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Application of response surface methodology for optimization of cadmium biosorption in an aqueous solution by *Saccharomyces cerevisiae*

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ABSTRACT

Optimization of a cadmium biosorption process was performed by varying three independent parameters (initial pH, initial cadmium ion concentration, *Saccharomyces cerevisiae* dosage) using a central composite design (CCD) under response surface methodology (RSM). For the maximum biosorption of cadmium ion in an aqueous solution by *S. cerevisiae*, a total of 20 experimental runs were set and the experimental data fitted to the empirical second-order polynomial model of a suitable degree. The potential of *S. cerevisiae* as a bioadsorbent was evaluated as a pretreated material with 700 g/L of ethanol. Furthermore, the quantitative relationship between the heavy metal uptake (q) and different levels of these factors was used to work out optimized levels of these parameters by a full factorial design (2^3). The analysis of variance (ANOVA) of the quadratic model demonstrates that the model was highly significant. The best set required 5 as initial pH, 3.8 g/L *S. cerevisiae* and 19 mg/L cadmium ion concentration within 240 min of contact time. Three dimensional plots demonstrate relationships between the cadmium ion uptake with the paired factors (when other factor was kept at its optimal level), describing the behavior of biosorption system in a batch process. The model showed that cadmium uptake in aqueous solution was affected by all the three factors studied. An optimum cadmium uptake of 6.71 mg/g biomass was achieved at initial cadmium ion concentration of 26.46 mg/L and *S. cerevisiae* dosage of 2.13 g/L. The process kinetic was also evaluated by isotherm, pseudo-second-order and intra-particle diffusion models. It showed that both monolayer adsorption and intra-particle diffusion mechanisms were effective in the cadmium biosorption process. Therefore, it is apparent that the response surface methodology not only gives valuable information on interactions between the factors but also leads to identification of feasible optimum values of the studied factors.

1. Introduction

The pollution of the environment with toxic heavy metals is reaching hazardous levels and spreading through the world along with industrial progress [1–3]. Heavy metals are major pollutants in oceans, marines, lakes, rivers, ground industrial and even treated wastewaters [4]. Different developed methods that could be used to remove dissolved heavy metal ions from wastewaters include chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment membrane technologies, adsorption on activated carbon and evaporative recovery [5]. These techniques have significant disadvantages including incomplete metal removal, the need for expensive equipment and monitoring system, high reagent or energy requirement or generation of toxic sludge or other waste products that require disposal [6]. They also are ineffective when metal ion concentration in aqueous solution is as low as ppm (parts per millions) levels. Although, an alternative process is biosorption, which used various natural materials of biological origin, including bacteria, fungi, yeasts, algae, molds and composting materials [7]. Metal-sequestering properties of nonviable biomass provide a basis for new approach to recover, at very low cost, even small amounts of toxic heavy metals from industrial effluents. They can effectively sequester dissolved metal ions out of dilute complex solution with high efficiency and quickly [5]. Therefore, biosorption is an ideal candidate which has been extensively used for the treatment of high volume and low concentration complex wastewaters during the several decades [8].

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Indeed, some types of potential biomaterials, which are very effective in accumulating heavy metals, with different metal-binding capacity have been investigated [9,10].

Marine algae (e.g. *Sargassum natans*), bacteria (e.g. *Bacillus subtilis*), fungi (e.g. *Rhizopus arrhizus*), yeast (e.g. *Saccharomyces cerevisiae*) as a waste biomass resulted from fermentation process and some food industries have been suggested as potential heavy metal biosorbents [6,11]. For the economical reason, researchers have paid much attention to various by-products from fermentation industry, because they are produced in large quantities. Although *S. cerevisiae* is a mediocre biosorbent, it is examined as a biomaterial in biosorption study for heavy metal removal [9]. Furthermore, Vieira and Volesky [9], Kapoor and Viraraghavan [12] and Jianlong and Can [13] have shown that the questioned yeast has commercial application as biosorbent on the following major fields. At first *S. cerevisiae* is easy to cultivate at large scale. It can grow with unsophisticated fermentation techniques and inexpensive growth media. Second, the biomass of S. *cerevisiae* can be obtained from various food and beverage industries. Third, *S. cerevisiae* is not usually a waste, but a commercial commodity and considered safe. Therefore, biosorbent made from *S. cerevisiae* may be easily accepted by the public when applied in practice as it can be used at large scale with low cost, especially for treating of large amount of wastewater containing heavy metal in low concentration. Fourth attempt is to use *S. cerevisiae* as biosorbent, but not the last, is an ideal model organism to identify the kinetics of the biosorption in metal ion removal, especially to investigate the interactions of metal–microbe at molecular level [11]. However, the desorption efficiency of heavy metal loaded biosorbent was investigated for feasibility of applying *S. cerevisiae* in practical heavy metal removal processes [14,15]. Wang and Chen [11] have investigated metal ions bound on the surface can be eluted by the other ions, chelating agents and acids. Therefore, regeneration of biomass for heavy metal recovery and biomass reuses have been suggested by utilizing various desorption agents, such as HCl, H_2SO_4, Na_2CO_3, EDTA, and β-mercaptoethanol [16,17].

Optimization of biosorption of heavy metals by the classical method involves changing one independent variable (i.e. *S. cerevisiae* dosages, pH, heavy metal concentration, temperature) while maintaining all others at a fixed level which is extremely time consuming and expensive for a large number of variables. To overcome this difficulty, experimental factorial design and response methodology can be employed to optimize the biosorption of heavy metal. The objective of the present study is to optimize biosorption of cadmium(II) ions in aqueous solution onto pretreated *S. cerevisiae* in a batch experiment. For better understanding of different stages of biosorption at varying heavy metal concentration, pH and sorbent dosages, RSM was used to optimize heavy metal uptake.

