

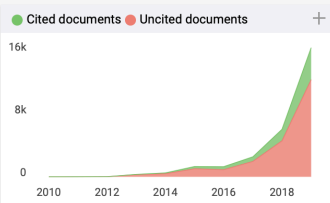
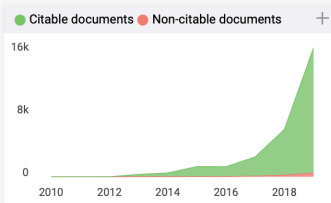
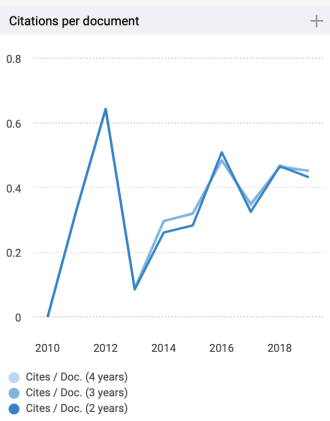
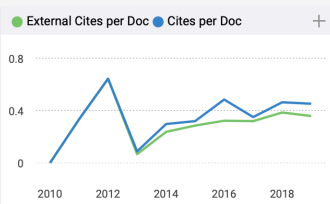
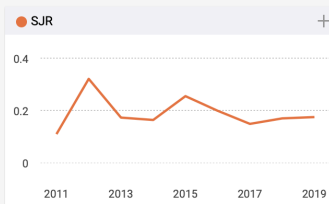


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The effect of biomass concentration on polymer alginate in the immobilized biosorbent formation during the sorption process of heavy metal Cu^{2+}

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Abstract. A research in environmental biotechnology has been done to analysis adsorption of ion Cu^{2+} by biomass of microalgae (*Chlorella sp*, *Ankistrodesmus braunii*, *Scenedesmus quadricauda*) and *Saccharomyces cerevisiae* onto alginate polymer as immobilized biosorbent on laboratory scale. The purpose of this study is to achieve the optimum biomass concentration which gives the best biosorption performance. Biosorption of Cu^{2+} was carried out in continuous fixed-bed column reactor system, volume of 1.5 L, equipped with peristaltic pump with a flow rate of 13 mL/min. Biosorption of Cu^{2+} was investigated using immobilized biosorbent with concentration of (g biomass/g polymer) 0.25; 0.5; 1, at pH4, initial concentration Cu^{2+} 15 mg/L and $26^\circ\text{C}\pm 1$. The results of this study showed that the increasing of biomass concentration (0 to 0.5 g/g) would result in better biosorption performance but soon decreased its performance at biomass concentration of 1 g/g. Biosorption capacity and highest removal efficiency of 0.1025 mg Cu^{2+} /g biosorbent and 66.36% occurred by immobilized biosorbent with 0.5 g/g concentration. The connection between the variation of biomass concentration in alginate to the biosorption performance by immobilized biosorbent shown by breakthrough curve, total adsorbed metal mass (q_{total}), efficiency of removal (%R) and biosorption capacity at breakthrough (q_e). Excessive biomass concentrations lead to reduced porosity of the beads thus slowing down the adsorption process.

Keywords: alginate polymer, biosorbent, biosorption, breakthrough curve, green microalgae

1. Introduction

The increasing concern for heavy metals danger in the neighbourhood encourages an alternative technology search to remove dangerous substances of heavy metals which is environmentally friendly. Biosorption is process of binding as well as selected unwanted ions or molecules from heavy metals utilizes biological materials like yeast and microalgae acting as biosorbent. The applianse of biomass microalgae as biosorbent appears as an interesting choice, economical and effective because some of its advantages than others [1]. Those benefits include low needs nutrients, high biomass production because of its autotroph, and unlike others as bacteria and fungi, microalgae generally not producing dangerous compound.

The biomass ability of binding metal ions influenced by several factors, namely temperature, acidity degrees (pH), biosorbent concentration, early sorbate concentration, contact time and the existence of other metal ions [2]. Nevertheless, free or mobile biosorbent when used in a reactor



column on a continuous way by low density and size potentially causing blockage (clogging) on reactor so that causes pressure to be drastically decreasing. Biosorbent Immobilization can resolve these problems so that biosorbent can be used in the continuous reactor system to absorb heavy metals. Immobilization can be defined as confinement physical and localization cells microbes at a certain room in such a way as to limit migration microbes freely. Immobilised Cells show different hydrodynamics characteristic from environment but still able to maintain catalyst activity to be reused [3], [4].

