Optimization of Cu and Pb biosorption by *Aphanizomenon ovalisporum* using Taguchi approach: kinetics and equilibrium modeling

Roula Flouty[a,b,*], Jessica El-Khoury[a], Elias Maatouk[a], Antoine El-Samrani[a]

[a]Laboratory of Biosorption and Bioaccumulation Processes (L2GE group), Lebanese University, Faculty of Sciences II, Fanar Campus, PO Box 90656, Jdeidet, Lebanon, Tel. 961-3-349068; email: rflouty@ul.edu.lb (R. Flouty)
[b]National Centre for Marine Sciences, Batroun, Lebanon

ABSTRACT

In this study, the cyanobacterium *Aphanizomenon ovalisporum* collected from the Litany River in Lebanon was used as an efficient biosorbent for the removal of copper and lead metal ions from aqueous solutions. Scanning electron microscopy (SEM) revealed the change of the surface morphology due to sorption of lead and copper metal ions on algal surface sites. In addition, the elemental composition of native algal biomass showed mainly the presence of carbon, nitrogen and oxygen as 66.15%, 14.58% and 16.79% respectively. The percentage composition of C decreases while the percentages of N and O increase after biosorption of Cu and Pb, which indicates the interaction of metal ions with nitrogen and oxy functional groups on algal cell surface. Fourier transform infrared spectroscopy (FTIR) has proved the presence of the amine, phosphate, carboxyl and carbonyl groups as binding surface sites for metal ions. The optimum conditions were determined using Taguchi statistical approach. The maximum removal efficiency values (87.59% Pb; 69.65% Cu) were obtained at pH 7.5 in 75 min with 0.7 g L^{-1} of algal biomass and initial metal concentration of 200 mg L^{-1} for Pb and 100 mg L^{-1} for Cu. The most influential factor on biosorption is pH with a percentage contribution of 40.8% and 31.5% for Cu and Pb, respectively. The kinetic biosorption followed the pseudo-second-order model for Cu and the pseudo-first-order model for Pb. Biosorption equilibrium was well described by Langmuir adsorption isotherm for both metals.

Keywords: Biosorption; Taguchi method; *Cyanobacteria*; Heavy metal; Kinetics; Equilibrium models

1. Introduction

Aquatic systems and human health are threatened by heavy metal pollution, which is a severe environmental issue. Unlike organic contaminants, heavy metals such as, copper and lead are main pollutants of freshwater due to their inherent nature of being persistent, toxic, recalcitrant and non-biodegradable [1]. At low concentrations copper is essential to human life since it is found in a variety of enzymes and is used for biological electron transport. However, like all heavy metals, it is potentially toxic, especially at high concentrations. On the other hand, the most toxic heavy metals affecting the environment is lead even at very low concentrations, lead may cause severe damage on living organisms and even death [2,3]. Recently, the most promising effective method for the treatment of metal pollution is biosorption process mainly because of its low cost, high efficiency to eliminate low concentrated metal ion solution, a small quantity of spent biosorbent for final discharge and easy to immobilize the dead cells for continuous metal ions removal and environmental friendly [4–6]. A wide variety of biological materials have been employed as biosorbents mainly dry algal biomass and other microorganisms [7–13]. These biosorbents can passively bind metal ions with a combination of several mechanisms including electrostatic attraction, complexation, ion exchange, chelating, and microprecipitation. Heavy metal uptake

* Corresponding author.

capacity of algal biomass has proved to be the highest due to the presence of polymers such as polysaccharides, proteins or lipids on the cell wall surface containing functional groups such as amino, hydroxyl, carboxyl and sulfate, which can act as binding sites for metals [14,15]. Cyanobacteria is blue-green algae, which have a significant influence on physical, chemical and biological properties of many ecosystems [16,17]. These algal cells can effectively sequester dissolved metal ions from dilute aqueous solutions with high efficiency and quickly due to many features such as high tolerance to heavy metals, ability to grow autotrophically and heterotrophically, large surface area/volume ratios with great binding affinity, phytochelatin expression and potential for genetic manipulation [18–20]. Many studies have shown that biosorption process is strongly dependent on several experimental factors such as the pH of the solution, the nature and concentration of the metal ion, the type and dose of biosorbent and also the contact time and the kinetics of the uptake [21–26]. Although, biosorption process has proved to be more effective than the conventional metal removal technologies, great efforts are required to optimize the experimental factors for the maximum efficiency of metal removal, which is expected to lead to its large scale and potential applications. However, the traditional pattern of performing optimization studies by varying one parameter while keeping all others at a predetermined level is very inefficient in many cases, since it is considered an exhaustive and expensive ordeal. Taguchi method is mainly a unique and powerful optimization discipline that allows optimization with a minimum number of experiments [27]. The Taguchi experimental design reduces cost, improves quality and provides robust design solutions. The advantages of Taguchi method over the other methods are that numerous factors can be simultaneously optimized and more quantitative information can be extracted from fewer experimental trials. Therefore, it is less laborious and time-consuming than other methodologies [28,29]. Recently, limited number of studies is available on the application of Taguchi approach for optimization of adsorption processes [30–36]. In this study, the cyanobacterium *Aphanizomenon ovalisporum* collected from the 'Litani River', in Lebanon, is used as a biosorbent for the removal of Cu(II) and Pb(II) from aqueous solutions with the aim of exploring the capacity of freshwater algal biomass for heavy metal removal from aqueous solutions. The main objective of this work is the use of the Taguchi approach to optimize the biosorption controllable factors for the maximum metal removal efficiency of algal biomass. Lead and copper were selected because of their contrasting toxicity and essentiality. Furthermore, isotherm and kinetic modeling of metal adsorption and surface chemistry of the biosorbent have been investigated. To the best of our knowledge, *A. ovalisporum* has not been reported as biosorbent for the removal of heavy metals from aqueous medium under Taguchi optimum conditions and hence this study would be of great importance in the bioremediation of metals from aqueous environment.

2. Materials and methods

2.1. Biosorbent

Fresh algal biomass was collected from the 'Litani River' in Lebanon. *Aphanizomenon ovalisporum* is a cyanobacterium,

which produces a toxin called cylindrospermopsin that have been reported in several Lakes and reservoirs in Lebanon [37]. Before use, it was kept at –4°C after addition of formaldehyde (4%) for chemical fixation [38]. Algal biomass was recovered by centrifugation (4,000 rpm, 30 min) using the centrifuge (NF800). The initial surface-bound metal was extracted by washing algal cells with 10^{-3} M EDTA (ethylenediaminetetraacetic acid) [15]. After washing, the algae were lyophilized (CHAIST, –80°C) for 30 h and ground to a particle size of 1 mm and then sieved to a size of 200 μm.

2.2. Chemicals

All materials and chemicals were of analytical grade and used as purchased without further purification. Experimental solutions were prepared in 10^{-2} M MES [2-(*N*-morpholino) ethanesulfonic acid, Sigma] as the pH buffer. The test solutions of various concentrations were prepared by diluting the stock solutions of $Cu(NO_3)_2.3H_2O$ and $Pb(NO_3)_2$ (1,000 mg L^{-1}) in MES. The solutions were then diluted to the desired concentrations. All MES media were sterilized. For pH adjustment, concentrated solutions of hydrochloric acid (HCl, 37%) (Suprapur, Sigma) were used. All experimental solutions were equilibrated one day prior to use. This period was sufficient to reach the metal speciation. Under these conditions, in the absence of added ligand, free metals (Pb^{2+}, Cu^{2+}) represented >96% of the total metal in solution.

