

Investigating the capability of chromium heavy metal removal and biodiesel production by three species of algae: *Scenedesmus acutus*, *Scenedesmus incrassatulus*, *Scenedesmus obliquus*

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ABSTRACT

One of the biggest problems which people face, is the crisis due to water shortage, which unfortunately our country is also at the center of this crisis. One of the methods to control this crisis is the reuse of water resources called wastewater treatment. Water pollution with heavy metals is one of the most serious environmental problems. Due to the inefficiency, high cost and high energy requirement of various heavy metal removal methods, the use of bio-absorbents such as microalgae has been considered. In this study, the efficiency of three species of microalgae including *Scenedesmus acutus*, *Scenedesmus incrassatulus* and *Scenedesmus obliquus* in absorption of chromium heavy metal was investigated. In order to know the effect of different parameters, different pH values (3 to 7), temperature (15 to 35 °C), absorbent contact time (30 to 150 minutes), amount of bio-absorbent (0.25 to 2 g) and metal concentration (20 Up to 200 mg / L) was tested. The results showed that the rate of heavy metals absorption by these three species was not significantly different. However, pH, algae density, pollutant concentration, time and temperature had a significant effect on the efficiency of algae in removing contaminants from the environment. The highest absorption of chromium occurred at pH of 6, temperature of 25 °C, density of 1.5 g microalgae, concentration of 150 mg / L chromium and 120 minutes of contact. Chromium absorption had no effect on lipid and biodiesel production by microalgae.

Keywords

Heavy Metals, Microalgae, *Scenedesmus*, Chromium

Introduction

Water pollution with heavy metals is one of the most serious environmental problems (Salam et al., 2011). Industries introduce many heavy metals such as lead, cadmium, copper, nickel, chromium, and zinc into various environments, including the aquatic environment (Gupta and Rastogi, 2008). Among these metals, more attention has been paid to chromium in recent years. Chromium is a heavy metal and due to its high hardness, resistance to corrosion and abrasion, it is widely used in manufacturing various alloys, transport industries, buildings and machinery, tanning and electronic industries. Chromium is often found as trivalent and hexavalent chromium. Trivalent chromium is a non-toxic species of chromium that is essential for the normal metabolism of carbohydrates and for regulating mammals blood sugar level in very small amounts (Li et al., 2006). Hexavalent chromium is much more toxic than trivalent chromium and has a higher solubility (Yilleng et al., 2013). The national standard of Iran has announced the allowed amount of chromium in drinking water as 0.05 mg / l (Eghbalpour and Manafi, 2013). Heavy metals do not die naturally and are very stable and become more toxic and dangerous compounds by passing the time. Therefore, they must be removed from contaminated environments by different methods (Iqbalpour and Manafi, 2013).

Common methods to remove this metal from wastewaters are the use of chemical reactions such as reduction and precipitation, reverse osmosis, electrodialysis, ultrafiltration, evaporation and ion exchange, in most of which large amounts of sludge containing toxic substances are produced. Other chemical methods are usually very expensive, inefficient and unsuitable (Sadough and Talaei, 2005). Also, these methods are not effective for concentrations of 1 to 100 mg / l (Yilleng et al., 2013). In recent years, bio-sorbents have been used to remove heavy metals in most researches (Han et al., 2007). Many species of algae are able to store large amounts of heavy metals, but green algae are of attention for their special nature in absorption and high surface-to-volume ratio (Han et al., 2007).

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Microalgae are a very diverse group of microorganisms that are mostly single-celled and photoautotrophic and are known as large producers in oceans and freshwater (Olaizola, 2003; Hoa et al., 2011). The rate of photosynthesis of microalgae is 10 to 50 times that of terrestrial plants (Chinnasamy et al., 2009) and the biomass of microalgae doubles in less than a day (Sudhakar and Premalatha, 2012). *Sandmus* microalgae is a green alga of the chlorophyll branch, which is a common species in freshwater. There are currently 171 known species of *Sandmus* algae, each with sub-branches. This microalga is present in the form of 4 to 8 colonies and has a eukaryotic structure. The optimum growth temperature of *Sandmus* is 20 ° C. Most algae grow best at 25 to 27 degrees Celsius, but *Sandmus* has also the ability to grow at low temperatures (Schmitts and Birage, 2012). This alga is commonly used as a standard microorganism in many marine and aquatic sciences technologies and water management studies (Zachleder et al., 1986).

