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RESEARCH ARTICLE

COMPARATIVE BIODEGRADATION OF HDPE AND PET BY RIVERINE MICROBIAL COMMUNITIES: IMPLICATIONS FOR PLASTIC POLLUTION CONTROL IN THE TIGRIS RIVER

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ABSTRACT

Understanding how local bacterial communities interact with different plastic types in freshwater ecocystems remains limited, despite the continuous accumulation of synthetic polymers. The objective of this study was to use a raw microbial community from the Tigris River as a natural inoculum to evaluate its biodegradation potential. As the sole carbon sources, High-density polyethylene (HDPE) and polyethene terephthalate (PET) were subjected to 90 days of controlled laboratory conditions. Analysis of colony-forming units (CFU/ml) revealed significant differences in colonization dynamics ($p \leq 0.001$). HDPE showed irregular semistable growth, while PET supported continuous bacterial increase. 16S rRNA sequencing showed a consortium dominated by *Pseudomonas aeruginosa* (GJ-TIG1), *Bacillus subtilis* (GJ-TIG2), and *Bacillus subtilis* (GJ-TIG3), suggesting functional cooperation. The GJ-TIG1 strain likely acts as the first colonizer by oxidizing the plastic surface, which enables GJ-TIG2 and GJ-TIG3 to use enzymatic hydrolysis of ester bonds. These biological findings were confirmed by physical and chemical analyses. Field-emission scanning electron microscopy (FESEM) showed distinct damage in PET compared with limited surface changes in HDPE. Fourier transform infrared (FTIR) and gas chromatography-mass spectrometry (GC–MS) analyses supported this difference by detecting breaking of the ester bonds and the formation of by-products such as phthalates and hydrocarbons. These results indicate partial biodegradation by a natural consortium, with its effectiveness deppending on the type of polymer. This study therefore provides basic insights into bioremediation of microplastic contamination in the Tigris River, which often suffer from a scarcity of such studies.

KEYWORDS

Microbial community; Consortium; Biodegradation; Plastic; Freshwater; Bacillus; Pseudomonas; Bioremediation.

1. INTRODUCTION

Rivers are no longer merely conduits for transporting plastic debris from land to sea. They are active sites of interaction that determine the fate of plastics through physical, chemical and microbial processes (Kumar et al., 2021; Salim et al., 2025). Among the most common polymers in freshwater environments are HDPE and PET, both of which are characterized by a stable structure that grants them high resistance to natural degradation (Olam, 2023). Despite this durability, plastic surfaces are rapidly colonized by specialized microbial communities that form what is known as the plastisphere (Zhai et al., 2023; Sharma et al., 2025). These freshwater environments rich provide good conditions for microbial growth. Plastic surfaces act as substrates where microbial communities can form and adapt to the polymer and its biodegradation potential (Bocci et al., 2024; He et al., 2026). This dynamic is clearly observed in the Tigris River, where high levels of MPs have been reported (Shukur et al., 2023; Al-Azzawi et al., 2024). Evidence of ongoing pollution is reflected in water quality levels (Al-Sudani, 2021). Such close associations of MPs with microorganisms and biological tissues further highlight the critical role of biological interactions in shaping their environmental fate (Salih et al., 2026). They also highlight potential biodegradation processes (Zahid et al., 2024). Despite the increasing environmental risks within these systems, local studies using native strains remain scarce and limited in scope. This gap continues even though isolation bacterial strains from contaminated environments has shown promise for degrading PET (Mukhaifi et al., 2023). Addressing this knowledge gap requires a deeper understanding of how different polymers respond to local microbial activity. Therefore, The objective of this study is to assess the ability of local bacterial communities in the Tigris River to degrade HDPE and PET under controlled laboratory conditions. It also compares the structural changes between a hydrocarbon polymer and another containing ester bonds. This study also highlights the potential role of local microbial activity in reducing plastic debris within freshwater environments.

