Utilization of Eco-Enzyme Technology from Tomato Waste as Raw Material for Making Hand Sanitizer and Testing Its Inhibitory Power Against Staphylococcus Aureus Bacteria

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Abstract: Based on SIPSN (National Waste Management Information System) data from the Ministry of Environment and Forestry, in 2020 the national waste stockpile will reach 36.74 million tonnes/year, with 68.05% of waste being manageable. The waste that is managed is in the form of organic waste, most of the organic waste is only seen as residue without economic value, this large percentage makes organic waste a threat to the environment. The large amount of organic waste that has been accumulated can decompose, causing a foul smell and the evaporation of methane gas (CH4) into the atmosphere which causes global warming. CO2 gas released into the stratosphere can also act as a greenhouse gas (GHG). To minimize this impact, organic waste can be used as raw material for eco-enzymes, one of which is tomato waste. Tomatoes have a high organic content and contain various biocatalytic enzyme activities, namely lipase, amylase and protease. The resulting eco-enzymes will be used as raw materials for making hand sanitizers with distilled water, namely 1:400. EcoEnzym is very safe to use as a mixer for antiseptic products because the alcohol produced is organic alcohol from the fermentation of organic waste, and sugar is a carbohydrate producer which produces microbes in the fermentation process. The anti-bacterial test was carried out using a disc test on Staphylococcus aureus bacteria.

Keywords: eco-enzyme, organic waste, fermentation, hand sanitizer, Staphylococcus aureus

Introduction

Indonesia is a developing country with a large population. The greater the population, the greater the amount of waste produced. Industrial and technological developments can also have negative impacts, including increasing the volume, types and characteristics of increasingly diverse waste, one of the main causes of waste generation comes from household and industrial waste in the form of organic waste. Organic waste is waste that comes from household waste or the remains of living things that can be recycled into other forms that can bring prosperity to humanity (Puger, 2018)

Organic waste usually consists of vegetables, fruit or anything that comes from nature and can be broken down by bacteria. Without realizing it, this waste that seems biodegradable actually has a negative impact on the environment, where the accumulated organic waste can produce gas methane (CH4) 21 times stronger than CO2 so it has a big influence in reflecting heat from the earth back to the earth (Puger, 2018). CH4 can impact the atmosphere as a greenhouse gas (GHG) agent that can damage the atmosphere, as one form subtraction waste that is with recycling method, which aims to reduce the amount of organic waste in final disposal sites (TPA)

Judging from the total amount of waste produced, which is generally organic waste, which some people see as just waste and has no economic value. The rest is inorganic waste consisting of synthetic materials that are difficult or cannot be degraded by microbes. The large percentage of organic waste causes this waste to become a threat to the environment. So, we need a way to overcome this problem by managing organic waste using eco enzyme technology. Eco enzyme is a liquid product

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obtained from the fermentation of organic fruit and vegetable waste from households, plantations, and agriculture (Tangapo, 2021). Dr Rasukon Poompanvong was the first person to introduce eco-enzymes from Thailand. Eco-enzymes are enzymes produced during the fermentation process of natural ingredients such as vegetable proteins, minerals, and hormones (Panataria et al, 2022).

When making eco-enzymes, which is done by anaerobic fermentation process, organic waste, namely by using the main ingredient of palm sugar, because it functions as an organic material that is high in carbohydrates with a sucrose content of 84% and acts as a substrate, during the fermentation process. (Yuliono, 2022). Eco enzyme content, namely form sour acetate (H3COOH) which has ability kill germs, viruses and bacteria as well as can used in biotechnology simple for produce product cleaner. During fermentation, carbohydrates are converted into volatile acids, and because the pH of the waste enzymes is acidic, the organic acids present in the waste also dissolve into the fermentation liquid. During the fermentation process, glucose is broken down into pyruvat. Pyruvate is degraded to acetaldehyde by pyruvate decarboxylase under anaerobic conditions, then acetaldehyde is converted to ethanol and carbon dioxide by alcohol dehydrogenase, Acetobacter bacteria convert alcohol to acetaldehyde and air, and then Acetobacter bacteria convert acetaldehyde to acetic acid (Rusdianasari et al, 2021)