One biomass immobilization technique often used to biosorption research is entrapment or coating in polymer matrix. Entrapment is the irreversible method; namely immobilized biomass which is entrapped in a supportive matrix or in fiber. This technique creates protective around immobilized microalgae to ensure the prolonged survival, not just for the process but also the stowage in polymers [5]. Alginate is one of its polymers compounds often used as supportive matrix to biomass immobilization using entrapment technique. Based on this description, this research is aimed at determining the influence of varied biomass concentration addition to polymer alginate in the formation of immobilized biosorbent in efforts to improve efficiency and the absorption capacity of heavy metal Cu^{2+} .

2. Research Method

The heavy metal Cu^{2+} used as an adsorbate derives from the liquid wastewater of the metal coating industry.

2.1. Biomass cultivation and its production

Biosorbent raw materials are *Chlorella* sp. microalgae biomass, *Ankistrodesmus braunii*, *Scenedesmus quadricauda* and *Saccharomyces cerevisiae*, which is isolated from the waters of Setiabudi-Jakarta Reservoir. Biomass cultivation is done in vertical column photobioreactor with 10 L capacity, filled with artificial growth medium of PHM (Provasolli Haematococcus Media) to obtain optimum condition for microalgae growth. The operational conditions are controlled at 25°C with 2500 lux lighting intensity, airflow rate of 800 mL/min, bright periods: dark (24h: 0), to obtain optimum biomass growth [1].

2.2. Mobile Biosorbent Production

Microalgae biomass cultures and *Saccharomyces cerevisiae* which are in the exponential phase, are harvested by centrifugation method with a rotational speed of 4000 rpm for 15 minutes. Centrifugation is done to separate biomass from its growth medium. The insoluble precipitated biomass is poured into a petri dish then dried in an oven. Dry biomass is crushed with mortar to become a biomass powder. Microalgae powder (MA) and *Saccharomyces cerevisiae* (SC) were mixed with the MA: SC ratio of 3: 1, forming immobilized biosorbent ready for research.

2.3. Operational Research Condition

The mobile biosorbent was weighed to obtain a load of 2 g, 4 g, and 8 g. The weighed biomass was immobilized with 400 ml of 2% Na-alginate solution to form beads. These beads are called immobilized biosorbents. Immobilized biosorbent was arranged into a continuous fixed-bed column reactor system with a capacity of 1.5 L, to reach a bed height of 5 cm. The feeding solution with an initial concentration of Cu^{2+} 15 mg/L, pH 4, was flowed into a fixed-bed column reactor vertically up-flow to prevent the channel effect [5], using a peristaltic pump with discharge capacity of 13 ml/min, controlled at room temperature 25°C. Samples were taken from the point of effluent periodically with the capture time range 0, 10, 20, 30, 45, 60, 80, 100, 125 and 150 minutes. Periodic effluent sampling was done to determine the change of concentration of Cu^{2+} gradually. The percentage of Cu^{2+} removal in the sample solution was measured using Atomic Absorption Spectrophotometry (AAS).

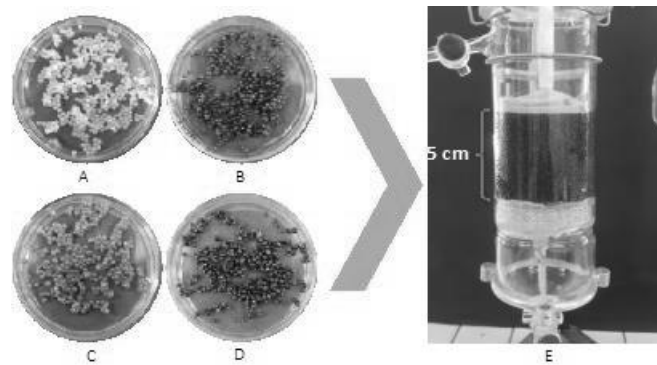


Figure 1. A) Sorbent Ca-alginate without biomass (control); B) immobilized biosorbent with (g biomass/g polymer) 0.25 g/g concentration; C) immobilized biosorbent 0.5 g/g; D) immobilized biosorbent 1 g/g; E) immobilized biosorbent is arranged into continuous fixed-bed column reactor system with capacity of 1.5 L, until reach the *bed* height of 5 cm.

2.4. Breakthrough Curve

The breakthrough curve is a curve that describes the relation between changes in the concentration of adsorbate (Cu^{2+}) in effluent with time (t). The change in the concentration of adsorbate begins to be recorded when the solution reaches the effluent point where $t = 0$. Although the analysis results note that the concentration of adsorbate in the effluent is not equal to zero, but to illustrate the principle of biosorption in the column of adsorbate concentration when $t = 0$ is considered zero. This concentration change describes the biosorption events occurring in the fixed-bed column reactor, i.e. the absorption of adsorbate at the beginning of the column when the feed solution passes through a column already filled by immobilized biosorbent. The adsorption reaction zone is increasingly moving up along with the saturated immobilized biosorbent at the bottom of the column. When the adsorption zone reaches the effluent point, the breakthrough concentration is reached ($10\% C_0$). The time required to reach the breakthrough point (t_b) becomes an important parameter in this study.

