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Accelerated degradation of low-density polyethylene by bacteria isolates: Insights from Surabaya River, Indonesia

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ABSTRACT

Low-density polyethylene (LDPE) plastic bags were the dominant type of waste in Surabaya River, accounting for 21.16 particles/m^3 in 2021. For both the environment and human health, LDPE poses a significant risk, as it contains plastic additives (plasticizers) with the ability to bind heavy metals such as copper (Cu), zinc (Zn), nickel (Ni), and lead (Pb). Therefore, this study aimed to compare the plastic degradation abilities of different bacteria isolates in the Surabaya River. Analysis was carried out to explore bacteria growth on plastic surfaces, changes in the chemical structure of the plastic, and the morphological variations of the plastic surface over a 30-day incubation period. Bacteria isolates were collected from a depth of 1.5 meters in the Surabaya River. The results showed that M2 had the highest bacteria growth on the plastic surface, reaching 42×10^4 cfu/mL. Additionally, isolates M2, M3, and M4 were able to stretch the plastic's chemical structure affecting O-H, C-H, C=C, and C-O groups, thereby causing visible damage to the plastic surface. The 16S RNA sequencing identified isolates M2, M3, and M4 as *Bacillus cereus* and *Pseudomonas sihuiensis*. Based on bacteria growth, changes in the plastic chemical structure, and surface damage, the results suggested that *Bacillus cereus* was more effective in degrading plastic than *Pseudomonas sihuiensis*.

Keywords: *Bacillus cereus*, low-density polyethylene, plastic degradation, *Pseudomonas sihuiensis*, Surabaya River.

INTRODUCTION

Indonesia is ranked third worldwide in plastic waste production, with Surabaya producing approximately 111,300 tons of waste in 2021. As the third-largest city in the country, Surabaya generated a significant amount of waste in 2022 comprising plastic bags, single-use food containers, sanitary products, drinking bottles, styrofoam, trays, and plastic bottles. This waste pollute the river severely, containing 21.16 microplastic particles per cubic meter made of Low-Density Polyethylene (LDPE) (Lestari et al., 2020). As thermoplastic produced by Imperial Chemical Industries (ICI) in 1933, LDPE is composed of long chains of ethylene molecules, with a melting point of 115 °C and high resistance to chemical reactions at temperatures below 60 °C. Furthermore, LDPE is approximately 50–60% crystalline at a density ranging from 0.91 to 0.94 g/cm^3 (Uthpalani et al., 2022). In 2015, global LDPE production was 64 million tons per year (Geyer et al., 2017). This figure is expected to rise because of daily application in products such as plastic bags, food packaging, milk carton liners, beverage cups, plastic wraps, and wire insulation. LDPE constitutes approximately 23% of the total plastic waste disposed of in landfills (Sánchez-Duque et al., 2020). The strong chemical structure including long chains

of carbon-carbon and carbon-hydrogen bonds, makes LDPE resistant to natural degradation. It can also absorb heavy metal contaminants such as copper, zinc, nickel, and lead, which are often present in water (Khadija et al., 2021; Lin et al., 2023), potentially introducing toxicity into the food chain (Lin et al., 2021). LDPE contains plasticizers called phthalates, which increase the strength and flexibility of plastics. However, phthalates like diethyl phthalate (DEP), dimethyl phthalate (DMP), and dibutyl phthalate (DBP) can affect humans and aquatic life (Bridson et al., 2021), potentially damaging the endocrine systems of aquatic organisms (Mathieu-Denoncourt et al., 2015).

Although plastic is difficult to biodegrade due to the robust polymer structure, certain microorganisms can break down (Atanasova et al., 2021; Jeon et al., 2021; Ru et al., 2020; Wani et al., 2016; Wu et al., 2023). Enzymes such as lipases, alkane hydroxylases, lignolytic enzymes, laccases, serine hydrolases, esterase, and proteinase K are produced by microorganisms (Salinas et al., 2024; Stepczyńska and Rytlewski, 2018) to break down polymers into smaller molecules such as oligomers, dimers, and monomers, which release carbon dioxide into the environment or bacteria medium (Rana et al., 2022; Rauscher et al., 2023). Therefore, this study aimed to compare the plastic degradation abilities of different bacteria isolates in the Surabaya River. The analysis provided valuable insights into the biodegradation process by exploring the of *Bacillus cereus* and *Pseudomonas sihuiensis* to degrade LDPE. The results showed the effectiveness of certain bacteria isolates in breaking down plastic and provided strategies for mitigating plastic pollution as well as the harmful effects on ecosystems and human health.

