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Immobilization of *Pseudomonas Aeruginosa* Fncc-0063 on Calcium Alginate And Its Application As A Biosorbent For Cu (II) in Water

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Abstract: *Biosorption of Cu (II) using bacteria Pseudomonas aeruginosa FNCC-0063 was immobilized on calcium alginate (PAI). This research examined the effect of various parameters such as pH, contact time, and initial Cu (II) concentration. The biosorption mechanism of Cu (II) was studied by sequential desorption with H_2O, KNO_3 1 M, HNO_3 0.5 M and Na_2EDTA 0.1 M. Cu (II) concentration was analyzed using atomic absorption spectrophotometer (AAS). The results revealed that optimum Cu (II) ion biosorption occurred at a pH biosorption rate constant of 0.03724 g mg-1.min-1. The kinetics studies revealed that Cu (II) biosorption follows pseudo-second-order. The biosorption capacities of 36.60 mg/g. Cu (II) Biosorption followed the Freundlich equation, as shown by a high correlation coefficient (R2) of about 0.99. Ionic bonds dominated the biosorption mechanism of Cu (II) ion on immobilized PAI.*

Keywords*: Pseudomonas aeruginosa*; *Ca-alginate; immobilized; Cu(II); Freundlich*

INTRODUCTION

The development of industrial activities and technological advances carries a positive impact as well as its negative impact (El-Amier et al., 2021). The rapid growth of industry means an increasing amount of produced waste and causes complex problems. Therefore, the process of handling waste is essential. Heavy metals(Masood et al., 2023) are classified as B3 waste (Toxic and Hazardous Materials), which at certain levels can harm the surrounding environment because they are toxic to plants, animals and humans.(Pratidina et al., 2022)

Copper is an essential element for all organism (Priessner et al., 2021). At high concentrations, copper is strongly toxic because it interferes with physiological processes due to forming organo-copper complexes. Waste containing copper can be generated from the paint industry, metal coating, textile industry and metal mining (Yuan et al., 2023). In water, copper is generally found as copper (I) and copper (II), but the most abundant is copper with an oxidation state of +2 or copper (II) (Werr et al., 2021).

Biosorption is an alternative method that uses microorganisms to accumulate heavy metals from aquatic waste (Sukumar et al., 2014). In principle, the biosorption process is the binding of metal ions to the structure of microbial cells, especially cell walls (Amar et al., 2021; Saravanan et al., 2022) Biosorption has several advantages over other waste treatment methods (Sukumar et al., 2014). The advantages include cheap,

efficient, and able to minimize the occurrence of chemical and biological deposits. Without additional nutrients, sorbents can be regenerated and be able to recover metals. The ability of microorganisms to overcome heavy metal pollution, especially bioaccumulation through the biosorption mechanism, has the potential to be utilized in industrial waste treatment.(Nh et al., 2018; Sukumar et al., 2014).

The microorganism used in this study was the bacterium Pseudomonas aeruginosa FNCC-0063. However, suppose P. aeruginosa FNCC-0063 cells are used directly as biosorbents(Thi et al., 2020). In that case, they still have backwards, including being very soft and sticky when interacting with metal ion solutions, making it challenging to separate metal ions from the adsorbent. The biomass is easily damaged due to decomposition by other microorganisms. Therefore, to eliminate these weaknesses in this study, we tried to immobilize P. aeruginosa FNCC-0063 on Ca-alginate so the cells are not easily damaged by the decomposition of microorganisms, becoming an adsorbent material having the higher particle strength and chemical resistance.

RESEARCH METHODS

Materials and Tools

Pseudomonas aeruginosa FNCC-0063, $CaCl_2 \cdot 2H_2O$, Cu(II), KNO_3 HNO_3 Na_2EDTA, Na-alginat 2%, NaCl 0,85%.

Instrumentation

Spectrofotometer FTIR (Nicolet iS10), Spectrofotometer UV-Vis (Geneys 20).

