

Statistical analysis and isotherm study of uranium biosorption by *Padina* sp. algae biomass

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Received: 27 September 2010 / Accepted: 23 November 2010 / Published online: 19 December 2010
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Abstract

Introduction The application of response surface methodology is presented for optimizing the removal of U ions from aqueous solutions using *Padina* sp., a brown marine algal biomass.

Methods Box–Wilson central composite design was employed to assess individual and interactive effects of the four main parameters (pH and initial uranium concentration in solutions, contact time and temperature) on uranium uptake.

Results and discussion Response surface analysis showed that the data were adequately fitted to second-order polynomial model. Analysis of variance showed a high coefficient of determination value ($R^2=0.9746$) and satisfactory second-order regression model was derived.

Conclusion The optimum pH and initial uranium concentration in solutions, contact time and temperature were found to be 4.07, 778.48 mg/l, 74.31 min, and 37.47°C, respectively. Maximized uranium uptake was predicted and experimentally validated. The equilibrium data for biosorption of U onto the *Padina* sp. were well represented by the Langmuir isotherm, giving maximum monolayer adsorption capacity as high as 376.73 mg/g.

Keywords Biosorption · Uranium · Algae biomass · Isotherm · Experimental design · Optimization

Nomenclature

b the Langmuir adsorption constant ($l\text{ mg}^{-1}$)
 C_{eq} residual metal ion concentration at equilibrium (mg l^{-1})
 C_o initial metal ion concentration (mg l^{-1})
 C_t metal concentration after time t (mg l^{-1})

q_{eq} adsorbed metal ion quantity per g of alga at equilibrium (mg g^{-1})
 q_{exp} observed U uptake (mg/g)
 q_{pre} predicted U uptake (mg/g)
 q_t adsorbed metal ion quantity per g of alga at any time (mg g^{-1})
 $q_{t,\text{exp}}$ observed U uptake at any time (mg/g)
 $q_{t,\text{pre}}$ predicted U uptake at any time (mg/g)
 Q^0 the Langmuir adsorption constant (mg g^{-1})
 Q_{eq} adsorbed metal ion quantity per g of alga at equilibrium (mg g^{-1})
 M amount of the biosorbent (g)
 R^2 correlation coefficient
 t time (min)
 T solution temperature (°C)
 v volume of the solution (l)
 w alga concentration (g l^{-1})

1 Introduction

Uranium (U) is one of the most important natural radionuclides and can arrive in nature from the water supply, run off in combined systems, and wash water, especially in areas with high uranium content in the soil, or if the waste water system is used by facilities that process uranium, or clean clothing or equipment that is contaminated with uranium as well as to a number of human activities (e.g., mining, production, and use of phosphate fertilizers, copper metallurgy, and military activities; Bituh et al. 2009; Ragozzini et al. 1986). Uranium forms more than 160 mineral species and accounts for 5% of all known minerals (Kalin et al. 2005a). This element is possible harmful pollutant in the environment. Hence, investigating the concentration of uranium in the environment is significant because of its radioactivity and chemical toxicity.

Responsible editor: Philippe Garrigues

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Different methods have been employed for the purification of these wastewaters, including traditional adsorption, physico-chemical treatment, solvent extraction, ion-exchange, evaporation, and reverse osmosis (Bayramoglu et al. 2006). The high cost of the chemical reagents, the problems of secondary pollution and low efficiency at low metal concentrations make the above physicochemical methods rather limited in their application. In the last two decades, more studies have focused on using different microorganism to remove radionuclides from wastewaters. There are many biosorbents that have been used for uranium removal from water solutions (Kalin et al. 2005a; Bayramoglu et al. 2006; Parab et al. 2005; Li et al. 2004; Psareva et al. 2005; Sar et al. 2004; Genc et al. 2003; Yang and Volesky 1999; Khani et al. 2006, 2008). These studies document that various biomasses from fungi, yeast, algae, and unicellular bacteria are capable of uptake or binding of uranium greater than 15% of biomass and dry weight. A metal-loading capacity of greater than 15% of biomass and dry weight has been defined as an economic threshold for practical applications of biosorption compared to conventional methods (Kalin et al. 2005b; Sar and D'Souza 2001). Studies on the biosorption of uranium onto brown algae pieces such as *Cystoseria indica* and *Sargassum fluitans* show promise in removal of uranium (Yang and Volesky 1999; Khani et al. 2006, 2008). Several species of marine brown algae including *C. indica*, *Sargassum* sp., *Padina* sp., etc. are very abundant in the gulf of Oman on the coast of Chabahar, Iran. The aim of this study is to optimize of uranium biosorption by the brown marine algal biomass, *Padina* sp. and isotherm study at optimum conditions. Optimization of biosorption of heavy metals by the classical method involves changing one independent variable (i.e., biomass dosages, initial metal concentration, pH, temperature, or time of contact) 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 Box–Behnken design or central composite design under response surface methodology can be employed to optimize the biosorption of heavy metals (Massart et al. 2003). At the present study, response surface methodology (RSM) was used to optimize the uranium uptake by *Padina* sp. algae biomass.

