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RESEARCH PAPER

Study of the Bioaccumulation of UO_2^{2+} onto the Green Microalgae *Botryococcus braunii* Using Response Surface Methodology

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Abstract

The bioaccumulation of UO_2^{2+} (uranyl ion) was investigated by using dry biomass of *Botryococcus braunii*. Experiments were conducted based on a rotatable central composite design (CCD) and analyzed using response surface methodology (RSM), which was used to optimize the bioaccumulation process. In this goal, the effects of parameters such as initial uranium (VI) concentration, pH, temperature and contact time were studied. The optimum conditions for maximum removal (87%) of UO_2^{2+} from an aqueous solution were as follows: initial uranium (VI) concentration (55 mg/l), pH (3.6), temperature (46°C) and contact time (74 min). Langmuir and Freundlich isotherms and also thermodynamic parameters like Enthalpy (ΔH°), Entropy (ΔS°), standard Gibbs free energy (ΔG°) were investigated, as well.

Introduction

Uranium mining and mineral processing for nuclear power lead to accumulation of significant amounts of radioactive wastes with tremendous environmental impact. Uranium is one of the most serious contamination concerns attracting wide environmental attention due to its radioactivity, long half-life ($4.47x10^9$ years for U-238) and heavy-metal toxicity. Uranium occurs naturally in the +2, +3, +4, +5 and +6 valence states, but it is most commonly found in the hexavalent form. In nature, hexavalent uranium is commonly associated with oxygen as the uranyl ion, UO_2^{2+}(Wisser & Hartkopf, 2006). The uranyl ions (UO_2^{2+}) are easily soluble in water. Soluble uranium compounds, for example; such as $UO_2(NO_3)_2.6H_2O$, easily pass through the surface and groundwater, and consequently cannot be separated by ordinary physical means of separation. Heavy metals in industrial wastewater could be removed by some physicochemical methods, such as chemical precipitation, chemical oxidation or reduction, electrochemical treatment, evaporative recovery, filtration, adsorption, coagulation, ion exchange, membrane technologies, etc. (Gunatilake, 2015). Bioaccumulation is economically and environmentally more viable treatment alternative to some of these ineffective or expensive processes.

Removal of contaminants from aqueous solutions using biological materials has been defined as biosorption (Chen, 2010). Biosorption has the capacity to generally high selectivity and sorption as compared to ion-exchange resins or other adsorbents. Due to its low cost and high metal binding capacity, it has been used to treat industrial effluents (Arslan & Pehlivan, 2007). Although bioaccumulation is promising, its

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mechanism is not well elucidated. The type of metal and the biosorbent structure determine biosorption mechanisms (Brinza, Nygard, Dring, Gavrilescu, & Benning, 2009). There are effective biosorbents easily available in all the four groups: algae, fungi, bacteria and also plant biomass.

The bioaccumulation of uranyl by various biomaterials has been reported by different research groups. Bhat, Melo, Chaugule, and D'Souza (2008) characterized bioadsorption of uranium (VI) from aqueous medium onto *Catenella caespitosa* (With.) L.M.Irvine, a red alga. Marine macroalga *Padina pavonica* (L.) Thivy was successfully used to removing of UO_2^{2+} by Aytas et al. (Aytas, Gunduz, & Gok, 2014). Bai et al. (2014) reported that the uranium could be removed by immobilized cells of *Rhodotorula glutinis* (Fresen.) F.C. Harrison (Basidiomycota) from wastewaters. Lee et al. (2014) claimed that brown alga *Saccharina japonica* (Aresch.) C.E.Lane, Mayes, Druehl and G.W. Saunders could be used for adsorption of uranium (VI) ions. Sana, Roostaazad, and Yagmaei (2014) applied live and dead *Aspergillus niger* Tiegh:as biosorbent for removal of uranium from the aqueous medium. Yang and Volesky (1999) studied the adsorption of uranium from aqueous solutions using *Sargassum* biomass. Also, many researchers reported adsorption of uranium by using biosorbents such as fungi (Pang et al., 2011), alga (Ghasemi, Keshtkar, Dabbagh, & Jaber Safdari, 2011; Zhao, Wang, Liu, & Wu, 2013), bacteria (Sheng & Fein, 2013; Morcillo et al., 2014) and yeast (Omar, Merroun, Gonzalez Munoz, & Arias, 1996). Microalgae have higher photosynthetic efficiency than plants for the production of biomass (Benemann, 1997; Miao & Wu, 2006). They can be used for biofuel production, purification of wastewater, extractions of high added value foods and pharmaceutical products or as food for aquaculture (Spolaore, Joannis-Cassan, Duran, & Isambert, 2006). Moreover, microalga cells grown under different culture conditions have the ability to accumulate uranium (Manikandan, Prasath, & Prakash, 2011).

Response surface methodology (RSM) is a collection of mathematical and statistical techniques used for optimizing, improving and developing processes by analyzing the effects of several independent variables (Myers, Montgomery, & Anderson-Cook, 2009). This methodology is used to reduce the number of experiments or repeats and to improve the qualification of the information obtained from the results. This means considerably less process, time and cost. Previously, RSM for heavy metal adsorption has been successfully applied. Sert and Eral (2010) reported uranium adsorption studies on aminopropyl modified mesoporous sorbent using statistical design method, Cakir, İnan, and Altas (2014) have investigated strontium and uranium sorption onto zirconium-antimony oxide/polyacrylonitrile (Zr-Sb oxide/PAN) composite using an experimental design. Kütahyali, Çetinkaya, Acar, Işik, & Cireli (2012), reported biosorption of Ce(III) onto modified *Pinus brutia* leaf powder using central composite design, etc.

