

## Increasing Effectiveness of Heavy Metal Sorption by Biosorbent Microalgae Beads

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### ABSTRACT

This research was conducted to overcome the Cu<sup>2+</sup> heavy metal pollution in the environment through a biotechnological approach with heavy metal sorption process by microalgae beads. Biosorbent in form of beads was produced from *Chlorella sorokiniana*, *Monoraphidium* sp., and *Scenedesmus obliquus* tropical microalgae mobilized with Na-alginate polymer. The sorption process is observed on a controlled batch culture with variations of temperature (25, 35, and 45 °C), and observation periods (200<sup>th</sup>, 220<sup>th</sup>, 250<sup>th</sup>, 270<sup>th</sup> min) as contact time. The absorption efficiency on each temperature variation reaches more than 90%, but the highest absorption efficiency rate is at 92.20% on 35 °C temperature and 200 minutes of contact time. Biosorbent beads with 2–3 mm of diameters show the best sorption ability than the 3–4 mm and 4–5 mm ones. Sorption process is also evident with the existence of intensity alteration on amide, ketone, and sulfhydryl function groups which were consistently weakened until the end of the sorption process. The beads utilized in this research are potentially reusable as biosorbent. Thus, further examination is required to acknowledge the maximum reutilization rate of the beads as biosorbent on heavy metal absorption process.

**Keywords:** beads, sorbent, biosorption, functional groups, heavy metal.

### INTRODUCTION

Microalgae population explosion, also known as microalgae booming, might lead to unbalanced water ecosystem, even though microalgae can act as natural oxygen producer on water due to their ability to conduct photosynthesis and bring benefits for other creatures in water ecosystem. The excessive amounts of microalgae might weaken the sunlight penetration ability on water and inhibit the photosynthesis process. This would lower oxygen availability and increase acidity on water, causing unbalanced water ecosystem (Rinanti et al., 2018).

According to Edmundson and Huesemann (2015), the abundant availability of microalgae on water can be utilized as biomaterial on heavy metal absorption process in the environment due to industrial waste penetration on the environment.

To overcome the decline in environmental quality, the wastewater that contains heavy metals such as Cu must be well processed. If Cu<sup>2+</sup> manages to enter human body through water on high concentration level, it may lead to a number of diseases such as liver disease, mental illness and cancer (Liu et al., 2013). Therefore, processing of the wastewater that contains the Cu<sup>2+</sup> heavy metal is vital.

Precipitation methods such as mechanical filtration, ion exchange, oxidation reduction, and physical adsorption are some of the techniques that have been used to separate heavy metals from wastewater (Pratiwi and Prinajati, 2018). However, some of these methods have limitations such as relatively high costs, produce toxic deposits, and cause secondary pollution (Montalescot et al., 2015). One alternative method that can be employed to overcome the problem of heavy metal pollution is biosorption, which is the process of

removing heavy metals using biological materials or microorganisms from the solution (Nafie, 2010).

Biosorption is a sorption process utilizing the ability of biological materials or organisms, using macro and microorganisms, both in the form of dead organisms and living organisms that can accumulate heavy metals from a chemical physical solution. The basic principle of biosorption using microalgae is the substitution of functional groups with heavy metals, so that one of the elements in the functional group is replaced by heavy metal ions (Rinanti et al., 2017). The biosorption process has several advantages, including low operating costs, minimization of the volume of chemical or biological sludge to be disposed of, and high efficiency in detoxifying highly dilute effluents (Rinanti et al., 2017; Ameri et al., 2019).

The application of bacteria as biosorbent is more beneficial due to the small cell size, contributing to a large specific surface area, which has implications for its high biosorption ability. However, because of its small size, the biosorbents from bacterial cells are not suitable for direct application, especially in waste treatment units with continuous systems. To overcome these problems, in this study, the microalgae biomass was immobilized in a biopolymer matrix. This immobilization is one of the techniques to improve the biosorption process that still require further development (Ameri et al., 2019).