2.3. Characterization of the biosorbent

The surface morphology of the cyanobacterial biomass was observed using scanning electron microscope (SEM- Seron AIS2300C) operating at a working distance of 25 mm and accelerating voltage of 20 kV. This technique was coupled to energy dispersive X-ray (EDX) to get an elemental analysis of the samples before and after metal treatment. In addition, the presence of functional groups involved in the adsorption of metal ions was identified using a FTIR spectrophotometer (JASCO 6300 FT-IR, USA) within the range of 400–4,000 cm^{-1}. In order to form pellets, 5% of the biomass previously crushed was mixed to 95% of KBr. The resulting mixture was pressed in a mold (Φ = 7 mm) with a pressure of 10 tonnes cm^{-2}.

2.4. Biosorption studies

Biosorption experiments were performed in a water bath shaker (150 rpm) at room temperature around 25°C. The effects of contact time (A), initial metal concentration (B), algal dose (C) and pH (D) were studied. The four controllable factors having significant impact on metal biosorption with their four variable levels were listed in Table 1. At scheduled time intervals, samples were filtered and analyzed for metal concentrations. The concentrations of metal ions in the initial solutions (C_0) and after biosorption (C) were determined using a flame atomic absorption spectrophotometry (Rayleigh-WFX210). This technique is commonly used for detecting metals present in samples.

Table 1
Controllable factors and their levels

Factor	Description	Level 1	Level 2	Level 3	Level 4
A	Contact time (min)	50	75	100	120
B	Initial metal concentration (mg L^{-1})	10	50	100	200
C	Algal dose (g L^{-1})	0.25	0.5	0.7	1.5
D	pH	4	5	6	7.5

2.5. Optimization studies

The Taguchi method has been used to determine the optimum experimental conditions of metal biosorption by *A. ovalisporum*. Factors such as contact time (A), initial metal concentration (B), algal dose (C) and pH of the solution (D) have been changed in order to optimize the adsorption process (Table 1). 16 different experiments using L$_{16}$ (4^4) orthogonal array were performed in duplicate at room temperature. One of the advantages of the Taguchi method over the conventional experimental design methods is the ability to determine the optimum conditions with the least variability, which is expressed by signal-to-noise ratio (S/N) [33,34]. Three types of signal-to-noise ratio can be analyzed, (1) lower is better (LB), (2) nominal is better (NB), and (3) higher is better (HB). In this study, as the maximum metal removal efficiency (MRE) is required, the analysis of signal-to-noise with (HB) quality characteristic was processed to determine the optimum conditions for metal biosorption process and to estimate the MRE at the optimum conditions [30–32]. The MRE was calculated as follows:

$$\text{MRE} = \frac{\left(C_0 - C_e\right)}{C_0} \times 100 \tag{1}$$

where C_0 and C_e are the initial and equilibrium concentrations of the metal (mg L^{-1}), respectively. The S/N ratio was determined using Eq. (2):

$$\frac{S}{N} = -10\log_{10}\left[\frac{1}{n}\Sigma\left(\frac{1}{\text{MRE}_i}\right)^2\right] \tag{2}$$

where n is the number of repetitions under the same experimental conditions. In addition, the mean (M) of S/N for each factor at a certain level was also determined using Eq. (3):

$$\left(M\right)\text{Factor}^{\text{level}=i} = I = \frac{1}{n_{Ii}}\sum_{j=1}^{n_{Ii}}\left[\left(\frac{S}{N}\right)\text{Factor}^{\text{level}=i} = I\right] \tag{3}$$

where n_{Ii} is the number of appearances of factor I in the level i, $\left[\left(\frac{S}{N}\right)\text{Factor}^{\text{level}=i} = I\right]_j$ is the ratio of factor (I) in level (i) with its appearance sequence (j^{th}).

In order to validate the optimum conditions deduced from the experiments, certain confirmation tests were carried out in duplicate at the optimum conditions. The optimum conditions were considered for kinetics and equilibrium studies. On the other hand, the influence of each factor on the removal of Cu and Pb was analyzed using the analysis of variance statistical method [35]. The percentage contribution of each factor (R_F) is given by:

$$R_F = \frac{SS_F - \left(DOF_F V_{Er}\right)}{SS_T} \times 100 \tag{4}$$

In Eq. (4), DOF$_F$ represents the degree of freedom for each factor, which is obtained by subtracting one from the number of the level of each factor. The total sum of squares, SS$_T$, is given by:

$$SS_T = \sum_{j=1}^{m}\left(\sum_{j=1}^{n}(\text{MRE}_i)^2\right) - mn(\overline{\text{MRE}}_T)^2 \tag{5}$$

where $\overline{\text{MRE}}_T$ is the average of the total MRE$_i$, m represents the number of experiments carried out in this study, and n represents the number of repetitions. The following equation gives the factorial sum of squares, SS$_F$:

$$SS_F = \frac{mn}{L}\sum_{k=1}^{L}(\overline{\text{MRE}}_k^F - \overline{\text{MRE}}_T)^2 \tag{6}$$

where $\overline{\text{MRE}}_K^F$ is the average value of the measurement results of a certain factor F in the kth level. Additionally, the variance of error, V_{Er}, is given by:

$$V_{Er} = \frac{SS_T - \sum_{F=A}^{D} SS_F}{m\left(n-1\right)} \tag{7}$$

2.6. Equilibrium isotherm studies

For biosorption-equilibrium studies, a series of experiments were performed in monometallic solutions at a constant temperature (25°C) under optimum conditions. For both metal ions, experiments were carried out in a water shaker bath at 150 rpm with 0.7 g L^{-1} of algal biomass at pH 7.5. At the optimum contact time (75 min), samples were immediately filtered using a 0.45 µm filter for metal analysis. Langmuir and Freundlich isotherm models were used [39,40]. Metal biosorption capacity at equilibrium q_e (mg g^{-1}) was calculated using the following equation:

$$q_e = \frac{\left(C_0 - C_e\right) \times V}{m} \tag{8}$$

where C_0 and C_e are the initial and equilibrium concentrations of the heavy metals respectively, V is the volume of the solution (L), and m is the mass of dry adsorbent used (g). The Langmuir isotherm can be expressed using equation [38]:

$$\frac{1}{q_e} = \frac{1}{Q_{\max}} + \frac{1}{bQ_{\max}C_e} \tag{9}$$

where Q_{max} (mg g^{-1}) and b are the Langmuir constants related to the maximum monolayer adsorption capacity and to the free energy of adsorption, respectively. The logarithmic form of Freundlich model can be given by the following equation [39]:

$$\log q_e = \log K_F + \frac{1}{n}\log C_e \tag{10}$$

where K_F and n (mg L^{-1}) are the Freundlich constants indicators of the adsorption capacity and adsorption intensity, respectively.

