Exploring Microbial Solutions: Effective Biodegradation of Low-Density Polyethylene (LDPE) Plastic Using Bacteria Isolated from the Surabaya River, Indonesia

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***Abstract*—Low-Density Polyethylene (LDPE) is a compound composed of long chains of ethylene molecules (C_2H_4)n. LPDE can contaminate the environment and pose health risks due to the presence of additive plasticizers capable of bonding with heavy metals. Therefore, this study aimed to evaluate the efficiency of bacteria in degrading LDPE. The applied methods included analysis of the difference in the dry plastic weight and chemical structure changes, which was performed using Fourier Transform Infrared (FT-IR) instrument. Furthermore, bacterial isolates were obtained from the surface of Surabaya River in Indonesia and purified for plastic degradation tests over a period of 30 days. The results showed that isolate S2 had a more effective capacity to degrade LDPE compared to S1 and S3. Isolate S2 achieved a reduction in the dry weight of plastic by 5.979%, while S1 and S3 had reductions of 0.638% and 0.264%, respectively. This reduction was reflected in changes to LDPE chemical structure, marked by lower intensity in hydroxyl (-OH), C-H, C=C, and C-O bonds compared to pure LDPE. The results suggested the potential of bacteria as agents for bioremediation in addressing future plastic pollution issues.**

***Keywords*—biodegradation, LDPE, plastic pollution, Surabaya River**

I. Introduction

Low-Density Polyethylene (LDPE) plastic is among the most widely used plastic materials in daily life. This was first produced in 1933 by Imperial Chemical Industries (ICI) as a thermoplastic polyethylene type. The structure of LDPE consists of long molecular chains based on ethylene with the chemical formula (C_2H_4)n. The melting point is around 115°C and highly resistant to chemical compounds at temperatures below 60°C. Additionally, the relatively low density, ranging between 0.91–0.94 g cm^{-3}, leads to flexible and easy to shape. The semi-crystalline structure, comprising 50–60% crystalline parts, provides sufficient mechanical properties for various applications [1]. Global LDPE production steadily increased over the years considering that in 2015, the annual production reached 64 million tons. This increase is due to the widespread use in various products, such as shopping bags, food wrappers, beverage bottles, milk carton liners, and cable coatings [2]. The extensive production and use significantly contribute to global plastic waste. According to [3], approximately 23% of total plastic waste in landfills consists of LDPE, showing waste management inefficiencies.

LDPE has a unique chemical polymer structure with strong carbon-carbon (C-C) bonds, promoting resistance to decomposition by environmental factors such as microorganisms and ultraviolet light [4]. This property makes LDPE a primary contributor to the accumulation of plastic waste in the environment. A previous study [5] showed that PE adsorbed heavy metals such as Cu (Copper), Zn (Zinc), Ni (Nickel), and Pb (lead) from polluted water bodies. The heavy metals accumulated on LDPE surface are often found in the form of microplastics, which can enter the food chain and lead to bioaccumulation in organisms, including humans [6]. LDPE plastic often contains additives such as plasticizers to enhance flexibility and durability. However, plasticizers in the form of diethyl phthalate (DEP), dimethyl phthalate (DMP), and dibutyl phthalate (DBP) are toxic and capable of disrupting the endocrine systems of aquatic organisms and humans [7, 8]. These substances can leach into water bodies, pollute the environment, and harm ecosystems.

Investigations on plastic biodegradation by microorganisms offer a promising solution to the growing pollution issues. Some microorganisms have been proven to possess the ability to degrade plastic polymers, including LDPE, through enzymatic activity [9, 10]. Enzymes such as lipase, laccase, alkane hydroxylase, and serine hydrolase play a crucial role in breaking down the long polymer chains of LDPE into smaller molecules such as oligomers, dimers, and monomers [11]. These molecules can be metabolized by microorganisms into environmentally friendly end products such as carbon dioxide and water [12]. Recent studies suggest that the enzyme proteinase K can enhance LDPE biodegradation efficiency. Stepczyńska and Rytlewski [13] reported that the use of this enzyme in biodegradation processes accelerated the breakdown of polymer chains. Furthermore, Ru *et al.* [14] found that bacteria isolated from polluted aquatic environments had significant potential in degrading LDPE plastic. These results show that biodegradation processes can be optimized by using microorganisms adapted to plastic-rich environments.

