Removal of heavy metal (Cu2+) by immobilized microalgae biosorbent with effect of temperature and contact time

N A Lieswito, A Rinanti and M F Fachrul*
Department of Environmental Engineering, Faculty of Landscape Architecture and Environmental Technology, Universitas Trisakti, Jakarta, Indonesia

*melati@trisakti.ac.id

Abstract. The purpose of this research was to removed heavy metal of Copper (Cu2+) with biotechnology using mixed-microalgae of Chlorella sorokiniana, Monoraphidium sp. and Scenedesmus obliquus bound in Na-Alginate. The effect of contact time and temperature on biosorption rate to achieve the highest efficiency were studied. Mixed-microalgae were dried in the oven, hulled, and trapping onto alginate polymer to forming an immobilized biosorbent or beads. The decrease of Cu2+ concentration was analyzed using Atomic Absorption Spectroscopy (AAS). Optimization of parameters was conducted by photobioreactor in batch system using concentrations of 20 mg/L Cu which were containing wastewater, biosorbent concentration of 0.5 g/g, pH 4 with setting of contact time (60, 120, 180) minutes and temperature (25, 35.45) oC. The results show that the maximum biosorption occurred at pH 4, temperature 35oC, contact time 180 minutes and removal efficiency of 96.4% was achieved. The research proved that immobilized biosorbent was very effective in reducing Cu2+ in wastewater and able to be used as a competitive and environment-friendly alternative method to control heavy metal pollution in water.

1. Introduction
Industrial development has been increasing throughout the globe and this does not only produce useful products, but also lead to the production of wastewater containing several heavy metals like Copper (Cu2+). However, the ineffective management of this waste causes pollution and reduces environmental quality. In drinking water, the maximum content of Cu2+ allowed comply with the Regulation of Health Department of the Republic of Indonesia Number 429/2010 on Drinking Water Quality is 2 ppm [1]. It will toxic for plants if contains more than 0.1 ppm concentration and for sheep beyond 20 ppm [2] on Standard Quality of Wastewater [3], the highest content Cu2+ recommended for metal coating is 0.5 mg/L. Therefore, there is a need to manage wastewaters containing Cu metal.

Several methods have been developed to separate heavy metal from wastewater and they include chemical deposition, mechanic filtration, ion exchange, oxidation or reduction, membranous system, and physical absorption. However, each of these has various limits, such as relatively high cost, secondary pollution production due to the chemical agents used, and production of toxic deposit [4]. However, the increased attention to the environment has led to the search for more environment-friendly alternative technologies to separate heavy metal.

One of the usable alternative methods discovered is biosorption. Biosorption used by biological agent (biomass) or microorganism in solution [5]. Since biosorption occurs metabolically in a passive process, it is possible to absorb the pollutants in the biosorbent cellular structure. However, the number of
eliminable contaminants depends on the kinetic balance and cellular surface composition of the biosorbent [6]. This process has some advantages, including low operational cost, minimum chemical agent volume and/or disposed biological mud and, high efficiency in detoxifying the much-diluted waste [7]. Moreover, the use of microalgae is advantageous due to the environment-friendliness derived from the biological agent, recyclability, low maintenance cost, high growth rate, and ability not to produce toxic waste [6].

The biosorption process makes use of biomass such as microalgae, bacteria, and fungi. However, microalgae reported have the high ability to absorb and accumulate heavy metal contained in wastewater [8], due to the functional clusters, especially carboxyl, amina, sulphate and sulphonic, binding metal ion found in the cellular wall of its cytoplasm [9]. The biomass possible to used both in the living and dead conditions in the process of biosorption. However, they have different binding mechanism depending on the metabolism system. Small-sized living biomass particle has weaknesses such as low mechanic force, causing obstruction in reactor, and difficulty in regenerating. Biosorbent while immobilized ones have advantages such as not allowing toxic heavy metal to affect the capacity of biomass absorption, no need for nutrient supply, can be regenerated and reused for some cycles [10], and the ease with which it separates solid and liquid [11].

