Korean J. Chem. Eng., **30**(5), 1067-1075 (2013)
DOI: 10.1007/s11814-013-0012-0

Application of Doehlert experimental design for the optimization of cadmium biosorption in an aqueous solution by marine yeast biomass of *Yarrowia lipolytica*

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(*Received 20 September 2012 • accepted 30 January 2013*)

Abstract−A cadmium biosorption process was optimized by varying three independent variables pH (4.5-7.5), initial cadmium ion concentration (10-30 mg L^{-1}), and *Yarrowia lipolytica* dosage (3-5 g L^{-1}) by using a Doehlert experimental design (DD) involving response surface methodology (RSM). For the maximum biosorption of cadmium ion in an aqueous solution by *Y. lipolytica*, a total of fifteen experimental runs were set and the experimental data fitted to the empirical second-order polynomial model of a suitable degree. The analysis of variance of the quadratic model demonstrates that the model was highly significant. Three-dimensional plots demonstrate relationships between the cadmium ion uptake with the paired variables (when other variable 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 variables studied. The optimum values of the variables were found to be 6.43, 17.56 mg L^{-1} and 3.63 g L^{-1} for pH, initial cadmium ion concentration and biomass dosage, respectively, at a contact time of 40 min. At these optimal conditions, the maximum percentage biosorption of cadmium was predicted to be 48.89. The experimental values were in good agreement with predicted values and the correlation coefficient was found to be 0.9985. It showed that both monolayer adsorption and intra-particle diffusion mechanisms were effective in the cadmium biosorption process. Therefore, it is apparent that the DD involving RSM not only gives valuable information on interactions between the variables but also leads to identification of feasible optimum values of the studied variables.

Key words: Biosorption, Doehlert Experimental Design, Response Surface Methodology, *Yarrowia lipolytica*

INTRODUCTION

The presence of toxic heavy metals contaminated in aqueous streams, arising from the discharge of untreated metal containing effluent into water bodies, is one of the most important environmental issues [1]. Their presence in aquatic ecosystem causes a harmful effect on living organisms [2]. In recent years, increased attention has focused on the use of microorganisms for the removal and possible recovery of metal ions from industrial wastes. In fact, it represents a potential alternative to existing technologies (chemical precipitation, reverse osmosis, solvent extraction), which have significant disadvantages such as high reagent or energy requirements and generation of toxic sludge or other products that need disposal [3]. Biosorption, the passive binding of metals by living or dead biomass, can be used to remove toxic heavy metals from industrial wastewater. Cadmium is attracting wide attention of environmentalists as one of the most toxic heavy metals. The major sources of cadmium release into the environment by waste streams are electroplating, smelting, alloy manufacturing, pigments, plastic, battery, mining and refining processes [4]. Cadmium has been recognized for its negative effects on the environment where it readily accumulates in living systems. Adverse health effects due to cadmium are well documented, and it has been reported to cause renal disturbances, lung insufficiency, bone lesions, cancer and hypertension in humans [5,6].

In recent years, *Yarrowia lipolytica* has emerged as an important non-conventional yeast with significant biological relevance and biotechnological applications [7,8]. This yeast has been used in the remediation of various polluted environments [9-11] and is also applied in the degradation of different wastes [12-15]. *Y. lipolytica* is able to utilize a variety of renewable carbon sources, and the biomass of the fungus has been used as single cell protein or as single cell oil [16,17]. *Y. lipolytica* biomass, to the best of our knowledge, has not been used for the biosorption of Cd (II) ions, although this yeast can survive metal stress and accumulate chromium, copper, cobalt, cadmium, nickel, zinc and gold [18-22]. This microorganism, therefore, displays a potential for the bioremediation of metal polluted environments. Biosorption of cadmium ions by different living and nonliving biomass has been studied by several authors [23-27].

Optimization of biosorption of heavy metals by the classical method involves changing one independent variable (i.e., *Y. lipolytica* 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. These drawbacks of single parameter optimization can be eliminated by optimizing all the affected parameters collectively by Doehlert experimental design

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(DD) [28] of response surface methodology (RSM). Basically, this optimization process involves three major steps: performing the statistically designed experiments, estimating the coefficients in a mathematical model, predicting the response and checking the adequacy of the model. Several researchers have applied various designs for optimization of different process variables [29-32]. The objective of the present study is to optimize biosorption of cadmium (II) ions in aqueous solution onto *Y. lipolytica* 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.

MATERIALS AND METHODS

1. Microorganism and Growth Conditions

Yarrowia lipolytica NCIM 3589, obtained from National Chemical Laboratory, Pune, India, was used throughout the study. The culture was maintained on MGYP slants having the composition (g L^{-1}): malt extract 3, glucose 10, yeast extract 3, peptone 5 and agar agar 20. The pH of the medium was adjusted (by using 0.1 N NaOH or 0.1 N HCL) to 6.4-6.8 and culture was incubated at 30 °C for 48 h. Subculturing was carried out once in 2 weeks and the culture was stored at 4 °C.

2. Microbial Cultivation

The composition of culture medium was (grams per liter): peptone: 5; yeast extract: 3 and NaCl: 3 [33]. The medium was sterilized by autoclaving at a pressure of 1.5 atm and temperature of 121 °C for 20 min. The yeast cells were grown for 16 h (at end of exponential phase) and then filtered.

3. Preparation of Biosorbent

Yeast biomass was deactivated by heating in an oven at 80 °C for 24 h [34]. 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 3,600 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 [35]. 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 [36].

4. Preparation of Cadmium Solution

All the chemicals used are of analytical grade. Stock solution of 1,000 mgL^{-1} was prepared by dissolving appropriate amount of $3CdSO_4 \cdot 8H_2O$ in 1 L of deionized water. Cadmium solution of different concentrations was prepared by suitable dilution of the stock solution to known volumes.