After the fermentation process is complete, Ecoenzyme (dark brown liquid) is produced. One type of waste House frequent stairs and industry we Look is waste fruit tomato (*Lycopersicum esculentum Mill*), ripe tomatoes contain about 95% water so easy rotten or damaged in a way physical (Suhartati, 2015). This thing cause waste tomato pile up become waste industrial and domestic over time will become problem ona environment. Tomato Alone own content including alkaloids, solanine, saponins, folic acid, acid citrate, bioflavonoids, chlorine, and sulfur, tomato alkaloids as well as compounds that have anti-inflammatory and anti- inflammatory functions (Rasit et al, 2019). Meanwhile, saponins and alkaloids can Act as agent antibacterial (Tangapo, 2021). Because of its content the then eco enzyme from waste tomato can can used as material standard making eco enzymes. Eco enzyme is used as material base making product basic health as murderer germs, like disinfectant, antiseptic soap, and hand sanitizer from the application of eco enzyme technology, we will be able to create a clean environment and achieve Indonesia's goal of being waste-free and utilize waste into something that can have economic value and is also useful for the lives of the public.

Materials and Methods

Study area

This study aims to process tomato waste as raw material for eco enzymes, through a fermentation process. Making eco enzymes is done by mixing tomato waste, granulated sugar and water in a ratio of 3:1:10. then fermented for three months. then the eco enzyme effectiveness test was carried out using a pH meter, Apha 2540 B Standard, Apha2540 C Standard, Apha5210 B Standard, Apha 5220 C Standard, High Performance Liquid Chromatography Method (HPLC), Casein Digestion Unit (CDU), 3.5 Acid Method - Dinitrosalicylate (DNS), Titrimetric Method. In the anti-bacterial test, the disc method is used to determine the bacterial inhibition zone, the bacteria used is Staphylococcus Aureus

Figure 1. Tomato

Procedures Preparation for Making Eco Enzyme

In preparation for making eco enzymes is to prepare tools and materials, the tools used are jars with a size of 1.5 L, 300ml water bottles, 20 cm hoses and universal ph, (rukmini, 2023). while the main constituent ingredients of eco enzymes, namely fruit waste, sugar, and water in a ratio of 3:1:10. For example 300g fruit peel: 100g sugar: 1 liter of water.

The process of making it is very easy, namely, in the initial stage we use any sugar other than white sugar, it's best to use brown sugar. Then, use the remaining fruit waste or fruit peel, then pour all the ingredients into a bottle, you can also use a blender to chop the waste, then mix the sugar and water in the bottle. Store in a dry and cool place at home temperature. Leave it for 3 months and open it every day for the first 2 weeks, then once every 2-3 days, then once a week. In the first week there will be a lot of gas produced. Sometimes there is a white layer on the surface of the solution, and if worms appear, add a handful of sugar, stir well then cover. After 3 months, filter the eco enzyme using gauze or a filter. The residue can be used again for new batch of production by adding fresh waste. The residue can also be dried, then blended and buried in the soil as fertilizer (Hadi et al, 2022)

The fermentation process in making eco-enzyme is carried out for 3 (three) months. If the resulting liquid is characterized by a dark brown color and has a strong sweet and sour fermented aroma, this is a sign that it can be used. The ideal color of eco enzyme is dark brown. If it turns black, add the same amount of sugar to start the fermentation process again. Basically, eco-enzymes will not expire. And in the fermentation process, organic waste is used as raw material for making Eco-enzymes, with a fermentation process that uses a mixture of palm sugar and water, the fermentation process produces O3 (ozone) gas. During the fermentation process, alcohol will be produced in the first month, followed by vinegar or acetic acid in the second month and then enzymes in the third month (Nickil et al, 2023)

Characterization of Tomato Waste Enzymes

In this characterization process, the aim is to determine the enzyme content of tomato waste in *tomato eco enzyme* (TEE) preparations, a comparison is also used *orange eco enzyme* (OEE). This characterization process can use a pH meter to determine the pH value of the enzyme produced, and determine the activity of the enzyme that is active at that pH

Table. 1 Parameters and pH Measurement (Rasit et al, 2019)