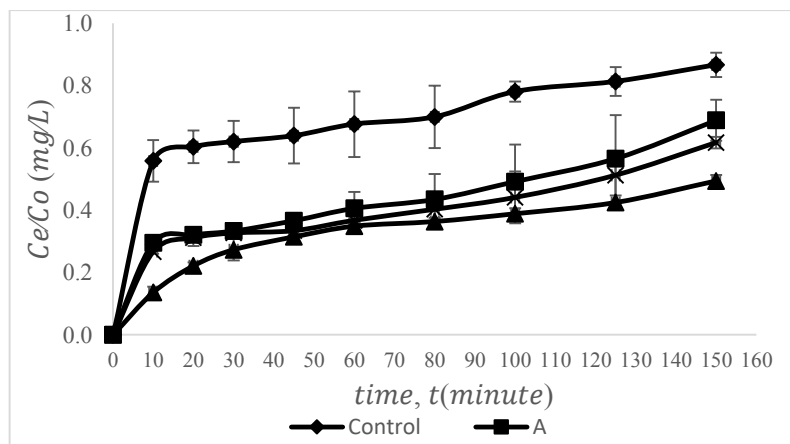
3. Results and Discussion

Cu^{2+} metal sorption results in a continuous system of fixed-bed column reactors are presented in Table 1. The plot of time-effected Cu^{2+} effluent concentration by immobilized biosorbent with different variations of biomass concentration on the polymer will form the breakthrough curve as in Figure 2.

The biomass concentration of polymer alginate in immobilized biosorbent formation influences the adsorption capacity of Cu^{2+} heavy metals occurring in fixed-bed column reactors that operate with continuous system (Figure 1). Based on the graph of the breakthrough curve in Figure 2, the concentration of Cu^{2+} in the effluent to the initial concentration of adsorbate (C_e/C_0) increases with time (t). This means the ability of adsorption by immobilized biosorbent increases with increasing of biomass concentration to polymer alginate. Curves formed by immobilized biosorbent (A) are steeper than those formed by immobilized biosorbent (B). Different performance is shown by immobilized biosorbent (C), where the curve formed is steeper than the curve formed by immobilized biosorbent (B) but not steeper than the curve formed by immobilized biosorbent (A). This suggests that the immobilized biosorbent adsorption capacity decreases with increasing biomass concentration of 0.5 g/g to 1 g/g polymer alginate.

Table 1. Parameter of breakthrough curve in biosorption process heavy metal Cu^{2+} by immobilized biosorbent with 5 cm bed height in continuous fixed-bed column reactor system.

immobilized biosorbent (g biomass/g polymer)	M (g)	t_b (menit)	m_{total} (mg)	q_{total} (mg)	q_e (mg/g)	R (%)
0 (control)	171.5	1.79	29.348	9.157	0.0534	31.20
(A) 0.25	183.5	3.38	29.348	16.584	0.0904	56.51
(B) 0.50	190.0	7.34	29.348	19.474	0.1025	66.36
(C) 1.00	203.8	3.75	29.348	17.681	0.0868	60.25

**Figure 2.** Breakthrough curve in heavy metal biosorption process Cu^{2+} by immobilized biosorbent with bed height of 5 cm in continuous fixed-bed column reactor system.

Based on the breakthrough curve formed, it can be identified that to reach the breakthrough concentration, it takes different (t_b) time. The steeper the curve forms, the faster the (t_b) required time to reach the breakthrough concentration. This is evidenced by the results of the calculation data in Table 1. Based on Table 1, the time required by the immobilized biosorbent column (A) to reach the breakthrough concentration ($C_e/C_0 = 0.1C_0$) is 3.38 minutes. Columns containing immobilized biosorbent (B) with the gentlest curve form shows the greatest result, i.e. the required time to reach the breakthrough is 7.34 minutes. The breakthrough time (t_b) achieved by the immobilized biosorbent (C) column is 3.75 minutes, greater than the immobilized biosorbent (A) but smaller than the immobilized biosorbent (B). The fastest breakthrough (t_b) time is achieved by control (alginate without biomass) with the steepest curve of 1.79 minutes. Breakthrough time is considered as an important parameter because it shows how fast a fixed-bed column is saturated. The longer breakthrough times indicate that the bed column is better because it takes longer to saturate.

