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Preparation and Identification of Local Microorganisms (LMOs) using Lake Toba Water and Their Utilization for Plastic Biodegradation

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Abstract

Local microorganisms (LMOs) are solutions made from anaerobic fermentation of sugar and complex carbohydrate sources using a local microorganism source medium. Generally, LMO solution possesses the potential to perform plastic biodegradation. This research aims to analyse the variation of the volume of Lake Toba water as a source of microorganisms in the preparation of LMO and to identify plastic degrading local microorganisms. The research steps consisted of making standard curves and growth curves, LMO preparation, testing the biodegradation of low-density polyethylene (LDPE) plastic, isolating microorganisms, performing biochemical test, testing the clear zone for plastic degrading microorganisms, and identifying microorganisms. LMO was made by mixing raw materials according to the ratio of Lake Toba water volume to substrate, namely 20:80 (% v/v); 30:70 (% v/v); and 40:60 (% v/v) which were fermented for 99 hours at 37 °C. The results showed that LMO pH before and after fermentation changed from 4.75; 4.9; and 4.94. to 3.46; 3.45; and 3.48. The decrease in pH was likely due to the activity of microorganisms that produce organic acids. The LDPE plastic degradation percentage of the resulting LMOs were 2.353% w/w; 3.012% w/w; and 4.023% w/w, respectively. For that reason, five microbe isolates obtained from fermentation of Lake Toba water volume to substrate ratio of 40:60 (% v/v) were further screened to validate their potential in degrading LDPE, which 2 isolates produced clear zones and identified as *Staphylococcus aureus* and *Streptococcus sp.*

Keywords: Biodegradation, fermentation, lake Toba water, LDPE, local microorganisms

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INTRODUCTION

Plastics are important materials, which mostly synthesized from petroleum used for various human needs, such as a basic material for packaging food and non-food products, medical equipment, automotive, and others. Indeed, plastics consumption continues to increase along with their increasing demand. As a consequence, generation of plastic waste also increase following its consumption trend. This raises numerous environment, health and social problems because most petroleum-based plastics are difficult to decompose (Lubis *et al*., 2018).

Various efforts have been made to reduce the impact of plastic pollution but they have yet to be

successful. The safest way to reduce plastic waste is to utilize microorganisms in plastic biodegradation (Filayani, 2019). Biodegradation is the process of breaking down complex compounds into simple compounds by utilizing microorganisms (Elfarisna & Nosa, 2014). However, for many reasons, the implementation of this method is never easy. Therefore, an effective and easy alternative to reduce plastic waste by local community is needed, namely by employing local microorganisms (LMOs). LMO is a fermentation solution that has generally been used as a starter and decomposer. In facts, LMO provides numerous advantages, such as being environmentally friendly, easy to be practiced, and low cost (Lubis Z. , 2020).

The ingredients used to produce LMO consist of several components, namely glucose, complex carbohydrates, and microorganisms' sources (Palupi, 2015). In North Sumatera, the common sources of complex carbohydrates are cassava dregs, rice washing water, stale rice, and potatoes. Meanwhile, the sources of glucose are brown sugar, granulated sugar, coconut water, and sugar water. Finally, fruit waste, vegetable waste, sea water, lake water, and anything that contains sources of bacteria can be used as the source of microorganisms. The fermentation process in making LMO takes place under anaerobic condition. Factors that affect fermentation are the type of microorganisms, nutrients, and fermentation media. However, the fermentation time varies from one type of material to another. The fermentation time is related to the availability of food used by microorganisms as an energy source. A longer the fermentation time means that the microorganisms consume less food to grow and maintain their cells' health (Jeksen & Mutiara, 2018). One of the potential sources of microorganisms to be utilized in the process of making local microorganisms is Lake Toba water. There are several types of microorganisms that have habitats in fresh water such as lake water, river water, and ponds. For instance, *Bacillus, Bacteroides, Lactobacillus, Staphylococcus, Clostridium, Micrococcus, Serratia, Thermus*, dan *Actinomycetes* (Reddy *et al*., 2003).

In this study, local microorganisms were made through a fermentation process that was further applied to degrade low density polyethylene (LDPE) plastic waste. Research on the provision of LMO to degrade plastic are still rare. To the best of our knowledge, the presence of microorganisms such as *Bacillus, Serratia*, and *Staphylococcus* in lake water that have the potential as plastic degrading agents can be used as a reference as a source of microorganisms in making LMO solutions with additional ingredients of complex carbohydrate sources and glucose sources in the form of cassava dregs, rice washing water, brown sugar, and coconut water. The resulting LMO will be tested for its degradation ability against LDPE plastic with a degradation period of 30 days, and the type of microorganism that has the greatest degradation ability against LDPE plastic will be isolated and identified.

MATERIALS AND METHOD

The work procedures in this study include the preparation of raw materials, standard curves and growth curves, preparation of local microorganisms, plastic biodegradation test, microorganisms' isolation, biochemical tests, clear zone test of LDPE degrading bacteria, and microorganisms' identification.

PREPARATION OF STANDARD CURVE

LMO was made by mixing cassava dregs, rice washing water, coconut water, brown sugar, and Lake Toba water into an Erlenmeyer flask and stirred evenly. Then dilution was carried out until the ninth dilution series. The stock solution was prepared by mixing 10 mL of LMO sample with 90 mL of sterile physiological NaCl (0.85% NaCl) and homogenized for 1 hour. The first serial dilution was prepared by mixing 1 mL of LMO from the stock solution into 9 mL of sterile physiological NaCl in a test tube. The second serial dilution was prepared by mixing 1 mL of LMO from the first serial dilution solution into 9 mL of sterile physiological NaCl in a test tube. The same dilution was carried out until the ninth serial dilution. The optical density (OD) of each dilution was measured using an OPTIMA SP-3000 nano spectrophotometer with a wavelength of 600 nm. Each dilution series was inoculated into Nutrient Agar (NA) media and incubated at 37 °C for 48 hours. The number of growing cells was counted and they were plotted as the abscissa and OD as the ordinate to obtain a standard curve.

