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Optimization of Pb (II) biosorption by Harvested Microalgae (*Chlorella vulgaris)* using response Surface Analysis

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Abstract

Biosorption has been defined as affinity characteristics of certain biomolecules present in dead/live biomass to passively bind selected ions from aqueous solutions. In this study, the bioremoval of Pb (II) was studied using dead biomass of green microalgae species, *Chlorella vulgaris*. Important individual and combined factors affecting the sorption process, such as initial concentration of Pb (II) (100-500 mgL^{-1}), pH (2.5-6.5), biomass concentration (0.1-0.5 gL^{-1}) of *C. vulgaris* and contact time (20-120 min) were investigated to achieve the maximum removal of lead ions from aqueous solutions. The parameters were optimized by the experimental design concept using central composite design (CCD). The statistical parameters of the derived model were R^2 = 87.91% and F value = 8.31. The response surface diagrams were plotted based on the defined model to show the effects of the variables on the Pb(II) bioremoval efficiency. A quadratic model was proposed by CCD which agreed well with the experimental data (R^2 = 87.91%). Also, the model was able to predict optimal conditions of pH= 2.5, C_0 = 100.0 mgL^{-1}, $C_{biomass}$ = 0.4 gL^{-1} and t = 120.0 min for the target bioremoval of Pb (II) (100%).

Keywords: Biosorption; Microalgae; Lead; Optimization, Response Surface Analysis

Introduction

The environmental heavy metal contamination increasingly threatens the human health and wild life. Industrial wastewaters (IWs) emerged as a new-born source of heavy metal ions cause serious environment pollution. The IWs carry heavy metal such as lead (II), copper (II), cadmium (II), zinc (II), and nickel (II) [1]. These heavy metals , even at low concentration, could be toxic to the living organisms as metals are potentially bioaccumulated [2]. According to the U.S. Environmental Protection Agency (USEPA), the threshold concentration level of Pb (II) is 15 μg L^{-1} in drinking water [3]. Several industries such as lead smelting and refining, battery manufacturing plants, rubber and plastic, printing industries and lead soldering release a considerable amount of Pb (II) into the environment as part of their waste streams [4].

The stringent restrictions and standards on the release of heavy metals to the environment, particularly Pb (II), indicate the necessity to develop reliable treatment methods for the IWs containing lead. A broad investigation have been done on the various treatment methods applied in the IWs by Talebi et al [5]. They reported that every process has its own strengths and limitations and, in some cases, integrating the different techniques could enhance the removal deficiency. The combination of multiple stages of physicochemical (membrane-based processes and chemical precipitation) and biological treatment methods (biosorption) could potentially decrease the harmful impact as well as the costs. The conventional non-biological methods exploiting to remove heavy metal ions are including membrane processes such as electrodialysis and reverse osmosis [6] and adsorption [7]. These methods often impose high cost and produce large amount of toxic sludge and brine, while biological methods are safer for the environment, more cost effective and efficient for IW treatment, especially in low concentration of heavy metal ions [8]. Besides, the biomass could be separated from solution and can be burned in incinerator in order to recover heavy metal ions from ash [9] and also to generate bioenergy. Moreover, the biological origin materials known as biosorbents such as microorganisms, algae and lignocellulosic compounds are able to easily bond with metals ions in aqueous. These compounds are basically originated in nature with no human interference, and can be used for the industrial purposes. The biosorption mechanisms of heavy metal ions consist of complexation, electrostatic interaction and precipitation in micro scale [7]. Among these processes, ion exchange is the main mechanism responsible for binding of heavy metal ions to the functional groups presenting on the algal cell wall surface [10]. Following the cell wall, the mucilage layer containing alginate and fucoidan-enrich [11], is the second main obstruction against the sorption of heavy metal ions [12]. The structure of this major barrier is composed of polysaccharides and proteins that provide numerous sites for linkage of heavy metal ions to the cell wall [13].

Among these materials, algal cells containing a variety of functional groups can effectively interact with the chemicals such as heavy metal ions in the aqueous solution and therefore are considered as a reliable biosorbent [10]. Several investigations have been demonstrated to measure the metal biosorption capacity of a number of microalgae such as *Chlorella vulgaris*. For example, in an investigation on the binary and ternary systems of Ni^{2+}, Zn^{2+}, and Pb^{2+} as competitive adsorbents, Rodrigues et al. [14] found that *C. vulgaris* can be used as an effective biosorbent for metal ions removal from wastewater. Also, Aung et al. reported the biosorption capacity of lead using *C. vulgaris* [15]; they found that optimum pH for the Pb (II) removal is 6 with 99.4% removal efficiency. The majority of studies have optimized the maximum bioremoval conditions by controlling only one factor, irrespective of the other influencing factors [16, 17] . Therefore, the main drawback is the lack of sufficient experimental and modeling data regarding the impacts of the parameters' interactions on the metal bioremoval efficiency. Despite the wide range of study on the bioremoval of heavy metals by various algae species, only a limited number of researches were conducted on the bioremoval of Pb (II) by *C. vulgaris*, and to the authors' knowledge, none has examined the effects of critical parameters, solely and combined, on the bioremoval efficiency of Pb (II) from aqueous solutions.

