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# Removal of lead from battery industry wastewater by *Chlorella vulgaris* as green micro-algae (Case study: Kerman, Iran)

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## ABSTRACT

Heavy metals are among the most common pollutants found in large concentrations in wastewater of industries, causing damage to the ecosystem. The aim of this study was to investigate the level of lead adsorption by *Chlorella vulgaris* from industrial wastewater. To determine the degree of lead adsorption by *Chlorella* adsorbent, the algae was brought in to contact under various conditions (pH, contact time, lead concentration, the adsorbent's level). Then, the residual amount was measured using atomic adsorption device. The experiments were performed under real conditions on a sample of battery industry wastewater in Kerman. The adsorption isotherms and the kinetic modeling of the metal ions on the adsorbent were determined based on Langmuir and Freundlich isotherm models as well as first-order and second-order kinetics. At the constant temperature of 25°C, optimal pH of 6, equilibrium contact time of 60 min, and adsorbent's level of 2 g/L, the lead removal efficiency in the real and synthetic samples was reported to be 89.26 and 93.2%, respectively. Based on the obtained results, lead adsorption followed Langmuir model and second-order kinetic equation. According to the results obtained from this study, it can be stated that Chlorella can be used effectively as an adsorbent for lead removal from industrial waste waters.

*Keywords:* Heavy metal; *Chlorella vulgaris algae*; Wastewater treatment; Battery industry; Isotherm models; Adsorption

## 1. Introduction

Emission of heavy metals in the environment, which is result of industrial development and population growth, is one of the major environmental problems in many countries. These metals are released in different industrial processes including melting, purification, and extraction of metals through emission of polluted gases or industrial waste waters in the environment. These metals are not biodegradable and their toxic effects are chronic in the body of living creatures [1].

Lead is a systemic toxin which attacks the majority of body organs following absorption. This element has a great tendency to blend with sulfuryl group (SH). The activity of the enzymes that are dependent on SH group halts or diminishes under its influence. This element acts in the place of calcium in the biological system of the body, such that around 90% of it accumulates in human skeleton. Further, it reacts with nucleic acid, thereby affecting protein levels and the biosynthesis. In children, lead mainly affects the central nervous system. It also influences the digestive system and causes colitis, loss of appetite, nausea, vomiting, and, constipation [2–5].

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The typical methods used in the treatment of wastewater containing heavy metals include chemical deposition, coagulation, ion exchange, solvent extraction, absorption and adsorption processes, microbial desalination cell (MDC), and reverse osmosis [6–8]. As physical and chemical methods are not efficient in eliminating low concentrations of heavy metals and are also very expensive [9], therefore in recent years, biological methods have been recommended for removing toxic metals [10,11].

Biological adsorption has attracted a great deal of attention thanks to the metal attachment capacities of various biological compounds such as algae, fungi, bacteria, and yeast which are highly efficient and cost effective especially at low concentrations of metals. Among these biological compounds, the capacity for adsorbing heavy metals has been proven to be greater through use of micro-algae [12,13].

Algae are employed considering their availability, high adsorptivity of metals, and inexpensiveness [6–14]. Malakootian et al. studied the efficiency of lead biosorption from industrial wastewater by *micro-alga Spirulina platensis* [15]. Al-Homaidan et al. studied the efficiency of *Spirulina platensis alga* in copper removal from aqueous solutions [16]. Qiao-Hui Shen et al. employed nonliving *Chlorella vulgaris* for hexavalent chromium detoxification [17]. Malakootian et al. used Ulothrix Zonata algae for removing heavy metals from industrial wastewater [18]. Rodrigues et al. in a study in Brazil compared dry biomass of Chlorella vulgaris and Spirulina platensis in removal of heavy metals [19]. Similarly, Ferreiraa et al. studied the efficacy of *Chlorella vulgaris* in heavy metal removal [7]. Aksu and Donmez examined the effect of concurrent adsorption of cadmium (II) and nickel (II) on dry biomass of *Cholera vulgaris* in Turkey [20].

As industrial wastewater is contaminated with heavy metals and considering the significance of this issue, This research examines the efficiency of *Chlorella vulgaris* micro-algae in removing lead from industrial wastewater.

## 2. Methods

### 2.1. Materials

This study has been experimental, conducted on laboratory scale and at the Environmental Health Engineering Research Center of Kerman University of Medical Sciences. In this study, the green-bluish micro-algae of *Chlorella vulgaris* were prepared from the Research Center of Caspian Sea Ecology and then cultured in The Minouche Research Laboratories (TMRL) culture medium. Thereafter, the resulting medium was kept at 25 ± 2°C, optical intensity of 1500 lux with a lighting period of 14 h and 10 h of darkness with regular aeration.

The stock solution of lead 1000 ppm was prepared by dissolving 1.59 g $Pb(No_3)_2$ (Merck Co, Germany) in 1 L of distilled water (pH = 7, EC = 9 μS, TDS = 3 ppm). Next, this stock solution was employed in order to prepare different concentrations of the lead solution. For adjusting pH, NaOH and nitric acid 0.1 normal were used.

