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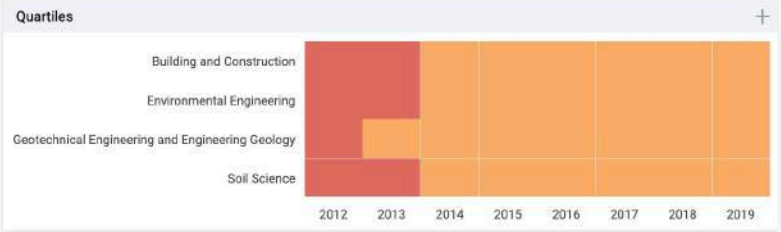
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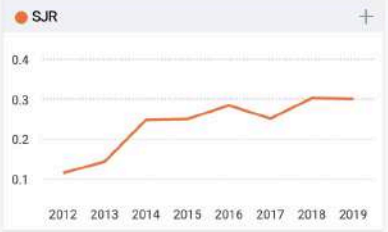
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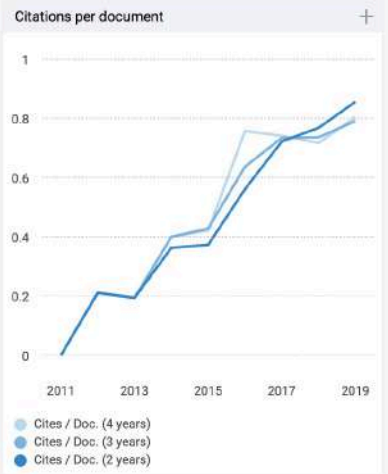
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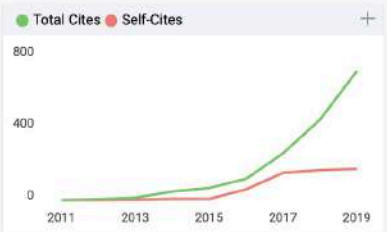
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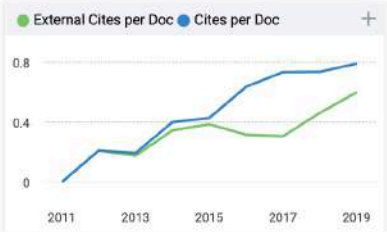
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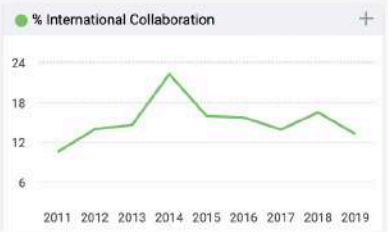
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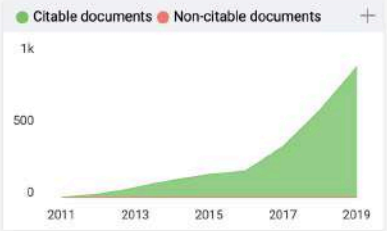
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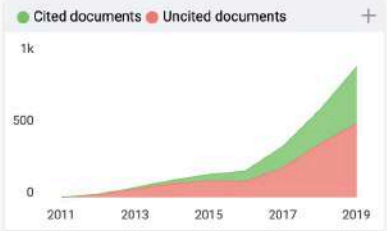
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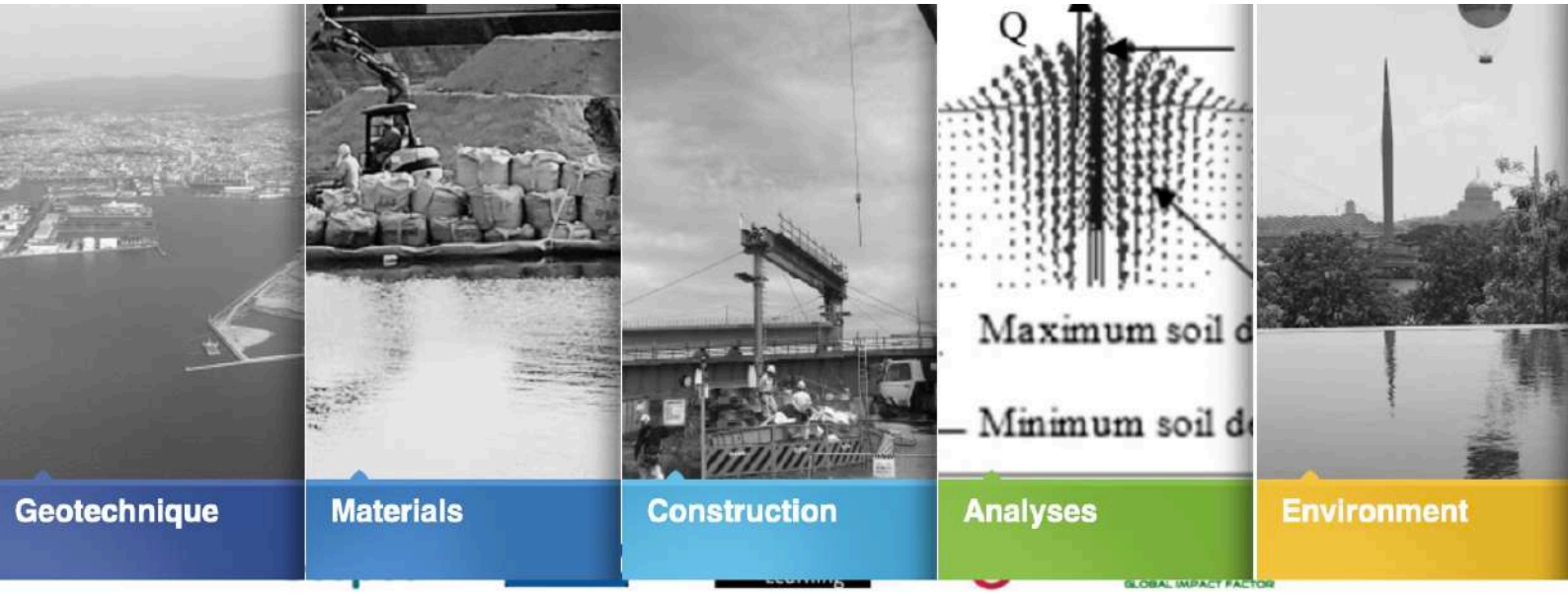

