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Characterization and Preliminary Polyethylene Microplastic Degradation Potential of Indigenous Bacteria from the Opak River

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Abstract: This study assesses the ability of indigenous bacterial consortia isolated from the Opak River (Yogyakarta, Indonesia) to cause early surface changes on polyethylene (PE) microplastics and to explain the related biofilm-polymer interfacial interactions. Five different stations were sampled and grown on Malt Extract Agar, which produced 24 bacterial isolates that were identified by macroscopic analysis and Gram staining. Two isolates (7 SM1 and 4 SM 1) had the largest clear zones on Mineral Salt Medium (MSM) with polyethylene glycol (3.791 ± 0.123 mm and 2.320 ± 0.174 mm, respectively) and were then combined to form a composite bacterial consortium. Over a 60-day incubation, the consortium caused a loss in mass of polyethylene (PE) of 2.270 ± 0.052%, whereas abiotic controls showed minimal loss (0.061 ± 0.065%), indicating substantially greater mass reduction under microbial activity. FTIR spectroscopy showed surface chemical changes that were evidence of oxidative reactions, which were represented by C=O and C–O functional groups; these results indicated that an interfacial change was caused by biofilm activity and not full mineralization of the polymer. Furthermore, SEM was used to verify progressive surface changes observed in the bacterial treatment, including pits, holes, microcracks, and delamination. Such morphological alterations are suggestive of initial stages of biodeterioration and surface oxidation, thus increasing microbial-polymer interactions. Overall, these findings suggest that indigenous freshwater bacteria can induce oxidative surface modification of polyethylene microplastics, thereby contributing to a better understanding of early microbe–polymer interactions relevant to microplastic degradation processes.

Keywords: indigenous bacteria; clear zone; surface oxidation; polyethylene; microplastics; Opak River.

1. Introduction

The widespread use of synthetic polymers in modern society is explained by their beneficial physicochemical characteristics, i.e., low density, high mechanical and chemical stability, and cost-effective production pathways [1]. Since the middle of the 20th century, plastic production has increased exponentially around the globe, reaching an estimated 8.3

billion metric tonnes and increasing at an annual rate [2]. However, conventional methods of waste management are not sufficient, and only 15–20% of plastic waste is recycled. This inefficiency has contributed to significant amounts of plastic in natural ecosystems, making plastic pollution a long-term and serious global environmental issue [3].

Once plastics are discharged into the environment, they are subjected to different abiotic and biotic stressors, such as ultraviolet radiation, mechanical wear, temperature changes, and biological processes, which gradually change their physicochemical properties [4]. These alterations facilitate the formation of microplastics, synthetic polymer particles measuring less than 5 mm in diameter, which are characterized by their significant persistence and resistance to degradation [5]. Microplastics have been observed in a wide range of matrices, including freshwater systems, sediments, soils, coastal areas, marine ecosystems, and even the atmosphere, thus highlighting their widespread environmental distribution [6–11]. Rivers are important pathways that carry microplastics to estuarine and marine systems [12]. Hydrodynamic regimes, land-use patterns, wastewater discharge, and anthropogenic activities that control deposition, resuspension, and consequent environmental changes therefore determine the presence and distribution of microplastics in river systems [13,14].

Microplastics have severe environmental and chemical effects on water bodies. The build-up of these particles in biota has been linked to physiological stress and impaired biological processes, especially in gastrointestinal tissues and gill structures [15,16]. The microplastic surfaces can adsorb persistent organic pollutants, heavy metals, and antibiotics. This process can promote the translocation of pollutants via trophic webs, which increases ecological risk [17]. It is also important to note that microplastic particles have been found in the human digestive system. Seafood consumption is a major pathway of human exposure to these particles [18,19].

