The influence of Ag concentration on the antibacterial properties of plastic made from silica immobilized with EDTA-Ag combined with chitosan

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Abstract. Antibacterial plastic is made by using composites silica gel immobilized EDTA-Ag combined with chitosan. Silica gel was formulated with three variations of Ag concentrations (i.e., 10^{-3}, 10^{-4}, and 10^{-5} M) and then tested for its antibacterial activity against Escherichia coli and Staphylococcus aureus. Result of the antibacterial test showed that the immobilized EDTA-Ag silica gel product could inhibit the growth of Staphylococcus aureus and Escherichia coli with the diameter of zone inhibition around 15.9 and 15.6 mm respectively at the lowest Ag concentration of 10^{-5}M. This result showed that higher concentration of Ag led to the decrease of antibacterial activity. The obtained products were then synthesized into plastic with variations of chitosan weight of 0.3 and 0.7 grams. The antibacterial test confirmed that the plastic had strong antibacterial properties with the diameter of zone inhibition for Staphylococcus aureus and Escherichia coli was 16.7 and 15.9 mm, respectively. The percentage of degradation was observed at 6.02 to 63.17% while water absorption test for plastic with 0.3 gram chitosan was 86.51% and for plastic with 0.7 gram chitosan was 16%. This indicated that more chitosan additions were able to expand antibacterial properties and reduce the absorption of plastic against water.

1. Introduction

Bacteria are microorganisms that can live and affect the life of cells in the human body. A lactic acid bacterium is an example of probiotic bacteria that play an essential role in human intestine [1]. In contrast, Escherichia Coli and Staphylococcus aureus bacteria are examples of pathogenic bacteria that can cause diseases in the human body. One of the efforts to overcome bacterial growth is by using antibacterial materials that can be synthesized from organic or inorganic materials. Inorganic metal ions such as Ag, Zn, Cu and Hg which are combined with porous materials like silica and clay are often used to produce antibacterial products such as antibacterial glass and medical tools [2].

Silica gel produced from rice husk has several advantages compared to other porous materials such as having finer grains, affordable at low cost, and high abundance of raw materials. Silica can be used as an antibacterial material through modification stages using other bactericidal substances.
Adoe et al. [3] used silica gel immobilized EDTA-Ag as an antibacterial material against E.coli. The results showed that silica gel immobilized EDTA-Ag was bactericidal against E.coli up to 99% at the concentration of 10 mg/mL. Wogo et al. [4] using silica gel immobilized EDTA-Ag and chitosan to produce products in the form of antibacterial plastic, which can inhibit the growth of S. aureus and E. coli up to 99.99%.

Biodegradable plastic is a type of plastic that can be decomposed in the environment and has good waterproof properties. A research conducted by Firdaus and Anwar [5] utilizes solid-liquid waste tapioca flour as a raw material for making biodegradable plastic films obtained that their plastic products can be degraded perfectly on the second week. Therefore the presence of biodegradable plastic will reduce industrial waste. Moreover, biodegradable plastic that has antibacterial properties will add the higher economic value of plastic especially in food and health packagings.

2. Experimental Procedures

2.1. General tools and materials
The tools used in this research are glassware, petri dish, 60 mesh sieve, magnetic stirrer, Laminar water flow, and Colony counter (SCAN® 500, version 6.1.2.0). While the materials used in this research are the rice husk, HCl, NaOH, CH₃COOH, AgNO₃, EDTA, Chitosan, the bacteria Escherichia coli, and Staphylococcus aureus strains.

2.2. Procedures
a. Rice husk ash preparation
Clean and dry rice husks were roasted to black and ignited for 4 hours at 700 °C. The ash obtained was crushed and then sifted with a 60 mesh sieve. 20 g of rice husk ash was washed with 120 mL of HCl 6 M continued with stirring for 1 hour, then rinsed with distilled water until neutral and then dried in an oven at 110 °C for ± 2 hours.

b. Preparation of sodium silicate
As much as 20 g of rice husk prepared were dissolved with 330 mL of 2M NaOH and then boiled up to thicken and were heated for 30 minutes at 500 °C. After that 200 mL of distilled water was added cooled overnight and filtered.

