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Isolation and characterization of new bacterial strains degrading low-density polyethylene

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Abstract. Plastics have become indispensable in everyday life due to their properties. For this reason, the accumulation of polymer waste in the natural environment is becoming a serious global problem. The aim of the research was to isolate microorganisms capable of biodegrading plastics. The studies focused on the biodegradation of low-density polyethylene as the most common polymer. Seven and five bacterial strains were isolated from the landfill and compost, respectively. The morphological and biochemical characteristics of the isolates were determined. These isolates were able to survive in an environment where the only carbon source was LDPE, but no increase in biomass was obtained. However, analysis of the spectra obtained by the ATR-FTIR method showed the formation of chemical changes on the polymer surface. Bacterial biofilm formation was visualized by scanning electron microscopy. The toxicity of plastic biodegradation products in a liquid environment was tested and their safety for plants was confirmed. However, these biodegradation products have acute lethal

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toxicity for the *Daphnia magna.*

LDPE films were pre-treated with H_2O_2, HNO_3, or heat. The biodegradation of HNO_3-treated LDPE by isolated bacteria was the most significant. The weight loss was approximately 8%, and 6%, for landfill and compost-isolated bacterial strains, respectively.

Keywords: LDPE, biodegradation, bacterial isolates, FTIR, SEM

1. INTRODUCTION

Plastics are synthetic, organic polymers produced on the basis of fossil fuels such as oil and natural gas. Due to their properties, these substances over time have become indispensable in everyday life and are increasingly replacing the previously used natural materials. Plastics are characterized by lightness, durability, strength, flexibility, and low production costs. These materials can also be relatively easily modified to specific requirements, which significantly affects their wider use in new areas of industry. The number of polymers produced is growing year by year: in 2020, global production of plastics amounted to 367 million tonnes, compared to 359 million tonnes in 2018.

With the increasing production of plastics, there is a global problem with the amount of synthetic waste, more so that, according to estimates, about 50% of polymer products are thrown away after a single use (Napper et al., 2019). The effective management of synthetic waste is a significant challenge, with the aim not only of reducing the amount of waste generated, but also of preventing its release into the environment (Fig. 1).

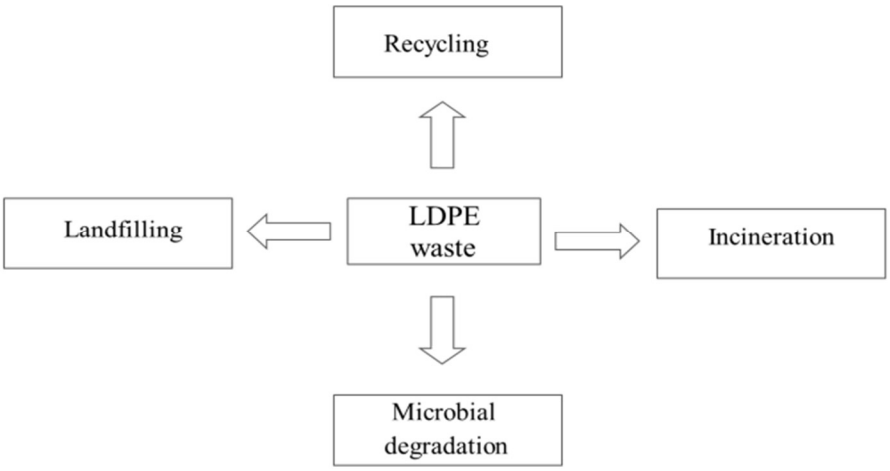

Figure 1. Methods of LDPE waste utilization (Jadaun et al., 2022).

Currently, synthetic polymers are widely used in every area of life, and it seems that there is no good alternative for them. The most popular plastic is polyethylene, which accounts for almost 30% of all polymers produced. This material is highly resistant to biodegradation due to the stable C-C and C-H covalent bonds present in the backbone and the lack of reactive functional groups, as well as high molecular weight and strong hydrophobic properties (Mohanan et al., 2020; Baldera-Moreno et al., 2022).

In the natural environment, plastics can degrade through both abiotic processes (chemical and physical degradation) and biodegradation. Biological methods are a promising alternative to removing plastic from the environment because they completely degrade pollutants and at the same time are relatively cheap and easy to use.

Aerobic biodegradation involves microorganisms that break down plastics into the water, carbon dioxide, and biomass. This complex process depends on many factors, such as environmental conditions (pH, temperature, operation), the chemical structure of the polymer, its molecular weight, the content of crystalline and amorphous particles, and the physical form of the polymer.

The entire degradation process of plastics, due to their physical and chemical properties, is a multi-stage complex and may involve a combination of different mechanisms. Often, the first stage involves changes in the physicochemical properties of polymers caused by the action of abiotic environmental factors, and the next stage is decomposition by microorganisms [Ali et al., 2021; Arutchelvi et al., 2008; Rani et al., 2022; Matjašič et al., 2021).

The presence of polymer waste in the natural environment caused many microorganisms to develop the ability to use them as a source of carbon and energy. The evolution of the metabolic systems of cells, which allows obtaining nutrients from polymers, somehow adapts microbes to life in the era of synthetic materials. Microorganisms showing the ability to degrade LDPE have been characterized in scientific studies, and the following bacteria were presented: *Bacillus licheniformis* SARR1, *Serratia* sp., *Stenotrophomonas* sp., *Pseudomonas* sp., *Ralstonia* sp. SKM2, *Bacillus* sp. SM1 and *Pseudomonas aeruginosa* (PAO1) (Nadeem et al., 2021; Rani et al., 2022; Biki et al., 2021; Kyaw et al., 2012). The objective of this study was to isolate and characterise novel microorganisms that degrade low-density polyethylene. Bacterial strains from two different sources, landfill and compost, were isolated and characterised. Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) were used to analyze the degradation process of LDPE.

2. MATERIALS AND METHODS

2.1. Polyethylene film preparation

The LDPE film was purchased from a retail store in Gliwice. The density and surface weight of the film were 921 - 926 kg/m3 and 36.8 ± 7.0 g/m3, respectively. The LDPE film was cut into small pieces of 3 cm x 3 cm, washed with 70% ethanol for 30 minutes, washed three times with sterilized distilled water, and dried in an oven at 60 °C overnight.

To test the biodegradability of LDPE, the basic mineral medium consisted of the following ingredients per 1 liter of distilled water: 0.7 g of KH_2PO_4, 0.7 g of K_2HPO_4, 0.7 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of NH_4NO_3, 0.005 g of NaCl, 0.002 g of $FeSO_4 \cdot 7H_2O$, 0.002 g of $ZnSO_4 \cdot 7H_2O$, and 0.001 g of $MnSO_4 \cdot H_2O$. The medium was autoclaved at 121°C for 20 minutes.

2.2. Sample collection and isolation of LDPE-degrading bacteria

Soil samples were collected from two sources: a landfill and commercial compost. The landfill site was situated in a location where plastic waste had been deposited for an extended time (10-20 years), which heightened the possibility of identifying bacteria capable of breaking down LDPE. Approximately 10 g of soil was collected from 10 different points (1 to 5 cm depth in the soil), placed in the sterile test tube and transported to the laboratory. Soil samples were stored at 4°C and used for experiments within 24 hours of collection. All soil samples were mixed and 10 g of soil was suspended in 90 ml of sterile water.

