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Biosorption by *Streptococcus mutans* Bacteria against Pollutant Metal Manganese (II)

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Abstract

The development of electroplating industries has increased the potential for environmental pollution due to heavy metal waste, such as manganese (Mn), which is toxic. One alternative for treating this waste is through biosorption using microorganisms. This study aims to evaluate the biosorption capability of *Streptococcus mutans* against Mn(II) and to determine the optimum concentration conditions. The experiment was conducted by culturing *Streptococcus mutans* in Nutrient Broth media exposed to manganese (Mn) at varying concentrations (12, 18, 24 ppm). Metal absorption was analyzed using an atomic absorption spectrophotometer (AAS). The results showed that *Streptococcus mutans* was able to absorb Mn at exposure concentrations (12, 18, 24 ppm), with the maximum absorption rate of 71.97% at 18 ppm over 6 days. The optimum absorption time for Mn was found to be 4 days for all concentration variations, with the optimum absorption of 63.43% at 12 ppm.

Keywords: Biosorption; Mangan; Heavy Metal; *Streptococcus mutans*

1. Introduction

Indonesia has enormous potential in mineral resources, but their utilization is still not optimal. One of the minerals with significant reserves is manganese ore, which is widely distributed across various regions. Manganese plays a vital role in several industrial sectors, including steel production, paint, fertilizers, livestock, chemicals, and batteries. With more efficient processing and utilization, manganese could become a significant driver for national economic growth. High-grade manganese, known as metallurgical grade, refers to manganese ore with a content exceeding 44%. This type is generally used in the steel industry, both as a raw material for ferromanganese and as an alloying element in the production of high-quality steel. Meanwhile, low-grade manganese ore is typically used outside the steel sector, such as in the production of manganese dioxide (MNO2), which serves as a depolarizer in dry batteries, as well as in the ceramic industry and the production of various chemical compounds [1].

Soil contamination due to heavy metals from mining activities is one of the most high-risk forms of anthropogenic pollution. Waste from the mining process generally contains various heavy metals, including manganese (Mn), which have the potential to contaminate soil, water, and air. In the manganese ore processing process, washing is carried out to remove impurities and unwanted minerals from the ore. This stage produces tailing waste in the form of a mixture of water and sediment, which is then initially stored in sedimentation ponds [2]. The presence of dissolved Mn in irrigation water in agricultural areas around industrial zones shows concentrations exceeding the allowed threshold, leading to the accumulation of Mn in agricultural crops [3].

The accumulation of heavy metals in the body in excessive amounts can lead to toxic effects that are harmful to health. These substances can inhibit growth, cause physical damage, and weaken cognitive function, the nervous system, and bones. Therefore, effective treatment efforts are needed to reduce the concentration of heavy metals in the environment, especially in water media, to prevent broader negative impacts. To mitigate the effects of heavy metal pollution in water,

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one of the methods that can be used is adsorption technique [4]. The microorganism's adsorption of heavy metal ions is a rapid and reversible process in which the cell wall serves as a binding site, which means that the microorganism does not even need to be alive for this purpose. Using dead microbial cells could be more cost-efficient because they do not require a supply of nutrients during the process [5]

As an effort to address this issue, researchers have conducted various studies to find solutions for environmental problems. In general, industries have procedures in place to manage the waste they generate. However, it is undeniable that some waste can accidentally pollute the environment, leading to accumulation in water channels and soil. To reduce the impact of pollution, several methods have been implemented, one of which is biosorption. Biosorption is a process that can be performed by bacteria through physicochemical interactions between metals and functional groups on microbial cell surfaces. This occurs based on physical adsorption, ion exchange, and chemical absorption, which are not dependent on cell metabolism. The bacterial biomass, which is primarily composed of polysaccharides, proteins, and lipids, has abundant metal-binding groups such as carboxyl, sulfate, phosphate, and amino groups [6].

In this study, the selection of *Streptococcus mutans* in this study is based on its characteristics as a Gram-positive bacterium with a cell wall rich in functional groups, which gives it a high potential for binding heavy metal ions. According to research conducted by [7], *Streptococcus mutans* was able to adsorb Cr (VI) metal ions up to 90.80% on the fourth day. Additionally, a study by [8] using Bacillus cereus bacteria demonstrated excellent adsorptive capacity for Mn (II) metal ions, reaching 98.9% on the fifth day. Based on these empirical results, a study was conducted to investigate the ability of *Streptococcus mutans* bacteria to absorb Manganese (Mn) by determining the percentage of Mn absorption in the bacterial medium using atomic absorption spectrophotometer instrumentation.

2. Methodology

2.1. Materials and Tools

The equipment used in this study included Measuring cylinder, Erlenmeyer flask, filter paper, autoclave, analytical balance, spatula, micropipette, dropper, graduated pipette, volumetric pipette, spray bottle, hotplate, magnetic stirrer, Bunsen burner, Buchner funnel, vacuum pump, sterile cotton swabs, incubator, Schott bottle, desiccator, laminar air flow and Atomic Absorption Spectrophotometer.