5. Experimental Procedure

2 g L^{-1} of biosorbent was added to 50 mL of the cadmium ion solution in each of 250 mL Erlenmeyer flasks. The flasks were incubated in orbital shaker at a speed of 180 rpm for different agitation times (1, 2, 5, 10, 20, 30, 40, 50, 60, 90, and 120 min). The resulting samples were filtered by Whatman filter paper and analyzed for cadmium concentration in flame atomic absorption spectrometer (novAA® 350, Analytikjena, Germany).

The percent cadmium biosorbed is calculated from the relation

$$= \frac{C_o - C_i}{C_o} \times 100 \quad (1)$$

where C_o=initial concentration of cadmium in the aqueous solution (mg L^{-1})

C_i=final concentration of cadmium in the aqueous solution (mg L^{-1})

Preliminary experimental runs for the biosorption of cadmium using *Yarrowia lipolytica* have been conducted by assigning three experimental strategies, as listed below, varying one parameter and keeping the other two parameters constant for an equilibrium agitation time and temperature at 303 K.

Strategy 1: pH was varied as 1, 2, 3, 4, 6 and 7 keeping the biosorbent dosage and initial cadmium ion concentration at constant values.

Strategy 2: Initial cadmium ion concentration was varied as (mg L^{-1}) 20, 50, 100, 150 and 200, keeping the other parameters constant.

Strategy 3: Keeping pH and initial cadmium ion concentration as constants, the biosorbent dosage was varied as (g L^{-1}) 1, 2, 3, 4 and 5.

6. Doehlert Experimental Design

Once the variables having a statistically significant influence on the responses were identified, a Doehlert experimental design [28] was used to optimize the values of these variables. The number of experiments required (N) is given by $N=n^2+n+n_0$, where n is the number of variables and n_0 is the number of center points. Replicates at the central level of the variables are performed in order to validate the model by means of an estimate of experimental variance. For statistical calculations, the natural variables X_i were coded as x_i according to Eq. (2):

$$x_i = \left(\frac{X_i - X_{oi}}{\Delta X_i}\right)\alpha_i \quad (2)$$

where x_i is the coded value of the i^{th} variable, X_i the natural value, X_{oi} the value at the center point, ΔX_i the step change value, and α_i is the maximum value of the coded variable (i.e., 1.0, 0.866 and 0.816 for five levels, seven levels, and three levels, respectively).

The second degree polynomial (Eq. (3)) was fitted to the experimental data by using the statistical software STATISTICA 6.0 (StatEase Inc., Tulsa, OK, USA) to estimate the response of the dependent variable and the regression coefficients.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 \quad (3)$$

where Y is predicted response, X_1, X_2, X_3 are independent variables, b_0 is offset term, b_1, b_2, b_3 are the coefficients for linear effects, b_{11}, b_{22}, b_{33} are the coefficients for squared effects and b_{12}, b_{23}, b_{13} are the coefficients for interaction terms.

Doehlert design offers the following advantages over the other designs of RSM. First, this design needs fewer experiments; second, the number of levels is not the same for all variables, which allows flexibility to assign a large or a small number of levels to the selected variables depending on their relative importance. Generally, it is preferable to choose the variable with the stronger effect as the variable with seven levels in order to obtain maximum information of the system. Third, considering that the efficiency of any

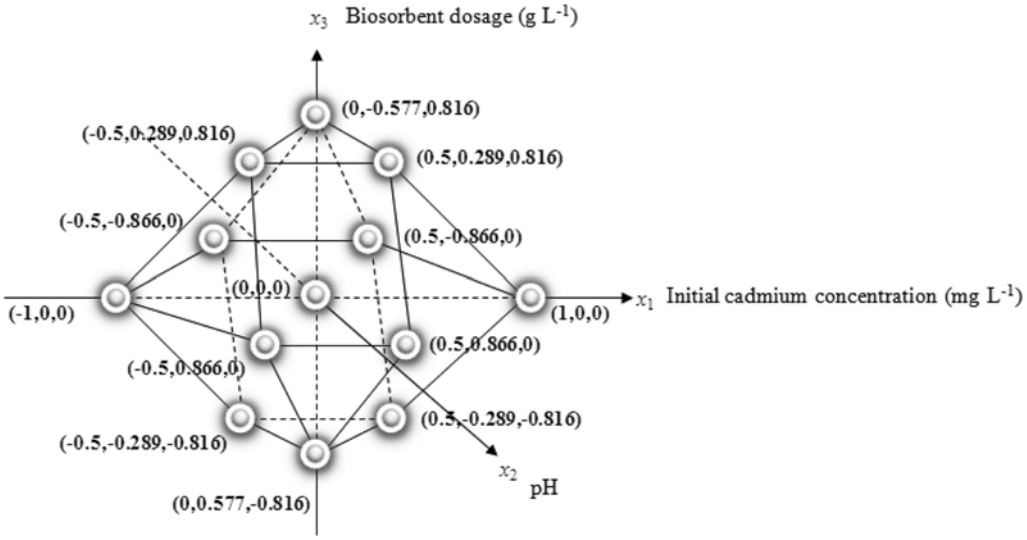

Fig. 1. 3D view of the experimental domain. The dots represent the experimental runs of Table 2.

experimental design is defined as the number of coefficients of the model divided by the number of experiments, Doehlert design is more efficient than Central Composite design or Box-Behnken design. Finally, Doehlert design is also more efficient in mapping the space: adjoining hexagons can fill a space completely and efficiently, since the hexagons fill space without overlap [37].