Temperature and contact time are some of the factors observed to be affecting the process of biosorption. Temperature gives important effect during the process of biosorption because of its effects on the rate at which the process occurs. Likewise, longer contact time causes sticking and makes the adsorbate molecule work better. However, biosorption based on temperature and contact time using microalgae can be different, depending on the variations of temperature and time, types of microalgae and metals used. According to Anita and Edwan, in a research conducted on biosorption at the temperature range of 25-35°C, removal efficiency of 65% was observed at an optimum temperature of 25°C [12]. In another research by Oboh et al., microalgae of Scenedesmus sp. was utilized to obtain the highest absorption efficiency of 92.10% in 180 minutes [4]. While Zhang et al. reported the highest removal efficiency of 96.83% for 180 minutes using Scenedesmus obliquus [13].

Therefore, the purpose of this research was to obtain the highest removal efficiency of Cu heavy metal through the use of mix culture of three types of microalgae immobilized by optimizing temperature and contact time of the biosorption process.

2. Research method

2.1. Cultivation of microalgae
Microalgae of Clorella sorokiniana, Monoraphidium sp., and Scenedesmus obliquus was cultivated by photobioreactor in batch system containing growth medium of Phovasoli Haematococcus Media (PHM) to obtain sufficient amount needed as raw material for the biosorption process. The total culture volume was 80% of usable photobioreactor. PHM was added at 90% of total culture volume while microalgae seed was at 10%. The microalgae stock was cultivated up to saturation (± 16 days) and the result was conditioned at temperature 25°C, pH 8, with a light intensity of 3500 lux derived from TL lamp [14]. Furthermore, an airflow rate of 900 mL/min and light: dark period at 24:0 hours were used up to the exponential phase [15] which was determined by observing the microalga growth through the use of UV-VIS Shimadzu 800 spectrophotometer at the wavelength of 680 nm every day [16].

2.2. Preparation of biosorbent powder
The cultivated microalgae were harvested and isolated by centrifuging at 4000-rpm speed for 20 minutes. The isolated biomass was kept in a porcelain cup then dried in an oven at 100 °C for 24 hours to produce the microalgae powder ready to be immobilized [17].

2.3. Biosorbent immobilized by Na-Alginate
Two grams of the microalgae powder obtained was mixed with 4 grams of sodium alginate and dissolved in 200 mL demineralized aqua through the use of a magnetic stirrer to obtain a biosorbent concentration
of 0.5 g biosorbent/g polymer. After mixed properly, the solution was dropped in a solution of CaCl$_2$, 0.1 M 2H$_2$O (14.7 gram of CaCl$_2$ in 1000 mL distilled water) by using 20 mL syringe while stirring to prevent sticking between beads. Furthermore, the beads formed were left in a 0.1 M solution of CaCl$_2$ for 30 minutes to achieve a complete formation. They were moved to the 5 mM CaCl$_2$ solution containing 0.735 gram of CaCl$_2$ in 1000 mL stored in the freezer for 24 hours. The mixture was put in a glass cup containing distilled water and stirred for 30 minutes. The solution was sieved by cloth and washed with distilled water (aquades) to form beads with general diameters of 3-4 mm [16].

2.4. Preparation of artificial waste
Artificial wastewater containing 1000 ppm Cu$^{2+}$ concentration was prepared by weighing and dissolving some amount of copper sulphate (CuSO$_4$.5H$_2$O) in demineralized water to form the main solution. This was further diluted to obtain different Cu$^{2+}$ concentration while the pH value was set by adding a 0.1 N HCl solution or 0.1 N NaOH [2].