The toxic nature of heavy metals does not affect the uptake capacity of biomass, does not require nutrient supply, as well as can be regenerated and be reused for several cycles (Wyk, 2011). Immobilized biomass is usually easier to handle, and easier to separate between solids and liquids (Ivánová et al., 2010). According to Siwi et al. (2017) and Daneshvar et al. (2017) immobilized biomass is able to absorb heavy metals better, if environmental conditions are controlled based on factors that affect the biosorption process.

Furthermore, the adsorbents that have bound heavy metals must be managed properly so as not to cause re-pollution to the environment because basically heavy metals have toxic properties. This research is vital in order to develop appropriate technology to absorb and release  $\text{Cu}^{2+}$  by environmentally friendly biosorbent agent. The specific purpose of this research is to obtain the highest efficiency level of sorption process based on the temperature ( $^{\circ}\text{C}$ ), contact time (Td) and biosorbent beads diameter supported by function groups analysis.

## MATERIALS AND METHODS

### Microalgae cultivation, harvesting, and powder making

*Chlorella sorokiniana*, *Monoraphidium* sp., and *Scenedesmus obliquus* microalgae were individually cultivated before mixed on a batch system photobioreactor filled with Phovasoli Haematococcus Media (PHM) as growth medium (Provasoli and Pintner, 1959) to obtain adequate microalgae amount as biosorbent material. Cultivation was implemented under the conditions of  $\pm 27^{\circ}\text{C}$  room temperature, pH level of 8, air flow rate of 900 mL/min, lighting intensity of 4000 lux obtained from TL lamp, with 16 hours of bright time and 8 hours of dark times for each day.

Single culture microalgae mixture was implemented on exponential phase with ratio of *Chlorella sorokiniana* : *Monoraphidium* sp. : *Scenedesmus obliquus* at 1:1:1 to produce 10% of the total culture volume. Microalgae were harvested on growth exponential phase with the centrifugation method, on a 2000 rpm spinning pace for 30 minutes. Centrifugation is conducted in order to separate biomass from its growth medium. Separated biomass is then placed on a porcelain cup and dried for 24 hours in an oven at a temperature of under  $90^{\circ}\text{C}$ . Dried biomass is then ground with mortar until it smoothens to obtain the microalgae powder ready to immobilized as biosorbent.

### Microalgae beads biosorbent preparation

The harvested microalgae biomass was washed, chemically activated using an acid and alkaline solution. As a control, biomass in contact with distilled water was used. The preparation of the biosorbent was carried out as follows: a certain amount of biomass was suspended and immersed for several hours in a 0.1 N HCl solution; 0.1 N NaOH, and distilled water ( $\text{dH}_2\text{O}$ ) The activated biomass was then dried in an oven at  $700^{\circ}\text{C}$  for 20 hours, pulverized using a mortar. The biomass powder was used as a biosorbent for the next research stage Figure 1. The biomass or microalgae powder was then mixed with sodium alginate and dissolved in demineralized aqua (aquadest). The mixing process was accelerated by using a magnetic stirrer to obtain immobilized microalgae in the form of beads as biosorbents.

Biosorbent immobilization was carried out using the “entrapment” method (Gracia et al, 2019). This method uses the principle of binding microalgae in gel beads to avoid fouling and sedimentation. A certain amount of biomass powder was suspended in 100 mL of 2% (w/v) sodium alginate solution. The suspension was dropped into 500 mL of 24% CaCl solution. When in contact with CaCl<sub>2</sub> solution, the biomass-sodium alginate suspension will polymerize to form sodium-alginate beads which trap the biomass in its structure.

### Biosorption by microalgae biosorbent beads

The microalgae biosorbent beads were then contacted with artificial Cu<sup>2+</sup> waste by making 1000 ppm CuSO<sub>4</sub> mother liquor by calculating the mass of CuSO<sub>4</sub> as much as 2.512 grams. Biosorption of heavy metal Cu<sup>2+</sup> by microalgae beads biosorbent was investigated using optimum physical parameters based on the results of previous studies, namely the pH value of 6 for CuSO<sub>4</sub> mother liquor and a temperature of 28 °C ± 2 Rinanti et al. (2017) and variations in contact time of 200, 220, 250 and 270 minutes.