2. Materials and methods

2.1. Biomass

Saccharomyces cereviciea (PTCC 5010) was provided from Research and Technology Department of Ministry of Sciences (Persian Type Culture Collection) in the form of freeze dry, and then cultured in sterilized medium. The composition of growth medium was (grams per liter): glucose, 15; $(NH_4)_2SO_4$, 9; $MgSO_4$, 2.5; yeast extract, 1; KH_2PO_4, 1; K_2HPO_4, 0.2. The medium was sterilized by autoclaving at a pressure of 1.5 atm and temperature of 121 °C for 20 min. While the temperature and pH of growth medium were at ambient temperature (25 °C) and 4.5 respectively without shaking. The yeast cells were grown for 16 h (at end of exponential phase) and then filtered (0.45 μm pore size).

Table 1
Partial composition of *Aspergillus niger*

Component	Content, %
Moisture	73.57
Dry content	26.43
Ash	11.37
Total nitrogen	9.34
Crude protein[a]	58.39

[a] Crude protein = 6.25 × total nitrogen.

2.2. Preparation of biomass

Yeast biomass was deactivated by heating in an oven at 80 °C for 24 h [18]. The dried yeast was ground and screened through a sieve with 100 mesh. The pretreatment of the biosorbent was carried out with nonviable yeast cells in 700 g/L ethanol solution for 20 min at room temperature. Then, it was centrifuged at 3600 rpm for 10 min and the ethanol solution was discarded. The ethanol-washed biomass was rinsed several times with deionized water to remove excess ethanol and adsorbed nutrient ions. The rinsed yeast was again centrifuged and the remaining biomass was dried at 70 °C for 12 h [19]. The dried cells were ground and screened as mentioned above. The purpose of grinding dried yeast was to make a homogenized yeast biomass in order to destroy biomass aggregates and increase uptake capacity [20]. The ground biomass was stocked in the refrigerator for use in biosorption studies. Scanning electron microscope (SEM, Phillips XL30, Holland) was used for the observation of *S. cerevisiae* before and after treatment by 70% ethanol. Fig. 1 shows scanning electron microscope of nonviable *S. cerevisiae* before (a) and after pretreatment (b). The surface layer of *S. cerevisiae* may exhibit a microstructure porosity for both untreated and pretreated biomass. Actually, the chemical treatment by using esterification of ethanol with carboxyl groups (–COOH) has not provided any pore structure on the biosorbent surface. Microporous active sites distinguished on the surface layer of nonviable *S. cerevisiae* may proceed faster action of biosorption. Theses *S. cerevisiae* particles with clean surface and high porosity may have application as biosorbent for heavy metal removal from wastewater effluents.

2.3. Analytical methods

Protein and total nitrogen contents of nonviable cells were determined by Kjeldahl determination (2300 Kjettec Analyzer Unit, Foss Tecator, Sweden). The TKN value (Total Kjeldahl Nitrogen) represents a total nitrogen concentration, which is the sum of organic nitrogen compounds and ammonium nitrogen (TKN = org-N + NH_4-N [mg/L]). The Nutrient Data Laboratory (NDL) derived the values for protein were calculated from the level of total nitrogen (N) in the food, using the conversion factors recommended by Jones [21]. The percentage of crude protein can be calculated by percentage of nitrogen multiplied by a nitrogen-to-protein factor (6.25) and the percentage of nitrogen in the dried *S. cerevisiae* biomass was estimated by the Kjeldahl method. The moisture and ash content of nonviable cells was determined by method detailed in standard method [22]. The characteristics and composition of *Saccharomyces cereviciea* is given in Table 1. The concentrations of residual cadmium(II) ions in the supernatant solutions were determined using flame atomic absorption spectrophotometer (Philips, PU9400, USA). Each determination was repeated three times and the results given are the average values. The deviation was less than 5%. The chemical used for this study was analytical grades of cadmium sulfate ($CdSO_4 \cdot 8/3\ H_2O$) supplied by Riedel-de Häen (Germany). A stock cadmium sulfate solution of 1000 mg/L was prepared by dissolving 196.6 g of cadmium sulfate in a 1000 mL of

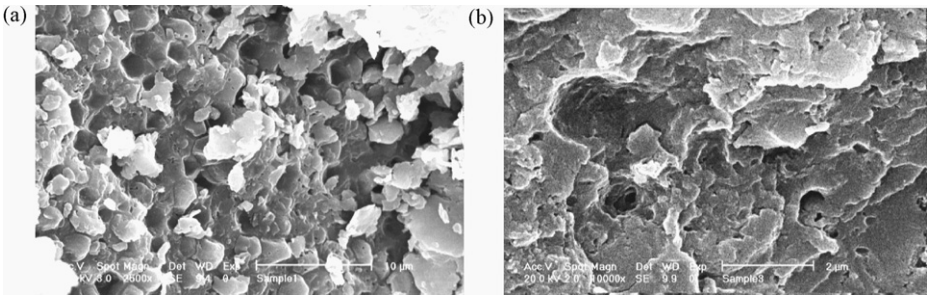

Fig. 1. Micrographs of scanning electron microscope, surface of *S. cerevisiae* before treatment with 10,000 magnification (a) and surface of *S. cerevisiae* after treatment with 20,000 magnification (b).

deionized water. The solution was diluted for different cadmium(II) concentration by deionized water as required working solutions. The initial pH of working solution was adjusted by addition of 2N HCl and 2N NaOH.