EXPERIMENTAL MATERIALS AND CHEMICALS

Materials

In this study, the materials used were plastic bags, nets, distilled water, nutrient agar (NA), nutrient broth (NB), $MgSO_4$ (99.5%), $CaCl_2$ (99%), KH_2PO_4 (99.5%), K_2HPO_4 (99%), NH_4NO_3 (99.8%), and $FeCl_2$ (99%). All chemicals were supplied by Merck and did not require additional purification.

Methods

In this study, several stages were included to explore the ability of bacteria isolates to degrade LDPE originating from the Surabaya River. The process started with sampling and all the methods are summarized in the flow chart (Figure 1).

Sampling methods

LDPE plastic bag waste was collected from the Surabaya River in Bambe Village, Driyoredjo District, Surabaya, Indonesia, at coordinates 7°21'06.3" S, 112°39'43.9" E (Figure 2). A net was placed across the middle of the river, covering a 3-meter stretch, for 7 days to capture plastic bag waste. The plastic bags were collected at a depth of 1.5 meters.

Bacteria isolation and purification

Plastic samples were swabbed using a sterile cotton bud, placed in a test tube containing 10 mL of sterile distilled water, and vortexed for 1 minute. From this suspension, 1 mL was transferred to a test tube with 9 mL of sterile distilled water for a 10^{-1} dilution and vortexed. Furthermore, 1 mL of the 10^{-1} dilution was transferred to another test tube containing 9 mL of sterile distilled water for a 10^{-2} dilution. The 10^{-2} dilution