Parameter	Method
PH	Ph meter
T.S	Apha 2540 B Standard Method
TDS	Apha2540 C Standard Method
Directors	Apha5210 B Standard Method
COD Citric Acid	Apham5220 C Standard Method High Performance Liquid Chromatography (HPLC) Method
Protease Activity	Casein Digestion Unit (CDU) Analysis Method
Amylase Activity	3,5- Dinitrosalicylic Acid (DNS) Method
Lipase Activity	Titrimetric Method

After three months of fermentation, the enzyme solution is filtered and separated from the fruit residue. The characteristics of tomato and orange enzyme solutions were analyzed using different standard methods as shown in Table 1 The enzyme activity test parameters for tomato eco enzyme include pH, total solids (TS), total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), citric acid concentration and enzyme activities which are protease, amylase, and lipase (Rasit et al, 2019)

Antibacterial Activity Test

The antibacterial activity test was carried out using the disc method, to see the enzyme inhibitory power of the Straphillococus aureus bacteria. Pour 12 ml of still liquid Mueller-Hinton agar at a temperature of 45° C (thickness ± 4-5 mm) into a sterile petri dish, shake and let it freeze. Staphylococcus aureus bacterial suspension with a bacterial density of 379 x 106 /mL was spread with a bent glass rod into 0.1 mL of frozen Mueller-Hinton medium, allow the agar plate to dry for 5 minutes, then place the paper disc on top of the agar using tweezers. Tomato waste ethanol extract was dripped with various concentrations using a

clinipete, then incubated at 37° C for 24 hours (Suhartati & Nuryanti, 2015)

Making Positive and Negative Control Bacteria

Apart from working on samples, positive controls (Mueller-Hinton media + bacterial suspension) and negative controls (Mueller-Hinton media) were also carried out. It was observed that there was an obstacle area in the form of a clear zone around the disc paper. How to calculate the diameter of the bacterial inhibition zone in tomato waste enzymes:

Inhibition zone diameter (mm) = overall zone diameter (mm) – paper disc diameter (mm). (Suhartati & Nuryanti, 2015)

Results and Discussion

Characterization of Tomato Waste Ph Eco Enzyme Test

Figure 2 presents lipase activity *tomato eco enzyme* (TEE) and *orange eco enzyme* (OEE) with different pH values. The graph states that *orange eco enzyme* (OEE) has higher lipase activity compared to *tomato eco enzyme* (TEE) because of its citric acid content. The citric acid contained in *orange eco enzyme* (OEE) will stimulate cell lysis to release more intracellular enzymes that carry out this activity. This results in higher enzyme activity found in *orange eco enzyme* (OEE). Figure 1 also shows that the lipase activity for both enzymes begins to increase at the initial pH value (pH 2.8) and reaches maximum activity (330 μ/ml for TEE and 690 μ/ml for OEE) at pH 8. Maximum lipase activity usually occurs at pH 8 and will continue to increase up to pH 9

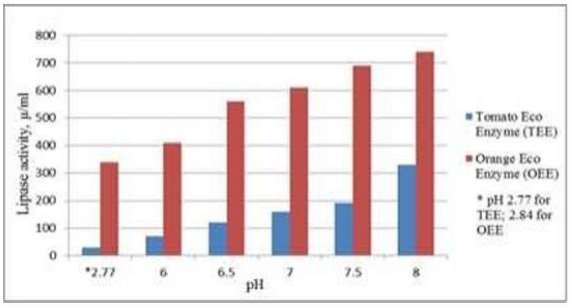

Figure 2 Lipase Activity at Different pH Values from Wasted Tomatoes (Rasit et al, 2019)

The higher catalytic properties of amylase will occur by maintaining a pH value ranging from 6 to 7. In Figure 3, both enzymes contain high amylase activity in the pH range, which states that maximum activity occurs at pH 6.5 for tomato *eco enzyme* TEE with value 2.62μ/ml. Meanwhile, *orange eco enzyme* OEE has 2.37μ/ml as maximum activity at pH 7.0

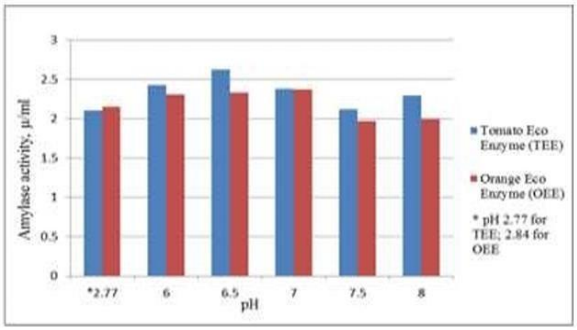

Figure 3 Amylase Activity at Different pH Values of Tomato Enzyme (Rasit et al, 2019)