Adsorption studies investigated the effect of different parameters including the concentration of the contaminant (50, 100, 150, 200, and 250 mg/L), the amount of the adsorbent (0.1, 0.5, 1, 1.5 and 2.0 g (dry weight)/L), contact time (5, 15, 30, 60, 120, and 180 min), and pH (3,4,5,6,7) in a batch system [21]. This range of parameters was determined having inspected the literature. All experiments were conducted on the synthetic wastewater with optimal conditions obtained. Then, under optimal conditions, the test was performed on a real battery wastewater.

A solution containing 90 mL of the contaminant and 10 mL of the adsorbent was poured into a flask with a capacity of 250 mL which was then placed on a shaker at 180 rpm within regular intervals.

Next, the contacted adsorbent was separated through a centrifuge device (centrifuge-150, Iran) and then the top liquid was passed through Wattman filter paper 0.45 micron. The residual concentration of lead in the experimented solutions was analyzed by flame atomic absorber device at the wavelengths of 283.3 nm (Youngling AAS 8020). The concentration of metals was stated in terms of mg/L in the liquid. The real sample was then taken from the wastewater equalizer reservoir of Kerman battery industry [15]. Thereafter, the quality of the wastewater was determined in terms of cations and anions present in it, as provided in Table 1.

To detect the functional groups involved in the adsorption by the adsorbent, Fourier transform infrared (FT-IR) spectroscopy device (VECTOR22, Bruker Co., Germany) was utilized, where the adsorbent was analyzed before and after contact with lead.

All of the experiments were performed based on the standard methods of physical and chemical experiments of water and wastewater, 20th edition (1998). For data analysis, SPSS 18 was employed, where first the data normality was determined and if the data were normal, Pearson test was used with an error percentage of 5%. To determine the adsorption isotherm, Langmuir and Freundlich adsorption isotherms were used, calculated in the form of Eqs. (1) and (2).

$$\frac{C_e}{q_e} = \frac{1}{(q_m.k)} + \frac{c_e}{q_m} \tag{1}$$

where $q_e$ represents the adsorption capacity at the time of equilibrium in terms of mg/g, $c_e$ denotes the equilibrium concentration (mg/L), $q_m$ is the adsorption capacity, and $k$ shows the Langmuir constant.

Table 1
The quality of wastewater of Kerman battery industry in terms of the anions and cations present in it

| Parameters of battery industry wastewater | Amounts |
|---|---|
| $Pb^{2+}$ (mg/L) | 52 ± 3 |
| $Cd^{2+}$ (mg/L) | 20.32 ± 1 |
| $Cu^{2+}$ (mg/L) | 2.8 ± 0.50 |
| Temperature (°C) | 22 ± 0.50 |
| pH | 5.5 ± 0.50 |
| BOD (mg/L) | 180 ± 15 |
| COD (mg/L) | 350 ± 19 |
| TDS (mg/L) | 1100 ± 116 |

$$\log q_e = \log k + \frac{1}{n} \log c_e \tag{2}$$

where, $K$ and $n$ are the Freundlich constants [22]. The adsorption kinetics was obtained by analyzing pseudo-first order and second-order kinetic models. Eq. (3) was used to determine the pseudo-first order kinetics:

$$\ln\left(1 - \frac{q_t}{q_e}\right) = -k_t \tag{3}$$

where, $q_e$ and $q_t$ represent the adsorption capacity at the time of equilibrium and the time of $t$, respectively, with $k_1$ being the constant coefficient. Eq. (4) was employed to determine the pseudo-second order kinetics [23,24]:

$$\frac{t}{q_t} = \frac{1}{\left(k_2 q_2\right)} + \left(\frac{1}{q_e}\right)t \tag{4}$$

where, $k_2$ represents the pseudo-second order adsorption constant rate (g/mg min).

### 2.2. Measurement methods

To measure the concentration of lead before and after contact with the adsorbent, atomic adsorption device (youngling AAS 8020) was used. This device has a gas tank on which two valves are devised specifically designed for turning the gas flow on and off and adjusting the pressure. As the first valve opens, the gas flow starts. Once the specific lamp of each metal with a specific number was installed on the device, in the part of adjusting the wavelength and flow of each lamp, the wavelength (nm) and specific current (mA) of the experimented metal were adjusted according to the catalog of the device.

After half an hour, when the device was warmed up, the degree of absorption of metal ions was measured by the device, where the standard curve was used for calculating the concentration of every metal ion. To detect the functional groups involved in the absorption by the adsorbent, FT-IR spectroscopy device (VECTOR22, Bruker, Germany) was used to analyze the adsorbent before and after contact with lead. FTIR spectrum is usually obtained from samples in the form of a film, which is usually thinner than 50 µm.

To prepare a suitable film out of the thicker samples or granules, this sample was heated up to above the softening temperature. It was then pressed in order to prepare films thin enough to be used directly in FTIR spectroscopy. Notably, one can also use solvent films. In this state, a small piece of the sample of interest is dissolved in a suitable solvent and by putting it on potassium bromide pills with complete evaporation of the solvent, the thin film of the sample directly forms on the KBr pill, since KBr has no absorption within the infrared region.

## 3. Results and discussion

### 3.1. The effect of pH on removal efficiency

The effect of pH on the capacity of lead adsorption is indicated in Fig. 1.

