

Response surface methodology for lead biosorption on *Aspergillus terreus*

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ABSTRACT: A central composite face-centered design was used to study and to optimize lead biosorption from aqueous solution on *Aspergillus terreus* biomass. Four factors such as stirring speed, temperature, solution pH and biomass dose at different levels were studied. The hierarchical quadratic model were established by adding replicates at the central point and axial points to the initial full factorial design (2^4). The percentage removal of lead was affected by biomass dose, pH, and interactions between pH and biomass dose, pH and stirring speed, pH and temperature. The hierarchical quadratic model described adequately the response surface based on the adjusted determination coefficient ($R^2_{Adj} = 0.97$) and the adequate precision ratio (42.21). According to this model, the optimal conditions to remove lead completely from aqueous solutions (at initial lead concentration of 50 mg/L and solutions of 100 mL) with *Aspergillus terreus* were at pH 5.2, 50 °C, stirring speed of 102/min and a biomass dose of 139 mg. The response surface methodology can be used to determine the optimal conditions for metal adsorption on several adsorbents. In addition, results reported in this research demonstrated the feasibility of employing *A. terreus* as biosorbent for lead removal.

Keywords: Biosorbent; Central composite face-centered design; Heavy metals; Optimization; Wastewater

INTRODUCTION

Heavy metals are of great concern because they are considered as the most toxic environment pollutants (Regine and Volesky, 2000). Metals enter the environment when metal-bearing streams are not treated properly. Metals can indefinitely persist in nature and accumulate through the food chain causing a serious risk to the environment and public health (Ahalya *et al.*, 2003; Nouri *et al.*, 2011). The major toxic and hazardous metals for humans as well as other forms of life are mercury, cadmium, lead, chromium, nickel, zinc, copper, etc. Several techniques have been applied to treat metal-containing wastewaters, including reverse osmosis, electrodialysis, ultrafiltration, ion exchange, chemical precipitation, adsorption and phytoremediation, among others. However, the major disadvantages of these techniques include incomplete metal removal, high reagent or energy requirements and sludge generation that requires to be disposed (Ahalya *et al.*, 2003; Gueu *et al.*, 2007). In recent

decades, a number of research studies demonstrated that biosorption can be used to remove metals from aqueous solutions. Biosorption is an emerging technology that uses biological materials, such as biosorbents, for metal removal from water. In addition, biosorption is a cost-effective and an eco-friendly alternative for metal adsorption (Volesky, 2001). Biosorbents commonly used for metal removal include agro-waste materials (García-Reyes *et al.*, 2009; García-Reyes and Rangel-Mendez, 2009), bacteria (Selatnia *et al.*, 2004; Iyer *et al.*, 2005), yeast (Goksungur *et al.*, 2005; Seki *et al.*, 2005), fungi (Pal *et al.*, 2006; Tunali *et al.*, 2006) and algae (Lodeiro *et al.*, 2005; Almaguer-Cantú *et al.*, 2008), among others.

Fungal biomass has proved to be a potential metal biosorbent. Fungi cell walls contain polysaccharides such as glycans (1-6 and 1-3 linked D-glucose residues), chitin (1-4 N-acetyl-D-glucosamine), chitosan (1-4 D-glucosamine), mannans (1-4 mannose), phosphomannans (phosphorylated mannans) (Yan and Viraraghavan, 2000) and glycoproteins have different

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potential metal binding groups such as amine, imidazole, phosphate, sulfhydryl and hydroxyl (Crist et al., 1981).

Filamentous fungi are used in fermentation processes to produce metabolites such as enzymes, antibiotics, organic acids and other bio-molecules. In these processes, thousands of tons of fungi biomass per year are produced and burned or disposed inappropriately. Nevertheless, these by-products have the potential to be used as biosorbent to remove metals from contaminated water (Fourest and Roux, 1992). For example, *Aspergillus terreus* biomass, generated in fermentation processes to obtain enzymes and secondary metabolites (Gulati et al., 1999; Ruchi et al., 2003; Kaur et al., 2009) is recognized to be a competitive copper biosorbent (Ruchi et al., 2003; Cerino-Córdova et al., 2011).