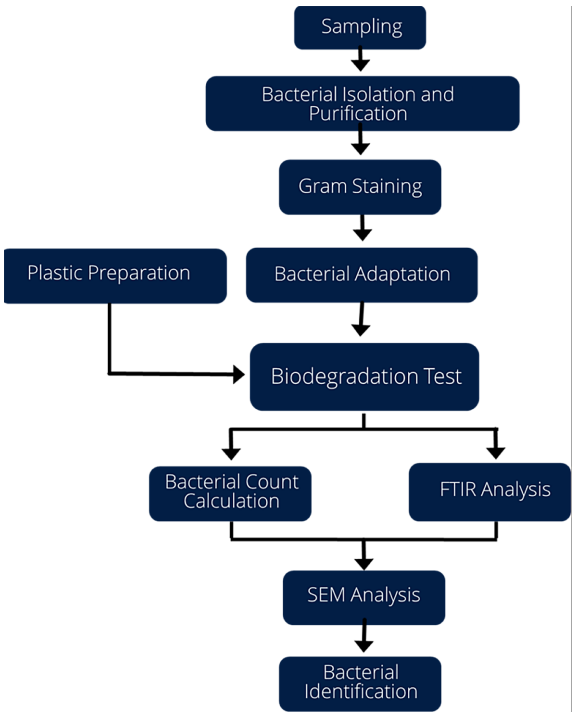

Figure 1. Flow chart biodegradation LDPE analysis

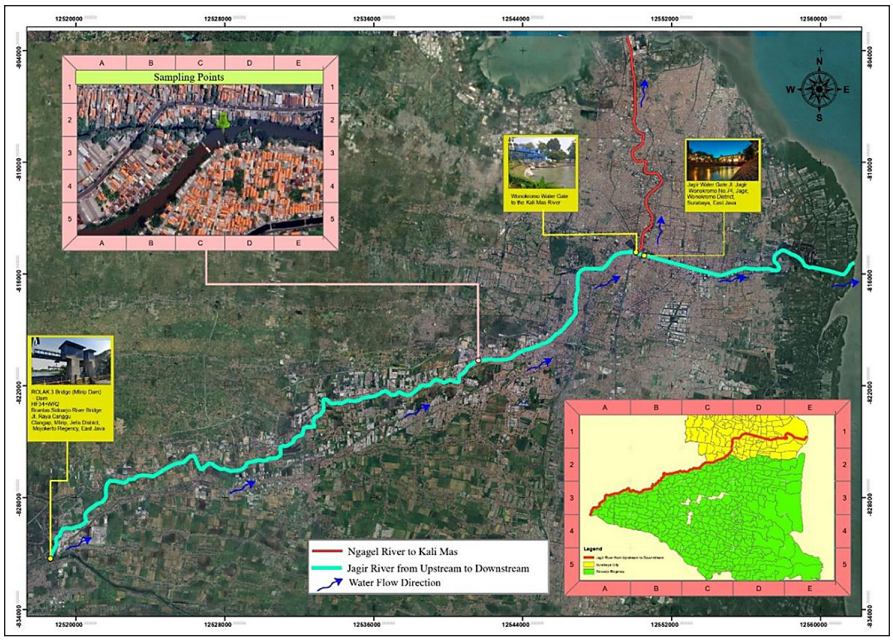

Figure 2. Sampling location Bambe Village, Driyoredjo District, Gresik Regency, East Java Province, Indonesia

was inoculated onto NA medium using the spread plate method. The growing isolates were purified using the streak plate method until a pure colony was obtained.

Gram staining

Distilled water was dropped into the center of a glass slide, followed by the addition of an inoculation loop of bacteria culture, which was spread in a zigzag pattern. The prepared sample was fixed by passing it over a bunsen burner until the water dried. This was followed was followed staining with crystal violet for one minute, rinsing with distilled water, and treating with iodine for another minute. After one minute, the prepared sample was rinsed with distilled water, followed by washing with 70% alcohol. The prepared sample was rinsed again with distilled water, stained with safranin for 45 seconds, dried, and observed under a microscope.

Bacteria adaptation

For bacteria adaptation, NB and mineral salt medium (MSM) were mixed in a 1:1 and 1:2 ratio. MSM was prepared with the following composition: $MgSO_4$ (0.2 g/L), $CaCl_2$ (0.02 g/L), KH_2PO_4 (1 g/L), K_2HPO_4 (1 g/L), NH_4NO_3 (1 g/L), and $FeCl_2$ (0.02 g/L), with a final volume of 150 mL. Pure bacteria isolates were first cultured in 100 mL of NB for 24 hours. Subsequently, 15 mL of the culture was transferred to the 1:1 medium and incubated for 2 days. The culture was transferred to the 1:2 medium for an additional 2 days of incubation. The adapted isolates were ready for further testing.

Plastic preparation

LDPE plastic bags were cut into 1x1 cm pieces, sterilized with 70% alcohol, and exposed to UV light for 1 hour. The sterilized plastic pieces were dried in a desiccator for 24 hours.

Biodegradation test

A total of 15 mL bacteria isolates were taken and put into a 150 mL Erlenmeyer flask containing 135 ml of sterile MSM (the volume of bacteria isolate was 10% of the total volume). A total of 10 pieces of sterilized plastic were placed into the flask, which was incubated at room temperature (25–30 °C) with stirring at a speed of 125 rotary per minute for 30 days. To determine the effect of isolates on plastic degradation, treatment samples were compared to the control (without isolates), and the process was repeated three times.

Bacteria count calculation

After incubation, the plastic samples were aseptically removed and placed in a test tube containing 10 mL of sterile biological solution.

The mixture was homogenized by vortexing for 5 minutes. Subsequently, 1 mL of the suspension was transferred to a test tube containing 9 mL of sterile biological solution for a 10^{-1} dilution, and dilutions were made to approximately 10^{-4}. At the 10^{-4} dilution, 1 mL of the suspension was inoculated into Petri dishes containing NA medium using the pour plate method.

Fourier transform infrared (FTIR) analysis

The plastic samples were analyzed using an Agilent Cary 630 FTIR spectrophotometer. Absorbance was measured in the mid-IR range, covering wavelengths from 400-4000 cm^{-1}. The analysis was conducted using Agilent Microlab software.

Scanning electron microscope (SEM) analysis

The morphological changes of the plastic film due to microbial degradation were observed using a SEM Hitachi SU1000. The plastic samples were coated with a thin layer of gold (approximately 9.8 nm) to improve conductivity, and the surface was examined at magnifications ranging from 2.0 K to 40.0 K.

Bacteria identification

Identification of the three most promising bacteria isolates was carried out using the 16S rRNA method, with primers 27F and 1492R. Bacteria identification process was performed at the Equipment and Reagent Laboratory of PT Biotek Prima Indoplus, Sidoarjo, Indonesia.