Table 1. Number of Microorganisms and Substrate

Variation	Ratio of microorganism volume to substrate (% v/v)	Amount of microorganism (mL)	Amount of substrate (mL)
1	20:80	100	400 (100 mL rice washing water + 100 mL coconut water + 100 g brown sugar + 100 g cassava dregs)
2	30:70	150	350 (87,5 mL rice washing water + 87,5 mL coconut water + 87,5 g brown sugar + 87,5 g cassava dregs)
3	40:60	200	300 (75 mL rice washing water + 75 mL coconut water + 75 g brown sugar + 75 g cassava dregs)

PREPARATION OF GROWTH CURVE

The LMO made was incubated in a water bath shaker at 37 °C for 72 hours to obtain local microorganism cultures. Then OD was measured every 3 hours using a spectrophotometer with a wavelength of 600 nm. The OD results were converted into the number of cells through the equation of the line obtained on the standard curve. The growth curve

was obtained by plotting the number of cells as the ordinate and time as the abscissa.

PREPARATION OF LMO

LMO was prepared by mixing cassava dregs, rice washing water, coconut water, brown sugar, and Lake Toba water into an Erlenmeyer flask and stirred evenly. The amount of each material can be seen in Table 1. Then the Erlenmeyer flask was then covered with fat cotton and aluminum foil and fermented for 99 hours based on the results of the growth curve obtained. Upon the completion of fermentation process, observations will be made on the parameters of the LMO obtained.

PLASTIC BIODEGRADATION TEST

Biodegradation test was subjected to LDPE plastic measuring 2 cm × 2 cm. The weight of the plastic was weighed before sterilization to observe the ability of microbial isolates to break down plastic polymers. Sterilization was carried out by soaking the plastic in 70% aqueous ethanol for 30 minutes and then drying it in a laminar air flow. Then the plastic was put into a sterilized Erlenmeyer flask containing 50 mL of microbial isolates. Afterward, the Erlenmeyer was closed using fat cotton to avoid microbial contamination from the environment.

Biodegradation test was carried out for 30 days. After 30 days, the plastic samples were washed with sterile distilled water and sprayed using 70% aqueous ethanol. Then the plastic samples were dried in the open air and their final weight was weighed. Measurement of plastic weight loss was carried out by calculating the difference in plastic weight, namely the initial dry weight of the plastic before being put into the Erlenmeyer with the final dry weight of the plastic after 30 days.

MICROORGANISMS ISOLATION

Upon the completion of the plastic biodegradation process, the next step is to isolate the microorganisms contained in the local microorganisms. Isolation of microorganisms was carried out on samples that have the greatest ability to degrade LDPE plastic. Isolation of microorganisms is carried out using the spread plate method into Mineral Salt Medium Agar (MSMA) media mixed with LDPE powder. The media was then incubated in an incubator at a 37 °C for 7 days.

BIOCHEMICAL TEST

The single colony results obtained from the isolation of microorganisms are then regrown into NA media so that biochemical tests can be carried out. Biochemical tests that will be carried out include starch hydrolysis tests, catalase tests, carbohydrate fermentation tests, and triple sugar iron (TSI) tests.

Starch hydrolysis test was conducted to determine the ability of bacteria to hydrolyze starch by producing amylase enzyme. Bacteria were inoculated into starch media and incubated for 48 hours. After 48 hours, the media was dripped with iodine reagent. Then the presence of clear color was observed as a positive result.

Catalase test was conducted to determine the ability of bacteria to produce catalase enzyme. The presence of catalase enzyme that can break down peroxide compound into water and oxygen allows bacteria to survive the threat of poisoning. Bacteria were grown on NA media and incubated for 48 hours. After 48 hours, the media was dripped with 3% H_2O_2 reagent. Then, observation of air bubble formation was carried out as a positive result.

In the carbohydrate fermentation test, the medium containing simple carbohydrate compounds (sugar) is broken down by microbial enzymes into final products in the form of organic acid compounds that can lower pH and possibly produce CO_2 gas. The specialty of this test is that each microbe has different sugar fermentation capabilities ranging from simple sugars to complex sugars. Bacteria are inoculated into each of the 1% sucrose broth media, 1% glucose, and 1% lactose and incubated for 48 hours. Then, the media color is observed to turn yellow and the presence of gas bubbles in the Durham tube as a positive result.

The triple sugar iron (TSI) test is designed to distinguish between Enterobacteriaceae groups that have a gram-negative bacillus shape and produce positive glucose fermentation results with acid formation. Bacteria are inoculated on TSI agar media and incubated for 48 hours. After 48 hours, phenol red reagent is dripped onto the media. Then, observations are made on the color change of the media as a positive result.

CLEAR ZONE OF LDPE DEGRADING BACTERIA TEST

Each pure isolate was grown in MSMA media mixed with LDPE powder using the streak method. Then, incubation was carried out at 37 °C for 7 days. After the incubation period was complete, bacteria that were able to grow in MSMA media were selected to undergo a clear zone test (Kunlere *et al*., 2019). The selected bacterial isolates were then recultivated in an MSMA media mixed with LDPE powder using the dot method. All isolates were incubated at 37°C for 7 days. After the incubation period was complete, each petri dish was stained with 0.1% Coomassie Brilliant Blue solution and destained to visualize the clear zone around the colony. The Coomassie Brilliant Blue solution was made by dissolving 0.1% (w/v) Coomassie Blue into 40% (v/v) methanol and 10% (v/v) acetic acid. The destaining solution was prepared by adding 40% (v/v) methanol to 10% (v/v) acetic acid. The agar medium was flooded with 0.1% Coomassie Blue R-250 solution for 20 minutes. The Coomassie Blue solution was poured off, and the medium was flooded with the destaining solution for 20 minutes. Bacteria that produced a clear zone with a blue background were considered as LDPE decomposers (Gupta *et al*., 2016). Bacteria that produced a clear zone diameter were then used for the identification.

MICROORGANISMS IDENTIFICATION

Identification of microorganisms was conducted by morphological observation. Morphological observation of microorganisms can be done on microorganisms in living or dead conditions. Observation of microorganisms is done through gram staining techniques.

RESULTS AND DISCUSSION

PREPARATION OF STANDARD CURVE

The LMO standard curve is a curve used to calculate the number of bacterial cells indirectly, namely by regressing the absorbance value and the number of colonies into the standard curve line equation y = ax + b where y is the absorbance value and x is the number of cells.