To test the potential of bacteria present in commercial compost to break down LDPE, an LDPE film was buried in a container containing compost purchased from a local garden store. After a 10-month incubation period, the LDPE was removed from the compost and rinsed with sterile basal medium.

Isolation of bacteria was done by serial dilution and spread plate technique using agar plates. For isolation of the LDPE-degrading bacteria the agar plates with 0.1% LDPE powder (Thermo Fisher Scientific) were prepared. After inoculation plates were incubated at 30°C until bacterial growth was observed. All morphologically distinct colonies were separated to get pure isolates. Isolated bacterial strains were tested for LDPE degradation ability.

2.3. Physiological and biochemical characteristics of isolated bacteria

After incubation on agar plates with LDPE powder as a carbon source, morphologically and biochemically distinct isolates were obtained. All pure isolates were tested for their physiological and biochemical properties. Biochemical studies were carried out after 24 hours of incubation of the cultures on agar plates at 30°C. The Gram reaction and culture characteristics such as colour, colony shape, colony size, etc. were described. Selected biochemical tests such as catalase test, oxidase test, motility test, casein hydrolysis test, starch hydrolysis test, lecithinase test, and potato pathogenicity test were performed.

2.4. Biodegradation of LDPE

For biodegradation tests, 6 pieces of LDPE foil were weighted and placed in 500 ml Erlenmeyer flasks containing 200 ml of basic mineral medium. Flasks were inoculated with selected bacterial strains isolated from landfill (T1, T2, T3) and compost (K2, K4, K5). The consortium of bacteria, consisting of strains K2, K4 and K5, was also used for LDPE degradation tests. The cultures were incubated for 60 days at 30°C on a rotary shaker with rotation at 130 rpm. Inoculum and incubation were performed under fully aseptic conditions.

2.5. LDPE weight loss

After 2 months, the LDPE pieces were removed from the culture. The LDPE films were washed 3 times with 75% ethanol, sterilized water, and then immersed in 30 ml of a 10% SDS solution for 24 hours. After 24 hours of drying at 65°C, the weight of the residue was determined using a MAX 50/1/NH moisture analyzer (Radwag). The amount of mass lost by the polymer was calculated as:

$$weight\ loss\ [\%] = \frac{W_0 - W}{W_0} \cdot 100\% \quad (1)$$

where W_0 and W are the initial and final weights of the polymer, respectively.

Different pre-treatments were used to increase the susceptibility of LDPE film to biodegradation. The LDPE pieces were treated by temperature (80 °C) or immersed in 50%

HNO_3 or 30% H_2O_2 for 120 min. Then was prepared as described above for biodegradation tests. The LDPE foil was weighted and placed in 500 ml Erlenmayer flasks containing 200 ml of basic mineral medium. The flasks were inoculated with the bacteria isolated from the landfill. The rest of the pre-treated LDPE was buried in the compost. After 60 days the weight was determined.

2.6. Contact angle

The hydrophobicity of the sample surface can be assessed by measuring the contact angle. The contact angle is the angle between a solid surface and a drop of liquid falling on it. The hydrophobicity of LDPE was measured before and after incubation with the isolated bacterial strains. It is assumed that the contact angle of the hydrophilic materials is less than 90° and that the hydrophobic materials have a contact angle greater than 90°. In the present studies, deionized water was used for contact angle measurements using a video camera (JVC™ GZ-EX355 Everio). Contact angles were measured at room temperature. An average of three measurements was reported.

2.7. Hydrophobicity of bacterial cells

The BATH (bacterial adhesion to hydrocarbon) test (Rosenberg et al. 1980) was used to determine the hydrophobicity of the bacterial cell surface of the isolated bacteria. A 24-hour culture (5 mL) in nutrient broth was centrifuged at 10,000 rpm for 15 min at 4°C and washed twice with phosphate-urea-magnesium (PUM) buffer. After centrifugation, the supernatant was discarded and the pellet was resuspended in PUM buffer with an optical density of 0.6 at 550 nm. 0.2 mL hexadecane was added to the suspension and vortexed for 20 minutes. The tubes were allowed to stand for 5 minutes to facilitate phase separation. The absorbance of the aqueous layer was then measured at 550 nm. The culture-free buffer was used as a blank. The percentage of hydrophobicity was calculated as follows:

$$hydrophobicity\ [\%] = \frac{Initial\ OD - Final\ OD}{Initial\ OD} \times 100\% \quad (2)$$

2.8. Clear zone assay

A clear zone method was used to screen bacterial isolates for LDPE degradation. Agar plates containing LDPE powder as a carbon and energy source were prepared and inoculated with

bacteria isolated from compost. After 48 hours of incubation, a clear zone around the colonies was visualized by staining the plates with 0.1% Coomassie Brilliant Blue and destaining as described by Nademo et al. (2023). Coomassie Blue was dissolved in 40% (v/v) methanol and 10% (v/v) acetic acid to prepare a 0.1% Coomassie Brilliant Blue solution. The destaining solution was prepared by mixing 40% methanol with 10% acetic acid. The agar plates were first stained with the Coomassie Blue solution for 20 min and then the pigment was washed off with the destaining solution for 20 min. A transparent zone around the colony indicates that the bacterial strains can be considered LDPE-degrading isolates.

2.9. Fourier-transform infrared spectroscopy

The most commonly used technique to determine the impact of microorganisms on plastics is Fourier-transform infrared spectroscopy (FTIR). FTIR allows for the assessment of chemical changes occurring on the surface of the polymer. The carbonyl index (CI) can be used to measure the degree of degradation of polyethylene because its value depends on the amount of degraded carboxylic bonds. The carbonyl index is calculated according to the formula:

$$\mathrm{CI} = \frac{\text{absorption in the range of } 1650-1780\ \mathrm{cm}^{-1}}{\text{absorption in the range of } 1440-1485\ \mathrm{cm}^{-1}} \quad (3)$$

where the range 1650-1780cm^{-1} corresponds to the carboxyl group and 1440-1485 cm^{-1} corresponds to the methyl group. The LDPE films after exposition do isolated bacteria were analyzed by FTIR-ATR spectrophotometer (Nicolet 6700, Thermo Electron Corporation) at regular intervals in the frequency range of 400-4000) cm^{-1}.

2.10. Scanning electron microscopy

Changes in the surface morphology of the LDPE films incubated with isolated bacteria were examined by scanning electron microscopy (SEM) (Phenome Pure, Thermo Fisher Scientific). The LDPE films were removed from the cultures and fixed overnight with 3% glutaraldehyde (0.1 M PBS, pH 7.4). The LDPE was rinsed with 0.1 M PBS before dehydration in 50, 70, 90 and 96% ethanol and twice in 100% acetone. The films were dried overnight and sputter-coated with gold prior to imaging.