The application of LDPE biodegradation on a large scale still faces several challenges. Factors such as temperature, pH, oxygen availability, and enzyme concentration influence the efficiency of natural biodegradation processes [15]. Moreover, the development of genetically engineered microorganisms with higher degradation efficiency is in the early stages. A multidisciplinary method including biotechnology, environmental chemistry, and material engineering is essential to address these challenges. In the

context of sustainability, plastic waste management based on biodegradation corresponds with the circular economy principles. The use of plastic waste as raw material for the production of new products reduces the waste accumulation and supports resource efficiency [16]. Biodegradation technology application in plastic waste management can be a crucial stage toward achieving Sustainable Development Goals (SDGs), particularly in reducing marine and aquatic ecosystem pollution, which shows a need for investigations on LDPE plastic biodegradation using bacteria. Therefore, this study aimed to provide solutions to the issue of plastic waste accumulation and offers an alternative for more environmentally friendly waste management. New insights will be provided into the potential of bacteria isolated from the surface water of Surabaya River in efficiently degrading LDPE plastic. The promising results can serve as a foundation for the development of future biodegradation technologies.

II. Experimental Materials and Chemicals

A. Material

The chemicals used in this study included Plastic (LDPE), Distilled Water (aquades), Nutrient Agar (NA), Nutrient Broth (NB), magnesium sulfate ($MgSO_4$), calcium chloride ($CaCl_2$), potassium dihydrogen phosphate (KH_2PO_4), potassium hydrogen phosphate (K_2HPO_4), ammonium nitrate (NH_4NO_3), and iron(II) chloride ($FeCl_2$). Moreover, all the materials were of analytical grade and did not require further purification.

B. Sampling Methods

LDPE plastic waste was collected from Surabaya River, located in Bambe Village, Driyorejo District, Surabaya, Indonesia, at coordinates 7°21'06.3"S and 112°39'43.9"E (Fig. 1). To collect the samples, a net was installed across the river, extending 3 m towards the center. The net was left in position for 1 week to capture LDPE plastic bags, among which those trapped at a depth of 0.5 m were retrieved and stored in an ice box for transport to the laboratory.

C. Initial Plastic Sample Preparation

LDPE plastic samples were sterilized through swabbing with a sterile cotton bud dipped in a test tube containing 10 mL of sterile distilled water, followed by vortexing for 1 minute. A 1 mL aliquot of the suspension was transferred into a second test tube containing 9 mL of sterile distilled water and vortexed (10^{-1} dilution). Subsequently, 1 mL from the first dilution was transferred to another test tube containing 9 mL of sterile distilled water and vortexed (10^{-2} dilution). A 1 mL aliquot of the second dilution was inoculated onto NA medium using the spread plate method. The resulting colonies were then purified using a 16-streak method until pure isolates were obtained.

