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(RESEARCH ARTICLE)

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Biosorpion of Cu (II) Heavy Metal by *Streptococcus mutans*

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Abstract

The rapid development of electroplating industries has increased the risk of environmental pollution due to toxic heavy metal waste, such as copper Cu (II). Biosorption using microorganisms is considered an effective and environmentally friendly alternative for heavy metal removal. This study aims to evaluate the biosorption capacity of *Streptococcus mutans* toward Cu (II) ions and to determine the optimum concentration conditions. The experiments were conducted by culturing *Streptococcus mutans* in Luria–Bertani medium exposed to Cu (II) at concentrations of 3, 6, 9, 12, and 15 ppm. The residual Cu (II) concentration was analyzed using atomic absorption spectrophotometry. The results indicate that *Streptococcus mutans* is capable of biosorbing Cu (II) at all tested concentrations, with a maximum biosorption efficiency of 86.82% at 3 ppm after 6 days. The optimum biosorption time was observed at 4 days for all concentration variations, yielding an optimum absorption of 38.26% at 3 ppm.

Keywords: Biosorption; Copper; Spectrophotometry; *Streptococcus mutans*

1. Introduction

Rapid technological development has led to significant changes in the industrial sector. While these advancements provide numerous benefits, they also generate adverse impacts on human life. One of the most evident negative effects is the generation of waste from industrial processes [1]. The electroplating industry is one of the major contributors to environmental waste. The rapid growth of electroplating industries, driven by increasing societal demand, has resulted in a corresponding increase in waste generation. Waste produced by this industry contains heavy metals and is classified as hazardous and toxic material (B3). The direct discharge of untreated waste can cause environmental ecosystem degradation and pose serious risks to human health, affecting both industrial workers and surrounding communities. Although the volume of waste generated may be relatively small, electroplating waste exhibits high toxicity levels. One of the heavy metals commonly present in electroplating waste is copper (Cu) [2].

In relation to industrial waste generation, heavy metals are among the most hazardous pollutants in ecosystems due to their non-biodegradable nature, toxicity, and ability to bioaccumulate within food chains. The accumulation of heavy metals can cause long-term adverse effects on living organisms, while their persistent characteristics allow them to remain in the environment for extended periods. Environmental pollution by heavy metals is closely associated with their use in human activities and the intentional or unintentional discharge of metal-containing waste into the environment. The presence of certain heavy metals at high concentrations poses a serious threat to aquatic, terrestrial, and atmospheric ecosystems. One of the most hazardous heavy metals commonly found in industrial waste, particularly electroplating effluents, is copper (Cu) [3].

Copper (Cu) is an essential metal required by organisms in trace amounts; however, it can exert toxic effects at elevated concentrations. At a concentration of 0.01 ppm, Cu can be lethal to phytoplankton, while concentrations of 2.5–3 ppm

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in Cu-containing waste can cause fish mortality. Sources of Cu contamination originate from industrial, agricultural, and domestic wastewater. Therefore, the development of effective technologies for the treatment of heavy metal–containing waste is required [4].

In response to the toxicity of copper (Cu) at elevated concentrations, biosorption has emerged as an environmentally friendly and cost-effective technology for heavy metal removal. The reduction of Cu (II) concentration through biosorption occurs due to the presence of biosorbents with a high affinity toward metal ions in aqueous solutions, enabling effective metal binding. This binding process primarily involves ion exchange mechanisms, in which ions on the microbial cell wall are replaced or exchanged with heavy metal ions [5]. Among various biosorbents, *Streptococcus mutans* has been identified as a potential microorganism for heavy metal biosorption.

Based on the above considerations, this study was conducted to evaluate the ability of Streptococcus mutans to adsorb copper (Cu) by determining the percentage of Cu uptake in bacterial growth media using atomic absorption spectrophotometry. The data obtained provide information regarding the adsorption capacity and optimum adsorption conditions of Cu by Streptococcus mutans.