2.11. The toxicity of the biodegradation products

The phytotoxicity of cell-free culture supernatant was evaluated in a static test (Mendes et al. 2021). Seeds were purchased from a local company. Their germination potential was examined at 22 ± 2°C in darkness, prior to the assays as a control for the (90% guaranteed) viability of the seeds. The static test was based on root elongation and seed germination of *Lepidium sativum* and *Triticum aestivum* L. 10 seeds were placed on each plate to the filter paper and 4 ml of the cell-free culture supernatant or water was added. Seed germination and root elongation (≥5 mm) were determined after 5 days of incubation in the dark. Relative seed germination, relative root length, and germination index were then determined as seen below:

$$\text{Relative seed germination} = \frac{\text{number of seeds germinated in the supernatant}}{\text{number of seeds germinated in the control}} \cdot 100\% \quad (4)$$

$$\text{Relative root length} = \frac{\text{mean root length in the supernatant}}{\text{mean root length in the control}} \cdot 100\% \quad (5)$$

Toxicity studies were performed in a fermented medium without bacterial cells (centrifugation at 4 °C, 15 min, 5000 g). Toxicity tests using the microcrustacean *Daphnia magna* were performed on organisms aged from 6 to 24 hours. Toxicity was measured by the effect on mortality after 24 and 48 hours of exposure (Persoone et al. 2009).

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. All the experiments with plants and microcrustaceans were carried out in five replicates.

3. RESULTS AND DISCUSSION

3.1. Isolation and characterization of LDPE-degrading bacterial strains

LDPE-degrading bacterial strains were isolated from soils with long-lasting polymers. One source of the bacteria was a landfill that had been a plastic landfill for many years, the other source was commercially purchased compost in which LDPE had been placed (Fig. 2).

Seven bacterial strains (T1–T7) were obtained from the landfill site and five different bacteria (K1–K5) were isolated from the compost. Morphological and biochemical characterization of the isolated bacterial strains was conducted. Each colony formed after purification was

characterized by colonial morphology, including edge shape, colour, and colony surface. The isolates demonstrated significant diversity in terms of both morphology and biochemistry (Table 1).

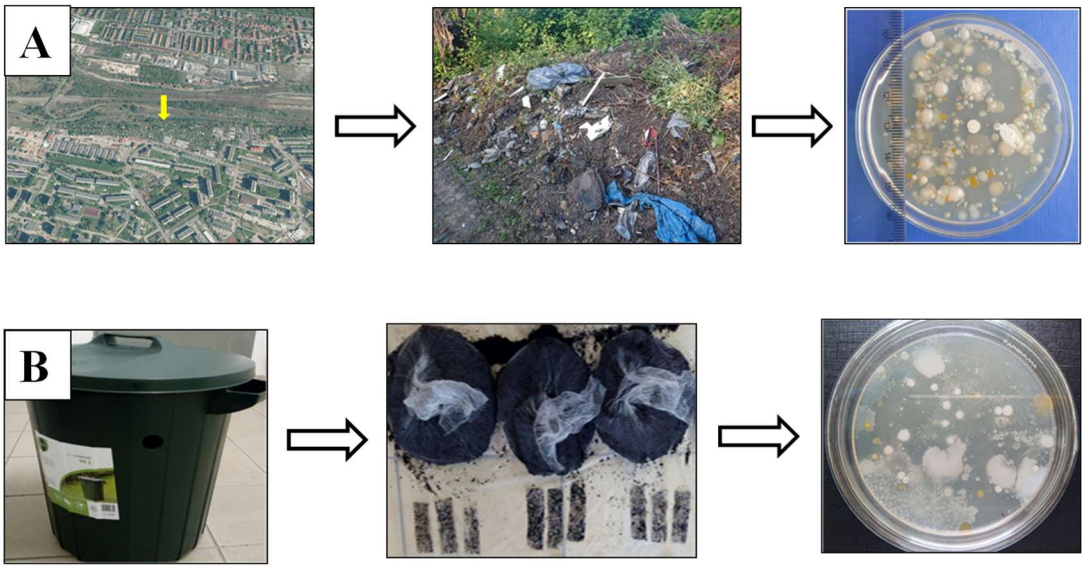

Figure 2. Isolation of bacteria from plastic-contaminated soil. A-isolation from landfill, B-isolation from compost.

Gram staining revealed that five soil isolates were gram-negative and two were gram-positive. Among the bacterial strains isolated from the compost, only strain K3 was gram-negative, while the other isolates were gram-positive. The colour of the colonies ranged from brown to light cream and the size of colonies was generally small to medium. The biochemical profile of the bacteria was examined to determine their potential wider applications. The following tests were performed: catalase and oxidase activity, the ability to hydrolyze casein and gelatin, and lecithinase activity. Additionally, the amylase test was conducted. Of all the isolates, only strain K4 was positive for the lipase test. Strain K3 exhibited different characteristics compared to other bacteria obtained from compost. It was the sole bacteria isolate that tested positive for casein and gelatin hydrolysis. During the investigation of bacterial phytopathogenicity, it was discovered that only T4 strain isolated from a landfill site could cause potato diseases. All test results are shown in Table 2.

270 Table1. Morphological features of bacterial isolates from the soil.

Bacterial isolates	Morphology	Pigmentation	Diameter, mm
T1	Colonies are round, flat-convex, flat, transparent, shiny; the contour of the edge is even; the structure is uniform; the consistency is paste-like	light cream	5
T2	Colonies are round, flat-convex, opaque, smooth, and shiny; the contour of the edge is even; the consistency is paste-like	light yellow	1-2
T3	Colonies of irregular shape, cloudy, flat-convex, the surface is radially striated; the contour of the edge is jagged; the consistency is paste-like.	cream	5-8
T4	Colonies are round, flat-convex with a raised center, the surface is rounded, shiny with a shine, and transparent; the contour of the edge is wavy; the structure is uniform; the consistency is paste-like	yellow	1-2
T5	Colonies are round, drop-shaped, smooth, shiny, and opaque, the contour of the edge is even; the consistency is pasty	orange	1-2
T6	Colonies are rhizoidal, bent, not smooth, opaque, the contour of the edge is wavy; the consistency is brittle, dry	white	5-6
T7	Colonies are round, convex, smooth, shiny, and opaque, the contour of the edge is even; the consistency is pasty	brown	1
K2	Colonies are round, flat-convex with a raised center, opaque, smooth, the contour of the edge is even, embedded in the agar, producing pigment.	grey-white	1-3
K3	Colonies are round, flat, transparent, shiny; the contour of the edge is even; the structure is uniform; the consistency is paste-like	light cream	1
K4	Colonies are round, flat-convex with a convex center, opaque, smooth, shiny, edge contour even, embedded in the agar	cream	2-4
K5	Colonies of irregular shape, cloudy, flat-convex, the surface is radially striated; the contour of the edge is wavy; the consistency is paste-like, producing pigment.	cream	2-3
K6	Colonies of irregular shape, cloudy, flat-convex, the surface is radially striated; , the contour of the edge is wavy; the consistency is paste-like, producing pigment.	cream	2-3

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Table 2. Biochemical characterization of bacterial isolates from the soil.