The calculation of the number of LMO cells in making the standard curve in this study was carried out using the cup count method (Yunita *et al*., 2015) and using a spectrophotometer to observe the level of turbidity (Optical Density) which is read through the absorbance value produced (Septiani & Wijayanti, 2017). The LMO standard curve obtained in this study is presented in Figure 1.

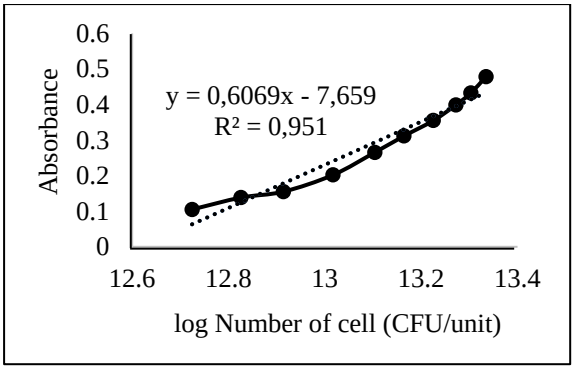


Figure 1. Standard Curve of LMO

From Figure 4.1, a linear regression equation is obtained y = 0.6069x – 7.659 which will be used to calculate the number of cells on the LMO growth curve where y is the absorbance of the cells and x is the logarithm of the number of cells.

PREPARATION OF GROWTH CURVE

The preparation of a growth curve was carried out to determine the growth phase of the LMO culture. The phases in the growth curve consist of several phases, namely the lag phase (adaptation), the exponential phase (log), the stationary phase, and the death phase (Wahyuningsih & Zulaika, 2018). The growth curve was calculated using the turbidimetry method. This method has the advantage of being a fast and inexpensive process (Iswadi, 2016). The LMO growth curve is presented in Figure 2.

The growth curve shows that the LMO lag phase occurs at 0 to 6 hours. In this phase, slow growth occurs because it is influenced by bacterial activity in carrying out the process of adjusting to environmental conditions such as pH, temperature, and nutrition (Mardalena, 2016). The lag phase occurs during the first six hours of incubation of the early growth period.

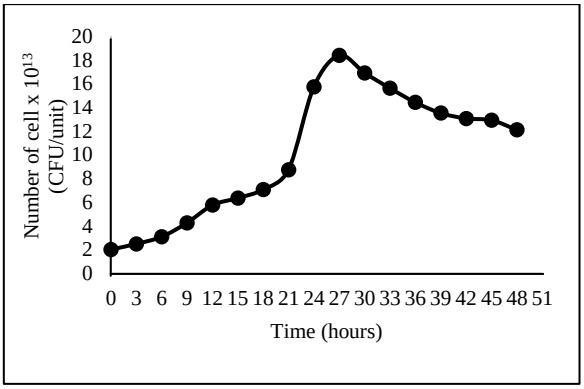


Figure 2. Growth Curve of LMO

Furthermore, the exponential phase occurs in the next twenty-one hours. The exponential phase occurs at 6 to 27 hours. The exponential phase is a phase where there is an increase in the activity of microorganisms that experience changes in shape and an increase in the number of cells to the maximum speed so that a curve is obtained in exponential form. In this study, the peak of the exponential phase occurred at 27 hours. Factors that influence growth in the exponential phase consist of two factors, namely biological and non-biological factors. In biological factors influenced by the nature and form of microbes to the environment and the unity of life among related microorganisms. Non-biological factors are the nutrient value contained in the growth medium, temperature conditions, and pH of the media (Rolfe *et al*., 2012).

Furthermore, at 30 to 48 hours, microorganisms enter the death phase indicated by a decrease in cell count data indicating no more microorganism growth.

PREPARATION OF LMO

The parameters of the three LMO variations observed in this study included the number of microorganisms, pH, presence or absence of gas bubbles, aroma, and color of LMO, which are presented in Table 2.

NUMBER OF COLONIES

The number colonies are one of the parameters of the LMO solution observed. After calculating the number of colonies in the LMO solution, the data obtained were as presented in Figure 3.

From Figure 3, the number of colonies in LMO solution is influenced by the ratio of the volume of microorganisms.

The greater the ratio of the volume of microorganisms to the substrate used in making LMO, the greater the number of colonies. The highest number of colonies is found in the variation of the ratio 40:60 (% v/v) which is 31×10^7 CFU/mL.

Table 2. Characteristics of LMO Solution

LMO Parameters	Ratio of Microorganism Volume to Substrate 20:80 (% v/v)		Ratio of Microorganism Volume to Substrate 30:70 (% v/v)		Ratio of Microorganism Volume to Substrate 40:60 (% v/v)	
	Before Fermentation	After Fermentation	Before Fermentation	After Fermentation	Before Fermentation	After Fermentation
Number of colonies (CFU/mL)	15×10^7		20×10^7		31×10^7	
pH	4,75	3,46	4,90	3,45	4,94	3,48
Presence of gas bubbles	-	+	-	+	-	+
Aroma	Aroma of the raw material	Aroma of the fermented tapai	Aroma of the raw material	Aroma of the fermented tapai	Aroma of the raw material	Aroma of the fermented tapai
Color	Dark reddish brown	Light reddish brown	Dark reddish brown	Light reddish brown	Dark reddish brown	Light reddish brown

This occurs because microorganisms utilize the substrate in making LMO as a source of nutrition for their growth. Carbohydrates contained in the substrate can trigger the growth of amylolytic bacteria (Marzuqi, 2015).

Amylolytic bacteria are a type of bacteria that can produce amylase enzymes and are able to break down starch (Türker & Özcan, 2015). These microorganisms can inhabit freshwater habitats such as lakes, rivers, and ponds (Waluyo, Mikrobiologi Lingkungan, 2009). The genera of amylolytic bacteria that are quite widely known are *Bacillus*, *Bacteroides*, *Lactobacillus*, *Clostridium*, *Micrococcus*, *Thermus* dan *Actinomycetes* (Reddy *et al*., 2003).

