

Application of response surface methodology for optimization of lead biosorption in an aqueous solution by *Aspergillus niger*

Malihe Amini^a, Habibollah Younesi^{a,*}, Nader Bahramifar^a, Ali Akbar Zinatizadeh Lorestani^b, Farshid Ghorbani^a, Ali Daneshi^a, Mazyar Sharifzadeh^c

^a Department of Environmental Science, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Imam Reza Street, P.O. Box 46414-356, Noor, Iran

^b Material and Energy Research Center, P.O. Box 14155-4777, Tehran, Iran

^c Department of Chemical Engineering, Faculty of Engineering, Islamic Azad University, Amol Branched, Iran

Received 6 August 2007; received in revised form 24 September 2007; accepted 21 October 2007

Available online 20 February 2008

Abstract

Response surface methodology was applied to optimize the removal of lead ion by *Aspergillus niger* in an aqueous solution. Experiments were conducted based on a rotatable central composite design (CCD) and analyzed using response surface methodology (RSM). The biosorption process was investigated as a function of three independent factors viz. initial solution pH (2.8–7.2), initial lead concentration (8–30 mg/l) and biomass dosage (1.6–6 g/l). The optimum conditions for the lead biosorption were found to be 3.44, 19.28 mg/l and 3.74 g/l, respectively, for initial solution pH, initial lead ion concentration and biomass dosage. Lead biosorption capacity on dead *A. niger* fungal biomass was enhanced by pretreatment using NaOH. Under these conditions, maximum biosorption capacity of the biomass for removal of lead ions was obtained to 96.21%. The desirability function was used to evaluate all the factors and response in the biosorption experiments in order to find an optimum point where the desired conditions could be obtained. The *A. niger* particles with clean surface and high porosity may have application as biosorbent for heavy metal removal from wastewater effluents.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Biosorption; *Aspergillus niger*; Response surface methodology; Desirability function

1. Introduction

Heavy metal pollution has become one of the serious environmental problems of worldwide concern. Heavy metals released into the environment have been increased continuously as a result of the rapid development of various industrial activities and technologies, posing a significant threat to the environment and public health because of their toxicity, bioaccumulation and bioaugmentation in the food chain and persistence in nature [1]. The heavy metals like lead, mercury, copper, cadmium, zinc, nickel, chromium are among the most common pollutants found in industrial effluents which cause serious threat to environment, animals and human for their extreme toxicity [1,2]. These metals have been harmful to the environment

as their concentrations reach a certain level. Among aforementioned heavy metals, lead is extremely toxic to organisms even at low concentration and can damage to the nervous system, gastrointestinal track, encephalopathy with permanent damage, kidneys and reproductive system, particularly in children [3]. Many industrial such as mining and smelting metalliferous, surface finishing industry, energy and fuel production, fertilizer and pesticide industry, electroplating, electrolysis, electro-osmosis, coating and metal surface finishing, automotive, aeronautical and steel industrial, atomic energy installation, leatherworking, photography, electric appliance manufacturing discharge large quantities of wastewater containing various concentrations of heavy metals into the environment increasingly [4,5]. These concentrations are usually too low to be treated by standard methods [6]. Some studies indicated that moderate lead poisoning can result in neurobehavioral and intelligence deficits [7,8].

The removal and recovery of heavy metals is important from the point of resource recovery as metals are non-renewable [9].

* Corresponding author. Tel.: +98 122 625 3101 3; fax: +98 122 625 3499.
E-mail addresses: hunesi@modares.ac.ir, hunesi@yahoo.com (H. Younesi).

It therefore is regulated by many countries that the heavy metal ions must be treated to certain levels before their discharges into watercourses [8]. There are conventional methods for removing of heavy metals from aqueous solution such as chemical precipitation, membrane technologies, ion exchange, electrochemical treatment and adsorption on activated carbon [8,9]. Conventional treatment processes for industrial effluents are neither effective nor economical. Nevertheless, chemical precipitation of heavy metals produces large amount of sludge and is ineffective, when metals ion concentration in aqueous solution are as low as 100 mg/l [5]. Solvent extraction techniques are not suitable for effluents containing less than 1 g/l of targeted heavy metals [10]. Ion exchange, membrane technologies, activated carbon adsorption processes are too expensive due to the high cost of synthetic resins [10] and when large amount of water and wastewater containing heavy metal in low concentration [5].

The following reviews present a report of the performance of the above-mentioned methods to remove lead. Lead removal by a new ligand (benzenediamidoethanethiol) resulted 99.9% at a pH of 4.0 and initial lead concentration of 50 mg/l in a chemical precipitation process [11]. Lead hydroxide co-precipitated with alum sludge (180 mg/l dose) at pH of 11.0 led to 98% lead removal in a coagulation process [12]. Separation of lead by potato starch and dextrans resulted 83% removal at pH 8.2 of flotation [13]. A maximum lead removal by bentonite reached 100% at ambient temperature and 100 rpm agitation speed in an ion exchanged process [14]. Lead removal of 99.5% obtained after 5 min contact time by using emulsion liquid membrane [15].

A considerable alternative process is biosorption because of low cost and good performance [16,17], which utilizes the ability of various biological materials to bind and sequester heavy metals from aqueous solutions [18]. Biosorption processes are based in the ability of a microorganism to adsorb a metal in an aqueous solution by several physical–chemical processes through the cell's wall; this process does not always involve biomass metabolism, and that is why it does not matter if the microorganisms are dead or alive for the development of such processes [19,20]. A variety of natural materials of biological origin including bacteria, fungi [21,22], algae, mosses, macrophytes and higher plants [23] can decrease the concentration of heavy metal ions from aqueous solution from ppt to ppb level [24,25]. Different from of inexpensive non-living plant material such as rice husk, sawdust, and pine bark and canola meal have been widely and Scots pine cones newly investigated as potential biosorbents for heavy metals [25,26]. Biosorption by *Aspergillus niger* has been identified for its potential to remove metals due to its availability from various industrial fermentation processes [27]. Kapoor and Viraraghavan [28,29] and Kapoor et al. [30] performed experiments with raw and pretreated *A. niger* to determine the effectiveness of the biomass for removal of lead, cadmium, copper and nickel. They showed that the various functional groups in the cell wall of *A. niger* are responsible for biosorption.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing the

effects of several independent variables on the response [31]. RSM has an important application in the process design and optimization as well as the improvement of existing design. This methodology is more practical compared to the approaches mentioned above as it arises from experimental methodology which includes interactive effects among the variables and, eventually, it depicts the overall effects of the parameters on the process [32].