Test	Isolates from landfill							Isolates from compost				
	T1	T2	T3	T4	T5	T6	T7	K2	K3	K4	K5	K6
Gram test	-	+	+	-	-	-	-	+	-	+	+	+
Catalase test	-	-	+	+	-	+	+	-	+	-	+	+
Oxidase-test	+	+	+	+	-	-	+	-	-	-	+	-
Casein hydrolysis test	-	-	-	+	+	+	+	-	+	-	-	-
Gelatin hydrolysis test	-	-	-	-	+	+	+	-	+	-	-	-
Amylase test (starch hydrolysis test)	-	-	-	-	+	+	-	+	+	+	+	+
Lecithinase test	-	-	+	-	-	-	-	-	-	-	+	+
Phytopathogenicity test	-	-	-	+	-	-	-	-	-	-	-	-
Lipase test	-	-	-	-	-	-	-	-	-	+	-	-

3.2.Determination of weight loss

The common approach to assessing the biodegradation of LDPE is to estimate its weight loss. After 60 days of incubation of LDPE with bacteria, the weight was measured and the weight loss was calculated (refer to Table 3). It was found that the microorganisms isolated from the compost showed a greater weight reduction than those isolated from the landfill. Table 3 presents the results of the biodegradation tests carried out without pre-treatment, and no reduction in biomass was observed in the control sample. The study's conclusions were consistent with previous research by Gupta and Devi (2020), who identified three bacterial strains (ISJ36, ISJ38, and ISJ40) isolated from soil-adherent polyethylene film collected from landfill sites. Khandare et al. (2021) noted that over a period of 90 days, four marine bacterial isolates (H-237, H-255, H-256, and H-265) experienced weight loss of 1.4%, 1.72%, 1.26%, and 0.97%, respectively. Both studies yielded outcomes without the use of a pretreatment approach. Other studies have demonstrated the possibility of isolating bacterial strains with higher LDPE-degrading efficiencies. It was found that *Serratia* sp. was able to reduce the weight of the LDPE plastic pieces by up to 40% and *Nocardiopsis alba* also achieved a 32.25% reduction in polymer weight. The origin of the bacterial isolates, the nature of the LDPE, and the culture conditions, such as incubation time, may explain the differences in the percentage of body weight loss in our study compared with the literature. The biodegradability of LDPE depends on its chemical and physical characteristics, including its hydrophilic/hydrophobic properties, crystallinity, and form (i.e., whether it is in film or

powder form). These factors should be considered when assessing LDPE's biodegradability (Ali et al. 2021; Auta et al. 2018; Maroof et al. 2021; Matjasic et al. 2021). Previous research has emphasized the significance of the origin of isolated bacteria, as well as the effects of distinctive environmental conditions on the ability of diverse microorganisms to biodegrade plastics (Nakei et al., 2022; Zhang et al., 2022). To provide an example of the significance of the origin of the bacteria, three isolates outlined by Nanda et al. (2010) should be taken into consideration. A comparison of three strains of *Pseudomonas* sp. from different sources of isolation indicated that the *Pseudomonas* sp. obtained from a sewage sludge dump (P1) was capable of polyethylene degradation with an efficiency of 29.1%. *Pseudomonas* sp. isolated from a textile sewage site showed a polyethylene biodegradability of 19.6%, while *Pseudomonas* sp. isolated from a domestic waste landfill (P2) showed the lowest PE biodegradability of 16.3%. Similarly Maroof and colleagues (2021) isolated *Bacillus subtilis* from waste disposal sites and found that the efficiency of this strain was roughly 20% lower than that of the *Bacillus subtilis* indigenous to the mangrove soil of the Niger Delta, as reported by Ibiene and colleagues (2013). The origin of the bacteria could have caused the difference in LDPE degradation.

To investigate the elimination of LDPE from the environment, not only pure bacterial strains of microorganisms were used, but also a bacterial consortium consisting of strains K2, K4, and K5. While various prior studies have indicated that mixed cultures exhibit higher efficacy in plastic degradation (Cada et al. 2019; Zhang et al. 2023), the LDPE degradation by the consortium K2, K4, and K5 examined was lower than that of single bacterial strains. Interactions between microorganisms seemed to limit LDPE degradation to some extent and competition for substrate uptake between bacteria in mixed cultures was unfavourable. It is possible that competition for nutrients and space intensified in the consortium used in the presented study. In addition, certain substances produced by the bacteria did not favour PE degradation. Similarly, the reduced degradation efficiency of hydrocarbons by mixed cultures was shown in a study by Al-Kaabi et al. (2022). They isolated three bacterial strains from the Dukhan site and tested their ability to degrade hydrocarbons. The effectiveness of single strains was greater than the combination of *B. licheniformis* D1D2 with either *P. aeruginosa* D5D1 or *P. aeruginosa* D7S1. Using these combinations results in a nearly 20% decrease in performance compared to that of pure bacterial strain.

Table 3. Weight loss of low-density polyethylene after incubation with isolated bacteria.

Isolated bacteria	T1	T2	T3	K2	K4	K5	Consortium
Weight loss [%]	0.67	0.66	0.38	0.9	0.93	1.01	0.2

Plastics possess properties of high durability and resistance to biodegradation, thus pre-treatment is frequently required to enable the breakdown of polymers by microorganisms. The objective of such treatments is to decrease the average polymer chain length or modify its surface. In our experiments, we utilized two different pre-treatment methods: thermal and chemical (using HNO_3 and H_2O_2). According to Table 4, the most effective technique for increasing biodegradation efficiency in the conducted tests was treating the polymers with a nitric acid solution. Rajandas et al. (2012) also reported on the efficacy of treating LDPE with nitric acid, which enabled the effective degradation of polyethylene by *Microbacterium paraoxydans*. The authors suggested that out of the various pre-treatment methods that exist, the incorporation of carbonyl groups into the backbone of the polymer using nitric acid is a potent strategy to increase the degradation rate of PE. Thermally pre-treated LDPE was used in the study, but no increased biodegradability was observed. In contrast, Kalia and Dhanya (2022) observed that *Lysinibacillus fusiformis* TPB was able to consume thermally pre-treated LDPE 35.54% more efficiently than untreated LDPE film

Table 4. Weight loss of LDPE after pretreatment and incubation with isolated bacteria.

Pre-treatment	T1	T2	T3	Placed in compost
Temperature	0.36	0.78	1.75	0
H_2O_2	0.34	0.79	0.9	0.45
HNO_3	7.38	8.04	8.01	5.6

3.3. LDPE hydrophobicity

BATH tests were conducted to assess the hydrophobicity of cell surfaces in the isolated bacteria. The reference organisms used were *Rhodococcus erythropolis* and *Pseudomonas aeruginosa*. *Rhodococcus erythropolis* exhibited high hydrophobicity, while *Pseudomonas aeruginosa* was a hydrophilic organism. The polymer surface's hydrophobicity is a critical factor in biodegradation research, and the substrate's affinity for microorganisms is crucial for colonizing the polymer surface. Bacterial cell adhesion to the substrate is a key factor in

allowing isolates to use the substrate as a carbon and energy source. Thus, hydrophobic bacteria are inclined to adhere to hydrophobic surfaces, while hydrophilic bacteria prefer to attach to hydrophilic substrates. Due to LDPE's hydrophobicity, it is thought that hydrophobic cells bind to the polymer more readily compared to hydrophilic isolates.

