Innovation in Plastic Waste Management: Toward the Biological Decomposition of LDPE for a Circular Economy in Andralanitra, Antananarivo, Madagascar

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Abstract

A polymer formed from long chains of ethylene molecules (C2H4)n is widely used as a plastic material whose persistence in the environment makes it a major source of pollution in Antananarivo. This problem is aggravated by plasticizing additives capable of binding heavy metals, thereby creating serious health risks.

Objective: This study evaluates the ability of specific bacterial strains to degrade LDPE in order to propose a natural bioremediation solution that reduces plastic pollution and improves its potential for energy recovery.

Methodology: Bacteria were isolated from polluted environments and tested over 30 days. Degradation efficiency was assessed by measuring plastic dry mass loss and analyzing chemical structure changes using Fourier Transform Infrared Spectroscopy (FT-IR).

Results: Isolate AND02 showed the highest performance, achieving a 7.42% reduction in dry mass within one month (compared to 0.85% for AND01 and 0.41% for AND03). FT-IR analyses confirmed deep structural degradation, with decreased intensity of key bonds (–OH, C–H, C=C, C–O), demonstrating real breakdown of plastic molecules.

Conclusion: These results highlight the strong potential of bacteria as bioremediation agents. Applied at the Andralanitra site, this approach could treat approximately 15% of plastic waste and prepare it for optimized conversion into biofuel, supporting a circular economy strategy for Madagascar.

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Keywords: Environmental Pollution, Plastic Material, Fourier Transform Infrared Spectroscopy, Andralanitra

1. Introduction

Beyond its structural features, LDPE is characterized by a relatively low density (0.91–0.94 g·cm^{-3}), providing the material with its signature flexibility and ease of processing. Its semi-crystalline nature, typically featuring a 50–60% crystalline fraction, ensures the mechanical integrity required for diverse industrial uses (Callister & Rethwisch, 2014) [5]. Since 2015, global manufacturing volumes have surged to approximately 64 million tonnes annually, driven by the ubiquitous demand for packaging films, grocery bags, beverage containers, and protective cable sheathing (PlasticsEurope, 2016) [13]. However, this massive production scale poses a severe environmental challenge; LDPE constitutes nearly 23% of the plastic debris found in landfills, a figure that underscores significant gaps in current waste recovery strategies (Geyer *et al*., 2017) [7].

The chemical stability of LDPE stems from its robust carbon–carbon (C–C) backbone, which resists natural breakdown from microbial action or UV exposure (Andrady, 2011) [1]. Consequently, it persists in the environment, where it has been found to act as a substrate for hazardous pollutants. Research indicates that polyethylene surfaces can adsorb heavy metals—including lead (Pb), nickel (Ni), zinc (Zn), and copper (Cu)—from contaminated waters (Holmes *et al*., 2012) [8]. As these plastics fragment into microplastics, they facilitate the bioaccumulation of toxins within the food web, eventually reaching human populations (Wright *et al*., 2013) [21]. Furthermore, the leaching of integrated additives, specifically phthalates such as DEP, DMP, and DBP, presents a chemical risk due to their role as endocrine disruptors in both humans and aquatic life (Oehlmann *et al*., 2009) [12]; (Net *et al*., 2015) [11].

Biotechnological Solutions and Challenges

Microbial biodegradation represents a sustainable pathway for mitigating plastic pollution. Specific microorganisms utilize enzymatic toolkits—including laccases, lipases, and various hydrolases—to depolymerize LDPE chains (Shah *et al*., 2008) [14]; (Sivan *et al*., 2006) [15]; (Urbanek *et al*., 2018) [19]. These biochemical processes break down complex polymers into simpler monomers or oligomers, which are subsequently mineralized into carbon dioxide (CO_2) and water (H_2O) (Gewert *et al*., 2015) [6]. Innovative studies have highlighted the potential of enzymes such as proteinase K to accelerate chain scission (Tokiwa *et al*., 2009) [18]. Moreover, indigenous bacteria isolated from heavily polluted sites have shown a specialized capacity for LDPE degradation, suggesting that environmental adaptation can be leveraged for bioremediation (Yoshida *et al*., 2016) [23].