Procedures

Production of Immobilized P. aeruginosa Biomass

P. aeruginosa pellet was put into 2% Na-alginate then dripped by flowing it through an infusion tube into 500 mL of 0.1 M $CaCl_2 \cdot 2H_2O$ (stirring slowly with a stirrer). The beads formed were allowed to harden for 30 minutes and then filtered and washed with 0.85% NaCl. Beads were stored at 4 C in 5 mM $CaCl_2 \cdot 2H_2O$ solution. (de Lima et al., 2018)

Biosorption Study with Batch. Method

Effect of pH on Cu (II) ion biosorption

0.1 gram of immobilized P. aeruginosa biomass interacted with 25 mL of 40 ppm Cu(II) solution with a pH variation of 3; 3.5; 4; 4.5; 5; and 5.5, then shaken with an incubator shaker for 60 minutes. The mixture was filtered, and then the concentration of Cu (II) ions in the filtrate were determined by ASS.(Zhao et al., 2018)

Effect of biosorption time

0.1 gram of immobilized *P. aeruginosa* biomass interacted with 25 mL of 40 ppm Cu(II) solution at optimum pH with variations in contact time (0, 10, 30, 60, 90, 120, 180, 240, 300, 360 minutes). The mixture was filtered, and then the concentration of Cu(II) ions in the filtrate were determined by AAS.

Effect of initial concentration of Cu (II) ion

0.1 gram of immobilized *P. aeruginosa* biomass interacted with Cu(II) solution at various initial concentrations, namely; (0, 10, 20, 40, 60, 80, 100, 150, 200, 250, and 300 ppm) were then shaken for the optimum contact time. The mixture was filtered, and then the concentration of Cu(II) ions in the filtrate were determined by AAS.(Nh et al., 2018)

Sequential Desorption of Cu (II) Ions in Immobilized *P. aeruginosa* Biomass

0.1gram *P. aeruginosa* biomass interacted with 300 ppm Cu (II) solution at optimum pH conditions. Then, it is shaken with a shaker for 24 hours, 150 rpm, and the filtrate is separated the residue. Atomic Absorption Spectroscopy measured the remaining Cu (II) content in the filtrate. The absorbed beads of Cu (II) metal are dried overnight at 50 C, desorbed, and then interacted with 50 mL H_2O for 30 minutes. The filtrate and beads were separated, and AAS measured the Cu (II) content in the filtrate. The separated beads were dried in an oven for 12 hours, desorbed again with 50 mL 1 M KNO_3 solution, and shaken at 150 rpm for 3 hours. AAS measured Cu (II) levels in the filtrate. Then the dried be ads were desorbed again with 50 mL of 0.5 M HNO_3 solution and shaken at 150 rpm for 3 hours. The filtrate and precipitate were separated. AAS measured the level of Cu (II) in the filtrate. Beads were dried in an oven for 12 hours. Then the residue interacted with a solution of 50 mL 0.1 M Na_2EDTA and shaken at 150 rpm for 20 hours. Furthermore, the level of Cu (II) in the filtrate was measured by AAS.(Yasir et al., 2020)

RESULTS AND DISCUSSION

Identification of Biosorbent Functional Groups

Pseudomonas aeruginosa Biomass

The Infrared spectrum of *P. aeruginosa*(Pérez-Cid et al., 2020) is presented in Figure 1. The picture reveals a broad absorption in the area of 3425.58 cm^{-1}, that stretching vibration of the –OH group. The strong absorption in the area of 1658.78 cm^{-1} shows the asymmetric stretching vibration –COO- while the symmetric stretching vibration appears weak at 1404.18 cm^{-1}.