2 Methods

2.1 Preparation of biosorbent

Padina sp., a marine brown algae biomass, obtained from the gulf of Oman on the coast of Chabahar, Iran, was extensively washed with distilled water and sundried on the beach and in an oven at 50°C overnight. Dried biomasses

was ground in a laboratory blender and sorted by sieving using the standard test sieves. The batch of biomass with particle size 1.0–1.25 mm was selected for subsequent pretreatments. Pretreatment of the biomass was carried out as follows: a sample of 10 g of biomass was treated with 0.1 N HCl solutions (1,000 ml) for 12 h under slow stirring. After 12 h of contacting with acid, the biomass was rinsed with deionized water in the same volume many times until a stable wash solution pH was reached. The biomasses were then dried in an oven at 50°C overnight. So, prepared biomasses were stored for later uses.

2.2 Preparation of uranium solution

Uranium solutions were prepared by diluting 1,000 mg uranium/l stock solution which was obtained by dissolving a weighed quantity of uranium in nitrate salt form, $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, analytical grade. Diluted solutions were prepared at room temperature in distilled and deionized water. The range of concentrations of prepared solutions changed between 150 and 1,000 mg l^{-1} . The pH of each solution was adjusted with diluted or concentrated HCl and NaOH solutions before mixing with the biosorbent.

2.3 Batch sorption program and analysis of uranium concentration

On a shaker incubator, 0.1 g of biosorbent was mixed with 50 ml $\text{UO}_2(\text{NO}_3)_2$ solution with known initial uranium concentration, pH, and temperature at 150 rpm. The pH was adjusted to the desired values by adding 0.05 N HCl or NaOH during the sorption process and the volume of HCl or NaOH added was recorded. After predefined contact time, according to the experimental design, solution was filtered. A series of 0.5 mL samples of solution were removed from the vessel. After appropriate dilution, the samples were analyzed by the ICP-AES for metal concentrations.

The q_t (mg/g), amount of uranium ions adsorbed per unit of empty sorbent at time t , was obtained by using the following expression:

$$q_t = (C_0 - C_t) \cdot V / M \quad (1)$$

where C_0 is the initial metal concentration (mg/l), C_t is the metal concentration after time t (mg/l), V is the volume of the solution (l), and M is the amount of the biosorbent (g).

2.4 Statistical design and analysis

The RSM was employed to statistical design of experiments. This method allows us to estimate interaction and even quadratic effects, and therefore give us an idea of the

(local) shape of the response surface we are investigating. Box–Behnken, central composite, and Doehlert designs (Massart et al. 2003; Ferreira et al. 2004; NIST/SEMATECH e-Handbook of Statistical Methods) are among the principal response surface methodologies used in experimental design. The Box–Behnken design is an independent quadratic design in that it does not contain an embedded factorial or fractional factorial design. In this design, the treatment combinations are at the midpoints of edges of the process space and at the center. This design are rotatable (or near rotatable) and require three levels of each factor. It has been applied for optimization of several chemical and physical processes (Tan et al. 2008; Hameed et al. 2008; Tarangini et al. 2009). The Box–Behnken and Doehlert designs have limited capability for orthogonal blocking compared to the Box–Wilson central composite design. A Box–Wilson central composite design, commonly called “Central Composite Design”, contains an imbedded factorial or fractional factorial design with center points that is augmented with a group of ‘star points’ that allow estimation of curvature. If the distance from the center of the design space to a factorial point is ± 1 unit for each factor, the distance from the center of the design space to a star point is $\pm \alpha$ with $|\alpha| > 1$. The precise value of α depends on certain properties desired for the design and on the number of factors involved. At the present study, the central composite design was used for optimization of the bio-sorption process and to evaluate the effects and interactions of the process variables.

It is important to include the second-order model to provide good prediction throughout the region of interest. The second-order response surface design is rotatable; this means that the variance of the predicted response is the same at all points. Rotatability is a reasonable basis for the selection of response surface design. Because the purpose of RSM is optimization and as the location of the optimum is unknown prior to running the experiment, it makes sense to use design that provides equal precisions of estimation in all directions (Tan et al. 2008).

The experimental data was analyzed using the statistical software, Design-Expert software version 8.0.2 (STAT-EASE Inc., Minneapolis, MN, USA), for regression analysis to fit the equations developed and also for the evaluation of the statistical significance of the equations.

3 Results and discussion

3.1 Statistical analysis

Uranium removal by algae in a batch system usually depends on several factors, such as biosorbent concentration in solution (w), pH of the aqueous solution, temperature (T), contact time (t), initial concentration of metal ion