2. Experimental

2.1. Equipment's and materials

2.1.1. Equipment

The equipment used in this study included an autoclave, stirring rod, Schott bottles, spray bottles, weighing bottles, Bunsen burner, beakers, graduated cylinders, hot plate stirrer, sterile cotton swabs, gauze, Erlenmeyer flasks, volumetric flasks, laminar airflow cabinet, magnetic stirrer, analytical balance, micropipettes, dropper pipettes, measuring pipettes, volumetric pipettes, shaker, spatula, test tubes, pipette tips, thermometer, test tube rack, and an atomic absorption spectrophotometer.

2.1.2. Material

The materials used in this study included distilled water, Streptococcus mutans, a 1000 ppm Cu stock solution, 65% (v/v) concentrated HNO_3, NaCl, tryptone, yeast extract, and a 0.2 μm Millipore membrane.

2.1.3. Tool Sterilization

Glassware used in this study, including graduated cylinders, Erlenmeyer flasks, measuring pipettes, and volumetric pipettes, was first washed thoroughly and dried. The equipment was then wrapped with paper and plastic and sterilized using an autoclave at 121 °C for 1 hour.

3. Methods

3.1. Preparation of Luria Broth

Six Schott bottles were each prepared by adding 1% NaCl, 1% tryptone, and 0.5% yeast extract. The components were dissolved in 200 mL of distilled water and homogenized using a magnetic stirrer. The prepared Luria–Bertani medium was sterilized in an autoclave at 121 °C for 30 minutes and stored at 4 °C prior to use.

3.2. Bacterial Regeneration

Ten milliliters of sterile Luria–Bertani medium were transferred into test tubes, and 10 μL of Streptococcus mutans culture was inoculated into each tube under aseptic conditions in a laminar airflow cabinet. The bacterial starter culture was incubated at 37 °C for 24 hours before use.

3.3. Determination of OD (Optical Density) Value

One milliliter of the bacterial starter culture was collected for absorbance measurement. The absorbance was measured twice, namely before incubation and after 24 hours of incubation. Measurements were performed using a visible spectrophotometer at a wavelength of 610 nm.

3.4. Biosorption of Cu (II) Ion by Streptococcus mutans.

Biosorption experiments were conducted using Luria–Bertani medium exposed to Cu (II) at various concentrations. Six Schott bottles containing LB medium were each supplemented with 0, 7.5, 15, 22.5, 30, and 37.5 mL of a 100 ppm Cu standard solution and diluted with distilled water to a final volume of 250 mL, resulting in Cu (II) concentrations of 0, 3, 6, 9, 12, and 15 ppm. Subsequently, 1 mL of Streptococcus mutans starter culture was inoculated into each medium. The cultures were incubated in a shaker at 37 °C during the biosorption process.

3.5. Determination of Cu (II) Ion Concentration

Samples from each Cu (II) concentration variation (0, 3, 6, 9, 12, and 15 ppm) were collected periodically. Ten milliliters of each bacterial culture were withdrawn and filtered using a 0.2 µm Millipore membrane into 1.5 mL microtubes. The filtrate was then analyzed to determine the residual Cu (II) concentration using an atomic absorption spectrophotometer. Cu (II) concentration measurements were performed every 48 hours.

4. Analysis data

4.1. Linearity Determination

Data analysis was performed by constructing a standard calibration curve correlating absorbance (y-axis) with Cu (II) concentration (x-axis) using a series of standard Cu solutions with concentrations of 0, 0.2, 0.5, 1, 2, 4, and 5 ppm. Linear regression analysis was applied to obtain the calibration equation, as expressed in Eq. (1)

$$y = ax + b \qquad 1)$$

Description

- a = intercept
- b = slope
- x = sample concentration (ppm) y = absorbance

4.2. Biosorption Percentage

The percentage of Cu (II) biosorption was calculated based on the difference between the initial Cu (II) concentration before biosorption and the residual concentration after biosorption, as shown in Eq.(2)

$$\% \text{ Biosorpsi} = \frac{C_a - C_b}{C_a} \times 100\%$$

- Description
    - Ca = Initial Cu (II) concentration (ppm)
    - Cb = Cu (II) concentration after biosorption (ppm)

5. Results and discussion

5.1. The Regeneration of Streptococcus mutans

5.1.1. Determination of Optical Density (OD)

Optical density (OD) measurement was performed to evaluate the growth of Streptococcus mutans in the starter medium. The absorbance values obtained before and after incubation are presented in Table 1.