Fig. 1 shows the graphical representation of this network in the space defined by the factors. This uniform distribution of experimental points (shown as dots) allows interpolation by the mathematical model of responses anywhere within the experimental domain. Furthermore, the quality of the interpolation remains constant since the network is uniform. The Doehlert design shows great flexibility compared to other classical designs used in the process optimization.

RESULTS AND DISCUSSION

1. Effect of Contact Time

Time course profile for the biosorption of Cd (II) for a solution of 20 mg L^{-1} is shown in Fig. 2. The data show that a contact time of 40 min was required to achieve an optimum biosorption and there was no significant change in removal of the metal ions with further increase in contact time. Therefore, the uptake and unadsorbed cadmium concentrations at the end of 40 min are given as the equilibrium values, q_{eq} (mg g^{-1}) and C_{eq} (mg L^{-1}). For further studies of biosorption with other variable parameters the optimum time of 40 min for Cd (II) has been chosen for the contact period.

2. Effect of pH

Earlier studies on heavy metal biosorption have shown that pH of the solution is the most important physical parameter influencing the adsorption process. Batch biosorption experiments were conducted at different pH (1.0-7.0) values and the results are depicted in Fig. 3. There was an increase in biosorption capacity of *Y. lipolytica* with increasing pH from 2.0 to 6.0 for Cd (II), and maximum adsorption capacity was observed at pH 6.0. The pH dependency may be due to the binding of the metal through an ion-exchange mechanism through functional groups or positively charged ligands with *Y. lipolytica*.

3. Effect of Initial Cadmium Ion Concentration

The relationship between the percentage biosorption of Cd (II)

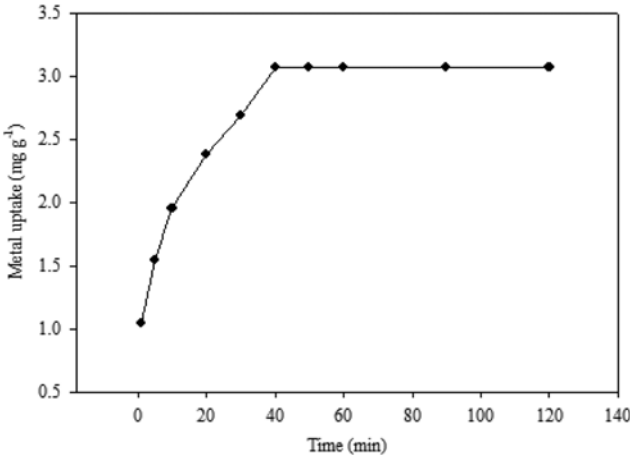

Fig. 2. Effect of contact time on biosorption of cadmium by *Y. lipolytica*.

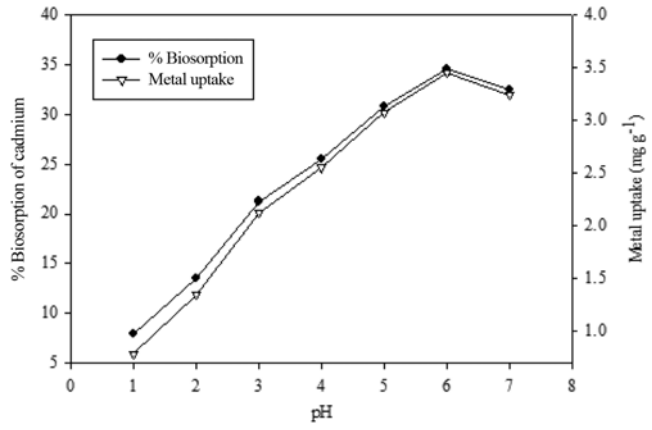

Fig. 3. Effect of pH on cadmium biosorption for 20 mg/L of metal and 2 g/L of yeast biomass concentration.

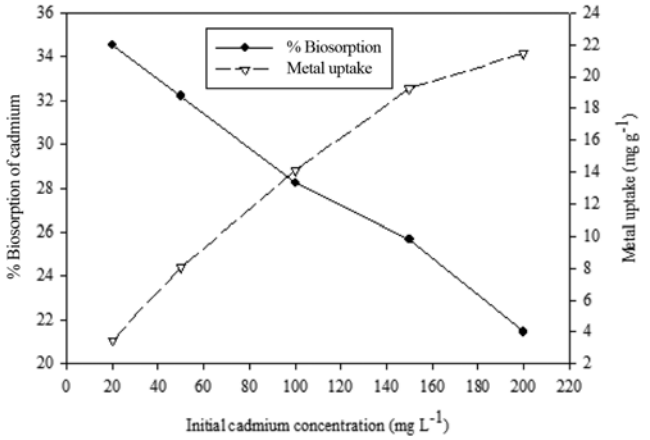

Fig. 4. Effect of metal ion concentrations on biosorption of cadmium by *Y. lipolytica* at 2 g/L of biosorbent concentration (pH 6).