The aim of the present research is to study the effect of pH, initial uranium (VI) concentration, contact time and temperature on the bioaccumulation of uranyl using *Botryococcus braunii* Kütz. biomass. Optimum conditions for the elimination of UO_2^{2+} was determined with RSM technique by using Design Expert software Version 9.0.0 (State-Ease, USA).

Materials and Methods

Chemicals

All chemicals used in the experiments are of analytical grade. Bi-distilled deionized water obtained by Milli-Q water system (resistivity ≥ 18.2 MΩ.cm at 298 K) was used in all experiments. Uranyl stock solution of 1000 mg/l was prepared by dissolving a required amount of uranyl nitrate hexahydrate, $UO_2(NO_3)_2.6H_2O$ (Merck, Germany) in distilled water at room temperature and then diluted to the desired concentration as required. The pH value of all solutions used in this study was adjusted with ammonia and nitric acid solutions.

Preparation of Biosorbent

B. braunii biomass used in this study were obtained from the alga culture collection of Utex (The University of Texas, TX). Cells were maintained in the medium of Modified Bristol solution whose composition was as follows: (per liter) 0.025 g NaCl, 0.25 g $NaNO_3$, 0.075 g $MgSO_4.7H_2O$, 0.025 g $CaCl_2.2H_2O$, 0.175 g KH_2PO_4, 0.075 g K_2HPO_4, and 40 ml soil water extract. Nutrient media were placed in Erlenmeyer flasks and their pH values were adjusted to 7.5. After inoculation of 10 ml of alga into each flask, they were cultured for 20 days in the laboratory under 33.6 µmol photon $m^{-2}.s^{-1}$ light intensity. Continuously aerated and the temperature was kept constant at 26ºC. At the end of the 20-day production period, all alga were harvested by centrifugation at 4000 rpm for 10 minutes. Biomass remaining in the centrifuge vials were rinsed with distilled water and dried for 12 hours in the oven set to 100°C (Lee, Yoon, & Oh, 1998).

Nomenclature of *Botryococcus braunii*

B. braunii which is a green, colonial, slow-growing microalga is widespread in freshwater, brackish lakes, reservoirs, and ponds. During observation under a light microscope, *B. braunii* colonies appear as a cluster of cells (Orpez et al., 2009). The pyriform shaped cells are usually 13 µm x 7 µm. This species is characterized by an original organization of colonies and an unusual capacity to produce unsaturated long-chain hydrocarbons, reaching contents ranging from 15% to 75% of its dry weight (Metzger, Largeau, & Casadevall, 1991). Nomenclature of Microalgae information used in the sorption is listed below according to Guiry and Guiry (2018).

Phylum : *Chlorophyta*
Classis : *Trebouxiophyceae*
Ordo : *Trebouxiales*
Family : *Botryococcaceae*
Genus : *Botryococcus*
Species : *Botryococcus braunii*

Sorption Experiments

A batch technique using a thermostated shaker bath (GFL-1083) at 150 rpm has been used for the adsorption experiments. The amounts of uranyl before and after process were calculated by estimating the concentration of uranium with the Arsenazo III spectrophotometric method by measuring the absorbance at 653 nm using Shimadzu UV–vis 1601 Model spectrophotometer (Niazi, Ghasemi, Goodarzi, & Ebadi, 2007). The pH measurements were made using a digital pH meter calibrated with pH 4 and 7 buffers.

The batch experiments were performed in 15 ml stoppered polypropylene (PP) tubes where 0.01 g of *B. braunii* and 10 ml of U (VI) solution at a desired initial concentration (20–100 mg/l) were added. The PP tubes were agitated at 150 rpm until desired contact time (15–155 min). The supernatant was separated from the biomass by centrifugation. Residual concentration of U (VI) ions in the supernatant were determined using spectrophotometric Arsenazo-III method.

In order to optimize the process, the batch experiments were dealt with according to the Central Composite Design (CCD). The response of experiment, bioaccumulation percentage was calculated as

$$\text{Bioaccumulation percentage (\%)} = \frac{C_0 - C_e}{C_e} x100 \qquad (1)$$

where, C_0 and C_e are initial concentration of solute (mg/l) and concentration of solute at equilibrium (mg/l), respectively. Each experiment was performed twice and the mean values were calculated. The standard deviation was found as about ±2%.

Experimental Design for Optimization of Experiment Parameters

The optimization of U (VI) bioaccumulation was realized by four selected independent process variables, pH (X_1), the initial concentration of uranium (VI) (X_2), contact time (X_3) and temperature (X_4) with six replicates at center points according to Central Composite Design (CCD). The lowest and highest levels of the variables were pH 2 and 6, the initial concentration of uranium (VI) 20 and 100 mg/l, contact time 15 and 155 min and temperature 15 and 75ºC, respectively. Based on the preliminary experiments, the ranges of variables were selected and were given in Table 1. The broad ranges were used for each variable. The design matrix is designed to be in five level (-2, -1, 0, +1, +2) and four variables (X_1, X_2, X_3, X_4, X_5).

The metal bioaccumulation percentage of *Botryococcus branuii* biomass was multiply regressed with respect to the different parameters by the least square methods as follows:

$$Y_i = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta X_i X_j + \varepsilon \qquad (2)$$

where Y_i is the predicted response variable.