The effect of pH on Pb uptake capacity by algae *Chlorella vulgaris* was studied at a concentration of 50 mg/L, 25°C, 60 min contact time and adsorbents' concentration 2 g/L. The results of investigation of pH on the adsorption capacity of lead on the *Chlorella vulgaris* algae indicated that the greatest degree of removal and adsorption capacity occurred at pH = 6 (removal efficiency = 93.2%), while at values above and below this level, the adsorption capacity diminished.

Statistical analysis showed a significant relationship between pH and adsorption efficiency (*pv* = 0.26).

The pH exerts its influence on adsorption mechanism through affecting levels of cells, representing physiochemical-like reactions in the solution and the adsorption sites of the cells. Various strains of the algae might have different optimal pHs. Further, the optimal pH of adsorption is an organism-dependent parameter, as different microorganisms have also different adsorption sites [25].

Numerous studies have suggested that pH is the most important factor influencing the adsorbent's capacity. The effect of pH is usually determined considering the type of the adsorbate and the functional groups on the adsorbent [26]. Malakootian et al. examined copper removal using *Chlorella vulgaris* microscopic green algae from industrial wastewater. Congruent with the results of this research, they observed that at pH = 6, the adsorption of copper had the greatest level [27]. Mohseni et al. investigated the role of *Chlorella algae* and removing lead contamination as well as the parameters influencing it. They found that at pH = 6, the lead adsorption degree had the greatest efficiency [28].

### 3.2. The effect of contact time on removal efficiency

The effect of contact time on removing metal ions is demonstrated in Fig. 2.

With lengthening the contact time between the adsorbent and the lead-containing solutions, the adsorption efficiency was enhanced. This was due to increased contact and greater collision between the adsorbent body and lead, where 50% of the lead metal ions were removed in the first five minutes of contact. The most optimal degree of adsorption of lead ions occurred within 60 min of contact. The statistical analysis revealed no significant relationship between the contact time and adsorption efficiency (*pv* = 0.92).

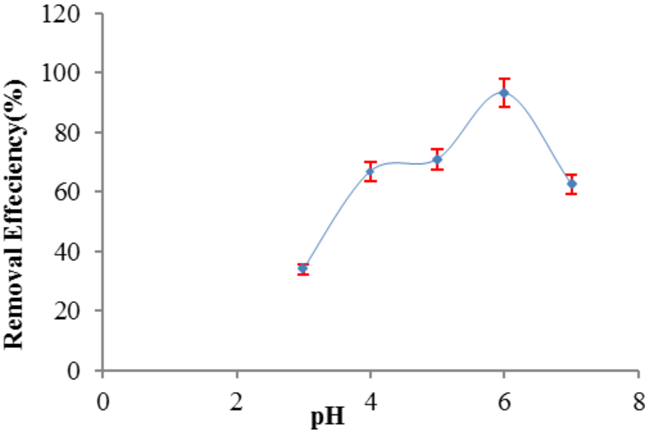

Fig. 1. The effect of pH on removing the lead ions ($t$ = 60 min, $m$ = 2 g/L and C = 50 mg/L).

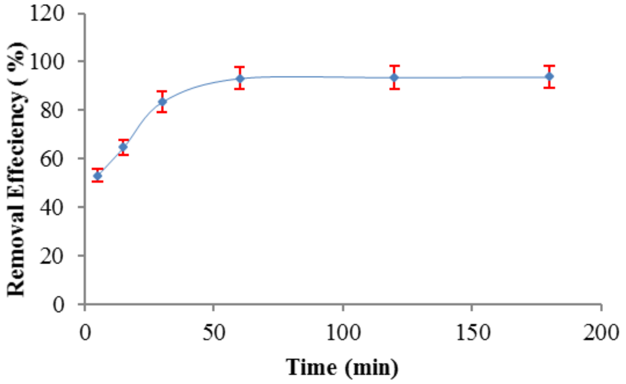

Fig. 2. The effect of contact time (min) on removing lead at pH = 6, adsorbent dose = 2 g/L, and initial concentration of lead = 50 mg/L.

In the research by Shokuhi et al. [29], it was also found that with the increase in the contact time, removal efficiency grew, and after 60 min it reached equilibrium. Across all concentrations over 0.85, the adsorption happened within the first 5 min [29]. Malakootian et al. indicated that the efficiency of zinc ($Zn^{2+}$) removal by *Chlorella vulgaris algae* reached its maximum within 60 min [30].

### 3.3. The effect of initial concentration on removal efficiency

To determine the maximum capacity of lead adsorption by *Chlorella algae*, the adsorption equilibrium data were investigated across different concentrations of the metal (Fig. 3).

As the initial concentration of lead increased from 50 to 250 mg/L, the degree of lead removal diminished from 93.2 to 38.56%. The statistical analysis revealed a significant relationship between initial concentration of lead and adsorption efficiency (*pv* = 0.001).

It was also observed that any elevation in the initial concentration of lead results in increased residual level of this metal in the solution. The reason might be the fact that with the increase in the superficial charge (adsorbate compounds) on the adsorbent, the adsorbent sites of the top levels on the adsorbent become saturated, and hence the removal efficiency of the adsorbent declines rapidly [31].