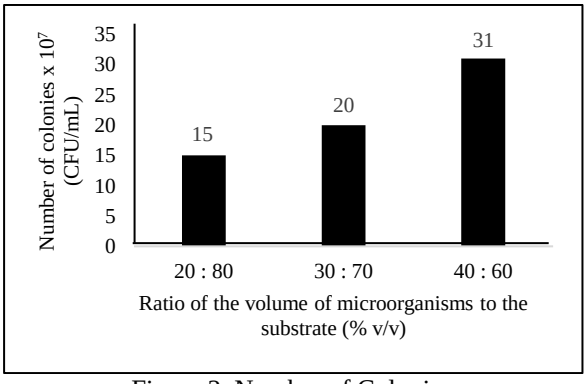


Figure 2. Number of Colonies

The source of microorganisms used in the manufacture of LMO solutions is one of the factors that influence the success of the fermentation process. In addition, other factors that influence are the substrate used, temperature, and oxygen levels (Masiningsih *et al*., 2015).

pH OF LMO SOLUTION

The second parameter observed was the pH of the LMO solution. Based on the research conducted, all three LMO variations experienced a decrease in pH during the incubation period. Changes in the pH of the LMO solution before and after fermentation are presented in Figure 4.

From Figure 4, the variation of the 20:80 ratio (% v/v) experienced a decrease in pH of 1.29 from 4.75 to 3.46. The variation of the 30:70 ratio (% v/v) experienced a decrease in pH of 1.45 from 4.9 to 3.45. The variation of the 40:60 ratio (% v/v) experienced a decrease in pH of 1.46 from 4.94 to 3.48. The decrease in pH occurs due to the activity of microorganisms that produce organic acids.

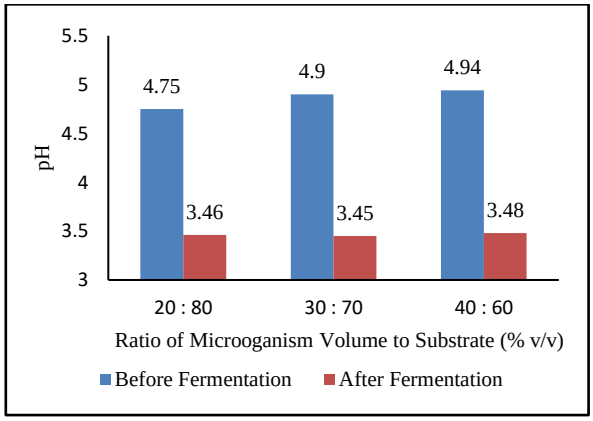


Figure 3. pH of LMO Solution

From these results, the greater the number of microorganism sources, the greater the decrease in pH. This is due to the higher activity of microorganisms hydrolyzing carbohydrates so that the production of organic acids will increase, resulting in a decrease in pH. Marsiningsih *et al.* (2015) explained that the acidity of LMO products is closely related to the production of organic acids by microorganisms that can lower pH. In the process, there is a release of hydrogen ions which can change the balance of the solution so that the pH becomes low.

THE PRESENCE OF GAS BUBBLES

The presence of gas bubbles in the LMO solution is also one of the parameters for the success of making LMO solutions. Based on the research conducted, the three LMO variations showed the presence of gas bubbles after fermentation took place. This indicates the activity of microorganisms that produce carbon dioxide (CO_2) gas. Fitrianto *et al*. (2016) explained that the formation of air bubbles in the LMO solution indicates that there is pressure due to the formation of CO_2 gas from the anaerobic catabolism process as a sign that fermentation is taking place. The absence of air bubbles indicates that LMO fermentation is complete. The presence of gas bubbles in the LMO solution before and after fermentation can be observed in Figure 5.

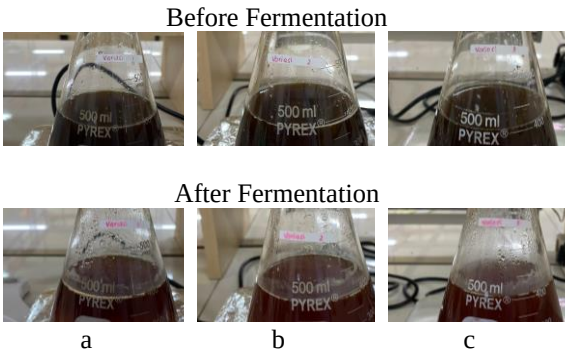


Figure 4. Observation of Gas Bubble Before and After Fermentation: (a) Variation 1 (b) Variation 2 (c) Variation 3

Aroma of LMO Solution

The next parameter observed was the aroma of the LMO solution. The aroma of the LMO solution before fermentation in all treatments showed the typical aroma of the raw materials used, namely the aroma of brown sugar and cassava dregs. The aroma after fermentation changed to a sour aroma like the aroma of fermented tapai. This shows that the LMO solution has been fermented well. Yuliana (2021) reported that LMO that is ready to use and has been cooked is characterized by a sour aroma like tapai.

LMO is a fermentation result that produces organic acids. According to Yunilas *et al.* (2022), the organic acids produced are the result of the activity of microorganisms that hydrolyze carbohydrates to form organic acids in the form of lactic acid and alcohol.

Color of LMO Solution

The fifth parameter observed is the color of the LMO solution. Before fermentation is carried out, the color of the LMO solution made is a combination of the color of the LMO raw materials in colloidal form. This color will not change even if it undergoes filtration or centrifugation (Effendi, 2003). All LMO with plant sources will produce sediment. The color of this sediment depends on the color of the organic material used. Changes in the color of the LMO solution before and after fermentation can be seen in Figure 6.

As depicted in Figure 6, before fermentation, the three variations of LMO solution had a dark reddish-brown color and had a brownish white cassava pulp sediment. However, after fermentation was complete, the color of the LMO solution changed to light reddish brown and the color of the cassava pulp sediment became whiter than before.