Figure 4 shows higher protease activity for both enzymes achieved in the pH range of 6.5 to 7.5. However, its activity greatly decreases at low pH and pH higher than 7.5 because its optimum pH is in the range of 6.5 to 7

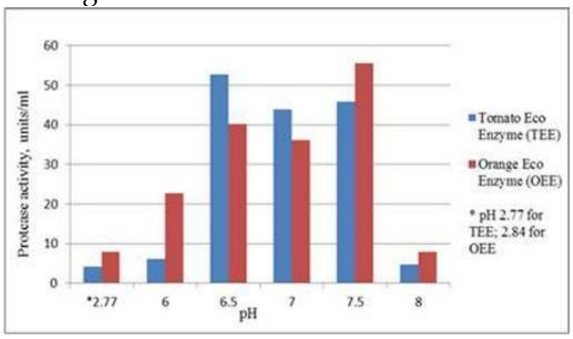

Figure 4. Protease Activity at Different pH Values from Wasted Tomatoes

Table 2. Characteristics of Environmentally Friendly Enzymes in Tomatoes.

Parameter	**Eco enzyme Tomato**	**Eco enzyme Orange**
PH	2.79	2.86
TDS	14,000	14,000
T.S	17,000	19,000
BOD 5	40	33
COD	80,000	96,000
CITRIC ACID	14,130	35,281

Table 3 displays the characteristics of *tomato eco enzyme* (TEE) and *orange eco enzyme* (OEE) after 3 months of fermentation. From the table, it can be seen that both eco enzymes are acidic with a low pH value of 2.79 for TEE. Thus, eco enzymes have a low pH value in this study as a result of the high content of organic acids such as acetic acid or citric acid, the BOD5 value from TEE is 40 mg/l. Apart from that, the TEE COD value is 80,000 mg/l. BOD5 and high COD indicates that the enzyme solution contains a large amount of organic material caused by fruit waste and brown sugar added as substrates in the fermentation process. On the characteristics of tomato eco enzyme used orange eco enzyme comparison where is the orange eco enzyme own sufficient results, however availability waste orange seldom found because orange own layer protector that is enough skin thick for hinder damage, or decay consequence microorganisms. Matter this compare backwards with waste tomatoes piled up in industry, supermarkets and restaurant consequence from tomato own layer thin and vulnerable skin putrefaction, so possible processing waste tomato as material basic eco enzyme

Antibacterial Activity Test

Table 3. Tomato Waste Extract Inhibition Zone (Suhartati & Nuryanti, 2015)

Concentration variations	Disc (mm)	Overall zone		Inhibition zone diameter (mm)		Averag (mı
		Deuteronomy 1	Deuteronomy 2	Deuteronomy 1	Deuteronomy 2	
10%	6	6	6	0	0	0
20%	6	6	6	0	0	0
30%	6	6	6	0	0	0
40%	6	6	6	0	0	0
50%	6	7.7	7.4	1.7	1.4	1.5
60%	6	8.7	7.9	2.7	1.9	2,3
70%	6	10.4	10.1	4.4	4.1	4.2
80%	6	11.7	11.2	5.7	5.2	5.4
90%	6	12.7	12.1	6,7	6.1	6.4
100%	6	13.9	13.9	7.9	7.9	7.9

Based on research results inhibitory power of tomato waste ethanol extract (Lycopersicum esculentum Mill) against growth of Staphylococcus aureus bacteria in-vitro, is formed inhibition zone or clear area on media so that it is not overgrown by bacteria due to the presence of antibacterial substances from ethanol extract of tomato waste which can be diffuses on the agar medium, so that contact with bacteria and inhibit bacterial growth and inhibit growth of Staphylococcus bacteria aureus. Based on measurement results inhibition zone of tomato waste extract the results it can be seen that the minimum inhibitory concentration of tomato waste ethanol extract on the growth of Staphylococcus aureus bacteria is the inhibition zone of tomato ethanol extract at a concentration of 50% with an inhibitory zone diameter of 1.55 mm. Followed by inhibition zones at concentrations of 60%, 70%, 80%, 90%, and 100%. Meanwhile, concentrations of 10%, 20%, 30%, 40% do not show an inhibition zone at all, this is because the antimicrobial substances contained in these concentrations are not able to inhibit the growth of Staphylococcus aureus bacteria (Suhartati & Nuryanti, 2015)

