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SCREENING OF LDPE PLASTIC-WASTE-DEGRADING BACTERIA USING WINOGRADSKY COLUMN

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ABSTRACT

This study aims to screen Low-Density Polyethylene (LDPE)-degrading bacteria from three highly plastic-contaminated environments: the Jatimalang River stream, Bekonang Market soil, and Joho Village Waste Disposal Site. The research used the Winogradsky column method with two incubation periods: 1 month and 2 months. This research was conducted with 3 repetitions using a descriptive-quantitative data analysis approach, and was a preliminary screening. Observed parameters included the percentage of plastic degradation, biofilm thickness, physical changes in LDPE, and bacterial morphology isolated using Nutrient Agar. The results showed that the highest degradation occurred in the waste-disposal soil (TPS), with values of 34.37% (1 month) and 40.62% (2 months), followed by river soil (22.72% and 27.27%) and market soil (13.63% and 18.18%). Biofilm thickness was also most significant in TPS samples, reaching 0.57 ± 0.01 and 0.91 ± 0.03 µm. Physical changes included yellowish to brownish discoloration, increased roughness, and enhanced elasticity of LDPE. All bacterial isolates exhibited irregular colony forms, lobate margins, flat elevations, and cream-to-yellow pigmentation. Gram staining identified all isolates as Gram-positive bacteria. These findings indicate that plastic-contaminated environments hold strong potential as natural sources of LDPE-degrading bacteria

INTRODUCTION

Plastic pollution is a significant global environmental issue, with Low-Density Polyethylene (LDPE) accounting for approximately 18.9% of total plastic waste (Singh

et al., 2024). Its crystalline structure, high molecular weight, and hydrophobicity make LDPE highly resistant to natural degradation, allowing it to persist for centuries in the environment (Dailin et al., 2024). This issue is more severe in developing countries, including Indonesia, where inadequate waste management, low recycling rates (approximately 7% globally), and direct disposal into rivers and soil result in the accumulation of LDPE (Chigwada & Tekere, 2023). Rivers, market-area soils, and landfills have become significant reservoirs of LDPE, posing a threat to ecosystems and public health (Chigwada & Tekere, 2023).

Biodegradation by microorganisms has recently emerged as a promising alternative solution. Several studies have successfully isolated LDPE-degrading bacteria from mangrove habitats, marine plastisphere communities, and contaminated soils. Singh et al. (2024) reported that halophilic bacteria from Malaysian mangroves can degrade LDPE up to 4.64% in 30 days through laccase and lipase activity. Febria et al. (2024) isolated four marine plastisphere strains that achieved 10–15% degradation in 35 days, accompanied by surface erosion and changes in FTIR spectra. Dailin et al. (2024) also identified two *Streptomyces* strains capable of degrading LDPE, with *S. indiaensis* WICC-B67 achieving 0.83% degradation in 30 days.

The biodegradation of LDPE occurs through enzymatic oxidation and hydrolysis mediated by laccases, lipases, esterases, proteases, and other hydrolases, which introduce carbonyl and hydroxyl groups into the polymer chain, reducing hydrophobicity and promoting depolymerization (Patel et al., 2024). Evidence such as biofilm formation, surface cracks, holes, and the appearance of new functional groups supports the role of these initial oxidative and hydrolytic processes in enhancing polymer susceptibility (Febria et al., 2024). Environments heavily exposed to plastics, such as river sediments, contaminated soils, and waste disposal sites, serve as selective niches for microbes that tolerate or utilize polymers (Afianti et al., 2024). Studies have identified biofilm-forming, polyethylene-interacting bacteria from mangrove sediments and plastisphere communities, as well as genera like *Bacillus*, *Pseudomonas*, *Lysinibacillus*, and *Streptomyces*, highlighting microbial adaptation in plastic-rich habitats (Febria et al., 2024; Mendoza et al., 2025).