(C_0), and the shaking rpm. In the present study, the shaking rate and biosorbent concentration was kept constant. In order to study the combined effect of these factors, experiments were performed for different combinations of the parameters using statistically designed experiments. The experiments were designed based on a five level (axial points, factorial points and center point) four factor Box–Wilson Central Composite Design. Initial uranium concentration of the aqueous solution (150–1,000 mg/L), pH of the aqueous solution (2–7), temperature (10–50°C) and contact time between biosorbent and sorbate (10–100 min) were kept as variable input parameters (factors) within the ranges defined, while biosorbent dosage (2 g/L) and shaking rate (150 rpm) was kept as constant input parameters. The factors levels were coded as -2 (low axial point), -1 (low factorial point) 0 (central point), $+1$ (high factorial point), and $+2$ (high axial point). The input parameters and experimental design levels used in the present work are shown in Table 1. Response surface methodology was applied to the experimental data using statistical software, Design-Expert V8.0.2. Linear and second order polynomials were fitted to the experimental data to obtain the regression equations. The sequential F test, lack-of-fit test, and other adequacy measures were used for selecting the best model. A manual regression method was used to fit the second-order polynomial (Box and Hunter 1957) to the experimental data and to identify the relevant model terms. Considering all the linear terms, square terms, and linear by linear interaction items, the quadratic response model can be described. A natural log transformation should be applied to the experimental data, because the ratio of minimum to maximum uptake of uranium is greater than 10 suggesting that transformation was required in the present system. This can be written as follows:

$$q' = \ln(q_t + y) \quad (2)$$

where y is constant and set to zero. Table 2 shows the results of responses (q_t of uranium onto biosorbent), which were measured according to the Design-Expert software. Using the point prediction option in the software, the response of the validation experiments were predicted using the developed models. The predicted results for q_t ($q_{t,pre}$) are also shown in Table 2.

Analyzing the measured responses, three different tests such as the sequential model sum of squares, lack of fit tests, and model summary statistics were carried out. The fit summary output indicated that the quadratic polynomial model was significant for the present system as shown in Table 3. As a natural log transformation was applied to the experimental data, the interaction of two factors (2FI) and the linear model were suggested to be insignificant using the response surface methodology. However, the p value for the linear and two factor interaction models was found to be

Table 1 Input parameters and experimental design levels

Factors	Unit	Coded levels				
		-2	-1	0	+1	+2
pH	–	2	3.25	4.5	5.75	7
<i>t</i> (time)	min	10	32.5	55	77.5	100
<i>C</i> ₀ (initial metal concentration)	mg/l	150	362.5	575	787.5	1,000
<i>T</i> (temperature)	°C	10	20	30	40	50

less than 0.01. The model summary statistic showed the regression coefficient is found to be highest ($R^2=0.9746$) for the quadratic model with minimum standard deviation (0.13). Analysis of variance (ANOVA) analysis confirmed that the form of the model chosen to explain the relationship between the factors and the response is correct. Table 4 shows the ANOVA result for uranium removal onto

the *Padina* sp. algae biomass, with a model F-value of 35.57, implying that the model is significant (at $p<0.05$).

By selecting the manual regression method, which eliminated the insignificant model terms automatically, the resulting ANOVA for the reduced quadratic models (Table 4) summarizes the analysis of variance of each response and shows the significant model terms. Values of

Table 2 Predicted results of responses (q_t of uranium onto biosorbent) according to the Box–Wilson central composite design together with experimental values

Std	Run	Parameters				Response	
		<i>C</i> ₀ (mg/l)	pH	<i>t</i> (min)	<i>T</i> (°C)	$q_{t,exp}$ (mg/g)	$q_{t,pre}$ (mg/g)
17	1	575.00	4.50	55.00	30.00	256.00	233.13
15	2	787.50	3.25	77.50	40.00	298.00	258.55
3	3	362.50	3.25	77.50	20.00	134.01	121.82
8	4	787.50	5.75	77.50	20.00	191.02	142.30
2	5	362.50	5.75	32.50	20.00	93.01	85.75
9	6	362.50	3.25	32.50	40.00	122.50	124.65
14	7	787.50	5.75	32.50	40.00	166.00	165.23
12	8	362.50	5.75	77.50	40.00	91.02	79.59
18	9	575.00	4.50	55.00	30.00	255.90	233.13
5	10	787.50	3.25	32.50	20.00	254.00	248.45
19	11	575.00	4.50	55.00	30.00	256.00	233.13
16	12	787.50	5.75	77.50	40.00	230.01	170.13
20	13	575.00	4.50	55.00	30.00	256.00	233.13
7	14	787.50	3.25	77.50	20.00	259.09	217.56
6	15	787.50	5.75	32.50	20.00	180.08	156.76
13	16	787.50	3.25	32.50	40.00	233.00	260.30
4	17	362.50	5.75	77.50	20.00	98.40	77.84
10	18	362.50	5.75	32.50	40.00	87.60	77.29
1	19	362.50	3.25	32.50	20.00	132.00	139.12
11	20	362.50	3.25	77.50	40.00	128.50	123.81
24	21	575.00	4.50	100.00	30.00	267.01	202.09
30	22	575.00	4.50	55.00	30.00	256.10	233.13
21	23	575.00	2.00	55.00	30.00	114.02	82.19
23	24	575.00	4.50	10.00	30.00	200.00	224.13
28	25	575.00	4.50	55.00	50.00	234.40	247.65
22	26	575.00	7.00	55.00	30.00	28.51	33.34
29	27	575.00	4.50	55.00	30.00	256.00	233.13
25	28	150.00	4.50	55.00	30.00	71.61	75.96
27	29	575.00	4.50	55.00	10.00	288.00	231.18
26	30	1,000.00	4.50	55.00	30.00	368.02	289.99