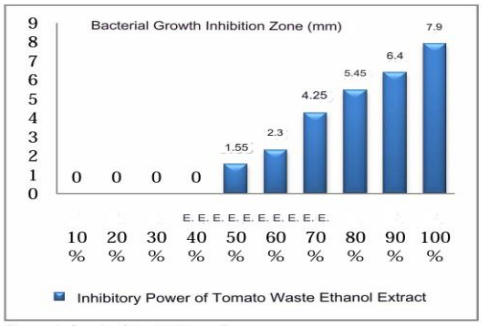

Figure 5. Graph of the Inhibitory Zone for the growth of S. aureus bacteria (Suhartati & Nuryanti, 2015)

In Figure 5 it can be seen that the higher the concentration of the extract, the greater the inhibition zone for the growth of S. aureus bacteria, this shows that the potential of the active substance which is antibacterial is lower in line with the smaller the concentration of the ethanol extract from tomato waste.

Discussion

Eco Enzyme is a product in the form of a liquid from processed organic waste from fresh vegetables and fruit which is fermented using brown sugar (Rochyani et al, 2020). Tomato waste is obtained by utilizing the remaining parts that are still intact from tomato waste originating from the market. The type of tomato used is an ordinary vegetable tomato (Lycopersicum esculentum Mill)

because this type of tomato waste is often found on the market. The unused or rotten parts of the tomato waste are discarded, then the selected tomato parts are cleaned with running water, then cut into pieces. into several parts after which the tomatoes are dried in the oven at a temperature of 60-80°C. This drying is intended to reduce the water content and deactivate the enzyme, because it is feared that enzyme activity will affect the examination results. Table 2 displays the characteristics of tomato eco enzyme (TEE) and orange eco enzyme (OEE) after 3 months of fermentation. From the table it can be seen that both eco enzymes are acidic with low pH values, namely 2.79 for TEE and 2.86 for OEE. Organic acids are an important key in determining acidity, meaning the higher the acidity.

Thus, eco enzymes have a low pH value in this study due to the high content of various organic acids such as acetic or citric acid. OEE parameters are slightly higher than TEE except biological oxygen value (BOD5). The BOD5 TEE value is 40 mg/l while the OEE is 33 mg/l. Apart from that, OEE has a high COD value of 173 96,000 mg/l while TEE's COD value is 80,000 mg/l. High BOD5 and COD values indicate that the enzyme solution contains a large amount of organic material produced by fruit waste and additional brown sugar, as a substrate in it. In the fermentation process, the remaining fermented solid material which is considered organic waste is also a factor in TS and High TDS on eco enzymes. The two environmentally friendly enzymes are also the same with a TDS value of 177 14,000 mg/l and OEE containing 19,000 mg/l TS and 17,000 mg/l for TEE. Eco enzymes contain Lipase, Trypsin, and Amylase which can prevent pathogenic bacteria. In other research, it was stated that eco enzymes have a high ability to kill E. coli, S. aureus, S. Typhi, C. Albicans, and viruses (Aruna & Sivashanmugam, 2015).

This has been confirmed by the inhibition test using the disk method. where the phytochemical test results showed positive results for saponin compounds, marked by the formation of foam which did not disappear for 30 minutes after shaking, and the alkaloid compound test results also showed positive results, marked by the formation of a precipitate after extracting, then dripping with Wagner's reagent. The nature of bacterial growth can be influenced by certain chemicals, including the active substances saponins and alkaloids contained in tomatoes. Saponin is a surface-active compound and has soapy properties. similar properties and can be detected based on their ability to form foam.