2.7. Kinetic studies

The biosorption kinetic experiments were performed in a water shaker bath (150 rpm) at 25°C under optimum conditions. Each sample contains 0.7 g L^{-1} of algal dose and an initial metal concentration of 200 mg L^{-1} for Pb and 100 mg L^{-1} for Cu at pH 7.5. At selected time intervals, samples were filtered for metal analysis. Two models were used to describe the kinetics of heavy metal biosorption [41,42]. The pseudo first order (Eq. (11)) and the pseudo second-order (Eq. (12)):

$$\log\left(q_e - q_t\right) = \log q_e - \frac{K_{1,ads}}{2.0303}t \tag{11}$$

$$\frac{t}{q} = \frac{1}{K_{2,ads}q_e^2} + \frac{1}{q_e}t \tag{12}$$

where q_e (mg g^{-1}) and q_t (mg g^{-1}) are the metal biosorprtion capacities at equilibrium and at time t respectively, $K_{1,ads}$ (min^{-1}) and $K_{2,ads}$ (g mg min^{-1}) are the rate constants of the pseudo first and second order adsorption, respectively.

3. Results and discussion

3.1. Characterization of the biosorbent

3.1.1. SEM and elemental analysis

The surface texture and morphology of the biosorbent were clearly revealed by SEM. The native algal biomass showed a tangled mass of filaments in a porous structure with lot of irregular surface format (Fig. 1(a)) which showed a good possibility for Pb(II) and Cu(II) metal ion to be adsorbed into these pores. Its filamentous morphology and colony size offer protection against grazing. It produces cylindrospermopsin, a toxin that causes serious threats to human and environmental health [43]. From the micrographs, it is evident that cell morphology is affected with metal loaded cells. As shown in Fig. 1, the cell surface morphology considerably changed after Cu (Fig. 1(b)) and Pb (Fig 1(c)) biosorption. The number of pores seems to be reduced due sorption of lead and copper metal ions on algal surface sites. The change of biosorbent cell morphology in the presence of metal was also observed in the literatures [19,22,44–46]. In addition, the elemental composition of native algal biomass
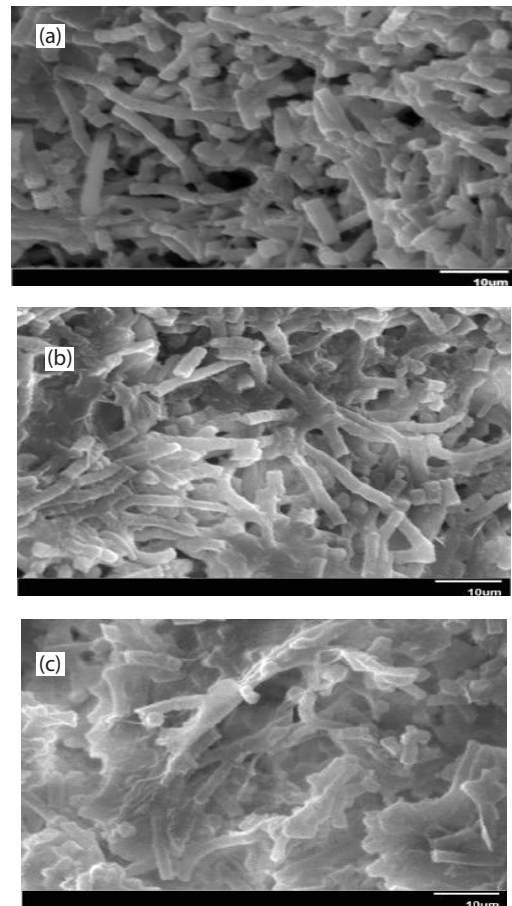

Fig. 1. SEM photos of (a) Native algal biomass, (b) Cu treated algal biomass, and (c) Pb treated algal biomass. [Pb^{2+}] = [Cu^{2+}] = 100 ppm, algal dose = 0.25g L^{-1} in 120 min at pH 7.5 and 25°C. Magnification 3.0 Kx.

showed mainly the presence of carbon, nitrogen and oxygen as 66.15%, 14.58%, and 16.79% respectively. Table 2 shows that the percentage of C decreases while the percentages of N and O increase after metal biosorption (Table 2). This result indicates the presence of nitrogen and oxy functional groups responsible of metal binding on algal cell surface.

3.1.2. FT-IR

The analysis of the FT-IR spectra has proved the presence of amino, carboxylic, hydroxyl and carbonyl groups on the surface of algal biomass (Fig. 2). As shown in Fig. 2, the characteristics peaks present in the native cyanobacterial biomass are NH$_2$, P–O–C stretching vibration, C=O stretching vibration and C–H, at the wave numbers of 706, 1,048, 1,272 and 2,620 cm^{-1}, respectively [19,22]. The strong broad band observed in the region 3,400–3,300 cm^{-1} is indicative of the –OH and –NH stretching vibrations [19,22]. As shown in Fig. 2, for metal loaded biomass, P–O–C and C=O peaks shift to the wave numbers of 1,015 and 1,267 cm^{-1}, respectively. The shifts or changes of these groups indicate the interaction between cyanobacterial cell and metal ions. NH$_2$ bond at 706 cm^{-1} has not been seen in the lead and copper loaded samples. However, O–H bond remains the same as that of native cyanobacterial

biomass (Fig. 2). The functional groups present on the cell wall are primarily responsible for binding metal ions [9,14,20]. Similar behavior has been described in the literatures [45–49]. These results confirm that the cell wall of algal biomass contains complex polymers as polysaccharides, proteins or lipids providing amino, hydroxyl, and carboxyl groups as surface binding sites for metal ions. Recently, Fang et al. [48] investigated the binding characteristics of Cu(II) and Cd(II) by the cyanobacterial biomass *Spirulina platensis*. They demonstrated that the carboxyl groups play a vital role in the adsorption of metal ions to the cyanobacterium *S. platensis*. Ion exchange and complexation are the dominating mechanisms for metals adsorption [46].

3.2. Optimization of biosorption process

3.2.1. Taguchi approach

The L_{16} (4^4) orthogonal array with Pb and Cu metal removal efficiency and signal to noise ratio of tests 1–16 determined according to Taguchi method are shown in Table 3. MRE_1 and MRE_2 for both metal ions are duplicate results for each test. As shown in Table 3, the highest MRE

values for both metal ions are obtained in Test 9. The average MRE is 87.59% for Pb and 69.65% for Cu. In addition, the maximum S/N ratios obtained in tests 9 are 38.84 and 36.85 for Pb and Cu, respectively. These maximum results (referred on boldfaces in Table 3) are obtained in solutions containing 10 mg L^{-1} of initial metal concentration (B) and 0.7 g L^{-1} of algal dose (C) at pH 7.5 (D) for a contact time of 100 min (A) (Test 9). The mean of the S/N ratios of each factor in the ith level $(M)_{\text{Factor}}^{\text{Level}}$ measured using equation (Eq. 3) are shown in Table 4 for Pb and Table 5 for Cu. The maximum value of the mean of the S/N ratios of each factor among the four levels indicates the optimum biosorption conditions.