Despite this potential, scaling these biological solutions remains difficult due to variables such as pH levels, thermal conditions, and oxygen solubility (Montazer *et al*., 2020) [10]. Future progress necessitates a cross-disciplinary strategy combining genetic engineering and materials science to optimize degradation rates. Transitioning toward enzyme-based waste management not only aligns with the circular economy but also supports the United Nations Sustainable Development Goals (SDGs) regarding marine conservation. This research seeks to advance these eco-friendly alternatives to address the global crisis of plastic persistence.

This study provides new perspectives on the potential of specific bacterial strains to effectively degrade LDPE plastic within the context of Andralanitra. These promising results serve as a scientific foundation for developing innovative biodegradation and energy recovery technologies, aimed at transforming plastic waste in Antananarivo into sustainable biofuels.

2. Experimental Materials and Chemical Study

2.1. Materials and Reagents

The experimental setup employed Low-Density Polyethylene (LDPE) as the primary substrate (Shah *et al*., 2008) [14]; (Sivan *et al*., 2006) [15]. For microbial cultivation and the preparation of mineral salt media, the following reagents were used: Nutrient Agar (NA) and Nutrient Broth (NB) (Atlas, 2010) [3], 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 ($FeCl_2$) (Stanier *et al*., 1986) [16]; (Madigan *et al*., 2015) [9]. All chemical compounds were of analytical grade (AR grade) and were used without further purification (Vogel, 2000) [20]. Distilled water (aquades) was employed for the preparation of all solutions (APHA, 2017) [2].

2.2. Sampling Methods

LDPE plastic waste samples were collected at the Andralanitra landfill site, located in Antananarivo, Madagascar, at the approximate coordinates 18°52'45"S and 47°29'50"E (Fig. 1). Sample collection was carried out manually at several points across the site, particularly in humid and partially buried areas. The plastic fragments were retrieved from a depth ranging between 5 and 10 cm below the surface. All samples were placed in sterile bags and stored in a cooler prior to transportation to the laboratory for further analysis.