RESULTS AND DISCUSSION

Isolation and purification of bacteria strain from LDPE waste in Surabaya River

In this study, five bacteria isolates were successfully obtained from plastic bag (LDPE) waste collected from the Surabaya River. Among these, one isolate was coccus-shaped, and the remaining were rod-shaped (Table 1). As observed through Gram staining, there are two Gram-positive and three Gram-negative bacteria isolates (Figure 3). The distinction between both samples is based on the thickness of the peptidoglycan in bacteria cell wall. Gram-negative samples have a layer of peptidoglycan strands that is only a few nanometer thick. Meanwhile, Gram-positive has a layer with thickness ranging from 30 to 100 nm (Rohde, 2019).

The difference in cell wall composition is essential for understanding bacteria behavior, particularly in their ability to degrade plastic. Before the biodegradation test was conducted, the five bacteria isolates were adapted to the MSM, with the results shown in Table 2. Based on Table 2, five bacteria isolates continued to grow in msm (1:2). However, the growth rate decreased because bacteria cells required time to adapt to a medium

Table 1. Plastic degrading bacteria isolates from Surabaya River

Isolate	Color	Elevation	Colony	Margin	Shape	Gram
M1	White	Convex	Irregular	Undulate	Coccus	-
M2	Crem	Umbonate	Circullar	Entire	Rod	+
M3	Crem	Convex	Circullar	Entire	Rod	-
M4	Crem	Convex	Circullar	Entire	Rod	-
M5	Crem	Convex	Circullar	Entire	Rod	+

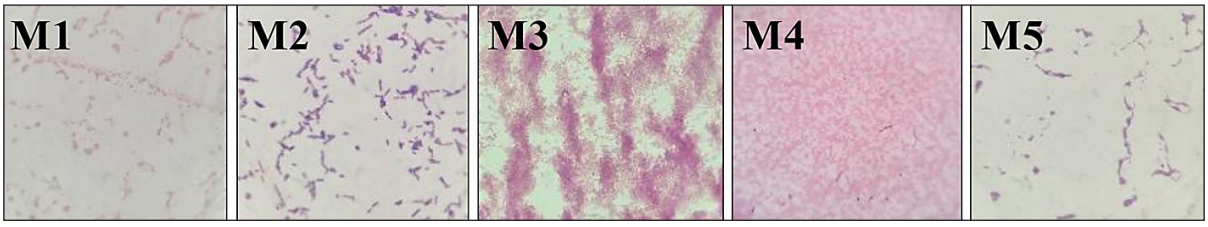

Figure 3. Microscopy of plastic degrading isolates. Red color shows lipid bonding of bacteria membrane with safranin. Blue/bronze color shows peptidoglycan bonding with crystal violet

Table 2. Optical density value during adaptation

Isolates	Volume Rasio of NB and MSM		
	1:0	1:1	1:2
M1	1.143	1.091	0.866
M2	0.740	0.865	0.979
M3	0.752	0.704	0.570
M4	0.784	0.644	0.246
M5	0.516	0.682	0.483

with a low carbon concentration. Bacteria isolates obtained were used for biodegradation tests.

Plastic degradation potential of bacteria isolates

Bacteria isolates showed varying abilities to degrade plastic, a property directly related to the specific enzymes produced (Mohanan et al., 2020). As bacteria isolates grow and adapt during the incubation period, their ability to degrade plastic is shown in the colony count on the plastic surface (Figure 4). However, the growth rate differed, with isolates M2, M1, M3, M4, and M5 showing colony counts of 45×10^4, 42×10^4, 36×10^4, 36×10^4, and 19×10^2 cfu/mL, respectively.

The results showed that M2 had the highest growth potential, followed by M1, M3, M4, and M5. This variation suggested that different bacteria strains used distinct enzymatic mechanisms for plastic degradation, contributing to their varying abilities to adapt and thrive on plastic surfaces. Based on López et al., (2024), a higher bacteria concentration correlated with a greater reduction in plastic mass. FTIR testing was carried out on the plastic biodegradation test on each isolate.

Changes in the chemical structure of LDPE

As shown in Figure 5, changes in the chemical structure of LDPE plastic confirmed bacteria activity on plastic surfaces based on FTIR spectroscopy. At specific wavelength ranges, there were significant peaks, showing alterations in the chemical groups of the plastic. Specifically, a decrease in the hydroxyl group (–OH) was detected at 3392–2994 cm^{-1} (Gao and Sun, 2021; Yang et al., 2021), and stretching of the C-H bond occurred between 2930–2835 cm^{-1} (Jung et al., 2018; Shah et al., 2008). Additional changes were observed in the C=C and C-O bond stretching at 1476–1446 cm^{-1} and 1294–811 cm^{-1}, respectively (Gao et al., 2022; Negi et al., 2011).

