Copper biosorption using beads biosorbent of mixed culture microalgae

T Wilan, R Hadisoebroto* and A Rinanti
Environmental Engineering Department, Faculty of Landscape Architecture and Environmental Technology, Universitas Trisakti, Jakarta, Indonesia
*rositayanti@trisakti.ac.id

Abstract. Research on the sorption process of copper metal (Cu2+) from artificial electroplating wastes using a biotechnology approach was studied using beads biosorbent of mixed culture microalgae Chlorella vulgaris, Chlorococcum sp., and Scenedesmus obliquus conducted in batch culture to determine the highest efficiency of Cu2+ adsorption as an effort to control Cu2+ pollution from the environment. Biosorbent is made into beads by mixing 0.5 gr (dead biomass)/gr (Na-alginate polymer). The beads biosorbent of these microalgae are contacted with Cu2+ in the pH range 4-6 and contact time 0-180 minutes. The highest sorption efficiency of Cu2+ is 92% obtained at pH 4, 120 minutes’ contact time, in temperature of 25 ºC, and with an initial waste concentration of 25 mg/L. The sorption process of this research follows Langmuir Isotherm with R2 value of 0.9991. It is proven that beads biosorbent of mixed culture microalgae can be used as an environmental-friendly solution to control Cu2+ pollution in the environment.

1. Introduction
Mining activities which produce various wastes, such as heavy metals, have significantly increased due to industrialization. One research Adji show that in the Rancaekek sub-district and its surrounding there are some textile industry areas with a total of up to 30 factories and from sludge analysis in one of the largest textile factories, it is reported there were 15.61 ppm Pb and 413 ppm Cr [1]. An example of common wastes in these areas is Copper, a heavy metal often used in the industries [2]. Copper is very useful to living organisms when in small amounts but very dangerous and toxic in excess [3]. According to the Waste Water Quality Standard in the regulations of Indonesian Minister of Environment No.5 of 2014, the allowable Cu2+ content is only 0.5 mg/L [4]. Apart from being dangerous and toxic, generally it is corrosive and difficult to degrade. Therefore, efforts are needed to treat the waste containing copper metal in order to reduce the excessive concentration in the environment.

Several methods based on physics, chemistry and biology are used to address this problem. In physical-chemical methods, the copper-containing wastes could be treated with several approaches such as precipitating it as hydroxide or sulfide, capturing Cu2+ with certain resins in the ion exchange process [5]. The process is, however, not effective in the concentration of heavy metals in waters less than 50 mg/L and produces large amounts of mud which is difficult to overcome [6]. Alternatively, the biological processes using microbes significantly reduce the problem of heavy metal pollution. This is a biological approach using the microbes, either living or dead, to carry out ion exchange, complex formation and the absorption of heavy metals. The best microbes for this role are algae, the natural ingredients with active groups, which play a significant role in binding the metal ions. The organisms
are very abundant in both Indonesian freshwater and seawater. However, other studies suggest microalgae (live or die) may eliminate the heavy metals even in low concentrations [7]. Generally, the algal biomass contains several functional groups acting as ligands in metal ions [8,9]. The main functional groups are carboxyl groups, hydroxyl, sulfhydryl, amino, imidazole, sulfate, and sulfonates contained in cell walls within the cytoplasm. Importantly, there are several factors influencing the biosorption process, including acidity (pH), initial waste concentration, temperature, and biomass concentration in solution [10], and contact time (td).

According to research Aksu and Kutsal, the use of Chlorella vulgaris as biosorbent at pH 4 absorb the copper metal by 37.6 mg/g [11]. With the same biosorbent at pH 5, another research Dönmez et al. reported that the copper metal uptake was only 1.8 mg/g [12], while according to the research Romera, the absorption of Cu2+ could reach 34.89 mg/g [13]. Scenedesmus obliquus is a biosorbent with the ability to absorb Cu2+ up to 20 mg/g [12]. The mixed cultures of microalgae have the ability to absorb Cu2+ higher than a single culture [14].