The main objective of present study is to evaluate the effects of critical parameters, solely and combined, on the Pb (II) biosorpotion process using *Chlorella vulgaris*, a unicellular green algae, as a biosorbent. This has been conducted by optimization of the critical parameters including pH, contact time, biomass concentration and metal concentration on the efficiency of lead bioremoval. All attempts have been made to optimize these parameters by using the response surface methodology (RSM). In addition, the interactions among the critical parameters and their impacts on the Pb (II) bioremoval were investigated. Batch biosorption of Pb (II) was carried out according to the design of experiments incorporated in Minitab® 16 Statistical software.

Materials and methods

Microalgae cultivation and biosorbent preparation

In this study *C. vulgaris* (CCAP 211/11B) was received from Semnan University, and cultivated in Bold's Basal Medium (BBM). The BBM was prepared according to the standard operation procedure described in literature [18]. Composition of BBM was (mgL^{-1} medium): $NaNO_3$ (250), K_2HPO_4 (75), KH_2PO_4 (175), $MgSO_4.7H_2O$ (75), $CaCl_2.2H_2O$ (25), NaCl (25), Na_4EDTA (50), $FeSO_4.7H_2O$ (5), H_3BO_3 (11), Co $(NO_3)_2.6H_2O$ (0.08), $MnCl_2.4H_2O$ (0.23), $Na_2MoO_4.2H_2O$ (0.19), $CuSO_4.5H_2O$ (0.25), $ZnSO_4.7H_2O$ (1.41). Initial pH of culture medium was adjusted at 6.8±0.3 by adding a proper dosage of NaOH and HNO_3. Algal cultures were maintained in the 5 L Erlenmeyer flasks and aerated accordingly to aid the gas exchange and to keep the algal cells in suspension.

The Erlenmeyer flasks containing culture medium were incubated at the laboratory conditions including an average light intensity of 4000 lux in light-dark cycle 12/12 h at the room temperature (25± 2°C). The cell growth was monitored by measuring optical density using Uv- Vis spectrophotometer (Agilent 480) at 680 nm. Once the algal cells were reached stationary phase, they were harvested by centrifugation (Behdad) at 2000 rpm and the obtained pellets were washed three times with deionized water to remove any residues adsorbed on the algae cell wall. Subsequently, the washed cells were dried in an oven (Tegh Fan) at 60°C for 24 hours. Finally, the dried C*. vulgaris* powder biomass was used as a biosorbent for the Pb (II) biosorption.

Metal solutions preparation

In order to investigate the bioremediation capabilities of the dry biomass of *C. vulgaris*, the media was inoculated with different concentrations of Pb (II) using the $Pb(NO_3)_2$ stock solutions (1000 ppm). The stock solutions were then diluted to the desired concentrations for the subsequent analysis. In order to prevent hydrolysis of Pb (II), stock solution was prepared using deionized water containing 0.5% HNO_3 (V/V).

Bioremoval procedure

The experiment design was performed using central composite design (CCD) in batch system applying Minitab software (version 16.2.0; 2010 Minitab Inc, State College, Pennsylvania, USA). The simultaneous effects of the studied parameters on the efficiency of Pb (II) bioremoval were interpreted accordingly.

Microalgae, *C.vulgaris,* was harvested by centrifugation at 2000 rpm for 5 min (Behdad) and the biomass was washed three times with doinezed water. The harvested microalgae was dried using oven overnight. Subsequently, dried microalgae was converted to powder and non-living cells were resuspended in dionized water in order to reach a cell concentration of 1g L^{-1}. The biosorption experiments were carried out in a 100 ml Erlenmeyer flask containing 25 ml of batch solutions. The sample containers were shaken on orbital shaker (Fara Azma) at 120 rpm followed by centrifugation (Beckman L2-65B) at 10000 rpm for 5 min to separate biosorbent from lead ions solution. Initial pH of all solutions were adjusted using HNO_3 and NaOH solutions. The entire experiments were performed at room temperature (25±2°C). The residual Pb (II) in the supernatant solution was determined using flame atomic absorption spectrophotometry (AAS) (Shimadzu AA-670). The percentage of bioremoval of Pb (II) was calculated using Eq.1:

$$R = \frac{C_i - C_f}{C_i} \times 100 \qquad \text{(Eq.1)}$$

Where R is bioremoval percent, C_i and C_f are the initial and final concentrations of Pb (II) in solution, respectively.