2.4. Cadmium adsorption studies

Batch adsorption experiments were conducted at room temperature (25 °C) to study the effect of solution pH, initial cadmium ion concentration and the dosage of biomass. Each experiment was carried out in Erlenmeyer flasks containing 100 mL cadmium(II) solution by shaking the flasks at 120 rpm for period contact time of 240 min. Samples were withdrawn at predetermined time intervals (2, 5, 15, 30, 60, 90, 120 and 240 min) and filtered through 0.25 μm filters. Filtered samples were analyzed for residual cadmium ion concentration. Metal uptake by *S. cerevisiae* was determined according to Eq. (1):

$$q = \frac{V(C_\mathrm{i} - C_\mathrm{e})}{S} \tag{1}$$

where q (metal uptake) is the amount of metal ions adsorbed on the biosorbent in mg/g, V is the volume of metal containing solution in contact with the biosorbent in mL, C_i and C_e are the initial and equilibrium (residual) concentration of metal in the solution in mg/L, respectively and S is the amount of added biosorbent on dry basis in g [19].

2.5. Experimentation and optimization of biosorption

Optimum condition for the biosorption of cadmium by *S. cerevisiae* was determined by means of central composite design (CCD) under 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 *S. cerevisiae* doses, initial cadmium ion concentrations and pH of solutions [23–25]. The chosen independent variables used in this study were coded according to Eq. (2):

$$x_i = \frac{X_i - X_0}{\Delta x} \tag{2}$$

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

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_1 X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=1}^{k} \beta_{ij} X_i X_j + \varepsilon \tag{3}$$

where Y is the predicted response, X_i, X_j, . . ., X_k are the input variables, which affect the response Y, $X_i^2, X_j^2, \ldots, X_k^2$ are the square effects, X_iX_j, X_iX_k and X_jX_k are the interaction effects, β_0 is the intercept term, β_i (i = 1, 2, . . ., k) is the linear effect, β_{ii} (i = 1, 2, . . ., k) is the squared effect, β_{ij} (i = 1, 2, . . ., k; j = 1, 2,. . ., k) is the interaction effect and ε is a random error [26,27].

The DESIGN EXPERT 7.0 (Stat-Ease, Inc, Minneapolis, MN, USA) software was used for regression and graphical analysis of the obtained data. A design of 20 experiments was formulated for three factorial (2^3) designs and six replicates at the central points, four star points were employed to the second-order polynomial model. The optimum values of the selected variables were obtained by solving the regression equation and also by analyzing the response surface plots. 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 *S. cerevisiae* [27].

2.6. Isotherm models

The biosorption data were fitted to both Freundlich and Langmuir isotherm equations. The equilibrium adsorption was firstly described by Freundlich equation, which is must commonly written as [28]:

$$q_\mathrm{e} = K_\mathrm{f} C_\mathrm{e}^{1/n} \tag{4}$$

where q_e is the equilibrium biosorption capacity of the biomass in mg Cd^{2+}/g biomass, C_e is the equilibrium concentration of cadmium ion in mg/L and K_f (in mg/L) and $1/n$ are constants related to the sorption capacity and intensity, respectively. Nonlinear regression analysis was carried out in SigmaPlot software (SigmaPlot 2000, SPSS Inc., USA) in order to determine K_f and n values. The Langmuir equation is also employed to model the biosorption process. The equation is written as follows [28]:

$$q_\mathrm{e} = \frac{q_\mathrm{max} b C_\mathrm{e}}{1 + bC_\mathrm{e}} \tag{5}$$

where q_e is the equilibrium biosorption capacity of biomass in mg Cd^{2+}/g biomass, C_e is the equilibrium concentration of cadmium ion in mg/L, q_max is the maximum amount of metal sorbed in mg Cd^{2+}/g biomass and b is the constant that is referred to the bonding energy of sorption in mg/L. Likewise, nonlinear regression analysis was performed in SigmaPlot 2000. Using the mean of three repetitions, the equilibrium data were used to construct Freundlich and Langmuir isotherms.

2.7. Biosorption kinetics

The pseudo-second-order biosorption and the intra-particle diffusion model were applied to describe the kinetics of biosorption.

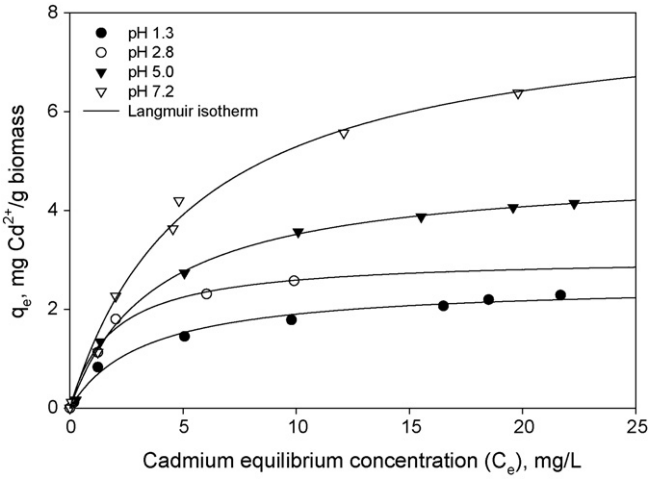

Fig. 2. Isotherm of cadmium biosorption by *S. cerevisiae* with simulation of Langmuir isotherm at various initial solution pH, nonviable yeast dosage concentration was 3.8 g/L in 100 mL aqueous solution.