The use of microalgae biomass as biosorbent applies in living or dead conditions. Generally, the live biosorbent has several disadvantages, such as low biosorbent density and perishability. Nonetheless, immobilizing the biomass helps to overcome this weakness. Immobilization is a method of binding cells to the supporting matrix in order to improve the stability of the cells and could be used continuously [15]. This process creates a biosorbent structure in the beads. The immobilized biosorbent has the adsorption ability of Cu2+ + 71.91%. In this condition, the immobilized biosorbents have better adsorption ability than the mobile [14]. The research established that alginate is a good immobilization matrix because it is efficient, easy to use, can be modified, does not cause allergic reactions and is nontoxic [16]. The results of other research also prove that alginate has the ability to dissolve in water and increase the viscosity of the solution, the ability to form gels, films (sodium and calcium alginate) and fiber (calcium alginate) [17]. The Chlorella vulgaris immobilized using sodium alginate at pH 4.5 is able to absorb Cu2+ by 63.08 mg/g [18].

This study aims to absorb Cu2+ ions using biosorbent beads of freshwater microalgae in mixed cultures consisting of Chlorella vulgaris, Chlorococcum sp., and Scenedesmus obliquus. The results were expected to be used as an alternative solution to Cu2+ heavy metal pollution in the environment.

2. Research method
The Cu2+ biosorption process was carried out using a mixed culture microalgae immobilized in beads as biosorbent to absorb the metal ions in the waste.

2.1. Microalgae cultivation

The Microalgae Chlorella vulgaris, Chlorococcum sp., and Scenedesmus obliquus were grown in batch culture using the artificial growth media of PHM (Provasoli Haematococcus Media). The microalgae cultivation was carried out on 30 L photobioreactor and 1 L Erlenmeyer flask. The ratio between microalgae and PHM media was 1:9. The environmental conditions were set at a room temperature 25±2°C, pH 6, lighting intensity 3500 lux, and the air flow rate of 0.3 L/sec. The growth of microalgae in this mixed culture was determined using the optical density method. In measuring the absorbance value, UV-Vis spectrophotometer with a wavelength of 680 nm was used. After absorbance value was obtained, a growth curve was made to get the exponential phase.

2.2. Biosorbent microalgae beads preparation

The cultivated microalgae were harvested and separated in 2 ways on the 6th day by centrifuging at a speed of 2000 rpm for 20 minutes and with biofloculation which was a deposition of microalgae by gravity without aeration. The separated biomass was then put into a porcelain dish to be dried in oven for 24 hours at 80°C. To obtain the dry weight, the biomass was weighed using an analytical balance and ground finely to produce its powder.

The powdered biomass obtained were weighed of 2 grams and mixed with 4 grams of sodium alginate 2% before dissolving in 100 mL of distilled water. After being completely mixed, the solution was
dropped by gravity using a 20 mL (no needle) syringe into 1M CaCl$_2$ solution to form the beads ± 0.5 cm in diameter. The biosorbent beads were stored in a 5mM CaCl$_2$ solution at 4ºC for 24 hours. These beads were then washed and stored in distilled water at 4ºC until they were ready for use.

2.3. Biosorption process

In order to determine the highest Cu$^{2+}$ absorption and adsorption capacity, the biosorption process was carried out by adjusting the pH value and contact time using HCl and NaOH solutions to the pH 4, 5, and 6. The concentration of Cu$^{2+}$ solution in waste was 25 mg/L, the weight of biosorbent beads was 10 grams, and the temperature 25 ± 2ºC. The biosorbent beads and waste were contacted in a photobioreactor and aerated from below using a hose to make link between biosorbent beads and waste solution. This process was carried out with contact times of 60, 120 and 180 minutes. The empty biosorbent (sodium alginate beads without microalgae biomass) was also used in this experiment as a control. After the biosorption process was completed, the biosorbent beads and waste solution were separated and examined using AAS (Atomic Absorption Spectrophotometer) to determine the concentration of Cu$^{2+}$ ions contained in the waste at the beginning and end of the procedure.