Ferreira et al. observed that increased initial concentration of each of the metals including lead, zinc, and nickel caused decreased removal efficiency and increased adsorption capacity of them [7]. Malakootian et al. examined lead adsorption from industrial waste waters by the green micro algae *Spirulina Platensis*. They observed that with the increase in the initial concentration of lead, the removal efficiency diminished while the adsorption capacity rose [15].

### 3.4. The effect of the amount of adsorbent on removal efficiency

The effect of adsorbent level on the lead removal is indicated in Fig. 4.

In any adsorption process, another important parameter is the amount of adsorbent. With the increase in the amount of the adsorbent, the removal efficiency and the adsorption capacity grew, such that with the increase in the adsorbent's level from 0.1 to 2 g, the degree of lead removal rose from 44.5 to 93.2%. At the adsorbent level of 0.1 g, the removal level was very low and equilibrium was obtained more rapidly. At lower doses, the adsorption sites are fewer, resulting in less adsorption [33].

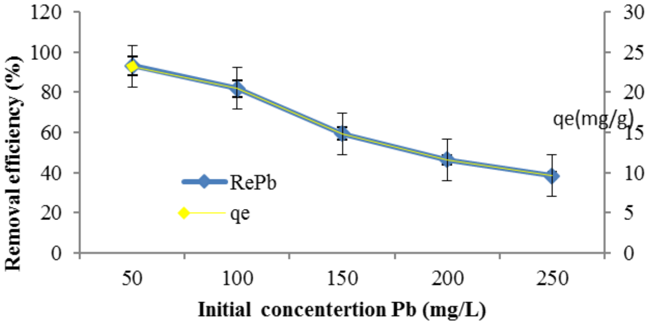

Fig. 3. The effect of the initial concentration of metal ions on the adsorbent at 60 min of contact time, adsorbent dose of 2 g/L, and the optimum pH.

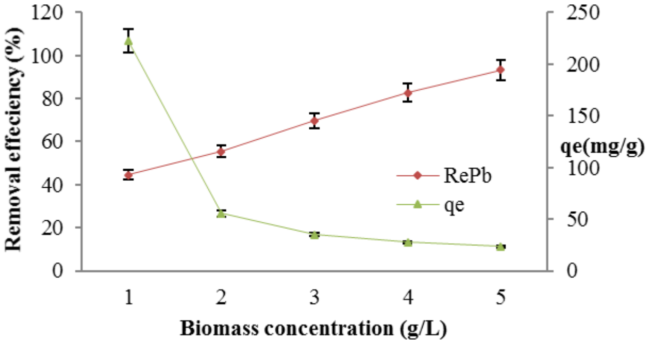

Fig. 4. The effect of the adsorbent weight (g/L) on the adsorption at the contact time = 60 min, lead initial concentration = 50 mg/L, and pH = 6.

Malakootian et al. investigated zinc removal in industrial wastewater using the microscopic green algae of *Chlorella vulgaris*, and reported 2 g/L as the optimal value of the adsorbent. They also showed that with the increase in the amount of the adsorbent, the removal efficiency also developed [30]. Further, the greatest level of adsorption was achieved at the adsorbent value of 2 g. The similar results obtained by Rezaei et al. confirm the validity of this claim [33].

### 3.5. Lead removal efficiency in the real sample

Table 1 presents the results obtained from investigating the wastewater quality of Kerman Battery Manufacturing Factory in terms of the anions and cations present in it. According to this table, the level of lead in raw wastewater is 52 mg/L. By applying optimal conditions (pH = 6, contact time = 60 min, adsorbent value = 2 g/L) to it, the lead removal efficiency in this factory was reported to be 89.26%. The efficiency of removing metal ions in the real wastewater sample has been lower compared to removing the synthetic sample. This is due to the competitive effect of metal ions with each other at adsorption sites on the adsorbent [15].

### *3.6. Equilibrium and kinetic studies*

For equilibrium studies, Langmuir and Freundlich isotherm models were used for lead removal efficiency. Fig. 5 displays the Langmuir and Freundlich isotherm models.

Comparing Langmuir and Freundlich isotherm models based on Fig. 5, it can be concluded that in removing lead by the microscopic green algae *Chlorella vulgaris* with $R^2 = 0.99$, it mainly follows the Langmuir model. Table 2 summarizes the parameters of isotherm models.

The equilibrium information obtained from adsorption of lead on *Chlorella* indicates that the process of lead adsorption mainly follows Langmuir rather than Freundlich model ($R^2 = 0.99$). Naeimi et al. in Iran investigated the degree of biological adsorption and the physiological effects of lead in *green algae Chlorella*. They concluded that the adsorption isotherm followed Langmuir model [35]. The $q_m$ obtained from Langmuir models represents the degree of adsorption of metal ions required for mono layer formation. This value for lead adsorption was 49.26 mg/g. In comparison with other adsorbents, it has a high adsorption capacity. Table 3 outlines this comparison.

The value of $1/n$ represents the intensity of adsorption. If it is equal to zero, then this is irreversible, while if it is between zero and one, then this is desirable. Finally, if is above 1, then this is undesirable [38]. The value of $1/n$ in this research has been below 1, suggesting desirable adsorption. Fig. 6 illustrates the pseudo-first-order and second-order kinetics.