Biosorption capacity is highly influenced by some operational conditions, for instance temperature, pH solution, metal and biomass concentration, stirring speed, and particle size, among others. In addition, the physical-chemical properties of biosorbents and pollutants play a major role in biosorption processes (Garcia-Reyes et al., 2009). Several researchers have reported the effects of those operational conditions individually. However, just a few researchers explored the influence of the interactions between the operational conditions (Freitas et al., 2009; Cerino-Córdova et al., 2011). These interactions are required to generate a statistical model that represents adequately the response surfaces of biosorption processes. These response surfaces are useful to investigate the influence of different variables in biosorption processes and to determine the optimal conditions.

In this study, a response surface analysis was performed to understand which experimental conditions (temperature, pH, biomass dose and stirring speed), as well as their interactions, play a major role in lead biosorption on *Aspergillus terreus*. Central composite face-centered design (CCFD) was used to determine the optimal conditions of lead biosorption. This research was conducted at the Facultad de Ciencias Químicas, Universidad Autónoma de Nuevo León, México during 2007-2008.

MATERIALS AND METHODS

Biosorbent

Aspergillus terreus (ATCC 20516) was grown and stored on Sabouraud maltose agar in sealed screw-cap

tubes at 4 °C. The microorganism was cultured by two-stages protocol in a medium of 20 g of dextrose, 5 g of soybean meal, 5 g of yeast extract, 5 g of NaCl, and 5 g of K₂HPO₄ in 1 L of distilled water adjusted to pH 7 with 6 N HCl. Medium was sterilized by autoclaving at 121 °C for 20 min. During stage I, 50 mL of culture medium were inoculated with an *A. terreus* spore suspension. Incubations were conducted at 28 °C on an orbital shaker at 150/min for 48 h. Culture medium (500 mL, stage II) was inoculated with 5 % (v/v) cell suspension, obtained from the first stage culture and incubated as previously described. After 48 h, the pellets obtained from stage II culture were separated by filtration (Whatman 40 filter paper) and washed several times with NaCl (0.9 % w/v) and finally washed and rinsed with deionized water. Biomass was dried at 60 °C for 4 h, powdered, and kept at -11 °C previous to biosorption experiments.

Biosorption experiments

To determine the removal percentage of lead on *A. terreus*, samples of fungi biomass were added to 100 mL of lead of 50 mg/L and stirred for 5 h. Other operational conditions (i.e. temperature, pH, stirring speed and biomass dose) will be described in the following sections. The initial (C_0) and final (C_f) lead and copper concentrations were determined by using an atomic absorption spectrometer (GBC Scientific 932AA, acetylene-air flame) at wavelength of 217 nm for lead concentrations between 2.5-20 mg/L, respectively. All experiments were conducted in triplicate. The lead removal percentage (R) was calculated according the following equation:

$$R = \left(\frac{C_0 - C_f}{C_0} \right) \times 100 \quad (1)$$

Full factorial design

The experimental design for optimization of lead biosorption on *A. terreus* includes a number of steps according to the statistical methodology (Montgomery, 2001) discussed in the following sections. Design Expert (2000; version 6.0.1, Stat-Ease, Inc., MN) software was used for statistical data analysis.

Full factorial design was used to identify which independent process variables (factors) and their interactions have a significant effect on lead

removal (response variable) by *A. terreus*. A 2^4 full factorial experimental design with four independent variables (stirring speed, temperature, pH and biomass dose) at two levels was carried out initially. Table 1 shows ranges and levels (coded as -1 and +1) of the selected variables that play a major role in adsorption processes.