Although numerous studies have demonstrated the potential of bacterial LDPE biodegradation, several critical knowledge gaps remain. Recent reviews emphasize the

challenges in confirming actual biodegradation, particularly in distinguishing between surface oxidation and actual mineralization, as well as in optimizing environmental conditions, identifying specific enzymes involved, elucidating degradation mechanisms, and understanding microbial interactions or consortium effects under natural conditions (Patel et al., 2024). Additionally, most existing studies have centered on marine environments, mangrove ecosystems, or controlled laboratory experiments, while research exploring terrestrial sources of LDPE-degrading bacteria in Indonesia remains scarce. To address these gaps, the Winogradsky column was employed as it enables the simulation of natural environmental gradients (e.g., oxygen, nutrients, and redox conditions) and supports the enrichment of diverse microbial consortia under conditions more closely resembling those of real ecosystems. In particular, investigations targeting rivers, market soils, and waste-disposal sites contaminated with plastic waste are still minimal.

This study introduces a novel approach by screening LDPE-degrading bacteria from three strategic locations in Indonesia: the Jatimalang River stream, Bekonang Market soil, and Joho Village Waste Disposal Site. These sites were selected because they represent environments with continuous and intensive plastic exposure, making them likely habitats for microorganisms that have developed adaptive capabilities to degrade LDPE.. This study aims to screen and comprehensively characterize the LDPE-degrading potential of bacterial isolates by integrating enrichment, isolation, and evaluation steps, including enrichment using a Winogradsky column, isolation on Nutrient Agar, and assessment of degradation performance through measurements of plastic weight loss, biofilm thickness, and changes in the physical condition of LDPE after incubation.

MATERIALS AND METHODS

Research Subject

The research subjects were bacteria associated with Low-Density Polyethylene (LDPE) plastic waste originating from three soil sources: Jatimalang River soil (TG), Bekonang Market soil (TP), and Joho Village waste-disposal site soil (TS). The study employed a quantitative experimental approach using a completely randomized design (CRD) with a single experimental factor, namely LDPE-degrading bacteria. The

treatments consisted of soil type and incubation time, including 1 month and 2 month incubation periods with the description of control Jatimalang River Soil (T0), 1 month incubation River Soil (TG1), 2 month incubation River Soil (TG2), control Bekonang Market Soil (TP0), 1 month incubation Market Soil (TP1), 2 month incubation Market Soil (TP2), Joho Landfill control (TS0), 1 month incubation Joho Landfill (TS1), 2 month incubation Joho Landfill (TS2). The study took place from June to August 2025 at the Microbiology Laboratory, Department of Biology Education, FKIP UNS.

Tools and Materials Used

The tools used in this research included 10 mL measuring cylinders, test tubes, petri dishes, beakers, analytical balances, micropipettes, vortex mixers, spectrophotometers, and autoclaves. The materials used consisted of clear LDPE plastic, soil samples from three locations, distilled water, Nutrient Agar (NA), and 70% alcohol. The procedure sterilized all tools and materials in an autoclave before use to minimize contamination.

Research Procedure

The stages of this research are soil sampling, preparation of LDPE plastic, biodegradation, sterilization of tools and materials, data collection, bacterial isolation, and morphological observation.

Soil sampling

The study collected riverbank soil samples from the Jatimalang River in Jatimalang Village, Mojolaban, Sukoharjo (latitude −7.586051°, longitude 110.883475°). The study collected market soil samples from Bekonang Market in Bekonang Village, Mojolaban, Sukoharjo (latitude −7.608791°, longitude 110.873423°). The study collected waste soil samples from the Joho Village landfill in Mojolaban, Sukoharjo (latitude −7.591659°, longitude 110.898586°). All three samples were collected from the soil surface using purposive sampling, selecting three sampling points at each location. The study then combined the soil samples from each location.