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IMPROVING BIOSORPTION OF CU(II)-ION ON ARTIFICIAL WASTEWATER BY IMMOBILIZED BIOSORBENT OF TROPICAL MICROALGAE

*Astri Rinanti, Melati Ferianita Fachrul, Rositayanti Hadisoebroto, Mawar Silalahi

Faculty of Landscape Architecture and Environmental Technology, Trisakti University, Indonesia

Corresponding Author, Received: 15 June 2016, Revised: 30 Nov. 2016, Accepted: 20 Dec. 2016

ABSTRACT: This research purposes to study the role of microalgae in tropical environment – isolated from Wastewater Treatment Plant (WWTP) Setiabudi, Jakarta, Indonesia – on biosorption of Cu(II) ion in heavy metal wastewater. The effects of pH and contact time on the rate of metallic biosorption were examined to reach the greatest biosorption efficiency. Microalgae diversity analysis through phenotypic approaches showed that the microalgae community was comprised of 3 species of Chloropyceae i.e *Ankistrodesmus braunii*, *Chlorella* sp., and *Scenedesmus quadricauda var quadrispina*. Immobilized biosorbent from microalgae was prepared by oven-drying, grinding, and entrapping the microalgae biomass into polymeric matrix of alginate. The sorption properties of biosorbent was characterized by using infrared spectroscopy and SEM micrograph analysis. The optimization of sorption parameters was conducted in batch systems using Cu(II)-artificial wastewater of 300 ppm with pH arrangement of 2-7 and the contact time arrangement of 60, 90, 120, 180, 240 minutes. During the experiment, the Chlorophyceae free and Na-alginat immobilized biomass were added. The research showed that either free and immobilized biomass could adsorb Cu(II) metal ion and reduce its concentration into 25-50 ppm. A maximum biosorption by the alginate-immobilized biosorbent obtained at pH 3,0 and contact time of 180 minutes with 43% absorption efficiency. Sorption properties of microalgae biomasses were indicated by various functional groups presence on biosorbent that could bind heavy metals compared to others. The research proved that the alginate-immobilized biosorbent was highly effective for the treatment of Cu(II)-artificial wastewater and that tolerant cholorophyceae could act as an effective biosorbent in further optimization.