The experimental results indicated that the adsorption kinetics with $R^2 = 0.99$ had the greatest correspondence with pseudo-second order reaction. Table 4 provides the parameters of the kinetic reactions.

Table 4 presents $K_1$ and $q_e$ values obtained from the log graph ($q_e - q_t$) versus $t$ as well as the correlation coefficient ($R^2$) along with $K_2$, $q_e$ and correlation coefficient by plotting the $t/q_t$ graph against $t$. $q_e$ and $q_t$ represent the adsorption capacity at time of equilibrium and time $t$ with $k_1$ constant coefficient, respectively. $k_2$ denotes the second-order constant rate of adsorption (g/mg min) [15]. The experimental results indicated that the adsorption kinetics with $R^2 = 0.99$ had the greatest correspondence with pseudo-second order reaction. The correspondence of the adsorption process with pseudo-second order model suggests that in the adsorption process, both reactions in parallel are effective in adsorbing the adsorbate on the adsorbent, rapidly reaching equilibrium. The second has a slow rate and continues for a longer time [32]. Malakootian et al. investigated adsorption of lead from industrial wastewater using the microscopic green algae of *Spirulina Platensis*. They found that adsorption of lead by this algae followed second-order kinetic adsorption model [15]. Naeimi et al. investigated the biological adsorption and physiological effects of lead in *green algae Chlorella*. They observed that adsorption of lead by these algae followed second-order kinetic adsorption model [35]. In the study by Chen et al. to determine the kinetic behavior of the adsorption of metals, it was found that adsorption of heavy metals in aqueous solutions followed second-order kinetics [39].

Table 2
The constant coefficients and correlation coefficients of Freundlich and Langmuir isotherms in the adsorption of lead

| Freundlich isotherm | | | Langmuir isotherm | | |
|---|---|---|---|---|---|
| $1/n$ | $K_f$ (mmol/g) | $R^2$ | $q_m$ (mg/g) | $k$ | $R^2$ |
| 0.18 | 0.11 | 0.87 | 49.26 | 0.22 | 0.99 |

Table 3
Comparison of adsorption capacity of *Chlorella vulgaris* with that of other adsorbents

| Entry | Adsorbent | Adsorption capacity (mmol/g) | References |
|---|---|---|---|
| 1 | *Pseudomonas aeruginosa* | 0.33 | [36] |
| 2 | *Rhizopus arrhizus* | 0.15 | [36] |
| 3 | *Chlorella vulgaris* | 0.47 | [current study] |
| 4 | *Brown algae Cladophora glomerata* | 0.35 | [37] |
| 5 | *Galaxaura marginata* | 0.12 | [36] |
| 6 | *Red algae Gracilaria corticata* | 0.26–0.20 | [37] |

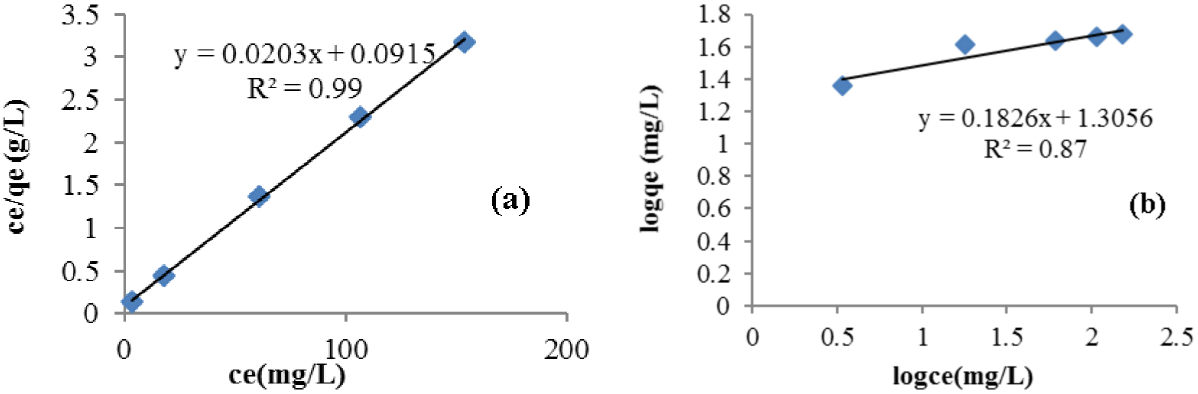

Fig. 5. Langmuir (a) and Freundlich (b) isotherm models for lead removal efficiency.