The modifications show the breakdown of the polymer chain, suggesting microbial degradation of plastic material (Ali et al., 2023; Khampratueng et al., 2024). The presence of enzymatic processes, such as the reduction or addition of hydroxyl groups, further supports bacteria degradation mechanism (Jin et al., 2023). Based on the results, M2 showed the greatest change in polymer structure. This shows plastic degradation, indicating the need for SEM test.

Surface morphological changes of LDPE

To determine bacteria enzyme activity, changes in plastic surface were analyzed using SEM. As presented by SEM images in Figure 6, plastic exposed to isolates M2 showed significant surface damage. The plastic surface exposed to M2 showed higher plastic surface damage than the control. Bacteria colonization and extracellular enzyme activity on the plastic surface are

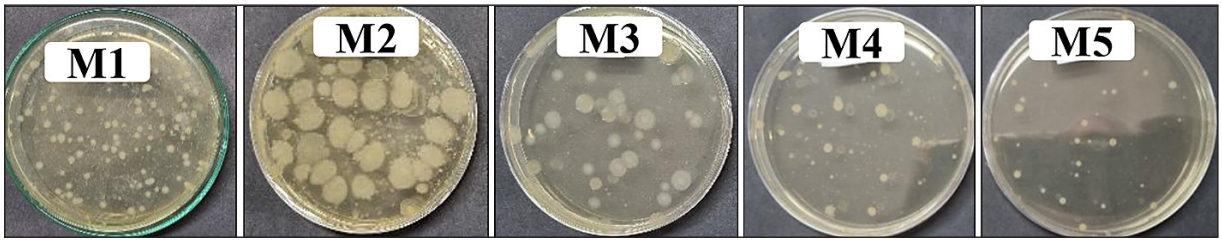

Figure 4. Growth of bacteria isolates after incubation. White colony color in M1 and crem color in other isolates

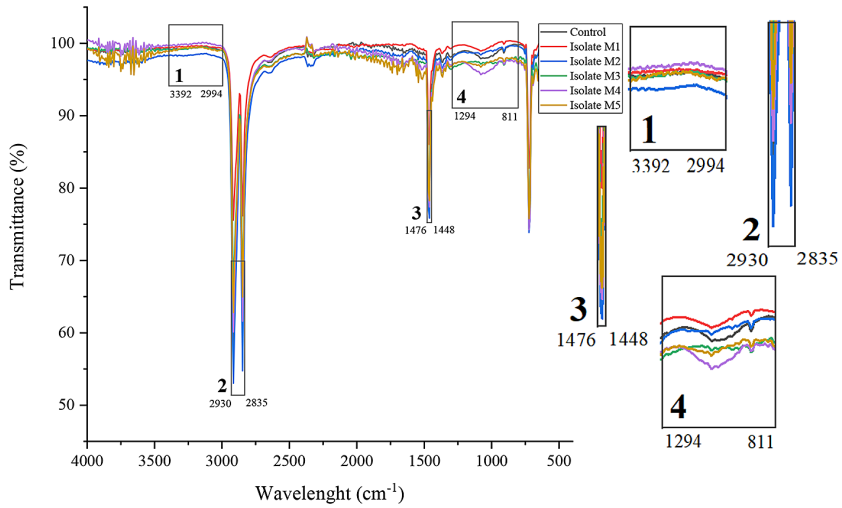

Figure 5. FTIR of plastic bags with bacteria isolates from Surabaya River after 30 days. (1) wavelength 3392–2996 cm^{-1} (O-H group), (2) wavelength 2930–2835 cm^{-1} (C-H group), (3) wavelength 1476–1448 cm^{-1} (C=C group), (4) Wavelength 1294–811 cm^{-1} (C-O group)