The total metal mass value entered the column (m_{total}) shows a fixed value of 29.348 mg Cu^{2+} . That is, the total mass of Cu^{2+} metal entering the column is the same for each variation of the biosorbent concentration. The result is obtained from the initial concentration of Cu^{2+} metal multiplied by total reactor operating time of 150 min. The total mass of the adsorbed metal (q_{total}) also increased by 9.157; 16.584; 19.474 mg Cu^{2+} for control, immobilized biosorbent (A) and (B). However, different results are shown by immobilized biosorbent (C) with q_{total} value decreasing to 17,681 mg Cu^{2+} . In addition, the biosorption capacity (q_e) also shows a change as the total mass of the adsorbed metal (q_{total}) increases.

Biosorption capacity increased respectively 0,0534; 0.0904; 0.1025 mg Cu^{2+} /g biosorbent for control, immobilized biosorbent (A) and (B). However, different results were shown by immobilized

biosorbent (C) with q_e value decreasing to 0.0868 mg Cu²⁺/g biosorbent. The results of this study indicated that the capacity of biosorption (q_e) increased from 0.0534 to 0.1025 mg Cu²⁺ / g biosorbent as the immobilized biosorbent concentration increased (0 to 0.5 g/g). The decreased biosorption capacity (q_e) of 0.1025 to 0.0868 mg Cu²⁺/g biosorbent occurred when the immobilized biosorbent concentration was increased from 0.5 g/g to 1 g/g.

The efficiency of heavy metals removal of Cu²⁺ increased by 31.2%; 56.51%; 66.36% for control, immobilized biosorbent (A) and (B). However, the removal efficiency by immobilized biosorbent (C) appeared to be smaller or decreased to 60.25%. The results of this study indicated that the removal efficiency (%R) increased by 31.2% to 66.36% as the immobilized biosorbent concentration increased from 0 to 0.5 g/g. Decrease in removal efficiency (%R) by 66.36% to 60.25% occurred when the immobilized biosorbent concentration was increased from 0.5 g/g to 1 g/g.

This study revealed the existence of interrelation between a variation in the concentration of biomass in the polymer alginate to the performance of biosorption by immobilized biosorbent indicated by the breakthrough curve shape, the total mass of metal adsorbed (q_{total}), the removal efficiency (% R) and a capacity of biosorption at the time of breakthrough (q_e). The greater the concentration of immobilized biosorbent (0 to 0.5 g/g) the steeper the breakthrough curve will be so that breakthrough time taken getting bigger, the total mass of the adsorbate which absorbed the greater, the greater removal efficiency and greater biosorption capacity as well. Biosorption capacity and the highest removal efficiency of 0.1025 mg Cu²⁺/g biosorbent and 66.36% occurred by immobilized biosorbent (B). Lowest biosorption capacity found by immobilized biosorbent (A) with a capacity of biosorption 0.0904 mg Cu²⁺/g biosorbent and Ca-alginate without biomass as a control with a capacity of 0.0534 mg Cu²⁺/g biosorbent. The lowest allowance efficiency of 31.2% and 56.51% existed by control and immobilized biosorbent (A)

Ca-alginate beads can remove adsorbate. The ability of Ca-alginate to absorb heavy metals was evidenced by decreasing concentrations of adsorbate passing through fixed-bed column starting initial concentration of Cu²⁺ 15 mg/L to 9 mg/L at 10 minute. The breakthrough curves formed by Ca-alginate beads looked steeper than those of immobilized biosorbent beads. Based on the previous analysis, the steeper the curve the faster the breakthrough time, the smaller the biosorption capacity. This was evidenced by Ca-alginate biosorption capacity of 0.0534 mg Cu²⁺/g biosorbent which is smaller than the biosorption capacity of immobilized biosorbents with biosorbent concentration therein. The ability of Ca-alginate beads to absorb heavy metals was obtained from the active side found in alginate polymer compounds Beads.

The concentration of biomass in polymer solution can directly affect the immobilized biosorbent ability to adsorb the adsorbate. Increased biosorption capacity from 0.0534 to 0.1025 mg Cu²⁺/g biosorbent turned out when the immobilized biosorbent concentration was increased from 0 to 0.5 g/g. This increased capacity was due to the increased surface area of the adsorbent and the availability of more active adsorption side along with the increased concentration of biosorbent [6]. A further increase of immobilized biosorbent concentrations from 0.5 to 1 g/g showed a decrease in biosorption capacity from 0.1025 to 0.0868 mg Cu²⁺/g biosorbent. This is due to the disruption of the bond between immobilized biomass with Ca-alginate and decreased permeability of beads.