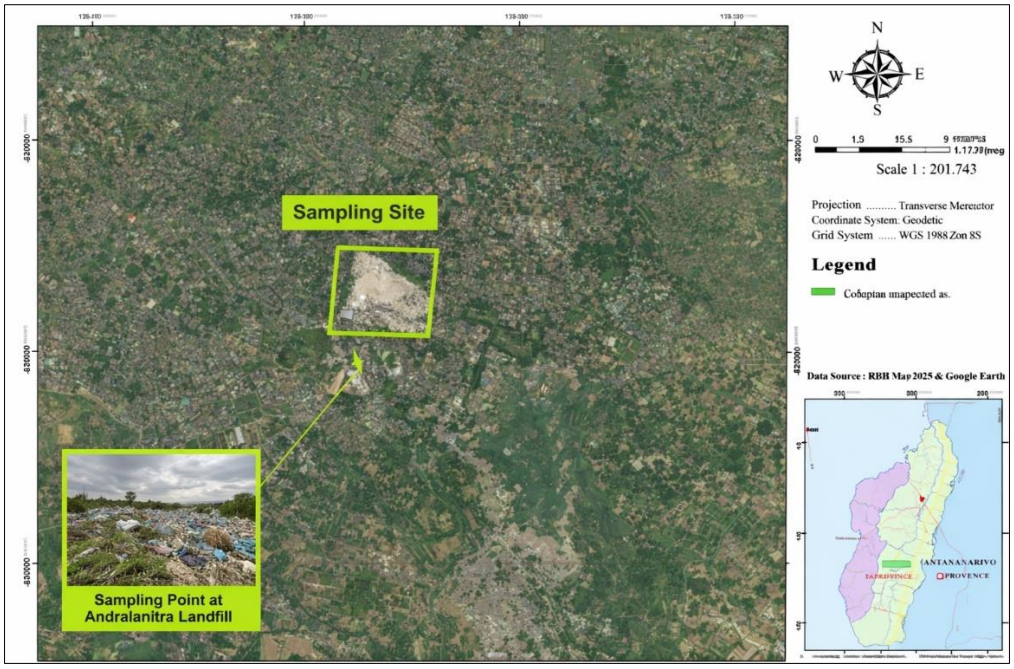

Fig 1: Location of the sampling site at Andralanitra using ArcGIS 10.8

2.3. Morphological Characterization by Gram Staining

The Gram reaction of the isolated bacterial strains was determined using a conventional staining method. Briefly, a smear was prepared by mixing a loopful of a pure culture with a drop of sterile distilled water on a clean glass slide and spreading it in a zigzag pattern. The smear was heat-fixed by gently passing the slide through a Bunsen burner flame until dry. Crystal violet was applied as the primary stain for 60 s, followed by rinsing with distilled water. Gram's iodine was then added for 1 min as a mordant. After rinsing, decolorization was carried out using 70% ethanol. The smear was subsequently counterstained with safranin for 45 s, air-dried, and observed under a light microscope to differentiate Gram-positive and Gram-negative bacteria based on cell wall structure (Beveridge, 2001; Madigan *et al*., 2015) [4; 9].

2.4. Bacterial Acclimatization Process

To enhance the ability of the bacterial isolates to degrade plastic, a gradual acclimatization procedure was performed using Nutrient Broth (NB) and Mineral Salt Medium (MSM). The MSM was prepared with the following composition ($g \cdot L^{-1}$): magnesium sulfate ($MgSO_4$, 0.2), calcium chloride ($CaCl_2$, 0.02), potassium dihydrogen phosphate (KH_2PO_4, 1), potassium hydrogen phosphate (K_2HPO_4, 1), ammonium nitrate (NH_4NO_3, 1), and iron(II) chloride ($FeCl_2$, 0.02).

2.5. Bacterial Acclimatization Process

To prepare the bacterial isolates for plastic degradation, a gradual acclimatization procedure was carried out using Nutrient Broth (NB) and Mineral Salt Medium (MSM) (Atlas, 2010). The MSM was prepared with the following composition ($g \cdot L^{-1}$): magnesium sulfate ($MgSO_4$, 0.2), calcium chloride ($CaCl_2$, 0.02), potassium dihydrogen phosphate (KH_2PO_4, 1), potassium hydrogen phosphate (K_2HPO_4, 1), ammonium nitrate (NH_4NO_3, 1), and iron(II) chloride ($FeCl_2$, 0.02).

The acclimatization process was performed in successive steps. Initially, pure bacterial isolates were inoculated into 100 mL of NB and incubated for 48 h to obtain actively growing cultures. In the first adaptation phase, 25 mL of the initial culture was transferred into 180 mL of a NB/MSM mixture at a 1:1 ratio and incubated for 48 h. In the second adaptation phase, 15 mL from Phase I was introduced into 180 mL of a medium containing a higher proportion of MSM (NB/MSM ratio of 1:2) and incubated for an additional 72 h (Shah *et al*., 2008) [14].

The resulting acclimatized strains, conditioned to mineral-rich environments, were subsequently used for biodegradation experiments conducted in the framework of the Andralanitra project.

2.6. Plastic Preparation

LDPE plastic samples were cut into pieces measuring 11 cm in length (10 pieces) and weighed using an analytical balance to determine their initial mass (Shah *et al*., 2008) [14]. The plastic pieces were sterilized with 70% ethanol, followed by exposure to ultraviolet (UV) radiation for 1 h, and subsequently dried in a desiccator for 24 h prior to use in the biodegradation experiments (Urbanek *et al*., 2018) [19].