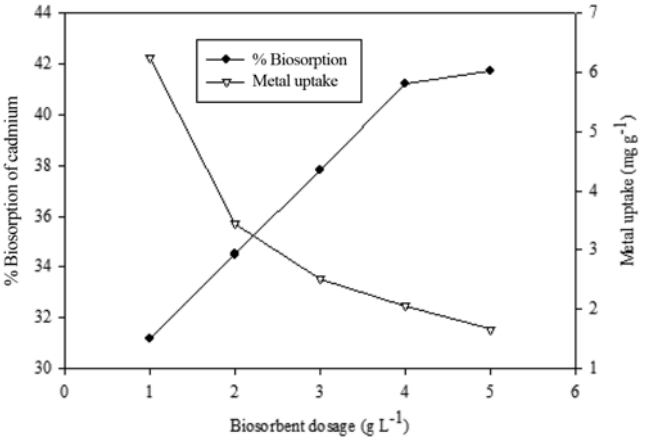

Fig. 5. Effect of biosorbent dosage on biosorption of cadmium for 20 mg/L of metal concentration (pH 6).

and its initial concentrations using different dosages of yeast biomass is presented in Fig. 4. The initial metal ion concentration remarkably influenced the equilibrium metal uptake and biosorption yield. The amount of metal ion adsorbed increased with increase in initial concentrations (due to higher availability of metal ions for sorption), while the percentage biosorption of metal ion decreased with an increase in initial metal ion concentration. The increase of metal uptake is a result of the increase in the driving force, i.e., concentration gradient, with an increase in the initial metal ion concentrations (from 20 to 200 mg L^{-1}). However, the percentage biosorption of Cd (II) on yeast biomass decreased from 34 to 21%. Though an increase in metal uptake was observed, the decrease in percentage biosorption may be attributed to lack of sufficient surface area to accommodate much more metal available in the solution. At lower concentrations, all metal ions present in solution could interact with the binding sites, and thus the percentage biosorption was higher than that at higher initial metal ion concentrations. At higher concentrations, lower biosorption yield is due to the saturation of biosorption sites.

4. Effect of Biosorbent Dosage

Studies on the effect of yeast biomass dosage for Cd (II) removal are important to get the trade-off between the adsorbent capacity and percent removal of metal ions resulting in an optimum biosorbent concentration. The percent of biosorption and adsorption capacity for Cd (II) as a function of biosorbent dosage was investigated (Fig. 5). The removal of cadmium was also dependent on the concentration of biomass used in the biosorption medium. The result shows that with the increase in the yeast biomass dosage, the metal removal efficiency also increased. The percentage biosorption of cadmium increased 31 to 41%. Results show that the biosorption efficiency is highly dependent on the increase in biomass dosage of the solution. This is expected because higher dosage of adsorbent in the solution increases the availability of exchangeable sites for the ions [38]. It was earlier reported that the increase in the efficiency of metal ions removal with an increase in the biosorbent dosage was due to the increase in the number of adsorption sites [39]. The drop in adsorption capacity is basically due to the sites remaining unsaturated during the adsorption process.

5. Optimization of the Medium Constituents Using DD

The selection of the range for process variables is extremely important when planning the experimental design; otherwise, after completion of the experimental runs, the optimal conditions, obtained either by response surface methodology, might not be found inside the experimental region. The following experiments were carried out to study the variables in such a range so that reasonable percentage removal of cadmium would be achieved within that range.

From the results of preliminary experimental runs, the three variables (pH, initial cadmium concentration, and biosorbent dosage) were identified as the potential variables for the percentage biosorption of cadmium. Out of them, pH showed stronger effect on percentage biosorption of cadmium, and hence it was assigned seven levels, followed by initial cadmium concentration which was assigned five levels and biosorbent dosage was assigned three levels. A summary of the independent variables and their range and levels is presented in Table 1.

Table 1. Experimental range and levels of the variables

Variables	Range and levels						
Coded variable, x_1	−1	−0.5	0	0.5	1		
Initial cadmium concentration, X_1 (mg L^{-1})	10	15	20	25	30		
Coded variable, x_2	−0.866	−0.577	−0.288	0	0.288	0.577	0.866
pH, X_2	4.5	5.0	5.5	6.0	6.5	7.0	7.5
Coded variable, x_3	−0.816	0	0.816				
Biosorbent dosage, X_3 (g L^{-1})	3.0	4.0	5.0				

Table 2. Doehlert three variable experimental design along with experimental and predicted values

Experiment number	Coded values			Natural values			% Biosorption of cadmium	
	x_1	x_2	x_3	X_1	X_2	X_3	Experimental	Predicted
1	1	0	0	30	6.0	4.0	32.18	31.6600
2	−1	0	0	10	6.0	4.0	39.23	39.7500
3	0.5	0.866	0	25	7.5	4.0	33.67	33.9362
4	−0.5	−0.866	0	15	4.5	4.0	32.53	32.2637
5	0.5	−0.866	0	25	4.5	4.0	31.45	31.7337
6	−0.5	0.866	0	15	7.5	4.0	41.78	41.4962
7	0.5	0.288	0.816	25	6.5	5.0	29.43	29.6837
8	−0.5	−0.288	−0.816	15	5.5	3.0	40.24	39.9862
9	0.5	−0.288	−0.816	25	5.5	3.0	37.59	37.8262
10	0	0.577	−0.816	20	7.0	3.0	41.65	41.6675
11	−0.5	0.288	0.816	15	6.5	5.0	35.85	35.6137
12	0	−0.577	0.816	20	5.0	5.0	29.71	29.6925
13	0	0	0	20	6.0	4.0	45.32	45.5533
14	0	0	0	20	6.0	4.0	45.98	45.5533
15	0	0	0	20	6.0	4.0	45.36	45.5533

X_1=initial cadmium concentration (mg L^{-1}), X_2=pH, X_3=biosorbent dosage (g L^{-1})

Fifteen experimental runs (Table 2) including three replicates at the center point were carried out for 40 minutes of contact time. By using multiple regression analysis (STATISTICA 6.0) the coefficients of Eq. (4) were estimated, and gave the following equation.

$$Y=-250.781+5.226X_1+50.999X_2+48.783X_3-0.098X_1^2-3.659X_2^2 -6.337X_3^2-0.234X_1X_2-0.123X_2X_3-0.071X_1X_3 \quad (4)$$