Table 1 Optical density value

Treatment	Time (Hour)		
	1	12	24
Before Incubation (610 nm)	0.004		
After Incubation (610 nm)	0.027	0.614	0.550

Based on the results of Table 1, an increase in absorbance after incubation indicates an increase in turbidity, suggesting successful bacterial growth. This result demonstrates that *Streptococcus mutans* was able to grow well in the Luria–Bertani medium and was suitable for use in subsequent biosorption experiments

5.1.2. Preparation of Standard Curve of Cu (II)

The standard calibration curve of Cu (II) was constructed to determine Cu (II) concentrations based on absorbance values measured by atomic absorption spectrophotometry. The results showed that the absorbance increased linearly with increasing Cu (II) concentration, indicating a positive correlation between concentration and absorbance. Linear regression analysis yielded the equation y=0.1084x + 0.0043 with a coefficient of determination R^2 = 0.9993 with slope = 0.1084 and intercept = 0.043 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 [6].

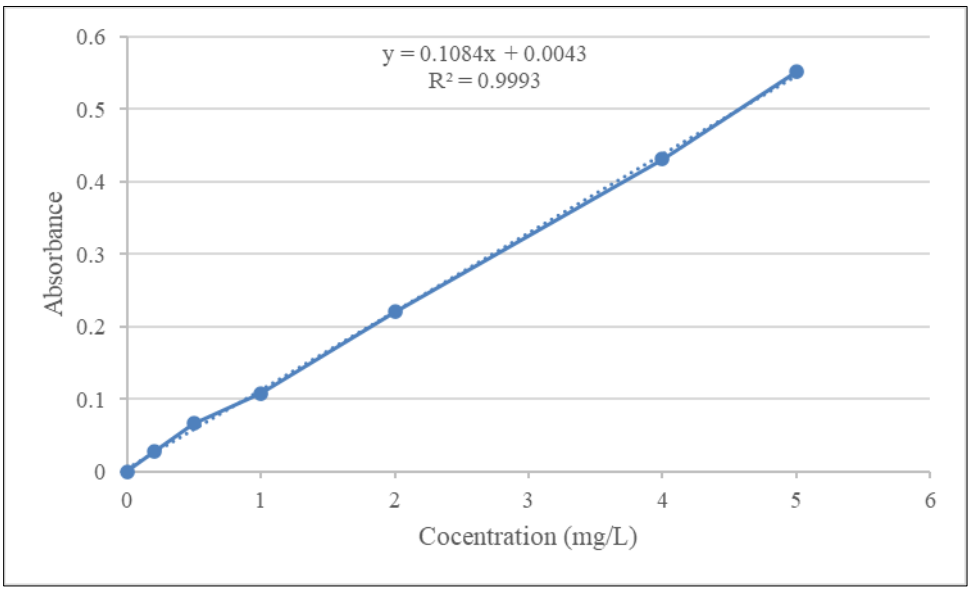

Figure 1 Cu (II) concentration curve against concentration

5.1.3. Biosorption of Cu (II) by Streptococcus mutans.

Cu (II) biosorption was carried out using *Streptococcus mutans*, which is a microorganism capable of adsorbing Cu (II) ions. *Streptococcus mutans* is a Gram-positive bacterium characterized by a thick cell wall structure rich in peptidoglycan. The Gram-positive cell wall consists of a thick peptidoglycan layer containing numerous negatively charged functional groups, such as phosphate and carboxyl groups, particularly within teichoic acid and lipoteichoic acid molecules [7]. The results of the Cu (II) biosorption analysis on the growth media of *Streptococcus mutans*. can be seen in Table 2.