In a system involving three independent variables, 14 coefficients have to be determined, as are coefficients of the 6 interaction effects, 4 quadratic effects, 4 main effects and 1 intercept term. β_0 is the intercept term, β_i (i =1, 2, ..., k) are the linear coefficient, β_{ii}(i =1, 2, ..., k) are the quadratic coefficient, β_{ij} (i =1, 2, ..., k ; (j =1, 2, ..., k) are the interaction coefficient, $\boldsymbol{\varepsilon}$ is a random error, X_i and X_j represent the coded independent variables in this model. In this study, Eq. (3) can be given as a second-order polynomial equation.

$$y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 \qquad (3)$$

The coefficient of determination (R^2) and F value was used to evaluate the accuracy and fitness of the above model. The predicted values for uranium sorption were obtained by applying quadratic model (Design-Expert® Software Version 9 Free Trial). The optimum values of the variable parameters for metal sorption were obtained by solving the regression equation, analyzing the contour plots and constraints for the variable parameters using the same software. Bioaccumulation percentage was studied with a standard RSM design called CCD (Montgomery, 2001). With solving the regression equation and analyzing the response surface contour plots the optimum values of the selected variables were obtained. The variability independent variables were explained by the multiple coefficients of determination, R^2 and the model equation was used to predict the optimum value and then to elucidate the interaction between the factors within the specified range (Elibol & Ozer, 2002).

Results and Discussion

Fourier Transform Infrared Analysis of Biomass (FTIR-ATR):

In order to identify the structure of biomass and clarify the situation of accumulated uranium, IR analysis was performed with a Fourier transform infrared spectroscopy (Thermo Scientific Nicolet iS5 FTIR-ATR spectrometer). Before and after treatments of the spectra of bioadsorbents were measured within

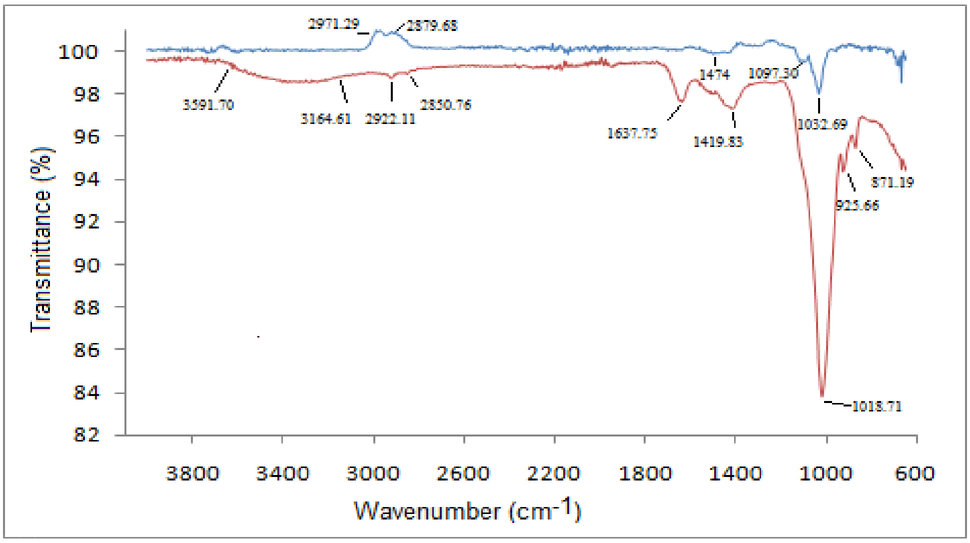

Figure 1. (a) The FTIR-ATR spectra of *Botryococcus braunii* biomass, (b) $(UO_2)^{+2}$ adsorbed on *Botryococcus braunii* biomass.

Table 1. Independent variables and their levels in the experimental design

Factors	Factor code	Level and range (coded)				
		-2	-1	0	+1	+2
pH	X_1	2	3	4	5	6
Initial concentration of uranium (VI) (mg/l)	X_2	20	40	60	80	100
Contact time (min)	X_3	15	50	85	120	155
Temperature (°C)	X_4	15	30	45	60	75

the range of 4000–600 cm^{-1} wavenumbers. The obtained vibrational frequency changes were used to enlighten the functional groups in the *B. braunii* biomass. The FTIR-ATR spectra of *B. braunii* biomass with and without UO_2^{2+} are shown in Figure 1 a,b.

The outer walls of the green alga *B. braunii* show a complex structure. It contains extraordinarily high hydrocarbon levels (15-75% of dry wt.) in structure. Light peaks observed at 2922 and 2850 cm^{-1} can be interpreted as vibration peaks of the CH_2 chain in the green alga. Moreover, *B. braunii* contains fewer hydroxyl groups in the broadband between 3700-3100 cm^{-1} (Berkaloff et al., 1983; Toyoda, Gishi, & Ihara, 2011).

The observed wide absorption band at 3591–3164 cm^{-1} can be used as an evidence for the formation of intermolecular bonding of the hydroxyl groups on the uranium ions (Bellamy, 1975; Aslani, Yusan, Yenil, & Kuzu, 2012; Yusan, Yenil, Kuzu, & Aslani, 2011). The observed strong peaks at 1000 and 1100 cm^{-1} also confirmed the presence of carboxyl ion groups in the alga biosorbent (Gok, Turkozu, & Aytas, 2011). The peaks indicate the asymmetrical stretching band of the ionized carboxylate (COO−) groups at 1637-1419 cm^{-1}.The spectra of vibration arising from the amino groups is observed at around 1470 cm^{-1} (Socrates, 2004). The main distinguishing feature observed on the *B. braunii* biomass after the accumulation of UO_2^{2+} is the presence of additional peaks at 925 and 871 cm^{-1} (Figure 1b).

Experimental Design Results:

Model Selection and Statistical Evaluation

The different parameters such as pH (2, 3, 4, 5 and 6), initial uranium (VI) concentration (20, 40, 60, 80 and 100 mg/l), contact time (15, 50, 85, 120 and 155 min) and temperature (15, 30, 45, 60 and 75°C) were chosen as the critical variables and respectively designated as X_1, X_2, X_3 and X_4. The levels of each variable were respectively designated as -2, -1, 0, +1 and +2, and tabulated in Table 1. All the experiments were carried out in a random order by using different parameters and the low and high levels of the factors were selected according to some preliminary experiments.