Decreased biosorption capacity at a 0.5 to 1 g / g immobilized biosorbent concentration was also due to the viscosity of the slurry formed by a mixture of biomass with an alginate polymer solution. The quantity of biomass mixed into the alginate solution may affect the viscosity / thickness of the solution. The more the biomass load in the polymer solution the more difficult it is to form beads. Excessive concentrations of biomass can lead to reduced porosity of the beads thus slowing down the adsorption process. The porous structure will provide opportunities for biomass that are within the beads to participate in metal ion absorption. Biosorbent with a concentration of 0.5 g/g of polymer has a lower capacity due to the limited diffusion of metal ions by functional groups on the cell surface. The results of this study are similar to the research of Abdel-Razek [6] which shows that the biosorption capacity increases to the maximum along with increasing the biomass concentration up to 0.75 g/g, then increasing the biomass concentration in the polymer further leads to decreased biosorption capacity. Researchers [6], [7] suggests that this event may be due to an interaction between the active

side of excessive biomass and the active side of the polymer, so that the free active side of the biomass and the polymer is decreasing.

4. Conclusion

Biomass concentrations trapped by alginate polymers in immobilized biosorbent formation have an effect on the absorption of Cu^{2+} metals. The highest removal efficiency of heavy metals Cu^{2+} of 90.69% occurred by immobilized biosorbent with a concentration of 0.5 g/g. Biomass concentration also influences the biosorption capacity, i.e. the higher the biomass concentration of 0.25 to 0.5 g/g, the greater the biosorption capacity reaches 0.0068 mg/g, but the capacity decreases with increasing biomass concentration of 0, 5-1 g/g of polymer. The result of SEM analysis shows the morphological changes of the immobilized biosorbent surface before and after the process of heavy metal biosorption of Cu^{2+} . Morphological changes occur because of the interaction between heavy metal ions Cu^{2+} and the active side on the immobilized biosorbent surface. These changes cause the pore to surface in the immobilized biosorbent by biomass so that the pore structure appears to be getting smaller.

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1. Introduction

The increasing concern for heavy metals danger in the neighbourhood encourages an alternative technology search to remove dangerous substances of heavy metals which is environmentally friendly. Biosorption is process of binding as well as selected unwanted ions or molecules from heavy metals utilizes biological materials like yeast and microalgae acting as biosorbent. The appliance of biomass microalgae as biosorbent appears as an interesting choice, economical and effective because some of its advantages than others [1]. Those benefits include low needs nutrients, high biomass production because of its autotroph, and unlike others as bacteria and fungi, microalgae generally not producing dangerous compound.

The biomass ability of binding metal ions influenced by several factors, namely temperature, acidity degrees (pH), biosorbent concentration, early sorbate concentration, contact time and the existence of other metal ions [2]. Nevertheless, free or mobile biosorbent when used in a reactor



column on a continuous way by low density and size potentially causing blockage (clogging) on reactor so that causes pressure to be drastically decreasing. Biosorbent Immobilization can resolve these problems so that biosorbent can be used in the continuous reactor system to absorb heavy metals. Immobilization can be defined as confinement physical and localization cells microbes at a certain room in such a way as to limit migration microbes freely. Immobilised Cells show different hydrodynamics characteristic from environment but still able to maintain catalyst activity to be reused [3], [4].

One biomass immobilization technique often used to biosorption research is entrapment or coating in polymer matrix. Entrapment is the irreversible method; namely immobilized biomass which is entrapped in a supportive matrix or in fiber. This technique creates protective around immobilized microalgae to ensure the prolonged survival, not just for the process but also the stowage in polymers [5]. Alginate is one of its polymers compounds often used as supportive matrix to biomass immobilization using entrapment technique. Based on this description, this research is aimed at determining the influence of varied biomass concentration addition to polymer alginate in the formation of immobilized biosorbent in efforts to improve efficiency and the absorption capacity of heavy metal Cu²⁺.

2. Research Method

The heavy metal Cu²⁺ used as an adsorbate derives from the liquid wastewater of the metal coating industry.

2.1. Biomass cultivation and its production

Biosorbent raw materials are *Chlorella* sp. microalgae biomass, *Ankistrodesmus braunii*, *Scenedesmus quadricauda* and *Saccharomyces cerevisiae*, which is isolated from the waters of Setiabudi-Jakarta Reservoir. Biomass cultivation is done in vertical column photobioreactor with 10 L capacity, filled with artificial growth medium of PHM (Provasolli Haematococcus Media) to obtain optimum condition for microalgae growth. The operational conditions are controlled at 25°C with 2500 lux lighting intensity, airflow rate of 800 mL/min, bright periods: dark (24h: 0), to obtain optimum biomass growth [1].