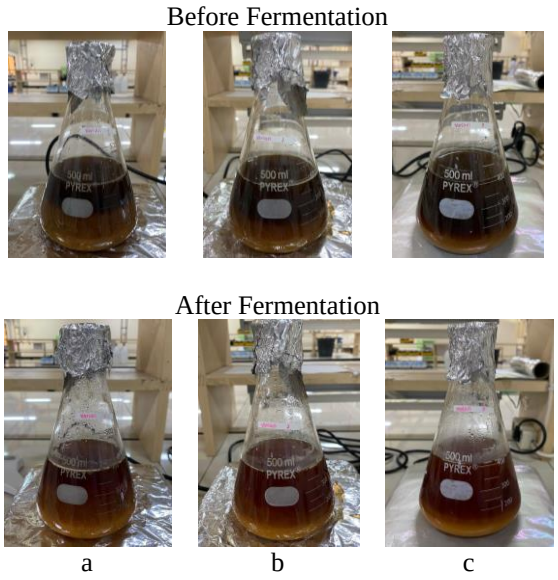


Figure 5 Observation of LMO Color Before and After Fermentation: (a) Variation 1 (b) Variation 2 (c) Variation 3

The change in color of the LMO solution was caused by the organic materials contained in the LMO solution, namely brown sugar, and cassava pulp, which had undergone a decomposition, thus affecting the color of the LMO. Arief *et al.* (2011) stated that microorganisms break down carbohydrate compounds into simple compounds in the form of water, carbon dioxide, alcohol, and organic acids.

Plastic Biodegradation Test

The plastic biodegradation test in this study was conducted for 30 days using Low Density Polyethylene (LDPE) plastic samples. The LDPE plastic biodegradation process is seen from the decrease in plastic weight. Data on the percentage of LDPE plastic degradation can be seen in Figure 7.

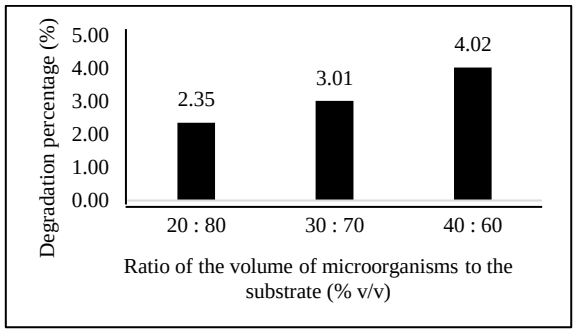


Figure 6. LDPE Plastic Degradation Percentage

Figure 7 illustrates that there is a decrease in the dry weight of LDPE plastic after the biodegradation process. Based on the results of the research conducted, the ratio variation that has the greatest potential in degrading LDPE plastic is the ratio of 40:60 (% v/v) with a percentage of dry weight loss of LDPE plastic of 4.02% w/w. The ratio variations of 30:70 (% v/v) and 20:80 (% v/v) have a percentage of dry weight loss of LDPE plastic of 3.01% w/w and 2.35% w/w, respectively.

Based on the biodegradation results obtained in this study, the LMO formulation that has been made could degrade LDPE plastic. During its growth, LMO utilizes the carbon source in the test plastic in the metabolic process to produce energy.

The decrease in the weight of LDPE plastic during the incubation time by microorganisms is due to the presence of enzymes produced by the microorganisms. The enzyme attaches to the surface of the test plastic and then undergoes a hydrolysis process which can erode the polymer surface, resulting in a loss of polymer weight and a decrease in the percentage of dry weight of the test plastic (Zusfahair *et al*., 2007).

MICROORGANISMS' ISOLATION

The LMO formulation with a ratio of 40:60 (% v/v) is the sample that produces the largest percentage of LDPE plastic degradation. The sample was then diluted to reduce the density of bacterial colonies in the isolation media and to represent all types of bacteria in the sample.

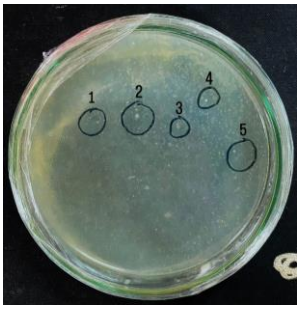


Figure 7. Microorganisms' isolation

Furthermore, bacterial isolation was carried out by pouring the dilution suspension into a petri dish containing selective media, namely MSMA (Mineral Salt Medium Agar) media added with 0.2% w/v LDPE powder. The addition of LDPE powder aims to test the ability of isolates to grow in an environment containing polyethylene plastic as the base material. Normal bacterial growth in media containing plastic (polymer) as the base material can indicate that bacteria are able to use the carbon requirements of the polymer to meet the carbon requirements in their nutrition (Marjayandari & Maya, 2015). The media containing the suspension was then incubated for 7 days. The results of the isolation of the LMO formulation sample with a ratio of 40:60 (% v/v) can be seen in Figure 8.

As seen in Figure 8, there are 5 isolates that were successfully grown on MSMA media. Then these five isolates were regrown separately for biochemical tests.

Biochemical Test

The five isolates that were successfully isolated were then subjected to microorganism biochemical tests. The biochemical tests carried out included starch hydrolysis test, catalase test, carbohydrate fermentation test, and Triple Sugar Iron (TSI) test. The results of the biochemical tests of the five isolates are presented in Table 3.

Table 3 Microorganisms Biochemical Tests

Isolate	Starch Hydrolysis Test	Catalase Test	Carbohydrate Fermentation Test	TSI Test
Isolate 1	+	-	+	+
Isolate 2	+	+	+	+
Isolate 3	+	+	+	+
Isolate 4	+	-	+	+
Isolate 5	+	-	+	+

Description:
Starch hydrolysis test (+) = formation of a clear zone around the starch media
Catalase test (+) = formation of gas bubbles in the media
Carbohydrate fermentation test (+) = color change in the media and gas bubbles formed in the Durham tube
TSI test (+) = color change in the media to yellow

The starch hydrolysis test aims to identify microorganisms that can produce alpha amylase and oligo-1,6-glucosidase which cause starch hydrolysis (Hemraj *et al*., 2013). Starch is a high molecular weight branched polymer consisting of glucose molecules linked to each other through glycosidic bonds. The degradation of this macro molecule requires the presence of extracellular amylase enzymes for its hydrolysis into shorter polysaccharides, namely dextrin and finally into maltose molecules. The final hydrolysis of this disaccharide catalyzed by maltase will produce low molecular weight glucose molecules that can be transported into cells and used for energy production through the glycolysis process. In the procedure, starch agar is used to demonstrate the hydrolytic activity of this exoenzyme. The medium consists of nutrient agar supplemented with starch which functions as a polysaccharide substrate. Detection of hydrolytic activity after the incubation period is carried out by conducting a starch test to determine the presence or absence of starch in the medium. Starch with iodine will give a blackish blue color to the media indicating the absence of starch-degrading enzymes and indicating a negative result. If the starch has been hydrolyzed, a clear zone of hydrolysis will surround the growth of microorganisms indicating a positive result (Cappucino & Sherman, 1987).