In the last few years, RSM has been applied to optimize and evaluate interactive effects of independent factors in numerous chemical and biochemical processes. Response surface methodology was applied to optimize nickel (Ni(II)) removal by *Pinus sylvestris* [33]. The optimum pH, biomass concentration and initial Ni ion concentration were found to be 6.17, 18.8 g/l and 11.18 mg/l, respectively. Under these conditions, the removal efficiency of Ni ion was 99.91%.

The objective of the present research was to study the effect of initial lead concentration, pH and biomass concentration on biosorption of lead using *A. niger* biomass. Optimum conditions for elimination of Pb(II) was determined with RSM under DESIGN EXPERT software. The main aim of this work was to find the biosorption characteristics of *A. niger* for the removal of lead ions in a aqueous solution.

2. Materials and method

2.1. Biomass

A. niger (DSMZ 823) that obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) in the form of freeze dry was used. Then it was cultured in sterilized medium and maintained in nutrient agar at 4 °C. The composition of growth medium was (grams per liter): sucrose, 50; NH₄NO₃, 2; KH₂PO₄, 0.15; MgSO₄, 0.15. The medium was sterilized by autoclaving at a pressure 1.5 atm and temperature of 121 °C for 20 min. Temperature and pH of growth medium were respectively ambient temperature (30 °C) and 5.5 without shaking. The fungus cells were grown for 5 days (end of exponential phase) and then filtered (0.45 μm pore size).

2.2. Preparation of biomass

Fungus biomass was deactivated by heating in an autoclave at 121 °C for 15 min [34,35]. The drying fungus was ground and screened through a set of sieve with 100 mesh. The pretreatment of the biosorbent was carried with non-viable of fungus cells into 0.5N sodium hydroxide solution for 20 min at boiling temperature. Then it was centrifuged at 4000 rpm for 10 min and the supernatant was discharged. Then the sodium hydroxide-washed fungus was rinsed several times with deionized water to remove excess NaOH and adsorbed nutrient ions. The rinsed fungus is again centrifuged and the remaining biomass was dried at 50 °C for 24 h [36]. It is important to note that yield of the adsorbent (treated *A. niger*) in terms of percentage of original biomass quantity taken was about 40% while the ion exchange capacity values (q_m , mg/g) of the untreated and NaOH-treated *A. niger* biomass were increased from 1.45 to 6.78 mg Pb²⁺/g, respec-

Table 1
Partial composition of *Aspergillus niger*

Component	<i>A. niger</i> (%)
Moisture	96.12
Dry content	2.55
Ash	1.33
Total nitrogen	3.23
Crude protein	20.2

tively. The dried cells ground and screened according mention above. This was produced a uniform material which was stocked in the refrigerator.

2.3. Scanning electron microscope (SEM)

SEM was used to study the outer surface, microporosity and pores size of the non-viable *A. niger* after treatment by NaOH. The samples were dried, coated with gold and observed with a (Phillips XL30, Holland) microscope. Finally, the samples were analyzed under SEM at 2000× magnification [33,37].

2.4. Analytical methods

Protein and total nitrogen contents of unviable cells were analyzed by Kjeldahl determination (2300 Kjettac Analyzer Unit, Foss Tecator, Sweden). The moisture and ash content of unviable cell was determined by method according to standard method [38]. The characteristics and composition of *A. niger* is given in Table 1. The concentrations of residual lead(II) ions in the supernatant solutions were determined using atomic absorption spectrophotometer (Philips, PU9400, USA). Each determination was repeated three times and the results given were the average values. The deviation was less than 5%.

The chemical used for this study was analytical grades of lead nitrate ($\text{Pb}(\text{NO}_3)_2$) supplied by Riedel-de Häen (Germany). A stock lead nitrate solution of 1000 mg/l was prepared by dissolving 1.5980 g of lead nitrate in a 1000 ml of deionized water. The solution was diluted for different lead(II) concentration as required working solutions. The initial pH of working solution was adjusted by addition of 1N H_2SO_4 and 1N NaOH solutions.

2.5. Lead adsorption studies

Twenty batch adsorption experiments designed by RSM were conducted at the equilibrium time of 240 min, agitation speed of 200 rpm and experimental temperature of 30 °C to study the effect of solution pH, initial lead ion concentration and the dosage of biomass on lead removal. Each experiment was carried out in Erlenmeyer flasks containing 100 ml lead(II) solution by shaking the flasks at 120 rpm for period contact time of 1440 min. Samples were withdrawn at pre-determined time intervals (2, 5, 15, 30, 60, 90, 120 and 1440 min) and filtered through 0.25 μm filters. Filtered samples were analyzed for residual lead ion concentration. Metal removal by *A. niger* was

determined as according Eq. (1):

$$R = \frac{P_0 - P_e}{P_0} \times 100 \quad (1)$$

where R is the percentage of lead adsorbed by biomass in percentage, P_0 is the initial concentration of metal ion in mg/l and P_e is the final concentration of metal ion in mg/L [36].