The predicted percentage biosorption of cadmium that resulted from Eq. (4) is in close agreement with the experimental values, as evident from last column of Table 2, and hence the above equation was deemed to be adequate in representing the percentage biosorption of cadmium under the specified range of experiments. For quadratic models, the optimum point can be characterized as maximum, minimum, or saddle. It is possible to calculate the coordinates of the optimum point through the first derivative of the mathematical function, which describes the response surface and equates it to zero. The above quadratic equation obtained for three variables is described below:

$$\frac{\partial Y}{\partial X_1}=5.226-0.196X_1-0.234X_2-0.071X_3=0 \quad (5)$$

$$\frac{\partial Y}{\partial X_2}=50.999-0.234X_1-7.318X_2-0.123X_3=0 \quad (6)$$

$$\frac{\partial Y}{\partial X_3}=48.783-0.071X_1-0.123X_2-12.674X_3=0 \quad (7)$$

Thus, to calculate the coordinate of the optimum point, it is necessary to solve the first grade system formed by Eqs. (5), (6) and (7) and to find the $X_{1,opt}$, $X_{2,opt}$ and $X_{3,opt}$ values. The optimal set of conditions for maximum percentage biosorption of cadmium is pH=6.43, initial cadmium concentration=17.56 mg L^{-1}, and biosorbent dosage=3.63 g L^{-1}. The extent of biosorption of cadmium at these optimum conditions was 48.89%. An experiment was conducted at the optimum conditions of the above process variables; the experimental value of percentage biosorption of cadmium (48.12) was found to be very close to the value predicted by Doehlert experi-

Table 3. Model coefficients estimated by multiple linear regression (significance of regression coefficients)

Term	Coefficient	Value	Standard error of coefficient	*t*-Value	*p*-Value
Constant	b_0	−250.781	13.36738	−18.7606	0.000008*
Initial cadmium concentration	b_1	5.226	0.31575	16.5516	0.000015*
pH	b_2	50.999	2.58929	19.6962	0.000006*
Biosorbent dosage	b_3	48.783	3.26676	14.9331	0.000024*
Initial cadmium concentration×initial cadmium concentration	b_{11}	−0.098	0.00476	−20.6971	0.000005*
pH×pH	b_{22}	−3.659	0.15861	−23.0719	0.000003*
Biosorbent dosage×biosorbent dosage	b_{33}	−6.337	0.30094	−21.0579	0.000004*
Initial cadmium concentration×pH	b_{12}	−0.234	0.03475	−6.7434	0.001088*
pH×biosorbent dosage	b_{23}	−0.123	0.31722	−0.3888	0.713434
Biosorbent dosage×initial cadmium concentration	b_{31}	−0.071	0.05494	−1.2983	0.250845

* Significant at $p \leq 0.05$

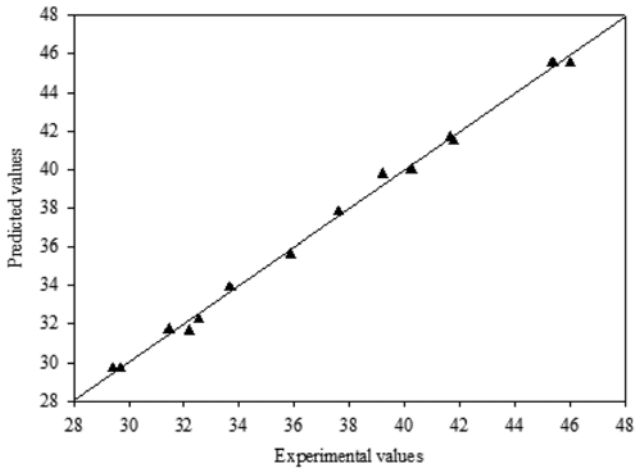

Fig. 6. Parity plot showing the distribution of experimental vs. predicted values of percentage biosorption of cadmium.

mental design.

The significance of each coefficient in Eq. (4) was determined by student's *t*-test and *p*-values, which are also listed in Table 3. The larger the magnitude of the *t*-value and smaller the *p*-value, the more significant is the corresponding coefficient [40]. This implies that the linear and quadratic effects of initial cadmium concentration, pH and biosorbent dosage were highly significant, as is evident from their respective *p*-values. The interaction effect of initial cadmium concentration and pH was found to be significant ($p \leq 0.05$). The remaining two interaction terms, pH×biosorbent dosage and biosorbent dosage×initial cadmium concentration, were found to be insignificant ($p > 0.05$), which was also presented in Table 3. The parity plot (Fig. 6) shows a satisfactory correlation between the experimental and predicted values of percentage biosorption of cadmium, wherein the points cluster around the diagonal line, which indicates the good fit of the model because the deviation between the experimental and predicted values is less.

The results of the second order response surface model fitting in the form of Analysis of Variance (ANOVA) are given in Table 4. It is required to test the significance and adequacy of the model. The Fisher variance ratio, the *F*-value ($=S_r^2/S_e^2$), is a statistically valid measure of how well the factors describe the variation in the data about its mean. The greater the *F*-value is from unity, the more certain it is that the factors explain adequately the variation in the data about

Table 4. ANOVA for the entire quadratic model

Source of variation	Sum of squares (SS)	Degree of freedom (d.f.)	Mean squares (MS)	*F*-value	Probe>*F*
Model	472.3209	9	52.48010	193.1558	0.000008
Error	1.3585	5	0.27170		
Total	473.6794	14			

