

Determination of Optimum Condition of Lead (Pb) Biosorption Using Dried Biomass Microalgae *Aphanothece* sp.

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Received: 16 February 2020, Accepted: 27 April 2020, Published online: 07 August 2020

Abstract

Contamination of lead (Pb) in water due to domestic and industrial activities can endanger the environment and human health. One of the heavy metal waste treatments is adsorption using microorganisms (biosorption). In this study, dried microalgae *Aphanothece* sp. used as biosorbent for binding Pb in aqueous solution. Biosorbent was prepared from the 14 days cultivation of microalgae in a photobioreactor system which was then dried and mashed to the size of 45 mesh. Pb metal biosorption experiments were carried out in a batch system at various initial concentration variations (3.9–18.6 mg/L) and contact times (30–180 minutes) to find the optimum conditions of the biosorption process. The concentration of Pb in solution was analysed using the Atomic Absorption Spectroscopy (AAS). The results of the experiment showed that the highest removal efficiency of Pb metal in the initial concentration variation of 18.6 mg/L and contact time of 30 minutes was 99.9 % with an absorption capacity of 185.64 mg/g. Pb metal adsorption data at equilibrium conditions follows the Langmuir isotherm model equation with $R^2 > 0.9$. Biosorption kinetics using dried biomass of *Aphanothece* sp. following the pseudo second-order kinetics model. The results of this study provide an overview of the potential microalgae as Pb metal biosorbent in wastewater treatment on a larger scale.

Keywords

Aphanothece sp., biosorption, Pb metal, microalgae

1 Introduction

In general, heavy metal waste can come from various human activities such as the production process (electronic industry, electroplating, paint, paper, etc.) as well as the use of construction materials, fuels, medicines and cosmetics [1]. Waste management that has not been optimum causes heavy metal contamination to water bodies. Some heavy metals found in aquatic environments include Cu, Ni, Zn, Cr, Cd and Pb [2].

Although in low concentrations, Pb metal is a toxic heavy metal. According to the Minister of Environment Regulation No. 5 of 2014 concerning Wastewater Quality Standards for the Electronics Industry, the Pb concentration threshold limit in waters is 0.1 mg/L. The concentration of Pb in water required by WHO is 0.05 mg/L [3]. Pb metal contamination in the human body can cause headaches, stomachaches, insomnia, hallucinations, kidney dysfunction, hypertension and arthritis, in pregnant women can cause fetal miscarriage [4]. In addition, high Pb levels are also dangerous for marine life. Pb levels of 0.04–0.2 mg/L have been able to

cause poisoning in certain types of fish [5]. Aquatic biota such as crustaceans will experience death after 245 hours, if in aquatic bodies where the biota is dissolved Pb at a concentration of 2.75–49 mg/L [6]. Therefore, we need various efforts to overcome the pollution of Pb heavy metals before finally being released into water bodies.

Adsorption is a more effective and economical method of waste separation when compared to other processes such as chemical deposition, ion exchange, reverse osmosis and electrolysis. The adsorption process provides flexibility in design and operation. In addition, the adsorption process is generally reversible so that the adsorbent can be recovered with an appropriate desorption process [7].

The process of adsorption using biomaterial is called biosorption [8]. Types of native's biomass that have been used for preparing biosorbents are bacteria, fungi, algae, sea sponge, industrial wastes, agricultural wastes, and natural residues [9, 10]. Biosorption using microorganisms can be carried out directly or immobilized on natural

or synthetic adsorbents. Various solid-phase sorbents, including Diaion and Amberlite resins have been used for the preconcentration/separation of heavy metals at trace levels prior to their instrumental detection [11, 12].

One of microorganisms group that have biosorption ability are microalgae. Microalgae is relatively easy to grow, and several types of algae have been sold in large quantities. Metal ion can be adsorbed by microalgae because on the surface of microalgae cell walls contain many functional groups that act as metal binding sites. Some of these functional groups are hydroxyl, phosphoryl, amino, carboxyl, sulfidryl and other groups [13]. Different algae groups will produce different adsorption abilities of heavy metals, both in terms of capacity and type of heavy metal [14].

Microalgae used as biosorbents can be either living microalgae or dead biomass that has been dried. Some considerations that become an advantage of the use of dried biomass as biosorbent including: there is no limitation of toxicity due to metal adsorption, the range of operating conditions can be wider, dead biomass acts as an ion exchanger, the metal can be desorbed faster, and not limited by the physical properties of living microorganisms [15]. In addition, dried biomass of microalgae can be made in uniform sizes according to the need for a larger surface area. The drawback of using dead biomass is the process of biosorbent preparation such as filtering and drying.