A first-order design was built to determine if the response surface could be a plane in the range of the tested factors. The model represented in Eq. 2 includes simple factors, their interactions and the quadratic factors. This model was used to predict the response variable and to explore the design surface.

$$Y = \beta_0 + \sum_{i=1}^l \beta_i x_i + \sum_{i=1}^l \sum_{j=1}^l \beta_{ij} x_i x_j + \sum_{i=1}^l \sum_{j=1}^l \sum_{k=1}^l \beta_{ijk} x_i x_j x_k + \sum_{i=1}^l \beta_{ii} x_i^2 + \varepsilon \quad (2)$$

$\hat{a}_i, \hat{a}_{ij}, \hat{a}_{ijk}, \hat{a}_{ii}$ coefficients of the linear effect, double, triple interaction, and quadratic effects.

Where, Y is response variable, \hat{a}_0 is constant coefficient, x_i, x_j, x_k are independent variables or factors and ε is error.

To measure the variability of the linear model with the experimental data, the adjusted linear determination coefficient (R^2_{Adj}) was calculated.

The 2^4 full factorial design was modified by adding replicates at the central point (coded as 0 in Table 1). This modification was performed to evaluate the standard deviation of each factor and to find any inflection point, which is related to a curvature in the response surface. This design included 55 experiments: 3 replicates of 16 factorial points and 7 replicates at the central point (Table 2).

Table 1: Experimental factor levels used in the factorial design

Independent variable	Factor	Coded levels		
		-1	0	+1
		Actual levels		
Stirring speed (1/min)	X_1	50	100	150
Temperature (°C)	X_2	30	40	50
pH	X_3	4	5	6
Biomass dose (mg)	X_4	10	92.5	175

CCFCD

Axial points were added to the full factorial design with a central point to predict the optimal conditions for lead removal from aqueous solutions on *A. terreus* biomass. CCFCD included 55 experiments (previously mentioned) and 8 axial points ($\alpha=1$) (Montgomery, 2001) that were selected according to the combinations of the variables shown in Table 1.

The prediction of optimal conditions was performed by using the quadratic model (Eq. 2) that includes four independent variables, the interaction between each other and the quadratic variables.

This equation was used to explore the space design (a three-dimensional response plot) and to predict the optimal conditions for lead biosorption on *A. terreus*.

The variability of the response variable was determined by the adjusted multiple coefficient of determination (R^2_{Adj}). In addition, the adequate precision ratio (Box et al. 1978) was calculated by Eq. 3 to verify that the quadratic regression model is adequate.

$$\text{Adequate precision} = \frac{\text{Max}(\hat{Y}) - \text{Min}(\hat{Y})}{\sqrt{\bar{V}(\hat{Y})}} \quad (3)$$

$$= \frac{\text{Max}(\hat{Y}) - \text{Min}(\hat{Y})}{\sqrt{\frac{p\sigma^2}{n}}}$$

$\text{Max}(\hat{Y})$ is maximal predicted value,

$\text{Min}(\hat{Y})$ is minimal predicted value,

$\bar{V}(\hat{Y})$ is average variance of predicted values,

p is number of model parameters (including \hat{a}_0),

σ^2 is residual mean square, and

n is number of experiments.

RESULTS AND DISCUSSION

Linear model for lead biosorption process

Table 2 shows the experimental data and the predicted values of lead biosorption. *A. terreus* achieved the highest lead percentage removal (86.1 %) at pH 4, biomass dose of 175 mg, 30 °C and stirring speed of 50/min, compared to the other combinations. However, statistical analysis of the experimental data is required to establish the optimal conditions of lead biosorption in the range of the

Table 2: Experimental and predicted data of lead removal efficiency in experimental designs