Based on equilibrium biosorption, the pseudo-second-order kinetic equation is expressed as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{6}$$

where q_t and q_e (in mg Cd^{2+}/g biomass) are the amount of cadmium ion sorbed on biomass at equilibrium and at time t, respectively, and k_2 is the pseudo-second-order rate constant in g mg^{-1} min^{-1}. From Eq. (6), the amount of cadmium ions adsorbed on the biomass (mg Cd^{2+}/g biomass) at time t can be rearranged as:

$$\frac{1}{q_t} = \frac{t}{(1/k_2 q_e^2) + (t/q_e)} \tag{7}$$

The intra-particle diffusion model proposed by Weber and Morris is tested for the diffusion mechanism. The intra-particle equation can be described as:

$$q_t = k_i t^{1/2} + \frac{1}{C} \tag{8}$$

where k_i is the intra-particle diffusion rate constant in $mg\,g^{-1}\,min^{-1}$ and C is the constant that gives intra-particle accumulation in the boundary layer in mg Cd^{2+}/g. This suggested that biosorption of heavy metal by nonviable biomass was probably not due to cell surface-binding, but occurred also via intra-particle accumulation. Then, combination of the pseudo-second-order equation and intra-particle diffusion equation can be taken into account for the both mechanisms as follows:

$$q_t = k_i t^{1/2} + \frac{t}{(1/k_2 q_e^2) + (t/q_e)} \tag{9}$$

3. Results and discussion

3.1. Isotherm models and biosorption kinetics

Fig. 2 shows the result of the curve fitting with Langmuir equation at various initial solution pH. However, Fig. 2 does not show the results of the curve fitting with Freundlich equation as this fit was worse and this type of isotherm says less about the mechanism of the adsorption that Langmuir isotherm. Mean value of three repetitions was used for the analysis of the equilibrium data obtain in order to conduct the Freundlich and Langmuir isotherms, keeping the sorbent concentration constant at 3.8 g/L. Langmuir isotherm was in good agreement with the data for cadmium ion biosorption, evidenced by higher R^2 values (greater than 0.988). From Fig. 2, the Freundlich isotherm describes cadmium ion biosorption by indicating that the affinity for adsorption decreases exponentially as adsorption increases, while Langmuir isotherm considered monolayer adsorption, which assumes a constant binding energy until all available adsorptive active sites on the biomass are occupied. On the other hand, cadmium ion adsorption by *S. cerevisiae* was more likely monolayer sorption, instead of heterogeneous surface adsorption. The adsorption constants estimated from Freundlich and Langmuir isotherms are summarized in Table 2. Each of the equations used in the curve fitting indicated significant differences ($P < 0.0001$). Higher biosorption at moderate pH indicated that *S. cerevisiae* will have a better potential for cadmium biosorption. The highest capacity of cadmium biosorption (q_{max}) was found at the pH 7.2 as shown in Table 2. However, influence of pH at 1.3–7.2 showed an increase of K_f values for *S. cerevisiae*.

Table 2
Constants of Langmuir and Freundlich isotherms for cadmium biosorption by *S. cerevisiae*

pH	Langmuir			Freundlich		
	b	q_{max}	R^2	$1/n$	K_f	R^2
1.3	0.30	2.53	0.992	0.39	0.71	0.986
2.8	0.55	3.06	0.988	0.40	1.07	0.944
5.0	0.26	4.84	0.998	0.41	1.25	0.969
7.2	0.18	8.17	0.993	0.47	1.68	0.966

Fig. 3 shows simulations results of Eq. (9) with the experimental data. The model was able to predict the data quite well. It suggested that the combined equation describes cadmium ion uptake by nonviable biomass considering the pseudo-second-order model and intra-particle diffusion. It was thought that precipitation of $Cd(OH)_2$ may occur in an alkali zone. The biosorption capacity was decreased in an increase with the initial solution pH as shown in Fig. 3(a) and (b). A possible explanation can be that in the moderate alkali, pH influenced the binding of the divalent Cd^{2+} ions, therefore reducing the biosorption capacity. Time period taken by the biosorption process showed that cadmium ion sorption by *S. cerevisiae* occurred rapidly within the first 50 min. From Fig. 3(a) and (b), it is suspected that the cadmium uptake by *S. cerevisiae* was not only due to monolayer adsorption, but also via intra-particle accumulation. It might be concluded that the model Eq. (9) could be used to describe cadmium uptake by *S. cerevisiae* by considering both monolayer adsorption and intra-particle diffusion. The kinetic constants obtained from the model Eq. (9) are given in Table 3. It was observed that the coefficient of intra-particle diffusion rate constant (k_i) was very small in the acidic pH. The larger value of k_i (0.0343 $mg\,g^{-1}\,min^{-0.5}$) at moderate acidic pH suggested that it might be due to the possibility of intra-particle accumulation that apparently being higher for cadmium ion sorption by *S. cerevisiae*.