The reason for researchers' attention to these algae is the rapid growth rate, high production and ability to bioremediate wastewater (Mohamed et al., 2017). So far, several studies have shown the ability of this microalga to remove heavy metals. Studies have investigated the ability to remove the heavy metal cadmium using the alga *Scenedesmus obliquus* (Chen et al., 2014; Monteiro et al., 2009). The removal of divalent cadmium from aqueous solutions by *Scenedesmus cerevisiae* has also been investigated (Helihor et al., 2014). A study by Perales-Vela et al. (2006) found that *Scenedesmus* was able to remove divalent cadmium, divalent iron, and divalent zinc. Removal of lead from aquatic solutions has been done by the alga *Sandmus vadricuada* (Derakhshan-e-Booroojeni and mirgaffari2017). Soltani et. Al. in a study showed the capability of *Scenedesmus quadricuada* microalgae to remove the heavy metal chromium. Senturk and Yildiz (2008) used algae *Chlorella vulgaris* and *Scenedesmus* sp. to remove heavy metals antimony, manganese, copper and nickel. Malakootian et al. (2016) showed that the alga *Chlorella vulgaris* has a high efficiency in removing copper from industrial wastewater.

In this study, the efficiency of three species of microalgae *Scenedesmus acutus*, *Scenedesmus incrassatulus* and *Scenedesmus obliquus* in absorption of chromium heavy metal was investigated. The effect of chromium absorption on lipid and biodiesel production by algae was also investigated.

Method

Heavy metal preparation:

To prepare the parent solution of the heavy metal chromium, potassium dichromate salt $K_2Cr_2O_7$ produced by the German company Merck was used. By adding one liter of water, the parent solution was prepared to create the required concentrations.

Absorbent preparation

Arian Gostar stocks were used to prepare *Scenedesmus acutus*, *Scenedesmus incrassatulus* and *Scenedesmus obliquus* microalgae. These stocks were stored in an isolated environment after transferring to laboratory due to the sensitivity of the stocks, and after sterilization of the medium and preparation of the culture medium the stocks were transferred into culture medium, all of which used BBM (Andersen, 2005). Control of environmental factors in the second stage of production was performed using a vertical tube photoreactor (Figure 1) (Afsharbaksh et al., 2020). During 15 days, all production factors were adjusted and controlled to be the same for all three algae in accordance with Table (1) (Farhadian and Jafari, 2015).



Figure 1- Vertical tube photobioreactor

Table 1 - Basic conditions for the production of three microalgae to use as absorbents for heavy metals

Culture medium	Nitrate composition of culture medium	Harvest time (Day)	Temperature (Celsius)	PH	Light duration (hours)	Light intensity (luxury)	CO ₂ (%)	production system
BBM	NaNO ₃	13	25	8±0.5	14	3000	15	Vertical photobioreactor

Biomass was collected after a two-week growth period. To avoid the effects of salt, the alga biomass was washed five times with distilled water. Samples were dewatered using a centrifuge. The samples were then dried in an oven at 40 ° C and sieved through a 200 microns sieve and used as an absorbent in the later stages of the experiment.

After preparing the absorbents to investigate the effect of parameters such as pH, etc. on the absorption of chromium by the microalgae *Scenedesmus acutus*, *Scenedesmus incass atulus* and *Scenedesmus opliquus*, a set of different values of these variables was tested (Table 2).

