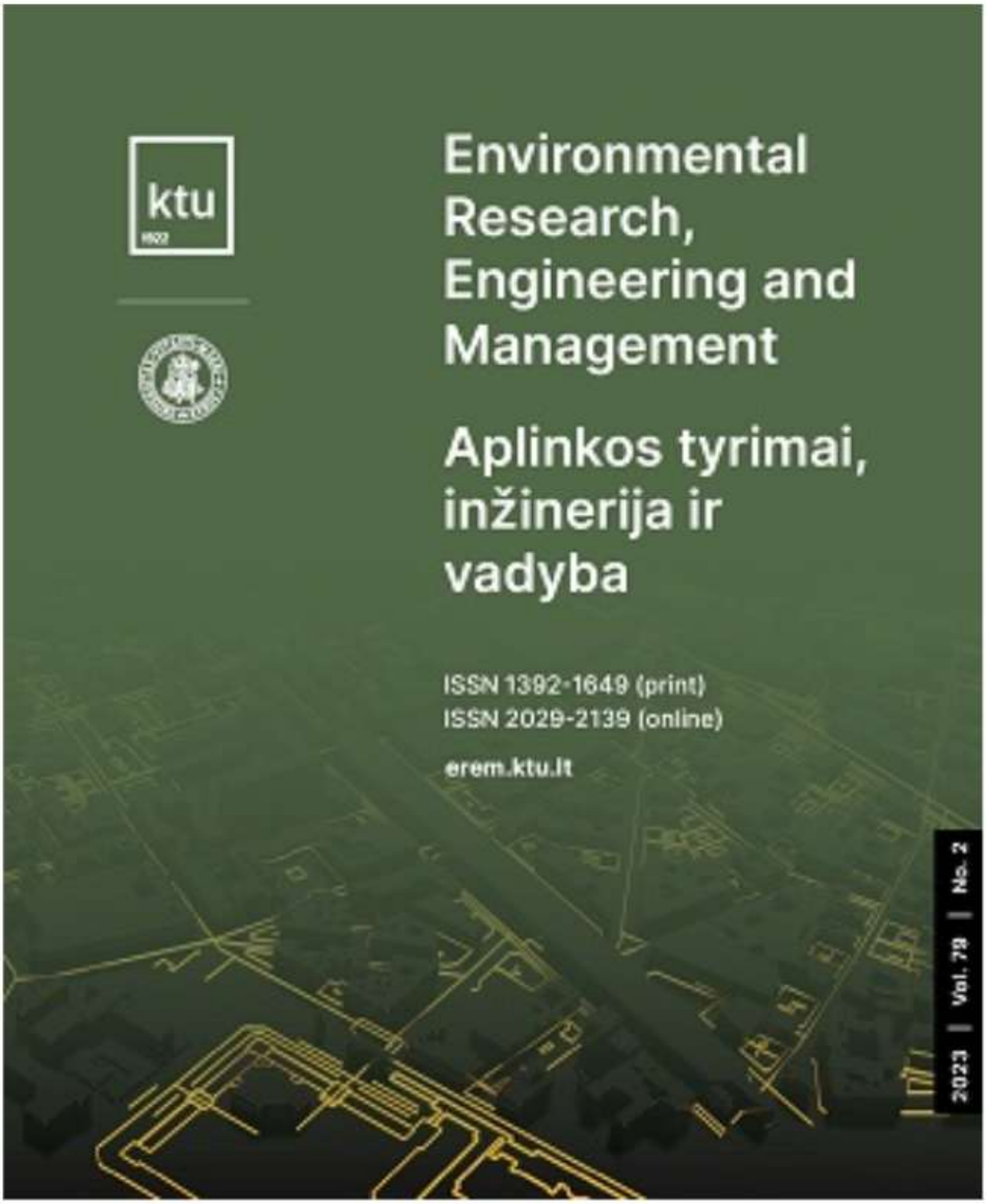
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Fe and Mn Removal from Acid Mine Drainage by Utilizing *Chlorella sorokiniana* and *Monoraphidium neglectum* as Biosorbent

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Fe and Mn Removal from Acid Mine Drainage by Utilizing *Chlorella sorokiniana* and *Monoraphidium neglectum* as Biosorbent

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The mining industry generates acid mine drainage (AMD) characterized with a low pH value and high dissolved metal concentration that leads to the negative impacts on the environment and human health. The objectives of this research were to investigate the growth response of mixed culture of microalgae *Chlorella sorokiniana* and *Monoraphidium neglectum* in a liquid media contaminated with AMD; generate the optimum environmental conditions (pH value and contact time) to determine the efficiency biosorption of iron and manganese contained in the solution of AMD into the consortium of microalgae; and quantify the maximum removal amount of iron and manganese contained in the solution of AMD by utilizing microalgae consortium of *Chlorella sorokiniana* and *Monoraphidium neglectum* as biosorbent. AMD used in this research was characterized with a pH value of 1.65 with iron and manganese concentrations of 8.28 mg/L and 4.57 mg/L. The research of biosorption was conducted in 150 rpm with pH level variations of 4, 5, and 6, and contact time variations of 60, 120, and 180 min. The maximum value of iron and manganese removals occurred when pH level reached 5 at 180 min of contact time with removal efficiency of 89.73% for iron and 94.53% for manganese. The results proved that the mixed culture of microalgae namely *Chlorella sorokiniana* and *Monoraphidium neglectum* can be utilized to remove iron and manganese contained in acid mine drainage.

Keywords: acid mine drainage, heavy metal, microalgae, *Chlorella sorokiniana, Monoraphidium neglectum.*

Introduction

Acid mine drainage (AMD) is characterized by low pH value of 1.5 to 4, which is mostly produced by mining industries. Since AMD may contain high dissolved metal compounds such as iron, aluminum, manganese, cadmium, copper, lead, zinc, arsenic and mercury, AMD is classified as a type of wastewater. A highly acidic solution can cause corrosion on pipes and buildings; moreover, this acidic water is also dangerous for organisms. Heavy metal compounds contained in AMD can potentially threaten human life due to their toxicity, persistence and ability to accumulate in the human body (Wahyudin et al., 2018; Rinanti et al., 2021).

Meanwhile, AMD can be basically treated using physical, chemical, and biological methods (Tong et al., 2021). However, further processing stages must be conducted before dumping it into the environment. Previous research has shown that a physical processing method experienced a couple of weaknesses such as a high processing cost and a low removal capacity on low AMD concentrations (less than 100 mg/L) (Rinanti, 2018). The treatment process of AMD using a biological method is commonly practiced through the utilization of sulphate reducer, yet it requires a long period of residence time and adequate organic substrate supply to reach the maximum removal effectiveness (Mang and Ntushelo, 2020). In the context of reducing the negative impact of AMD, the development of green technology, such as the increasing utilization of microalgae, is essential to uncover.