Previous study using dried microalgae biomass have been conducted by Satya et al. [16] using *Aphanothece* sp. for the cadmium (Cd) metal biosorption. The optimum conditions obtained were pH 8.0, temperature of 30 °C, biomass concentration of 1 g/L and contact time of 60 minutes with adsorption efficiency of more than 90 %.

In this study the biosorption of lead metal (Pb) in solution in a batch system was carried out using dried biomass of microalgae *Aphanothece* sp. Biosorption experiments were conducted at various initial concentrations and contact times to obtain optimum conditions and biosorption equilibrium. The Pb metal absorption data is then fitted with several isotherm and kinetics equilibrium models to find the most suitable equation.

2 Methods

2.1 Cultivation of *Aphanothece* sp.

The cultivation medium of microalgae *Aphanothece* sp. is a BG–11 medium made by dissolving the constituent materials into aqua DM and stirring with a magnetic stirrer. The composition of BG–11 medium is NaNO₃ 1.5 g/L, KH₂PO₄ 0.04 g/L, MgSO₄·7H₂O 0.075 g/L,

CaCl₂·2H₂O 0.036 g/L, Na₂CO₃ 0.02 g/L, citric acid 0.006 g/L, Na₂-EDTA 0.001 g/L, and ferric ammonium citrate 0.006 g/L [17]. All the chemicals used for cultivation preparation medium were of analytical reagent grade and purchased from Merck (Germany) or Sigma–Aldrich (Germany). BG–11 medium that has been made is then autoclaved for sterilization purposes.

A total of 100 mL microalgae inoculums were transferred into a photobioreactor containing 900 mL of BG–11 medium. To the photobioreactor then added 1 mL of A5 trace metal mix solution. After that, the air is flowed into the photobioreactor at a rate of 3 L/min and illuminated by light with a lamp set at 5700 lux illumination. This cultivation is carried out for 14 days then harvested to be used as biosorbent.

2.2 Preparation of biosorbent

Growing microalgae are centrifuged at 6000 rpm for 5 minutes at 25 °C. After that, the solution containing microalgae is decanted and washed with aqua DM to remove the remaining media. Then the microalgae were dried using an oven at 60 °C for 7 days. Dried microalgae are crushed with mortar and then sieved with sieve no. 45 (354 μm). After that, the biosorbent is stored in a desiccator and ready to use.

2.3 Preparation of synthetic Pb waste solution

The Pb synthesis solution of 1000 mg/L was prepared by dissolving 1.5985 g of Pb (NO₃)₂ (Sigma-Aldrich, Germany) in 20 ml of 1:1 HCl solution (Merck, Germany) then added aqua DM until the volume was exactly 1 L. The experimental work solution was made from diluting Pb stock solution 100 mg/L into the desired concentration variation and added buffer pH = 6 made from a mixture of KH₂PO₄ and K₂HPO₄ (Merck, Germany). The solution is then stirred until homogeneous. This pH was chosen based on optimum condition achieved from previous Pb biosorption studies [18, 19]. There are several concentrations of synthetic Pb solution that must be made according to the variation of the experiment at low to medium range concentration, which are 3.9; 7.7; 12.8; 14.9 and 18.6 mg/L. This concentration range was chosen to better suit with actual range of Pb in industrial wastewater [20].

2.4 Biosorption of Pb using dried biomass

Aphanothece sp.

The main Pb metal biosorption experiments were carried out on a batch system in an Erlenmeyer flask with a working volume of Pb solution of 250 mL. The addition of

biosorbent as much as 0.1 g/L working solution and shaken with an agitation rate of 120 rpm. The parameters varied include contact time to determine the optimum conditions of adsorption as well as variations in the initial Pb concentration to determine the optimum capacity of Pb biosorption by dried microalgae.

2.5 Analysis of biosorption results

Solution of biosorption experiments were centrifuged at 4500 rpm for 3 minutes then supernatant fluid was analyzed using flame atomic absorption spectrometry, SavantAA model from GBC Scientific Equipment Pty Ltd (Australia). The type of flame used is air-acetylene with a lamp current of 5.0 mA and a maximum wavelength (λ_{max}) of 217 nm and a slit width of 0.5 nm. Before measuring the concentration of Pb in sample water, it is necessary to prepare a blank solution and a standard Pb solution. These solutions are necessary for determination of calibration curve to ensure that the analytical instrument is working properly, and the methodology is being practiced properly.