Strain K3 exhibited the greatest hydrophobicity, while the remaining isolated bacteria were more hydrophilic (Table 5). All the isolated microorganisms were found to be less hydrophobic than bacterial strains ISJ40 (28.7%), ISJ36 (13.3%), and ISJ38 (19.7%), as described by Gupta and Devi (2020). Nonetheless, the observed LDPE degradation ability of the aforementioned bacteria was not significantly lower than those reported in previous studies. The bacteria's affinity to the substrate is crucial for LDPE biodegradation, but not the only one affecting biodegradation.

Table 5. Hydrophobicity of the isolated bacteria.

Isolated bacteria	K2	K3	K4	K5	K6	R. erythropolis	P. aeruginosa
Hydrophobicity, %	2,9	14	2,2	1,9	2,26	40	0,37

3.4.Contact angle

The importance of the hydrophobicity of LDPE in the initiation of biofilm development can be determined by measuring the contact angle. This is a useful parameter for assessing the hydrophobicity/hydrophilicity of a specific surface. A lower contact angle value indicates greater hydrophilicity and makes it easier for microorganisms to colonize the surface. Zhang et al. (2022) suggested that the increase in hydrophilic properties of LDPE was the result of increasing the amount of oxygen on the polymer surface as a result of oxidative processes carried out by *Acinetobacter* sp. LW-1. In the experiments carried out, the contact angle of low-density polyethylene (LDPE) was measured after exposure to different strains of bacteria. The outcomes are presented in Table 6, indicating that the emergence of bacteria caused a shift in the foil's characteristics towards a more hydrophilic nature, promoting cellular adhesion and biofilm formation. Consequently, this enhanced the susceptibility of LDPE to biodegradation. *Bacillus tropicus* (MK318648) displayed comparable outcomes, wherein the contact angle reduced from 98.7 to 69.5 after bacterial treatment (Samanta et al., 2020). Furthermore, according to Han et al. (2020), hydrophilicity could be enhanced by 2.7% and 5.3%, respectively, through the use of *Arthrobacter sp*. and *Streptomyces sp*.

3.5. Clear zone

The study used the clear zone method to investigate the ability of bacterial strains isolated from compost to consume LDPE as a carbon source. The formation of the clear zone confirms the biodegradation of the polymer, which was further demonstrated by Augusta (1993) and Rafiq et al. (2018). Clear zone-forming bacteria are thought to have a greater ability to degrade polyethylene than other microorganisms. The reason for this is the secretion of extracellular enzymes that are responsible for the hydrolysis of LDPE (Nademo et al., 2023; Nakei et al., 2022; Rafiq et al., 2018). In this study, inoculated agar plates containing LDPE powder were stained with Coomassie Brilliant Blue. After decolorization with a destaining solution, clear zones were visible around LDPE-degrading colonies. The clear zone was observed around bacterial strains inoculated on agar plates, and confirmed the ability of tested isolates to degrade polyethylene (Fig. 3).

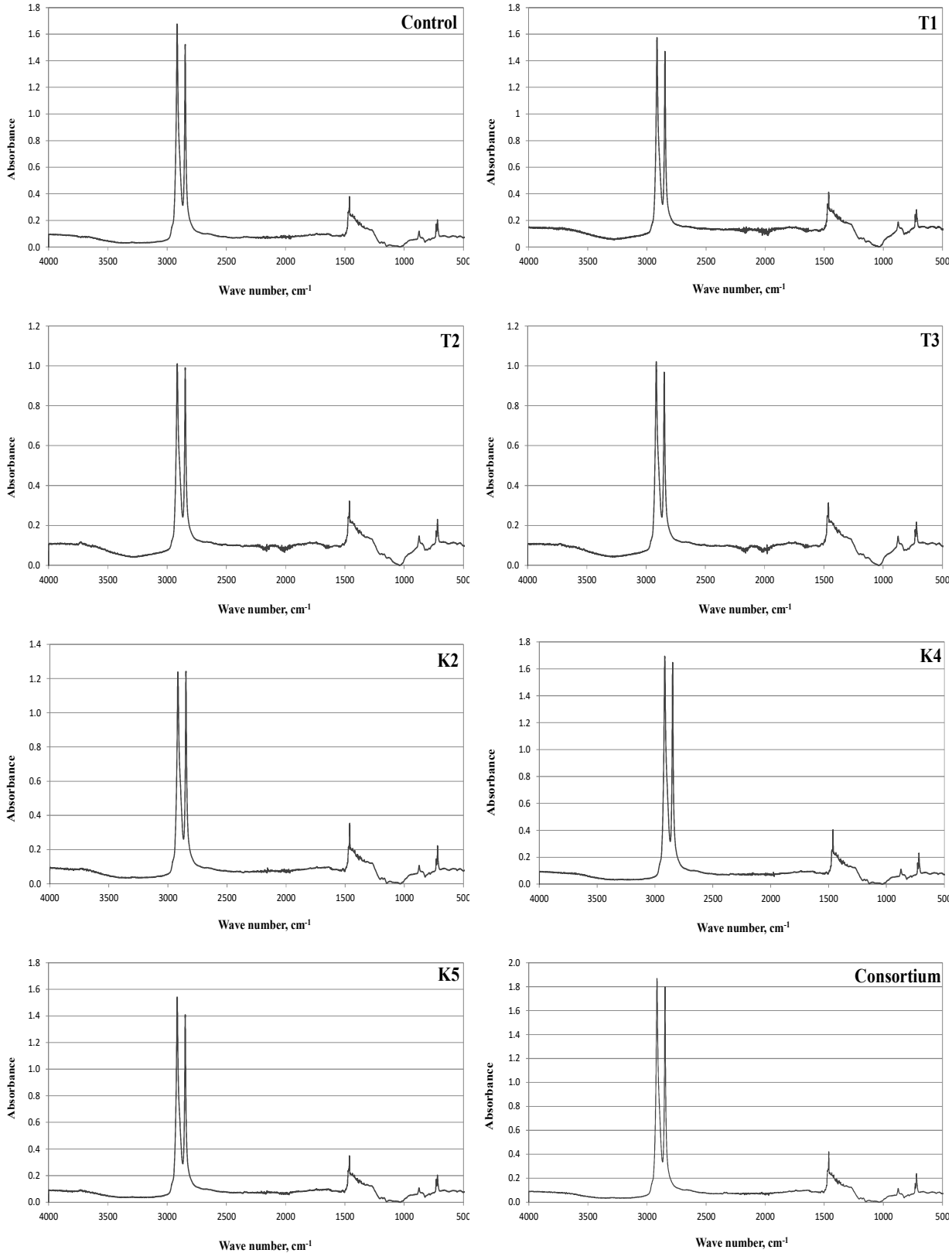
Figure 3. Clear zones formed by isolated bacteria.