2. MATERIALS AND METHODS

2.1 Sample collection and preparation of HDPE and PET

Water samples were collected from the Tigris River in Baghdad. The river is constantly discharged with wastewater, industrial waste, and surface

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runoff. These environments provide diverse microbial communities that are able to adapt to pollutants and potentially utilize polymers as a carbon source (Yang et al. 2020). Samples were collected from the banks of the river at three distinct locations: Al-Muthanna Bridge(Longitude 44°2"47.2"E, Latitude 33°2"40.9"N), Bab Al-Muadham (Longitude 44°2"45.4"E, Latitude 33°2"35.5"N), and Al-Zafraniya(Longitude 44°2"40.3"E, Latitude 33°1"47.3"N), To insure representative sampling of the mesosphere, sterile 500 mL plastic bottles at a depth of 30 cm below the water surface. Based on general monthly monitoring of the study sites, the environmental baseline was characterized by a mean pH 8.05 ± 0.5, Dissolved Oxygen (DO) 7.33 ± 0.4 mg/L, and a total bacterial load 4.27 ± 0.1 log cfu/mL. Samples were then kept in separate coolers at 4 °C and transported to the laboratory within a few hours of collection. The polymers used in this study consisted of HDPE and PET pellets and were selected to provide a strategic chemical contrast between recalcitrant hydrocarbon chains (HDPE) and ester-linked polymers (PET). These substrates allow for a comparative evaluation of the selective degradation capacity of the local microbial community. The pellets were sterilized by soaking in 70% ethanol solution for 30 min. They were then dried inside a biosafety cabinet at room temperature to ensure the remove surface microbial contamination while preserving the chemical structure of the polymer.

2.2 Experimental Design

This experiment was designed to evaluate the ability of environmental microbial communities to colonize the surface of a polymer. The polymer was used as the sole carbon source under controlled lab conditions. The medium (MSM) followed the recipe of Roberts et al. (2020). In addition, trace minerals were added to the medium at set amounts to stimulate bacterial growth and enhance degradation activity. The final composition per liter of distilled water was as follows: KH_2PO_4 (0.7 g), K_2HPO_4 (0.7 g), $MgSO_4.7H_2O$ (0.7 g), NH_4NO_3 (1.0 g), NaCl (0.005 g), and $FeSO_4.7H_2O$ (0.002 g), in addition to the following trace metals: H_3BO_3 (0.3 g), $CoCl_2.6H_2O$ (0.2 g), $ZnSO_4.7H_2O$ (0.1 g), $MnCl_2.4H_2O$ (0.03 g), $Na_2MoO_4.2H_2O$ (0.03 g), $NiCl_2.2H_2O$ (0.02 g), and $CuSO_4.6H_2O$ (0.01 g). The pH was adjusted to 7.0 using NaOH to apply selective pressure for polymer utilization. Sterile glass vials (10 mL) were autoclaved at 121 °C for 15 min and filled with 6 mL of MSM solution. Polymer pellets were added with sterile forceps to prevent contamination. Two experiment groups were prepared for each type of plastic, with three replicates: a control group containing MSM and sterile pellets and a treatment group containing MSM and sterile pellets supplemented with 100 µL of unfiltered river water as a inoculum. All viales were sealed and wrapped in sterile aluminum foil to protect them from light and incubated statically at 37 °C for 90 days. This temperature was chosen to optimize metabolic activity and enzyme secretion of the local mesophilic bacteria. Additionally, it served as an accelerated changes within the experimental periode.

2.3 Monitoring of microbial activity

2.3.1 Bacterial enumeration

During the incubation period, microbial activity was checked every 30 days using a counting assay. At each time point, vials were opened in a biosafety cabinet and mixed gently. Serial dilutions were prepared with sterile solution, 100 µL was plated on nutrient agar. The plates were incubated at 37 °C for 24h, after which the colonies were counted. A dilution resulting in 30–300 colonies were used for CFU calculation. All the measurements were done in triplicate and recorded for analysis.

2.3.2 Statistical Analysis

The results are shown as the mean values ± standard deviations (SD). The data did not follow normal distribution (Shapiro-Wilk test). Therefore, the non-parametric Kruskal-Wallis test was used to check differences in bacterial communities across the incubation times (0, 30, 60, and 90 days). Dunn's post hoc test with Bonferroni and Holm corrections was subsequently used to determine specific pairwise differences between time points. All the statistical analyses were conducted using Jamovi software (Version 2.3).

2.4 Fourier Transform Infrared Red (FTIR) Analysis

Spectra were recorded by means of a Shimadzu IRPrestige-21 spectrometer (Shimadzu, Japan) with a ZnSe crystal in an ATR accessory. Plastic samples from the treatment and control groups were collected after 90 days of incubation, gently washed with sterile normal saline and then dried at room temperature.