Table 3 Fit summary output as the sequential model sum of squares, lack of fit tests, and model summary statistics

Sequential model sum of square						
Source	Sum of squares	df	Mean squares	F value	P value	Remark
Mean	793.96	1	793.98	–	–	–
Linear	4.65	4	1.16	6.15	0.0016	
2FI	0.043	6	7.22×10^{-3}	0.029	0.9999	
Quadratic	4	1.02		57.83	≤ 0.0001	Suggested
Cubic	0.23	8		0.029	333.11	Aliased
Residual	5	8.565×10^{-5}		–	–	–
Total	30	26.77		–	–	–
Lack of fit test						
Source	Sum of squares	df	Mean squares	F value	P value	Remark
Linear	4.34	20	0.22	5.279×10^2	< 0.0001	–
2FI	4.30	14	0.31	7.439×10^2	< 0.0001	–
Quadratic	0.23	10	0.023	5.519×10^1	≤ 0.0001	Suggested
Cubic	4.283×10^{-4}	2	2.141×10^{-4}	0.514	< 0.0001	Aliased
Pure error	1.25×10^{-3}	3	4.167×10^{-4}	–	–	–
Model summary statistic						
Source	SD	R ²	Adj. R ²	Pre. R ²	PRESS	Remark
Linear	0.43	0.5170	0.4330	0.1689	7.47	–
2FI	0.5	0.5218	0.2406	0.0174	8.83	–
Quadratic	0.13	0.9746	0.9472	0.7926	1.86	Suggested
Cubic	9.255×10^{-3}	1.0000	0.9997	0.9656	0.31	Aliased

“Prob > F” (*p* values) less than 0.0500 indicate model terms are significant. There is only a 0.01% chance that a “Model F Value” this large could occur due to noise. In this case pH, C₀ (initial metal concentration), pH², C₀² are highly

significant model terms, *t* (time) is a significant model term, while model *p* values greater than 0.10 indicated that the model terms were not significant. For example *T* (temperature) was a not significant term. The values of the

Table 4 Analysis of variance (ANOVA) table for Response Surface Quadratic Model for uranium biosorption by *Padina* sp. algae biomass

Source	Sum of squares	d.f.	Mean square	F value	p Value	Remark
Model	8.76	14	0.63	35.57	< 0.0001	a
pH	1.20	1	1.20	68.14	< 0.0001	a
t	0.080	1	0.080	4.56	0.0523	a
C ₀	3.36	1	3.36	190.83	< 0.0001	a
T	0.011	1	0.011	0.64	0.4385	c
pH.t	1.320×10^{-3}	1	1.320×10^{-3}	0.075	0.7885	c
pH.C ₀	5.429×10^{-4}	1	5.429×10^{-4}	0.031	0.8633	c
pH.T	3.785×10^{-5}	1	3.785×10^{-5}	2.152×10^{-3}	0.9637	c
t.C ₀	0.015	1	0.015	0.85	0.3737	c
t.T	0.016	1	0.016	0.92	0.3552	c
C ₀ .T	0.010	1	0.010	0.59	0.4565	c
pH ²	3.82	1	3.82	217.15	< 0.0001	a
t ²	0.014	1	0.014	0.81	0.3842	c
C ₀ ²	0.34	1	0.34	19.37	0.0007	b
T ²	1.176×10^{-3}	1	1.176×10^{-3}	0.067	0.8000	c
Residual	0.23	13	0.018	–	–	–
Lack of Fit	0.23	10	0.023	–	–	–
Pure Error	1.25×10^{-3}	3	4.167×10^{-4}	–	–	–
Cor Total	8.99	29	–	–	–	–

a Highly significant, b significant, c not significant

predicted and adjusted R^2 are 0.7926 and 0.9472, respectively, so have a reasonable agreement together. The high value of adequate precision (22.454 for the uranium–algae system) is desirable in supporting the fitness of the model. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable (Kumar et al. 2007). It means that this model can be used to navigate the design space.

As shown in Table 3, the cubic model was found to be aliased and could not be used for further modeling of experimental data. It means that the model has more terms in it than can be estimated from the data supplied, some of them will be aliased with other terms and cannot be estimated separately. The model is aliased means that the model is inappropriate for further investigations (Kumar et al. 2009). However, the model summary statistics of the present biosorption system showed that the regression coefficient was found to be very high ($R^2=0.9999$) when compared with the quadratic model ($R^2=0.9746$).