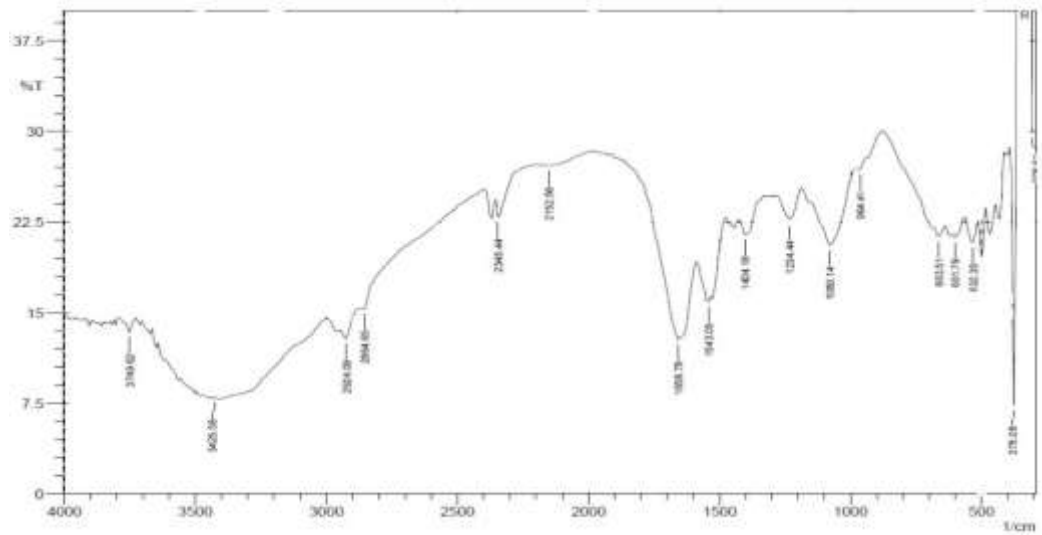

Figure 1. *P. aeruginosa* Biomassa spectra

Pseudomonas aeruginosa Immobilized Alginate (PAI)

The infrared spectra showed almost similar to those of P. aeruginosa that were not immobilized. Still, in these spectra, the two groups, namely the hydroxyl group (-OH) and the carboxyl group (-COO-), were sharply absorbed in the area of 3448.72 cm^{-1} and

1635.64 cm^{-1} increased in intensity, which indicated an increase in the number of active groups in the P. aeruginosa biosorbent after immobilization. (Sui et al., 2012)The infrared spectra of immobilized P. aeruginosa (PAI) are presented in Figure 2.

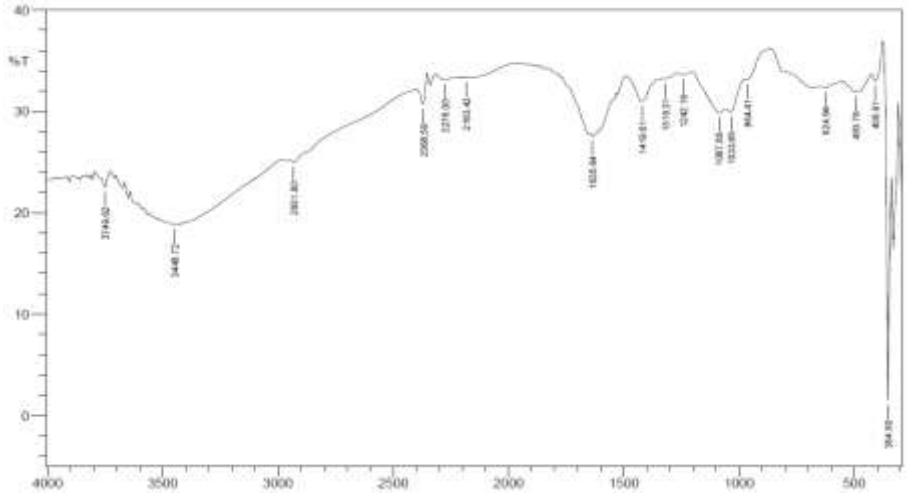

Figure 2. *P.aeruginosa* Immobilized Alginate Spectra

In this study 0.1 M $CaCl_2$ was used as a source of Ca^{2+} ions (Katircioğlu et al., 2008). Gel formation between alginate and Ca^{2+} ions is the process of forming a chelate bond between sodium alginate and calcium chloride through a chain mechanism or interchain mechanism (Kim et al., 2021). In this gel, Ca^{2+} ions will interact with the carboxyl group, where Ca^{2+} binds to the oxygen atom of the carboxyl group. Oxygen atoms with high electronegativity will bond with Ca^{2+} ions and form a lump (gel). Pseudomonas aeruginosa cells were physically trapped in Ca-alginate gel medium (Martínez-Arcos et al., 2021).