Polyethylene (PE) is the most commonly encountered polymer in aquatic microplastic assemblages, indicative of its mass production and extensive use [20]. In 2023, the world produced about 413.8 million metric tonnes of plastic, with PE taking almost 26% of the total [21]. PE has a semi-crystalline structure and is hydrophobic, which provides it with resistance to hydrolytic and enzymatic degradation, leading to low inherent degradability and long environmental persistence [22,23]. These properties make PE a widely used polymer to study microbe-polymer interfacial interactions and surface transformation processes in the laboratory.

The widespread use of plastics and high population density in Indonesia make river systems such as the Opak River in Yogyakarta important natural laboratories for studying microplastic dynamics [24–26]. Furthermore, the area's tropical climate, marked by significant rainfall and surface runoff, facilitates the transport of plastic waste into estuarine and coastal environments [27]. Within these aquatic environments, polyethylene constitutes a significant fraction of the plastic pollution, a consequence of its extensive application and durability [20–23,28,29]. Microplastic particles provide surfaces for microbial attachment and biofilm formation, thereby establishing dynamic interactions between plastic substrates and microbial communities [30–33]. The maturation of biofilms may modify the surface characteristics of roughness, polarity, and chemical reactivity, and thus affect the interactions between microorganisms and polymer substrates at the solid–liquid interface [34].

The microbial and environmental degradation of polymers follows a series of steps, including biodeterioration, surface oxidation, and total biodegradation [35,36].

Biodeterioration refers to early physicochemical alterations of polymer surfaces caused by microbial colonization or abiotic weathering, which is generally characterized by surface roughening, crack propagation, and functionalization of the polymer surface [37,38]. Surface oxidation can introduce oxygen-containing functional groups onto the polymer backbone, and full biodegradation requires depolymerization and mineralization into carbon dioxide, water, and microbial biomass [39]. Several bacterial groups have been reported to modify the surface of polyethylene through oxidative mechanisms that form oxygen-containing functional groups [40]. However, the efficiencies with individual bacterial isolates remain low, indicating that the transformations are mainly surface oxidation and partial depolymerization rather than total mineralization [41].

Microbial consortia may enhance these degradation pathways by enabling cooperative metabolic interactions among different bacterial species, thereby broadening the range of enzymatic activities involved in polymer transformation [42,43]. In the natural environment, biofilms are usually multispecies communities whose overall metabolic functions can be more effective at polymer surface modification than those of single isolates [44,45]. Although increasing studies have been conducted on plastic-related microorganisms, there is a dearth of systematic studies targeting indigenous freshwater bacterial communities in tropical river systems. Previous research has mainly concentrated on single bacterial isolates or marine systems [46–48]. This study aims to isolate culturable indigenous bacteria from different sites along the Opak River and to determine their ability to modify polyethylene surfaces, thereby helping better to understand mediated plastic transformation in tropical freshwater ecosystems. This study hypothesizes that freshwater bacterial consortia can trigger quantifiable early-stage surface alterations in polyethylene via biofilm–mediated interactions at the polymer–water interface.

2. Materials and Methods

2.1. Chemicals and reagents.

Polyethylene (PE) sheets consisting of transparent low-density polyethylene (LDPE) film were used as the model polymer substrate. The polyethylene used in this study was LDPE film with a thickness of 50 µm and a density of 0.92 g/cm³. No additives were reported by the manufacturer. The films were cut into two sizes depending on the analytical purpose: 0.5 × 0.5 cm fragments for surface morphology analysis using SEM and 2 × 2 cm fragments for FTIR analysis and gravimetric weight-loss measurements.

The chemicals used in this study included $(NH_4)_2SO_4$, K_2HPO_4, KH_2PO_4, NaCl, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, and $FeSO_4$ (Merck, Germany) as components of the Mineral Salt Medium (MSM). Polyethylene glycol 1000 (Merck, Germany) was used as a model polymer-related carbon source during the preliminary screening of bacterial isolates. Malt Extract Broth (Merck, Germany) was used for bacterial cultivation and inoculum preparation. Additional reagents used in the experiments included Coomassie Brilliant Blue G-250 (Himedia, India), methanol (Merck, Germany), glacial acetic acid (Merck, Germany), sodium dodecyl sulfate (Sigma-Aldrich, USA), ethanol (Merck, Germany), and analytical-grade reagents for Gram staining including crystal violet (Sigma-Aldrich, USA), Lugol's iodine solution (Merck, Germany), and safranin (BDH, United Kingdom). Sterile distilled water and deionized water were used throughout the experiments for media preparation and washing procedures. All chemicals used in this study were of analytical grade.