The spectra were recorded in the 400–4000 cm^{-1} wavenumber range with a spectral resolution of 4 cm^{-1}, and each spectrum was based on the average of 32 scans. This technique was used to monitor changes in chemical bonding by observing shifts in the intensity or position of characteristic functional peaks, indicating structural changes on the plastic surface.

2.5 Field Emission Scanning Electron Microscopy (FESEM) Analysis

FESEM analysis was performed via an Inspect™ F50 (FEI, USA) to observe nanometric topographical changes in the polymer surfaces induced by microbial activity. Plastic samples incubated with bacterial isolates for 90 days were subsequently washed in sterile saline, air-dried, cut into sections, fixed on aluminum stubs with conductive carbon tape, and sputter-coated with gold to ensure conductivity. Imaging was carried out with accelerating voltages of 5–30 kV at different magnifications (×15,000–×60,000) to visualize cracks, pits, deformations, microbial attachment, and adhesion zones.

2.6 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

GC–MS analysis was conducted to identify the low-molecular-weight byproducts of plastic biodegradation, including alcohols, acids, ketones, aldehydes, and hydrocarbons. The control and treated samples were extracted via liquid–liquid extraction with dichloromethane (DCM), dried over anhydrous sodium sulfate, concentrated and re-dissolved in DCM before injection. Analyses were performed with a Shimadzu GCMS-QP2010 Ultra (Shimadzu, Japan) fitted with an AOC-20i auto injector and a capillary column RTX-5MS (30 m×0.25 mm ID× 0.25 µm film thickness). The injector temperature was 250 °C, and the oven program was as follows: 60 °C, 2 min, ramping at 10 °C/min to 280 °C and holding for 10 min. The carrier gas was helium, 1 mL/min. The ion source and interface temperatures were 200 °C and 280 °C, respectively. Mass spectra were scanned from 40 to 600 m/z to detect all possible degradation products.

2.7 Isolation and Purification of Biofilm-Associated Bacteria

After the incubation period, the plastic pellets were removed from the vials by placing each pellet in a sterile tubes with 900 µL of deionized water. The samples were then vortexed for 20 s to detach the bacteria from the polymer surface without affecting it. This procedure is consistent with previous reports indicating that vortex mixing is one of the most effective separation methods (Stevenson et al., 2023). The suspensions were then diluted and plated on nutrient agar. After 24 h of incubation, distinct colonies with the highest density on the nutrient agar plates were selected and purified by repeated streaking. These isolates were subjected to subsequent molecular identification.

2.8 16S rRNA gene amplification and PCR product verification

The 16S rRNA gene was amplified using the universal bacterial primer pair 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′), which target a conserved region of the bacterial genome and produce an amplicon of 1500 bp (Weisburg et al., 1991). PCR amplification was performed in a total volume of 25 µL containing 12.5 µL of 2X GoTaq® Green Master Mix (Promega, USA), 1 µL of each primer (10 pmol/µL), 1 µL of template DNA, and nuclease-free water. The thermal cycling conditions involved initial denaturation at 95 °C for 5 min, followed by 30 cycles (95 °C for 30 s, 55 °C for 30 s, 72 °C for 90 s) and a final extension at 72 °C for 7 min.The PCR products were checked on a 1.2% (w/v) agarose gel in 1x TAE buffer, stained with ethidium bromide (EtBr), and run at 90 V for 45 min. Bands at 1500bp under UV illumination (Model YR06207, Kalstein, China) confirmed successful amplification.

2.9 Purification and Sanger Sequencing

The confirmed PCR products were subsequently purified using the GF-1 PCR Clean-Up Kit (Vivantis Technologies, Malaysia) according to the manufacturer's instructions. Purified DNA was eluted in nuclease-free water and stored at −20 °C. For taxonomic identification, the purified products were subjected to Sanger sequencing performed by Macrogen Inc. (Seoul, South Korea) using both forward and reverse directions to ensure complete coverage of the 1500 bp fragment. Chromatograms were quality checked before being processed for species-level identification using the Basic Local Alignment Search Tool (BLASTn) against the NCBI database.