The dependence of yield on individual parameters as well as interactions for simultaneous variations of parameters was calculated from the regression equation developed from different sets of the experiment. The batch technique experimental results of uranium accumulation on the *B. braunii* biomass were tabulated in Table 2.

The variability of four dependent variables were explained by the multiple coefficients of determination, R^2, and the model equation was used to predict the

Table 2. Experimental data points used in CCD statistical design and observed and predicted values for Uranium uptake capacity of *Botryococcus braunii*

Run no.	Coded values of independent variables				Responses (% adsorption)		Residuals values
	X_1	X_2	X_3	X_4	Experimental values	Predicted values	
1	1	1	-1	-1	73.62	69.50	4.12
2	1	-1	-1	-1	71.69	77.03	-5.34
3	0	0	0	0	79.70	87.84	-8.14
4	-1	1	1	-1	97.98	104.23	-6.25
5	-1	1	-1	-1	98.54	96.16	2.38
6	-1	1	1	1	98.73	92.50	6.23
7	0	0	0	0	88.49	87.24	1.25
8	-1	-1	-1	-1	97.60	101.56	-3.96
9	1	-1	-1	1	90.33	83.20	7.13
10	1	-1	1	1	87.86	89.35	-1.49
11	1	1	1	-1	83.65	83.05	0.6
12	-1	-1	-1	1	96.44	96.15	0.29
13	-1	1	-1	1	68.99	73.74	-4.75
14	-1	-1	1	1	93.58	96.82	-3.24
15	1	-1	1	-1	78.11	72.49	5.62
16	0	0	0	0	90.00	87.24	2.76
17	-1	-1	1	-1	98.69	91.55	7.14
18	1	1	-1	1	52.43	58.67	-6.24
19	0	0	0	0	88.65	87.24	1.41
20	1	1	1	1	87.75	82.91	4.84
21	0	0	0	0	90.06	87.84	2.22
22	0	0	0	2	87.79	88.29	-0.5
23	0	2	0	0	79.66	79.24	0.42
24	0	0	2	0	94.06	99.90	-5.84
25	0	-2	0	0	88.90	91.09	-2.19
26	0	0	0	0	90.11	87.24	2.87
27	0	0	-2	0	89.75	85.68	4.07
28	0	0	0	-2	92.57	93.85	-1.28
29	2	0	0	0	52.39	56.13	-3.74
30	-2	0	0	0	92.22	90.26	1.96

Table 3. Model Summary Statistics

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	Multiple R	
Linear	8.79	0.5446	0.4717	0.3003	0.7379	
2FI	7.36	0.7568	0.6289	0.2820	0.8699	
Quadratic	5.97	0.8740	0.7564	0.3580	0.9349	Suggested
Cubic	3.62	0.9784	0.9105	0.6505	0.9891	Aliased

optimum value and to subsequently elucidate the interaction between the factors within a specified range (Elibol & Ozer, 2002).

As a result of analysis of the experimental data quadratic model was reckoned appropriately to obtaine the suitable regression equation (Table 3). The value of R^2 is close to 1.0 which has advocated a high correlation between the observed values and the predicted values. The presence of multiple R-value 0.93, regression is statistically significant, and only 7% of the total variable portion cannot be explained with this model.

The application of the RSM based on the estimates of the parameters indicated an empirical relationship between the input variables and response expressed by the following quadratic model Eq. (4):

$$UO_2^{2+} \text{ accumulation percentage} = +87.84 - 8{,}53.X_1 - 2.96.X_2 + 3.56\,X_3 - 1.39\,X_4 - 3.66.X_1^2 - 0.67\,X_2^2 + 1.24.X_3^2 + 0.81.X_4^2 - 0.53\,X_1X_2 + 1.37\,X_1X_3 + 2.90\,X_1X_4 + 4.52.X_2X_3 - 4.25\,X_2X_4 + 2.67\,X_3X_4 \quad (4)$$

Significance of each coefficient presented in Eq. (4) is determined by the p-values and F values (Montgomery, 2001). F-test for the significance level of data gave $P<0.05$ (<0.0001) with a model F value of 16.65 revealing that this regression is statistically significant. The results of the quadratic model for percentage accumulation in the form of analysis of variance (ANOVA) are given in Table 4.

The regression model provides an excellent explanation of the relationship between the independent variables (factors) and the response values (% bioaccumulation). The larger and smaller values of F and P are more significant for the

Table 4. Analysis of variance (ANOVA) for a quadratic model for U(VI) adsorption on the *Botryococcus braunii*

Sources of squares variation	Sum of squares	df	Mean square	F-Value	Probability>
Model	4838.61	14	345.61	16.65	<0.0001 significant
Residual	290.68	14	20.76		
Lack of Fit	271.72	10	27.17	5.73	0.0535 not significant
Pure error	18.96	4	4.74		
Cor Total	5129.28	28			

R^2 = 0.9433, CV=5.81%, df= degree of freedom

Table 5. Estimated regressioncoefficient and corresponding F and P values

Regression	Coefficients	Standard error	F values	P values
Intercept	87.84	2.44	7.43	0.0002
X_1	-8.53	1.22	*49.10*	*<0.0001*
X_2	-2.96	1.22	*5.92*	*0.0280*
X_3	3.56	1.22	*8.53*	*0.0106*
X_4	-1.39	1.22	*1.30*	*0.2720*
X_1^2	-3.66	1.14	*10.33*	*0.0058*
X_2^2	-0,67	1.14	*0.34*	*0.5670*
X_3^2	1.24	1.14	*1.18*	*0.2937*
X_4^2	0.81	1.14	*0.50*	*0.4888*
X_1X_2	-0.53	1.49	*0.13*	*0.7276*
X_1X_3	1.37	1.49	*0.84*	*0.3734*
X_1X_4	2.90	1.49	*3.78*	*0.0710*
X_2X_3	4.52	1.49	*9.19*	*0.0058*
X_2X_4	-4.25	1.49	*8.12*	*0.0122*
X_3X_4	2.67	1.49	*3.31*	*0.0934*