Trial	Coded value				Replicates			Lead removal (%)		
	X_1	X_2	X_3	X_4	1 st	2 nd	3 rd	Average	Predicted	
									Linear model	Quadratic model
1	-1	-1	-1	-1	22.37	20.68	18.45	20.50	23.08	23.20
2	+1	-1	-1	-1	18.98	17.05	16.48	17.50	16.02	19.58
3	-1	+1	-1	-1	22.26	18.35	13.18	17.93	17.48	18.80
4	+1	+1	-1	-1	18.54	17.94	17.67	18.05	17.42	15.18
5	-1	-1	+1	-1	39.34	35.44	34.39	36.39	36.42	38.38
6	+1	-1	+1	-1	44.00	42.30	38.07	41.46	40.36	41.68
7	-1	+1	+1	-1	45.85	45.52	40.21	43.86	44.18	43.26
8	+1	+1	+1	-1	49.04	46.83	42.64	46.17	46.96	46.56
9	-1	-1	-1	+1	88.23	85.59	84.56	86.13	83.54	80.90
10	+1	-1	-1	+1	75.59	74.93	74.43	74.98	76.48	77.28
11	-1	+1	-1	+1	74.15	73.59	71.44	73.06	73.50	76.50
12	+1	+1	-1	+1	73.88	73.50	71.02	72.80	73.44	72.88
13	-1	-1	+1	+1	74.48	74.04	73.36	73.96	73.92	73.12
14	+1	-1	+1	+1	77.95	76.54	75.74	76.75	77.86	76.42
15	-1	+1	+1	+1	80.12	78.47	74.03	77.54	77.24	78.00
16	+1	+1	+1	+1	82.87	79.91	79.60	80.80	80.02	81.30
					88.15	86.57	87.60			
17	0	0	0	0	87.87	91.56	87.06	88.31	----	93.90
					89.39					
18	0	0	0	-1	65.03	----	----	65.03	----	50.67
19	0	0	0	1	98.06	----	----	98.06	----	96.89
20	0	0	-1	0	84.30	----	----	84.30	----	74.12
21	0	0	1	0	91.27	----	----	91.27	----	85.92
22	0	-1	0	0	98.78	----	----	98.78	----	93.78
23	0	1	0	0	97.12	----	----	97.12	----	94.02
24	-1	0	0	0	96.34	----	----	96.34	----	88.02
25	1	0	0	0	95.05	----	----	95.05	----	87.86

studied variables. Table 3 shows the Analysis of variance (ANOVA) results of the full factorial design by using the experimental data (Table 2). According to the results obtained from the ANOVA analysis (Table 3), the following codified hierarchical linear model was built using trials 1 to 16 as shows in the Table 2.

$$\begin{aligned} \hat{Y} = & 53.62 - 0.054X_1 + 0.16X_2 + 6X_3 + 23.38X_4 \\ & + 0.73X_1X_2 + 1.73X_1X_3 + 2.32X_2X_3 \\ & - 1.11X_2X_4 - 5.74X_3X_4 - 1.02X_1X_2X_3 \end{aligned} \quad (4)$$

The linear model included the most significant variables (i.e. $P < 0.05$) that affect lead removal on *A. terreus*. The selected model described adequately the response surface based on the high significant confidence level (99 %) and the adjusted determination coefficient ($R^2_{Adj} = 0.99$). Table 2 shows that predicted values are slightly different to the experimental values due to the lack of fit of the model. In other words, Eq. 4

is not enough to represent the nonlinear nature of the data since the lack of fit was significant at 95 % of confidence level ($P = 0.0481$). Therefore, a curvature test is required to explore the space design and to find the optimal conditions of the biosorption process.

Curvature test

The results of full factorial design showed that the system did not display a linear behaviour; therefore to test if any curvature in the response surface is present, seven replicates at central point (coded as 0 in Table 1) were added to the initial full factorial design to obtain an improved model. Lead removal percentage on *A. terreus* at the central point is shown in Table 2. Differences between experimental and predicted results can be explained by the heterogeneous nature of the biosorbent.

Table 4 shows the ANOVA analysis performed with the experimental results (full factorial design with the replicates at central point). The non-significant value of lack of fit ($P = 0.1812$ shown in

Table 3: Analysis of variance of the linear model

Variation source	Sum of square	Degree of freedom	Mean square	F-Value	P
Model	30093.46	10	3009.35	461.52	< 0.0001
Residual	241.26	37	6.52		
Lack of fit	68.54	5	13.71	2.54	0.0481
Pure error	172.72	32	5.40		
Total	30334.72	47			

Table 4) indicates that the linear model could be valid. However, a curvature of the responses between the maximal and the minimal values of the factor levels was detected by the significance of the curvature ($P < 0.0001$ shown in Table 4). For the aforementioned reason, a quadratic model must be applied to represent the design space and to find the maximal value of the response variable in the range of the studied variables.