The materials used in this study include *Streptococcus mutans* bacteria, Mn^{2+} solution 1000 ppm, HNO_3 solution, Nutrient Broth, distilled water, and 0.2 MM Millipore membrane.

2.2. Research Procedures

2.2.1. Standard of Mn (II) Curve Creation

A standard solution of Mn (0.1; 0.25; 0.5; 1; 1.5) ppm, 50 mL, was placed into an Erlenmeyer flask. Then, 5 mL of Nitric acid was added. The solution was homogenized. The absorbance of the solution was measured using an atomic absorption spectrophotometer at a wavelength of 279.5 nm (SNI 06-6989 5-2004).

2.2.2. Regeneration of Streptococcus mutans bacteria

Add 1 gram of nutrient broth to 6 Erlenmeyer flasks (200 mL) and dissolve using 200 mL of distilled water. Boil using a hotplate at 60°C while homogenizing. Sterilize the medium using an autoclave at 121°C for 3 hours. Take 10 mL of the sterilized medium and place it into a 50 mL Erlenmeyer flask. Inoculate 10 µL of *Streptococcus mutans* bacteria into the medium. Incubate the medium at 37°C for 24 hours before use.

2.2.3. Biosorption of Mn (II) Metals (12, 18 and 24 ppm) by Streptococcus mutans Bacteria

Erlenmeyer flasks containing 200 mL of Nutrient broth medium were each added with (24 mL; 36 mL; 48 mL) of a 100 ppm Mn^{2+} standard solution and then homogenized. Next, 1 mL of *Streptococcus mutans* bacterial starter was inoculated into each medium. The *Streptococcus mutans* bacterial medium was then placed in an incubator at a temperature of 37°C. The metal concentration was measured every 48 hours using an Atomic Absorption Spectrophotometer.

2.2.4. Testing for Mn (II) in metals

Sample solutions of bacterial medium from each metal concentration of Mn (12, 18, 24) ppm, 10 mL each, were taken into a beaker glass and filtered for each concentration using a 0.2 µm Millipore membrane. Then, the filtrate from the

filtration was measured for its absorbance using an Atomic Absorption Spectrophotometer at a wavelength of 279.5 nm (SNI 06-6989 5-2004).

2.2.5. Analysis of Results

The data were analyzed based on a standard curve created by plotting the absorbance on the y-axis and the concentration on the x-axis using a series of standard Mn solutions with concentrations of (0.1; 0.25; 0.5; 1; 1.5). This resulted in the following equation

$$y = ax \pm b$$

Description

- a = Slope
- b = Intercept
- y = Sample Absorbance
- x = Sample Concentration (ppm)

The biosorption percentage is obtained by calculating the difference between the metal concentration before biosorption and after biosorption using the following equation

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

Description

- Ca = Metal concentration before biosorption (ppm)
- Cb = Metal concentration after biosorption (ppm)

3. Results and Discussion

3.1. Determination of the Standard Curve of Mn(II) Solution

Based on SNI 6989.5-2009, the determination of Mn(II) concentration is performed using the atomic absorption spectrophotometry method with flame by preparing standard solutions and working solutions to form a standard curve. The measurement is carried out with an atomic absorption spectrophotometer at the characteristic wavelength of manganese. The principle of this method is based on the ability of free manganese atoms in the flame to absorb radiation at a specific wavelength, where the amount of measured atomic absorption is proportional to the concentration of manganese in the solution.