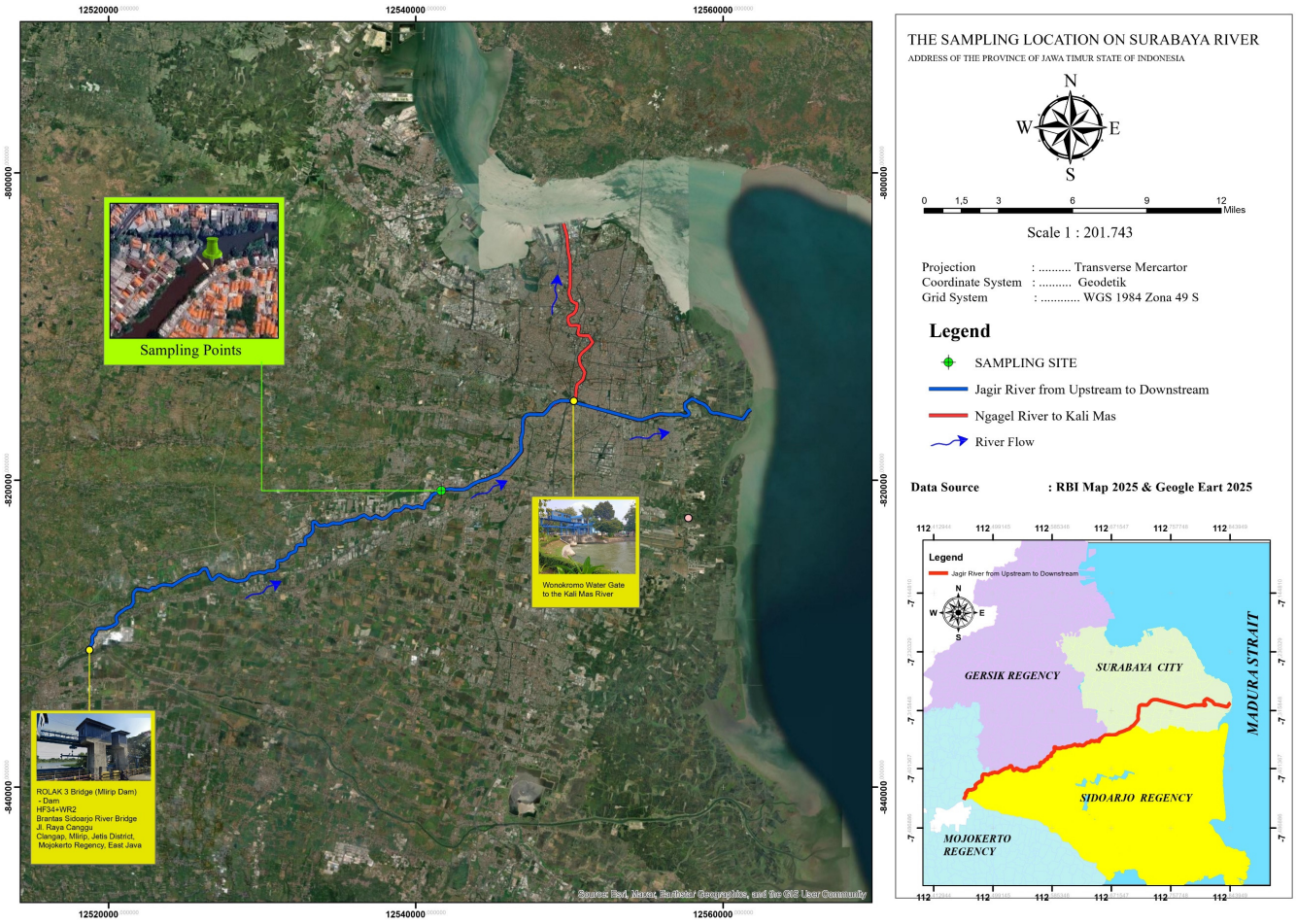

Fig. 1. The sampling location on Surabaya River (ArcGIS 10.8).

D. Gram Staining

A drop of sterile distilled water was positioned in the center of a glass slide, followed by the addition of an inoculation loop of bacterial culture through spreading in a

zigzag pattern. The prepared sample was fixed by passing it over a Bunsen burner until the water dried, then stained with crystal violet for 1 min, rinsed using distilled water, and treated with iodine for another minute. This sample was rinsed with distilled water, washed in 70% alcohol, rinsed again using distilled water, stained with safranin for 45 s, dried, and observed under a microscope.

E. Bacteria Adaptation

Bacterial adaptation process used NB and Mineral Salt Medium (MSM) in ratios of 1:1 and 1:2, with a final volume of 150 mL for each medium. MSM was prepared with the following composition (g L^{-1}), including $MgSO_4$ 0.2 g, $CaCl_2$ 0.02 g, KH_2PO_4 1 g, K_2HPO_4 1 g, NH_4NO_3 1 g, and $FeCl_2$ 0.02 g. Pure isolates were inoculated into 100 mL of NB and incubated for 24 h. Approximately 15 mL of this culture was transferred to the 1:1 medium and incubated for 2 days. Another 15 mL was transferred to the 1:2 medium and incubated for 2 days, then the adapted isolates were used for further experiments.

F. Plastic Preparation

LDPE plastic samples were cut into 11 cm (10 pieces) and measured using an analytical balance to determine the initial weight. The pieces were sterilized with 70% alcohol, followed by exposure to UV light for 1 hour, and drying in a desiccator for 24 h.