Furthermore, the utilization of microalgae to remove heavy metal contained in AMD is determined by many factors such as pH, temperature, contact time, and nutrition (Sunaryo et al., 2019). A number of previous researchers have claimed that the utilized microalgae biomasses were usually the dead ones. However, in this research, the utilized microalgae biomass is a living one, since a living microalgae biomass can remove heavy metal through various mechanisms such as bioaccumulation, biotransformation, or biodegradation that would lead to a higher level of removal efficiency. Another previous research has also stated that microalgae biosorbent has the ability to remove heavy metals up to 100% on certain conditions (Zeraatkar et al., 2021). Liang et al. (2017) have demonstrated the ability of *Chlorella sorokiniana* as a microalgae biosorbent, and showed that it was able to remove 51.90% of heavy metal Pb in wastewater at pH 6.7 and initial microalgae density of 0.739×10^{10} cells/L. Meanwhile, *Monoraphidium griffithii* also has an adsorption ability of 29.7 mg/g and zinc removal of 84.8% at pH 7–7.5, temperature 24°C, initial zinc concentration 10 mg/L (Bácsi al., 2015). According to León et al. (2021), *Chorella sorokiniana* has performed ideal microalgal traits to provide a study for bioremediation, through its ability to grow under unfavorable conditions and surpass other microalgae. The freshwater microalga *Monoraphidium* sp. was used in the research of Novak et al. (2020) to show the higher level of tolerance to heavy metals than other strains of freshwater microalgae such *Desmodesmus* sp.

Hence, this research aims to treat AMD attributed with low pH and containing iron (Fe) and manganese (Mn) using microalgae biosorbent. In order to achieve the expected results, three essential steps were conducted, such as investigating the growth response of mixed culture of microalgae *Chlorella sorokiniana* and *Monoraphidium neglectum* in a liquid media contaminated with AMD; generating the optimum environmental conditions (pH value and contact time) to determine the efficiency biosorption of iron and manganese contained in the solution of AMD into the consortium of microalgae; and quantifying the maximum removal amount of iron and manganese contained in the solution of AMD by utilizing microalgae consortium of *Chlorella sorokiniana* and *Monoraphidium neglectum* as biosorbent.

Methods

Microalgae culture and nutritions

This research used a microalgae culture as a consortium that consists of *Chlorella sorokiniana* and *Monoraphidium neglectum* purchased from the Laboratory of Indonesian Culture Collection (InaCC). The microalgae were cultivated in a media of Provasoli Haematococcus Medium (PHM). The composition elements for analysis (p.a), produced by Supelco and purchased from online stores in Indonesia, were added two drops of Fe stock and two drops of a trace element into 1 L of distilled water. 1 L of Fe stock contains 189 g of EDTA and 24.4 g of $FeCl_3.6H_2O$. Meanwhile, 500 mL of a trace element solution contain 2.05 mg of $ZnCl_2$, 30.5 mg of H_3BO_3, 2.55 mg of $CaCl_2.6H_2O$, 3 mg of $CuSO_4.5H_2O$, 2.05 mg of $MnCl_2.4H_2O$ and 19 mg of $(NH_4)_6 Mo_7.O_{24}.4H_2O$. The microalgae consortium was utilized as a biosorbent to treat artificial AMD by mixing 8.28 mg of $FeSO_4$, 4.57 mg of $MnSO_4$, and 0.2 N H_2SO_4 into 1 L of distilled water until it reached pH value of 1.65.

Acid mine drainage preparation

The acid mine drainage used in this study was an artificial AMD that was made according to the characteristics of AMD generated by the coal mining in East Kalimantan, Indonesia, with the value of pH 1.65, the concentration of iron 8.28 mg/L and manganese 4.57 mg/L (Rizki, 2013).

In order to create an artificial AMD, 8.28 mg $FeSO_4$ and 4.57 mg $MnSO_4$ were respectively dissolved into 1 L of distilled water. pH of AMD was then adjusted by adding 0.2N H_2SO_4 solution to reach a pH of 1.65. Then, 36 mL of AMD solution was contacted with 10% biosorbent in a 50 mL Erlenmeyer flask.

Microalgae cultivation

Chlorella sorokiniana and *Monoraphidium neglectum* were each cultivated in a series of 100 mL Erlenmeyer flasks with a total culture volume of 80% of the container volume used (80 mL of total culture volume), and the volume of media was 90% of the total culture volume (72 mL of media volume).

Chlorella sorokiniana and *Monoraphidium neglectum* microalgae were mixed in a composition ratio of 1:1 and cultivated in a 50 mL Erlenmeyer flask. The culture had the composition ratio 9:1 (media: microalgae) which only occupied 80% of the total given volume of the utilized Erlenmeyer flask, set at a temperature of 30°C, pH value of 7, and cultivated permanently under 3500 lux in the Laboratory of Environmental Microbiology. When microalgae biomass reached its exponential phase, the microalgae were then harvested to be utilized on the biosorption study.

Biosorption study

Biosorption study was initiated by contacting a 10% concentration of microalgae biosorbent in a 50 mL Erlenmeyer flask that contained media contaminated by AMD, inserted in a shaker with the rotation of 150 rpm and set at the temperature of 30°C. The variations of pH in the biosorption study were designated at the value of 4, 5, and 6, which were obtained by adding 0.1N NaOH or 0.1N H_2SO_4 until the solution reached the intended pH value. After measuring the optimum pH, the study was continued by measuring the optimum contact time on the optimum pH. Meanwhile, the contact time variations were 60, 120, and 180 min.

Afterwards, the study used atomic absorption spectrometry (AAS) to measure the concentration of heavy metals contained on microalgae biosorbent in the media. The removal efficiency of iron and manganese can be calculated by using the following formula:

$$\text{Removal efficiency (\%)} = \frac{Co-Ce}{Co}\ x\ 100\% \qquad (1)$$

Where: Co – initial heavy metal concentration in a solution; Ce – final heavy metal concentration in solution.

On the other hand, the heavy metal adsorption capacity can be measured by using the following formula:

$$Qe= \frac{(Ce-Co)\ x\ V}{Wg} \qquad (2)$$

Where: Qe – adsorption capacity (mg/g); Co – heavy metal concentration before adsorption (mg/L); Ce – heavy metal concentration after adsorption (mg/L); V – solution volume (L); Wg – adsorbent dose (g).

Adsorption isotherms

This research used the isotherm model, namely the Langmuir and Freundlich isotherm models. Langmuir isotherm explains the surface layering by balancing adsorption and desorption rates. The formula is as follows:

$$\frac{Ce}{qe} = \frac{1}{qm.K_L} + \frac{Ce}{qm} \qquad (3)$$

Where: Ce – adsorbate concentration on balance (mg/g); KL – Langmuir constant (mg/g); qe – number of adsorbates on balance (mg/g); qm – the maximum adsorption capacity (mg/mL).

The adsorption mechanism used Freundlich isotherm especially with the heterogenous surface of sorbent. The formula of Freundlich isotherm is as follows:

$$\text{Log}\ q_e = \log K_F + \frac{1}{n}\log C_e \qquad (4)$$

Where: Kf – adsorption capacity (L/mg); 1/n – adsorption intensity; Ce – adsorbate concentration on balance (mg/g); qe – number of adsorbates on balance (mg/g).