Preparation of LDPE plastic

The plastic used was clear LDPE, a type commonly used for food packaging. The study then cut the plastic into 60 uniform sheets measuring 8 cm × 2 cm. Before

treatment, the plastic was sterilized using 70% alcohol and dried. Then, it was weighed using an analytical balance to determine its initial weight (Patel et al., 2024).

Biodegradation

The study conducted biodegradation using the Winogradsky column method in 1.5-L mineral water bottles. The column was composed of six bottles, each containing a solution of 500 grams of soil sample from each location and water, stirred until homogeneous. The study labeled each bottle according to the specific soil type and incubation time. The soil sample solution from each location was poured into the bottles according to the label, filling them to approximately two-thirds of their capacity. The study then covered the bottles with plastic and secured them with rubber bands. The Winogradsky column was placed in an open area to receive sufficient sunlight for one week. After 1 week, pieces of LDPE plastic were inserted into each column to be incubated for 1 month and 2 months (Madigan et al., 2021).

Sterilization of Tools and Materials

The study sterilized 10-ml measuring cylinders, test tubes, petri dishes, beakers, distilled water, and Nutrient Agar (NA) solution in an autoclave. The study performed sterilization to reduce potential contaminants in the research samples.

Bacterial isolation

The study diluted the homogenized biofilm to a concentration of 10^{-4}. At dilutions of 10^{-3} and 10^{-4}, the study transferred 0.1 ml of the suspension using a micropipette onto Nutrient Agar and evenly spread it across the Petri dish with a Drigalski spreader. The petri dish was then tightly covered with plastic wrap to prevent air from entering and minimize contamination. The bacterial isolation stage was repeated 3 times for each treatment, resulting in a total of 18 petri dishes. The study incubated the Petri dishes containing the bacterial biofilm suspension for 24 hours. After observing bacterial colony growth, the study inoculated each isolate onto fresh Nutrient Agar (NA) using the streak plate method to assess morphology and then incubated the plates for 24 hours.

Morphological observation

Morphological observations on the incubated inoculum consisted of observations of shape, edge, elevation, color, and Gram type through Gram-positive and Gram-negative staining (Madigan et al., 2021).

Data Analysis and Interpretation

The parameters measured in this study to evaluate LDPE biodegradation included: (1) the percentage of plastic degradation, determined by the reduction in plastic weight after incubation; (2) biofilm thickness formed on the plastic surface, as an indicator of microbial colonization; and (3) changes in the physical condition of the plastic, such as the presence of cracks, holes, or surface roughness observed after incubation.

Percentage of plastic degradation

The study calculated the percentage of plastic degradation by subtracting the final weight from the initial weight (Skariyachan et al., 2021).

$$\%\, plastic\ degradation = \frac{Wi - Wf}{Wi} \times 100\%$$

Wi = weight before degradation (g)
Wf = weight after degradation (g)

Biofilm thickness

The degraded plastic was placed in 10 mL of sterile distilled water and homogenized using a vortex at 2000 rpm for 4 cycles of 30 seconds each. The study dried the biofilm-free plastic to determine its final weight and measured the thickness of the homogenized biofilm using a spectrophotometer at 600 nm. The study performed three replicate measurements. Biofilm thickness can indicate the number or concentration of LDPE plastic-degrading bacteria (Harshvardhan & Jha, 2013).

Physical condition of plastic

Plastic pieces before and after degradation were observed for physical changes, such as shape, color, and texture, to determine bacterial activity on LDPE (Auta et al., 2017).

RESULTS AND DISCUSSION

The study conducted the plastic biodegradation test using the Winogradsky column method, which employs artificial columns containing a mixture of soil and water as a medium for microbial enrichment. This method enables the observation of microbial ecology and stratification in sedimentary environments, including the distribution of organisms based on the availability of electron donors within each column layer (Jiang et al., 2018).