Quadratic model for lead biosorption process

To predict the optimal conditions for lead removal from aqueous solutions on *A. terreus* biomass, axial points were added to the full factorial design with a central point. Table 2 shows the experimental data of the added axial points. Lead removal percentage varied from 65.03 % to 98.78 %.

For ANOVA analysis (Table 5), all experimental results of response variable of the full factorial design, replicates at the central point, and axial points were used. According to the ANOVA analysis, the factors included in the hierarchical quadratic model (shown below) are significant ($P < 0.0001$) in this process.

$$\hat{Y} = 93.90 - 0.078X_1 + 0.12X_2 + 5.90X_3 + 23.11X_4 - 5.96X_1^2 - 13.88X_3^2 - 20.12X_4^2 + 1.73X_1X_3 + 2.32X_2X_3 - 5.74X_3X_4 \quad (5)$$

This equation reveals how the individual variables (linear and quadratic) or double interaction affected lead biosorption from aqueous solution on *A. terreus*

biomass. Negative coefficient values indicate that individual or double interactions factors negatively affect lead biosorption (i.e. removal percentage decreases), whereas positive coefficient values mean that factors increase lead removal in the tested range. For instance, among all linear factors, stirring speed had a negative effect, while temperature, pH and biomass dose had a positive effect on lead removal (Eq. 5). The most important individual effect was the biomass dose ($P < 0.0001$), followed by the solution pH ($P < 0.0001$). In addition, the most important double effect was the interaction between pH and biomass dose, which was very significant ($P < 0.0001$). The second most important effect is the interaction between temperature and pH ($P = 0.0011$), and the interaction between stirring speed and pH ($P = 0.0125$). It is important to note that individual factors such as stirring speed and temperature are non-significant effects (at confidence level of 5 %); nonetheless, these factors have a strong interaction with biomass dose and pH. These results can be explained because biomass dose and pH (both linear and quadratic factors) have the most significant effect on lead removal.

A high value of the adjusted determination coefficient ($R^2_{Adj} = 0.9711$) was estimated. This result means that 97 % of the total variation on lead biosorption data (Table 2) can be described by the selected model. The adequate precision ratio of 42.21, for the quadratic model, indicates an appropriated signal to noise ratio. Because the adjusted

Table 4: Analysis of variance of the linear model with central point for testing curvature on the response surface

Variation source	Sum of square	Degree of freedom	Mean square	F-Value	P
Model	30114.80	9	3346.09	621.24	< 0.0001
Curvature	7354.06	1	7354.06	1365.37	< 0.0001
Residual	236.99	44	5.39		
Lack of fit	47.20	6	7.87	1.57	0.1812
Pure error	189.79	38	4.99		
Total	37705.85	54			

Table 5: Analysis of variance of the quadratic model

Variation source	Sum of square	Degree of freedom	Mean square	F-Value	P
Model	44974.50	10	4497.45	209.55	< 0.0001
Residual	1116.04	52	21.46		
Lack of fit	926.25	14	66.16	13.25	< 0.0001
Pure error	189.79	38	4.99		
Total	46090.55	62			

determination coefficient and adequate precision ratio exceeded 70 % and 4, respectively, the quadratic model can be used to explore the design space and to find the optimal conditions of this process.