Table 2: Variables used to adsorb heavy metals by selected microalgae

Contact time	Temperature	PH	Biosorbent amount	metal concentration	Variable
minutes	Celsius	---	gram	mg per liter	Unit
30,60,90, 120,150	15,20,25,30,35	3,4,5,6,7	0.25,0.5, 1 1.5,2, 2.5	20,50,100,150,200	Executive range

All absorption experiments were performed by contact of biomass with metal ions identified in Erlenmeyer. Adjustment of pH of metal solutions was performed using one tenth of a molar of HCL hydrochloric acid and one

tenth of a molar of sodium hydroxide solution (Zulkali et al., 2009). Then certain amounts of algae were added to metal solutions with specific concentrations.

In the experiments, , amount of bio-absorbent, pH of solution, temperature of solution and time of bio-absorbent were examined, with respect to concentration. First, the metal with concentrations ranging from 20 to 200 mg / l as shown in the table above was considered and the rest of the factors were constant and an incubator shaker was used at 150 rpm for uniformity to obtain the best concentration for absorption and Then, according to the order mentioned above, this was done for the other variables.

After performing the tests, the samples were transferred to the atomic absorption laboratory to evaluate the absorption rate and the absorption rate of heavy metals was measured by the Elmer3110 Perkin atomic absorption device.

The following formula was used to calculate the absorption percentage:

$$\text{Biosorption}(\%) = \frac{C_f - C_i}{C_i} \times 100$$

Where C_i is the initial concentration (mg / L), C_f is the final concentration (mg / L) (Mirghaffari et al., 2015).

Algae harvest:

In this study, the mechanical method of centrifugation was used. In this method, algae were collected by Arsan cream Turkey separator centrifuge, which is used for separating suspended solids, and was in fact a cream separator device with a high volume and acceleration of 12,000 grams. After the algae precipitated and shrunk inside the rotor of the device, the condensed algae were collected and transferred to separate sterile containers. Due to the high number of samples, the replicates were removed and the result of three replications was used to evaluate the amount of biomass produced, the amount of lipids and biodiesel.

Lipid extraction

In the extraction process, the microalga biomass is contacted with a solvent to remove the oil from its cellular structure. Lipid extraction from microalgae is usually done by chemical and mechanical methods. Chemical methods include the use of traditional organic solvents (hexane, ether, chloroform, etc.), the use of supercritical fluid (SC-CO₂), extraction using the Soxhlet device. Among these methods, extraction using Soxhlet device (Figure 2) was used.

10 g of each dried biomass sample was slowly homogenized by a mortar and lipid extraction was performed by Soxtec 2050 according to the program, with each cycle including 25 minutes of boiling, 40 minutes of lipid extraction and 15 minutes of solvent recovery. All samples were extracted equally in three cycles. For extraction, three common solvents, diethyl ether, n-hexane and n-pentane with different boiling points and high purity (HPLC grade) were considered. To remove microalgae residues, the extracted lipid was filtered through a 45 µm filter. Each step of drying and lipid extraction was performed with three repetitions and the results were reported as average. After determining the lipid content, the dried lipid was dissolved in 4 ml of isopropyl alcohol and the amount of triglyceride in the lipid was measured (Xin et al, 2010).



Figure 2. Soxhlet device used to extract lipids

Biodiesel extraction:

Transesterification of fatty acids was performed in flasks containing sulfuric acid as a catalyst with a molar ratio of 1:40 methanol to oil extracted at 180 rpm for 5 hours. After that, two layers were formed, the upper layer containing biofuels and separated by petroleum ether (Dai et al., 2007).

Determining the percentage of lipid production:

The following formula was used to determine the above values (El-Fadaly et al., 2009).

$\text{weight of extracted oil} / \text{dry weight of biomass} \times 100 = \text{percentage of lipid production or lipid content}$

Determining the percentage of biodiesel production:

$\text{weight of extracted biodiesel} / \text{weight of lipid} \times 100 = \text{percentage of biodiesel production}$

The amount of lipid and biodiesel was measured before and after heavy metal absorption by algae and its difference was statistically analyzed. Expert Design software was used for statistical analysis (Soltani et al., 2015).