The final mathematical model in terms of actual factors as determined by Design-Expert software is shown below:

$$\begin{aligned} \ln(q_t) = & 0.326 + 1.93667\text{pH} - 1.84 \times 10^{-3}t + 3.8 \times 10^{-3}C_0 \\ & - 0.021T + 3.2 \times 10^{-4}\text{pH}\cdot t + 2.2 \times 10^{-5}\text{pH}\cdot C_0 + 1.2 \\ & \times 10^{-4}\text{pH}\cdot T + 6.4 \times 10^{-6}T\cdot C_0 + 1.4 \times 10^{-4}t\cdot T \\ & + 1.2 \times 10^{-5}C_0\cdot T - 0.239\text{pH}^2 - 4.5 \times 10^{-5}t^2 - 2.5 \\ & \times 10^{-6}C_0^2 + 6.5 \times 10^{-5}T^2 \end{aligned} \quad (3)$$

By eliminating the insignificant terms and refining the model, the above empirical model can be reduced to following equation:

$$\begin{aligned} \ln(q_t) = & -0.431 + 1.959\text{pH} + 2.6 \times 10^{-3}t + 4.5 \\ & \times 10^{-3}C_0 - 0.237\text{pH}^2 - 2.4 \times 10^{-6}C_0^2 \end{aligned} \quad (4)$$

The empirical Eq. 4 shows that the $\ln(q_t)$ was directly related to pH, C_0 , pH^2 , C_0^2 , and t or uranium removal. It was clear from Eq. 4 that there was a pH to pH and C_0 to C_0 interaction (quadratic effect with respect to pH and C_0) for removal of uranium in the present system.

A normal plot of residuals, which show how the model satisfies the assumptions of the analysis of variance, is shown in Fig. 1. The points on this plot indicate whether the residuals follow a normal distribution, in which the points have been followed a straight line, expect some scatter even with normal data. Also, a graph of the predicted response values versus the actual response values is shown in Fig. 2. It helps to detect a value, or group of values, that are not easily predicted by the model. Figure 2 shows that the developed models were adequate because the residuals for the prediction of each response are in minimum.

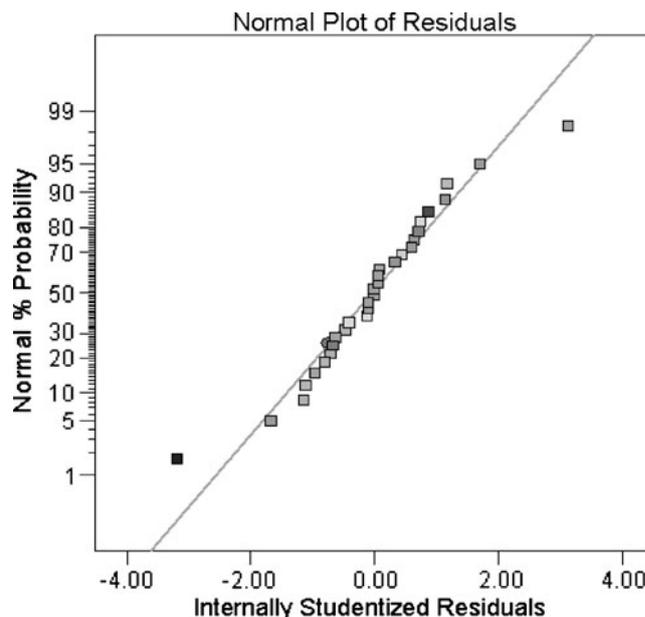


Fig. 1 Normal plot of probability (%) versus studentized residuals

In order to show how any two factors (pH– C_0 , pH– t , pH– T , C_0 – t , C_0 – T , and t – T) affect the response, the 3D response surface plots have been shown in Figs. 3, 4, and 5. It is important to focus on the effects of the significant terms. Therefore, the 3D response surface plots of the effect of pH– T , C_0 – T , and t – T were not shown.

3.1.1 Effect of pH and pH dependent mechanism

From the 3D graphs obtained from Design-Expert software, it is clear that the removal of uranium decreased at low and

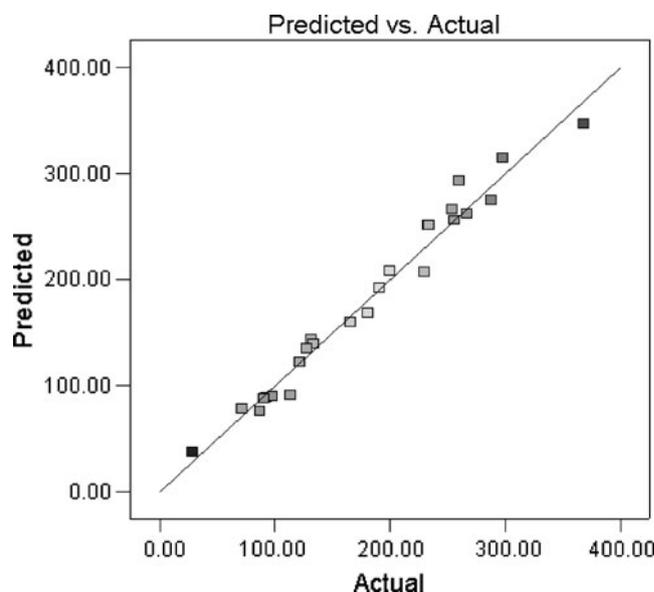


Fig. 2 Scatter graph of the predicted response values versus the actual response values for the uranium–algae biosorption system