2.6 Calculation

The absorption capacity (q_e) and the absorption efficiency of Pb (R) are determined using Eqs. (1) and (2) [21]:

$$q_e = (C_0 - C_e)V / m, \quad (1)$$

$$R = (C_0 - C_e) / C_0 \times 100 \%, \quad (2)$$

where q_e is the absorption capacity (mg/g) of Pb absorbed by dried biomass of microalgae (biosorbents), C_0 and C_e is the concentration of Pb metal ions (mg/L) in solution before and after absorption, V is the volume (L) of the solution and m is the mass (g) of biosorbent used in the study.

3 Results and discussion

3.1 Biosorbent characteristic

Biosorbent powder with a diameter of 354 μm has been obtained from the cultivation of microalgae *Aphanothece* sp. for 14 days in a photobioreactor. Based on the results of the Fourier Transforms Infra-Red spectroscopy (FTIR) analysis conducted by Satya et al. [16], on the biosorbent surface of *Aphanothece* sp. contains ionizable functional groups such as carboxyl, amino, amides, and hydroxyl that are able to interact with protons or ions. These functional groups play an active role in the biosorption mechanism for binding heavy metal including Pb ions in solution.

3.2 Effect of initial concentration in biosorption

Based on experiments, the concentration of lead (Pb) heavy metal contained in the solution decreased significantly after adding biosorbents in the form of dried biomass of microalgae *Aphanothece* sp. This significant decrease occurred in all variations in this study. This is due to the large difference between the concentration of Pb in solution and the biosorbent (driving force) at the beginning of the experiment.

Determination of optimum Pb concentration that can be absorbed by biosorbent *Aphanothece* sp. important to do before real application on a larger scale. By using variations in the Pb concentration in the range 3.9–18.6 mg/L will be analyzed the ability of dried biomass *Aphanothece* sp. to handle Pb waste. The ability of biosorption is determined by the parameters of capacity (q_e) and biosorption efficiency (R).

Effect of initial concentration on the capacity and efficiency of the Pb metal biosorption process using dried biomass of microalgae *Aphanothece* sp. can be seen in Fig. 1. It can be observed that as the initial concentration of Pb increases in the feed, the value of capacity and biosorption efficiency will also increase. This is because an increase in the concentration of Pb in the feed will cause a greater concentration difference between the surface of the biosorbent and the surrounding environment. Thus, the greater the initial concentration value of the feed will lead to the greater number of Pb adsorbed, so the value of biosorption capacity and efficiency will also increase at the same amount of biosorbent and contact time [22].

It can be seen from Fig. 1 that in the initial variation of Pb concentration of 18.6 mg/l after 180 minutes of contact, biosorption efficiency reached 98.76 %. This means that almost all of the Pb content in the solution is absorbed in the biosorbent particles. The maximum absorption capacity obtained was 183.53 mg/g biosorbent. However, adsorption

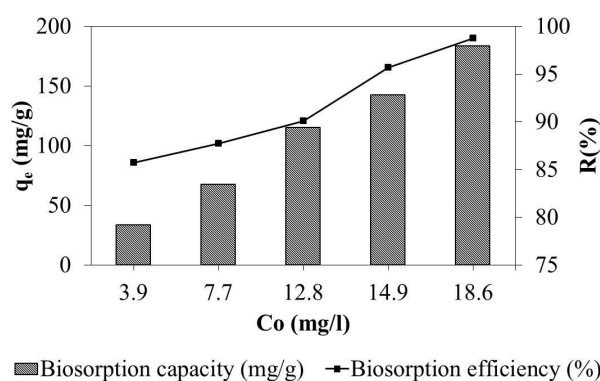


Fig. 1 Effect of initial concentration on the capacity and efficiency of Pb metal biosorption by *Aphanothece* sp. biomass (contact time 180 minutes)

capacity at a higher concentration level has decreased because all of biosorbent's active sites have been bound by metal ions in solution so that saturation occurs [12, 13].

3.3 Effect of contact time in biosorption

In the biosorption process, biosorbents will reach the saturation point or cannot absorb Pb metal anymore. Therefore, variation of contact time was carried out at the initial concentration of the solution which yielded the maximum biosorption capacity and efficiency from the previous experiment which was 18.6 mg/L to determine the biosorption capacity and efficiency profile over time. Effect of contact time on capacity and efficiency of Pb metal biosorption processes using dried biomass of microalgae *Aphanothece* sp. can be seen in Fig. 2.