c. Synthesis of EDTA-Ag immobilized silica gel
A total of 20 mL sodium silicate solution was put into a plastic container, 5 mL of EDTA 0.005 M solution and 5 mL of AgNO₃ 10⁻³ solution, 10⁻⁴ M, 10⁻⁵ M was added. The solution mixture was homogenized and HCl solution 3 M was added dropwise until gel was formed. The gel was left overnight, filtered, washed with distilled water until neutral and dried in an oven for 1 hour at 110 °C. The obtained product was crushed, sifted with a 60 mesh sieve and tested for antibacterial activity.

d. Antibacterial assay on Escherichia coli and Staphylococcus Aureus
A total of 25 g NA was dissolved in distilled water and heated to thicken and placed in an Erlenmeyer. Furthermore, the tools and materials were sterilized at 121 °C for 30 minutes and stored in Laminar airflow. Pure cultures of Escherichia coli and Staphylococcus aureus bacteria were dissolved in a test tube containing 2 mL NA media and grown in an incubator for 18-24 hours at 37 °C. Bacteria produced by culture were taken 1 eye dose and put in 9 mL NaCl 0.9% then diluted. The results of the dilution were transferred 1 mL into the petri dish and then poured the media so that it was 1 cm thick and left.

e. Preparation of antibacterial plastics
A total of 0.3 g and 0.7 g chitosan each dissolved in 50 mL CH₃COOH 1% stirring for 24 hours then added silica gel solids result of the procedure c. The mixture solution was filtered and poured into a
petri dish (15 cm in diameter) and dried at 65 °C for 7 hours. The formed plastic was released from the petri dish using 3 M NaOH and washed with distilled water until the pH is neutral then dried at room temperature. Plastics were tested for antibacterial activity using the paper disc method.

f. Qualitative test of plastic products
1. Biodegradation test
   Carried out in vitro in the ground for a certain period of time. The samples were then removed, cleaned with distilled water, dried, weighed by the weight of the remaining samples and calculated as percent degradation using the formula [6]:

   \[
   \% \text{ Degradation} = \frac{w_1 - w_2}{w_1} \times 100 \% \tag{1}
   \]

   With \( w_1 \) is the weight of the plastic before the degradation process, and \( w_2 \) is the weight of the plastic after the degradation process.

2. Test of water absorption on plastic products
   The samples were weighed and then dripped with water 5 times along the surface of the sample and left to stand for 2 minutes, then drained with tissue and weighed the final mass.

   \[
   \% \text{ Excess weight} = \frac{w_4 - w_2}{w_4} \times 100 \% \tag{2}
   \]

   With \( w_1 \) is the initial sample weight and \( w_2 \) is the final sample weight.

3. Results and Discussion

a. Rice husk and ash preparation
   The rice husk was collected from Noelbaki Village in Kupang eastern Indonesia. Changing the black color from the results of casting (Figure 1) indicates that these organic compounds have not been oxidized completely.

   \[\text{Figure 1. Results of rice husk processing}\]

   According to Kurniawati [7] ignition at temperatures above 700 °C will produce silica in the form of stable crystals. While combustion at temperatures below 700 °C will produce very low content of silica. Kalapathy and Proctor [8] stated that silica reactivity is influenced by the temperature of ignition with its optimum reactivity at a temperature of 550 - 700 °C. Therefore ignition was carried out at 700 °C resulting in unstable amorphous silica. The result of white ignition as shown in Figure 2 according to Bolle's statement [9] that the white color of the husk ash produced indicates high silica content.
Rice husk ash was activated using HCl 6 M to dissolve metal and non-metal oxides which are impurities in husk ash such as Na2O, MgO, K2O, CaO, P2O5 and Fe2O3 [10]. Then wash it to neutral pH with distilled water to release ash from chloride ions.

b. Preparation of sodium silicate solution
The rice husk ash was reacted with 2 M NaOH solution. According to Ngatidjo et al., [11] the initial indicator of sodium silicate solution was that it was slippery when the solution hits the surface of the skin. The mechanism for the reaction of sodium silicate formation is illustrated in Figure 3.