3.6. FTIR spectroscopy analysis

The FTIR analysis of LDPE films was used to reveal the formation of new or vanishing functional groups. The changes in the LDPE structure after incubation with bacterial strains were determined using FTIR spectroscopy (Nicolet 6700, Thermo Electron Corporation) in the frequency range of 400–4000 cm^{-1}. The FTIR spectra of the biologically treated polyethylene after a period of 60 days in aqueous media are shown in Figure 4. A variety of peaks that indicate the complex nature of LDPE were observed in the FTIR spectra of the PE film. Characteristic peaks at 2915 cm-1 and 2848 cm-1 were found to be indicative of asymmetric and symmetric C-H stretching, respectively. The LDPE strips exhibited

absorbance bands at 718 cm^{-1} prior to and post-incubation, which confirms the existence of =C–H bending bond (mono). Furthermore, characteristic absorption bands were observed at 1465 cm^{-1} for the C=C stretch. In this study, FTIR analysis showed that the band at 1465 cm^{-1} became significantly weaker after microbial treatment, indicating C=C bending deformation. The intensity of the peaks at 718 cm^{-1}, designated as C–H bending mono, decreased due to the microbial action of the isolated bacteria. The study showed that the isolated bacterial strains degraded polyethylene film, possibly mediated by enzymatic action. Enzymes are critical in catalyzing a precise sequence of reactions that result in a variety of molecular changes, including oxidation, reduction, hydrolysis, and esterification. In addition, enzymes play a crucial role in the biodegradation of polyethylene by facilitating internal molecular transformations. The identical findings have been documented by previous researchers who have monitored the formation and disappearance of functional groups in order to explain the mechanism of the biodegradation process. Changes in peak sizes and functional groups confirmed the modification of the polymer surface after biological treatment. The formation of keto, ester, vinyl, and internal double bonds were observed by FTIR spectra and indicated the bacterial degradation of the treated polymer (Cada et al., 2018; Rani et al., 2022; Samanta et al., 2020)

The Carbonyl Index (CI) was determined and is presented in Table 6. CI reflects changes in carbonyl groups and is the most important index used to evaluate the oxidation of LDPE during the biodegradation process. The studies presented indicate that the K4 isolate and consortium from compost caused a decrease in CI, whereas increased carbonyl indices were computed for bacteria isolated from landfills and strains K2 and K5 from compost. This aligns with similar results presented by Cada et al. (2019). A decrease in the CI for strain *Bacillus pseudofirmus* 17 and an increase in CI were observed after 60 days of incubation of the LDPE with *Bacillus agaradhaerens* I9. The lower carbonyl index was attributed to the use of oxidation products such as carboxylic acids by the inoculated bacteria, while the higher CI was due to the formation of ketone or aldehyde C=O groups during the degradation of LDPE, as suggested by the authors.



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445 Figure 4. FTIR spectra for control and bacterial-treated LDPE.

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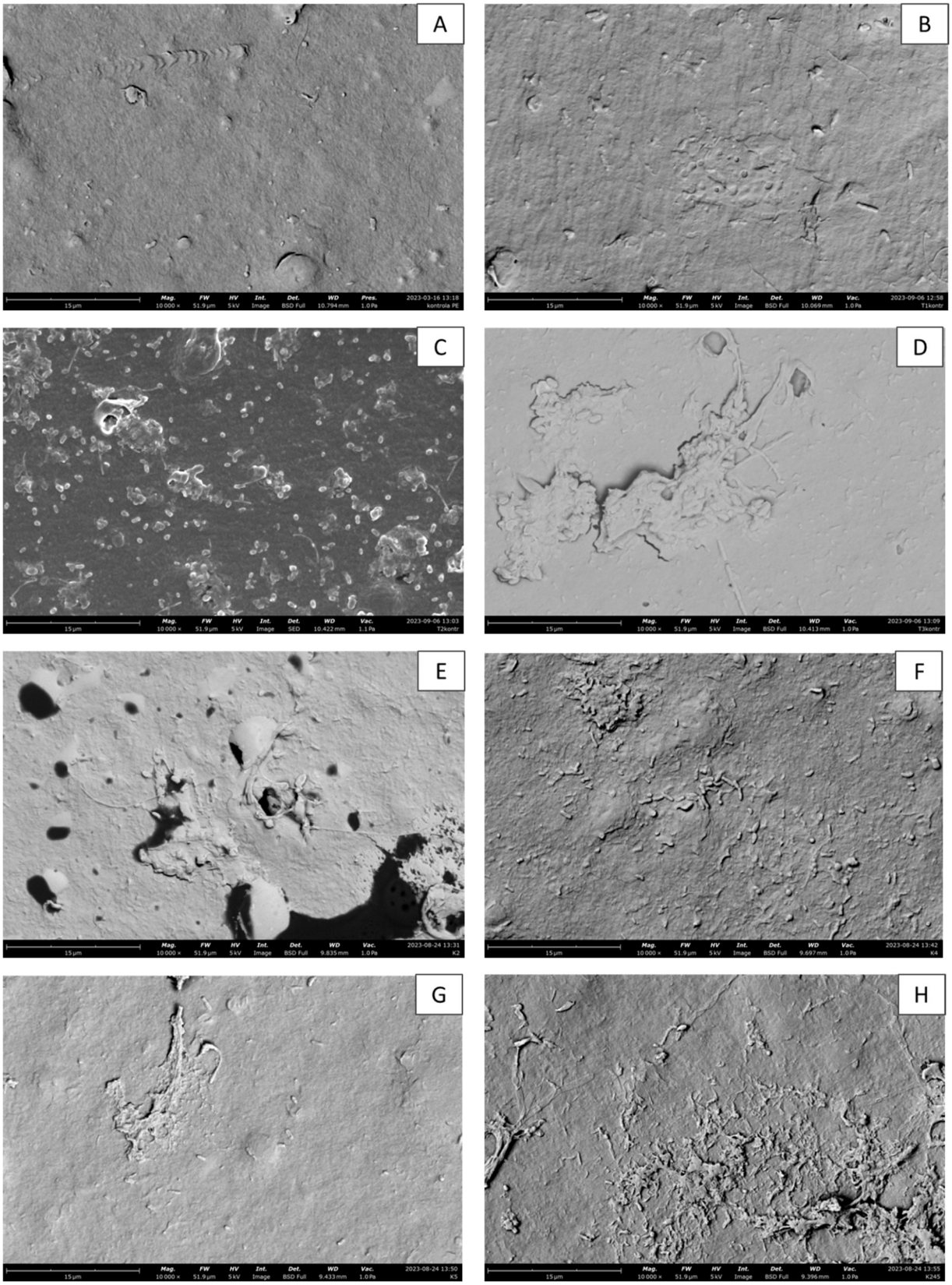
Table 6. Hydrophilicity of the polymer surface and Carbonyl Index of the polymer after bacterial treatment.