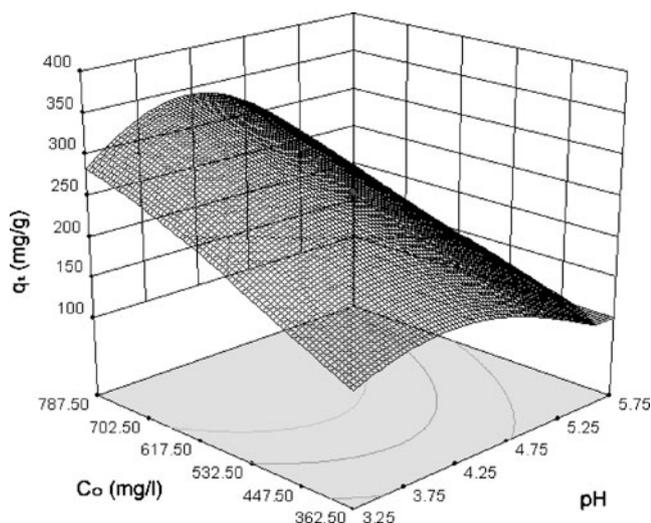


Fig. 3 Response surface 3D graph for removal of uranium by *Padina* sp. algae versus pH and initial uranium concentration at $t=55$ min and $T=30^\circ\text{C}$

high pH values for wide ranges of uranium concentration and contact time as shown in Figs. 3 and 5, respectively. According to these figures, the solution pH strongly affects biosorption of uranium by *Padina* sp. algae. Various reasons might be attributed to the metal adsorption behavior of the biosorbent relative to solution pH. The cell wall of algae contains a large number of surface active sites. The pH dependence of metal uptake can largely be related to these active sites and also to the chemistry of the metal in solution.

Several functional groups on the cell wall of brown algae are known to contribute to metal uptake; these include the

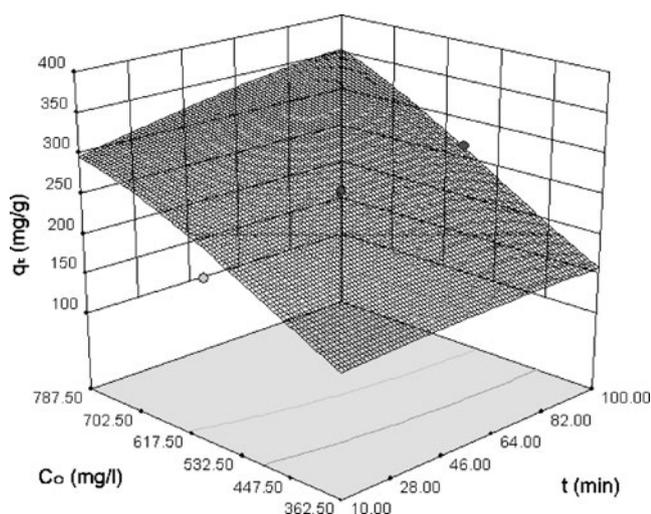


Fig. 4 Response surface 3D graph for removal of uranium by *Padina* sp. algae versus contact time and initial uranium concentration at $\text{pH}=4.5$ and $T=30^\circ\text{C}$

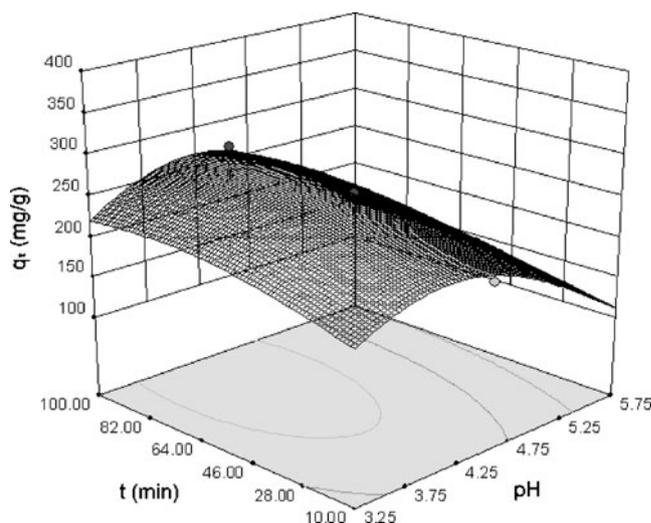


Fig. 5 Response surface 3D graph for removal of uranium by *Padina* sp. algae versus contact time and pH at $C_0=575$ mg/g and $T=30^\circ\text{C}$

carboxyl, sulfonate, and hydroxyl groups (Figueira et al. 1999). Both carboxyl and sulfate groups are weakly acidic groups whose availability of free binding sites is dependent on pH (Silke and Volesky 1995). FT-IR spectra of *Padina* sp. by Heng et al. (2004) represent the high content of carboxylate salt COO^-M , where M denotes the metal cation such as Na^+ , K^+ , Ca^{2+} , and Mg^{2+} that may exist naturally in the untreated *Padina* sp.. Carboxyl groups on the cell wall are ionizable groups, which suggest that the biosorption process could be affected by changes in the solution pH (Davis et al. 2003). The protons and the metal cations of carboxyl groups on the cell wall of *Padina* sp. algae can be exchanged with the uranium cations in solution.