Optimal conditions for lead biosorption process

The hierarchical quadratic model was used to represent the response surface in three-dimensional plots and to find the optimal conditions for lead removal on *A. terreus* biomass. Response surface space was built taking into account the solution pH with the other factors (biomass dose, stirring speed and temperature)

Fig. 1 shows the pH and biomass dose interaction that had the most significant effect on lead removal. At pH 5.2 and biomass dose of 139 mg, the model predicted lead removal efficiency close to 100 % by *A. terreus* biomass. At these predicted optimal conditions, *A. terreus* biomass achieved a lead biosorption capacity of 35.97 mg/g that is higher than the lead biosorption capacity on *A. niger* biomass (4.96 mg/g) at the optimal conditions (pH 3.44, initial concentration 19.28 mg/L and biomass dosage of 3.74 g/L) (Amini et al., 2008). These dissimilar results can be due to the basic treatment applied to the *A. niger* biomass prior to the biosorption experiments. However, lead biosorption on *A. terreus* biomass is lower than the maximal adsorption capacity on activated carbon (95.2 mg/g) (Issabayeva et al., 2006) but still *A. terreus* biomass seems to be a promising low-cost biosorbent that can be used for metal removal from water.

Lead removal efficiency depends on the *A. terreus* dosage. Contour plot (Fig. 1b) shows an increase on lead removal when biomass dose was increased from 10 to 139 mg (optimal biomass dose). Nevertheless, lead removal decreased at biomass dose higher than the optimal value probably due to biomass agglomeration. A similar effect was reported on

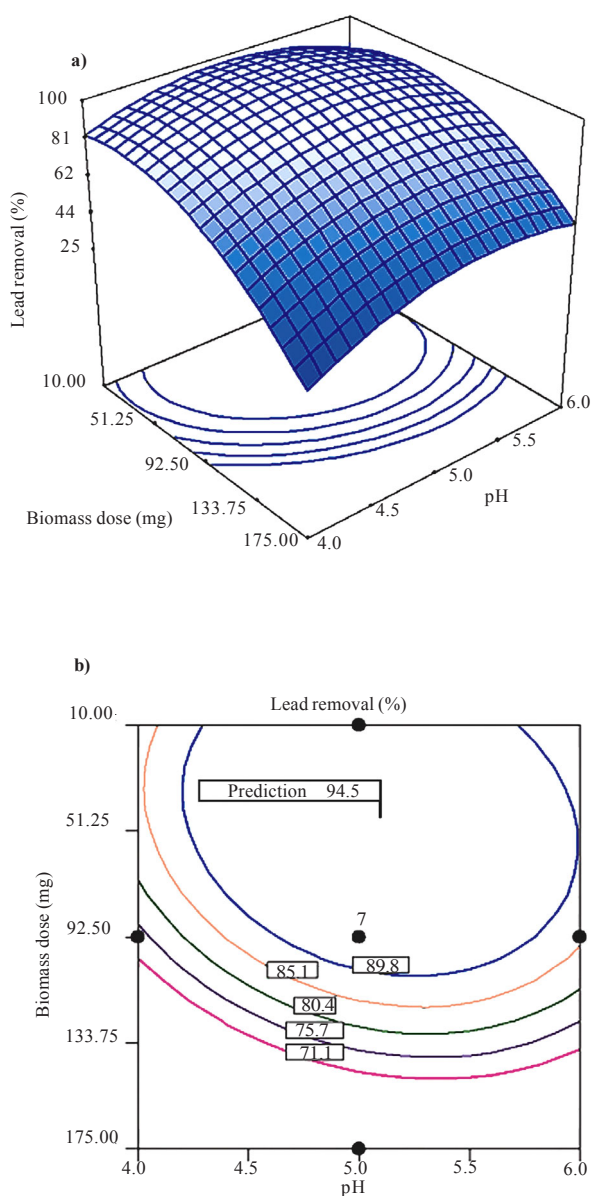


Fig. 1: Effect of the interaction between pH and biomass dose on lead removal at 100/min and 40 °C: a) Response surface plot, b) Contour plot

cadmium biosorption by using *Aspergillus niger* biomass (Amini *et al.*, 2009).