Table 2 Biosorption of Cu (II) by *Streptococcus mutans*

No	Initial Concentration of Cu (II) (ppm)		The concentration of Cu (II) after the biosorption process of *Streptococcus mutans* on day (ppm)		
	Solution	**Detected**	**2**	**4**	**6**
1.	0	0.04	0.04	0.04	0.04
2.	3	3.11	1.98	0.79	0.56
3.	6	5.88	5.12	2.22	0.83
4.	9	9.16	7.55	4.14	1.12
5.	12	11.78	10.07	7.35	4.11
6.	15	14.82	12.78	9.75	6.48

Based on Table 2, it can be seen that the variation of Cu (II) concentration is (3, 5, 9, 12, and 15) ppm and the variation of exposure time is (2, 4, and 6). day. The results obtained in the Cu (II) biosorption process by *Streptococcus mutans* decreased in concentration along with the increase in the days that had been set. This result indicates that *Streptococcus mutans* has the capability to absorb copper (Cu), as evidenced by the decrease in Cu concentration with increasing exposure time. These findings demonstrate that *Streptococcus mutans* can be effectively utilized as a biosorbent for the removal of copper as a heavy metal. According to Dewi (2007), biosorption occurs due to the presence of the amino acid cysteine in the bacterial body, the role of metallothionein protein in amino cysteine is able to bind essential and non-essential heavy metals [8]. Binz (2000) suggested that the use of metallothionein 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 [9]. The metal binding process occurs through two main stages, ion exchange, in which ions present on the microbial cell wall are replaced by heavy metal ions, followed by the formation of metal–functional group complexes. [5]

5.1.4. Optimum Concentration and Biosorption Time of Cu (II) by Streptococcus mutans.

The following Fig. 2 shows a comparison graph of the percent biosorption of each concentration variation against time (days).

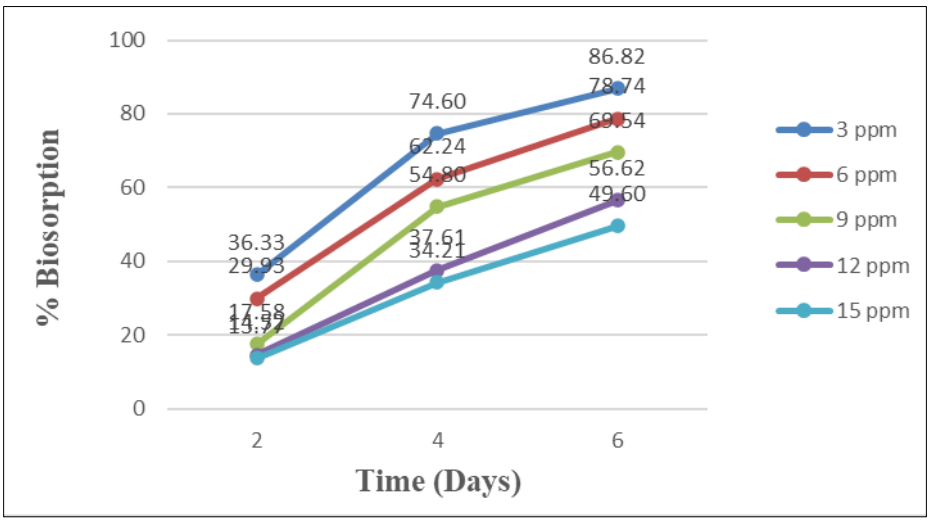

Figure 2 Comparison of % Biosorption of Cu (II) by *Streptococcus mutans* each variation of concentration against time (days)

Fig. 2 illustrates the biosorption of Cu (II) by *Streptococcus mutans* in growth media exposed to varying Cu (II) (3, 6, 9, 12, and 15) ppm over an incubation period from day 0 to day 6 with, the result indicate that *Streptococcus mutans* decreased in concentration which indicated that Cu (II) could be absorbed and obtained the percent biosorption, namely at a concentration of 3 ppm of 86.82%, a concentration of 6 ppm of 78.74%, a concentration of 9 ppm of 69.54%, a

concentration of 12 ppm of 56.62%, and a concentration of 15 ppm of 49.60%. In this study, it was shown that the higher concentration of Cu (II) solution would affect the percentage decrease in Cu (II) biosorption by *Streptococcus mutans*. This phenomenon may be attributed to changes in the pH of the medium, which can influence the biosorption process and result in reduced metal uptake at higher concentrations and longer exposure times.