3. Results and Discussion

3.1 Bacterial count (CFU/mL)

A marked difference in microbial colonization dynamics between HDPE and PET across time periods was detected (Kruskal-Wallis Test, p <.001). HDPE exhibited a slow initial pattern, the difference between day 0 (46.11 ± 10.29) and day 30 (286.56 ± 222.61)was statistically significant (p < 0.001)with a period near stabilization midway through the experiment, as there was no significant difference between 30 and 60 days (p = 0.447), whereas the duration of the late stage increased toward the end of the

incubation period, Group c (Fig. 1-A, S1). In contrast, PET displayed a more regular and continuous pattern, with microbial colonization increasing gradually. Dunn's Post Hoc Comparisons analysis confirmed that for PET, also the increase became significantly distinct starting from 0 day (Group b), also the difference between day 0 (46.11 ± 10.29) and day 30 (184.00 ± 29.84) was statistically significant (p = 0.002), suggesting a steady progression in surface utilization (Fig. 1-B, S2). The two types of MPs has no significant difference between 30 days and 60 , 90 days, These dynamics are likely facilitated by the presence of ester bonds that are more susceptible to microbial activity (Yoshida et al., 2016; Chen et al., 2022). This interpretation agreed with the outcomes who reported that the composition and dynamics of microbial communities in aquatic systems differ according to plastic type of Di Pippo et al., 2023). This highlights the influence of plastic surfaces and chemical traits on the colonization process. The study revealed that plastic type is a key determinant of microbial colonization patterns (Hong et al., 2024). Therefore, the observed differences between HDPE and PET in this study can be explained within the context of the effect of the distinctive physical and chemical properties of each polymer on microbial interactions (Table 1).

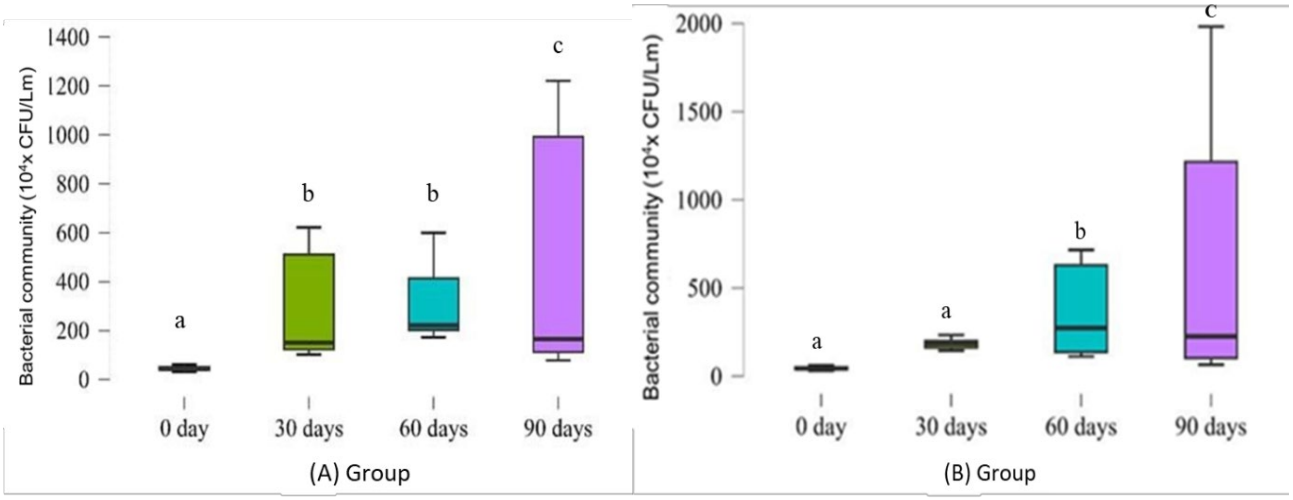

Figure 1: Microbial growth within 90 days: (A) The initial trend of high-density polyethylene (HDPE) was gradual, with a phase of relative stability occurring midway through the experiment before a marked increase was observed toward the end of the incubation period. (B) PET showed a more regular and continuous trend with gradually increasing colonization and a steady trend from the early stages to the end of the experiment.

Table 1: Comparative statistics of bacterial colonization on HDPE and PET.