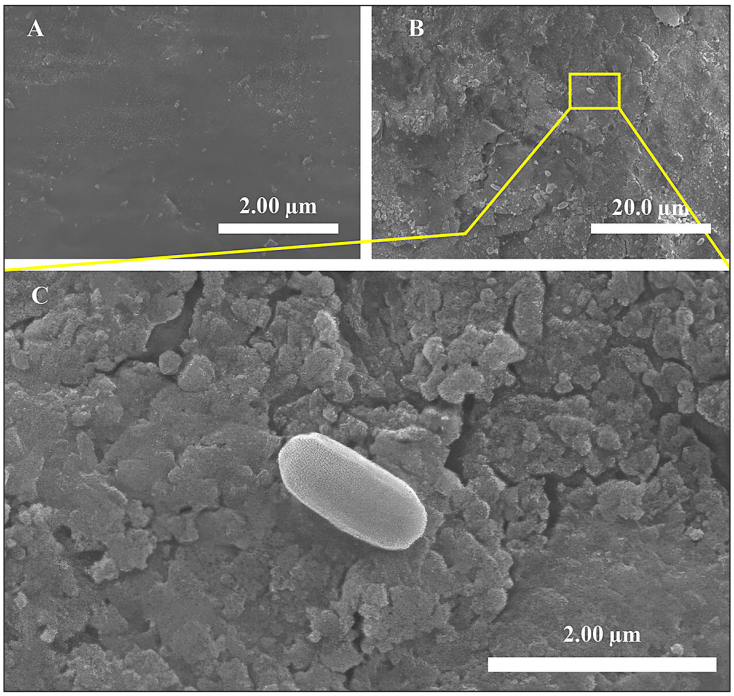

Figure 6. Surface morphology of plastics after 30 days of incubation; (a) control (without bacteria); (b, c) are plastics show cracks on the plastic surface by M2

indicative of microbial influence in degradation of the polymer structure (Tamoor et al., 2021). Combining FTIR and SEM analysis provides a complete understanding of the biodegradation process. Both analyses help assess how effectively bacteria isolates degrade LDPE by providing evidence of chemical and physical structural changes from the decomposition process. Subsequently, the potential bacteria isolates obtained are identified using the 16s RNA.

Identification of plastic-degrading bacteria from Surabaya River

To identify bacterial isolates responsible for plastic degradation, 16S rRNA gene sequencing was performed on the three most promising isolates (M2, M3, and M4). The results identified *Bacillus cereus* as the dominant species in M2 with a 99.86% similarity. Meanwhile, M3 and M4 were identified as *Pseudomonas sihuiensis*

with 99.86% and 99.73% similarity, respectively (Figure 7). The role of *Bacillus cereus* in degrading plastic has been identified in previous studies. These included the ability to reduce dry weight, alter the chemical structure, and cause cracking of LDPE surface (Jayan et al., 2023; Jebashalomi et al., 2024). *Bacillus cereus* possess monooxygenase and dioxygenase enzymes that add oxygen to the carbon chains of polyethylene (PE) or polystyrene (PS), initiating the oxidation (Nyamjav et al., 2023). Additionally, lipase is a group of hydrolase enzymes capable of degrading the carbon backbone of synthetic polymers. The degrading process facilitates the hydrolysis of chemical bonds in the polymeric material. The long carbon chain seen in polymeric material is broken down by lipases (Safdar et al., 2024). According to (Khampratueng et al., 2024), degradation of LDPE by *Bacillus cereus* forms intermediate products such as alcohol and carboxylic acids (Figure 8). *Pseudomonas* species have been shown to degrade PE plastics effectively (Gupta and Devi, 2020; Hou et al., 2022). Specifically, *Pseudomonas* and *Bacillus* species have been found to work synergistically in degrading plastic, cleaving bonds such as the 2-hydroxyethyl terephthalic acid bond in PET, and using the fragments as a carbon source (Roberts et al., 2020).

Additionally, *Pseudomonas* species have been shown to be biofragment and bioassimilate LDPE, turning it into biomass while producing alkane