Fig. 2 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 span with a biosorption percentage of 36.33% to 74.60%. The optimum concentration and time of absorption of *Streptococcus mutans* on Cu (II) occurred at a concentration of 3 ppm with a biosorption percent of 74.6% on the 4th day. This is due to a significant and high increase in biosorption capacity, which means that during this time, *Streptococcus mutans* bacteria work optimally in absorption Cu (II).

According to Diantariani (2008), the concentration of Cu (II) is inversely proportional to the biosorption capacity of *Streptococcus mutans*, where higher metal concentrations lead to a decrease in biosorption efficiency. Increased Cu (II) levels also enhance metal toxicity toward bacterial cells, thereby reducing their ability to bind copper ions

According to Diantariani (2008), the concentration of Cu (II) is inversely proportional to the biosorption capacity of *Streptococcus mutans*, where higher metal concentrations lead to a decrease in biosorption efficiency. Increased Cu (II) levels also enhance metal toxicity toward bacterial cells, thereby reducing their ability to bind copper ions [10]. Conversely, Abbas (2014) reported that the initial metal concentration plays a significant role in the biosorption process. Higher initial concentrations reduce biosorption efficiency due to saturation of active binding sites once equilibrium is reached. In contrast, lower initial metal concentrations promote stronger interactions between metal ions and functional groups on the biosorbent, resulting in enhanced metal uptake. [11].

6. Conclusion

Biosorption of Streptococcus mutans of Cu (II) at a concentration of 3 ppm was 86.82%, a concentration of 6 ppm was 78.74%, a concentration of 9 ppm was 69.54%, a concentration of 12 ppm was 56.62% and a concentration of 15 ppm was 49.60%. The optimum concentration and time of absorption of Streptococcus mutans against Cu (II). The optimum concentration occurred at a concentration of 15 ppm with a decrease of 5.131 ppm. The optimum time occurred on days 2 to 4 at a concentration of 3 ppm absorption of 38.26% occurred.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] A. D. Setiawan, R. Kartika, and R. Gunawan. AIP Conf. Proc. 2668 (2022)

[2] A. A. Prasetiyo, Y. Kinasih, and M. Q. Qodiahtadavi, Kohesi: Jurnal Teknologi dan Sains (2024)

[3] S. W. Pratomo, F. W. Mahatmanti, and Sulistyaningsih, Indonesian Journal of Chemical Science (2017)

[4] R. Adhani and Husaini, "Logam Berat Sekitar Manusia" (Lambung Mangkurat University Press, 2017)

[5] E, Ratnawati, R. Ermawati, and S. Naimah, Jurnal Kimia dan Kemasan (2010)

[6] L. Kurniasari, Momentum 6, 5 – 8 (2010).

[7] Y. N. Slavin, J. Asnis, U. O. Häfeli, and H. Bach, Journal of Nanobiotechnology (2017).

[8] N. K. Dewi, "Metallothionein," (Universitas Negeri Semarang, Semarang, 2017).

[9] P. A. Binz, J. H. R. Kagi, "Metallothione: molecular evolution classification," in Metallothionein IV, edited by

[10] C. D. Klaassen (Springer Basel AG,1999), pp. 7–13.

[11] N. P. Diantariani, I. W. Sudiarta, and N. K. Elantiani, Jurnal Kimia (2008).

[12] S. H. Abbas, I. M. Ismail, T. M. Mostafa, and A. H. Sulaymon, Journal of Chemical Science and Technology (2014).