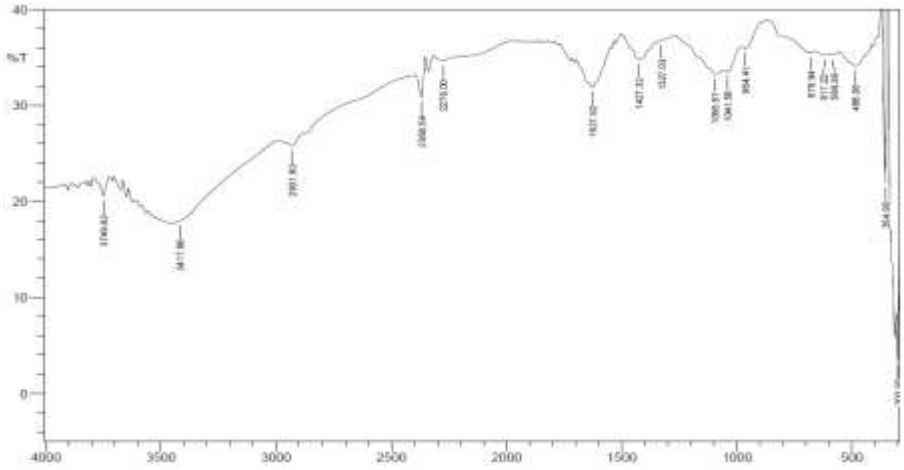

Fig 3. Immobilized P. aeruginosa after adsorption of Cu Spectra

Figure 3 shows the infrared spectra of immobilized *P. aeruginosa* after adsorption of Cu (II) ions(de Lima et al., 2018). The peaks in Figure 2 a and Figure 3, but some absorption bands have shifted; namely, the peak at 3448.72 cm^{-1} shifted to 3417.86 cm^{-1}, which is a hydroxyl vibration (-OH) as well as at the peak 1635.64 cm^{-1} shifted to 1627.92 cm^{-1} which is a carboxylic group (-COO-). This shift indicates the interaction process of Cu (II) metal ions and –OH and (-COO-) groups on the surface of the biosorbent. This was confirmed by the appearance of spectra in the area around 586.36 cm^{-1}, which identified the presence of Cu-O bonds.(Lee et al., 2021)

Biosorption Study

Effect of pH on Cu (II) ion biosorption

The pH of the solution is an essential factor affecting the adsorption of heavy metals from a solution, not only the solubility of heavy metal ions but also the ionization of functional groups on the cell wall of the biosorbent. This section examines the effect of solution pH on the number of Cu ions adsorbed by P. aeruginosa immobilized alginate. The effect of pH on the biosorption of 40 mg/L Cu (II) ions with a pH variation of 3.0 – 5.5 adjusted with Britton-Robinson buffer (BR) and a contact time of 60 minutes at room temperature.(Rasheed et al., 2020) The graph of the effect of pH can be seen in Figure 4.
In Figure 4, it is revealed that the biosorption of Cu metal generally increases with the increase in pH. The biosorption increases sharply in the pH 3.0 - 4.0 region with a maximum adsorption at pH 4 and begins to decrease at pH 5.