3.2.2. Optimum bisorption conditions

The plots of the mean S/N ratio as a function of the four levels for each metal biosorption factor are shown in Fig. 3 (Pb) and Fig. 4 (Cu). The optimum biosorption conditions for both metal ions are 0.7 g L^{-1} of algal biomass in 75 min at pH 7.5. The optimum initial metal concentration is 200 mg L^{-1} for Pb (Fig. 3(a)) and 100 mg L^{-1} for Cu (Fig. 4(a)). The effect

Table 2
Elemental composition of algal biomass before and after metal treatment

Elements (%)	C	N	O	P	Na	S	Cl	K	Total
Native	66.15	14.58	16.79	1.42	0.09	0.86	0.03	0.07	100
Cu treated	56.85	17.62	25.53	n.d[a]	n.d	n.d	n.d	n.d	100
Pb treated	56.97	18.53	24.50	n.d	n.d	n.d	n.d	n.d	100

[a]not determined.

Table 3
L16 (4^4) orthogonal array with MRE and S/N ratio of each test

Tests	Factor				MRE (%)				S/N	
	A	B	C	D	Pb(II)		Cu(II)		Pb(II)	Cu(II)
					MRE$_1$	MRE2	MRE$_1$	MRE2		
Tests 1	50	10	0.3	4	3.69	2.83	3.178	2.93	10.03	9.67
Tests 2	50	50	0.5	5	18.71	17.25	21.97	20.15	25.07	26.45
Tests 3	50	100	0.7	6	41.30	45.63	23.03	22.96	32.73	27.24
Tests 4	50	200	1.5	7.5	19.23	21.76	38.73	35.05	26.18	31.31
Tests 5	75	10	0.5	6	72.25	74.60	37.37	36.8	37.31	31.38
Tests 6	75	50	0.3	7.5	31.68	31.70	39.48	37.79	30.01	31.73
Tests 7	75	100	1.5	4	15.42	15.45	39.03	40.74	23.77	32.01
Tests 8	75	200	0.7	5	56.39	59.61	16.32	19.6	35.26	24.98
Tests 9	100	10	0.7	7.5	**88.37**	**86.81**	**68.63**	**70.68**	**38.84**	**36.85**
Tests10	100	50	1.5	6	13.38	15.04	10.75	12.58	23.01	21.25
Tests 11	100	100	0.3	5	2.34	3.13	12.31	13.33	8.45	22.13
Tests 12	100	200	0.5	4	26.23	28.96	11.42	11.65	28.78	21.24
Tests 13	120	10	1.5	5	3.26	2.73	9.6	8.95	9.42	19.33
Tests 14	120	50	0.7	4	11.50	12.74	48.96	48.48	21.63	33.75
Tests 15	120	100	0.5	7.5	47.63	47.64	41.47	39.64	33.56	32.15
Tests 16	120	200	0.3	6	46.61	48.65	17.33	18.3	33.55	25.01

Table 4
Mean of S/N ratio for Pb

Factor/level	$\left[\left(\dfrac{S}{N}\right)_{Factor}^{Level}\right]_j$				$(M)_{Factor}^{Level}$
	$j=1$	$j=2$	$j=3$	$j=4$	
A/1	10.03	25.07	32.73	26.18	23.50
A/2	37.31	30.01	23.77	35.26	31.58
A/3	38.84	23.01	8.45	28.78	24.77
A/4	9.42	21.63	33.56	33.55	24.54
B/1	10.03	37.31	38.84	9.42	23.90
B/2	25.07	30.01	23.01	21.63	24.93
B/3	32.73	23.77	8.45	33.56	24.62
B/4	26.18	35.26	28.78	33.55	30.94
C/1	10.03	30.01	8.45	33.55	20.51
C/2	25.07	37.31	28.78	33.56	31.18
C/3	32.73	35.26	38.84	21.63	32.11
C/4	26.18	23.77	23.01	9.42	20.59
D/1	10.03	23.77	28.78	21.63	21.05
D/2	25.07	35.26	8.45	9.42	19.55
D/3	32.73	37.31	23.01	33.55	31.65
D/4	26.18	30.01	38.84	33.56	32.14

Table 5
Mean of S/N ratio for Cu

Factor/level	$\left[\left(\dfrac{S}{N}\right)_{Factor}^{Level}\right]_j$				$(M)_{Factor}^{Level}$
	$j=1$	$j=2$	$j=3$	$j=4$	
A/1	9.67	26.45	27.24	31.31	23.66
A/2	31.38	31.73	32.01	24.98	30.02
A/3	36.85	21.25	22.13	21.24	25.36
A/4	19.33	33.75	32.15	25.01	27.56
B/1	9.67	31.38	36.85	19.33	24.30
B/2	26.45	31.73	21.25	33.75	28.29
B/3	27.24	32.01	22.13	32.15	28.38
B/4	31.31	24.98	21.24	25.01	25.63
C/1	9.67	31.73	22.13	25.01	22.13
C/2	26.45	31.38	21.24	32.15	27.81
C/3	27.24	24.98	36.85	33.75	30.71
C/4	31.31	32.01	21.25	19.33	25.97
D/1	9.67	32.01	21.24	33.75	24.16
D/2	26.45	24.98	19.33	22.13	23.22
D/3	27.24	31.38	21.25	25.01	26.22
D/4	31.31	31.73	36.85	32.15	33.01

of these factors on metal biosorption capacity was widely investigated in the literatures. The initial metal ion concentration is one of the most important factors in biosorption processes. Many studies have shown that the biosorption capacity of algal biomass increases first with increasing the initial concentration of metal ions and reached a saturation plateau [49–52]. At lower initial metal concentrations, the ratio of the initial amount of metal ions to the available surface area is low; subsequently, the fractional sorption becomes independent of the initial concentration. However, at higher concentrations, the sites available for sorption become fewer compared with the moles of metal ions present and hence, the removal of metal is strongly dependent upon its initial solute concentration [22]. In addition, the distribution of metal ions between the biosorbent surface and the solution is significantly influenced by the contact time. According to the literatures [23–26, 49–52], the optimum contact time obtained for metal biosorption process can be attributed to the fact that during the initial stage of contact time there is a lot of vacancy for the adsorption site on the biosorbent surface but after an optimum contact time, it becomes difficult for the metal ion to be adsorbed due to the repulsive forces between metal ions attached on the surface of algal biomass and those found in the bulk liquid phase [34]. Biosorbent dose also has a significant influence on metal removal efficiency due to the number of binding sites available for metal ions removal at a specified initial concentration [30–35]. The mean S/N ratio increases as the sorbent amount increases to a certain limit in the solution with a fixed initial metal concentration. In fact, increasing the amount of biosorbent provides greater surface area at a constant initial concentration of metal ion [30–35]. On the other hand, the maximum value of the mean of the S/N is observed at pH 7.5 for both metals. This can be explained by the decrease in competition between proton and metal ions on the surface active sites and also by the decrease of the surface positive charge of algal biomass with increasing the pH. Similar findings were observed in the literatures [22,52,53]. The decrease in metal sorption was generally associated with acidic pH (<2–3) and the protonation of functional groups found on cell surfaces. In addition, metal speciation is significantly altered with the change in pH, which in turn affects the removal efficiency of the biosorbent [22]. At high alkaline pH, the hydrolyzed species
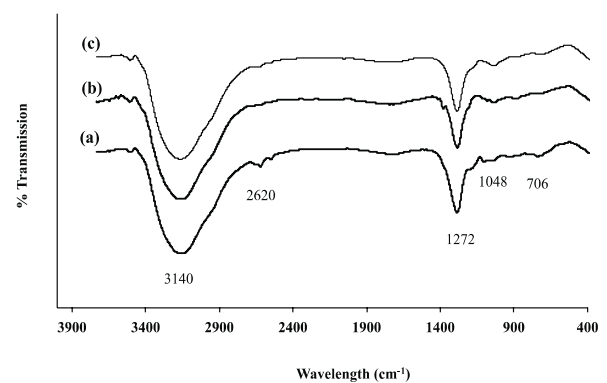

