Original Article

Adsorption of lead by Microalgae Chaetoceros Sp. and Chlorella Sp. from Aqueous Solution

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Abstract

Introduction: Using bio-sorbents is regarded as one of the effective methods to remove heavy metals. Therefore, this study aimed to investigate Pb adsorption from contaminated water by Chaetoceros sp. and Chlorella sp. algae.

Materials & Methods: This experimental study was conducted in a batch reactor. The amount of Pb adsorption by Chaetoceros sp. and Chlorella sp. algae was studied under such conditions as different adsorbent dosages (0.2, 0.5, 1.5 and 2 g/L), different pH (3, 4, 5, 6, 7, 8), different temperatures (20, 25, 30, 35, 40 °C), different contact times (30, 60, 90, 180, 360 minutes), different particle sizes (20, 40, 60, 100, 140 mesh) and different Pb concentrations (20, 40, 60 mg/L). Then adsorption isotherms and kinetic models of metal ions were determined based on Langmuir and Freundlich isotherms as well as first and second order kinetic models.

Results: The efficiency of Pb removal by Chlorella sp. and Chaetoceros sp. algae was reported 78% and 60% respectively. The maximum adsorption was observed at optimal conditions including 25°C, pH of 6, 180 minutes of contact time, particle size of 20 mesh, 1.5 gr/L of adsorbent and 20 mg/L concentration of Pb. Chlorella algae was observed to remove Pb more than Chaetoceros algae. Based on the study results, Pb adsorption by Chlorella algae complies with Langmuir adsorption isotherm model, whereas Chaetoceros algae follows Freundlich model. The kinetic models of algae adsorption followed a second order equation.

Conclusion: The study findings revealed that Chlorella algae can be effectively used in order to adsorb Pb from contaminated water due to its high efficiency of Pb adsorption.

Keywords: Adsorption; Algae; Chaetoceros sp; Chlorella sp; Pb

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Introduction

Heavy metals are components of the earth's crust. The most important aspect that distinguishes heavy metals from other dangerous wastes is their non-degradability. Therefore, to avoid environmental damage, it is necessary to prevent their discharge into running water. Lead is a heavy metal used in armaments, cosmetics, paint, battery making industries and building materials. Generally, water is one of the main sources contaminated by lead. Continuous adsorption of this element through various ways can cause such physical injuries as renal impairment. The standards set by the Environmental Protection Agency and the World Health Organization for discharging wastewater into the environment is 0.05 mg/L. Lead can be purified applying various techniques such as deposition in an alkaline environment and adsorption by natural and artificial adsorbents. Some algae can adsorb heavy metal ions and organic pollutants from the polluted waters. Output wastewater from fish farms often contains high levels of waste, which can cause problems for the adjacent farms and other aquatic environments. Algae use most of this waste as a food source and thus, it has been attempted to grow these algae on the wastewater of fish farms. Many algae demonstrate suitable capabilities for lead adsorption and as a result, a significant potential for wastewater purification.

Anastopoulos and Kyzas (2015) investigated the biosorption of heavy metals by algae and discussed about the alternative use of different algae (micro and macro) in raw or modified form as promising biosorbents for water or wastewater decontamination. Birungi and Chirwa (2015), in a study carried out on thallium adsorption by green micro-algae from water in South Africa, observed that these algae can be significantly effective in removing thallium from aqueous solutions. In another study conducted on the brown algae, Lou et al. (2015) found that Laminaria japonica brown algae is also effective in wastewater treatment. These algae tend to adsorb molybdenum and rhenium from the wastewater. Mitrogiannis et al. (2015) in Greece used Arthrospiraplatensis to biosorption of methylene blue from aqueous solutions. The biosorption results showed that A. Platensis could be employed as an efficient and eco-friendly biosorbent regarding the removal of cationic dyes. Temilselvan et al. (2011) studied the selective adsorption of heavy metals using algae species. In developing countries like Iran, urban and industrial wastewater together normally create municipal waste. Along with the development of urban wastewater systems, there is a necessity to seek appropriate methods in regard with the purification. There are many potential resources in nature to be applied for this purpose. Since algae have the ability to adsorb heavy metals, the present study intended to investigate the removal of lead as a heavy metal by Chlorella sp. And Chaetoceros sp. algae.