Results

The amount of chromium absorption by different algae was not significantly different (Figure 3). Changes in pH, temperature, alga density, pollutant concentration and time had a significant effect on metal absorption. The highest absorption of chromium occurred at pH 6 and the lowest absorption of chromium at pH 7 (Figure 4). The highest absorption of chromium was observed at 25 °C and the lowest absorption at 20 °C (Figure 5). The highest absorption of chromium metal was observed at a density of 1 g of alga and the lowest absorption of chromium metal was observed at a density of 150 mg / l chromium (Figure 7). The highest absorption of chromium occurred after 120 minutes of contact of microalgae with chromium metal (Figure 8). The interaction of pH and time, temperature and algal density and temperature and time on the amount of absorption was significant (Figure 9).

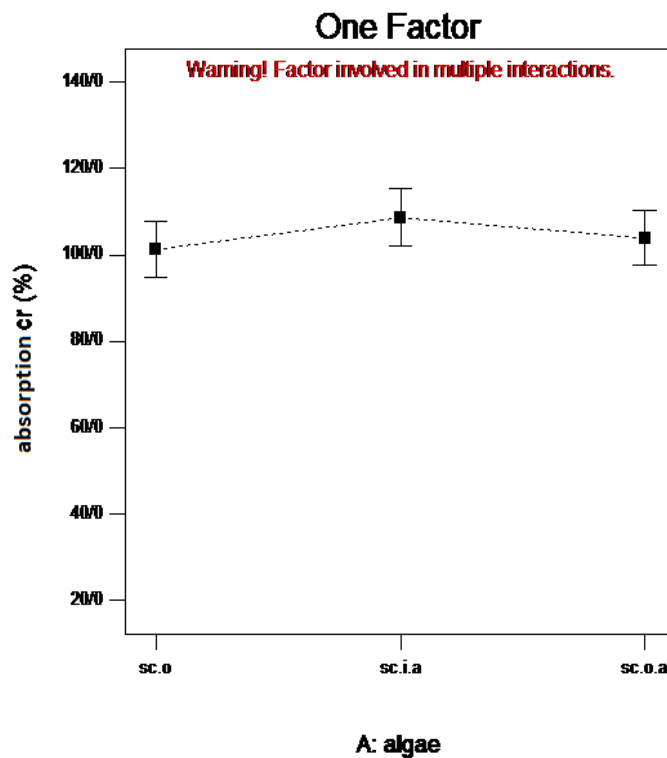


Figure 3 - The amount of chromium metal absorption by three species of microalgae tested