Fig. 2. FT-IR spectra of (a) Native, (b) Pb treated and (c) Cu treated algal biomass. [Cu] = [Pb] = 100 mg L⁻¹, algal dose = 0.25 g L⁻¹ in 120 min at pH 7.5 and 25°C.
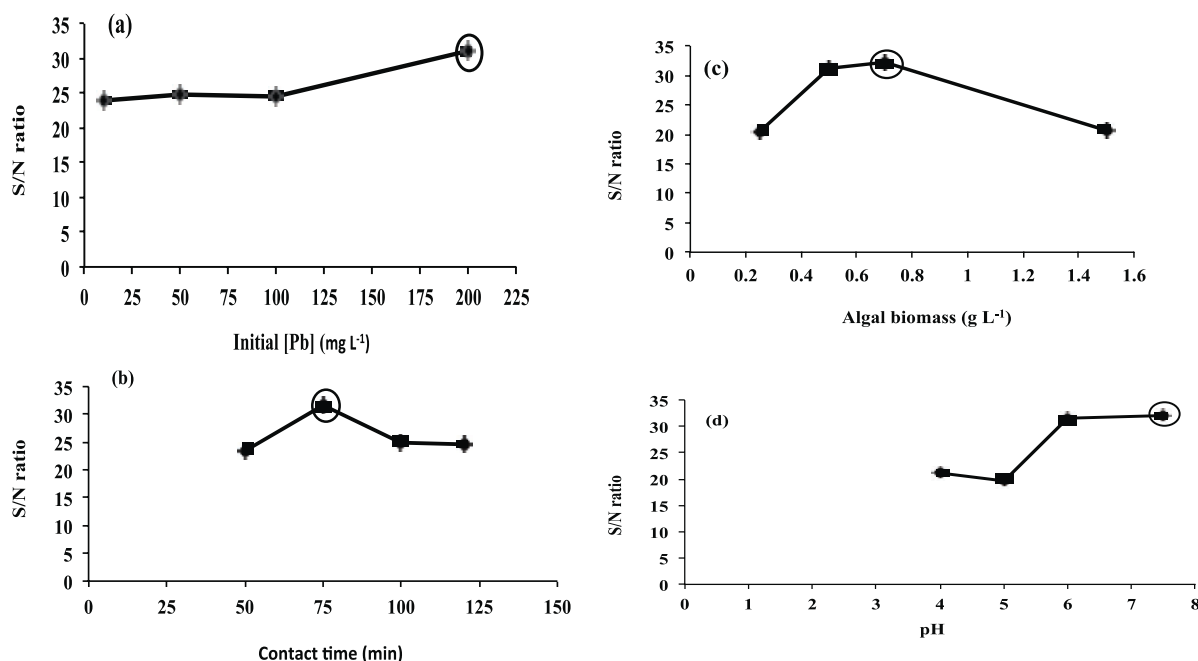
Fig. 3. Optimum conditions for the biosorption of Pb, (a) initial metal concentration, (b) contact time, (c) algal dose, and (d) pH. Circles indicate optimum level of each factor.
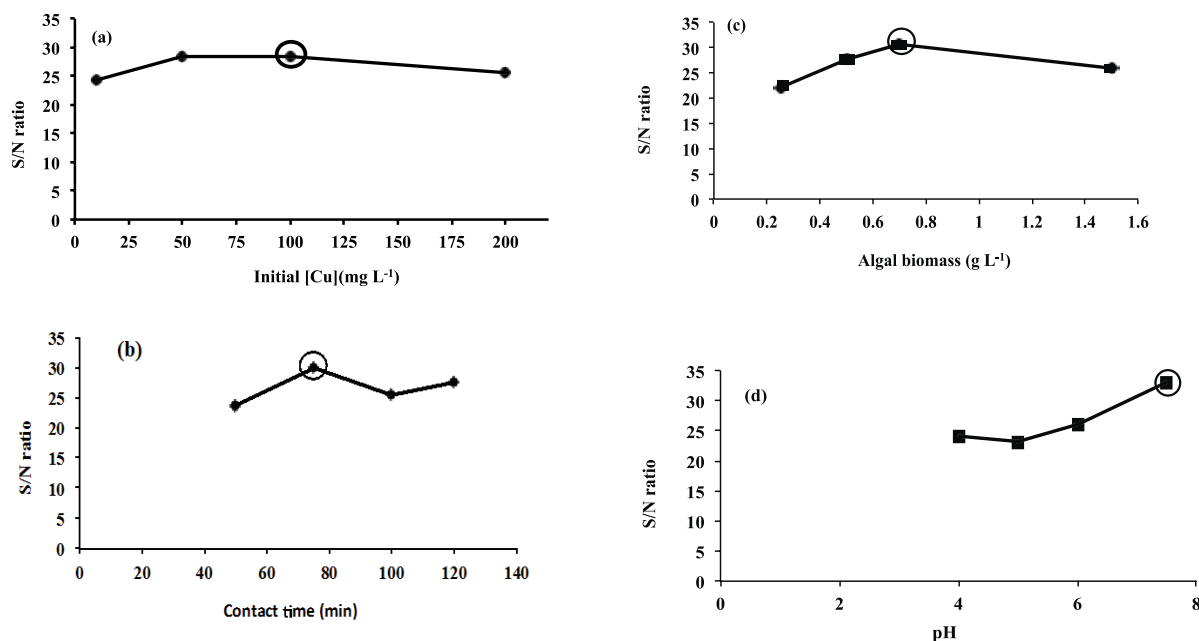

Fig. 4. Optimum conditions for the biosorption of Cu, (a) initial metal concentration (b) contact time, (c) algal dose, and (d) pH. Circles indicate optimum level of each factor.

of metal mainly Pb such as lead hydroxide will be formed. The hydrolysis and precipitation of metal ions may affect the removal process [22]. In this study, as the pH increased, metal adsorption was also increased and the maximum adsorption took place at pH 7.5. As shown in Table 4, the mean values of S/N ratio obtained for Pb at pH 6 and 7.5 were 31.65 and 32.14, respectively (Fig 3(d)). The increase in biosorption is due to the strong relation of sorption of lead on to the cell

surface negative charges and which in turn is dependent on the dissociation of functional groups [22]. On the other hand, many studies have shown that optimum biosorption conditions vary with the type and structure of the biosorbent. Zolfaghari et al. [31] have used the Taguchi optimization approach for the removal of Pb (II) and Hg (II) from aqueous solutions by modified mesoporous carbon. The maximum metal removal efficiency was obtained in 120 min with

10 mg L^{-1} of initial metal concentration, 0.7 g L^{-1} of sorbent dose at pH 6 [31]. Ibrahim et al. [32] also showed that the maximum metal adsorption by *ulva lactuca* activated carbon was obtained with 0.8 g L^{-1} of adsorbent at pH 5 for Cu^{2+} and 6 for Pb^{2+}. In addition, the optimum conditions for the biosorption of hydrazine by *Sargassum ilicifolium* determined by Taguchi method were initial hydrazine concentration of 135 mg L^{-1}, pH 7.5, biosorbent dose of 0.1 g L^{-1} in 90 min [33]. The Taguchi optimized conditions for the maximum removal efficiency of Cu and Ni by *Aspergillus sp*. were found to be 15% v/v biosorbent concentration, initial metal concentration of 50 mg L^{-1} at pH 4 and 30°C [34]. On the other hand, the best conditions for biosorption of Cd (II) by *Carpinus betulus* tree leaves found by Taguchi were pH 4.8, sorbent dose of 2 g L^{-1}, initial metal concentration of 140 mg L^{-1}, and sorbent size of 0.12–0.17 mm at 25°C [35].