Catalase test is conducted to identify microorganisms that produce catalase enzyme. Catalase enzyme produced by this bacterium will

neutralize hydrogen peroxide and will produce gas bubbles indicating a positive test result. Catalase enzyme is mostly produced by obligate aerobic bacteria and facultative anaerobic bacteria. The test is conducted by dripping 3% H_2O_2 into the media and observing the formation of gas bubbles in the media (Facklam & Elliott, 1995).

The carbohydrate fermentation test aims to identify bacteria that can ferment carbohydrates or those that cannot ferment carbohydrates. This test is based on the principle of acid or gas production. The media used in this test is glucose (Hemraj *et al*., 2013). Fermentation is carried out in a test tube containing a Durham tube, the inner bottle is inverted to detect gas production. The pH indicator used is phenol red which is red at pH 7 and turns yellow at acidic pH. After incubation, fermented carbohydrates with acid waste production will cause phenol red to turn yellow, indicating a positive test result.

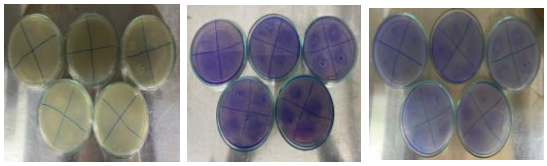
In some cases, acid production is accompanied by the release of carbon dioxide gas which will be seen as bubbles in the Durham tube. Cultures that are unable to ferment carbohydrate substrates will not change their indicators and the media will appear red, and no gas in the Durham tube indicates a negative test result (Cappucino & Sherman, 1987).

The TSI test aims to identify the Enterobacteriaceae bacterial group. TSI media consists of 3 types of sugar, namely 0.1% glucose, 1% lactose and sucrose each. Phenol red is used as an indicator. The media used is slant media. The inoculated bacteria will utilize glucose and glucose levels must be kept low compared to other sugars. If the bacteria can utilize glucose in both aerobic and anaerobic conditions, the color of the slant media will change to yellow due to acid production by the bacteria after incubation. If the bacteria can utilize sucrose and lactose, then acid production will continue, and the color of the media will remain yellow. If the bacteria cannot utilize sucrose or lactose, then the bacteria will utilize amino acids and make the media alkaline which will change the color of the media to red because of phenol red (Harley, 2005).

CLEAR ZONE TEST OF LDPE DEGRADING BACTERIA

Screening of plastic degrading bacterial isolates was carried out using the clear zone method. Of the 5 bacterial isolates obtained, there were 2 isolates that formed clear zones around their colonies. The results of the clear zone test of plastic degrading bacteria are presented in Figure 9.

From Figure 9, it is obtained that there are two isolates that produce clear zones, namely isolate 3 and isolate 4. Augusta *et al*. (1993) have reported that the clear zone around the colony is caused by extracellular hydrolysis enzymes secreted by the target microorganism into the media. All minerals are supplied together with polyethylene powder as a carbon source for the growth of microorganisms. Usha *et al*. (2011) stated that the ability of bacterial growth is the ability of bacterial isolates to utilize LDPE in MSMA media as a carbon source. The isolates that produce clear zones will then be identified to determine the morphology of the isolate.



(a) (b) (c)

Description:

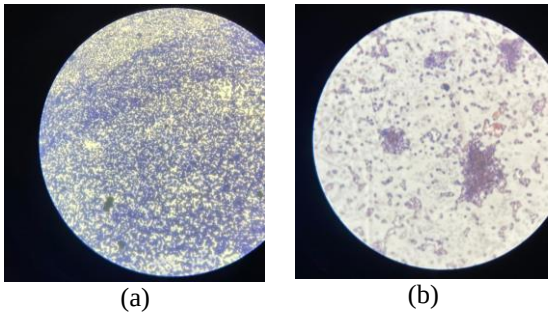
Petri dish top left: Isolate 1 Petri dish top middle: Isolate 2
Petri dish top right: Isolate 3 Petri dish bottom left: Isolate 4
Petri dish bottom right: Isolate 5

Figure 8. Clear Zone Testing: (a) Before being dripped with coomassie blue solution (b) After being dripped with coomassie blue solution (c) After being dripped with destaining solution

MICROORGANISMS IDENTIFICATION

Both isolates that have the potential to degrade plastic were identified using gram staining techniques. Gram staining is a staining technique used to classify gram-positive and gram-negative bacteria. Gram-positive bacteria will retain the crystal violet dye and will appear dark purple under a microscope. While gram-negative bacteria will lose their crystal violet dye after being washed with alcohol, and if given safranin dye, they will appear red (Walyono, 2007).

The result of gram staining isolate 3 and isolate 4 are presented in Figure 10. Based on the results of gram staining, it was found that isolate 3 and isolate 4 were gram-positive bacteria characterized by the formation of purple color in bacterial cells and the shape of isolate 3 and isolate 4 was coccus (round).



(a) (b)

Figure 9. Gram Staining (a) Isolate 3 (b) Isolate 4

The peptidoglycan layer on the cell wall of gram-positive bacteria is abundant and the lipid content is low, while in gram-negative bacteria the peptidoglycan content is low, and the lipid content is high. The purple color in gram-positive bacteria is caused because these bacteria have low lipid content, so that the cell wall will be more easily dehydrated due to the addition of alcohol. Dehydrated cell walls cause the size of the cell pores to become small and the

permeability is reduced so that the main dye cannot exit the cell and the cell will remain purple. Gram-negative bacteria will give a red color to gram staining. Giving alcohol to gram-negative bacterial cells can increase the porosity of the cell wall in dissolving lipids in the outer layer of bacterial cells (Sachan *et al*., 2018). This causes crystal violet that is not bound to the cell wall to be easily released when washed with alcohol. Therefore, gram-negative bacteria lose their main dye, namely crystal violet, so that the bacterial cells absorb the color from safranin as a comparison color. The absorption of safranin by bacterial cells causes the color of gram-negative bacteria to turn pink.