The ANOVA analysis shows that the stirring speed had a non-significant individual effect on lead removal (Section 3.3 and Table 5). However, stirring speed and pH interaction showed a significant effect on the lead removal (Fig. 2). According to the results shown in

contour plot (Fig. 2b), a relatively high lead removal (94.53 %) was predicted at pH 5.2 and 102/min. Lead removal slightly increased when the stirring speed was risen from 50-102/min and, also, lead removal increased with increasing the solution pH from 4.0 to 5.2, whereas lead removal slightly decreased with increments on stirring speed (102 to 150/min) and solution pH (5.2 to

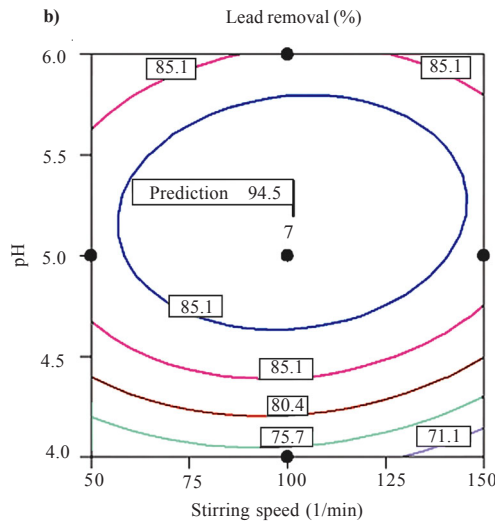
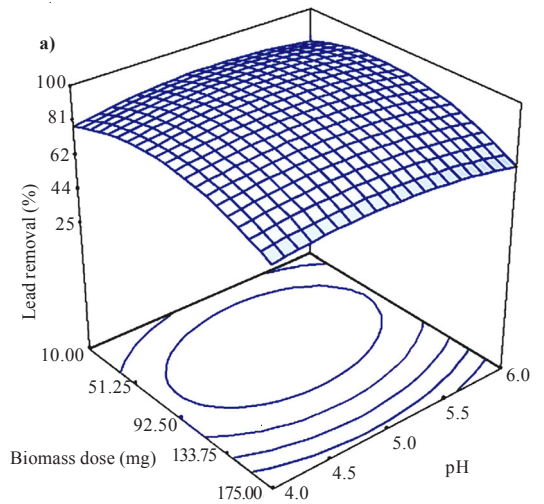


Fig. 2: Effect of the interaction between pH and stirring speed on lead removal at 92.5 mg of biomass and 40 °C: a) Response surface plot, b) Contour plot

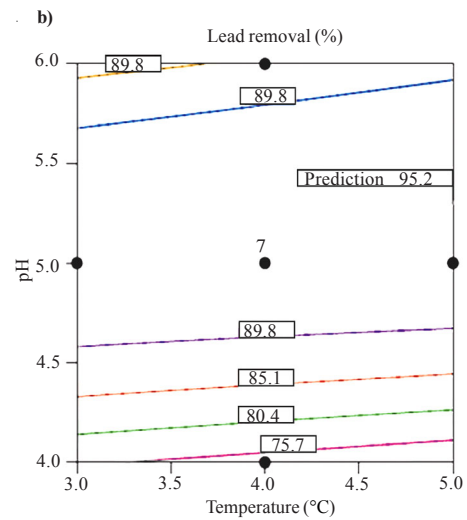
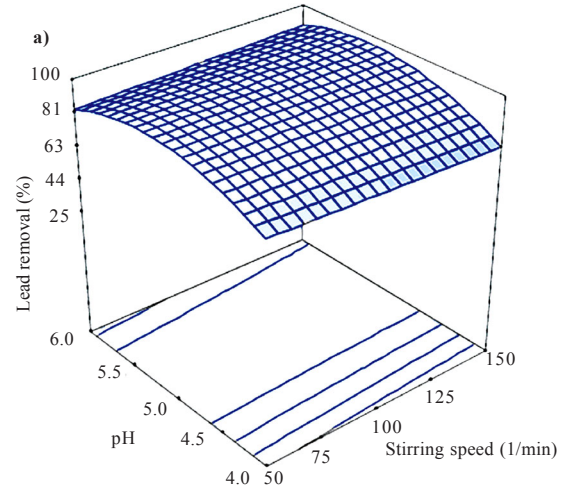