Experimental design and response surface methodology

In this study, effective factors on the bioremoval of Pb(II) were selected from literature [19, 20]. The bioremoval factors were optimized by applying Response Surface Method (RSM). The CCD with four variable factors at three replicates was used to optimize the experimental factors. Initial pH, Pb (II) concentration, biomass concentration and contact time were coded as X_1, X_2, X_3 and X_4, respectively.

Each factor was coded at five levels: -α, -1, 0, +1 and +α. These points represent the center point (0), factorial point (±1), axial point (±α). Each axial point assigned one factor at level of ± α and the remaining factors at level zero [21]. The value of α was calculated from $\alpha = (F)^{1/4}$ [22], where F is the number of factorial points which is 16 and thus the coded value of α will be 2. Table 1 shows the selected range and levels of the factors.

Variables	***Unit***	***Levels***				
		-α	***-1***	***0***	***1***	***+α***
pH	--- (X_1)	2.5	3.5	4.5	5.5	6.5
Pb(II)	mg L^{-1} (X_2)	100	200	300	400	500
Biomass;	g L^{-1} (X_3)	0.1	0.2	0.3	0.4	0.5
Time	min (X_4)	20	45	70	95	120

Table 1: Variables and their levels used in the model.

The matrix of experiments consists of the uncoded values of the variables namely including the initial pH, initial lead ion concentration, biomass concentration and contact time. These factors, presented in Table 2, were evaluated during the optimization of the bioremoval of Pb (II). All analyses were performed in triplicates. Also, the predicted responses of new design points using model for bioremoval percent are shown in Table 2.

Run	point	Real values [a]				Bioremoval Pb (II) (%)	
		X_1	X_2	X_3	X_4	Experimental	Predicted
1	+1	3.5	200	0.2	45	27.3	21.96
2	+1	5.5	200	0.2	45	25.2	31.75
3	-1	3.5	400	0.2	45	20.7	23.66
4	+1	5.5	400	0.2	45	21.1	30.53
5	-1	3.5	200	0.4	45	38.4	48.27
6	+1	5.5	200	0.4	45	80.6	68.47
7	+1	3.5	400	0.4	45	17.1	16.9
8	-1	5.5	400	0.4	45	31.5	34.18
9	-1	3.5	200	0.2	95	19.4	27.05
10	+1	5.5	200	0.2	95	45.0	36.41
11	+1	3.5	400	0.2	95	13.8	17.13
12	+1	5.5	400	0.2	95	23.1	23.57
13	+1	3.5	200	0.4	95	87.1	68.88
14	+1	5.5	200	0.4	95	81.3	88.67
15	+1	3.5	400	0.4	95	22.1	25.88
16	+1	5.5	400	0.4	95	46.2	42.75
17	-∞	2.5	300	0.3	70	29.6	28.45
18	+∞	6.5	300	0.3	70	55.5	55.10
19	-∞	4.5	100	0.3	70	50.0	57.19
20	+∞	4.5	500	0.3	70	21.7	12.97
21	-∞	4.5	300	0.1	70	29.1	21.64
22	+∞	4.5	300	0.5	70	61.2	67.12
23	-∞	4.5	300	0.3	20	29.6	23.45
24	+∞	4.5	300	0.3	120	32.5	37.10
25	0	4.5	300	0.3	70	35.1	33.79
26	0	4.5	300	0.3	70	33.9	33.79
27	0	4.5	300	0.3	70	36.4	33.79
28	0	4.5	300	0.3	70	33.2	33.79
29	0	4.5	300	0.3	70	32.9	33.79
30	0	4.5	300	0.3	70	30.2	33.79
31	0	4.5	300	0.3	70	34.8	33.79

[a] X_1 : pH; X_2: initial Pb(II) concentration (mgL^{-1}); X_3: biomass concentration (gL^{-1}); X_4: contact time (min).

Table 2: Central composite matrix along with Experimental and predicted values for bioremoval (%) of Pb(II) using *C. vulgaris* biomass.