It can be seen in Fig. 2 that in the contact time range of 30 - 180 minutes the biosorption capacity value is already in the optimum biosorption capacity range so that the value does not differ greatly that is 182.2–185.6 mg/g with biosorption efficiency reaching 98–99.9 %.

At the beginning of the process, a large number of active sites are available for the adsorption process to occur. The longer the interaction between the solution and the biosorbent, the more the amount of metal ions absorbed on the surface of the biosorbent particle, so that the active site which was originally available is pretty much reduced. It can be seen in the contact time range of 30–120 minutes the value of capacity and biosorption efficiency has decreased. Decreased absorbed metal ions caused by the instability of the bond between biosorbent with metal ions so that a small portion of metal ions will be released again [23].

3.4 Isotherm of biosorption

The biosorption equilibrium model was created to provide an overview of the resulting test data. Pb biosorption equilibrium

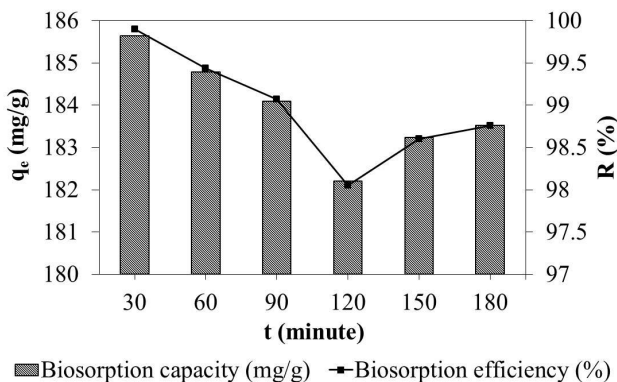


Fig. 2 Effect of contact time on the capacity and efficiency of Pb metal biosorption by *Aphanothece* sp. biomass (Co=18.6 mg/l)

data by dried biomass of microalgae *Aphanothece* sp. obtained from the experimental results contact time 30–180 minutes with an initial Pb concentration of 18.6 mg/L and analyzed with the Langmuir and Freundlich isotherms. The Langmuir Isotherm assumes adsorption occurs in monolayer on a homogeneous surface with the number of sites or sites of absorption, after the site is filled, no further adsorption occurs at that site. The linearization of the Langmuir equation can be described as follows in Eq. (3) [24]:

$$\frac{C_e}{q_e} = \frac{1}{q_m} \times C_e + \frac{1}{K_L \times q_m}, \quad (3)$$

where K_L is the Langmuir constant related to adsorption energy (L/mg), q_m is the optimum absorption capacity (mg/g), and C_e is the concentration of metal ions in solution after absorption.

The Freundlich isotherm assumes that adsorption occurs multilayer or heterogeneous. The Freundlich isotherm has the following form of linear equation (Eq. (4)) [24]:

$$\ln q_e = \ln K_f + 1/n(\ln C_e), \quad (4)$$

where K_f and n are Freundlich's constants, each showing adsorption capacity and adsorption intensity. If $n = 1$ then adsorption is linear, if $n < 1$ chemical adsorption and if $n > 1$ physical adsorption. The plot of Langmuir and Freundlich Isotherm model for Pb biosorption using dried *Aphanothece* sp. was shown by Fig. 3.

The coefficient of determination (R^2) is considered as a measure to determine the isotherm model that is suitable for the adsorption process. The coefficient of determination (R^2) and optimum adsorption capacity of the two isotherm models show that this experiment tends to follow the Langmuir isotherm rather than the Freundlich isotherm. This suggests that the active site is homogeneously dispersed in the dried biomass of *Aphanothece* sp. where Pb covers the surface of the biosorbent by forming a single layer (monolayer) and is bound to the chemically active site of the biosorbent. The optimum Pb absorption capacity (q_m) based on the Langmuir equation is 181.8 mg/g. These results didn't much different when compared to the optimum absorption capacity obtained from the experimental results which can be seen in the optimum Pb absorption capacity for the initial concentration variation of 183.5 mg/g.