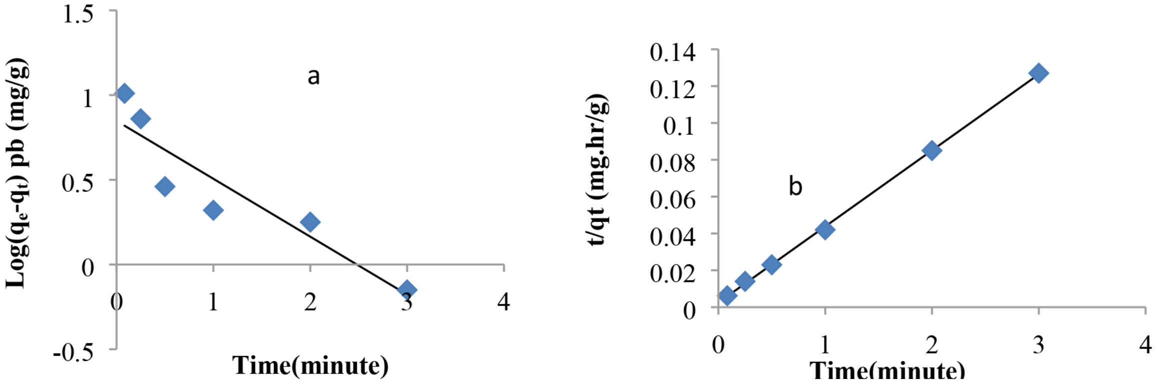

Fig. 6. First-order kinetics (a), second-order kinetics (b) of the lead removal reaction by *Chlorella vulgaris algae.*

Table 4
The constant coefficients and correlation coefficients of the pseudo-first-order and second-order kinetic model in the adsorption of lead

| Pseudo-first order kinetics | | | Pseudo-second order kinetics | | |
|---|---|---|---|---|---|
| $k_1$ (1/min) | $q_e$(mg/g) | $R^2$ | $k_2$(g/mg min) | $q_e$(mg/g) | $R^2$ |
| 0.78 | 7.03 | 0.84 | 0.68 | 24.21 | 0.99 |

### *3.7. FT-IR analysis*

The results of *Chlorella vulgaris* FT-IR analysis before and after lead adsorption are represented in Fig. 7.

The peaks at the wavelength of 3430 cm$^{-1}$ represent the presence of OH-stretching vibration group, which is a type of hydroxyl group. The picks at the wavelengths of 2984–2780 cm$^{-1}$ reflect the CH-stretching vibration group. The strong bands at the wavelengths of 1651.92 and 1545.25 cm$^{-1}$ can be attributed to types I and II vibrating amid. At 1455.82 cm$^{-1}$, COO- group, at the wavelengths of 1820–1660 cm$^{-1}$ carbonyl group, at the wavelengths of 2600–1800 cm$^{-1}$ C=N and C=O groups, at the peaks of 1250–1050 cm$^{-1}$ C-C and C-O groups, and at peaks lower than 1000 cm$^{-1}$ phosphate, -PO, O-P-O and C-X groups are active [7,40].

The comparison of the two spectra of the adsorbent before and after adsorption of the metal ions indicates that the reason of OH band widening is the high adsorption of lead. On the other hand, metal cations can develop one or several dual bonds simultaneously (i.e. development of ligand). The reduction in the relative intensity of 1649 cm$^{-1}$ can be a result of the interaction between the cation and non-bonding electron pairs of oxygen as well as nitrogen atoms, carboxyl group, and amide. The transformation and displacement of the bands of phosphate and carbohydrate groups is the result of metal adsorption in the spectra. The changes in the coordination of phosphate and hydroxyl groups might have happened due to attachment with the metal. Eventually, it can be stated that with the increase in the OH bond, the hydroxyl group could have been more involved in lead absorption. Based on this research and other studies performed on the adsorption of metal ions by the *micro algae green Chlorella vulgaris* and other algae in aqueous solutions, it can be concluded that adsorption of lead ions on the adsorbent is mainly dependent on existence of the functional groups including hydroxyl (OH$^-$), carboxyl (–COO–), amide, and phosphate [7,15,40].

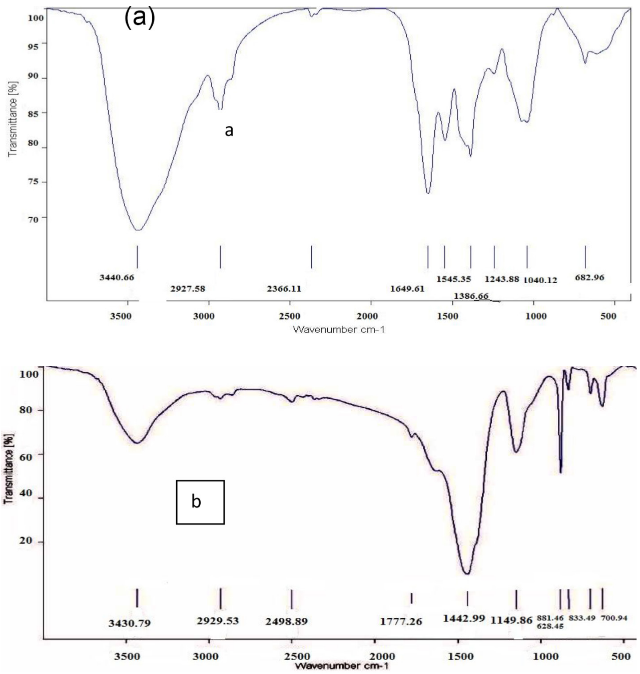

Fig. 7. FTIR spectra of algae *Chlorella vulgaris* before (a) and after (b) the adsorption.