Through the results of morphological and biochemical tests, isolate 3 was identified as *Staphylococcus aureus*. The results of this study were compared with previous studies conducted by Dey *et al*. (2016) where they reported that *Staphylococcus* sp. and consortium were able to grow on minimal media containing plastic as the sole carbon source. Degradation by bacteria was monitored by screening followed by a decrease in plastic weight. The percentage of plastic degradation by *Staphylococcus* sp. with a degradation period of 30 days was 8.3% while plastic degradation by consortium with a degradation period of 30 days was 9.09%.

Based on the biochemical tests conducted in this study, it showed that isolate 3 produced a positive test in the catalase test, starch hydrolysis test, carbohydrate fermentation test and TSI test. The same results were stated by Dey *et al*. (2016) in their study on the biodegradation ability of bacteria in plastic cups and thermocol that *Staphylococcus* sp. bacteria. is a gram-positive bacterium and its shape is coccus. The catalase test of *Staphylococcus* sp. bacteria is positive. Subhankari *et al*. (2011) in their research on the biochemical characteristics and antibiotic susceptibility of *Staphylococcus aureus* isolates also found that the starch hydrolysis test of *Staphylococcus aureus* bacteria was positive. *Staphylococcus* sp. is also able to ferment sugar contained in TSI media, thereby increasing the acid content, and changing the color of the solution to yellow (Singh & Prakash, 2008).

Then through the results of morphological and biochemical tests, isolate 4 was identified as Streptococcus sp. The results of this study were also compared with previous research conducted by Kathiresan (2003) regarding polyethylene and plastic decomposing microbes from Mangrove soil reported that Streptococcus sp. bacteria can degrade plastic with a degradation percentage of 1.07% with a degradation period of 30 days.

Based on the biochemical tests carried out in this study, it showed that isolate 4 produced a positive test in the starch hydrolysis test, carbohydrate fermentation test and TSI test but produced a negative test in the catalase test. The same results were stated by Toelle & Lenda (2014) that *Streptococcus* sp. is a gram-positive bacterium, and its shape is coccus. The study also stated that *Streptococcus* sp. produced a negative catalase test. The same results were also stated by Ni Kadek *et al.* (2018) that *Streptococcus* sp. bacteria produced a positive test in the TSI test and carbohydrate fermentation test. *Streptococcus* sp. also produces a clear zone in the starch hydrolysis test which indicates a positive test (Evans *et al*., 2004).

CONCLUSION

Based on the observation obtained in this research, it can be concluded that Lake Toba water has the potential to be used as a source of microorganisms in the preparation of LMO. A larger ratio Lake Toba water volume to the substrate used for anaerobic fermentation resulted in higher percentage of degradation of LDPE plastic. LMO made with a ratio of microorganism source to substrate of 0.4:0.6 has the highest percentage of degradation of LDPE plastic, which is 4.02%. Two of five microorganism isolates obtained from LMO made with a ratio of microorganism source to substrate of 0.4:0.6 that produced clear zones were gram-positive and coccus-shaped bacteria identified as *Staphylococcus aureus* dan *Streptococcus* sp.

REFERENCES

Arief, R., Irawati, I. dan Yusmasari. (2011). Penurunan Kadar Asam Fitat Tepung Jagung Selama Proses Fermentasi menggunakan Ragi Tape. Lampung, Seminar Nasional Serelia 2011. pp. 590-597

Augusta, J., Muller, R. and Widdecke, H. A. (1993). Rapid Evaluation Plate-Test for the Biodegradability of Plastics. Applied microbiology and biotechnology, 39, pp.673-678.

Cappucino, J. and Sherman, N. (1987). Microbiology: A Laboratory Manual 10th. California. Cummings Publishing Company. pp.161-201

Dey, S., Ashok, K. and Singh, G. (2016). Biodegradation Ability of Bacteria on Plastic and Thermocol Cups. European Journal of Biomedical and Pharmaceutical Sciences, 3(10), pp. 272-277.

Effendi, H. (2003). Telaah Kualitas Air Bagi Pengelolaan Sumber Daya dan Lingkungan Perairan. Yogyakarta. Kanisius, pp. 59-61.

Elfarisna, R.T.P. dan Nosa, T.P. (2014). Isolasi Mikroba yang Dapat Menghilangkan Bau pada Pupuk Organik Air Limbah Cucian Beras. Jurnal Matematika, Sains, dan Teknologi, pp. 91-96.

Evans, J.J., Klesius, P.H. and Shoemaker, C.A., (2004). Starch hydrolysis testing of multiple isolates for rapid differentiation of Streptococcus iniae. Bulletin-European Association of Fish Pathologists, 24(5), pp.231-239.

Facklam, R. and Elliott, J.A., (1995). Identification, classification, and clinical relevance of catalase-negative, gram-positive cocci, excluding the streptococci and enterococci. Clinical microbiology reviews, 8(4), pp.479-495.

Filayani, M.I., (2019), September. Proses Degradasi Plastik Jenis Polietilen Menggunakan Tanah Tempat Pembuangan Sampah (TPS) Iain Tulungagung Dan Yakult Menggunakan Kolom Winogradsky. In Prosiding Seminar Nasional Hayati, 7, pp. 40-45.

Fitrianto, L., Sukarsono dan Rofieq, A. (2016). Pengaruh Konsentrasi Mikroorganisme Lokal Feses Sapi Terhadap Kandungan Gas Metan dari Sampah Organik Pasar. In Prosiding Seminar Nasional, 2, pp. 915-920.

Gupta, K., Deepa, D. and Deepanshu, R. (2016). Isolation and Screening of Low Density Polyethylene (LDPE) Degrading Bacterial Strains from Waste Disposal Sites. 5(11), pp. 1633-1643.

Harley, J. (2005). Laboratory Exercises in Microbiology 6 ed. New York. McGraw Hill New York, pp. 133-137.

Hemraj, V., Diksha, S. and Avneet, G. (2013). A Review on Commonly Used Biochemical Test for Bacteria. Innovate Journal of Life Sciences, 1, pp. 1-7.

Iswadi. (2016). Fage Litik Spesifik Escherichia coli Pada Limbah Cair Pasar Tradisional di Kota Banda Aceh. Jurnal Biotik, 4(2), pp. 95-99.

Jeksen, J. dan Mutiara, C. (2018). Pengaruh Sumber Bahan Organik yang Berbeda terhadap Kualitas Pembuatan Mikroorganisme Lokal (LMO). AGRICA, 11, pp. 60-72.