Materials and Methods

This was an experimental study conducted in a batch reactor. The algae samples were prepared...
in order to evaluate the effect of two types of algae on the lead removal from the contaminated water. *Chlorella* algae from the green algae and *Chaetoceros* algae from the brown algae were chosen out of the total available different algae in fish breeding and research centers. The prototype algae were taken from the Fisheries Aquatic Center of Hormozgan, IRAN. Canvey medium was used for cultivating the algae, each of which was added separately to this medium until the algae thrived. Large quantities of white light and proper aeration at 26°C were used to grow the algae. The optimum conditions such as temperature, salinity, turbulence, pH and culture medium was used to obtain maximum cell density of algae. Deposition and centrifugation methods were used to separate the algae from the solution and then the algae were dried in an oven. Characterization of functional groups in *Chlorella* and *Chaetoceros* algae was carried out by Fourier transform infrared spectroscopic (FTIR RX-1, Perkin Elmer, USA) at the room temperature.

Then the values of 0.2, 0.5, 1, 1.5, 2 g of dried algae were prepared separately and were applied on synthetic solution samples of lead with concentrations of 20, 40 and 60 mg/L. The effective parameters on the adsorption process were studied, including contact time (30, 60, 90, 180, 360 minutes), pH of solution (3, 4, 5, 6, 7, 8), temperature (20, 25, 30, 35, 40 °C) and the particle size of adsorbents (20, 40, 60, 100 mesh). In each test, one parameter changed and the other parameters were fixed at the optimal dose. Each test was repeated three times, and totally, 168 tests were conducted. Hydrochloric acid 0.1 N and NaOH were utilized to adjust the pH of the solution. After preparing solutions with an initial concentration of 20, 40 and 60 mg/L of lead by PbSO₄ at pH=6, 1.5 g of algae was added and adsorption test was conducted at time intervals of 30 to 360 minutes. At this stage, the optimum contact time was 180 minutes. Two types of algae (*Chaetoceros* and *Chlorella*) were applied to study the effect of different lead concentrations (20, 40, 60 mg/L), taking into consideration a contact time of 180 minutes and pH of 6. The experiments were carried out according to the standard methods for water and wastewater treatment[17]. Lead solution concentration was measured applying flame atomic absorption model (Shimadzu / AA-670)[18]. Thus, in order to reach the optimal conditions, experiments were conducted under different conditions of temperature, time, pH, lead solution concentration, adsorbent dosage level and the size of adsorbent. The removal efficiency of lead was also investigated at optimum conditions calculated by the equation 1[19].

\[
\text{%RE} = \frac{C_0 - C_t}{C_0} \times 100 \quad \text{(Eq. 1)}
\]

RE: Removal efficiency

\(C_0\): Initial lead concentrations (mg/L)

\(C_t\): Lead concentrations at the desired time(mg/L)

**Adsorption isotherm models**

There are a great many isotherm models concerning analysis of experimental data and description of adsorption equilibrium, such as Langmuir, Freundlich and Temkin. Freundlich isotherm model is derived for describing single-component adsorption equilibria on heterogeneous surfaces. Langmuir isotherm represents a single layer and uniform
adsorbent without interactions between adsorbed molecules. In the current study, the Freundlich (Equation 2) and Langmuir (Equation 3) models were used to determine the concentration of the adsorbed material\cite{20-21}.

\[ \log q_{eq} = \log K_f + \frac{1}{n} \log C_e \] (Eq. 2)

\[ K_f: \text{Adsorption capacity at unit concentration} \]
\[ \frac{1}{n}: \text{Intensity of adsorption} \]
\[ \text{If } \frac{1}{n} = 0, \text{the adsorption process is irreversible.} \]
\[ \text{If } 0 < \frac{1}{n} < 1, \text{it is desired.} \]
\[ \text{If } \frac{1}{n} > 1, \text{it is undesirable.} \]

\[ \frac{C_e}{q_{eq}} = \frac{1}{Q^0} + \frac{C_e}{Q^0} \] (Eq. 3)

In the Langmuir model, \( Q^0 \) and \( b \) are Langmuir parameters, which are the maximum adsorption capacity and associated energy, respectively. The equilibrium parameter (\( R_L \)) is the basis of the Langmuir isotherm, which is defined by equation,\( R_L = \frac{1}{1 + bC_0} \)\cite{22-23}. In this equation, \( C_0 \) is the initial concentration and \( R_L \) is the type of isotherms. \( 1 < R_L < 0 \) is optimal adsorption, \( R_L = 1 \) is for undesirable adsorption, \( R_L = 1 \) shows linear adsorption, and \( R_L = 0 \) demonstrates irreversible adsorption.