![Figure 3. Formation reaction mechanism](image)

In the silica gel, there is a coordination bond between the metal Ag and the electronegative oxygen atom from the OH-group on the surface of silica and EDTA. EDTA also acts as a ligand carrying Ag⁺ ions into the bacterial environment so that the exchange of EDTA ligand will occur with bacterial...
biological ligands [12]. Oxygen atom acts as a Lewis base that will donate a pair of electrons to the metal Ag which acts as a Lewis acid by providing empty orbitals and forming Ag-O complex bonds. The estimated mechanism of binding of Ag metal to silica and EDTA is illustrated in Figure 5.

![Figure 5. Estimated bound mechanism metal Ag on silica and EDTA](image)

d. Antibacterial test against *Staphylococcus aureus* and *Escherichia coli*
Tests were carried out using the paper disc method with the aim of knowing the Large Area Diameter Obstacle (DDH). The results of the antibacterial activity test can be seen in Table 1.

**Table 1. Regional diameter inhibits bacterial growth in EDTA-Ag immobilized silica gel products.**

| Bacterial Type       | Inhibited Area Diameter (mm) |
|----------------------|------------------------------|
|                      | Sample type | Positive control | Negative Control |
|                      | A          | B          | C          | (Tetracycline) | (Aquades) |
| *Staphylococcus aureus* | 13,7       | 14,8       | 15,9       | 17,8          | 0         |
| *Escherichia coli*    | 12,2       | 14,1       | 15,6       | 17,4          | 0         |

Information: A, B, and C respectively are immobilized EDTA-Ag products with variations in concentration Ag $10^{-3}$ M, $10^{-4}$ M, and $10^{-5}$ M.

Based on the available data it can be seen that the inhibitory power in *S. aureus* bacteria is greater than *E. coli*. This is due to differences in the structure of cell walls that determine the amount of activity of antibacterial compounds. Gram-positive bacteria have a simpler cell wall structure than gram-negative bacteria, making it easier for antibacterial compounds to enter the cell. Gram-negative bacteria contain outer membranes that can block the entry of large molecules [13].

The results obtained have the potential as a strong antibacterial in accordance with the classification of responses to bacterial growth inhibition areas proposed by Pratama [14] that if the inhibition area diameter <5 (weak), 5-10 (medium), 10-20 (strong), > 20 (very strong).

e. Antibacterial plastic products and antibacterial tests on *Staphylococcus aureus* and *Escherichia coli*.

1. Antibacterial plastic products
The results of antibacterial testing with the greatest inhibitory power were then used to inhibit or kill the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria. Each chitosan amount was dissolved in 50 mL of 1% acetic acid with stirring for 24 hours with the aim that chitosan could dissolve completely in acetic acid. The use of acetic acid as a solvent was based on the character of chitosan which is alkaline and mostly dissolves in the acidic type organic or dilute minerals through protonation of free amino groups at pH > 6.5.

The next step is to add each mixture of acetic acid-chitosan solution with silica solids immobilized EDTA-Ag with the optimum concentration of Ag in antibacterial testing is $10^{-5}$ M. Then
filtering is carried out in order to separate the solution from the sample’s sediment that may not dissolve. Then printed into a petri dish and dried in an oven at 65 °C for 7 hours so that the solution can form plastic, then released using 3 M NaOH. The resulting plastic is then washed using distilled water periodically in order to neutralize the plastic sample previously removed using NaOH. The plastic is then dried at room temperature so that it is free of excess water content caused by the previous washing process, in addition to not damaging the plastic structure produced. The plastic obtained can be seen in Figure 6.

![Figure 6. Plastic (a) 0.3 gram chitosan and (b) 0.7 gram chitosan](image)

Estimates of the mechanism that occurs between immobilized EDTA-Ag and chitosan products is illustrated in Figure 7.