$R=0.99856499$; $R^2=0.99713204$; Adjusted $R^2=0.99196972$

$P_{model}>F=0.000008$

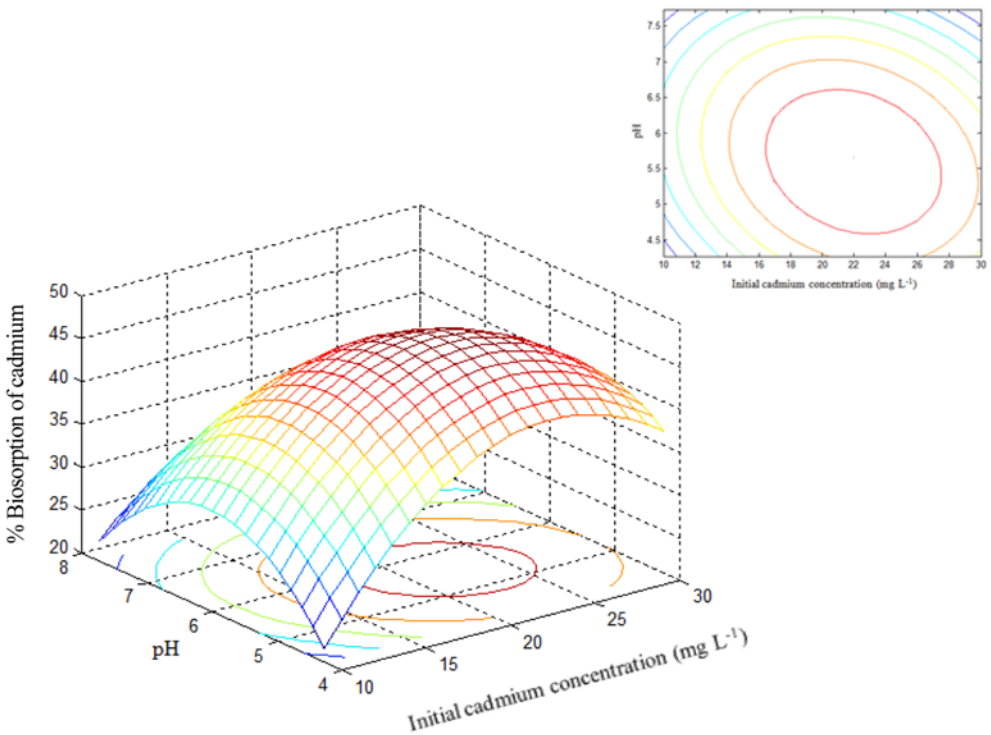

Fig. 7. Response surface and contour plot of initial cadmium concentration vs. pH on percentage biosorption of cadmium (biosorbent dosage was kept constant at (4 g L^{-1})).

its mean, and the estimated factor effects are real. The ANOVA of the regression model demonstrates that the model is highly significant, as is evident from Fisher's *F*-test (F_{model}=193.1558) and a very low probability value (P_{model}>F=0.000008).

The goodness of the fit of the model was checked by the determination coefficient (R^2). The R^2 value provides a measure of how much variability in the observed response values can be explained by the experimental variables and their interactions. The R^2 value is always between 0 and 1. The closer the R^2 value is to 1, the stronger the model is and the better it predicts the response. In this case, the value of the determination coefficient (R^2=0. 9971) indicates that 99.71% of the variability in the response could be explained by the model. In addition, the value of the adjusted determination coefficient (Adj R^2=0.9919) is also very high to advocate for a high significance of the model. Also, a higher value of the correlation coefficient (R=0.9985) justifies an excellent correlation between the

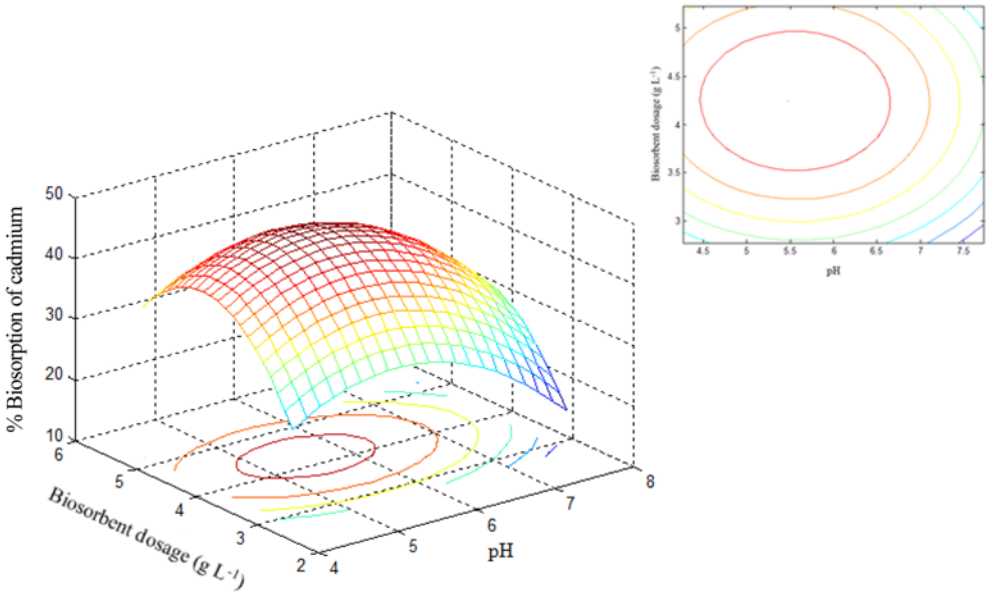

Fig. 8. Response surface and contour plot of pH vs. biosorbent dosage on percentage biosorption of cadmium (initial cadmium concentration was kept constant at (20 mg L^{-1})).