Kathiresan, K. (2003). Polythene and Plastics Degrading Microbes from the Mangrove Soil. Revista de Biologia Tropical, 51, pp. 3-4.

Kunlere, I.O., Fagade, O.E. and Nwadike, B.I., (2019). Biodegradation of low density polyethylene (LDPE) by certain indigenous bacteria and fungi. International Journal of Environmental Studies, 76(3), pp.428-440.

Lubis, M., Harahap, M.B., Ginting, M.H.S., Sartika, M. and Azmi, H. (2018). Production of bioplastics from avocado seed starch reinforced with microcrystalline. Journal of Engineering Science and Technology (JESTEC), 13(2), pp. 381-393.

Lubis, Z. (2020). Pemanfaatan Mikroorganisme Lokal (LMO) dalam Pembuatan Kompos. Prosiding Seminar Nasional Hasil Pengabdian 2020, p. 362.

Mardalena, (2016). Fase Pertumbuhan Isolat Bakteri Asam Laktat (BAL) tempoyak asal jambi yang disimpan pada suhu kamar. Jurnal Sains Peternakan Indonesia (JSPI), 11(1), pp. 58-66.

Marjayandari, L. dan Maya, S. (2015). Potensi Bakteri Bacillus sp. dalam Mendegradasi Plastik. Jurnal Sains dan Seni ITS, 2, pp. 59-62.

Marzuqi, M., Kasa, I.W. and Giri, N.A., (2019). Respons pertumbuhan dan aktivitas enzim amilase benih ikan bandeng (Chanos chanos Forsskal) yang diberi pakan dengan kandungan karbohidrat yang berbeda. Media Akuakultur, 14(1), p.31.

Marsiningsih, N., Suwastika, A. dan Sutari, N. (2015). Analisis Kualitas Larutan LMO (Mikroorganisme Lokal) Berbasis Ampas Tahu. E-Jurnal Agroekoteknologi, 4, pp. 180-190.

Ni Kadek, M. S., I Gusti, K. S. dan I Nengah, K. B. (2018). Isolasi dan Identifikasi Bakteri Streptococcus sp. pada Babi Penderita Porcine Respiratory Disease Complex. Buletin Veteriner Udayana, 13, pp. 174-181.

Palupi, N.P., (2015). Karakter kimia kompos dengan dekomposer mikroorganisme lokal asal limbah sayuran. Ziraa'ah Majalah Ilmiah Pertanian, 40(1), pp.54-60.

Reddy, N.S., Nimmagadda, A. and Rao, K.S., (2003). An overview of the microbial α-amylase family. African journal of biotechnology, 2(12), pp.645-648.

Rolfe, M.D., Rice, J.C.J., Lucchini, S., Pin, C., Thompson, A., Cameron, A.D.S., Alston, M., Stringer, M.F., Betts, R.P., Baranyi, J., Peck, M.W. and Hintona, J.C.D. (2012). Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. Journal of Bacteriology, 194(3), pp. 686-701.

Sachan, S., Iqbal, M. and Singh, A. (2018). Extracellular Lipase from Pseudomonas Aeruginosa JCM5962(T): Isolation, Identification and Characterization. International Microbiology, 4, pp. 197-205.

Septiani, D. E. N. dan Wijayanti, I. (2017). Aktivitas Antibakteri Ekstrak Lamun (Cymodocea rotundata) terhadap Bakteri Staphylococcus aureus dan Escherichia coli. Saintek Perikanan, 13(1), pp. 1-6.

Singh, P. and Prakash, A., (2008). Isolation of Escherichia coli, Staphylococcus aureus and Listeria monocytogenes from milk products sold under market conditions at Agra region. Acta agriculturae Slovenica, 92(1), pp.83-88.

Subhankari, P. C., Santanu, K. M. and Somenath, R. (2011). Biochemical Characters and Antibiotic Susceptibility of Staphylococcus aureus Isolates.
Toelle, N. N. dan Lenda, V. (2014). Identifikasi dan Karakteristik Staphylococcus sp. dan Streptococcus sp. dari Infeksi Ovarium pada Ayam Petelur Komersial. Jurnal Ilmu Ternak, 1, pp. 32-37.

Türker, C. and Özcan, B.D., (2015). Isolation of Alpha-amylase producing thermophilic bacillus strains and partial characterization of the enzymes. annals of agricultural sciences, 3(6), pp.387-393.

Usha, R., Sangeetha, T. and Palaniswamy, M., (2011). Screening of polyethylene degrading microorganisms from garbage soil. Libyan agriculture research center journal international, 2(4), pp.200-204.

Wahyuningsih, N. dan Zulaika, E. (2018). Perbandingan Pertumbuhan Bakteri Selulolitik Pada Media Nutrient Broth dan Carboxy Methyl Cellulose. Jurnal Sains dan Seni ITS, 7(2), pp. E36-E38.

Waluyo, L. 2009. Mikrobiologi Lingkungan. Malang. UMM Press, pp. 266-270.

Walyono, L. (2007). General Microbiology. Revised Edition. Universitas Muhammadiyah, pp. 148.

Yuliana, M., (2021). The effect of local microorganism (MOL) as liquid organic fertilizer to the growth of Ipomea reptans Poir. Jurnal Biota, 7(1), pp.51-56.

Yunilas, Y., Siregar, A.Z., Mirwhandhono, E., Purba, A., Fati, N. and Malvin, T., (2022). Potensi dan karakteristik larutan mikroorganisme lokal (MOL) berbasis limbah sayur sebagai bioaktivator dalam fermentasi. Journal of Livestock and Animal Health, 5(2), pp.53-59.

Yunita, M., Hendrawan, Y. and Yulianingsih, R., (2015). Analisis kuantitatif mikrobiologi pada makanan penerbangan (Aerofood ACS) garuda Indonesia berdasarkan TPC (Total Plate Count) dengan metode pour plate. Journal of Tropical Agricultural Engineering and Biosystems-Jurnal Keteknikan Pertanian Tropis dan Biosistem, 3(3), pp.237-248.

Zusfahair, Z., Lestari, P., Ningsih, D.R. and Widyaningsih, S., (2007). Biodegradasi polietilena menggunakan bakteri dari TPA (tempat pembuangan akhir) Gunung Tugel Kabupaten Banyumas. Molekul, 2(2), pp.98-106.