Adsorption kinetics

Adsorption kinetics is the curve (or line) that describe the retention rate or substance release from a solid phase surface at a certain adsorbent dose, temperature, flow rate, and pH. In this research, the studied adsorption kinetics were the reaction of the first and second order kinetics. The results of both kinetics were plotted to obtain the R^2 value. The curve with the R^2 value higher than 0.99 is considered as the most suitable curve (Michalak et al., 2013). The linear mathematical formula of order reaction one is as follows:

$$\mathrm{Ln}\,(Qe - Qt) = \ln Qe - k_1 t \quad (5)$$

Where: Qe – number of adsorbed compounds on balance (mg/g); Qt – number of adsorbed compounds at t time; k_1 – order one kinetics; t – time.

Meanwhile, the linear mathematical formula for reaction order two is as follows:

$$\frac{t}{Qt} = \frac{1}{k_2.Qe^2} + \frac{t}{Qe} \quad (6)$$

Where: Qt – number of adsorbed compounds at t time; Qe – number of adsorbed compounds on balance (mg/g); k_2 – order two kinetics rate; t – time.

Results and Discussion

The observation of the growth of mixed culture of microalgae was carried out for 22 days and measured for every two days. As shown in *Fig. 1(a)*, the microalgae reached the exponential phase on day 14. Similar to the findings of the preliminary study, the mixed culture of microalgae experienced four growth phases (Krishnan et al., 2015; Price and Farag, 2013). According to *Fig. 1*, on day 0 to day 10, the mixed culture of microalgae was in the lag phase (adaptation period) yet showed a little to no increase in absorbance. Then on day 10 to day 20, the mixed culture of microalgae began to enter the exponential phase, as seen from a significant increase in absorbance.

The cultivation of mixed cultures on liquid media contaminated with acid mine drainage showed different results. As it can be seen in *Fig. 1 (b)*, the mixed culture of microalgae directly entered the exponential phase on day 0 to day 2 without going through the lag phase (adaptation period) first. This indicates that the mixed culture of microalgae was able to utilize metal compounds of iron and manganese contained in acid mine drainage as nutrients to support the microalgae growth. As mentioned, microalgae utilize heavy metal compounds such as boron (B), cobalt (Co), copper (Cu), iron (Fe), molybdenum (Mo), manganese (Mn), and zinc (Zn) as trace elements in the enzymatic process and cell metabolism (Leong and Chang, 2020).

However, the value of maximum absorbance for the mixed culture of microalgae on the liquid media contaminated with AMD was ten times lower than the absorbance of the mixed culture of microalgae on the media that was not contaminated with AMD. The low absorbance value of the microalgae was presumed as the result of a longer exposure period to heavy metals

Fig. 1. *Growth curve in (a) PHM media; (b) PHM media contaminated by AMD*

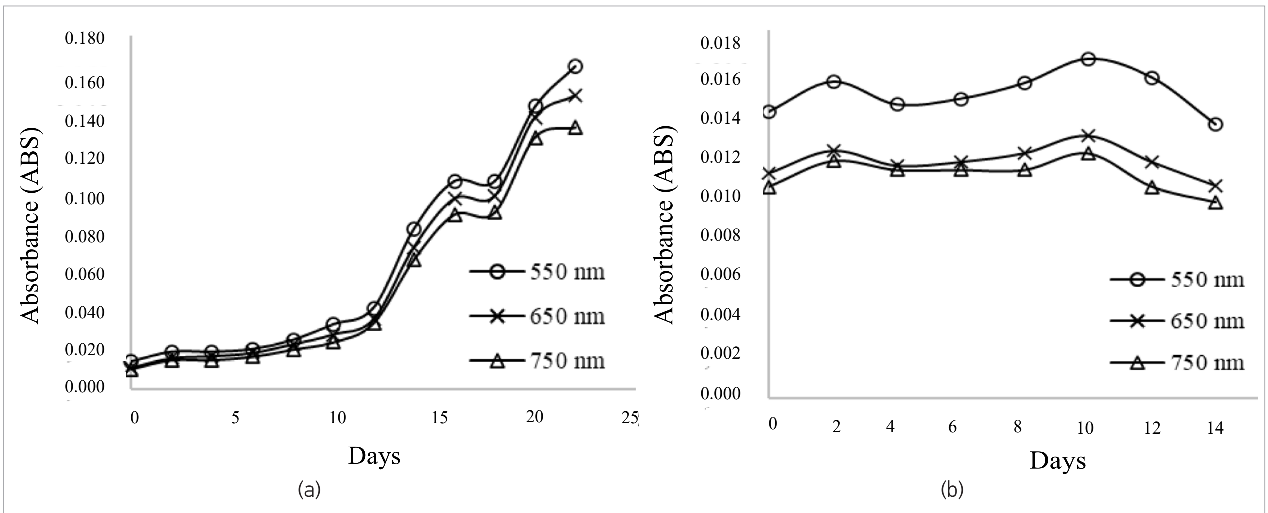


Table 1. *Iron metal (Fe) biosorption with pH variations*

pH	Initial concentration (Co) (mg/L)	Final concentration (Ce) (mg/L)	Co–Ce (mg/L)	Removal efficiency (%)	Adsorption capacity (Qe) (mg/mL)
4	8.28	2.10	6.18	74.64	0.062
5		1.48	6.81	82.19	0.068
6		1.70	6.58	79.47	0.066

Table 2. *Manganese metal (Mn) biosorption with pH variations*

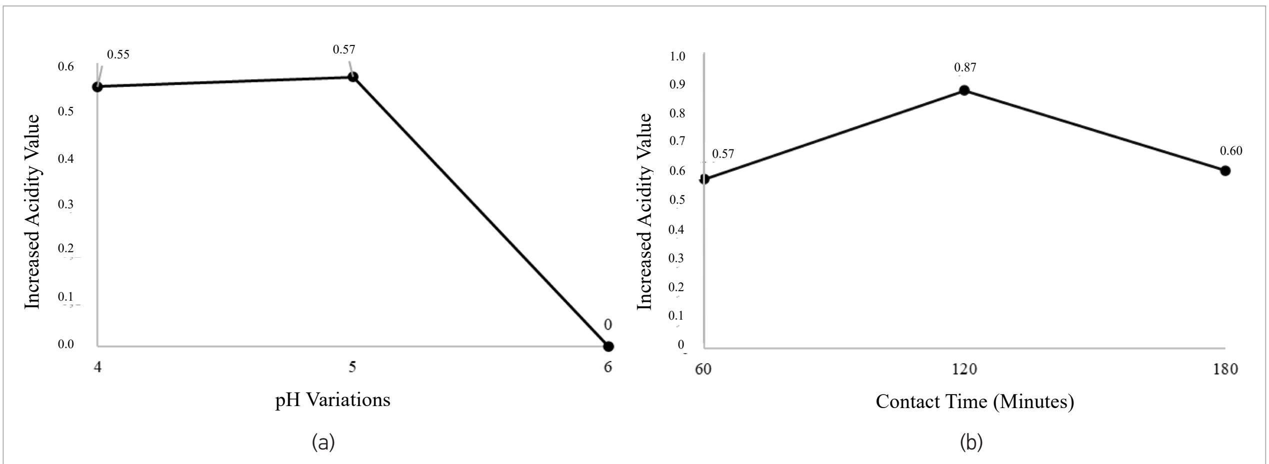
pH	Initial concentration (Co) (mg/L)	Final concentration (Ce) (mg/L)	Co–Ce (mg/L)	Removal efficiency (%)	Adsorption capacity (Qe) (mg/mL)
4	4.57	0.25	4.32	94.53	0.0432
5		0.25	4.32	94.53	0.0432
6		0.25	4.32	94.53	0.0432