2.2. Water sample collection.

The bacteria used in this study were obtained as riverine samples collected from the Opak River in Yogyakarta, Indonesia. The location is a tropical fluvial system that is still affected by the continued anthropogenic stressors. The river continuum was divided into five different stations to record spatial variability in physicochemical parameters and pollutant loads adequately. The sampling sites were stratified into downstream (Sites 1–3) and midstream (Sites 4–5) areas based on the land-use trends in the region and accessibility to manufactured sources. This geometrical arrangement, complemented by relevant physicochemical characteristics, provides the ecological context for the intentional selection of bacterial populations that can serve as precursors for early-stage polyethylene surface modification (Figure 1; Table 1).

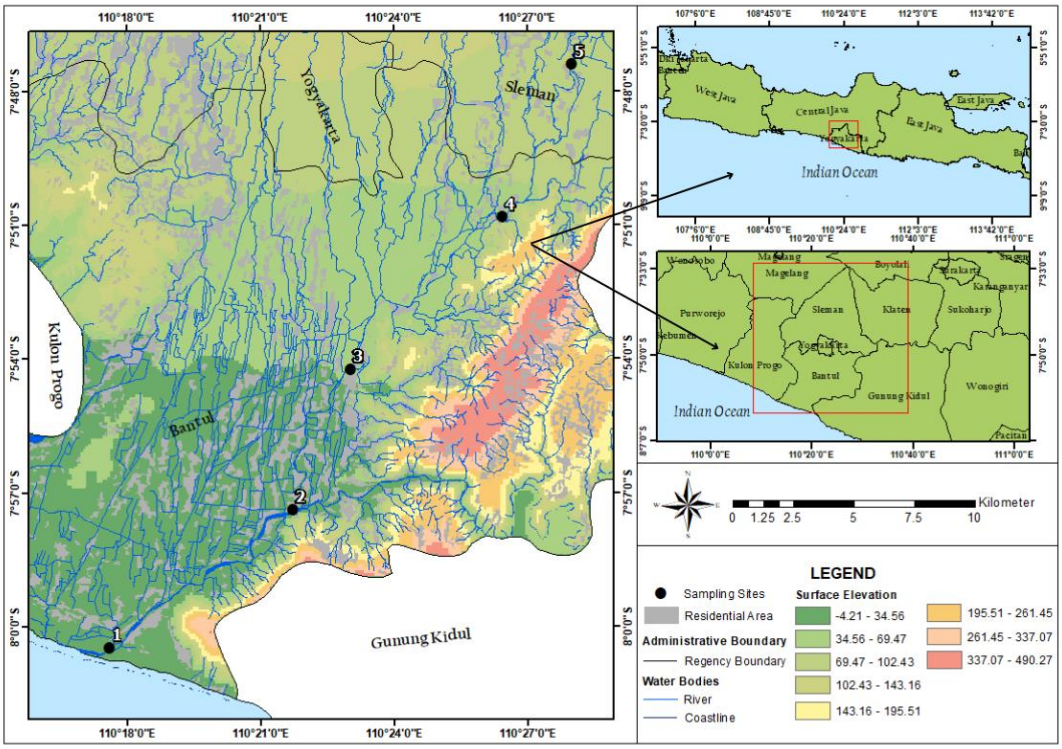

Figure 1. Map of the sampling locations along the Opak River, Special Region of Yogyakarta, Indonesia. 5 sampling sites (1–5) were established along the river continuum from the downstream estuary to the upstream area. The map also shows river networks, watershed topography represented by surface elevation (m), and administrative boundaries.