3.5 Biosorption kinetics

The biosorption kinetics depends on the surface area of the particle, the type of functional group and also the concentration [25]. To find out the mechanism of the reaction and the biosorption rate constant, the reaction kinetics of

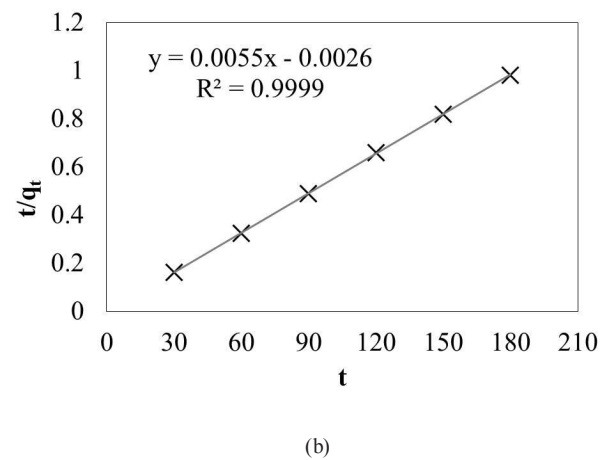
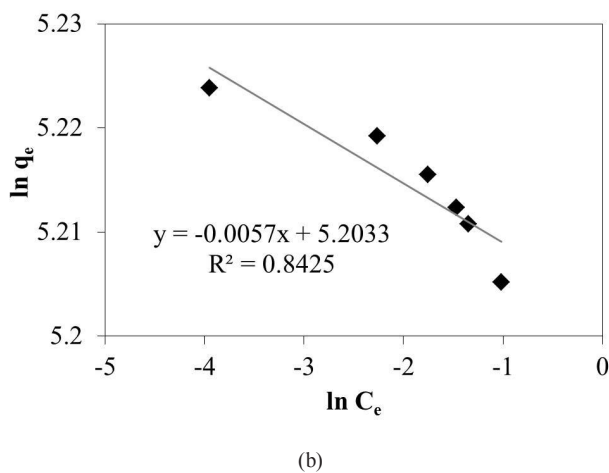
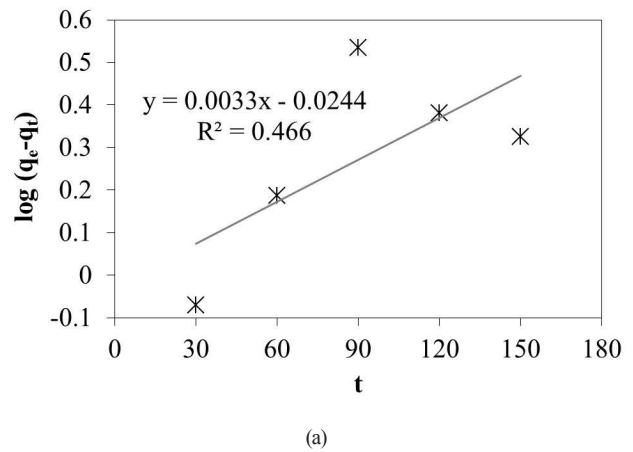
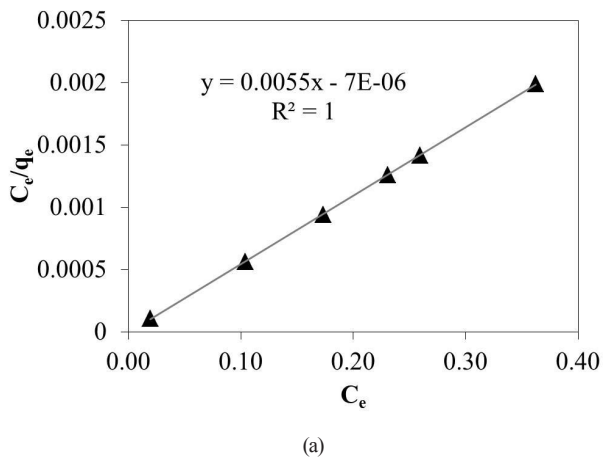


Fig. 3 (a) Langmuir and (b) Freundlich isotherm plot for Pb biosorption using dried *Aphanothece* sp. biomass ($C_0=18.6$ mg/l)

Fig. 4 Kinetics model plot for Pb biosorption (a) pseudo first-order and (b) pseudo second-order

the first-order and second-order pseudo-reaction models are used. The kinetics of pseudo first-order model is explained by Lagergren with the following linear equation (Eq. (5)) [26]:

$$\log q_e - q_t = \log q_e - (k_1 / 2,303) \times t, \quad (5)$$

where q_e and q_t are the number of Pb metal ions adsorbed (mg/g) to the biosorbent at equilibrium and at time t ; k_1 (min^{-1}) is the pseudo first-order reaction rate constant and t is time (minutes). The value of k_1 is obtained from the linear equation by plotting the log value ($q_e - q_t$) versus t .