3.2. Effect of biomass pretreatment

Prior to biosorption, an appropriate pretreatment system for removal of heavy metal from wastewater is required. Various pretreatment methods have been reported to deal with the yeast cell of *S. cerevisiae* [11]. In the biosorption of heavy metal by nonviable biomass, some pretreatment methods have been developed for uptake capacity of biomass which are physical methods, such as; freeze-drying and boiling [29,30], drying [1,31], heating and autoclaving [32], besides mechanical disruption [20] and chemical methods, such as treatment with various acid and caustic organic and inorganic reagents [33], methanol [34], formaldehyde [20,35], and ethanol [1,19]. Lee and Lee [1] have found that size of cavities on *Phanerochaete chrysosporium* at lower temperature (e.g. 40–60 °C)

Table 3
Pseudo-second-order biosorption and intra-particle diffusion summary for selected experimental runs

Parameters	Fig. 3(a)				Fig. 3(b)			
	Run #7	Run #8	Run #9	Run #15	Run #6	Run #10	Run #12	Run #14
k_i (mg g^{-1} min$^{-0.5}$)	0.0029	0.0135	0.0025	0.0343	0.0073	0.0191	0.0078	0.0344
k_2 (mg g^{-1} min^{-1})	0.78	0.02	0.74	0.08	1.21	0.23	0.06	0.22
q_e (mg/g)	0.62	4.10	1.34	5.82	1.08	3.94	6.20	4.24
R^2	0.957	0.994	0.999	0.986	0.998	0.986	0.988	0.999

were smaller with much rougher surface. However, metal-binding sites may get destroyed during heat and autoclave treatment [36]. Alkali treatment of biomass has significantly increased the uptake capacity, whereas acid treatment of biomass almost has not shown any influence on metal biosorption [32,37]. However, inactivating and temperature of heating biomass has a significant effect on biosorption capacity of metal ions [30,38,39]. Furthermore, non-viable biomass does not need nutrient supplement for cell growth and can be easily produced as a waste produced from industrial fermentation processes and is not sensitive to operation condition like temperature [40]. In the present study, it was observed that ethanol pretreated *S. cerevisiae* biomass increased cadmium biosorption capacity and the cadmium removal was two times greater than original *S. cerevisiae*. The higher metal uptake values obtained by ethanol treated yeast cells may be explained by the increase in the accessibility of metal ions to the metal-binding sites on the biomass

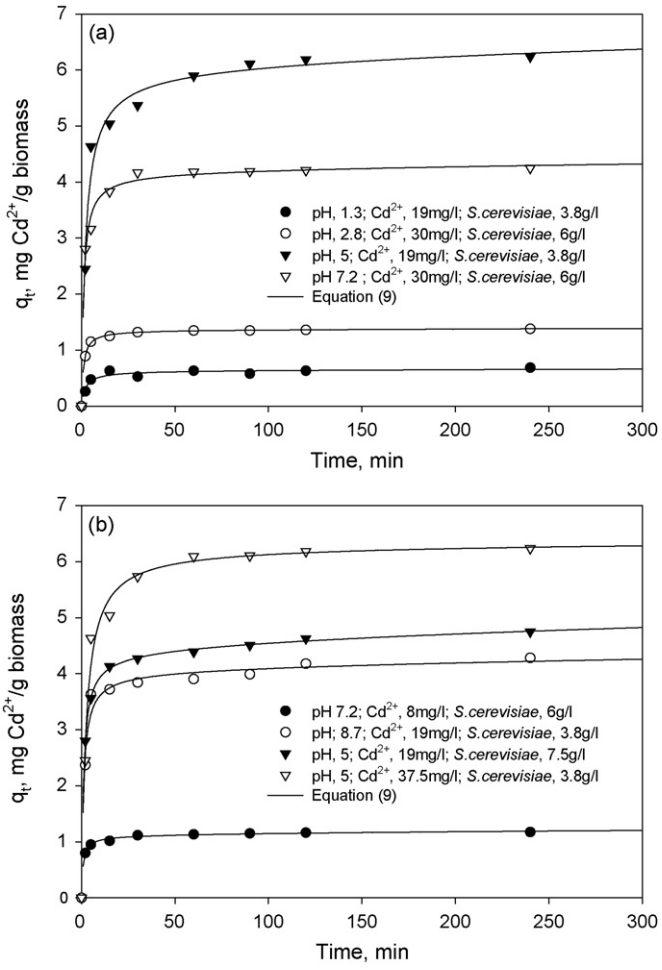

Fig. 3. Combination of intra-particle diffusion and pseudo-second-order equations for cadmium adsorption by *S. cerevisiae*: (a) effect of initial pH solution and biomass dosage; (b) various initial cadmium ion concentration and biomass dosage at pH 5.

Table 4
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 cadmium ion concentration, mg/L (X_2)	0.5	8	19	30	37.5
A. niger dosage, g/L (X_2)	0.1	1.6	3.8	6	7.5

[19]. However, the effect of caustic, ethanol and heat pretreatments on Cu^{2+} biosorption capacity of *S. cerevisiae* cells were investigated [41]. The highest metal uptake was obtained with caustic treated yeast cells and the effect of caustic treatment on metal uptake was explained by the removal of protein functional groups of the cell wall that make non-adsorbable protein complexes with Cu^{2+} ions [41].

3.3. Response surface methodology

A prior knowledge and understanding of the process and the process variables under investigation are necessary for achieving a more realistic model. The range and level of variables that were used in this experimental design were decided on the basis of literature reports for biosorption by *S. cerevisiae* [5,19,42–44]. Consequently, these variables were selected to analyze the optimum condition of higher biosorption efficiency using central composite design under response surface methodology. The range and level of experimental variables investigated in this study are shown in Table 4. The experiments were carried out with the 2^3 factorial designs as per central composite design (Table 5) and the max-

Table 5
Experimental design based on central composite design (CCD) used in this study