2.6. Experimental and optimization of biosorption

Optimum condition for the biosorption of lead by *A. niger* was determined by means of central composite design (CCD) and response surface methodology (RSM). The RSM consists of a group of empirical techniques devoted to the evaluation of relationship existing between a cluster of controlled experimental factors and measured responses according to one or more selected criteria. Optimization studies were carried out by studying the effect of three variables including *A. niger* doses, initial lead ion concentrations and pH of solutions [39–41]. The chosen independent variables used in this study were coded according to Eq. (2):

$$x_i = \frac{X_i - X_0}{\Delta X} \quad (2)$$

where x_i is the dimensionless coded value of the i th independent variable, X_0 is the value of X_i at the center point and ΔX is the step change value. The behavior of system is explained by the following empirical second-order polynomial model Eq. (3):

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^k \beta_{ij} x_i x_j + \varepsilon \quad (3)$$

where Y is the predicted response, x_i, x_j, \dots, x_k are the input variables, which affect the response Y , $x_i^2, x_j^2, \dots, x_k^2$ are the square effects, $x_i x_j, x_i x_k$ and $x_j x_k$ are the interaction effects, β_0 is the intercept term, β_i ($i=1, 2, \dots, k$) is the linear effect, β_{ii} ($i=1, 2, \dots, k$) is the squared effect, β_{ij} ($i=1, 2, \dots, k; j=1, 2, \dots, k$) is the interaction effect and ε is a random error [36,42,43].

The DESIGN EXPERT 6.07 (Stat-Ease Inc., Minneapolis, MN, USA) software was used for regression and graphical analysis of the obtained data. The central composite design (CCD) is the most frequently used under RSM design. The study carried out involved the employment of central composite design to optimize the biosorption process due to its suitability to fit quadratic surface which usually works well for process optimization. A design of 20 experiments was formulated for three factorial (2^3) designs and six replicates at the central points, six star points were employed to the second-order polynomial model. The optimum values of the selected variables were obtained by solving the regression equation at desired values of the process responses as the optimization criteria. Each of the parameters was coded at five levels: $-\alpha, -1, 0, +1$ and $+\alpha$. The range of variables was decided on the basis of literature reports for heavy metals biosorption by *A. niger* [36]. The range and level

Table 2
Experimental ranges and levels of the independent variables

Independent variables	Range and level				
	$-\alpha$	-1	0	+1	$+\alpha$
pH (X_1)	1.3	2.8	5	7.2	8.7
Initial lead ion concentration, mg/l (X_2)	0.5	8	19	30	37.5
<i>A. niger</i> dosage, g/l (X_2)	0.1	1.6	3.8	6	7.5

of the variable in coded units from RSM studies are given in Table 2.

3. Results and discussion

3.1. Effect of biomass pretreatment on the lead ion removal

Adsorption capacity increased in alkaline pretreated biomass [22,44,45]. There are three hypotheses that justifying the improved adsorption capacity, which are (1) increase in the number of available sites for metal ions, (2) the high degree of deprotonation of the binding groups, and (3) the subsequent alkalization of the environment [7,22,44]. Akar et al. [46] and Sampedro et al. [45] found a dependence biosorption of the Pb(II) uptake capacity by *Phomidium laminosum* and *Botrytis cinerea* with NaOH pretreatment method. However, it has been reported that the maximum metal uptake (q_m , mg/g) values of treated and untreated *A. niger* biomass were found to be 2.25 and 7.42 mg/g for Pb²⁺, respectively.

Fig. 1 shows a general structure of scanning electron microscope (SEM) of non-viable *A. niger* used in this study. SEM images of the untreated biomass, NaOH-treated biomass and lead-loaded biomass did not show any significant changes on

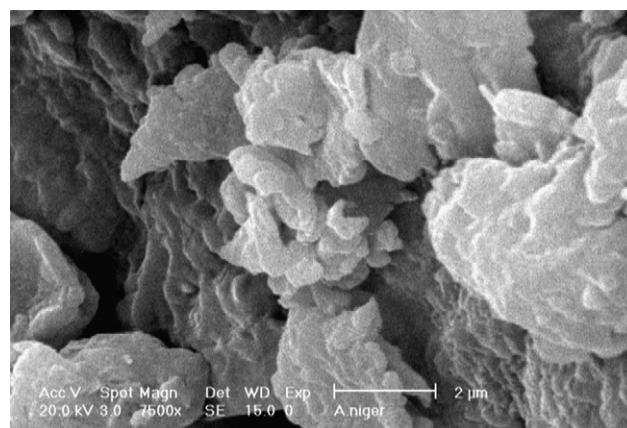


Fig. 1. SEM micrograph of pretreated *Aspergillus niger*.

surface morphology. As can be seen in the figure, surface layer of the treated *A. niger* exhibited microcavities and porous structure. Alternatively, SEM micrograph of the *A. niger* indicates that the inner surface of the bioadsorbent is also seemed to have multi-layered walls available for the adsorption process. The active microsites distinguished on the surface layer of non-viable *A. niger* may proceed faster the biosorption process [35,36]. The pore size of the bioadsorbent was approximately 0.44–0.77 μm as calculated from SEM analysis.

3.2. Lead biosorption process

The coded and actual values of the test variables (Table 3) as well as lead removal percentage as the process response were used to optimize the process as presented in Table 4. Adsorption solution pH influences both cell surface metal binding sites and

Table 3
Experimental design based on central composite design (CCD) used in this study

Run no.	Independent values				Categorical factor levels		
	Coded values		Real values				
1	-1	-1	-1	2.8	8	1.6	Fractoinal 2 ³⁻¹ fractional factorial points
2	+1	-1	-1	7.2	8	1.6	
3	-1	1	-1	2.8	30	1.6	
4	+1	+1	-1	7.2	30	1.6	
5	-1	-1	+1	2.8	8	6	
6	+1	-1	+1	7.2	8	6	
7	-1	1	+1	2.8	30	6	
8	+1	+1	+1	7.2	30	6	
9	$-\alpha$	0	0	1.3	19	3.8	Star points (6 points)
10	$+\alpha$	0	0	8.7	19	3.8	
11	0	$-\alpha$	0	5	0.5	3.8	Central points (6 points)
12	0	$+\alpha$	0	5	37.5	3.8	
13	0	0	$-\alpha$	5	19	0.1	
14	0	0	$+\alpha$	5	19	7.5	
15	0	0	0	5	19	3.8	
16	0	0	0	5	19	3.8	
17	0	0	0	5	19	3.8	
18	0	0	0	5	19	3.8	
19	0	0	0	5	19	3.8	
20	0	0	0	5	19	3.8	