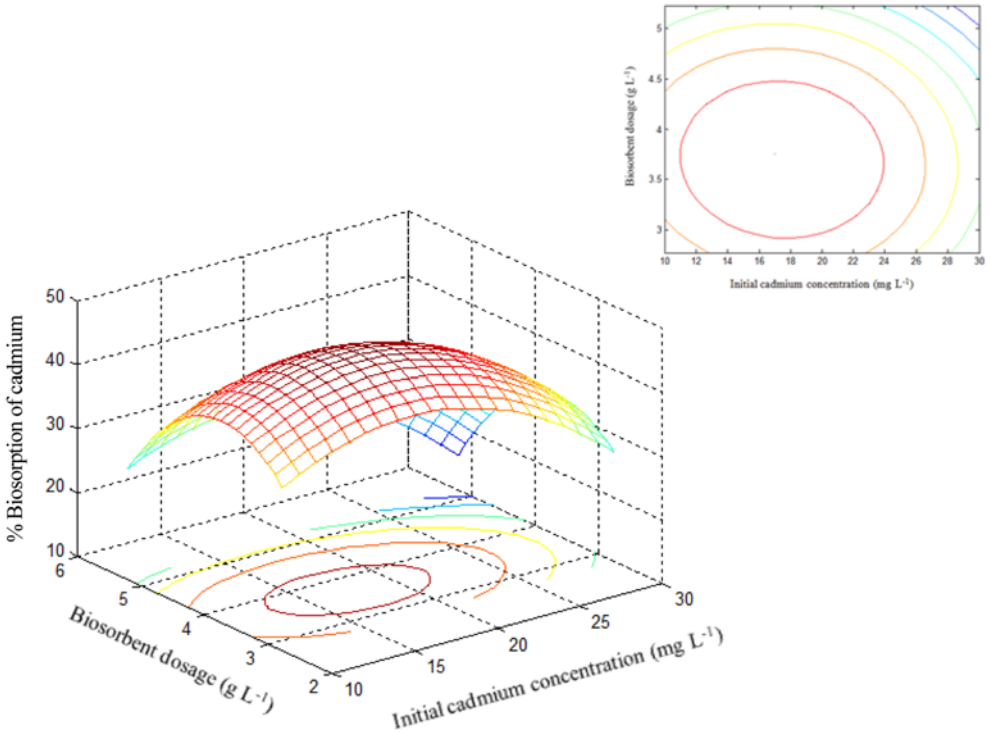

Fig. 9. Response surface and contour plot of biosorbent dosage vs. initial cadmium concentration on percentage biosorption of cadmium (pH was kept constant at 6).

independent process variables.

Based on the above experimental results, surface contours were generated to understand the impact of one variable on the other using fitness function with the help of MATLAB 7.0. Figs. 7-9 represent the surface contour plots for the optimization of percentage biosorption of cadmium. Fig. 7 shows the interaction effect of initial cadmium concentration and pH on percentage biosorption of cadmium at the fixed biosorbent dosage (4 g L^{-1}). It is evident that percentage biosorption of cadmium significantly increased with the initial cadmium concentration up to about 17 mg L^{-1}, and reached the maximum percentage biosorption of cadmium at some 16-18 mg L^{-1} initial cadmium concentration, but decreased slowly beyond this concentration. Similarly, the percentage biosorption of cadmium drastically increased with increase in the pH in the range 5-7, and then steadily decreased beyond that range. Fig. 8 depicts the surface contour plot of the effects of pH and biosorbent dosage on percentage biosorption of cadmium at a fixed initial cadmium concentration (20 mg L^{-1}). As can be seen, with the increase of biosorbent dosage from 3.5 to 4.5 g L^{-1}, the percentage biosorption of cadmium increased gradually from 43 to 44 but the trend is negligible beyond 4.5 g L^{-1}. Fig. 9 shows the effects of interaction of biosorbent dosage and initial cadmium concentration on percentage biosorption of cadmium at a fixed pH (6). With the concentration of initial cadmium increasing, the percentage biosorption of cadmium increased in the biosorbent dosage of 3.5-4.5 g L^{-1}, but decreased slowly beyond that range.

CONCLUSIONS

The present study involves the use of Doehlert experimental designs to optimize process conditions for maximal biosorption of cadmium from aqueous solutions using *Yarrowia lipolytica* NCIM 3589 biomass as biosorbent. Three variables--pH, initial cadmium concentration, and biosorbent dosage--were identified by preliminary experimental runs as significant for percentage biosorption of cadmium. These variables are optimized with Doehlert experimental design using STATISTICA 6.0 for the necessary computations. All the three variables showed significant influence on the percentage removal of cadmium. Significant interactions between the three variables were also observed from the response surface and contour plots. The maximum percentage biosorption of cadmium was predicted to be 48.89 when the optimized process conditions were set as follows: initial cadmium concentration 17.56 mg L^{-1}, pH 6.43, and biosorbent dosage 3.63 g L^{-1}. When an experiment was conducted at the optimum conditions of above process variables, the experimental value of percentage biosorption of cadmium (48.12) was found to be very close to the value predicted by Doehlert experimental design. Thus the present data confirms that marine yeast biomass can be used as efficient biosorbent for the removal of cadmium (II) ions from aqueous solution, and that this methodology could be effectively used to study the importance of individual, cumulative and interactive effects of the test parameters in biosorption and other processes.

ACKNOWLEDGEMENTS

The author **(Dr. Sarat Babu Imandi)** is grateful for the funding received under the Fast Track Proposals for Young Scientists Scheme **(SR/FTP/ETA-109/2010)**, Department of Science and Technology, Government of India, New Delhi for carrying out this research. The authors are grateful to the Management, GITAM University and the Department of Biotechnology, GIT for extending their support during the implementation of the work.