Run no.	Independent values						Categorical factor levels
	Coded values			Real values			
	X_1	X_2	X_3	X_1	X_2	X_3	
1	−1	−1	−1	2.8	8	1.6	Fractoinal
2	+1	−1	−1	7.2	8	1.6	2^{3-1}
3	−1	+1	−1	2.8	30	1.6	Frac-
4	+1	+1	−1	7.2	30	1.6	tional
5	−1	−1	+1	2.8	8	6.0	fac-
6	+1	−1	+1	7.2	8	6.0	to-
7	−1	+1	+1	2.8	30	6.0	rial
8	+1	+1	+1	7.2	30	6.0	points
9	$-\alpha$	0	0	1.3	19	3.8	
10	$+(\alpha$	0	0	8.7	19	3.8	Star
11	0	$-\alpha$	0	5	0.5	3.8	points
12	0	$+(\alpha$	0	5	37.5	3.8	(6
13	0	0	$-\alpha$	5	19	0.1	points)
14	0	0	$+(\alpha$	5	19	7.5	
15	0	0	0	5	19	3.8	
16	0	0	0	5	19	3.8	Central
17	0	0	0	5	19	3.8	points
18	0	0	0	5	19	3.8	(6
19	0	0	0	5	19	3.8	points)
20	0	0	0	5	19	3.8	

Table 6
Obseved and predicted values

Run no.	Observed values	Predicted value	Residual
1	4.793	4.774	0.019
2	5.015	5.32	−0.305
3	7.25	7.869	−0.619
4	8.475	9.028	−0.553
5	0.537	0.224	0.313
6	1.13	0.751	0.379
7	0.63	0.566	0.064
8	1.446	1.706	−0.26
9	0.468	0.451	0.017
10	2.192	1.869	0.323
11	0.078	0.435	−0.357
12	4.537	3.84	0.697
13	13.667	12.916	0.751
14	2.523	2.933	−0.41
15	3.121	3.098	0.023
16	3.324	3.098	0.226
17	3.163	3.098	0.065
18	2.961	3.098	−0.137
19	2.974	3.098	−0.124
20	2.987	3.098	−0.111

imum metal uptake rate obtained after 240 min biosorption with 20 experiments in replicate are shown in Table 6. The application of response surface methodology expressed in the following regression Eq. (4), is an empirical relationship between metal uptake (q) and tested variables take in coded unit.

$$q = 3.03 - 0.51X_1^2 - 0.31X_2^2 + 1.74X_3^2 + 0.15X_1 + 1.31X_2 - 3.24X_3 0.097X_1X_2 + 0.5X_1X_3 - 1.19X_2X_3 \quad (10)$$

where q is the response, i.e. metal uptake, and X_1, X_2 and X_3 are the coded values of the main effects initial pH, initial cadmium ion concentration and *S. cerevisiae* dosage, respectively. Whereas the variables X_1X_2, X_1X_3 and X_2X_3 represent the interaction effect of initial pH initial cadmium ion concentration, initial pH *S. cerevisiae* dosage and initial cadmium ions concentration *S. cerevisiae* dosage, respectively. X_1^2, X_2^2, and X_3^2 are the measures of the main effect of variables initial pH, initial cadmium ion concentration and *S. cerevisiae* dosage, respectively. The central point was replicated six times. The results of second-order response surface model in the form of analysis of variance (ANOVA) are shown in Table 7. The statistical significance of the model equation was evaluated by the *F*-test ANOVA. The significance of each coefficient was determined by *F*-values and *P*-values. It was observed from Table 7, the coefficients for the main and square effects were highly significant ($P < 0.0001$) in comparison with interaction effects. Table 8 shows ANOVA for the response surface quadratic model. The *F*-value (193.52) with a low probability value ($P < 0.0001$) demonstrates a high significance for the regression model. The goodness of the fit of the model was also checked by the multiple correlation coefficient

Table 7
Regression analysis using the 2^3 factorial central composite design

Model term	Coefficient estimate	Standard error	*F*-value	*P*-value
X_1	+0.42	0.139209	9.16912	0.0127
X_2	+1.01	0.139209	52.88377	<0.0001
X_3	−2.97	0.139209	454.52650	<0.0001
x_1^2	−0.69	0.135517	25.56506	0.0005
x_2^2	−0.34	0.135517	6.27924	0.0311
x_3^2	+1.71	0.135517	158.57900	<0.0001
x_1x_2	+0.15	0.181886	0.70936	0.4193
x_1x_3	−0.0047	0.181886	0.00068	0.9798
x_2x_3	−0.69	0.181886	14.32783	0.0036

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.

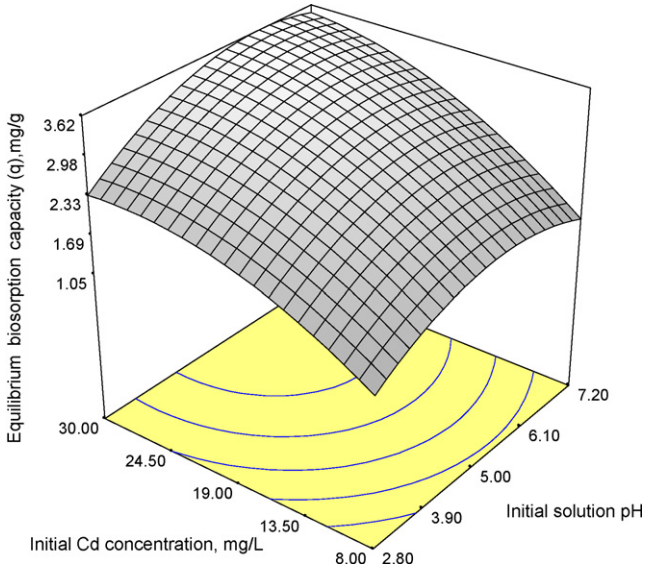

Fig. 4. Response surface plot showing the effect on Cd ions concentration and pH and their mutual effect on the metal uptake (q) while the remaining respective variable (biomass dosage) was at their respective zero levels.