The mathematical model for this system is shown in Eq. 2:

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

Where Y is the predicted response, x_i, x_j, ..., x_K are the variables which affect the response. The parameters are $x_i^2, x_j^2, \ldots, x_k^2$ the squared effects, x_i, x_j, x_j, x_K and x_j, x_K are the interaction effects, is the intercept term, $\beta_{i(i=1,2,\ldots,k)}$ is the linear coefficient, $\beta_{ii\ (i=1,2,\ldots,k)}$ is the squared coefficient, $\beta_{ij\ (i=1,2,\ldots,k;\ j=1,2,\ldots,k)}$ is the interaction coefficient and ε is a random error [23].

Results and discussion

Modeling and statistical analysis

Table 2 shows the matrix of experimental design and responses factors obtained in this study. The linear factors used in this study were initial pH (X_1), initial concentration of Pb (II) (X_2) in mgL^{-1}, concentration of biomass (X_3) in gL^{-1} and contact time (X_4) in minutes. The bioremoval percentage of Pb (II), Y, was the model outcome. A multiple estimated regression equation was used to evaluate the bioremoval of Pb (II) by *C.vulgaris* biomass. The estimated regression equation used for the evaluation of Pb (II) bioremoval is:

$$Y= 33.79 + 6.66 X_1 - 11.05 X_2 + 11.37 X_3 + 3.41 X_4 + 2X_1^2 + 0.32 X_2^2 + 2.64 X_3^2 - 0.88 X_4^2 - 0.73 X_1X_2 + 2.61 X_1X_3 -0.11\ X_1X_4 - 8.27 X_2X_3 - 2.91 X_2X_4 + 3.88X_3X_4 \qquad \text{(Eq. 3)}$$

According to Eq.3, a strong correlation exists among the variables with a regression coefficient (R2) of 87.91%. The modeling information of each factor are shown in Table 3. Hypothesis testing at a 5% significance level indicates that the effects of X_1, X_2, X_3, and X_2X_3 were significant in model as *p*-values were well below 0.05.

Source	*Estimate coefficient*	*Standard Error coefficient*	*T-value*	*P-value*
intercept	33.79	3.38	9.997	0.000
X_1	6.66	1.82	3.650	0.002
X_2	-11.05	1.82	-6.056	0.000
X_3	11.37	1.82	6.230	0.000
X_4	3.41	1.82	1.870	0.080
X_1X_2	-0.7312	2.235	-0.327	0.748
X_1X_3	2.6063	2.235	1.166	0.261
X_1X_4	-0.1063	2.235	-0.048	0.963
X_2X_3	-8.2688	2.235	-3.699	0.002
X_2X_4	-2.9063	2.235	-1.300	0.212
X_3X_4	3.8813	2.235	1.736	0.102
X_1^2	1.9984	1.672	1.195	0.249
X_2^2	0.3234	1.672	0.193	0.849

X_3^2	2.6484	1.672	1.584	0.133
X_4^2	-0.8766	1.672	-0.524	0.607

X_1: pH, X_2: initial Pb(II) concentration (mg/L); X_3: biomass concentration (g/L); X4: contact time (min).

Table 3: Estimate coefficients, standard error coefficient and corresponding T-value and *p-values* for Pb(II) bioremoval percent using *C. vulgaris* biomass.

The intercept in Eq. 3 indicates that the mean percentage removal of Pb (II) by C. *vulgaris*, which is 33.79, is independent of the variables and interactions among the variables. The modeling was carried out through analyzing of coefficients of model via evaluation of F-values and P-values. The large F-value (8.31) of regression indicates that the response variation can be explained by the model [24], and compared to the pure error is not significant.

As shown in Table 4, *p*-values of components in the model are all well below 0.05 excluding the square effects (p-value=0.411). This indicates that the estimated model is significantly fit to the variables. The analysis of variance also shows that the interaction effect is significant (*P*-value=0.026). The sum of square for each term indicates the amount of contribution of factors in the bioremoval efficiency of lead ions [20]. In Table 4, the main factors such as pH, initial Pb (II) concentration and biomass concentration have the most contribution in regression. Thus, they have the most contribution in prediction of lead ions bioremoval efficiency. In addition, the contributions of the interactions among the main factors were influential in the final biosorption capacity.

Source	***DF***	***SS***	***MS***	***F-value***	***P-value***
Regression	14	9305.06	664.65	8.31	0.000
Main effects	4	7380.59	1845.15	23.08	0.000
Square effects	4	336.93	84.23	1.05	0.411
Interaction effects	6	1587.54	264.59	3.31	0.026
Residual error	16	1279.32	79.96		
Pure error	6	23.59	3.93		

S = 8.94; R^2 = 87.91% ; R^2 (adj) = 77.34%

Table 4: Analysis of variance (ANOVA) of quadratic Pb (II) removal model.