X_1, X_2, X_3 and X_4 are the main effects; X_1^2, X_2^2, X_3^2andX_4^2 are the square effects; X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4andX_3X_4 are the interaction effects.

corresponding coefficient terms. The associated Prob. > F value for the model is lower than 0.05 indicating that the model is considered as statistically significant. The non-significant value of lack of fit (more than 0.05) showed that the quadratic model was valid for the present study. The lack-of-fit term is non-significant as it is desired (Hamsaveni, Prapulla, & Divakar, 2001). The results also suggest that the selected quadratic model were adequate in predicting the response variables for the experimental data.

Statistical analysis of the main parameters effect, main parameters affect squares and interactions between them were performed, as well. The coefficients of independent variables F and P values according to the investigated parameters are given in Table 5.

The three-dimensional response surface plots, obtained as a function of two factors with maintaining all other factors constant, are used in evaluating both the main effects and the interaction effects of these two factors (Adinarayana & Ellaiah, 2002). The obtained interferences were an explained below.

Effects of Main Parameters on UO_2^{2+} Bioaccumulation

At the studies on the adsorption of metal ions, the solution pH is one of the most important factors. Based on the analysis of data obtained in this study, the bioaccumulation of UO_2^{2+} onto the *B. braunii* biomass was investigated at varied pH values between 2 to 6. The pH parameter was found statistically significant (P = 0.0001, P<0.05). The negative coefficient value belonging to pH (X_1= -8.53) indicates that the pH has a negative effect on bioaccumulation of UO_2^{2+} from aqueous solution. It means to decrease the uptake of uranyl from solution while increasing pH of the solution. Figure 2a illustrates the pH effect of UO_2^{2+} accumulation onto *B. braunii* provided that the other variables are constant.

The bioaccumulation of UO_2^{2+} on the *B. braunii* was determined at different initial uranium (VI) concentration values ranging from 20 mg/l to 100 mg/l. Initial uranium (VI) concentration was found statistically significant (P=0.0280, P<0.05). The negative value of coefficient (X_2 = -2.96) belonging to initial U (VI) concentration represents that this parameter has a negative effect on the process. It means that increasing initial metal concentration of the solution decreases the bioaccumulation percentage of uranium from solution. According to Figure 2b, while initial uranium (VI) concentration increased, bioaccumulation percentage decreased.

Different contact time values ranging from 15 min. to 155 min. were also considered as another parameter which affect the process. The positive value of coefficient belonging to the contact time (X_3= +3.56)