The temperature was controlled with variations (25, 35, and 45 °C), and each treatment based on temperature variations was observed at the 200<sup>th</sup>, 220<sup>th</sup>, 250<sup>th</sup>, 270<sup>th</sup> minutes as contact time variations. Optimization of contact time and variations in the diameter of different beads (2–3 mm; 3–4 mm; and 4–5 mm) was carried out by contacting 10 g of immobilized biosorbent with 50 mL of 20 mg/L Cu<sup>2+</sup> solution in a 150 mL Erlenmeyer flask. Erlenmeyer flask was placed in a shaker incubator at 180 rpm. The Cu<sup>2+</sup> metal remaining in the solution at a contact time of 200 to 270 minutes was analyzed using an AAS spectrophotometer (Figure 4). After the biosorption process was completed, the biosorbent beads and the solution containing Cu<sup>2+</sup> were separated. The solution was analyzed using AAS to determine the concentration of copper. The absorption efficiency of Cu<sup>2+</sup> during the biosorption process can be calculated using equation (1) and equation (2).

$$\text{Absorption Efficiency (\%)} = \frac{C_o - C_e}{C_o} \times 100\% \quad (1)$$

$$q \left( \frac{\text{mg}}{\text{g}} \right) = \frac{C_o - C_e}{W} \times V \quad (2)$$

where: C<sub>o</sub> – initial water concentration that contains Cu<sup>2+</sup>; C<sub>e</sub> – Final concentration of the solution that contains Cu<sup>2+</sup>; q – metal adsorption capacity (mg/g); V – solution volume used in this research (Liter); W – the utilized biosorbent weight (gram).

## RESULTS

### Cu<sup>2+</sup> biosorption by biosorbent beads

Research on the sorption of Cu<sup>2+</sup> by biosorbent beads with a diameter of 3–4 mm has been carried out to determine the optimum temperature and contact time. The absorption efficiency of Cu<sup>2+</sup> is presented in Figure 2. The temperature of the biosorption process can affect the absorption ability of the biosorbent beads. Sorption efficiency at various temperature and contact time values ranges from 77% to 92%.

When viewed based on the temperature difference at any time, the absorption efficiency at 25 °C ranges from 77.50%–80.71%, at 35 °C it ranges from 87.26–92.21%, and at 45 °C it ranges from 79.59–85.47%. On the basis of the difference of the three temperatures, the highest absorption efficiency occurs at a temperature of 35 °C. The largest absorption occurred at a temperature of 35 °C for 200 minutes with an absorption efficiency of 92.21%. After reaching a temperature of 35 °C, the biosorption process decreased as temperature increased. The absorption efficiency at 35 °C reaches 92.21% then decreases to 85.47% at 45 °C. Figure 1 also explains that based on the difference in contact time, the highest absorption efficiency occurs at 200 minutes. At temperatures of 25, 35, and 45 °C respectively, the highest absorption was shown at 200 minutes, reaching 80.71%; 92.21% and 85.47%.

### The influence of beads diameter on heavy metal removal efficiency

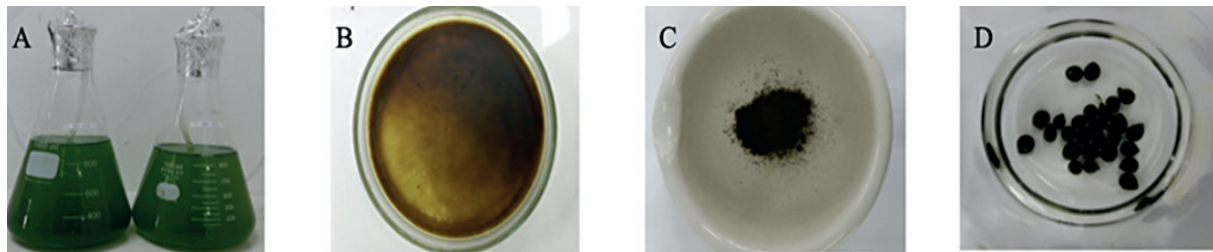
This research has also managed to generate immobilized biosorbent by certain Na-alginate polymer matrix (Figure 3) on variations of diameter namely 2–3 mm, 3–4 mm, and 4–5 mm. The research continued to acknowledge the influence of beads diameter on heavy metal absorption capacity. Immobile biosorbent with

2–3 mm and 4–5 mm diameters were applied on a fixed bed column.