		Landfill			Compost			
Isolated bacteria	Control	T1	T2	T3	K2	K4	K5	Consortium
Contact angle, º	98	68	72	72	84	84	83	86
CI	0.273	0.368	0.364	0.378	0.283	0.247	0.287	0.241

3.7. Scanning Electron Microscopy

Physical changes on the plastic surface can be observed by visualizing the plastic surface using a scanning electron microscope (SEM), which is commonly used for analysis purposes. Microbial activity can cause cracks, wrinkles, holes, and pores on the plastic surface. Scanning microscopy can also be used to assess the biofilm that has formed on the polymer surface. The presented research exhibits that the control samples maintained smooth surfaces without any significant changes observed. However, the scanning electron microscope images of the polyethylene film showed the presence of a biofilm on its surface after 60 days of bacterial treatment (Fig. 5). The biofilm present on the film indicated the ability of the isolated bacteria to adhere to the PE surface. The biofilm layer varied among the tested bacteria. Some microorganisms, such as strain T1 and K4, formed a thin biofilm layer, whereas others were able to cover the LDPE surface with a dense biofilm layer. Efficient microbial degradation of non-soluble substrates, such as polyethylene, requires the creation of a biofilm on the polymer surface. The biofilm's thickness depending on the adsorption potential of the isolated bacteria on the polymer. As reported by Gilan, isolate C208 effectively colonized the polyethylene surface and biodegraded polyethylene relatively fast, whereas three other isolates from the same consortium did not form a notable biofilm and were less effective at degrading polyethylene.

SEM images of LDPE revealed degradation in the area surrounding the bacterial cells, and cellular patterns were also observed on the polyethylene film. The changes on the LDPE surface could be ascribed to the bacteria's production of extracellular enzymes and metabolites. These findings imply that LDPE was a carbon source, confirming the ability of the isolated bacteria to degrade polyethylene. The formation of the biofilm layer and changes in LDPE surfaces were previously reported by Harshvardhan and Jha (2013), Gupta and Devi (2020), and Rani et al. (2021).



476 Figure 5. Biofilm formation and changes in surface topography of the LDPE film after
477 biological treatment. A-without-treatment, B-T1 strain, C-T2 strain, D-T3 strain, E-K2 strain,
478 F-K4 strain, G-K5 strain, H-Consortium.
479

3.8. The toxicity of the biodegradation products

The toxicity of plastic biodegradation products (filtrates) was investigated and the influence of leachates on relative root length, relative seed germination for wheat (*Triticum aestivum* L), and degree of toxicity for *Daphnia magna* was determined (Table 7). For this purpose, under similar conditions (OD 0.1, 28 days, 30°C, 130 rpm), bacteria were cultivated in the presence of LDPE. After centrifugation, the toxicity of the supernatant was measured. The safety of polyethylene biodegradation products for wheat was established. The degree of their toxicity does not exceed 20% for strains K2, K3, K4, and consortium, and the toxicity of bacteria isolated from landfill was less than 40%.

Similarly, Rani and colleagues (2022) reported that compounds generated from bacterial degradation of LDPE using *Bacillus licheniformis* SARR1 were non-toxic to *Vigna radiate*. Toxicity was observed for the crustacean Daphnia magna, indicating that plastic poses a hazard to water environments and its decomposed products could be harmful to aquatic organisms.

Table 7. Phytotoxicity and toxicity of cell-free culture supernatant after biodegradation of polyethylene.

			Control	T1	T2	T3	K2	K4	K5	Consortium
Phytotoxicity	Wheat (*Triticum aestivum* L)	Relative root length, [%]	65	75	68	64	85	82	80	98
		Relative seed germination, [%]	84,6	99	99	95	100	100	92,3	92,3
Toxic effect	*Daphnia magna*	Degree of toxicity, [%]	100	---	---	---	73	50	63	47

4. CONCLUSIONS

The study aimed to investigate the degradation of low-density polyethylene by novel bacterial strains. Morphological and biochemical characterization was carried out on 12 different bacteria isolated during tests. The microorganisms demonstrated the ability to utilize LDPE as the only carbon and energy source. Furthermore, the biodegradability of LDPE was significantly enhanced by nitric acid pretreatment. Chemical and physical modifications of

LDPE were detected after incubation of polyethylene with isolated bacteria. The FTIR analysis of LDPE films revealed the formation of new and vanishing functional groups. The research confirmed that the isolated bacteria formed a biofilm layer on the polymer surface, which enables microorganisms to utilize the insoluble substrate effectively. SEM images of LDPE showed decomposition in the region surrounding the bacterial cells, and cellular patterns were also detected on the polyethylene film. Extracellular enzymes and metabolites produced by the bacteria may be responsible for these changes on the LDPE surface. Plastic biodegradation products were tested for toxicity in a liquid environment and found safe for plants. Nonetheless, these products were observed to have acute and lethal toxicity towards the *Daphnia magna*. The research findings indicated that the isolated bacteria could have the potential to enhance the process of managing polymer waste.

SYMBOLS

W_0- initial weight of the polymer, g

W- final weight of the polymer, g

REFERENCES

1. Al-Kaabi N., Al Disi Z., Al-Ghouti M.A., Solling T. I., Zouari N., 2022. Interaction between indigenous hydrocarbon-degrading bacteria in reconstituted mixtures for remediation of weathered oil in soil. Biotechnol. Rep., 36, e00767. DOI: 10.1016/j.btre.2022.e00767.
2. Ali S.S., Elsamahy T., Al-Tohamy R., Zhu D., Mahmoud Y.A., Koutra E., Metwally M.A., Kornaros M., Sun J., 2021. Plastic wastes biodegradation: Mechanisms, challenges and future prospects. Sci. Total. Environ., 780, 146590. DOI: 10.1016/j.scitotenv.2021.146590.
3. Arutchelvi J., Sudhakar M., Arkatkar A., Doble M., Bhaduri S., Uppara P., 2008. Biodegradation of polyethylene and polypropylene. Indian J. Biotechnol., 7, 9-22.
4. Augusta J., Müller RJ., Widdecke H., 1993. A rapid evaluation plate-test for the biodegradability of plastics. Appl. Microbiol. Biotechnol., 39, 673–678. DOI: 1007/BF00205073.
5. Auta H.S., Emenike C.U., Jayanthi B., Fauziah S.H., 2018. Growth kinetics and biodeterioration of polypropylene microplastics by *Bacillus* sp. and *Rhodococcus* sp. isolated from mangrove sediment, Mar. Pollut. Bull., 127, 15-21. DOI: 10.1016/j.marpolbul.2017.11.036.

