Adsoprtion of Cu(II) Ion in Aqueous Solution by *Pseudomonas* sp. Biosorbent

Achmad Dwi Setiawan[a)], Rudi Kartika, Rahmat Gunawan

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Jalan Barong Tongkok No.4, Samarinda 75123, Indonesia

[a)] Corresponding author: achmaddwisetiawan@gmail.com

Abstract. Cu(II) waste pollution in the environment is commonly found in the electroplating industry. Cu(II) waste in the electroplating process has a high concentration of potential to pollute the environment, therefore it is necessary to use a method to overcome environmental pollution with an easy process. This study used the metal ion absorption process by *Pseudomonas* sp. bacteria which aims to determine the ability of *Pseudomonas* sp. bacteria to absorb Cu(II) based on variations in the optimum concentration and time required for bacteria in the Cu(II) ion biosorption process. The study used a visible spectrophotometer with a maximum wavelength of Cu(II) standard solution of 660 nm. The results of the test, *Pseudomonas* sp. bacteria were able to absorb Cu(II) at concentrations (3; 5; 7; 9; and 11) ppm with a percentage (%) of (84,447; 72,023; 66,614; 62,052 and 50,761) , respectively. the concentration of Cu(II) is quite significant.

INTRODUCTION

The development of the electroplating process is growing rapidly in line with the needs of the community. This increasingly rapid development in addition to providing benefits but also has a negative impact on the waste produced. Waste from the electroplating industry is in the form of waste containing heavy metals which are classified as Hazardous and Toxic Substances waste (Hazardous Toxic Materials). Direct disposal of waste in the electroplating process without any prior treatment to the environment causes environmental pollution that can pollute the environmental ecosystem in the form of colloids, solutions or other particles [1]. One of the electroplating process wastewater containing high heavy metals is copper or Cu. According to the Ministry of Environment No. 51 of 1995 concerning the quality standards of liquid waste in industrial activities, the threshold for copper concentration in industrial waste of 3 mg/L, if the Cu concentration exceeds the quality standard it will have a negative impact on human health and environment [2].

Heavy metals are hazardous pollutants and are included in Hazardous and Toxic Substances waste, this is because heavy metals have properties that cannot be degraded naturally and tend to accumulate in water, organism bodies and bottom sediments [3]. Heavy metals are toxic metals that are very dangerous if they enter the human body that exceeds the threshold. Heavy metals become dangerous because of the bioaccumulation process through the food chain [4]. Copper is an essential metal for organisms in certain amounts needed in metabolic, physiological processes in animals and the formation of hemoglobin [5]. If the concentration is excessive and exceeds the copper metal threshold in the body, it can cause health problems such as lung disorders, blood vessel damage, cancer and even death. Heavy metal pollution, especially copper, requires processing before the waste is discharged into the environment. One method that can be used to treat heavy metal waste is the adsorption method [6].

Adsorption method is a method of absorbing atoms, ions or molecules in solution on the surface of the adsorbent. Where the adsorbent is the substance that absorbs and the adsorbate is the substance that is absorption [7]. In general, the adsorbent that is often used in the separation of heavy metals in liquid waste is activated carbon, but in terms of price, it is quite expensive and its effectiveness against heavy metals is not too high and cannot be

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regenerated. Therefore, one alternative in the treatment of heavy metal waste is to use biological materials as adsorbents [8]. Microorganisms can be used as adsorbents in the treatment of heavy metal waste in a live or dead state called biosorbent. The use of microorganisms as adsorbents has the advantage of having high heavy metal binding efficiency, regenerative, the ease and abundance of microorganisms that can be used as biosorbents [9]. One of the bacteria that is often used as a biosorbent is *Pseudomonas* sp..

Based on the description above, this study used the bacterium *Pseudomonas* sp.. because it can absorb heavy metals in neutral pH conditions (4-7) and can be used as an absorber of various types of heavy metals such as copper (Cu), lead (Pb), zinc (Zn), cadmium (Cd) and chromium (Cr). This study aims to determine the effectiveness of *Pseudomonas* sp.. In absorption Cu (II) in aqueous water by using variations in concentration (3; 5; 7; 9; 11) ppm and knowing the concentration and optimum time for Cu (II) absorption.

EXPERIMENTAL

Equipments and materials

Equipments

The equipment used in the study were glassware, stirring rod, spatula, sterile cotton swab, Bunsen, ose needle, eppendorf micropipette, autoclave, incubator, water bath, pH meter, hot plate with magnetic stirrer, laminar air flow and Visible Spectrophotometer (Rayleight Vis 720G).