The pseudo-first order (Equation 4) and the pseudo-second order equations (Equation 5) were used in order to study the adsorbing kinetics\cite{24}.

\[ \ln (q_{eq} - q) = \ln q_{eq} - \frac{K_1}{230} t \] (Eq. 4)

\[ \frac{t}{q} = \frac{1}{K_2q_{eq}^2} + \frac{1}{q_{eq} t} \] (Eq. 5)

In this equation, \( q \) and \( q_{eq} \) represent the lead amount adsorbed for each gram of adsorbent at a specified time and equilibrium state. In addition, \( K_1 \) and \( K_2 \) are the first order and second order kinetic constants, respectively.

**Results**

**Characterization of the Adsorbents**

Characterization of these adsorbents were carried out via FTIR studies in order to identify the functional groups in the 4000–400 nm range. The obtained spectra indicate a number of adsorption peaks showing the complex nature of *Chaetoceros* and *Chlorella* algae.

The band observed at about 3430 cm\(^{-1}\) shows the presence of O-H stretching vibration group which is a type of hydroxyl group. The present picks at 2984–2780 cm\(^{-1}\) demonstrate C-H stretching vibration group. Strong bands at wavelengths of 1651.92 and 1545.25 cm\(^{-1}\) can be attributed to types I and II vibrating amid.

The \(-\text{COO} \) group was found to have peak at 1455.82 cm\(^{-1}\), the wave number (from 1660 to 1820 cm\(^{-1}\)) for carbonyl group, the wave number (from 1800 to 2600 cm\(^{-1}\)) for C=N and C=O groups, the wave number (from 1050 to 1250 cm\(^{-1}\)) for C-C and C-O groups, and the picks lower than 1000 cm\(^{-1}\) was found for phosphate, -PO, O-P-O and C-X groups\cite{25-26}. The different types of functional groups as observed during FTIR studies of *Chaetoceros* and *Chlorella* algae participate in the adsorption process. In the present study, the wavelength of hydroxyl decreased after adsorption, while that of carboxyl group increased and amid and phosphate groups were created. The presence of these functional groups and their chemical substances content caused electron cloud with negative bar that was of a great importance in adsorbing lead positive ions. The present study
and other studies related to adsorption of metal ions by *Chaetoceros* and *Chlorella* algae in aqueous solutions show that adsorption of lead ions onto adsorbent is mainly dependent on the presence of -OH, -COO, amide, phosphate and amine functional groups.

**Effect of contact time on Adsorption of lead**

The results of the present study regarding the effect of contact time on lead removal by *Chaetoceros* and *Chlorella* algae are depicted in Figure 1. In fact, the lead removal was increased by making an increase in the contact time. The maximum lead removal efficiency at 180 minutes (equilibrium time) was 78% and 60% by *Chlorella* and *Chaetoceros* algae respectively. The study data were analyzed applying linear regression, which showed a 3% removal rate increase by resulting from 30 minutes increase of contact time.

**Figure 1.** The effect of contact time on lead removal efficiency by *Chaetoceros* and *Chlorella* algae (adsorbent dose= 1.5 g/L, pH=6, T= 25 °C and Pb= 20 mg/L)

**Effect of pH on adsorption of lead**

The effect of pH on lead removal using *Chlorella* and *Chaetoceros* algae is demonstrated in Figure 2. The maximum lead removal by *Chlorella* algae was 78% at pH 6, at equilibrium time of 180 min. The lead removal decreased at pH>6 in the two algae, which this reduction reached 42% in the *Chaetoceros* algae. The results of one-way ANOVA revealed that pH has a significant effect on lead removal from aqueous solutions by the two algae.
**Figure 2.** The effect of pH on lead removal efficiency by *Chaetoceros* and *Chlorella* algae (adsorbent dose = 1.5 g/L, contact time = 180 min, T = 25 °C and Pb = 20 mg/L)

![Graph showing the effect of pH on lead removal efficiency by *Chaetoceros* and *Chlorella* algae.]