Table 4
Observed and predicted values

Run no.	Observed values	Predicted value	Residual
1	66.71	65.79	0.92
2	93.63	89.67	3.96
3	34.53	32.67	1.86
4	87.33	89.67	-2.34
5	54.84	52.78	2.06
6	36.07	38.12	-2.05
7	96.21	98.05	-1.84
8	46.54	45.60	0.95
9	90.94	89.67	1.27
10	85.90	89.67	-3.78
11	88.06	89.67	-1.61
12	70.35	68.56	1.79
13	45.86	44.84	1.02
14	32.14	34.31	-2.16
15	41.58	43.38	-1.81
16	89.30	91.43	-2.13
17	92.86	89.67	3.18
18	12.00	13.92	-1.92
19	51.83	49.95	1.88
20	48.48	47.73	0.75

metal chemistry in water. Functional groups such as weak acidic carboxyl ($R-COO^-$), phosphate and amino groups of cell wall *A. niger* constituents were observed to be responsible sites in biosorption of heavy metals [47,48]. This indicated that adsorption mechanism of lead (Pb^{2+}) by sorbent (i.e., *A. niger*) in aqueous solution may take place via ion exchange mechanism. In the ion exchange mechanism, Pb^{2+} binds to anionic active sites by replacing two sodium ions (Na^+) from anionic sites at high pH or two protons (H^+) from acidic group at low pH [49]. However, the amount of lead adsorption from aqueous solutions is strongly influenced by the chemistry and surface morphology of the sorbent.

Fig. 2a shows the simultaneous effect of initial solution pH and the initial concentration of Pb(II) in the aqueous solution on lead removal efficiency. Pb(II) removal showed to be very sensitive to changes in the solution pH. The removal capacity of *A. niger* was sharply increased when the pH of the solution increased from 2.8 to 5. The results showed that the maximum removal of lead ions was achieved at pH 5.07 whereas under highly acidic and moderate basic conditions little biosorption was occurred. At highly acidic pH, the overall surface charge on the active sites became positive and metal cations and protons compete for binding sites on cell wall, which results in lower uptake of metal [30,48,50]. About 12% lead biosorption removal was obtained at pH 8.70. In this condition, lead ions were precipitated in the form of $Pb(OH)_2$. As can be seen, initial Pb concentration has little effect on the response. One reason that could be given was that the range used for Pb concentration (8–30 mg/l) has not had a significant effect on the response. At pH values above the isoelectric point, there is a net negative charge on the cell surface and the ionic state of ligands such as carboxyl, phosphate and amino groups will be such that so as to promote reaction with metal ions, hence the rapid binding efficiency was obtained [48]. Fig. 2b depicts

the lead ion removal efficiency as a function of the *A. niger* dosage and the initial concentration of Pb(II) in the aqueous solution. As mentioned earlier, Pb concentration showed a little effect while a remarkable effect of *A. niger* dosage is seen in Fig. 2b. This figure shows a reverse impact of *A. niger* dosage on the response so that the increase in *A. niger* dosage brings about an increase in the response up to a concentration of 3.8 g/l while at the concentration larger than 3.8 g/l showed a decreasing effect. Agglomeration of the biomass was a reason that could be given for the decrease in the removal efficiency at high concentration of biomass. However, the maximum removal capacities of *A. niger* was determined 103 mg lead/g biomass, and the quantity of adsorbed biomass in this value and pH were 3.76 and 3.44 g/l, respectively. Lead ion biosorption have also tested by *Penicillium chrysogenum* and *Rhizopus arrhizus* in an aqueous solution. Biosorption capacities of lead ion by *P. chrysogenum* and *R. arrhizus* have reported to be, respectively, 116 and 104 mg Pb^{2+} /g biomass [51,52]. The lead removal as a function of biomass dosage and initial solution pH is given in Fig. 2c. The results showed that pH was an important parameter affecting the biosorption of heavy metals. Conventional isotherm study has been conducted to study the sorption of lead using NaOH-treated *A. niger* biomass [28,29]. Maximum lead removal capacity was obtained to 7.24 mg Pb^{2+} /g biomass (98%) which was comparable with the present study. Dursun [48] and Holan and Volesky [53] have observed that adsorption of lead ion on *Rhizopus arrhizus* showed a removal capacity of 8 mg Pb^{2+} /g. This means that the preference of lead sorption on *A. niger* and *R. arrhizus* was observed to be similar. From the figure, maximum removal efficiency (90.42%) was achieved at biomass concentration of 3.54 g/l and initial pH of 4.55, while lead concentration was 19 mg/l. These optimal conditions can be used in wastewater for lead ion removal. This reason is due to influence of adsorption medium pH on the sorption capacity. This means that the initial pH of the adsorption medium is related to the adsorption mechanisms onto the adsorbent surface from water and reflects the nature of the physicochemical interaction of the species in solution and the adsorptive sites of adsorbent [43].