Material

The materials used in this study are $CuSO_4$ solids, ascorbic acid, Methylene Blue solution, buffer solution (citric acid-Na_2PHO_4) pH 2.2, distilled water, liquid media Nutrient Broth (NB), and *Pseudomonas* sp..

Tool Sterilization

The tools used such as Erlenmeyer, ose needle and measuring pipette were washed thoroughly. Then the glass equipment was sterilized using an autoclave for 1 hour at a temperature of 121 ºC.

Methods

Preparation of Nutrient Broth

3.5 g of nutrient broth solids were put into 6 pieces of 250 mL Erlenmeyer, added with 200 mL of distilled water. Then heated using a hot plate at a temperature of 60 ºC and homogenized with a magnetic stirrer. Nutrient Broth was sterilized by autoclave at 121 ºC for 15 minutes. Nutrient Broth medium was put in an incubator at 30 ºC for 24 hours.

Bacterial Regeneration

A total of 6 Erlenmeyer 250 mL containing Medium Nutrient Broth, each inoculated with *Pseudomonas* sp. as much as 2 needles ose. The medium was incubated at 37 ºC for 24 hours before use.

Determination of OD (Optical Density) Value

Pseudomonas sp. as much as 1 mL were taken from each of 6 Erlenmayer pieces and put into a cuvette. The absorbance value was measured in the range of 610 nm on a Vis Spectrophotometer and measurements before and after incubation in the range of 1 hour, 12 hours and 24 hours.

Biosorption of Cu(II) Ion by Pseudomonas sp..

250 mL Erlenmeyer flask containing 6 nutrient broth was added with 0 ; 3 ; 5 ; 7 ; 9 ; and 11 mL of 250 ppm Cu(II) standard solution, added nutrient broth to the mark and homogenized. Then 6 Erlenmeyer containing nutrient broth were inoculated with *Pseudomonas* sp.. as much as 1 mL. *Pseudomonas* sp. medium placed in an incubator at 37 ºC.

Determination of Cu(II) Ion Concentration

The sample solution containing *Pseudomonas* sp. was exposed to Cu(II) with concentrations (0; 3; 5; 7; 9 and 11) ppm. The sample solution was taken as much as 10 mL and filtered using 0.45 millipore membrane filter paper. Then diluted with a buffer solution (citric acid-$Na_2HPO_4.7H_2O$) pH 2.2 in a 50 mL volumetric flask. Then 3 mL of ascorbic acid solution was added and the temperature was kept constant at 32 ºC for 3 minutes using a water bath. Then, 2 mL of methylene blue solution was added. After that, it was allowed to stand for 2 minutes and measured at the maximum wavelength. Cu(II) concentrations were measured every 48 hours.

Analysis Data

Linearity Determination

Standard solution of Cu(II) with a concentration of 10 ppm, then taken into each 50 mL volumetric flask by pipetting (0; 1.5; 2.5; 3.5; 4.5; 5.5) mL, diluted using a buffer solution (citric acid-$Na_2HPO_4.7H_2O$) pH 2.2 so that the concentration of the standard Cu(II) solution is obtained successively (0; 0.3; 0.5; 0.7; 0.9; 1 ,1) ppm. 3 mL of ascorbic acid solution of was added and the temperature was kept constant at 32 ºC for 3 minutes using a water bath. Then, 2 mL of methylene blue solution was added. After that, it was allowed to stand for 2 minutes and measured at the maximum wavelength. Measurements were repeated 3 times. With the following formula [10]:

$$y = ax + b \quad (1)$$

Information:
a = intersep
b = slope
x = sample concentration (ppm)
y = absorbance

Limit of Detection and Limit of Quantity

The detection limit of this study can be determined from the data for determining the standard curve of Cu(II) so that the regression equation is obtained, then the standard deviation is calculated, so that the LOD and LOQ values are obtained.

$$LOD = 3.3 \times \frac{SD}{Slope} \quad (2)$$

$$LOQ = 10 \times \frac{SD}{Slope} \quad (3)$$

Where SD is the standard deviation [11].

Precision

Precision testing was carried out using a sample solution that had been prepared and then analyzed using a Visible Spectrophotometer. This test was performed 7 times the sample repetition. From the data obtained, the

average value ($\bar{x}$), standard deviation (SB), and relative standard deviation (SBR) was calculated and compared with the acceptability conditions [10].