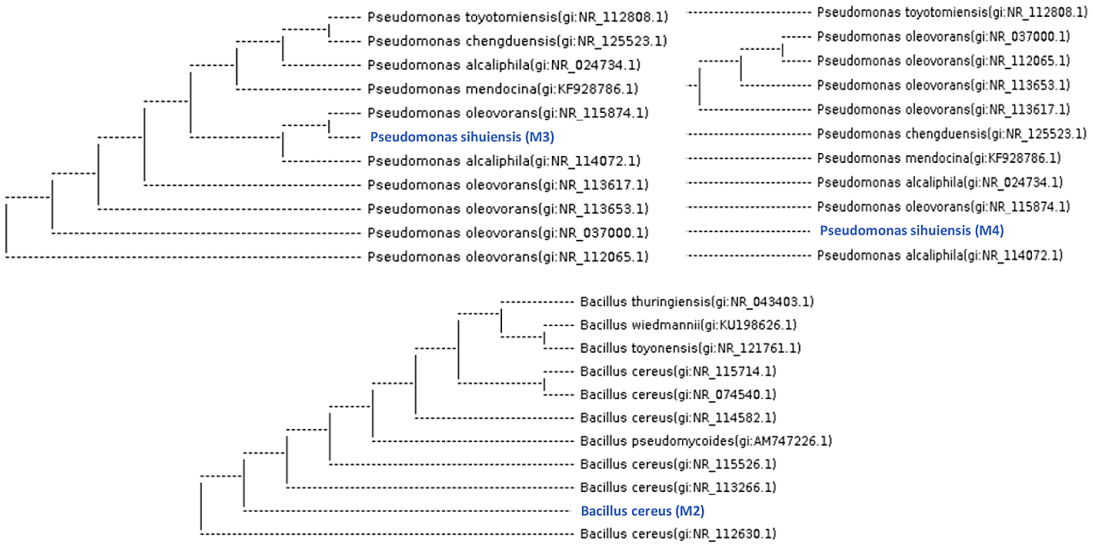

Figure 7. 16S rRNA gene sequencing for M2, M3, and M4

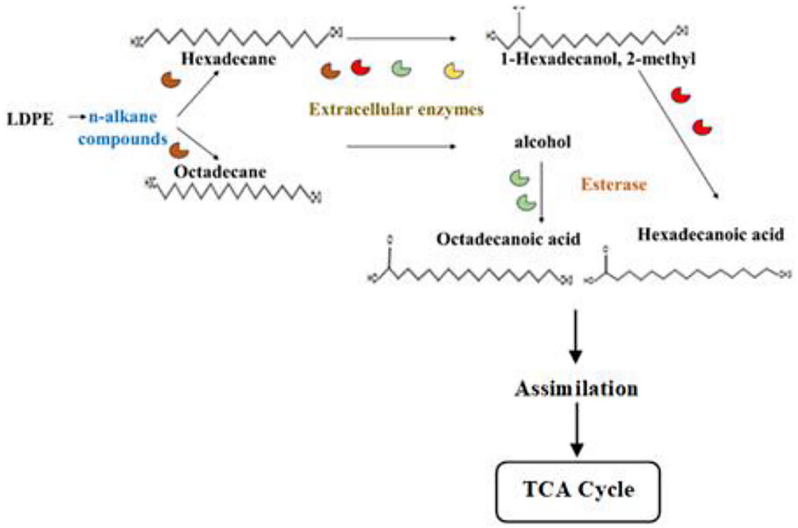

Figure 8. Biodegradation LDPE pathway by *Bacillus* sp.

hydrolysis products (Montazer et al., 2019). The ability of *Pseudomonas* species to degrade LDPE was determined by weight loss, morphological changes, mechanical variations, and spectroscopy. Coumponds eluted after degradation such as benzene, methyl-, tetrachloroethylene, benzene, 1,3-dimethyl, octadecane, 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione, hexadecanoic acid, ethyl ester, eicosane, octadenoic acid, docosane, 3-chloropropionic acid, heptadecyl ester, tricosane, butyl ester, 1-nonadecene, tetracosane, pentacosane, 1,2-benxenedicarboxylic acid, diisoostyl ester, hexacosane (Kyaw et al., 2012). This further emphasizes the potential of *Bacillus cereus* and *Pseudomonas sihuiensis* in plastic biodegradation and application in managing plastic pollution.

CONCLUSIONS

In conclusion, this study showed that *Bacillus cereus* and *Pseudomonas sihuiensis* could degrade LDPE plastic waste from the Surabaya River. The results showed that *Bacillus cereus* (M2) had the highest degradation ability, followed by *Pseudomonas sihuiensis* (M3 and M4). FTIR and SEM analysis also confirmed microbial breaking down of LDPE, showing the potential of these bacteria strain for natural plastic waste degradation.

By examining the effectiveness of bacteria, the results addressed a significant knowledge gap in understanding microbial solutions for LDPE degradation. Despite the unique contribution, this study had several limitations, including the need for controlled laboratory conditions that could not replicate the environment. Therefore, future studies should focus on optimizing bacteria strain for large-scale application to mitigate the environmental impact of plastic pollution.

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