contained in the AMD solution, which may affect the growth of microalgae. According to the preliminary study, the concentration of heavy metals and the exposure time are taken into account for the impact of the toxic nature of a heavy metal to microbes (Ouyang et al., 2012). Moreover, the blocking of important molecular functional groups such as enzymes and transport systems for nutrients and ions by heavy metals may lead to the inclination of toxicity of heavy metals to microbes (Naorbe and Serrano, 2012; Siwi et al., 2018).

The mixed culture of microalgae used in this research was contacted with a pollutant load (AMD) at the peak of the exponential phase on day 14. Since microalgae is principally attributed with the most active cell walls at the exponential phase, they therefore are able to carry out the biosorption process of iron and manganese metals (Wilan et al., 2019). During the biosorption process, there was an increase in the value of acidity in the liquid media contaminated with AMD. The increase in the acidity value of the AMD contained in a liquid media reached the maximum value at pH 5 which was shown by the increase of the acidity value for 0.57 units. The research was subsequently continued to determine the maximum contact time to increase the acidity value. *Fig. 2* shows the results of increasing acidity values by the mixed culture of microalgae with the selected contact time variations of 60, 120, and 180 min.

Based on *Fig. 2*, it can be seen that the increase in the acidity value of the AMD contaminated liquid media reached a maximum at the initial pH value of 5 and the

Fig. 2. *The increase of the acidity value with variations of (a) initial pH; (b) contact time*



contact time of 120 min, with the pH value increasing by 0.87 units (17.40%). In accordance with the preliminary findings, the acidity value will increase along with the growth of microalgae, due to the reduced dissolved carbon caused by photosynthesis process (Gao et al., 2013; Yu et al., 2022). At the contact time of 180 min, there was a decrease in the acidity value which may be prompted by the heavy metal biosorption mechanism by microalgae. The release of initial metal ions (Na^+, K^+, Ca^{2+}) attached to the surface of microalgae can eventually lead the hydrolysis of heavy metal ions to generate lower pH (Tang et al., 2003).

Moreover, this research used pH variations as an independent variable to observe the biosorption efficiency of iron and manganese as shown in *Table 1* and *Table 2*. The study showed that the pH variations had a significant impact on the removal efficiency of iron (Fe), while changes in pH did not affect the removal efficiency of manganese (Mn). The optimum pH for the biosorption of iron (Fe) and manganese (Mn) using the mixed culture of microalgae as a biosorbent occurred at the pH value of 5 with the absorption efficiency of Fe 82.19% and manganese 94.53%. At pH 4, the efficiency of iron metal removal was 74.64% showing a lower value than the result of removal efficiency at pH 5. The low value of pH will lead to the competition between metal ions and protons to attach to the binding sites of the microalgae surface that eventually inhibits the process of biosorption to optimally run (Olal, 2016). Along with the increase in pH, the concentration of protons on the surface of the microalgae biosorbent will decrease and be deprotonated, which generates the presence of negative functional groups on the surface of the microalgae biosorbent that may cause the binding of metal ions to the surface of the microalgae to increase and the biosorption process run at the optimum condition (pH 5) (Widyaningrum et al., 2021). The removal efficiency of iron at pH 6 reached a value of 79.47% showing a decreased value when compared with the removal efficiency at pH 5. Moreover, at a further increase in pH, iron metal will form a complex compound such as $Fe(OH_3)^{-}$ and $Fe(OH_4)^{2-}$ that cause the decrease of removal efficiency (Kanamarlapudi and Muddada, 2020)

After defining the optimum pH for the biosorption process, the research was continued to determine the optimum contact time. The variations of contact time used in this study were 60, 120, and 180 min. *Table 3* and *Table 4* show the results of the biosorption of iron and manganese using variations in contact time.

The variation of contact time affects the efficiency of iron metal removal but it may not affect the efficiency of manganese metal removal. At a contact time of 60 min, the efficiency of iron metal removal reached 82.19%, then increased to 88.22% at a contact time of 120 min and reached a maximum value of 89.73% at a contact time of 180 min. Meanwhile, the removal efficiency of manganese metal was being constant through the variations of contact time with a maximum removal efficiency of 94.53% after 60 min.

At a contact time of 60 min, the biosorption process took place briefly due to the availability of active sites

Table 3. *Iron metal (Fe) biosorption with contact time variations*

Contact time (min)	Initial concentration (Co)	Final concentration (Ce)	Co–Ce	Removal efficiency	Adsorption capacity (Qe)
	(mg/L)	(mg/L)	(mg/L)	(%)	(mg/mL)
60	8.28	1.475	6.805	82.19	0.0681
120		0.975	7.305	88.22	0.0731
180		0.850	7.430	89.73	0.0743

Table 4. *Manganese metal (Mn) biosorption with contact time variations*

Contact time (min)	Initial concentration (Co)	Final concentration (Ce)	Co–e	Removal efficiency	Adsorption capacity (Qe)
	(mg/L)	(mg/L)	(mg/L)	(%)	(mg/mL)
60	4.57	0.25	4.32	94.53	0.0432
120		0.25	4.32	94.53	0.0432
180		0.25	4.32	94.53	0.0432