2.4. Removal efficiency analysis and adsorption capacity

The adsorption capacity/the amount of copper ions absorbed by biosorbent beads was calculated using the following equation:

$$q = \frac{[(C_o-C).V]}{W}$$ (1)

Where $q$ is the amount of copper ions absorbed by biosorbent (mg / g). The initial waste $C_o$ and final one contains ion concentrations (mg/L). $V$ is the initial volume of waste containing solution copper (L) and $W$ is the biosorbent weight beads (gr). The allowance efficiency may also be determined using the following equation (2):

$$RE = \frac{[(C_o-C) / C_o] x 100\%}$$ (2)

RE is the efficiency of the elimination of the absorbed copper metal ions (%), the initial waste $C_o$ contains copper ion concentration (mg / L) while the final had concentration after adsorption (mg / L).

2.5. Adsorption isotherm

The isotherm adsorption study showed the biosorbent beads capacity for the adsorption. This is explained by the equilibrium sorption isotherm indicated by certain constants expressing the surface properties and affinity of the biosorbents. This study used 2 models of isotherm as adsorption, Langmuir and Freundlich.

In Langmuir isotherm, plotting C to C/qe led to constant $b$ and $q_m$ where $1 / b$ was a slope and $1/q_m$.b the intercept. The maximum capacity of adsorption and $q_m$ was obtained from the intercept and slope [6]. The equation of this model was:

$$\frac{C}{qe} = \frac{1}{b.q_m} + \frac{C}{q_m}$$ (3)

$C$ was the final waste concentration after the biosorption process (mg / L), $qe$ the amount of copper ion adsorbed (mg/g), the coefficient of $b$ Langmuir, and $q_m$ the adsorption capacity of biosorbent beads (mg /g).

The Freundlich Isotherm model assumes the adsorption runs in physics. The equation for getting the Freundlich constant is:

$$qe = k. C^{1/n}$$ (4)

$$\log qe = \log k + (1/n . \log C)$$ (5)

The $k$ and $n$ are Freundlich's constants, system characteristics, indicators of adsorption capacity and intensity.
3. Results and discussion

3.1. Microalgae cultivation
From the growth curve shown in Figure 1, it is evident the mixed culture microalgae reached the exponential phase on day 6.

Figure 1. Growth curve of mixed culture microalgae of Chlorella vulgaris, Scenedesmus obliquus, and Chlorococcum sp.

In the exponential phase, the photosynthetic activity occurs very high and it is useful in forming proteins during the growth period [19]. Harvesting of microalgae was carried out on the 11th day, or at the end of the exponential phase, using the centrifugation and bioflocculation methods. The results were dried and made beads for use in the biosorption process.

3.2. Biosorption process
The adsorption of Cu$^{2+}$ ions in the biosorption process as a function of pH and time was as shown in Figure 2. The pH value and contact time were some of the factors which influenced the biosorption process.

Figure 2. Cu$^{2+}$ removal efficiency with variation of pH and contact time using beads biosorbent of mixed culture microalgae (initial Cu$^{2+}$ concentration 25 mg/L; beads biosorbent weight 10 gr).

The Cu$^{2+}$ ions adsorbed at pH 4 tend to be higher at contact times 120 and 280 minutes (Figure 2). The maximum absorption of Cu$^{2+}$ occurs at pH 4 with a contact time of 120 minutes, and the removal efficiency reaching 92%. When compared with the results of research from researchers Siwi using a mixed culture of S. cerevisiae and Chlorella sp. immobilized with pH 4 and contact time of 120 minutes to absorb Cu$^{2+}$ with removal efficiency of 81.05%, this study was better and efficient in absorbing Cu$^{2+}$ [14].