Accuracy

In the accuracy test used to measure accuracy in this study, the spike solution was prepared and repeated 7 times. The average value is used as a reference to determine the % recovery. The calculation of % recovery is determined by the following formula [10]:

$$R = \frac{\bar{X}}{\mu} \times 100\% \quad (4)$$

Information:
R = percent recovery
$\bar{X}$ = the average concentration value of the measurement results
μ = actual concentration value

RESULTS AND DISCUSSION

The Regeneration of *Pseudomonas* sp.

Determination of Optical Density (OD)

Optical density (OD) aims to determine the turbidity value of the sample based on the read absorbance. The absorbance obtained from the bacterial starter media before and after incubation is shown in Table 1.

TABLE 1. Optical density value

Treatment	Time (Hour)		
	1	**12**	24
Before Incubation (nm)	1,659		
After Incubation (nm)	1,709	1,721	1,749

Based on the results of Table 1, the absorbance values obtained before and after incubation have increased, this indicates that the absorption of light by bacteria is increasing and the light that is passed is very little. So it can be concluded that the difference in the turbidity level of the starter media indicates the growth of *Pseudomonas* sp. goes well.

Preparation of Standard Curve of Cu(II)

The standard curve of Cu(II) is used to construct a linear regression equation which is used to measure the concentration by using the absorbance value. Based on the results of standard measurements of Cu(II) solution, the absorbance value decreased linearly with increasing concentration of Cu(II) solution. The measurement results obtained the value of y = -0.1845x + 0.4026 and the correlation coefficient value from the linear regression equation, namely R^2 = 0.996 with slope = -0.1845 and intercept = 0.4026 which is shown in Fig. 1. The value of the coefficient of determination (R^2) is close to 1 then the resulting calibration curve has a good linearity [10].

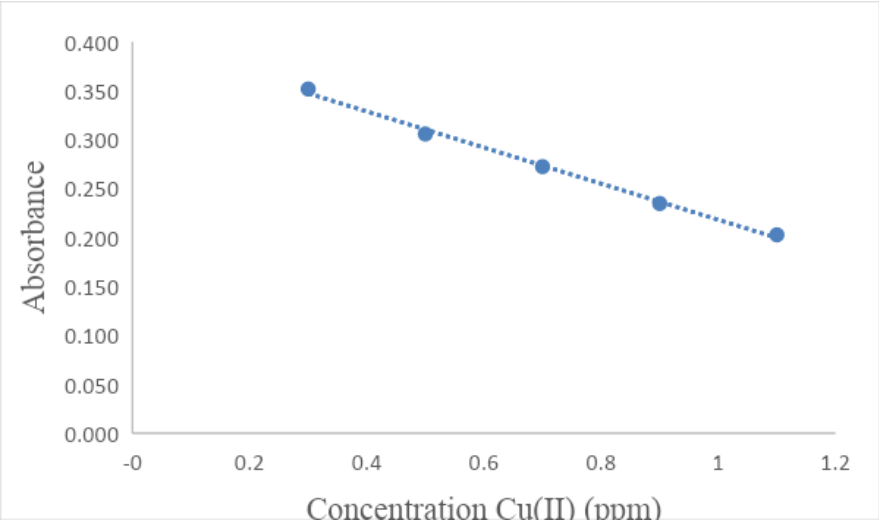

FIGURE 1. Cu(II) concentration curve against concentration

Biosorption of Cu(II) by Pseudomonas sp.

Cu(II) biosorption was carried out using *Pseudomonas* sp. which is one of the biosorbents that can absorb Cu(II). *Pseudomonas* sp. is a type of gram-negative bacteria whose cell walls are mostly composed of phosphates, amines, carboxylic groups and hydroxides [12]. The results of the Cu(II) biosorption analysis on the growth media of *Pseudomonas* sp. can be seen in Table 2.

TABLE 2. Biosorption of Cu(II) by *Pseudomonas* sp.

No	Initial Concentration of Cu(II) (ppm)		The concentration of Cu(II) after the biosorption process of *Pseudomonas* sp. on day (ppm)							
	Solution	Detected	2	4	6	8	10	12	14	16
1.	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.	3	3,241	2,564	1,561	1,127	0.938	0.775	0.585	0.531	0.504
3.	5	5,192	4,596	3,404	2,970	2,455	2,130	1,778	1,561	1,453
4.	7	6.916	6,428	5,453	4,748	3,718	3.0718	2,526	2,417	2,363
5.	9	9,084	8,650	7.566	6,049	4,640	4,206	3,772	3,556	3,339
6.	11	11,360	10,818	8,992	7,610	6,634	6,244	5,919	5,702	5.593