2.2. Mobile Biosorbent Production

Microalgae biomass cultures and *Saccharomyces cerevisiae* which are in the exponential phase, are harvested by centrifugation method with a rotational speed of 4000 rpm for 15 minutes. Centrifugation is done to separate biomass from its growth medium. The insoluble precipitated biomass is poured into a petri dish then dried in an oven. Dry biomass is crushed with mortar to become a biomass powder. Microalgae powder (MA) and *Saccharomyces cerevisiae* (SC) were mixed with the MA: SC ratio of 3: 1, forming immobilized biosorbent ready for research.

2.3. Operational Research Condition

The mobile biosorbent was weighed to obtain a load of 2 g, 4 g, and 8 g. The weighed biomass was immobilized with 400 ml of 2% Na-alginate solution to form beads. These beads are called immobilized biosorbents. Immobilized biosorbent was arranged into a continuous fixed-bed column reactor system with a capacity of 1.5 L, to reach a bed height of 5 cm. The feeding solution with an initial concentration of Cu²⁺ 15 mg/L, pH 4, was flowed into a fixed-bed column reactor vertically up-flow to prevent the channel effect [5], using a peristaltic pump with discharge capacity of 13 ml/min, controlled at room temperature 25°C. Samples were taken from the point of effluent periodically with the capture time range 0, 10, 20, 30, 45, 60, 80, 100, 125 and 150 minutes. Periodic effluent sampling was done to determine the change of concentration of Cu²⁺ gradually. The percentage of Cu²⁺ removal in the sample solution was measured using Atomic Absorption Spectrophotometry (AAS).

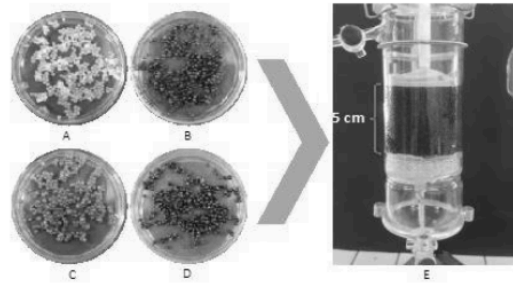


Figure 1. A) Sorbent Ca-alginate without biomass (control); B) immobilized biosorbent with (g biomass/g polymer) 0.25 g/g concentration; C) immobilized biosorbent 0.5 g/g; D) immobilized biosorbent 1 g/g; E) immobilized biosorbent is arranged into continuous fixed-bed column reactor system with capacity of 1.5 L, until reach the *bed* height of 5 cm.

2.4. Breakthrough Curve

The breakthrough curve is a curve that describes the relation between changes in the concentration of adsorbate (Cu^{2+}) in effluent with time (t). The change in the concentration of adsorbate begins to be recorded when the solution reaches the effluent point where $t = 0$. Although the analysis results note that the concentration of adsorbate in the effluent is not equal to zero, but to illustrate the principle of biosorption in the column of adsorbate concentration when $t = 0$ is considered zero. This concentration change describes the biosorption events occurring in the fixed-bed column reactor, i.e. the absorption of adsorbate at the beginning of the column when the feed solution passes through a column already filled by immobilized biosorbent. The adsorption reaction zone is increasingly moving up along with the saturated immobilized biosorbent at the bottom of the column. When the adsorption zone reaches the effluent point, the breakthrough concentration is reached (10% C_0). The time required to reach the breakthrough point (t_b) becomes an important parameter in this study.

3. Results and Discussion

Cu^{2+} metal sorption results in a continuous system of fixed-bed column reactors are presented in Table 1. The plot of time-effected Cu^{2+} effluent concentration by immobilized biosorbent with different variations of biomass concentration on the polymer will form the breakthrough curve as in Figure 2.

The biomass concentration of polymer alginate in immobilized biosorbent formation influences the adsorption capacity of Cu^{2+} heavy metals occurring in fixed-bed column reactors that operate with continuous system (Figure 1). Based on the graph of the breakthrough curve in Figure 2, the concentration of Cu^{2+} in the effluent to the initial concentration of adsorbate (C_e/C_0) increases with time (t). This means the ability of adsorption by immobilized biosorbent increases with increasing of biomass concentration to polymer alginate. Curves formed by immobilized biosorbent (A) are steeper than those formed by immobilized biosorbent (B). Different performance is shown by immobilized biosorbent (C), where the curve formed is steeper than the curve formed by immobilized biosorbent (B) but not steeper than the curve formed by immobilized biosorbent (A). This suggests that the immobilized biosorbent adsorption capacity decreases with increasing biomass concentration of 0.5 g/g to 1 g/g polymer alginate.