In this study, the researchers constructed Winogradsky columns using three types of soil exposed to plastic waste: soil from the Jatimalang River, soil from Bekonang Market, and soil from the Joho Village Waste Disposal Site (TPS). Each column contained 500 grams of soil mixed with water until homogeneous (Figures 1 and 2). Clear LDPE plastic, measuring 8 x 2 cm, was then immersed in the column for incubation over 1 and 2 months, allowing soil organisms to utilize the plastic as the sole additional carbon source (Febria et al., 2024).

LDPE plastic has a long-chain polymer structure that is hydrophobic and highly stable, making it difficult to degrade naturally (Gewert et al., 2015). According to Lucas et al. (2008), microorganisms play a crucial role in the biological degradation of polymers by breaking down complex structures into simpler molecules through enzymatic activity. This breakdown enables microbes to utilize polymer fragments as an energy or carbon source, particularly in environments with limited carbon sources (Wilkes & Aristilde, 2017).

Under these conditions, the use of a Winogradsky column in this study aims to optimize LDPE biodegradation. Sridharan et al (2021) explained that enrichment systems, such as the Winogradsky column, enable the formation of microbial communities with diverse metabolic capabilities in each layer, including organisms capable of utilizing complex or unusual compounds as carbon sources. This finding aligns with research indicating that each soil type produces distinct levels of degradation and biofilm formation, as evidenced by the physical changes in plastic and corresponding degradation percentage data (Paço et al., 2017).

Figure 1. The study conducted a biodegradation test using the Winogradsky column method with a 1-month incubation period and three replicates per column. Caption: a. Bekonang Market soil (TP) column. b. Joho Landfill (TS) column. c. Jatimalang River soil (TG) column.

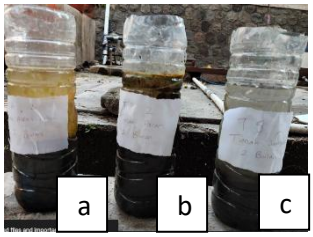

Figure 2. Biodegradation test using the Winogradsky column method with an incubation period of 2 months. Caption: a. Bekonang Market soil (TP) column. b. Joho Landfill (TS) column. c. Jatimalang River soil (TG) column.

The study observed changes in LDPE plastic after incubation in a Winogradsky column for one and two months, focusing on color, texture, and size. The plastic, which was initially clear white, changed to a yellowish color after 1 month of incubation and to a brownish color in two months of incubation, especially in the TS and TG treatments (Figure 3). This color change indicates surface oxidation resulting from microorganism enzymatic activity, which continues to increase with incubation time (Patel et al., 2024).

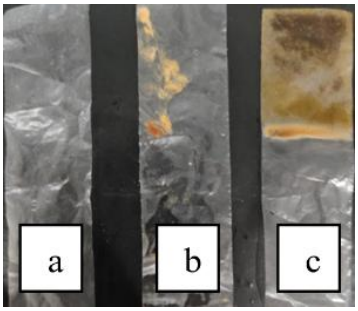

Figure 3. Physical condition of plastic after degradation. Caption: a. Plastic degraded in the Bekonang Market soil (TP) column. b. Plastic degraded in the Joho Landfill (TS) column. c. Plastic degraded in the Jatimalang River soil (TG) column.

The soil treatment at Joho Landfill (TS) showed the greatest changes among the treatments. The weight of TS plastic decreased from 0.032 g (TS0) to 0.021 g after one month of incubation and to 0.019 g after two months, indicating a greater mass reduction compared to the Jatimalang River soil (TG) and Bekonang Market soil (TP) treatments. Furthermore, the shrinkage in plastic length in the TS treatment was also more pronounced, decreasing from 8 cm to 7.8 cm after one month and to 7.3 cm after two months, with the color changing from yellowish to brownish and the texture becoming increasingly rough and elastic (Table 1). Compared to the TG and TP treatments, the physical changes in the TS treatment showed a more intense level of degradation, consistent with the large percentage of degradation and biofilm thickness in the other tables. It indicates that the Joho Landfill soil has a microbial community most adapted to LDPE and is capable of producing the strongest degradation activity; therefore, the TS treatment can be considered the most significant treatment for degrading LDPE plastic.