Based on Table 2, it can be seen that the variation of Cu(II) concentration is (3, 5, 7, 9 and 11) ppm and the variation of exposure time is (2, 4, 6, 8, 10, 12, 14, and 16). day. The results obtained in the Cu(II) biosorption process by *Pseudomonas* sp. decreased in concentration along with the increase in the days that had been set. This shows that the bacteria *Pseudomonas* sp. can be a bisorben in reducing Cu(II) waste and bacteria containing cysteine acid in their cell walls. According to Dewi (2007), biosorption occurs due to the presence of the amino acid cysteine in the bacterial body, the role of metallothiein protein in amino cysteine is able to bind essential and non-essential heavy metals [13]. In research Binz (2000) suggested that the use of metallothiein in the body of microorganisms has an important role in the process of metabolic mechanisms and cellular transitions in metal processing in the body, especially heavy metal ions [14]. The process of microorganisms binding to metals is through ion exchange, in which the ions in the cell walls of microorganisms will be replaced by heavy metal ions [15].

The following Fig. 3 shows a comparison graph of the percent biosorption of each concentration variation against time (days).

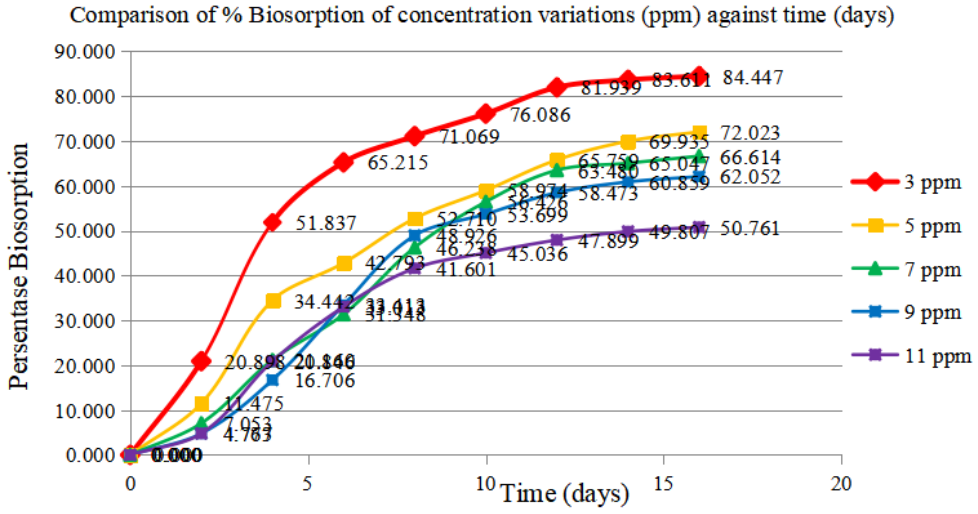

FIGURE 2. Comparison of % Biosorption of Cu(II) by *Pseudomonas* sp. each variation of concentration against time (days)

Fig. 2 is a medium exposed to Cu(II) with varying concentrations (3, 5, 7, 9, and 11) ppm with absorption from day 0 to day 16, indicating that *Pseudomonas* sp. decreased in concentration which indicated that Cu(II) could be absorbed and obtained the percent biosorption, namely at a concentration of 3 ppm of 84.447%, a concentration of 5 ppm of 72.023%, a concentration of 7 ppm of 66.614%, a concentration of 9 ppm of 62.052% and a concentration of 11 ppm of 50.761%. In this study, it was shown that the higher concentration of Cu(II) solution would affect the percentage decrease in Cu(II) biosorption by *Pseudomonas* sp. It is suspected that the pH of the medium affects the process of biosorption so that the absorption that occurs at variations in concentration and time decreases.

According to Rahmawati (2006), at a certain time, an equilibrium will occur between the adsorbent (*Pseudomonas* sp.) and the adsorbate (Cu(II)) which is called the optimum time for metal absorption [16]. Fig. 3 shows that at a concentration of 3 ppm there is a good increase in biosorption efficiency on days 2 to 4 which indicates that the biosorption process is running well in that time sp.an with a biosorption percentage of 20.898% to 51.837%. The optimum concentration and time of absorption of *Pseudomonas* sp. on Cu(II) occurred at a concentration of 3 ppm with a biosorption percent of 51,837% on the 4th day. This is due to a significant and high increase in biosorption capacity, which means that during this time, *Pseudomonas* sp. bacteria work optimally in absorption Cu(II) [17]. Then, on the 12th to the 16th day, the percentage of biosorption was obtained from 36.388% to 36.874% and the biosorption process went to a constant condition and was expected to stop the next day.