The normality of the data can be checked by plotting the normal probability plot (NPP) of the residuals. The NPP is a graphical technique to identify real deviation of a set of data from normal distribution [24, 25]. The difference between the observed and predicted values in the regression is called residual. If the data on the plot lie close to the straight line then the data are normally distributed. As shown in Fig. 1(a) the experimental results were reasonably aligned where expressing a normal distribution. Fig. 1(b) plots the residuals versus the fitted values (predicted responses). The plot showed the normal scatter residuals at zero where the errors have a constant variance. Fig. 1(c) shows the histograms of the residuals. Histograms of the residuals indicate approximately the normal distributions of residuals for all observations. The histogram of the residuals should be bell shaped. Fig. 1(d) plots the residuals in the order of the corresponding observations. The residuals in the plot are shown in a random pattern around the centerline.

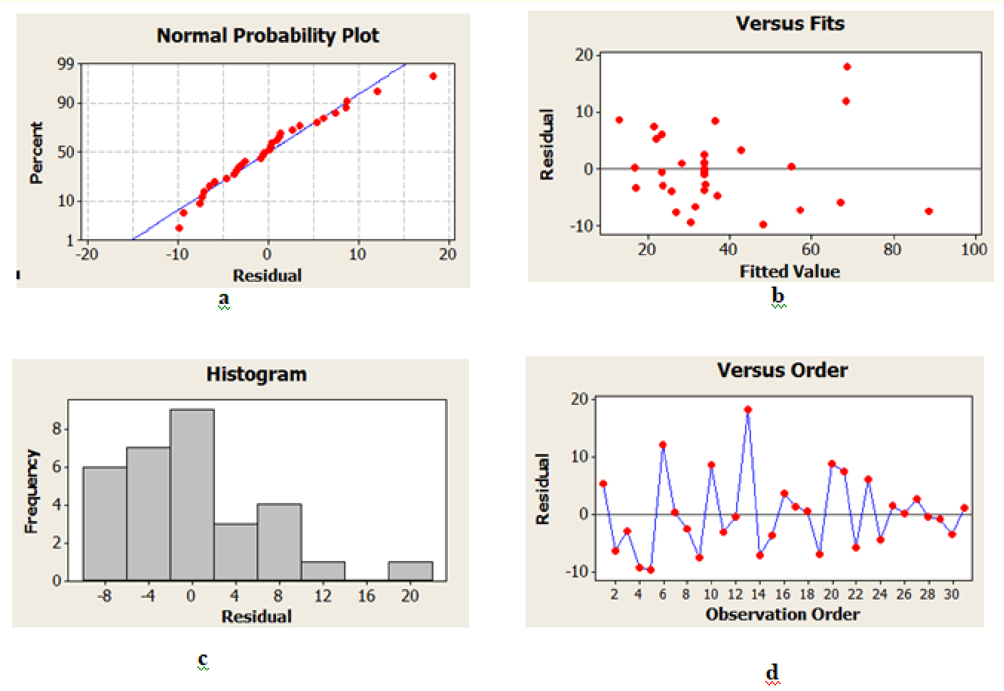

Figure 1: Standardized residual plots; a) Normal probability plot of the residuals, b) Residuals vs. plotted fitted values, c) Histogram of the residuals, d) Observation order.

Parameters' effects on Pb (II) bioremoval

Response optimization is a useful method to distinguish and identify the factor settings (operating conditions) that could optimize response. In this study, the main goal was to obtain a value at or near the target value of 100% Pb (II) bioremoval, therefore bioremoval values greater than 100% were unacceptable. The global solution, defined as the best combination of factor settings with the desired response, was found to be at pH = 2.5, C_0 = 100.0 mgL^{-1}, $C_{biomass}$ = 0.4 gL^{-1} and t = 120.0 minutes with a desirability score (D) of 1.00 (Fig. 2). Thus a maximum bioremoval of Pb (II) can be achieved by *C. vulgaris* under the studied conditions. Other desired values can also be predicted by changing the Current Factor level setting in the optimization plot. In general, there are many advantages of applying optimization plot including (i) to achieve predicted response with higher desirability score, (ii) lower cost factor settings near optimal properties,(iii) study the sensitivity of response variables with altering the factor settings (iv) and to obtain required responses for factor setting of interest [25]. Figure 2 shows the hundred bioremoval percentage which was estimated via optimized values obtained from Eq.3. According to Eq. 3 optimized value for each factor to achieve the hundred bioremoval percentage of lead ions was estimated and reported in Fig 2. These optimal values, pH= 2.5, C_0 = 100.0 mgL^{-1}, $C_{biomass}$ = 0.4 gL^{-1} and t = 120.0 minutes, were achieved via Eq.3 corresponding to the 100% of Pb (II) bioremoval. These optimal conditions can be used to remove lead ions from the aqueous solutions including the contaminated industrial wastewater streams.