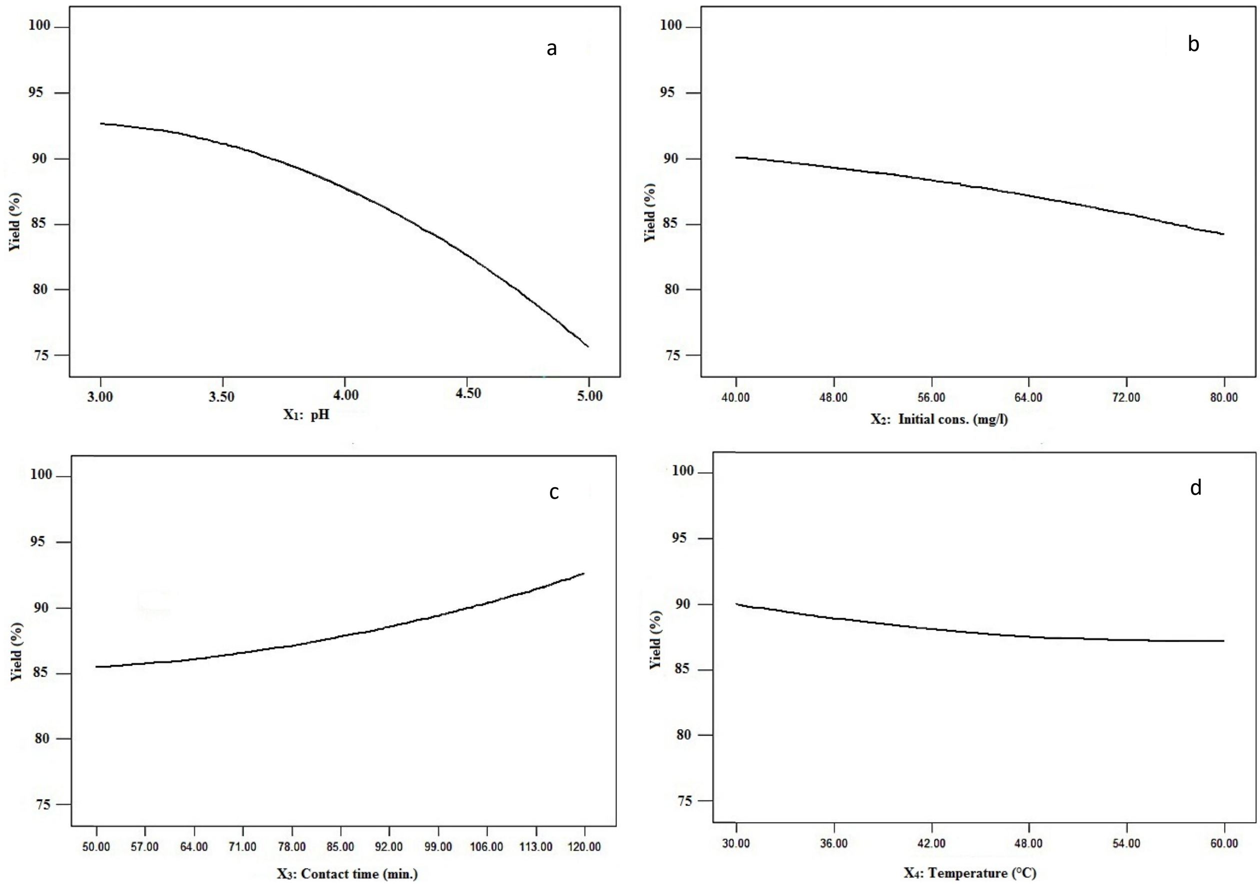

Figure 2. Effect of investigated parameters on U(VI) adsorption onto *Botryococcus Braunii.* (a) Effect of a solution pH, (b) initial U(VI) concentration, (c) contact time and (d) temperature.

indicates that the contact time has a positive effect on the process (Table 5). It means that the uptake of UO_2^{2+} increased while contact time was increased. Contact time was found statistically significant (P=0.0106, P<0.05) for this biomass. Figure 2c shows the contact time effect of UO_2^{2+} adsorption onto *B. braunii* with other variables being at fixed levels.

The bioaccumulation of UO_2^{2+} on the *B. braunii* was also determined at different process temperature values ranging from 15°C to 75°C. According to Table 5, the negative value (X_4= - 1.39) of coefficient belongs to the media temperature indicates that the temperature has a negative effect on bioaccumulation of UO_2^{2+} from aqueous solution. It means that the uptake of UO_2^{2+} decreases while solution temperature increases. Process temperature was found statistically nonsignificant (P=0.2720, P>0.05). Figure 2d shows the effect of the process temperature with the other variables being at fixed levels.

Three-Dimensional Response Surface Plots

In order to understand the main and the interaction effects of each of the two factors, response surface plots as a function of two factors were used simultaneously, while maintaining all the other factors at their fixed levels. These plots can be obtained by calculating the regression model and the values taken by one factor where the second varies with the constraint of a given uranyl ions (UO_2^{2+}) bioaccumulation percentage value. The percentage values for different concentrations of the variables can also be predicted from the respective response surface plots (Figure 3). The maximum predicted yield was indicated by the surface confined in the response surface diagram.

The independent variable interactions are displayed in Figure 3 in three dimensions (3D) surface plots with other variables being at their fixed levels. A combined effect of initial uranium (VI) concentration (X_2) and contact time (X_3) was found statistically significant (P = 0.0058, P<0,05). Figure 3a shows the combined effect of two independent variables of initial uranium (VI) concentration (X_2) and contact time (X_3). The rate of UO_2^{2+} bioaccumulation showed to be significantly dependent on the initial uranium (VI) concentration and the contact time. It was observed that percentage metal ions removal increased with increasing initial concentration of uranium (VI). Figure 3a shows that the maximum uranyl removal was predicted at an initial uranium (VI) concentration of 55 mg/l and contact time 74 minutes. Beyond this range, there was a significant decrease in the uranyl removal due to a decrease in adsorptive capacity.

Figure 3b shows the interaction between contact time (X_3) and temperature (X_4). It was observed that