The Cu$^{2+}$ absorption capacity at various pH and contact times is shown in Table 1. The pH optimization showed the most effective biosorbents for absorb copper ions. According to Aksu and Kutsal, pH greatly affects the protonation of metal binding sites exposed to the cell surface [11]. At 60 minutes, the percentage of Cu$^{2+}$ removal was higher than other contact times. For 120 and 180 minutes at pH 5 and 6, the percentage allowance was lower compared to the biosorption process at 60 minutes. At pH 4, the percentage allowance increased by 4.4% from the contact time of 60 minutes to 120 minutes. A fast time balance was probably caused by finer particle size of the biomass [20].
Table 1. Cu\(^{2+}\) Adsorption efficiency using beads biosorbent of mixed culture microalgae.

| pH 4 | Contact Time (Minutes) | Initial Waste Concentration (mg/L) | Final Waste Concentration (mg/L) | Cu\(^{2+}\) Adsorbed (mg/L) | Removal Efficiency (%) | Adsorption Capacity (mg/gr) |
|------|------------------------|-----------------------------------|---------------------------------|---------------------------|------------------------|---------------------------|
| 60   | 3.1                    | 21.9                              |                                 | 87.6                      | 0.219                  |
| 120  | 2.0                    | 23.0                              |                                 | 92.0                      | 0.230                  |
| 180  | 2.5                    | 22.5                              |                                 | 90.0                      | 0.225                  |
| 60   | 2.6                    | 19.7                              |                                 | 89.6                      | 0.224                  |
| 120  | 3.0                    | 22.0                              |                                 | 88.0                      | 0.220                  |
| 180  | 3.8                    | 21.2                              |                                 | 84.8                      | 0.212                  |
| 60   | 2.7                    | 20.8                              |                                 | 89.2                      | 0.223                  |
| 120  | 3.1                    | 21.9                              |                                 | 87.6                      | 0.219                  |
| 180  | 4.0                    | 21.4                              |                                 | 84.0                      | 0.210                  |

3.3. Adsorption isotherm

Figure 3 describes the study of isotherms in adsorption of Cu\(^{2+}\) ions by microalgae beads obtained using the optimum pH value 4 and contact time 120 minutes.

![Langmuir Isotherm graphic on Cu\(^{2+}\) biosorption process.](image1)

![Freundlich Isotherm graphic on Cu\(^{2+}\) biosorption process](image2)

The figure also shows the Langmuir constants and correlation regression coefficients of the copper biosorption process in this study. The value of R\(^2\) for the Langmuir isotherm was 0.9991, the constant b was 2.76121 and the adsorption capacity of biosorbent beads to absorb Cu\(^{2+}\) was 0.19226 mg/g. Furthermore, Figure 4 shows the Freundlich constant and correlation regression coefficient of the copper biosorption process. The value of R\(^2\) for the Freundlich isotherm was 0.9828, k was 0.004595 and n was -7.5018. The R\(^2\) value was used as a criterion for both isotherm models [21]. Langmuir's isotherm R\(^2\) value was greater than the Freundlich's isotherm. Therefore, the biosorption process follows Langmuir's isotherm and stops on a monolayer consistent with the specific ones to the functional binding sites [22].

4. Conclusion

The mixed culture microalgae biosorbent beads of Chlorella vulgaris, Chlorococcum sp., and Scenedesmus obliquus are suitable in reducing Cu\(^{2+}\) from the wastewater solution due to the high removal efficiency of the biosorption process. These microalgae reach the exponential phase on the 11th day. The percentage of maximum allowance for Cu\(^{2+}\) using microalgae beads occurs at pH 4 with a contact time of 120 minutes. The biosorption process is well explained by the Langmuir Isotherm model with the R\(^2\) value of 0.9991, constant b of 2.76121, and the biosorbent adsorption capacity of beads to absorb Cu\(^{2+}\) is 0.19226 mg/g.

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