G. Biodegradation Test

A total of 15 mL adapted bacterial culture was transferred into an Erlenmeyer flask containing 135 mL of MSM, which had a pH of 7 and did not contain any carbon source. The 10 sterilized plastic pieces were added to the Erlenmeyer flask and incubated at room temperature (25–30°C) with 125 rpm agitation for 30 days, then the process was repeated in triplicate.

H. Total Plate Count (TPC) Analysis

Incubated plastic samples were aseptically removed and transferred into test tubes containing 10 mL of sterile solution, then vortexed for 5 minutes. A 1 mL suspension was transferred into another test tube containing 9 mL of sterile solution and vortexed (10^{-1} dilution). A series of dilutions were performed until 10^{-4}, and 1 mL suspension of the last dilution was inoculated into Petri dishes containing liquid NA medium using the pour plate method.

I. Weight Loss Analysis

The plastic samples were dried in an oven at 80°C for 24 h and weighed using a high-precision analytical balance. Subsequently, the weight loss was calculated using the following Eq. (1).

$$Weight\ loss = \frac{W_0 - W_t}{W_0} \times 100\% \quad (1)$$

W_0: initial weight (mg)
W_t: final weight (mg)

J. Fourier Transform Infrared (FT-IR) Analysis

A single piece of incubated plastic was analyzed using FT-IR spectrophotometer (Nicolet Is50, Thermo Scientific, Massachusetts, USA). The absorbance was measured in the mid-IR region with a wavelength range of 400-4000 cm^{-1}.

III. Result and Discussion

This study successfully separated and purified three isolates, consisting of two coccus-shaped and one rod-shaped bacteria. Based on Gram staining results, two isolates were classified as Gram-positive, while the other was Gram-negative, as presented in Table 1 and Fig. 2. The distinction between Gram-positive and Gram-negative bacteria is based on the peptidoglycan layer thickness in the cell walls. Gram-positive bacteria possess a significantly thicker peptidoglycan, comprising more than 30 layers, compared to Gram-negative which have a thinner peptidoglycan layer covered by an outer membrane.

Table 1. Bacteria isolated from Surabaya River

Isolate	Color	Elevation	Colony	Margin	Shape	Gram
S1	Cream	Convex	Round	Lobate	rod	-
S2	White	Raised	Round	Entire	coccus	+
S3	Cream	Convex	Round	Entire	coccus	+

The thicker peptidoglycan layer in Gram-positive bacteria provides an advantage in certain environmental conditions, such as during the plastic biodegradation process. The robust Gram-positive cell wall structure facilitated the production of enzymes needed to degrade plastic polymers. The ability of bacteria to degrade plastics largely depends on the specific enzymes produced [10].

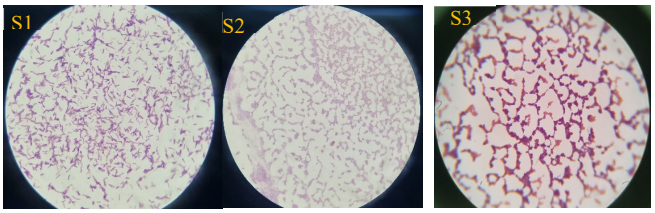

Fig. 2. Gram bacteria isolated from Surabaya River.

Three isolates were acclimatized/adapted before being used in biodegradation test. Additionally, bacterial cell density as a growth indicator is determined through Optical Density (OD) test, and the acclimatization results are presented in Table 2.

Table 2. Absorbance of adaptation stage

Isolates	Volume Rasio of NB and MSM		
	1:0	1:1	1:2
S1	0.806	1.093	0.015
S2	1.256	1.150	0.016
S3	1.311	1.363	0.068

The acclimatization stage is essential for bacteria application in biodegradation process but often influenced by several factors such as the age of isolates, nutrients, and growth-limiting factors [17]. Three isolates showed large OD values at 1:1 and 1:2 with an incubation time of 48 hours. This suggests that bacteria are metabolically ready even though the amount of carbon in the medium is decreasing. The increase in OD values occurs because the cells can adapt and utilize the available carbon.