## 4. Conclusion

According to the obtained results, the lead removal by green algae *Chlorella vulgaris* followed Langmuir model and the second-order kinetic equation. These algae have a suitable efficiency for removing lead from the wastewater of the battery industry. Furthermore, as the degree of adsorption of metals using this adsorbent is highly dependent on the pH of the solution, the initial concentration of the metal ion, contact time, and adsorbent levels, it can be concluded that adsorption of lead ions on the adsorbent is mainly dependent on existence of the functional groups of hydroxyl ($OH^-$), carboxyl (–COO–), amide, and phosphate. It is suggested that for achieving a greater removal efficiency, the optimal states of the mentioned variables be investigated when using *Chlorella vulgaris* as the biological adsorbent in removing heavy metals from other industrial waste waters.

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## References

[1] S.R. Shukla, R.S. Pai, Adsorption of Cu(II), Ni(II) and Zn(II) on modified jute fibers, Bioresour. Technol., 96 (2005) 1430–1438.

[2] N.Y. Salkhouri, A. Nouhi, Optimization of the conditions for removing lead by Saccharomysis Servisiae under laboratory conditions, J. Ghazvin. Univ. Med. Sci., 15(2) (2011) 13–21.

[3] M. Malakootian, Z. Darabi-Fard, N. Amirmahani, A. Nasiri, Evaluation of arsenic, copper, lead, cadmium, and iron concentration in drinking water resources of central and Southern Bardsir plain, Iran, in 2014, J Kerman Univ Med Sci., 22(5) (2015) 542–554.

[4] M. Malakootian, N.A. Jaafarzadeh, H. Hossaini, Efficiency of perlite as a low cost adsorbent applied to removal of Pb and Cd from paint industry effluent, Desal. Water Treat., 26 (2011) 243–249.

[5] M. Malakootian, J. Nouri, H. Hossaini, Removal of heavy metals from paint industry's wastewater using Leca as an available adsorbent, Int J. Environ. Sci. Technol., 6 (2009) 183–190.

[6] M. Malakootian, M. Hashemi, A. Toolabi, A. Nasiri, Investigation of nickel removal using poly(amidoamine) generation 4 dendrimer (PAMAM G4) from aqueous solutions, J. Eng. Res., 6(2) (2018) 13–23.

[7] L.S. Ferreira, M.S. Rodrigues, J.C.M.d. Carvalho, A. Lodi, E. Finocchio, P. Perego, A. Converti, Adsorption of $Ni^{2+}$, $Zn^{2+}$ and $Pb^{2+}$ onto dry biomass of *Arthrospira (Spirulina) platensis* and *Chlorella vulgaris*. I. Single metal systems, Chem Eng J., 173 (2011) 326–333.

[8] M. Malakootian, H. Mahdizadeh, A. Nasiri, F. Mirzaienia, M. Hajhoseini, N. Amirmahani, Investigation of the efficiency of microbial desalination cell in removal of arsenic from aqueous solutions, Desalination, 438 (2018) 19–23.

[9] M. Malakootian, L.R. Kalankesh, M. Loloi, Efficiency of hybrid nanoparticles of $TiO_2/SiO_2$ in removal of lead from paint industry effluents, J. Mazandaran Univ. Med. Sci., 23 (2013) 244–254.

[10] N. Goyal, S.C. Jain, B. UC, Comparative studies on the microbial adsorption of heavy metals, Adv Environ Res., 7 (2003) 311–319.

[11] M. Malakootian, A. Almasi, H. Hossaini, Pb and Co removal from paint industries effluent using wood ash, Int. J. Environ. Sci. Technol., 5 ( 2008) 217–222.

[12] N. Akhtar, M. Iqbal, S.I. Zafar, J. Iqbal, Biosorption characteristics of unicellular green *alga Chlorella sorokiniana* immobilized in loofa sponge for removal of Cr(III), Environ Sci., 20 (2008) 231–239.

[13] M. Malakootian, A. Toolabi, S.G. Moussavi, M. Ahmadian, Equilibrium and kinetic modeling of heavy metals biosorption from three different real industrial waste waters on to *Ulothrix Zonata Algae*, Aust J. Basic Appl. Sci., 5 (2011d) 1030–1037.

[14] S.K. Singh, A. Bansal, M.K. Jha, A. Dey, An integrated approach to remove Cr(VI) using immobilized *Chlorella minutissima* grown in nutrient rich sewage wastewater, Bioresour. Technol., 104 (2012) 257–265.

[15] M. Malakootian, Z.K. Limoni, M. Malakootian, The efficiency of lead biosorption from industrial wastewater by *Micro-alga Spirulina platensis*, Int. J. Environ Res., 10(3) (2016) 357–366.

[16] A. Al-Homaidan, J. Al-Houri, A. Al-Hazzani, G .Elgaaly, N.M.S. Moubayed, Biosorption of copper ions from aqueous solutions by *Spirulina platensis* biomass, Arab J. Chem., 7 (2014) 57–62.

[17] Q.-H. Shen, T.-T. Zhi, L.-H. Cheng, X.-H. Xu, H.-L. Chen, Hexavalent chromium detoxification by nonliving *Chlorella vulgaris* cultivated under tuned conditions, Chem. Eng. J., 228 (2013) 993–1002.

[18] M. Malakootian, A. Tolabi, Gh. Moussavi, Kinetic study and bio-absorption isotherm of heavy metals using the algae *Ulothrix Zonata* from industrial waste waters, J. Ilam. Univ. Med. Sci., 26 (2012) 37–44.