**Effect of the adsorbent dosage on adsorption of lead**

The effect of adsorbent dosage of *Chlorella* and *Chaetoceros* algae on lead removal is shown in Figure 3. The study results revealed that increasing the adsorbent dosage from 0.2 to 2 g/L caused the amount of lead removal at 1.5 g/L of adsorbent to reach the maximum of 78%, 68%, and 60%, respectively. The maximum value of reported lead removal efficiency was 78%, which occurred at 1.5 g/L of adsorbent by *Chlorella* algae.

**Figure 3.** The effect of the adsorbent dosage on lead removal efficiency by *Chaetoceros* and *Chlorella* algae (contact time = 180 min, pH = 6, T = 25 °C, and Pb = 20 mg/L)

![Graph showing the effect of adsorbent dosage on lead removal efficiency by *Chaetoceros* and *Chlorella* algae.]

**Effect of temperature on lead adsorption**

The effect of temperature on lead adsorption by *Chlorella* and *Chaetoceros* algae is presented in Figure 4 at 20 to 40 °C, 1.5 g/L dose of algae, at equilibrium contact time of 180 minutes and pH of 6.
Figure 4. The effect of temperature on lead removal efficiency by *Chaetoceros* and *Chlorella* algae (adsorbent dose = 1.5 g/L, contact time = 180 min, pH = 6 and Pb = 20 mg/L).

The lead removal efficiency was reported to change by increasing the temperature from 20°C to 30°C. The maximum removal efficiency (78%) occurred at 30°C by the *Chlorella* algae.

**Effect of particle size on lead adsorption**

Effect of adsorbent particle size of *Chlorella* and *Chaetoceros* algae is demonstrated in Figure 5 on lead adsorption at 35°C, concentration of 1.5 g/L algae, at equilibrium contact time of 180 minutes and pH of 6.

Figure 5. The effect of adsorbent particle size on lead removal efficiency by *Chaetoceros* and *Chlorella* algae (adsorbent dose = 1.5 g/L, contact time = 180 min, pH = 6 and Pb = 20 mg/L).

**Effect of initial metal ion concentration variation on lead adsorption**

The effect of the initial concentrations of lead (20, 40 and 60 mg/L) on removal efficiency of lead is shown in Figure 6 at equilibrium contact time of 180 minutes, pH of 6, 25°C and with a mesh size of 20 by two types of *Chlorella* and *Chaetoceros* algae.
The study results indicated that the maximum lead adsorption efficiency occurred at the concentration of 20 mg/L. The lead removal efficiency decreased by increasing the initial concentrations of lead from 20 to 60 mg/L. Moreover, the results of Langmuir and Freundlich isotherms are presented in Table 1.

**Adsorption isotherms study**

The results of Langmuir and Freundlich isotherms are presented in Table 1.

**Table 1. Compliance of Chaetoceros and Chlorella algae equilibrium data via Langmuir and Freundlich models**

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Langmuir</th>
<th>Freundlich</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NSD</td>
<td>$R^2$</td>
</tr>
<tr>
<td>Chlorella sp.</td>
<td>0.498</td>
<td>0.9839</td>
</tr>
<tr>
<td>Chaetoceros sp.</td>
<td>91.663</td>
<td>0.9204</td>
</tr>
</tbody>
</table>

NSD: Normalized Standard Deviation

According to the correlation coefficient obtained ($R^2 = 0.98$), the adsorption process of Chlorella algae follows the Langmuir model; and Chaetoceros algae follows the Freundlich model.

**Adsorption kinetics study**

The results of kinetic studies on the lead adsorption by Chlorella and Chaetoceros algae are reported in Table 2.

**Table 2. The values and the constants of lead adsorption kinetic models adsorb by Chlorella and Chaetoceros algae**

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>pseudo-first order model</th>
<th>pseudo-second order model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NSD</td>
<td>$K_1$ (mg/g)</td>
</tr>
<tr>
<td>Chlorella sp.</td>
<td>82.2</td>
<td>0.0036</td>
</tr>
<tr>
<td>Chaetoceros sp.</td>
<td>104.7</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

NSD: Normalized Standard Deviation
According to the results of kinetic investigations, the maximum correlation coefficient is shown in the pseudo-second order model.