3.2.3. Confirmation tests

According to the obtained results, the optimum conditions for both metals removal are agitation time 75 min, algal dose 0.7 g L^{-1}, and pH 7.5. The optimum metal ion concentration is 200 mg L^{-1} for Pb and 100 mg L^{-1} for Cu. In order to verify the optimum conditions deduced from the experiments, confirmation tests were conducted for biosorption of Pb and Cu metal ions at optimal level of each process factor. The difference between the results obtained under optimum conditions and that of test 9 are shown in Table 6. The values of the S/N ratio under optimum conditions are 38.93 and 36.70 for Pb and Cu, respectively. These values are approximately the same as the values obtained in test 9 (38.84: Pb; 36.85: Cu). In addition, the average MRE obtained in test 9 (87.59%: Pb; 69.65%: Cu) are approximately the same as the average MRE obtained under optimum conditions (88.36%: Pb; 68.4%: Cu).

3.2.4. Percentage of contribution

The average value of the measurement results of each factor (F) in each level (K) and the average of total MRE for Pb(II) and Cu(II) are listed in Table 7. The $\overline{\text{MRE}}_K^F$ values were obtained from MRE$_i$ values shown in Table 3. The percentage contribution of each Factor R_F, was determined using equation (Eq. (4)) and the obtained values are presented in Table 8. Pb biosorption process by algal biomass seems to be mostly affected by the biosorbent dose and the pH of exposure solution with approximately the same percentage contributions of 34.54% and 31.57%, respectively. Lower contribution of the initial metal ion concentration (11.95%) and the contact time (10.57%) on Pb biosorption was observed (Fig. 5). On the other hand, the most influential factor on Cu biosorption is the pH. The percentage contribution of pH factor for copper is 40.85% higher than the percentage contribution of the biosorbent dose (16.79%). The initial metal concentration

Table 8
Percentage contribution of each factor (R_F (%))

Metal	Factor	DOF$_F$	SS$_F$	R_F (%)	SST	V$_{Er}$
Pb (II)	A	3	2353.37	10.57	19950.41	81.11
	B	3	2625.10	11.95		
	C	3	7133.06	34.54		
	D	3	6540.98	31.57		
Cu (II)	A	3	645.27	2.55	9528.97	133.91
	B	3	444.29	0.45		
	C	3	2001.93	16.79		
	D	3	4294.80	40.85		

Table 6
Confirmation of the optimum biosorption conditions for both metal ions

	A	B	C	D	MRE$_1$	MRE$_2$	S/N
Test 9 for Pb	100	10	0.7	7.5	88.37	86.81	38.84
Confirmation test for Pb	75	200	0.7	7.5	89.06	87.67	38.93
Test 9 for Cu	100	10	0.7	7.5	68.63	70.68	36.85
Confirmation test for Cu	75	100	0.7	7.5	67.22	69.58	36.70

Table 7
Average of the measurement results of each factor in the kth level and the average of total MRE for Pb(II) and Cu(II)

Metal	Level	$\overline{\text{MRE}}_K^A$	$\overline{\text{MRE}}_K^B$	$\overline{\text{MRE}}_K^C$	$\overline{\text{MRE}}_K^D$	$\overline{\text{MRE}}_T$
Pb(II)	1	21.30	41.817	21.327	14.600	31.64
	2	44.64	19.000	41.658	20.427	
	3	33.03	27.315	50.293	44.683	
	4	27.59	38.429	13.283	46.852	
Cu(II)	1	21.00	29.767	18.081	25.798	27.48
	2	33.39	30.02	27.558	15.278	
	3	26.42	29.063	39.832	22.39	
	4	29.09	21.05	24.428	46.433	

and the contact time seem to have no significant influence on the adsorption process of Cu by algal biomass (Fig. 5). The obtained results indicate that the pH of the exposure solution has the most important contribution on the adsorption of both metals among the four controllable factors. As presented in Table 3 at pH 4, the average of Pb removal efficiencies in tests 1, 7, 12, and 14 are 3.26%, 15.43%, 27.59% and 12.12%, respectively, and the average of all of them is 14.60%. The average Cu removal efficiencies at pH 4 are 3.05%, 39.88%, 11.53% and 48.72%, respectively, and the average of all of them is 25.79%. However at pH 7.5, the average of Pb removal efficiencies in tests 4, 6, 9 and 15 are 20.49, 31.69, 87.59 and 47.64, respectively, and the average of all of them is 46.85%. For Cu, the average removal efficiencies are 36.89%, 38.63%, 69.65% and 40.55%, respectively, and the average of all of them is 46.43%. In other words, the average MRE at pH 7.5 was about 3 times higher for Pb and 2 times higher for Cu than the average MRE values obtained at pH 4.
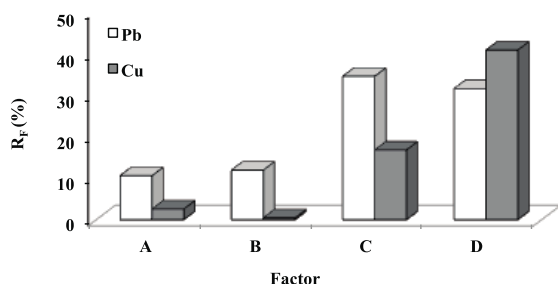
3.3. Equilibrium isotherms

Langmuir and Freundlich equilibrium adsorption isotherms were applied for the biosorption of Pb and Cu on algal biomass in order to characterize the interaction of metal ions with algal biomass (Fig. 6). Table 9 represents the estimated Langmuir and Freundlich sorption isotherm parameters along with their coefficient of determination (R^2) values for heavy metal removal by algal biomass at 25°C. The linearized forms of the Langmuir isotherm with the extremely high value of the correlation coefficient $R^2 > 0.99$ strongly support the fact that copper and lead biosorption process closely follows the Langmuir model of sorption (Table 9). This result suggests that a single surface reaction with constant activation energy is the predominant sorption step and possibly the predominant rate-controlling step [39]. However, the data are not well correlated to Freundlich correlation coefficients compared with the Langmuir correlation coefficients. Similar results were obtained in the literatures [18–22, 52–54]. It was shown that the equilibrium is well described by Langmuir isotherm with high correlation co-efficient values suggesting that metal ions are adsorbed in the form of a monolayer around the surface of the adsorbent [52–54]. Langmuir model assumes that the uptake of Pb and Cu occurs on a homogenous surface of the biosorbent with a finite number of adsorption sites, by monolayer adsorption without any interaction between adsorbed ions. Once a site is occupied, no further adsorption can occur at that site [52–54]. Other studies have shown fitting of the Freundlich model to the biosorption of Cu, Zn, Pb and Cd metal ions by *Nostoc muscorum* indicating that it followed the multilayer sorption theory-based Freundlich isotherm more accurately than the monolayer sorption theory-based Langmuir