2.7. Biodegradation Test

A volume of 20 mL of the acclimatized bacterial culture was inoculated into an Erlenmeyer flask containing 180 mL of Mineral Salt Medium (MSM) adjusted to pH 7.0 and devoid of any external carbon source (Shah *et al*., 2008) [14]. The ten sterilized LDPE pieces (11 cm each) were then introduced into the flask. The cultures were incubated at 28 ± 2 °C under continuous agitation at 120 rpm for a period of 30 days (Urbanek *et al*., 2018) [19]. All experiments were carried out in triplicate to ensure reproducibility.

2.8. Total Plate Count (TPC) Analysis

The incubated plastic samples were aseptically removed and transferred into test tubes containing 20 mL of sterile solution, then vortexed for 10 min to detach the adhered bacterial cells (Madigan *et al*., 2015) [9]. A 1 mL aliquot of the resulting suspension was transferred into another test tube containing 13 mL of sterile solution and vortexed to obtain a 10^{-1} dilution. Serial dilutions were subsequently prepared up to 10^{-4} (APHA, 2017) [2].

From the final dilution, 1 mL of the suspension was inoculated into sterile Petri dishes containing molten Nutrient Agar (NA) using the pour plate technique (Atlas, 2010) [3]. The plates were then allowed to solidify and incubated for colony enumeration.

2.9. Mass Loss Analysis

The plastic samples were dried in a hot air oven at 80 °C for 24 h and weighed using a high-precision analytical balance. The percentage of mass loss was then calculated using the following equation (Eq. 1).

$$Weight\ loss = \frac{W_0 - W_t}{W_0} \times 100\%$$

W_0: initial weight (mg)

W_t: final weight (mg)

J. Fourier Transform Infrared (FT-IR) Spectroscopy Analysis

The plastic samples were dried in a hot air oven at 80 °C for 24 h and weighed using a high-precision analytical balance (Shah *et al*., 2008) [14]. The percentage of mass loss was then calculated using the following equation (Urbanek *et al*., 2018) [19].

3. Results and Discussion

This study successfully enabled the isolation and purification of three bacterial strains, including two coccoid-shaped isolates and one bacillary-shaped isolate. Based on the Gram staining results, two isolates were identified as Gram-positive, while the remaining isolate was classified as Gram-negative, as shown in Table 1 and Fig. 2.

The differentiation between Gram-positive and Gram-negative bacteria is primarily based on the thickness of the peptidoglycan layer in the bacterial cell wall. Gram-positive bacteria possess a significantly thicker peptidoglycan layer, measuring approximately 20 to 80 nm in thickness (representing roughly 20–40 layers of cross-linked polymer), which retains the crystal violet stain. In contrast, Gram-negative bacteria are characterized by a much thinner peptidoglycan layer, typically 2 to 7 nm thick (comprising only 1–3 layers), which is situated between the inner cytoplasmic membrane and an asymmetrical outer membrane.

Isolate Code	Morphology	Gram Reaction	Peptidoglycan Est.	Final Weight Loss (%)	
AND-01	Bacillus	negative	~1–3 layers	0.85%	
AND-02	Coccus	Positive	~35–45 layers	7.42%	
AND-03	Coccus	Positive	~20–40 layers	0.41%	
Isolate	Color	Elevation	Colony shape	Margin	Gram
AND-01	Cream	Convex	Circular	Lobate	-
AND-02	White	Raised	Circular	Entire	+
AND-03	Cream	Convex	Circular	Entire	+

The thicker peptidoglycan layer of Gram-positive bacteria provides them with an advantage under certain environmental conditions, particularly during the plastic biodegradation process. The robust structure of the bacterial cell wall facilitates the production of enzymes required for the degradation of plastic polymers. The ability of bacteria to degrade plastics largely depends on the specific enzymes they produce.