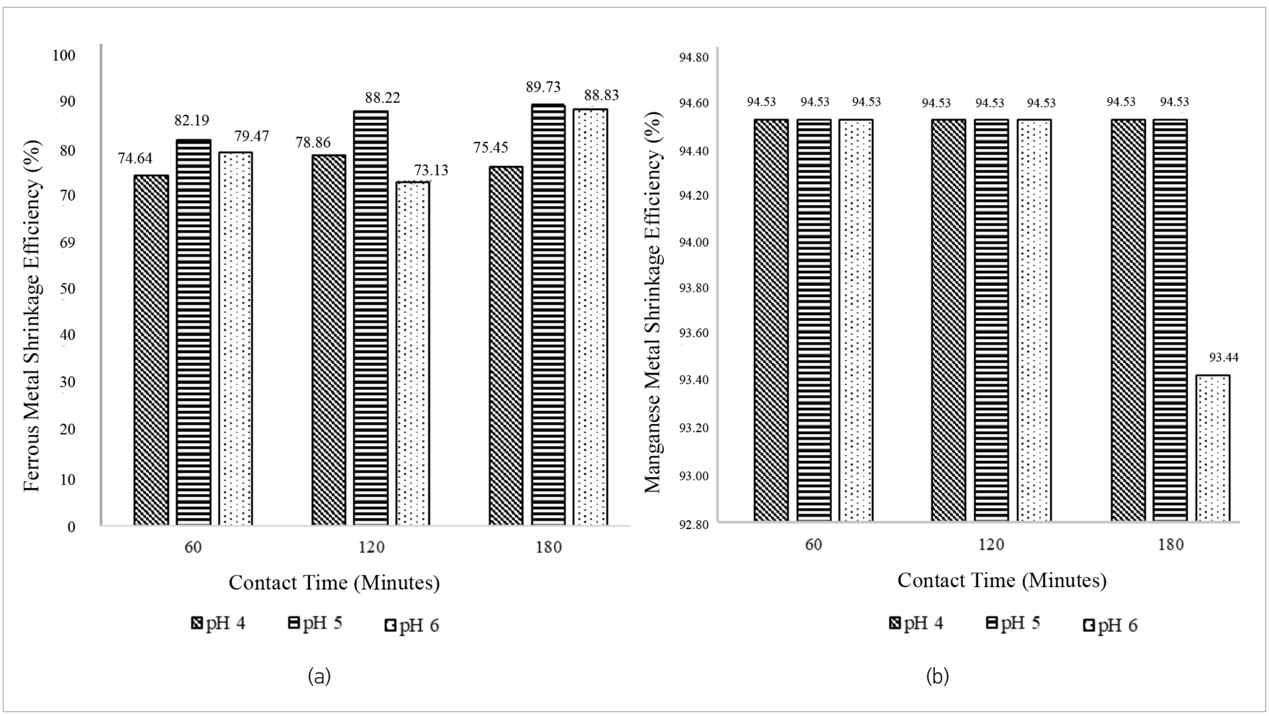
on the surface of the microalgae. Since these sites were filled with metals, the biosorption process therefore became slower and less efficient (Bouzit et al., 2018). The efficiency of iron metal removal was only 1.51% at the contact time 120 min and 180 min. A longer contact time will decrease the removal efficiency of heavy metal since it passes its equilibrium point that causes the saturation of the active sites on the surface of the microalgae (Nuban et al., 2021). Moreover, the addition of contact time reduces the level of removal efficiency of iron metal as shown in *Fig. 3(a)* at pH 4 and pH 6. Similar to this, *Fig. 3(b)* shows the removal efficiency of manganese metal decreased at a contact time of 180 min and at the value of pH 6.

The optimum environmental conditions for the biosorption of iron and manganese using the mixed culture of microalgae *Chlorella sorokiniana* and *Monoraphidium neglectum* were at pH 5 with a contact time of 180 min resulting in an efficiency of removal of iron metal of 89.73% and manganese metal of 94.53%. The maximum adsorption capacity of iron and manganese by the mixed culture of microalgae *Chlorella sorokiniana* and *Monoraphidium neglectum* were respectively 0.0743 mg/mL and 0.0432 mg/mL.

These results were slightly different than the research conducted by other researchers that generated 100% of iron removal efficiency in 20 min of the contact time at the temperature of 30°C and 80 rpm stirring speed by utilizing microalgae *Scenedesmus obliquus* (Bouzit et al., 2020). In an iron biosorption study using microalgae *Scenedesmus obliquus, Chlorella fusca, Chlorella saccharophila, Ankistodesmus braunii, and Leptolyngbya* on liquid media with iron concentration of 50 ppm (10^{-3}g/L), removal efficiency reached 99.9% in 4, 8, and 12 days of contact time (Zada et al., 2021). A manganese metal removal study was also conducted and utilized algae *Sargassum hystrix* at an initial concentration of 10 mg/L Mn(II), 10 g/L biosorbent dose and 120 min of contact time with removal efficiency of 85.6% (Ghasemi et al., 2016). Similarly, manganese removal efficiency of 75% was achieved by using *Ulva lactuca* dead microalgae biomass at pH value of 5, 60 min of contact time, and stirring speed of 100 rpm (Omar, 2008).

A number of previous biosorption studies using mixed cultures of microalgae *Chlorella sorokiniana* and *Monoraphidium neglectum* showed a higher removal efficiency for the removal of manganese. In contrast to previous studies, the removal efficiency of iron metal

Fig. 3. *Removal efficiency of metal (a) iron and (b) manganese with pH and contact time variations*



using a mixed culture of microalgae *Chlorella sorokiniana* and *Monoraphidium neglectum* showed a lower removal efficiency value and required a longer contact time. The difference in the value of this removal efficiency may be due to the different types of biosorbents used, since each type of biosorbent has different adsorption properties owing to the variations in dominant functional groups, surface area, pore size and volume of the biosorbent surface (Rinanti et al., 2017).

Fig. 4 shows a relationship between heavy metal concentration after a biosorption process (Ce) and heavy metal adsorption capacity (Qe) for Langmuir isotherm calculation, which shows only two different values for the Qe data series of manganese for different contact time and pH value resulting only in two value plots in the linear curve. *Fig. 5* shows a relationship between Log Ce and Log Qe utilized in Freundlich isotherm calculation. The correlation coefficient (R^2) for Langmuir isotherm was at 0.9972 for iron and 1 for manganese; meanwhile, the R^2 value for Freundlich isotherm was at 0.9811 for iron and 1 for manganese. The adsorption isotherm calculation results can be seen in *Table 5* and *Table 6*.