The uranium cation UO_2^{2+} is hydrolyzed in aqueous solutions within the range of the sorption system pH (Yang and Volesky 1999). The lower pH suppresses the enhancement of uranium biosorption occurring normally because of the hydrolyzed ions (UO_2OH^+ , $(\text{UO}_2)_3(\text{OH})_5^+$, and $(\text{UO}_2)_2(\text{OH})_2^{2+}$). When the pH becomes low enough, the divalent free UO_2^{2+} becomes the dominant ion form in the solution. Since the UO_2^{2+} is divalent, it can only replace two protons on the adjacent binding sites of the biomass but cannot react with those sites which are farther apart from each other. In other words, at low pH some binding sites are not available to the divalent UO_2^{2+} . On the other hand, the non-ion dissolved solid schoepite ($4\text{UO}_3 \cdot 9\text{H}_2\text{O}$) starts appearing in the solution when the pH is too high. So, the uranium sorption may be hindered by the decrease in ion concentration in this situation.

The strong pH dependence of uranium biosorption by *Padina* sp. algae with consideration to the active ionizable groups on the cell wall and hydrolyze behavior of uranium cations in solution indicated that the biosorption

process for uranium uptake can be attributed to ion exchange mechanism.

3.1.2 Effect of initial uranium concentration

Figures 3 and 4 shows that the uptake of uranium is increased with increasing initial uranium concentration for ranges of pH from 3 to 6 and contact time from 10 to 100 min, respectively. The initial concentration provides an important driving force to overcome all mass transfer resistance of uranium between the aqueous and solid phases. Hence a higher initial concentration of uranium will increase the adsorption rate. Since cells offer a finite number of surface binding sites, uptake showed saturation at higher metal ion concentrations.

3.1.3 Effect of contact time

Figure 4 shows that the uranium uptake is increased by increasing contact time between metal ions in solution and biosorbent until plateau values are reached. As can be seen from this figure, the uranium ions adsorption rate by algae biomass is high at the beginning but plateau values are reached for a wide uranium concentration range after about 100 min long. Also, the effect of t factor at a pH range of 3–6 was shown in the 3D graph of Fig. 5. This indicated that the biosorption process for uranium uptake with *Padina* sp. algae biomass was increased with increasing time for a wide range of pH values.

3.1.4 Effect of temperature

The model obtained, in terms of significant variables (Eq. 4) from the Design-Expert software show that the important process variable, temperature, was not coming into the equation, indicating a large block effect or possible problem with the model and/or data, in terms of thermodynamical considerations. This is due to the small effect of temperature on uranium uptake capacity of *Padina* sp. algae. As can be seen from Table 2, for an increase of temperature from 10 to 50°C, the U uptake of the algae is decreased from 288.00 to 234.40 mg/g (only 46 mg/g) at initial uranium solution concentration of 575 mg/l, pH of 4.5, and contact time of 55 min. While, other parameters have great affect on the biosorption capacity. For example,

the response for pH values of 4.5 and 7 at same conditions, respectively, are 256 and 28.5 mg/g, which show strong effect of pH on the biosorption process of uranium by *Padina* sp. algae (see Table 2). The literature revealed that the biosorption process for uranium uptake with another brown algae *C. indica* biomass was exothermic in nature (Khani et al. 2008). From the decreasing of uranium uptake capacity of *Padina* sp. algae with temperature, it can be result that the biosorption process of uranium by this brown alga also can be exothermic. Further studies are needed to determine the thermodynamic of this biosorption process.

3.2 Statistical optimization

The optimum values for initial metal concentration, temperature, time, and pH were estimated to be 778.48 g/L, 37.47°C, 74.31 min, and 4.07, respectively. The results for optimized parameters by Design-Expert software and validation experiments obtained for the process are given in Table 5.

3.3 Isotherm at optimized conditions

Equilibrium data, commonly known as adsorption isotherms, are basic requirements for the design of adsorption systems. Several isotherm equations have been used for the equilibrium modeling of biosorption systems. In this study, the classical Langmuir and Freundlich adsorption model were used to describe the equilibrium between adsorbed metal ions on the algal cell (Q_{eq}) and metal ions in solution (C_{eq}) at constant optimized conditions obtained by Design-Expert software (pH=4.07 and $T=37.47^\circ\text{C}$) and at previously obtained equilibrium temperature ($t=90$ min).

The Langmuir equation which is valid for monolayer sorption onto a surface with a finite number of identical sites is given by Eq. 5.

$$Q_{eq} = \frac{Q^0 b C_{eq}}{1 + b C_{eq}} \tag{5}$$

where, Q^0 is the maximum amount of the metal ion per unit weight of alga to form a complete monolayer on the surface bound at high C_{eq} , and b is a constant related to the affinity of the binding sites. The equilibrium parameters (Q^0 and b) can be determined from the regression analysis of non-

Table 5 Optimum values of the process parameters for maximum efficiency together with predicted and experimental (confirmative) values of response

Parameters	Process parameters (optimized)				Response (maximized U uptake)	
	pH	C ₀ (mg/l)	t (min)	T (°)	q _{pre} (mg/g)	q _{exp} (mg/g)
Value	4.07	787.48	74.31	37.47	372.83	357.42