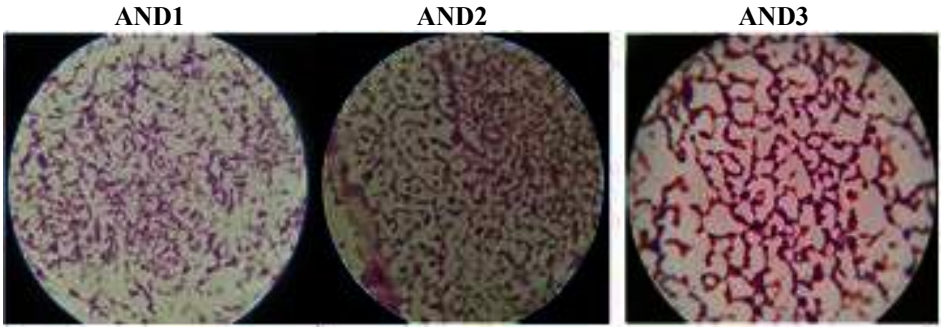

Fig 2: Gram-stained bacterial isolates

The three isolates were acclimatized and adapted prior to being used in the biodegradation test. In addition, bacterial cell density, as an indicator of growth, was determined by optical density (OD) measurement.

Isolates	NB/MSM Ratio 1:0	NB/MSM Ratio 1:1	NB/MSM Ratio 1:2
AND-01	1.285	1.390	0.075
AND-02	1.410	1.520	0.092
AND-03	0.950	1.050	0.020

The acclimatization stage is essential for the application of bacteria in the biodegradation process; however, it is often influenced by several factors such as the age of the isolates, nutrient availability, and growth-limiting conditions (Shah *et al*., 2008) [14]; (Montazer *et al*., 2020) [10]. The three isolates exhibited high optical density (OD) values at the 1:1 ratio; however, a significant decrease was observed at the 1:2 ratio. Despite this decline, the presence of measurable OD values suggests that the bacteria were attempting to adapt to the mineral-rich environment even as the carbon content decreased (Shah *et al*., 2008) [14]. The increase in OD values occurs because the cells are able to adapt and utilize the available carbon source (Madigan *et al*., 2015) [9].

The three isolates displayed variable abilities to degrade LDPE plastic, and according to Fig. 4, isolate AND02 exhibited the highest growth compared with AND01 and AND03 (Shah *et al*., 2008) [14]. The number of bacterial colonies formed on the surface of LDPE during incubation (Table 3) indicates differences in adaptation capacity among the isolates (Sivan *et al*., 2006) [15]. This adaptation enables the bacteria to use plastic as a source of energy and carbon, supported by enzymatic activity aimed at breaking down polymer chains into smaller molecules, including monomers and oligomers (Urbanek *et al*., 2018) [19]; (Tokiwa *et al*., 2009) [18].

Table 3: Number of bacterial colonies on the plastic surface

Isolates	Number of bacterial colonies (CFU/mL)
AND01	38×10^5
AND02	65×10^5
AND03	29×10^5

The results presented in Table 3 show that isolate AND02 produced the highest number of colonies, contributing to a higher rate of LDPE biodegradation compared with the other isolates. The LDPE degradation rate achieved by AND02 reached 7.42%, which is greater than the rates observed for AND01 (0.85%) and AND03 (0.41%), suggesting that AND02 has the greatest potential to utilize LDPE as a substrate.

According to a previous study, enzymes such as laccase and lipase play a critical role in the breakdown of plastic polymers into simpler compounds. Lipase belongs to the group of hydrolase enzymes and is essential for disrupting the carbon backbone of synthetic polymers. The degradation of polymeric materials is attributed to the assistance provided by lipase in the hydrolysis of chemical bonds. The long carbon chains present in polymeric materials are cleaved by lipases (Fig. 3).