Fig. 4. *Langmuir isotherm curve for (a) iron and (b) manganese*

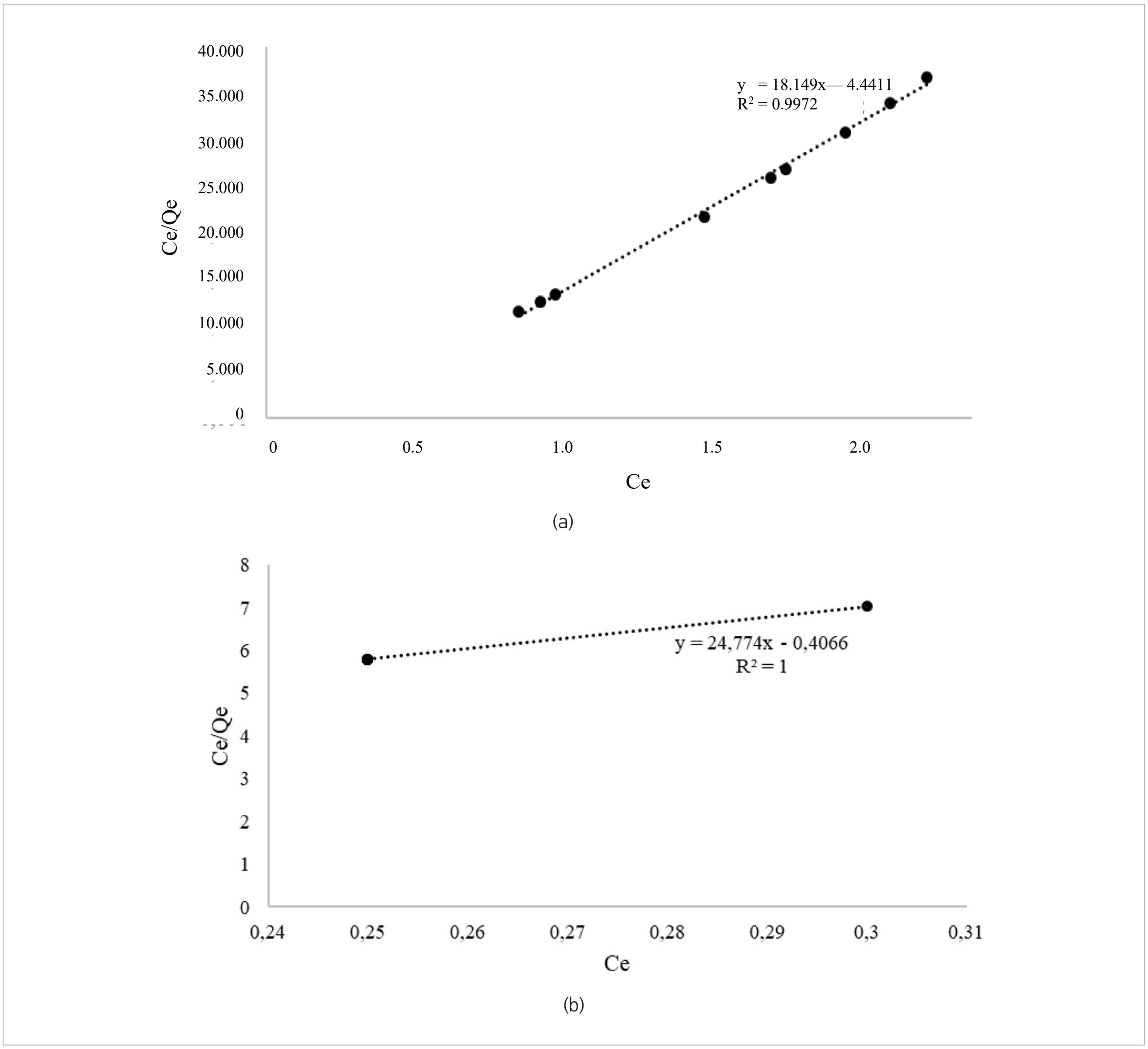


Fig. 5. *Freundlich isotherm curve for (a) iron and (b) manganese*

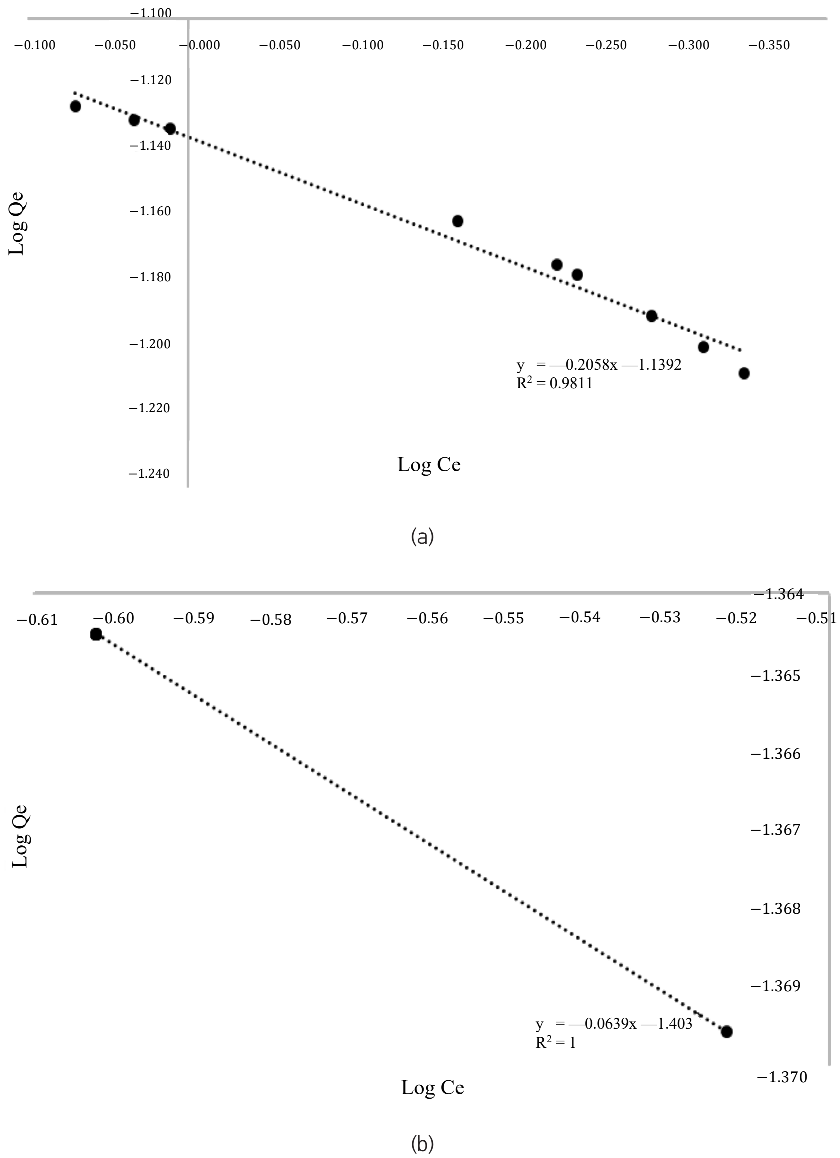


Table 5. *Iron metal adsorption isotherm calculation results*

Isotherm	R^2	Equation	Constant	Value
Langmuir	0.9972	$\frac{Ce}{Qe} = \frac{1}{-4.0870.055} + \frac{Ce}{0.055}$	Qm	0.055
			K_L	-4.087
Freundlich	0.9811	$\text{Log Qe} = \text{Log } 0.0726 - \frac{1}{4.859}\log Ce$	K_f	0.073
			n	-4.859

Table 6. *Manganese metal adsorption isotherm calculation results*

Isotherm	R^2	Equation	Constant	Value
Langmuir	1	$\frac{Ce}{Qe} = \frac{1}{-60.93.0.04} + \frac{Ce}{0.04}$	Qm	0.0400
			KL	-60.930
Freundlich	1	$\text{Log Qe} = \text{Log } 0.0395 - \frac{1}{-15.649} \log Ce$	Kf	0.0395
			n	-15.649

The maximum biosorption capacity of iron metal (Qm) based on the Langmuir isotherm was 0.055 mg iron/mL biosorbent with the Langmuir constant (KL) of -4.087. Based on the calculations, the value of the Freudlich constant (Kf) was obtained at 0.073 with a value of n of -4.859. The maximum adsorption capacity (Qm) based on Langmuir isotherm was 0.04 mg manganese/mL biosorbent with Langmuir constant -60.930 mL/mg, while the value of Freundlich constant was 0,0395 with a value of n of -15.649.