The optimization plots (Fig. 2) also elucidate the alteration in response of the four main investigated factors. As shown in Fig.2, factors such as initial biomass, pH and time exposure may have positive effects to enhance the lead bioremoval capacity. In contrary, the initial lead ion concentration has a negative effect which results in reduction of bioremoval capacity. These plots are represented based on the obtained model. The line, shown at the middle of the plot, indicates the target bioremoval capacity (100%). It also shows that the deviation of each studied factor from the target response is minimal. The 100% bioremoval line, shown in Fig.2 was assigned as the target line representing the optimum conditions of the RSM model.

Factor	pH	Pb (mgL^{-1})	Biomass($g\ L^{-1}$)	Time (min)
High	6.5	500.0	0.5	120.0
Current	2.5	100.0	0.4	120.0
Low	2.5	100.0	0.1	20.0

Removal %
Target=100
Y=100
D=1.00

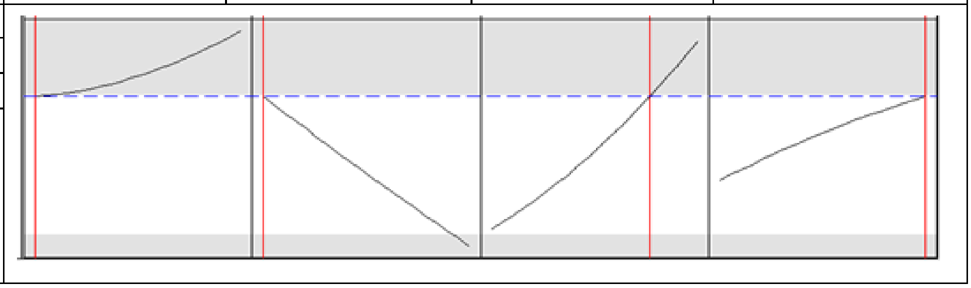

Figure 2: Optimization plots.

pH

pH is one of the most important factors in the biosorption of Pb (II) using algal biomass [26]. The influence of pH was investigated within the range of 2.5-6.5. The pH values greater than 6.5 result in the lead Pb (II) precipitation in the aqueous solution [25, 27], and therefore was not considered in this study. The initial pH of solution was adjusted using NaOH and HNO_3 solutions.

Interactions among the factors are critical and depicted in Figure 2. According to the coefficient of pH, bioremoval capacity is significantly dependent on pH, since any changes in the media acidity could result in the chemistry changes of Pb (II) in the solution. Moreover such changes potentially could alter the properties of a variety of functional groups existing on the algal cell wall that would have impacts on the biosorption capacity [26, 28]. At low pH, the sorption sites are protonated and consequently, the linkage of cations to binding sites would be restricted. As pH increases, functional groups are deprotonated and allows Pb (II) to bind to these vacant sites [26]. Another reason for increasing the bioremoval capacity of the metal ions with increasing pH is related to the isoelectric point (IEP) of the *C. vulgaris* cells. At the pH values more than IEP, it is expected that more ligands have negative charge. These negative charges would attract the positively charged metal ions [29]. The negative charge of the cell wall is controlled by the presence and status of carboxyl groups, phosphate, amine, and sulfonate residue on the cell wall [30]. This property is useful for biosorption of metal ions.

Initial Pb (II) concentration

In this study, the bioremoval potential of different concentrations of Pb (II) was studied (100, 200, 300,400 and 500 mg L^{-1}). It is noteworthy that the bioremoval capacity of Pb (II) by the *C. vulgaris* biomass depends on the initial Pb (II) concentration in the solution. The higher the Pb (II) concentration the lower the bioremoval capacity. As shown in Fig. 2, the increase of the Pb (II) concentration from 100 to 500 mg L^{-1} was accompanied by a slight decrease in the final metal bioremoval percentage. This could be due to the increasing of Pb (II) concentration that leads to binding the sites saturation [31]. Filling these active sites by higher concentration of metal ions results in metal biosorption being performed with lower metal biosorption efficiency compared to the lower concentration of Pb (II).

Minimum and maximum bioremoval capacities of Pb (II) observed in 500 and 100 mg L^{-1} of Pb (II), respectively. Generally, bioremoval of heavy metal ions performs with higher efficiency at lower concentrations. Similarly, Mehta and Gaur found that *C. vulgaris* is able to remove effectively metal ions (Ni and Cu) at low concentration from aqueous solution. They reported that in 2.5 mg L^{-1} of these metal ions concentration, C. *vulgaris* could adsorb 93 and 97% of Ni (II) and Cu (II) ions, respectively. However, these removal percentages reduced dramatically when the concentration of metals increased in the solution [32].