Keywords: Biosorption, Immobilized biosorbent, Cu(II), Microalgae diversity, Tropical environment

1. INTRODUCTION

The increasing of industrial development leads to heavy metal pollution in aquatic environment. Heavy metals are certainly needed by organisms to support the enzymatic process. However, in excessive amount, they can interfere the growth of the organisms. Copper or Cu is a heavy metal found in natural waters and is an essential matter to microalgae. Copper acts as a constituent of 13 plastocyanin, functioning in electron transport in photosynthesis [1]. Copper is encountered in the center of cytochrome c oxidase that makes up the superoxide dismutase enzyme and carries oxygen in hemocyanin pigments. Many kinds of enzymes contain copper [2]. Copper is considered to be toxic to plants at concentration above 0.1 ppm. Copper level in potable water should be not more than 1 ppm and is toxic to sheeps at concentration above 20 ppm. The presence of copper in wastewater ordinarily appears in the form of bivalent ion of Cu(II) as a hydrolytic product. Copper often found in wastewater of dyeing, paper, oil, and coating industry.

Conventional removal techniques for Cu(II) i.e precipitation, electrochemical, ion exchange, and membrane filtration, have their own limitations such as involving high installation cost, causing secondary pollution due to the chemicals use, and yielding toxic sludge [3], [4]. In addition, these processing techniques are also not able to meet the existing quality standards [5], [6]. Therefore, it is necessary to develop an alternative method of heavy metal removal to tackle the shortcoming mentioned above, among them is biosorption technology.

Biosorption is an absorption process utilizing the ability of biological materials (either macro or micromatters; either living or dead materials) to accumulate heavy metals from a solution metabolically or physicochemically [7]. Advantages gained from the use of microorganisms as biosorbent are low operational cost, high efficiency and high metal binding capacity, minimum sludge product leading to

minimum environmental impact, having a possibility of metal recovery as a result of desorption mechanism, having a regeneration mechanism (can be reused as biosorbent), as well as easily obtained, abundant raw materials [5].

Metal biosorption may occur due to the complexity of the positively charged metal ions with negatively charged active centers on the surface of the cell wall or on the extracellular polymers, such as proteins and polysaccharides as a source of functional groups playing important role in binding metal ions. This absorption process takes place rapidly in living cells as well as dead cells [7]

This absorption process does not depend on the metabolism, primarily because it occurs on the surface of the cell wall. Absorption by microorganisms is divided into two, namely metabolically independent absorption occuring on the surface of dead cells and metabolically dependent absorption taking place on the surface of living cells, that is slow and depends a lot on the nutrient availability and environmental conditions, e.g. solution pH and temperature [8]. Many factors can affect metal biosorption, among them are the type of biosorbent [5], pre-treatment of the biosorbent [6], solution pH [8], [9], [10], initial concentration of the adsorbate [11], also the influence of other ions in the solution [4].

The application of microalgae as a biosorbent is more favorable considering that small size of the cells provides larger specific surface that implicates on the higher absorption capacity. Moreover, high sorption ability is led by the presence of functional groups on the microalgae cell wall that are able to bind metal ions, particularly carboxyl, hydroxyl, amine, sulfhydryl, imidazole, sulfate and sulfonate chains [7]. As the raw material of biosorbent, microalgae is handily found, plenteously available, and does not need additional nutrients so that the operational cost can be cheaper [8].

On the other hand, the small size of the particles has disadvantages such as low mechanical strength, causing column plugging and clogging [8], the difficulty of biosorbent regeneration [12], also the requirement of complicated solid-liquid separation process [13]. To overcome these problems, immobilization of the tropical microalgae biomass in a biopolymeric matrix was applied in this study.

Immobilization can be defined as physical entrapment or localization of microalgae cell in such a way to limit the freely migration of microbes [14], while [15] explain that the biomass immobilization is a technique in which the target cell will be coated by porous polymeric layer that allows subtrate diffusion process into the cell. Several synthetic materials e.g. silica, polysulfone, polyurethane, polyvinyl alcohol, and acrylic polymers as well as natural polymers such as alginate, gelatin, agar, and cellulose derivatives can be used for the immobilization of microalgae biomass [16].

The objective of this study was to obtain the highest removal efficiency of heavy metal Cu(II) by utilizing immobilized tropical microalgae biomass in order to optimize the pH and contact time of biosorption.