(R^2). In this case, the value of the multiple correlation coefficient was 0.9867, which revealed that this regression is statistically significant and only 1.33% of the total variations is not explained by the model. The value of predicted multiple correlation coefficient (pred. $R^2 = 0.9747$) is in reasonable agreement with the value of the adjusted multiple correlation coefficient (adj. $R^2 = 0.9014$). At the same time, a relatively lower value of the coefficient of variance (CV = 2.94%) indicates a better precision and reliability of the experiments were carried out [27].

3.4. Effect of initial pH and initial metal ion concentration on the metal uptake

An attempt was made to improve the performance of the laboratory biosorption system with a view to understand better heavy metal removal and metal uptake efficiency. The effect of initial pH and initial cadmium ion concentrations on the cadmium uptake is shown in Fig. 4. The metal uptake increased with increase of initial solution pH ranging from 2.8 to 5 as well as with initial metal ion concentration ranging from 8 to 30 mg/L. Nevertheless, pH values higher than 5 reduced the biosorption efficiency. Therefore, the uptake of cadmium ion in aqueous solution was affected by pH 5. In the other hand, metal uptake was increased with increasing of initial cadmium ion concentration ranging from 8 to 30 mg/L but its obtained optimum value was about 19 mg/L. Comparatively the value of the factorial point of biomass dosage (*S. cerevisiae*) was 3.8 g/L. An increase of metal uptake by increasing initial metal ion concentration is a result of the increase in the driving force of the concentration gradient, rather than increase in the initial metal ion concentration. In the same condition, if the concentration of metal ions in the solution were higher, the active sites of *S. cerevisiae* would be surrounded by more metal ions, and the process of adsorption would be carried out more sufficiently. Therefore, the value of q increased with increasing of initial metal ions concentration [45]. However, several researchers have also investigated the effect of pH and cadmium ion concentration for biosorption of heavy metals by using different biomass and found similar results as with this study. Göksungur et al. [19] used *S. cerevisiae* for the removal of cadmium and lead ions. They have found that metal uptake increased with increase in medium pH and had a maxi-

Table 8
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	196.03	21.78	193.52	<0.0001
Main effects	3	136.72	45.47	172.2	<0.0043
Square effects	3	50.4	16.8	63.5	<0.011
Interaction effects	3	3.98	1.33	5.01	<0.47
Residual	10	2.65	0.26		
Total	19	198.68			

R^2, 0.9867; adjusted R^2, 0.9014; predicted R^2 0.9747; CV, 2.94.

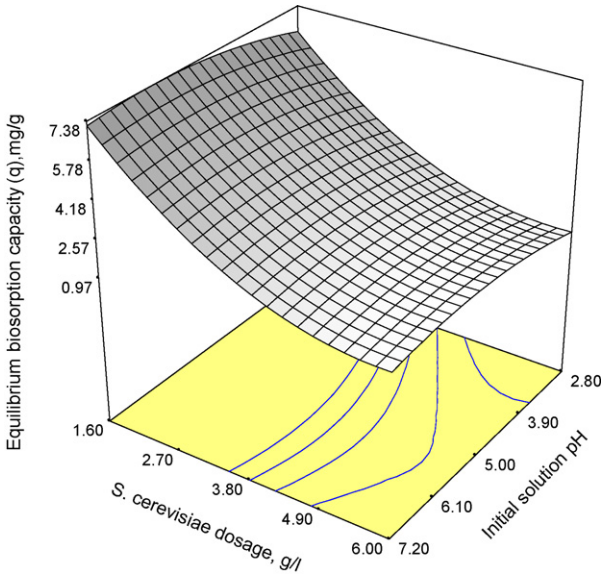

Fig. 5. Response surface plot showing the effect on *S. cerevisiae* and pH and their mutual effect on the metal uptake (q) while the remaining respective variable (initial cadmium ions concentration) was at their respective zero levels.

mum value at pH 6 and 5 for cadmium and lead ions, respectively. They also studied the effect of initial concentration of cadmium and lead ions on yeast cells treated with ethanol in a solution containing 5–25 mg/L metal ions and found that metal uptake increased with increase of ion concentrations. Han et al. [45] have stated that biosorption capacity of biomass increased with increasing of pH solution from 2 to 6 for copper and lead ions and the lowest metal uptake values were observed at pH < 2 for both metal ions. In their study the equilibrium uptake increased with increasing of initial metal ions concentration.

3.5. Effect of biosorbent dosage and initial pH on the metal uptake

The effect of *S. cerevisiae* dosage and initial pH solution on cadmium uptake is shown in Fig. 5. It was observed that the metal uptake decreased with increasing the amount of biomass from 1.6 to 6.0 g/L and its optimum value was 3.8 g/L. The optimum for the maximum cadmium uptake was found to be 4.91 g/g. In comparison, the value of actual factor (initial cadmium ion concentration) was 19 mg/L. To describe these experimental findings, though increasing adsorbent dosage can be attributed to increased biomass surface area and the availability of more adsorption sites, nevertheless, the values of metal uptake decreased with increasing the adsorbent dosage [45]. The primary factor explaining this characteristic is that adsorption sites remain unsaturated during the adsorption reaction whereas the number of sites available for adsorption increased by increasing the adsorbent dosage [46]. This means that the higher values of cadmium ions uptake obtained by the decrease in biomass dosage and increase in initial solution pH simultaneously. This may be explained by the increase in availability of binding sites at higher initial solution pH and this improved in the access of metal ions to the metal-binding sites of cell wall [16,30]. Similar observations were found in studies on Cu(II) biosorption using pretreated *A. niger* biomass [47] and in the case of copper and chromium uptake by *Aspergillus carbonarius* [48]. They reported that higher uptake at lower biomass dosage could be due to metal ions and biosorbent ratio, which decrease upon an increase in biomass dosage. The problem of high biomass dosage resulted aggregates of biomass and may cause interference between binding sites at higher biomass dosage or insufficiently of metal ions in the solution with respect to available binding sites [49]. It is likely that protons will then combine with metal ions for the ligands and thereby decrease the interaction of metal ions with the cell components [50]. Since these experiments were carried out, the work of Uslu and Tanyol [36] who studied biosorption of lead(II) and copper(II), ions both single component and binary systems in a solution using *Pseudomonas putida*, was published. They found that the initial adsorption rates decreased with increasing the biosorbent concentration. It should also be mentioned that Vasudevan et al. [51] reported that the cadmium ion adsorption capacity decreased with increase of biosorbent dosage. Their theory is similar to that advanced by Zou et al. [46] to account for the cell surface remaining unsaturated at higher biosorbent dosage.