3.3. Fitting the model

The statistical significance of the quadratic model was evaluated by the analysis of variance (ANOVA) as presented in Table 5. The results showed that this regression was statistically significant at F value of 414.05 and values of $prob > F$ (<0.0001). The fit of the model was checked by the determination of coefficient (R^2). In this case, the value of the determination coefficient ($R^2 = 0.9928$) indicated that only 0.72% of the total variable was not explained by the model. The closer the R^2 is to 1, the stronger the model and the better it predicts the response. The value of adjusted determination coefficient (adjusted $R^2 = 0.9864$) is also high, showing a high significance of the model. The value of predicted R^2 is also high to support for a high significance of the model. The predicted R^2 obtained 0.9695, indicating that the model does not explain only 4.05% of the total variations. This also revealed that predicted R^2 of 0.9695 is in reasonable

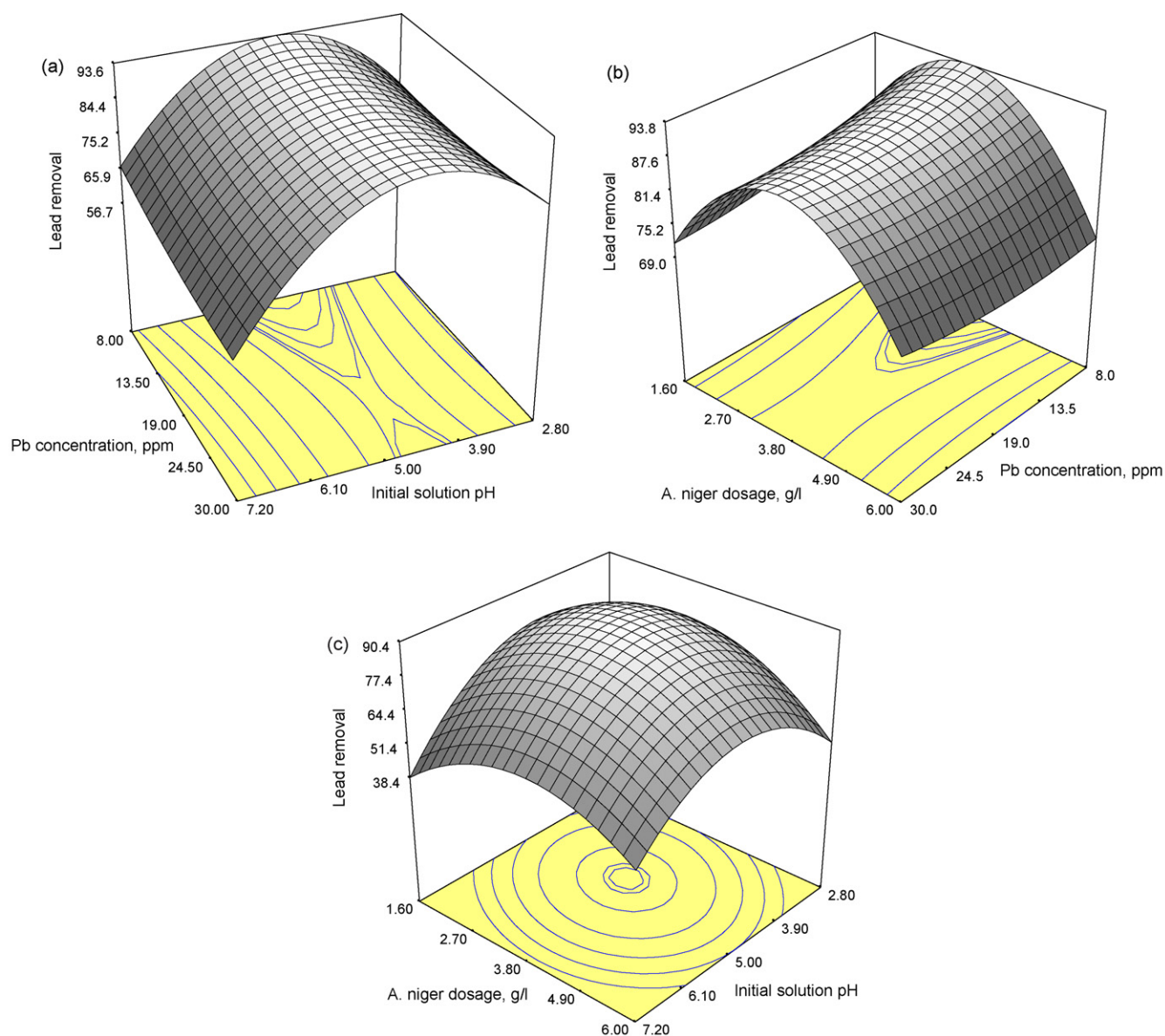


Fig. 2. Response surface plots for the effect of (a) initial solution pH and initial lead ion concentration (mg/l); (b) initial lead ion concentration (mg/l) and biomass dosage (g/l); (c) initial solution pH and biomass dosage (g/l) on the lead removal (%).

agreement with the adjusted R^2 of 0.9864. At the same time, a relatively low value of the coefficient of variation ($CV = 4.78$) indicates a good precision and reliability of the experiments [54,55]. The regression equation after the analysis of variances (ANOVA) gave the level of lead ion removal as a function of the

initial lead ion concentration, initial solution pH and *A. niger* dosage. By applying multiple regression analysis on the experimental data, the experimental results of the CCD design were fitted with a second-order full polynomial equation. The empirical relationship between lead ion removal (Y) and the three test

Table 5
Analysis of variance (ANOVA) for the response surface quadratic model

Source of variations	Degrees of freedom	Sum of squares	Mean square	F-Value	Probability (p)
Regression	9	12639.78	1404.42	153.85	<0.0001
Main effects	3	286.37	286.37	31.37	0.015
Square effects	3	3999.77	3999.77	438.18	0.016
Interaction effects	3	147.39	147.39	16.15	0.020
Residual	10	91.28	9.13		
Total	19	12731.07			

R^2 , 0.9928; adjusted R^2 , 0.9864; predicted R^2 0.9695; lack of fit, 41.61; CV, 4.78.