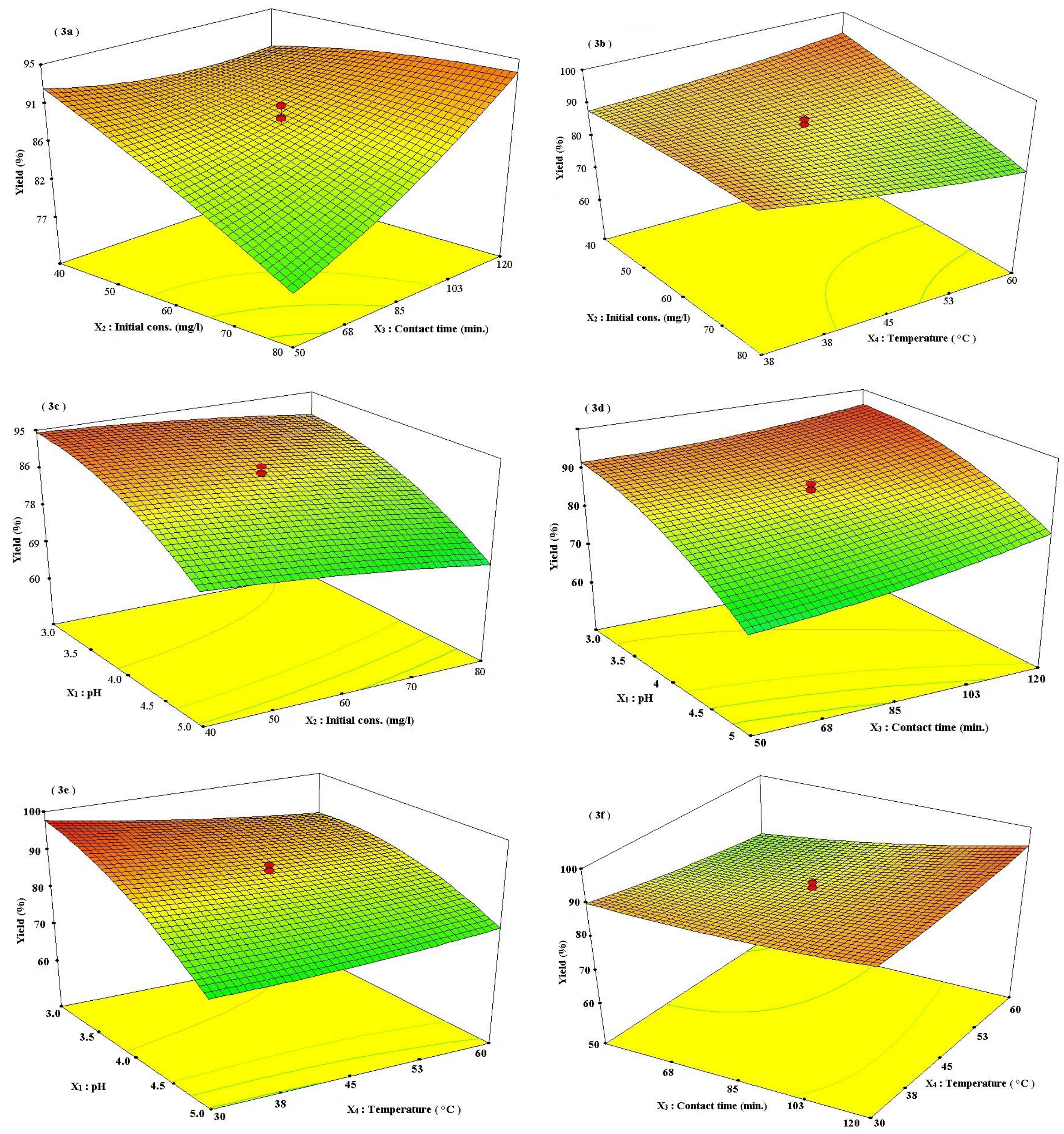

Figure 3. Response surface graphs for interactions of investigated parameters.

uranyl ions removal percentage increased with decreasing contact time of uranium and temperature. A combined effect of contact time (X_3) and temperature (X_4) was found statistically significant ($P=0.0122$, $P<0.05$) for uranyl bioaccumulation on the *B. braunii* biomass. From the contour plot and 3D plot (Figure 3b), the maximum uranium removal was predicted at a contact time of 74 min and temperature of 46°C.

Figures 3 c-f shows interaction effects between another two factors. The interaction effects were found statistically nonsignificant ($P>0.05$) for uranyl bioaccumulation by the *B. braunii*.

Optimal Conditions for Uranyl Uptake

The optimum condition of accumulation process was found as initial pH 3.6, temperature 46ºC, initial uranium (VI) concentration 55 mg/l and contact time 74 minutes. At this condition, uranyl ions accumulation yield was obtained as 89±2 %. And the value of regression coefficient (R^2) was found as 0.8740.

Accumulation Isotherms

For characterization of the accumulation process, concerned data were calculated by using Langmuir and Freundlich isotherms. Langmuir model is a theoretical model for monolayer adsorption while the Freundlich model allows multilayer adsorption at heterogeneous surfaces. Dönmez and Aksu (2002) implied that the sorption of chromate onto the biomass was complex and may involve more than one mechanism based on Langmuir and Freundlich model data.

Four series of tests were conducted with UO_2^{2+} solutions varying the initial uranium (VI) concentration between 30 and 140 mg/l. The pH value (3.6), temperature (46°C) and contact time (74 min) were maintained at optimum conditions. The Langmuir equation, which has been successfully applied to many sorption experiments, is given by (6) :

$$\frac{C_e}{q_e} = \frac{1}{b.q_m} + \frac{C_e}{q_m} \tag{5}$$

where q_e is the amount absorbed at equilibrium (mg/g), C_e is the equilibrium concentration (mg/l), q_m is the Langmuir constants related to monolayer maximum capacity and *b* is the equilibrium constant of the adsorption (l/mg). The monolayer coverage of the sorption surfaces is modeled by Langmuir sorption isotherm. This model assumed that sorption occurs on a structurally homogeneous adsorbent and all the sorption sites are energetically identical (Unlu & Ersoz, 2006).

The Freundlich equation, which was applied for the sorption of U (VI), is given as,

$$q_e = K_F.Ce^n \tag{6}$$

If equation (6) is rearranged, linear equation (7) can be obtained.

$$\text{Log } q_e = \log K_F + n \log C_e \tag{7}$$

where C_e is the equilibrium concentration, q_e is the amount of solute adsorbed per mass unit adsorbent and n and K_F are the ion-exchange intensity and the Freundlich constants related to the adsorption capacity of the adsorbent, respectively. The values of n greater than 1 show favorable ion exchange of metal on biomass. The Freundlich expression is an empirical equation based on a heterogeneous surface (Kutahyali, Cetinkaya, Acar, Isik, & Cireli, 2012). The corresponding Freundlich and Langmuir parameters along with correlation coefficients are reported in Table 6**.**

The correlation coefficients (R^2) indicate that the Langmuir model fits better than the Freundlich model for the UO_2^{2+} sorption onto *B. braunii* (Table 6). The Langmuir model has a much high value of R^2 (0.96). The adsorption of UO_2^{2+} onto *B. braunii* biomass fully obeys the Langmuir isotherm. q_m and b were also found to be 67.8 mg/g and 0.0125 l/mg, respectively.

Comparison with Other Alga Biosorbents

The theoretical biosorption capacity (q_m) for adsorption of UO_2^{2+} on onto *B. braunii* biomass in the present work compared with the literature values reported for other biosorbents. The reported q_m values for the adsorption of UO_2^{2+}on Green alga *Tetradesmus olbliquus* (Turpin) M.J.Wynne *34* (Xiaozhi, Shanggeng, Qun, Huaili, & Jinying, 1997*), Scenedesmus sp. LX1* (Li, Hu, Yu, & Zhao, 2016), Brown alga *Saccharina japonica* (Lee et al., 2014), *Sargassum fluitans* (Børgesen) Børgesen (Yang & Volesky, 1999), *Chlorella* sp. (Horikoshi, Nakajima, & Sakaguchi, 1979), *Chlamydomonas reinhardtii* P.A. Dangeard (Acikgoz, Arica, Akbulut, & Bayramoglu, 2014), *Sargassum sp*. biomass (Yang & Volesky, 1999), *Polycladia indica* (Thivy & Doshi) Draisma, Ballesteros, F.Rousseau & T.Thibaut (Khani, Keshtkar, Ghannadi, & Pahlavanzadeh, 2008), *Cystoserira sp*. and (Gok, Aytas, & Sezer, 2017) were found to be 75mg/g, 40.7 mg/g, 96.4 mg/g, 560 mg/g, 15.6 mg/g, 192.3 mg/g, 0.701 mmol/g, 233 mg/g and 37 mg/g, respectively. It is worth underlining that *B. braunii* biomass can be used for the biosorption of UO_2^{2+} ions with its adsorption capacity (67.8 mg/g).