Groups	N	HDPE (Mean±SD)	PET (Mean±SD)	p-value
0 days	3	46.11 ± 10.29	46.11 ± 10.29	≤ 0.001
30 days	3	286.56 ± 222.61	184.00 ± 29.84	
60 days	3	311.44 ± 150.67	353.33 ± 248.79	
90 days	3	461.67 ± 502.00	653.11 ± 778.24	

3.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis of the HDPE samples after bacterial exposure revealed spectral changes in surface chemistry. These alterations indicate limited surface oxidation compared with that of the control samples (Fig. 2, S3). A clear broadening in the hydroxyl region was observed at 3421.72 cm^{-1}, suggesting the possible addition of oxygenated functional groups, likely due to initial microbial oxidation. Also, a peak at 1637.56 cm^{-1} matches C = C stretching or carbonyl groups that may result from Norrish-type reactions during breaking of hydrocarbon chains. A peak at 1111 cm^{-1} linked to C–O bonds supports the onset of oxidative changes in the polymer chain. However, the main peaks of the CH_2 bending and stretching remained dominant, showing that HDPE degradation was limited to the surface. In contrast, PET samples showed stronger changes. A clear shift and change in intensity in the carbonyl band at 1728.22 cm^{-1}, along with changes in the C–O–C region (Fig. 3, S4), agree with partial breaking of ester bonds, possibly by enzymatic hydrolysis. These results suggest that PET exhibited more obvious spectral changes compared with HDPE. The hydrolysable ester bonds in PET may facilitate microbial interaction. In contrast, the chemically inert, saturated hydrocarbon structure of HDPE restricts such changes. This finding is consistent with a recent review showing that ester linkages in PET facilitate enzymatic attack (Satta et al., 2024). Conversely, the biodegradation of PE/HDPE, as saturated hydrocarbon polymers, is limited because their chemical structure is resistant to enzymatic attack (Kang et al., 2019; Ghatge et al., 2020).

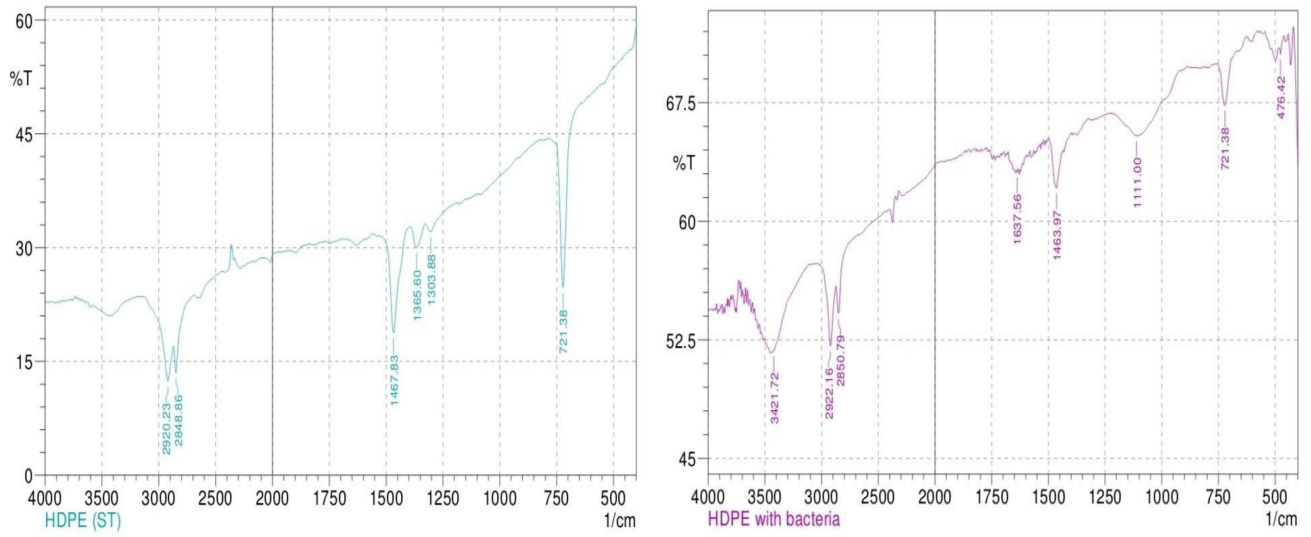

Figure 2: FTIR spectra of the HDPE polymer: The standard sample (ST) shows characteristic peaks of the saturated hydrocarbon structure, whereas the treated sample shows the appearance of new hydroxyl groups and oxidized bonds, indicating surface oxidation and partial chemical changes resulting from bacterial activity.