**Discussion**

In this study, lead adsorption efficiency increased by raising the exposure time. Contacts and collision between the adsorber and the metal can be stated as the probable reason for this increase, observed at the equilibrium time of 180 minutes. Although an increase in the contact time up to 180 minutes led to an increase in the adsorption efficiency, the adsorption rate did not increase and remained constant through the temperature of more than 180 minutes. This phenomenon may be related to the large number of empty surface sites on the adsorbent surface during the initial stages of the adsorption process. Over time, the adsorbent material can hardly adsorb the surface sites. Chonjnacka et al. (2005) in Poland investigated the mechanism of lead and cadmium adsorption by the green algae and found that increased contact time can raise the metal removal efficiency.

In addition, Malakootian et al. (2011) in Iran investigated the adsorption of heavy metals by *Ulothrix Zonata* algae and proposed that maximum adsorption occurred at 60 min. The results of other investigations concerning contact time are consistent with the results of the current study.

The solution pH was considered to be an important variable which controls the Pb(II) adsorption at the adsorbent–water interface, which can get increased via raising the solution pH. Maximum Pb(II) removal was obtained at pH 6, and the removal efficiency was found to be 78% for *Chlorella* at the optimum pH value. Therefore, all the following experiments on adsorption of Pb(II) from aqueous solution were carried out maintaining the solution at pH 6. The pH effect can be explained considering the surface charge on the adsorbent material. At low pH, due to high positive charge density caused by protons on the surface sites, adsorption of Pb(II) ions was intensively decreased. As a matter of fact, it is probably due to the competition of protons and Pb(II) ions towards a fixed number of adsorption sites. The increased pH leads to electrostatic repulsion decreases due to reduction of positive charge density on the sorption sites, thus resulting in an enhancement of metal adsorption. The Pb(II) ions in the aqueous solution may undergo solvation and hydrolysis. The process involved for Pb(II) is as follows:

\[
Pb^{2+} + nH_2O \rightarrow Pb(H_2O)^{2+}_n \tag{6}
\]

\[
Pb(H_2O)^{2+}_n \rightarrow Pb(H_2O)^{n-1} + H^+ \tag{7}
\]

\[
nPb^{2+} + mH_2O \rightarrow Pb(OH)^{2n-m}_m + mH^+ \tag{8}
\]

A perusal of the literature on Pb(II) speciation shows that the dominant species are Pb(OH)_2 at pH>6 as well as Pb^{2+} and Pb(OH)^+ at pH<6. Maximum removal of Pb(II) was observed at pH=6 in regard with the *Chlorella* adsorbent. On further increase of pH > 6, adsorption decreases, thought the lead removal increases due adsorption. Moreover, a rise can be detected regarding lead hydroxide formation which is precipitated. The optimum pH value for adsorption was found to be 6 where Pb(OH)_2 precipitation does not occur.
The adsorption of Pb(II) ions on the surface of Chlorella can probably be explained by an ion exchange mechanism based on the surface complex formation model facilitated by the dissociation of functional groups present in Chlorella as shown below.

\[
\begin{align*}
2(-R-OH) + Pb^{2+} & \rightarrow 2(RO)Pb + 2H^+ \quad (9) \\
-R-OH + Pb(OH)^+ & \rightarrow (-RO)PbOH + H^+ \quad (10)
\end{align*}
\]

where R represents the matrix present in Chlorella.

Increased adsorption as well as increased adsorbent dosage can be attributed to the availability of larger surface area and more adsorption sites. At very low adsorbent concentration, the adsorbent surface becomes saturated with the Pb\(^{2+}\) ions and the residual Pb\(^{2+}\) ion concentration in the solution is large. With an increase in adsorbent dosage, the metal ion removal increases as well. Removal rates decreased when adsorbent dosage were less than 1.5 g and adsorption efficiency rate decreased when dosage was greater than 1.5 g, yet, adsorption capacity was increased. The study results are in line with the results of Malakootian et al.’s study, (2011) in Iran examining adsorption of heavy metals by Ulothrix Zonata algae, who demonstrated that the highest adsorption was obtained at 30°C [28].