![Figure 7. Estimated reaction mechanism between immobilized EDTA-Ag and chitosan](image)

2. Antimicrobial test against *Staphylococcus aureus* and *Escherichia coli*

The synthesized plastic then tested for antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* bacteria. The complex formation reaction between chitosan and metal Ag occurs through covalent coordination by N atoms from NH$_2$ groups and –OH groups from chitosan. The Ag metal ion acts as a Lewis acid (electron acceptor), while nitrogen from chitosan acts as a Lewis base that will donate a pair of electrons to coordinate with the metal. After complex formation, the positive charge density on chitosan will increase and cause the antimicrobial activity of plastic to increase. The results of the antimicrobial activity test of plastic can be seen in Table 2.

| Bacterial Type          | Sample Type | Inhibited Area Diameter (mm) | Positive Control (Tetracycline) | Negative Control (Aquades) |
|-------------------------|-------------|-------------------------------|--------------------------------|---------------------------|
|                         | A (0,3 gr)  | B (0,7 gr)                    | 17,8                           | 0                         |
| *Staphylococcus aureus* | 13          | 16,7                          |                                |                           |
| *Escherichia coli*      | 14,1        | 15,9                          | 17,4                           | 0                         |
Based on the data in Table 2 shows that chitosan is one of the ingredients that have the potential as an antibacterial agent. This can be seen from the addition of chitosan which enlarges the inhibitory area of the bacteria. The factors that cause large inhibition in both bacteria are the cell wall structure factors of bacteria. Gram-negative bacteria have a double membrane containing little peptidoglycan which makes the cell wall thinner but has thick lipopolysaccharide resulting in difficult interactions between bacterial cell walls and antibacterial compounds while gram-positive bacteria are composed of 90% peptidoglycan and have a single membrane that makes the cell wall easily attacked by antibacterial compounds.

f. Plastic sample testing
In this study, two tests were carried out, namely biodegradation testing which aims to determine the time of degraded plastics using the method of landfilling in the soil and testing the absorption of water on plastic products which was carried out to determine the amount of absorption of plastic against water. Data from the biodegradation test and plastic product resistance test on water are shown in Tables 3 and 4.

**Table 3. Biodegradation test**

| Plastic type | Planting Variations | Initial Plastic Weight (w1), (gram) | Plastic Weight After Degradation Process (w2), (gram) | Degradation (%) |
|--------------|---------------------|------------------------------------|-----------------------------------------------|-----------------|
|              |                     | 1  | 2   | 3   | 1   | 2 | 3 | 1 | 2 | 3 |
| A            |                     | 0,0399 | 0,0822 | 0,1413 | 0,0375 | 0,0665 | 0,0981 | 6,02 | 19,10 | 30,57 |
| B            |                     | 0,1129 | 0,0723 | 0,2354 | 0,0964 | 0,0456 | 0,0867 | 14,61 | 36,93 | 63,17 |

Information: A and B are plastic with chitosan variations of 0.3 grams and 0.7 grams. While the numbers 1, 2 and 3 are plastic that is placed on the ground surface, piled up at a depth of 5 cm and 10 cm.

**Table 4. Water absorption test on plastic products**

| Plastic type | Plastic Size (cm) | Initial Weight (w1), (gram) | Final Weight (w2), (gram) | Excess Weight(%) |
|--------------|-------------------|------------------------------|---------------------------|-------------------|
| A            | 2 x 2             | 0,0341                       | 0,0636                    | 86,5103           |
| B            | 2 x 2             | 0,0488                       | 0,0567                    | 16,1885           |

Information: A and B are plastic with chitosan variations of 0.3 grams and 0.7 grams.

Based on the data in Table 3, it shows that the more variations in plastic are planted, the more plastic mass will decrease. This is because the depth of the soil is one of the factors that influence the degradation process. In Table 4, testing is carried out with the intention of obtaining plastic products that have low water absorption. Based on existing data, plastic with 0.7 gram chitosan content has lower water absorption than plastic with 0.3 gram chitosan content. In plastics with chitosan content, there is less chance that air is trapped in plastic, making plastic more absorb water.
4. Conclusion
The optimum concentration of Ag in antibacterial plastic products was $10^{-5}$M, with a large diameter inhibition zone of 15.9 mm for *Staphylococcus aureus* and 15.6 mm for *Escherichia coli*. Higher amount of chitosan led to the greater diameter of zone inhibition of *Staphylococcus aureus* from 13 mm to 16.7 mm and *Escherichia coli* from 14.1 mm to 15.9 mm. The absorption of water by plastic was 86.51% for plastic with 0.3 gram chitosan and 16.18% for 0.7 gram chitosan. Plastic made from immobilized silica gel composites EDTA-Ag and chitosan was able to be degraded in the environment within 14 days with the percentage of degradation around 60.02 - 63.17%.

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