At low pH, the surface of the biosorbent is positively charged so that the Cu (II) biosorption is very small. (Saravanan et al., 2022)Harris and Ramellow (1990) suspected that the zero point charge or the isoelectric point of the protein functional groups that make up organism cells occurs at pH 3. At pH less than 3 (pH < 3), the active surface sites of the cell walls of microorganisms have Positive charge, while negatively charged at above 3 (pH> 3).(Saravanan et al., 2022) The presence of this negative charge will cause an interaction between the positively charged Cu (II) ion with the active site on the negatively charged bacterial cell wall. So that there is an increase in the biosorption of Cu (II) ions at a pH greater than 3 (pH > 3). At low pH, the adsorption of Cu (II) metal ions is relatively more minor. This phenomenon is because, in acidic conditions, there is an increase in hydrogen ions (H^+) and hydronium ions (H_3O^+), so that the functional groups present in the adsorbent are protonated. On the other hand, at a relatively high pH, the active side of the biosorbent will be negatively charged so that it will facilitate the biosorption process. Still, at higher pH conditions, a hydrolysis process will occur to form a metal hydroxide complex, followed by the precipitation of metal hydroxides.(Yuan et al., 2023)

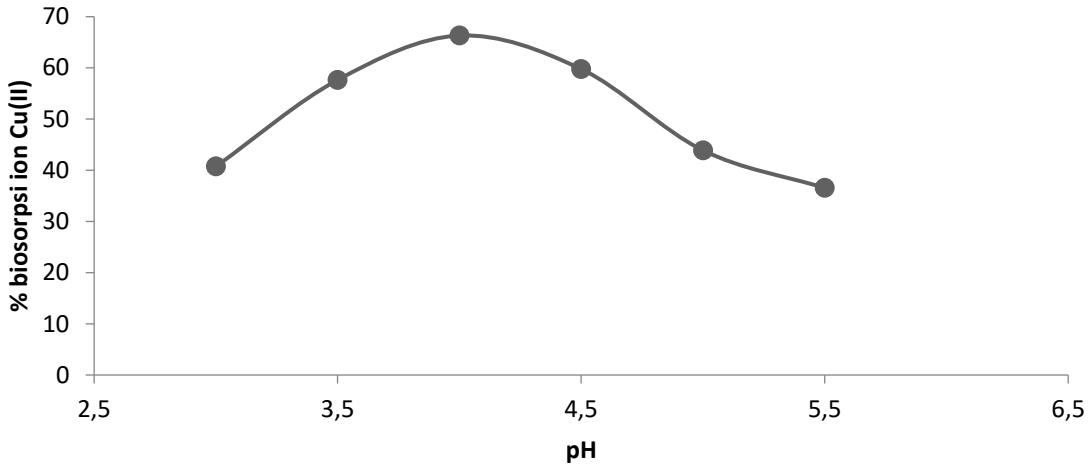

Fig 4. Effect of solution pH on the effectiveness of Cu(II) ion biosorption

Effect of contact time and biosorption kinetics

The Effect of contact time was observed at a time variation of 10 – 360 minutes, at pH 4. The results of the observation of contact time are presented in Figure 5. The results revealed that the biosorption of Cu(II) ions was 7.32 mg/g (73.74%) at the 300th minute. Kinetic parameters provide essential information in designing and modelling biosorption systems to predict biosorption rates. The biosorption of immobilized P. aeruginosa to Cu(II) ions was studied using reaction kinetics models of order-1, order-2, pseudo-order-1, and pseudo-order-2.(Nathan et al., 2021)