Table 1. Parameter of breakthrough curve in biosorption process heavy metal Cu^{2+} by immobilized biosorbent with 5 cm bed height in continuous fixed-bed column reactor system.

immobilized biosorbent (g biomass/g polimer)	M (g)	t_b (menit)	m_{total} (mg)	q_{total} (mg)	q_e (mg/g)	R (%)
0 (control)	171.5	1.79	29.348	9.157	0.0534	31.20
(A) 0.25	183.5	3.38	29.348	16.584	0.0904	56.51
(B) 0.50	190.0	7.34	29.348	19.474	0.1025	66.36
(C) 1.00	203.8	3.75	29.348	17.681	0.0868	60.25

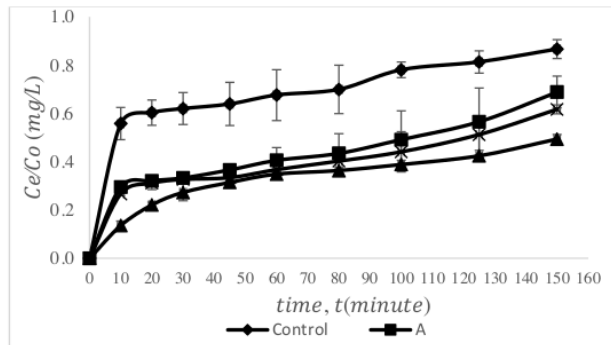


Figure 2. Breakthrough curve in heavy metal biosorption process Cu^{2+} by immobilized biosorbent with bed height of 5 cm in continuous fixed-bed column reactor system.

Based on the breakthrough curve formed, it can be identified that to reach the breakthrough concentration, it takes different (t_b) time. The steeper the curve forms, the faster the (t_b) required time to reach the breakthrough concentration. This is evidenced by the results of the calculation data in Table 1. Based on Table 1, the time required by the immobilized biosorbent column (A) to reach the breakthrough concentration ($C_e/C_0 = 0.1C_0$) is 3.38 minutes. Columns containing immobilized biosorbent (B) with the gentlest curve form shows the greatest result, i.e. the required time to reach the breakthrough is 7.34 minutes. The breakthrough time (t_b) achieved by the immobilized biosorbent (C) column is 3.75 minutes, greater than the immobilized biosorbent (A) but smaller than the immobilized biosorbent (B). The fastest breakthrough (t_b) time is achieved by control (alginate without biomass) with the steepest curve of 1.79 minutes. Breakthrough time is considered as an important parameter because it shows how fast a fixed-bed column is saturated. The longer breakthrough times indicate that the bed column is better because it takes longer to saturate.

The total metal mass value entered the column (m_{total}) shows a fixed value of 29.348 mg Cu^{2+} . That is, the total mass of Cu^{2+} metal entering the column is the same for each variation of the biosorbent concentration. The result is obtained from the initial concentration of Cu^{2+} metal multiplied by total reactor operating time of 150 min. The total mass of the adsorbed metal (q_{total}) also increased by 9.157; 16.584; 19.474 mg Cu^{2+} for control, immobilized biosorbent (A) and (B.) However, different results are shown by immobilized biosorbent (C) with q_{total} value decreasing to 17.681 mg Cu^{2+} . In addition, the biosorption capacity (q_e) also shows a change as the total mass of the adsorbed metal (q_{total}) increases.

Biosorption capacity increased respectively 0,0534; 0,0904; 0,1025 mg Cu^{2+} /g biosorbent for control, immobilized biosorbent (A) and (B). However, different results were shown by immobilized

biosorbent (C) with q_e value decreasing to 0.0868 mg Cu²⁺/g biosorbent. The results of this study indicated that the capacity of biosorption (q_e) increased from 0.0534 to 0.1025 mg Cu²⁺ / g biosorbent as the immobilized biosorbent concentration increased (0 to 0.5 g/g). The decreased biosorption capacity (q_e) of 0.1025 to 0.0868 mg Cu²⁺/g biosorbent occurred when the immobilized biosorbent concentration was increased from 0.5 g/g to 1 g/g.

The efficiency of heavy metals removal of Cu²⁺ increased by 31.2%; 56.51%; 66.36% for control, immobilized biosorbent (A) and (B). However, the removal efficiency by immobilized biosorbent (C) appeared to be smaller or decreased to 60.25%. The results of this study indicated that the removal efficiency (%R) increased by 31.2% to 66.36% as the immobilized biosorbent concentration increased from 0 to 0.5 g/g. Decrease in removal efficiency (%R) by 66.36% to 60.25% occurred when the immobilized biosorbent concentration was increased from 0.5 g/g to 1 g/g.