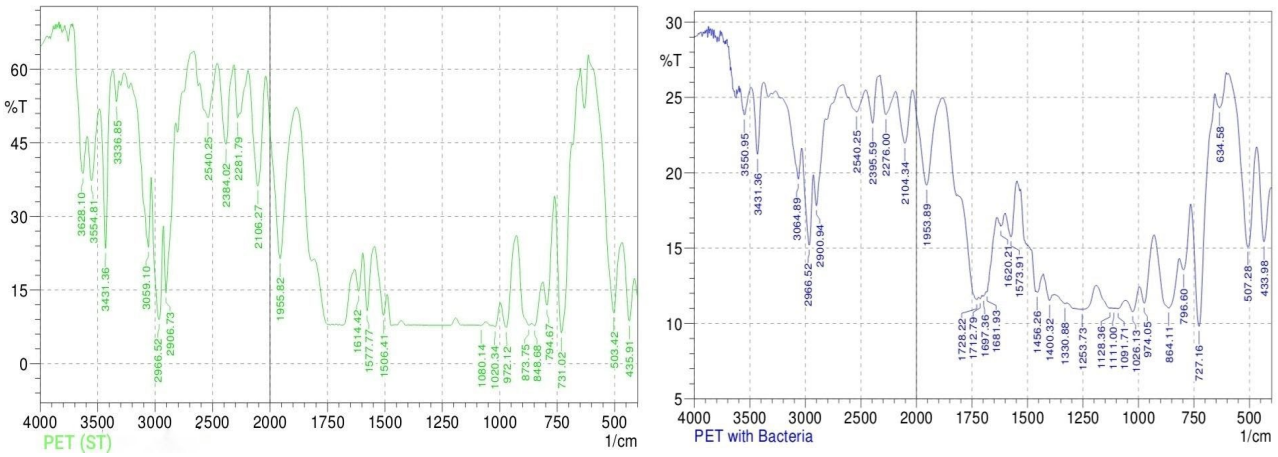

Figure 3: FTIR spectra of the PET polymer: The spectrum of the untreated sample shows the characteristic spectral signatures of ester bonds and aromatic rings, whereas those of the treated sample show changes in the carbonyl and hydroxyl bands and ester bonds, indicating partial biodegradation via the breaking of ester bonds with the aromatic structure remaining preserved.

3.3 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

Gas chromatography–mass spectrometry (GC–MS) analysis revealed distinct chemical differences between the HDPE and PET samples after 90 days of bacterial incubation. In both polymers, a remarkable increase in the relative abundance of the phenolic antioxidant derivative was observed; in PET, its proportion increased from 29.28% in the control sample to 49.07% in the treated sample, whereas in HDPE, it increased from 29.97% to 43.36%. This accumulation is best explained by leaching due to microbial erosion of the polymer matrix rather than direct chain scission. In the HDPE samples, the primary changes were associated with initial oxidative transformations. The proportion of n-hexadecanoic acid increased, and octadecanoic acid appeared exclusively in the treated sample. These results, which are consistent with the FTIR data indicating surface oxidation, suggest that the aliphatic backbone underwent initial bio-oxidation (S5 and S6). With respect to PET, degradation followed a more complex pattern. FTIR spectra revealed new peaks at 1728 cm^{-1} corresponding to ester functionalities, which aligned with the results of the GC–MS detection of novel compounds such as dibutyl maleate and phthalate derivatives (Dimethyl and Di-n-octyl). These products indicate partial ester bond scission and structural rearrangements within the aromatic framework. The reduction in spectral saturation allowed previously masked degradation-related compounds to be detected (S7, S8). As explained the degree of polymer degradation varies depending on the structure by (Gates and Crook, 2024). PET involves more complex pathways involving the oxidation and enzymatic degradation of ester bonds by specialized enzymes, which explains the diversity of products observed in the analysis. According to the GC–MS data in this study, the degradation of HDPE, an aliphatic polymer, is primarily dependent on the surface oxidation of aliphatic chains (Kopecká et al., 2022).

3.4 Field emission scanning electron microscopy (FESEM) analysis

FESEM images showed clear differences in the surface structure of both PET and HDPE before and after exposure to the natural bacterial community. For PET, the control samples maintained a smooth and intact surface (Fig. 4a, b). The treated samples exhibited significant roughness with deep, sharp edge cracks and the presence of bacterial cells adhering to the surface (Fig. 4c, d). These features suggest surface damage that may extend beyond superficial changes, which is consistent with the FTIR and GC–MS results, indicating partial ester bond rupture and the release of minor degradation products. This behavior is due to the ester bonds in PET, which facilitates the partial breakdown of the polymer chain compared with saturated hydrocarbon polymers (Fernández et al., 2022). The control samples of HDPE (Fig. 4a, b) showed no signs of weathering or microbial colonization, but the treated HDPE samples (Fig. 4c, d), showed localized changes, including slight surface roughening and cracks that were localized around areas of bacterial cells without any signs of significant structural breakdown. This reflects the high resistance of HDPE to microbial degradation, as noted by (Praveen and Mazumder, 2024). The changes were limited to surface oxidation, consistent with the FTIR results (weak carbonyl and hydroxyl peaks) and GC–MS findings (more fatty acids without significant ester degradation products).