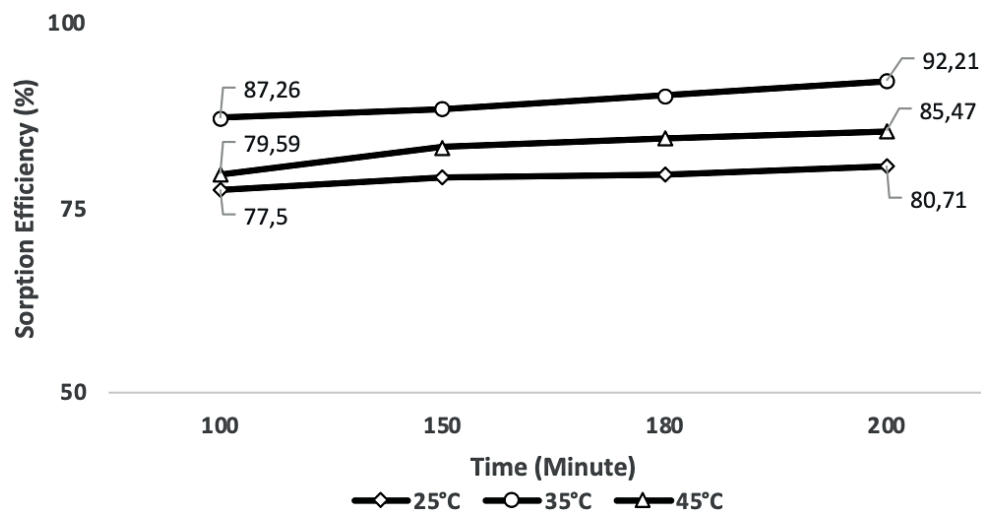
On the basis of Figure 4, it can be seen that the beads with the smallest diameter of 2–3 mm have a better sorption capacity for 200 to 250 minutes than the beads with a diameter of 3–4 mm and 4–5 mm. The results of this study indicate that the larger the surface area of the beads, the higher the absorption of heavy metals, which is indicated by the higher efficiency

of heavy metal removal. In this study, the microalgae biosorbent beads with 2–3 mm diameter can absorb  $\text{Cu}^{2+}$  up to 94.77% at a contact time of 220 minutes.

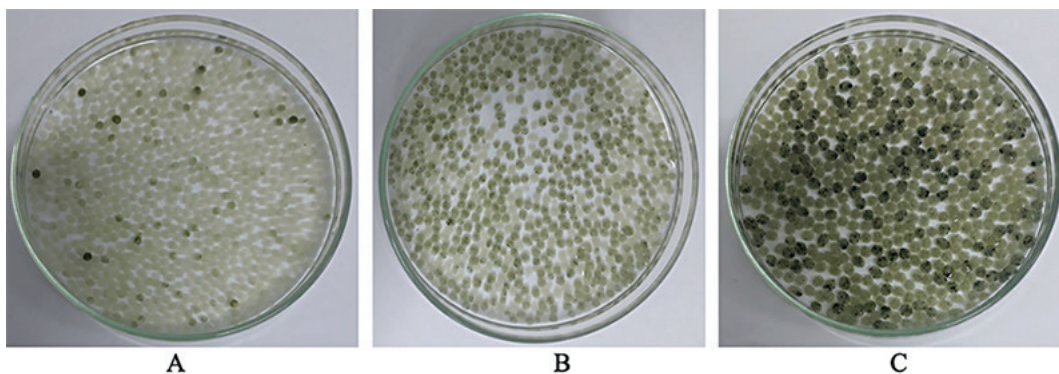
In this study, after 250 minutes, the absorption capacity of the beads with a diameter of 2–3 mm was not as good as that of the beads measuring 3–4 mm and 4–5 mm. It is suspected that the beads with a diameter of 2–3 mm have been saturated.