2. MATERIAL AND METHODS

2.1 Isolation and Identification of Tropical Microalgae

Isolation and sampling 3 species of Chloropyceae i.e *Chlorella* sp., *Ankistrodesmus braunii*, and *Scenedesmus quadricauda var quadri-spina* derived from algal-blooming took place in stabilization pond of Wastewater Treatment Plant (WWTP) Setiabudi, Jakarta, to be used as biosorbent. The method employed in identification of microalgae was phenotype approach, based on microscopical morphology analysis.

2.2 Preparation of Biosorbent

Washed microalgae biomass were activated chemically using acidic and alkali solution. Biomass rinsed with distilled water were used as the controls. Some biomass were suspended and soaked for 3 hours in a solution of 0,1 N HCl; 0,1 N NaOH, and distilled water (dH_2O) with a biomass to solution weight ratio of 1:3. Activated biomass then dried in an oven at 70°C for 20 hours, grinded using mortar, and screened using sieve size of 50-mesh (0,3 mm). Biomass powder sized ≤ 0,3 mm were used as biosorbent. This research was conducted in a batch reactor.

2.3 Immobilization of Biosorbent

Immobilization of biosorbent was carried out using entrapment method [17]. Some biomass powder were suspended in 100 mL solution of sodium alginate 2% (w/v). The suspension was dripped into a 500 mL solution of $CaCl_2$ 4%. When in contact with $CaCl_2$ solution, biomass suspension of sodium alginate were then polymerizing into bead forms—Na-alginate entrapped the biomass in its structure.

2.4 Preparation of Artificial Wastewater

Artificial wastewater containing Cu(II) (1,000 mgL^{-1}) was made by weighing a certain amount of copper sulphate ($CuSO_4 \cdot 5H_2O$) pro analysis and

dissolving it in a deionized distilled water to make a stock solution. To obtain different concentration of Cu(II), the stock solution was diluted in accordance to the necessary concentration. The value of pH was adjusted using HCl solution of 0,1 N and NaOH 0,1 N.

2.5 Optimization of pH

A total of 10 g immobilized biosorbent was contacted with 50 mL solution of Cu^{2+} 50 mg/L in an Erlenmeyer flask of 150 mL capacity. The value of pH was set in a range of 2, 3, 4, 5, 6, 7, 8. Furthermore, Selanjutnya, Erlenmeyer containing the mixture was stirred using a shaker with a speed of 180 rpm at optimum contact time and temperature. The metal (Cu^{2+}) remained in the solution was analyzed using AAS (Atomic Absorption Spectroscopy).

2.6 Optimization of Contact Time

A total of 10 g immobilized biosorbent was contacted with 50 mL solution of Cu^{2+} 20 mg/L in an Erlenmeyer of 150 mL volume. The Erlenmeyer then stirred using a shaker at 180 rpm. The metal Cu^{2+} remained in the solution at contact time of 60, 90, 180, and 240 minutes were then analyzed using AAS spectrophotometer.

2.7 Removal Efficiency of Cu(II)

Absorption efficiency of metal Cu(II) by the immobilized biomass of tropical microalgae was calculated using following formula:

$$\% \text{ Cu(II) removal} = [(Co - Ce) / Co] \times 100\% \quad (1)$$

Co = initial concentration of Cu (II) solution (mg/L)
Ce = equilibrium concentration (mg/L)

3. RESULTS AND DISCUSSION

3.1 Effect of the Biosorbent Immobilization

The effects of both immobilized and mobile (free) biosorbent are depicted in Fig.1.

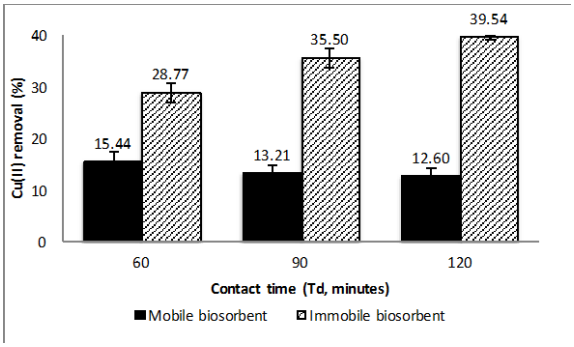

Fig. 1 Removal efficiency of heavy metal Cu(II) by mobile and immobilized tropical microalgae, at temperature 28 °C ± 2 and pH 3

The results show that both free and immobilized biosorbent have the ability to absorb heavy metal Cu(II) and reduce its concentration in the artificial wastewater. However, immobilized biosorbent delivered a better Cu(II) removal efficiency which is 2-3 times higher than mobile biosorbent efficiency.