Fig. 5. Percentage contribution of each factor on Pb and Cu biosorption by algal biomass.
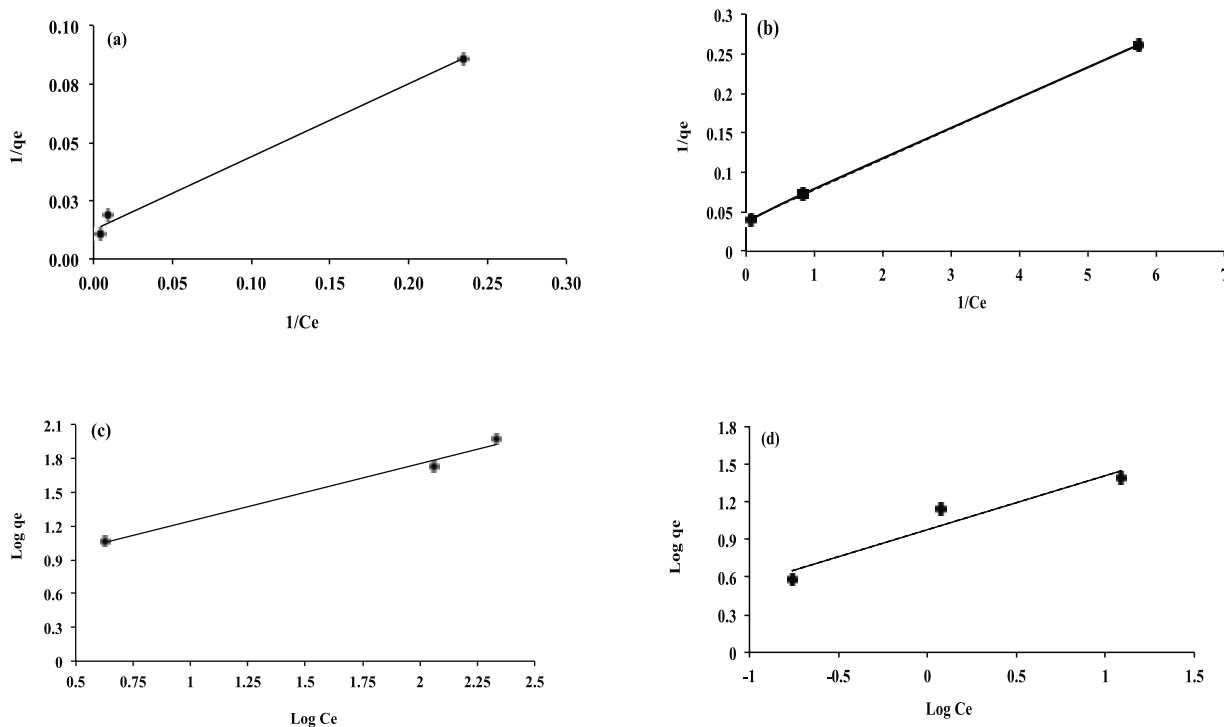
Fig. 6. Langmuir isotherms for Pb (a) and Cu (b) and Freundlich isotherms for Pb (c) and Cu (d) biosorption onto *A. ovalisporum*.

isotherm [16]. Winters et al. [51] reported that Cu and Ni ions sorption on living *Euglena gracilis* fitted Freundlich model. Also, the biosorption of copper (II) from aqueous solutions by *Aspergillus nige*r-treated rice straw was well described by Freundlich model [12]. On the other hand, Ferreira et al. [9] have studied the Langmuir and Freundlich biosorption isotherms of Ni^{2+}, Zn^{2+} and Pb^{2+} onto dry algal biomass, their determination coefficients of linear fitting values were sufficiently high to suggest that both models were able to well describe the sorption of metal ions, therefore, biosorption was likely to follow an intermediate behavior between a mono- and a multilayer adsorption mechanism [9]. On the

Table 9
Isotherm and kinetic parameters estimated for Pb and Cu biosorption by A. ovalisporum at 25°C

Isotherm model	Cu(II)	Pb(II)	Kinetic order	Cu(II)	Pb(II)
Freundlich			Pseudo-first		
n	2.320	1.958	K_1 (min^{-1})	$2.303.10^{-3}$	0.047
K_F (L g^{-1})	9.521	5.428	q_e (mg g^{-1})	64.10	87.57
R^2	0.9301	0.9872	R^2	0.904	0.993
Langmuir			Pseudo-second		
Q_m (mg g^{-1})	25.83	80	K_2 (g mg min^{-1})	0.021	1.506.10–3
b (L mg^{-1})	0.99	0.039	q_e (mg g^{-1})	2.78	9.04
R^2	0.999	0.993	R^2	0.997	0.948

Table 10
Maximum biosorption capacity based on the Langmuir isotherm of different adsorbents for Pb(II) and Cu(II)