**Figure 1.** Biosorbent preparation (A) *Chlorella* sp.; culture (B) dried *Chlorella* sp.; (C) *Chlorella* sp powder; (D) immobile *Chlorella* sp. (microalgae beads as biosorbent)



**Figure 2.** Sorption efficiency (%) based on temperature and contact time, on pH level of 4 and initial  $\text{Cu}^{2+}$  metal concentration of 38.62 mg/L



**Figure 3.** Microalgae biosorbent beads with diameters of (A) 2–3; (B) 3–4; and (C) 4–5 mm

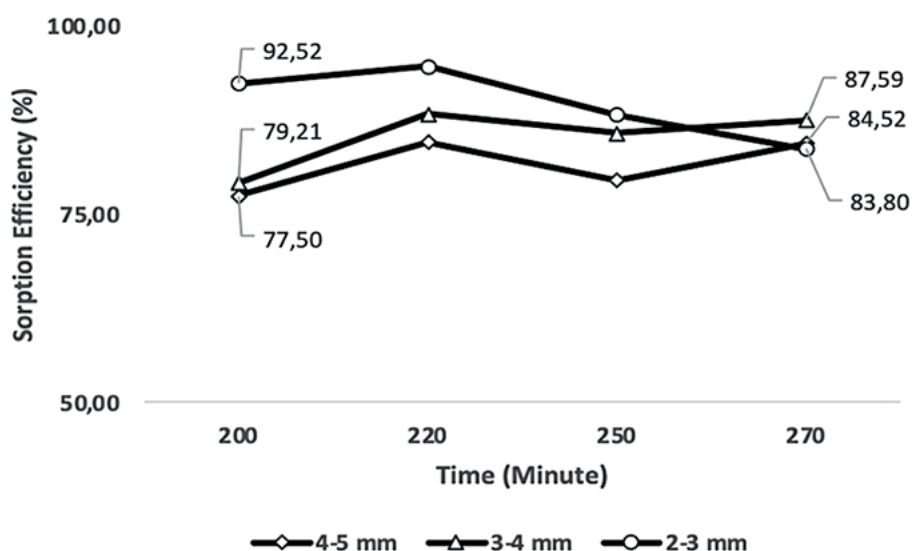


Figure 4. Biosorption by 3 diameter beads on different contact time between 200 to 270 minutes

### Biosorbent effectiveness based on function group intensity

The biosorbent beads in this study had the intensities ranging from 600–3500  $\text{cm}^{-1}$ , and functional groups were found including carboxylic acids (OH), alkyne ( $\text{C}\equiv\text{C}$ ), esters ( $\text{C}=\text{O}$ ), ethers

(C-O), amides ( $\text{C}=\text{N}$ ), aromatics (C-H), alkanes (CH) and alkenes ( $\text{C}=\text{H}$ ). These functional groups have wave numbers at their respective frequencies and produce different intensity values, as shown in Table 1. On the basis of Table 2, when viewed in terms of intensity, it appears that the functional groups of alkenes ( $\text{C}=\text{H}$ ), alkynes ( $\text{C}\equiv\text{C}$ ) and

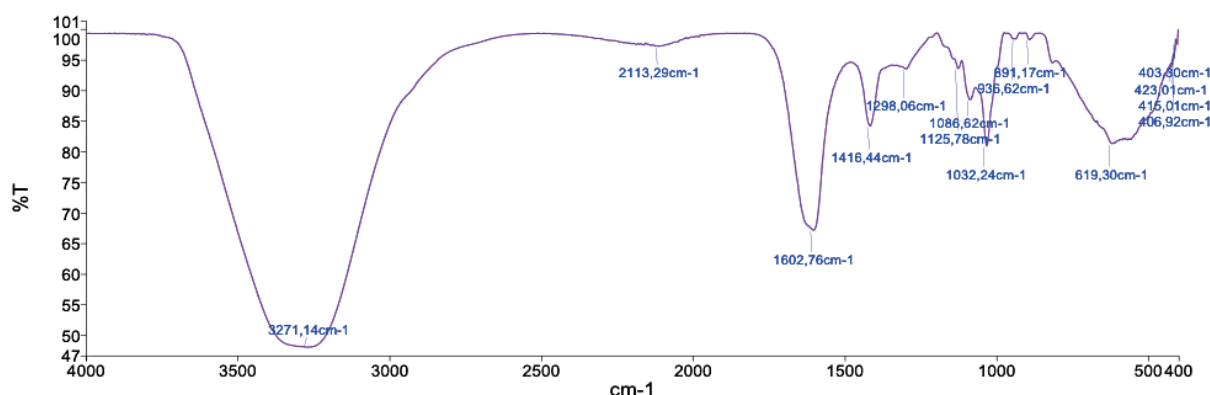


Figure 5. Mixed microalgae biosorbent ftir analysis result after the biosorption process