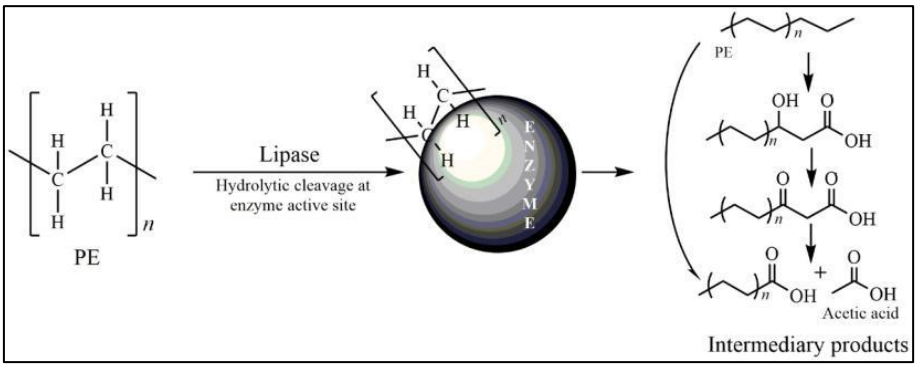

Fig 3: Pathway of polyethylene (PE) degradation by lipase [21].

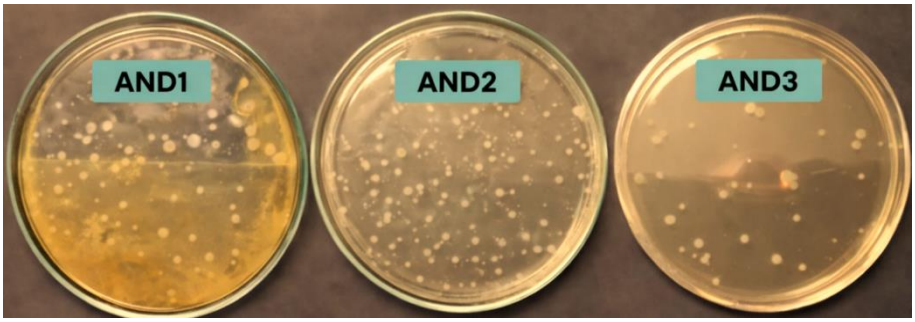

Fig 4: Bacterial growth on the surface of LDPE plastic.