Biomass concentration

To investigate the relationship between biomass concentration and bioremoval capacity of metal ions, 0.1, 0.2, 0.3, 0.4 and 0.5 g L^{-1} dried biomass were added into aqueous medium enriched by 100 - 500 mg L^{-1} of Pb (II). The results (Figure 2) clearly depicted that increase in biomass concentration is aligned with the enhancement of the final bioremoval percentage.

Based on the observed responses, the biomass concentration has positive effect on the final bioremoval of Pb (II). This due to the increasing of the free binding sites that resulted in adsorbing more ions [33]. Therefore, the bioremoval of heavy metal ions responds directly to the increasing of biomass concentration. The biosortion capacity can be calculated by using the equation of $q = \frac{(C_{in} - C_f)v}{m}$.

In this equation, q is Pb(II) biosorbed (mg/g), m is biomass concentration (g), C_{in} is the initial concentration (mg/L), C_f is the final or equilibrium concentration (mg/L), and v is the solution volume (L) [34]. According to this equation increasing in biomass concentration could result in decrease in the biosorption capacity at a fixed initial Pb (II) concentration. However, it could simultaneously enhance the bioremoval percentage of metal ions. This finding is in agreement with the obtained results from the present study.

Contact time

Contact time previously was introduced as an important factor in heavy metal biosorption [9]. Our results confirm this phenomena. According to Fig.2, this factor has a positive effect on final metal biosorption, however, the coefficient of contact time is small in Eq. 3 and it indicates that contact time has less contribution in the prediction of Pb (II) bioremoval capacity. Adsorption of Pb (II) process reached its half saturation at 20 min [35]. Increasing the exposure time from 20 to 120 minutes leads to establishing an equilibrium between the lead ions and the algal cell surface. By reaching the equilibrium state, the amount of bioremoval of lead ions did not change significantly during the longer incubation times (Figure 2). Similar observation was previously reported by Singh *et al.* [36]. They studied biosorption of lead and copper ions using *Spirogyra neglecta* biomass, and observed that the biosorption equilibrium was established during the first 20 minutes of bioremoval process.

Heavy metals bioremoval naturally occurs at two steps. At the first step, biosorption is performed using simple physical mechanisms where affinity between the biosorbent and sorbate determines the efficiency of the step [11]. Interestingly, the maximum biosorption occurs at this step. During the second step, bioaccumulation is achieved where the active metabolic transport and biochemical reactions are involved. Intracellular active biosorption occurs as a result of equilibrium between metal ions and cellular binding sites [37].

Effects of parameters' interactions

As shown in Table 4, interaction effects is statically significant (p-value=0.026). The interaction effects of factors on the Pb (II) bioremoval percent were studied by three-dimensional surface plot to find the optimum level of each factor for the target response. The surface plot indicating the interaction among variables on bioremoval percent of Pb (II) are presented in Fig. 3. Regression equations are represented by three-dimensional response surface plot and are typically used to demonstrate relationships between the response and experimental levels of each variable (Fig. 3.1-4). This analysis gives a better understanding of the influence of variables and their interactions on the response. To investigate the interactive effect of two factors on the bioremoval of lead ions, the response surface methodology-central composite design was used, and surface plots were drawn. Each interaction plot also represents the values of the remaining factors set including pH= 4.5, C_0 = 300.0 mgL^{-1}, $C_{biomass}$ = 0.3 gL^{-1} and t = 70.0 minutes. Figure 3 shows that interactions among the factors (pH, time, biomass concentration and Pb (II) concentration) on the bioremoval efficiency. These figures were prepared according to the computation via the calculated model. Figure 3-1 presents the Pb (II) bioremoval percent as a function of pH and biomass concentration. It demonstrated that increase in pH leads to amplifying the interaction between pH and biomass concentrations. Fig. 3-1 shows that regardless of the pH level, increasing initial biomass concentration enhanced the Pb (II) removal percentage.

Figure 3-2 depicts the interaction between pH and time on the Pb (II) bioremoval percentage. Interaction between pH and time explains a positive influence on the bioremoval efficiency. This is obvious from the figure where the maximum bioremoval percentage was achieved at the higher pH and longer retention time.