Table 1. Observation of the Physical Condition of LDPE Plastic Under Winogradsky Treatment

Treatment	Weight (g)	Size (cm)	Condition
TS0	0.032	p: 8 l: 2	White, smooth texture, slightly stiff
TS1	0.021	p: 7.8 l: 2	Yellowish white, slightly rough texture, slightly elastic
TS2	0.019	p: 7.3 l: 2	White with brownish tinge, slightly rough texture, elastic
TG0	0.022	p: 8 l: 2	White, smooth texture, slightly stiff
TG1	0.017	p: 7.9 l: 1.9	Yellowish white, slightly rough texture, slightly elastic
TG2	0.016	p: 7.3 l: 1.9	White with brownish tinge, slightly rough texture, elastic
TP0	0.022	p: 8 l: 2	White, smooth texture, slightly stiff
TP1	0.019	p: 7.8 l: 1.8	Yellowish white, slightly rough texture, slightly elastic
TP2	0.018	p: 7.4 l: 1.8	White with brownish tinge, slightly rough texture, elastic

The Joho Landfill (TS) had the highest LDPE plastic degradation capacity, reaching 34.37% after 1 month of incubation and 40.62% after 2 months. It indicates that the TPS soil samples have a microbial community that is more adaptive to plastic exposure, thus capable of more effective degradation. The degradation values for Jatimalang River soil (22.72–27.27%) and Bekonang Market soil (13.63–18.18%) increased with incubation

time, consistent with the theory that LDPE biodegradation proceeds slowly and depends on prolonged microbial contact with the polymer (Table 2).

This increase suggests that incubation time plays a crucial role in successful degradation, as bacteria require time to form biofilms, produce enzymes, and depolymerize polyethylene. This finding is consistent with research by Akram et al. (2024), which reported that LDPE degradation increases over time due to the activity of enzymes such as laccase, lipase, and esterase.

Table 2. Percentage of Plastic Degradation

Soil Type	Degradation Percentage (%)		
	1 month	2 months	Average
TS	34.37	40.62	37.50 ± 4.42
TP	13.63	18.18	15.91 ± 3.22
TG	22.72	27.27	25.00 ± 3.22

The thickest biofilm was observed in the TS treatment with thicknesses of 0.57±0.01 and 0.91±0.03, followed by TG (0.12–0.88) and TP (0.43–0.46) (Table 3). Thicker biofilms indicate a higher bacterial count and a stronger ability to adhere to the LDPE surface. This observation is consistent with recent findings that biofilm formation plays a crucial role in plastic biodegradation by enabling microorganisms to localize on the polymer surface and enhance degradation efficiency (Schneier et al., 2024). The positive relationship between biofilm thickness and degradation percentage indicates that biofilm formation is a key initial stage in LDPE biodegradation, during which microorganisms produce extracellular enzymes that alter surface properties, reducing hydrophobicity, introducing oxygen-containing functional groups, and ultimately causing structural damage such as cracks and surface erosion (Adithama et al., 2023).

Table 3. Biofilm Thickness

Soil Type	Average Biofilm Thickness	
	1 month	2 months
TS	0.57±0.01	0.91±0.03
TP	0.43±0.01	0.46±0.02
TG	0.12±0.01	0.88±0.04

The diluted biofilms from each treatment were then isolated on NA media (Figure 4). The three isolates from the Jatimalang River (TG), Joho TPS (TS), and Bekonang Market (TP) soils exhibited a distinctive colony growth pattern, namely irregular colony shapes *with* lobate edges *and flat* elevations. The difference lies in pigmentation, where colonies from TG appeared yellow, while isolates from TS and TP tended to be creamy white (Table 4). This color variation indicates differences in the types or metabolites

produced by microorganisms at each location. Colony density in the TS treatment appeared higher than in the other two locations, confirming the results for biofilm thickness and degradation percentage, which showed that the Joho TPS soil had the most intense microbial activity against LDPE (Pippo et al., 2023).