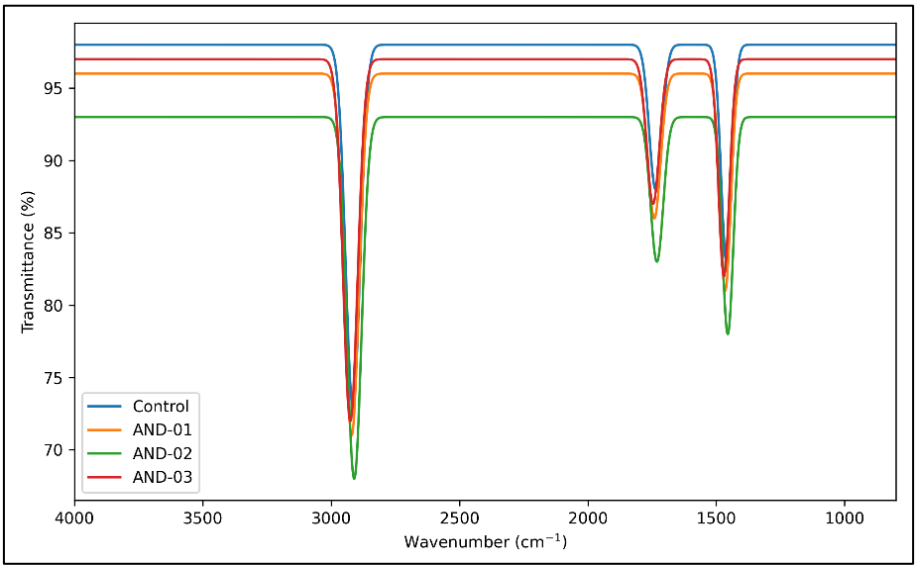

Fig 5: FT-IR spectra of LDPE plastic biodegradation by the isolated bacteria

Chemical analysis of LDPE plastic by FT-IR revealed structural changes in the polymer after the biodegradation process (Fig. 5) (Urbanek *et al*., 2018) [19]. These changes were evidenced by a reduction in peak intensities in specific FT-IR wavenumber regions. For instance, the decrease in intensity within the range of 3392–2994 cm^{-1} indicated alterations in hydroxyl (–OH) groups, reflecting enzymatic reactions acting on the plastic polymer (Stuart, 2004) [17]. Significant changes observed in the regions 2930–2835 cm^{-1} and 1476–1446 cm^{-1} corresponded to stretching vibrations of C–H and C=C bonds, suggesting the cleavage of polymer chains (Gewert *et al*., 2015) [6].

These results are consistent with previous studies demonstrating the role of enzymes such as alkane hydroxylase and serine hydrolase in plastic degradation (Tokiwa *et al*., 2009) [18]. Such enzymes are capable of cleaving LDPE polymer chains into smaller compounds that are more readily metabolized by bacteria (Urbanek *et al*., 2018) [19]. In addition, notable changes within the region 1294–811 cm^{-1} represent stretching of C–O bonds, which constitutes another indication of oxidative degradation (Stuart, 2004) [17]. Differences in transmittance in the FT-IR spectra suggest that the bacteria reduced the mass of the plastic and modified its chemical structure. This evidence demonstrates that bacterial biodegradation involves complex enzymatic reactions that transform both the physical and chemical properties of LDPE plastic (Shah *et al*., 2008) [14]. The biodegradation process minimizes the environmental impact of plastic waste and converts it into simpler and more environmentally friendly compounds (Urbanek *et al*., 2018) [19].

Isolate AND02 exhibited the highest potential as an LDPE

plastic biodegradation agent. This finding supports previous reports suggesting that Gram-positive bacteria are more efficient in degrading plastics due to their ability to produce larger quantities of enzymes (Shah *et al*., 2008) [14]. However, the efficiency of plastic biodegradation is strongly influenced by environmental conditions such as temperature, pH, and oxygen availability (Montazer *et al*., 2020) [10]. Wu *et al*. (2023) [22] reported that controlled environmental conditions can increase bacterial enzymatic activity by two to threefold. This study provides new insights into the potential of bacteria as an environmentally friendly solution for treating LDPE plastic waste.

4. Conclusion

In conclusion, this study successfully isolated and characterized three bacterial strains, including two Gram-positive coccoid-shaped bacteria and one Gram-negative bacillary-shaped bacterium. The distinct structural differences between Gram-positive and Gram-negative bacteria, particularly the thickness of the peptidoglycan layer, contributed to variations in the efficiency of plastic biodegradation. Among the isolates, AND02 exhibited the highest potential for LDPE degradation, achieving a degradation rate of 7.42%, which was markedly higher than those observed for AND01 (0.85%) and AND03 (0.41%). This result demonstrated that AND02 was able to utilize LDPE more efficiently as a source of carbon and energy.

FT-IR analysis revealed structural modifications in LDPE after bacterial degradation, including alterations of hydroxyl (–OH) groups, stretching of C–H and C=C bonds, and oxidative modifications of C–O bonds. These changes indicate enzymatic activity by the isolates, leading to the breakdown of polymer chains into smaller and simpler molecules, thereby reducing the mass of plastic waste through bacterial biodegradation and transforming it into environmentally friendly compounds.

The results highlight the strong potential of bacteria, particularly isolate AND02, as an effective agent for LDPE plastic biodegradation. This study contributes to the growing body of evidence supporting the use of microorganisms in sustainable plastic waste management. Furthermore, valuable insights were provided into the potential of bacterial isolates to address the global problem of plastic pollution. These findings form a basis for the development of environmentally friendly, biotechnology-based solutions aimed at mitigating plastic waste accumulation and promoting a sustainable approach to waste management.

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