The appropriate isotherm model is determined based on the value of the correlation coefficient (R^2) which is closer to 1 (Tahad et al., 2018). Since the Langmuir isotherm R^2 value was closer to 1 when compared with the R^2 value produced by the Freundlich isotherm model, the appropriate model to explain the biosorption process of iron and manganese using the mixed culture of microalgae *Chlorella sorokiniana* and *Monoraphidium neglectum* was therefore the Langmuir isotherm. In addition, the value of n for the Freundlich isotherm which was less than 0 indicated that the adsorption isotherm that occurred was in accordance with the normal Langmuir isotherm (Jodeh et al., 2015; Rinanti et al., 2021).

The Langmuir isotherm assumes that each active site only interacts with one adsorbate molecule, the surface of the biosorbent is homogeneous, and all binding sites are evenly distributed and have the same affinity (Sahu and Singh, 2019). The Langmuir isotherm also assumes that adsorption occurs on a homogeneous surface through a monolayer process and is in equilibrium when the adsorption rate is the same as the desorption rate (Woo et al., 2021; Perwitasari et al., 2021; Awaeri et al., 2017). Biosorption kinetics shows the rate of a heavy metal binding to the surface of the biosorbent (Bulgariu and Gavrilescu, 2015). This research used the biosorption kinetics of first-order and second-order reaction kinetics. The first-order adsorption kinetics curve can be seen in *Fig. 6* and the second-order adsorption kinetics can be seen in *Fig. 7*.

The value of the correlation coefficient and the reaction rate constant for the first and second order of iron metal can be seen in *Table 7*. The second-order R^2 value at pH 5 was the value that is closest to the value 1, which was 0.9999. The Qe values obtained from the second-order calculation for pH 4, 5, and 6 were 0.0641 mg/mL, 0.0779 mg/mL, and 0.0781 mg/mL, respectively. The second-order Qe value was closer to the actual Qe value at each of these pHs. The values of the reaction rate constant for pH 4, 5, and 6 obtained based on the second-order reaction in sequence were 21.513 mL/mg.min, 1.5254 mL/mg.min, and 1.5147 mL/mg.min. Based on that, it can be concluded that the biosorption kinetics of iron metal was in accordance with the second-order reaction kinetics. Second-order biosorption kinetics assumes that the rate of solute adsorption is proportional to the available sites on the adsorbent (William et al., 2019).

The results of the calculation of the manganese adsorption kinetics can be seen in *Table 8*. The value of Qe and the first-order reaction rate constant at pH 4 and 5 could not be determined because there was no change in the adsorption capacity with the addition of contact time. When compared with the first-order, the second-order R^2 value was closer to 1 for all pH values. The Qe values obtained from second-order calculations for pH 4, 5, and 6 were 0.0432 mg/mL, 0.0432 mg/mL, and 0.0425 mg/mL, respectively. The results of the second-order Qe calculation were closer to the actual Qe value in the manganese metal biosorption process. The second-order reaction rate constant at pH 6 was 17.0578 mL/mg.minute, while the second-order reaction rate constant for pH 4 and 5 could not be determined because the intercept value obtained based on the adsorption kinetics graph was 0. Based on that, the adsorption kinetics which related to the biosorption

Fig. 6. *First-order adsorption kinetics curve for (a) iron and (b) manganese*

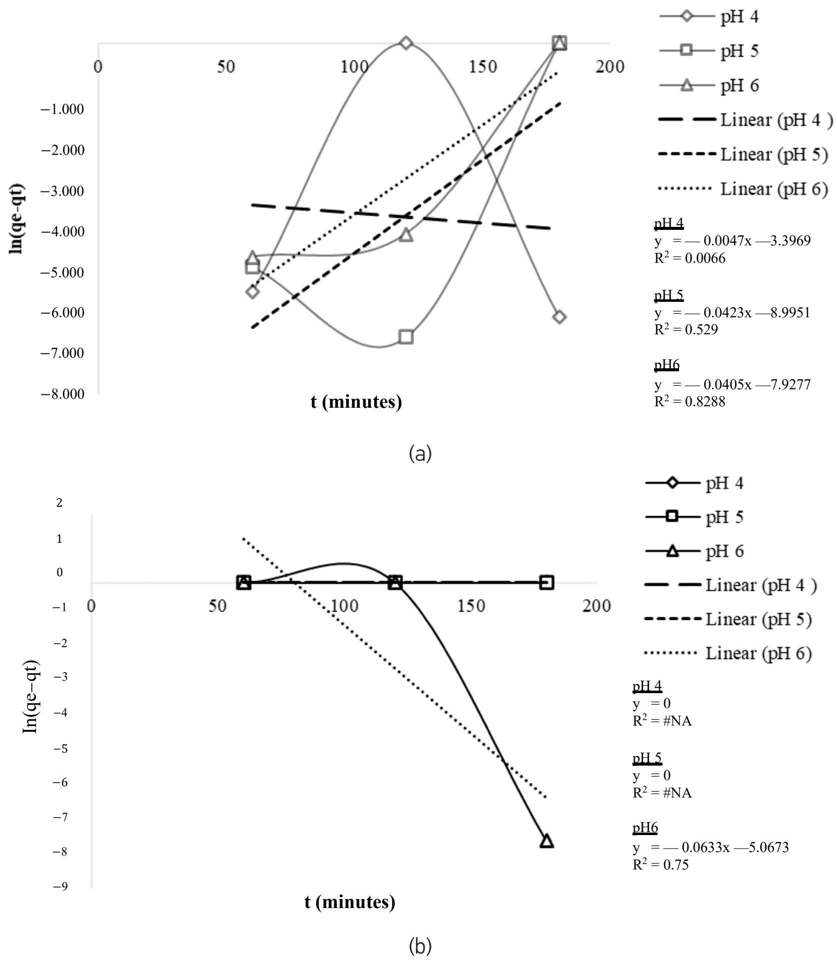


Table 7. *Calculation results of iron metal adsorption kinetics*

Order reaction	pH	R^2	Qe (mg/mL)	Reaction rate constant
First	4	0.0066	0.0335	0.0047 /min
	5	0.5290	0.0001	0.0423 /min
	6	0.8288	0.0004	0.0405 /min
Second	4	0.9982	0.0641	21.513 mL/mg.min
	5	0.9999	0.0779	1.5254 mL/mg.min
	6	0.9509	0.0781	1.5147 mL/mg.min