The pseudo second-order model assumes that the absorption rate at the biosorbent adsorption site is proportional to the square of the number of empty sites [1]. The linear form of this model is (Eq. (6)):

$$\frac{t}{q_t} = \frac{1}{q_e^2 \times k_2} + \frac{t}{q_e}, \quad (6)$$

where k_2 is the rate constant of the pseudo second-order reaction (g/mg.min). The values of q_e and k_2 are determined from the linear equation by plotting (t/q_t) versus t .

From the two linear regression equations of the kinetic model (Fig. 4), it can be seen that the coefficient of determination of the pseudo second-order kinetics model is higher ($R^2 > 0.99$). From the linear equation by plotting t/q_t versus t , the reaction rate constant value (k_2) is 0.01163 g/mg.min. The predicted value of adsorption capacity (q_e) of Pb in the pseudo second-order of 181.82 mg/g is closer to the results of the study which can be seen in the initial concentration variation for adsorption capacity of 183.53 mg/g. It can be concluded from R^2 and the similarity of the q_e experiment with the calculated q_e value, that the biosorption of Pb using dried biomass of *Aphanothece* sp. follows a pseudo second-order kinetics model.

Table 1 shows a performance comparison of the use of dried biomass of *Aphanothece* sp. against other biosorbents used for Pb absorption in aqueous solutions. The difference in q_m in some of these biosorbents is due to the characteristics of each biosorbent such as surface area and main functional groups in the structure of biosorbents.

From Table 1 the biosorption capacity using dried biomass of microalgae *Aphanothece* sp. was relatively high

Table 1 Comparison of biosorption capacities for Pb using different biosorbents derived from plant and algal biomass

Biosorbent	pH	Biosorption capacity (mg/g)	References
<i>Salacca sumatrana</i> peel ^a	4	83.33	[21]
Papaya leaves ^a	4	284.35	[3]
Petai peels ^a	5	36.01	[3]
<i>Ficus carcia</i> leaves	5	34.36	[24]
<i>Moringa oleifera</i> leaves ^b	5	209.54	[27]
Pine bark	4	76.8	[28]
Pomegranate peel	5.6	13.87	[18]
Rice husk	6	58.1	[29]
Sugar beet pulp	5	43.5	[30]
Grape stalk waste	5.5	49.9	[31]
<i>Ulva reticulata</i>	3	46.51	[32]
<i>Laminaria japonica</i> ^c	4.5	349.09	[33]
<i>Ecklonia maxima</i> ^c	5	227–243	[34]
<i>Aphanothece</i> sp.	6	185.64	This study

^aactivated with HNO₃; ^bchemically modified; ^cactivated with CaCl₂

compared to other biosorbents. The high biosorption capacity values were obtained without prior processing and treatment. Whereas other biomass which has a high absorption capacity value requires activation treatment either in the form of addition of HNO₃, CaCl₂, or citric acid [3, 21, 27, 33]. The treatment consequently needs extra time and process costs. This study proves that dried

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biomass of *Aphanothece* sp. can be used as an effective Pb biosorbent in wastewater treatment. Furthermore, for the efficiency of the process, the Pb metal desorption process might be necessary to be carried out to reuse biosorbents for the next several biosorption cycles.

4 Conclusion

The present study concluded that the dried biomass of microalgae *Aphanothece* sp. can be used as an effective biosorbent for removal of Pb metal ion from aqueous solutions. The results of the experiment showed that the highest removal efficiency of Pb metal in the initial concentration variation of 18.6 mg/L and contact time of 30 minutes was 99.9 % with an absorption capacity of 185.64 mg/g. The high biosorption capacity values were obtained without prior processing and pretreatment.

Pb metal adsorption data at equilibrium conditions are satisfactorily fitted the Langmuir isotherm model equation with $R^2 > 0.9$. Biosorption kinetics using dried biomass of microalgae *Aphanothece* sp. follows the pseudo second-order kinetics model. The results of this study provide an overview of the potential microalgae *Aphanothece* sp. as Pb metal biosorbent in wastewater treatment on a larger scale.

Acknowledgement

This research was funded by DIPA Politeknik Negeri Bandung with the scheme of Penelitian Mandiri.

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