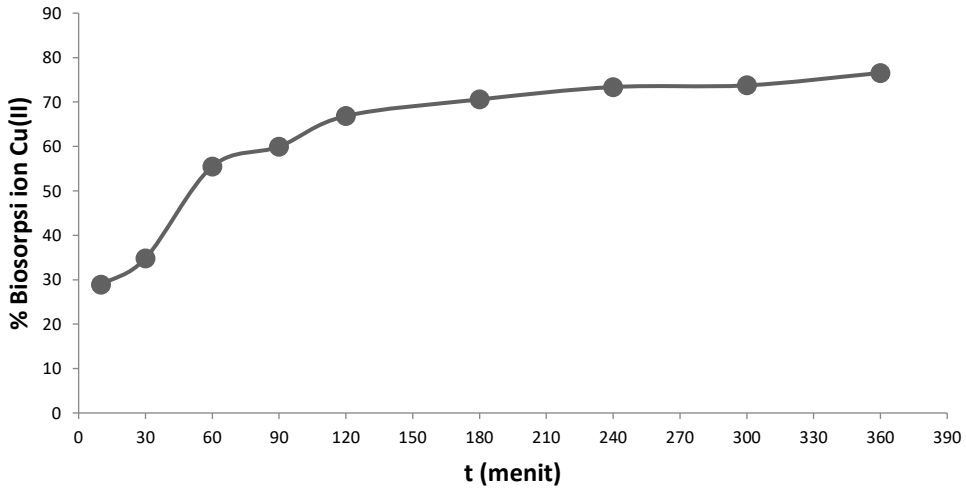

Figure 5. Effect of contact time on Cu(II) ion biosorption

Table 1. Biosorption Kinetic Model of Immobilized P. aeruginosa against Cu(II) Ions

Kinetic model	Rate Constant (k)	R^2
Orde-1	3 x 10^{-3} minute	0.8312
Orde-2	2 x 10^{-4} minute	0.9179
Pseudo orde-1	0.01036 minute	0.9578
Pseudo orde-2	0.03724 g/mg.minute	0.9976

Based on the data from Table 1, it is revealed that the linearity of the kinetics (R2) of Cu(II) ion biosorption by immobilized *P. aeruginosa*, which is closest to one, is pseudo-order 2. The pseudo-order two kinetic model illustrates that the adsorption process does not only involve biosorbent and biosorbate but there are reactants or other factors that influence the reaction rate, such as H^+ ions.

Effect of initial concentration of Cu(II) ion and sorption isotherm

This section examines the value of biosorption capacity, equilibrium constant and adsorption energy based on the Langmuir and Freundlich adsorption isotherm equation(da Costa et al., 2020). The Effect of metal ions' initial concentration on biosorption in the two biosorbents. At different concentrations ranging from 10 mg/L to 300 mg/L at pH 4.(Werr et al., 2021) The results are shown in Figure 6.

Determination of the biosorption pattern between metal ions and biosorbents, in this case, immobilized P. aeruginosa, was carried out by channeling the concentration of metal ions at equilibrium at various concentrations of adsorption capacity. The Langmuir isotherm model refers to the formation of a monolayer on the surface of the biosorbent. In contrast, the Freundlich isotherm model in equation (1) assumes the formation of a multilayer surface complex, namely the formation of bonds between more than one surface active group and metal ions.

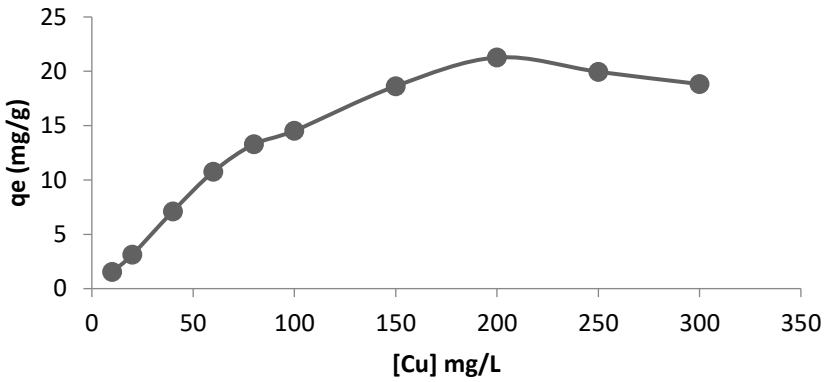

Figure 6. Effect of initial concentration on the number of Cu(II) ions adsorbed by immobilized P. aeruginosa

The Langmuir adsorption isotherm model is stated in equation (2) by making a curve of the relationship between the number of moles of Cu(II) metal ions adsorbed per gram of biosorbent with the concentration of metal ions at equilibrium.