REFERENCES

1. A. H. Hawari and C. N. Mulligan, *Process. Biochem.*, **41**, 187 (2006).
2. W. Xuejiang, C. Ling, X. Siqing, Z. Jianfu, J. M. Chovelon and N. J. Renault, *Miner. Eng.*, **19**, 968 (2006).
3. S. Bossrez, J. Remacle and J. Coyette, *J. Chem. Technol. Biotechnol.*, **70**, 45 (1997).
4. M. Tsezos, *Hydrometallurgy*, **59**, 241 (2001).
5. J. Yin and H. W. Blanch, *Biotechnol. Bioeng.*, **34**, 180 (1989).
6. Y. C. Sharma, *J. Appl. Interface Sci.*, **173**, 66 (1995).
7. G. Barth and C. Gaillardin, *FEMS Microbiol. Rev.*, **19**, 219 (1997).
8. P. Fickers, P. H. Benetti, Y. Waché, A. Marty, S. Mauersberger, M. S. Smit and J. M. Nicaud, *FEMS Yeast Res.*, **5**, 527 (2005).
9. R. Margesin and F. Schinner, *FEMS Microbiol. Ecol.*, **24**, 243 (1997).
10. S. S. Zinjarde and A. A. Pant, *Mar. Pollut. Bull.*, **44**, 118 (2002).
11. M. R. Jain, S. S. Zinjarde, D. D. Deobagkar and D. N. Deobagkar, *Mar. Pollut. Bull.*, **49**, 783 (2004).
12. V. Johnson, S. J. Patel, K. A. Patel and M. H. Mehta, *World J. Microbiol. Biotechnol.*, **10**, 524 (1994).
13. B. De Felice, G. Pontecorvo and M. Carfagna, *Acta Biotechnol.*, **17**, 231 (1997).
14. N. Oswal, P. M. Sharma, S. S. Zinjarde and A. Pant, *Bioresour. Technol.*, **85**, 35 (2002).
15. R. Lanciotti, A. Gianotti, D. Baldi, R. Angrisani, G. Suzzi, D. Mastrocola and M. E. Guerzoni, *Bioresour. Technol.*, **96**, 317 (2005).
16. B. Achremowicz, F. V. Kosikowski and K. Masuyama, *Acta Microbiol. Pol.*, **26**, 265 (1977).
17. S. Papanikolaou, I. Chevalot, M. Komaitis, I. Marc and G. Aggelis, *Appl. Microbiol. Biotechnol.*, **58**, 308 (2002).
18. A. A. Bankar, A. R. Kumar and S. S. Zinjarde, *J. Hazard. Mater.*, **170**, 487 (2009).
19. S. García, M. Prado, R. Dégano and A. Domínguez, *J. Biol. Chem.*, **277**, 37359 (2002).
20. M. Strouhal, R. Kizek, J. Vacek, L. Trnkova and M. Nemec, *Bioelectrochemistry*, **60**, 29 (2003).
21. H. Ito, M. Inouche, H. Tohoyama and M. Joho, *Biometals.* **20**, 773 (2007).
22. M. Agnihotri, S. Joshi, A. R. Kumar, S. Zinjarde and S. Kulkarni, *Mater. Lett.*, **63**, 1231 (2009).
23. A. Kapoor, T. Viraraghavan and D. R. Cullimore, *Bioresour. Technol.*, **70**, 95 (1999).
24. Z. Aksu, *Sep. Purif. Technol.*, **21**, 285 (2001).
25. P. Kaewsarn and Q. Yu, *Environ. Pollut.*, **112**, 209 (2001).
26. B. Benguella and H. Benaissa, *Water Res.*, **36**, 2463 (2002).
27. K. Y. H. Gin, Y. Z. Tang and M. A. Aziz, *Water Res.*, **36**, 1313 (2002).
28. D. H. Doehlert, *Appl. Stat.*, **19**, 231 (1970).
29. M. Amini, H. Younesi and N. Bahramifar, *Colloids and Surfaces A: Physicochem. Eng. Aspects.*, **337**, 67 (2009).
30. F. Ghorbani, H. Younesi, S. M. Ghasempouri, A. A. Zinatizadeh, M. Amini and A. Daneshi, *Chem. Eng. J.*, **145**, 267 (2008).

31. R. Singh, R. Chadetrik, R. Kumar, K. Bishnoi, D. Bhatia, A. Kumar, N. R. Bishnoi and N. Singh, *J. Hazard. Mater.*, **174**, 623 (2010).
32. M. Fereidouni, A. Daneshi and H. Younesi, *J. Hazard. Mater.*, **168**, 1437 (2009).
33. S. B. Imandi, V. V. R. Bandaru, S. R. Somalanka, S. R. Bandaru and H. R. Garapati, *Bioresour. Technol.*, **99**, 4445 (2008).
34. S. Schiewer and B. Volesky, *Environ. Sci. Technol.*, **29**, 3049 (1995).
35. Y. Gőksungur, S. Üren and U. Güvenç, *Bioresour. Technol.*, **96**, 103 (2005).
36. T. Bahadir, G. Bakan, L. Altas and H. Buyukgungor, *Enzyme Microb. Technol.*, **41**, 98 (2007).
37. S. B. Imandi, V. V. R. Bandaru, S. R. Somalanka and H. R. Garapati, *Enzyme Microb. Technol.*, **40**, 1367 (2007).
38. A. Esposito, F. Pagnanelli, A. Lodi, C. Solisio and F. Vegliò, *Hydrometallurgy*, **60**, 129 (2001).
39. V. T. P. Vinod, R. B. Sashidhar and B. Sreedhar, *J. Hazard. Mater.*, **178**, 851 (2010).
40. Ź. Lazić, *Design of experiments in chemical engineering*, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim (2004).