In another study conducted on adsorption of lead and cadmium from aqueous solutions by green algae, Sari et al. (2002) observed that metal adsorption rate increased by increasing the temperature [20].

The results revealed that the maximum percentage of lead removal (78%) was carried out by Chlorella alga and 20-mesh adsorbent particle size, and the results showed that a decrease in particle size causes an increase in the adsorption capacity of Chlorella and Chaetoceros algae. In the adsorption process, smaller particle size of adsorber gives to a large surface area for adsorbates. The findings of the current study are consistent with those of Kumar et al. (2006) conducted in India concerning lead adsorption by the green algae as well as those of Ozer et al. (2008) who studied the adsorption of heavy metals by the green algae [35-36].
In the adsorption systems, concentration of the adsorbed chemical plays an important role as a driving force to overcome the resistance caused by mass transfer between solid and liquid phase. In this study, three different concentrations were used and the highest removal efficiency was observed at the concentration of 20 mg/L. In other words, lead removal efficiency decreased resulted from the increased concentration of lead. This can be associated with the increase in ion exchange bands where the initial concentration of metals is lower. By increasing the surface charge of the adsorbent material on the adsorbent, the adsorption locations on the adsorbent are saturated and removal efficiency of the adsorbent decreases rapidly \([37-38]\). Aksu et al. observed that increasing the concentration from 50 mg/L to 500 mg/L caused a reduction in removal efficiency as well as an increase in adsorption capacity \([39]\).

Investigating lead adsorption isotherms by *Chlorella* and *Chaetoceros* algae are shown in Table 1, which indicates that lead adsorption by *Chlorella* and *Chaetoceros* algae comply with the Langmuir and Freundlich models, respectively.

\( R_L \) in the range between 0 and 1 indicates the desired lead adsorption by the two algae. Freundlich isotherm coefficient indicated that the adsorption process does not follow this model. The accuracy of the adsorption isotherm models was confirmed by the normalized standard deviation factor (NSD) between experimental data and estimates of adsorption capacity. Lower NSD represents a more accurate estimation of \( q_c \). NSD, that can be determined by Equation 11 \([40]\).

\[
\text{NSD}(\%) = 100 \sqrt{\frac{1}{N-1} \sum_{i=1}^{N} \left( \frac{q_{i \text{exp}} - q_{i \text{cal}}}{q_{i \text{exp}}} \right)^2}
\]

\( q_{i \text{exp}} \) and \( q_{i \text{cal}} \) are the experimental and estimated capacities respectively and \( N \) represents the number of experiments.

The kinetic study, carried out to predict the adsorption rate in order to design and model the process, showed that the pseudo-second order kinetic model is the best model to determine the rate of reaction with this adsorbent. The correlation coefficients in this model were more than 0.90 and the \( q_{i \text{cal}} \) value was close to \( q_{i \text{exp}} \).

The adsorption process followed the pseudo-second order model demonstrating that in the adsorption process, two reactions have parallel effects on adsorption. The first reaction quickly reached equilibrium, whereas the second reaction was slow and took a longer time \([40]\).

NSD in pseudo-second order model was less than that of the pseudo-first order model. Pseudo-second order model was more consistent with the experimental data. In a study conducted by Chen et al. (2008) in China, to determine the kinetic behavior of lead and nickel adsorption by the green algae, the researchers found that the adsorption of heavy metals in aqueous solutions obeys second order kinetics \([41]\). In two studies conducted in Iran by Malakootian et al. (2011) and Gholizade et al. (2012) conducted a study in order to determine the kinetic behavior of metals adsorption, who proposed that adsorption of heavy metals in aqueous solutions follows the second order kinetic model \([28-39]\).

**Conclusion**

Lead removal efficiency by *Chlorella* and *Chaetoceros* algae were 78% and 60%, respectively. As a result, using these biological...
adsorbents is recommended. Due to the high cost of other removal methods, applying the *Chlorella* alga is an effective way to remove lead from wastewater effluents. The amount of adsorption capacity depends on such factors as pH, temperature, initial metal ion concentration, adsorbent size, adsorbent dose and contact time. Therefore, applying the optimal is recommended in order to achieve higher removal efficiency.

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**References**