6. Baldera-Moreno Y., Pino V., Farres A., Banerjee A., Gordillo F., Andler R., 2022. Biotechnological Aspects and Mathematical Modeling of the Biodegradation of Plastics under Controlled Conditions. Polymers., 14, 375. DOI: 10.3390/polym14030375.
7. Biki S.P., Mahmud S., Akhter S., Rahman J., Rix J.J., Al Bachchu A., Ahmed M., 2021. Polyethylene degradation by *Ralstonia* sp. strain SKM2 and *Bacillus* sp. strain SM1 isolated from land fill soil site. Environ. Technol. Innov., 22, 101495, DOI: 10.1016/j.eti.2021.101495.
8. Cada, E. J. G., Muyot, M. L. C., Sison, J. M. C., & Baculi, R. Q., 2019. Enhanced in vitro biodegradation of low-density polyethylene using alkaliphilic bacterial consortium supplemented with iron oxide nanoparticles. Philipp. Sci. Lett, 12, 55-69.
9. Gupta, K. & Devi, D., 2020. Biofilm mediated degradation of commercially available LDPE films by bacterial strains isolated from partially degraded plastic. Rem. J., 30 (11). DOI: 10.1002/rem.21660.
10. Han Y.N., Wei M., Han F., Fang C., Wang D., Zhong Y.J., Guo C.L., Shi X.Y., Xie Z.K., Li F.M., 2020. Greater Biofilm Formation and Increased Biodegradation of Polyethylene Film by a Microbial Consortium of *Arthrobacter* sp. and *Streptomyces* sp., Microorganisms., 8, 1979. DOI:10.3390/microorganisms8121979.
11. Harshvardhan K., Jha B., 2013. Biodegradation of low-density polyethylene by marine bacteria from pelagic waters, Arabian Sea, India, Mar. Pollut. Bull., 77:1–2, pp. 100-106, ISSN 0025-326X, DOI: 10.1016/j.marpolbul.2013.10.025.
12. Jadaun J.S., Bansal S., Sonthalia A., Rai A.K., Singh S.P., 2022. Biodegradation of plastics for sustainable environment, Bioresour. Technol., 347, 126697. DOI: 10.1016/j.biortech.2022.126697.
13. Kalia A., and Dhanya M. S., 2022. Effect of Used Engine Oil and UV-Thermal Pretreatments on Biodegradation of Low-Density Polyethylene by *Lysinibacillus fusiformis* TPB, J. Sci. Ind. Res. 81, 606-612.
14. Khandare D., Chaudhary D.R., Jha B., 2021. Marine bacterial biodegradation of low-density polyethylene (LDPE) plastic. Biodegradation., 32, 127–143. DOI: 10.1007/s10532-021-09927-0.
15. Kyaw B.M., Champakalakshmi R., Sakharkar M.K., Lim C.S., Sakharkar K.R., 2012. Biodegradation of Low Density Polythene (LDPE) by *Pseudomonas* Species, Indian J Microbiol., 52, 411–419. DOI: 10.1007/s12088-012-0250-6.

16. Maroof L., Khan I., Yoo H.S., Kim S., Park H.T., Ahmad B., Azam S., 2021. Identification and characterization of low density polyethylene-degrading bacteria isolated from soils of waste disposal sites, Environ. Eng. Res., 26, 3: 200167, DOI: 10.4491/eer.2020.167.
17. Matjašič T., Simčič T., Medvešček N., Bajt O., Dreo T., Mori N., 2021. Critical evaluation of biodegradation studies on synthetic plastics through a systematic literature review, Sci. Total Environ., 752, 141959. DOI: 10.1016/j.scitotenv.2020.141959.
18. Mendes P.M., Ribeiro J.A., Martins G.A., Lucia T. Jr., Araujo T.R., Fuentes-Guevara M.D., Corrêa L.B., Corrêa É.K., 2021. Phytotoxicity test in check: Proposition of methodology for comparison of different method adaptations usually used worldwide. J. Environ. Manage., Aug 1; 291:112698. DOI: 10.1016/j.jenvman.2021.112698.
19. Mohanan N., Montazer Z., Sharma P.K., Levin D.B., 2020. Microbial and Enzymatic Degradation of Synthetic Plastics. Front. Microbiol., 11, 580709, 1-22. DOI: 10.3389/fmicb.2020.580709.
20. Nadeem H., Alia K.B, Muneer F., Rasul I., Siddique M.H., Azeem F., Zubair M., 2021. Isolation and identification of low-density polyethylene degrading novel bacterial strains, Arch Microbiol., 203, 5417–5423. DOI: 10.1007/s00203-021-02521-1.
21. Nademo, Z.M., Shibeshi, N.T. & Gemeda, M.T. 2023. Isolation and screening of low-density polyethylene (LDPE) bags degrading bacteria from Addis Ababa municipal solid waste disposal site “Koshe”. Ann. Microbiol., 73, 6. DOI: 10.1186/s13213-023-01711-0.
22. Nakei M.D., Misinzo G., Tindwa H., Semu E., 2022 Degradation of polyethylene plastic bags and bottles using microorganisms isolated from soils of Morogoro, Tanzania, Front. Microbiol., 13:1077588. DOI: 10.3389/fmicb.2022.1077588.
23. Nanda S., Sahu S., Abraham J., 2010. Studies on the biodegradation of natural and synthetic polyethylene by *Pseudomonas* spp. J. Appl. Sci. Environ. Manage., 14 (2) 57 – 60. DOI: 10.4314/jasem.v14i2.57839.
24. Napper I.E., Thompson R.C., 2019. Environmental Deterioration of Biodegradable, Oxo-biodegradable, Compostable, and Conventional Plastic Carrier Bags in the Sea, Soil, and Open-Air Over a 3-Year Period, Environ. Sci. Technol. 53, 4775−4783. DOI: 10.1021/acs.est.8b06984.
25. Persoone, G., Baudo, R., Cotman, M., Blaise, C., Thompson, K. C., Moreira-Santos, M., Vollat B., Törökne A., Han T., 2009. Review on the acute *Daphnia magna*

toxicity test–Evaluation of the sensitivity and the precision of assays performed with organisms from laboratory cultures or hatched from dormant eggs. Knowl. Managt. Aquatic Ecosyst., 393, 01, DOI: 10.1051/kmae/2009012.

26. Rafiq, S., Fathima, F., Shahina, Sk.J., Ramesh, K., 2018. Biodegradation of low density polyethylene (LDPE) by halophilic bacteria isolated from solar saltpans, Kovalam, Chennai. Nat. Environ. Pollut. Technol., 17, 1367-1371.
27. Rajandas H., Parimannan S., Sathasivam K., Ravichandran M., Yin L.S., 2012. A novel FTIR-ATR spectroscopy based technique for the estimation of low-density polyethylene biodegradation, Polym. Test., 31, 8: 1094-1099. DOI: 10.1016/j.polymertesting.2012.07.015.
28. Rani R., Rathee J., Kumari P., Singh N.P., Santal A.R., 2022. Biodegradation and detoxification of low-density polyethylene by an indigenous strain *Bacillus licheniformis*, J Appl Biol Biotech., 10(01), 9–21. DOI: 10.7324/JABB.2021.100102.
29. Samanta S., Datta D., Halder G., 2020, Biodegradation efficacy of soil inherent novel sp. *Bacillus tropicus* (MK318648) onto low density polyethylene matrix. J. Polym. Res., 27, 324. DOI: 10.1007/s10965-020-02296-x.
30. Zhang H., Lu Y., Wu H., Liu Q., Sun W., 2022. Effect of an *Acinetobacter pittobacter* on low-density polyethylene. Environ. Sci. Pollut. Res. DOI: 10.1007/s11356-022-22658-w.
31. Zhang, H., Wu, H., Liu, Q., Sun W., Yang F., Ma Y., 2023. Degradation of High-Density Polyethylene (HDPE) Film by Bacterial Consortium. JOM. DOI: 10.1007/s11837-023-06178-7.
32. Zhang Y., Pedersen J.N., Eser B. E., Guo Z., 2022. Biodegradation of polyethylene and polystyrene: From microbial deterioration to enzyme discovery. Biotechnol. Adv., 60, 107991. DOI: 10.1016/j.biotechadv.2022.107991.