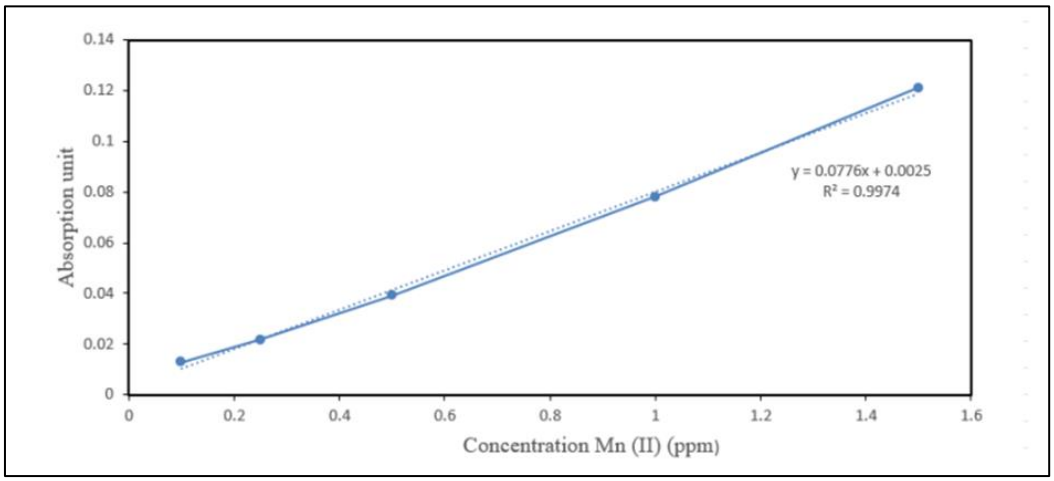

Figure 1 Standard Curve of Mn (II) Solution

The linear relationship between the concentration of the standard manganese and the resulting absorption value is used as the basis for determining the manganese content in the sample. The resulting graph is shown in Figure 1.

Based on the analysis results (Figure 1), it was found that the increase in absorbance is directly proportional to the increase in Mn metal concentration, indicating a positive relationship between concentration and absorbance values. A linear regression equation of y = 0.0776x + 0.0025 was obtained with a correlation coefficient of 0.9974. The results obtained meet the requirements of the Indonesian National Standard (SNI), indicating that the analytical system used is capable of providing good overall results. The regression equation generated was then used to determine the Mn metal concentration in the sample solution, with absorbance values as the y variable and concentration in the sample as the x variable.

3.2. Biosorption of Mn (II) by *Streptococcus mutans*

Biosorption is the process of heavy metal absorption using biosorbents, such as bacteria and fungi, which have a natural ability to accumulate pollutants in aquatic environments. This ability is based on the presence of functional groups on the cell walls of microorganisms that can bind with metal ions. In this study, *Streptococcus mutans* bacteria are used as biosorbents to absorb Mn metal. *Streptococcus mutans* is a Gram-positive bacterium that is facultatively anaerobic, spherical in shape with a diameter of approximately 0.5–2.0 µm, and can be arranged in pairs or form chains. This bacterium typically lives in the mouth, throat, and digestive tract. S. mutans is known as the main cause of dental caries due to its ability to form biofilms, adapt to acidic environments, and play a crucial role in pathogenicity [10]. According to [11], teichoic acid itself is a compound found in the bacterial cell wall that can be targeted by antibacterial compounds. Teichoic acid functions in the activation of enzymes, which are then used in protein synthesis for the bacteria. Teichoic acid on the cell wall acts as a negative ion that helps in ion exchange processes as a defense mechanism against exposure to heavy metals. The mechanism that occurs when Mn (II) metal ions bind with teichoic acid is as follows.

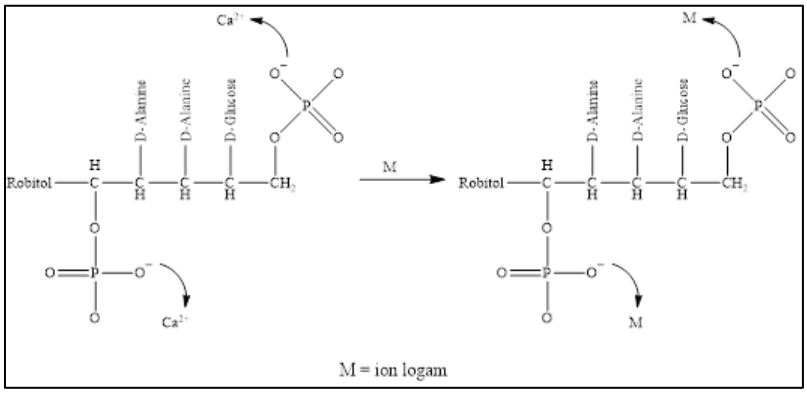

Figure 2 Metal ion substitution reaction on the cell wall

Based on Figure 4.2, the mechanism of this reaction occurs through an ion exchange process, where monovalent or divalent metal ions present on the bacterial cell wall, such as Mg^{2+}, Ca^{2+}, and Na^{+}, are replaced by Mn (II) metal ions. The displaced metal ions then form complexes with the functional groups on the bacterial surface, including amino, phosphate, hydroxyl, hydroxy-carboxyl, thiol, and carbonyl groups [12]

The results of the biosorption analysis of Mn metal on the growth medium of *Streptococcus mutans* can be seen in the following table:

Table 1 Percentage of Mn metal biosorption by *Streptococcus mutans* bacteria

No.	**Initial concentration of Mn(II) (ppm)**		**Measured concentration of Mn(II) metal after the biosorption process by *Streptococcus mutans* bacteria on day- (ppm)**		
	Solution	Detected	2	4	6
1	12	12	8.88	6.14	4.39
2	18	18	13.57	6.56	5.05
3	24	24	22.20	12.60	12.15

Based on Table 1, a measured decrease in Mn metal concentration was observed across variations of 12, 18, and 24 ppm on days 2, 4, and 6. This trend suggests that *Streptococcus mutans* can tolerate the relatively low toxicity of Mn. Specifically, at the 18-ppm concentration, although the residual metal was nominally higher than at 12 ppm, the absorbed amount and percentage were significantly higher. This indicates that the metal availability at 18 ppm supports more massive absorption before toxic effects limit the process. In contrast, at 24 ppm, toxicity effects began to inhibit absorption, resulting in a lower percentage compared to 18 ppm. In addition, the data shows that the absorption rate slows down when the remaining concentration approaches or falls below the 5-ppm threshold, as observed at the end of the observation for the 12 ppm concentration (residual 4.39 ppm) and 18 ppm concentration (residual 5.05 ppm).