Chlorella homospaera immobilized in alginate brought favorable system to lower the level of copper, cadmium, zinc, and gold in a contaminated water [1], meanwhile [17] stated that immobilized *Chlorella emersoni* cells was better in accumulating copper than mobile cells. Researcher [14] stated that adsorption capacity of *Spirulina* sp. microalgae biomass can reach 213 mg/g. This ability is considered to be exceptional because according to [5], the adsorption capacity of biomass has to exceed 150 mg/g to be able to compete with other technologies.

3.2 Optimization of pH

The effects of pH and contact time towards the rate of metal biosorption were examined to determine the highest biosorption efficiency. The value of pH is one of the crucial physicochemical parameters in biosorption process [8], [9], [10]. The effect of pH on the sorption of Cu(II) by the immobilized biosorbent of tropical microalgae can be seen in Fig. 2.

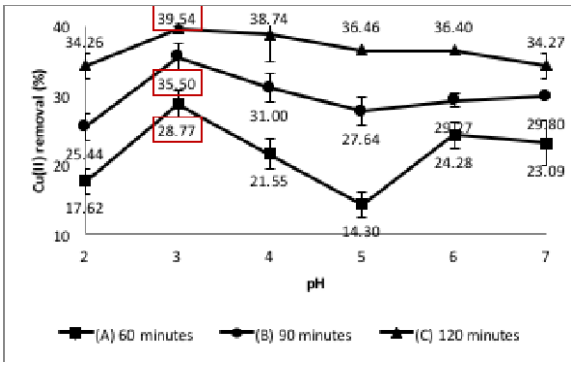

Fig. 2 The effect of pH towards Cu(II) biosorption by microalgae biosorbent, at temperature 28 °C ± 2 and contact time (Td) 60, 90, 120 minutes

Among the physicochemical factors affecting biosorption, apparently pH value is the key factor that was most responsible. The pH value of the solution affects metal speciation [5] and the ionization of biosorbent's functional chains [5], [18]. Most studies of metal biosorption by microalgae showed the dependence relationship to pH value (pH-dependent). At low pH, competition between protons and metal cations such as Cu occured to the active sites of biosorbent. As a result, the cation removal efficiency decreased at low pH. On the contrary, in the biosorption of metal anion such as SO_4^{2-}, the anion metal removal efficiency increased at low pH [18].

Figure 1 reflects that pH value greatly affect the biosorption process of Cu(II) by the biosorbent. The value of pH affects not only the charge of biosorbent functional groups, but also the metal ion speciation [19]. At pH value of 3, the biosorbent with all variation of contact time (60, 90, 120 minutes) removed Cu(II) of 28-40% more than the removal at higher pH. At low pH, the biosorbent surface becomes positively charged so that it can enhance the sorption of metal anion. Researcher [19] also proved that the highest removal of Cu(II) was at pH value of 1.5. The highest Cu(II) removal reached at a very acidic pH condition (≤ 2) was also reported by other researchers [10], [18], [20].

3.3 Optimizing the Contact Time

The effect of contact time towards the sorption of Cu(II) by phytoplankton biomass is shown in Fig 3 below. Figure 3 represents that in such condition, more than 25% of metal removal was obtained during the first 60 minutes in this study. This fast metal removal kinetics could be triggered by the stirring treatment that minimizes the mass transfer constraints. In addition, the rapid sorption kinetics could also indicate that the adsorption occured physically to the pores of the biosorbent.

The optimum percentage of Cu(II) removal was obtained after 180 minutes of contact time, whereas longer than that, the removal percentage proned to be stable with a slight decline (Figure 3). It shows that the sorption process began to reach equilibrium state between sorption rate and desorption rate. Based on that, the optimization of physicochemical parameter in the future studies will be carried out with a contact time of 180 minutes.