$$\log m = \log B + \frac{1}{n}\log C_{eq} \qquad (1)$$

$$\frac{C}{m} = \frac{1}{bK} + \frac{C}{b} \qquad (2)$$

Based on the study of Langmuir and Freundlich, adsorption isotherms. (Syauqiah et al., 2022)The study revealed that the biosorption of immobilized *P. aeruginosa* on Cu(II) ions is more suitable to follow the Freundlich adsorption isotherm model(Wijayanti & Kurniawati, 2019), as seen from the linear regression coefficient (R2), which is closer to one. Thus, it can be concluded that there is an interaction between Cu(II) ion molecules so that the biosorption of Cu(II) ions on the surface of the two biosorbents forms a multilayer layer or biosorption occurs not only on the surface active site. From the calculation results (Table 3), the biosorption capacity of Cu(II) ions in *Pseudomonas aeruginosa* immobilized alginate is 36.60 mg/g.

The biosorption energy values are presented in Table 3. In general, the biosorption energy for the immobilized P. aeruginosa biosorbent was in the range of (Ea) 23.63 kJ/mol. Based on the value of adsorption, energy (Ea) adsorption is divided into chemisorption (chemical adsorption) and physisorption (physical adsorption). According to Adamson (1990), the minimum limit of adsorption is categorized as chemisorption if it has adsorption energy of 20.92 kJ/mol. So if the adsorption energy on immobilized P. aeruginosa biomass refers to Adamson (1990), it is classified as a weak chemical interaction or chemical adsorption

Table 3. Data of Isoterm Langmuir and Freundlich

Biosorbent	Langmuir				Freundlich		
	Qmax (mg/g)	K x 10^3 L/mol	E (kJ/mol)	R^2	K_f (mg/g)	n	R^2
P. aeruginosa immobilized	36.60	11.85	23.63	0.98	15.43	2.68	0.99

Cu (II) Ions desorption at immobilize P. aeruginosa Biomass.

Determining the biosorption mechanism that plays a role in the biosorption of Cu (II) by immobilized P. aeruginosa alginate beads, sequential desorption was carried out on the adsorbent, which had previously been contacted with a Cu (II) solution. (Pescosolido et al., 2011)Furthermore, Cu (II) ions that have been adsorbed are desorbed with desorption agents sequentially: distilled water, 1 M KNO_3, 0.5 M HNO_3, and 0.1 M Na_2EDTA.

Table 4. Sequential desorption of Cu (II) metal ions on P. aeruginosa immobilized alginate

Desorbent	Amount of desorbed Cu (II) Ions (%) (%)
Aquadest	0.08571
KNO_3 1 M	2.15592
HNO_3 0.5 M	22.46171
Na_2EDTA 1 M	0.53485
Total	25.23819

Cu (II) ions which are adsorbed through the entrapment mechanism can be desorbed with distilled water. Potassium nitrate is used to desorb the adsorbed Cu (II) ions through an ion exchange mechanism, while nitric acid is used to desorb the bound Cu (II) ions through ionic bonds. Desorption of adsorbed Cu (II) ions through complex formation was used as chelating agent sodium EDTA. By observing at the percentage of desorption for each desorption agent on the adsorbent in Table 4, it can be seen that the most dominant mechanism of adsorption of Cu (II) ions is through the mechanism of ionic bond formation, because the percentage of desorption with HNO_3 is the largest.

CONCLUSIONS

The biosorption capacity of immobilized *P. aeruginosa* alginate ions to Cu(II) ions was 36.60 mg/g, following the Freundlich adsorption isotherm pattern. 1. Biosorption of Cu(II) ions with a concentration of 40 mg/L by 0.1 gram of P. aeruginosa immobilized alginate to reach the optimum at pH 4 with the optimum contact time at 300 minutes, following the pseudo reaction second order with a rate constant of 0.03724 g/mg.min.

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