[19] M.S. Rodrigues, L.S. Ferreira, J.C.M.D. Carvalho, A. Lodi, E. Finocchio, A. Converti, Metal biosorption onto dry biomass of *Arthrospira (Spirulina) platensis* and *Chlorella vulgaris*: Multimetal systems, J. Hazard. Mater., 217–218 (2012) 246–255.

[20] Z. Aksu, Binary biosorption of cadmium(II) and nickel(II) onto dried *Chlorella vulgaris*: Co-ion effect on mono-component, Process Biochem., 41 (2006) 860–868.

[21] Z. Moussavi, A. Rahmati, Removing cadmium from aqueous environments using the nano structure γ-alumina, Water Wastewater J., 9 (2011) 4–20.

[22] A. SarI, Equilibrium, thermodynamic and kinetic studies on aluminum biosorption from aqueous solution by brown algae (*Padina pavonica* biomass), J. Hazard Mater., 171 (2009) 973–979.

[23] C. Chen, X.K. Wang, Adsorption of Ni(II) from aqueous solution using oxidized multiwall carbon nanotubes, Ind. Eng. Chem. Res., 45 (2006) 9144–9149.

[24] V.K. Gupta, A. Rastogi, Sorption and desorption studies of chromium (VI) from nonviable cyanobacterium nostoc muscorum biomass, J. Hazard. Mater., 154 (2008) 347–354.

[25] Z. Aksu, Competitive biosorption of phenol and chromium (VI) from binary mixtures onto dried anaerobic activated sludge, Biochem. Eng. J., 7 (2001) 183–193.

[26] A. Gholizadeh, M. Kermani, M. Farzadkia, B. Kakavandi, A. Rastegar, Y. Poureshgh, Equilibrium and kinetic models for the absorption of phenolic compounds on chemically modified marine algae: Sistosira index, J. Northern Khorasan Univ. Med. Sci., 4(4) (2012) 683–693.

[27] M. Malakootian, Z. Yousefi, Z.K. Limoni, Investigation of removal of copper by the microscopic green algae of *Cholera vulgaris* from industrial wastewater, J. Gorgan. Univ. Med. Sci., 18(4) (2016) 1–7.

[28] N. Mohseni, A. Naeimi, J. Sarmad, The role of the cholera algae in removing lead contamination and investigation of the parameters influencing it, the national conference of Iranian environmental research, J. Shahid Mofateh Univ., (2013 ) 73–76.

[29] R. Shokuhi, M. Siboni, N. Ghamar, S. Saeidi, Removing the acid blue dye (AB113) from aqueous solution through absorption on activated red flower: a kinetic and equilibrium study, J. Kurdistan Univ. Med. Sci., 60(2) (2011) 55–65.

[30] M. Malakootian, Z. Yousefi, Z.K. Limoni, Removing zinc from industrial waste waters using the micro-algae green of *Chlorella vulgaris*, J. Ilam. Univ. Med. Sci., 23(6) (2015) 40–50.

[31] F. Banat, S. Al Asheh, O. Hayajneh, Adsorption of phenol by bentonite, Environ. Pollut., 107(3) (2000) 391–398.

[32] A.H. Mahvi, A. Maleki, A. Eslami, Potential of rice husk and rice husk ash for phenol removal in aqueous systems, Amer, J. Appl. Sci., 1(4) (2004) 321–326.

[33] H. Rezaei, Biosorption of chromium by using *Spirulina sp*, Arab J. Chem., 11 (2013) 1–8.

[34] M. Malakootian, A. Khazaei, Comparing the efficiency of the nanoparticles of the mixture of zero Valent iron and manganese in removing cadmium ion from aqueous solutions, J. Ilam. Univ. Med. Sci., 22(2) (2013) 93–103.

[35] A. Naeimi, J. Sarmad, N. Mohseni, Investigation of the degree of biological absorption and physiological effects of lead in the green algae *Chlorella*. MSc. Thesis at Guilan Univ., (2013) 50–56.

[36] J. Rincon, F. González, A. Ballester, M.L. Blázquez, J.A. Muñoz, Biosorption of heavy metals by chemically-activated alga Fucus vesiculosus, J. Chem. Technol. Biotechnol., 80 (2005) 1403–1407.

[37] E. Romera, F. Gonzalez, A. Ballester, M.L. Blazquez, J.A. Munoz, Biosorption with algae: a statistical review, Crit. Rev. Biotechnol., 26 (2006) 223–235.

[38] A. Mesdaghinia, A. Mahvi, M. Fazlzadeh, M. Dehghani, B. Heybati, Z. Daliri, Comparing the efficiency of natural Pamis and modified with iron in removing activated Black 5 dye, J. Northern Khorasan Univ. Med. Sci., 5(2) (2013) 449–457.

[39] ZMW. Chen, M. Han, Biosorption of nickel and copper onto treated alga (*Undaria pinnatifida*): application of isotherm and kinetic models, J. Hazard. Mater., 155 (2008) 327–333.

[40] A.M. Pistorius, W.J. DeGrip, T.A. Egorova-Zachernyuk, Monitoring of biomass composition from microbiological sources by means of FT-IR spectroscopy, Biotechnol. Bioeng., 103 (2009) 123–129.