This study revealed the existence of interrelation between a variation in the concentration of biomass in the polymer alginate to the performance of biosorption by immobilized biosorbent indicated by the breakthrough curve shape, the total mass of metal adsorbed (q_{total}), the removal efficiency (% R) and a capacity of biosorption at the time of breakthrough (q_e). The greater the concentration of immobilized biosorbent (0 to 0.5 g/g) the steeper the breakthrough curve will be so that breakthrough time taken getting bigger, the total mass of the adsorbate which absorbed the greater, the greater removal efficiency and greater biosorption capacity as well. Biosorption capacity and the highest removal efficiency of 0.1025 mg Cu²⁺/g biosorbent and 66.36% occurred by immobilized biosorbent (B). Lowest biosorption capacity found by immobilized biosorbent (A) with a capacity of biosorption 0.0904 mg Cu²⁺/g biosorbent and Ca-alginate without biomass as a control with a capacity of 0.0534 mg Cu²⁺/g biosorbent. The lowest allowance efficiency of 31.2% and 56.51% existed by control and immobilized biosorbent (A)

Ca-alginate beads can remove adsorbate. The ability of Ca-alginate to absorb heavy metals was evidenced by decreasing concentrations of adsorbate passing through fixed-bed column starting initial concentration of Cu²⁺ 15 mg/L to 9 mg/L at 10 minute. The breakthrough curves formed by Ca-alginate beads looked steeper than those of immobilized biosorbent beads. Based on the previous analysis, the steeper the curve the faster the breakthrough time, the smaller the biosorption capacity. This was evidenced by Ca-alginate biosorption capacity of 0.0534 mg Cu²⁺/g biosorbent which is smaller than the biosorption capacity of immobilized biosorbents with biosorbent concentration therein. The ability of Ca-alginate beads to absorb heavy metals was obtained from the active side found in alginate polymer compounds Beads.

The concentration of biomass in polymer solution can directly affect the immobilized biosorbent ability to adsorb the adsorbate. Increased biosorption capacity from 0.0534 to 0.1025 mg Cu²⁺/g biosorbent turned out when the immobilized biosorbent concentration was increased from 0 to 0.5 g/g. This increased capacity was due to the increased surface area of the adsorbent and the availability of more active adsorption side along with the increased concentration of biosorbent[6]. A further increase of immobilized biosorbent concentrations from 0.5 to 1 g/g showed a decrease in biosorption capacity from 0.1025 to 0.0868 mg Cu²⁺/g biosorbent. This is due to the disruption of the bond between immobilized biomass with Ca-alginate and decreased permeability of beads.

Decreased biosorption capacity at a 0.5 to 1 g / g immobilized biosorbent concentration was also due to the viscosity of the slurry formed by a mixture of biomass with an alginate polymer solution. The quantity of biomass mixed into the alginate solution may affect the viscosity / thickness of the solution. The more the biomass load in the polymer solution the more difficult it is to form beads. Excessive concentrations of biomass can lead to reduced porosity of the beads thus slowing down the adsorption process. The porous structure will provide opportunities for biomass that are within the beads to participate in metal ion absorption. Biosorbent with a concentration of 0.5 g/g of polymer has a lower capacity due to the limited diffusion of metal ions by functional groups on the cell surface. The results of this study are similar to the research of Abdel-Razek [6] which shows that the biosorption capacity increases to the maximum along with increasing the biomass concentration up to 0.75 g/g, then increasing the biomass concentration in the polymer further leads to decreased biosorption capacity. Researchers [6], [7] suggests that this event may be due to an interaction between the active

side of excessive biomass and the active side of the polymer, so that the free active side of the biomass and the polymer is decreasing.

4. Conclusion

Biomass concentrations trapped by alginate polymers in immobilized biosorbent formation have an effect on the absorption of Cu^{2+} metals. The highest removal efficiency of heavy metals Cu^{2+} of 90.69% occurred by immobilized biosorbent with a concentration of 0.5 g/g. Biomass concentration also influences the biosorption capacity, i.e. the higher the biomass concentration of 0.25 to 0.5 g/g, the greater the biosorption capacity reaches 0.0068 mg/g, but the capacity decreases with increasing biomass concentration of 0, 5-1 g/g of polymer. The result of SEM analysis shows the morphological changes of the immobilized biosorbent surface before and after the process of heavy metal biosorption of Cu^{2+} . Morphological changes occur because of the interaction between heavy metal ions Cu^{2+} and the active side on the immobilized biosorbent surface. These changes cause the pore to surface in the immobilized biosorbent by biomass so that the pore structure appears to be getting smaller.

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