Fig. 3: Effect of the interaction between pH and temperature on lead removal at 92.5 mg of biomass and 100/min: a) Response surface plot, b) Contour plot

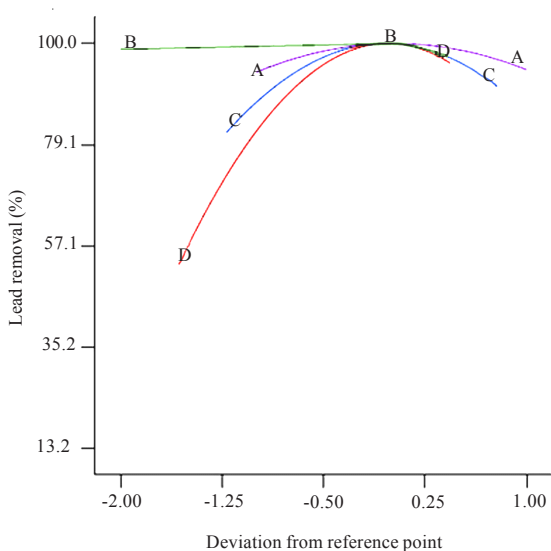


Fig. 4: Perturbation plot of lead removal at the optimal conditions: biomass dose of 139 mg (D), solution pH 5.2 (C), 102/min (A) and 50 °C (B)

6.0) (Fig. 2b).

Fig. 3 shows the effect of the interaction of pH and temperature on lead removal. At pH 5.2 and 50 °C, *A. terreus* biomass removed 95.2 % of lead from water. Lead removal was increased by rising temperature at pH values higher than 5.2. Furthermore, lead removal decreased by increasing temperature at pH values lower than 5.2. This behaviour can be due to protonation of the binding sites (i.e. carboxyl groups), and at acidic pH values, cell walls may be damaged (Khalaf, 2008).

A comparison of the effects of all factors at the optimal conditions on lead biosorption was performed by using a perturbation plot (Fig. 4). The steep curvature of biomass dose and pH indicates that lead removal is highly affected by these variables which agree with the results previously discussed.

Solution pH affects the surface charge of the biosorbent and the metal speciation. Functional groups of biosorbents can be protonated or deprotonated depending on their dissociation constant that is function of the solution pH. For example, carboxyl groups are ionized when solution pH is lower than 5 (Qiming and Kaewsarn, 1999;

Volesky, 2003). At this condition, positively charged metal species can be adsorbed on negatively charged surface functional groups by means of electrostatic forces (Garcia-Reyes *et al.*, 2009). Infrared analysis (data not shown) of *A. terreus* revealed that the biosorbent surface contains functional groups such as hydroxyl, carboxyl and amide groups (Silverstein and Webster, 1998). Nitrogen-containing groups can be used as metal-binding sites at pH above the dissociation constant, for example, amide groups in chitin biopolymers have electron pairs which can be form coordinated covalent bonds with metal cations and, therefore, amide groups can contribute to the metal removal. Although a response surface analysis is a useful tool for optimizing processes, this methodology does not explain physically the adsorption phenomena. Nevertheless, statistical analysis allows to determine the most significant factors involved in the process as well as to find the optimal conditions in the selected range of the variables.

CONCLUSION

Lead removal from aqueous solutions by *A. terreus* was studied with a CCFCFCD. According to the ANOVA analysis, lead removal is highly affected by biomass dose, pH and the interactions between pH with the other factors (biomass dose, stirring speed and temperature). The hierarchical quadratic model represents adequately the response surface space based on the adjusted determination coefficient ($R^2_{Adj} = 0.97$) and the adequate precision ratio (42.21). By using this model, optimal conditions are pH 5.2, 139 mg of biomass, 102/min and 50 °C. At these conditions, the predicted removal efficiency achieved near to 100 % of lead from aqueous solutions, when using *A. terreus* biomass. Finally, the reported results in this research demonstrate the feasibility of employing *A. terreus* as a biosorbent for lead removal.

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