Table 1. FTIR analysis result prior to the biosorption process

Wavenumber Area ( $\text{cm}^{-1}$ )	Wave Peak ( $\text{cm}^{-1}$ )	Function groups	Intensity
690 – 900	890.46	Aromatic (C-H)	0.983
675 – 995	936.64	Alkene (C=H)	0.987
1030 – 1070	1032.09	Ether (C-O)	0.780
1020 – 1220	1084.91	Amide (C=N)	0.864
1050 – 1300	1124.76	Ether (C-O)	0.928
1250 – 1300	1297.70	Esther (C-O)	0.934
1300 – 1500	1416.94	Alkane (C-H)	0.825
1600 – 1820	1601.96	Esther (C=O)	0.650
1990 – 2140	2112.81	Alkyne ( $\text{C}\equiv\text{C}$ )	0.975
2800 - 3400	3271.20	Carboxylate Acid (O-H)	0.486

**Table 2.** FTIR analysis result after the biosorption process

Wavenumber area (cm <sup>-1</sup> )	Wave peak (cm <sup>-1</sup> )	Function groups	Intensity
690–900	891.17	Aromatic (C-H)	0.984
675–995	936.62	Alkene (C=H)	0.985
1030–1070	1032.24	Ether (C-O)	0.810
1020–1220	1086.62	Amide (C=N)	0.886
1050–1300	1125.78	Ether (C-O)	0.937
1250–1300	1298.06	Esther (C-O)	0.936
1300–1500	1416.44	Alkane (C-H)	0.842
1600–1820	1602.76	Esther (C=O)	0.671
1990–2140	2113.29	Alkyne (C≡C)	0.974
2800 - 3400	3271.14	Carboxylate Acid (O-H)	0.479

carboxylic acids (OH) are weakened. In contrast, the functional group of ethers (C-O), esters (C=O), amides (C=N), aromatics (C-H), and alkanes (CH) are increased in intensity. Following this, the intensity value of functional groups after the biosorption process are shown in Figure 5.

## DISCUSSION

On the basis of temperature and contact time, the higher sorption efficiency is in line with the increasing value of adsorption capacity, namely the ability of biosorbent beads to absorb heavy metals. This proves that the biosorbent beads in this study are capable and very efficient at absorbing Cu<sup>2+</sup>, since the sorption efficiency is characterized by a high percentage of absorption above 50%. This is in accordance with the research of Abbas et al., (2014) which stated that the absorption efficiency of Cu<sup>2+</sup> metal by biosorbent beads reached 73.58% at 25°C and the absorption efficiency at 30 °C increased to 79.4%. Biosorption decreased the absorption efficiency at temperatures above 30 °C due to the biosorption process being exothermic, so that increasing temperature will cause damage to microbial cells, which in turn causes a decrease in adsorption ability (Zhang, 2014). The decrease in absorption efficiency at a temperature of 40 °C – 50 °C is caused by damage to the active site on the surface of the biosorbent due to bond disruption or due to weakening of the active site in binding heavy metals (Ilamathi et al., 2014; Al-Homaidan et al., 2014).

A similar study conducted by Zhang, (2014) showed that the absorption of Cu<sup>2+</sup> by the microalgae *Spirulina plantesis* had the highest efficiency of 84.1% occurring at 90 minutes and then

decreasing 30 minutes later. According to Rinanti et al. (2017), the difference in absorption efficiency and the most optimum contact time is caused by differences in the use of microalgae types as biosorbents, variations in functional groups, biosorbent surface area, amount of biosorbent used, and pore volume which can affect its ability to absorb Cu<sup>2+</sup> metal.