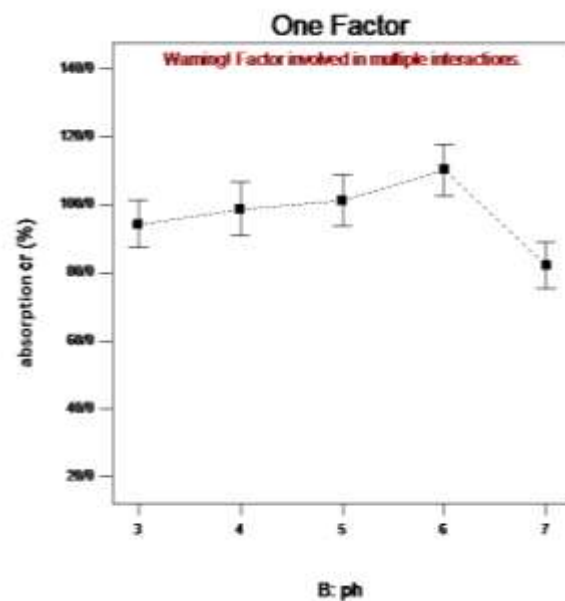


Figure 4 - The rate of absorption of chromium by microalgae at different pHs

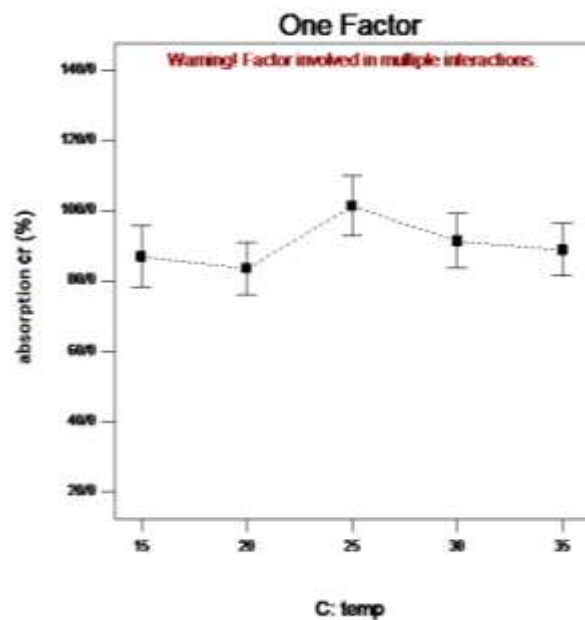


Figure 5 - The rate of absorption of chromium metal by microalgae at different temperatures

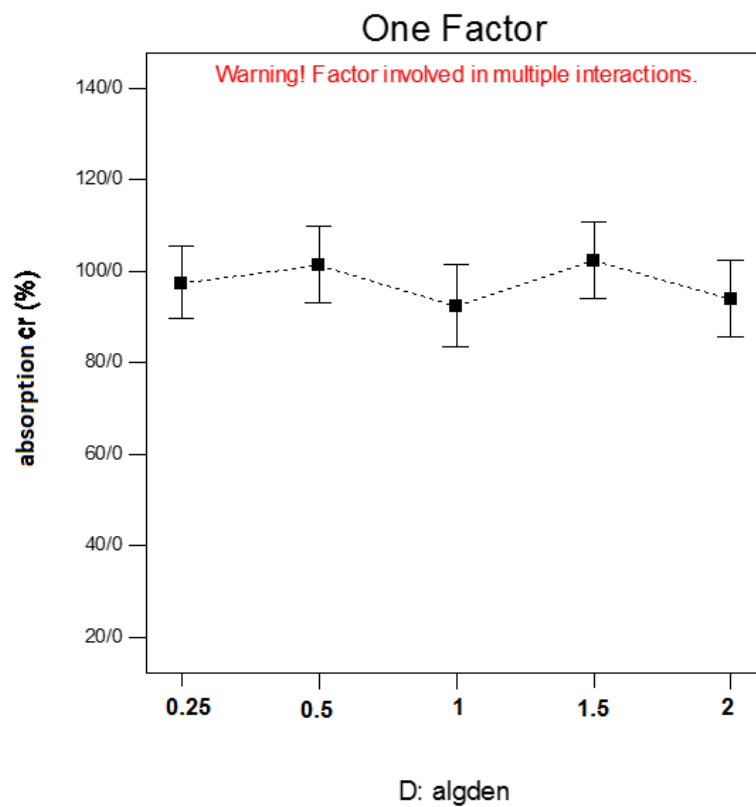


Figure 6 - The rate of absorption of chromium metal by microalgae in different densities of microalgae in terms of grams

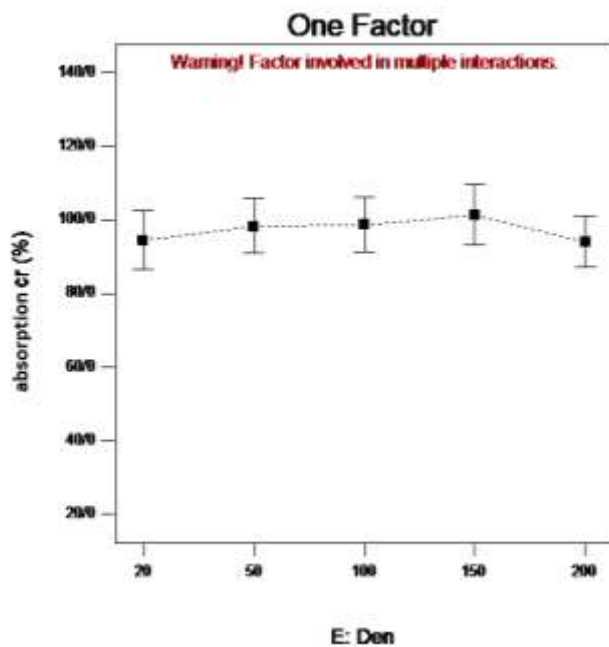


Figure 7- Amount of chromium absorption by algae in different concentrations of chromium (mg / l)

