/g)			Cr (mg/g)		
Variable	Effect	Standard error	t-Value	Effect	Standard error	*t*-Value	Effect	Standard error	*t*-Value
Mean	33.00	±2.03	—	0.98	±0.11	—	8.15	±0.63	—
X_1	–32.50	±4.54	–7.15[a]	–0.11	±0.25	–4.41[a]	–7.52	±1.42	–5.28[a]
X_2	–30.40	±4.54	–6.69[a]	–0.12	±0.25	–4.72[a]	–9.17	±1.42	–6.44[a]
X_3	–62.42	±4.54	13.73[a]	0.10	±0.25	3.91[b]	15.29	±1.42	10.74[a]
X_4	–0.87	±4.54	–0.19	–0.02	±0.25	–0.76	0.21	±1.42	0.15
X_1X_2	23.70	±4.54	–5.21[b]	0.09	±0.25	3.60[b]	5.78	±1.42	4.10[a]
X_1X_3	–32.52	±4.54	–7.15[a]	–0.06	±0.25	–2.59[b]	–6.84	±1.42	–4.81[a]
X_1X_4	1.92	±4.54	0.42	0.01	±0.25	0.05	0.01	±1.42	0.01
X_2X_3	–31.47	±4.54	–6.92[a]	–0.05	±0.25	–2.28	–8.26	±1.42	–5.81
X_2X_4	–2.52	±4.54	–0.55	0.09	±0.25	0.35	–0.44	±1.42	–0.31
X_3X_4	0.10	±4.54	0.02	0.01	±0.25	0.05	–0.28	±1.42	–0.20
$X_1X_2X_3$	26.22	±4.54	5.77[b]	0.06	±0.25	0.24[b]	5.54	±1.42	3.89[b]

[a] $p < 0.01$.
[b] $p < 0.05$.

Table 4
Analysis of Variance for Model Regression
That Represents Specific Metal Uptake

Source	Degrees of freedom	Sum of square			Mean square		
		Fe	Ni	Cr	Fe	Ni	Cr
Model	6	36,701.1	0.21	2215.9	5243.0	0.03	316.5
Residual	13	530.6	0.01	43.4	44.2	0.00	3.62
Total	19	37,231.7	0.22	2259.3			
F-ratio		118.6	37.5	87.43			
p-value		0.000	0.000	0.000			
R^2		0.98	0.95	0.98			

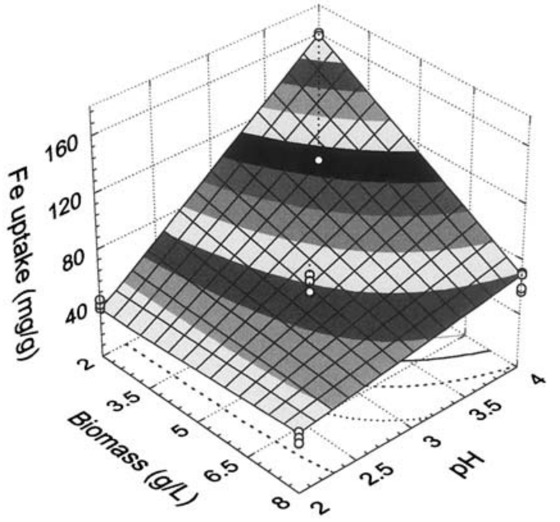

Fig. 1. Response surface described by the model $\hat{y}_1$ that represents specific Fe uptake by waste biomass.

mination coefficients ($R^2 > 0.95$). Thus, mathematical models representing the specific metal uptake in the range studied can be expressed by the following equations:

$$\hat{y}_1 = 33.0 - 16.2x_1 + 15.2x_2 + 31.2x_3 + 11.8x_1x_2 - 16.3x_1x_3 + 1.7x_2x_3 + 13.1x_1x_2x_3 \quad (2)$$

$$\hat{y}_2 = 0.09 - 0.5x_1 - 0.06x_2 + 0.05x_3 + 0.04x_1x_2 - 0.03x_1x_3 - 0.03x_2x_3 + 0.03x_1x_2x_3 \quad (3)$$

$$\hat{y}_3 = 8.1 - 3.8x_1 - 4.6x_2 + 7.6x_3 + 2.9x_1x_2 - 3.4x_1x_3 - 4.1x_2x_3 + 2.8x_1x_2x_3 \quad (4)$$

in which $\hat{y}_1$, $\hat{y}_2$, $\hat{y}_3$ are predicted values for Fe, Ni, and Cr uptake and x_1, x_2, and x_3 are coded values for Celite concentration, biomass concentration, and pH, respectively.