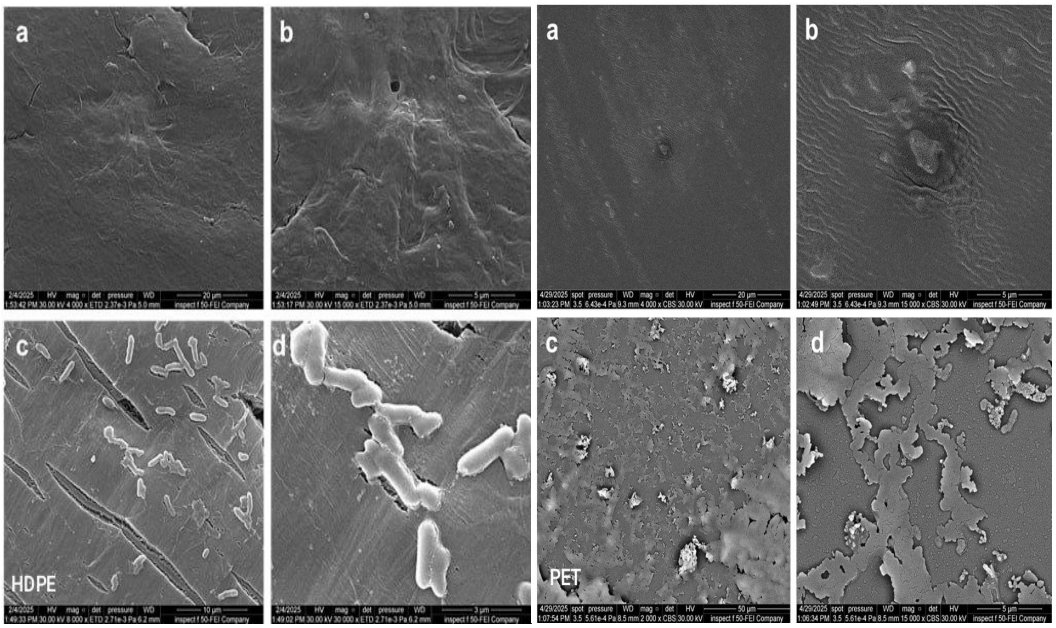

Figure 4: FESEM images of HDPE and PET samples before and after treatment with natural bacteria. Pretreatment images (a and b) show a relatively smooth surface, whereas posttreatment images (c and d) reveal limited surface changes with clear cracks around areas of bacterial accumulation in the HDPE. The PET samples showed marked roughness and deep cracks, as well as micropores, confirming the onset of structural corrosion due to microbial degradation.

3.5 Molecular Characterization of Plastic-Associated Bacteria and their Role in Polymer Biodegradation

The 16S rRNA gene sequencing showed that the dominant bacteria isolated from the plastic-associated bacterial community were three distinct isolates: *Pseudomonas aeruginosa* and two isolates of *Bacillus subtilis* (Table 2). The substantial differences in degradation efficiency can be explained by the link between the enzymatic specialization of microbial communities and the chemical structure of polymers, which is also affected by environmental and lab conditions (Sander et al., 2023). PET, as a semiaromatic polyester, has ester bonds in its main chain, a structure that can be easily broken down by enzymatic hydrolysis (Yoshida et al., 2016). *B. subtilis* isolates exhibit an exceptional ability to secrete lipases and esterases that target these bonds (Demirkan et al., 2020). This was chemically demonstrated by FTIR results showed significant changes in the carbonyl (C=O) bands and ester (C-O) bonds. These results were demonstrated by GC–MS analysis, which revealed the release of chain-scission products such as dimethyl phthalate and dibutyl maleate. These findings align with regarding the role of a bacterial consortium in providing the necessary hydrolases to overcome PET crystallinity and convert them into aromatic intermediates (Roberts et al., 2020).