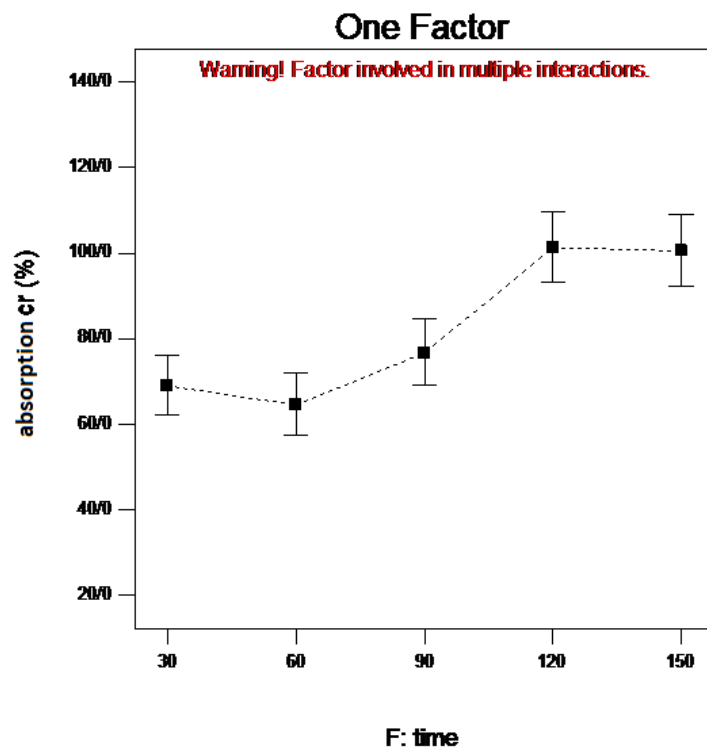


Figure 8- Absorption of chromium metal by microalgae over time (minutes)