Table 6
Regression analysis using the 2^3 factorial central composite design

Model term	Coefficient estimate	Standard error	F-Value	p-Value
Intercept	+89.67	1.23		
X_1	-7.19	0.82	77.42	<0.0001
X_2	-1.97	0.82	5.80	0.0368
X_3	-2.70	0.82	10.90	0.0080
x_1^2	-22.51	0.80	799.63	<0.0001
x_2^2	+1.79	0.80	5.06	0.0482
x_3^2	-17.97	0.80	509.84	<0.0001
x_1x_2	-3.07	1.07	8.28	0.0165
x_1x_3	+6.30	1.07	34.73	0.0002
x_2x_3	+2.49	1.07	5.43	0.0421

x_1, x_2 and x_3 are the main effects; x_1^2, x_2^2 and x_3^2 are the square effects; x_1x_2, x_1x_3 and x_2x_3 are the interaction effects.

variables in coded units obtained by the application of RSM is given by

$$Y = 89.67 - 7.19x_1 - 1.97x_2 - 2.70x_3 - 22.51x_1^2 + 1.79x_2^2 - 17.97x_3^2 - 3.07x_1x_2 + 6.30x_1x_3 + 2.49x_2x_3 \quad (4)$$

where Y is lead ion removal (response) in percentage, x_1, x_2 and x_3 are the coded values of the tests variables, initial solution pH (x_1), initial lead ion concentration (x_2) in mg/l and *A. niger* dosage (x_3) in g/l. The ANOVA has conducted on for second-order response surface model and the results are given in Table 6. The significant of each coefficient was determined by F -values and p -values, which are listed in Table 6. The larger the magnitude of the F -values and the smaller p -values, the more significant is the corresponding coefficients. Values of “prob > F ” less than 0.0500 also indicate high significant regression at 95% confidence level. In this case, the first-order main effects, square effects and interaction effects of initial solution pH, initial lead ion concentration and biomass dosage were significant model terms. However, the model F -value of 153.85 and values of prob > F (<0.0001) indicated that the model terms are significant.

3.4. Optimization using the desirability function

In the numerical optimization, we choose the desired goal for each factor and response from the menu. The possible goals are: maximize, minimize, target, within range, none (for responses only) and set to an exact value (factors only). A minimum and a maximum level must be provided for each parameter included. A weight can be assigned to each goal to adjust the shape of its particular desirability function. The goals are combined into an overall desirability function. Desirability is an objective function that ranges from zero outside of the limits to one at the goal. The program seeks to maximize this function. The goal seeking begins at a random starting point and proceeds up the steepest slope to a maximum. There may be two or more maximums because of curvature in the response surfaces and their combination into the desirability function. By starting from several points in the design space chances improve for finding the “best” local maximum. A multiple response method was applied

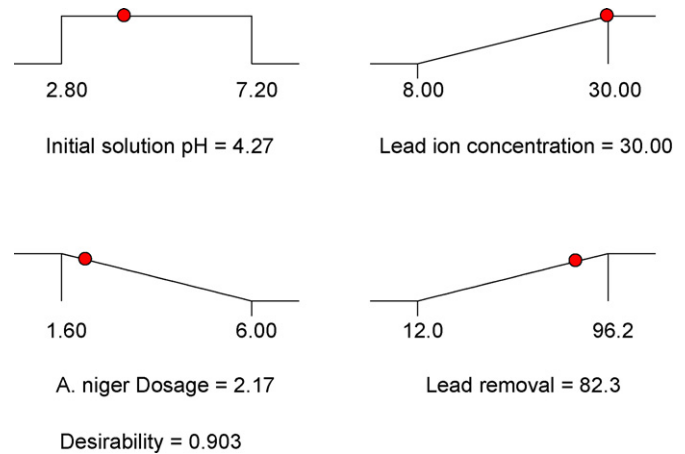


Fig. 3. Desirability ramp for numerical optimization of four goals, namely the initial solution pH, initial lead ion concentration, biomass dosage and lead removal.

for optimization any combination of four goals, namely the initial solution pH, initial lead ion concentration, biomass dosage and lead removal. The numerical optimization found a point that maximizes the desirability function. A minimum level of biomass dosage (1.6 g/l), maximum levels of initial lead ion concentration (30 mg/l) and lead removal (96.21%) and level of initial solution pH within range of 2.8–7.2 were set for maximum desirability. The importance of each goal was changed in relation to the other goals. Fig. 3 shows a ramp desirability that was generated from 10 optimum points via numerical optimization. By seeking from 10 starting points in the response surface changes, the best local maximum was found to be at initial solution pH 4.27, initial ion concentration of 30 mg/l, *A. niger* dosage of 2.17 g/l, lead removal of 82.3% and desirability of 0.903. Whereas optimum capacity of *A. niger* was 4.61 mg/g at pH value of 4.71 and 3.98 g biomass dosage. The equilibrium time, agitation speed and temperature were 240 min, 200 rpm and 30 °C, respectively. The obtained value of desirability (0.903) shows that the estimated function may represent the experimental model and desired conditions.

4. Conclusion

The results of present studies show that *A. niger* biomass treated with sodium hydroxide was an efficient biosorbent of lead ion in a dilute solutions. Experiments were performed as a function of initial solution pH, initial lead ion concentration and initial biomass dosage. The obtained results showed that *A. niger* is good adsorbing medium for lead ion and had high adsorption yields for the treatment of wastewater containing lead ions. However, optimization of biosorption from aqueous solution by response surface methodology resulted in 96.21% lead removal than that of the pre-optimized condition. The level of the three variable, initial solution pH, 4.27; initial ion concentration, 30 mg/l; *A. niger* dosage, 2.17 g/l, were found to be optimum for maximum lead ion removal. Scanning electron microscope of *A. niger* (SEM) shows surface of the bioadsorbent seems to have multi-layered walls available for the biosorbent and micropores.

According to these observations fungus biomass of *A. niger* is a suitable biosorbent for the removal of lead(II) from industrial wastewater.

Acknowledgements

The present research was made possible through an university grant, sponsored by Ministry of Science, Iran, Tarbiat Modares University (TMU). The authors wish to thank Mrs Haghdoust (Technical assistant of Environmental Laboratory), Tarbiat Modares University and Ministry of Science for their financial support.