| Metal ions	Adsorbent	Maximum adsorption capacity Q_m (mg g^{-1})	References
Pb(II)	Sawdust activated carbon	80.645	[53]
	Commercial activated carbon	20.3	[55]
	Nano-composite adsorbent	18.38	[56]
	Chitosan schiff's base@Fe$_3$O$_4$	83.33	[57]
	Multi-metal binding biosorbent	76.25	[21]
	Bacillus sp. PZ-1	9.30	[44]
	Bacillus sp. G1	76.8	[8]
	Arthrospira platensis	113.13	[9]
	Anabaena sphaerica	129.95	[58]
	Lepiota hystrix	3.89	[13]
	Spirogyra sp.	140.84	[50]
	A. ovalisporum	80	This study
Cu(II)	Activated carbon	43.47	[59]
	Nano-composite adsorbent	173.62	[60]
	Graphene oxide–chitosan	25.4	[61]
	Multi-metal binding biosorbent	41.06	[21]
	Bleached almond shell	18.71	[11]
	Fermented rice straw	23.8	[12]
	Activated watermelon shell	31.25	[54]
	Cystoseira indica	94.339	[24]
	Arthrospira platensis	40.65	[26]
	Lepiota hystrix	8.58	[13]
	Spirogyra sp.	151.57	[50]
	A. ovalisporum	25.83	This study
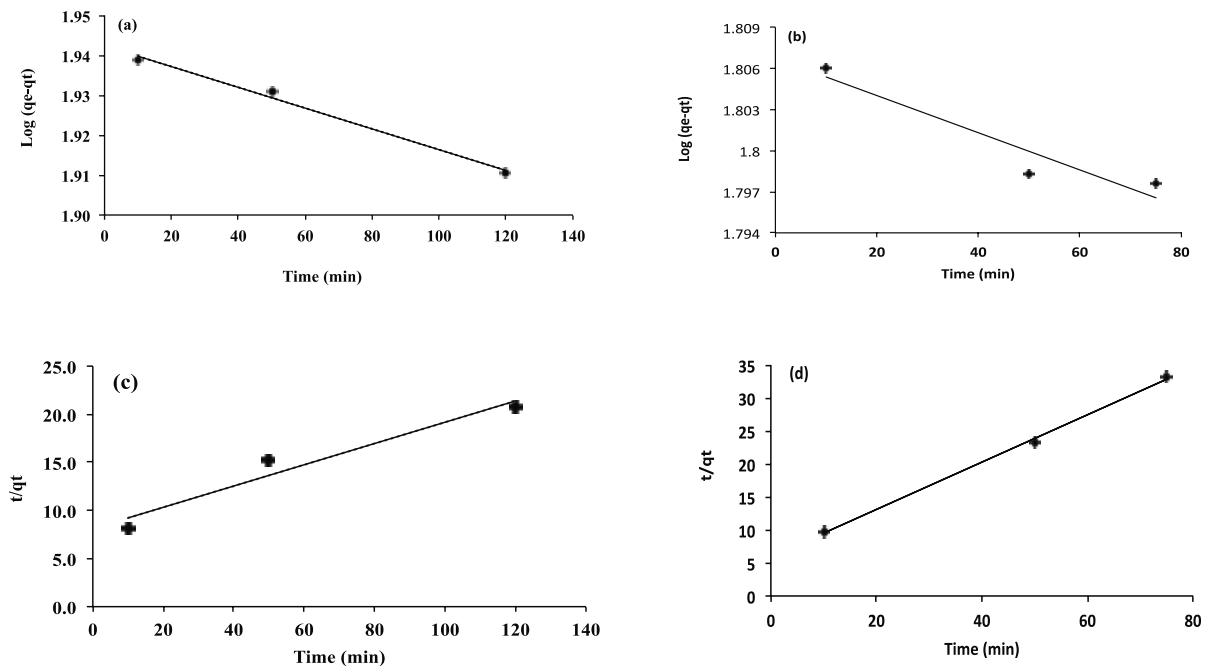
Fig. 7. Pseudo-first-order for Pb (a) and Cu (b) and pseudo-second-order for Pb (c) and Cu (d) biosorption onto *A. ovalisporum.*

other hand, the biosorption capacity of *A. ovalisporum* for Pb(II) (80 mg g^{-1}) was higher than for Cu(II) (25.83 mg g^{-1}), indicating the higher affinity of algal biomass for lead over copper ions. Table 10 shows the saturated monolayer sorption capacity of lead and copper on different adsorbents.

3.4. Kinetics

The kinetics of metals biosorption by *A. ovalisporum* were evaluated using Lagergren's pseudo-first-order kinetic [41] and Ho's pseudo-second-order kinetic models [42]. The results are shown in Fig. 7. The estimated kinetic parameters and the value of correlation coefficient R^2 corresponding to these models are listed in Table 9. These results show that the pseudo-second-order kinetic model accurately described the removal of Cu(II) by the algal biomass, where the value of correlation coefficient R^2 is relatively high (0.997). The obtained results are in agreement with the literatures [6,12,13,26,50]. The biosorption kinetic parameters of for Cu(II) and Pb(II) ion removal from aqueous solutions using the untreated *Anadara inaequivalvis* shells as a biosorbent were well described by the pseudo-second order kinetic model [6]. The pseudo-first-order and the pseudo-second-order were used to analyze the kinetic data of the adsorption process of Cu(II) from aqueous solutions by *Aspergillus niger*-treated rice straw and the data was fitted well with the pseudo-second-order kinetic model with R^2 values (close to the unity) [12]. The biosorption of copper onto *Lepiota hystrix* biomass follows second order kinetics [13]. According to the pseudo-second order kinetic model, the mechanism is in agreement with chemisorption or chemical adsorption as the rate-limiting step. In addition, Markou et al. [26] have shown that the biosorption of copper onto *A. platensis* biomass occurred mainly due to the mechanisms of ion exchange and complexation, and less

to physical adsorption. The linear regression analysis of the kinetic biosorption data showed that the model, which fitted best the experimental data, was the pseudo second-order model ($R^2 > 0.976$), while the pseudo first-order yielded relatively low R^2 value [26]. On the other hand, in the case of Pb(II) removal, the fit to the pseudo-first-order kinetic model was better with a value of 0.993 as correlation coefficient R^2 (Table 9). Recently, the pseudo first order describes the biosorption of Pb(II) from multi-metal aqueous solutions using multi-metal binding biosorbent (MMBB) [21]. Gunasundari and Kumar [52] have shown that the pseudo-first order model rather than the pseudo-second-order successfully described the biosorption kinetic data of Cu metal ions onto the dried algal biomass (*Spirulina platensis*). It was suggested that the adsorption data were well represented by pseudo-first-order and the rate-limiting step of Cu ions onto biosorbent may be physical adsorption or physisorption [52]. From the obtained results, it can be assumed that physisorption is the possible bisorption mechanism of Pb(II) by *A. ovalisporum* and the uptake rate is directly proportional to the amount of available active sites on the biosobent surface, whereas chemisorption is the possible mechanism of Cu(II) uptake by *A. ovalisporum* involving bond formation between the adsorbate and the adsorbent.

4. Conclusion

In the present study the cyanobacterial biomass *A. ovalisporum* has been found efficient for the removal of Pb(II) and Cu(II) from aqueous media. Scanning electron microscopy (SEM) showed that metal loaded cells affect the surface morphology of algal biomass. The change of the biosorbent surface morphology and elemental composition after metal treatment indicated the interaction between cyanobacterial cell wall and metal ions. The adsorption

mechanism of studied metal ions is probably due to an electrostatic attraction, a surface complex formation and chemical interaction between the metal ions and the functional groups of algal extracellular biopolymers. FT-IR analysis has proved that the amino, carboxyl, hydroxyl and carbonyl groups are the main binding sites for Pb(II) and Cu(II) on the surface of the biosorbent. Controllable factors such as initial metal ion concentration, solution pH, contact time and algal dose strongly affect the performance of the biosorbent. The maximum removal efficiencies of *A. ovalisporum* were 87.59% for Pb(II) and 69.65% for Cu(II) at optimum conditions. The optimum conditions found by the Taguchi method were initial concentration of 200 mg L^{-1} for Pb and 100 mg L^{-1} for Cu, algal dose 0.7 g L^{-1}, contact time 75 min and pH 7.5. The pH of solution seems to be the most significant factor with 40.85% and 31.57% contribution on the adsorption of Cu and Pb, respectively. The Langmuir model shows the best fit as equilibrium model compared with Freundlich model for both metal ions. The maximum monolayer adsorption capacities for the removal of Pb(II) and Cu(II) were 80 and 25.83 mg g^{-1}, respectively, at optimum conditions. The pseudo-second-order model kinetically describes the biosorption of copper while the pseudo-first-order describes the biosorption of lead. Further development of Taguchi's experimental design with a larger number of test runs and different set of parameters would provide a better understanding of the biosorption process and may increase the process efficiency.

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