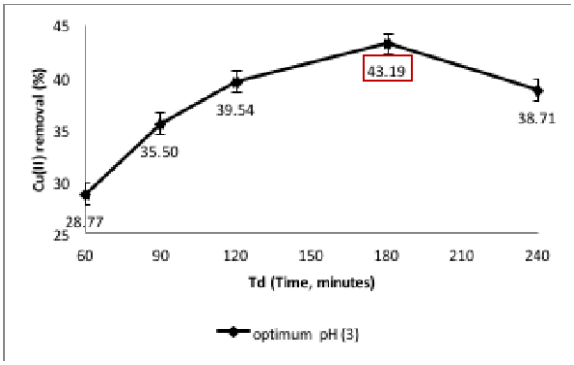

Fig. 3 The effect of contact time on the sorption of Cu(II) by microalgae biosorbent, at temperature 28°C ± 2 and pH 3

The optimum contact time generated in this study is longer compared to the research done by [20], which stated that initial Cu and Zn concentration of 20-27 ppm could be reduced by 99% within 60 minutes contact time and 90% decreased after 30 minutes. Presumably, the type of biosorbent affects its ability to restrain the adsorbate for different kind of biosorbents have different adsorption properties indeed. Such difference may be caused by variation of dominant functional groups, surface area, as well as size and pore volume, which in turn affects the ability to interact with the adsorbate.

4. CONCLUSION

Based on the results obtained in this study, it can be concluded that the biomass of microalgae consortium in tropical environments have a character as a biosorbent that potentially act as a binder of heavy metal Cu(II). Immobilized biosorbent of tropical microalgae consisting of *Ankistrodesmus braunii*, *Scenedesmus quadricauda var quadrispina*, and *Chlorella* sp., are considered to be promising biomaterial for it was able to set aside Cu(II) at pH value of 3 and optimum contact time of 180 minutes. Thus, immobilized biosorbent of microalgae was proven to increase sorption process compared to the free (mobile) biosorbent. It is necessary to conduct further research on the variations of temperature and initial concentration of biosorbent.

5. ACKNOWLEDGEMENTS

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IMPROVING BIOSORPTION OF CU(II)-ION ON ARTIFICIAL WASTEWATER BY IMMOBILIZED BIOSORBENT OF TROPICAL MICROALGAE

by Astri Rinanti

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*Astri Rinanti, Melati Ferianita Fachrul, Rositayanti Hadisoebroto, Mawar Silalahi
Faculty of Landscape Architecture and Environmental Technology, Trisakti University, Indonesia

Corresponding Author, Received: 15 June 2016, Revised: 30 Nov. 2016, Accepted: 20 Dec. 2016

ABSTRACT: This research purposes to study the role of microalgae in tropical environment – isolated from Wastewater Treatment Plant (WWTP) Setiabudi, Jakarta, Indonesia – on biosorption of Cu(II) ion in heavy metal wastewater. The effects of pH and contact time on the rate of metallic biosorption were examined to reach the greatest biosorption efficiency. Microalgae diversity analysis through phenotypic approaches showed that the microalgae community was comprised of 3 species of Chloropyceae i.e *Ankistrodesmus braunii*, *Chlorella* sp., and *Scenedesmus quadricauda var quadrispina*. Immobilized biosorbent from microalgae was prepared by oven-drying, grinding, and entrapping the microalgae biomass into polymeric matrix of alginate. The sorption properties of biosorbent was characterized by using infrared spectroscopy and SEM micrograph analysis. The optimization of sorption parameters was conducted in batch systems using Cu(II)-artificial wastewater of 300 ppm with pH arrangement of 2-7 and the contact time arrangement of 60, 90, 120, 180, 240 minutes. During the experiment, the Chlorophyceae free and Na-alginat immobilized biomass were added. The research showed that either free and immobilized biomass could adsorb Cu(II) metal ion and reduce its concentration into 25-50 ppm. A maximum biosorption by the alginate-immobilized biosorbent obtained at pH 3,0 and contact time of 180 minutes with 43% absorption efficiency. Sorption properties of microalgae biomasses were indicated by various functional groups presence on biosorbent that could bind heavy metals compared to others. The research proved that the alginate-immobilized biosorbent was highly effective for the treatment of Cu(II)-artificial wastewater and that tolerant cholorophyceae could act as an effective biosorbent in further optimization.

Keywords: Biosorption, Immobilized biosorbent, Cu(II), Microalgae diversity, Tropical environment

1. INTRODUCTION

The increasing of industrial development leads to heavy metal pollution in aquatic environment. Heavy metals are certainly needed by organisms to support the enzymatic process. However, in excessive amount, they can interfere the growth of the organisms. Copper or Cu is a heavy metal found in natural waters and is an essential matter to microalgae. Copper acts as a constituent of 13 plastocyanin, functioning in electron transport in photosynthesis [1]. Copper is encountered in the center of cytochrome c oxidase that makes up the superoxide dismutase enzyme and carries oxygen in hemocyanin pigments. Many kinds of enzymes contain copper [2]. Copper is considered to be toxic to plants at concentration above 0.1 ppm. Copper level in potable water should be not more than 1 ppm and is toxic to sheeps at concentration above 20 ppm. The presence of copper in wastewater ordinarily appears in the form of bivalent ion of Cu(II) as a hydrolytic product. Copper often found in wastewater of dyeing, paper, oil, and coating industry.