Saponins inhibit the growth or kill bacteria by interacting with membrane sterols. The main effect of saponins on bacteria is the release of proteins and enzymes from cells. Alkaloids are antibacterial because they have the ability to inhibit the work of enzymes in synthesizing bacterial proteins. Normal living cells have a large number of enzymes carrying out metabolic processes and other proteins, nucleic acids and other compounds. Disturbances in bacterial metabolism mean that energy needs are not met, resulting in permanent damage to bacterial cells which leads to the death of the bacteria.

In this case we can get knowledge that tomato waste can be a good choice of material for making eco-enzymes, because tomato content can inhibit the growth of Staphylococcusaureus bacteria in-vitro at a concentration of 50 - 100% with a minimum inhibitory concentration of 50%. In the process of bacterial activity test, tomato waste can produce ethanol extract. In Eco-Enzyme, which has been fermented for 90 days, it also contains alcohol and acetic acid. The following reaction occurs during the fermentation process.

$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + CO_2$ (1)

$CH_3CH_2OH + ½\ O_2$ Alcohol $\rightarrow CH_3CHO + H_2O$ acetaldehyde (2)

$CH_3CHO + ½\ O_2$ Acetaldehyde $\rightarrow CH_3COOH$ Acetic Acid (3)

Based on the chemical structure of the fermentation process, it involves the breakdown of carbohydrates into volatile and organic acids in the bacterial cell wall, which helps prevent or treat pathogens. Glucose is broken down resulting in the formation of pyruvate, which is converted to acetaldehyde, ethanol, and carbon dioxide by decarbosylase. Acetobacter converts alcohol to acetate and air, producing acetate (Supriani et al., 2020). Due to this process, alcohol is produced, so this content makes Eco Enzyme has disinfectant

properties or as anti-bacteria, these two compounds are able to destroy microbial cell walls and kill and inhibit the growth of living microorganisms. The enzyme content in eco enzyme consists of lipase, trypsin, amylase which can kill and prevent pathogenic bacteria. From this Eco Enzyme content, health products that are based on it as a germ killer can be made, one of which is Handsanitizer.

As for the factors that affect how eco enzyme works, namely, pH, the effectiveness of enzymes produced by microorganisms is very influential on the pH of eco enzyme where changes in pH can reduce the effectiveness of enzymes due to the denaturation process so that a good pH for eco enzyme is 4.5-5. Next, is the temperature factor, this factor greatly affects the activity of microorganisms where increasing temperature will accelerate the reaction of microbial enzymes (Xiaorui Xue et al, 2023)

One of the advantages of products produced from eco-enzyme is that there is no need to add alcohol because the eco-enzyme product itself already contains alcohol from fermentation, thereby reducing the incidence of irritation on the skin which is usually caused by synthetic alcohol when used frequently and over a long period of time. long time.

Therefore, eco-enzyme products are not only useful in preserving the environment but can also be used as a basis for making health products, because they have pharmacological activities, including antibacterial. To be able to see more potential from eco-enzyme results, testing is needed not only on bacteria, but also optimization regarding the stability of the pharmaceutical products being developed. It is hoped that in the future, efforts to improve eco-enzyme technology will be directly proportional to the level of reduction in organic waste so that Indonesia's goal of becoming waste free can be realized.

Conclusions

It can be concluded that, in the application of tomato eco enzymes to hansanitizers, namely by testing the inhibition of Staphylococcus Aureus bacteria in inhibiting their growth. The basic ingredients for making eco enzyme for making hand sanitizer are using tomato waste, which can kill bacteria, especially Staphylococcus aureus bacteria. Based on the observation of the data generated at a concentration of 50 - 100% with a minimum inhibitory concentration of 50%. The advantage of tomato waste used for the manufacture of eco-enzyme is that it has the result of ethanol extract, where the function of this ethanol can inhibit bacterial growth, in the application of making eco-enzyme also has a huge advantage, namely, there is no need to add alcohol because the eco-enzyme product itself already contains alcohol obtained from the fermentation results.

And from the research that has been carried out, we can know that eco-enzyme products are not only useful in preserving the environment but can also be used as a basis for making health products, because they have pharmacological activities, including antibacterial. To be able to see more potential from eco-enzyme results, testing is needed not only on bacteria, but also optimization regarding the stability of the pharmaceutical products being developed. It is hoped that in the future, efforts to improve eco-enzyme technology will be directly proportional to the level of reduction in organic waste so that Indonesia's dream of becoming waste free can be realized.

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