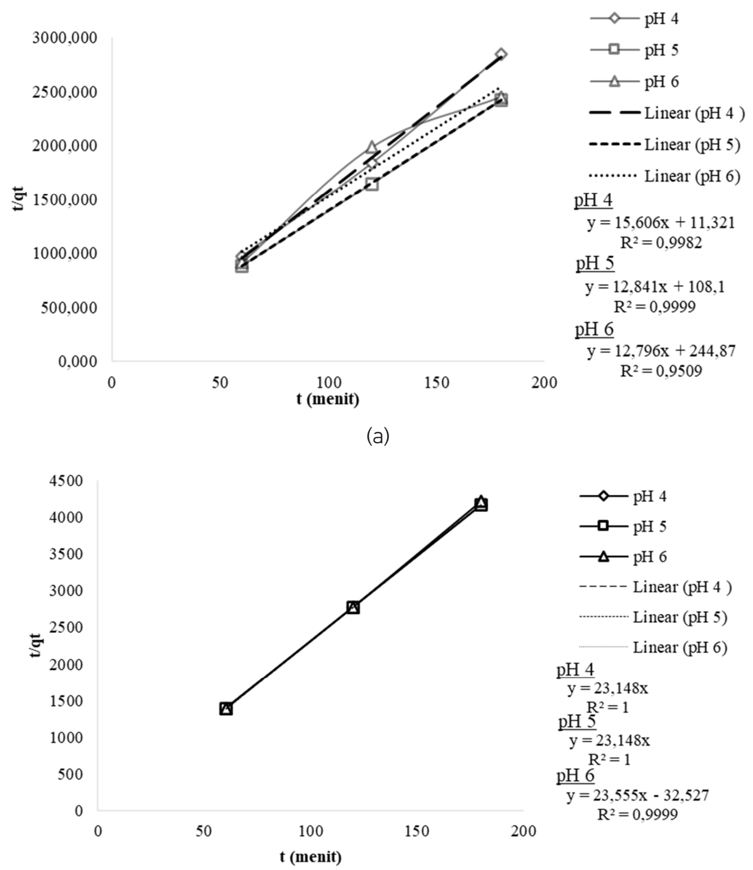
kinetics of manganese metal using the mixed culture of microalgae *Chlorella sorokiniana* and *Monoraphidium neglectum* was the second-order adsorption kinetics.

The results of this study were expected to be implemented on a larger scale. For this reason, a pilot scale implementation design was carried out to treat the 50% of AMD obtained from one of the coal mines in Indonesia which has a discharge of 146.88 m^3/day using a mixed culture of microalgae biosorbent *Chlorella sorokiniana* and *Monoraphidium neglectum* at its optimum pH of 5

Table 8. *Calculation results of manganese metal adsorption kinetics*

Order reaction	pH	R^2	Qe (mg/mL)	Reaction rate constant
First	4	—	—	—
	5	—	—	—
	6	0.7500	158.745	0.0633/min
Second	4	1.0000	0.04320	—
	5	1.0000	0.04320	—
	6	0.9999	0.04250	17.0578 mL/mg.min

Fig. 7. *Second-order adsorption kinetics curve for (a) iron and (b) manganese*



using a bioreactor (Indra et al., 2014). The treatment of AMD using a bioreactor requires a solution of AMD without suspended solids; thus, it requires a pre-treatment before pouring AMD into the bioreactor. Given the existing data of the concentration of iron and manganese of AMD in one of the coal mines in East Kalimantan, the respective value concentrations were 8.28 mg/L and 4.57 mg/L (Rizki, 2013). However, the concentration values exceeded the quality standard value stipulated in the Decree of the Minister of the Environment of the Republic of Indonesia No. 133 of 2003, which is 7 mg/L for iron metal and 4 mg/L for manganese metal.

Table 9 shows a pilot scale design and calculations; and the drawing of a pilot scale bioreactor design can be seen in *Fig. 8*.

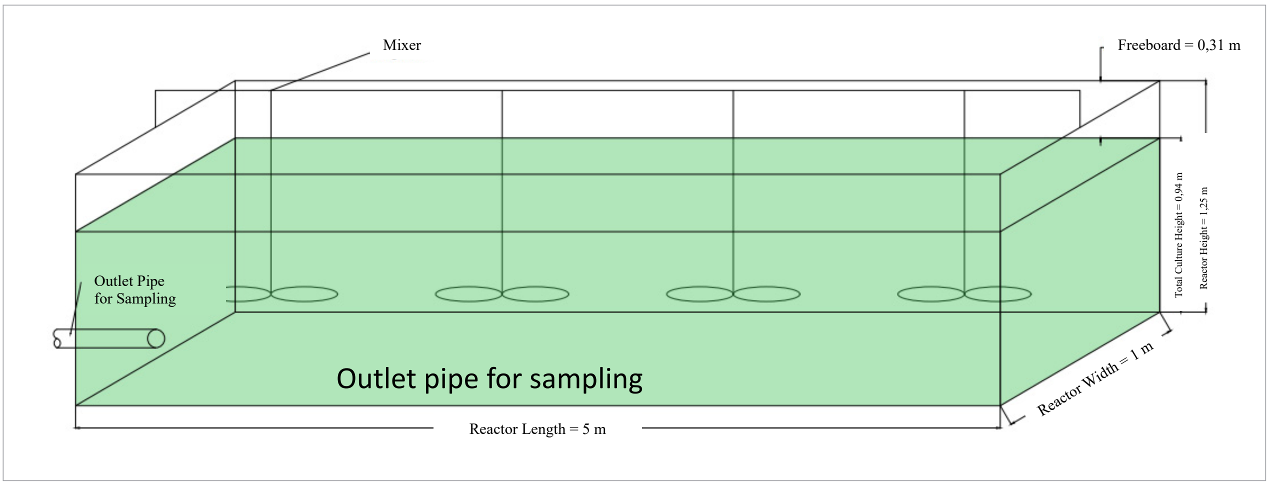
Table 9. *Pilot scale design calculation*

No.	Parameters	Value	Unit
1.	pH	5.00	—
2.	Processing debit (Q)	73.44	m^3/day
3.	Processing time (t)	22.95	min
4.	Pilot scale AMD volume (V_{AMD})	1.17	m^3
5.	Pilot scale total culture volume (Vk)	4.68	m^3
6.	Pilot scale reactor volume (V_R)	5.85	m^3
7.	Reactor length (P)	5.00	m
8.	Reactor width (L)	1.00	m
9.	Reactor height (T)	1.25	m
10.	Freeboard	0.31	m
11.	Stirring number	4	Pieces
12.	Stirring speed	4.97	rpm
13.	Required biosorbent volume ($V_{biosorbent}$)	0.066	m^3

Conclusions

The mixed culture of microalgae *Chlorella sorokiniana* and *Monoraphidium neglectum* was able to grow in AMD-contaminated media and reached a peak exponential phase on day 2. The optimum environmental conditions for the biosorption of iron and manganese heavy metals in AMD using a consortium of microalgae *Chlorella sorokiniana* and *Monoraphidium neglectum* were at pH 5 and a contact time of 180 min with a maximum removal efficiency of 89.73% for iron and 94.53% for manganese. The isotherm adsorption model that was suitable to describe the biosorption process of this study was the Langmuir isotherm model, which indicated that the biosorption process occurred on a homogeneous surface through a monolayer process. Meanwhile, second-order reaction kinetics with the Qe value from the calculation results was close to the actual Qe value, which illustrated that the solute adsorption rate was proportional to the available sites on the adsorbent.

Fig. 8. *Pilot scale bioreactor design*



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