The three isolates showed varying abilities to degrade LDPE plastic, and according to Fig. 4, S2 had the highest growth compared to S1 and S3. The number of bacterial colonies on LDPE plastic surface during incubation (Table 3) signifies differences in adaptation capabilities among the isolates. This adaptation enables bacteria to use plastic as a

source of energy and carbon, supported by enzymatic activity to break down plastic polymer chains into smaller molecules including monomers and oligomers [18, 19].

Table 3. Number of bacterial colonies on plastic surfaces

Isolates	Number of bacterial colonies (cfu/ml)
S1	32×10^5
S2	50×10^5
S3	42×10^5

The results in Table 3 showed that S2 generated the highest number of colonies, contributing to a higher rate of LDPE biodegradation compared to the other isolates. LDPE degradation rate by S2 reached 5.979%, which was higher than the rates observed for S1 (0.638%) and S3 (0.264%), suggesting the greater potential of S2 to use LDPE as a substrate. According to a previous study [20], enzymes such as laccase and lipase play a critical role in breaking down plastic polymers into simpler compounds. Lipase is a group of hydrolase enzymes essential in breaking down the carbon backbone of synthetic polymers. The degradation of polymeric materials is attributed to the generated assistance of lipase in chemical bond hydrolysis. The long carbon chains present in polymeric materials are broken down by lipases (Fig. 3).

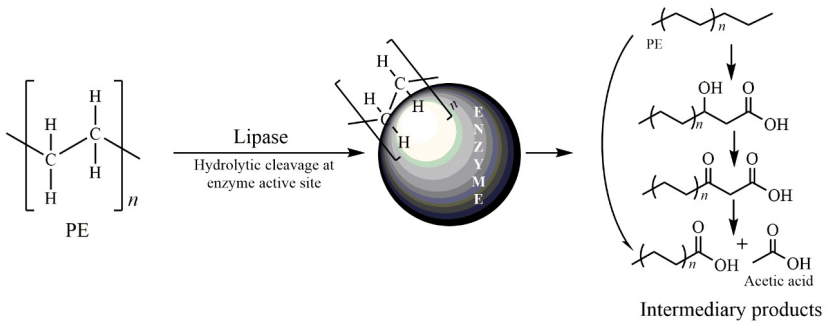

Fig. 3. Degradation pathway for PE by Lipase [21].

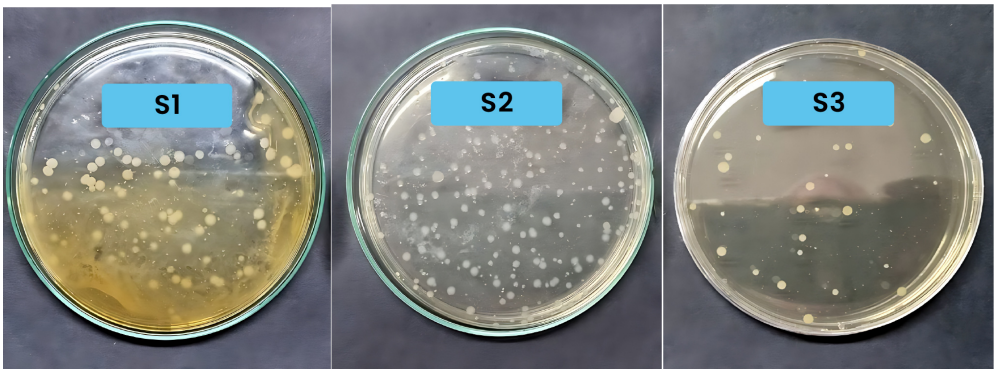

Fig. 4. Bacterial growth on the surface of LDPE plastic.

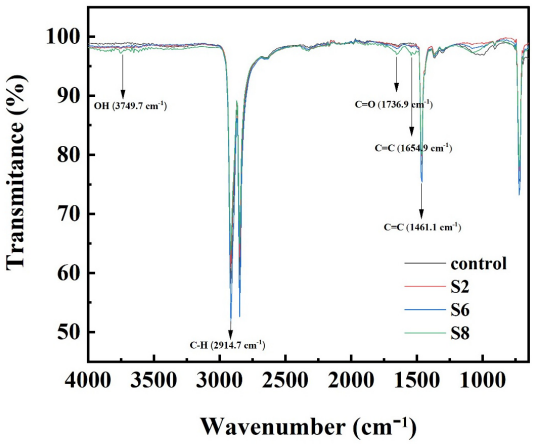

Fig. 5. FT-IR spectra of LDPE plastic biodegradation by isolated bacteria.