Conventional removal techniques for Cu(II) i.e precipitation, electrochemical, ion exchange, and membrane filtration, have their own limitations such as involving high installation cost, causing secondary pollution due to the chemicals use, and yielding toxic sludge [3], [4]. In addition, these processing techniques are also not able to meet the existing quality standards [5], [6]. Therefore, it is necessary to develop an alternative method of heavy metal removal to tackle the shortcoming mentioned above, among them is biosorption technology.

Biosorption is an absorption process utilizing the ability of biological materials (either macro or micromatters; either living or dead materials) to accumulate heavy metals from a solution metabolically or physicochemically [7]. Advantages gained from the use of microorganisms as biosorbent are low operational cost, high efficiency and high metal binding capacity, minimum sludge product leading to

minimum environmental impact, having a possibility of metal recovery as a result of desorption mechanism, having a regeneration mechanism (can be reused as biosorbent), as well as easily obtained, abundant raw materials [5].

Metal biosorption may occur due to the complexity of the positively charged metal ions with negatively charged active centers on the surface of the cell wall or on the extracellular polymers, such as proteins and polysaccharides as a source of functional groups playing important role in binding metal ions. This absorption process takes place rapidly in living cells as well as dead cells [7]

This absorption process does not depend on the metabolism, primarily because it occurs on the surface of the cell wall. Absorption by microorganisms is divided into two, namely metabolically independent absorption occuring on the surface of dead cells and metabolically dependent absorption taking place on the surface of living cells, that is slow and depends a lot on the nutrient availability and environmental conditions, e.g. solution pH and temperature [8]. Many factors can affect metal biosorption, among them are the type of biosorbent [5], pre-treatment of the biosorbent [6], solution pH [8], [9], [10], initial concentration of the adsorbate [11], also the influence of other ions in the solution [4].

The application of microalgae as a biosorbent is more favorable considering that small size of the cells provides larger specific surface that implicates on the higher absorption capacity. Moreover, high sorption ability is led by the presence of functional groups on the microalgae cell wall that are able to bind metal ions, particularly carboxyl, hydroxyl, amine, sulfhydryl, imidazole, sulfate and sulfonate chains [7]. As the raw material of biosorbent, microalgae is handily found, plenteously available, and does not need additional nutrients so that the operational cost can be cheaper [8].

On the other hand, the small size of the particles has disadvantages such as low mechanical strength, causing column plugging and clogging [8], the difficulty of biosorbent regeneration [12], also the requirement of complicated solid-liquid separation process [13]. To overcome these problems, immobilization of the tropical microalgae biomass in a biopolymeric matrix was applied in this study.

Immobilization can be defined as physical entrapment or localization of microalgae cell in such a way to limit the freely migration of microbes [14], while [15] explain that the biomass immobilization is a technique in which the target cell will be coated by porous polymeric layer that allows subtrate diffusion process into the cell. Several synthetic materials e.g. silica, polysulfone, polyurethane, polyvinyl alcohol, and acrylic polymers as well as natural polymers such as alginate, gelatin, agar, and cellulose derivatives can be used for the immobilization of microalgae biomass [16].

The objective of this study was to obtain the highest removal efficiency of heavy metal Cu(II) by utilizing immobilized tropical microalgae biomass in order to optimize the pH and contact time of biosorption.

2. MATERIAL AND METHODS

2.1 Isolation and Identification of Tropical Microalgae

Isolation and sampling 3 species of Chloropyceae i.e *Chlorella* sp., *Ankistrodesmus braunii*, and *Scenedesmus quadricauda var quadri-spina* derived from algal-blooming took place in stabilization pond of Wastewater Treatment Plant (WWTP) Setiabudi, Jakarta, to be used as biosorbent. The method employed in identification of microalgae was phenotype approach, based on microscopical morphology analysis.