In contrast, HDPE is characterized by a high-molecular-weight, fully saturated (C-C) hydrocarbon structure lacking hydrolyzable functional groups, making it more bioresistant (Shah et al., 2008). Therefore, its biodegradation relies on an oxidative pathway via *P. aeruginosa* (Chandra and Gothalwal, 2024). In addition, biosurfactants such as rhamnolipids increase surface hydrophilicity and microbial adhesion, facilitating the initial steps of degradation (Tribedi & Sil, 2013). This oxidative attack, catalyzed by the alkane hydroxylase (AlkB) system, explains the appearance of weak carbonyl and hydroxyl group peaks in the FTIR spectra of the treated HDPE, resulting from the introduction of oxygen atoms into the inert chain (Gorish et al., 2024; Shilpa et al., 2025). This mechanism was confirmed by the GC–MS results, which revealed n-hexadecanoic acid, a classic intermediate product of the β-oxidation of long carbon chains after their conversion to fatty acids (Trad et al., 2025). The observed synergy among the three isolates suggests a cooperative strategy showed that *P. aeruginosa* produces enzymes that increase biofilm formation, supporting its role as an early colonizer. Similarly, explained how microplastic biofilms prepare surfaces and affect pollutant behavior, highlighting cooperative strategies in colonization (Howard et al., 2025; Yan et al., 2024). This consortium-mediated cooperation provides a suitable environment for *Bacillus* strains to complete enzymatic breakdown, which explains the steady bacterial growth in PET media compared with the unstable growth in HDPE media due to the high energy needed to break C-C bonds.

Table 2: Bacterial identification of isolates by Sanger sequencing

Accession No.	Bacterial Species	Isolate ID
PZ299177	*Pseudomonas aeruginosa*	GJ-TIG1
PZ299178	*Bacillus subtilis*	GJ-TIG2
PZ299179	*Bacillus subtilis*	GJ-TIG3

4. Conclusion

The results show that the local microbial communities from the Tigris River have a clear metabolic response to synthetic polymers under laboratory selection pressure. In these conditions, the bacterial isolates utilized polymers as their only carbon source. The study revealed that biodegradation efficiency mainly depends on the chemical structure of the polymer and the combined activity of the microbial consortium, dominated by *P. aeruginosa* GJ-TIG *B. subtilis* GJ-TIG2, and *B. subtilis* GJ-TIG3. Microbial counts showed distinct colonization patterns. PET supported steady and continuous bacterial growth, while HDPE showed irregular growth. Analytical methods provided more insights into the mechanisms of this interaction. FESEM detected physical surface changes like cracks and erosion. FTIR detected new functional groups, pointing to hydrolytic cleavage in PET and oxidative changes in HDPE. Additionally, GC–MS analysis revealed intermediate compounds (phthalates and hydrocarbons), showing the potential of the consortium to initiate the breakdown of complex polymers into shorter chains. The results highlight that the biodegradation efficiency of bacterial communities in aquatiq environments depends on the compatibility between the structural properties of each plastic type and the metabolic capacity of the microbial community. This explains the differences in degradation responses between different polymer types. These findings provide preliminary insight into the interaction mechanisms between riverine microbial communities and different plastic types. They highlight the potential of local microbial consortia from the Tigris River for future bioremediation research. However, their applicability at field scale has not yet been demonstrated, this remains a limitation of the current study. Future research is needed to evaluate these isolates in natural environments.

Recommendations

I. The change in plastic weight should be measured in future studies to calculate the actual biodegradation rate and link it to the observed chemical changes.

II. Functional metagenomic sequencing should be conducted to identify specific genes within the consortium to provide direct genetic evidence for the proposed metabolic pathways.

III. The experiments should be expanded to cover different environmental conditions (temperature, salinity, and nutrients) to simulate aquatic systems, and their effects on microbial colonization and plastic degradation should be evaluated.

IV. Comparative studies of local and isolated microbial communities or genetically modified microorganisms are needed to improve biodegradation.

V. Future studies are recommended to include functional verification through enzyme assays (e.g., PETase, esterase, and alkane hydroxylase) to further support the proposed cooperative mechanism.

Declarations

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Competing interests: All the authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or nonfinancial interest in the subject matter or materials discussed in this manuscript.

Ethics approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Consent: Not applicable.

Data availability: Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions: All the authors participated in the concept and methodology of the research. They contributed to the preparation, writing, reviewing, and editing of the original draft. The manuscript was reviewed and approved for publication by all the authors.

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