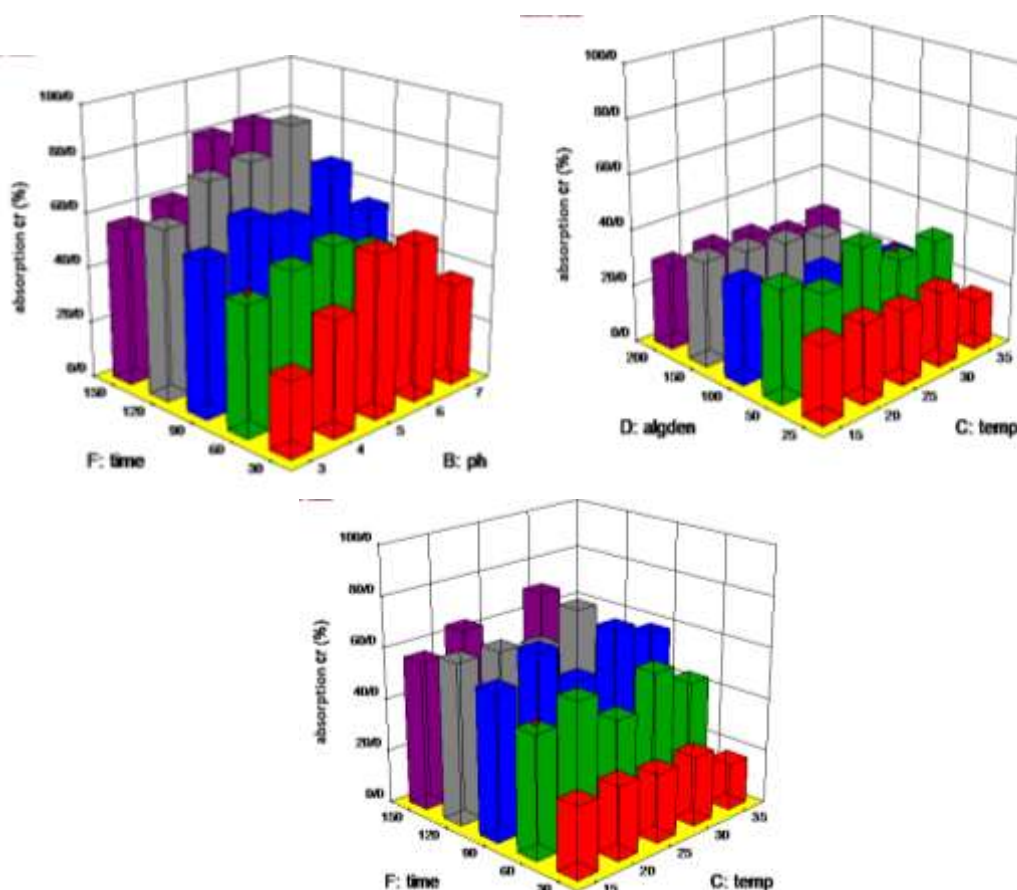


Figure 9 - Significant interactions on the absorption of chromium by microalgae

Comparison of lipids and biodiesel produced before and after chromium absorption showed that there was no significant difference between the two categories and the amount of lipids and biodiesel produced by algae used in absorb chromium and algae not used in absorb lead, doesn't differ significantly ($P > 0.05$).

Discussion

The three microalgae were not significantly different in the amount of chromium heavy metal absorption. Studies showed that factors such as pH, temperature, time, absorbent density and heavy metal density have effect on the absorption rate. The highest amount of algae absorption was after 120 minutes, while in the study conducted by Soltani et al. (2015), algae had the highest absorption of chromium after 110 minutes. One of the most important parameters in the bio- absorption of metals is pH. The highest absorption of chromium in this study was observed at pH equal to 6, while in the study of Soltani et al. (2015) the highest absorption was observed at pH equal to 5. Akhtar et al. (2008) reported that the rate of absorption and the amount of absorption of chromium decreased with increasing concentration of chromium in the environment. In this study absorption amount increased until the concentration of 150 mg/l and then absorption amount decreased.

In the study on the absorption of chromium by the green alga *Spirogerira porticalis* (Aseman and Sayyaf, 2017), the optimal pH value for chromium removal was 3 and the optimal metal concentration was 40 mg / l with a contact time of 60 minutes. The findings of this study are different. Sepehr et al. (2016) in studying the role of wastewater stabilization pond algae in the bio-absorption of hexavalent chromium found that the pH suitable for the absorption of chromium by algae was 5, which differs from the findings of this study, but like this study, the optimal time for chromium absorption by algae is 120 minutes. The optimal concentration of algae in Sepehr et al.'s (2016) study was 1 gram versus 1.5 grams found in this study. In a study on the performance of *Spirogerira* algae in the absorption of heavy metal chromium from aqueous media, Sayadi and Shokri (2015) observed that suitable pH for absorption of

chromium was 3, the initial concentration of chromium was 20 mg / l and the optimal duration was 15 minutes. Therefore, it can be concluded that the algae used in this study were able to optimally absorb chromium at higher pH. With increasing pH, the concentration of H⁺ and H₂O⁺ ions decreases and metal ions dominate in the environment and as a result, the absorption rate increases (Keshtkar and Kafshgari, 2014). In the bio-absorption process, the higher the initial concentration of metal in the solution, the higher the ability of the biomass to absorb the metal. As Ahuja et al. (1999) found, as the initial concentration of the metal increased, so did the absorption of the metal into the oscillator algae. In this study, by increasing the concentration of metal up to 150 mg / l, the amount of absorption by microalgae was increased.

at the end, it was observed that the absorption of chromium does not have a significant effect on the production of lipids and biodiesel in algae, so the algae used in the treatment of chromium-containing wastewaters can also be used as fuel.

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