Thermodynamic Results

The values of sorption enthalpy (ΔH) and entropy (ΔS) were calculated from the slope and intercept of the linear van't Hoff plot respectively, using the relation (8):

$$\ln K_d = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \tag{8}$$

Where, ΔS° and ΔH° are the standard entropy and enthalpy change for the sorption, respectively. Negative ΔH° values for UO_2^{2+} sorption onto *B. braunii* biosorbent indicate that adsorption of U(VI) is exothermic and those results are given in Table 7.

The values of free energy of specific adsorption, ΔG, at various temperatures were calculated by using Eq. (9)

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{9}$$

In the temperature range of 298-323 K, ΔG° of UO_2^{2+} ions sorption has negative values which point out the adsorption process occurred spontaneously. It also shows that the adsorbed uranyl ions onto the surface of *Botryococcus braunii* biomass is stable. The low ΔG° values indicate that the process is a physical adsorption, which was confirmed by slightly increasing ΔG° values as temperature rising from 298 to 323 K. The adsorption phenomenon triggered by increasing temperature. Moreover when the ΔG° values are between 20 and 0 kJ mol^{-1} the physical sorption occurred, spontaneously. For the present work ΔG° changes from –16.96 to – 15.19 kJ.mol^{-1}, while

Table 6. Sorption isotherm constants for the sorption of uranium onto *Botryococcus braunii* biomass

	Freundlich			Langmuir		
	R^2	n (l/mg)	K_F (mg/g)	R^2	q_m (mg/g)	b (l/mg)
Botryococcus braunii	0.61	3.6	114.5	0.96	67.8	0.0125

Table 7. Thermodynamic parameters for UO_2^{2+}sorption onto *Botryococcus braunii* biomass

	ΔH°(kJ/mol)	ΔS°(j/mol.K)	ΔG° (kj/mol)				
			298K	303K	308K	313K	323K
Botryococcus braunii	-38.09	-70.83	-16.96	-16.61	-16.26	-15.91	-15.19

temperature increased from 298 to 323 K. These values are higher than values related to chemisorption's (–80 to –400 kJ mol^{-1}), so the physisorption was dominated for uranyl-*Botryococcus braunii* biomass system. Physical adsorption is resulted mainly by Van der Waals forces and electrostatic forces between adsorbate molecules and atoms. As seen from Table 7, the above assertion about physisorption confirm with negative ΔH° value (–38.04 kJ.mol^{-1}) emphasis that the process has exothermic nature. The negative ΔS° value (–70.83 J.mol^{-1}.K^{-1}) suggests a decrease in the randomness at the solid-solution interface during the sorption process (Aslani, Celik, Yusan, & Kutahyali Aslani, 2017).

Conclusions

Botryococcus branuii biomass was used for the bioaccumulation of uranyl ions. The affecting parameters were statistically analyzed using CCD as the experimental design method. The four independent variables such as initial pH, temperature, initial uranium (VI) concentration and contact time were selected for this study. This experimental design and statistical analysis provide a polynomial equation which can be used to predict adsorption values within the range of the independent variables and also to draw the response surface plots. The ANOVA of the obtained model was carried out at 95% confidence level and checked to the fitting of experimental and predicted values, as well. The values of the F and P related to the model were calculated as 16.65 and <0.0001 (<0.05), respectively. These results showed that this model is statistically significant. The correlation coefficient value (R^2) to be obtained as 94% indicated that there is a high correlation between the predicted and the observed values. The main effects, main affects squares and the interactions between the main effects are statistically analyzed and their effects on the adsorption process are investigated. The influences of initial pH, contact time and initial U(VI) concentration on the adsorption process are considered statistically significant as main effects. On the other hand, interaction effects of initial uranium (VI) concentration and contact time, initial uranium (VI) concentration and temperature were considered as significant parameters on the accumulation process. The optimum condition of accumulation process was found to be at initial pH 3.6, temperature 46°C, initial uranium (VI) concentration 55 mg/l and contact time 74 minutes. At this condition, uranyl accumulation yield was obtained as 89±2%.

In order to the characterization of the sorption process, concerned data were calculated by using Freundlich and Langmuir isotherms. The results indicated that the sorption of uranyl on *B. braunii* was described as Langmuir isotherm monolayer type. The correlation coefficients (R^2) indicate that the Langmuir model fits better than the Freundlich model for the UO_2^{2+} sorption onto *B. braunii*. The Langmuir model has a much high value of R^2 (0.96). The adsorption of UO_2^{2+} onto *B. braunii* biomass fully obeys the Langmuir isotherm. q_m and b were also found to be 67.8 mg/g and 0.0125 l/mg, respectively.

Thermodynamic parameters (ΔG°, ΔH° and ΔS°) showed the overall process is found to be physical, exothermic and spontaneous in nature. As a consequence *B.braunii* can be used as a potential biosorbent for purification of Uranium (VI) and may be used for the efficient removal of this toxic ion from aqueous solutions.

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