2.2 Preparation of Biosorbent

Washed microalgae biomass were activated chemically using acidic and alkali solution. Biomass rinsed with distilled water were used as the controls. Some biomass were suspended and soaked for 3 hours in a solution of 0,1 N HCl; 0,1 N NaOH, and distilled water (dH_2O) with a biomass solution weight ratio of 1:3. Activated biomass then dried in an oven at 70°C for 20 hours, grinded using mortar, and screened using sieve size of 50-mesh (0,3 mm). Biomass powder sized ≤ 0,3 mm were used as biosorbent. This research was conducted in a batch reactor.

2.3 Immobilization of Biosorbent

Immobilization of biosorbent was carried out using entrapment method [17]. Some biomass powder were suspended in 100 mL solution of sodium alginate 2% (w/v). The suspension was dripped into a 500 mL solution of $CaCl_2$ 4%. When in contact with $CaCl_2$ solution, biomass suspension of sodium alginate were then polymerizing into bead forms—Na-alginate entrapped the biomass in its structure.

2.4 Preparation of Artificial Wastewater

Artificial wastewater containing Cu(II) (1,000 mgL^{-1}) was made by weighing a certain amount of copper sulphate ($CuSO_4 \cdot 5H_2O$) pro analysis and

dissolving it in a deionized distilled water to make a stock solution. To obtain different concentration of Cu(II), the stock solution was diluted in accordance to the necessary concentration. The value of pH was adjusted using HCl solution of 0,1 N and NaOH 0,1 N.

2.5 Optimization of pH

A total of 10 g immobilized biosorbent was contacted with 50 mL solution of Cu^{2+} 50 mg/L in an Erlenmeyer flask of 150 mL capacity. The value of pH was set in a range of 2, 3, 4, 5, 6, 7, 8. Furthermore, Selanjutnya, Erlenmeyer containing the mixture was stirred using a shaker with a speed of 180 rpm at optimum contact time and temperature. The metal (Cu^{2+}) remained in the solution was analyzed using AAS (Atomic Absorption Spectroscopy).

2.6 Optimization of Contact Time

A total of 10 g immobilized biosorbent was contacted with 50 mL solution of Cu^{2+} 20 mg/L in an Erlenmeyer of 150 mL volume. The Erlenmeyer then stirred using a shaker at 180 rpm. The metal Cu^{2+} remained in the solution at contact time of 60, 90, 180, and 240 minutes were then analyzed using AAS spectrophotometer.

2.7 Removal Efficiency of Cu(II)

Absorption efficiency of metal Cu(II) by the immobilized biomass of tropical microalgae was calculated using following formula:

$$\%\ \text{Cu(II) removal} = [(Co - Ce) / Co] \times 100\% \quad (1)$$

Co = initial concentration of Cu (II) solution (mg/L)
Ce = equilibrium concentration (mg/L)

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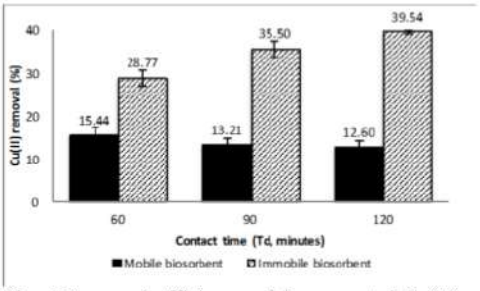

Fig. 1 Removal efficiency of heavy metal Cu(II) by mobile and immobilized tropical microalgae, at temperature 28 °C ± 2 and pH 3

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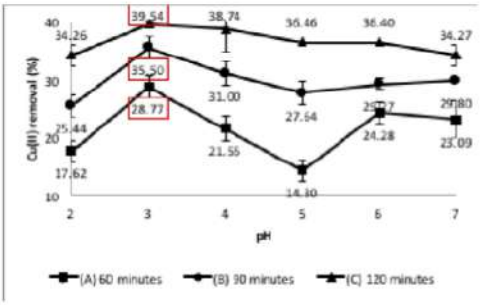

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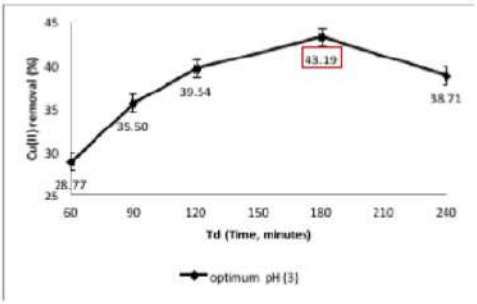

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