3.3. Optimum concentration and time for Mn(II) metal absorption by *Streptococcus mutans* bacteria

The results of the analysis of the percentage of Mn metal biosorption by *Streptococcus mutans* bacteria at each concentration variation over the exposure time can be seen in Figure 3 and Table 2 below.

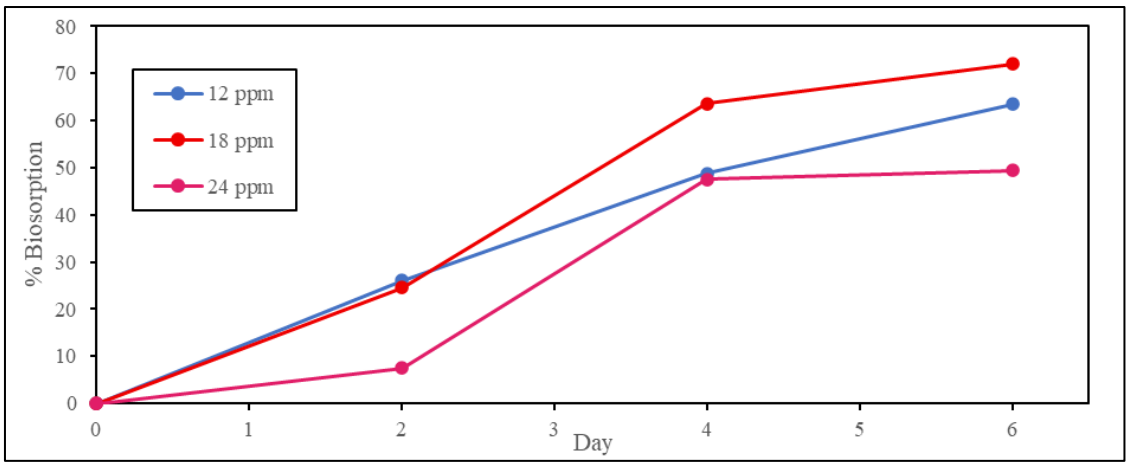

Figure 3 Comparison of Mn Biosorption Percentage by *Streptococcus mutans* Bacteria at Each Concentration Variation with Exposure Time

Table 2 Percentage of Mn metal biosorption by *Streptococcus mutans* bacteria

Percentage of Mn(II) Metal Biosorption					
No	**Initial concentration of Mn(II) (ppm)**	**% Biosorption with variation in time (Days)**			
		0	**2**	**4**	**6**
1	12	0	26.01	48.83	63.43
2	18	0	24.61	63.56	71.97
3	24	0	7.51	47.51	49.37

Based on Figure 3 and Table 2, it shows that the percentage of Mn metal absorption by *Streptococcus mutans* bacteria increases with increasing exposure time, where the maximum absorption by *Streptococcus mutans* bacteria was obtained at a concentration of 18 ppm with a biosorption percentage reaching 71.97%. According to [13], the duration of exposure time is directly related to the biosorption results, where the longer the exposure time, the more opportunity the biosorbent has to interact with metal ions, thus increasing the number of metal ions absorbed by the biosorbent

According to [14], the concentration of Mn (II) heavy metal is inversely proportional to the biosorption capacity of *Streptococcus mutans*, where the higher the concentration of the heavy metal exposed, the lower the biosorption capacity of the bacteria. An increase in heavy metal concentration also increases its toxicity to the bacteria, thus reducing the ability of *Streptococcus mutans* to absorb Mn metal. On the other hand, [15] state that the initial concentration of the metal in the biosorption process affects the metal absorption capacity of the biosorbent. At high initial concentrations, the absorption capacity decreases because the active sites on the biosorbent quickly become saturated, and the biosorption process reaches equilibrium. Conversely, at low initial concentrations, the biosorption

process becomes more efficient because the interaction between metal ions and functional groups on the biosorbent increases, thus enhancing the absorption capacity.

4. Conclusion

It can be concluded that *Streptococcus mutans* bacteria effectively absorb Mn (II) metals, with optimal absorption reaching 71.97% at 18 ppm after 6 days, with the highest absorption observed on day 4.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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