2.5. Optimization of temperature
The research concerning copper absorption with immobile biosorbent was conducted by preparing 20 mg/L wastewater concentration, at 120-minute contact time, pH 4, and variations of temperature between 25, 35, and 45°C. Furthermore, 10 g immobile biosorbent was contacted to 50 mL Cu$^{2+}$ solution in aerated Erlenmeyer flask on a hotplate with temperature set and controlled during the research. The aeration was used to ensure sufficient contact between biosorbent and wastewater solution during mixing. Moreover, the remaining Cu$^{2+}$ copper in the solution was analyzed using Atomic Absorption Spectrophotometry (AAS).

2.6. Optimization of contact time
10 g immobile biosorbent was contacted for 60, 120, and 180 minutes in a 100 mL solution containing Cu$^{2+}$ 20 mg/L in the aerated Erlenmeyer flask. In optimizing the contact time, the optimal temperature used was in reference to the previous biosorption process conducted and the sample of a solution containing Cu$^{2+}$ was analyzed using AAS.

2.7. Percentage of Cu$^{2+}$ heavy metal removal
After the AAS analysis, the following equation was used to calculate the removal efficiency for Cu$^{2+}$ heavy metal:

\[
\% \text{removal} = \left(\frac{C(a) - C(b)}{C(a)}\right) \times 100\%
\]

Where:
- $C(a)$ = Initial concentration of Cu$^{2+}$
- $C(b)$ = Final concentration of Cu$^{2+}$

3. Results and discussion

3.1. Cultivation of microalgae
Figure 1 showed that the microalgae experienced four growth phases which included lag, exponential, slow growth, and stationary phases in 16 days. The exponential phase was found to occur in day 8 with the microbe harvested by precipitation method and centrifuged and dried in an oven for 24 hours. The dried product was trapped by Na-Alginate polymer to form immobile microbe bio-sorbent.
3.2. Effect of temperature
The temperature where the copper biosorption experienced the highest efficiency was analyzed. The results indicate immobile biosorbent is able to absorb heavy metal (Cu$^{2+}$) and reduce concentration in artificial wastewater. Furthermore, temperature gave an important effect on metal absorption, because most of the chemical reaction rates were sensitive to change in temperature [6]. Figure 2 shows biosorbent could have removed Cu$^{2+}$ at temperature range of 25°C up to 45°C with the highest removal efficiency of 94.8% observed at 35°C.

![Figure 2. The effect of temperature of Cu$^{2+}$ removal.](image)

3.3. Effect of contact time
The contact time was optimized on the 35°C optimum temperature obtained in the previous phase. Figure 3 shows that from 60 up to 180 minutes it has been removal of Cu$^{2+}$ metal ions by biosorbents of more than 85%, the absorption process occurred physically in bio-sorbent pores at a relatively shorter time [2]. However, the optimum contact time produced in this research was shorter than the result obtained from the research by Rinanti et al. that Cu$^{2+}$ metal with 41.99% of specific initial concentration could be absorbed at a contact time of 240 minutes [18]. This variation may be associated with the use of different microalgal as biosorbent, variations of functional cluster group, wide biosorbent surface, total usable biosorbent, and pore volume affecting the ability to absorb Cu$^{2+}$ metal.

![Figure 3. Mixed microalgae growth curve.](image)
4. Conclusion
The results of the cultivation of the mixed microalgae in 30-L photobioreactor indicated it could live in synergy in the exponential phase in day 8. At this time, it was ready to be harvested and immobilized using Na-Alginate polymer as biosorbent. Furthermore, the most effective condition in the biosorption process occurred at 35°C and 180-minute contact time. The percentage of Cu\textsuperscript{2+} removal at 35°C for 120 minutes was 94.8%, whereas with a contact time of 180 minutes Cu\textsuperscript{2+} removal reached 96.4%. This shows that the longer biosorption process leads to higher removal percentage of Cu\textsuperscript{2+}. Therefore, this research proves immobilized microalgae to be a very effective biosorbent in reducing Cu\textsuperscript{2+} in wastewater.

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Figure 3. The Effect of Contact Time of Cu\textsuperscript{2+} Removal.
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