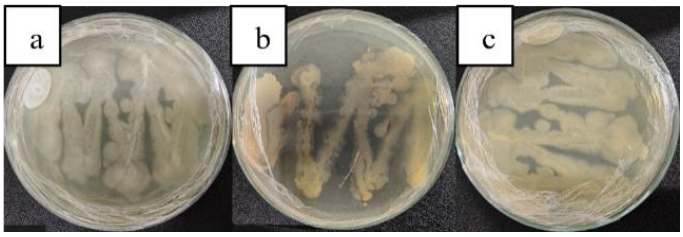

Figure 4. Purification of bacterial colonies using the streak plate method. Caption: a. Jatimalang River soil (TG) b. Joho Landfill (TS) c. Bekonang Market soil (TP)

After Gram staining, the three isolates from TG, TS, and TP showed purple staining, indicating that all were Gram-positive bacteria (Figure 5). Gram-positive characteristics indicate that the bacteria have thick peptidoglycan cell walls, making them more resistant to specific environmental conditions and capable of forming strong biofilms on hydrophobic surfaces such as LDPE. The presence of Gram-positive bacteria across all locations supports previous studies, reporting that genera such as *Bacillus*, *Lysinibacillus*, and *Streptomyces* are often found in plastic-polluted environments and have the potential to depolymerize LDPE (Amobonye et al., 2021). Thus, these results indicate that microorganisms isolated from the three locations exhibit morphological and staining characteristics consistent with Gram-positive bacteria commonly reported in plastic-associated environments; however, confirming their taxonomic identity and LDPE biodegradation potential requires further biochemical characterization, molecular identification, and specific degradation assays. (Wilkes & Aristilde, 2017).

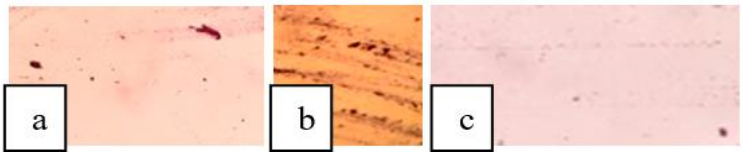

Figure 5. Gram staining of bacterial isolates. Caption: a. Jatimalang River soil (TG) b. Joho Landfill (TS) c. Bekonang Market soil (TP)

Table 4. Macroscopic and Microscopic Characteristics

Soil Type	Macroscopic				Microscopic	
	Form	Edge	Elevation	Color	Gram +	Gram -
TS	Irregular	Lobate	Flat	Creamy white	√	
TP	Irregular	Lobate	Flat	Beige	√	
TG	Irregular	Lobate	Flat	Yellow	√	

CONCLUSION

This study shows that environments contaminated with plastic waste have significant potential as sources of LDPE-degrading bacteria. The soil at the Joho landfill site (TPS Joho) exhibits the highest degradation capacity, with percentages ranging from 34.37% to 40.62%, accompanied by the thickest biofilm and the most pronounced physical changes in the plastic. The isolated bacteria exhibited Gram-positive characteristics with irregular colony morphology and lobate edges. However, these observations alone are not sufficient to confirm their ability to degrade hydrophobic polymers such as LDPE. Moreover, because the study used Nutrient Agar (NA), a non-selective medium without LDPE supplementation, the isolates represent general heterotrophic bacteria rather than specifically selected LDPE-degrading strains. Therefore, although biofilm formation and plastic-associated growth suggest possible interaction with LDPE, the current data do not allow a conclusive determination of the isolates' biodegradation capability. Further studies are required, including selective screening on LDPE-containing media, quantitative degradation assays, enzyme activity analysis, and molecular identification to verify their potential role in plastic biodegradation.

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