References

- [1] T. Bahadir, G. Bakan, L. Aitas, H. Buyukungor, The investigation of lead removal by biosorption: an application at storage battery industry wastewaters, *Enzyme Microb. Technol.* 41 (2007) 98–102.
- [2] Y.-G. Liu, T. Fan, G. Zeng, X. Li, Q. Tong, F. Ye, M. Zhou, W. Xu, Y. Huang, Removal of cadmium and zinc ions from aqueous solution by living *Aspergillus niger*, *Trans. Nonferr. Met. Soc. China* 16 (2006) 681–686.
- [3] Q. Feng, Q. Lin, F. Gong, S. Sugita, M. Shoya, Adsorption of lead and mercury by rice husk ash, *J. Colloid Interface Sci.* 278 (2004) 1–8.
- [4] R. Hana, H. Li, Y. Li, J. Zhang, H. Xiao, J. Shi, Biosorption of copper and lead ions by waste beer yeast, *J. Hazard. Mater. B* 137 (2006) 1569–1576.
- [5] J. Wang, C. Chen, Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review, *Biotechnol. Adv.* 24 (2006) 427–451.
- [6] M. Selatnia, M.Z. Bakhti, A. Madani, L. Kertous, Y. Mansouri, Biosorption of Cd^{2+} from aqueous solution by a NaOH-treated bacterial dead *Streptomyces rimosus* biomass, *Miner. Eng.* 17 (2004) 903–911.
- [7] A. Selatnia, N. Boukazoula, N. Kechid, M.Z. Bakhti, A. Chergui, Y. Kerchich, Biosorption of lead(II) from aqueous solution by a bacterial dead *Streptomyces rimosus* biomass, *Biochem. Eng. J.* 19 (2004) 127–135.
- [8] J. Chen, L. Wang, S. Zou, Determination of lead biosorption properties by experimental and modeling simulation study, *Chem. Eng. J.* 131 (2007) 209–215.
- [9] P. Puranik, J. Modak, K. Paknikar, A comparative study of the mass transfer kinetics of metal biosorption by microbial biomass, *Hydrometallurgy* 52 (1999) 189–197.
- [10] N. Mameri, N. Boudries, L. Addour, D. Belhocine, H. Lounici, H. Grib, A. Pauss, Batch zinc biosorption by a bacterial nonliving *Streptomyces rimosus* biomass, *Water Res.* 33 (1999) 1347–1354.
- [11] M.M. Matlock, B.S. Howerton, D.A. Atwood, Irreversible precipitation of mercury and lead, *J. Hazard. Mater.* 84 (2001) 72–83.
- [12] W. Chu, Lead metal removal by recycled alum sludge, *Water Res.* 33 (1999) 3019–3025.
- [13] J. Drzymala, J. Kapusniak, P. Tomasik, Removal of lead minerals from copper industrial flotation concentrates by xanthate flotation in the presence of dextrin, *Int. J. Miner. Process.* 70 (2003) 147–155.
- [14] V.J. Inglezakis, M.A. Stylianou, D. Gkantzou, M.D. Loizidou, Removal of Pb(II) from aqueous solutions by using clinoptilolite and bentonite as adsorbents, *Desalination* 210 (2007) 248–256.
- [15] R. Sabry, A. Hafez, M. Khedr, A. El-Hassanin, Removal of lead by an emulsion liquid membrane. Part I, *Desalination* 212 (2007) 165–175.
- [16] G. Ozdemir, N. Ceyhan, T. Ozturk, F. Akirmak, T. Cosar, Biosorption of chromium(VI), cadmium(II) and copper(II) by *Pantoea* sp. TEM18, *Chem. Eng. J.* 102 (2004) 249–253.
- [17] M. Pamukoglu, F. Kargi, Removal of copper(II) ions from aqueous medium by biosorption onto powdered waste sludge, *Process Biochem.* 41 (2006) 1047–1054.
- [18] A. Çabuk, T. Akar, S. Tunali, S. Gedikli, Biosorption of Pb(II) by industrial strain of *Saccharomyces cerevisiae* immobilized on the biomatrix of cone biomass of *Pinus nigra*: equilibrium and mechanism analysis, *Chem. Eng. J.* 131 (2007) 293–300.
- [19] M. Ramirez, M.P. da Silva, G. Selma, L. Ferreira, E.O. Vasco, Mathematical models applied to the Cr(III) and Cr(VI) breakthrough curves, *J. Hazard. Mater.* 146 (2007) 86–90.
- [20] M.N. Nourbakhsh, S. Kiliçarslan, S. Ilhan, H. Ozdag, Biosorption of Cr^{+6} , Pb^{+2} and Cu^{+2} ions in industrial waste water on *Bacillus* sp., *Chem. Eng. J.* 85 (2002) 351–355.
- [21] G.M. Gadd, Accumulation of metals by microorganisms and algae, in: H.J. Rehm, G. Reed (Eds.) *Biotechnology* 6 (1988) 401–433.
- [22] C.L. Brierley, Bioremediation of metal-contaminated surface and groundwaters, *Geomicrobiology* 8 (1991) 201–224.
- [23] L. Svecova, M. Spanelova, M. Kubal, E. Guibal, Cadmium, lead and mercury biosorption on waste fungal biomass issued from fermentation industry. Part I. Equilibrium studies, *Sep. Purif. Technol.* 52 (2006) 142–153.
- [24] M. Ansari, A. Malik, Biosorption of nickel and cadmium by metal resistant bacterial isolates from agricultural soil irrigated with industrial wastewater, *Bioresour. Technol.* 98 (2007) 3149–3153.
- [25] Y. Nuhoglu, E. Oguz, Removal of copper(II) from aqueous solutions by biosorption on the cone biomass of *Thuja orientalis*, *Process Biochem.* 38 (2003) 1627–1631.
- [26] L. Waihung, H. Chua, K. Lam, S. Bi, A comparative investigation on the biosorption of lead by filamentous fungal biomass, *Chemosphere* 39 (1999) 2723–2736.
- [27] M. Mukhopadhyay, S. Noronha, G. Suriaishkumar, Kinetic modeling for the biosorption of copper by pretreated *Aspergillus niger* biomass, *Bioresour. Technol.* 98 (2007) 1781–1787.
- [28] A. Kapoor, T. Viraraghavan, Biosorption of heavy metals on *Aspergillus niger*: Effect of pretreatment, *Bioresour. Technol.* 63 (1998) 109–113.
- [29] A. Kapoor, T. Viraraghavan, Heavy metal biosorption sites in *Aspergillus niger*, *Bioresour. Technol.* 61 (1997) 221–227.
- [30] A. Kapoor, T. Viraraghavan, D. Cullimore, Removal of heavy metals using the fungus *Aspergillus niger*, *Bioresour. Technol.* 70 (1999) 95–104.
- [31] G.E.P. Box, N.R. Draper, *Empirical Model-building and Response Surfaces*, John Wiley & Sons, New York, 1987.
- [32] D. Baş, İ.H. Boyacı, Modeling and optimisation. I. Usability of response surface methodology, *J. Food Eng.* 78 (2007) 836–845.
- [33] M. Yalvac Can, Y. Kaya, O.F. Algur, Response surface optimization of the removal of nickel from aqueous solution by cone biomass of *Pinus sylvestris*, short communication, *Bioresour. Technol.* 97 (2006) 1761–1765.
- [34] S. Schiewer, B. Volesky, Mathematical evaluation of the experimental and modeling errors in biosorption, *Biotechnol. Technol.* 9 (1995) 843–848.
- [35] S. Schiewer, B. Volesky, Modeling of the proton–metal ion exchange in biosorption, *Environ. Sci. Technol.* 29 (1995) 3049–3058.
- [36] Y. Goksungur, S. Uren, U. Guvenc, Biosorption of cadmium and lead ions by ethanol treated waste baker's yeast biomass, *Bioresour. Technol.* 96 (2005) 103–109.
- [37] J.Y. Lee, E.K. Lee, Drying temperature can change the specific surface area of *Phanerochaete chrysosporium* pellets for copper adsorption, *Biotechnol. Lett.* 20 (6) (1998) 531–533.
- [38] American Public Health Association (APHA), *Standard Methods for the Examination of Water and Wastewater*, 20th ed., American Public Health Association Publications, Washington, 1998.
- [39] E. Bayraktar, Response surface optimization of the separation of DL-tryptophan using an emulsion liquid membrane, *Process Biochem.* 137 (2000) 169–175.
- [40] A. Kunamneni, S. Singh, Response surface optimization of enzymatic hydrolysis of maize starch for higher glucose production, *Biochem. Eng. J.* 27 (2005) 179–190.
- [41] B. Preetha, T. Viruthagiri, Application of response surface methodology for the biosorption of copper using *Rhizopus arrhizus*, *J. Hazard. Mater.* 143 (2007) 506–510.
- [42] Z. Aksu, F. Gönen, Binary biosorption of phenol and chromium(VI) onto immobilized activated sludge in a packed bed: prediction of kinetic parameters and breakthrough curves, *Sep. Purif. Technol.* 49 (2006) 205–216.
- [43] Z. Aksu, F. Gönen, Z. Demircan, Biosorption of chromium (VI) ions by Mow-ital B₃OH resin immobilized activated sludge in a packed bed:

- comparison with granular activated carbon, *Process Biochem.* 38 (2002) 175–186.
- [44] E. Fourest, J.C. Roux, Heavy metal biosorption by fungal mycelial by-products: mechanisms and influence of pH, *Appl. Microbiol. Biotechnol.* 37 (1992) 399–403.
- [45] M. Sampedro, A. Blanco, J. Llama, J. Serra, Sorption of heavy metals to *Phomidium laminosum* biomass, *Biothechnol. Appl. Biochem.* 22 (1995) 355–365.
- [46] T. Akar, S. Tunalı, I. Kiran, *Botrytis cinerea* as a new fungal biosorbent for removal of Pb(II) from aqueous solutions, *Biochem. Eng. J.* 25 (2005) 227–235.
- [47] Md. Naseem Akthar, K. Sivarama Sastry, P.M. Mohan, Mechanism of metal ion biosorption by fungal biomass, *BioMetals* 9 (1996) 21–28.
- [48] A.Y. Dursun, A comparative study on determination of the equilibrium, kinetic and thermodynamic parameters of biosorption of copper(II) and lead(II) ions onto pretreated *Aspergillus niger*, *Biochem. Eng. J.* 28 (2006) 187–195.
- [49] X.M. Zhan, X. Zhao, Mechanism of lead adsorption from aqueous solutions using an adsorbent synthesized from natural, *Water Res.* 37 (2003) 3905–3912.
- [50] M. Iqbal, R. Edyvean, Biosorption of lead, copper and zinc ions on loofa sponge immobilized biomass of *Phanerochaete chrysosporium*, *Miner. Eng.* 17 (2004) 217–223.
- [51] H. Niu, X. Shu, J. Wang, B. Volesky, Removal of lead from aqueous solutions by *Penicillium* biomass, *Biotechnol. Bioen.* 42 (1993) 785–787.
- [52] J.M. Tobin, D.G. Cooper, R.J. Neufeld, Uptake of metal ions by *Rhizopus arrhizus* biomass, *J. Appl. Environ. Microbiol.* 47 (1984) 821–824.
- [53] Z.R. Holan, B. Volesky, Accumulation of cadmium, lead, and nickel by fungal and wood biosorbents, *Appl. Biochem. Biotechnol.* 53 (1995) 133–146.
- [54] A.I. Khuri, J.A. Cornell, *Response Surfaces: Design and Analyses*, 2nd ed., Marcel Dekker, New York, 1996.
- [55] R.O. Kuehl, *Design of Experiments: Statistical Principles of Research Design and Analysis*, 2nd ed., Duxbury press, Pacific Grove, CA, 2000, pp. 20–25.