3.6. Effect of biosorbent dosage and initial metal ion concentration on the metal uptake

The relationship between initial metal ion concentration and biomass dosage is shown in Fig. 6. It shows that pH is actual factor that determined pH 5. The optimum *S. cerevisiae* for the maximum uptake of metal was found to be 1.73 g/L. A higher ratio of the surface binding site on the biosorbent to the metal ion concentration could be obtained at lower biomass dosage and higher initial ion cadmium concentration. The results agree with the results of [50]. From Fig. 6, with the dose of biosorbent increasing, the uptake capacity of Cd(II) ion per unit mass of biosorbent (q_e, mg/g) was decreased. Increasing biosorbent dosage can be attributed to increased biosorbent surface area and the availability of more adsorption sites. But the values of uptake capacity (q_e) decreased with increasing the biosorbent dosage. The primary factor explaining this characteristic is that adsorption sites remain unsaturated during the adsorption reaction whereas the number of sites available for adsorption site increases by increasing the adsorbent dose [51]. However, the uptake capacity decreased with the decreases of biomass dosage. It seems that the particles aggregation all the time present. That is why no optimum value or at least plateau (even though at low biomass dosage) is observed. However, there seems to be an indirect relationship between uptake capacity of Cd(II) ion by the yeast biomass to the initial concentration. Moreover, there is a net negative charge on the cell wall biomass in moderate acidic and basic pH and the ionic state of ligands such as carboxyl, phosphate and amino

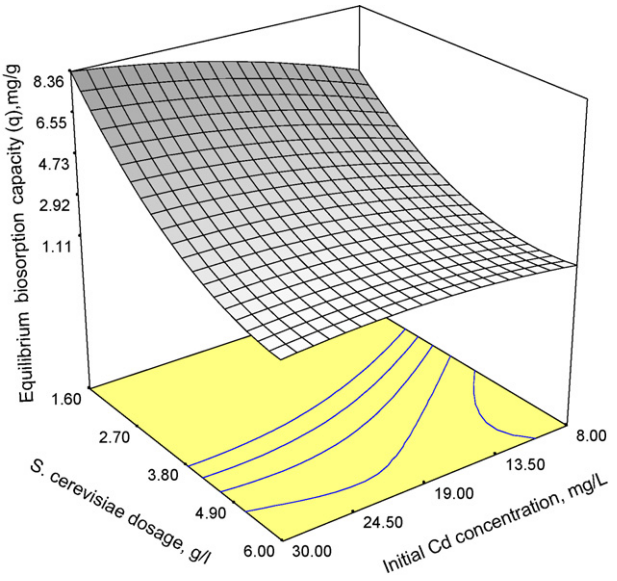

Fig. 6. Response surface plot showing the effect on *S. cerevisiae* and Cd ions concentration and their mutual effect on the metal uptake (q) while the remaining respective variable (initial solution pH) was at their respective zero levels.

groups could be as such to promote reaction with metal cations. As the pH was lower, the overall surface charge on the cells could be positive, which inhibited the approach of positively charged metal cations [19]. From Fig. 6, the optimum values of the experimental variables and the corresponding maximum metal uptake obtained were 6.71 mg/g biomass at initial cadmium ion concentration of 26.46 mg/L and *S. cerevisiae* dosage of 2.13 g/L.

4. Conclusion

The present study has demonstrated the use of central composite design by determining the conditions leading to high metal uptake efficiency. The results obtained for the removal of initial cadmium ion concentration in an aqueous solution using *S. cerevisiae* show a decrease of biosorption efficiency at low and high value initial pH. An optimum condition for cadmium uptake of 8.56 mg/g biomass was achieved with RSM under Design-Expert software at initial cadmium ion concentration of 30 mg/L and *S. cerevisiae* dosage of 1.6 g/L. In addition, it was showed that metal uptake increased with increasing cadmium ion concentrations but the optimum cadmium ion concentrations is 19 mg/L, and so with increasing biomass dosage the metal removal decreased. The points giving the maximum removal of cadmium ion were found to be at 3.8 g/L biomass concentration. The fit of the model was checked by the determination (R^2). In this case, the value of the multiple correlation coefficient ($R^2 = 0.9867$) indicates that 1.33% of the variation was not explained by the model. The process kinetic study showed that both monolayer adsorption and intra-particle diffusion mechanisms were effective in the cadmium biosorption process.

It was concluded that ethanol pretreated *S. cerevisiae* biomass may be used as a low-cost, natural and abundant source for the removal of heavy metal ions from wastewater and it may be an alternative to more costly methods such as activated carbon adsorption, solvent extraction and chemical oxidation applied for this purpose.

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