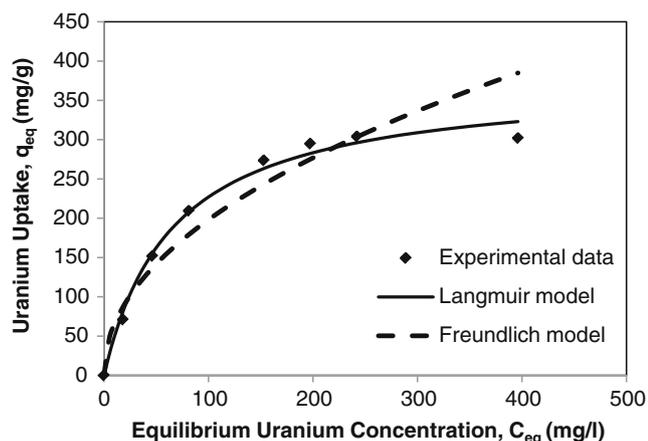


Fig. 6 Uranium biosorption isotherms: non-linear model regression at pH=4.07, $T=37.47^{\circ}\text{C}$

linear plot of Q_{eq} versus C_{eq} or linear plot of $C_{\text{eq}}/Q_{\text{eq}}$ versus C_{eq} . At present study, the non-linear regression was employed to determine Q° and b .

The Freundlich expression is an empirical equation based on sorption on a heterogeneous surface and is commonly presented as Eq. 6:

$$Q_{\text{eq}} = K_F C_{\text{eq}}^{1/n} \quad (6)$$

where, K_F (mg/g) and n are the Freundlich constants related to the adsorption capacity and intensity of the sorbent, respectively. The non-linear regression was employed to determine K_F and n .

The isotherm plots of Q_{eq} versus C_{eq} are given in Fig. 6. The Langmuir and Freundlich adsorption constants evaluated from the isotherms at optimized conditions obtained by Design-Expert software (pH=4.07 and $T=37.47^{\circ}\text{C}$) and at previously obtained equilibrium temperature ($t=90$ min) together with correlation coefficient are presented in Table 6. High regression correlation coefficient (>0.99) was found for Langmuir isotherm model, suggesting that this model is very suitable for describing the biosorption equilibrium of uranium by the *Padina* sp. algae cells in the studied concentration range. The applicability of the Langmuir isotherm model to the uranium–algae system implies that monolayer biosorption conditions exist under the experimental conditions used. Some authors explain that Langmuir isotherm corresponds to a dominant ion exchange mechanism while the Freundlich isotherm shows

adsorption–complexation reactions taking place in the adsorption process (Murugesan et al. 2006; Fiol et al. 2006). The maximum capacity Q° (376.73 mg/g) determined from the Langmuir isotherm defines the total capacity of the biosorbent for uranium. A higher value of b (0.01517 l/mg) also implied strong bonding of uranium to the dried *Padina* sp. algae biomass. These results show that the *Padina* sp. marine brown algae, with the maximum sorption capacity of 376 mg/g, can be competitive in uranium biosorption with the other marine brown algae such as *Sargassum* and *C. indica*, with the maximum uranium sorption capacity of 560 and 256 mg/g (in protonated state), respectively (Yang and Volesky 1999; Khani et al. 2008).

4 Conclusion

A Box–Wilson central composite design was conducted to find out and optimized the process of uranium biosorption onto *Padina* sp. algae biosorbent. The response surface methodology based on Box–Wilson central composite design was used to determine the effect of pH, initial concentration of U, contact time, and temperature on the biosorption of uranium and to develop mathematical models for predicting uranium removal by *Padina* sp. algae biomass. The results obtained show that the removal is highly sensitive to the pH and U concentration of the solution and contact time in the present study.

Also, the Box–Wilson central composite design can be employed to develop mathematical models for predicting U removal. Based on experimental results, an empirical relationship between the response and independent variables is obtained and expressed by the second-order polynomial equation. Analysis of variance showed a high coefficient of determination value ($R^2=0.9746$), thus ensuring a satisfactory adjustment of the second-order regression model with the experimental data.

The optimal process parameters were obtained as initial pH, initial concentration of U solution, contact time and temperature and these were found to be, respectively, 4.07, 778.48 mg/l, 74.31 min and 37.47°C , resulting in 372.83 mg/g of uranium uptake by algae, which is in good agreement with the experimental value obtained at optimized conditions.

Table 6 Isotherm sorption models constants together with correlation coefficient values

Conditions			Langmuir constants– R^2 value			Freundlich constants– R^2 value		
pH	T ($^{\circ}\text{C}$)	t (min)	Q° (mg/g)	b (l/mg)	R^2	K_F [(mg/g).(l/mg) $^{1/n}$]	n	R^2
4.1	37.5	90	376.73	0.01517	0.9902	21.52	2.07	0.9409

The equilibrium data at optimized conditions (optimum pH and temperature) were well represented by the Langmuir isotherm, giving maximum monolayer adsorption capacity as high as 376.73 mg/g.

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