The response surface described by the model equations are represented in Figs. 1–3, and the predicted values were found to be 155 mg/g for

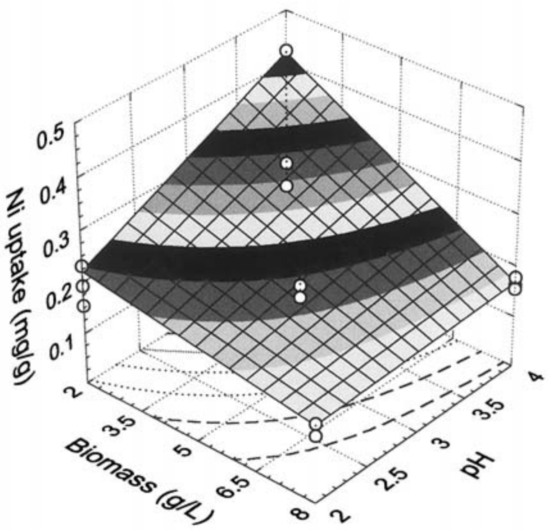

Fig. 2. Response surface described by the model $\hat{y}_2$ that represents specific Ni uptake by waste biomass.

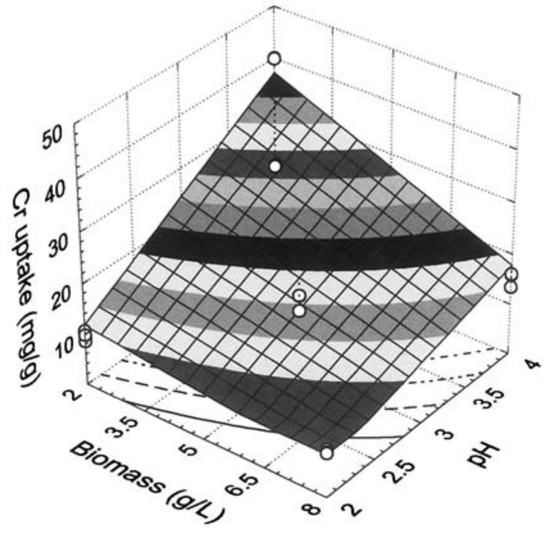

Fig. 3. Response surface described by the model $\hat{y}_3$ that represents specific Cr uptake by waste biomass.

Fe, 39 mg/g for Cr, and 0.4 mg/g for Ni working at a high level for pH, low level of biomass concentration, and absence of Celite.

Discussion

Biosorption of heavy metals to nonviable cells can be used as a potential technology for removal of toxic metals from industrial waste streams, in terms of efficiency and operational suitability. In the present study, waste biomass from the production of *cachaça* was used as a biosorbent to remove heavy metals from stainless steel production effluents. *Cachaça* is a typical Brazilian alcoholic beverage with an alcohol content in the range of 30–43%, and its manufacture by spontaneous fermentation of sugarcane juice is a traditional expertise in the state of Minas Gerais. Unlike fuel alcohol industries, the stillage generated by these distilleries has solid contents because the biomass is not recovered prior to the distillation step *(12,16)*. Therefore, the microbiota involved in the production of *aguardente* is very diversified including species from *Saccharomyces*, apiculate yeasts, *Candida*, and *Schizosaccharomyces* strains rendering to the waste biomass suitable characteristics to be applied to the removal of metals from industrial effluents, as shown in previous work *(12,13)*.

We extended the treatment of multimetal ion effluents by evaluating the effects of Celite and biomass concentrations, pH, and sorption time on the specific uptake (mg of metal/g of biomass as dry weight) by waste biomass employing a multivariate statistical approach. A 2^4 factorial design with center point was adopted for a full understanding of these effects and their interactions. The specific uptake was found to be dependent on both the operating conditions and metal species, although similar factor effects and interactions are found for each metal involved in this biosorption. Because the time course of biosorption runs was not involved in any significant value in terms of the models, the responses were well modeled by a linear function of the other three independent variables. The maximum metal uptake was obtained working at the highest level of pH (4.0), minimum level of biomass (2.0 g/L), and absence of Celite with a contact time of 3 h. Under such conditions, specific uptake values were found to be 155 mg/g for Fe, 38 mg/g for Cr, and 0.4 mg/g for Ni.

Sorption of multimetal ions by biomass is a complex function of the metal combination, levels of metal concentration, and specific metal uptake. In addition, there appears to be competition for adsorption sites on the biomass in such a way that higher specific metal uptake can be observed for single metal solutions.

In the particular case in which Fe was the dominant metal specie in the effluent solution, its removal was favored and lower adsorption of Cr was exhibited. This is typical antagonism behavior in which the effect of the mixture is less important than that of each individual effect of the constituents in the mixture *(1,2)*. On the other hand, no interaction between these metals with Ni was observed. According to the results obtained (reduction in the levels of about 57% for Fe, 25% for Ni, and 94% for Cr), two-stage treatment should be considered to remove completely the high level of Fe from this effluent stream.

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