In addition, the highest efficiency that occurs based on the length of contact time indicates that the sorption begins to reach equilibrium conditions at that time. One of the properties of the biosorption process is that a reversible reaction occurs which takes place very quickly but the biosorbent can experience saturation at certain times. After reaching the saturation point, the biosorbent will release the absorbed heavy metal again, and so on (Rinanti et al., 2017). In the research by Postma (2015) that utilized different sizes of beads diameter to remove NH<sub>4</sub><sup>+</sup> -N and phosphate pollutants, it was discovered that immobilized alginate beads with medium diameter (4 mm) and cell concentration of 1.5 × 10<sup>6</sup> cell/bead reached optimum result which is the perfect removal of NH<sub>4</sub><sup>+</sup> -N and 95% of phosphate removal, as opposed to the application of beads with smaller diameter namely at 2.8 mm and 6.0 mm. The addition of a contact time more than 220 minutes did not give higher adsorption results. This indicates that the microalgae biosorbent beads have reached the saturation point.

This finding is in accordance with the previous research that utilized low cost biosorbent made of keratin biomaterial to remove heavy metal; broader surface will show better biosorption capacity (Zhang, 2014; Ilamathi et al., 2014; Al-Homaidan et al., 2014) and adsorption capacity will increase when biosorbent dose is increased, because it will lead to greater surface

area (Al-Homaidan et al., 2014), and the surface area of the biosorbent affects the efficiency of heavy metal removal because a wider surface area will increase the biosorption capacitance under the same conditions (Zhang, 2014).

The research by Gracia et al (2019) compared the binding of two different cell types, namely algal cells and hybridoma cells. As a result, the algal cells with a diameter of ~10 m beads had a higher adsorbate concentration than the hybridoma cells with a diameter of ~15 m. The presence of a higher concentration of adsorbate will increase the adsorption capacity (Redha, 2020). The use of immobilized biosorbents in fixed bed columns was proven to increase the removal efficiency of  $\text{Cu}^{2+}$  metal ions and facilitate the regeneration of biosorbents.

The effectiveness of the biosorbent during the biosorption and desorption process can be seen based on the functional groups involved in the biosorbent beads. This indicates that there is a substitution between one of the elements contained in the carboxylic acid group with  $\text{Cu}^{2+}$  (Zhang, 2014). However, it is not possible to determine specifically what elements are substituted with  $\text{Cu}^{2+}$ , because this Fourier Transform Infrared (FTIR) analysis is only qualitative. According to Zhang and Wang (2015) the weakened intensity of the functional groups indicates that the biosorbent is not as good as the initial ones before it was used as a medium for heavy metal absorption, but the biosorbent can still be reused to absorb heavy metals, as seen with the changes in intensity which is not much different from the initial biosorption process.

Moreover, the findings of functional groups of carboxylic acids (OH), amides (C=N), aromatics (C=C), alkanes (CH) and alkenes (C=H) is in accordance with the statement of Al-Homaidan et al. (2014) that microalgae possess carboxylate, amine, phosphate, and hydroxyl functional groups. Previous research by Kumar et al. (2018) also mentioned that microalgae biosorbents have functional groups such as alkenes, amines, alkanes, aromatics, carboxylic acids, alkynes, and phenols, with carboxylic acid functional groups being the most dominant in the biosorption process. However, the functional groups found in this study are not all the same as the functional groups reported by previous researchers. The difference in functional groups is most likely influenced by the characteristics of the type of microalgae used as biosorbent.

## CONCLUSIONS

The sorption process is an effort to overcome the environmental problems caused by heavy metal pollution on water, which is conducted by reduced biosorbent preparation cost to create a more efficient technique, because the previously used biosorbent can be reutilized to absorb heavy metal compounds. The highest absorption efficiency is at 94.80% performed by 2–3 mm diameter biosorbent beads at a temperature of 35 °C for 220 minutes. The most dominant function groups in biosorption process were amide, ketone, and sulfhydryl groups that can be witnessed by their weakened intensity levels. The smaller the beads diameter, the higher the surface will be which leads to increased sorption capacity up until the 220th minute. The capacity of 2–3 mm beads becomes lower after 220th minute compared to 3–4 mm and 4–5 mm diameter beads, which probably caused by saturated beads.

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