Chemical analysis of LDPE plastic using FT-IR identified structural changes in the plastic after biodegradation process (Fig. 5). These changes were evident from the reduction in peak intensity in certain FT-IR wavelength ranges. For example, the decrease in intensity at 3392–2994 cm^{-1} showed alterations in hydroxyl (-OH) groups, signifying enzymatic reactions on the plastic polymer [22]. Significant changes in the 2930–2835 cm^{-1} and 1476–1446 cm^{-1} wavelength ranges implied stretching in C-H and C=C bonds, suggesting polymer chain breakdown [23].

The results were consistent with previous studies showing the role of enzymes such as alkane hydroxylase and serine hydrolase in plastic degradation. These enzymes can cleave LDPE polymer chains into smaller compounds suitable for easy metabolism by bacteria [19]. Furthermore, significant changes in the 1294–811 cm^{-1} wavelength range represent stretching in C-O bonds, which is another sign of oxidative degradation. The differences in transmission in FT-IR spectra suggest that bacteria reduce the mass of plastic and alter the chemical structure. This evidence shows bacterial biodegradation includes complex enzymatic reactions that transform the physical and chemical properties of LDPE plastic. Biodegradation process minimizes the environmental impact of plastic waste and converts it into simpler, more environmentally friendly compounds [18].

S2 showed the highest potential as an agent for LDPE plastic biodegradation. This supported previous results suggesting that Gram-positive bacteria were more effective at degrading plastics due to the ability to produce larger quantities of enzymes [18]. However, the effectiveness of

plastic biodegradation is influenced by environmental conditions such as temperature, pH, and oxygen availability. Z. Wu *et al.* (2023), reported that controlled environmental conditions could enhance bacterial enzyme activity by two to three times [15]. This study provides new insights into the potential of bacteria as an environmentally friendly solution to address LDPE plastic waste. Bacteria can be used as key agents to process plastic waste into more useful products through further development of integrated biodegradation technology. In the future, industrial-scale applications including bacteria such as isolate S2 tend to become an effective stage in implementing sustainable biotechnology-based plastic waste management.

IV. Conclusion

In conclusion, this study successfully isolated and characterized three strains, comprising two Gram-positive coccus-shaped and one Gram-negative rod-shaped bacteria. The distinct structural differences between Gram-positive and Gram-negative bacteria, particularly the peptidoglycan layer thickness, contributed to the varying effectiveness in plastic biodegradation. Among the isolates, S2 had the highest potential for degrading LDPE plastic, with a rate of 5.979%, significantly outperforming S1 (0.638%) and S3 (0.264%). This showed that S2 was capable of using LDPE as a carbon and energy source more efficiently. FT-IR analysis identified structural changes in LDPE plastic after bacterial degradation, including alterations in hydroxyl (-OH) groups, stretching of C-H and C=C bonds, as well as oxidative modifications in C-O bonds. These changes signified the enzymatic activity of the isolates which degraded polymer chains into smaller, simpler molecules, showing plastic waste mass reduction by bacterial biodegradation and the chemical transformation into environmentally friendly compounds. The results emphasized the potential of bacteria, particularly S2, as an effective agent for LDPE plastic biodegradation. This study would contribute to the growing body of evidence supporting the use of microorganisms in managing plastic waste sustainably. Additionally, valuable insights were provided into the potential of bacterial isolates for addressing the global issue of plastic pollution. The results formed a foundation for developing environmentally friendly, biotechnology-based solutions to mitigate the accumulation of plastic waste, promoting a sustainable approach to waste management.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All authors contributed to the study. M.S. and A.Y led and designed experiment. A.W and M.T contributed to the collection of samples, conducted degradation test, analyzed of the result and wrote the first draft of the manuscript. E.S.S provided general supervision of the work. All author had approved the final version.

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