Interaction effects of biomass concentration and Pb (II) concentration on the Pb (II) bioremoval percentage are shown in Fig. 3-3. It was observed that the low level of Pb (II) concentration (100 mg L^{-1}) and high level of biomass concentration (0.5g L^{-1}) result in a higher percent removal. According to Fig.2, in the low pollutant concentration (low concentration of heavy metal ions) any increase in biomass concentration results in a significant increase in the bioremoval percentage. These findings could potentially give an answer to the main limiting factors such as high costs of chemicals and incomplete removal of physicochemical approaches to remove heavy metal ions from wastewaters [38]. Bioremoval of toxic pollutants by algae can provide an ecologically safer, cheaper, and more efficient means to specific remediation at low concentration of metal ions in wastewater streams. Fig. 3-4 represents the interaction between biomass loading and time on the bioremoval percent of Pb (II). This surface plot explained that maximum bioremoval of Pb (II) was obtained at high level of biomass concentration (0.5 gL^{-1}) and time period (120 min).

Optimization of the critical parameters affecting biosorption capacity lead to the development of low-cost microalgae cultivation and overall improvement of bioremediation potential. Interaction between time and biomass concentration was positive and had coefficient of +1.10 (Fig. 3-4). The most preliminary information on the time and other factors interaction comes from the equilibrium sorption dynamics. In other words, ‘‘enough time’’ required for the contact between the sorbate and biosorbent is dependent on the size of sorption particles, pH, and the concentration [11].

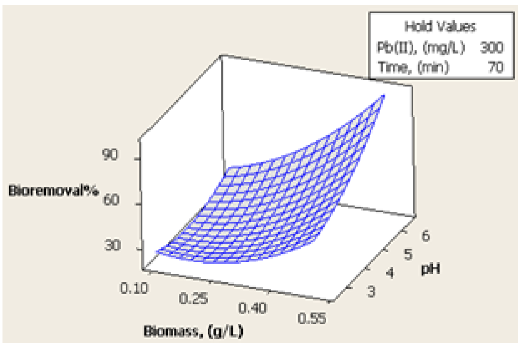

Fig. 3-1. Interaction of pH vs biomass

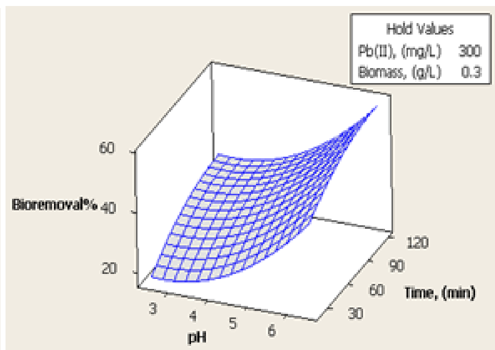

Fig. 3-2.Interaction of pH vs time

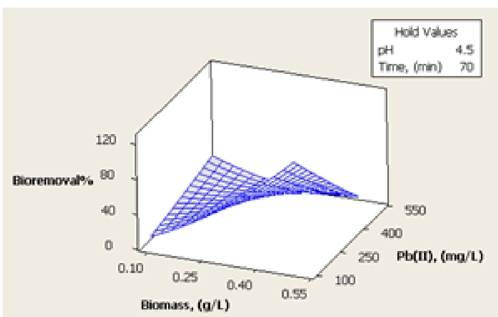

Fig. 3-3.Interaction of Pb (II) vs biomass

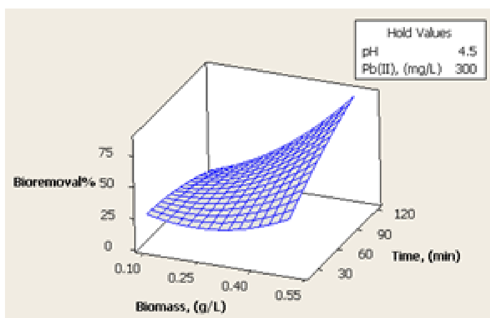

Fig. 3-4. Interaction of Biomass vs time

Conclusions

Response surface methodology (RSM) and central composite design (CCD) were used to determine the optimal conditions of Pb(II) ions bioremoval capacity by *C. vulgaris*. A quadratic model was successfully employed to predict and optimize the interactions of important factors including the individual and combined impacts on the Pb(II) bioremoval. The model indicated that the effects of selected parameters were significant in model (p-values < 0.05), and showed that *C. vulgaris* as an excellent biosorbent could remove Pb(II) ions efficiently when the optimal values of pH= 2.5, C_0 = 100 mgL^{-1}, $C_{biomass}$ = 0.4 gL^{-1} and t = 120 min were considered. The analysis of variance also revealed that a strong correlation exists among the variables (R^2 = 87.91) and the interaction effect is significant (P-value=0.026) where pH, initial Pb (II) concentration and biomass concentration factors have the most contributions in the prediction of Pb (II) bioremoval efficiency.

Acknowledgment

NA

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