Analysis Data

Linearity

Based on the obtained curve, it can be seen that the linear equation is y = -0.1845x + 0.4026 with a regression value of R^2 = 0.996 and r = 0.9979. The R^2 value of 0.996 indicates that there is a very strong correlation between concentration and absorbance.

Detection Limit

The detection limit is the smallest amount of analyte that can be detected in a sample that gives a significant response compared to the blank. The quantitation limit is the quantity of the smallest amount of analyte in a sample that can meet the careful and thorough criteria [10]. The results of the LOD and LOQ calculations can be seen in Table 3.

TABLE 3. Determination of detection limit

No.	Cu(II) standard	Absorbance	yi	y-yi	(y-yi)2
1	0.3	0.352	0.3472	0.005	2.6351x10-5
2.	0.5	0.306	0.3105	-0.004	1.7361x10-5
3.	0.7	0.273	0.2736	-0.001	8,7111x10-7
4.	0.9	0.235	0.2367	-0.001	1.8677x10-6
5.	1.1	0.204	0.2052	-0.002	2.3511x10-6
Sum (n = 5)					∑ 4.8802x10-5
SD					7.3637 x10-6
LOD					0.12 ppm
LOQ					0.4 ppm

From the data obtained in Table 3, it is known that the detection limit value of the Cu(II) determination obtained the detection limit value of 0.12 ppm and the quantization limit of 0.4 ppm. This value indicates that the amount of analyte can still be measured or detected, so it can be said that the analysis of the adsorption of Cu(II) ions in aqueous solution by *Pseudomonas* sp. bacteria can be used according to the specified value.

Precision

Test precision is a measure of the degree of concordance between individual results from the mean as measured by the spread of individual results from the mean if the procedure is applied repeatedly to samples taken from a homogeneous mixture. Careful criteria are given if the method provides a relative standard deviation (RSD) or coefficient of variation (CV) of 2% or less [10]. The results of the precision test calculation can be seen in Table 4.

TABLE 4. Determination of the Relative Standard Deviation (% RSD)

No.	Abs (y)	b	a	Measured concentration (ppm)
1	0.272	0.4026	-0.1845	0.708
2	0.275	0.4026	-0.1845	0.692
3	0.273	0.4026	-0.1845	0.702
4	0.275	0.4026	-0.1845	0.692
5	0.272	0.4026	-0.1845	0.708
6	0.272	0.4026	-0.1845	0.708
7	0.271	0.4026	-0.1845	0.713
Average	**0.273**			**0.703**
Standard Deviation (SD)				**0.008346**
% RSD				**1.187%**
% RSD				**Accepted**

From the results of the data obtained, it can be seen that the average test results have a relative standard deviation value (% RSD) ≤ 2% and in accordance with the terms of acceptance according to Riyanto (2014) [10]. So the results with the relative standard deviation (% RSD) of 1.187% and SD (Standard Deviation) of± 0.008346.

Accuracy

Accuracy is a value that indicates the degree of similarity of the analysis results with the actual state of the analyte. Accuracy is expressed as % recovery (percent recovery) of the analyte that is added therein [10]. The results of the calculation of the accuracy test can be seen in Table 5.

Table 5 Determination of accuracy (% Recovery)

% Recovery		
Reference Solution (ppm)	**Test Solution**	**%Recovery**
0.7	0.708	101.123
0.7	0.692	98,800
0.7	0.702	100,348
0.7	0.692	98,800
0.7	0.708	101.123
0.7	0.708	101.123
0.7	0.713	101,897
Average		**100.459**

Based on the data in Table 5, it can be seen that the standard concentration of Cu(II) analyzed has an average recovery (% recovery) of 100.459%. According to Riyanto (2014) the results of good accuracy if obtained values with a range of 90-107% [10]. Based on the data obtained, in this study the results of the accuracy test are still within the specified range. The use of method validation in this study aims to ensure that the analytical method is accurate, specific, reproducible and ensures that the analytical method is appropriate for its intended use.

CONCLUSION

Biosorption of *Pseudomonas* sp. of Cu(II) at a concentration of 3 ppm was 84,447%, a concentration of 5 ppm was 72,023 %, a concentration of 7 ppm was 65.831 %, a concentration of 9 ppm was 63,246% and a concentration of 11 ppm was 50,761%. The optimum concentration and time of absorption of *Pseudomonas* sp. against Cu(II). The optimum concentration occurred at a concentration of 11 ppm with a decrease of 5.767 ppm. The optimum time occurred on days 2 to 4 at a concentration of 3 ppm absorption of 30.939% occurred.

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