Table 1. Geographic coordinates, site characteristics, and river-zone classification of water sampling locations along the Opak River, Yogyakarta, Indonesia. Coordinates are presented in degrees (°), minutes (′), and seconds (″).

Sampling Sites	Location Description	Latitude	Longitude	River Zone
1	Deswita Opak, Karang, Tirtohargo Village, Kretek District, Bantul Regency	8°00′28.4"S	110°17′34.5"E	Downstream
2	Potrobayan, Srihardono Village, Pundong District, Bantul Regency	7°57′22.3"S	110°21′43.4"E	Downstream
3	Singosaren, Wukirsari Village, Imogiri District, Bantul Regency	7°54′14.1"S	110°23′01.2"E	Downstream
4	Pagergunung, Sitimulyo Village, Piyungan District, Bantul Regency	7°50′49.2"S	110°26′25.8"E	Midstream
5	Grembyangan, Mutihan, Madurejo Village, Prambanan District, Sleman Regency	7°47′24.2"S	110°27′59.6"E	Midstream

2.3. Isolation and purification of bacterial isolates.

Isolation of bacteria was performed through a ten-fold serial dilution protocol in accordance with standard operating procedure [49]. Briefly, 1 mL of river water was aseptically transferred into 9 mL of sterile distilled water to form a 10^{-1} dilution. Subsequent serial dilutions were made all the way to 10^{-5}, and 0.1 mL aliquots were placed on MEA plates. Even though MEA is traditionally used to cultivate fungi, previous research has confirmed its usefulness in promoting the growth of heterotrophic bacteria under appropriate incubation conditions [50]. Plates were incubated at 28°C for 24–72 h. Colonies that were morphologically different were picked and isolated through a four-quadrant streaking method on fresh MEA media.

2.4. Macroscopic and Gram staining characterization.

Macroscopic characterization of purified isolates was conducted by evaluating colony morphology, including color, shape, margin, elevation, surface texture, and opacity, according to standardized descriptive criteria [51]. The observations were done under a microscope (Zeiss Axiolab, Germany). Gram staining was performed according to a previously reported protocol with slight modifications [52]; heat-fixed smears were stained with crystal violet (1 min), Lugol's iodine (1 min), decolored with 96% acetone (10–15 s), and counter-stained with safranin (1 min). Observations were performed using a trinocular light microscope (Zeiss Axiolab, Germany) at 1000× magnification with oil immersion.

2.5. Screening for polyethylene-associated biodegradation potential using PEG on solid medium.

The ability of bacterial isolates to degrade polyethylene-related substrates was evaluated using Mineral Salt Medium (MSM) supplemented with polyethylene glycol (PEG 1000) as the primary carbon source. PEG was added at a final concentration of 1% (w/v) as a simplified polymer analog that is more readily utilized by microorganisms than conventional polyethylene. This screening step does not directly indicate polyethylene degradation capability but was used only to select isolates capable of utilizing structurally related carbon substrates [53]. MSM was prepared according to the following composition per liter: K_2HPO_4 (1 g), KH_2PO_4 (0.2 g), NaCl (1 g), $(NH_4)_2SO_4$ (1 g), $CaCl_2 \cdot 2H_2O$ (0.1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), $CuSO_4 \cdot 5H_2O$ (0.001 g), $ZnSO_4 \cdot 7H_2O$ (0.001 g), $MnSO_4 \cdot H_2O$ (0.001 g), and $FeSO_4 \cdot 7H_2O$ (0.01 g) [54]. Cycloheximide was added at 1% (v/v) of a 45 mg/mL stock solution as an antifungal agent.

Isolates were inoculated on MSM agar plates and incubated at 28°C for 3 days. All tests were performed in triplicate. Polymer utilization was assessed using a clear-zone formation assay [55]. Plates were stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 in 40% methanol and 10% acetic acid for 20 min, followed by destaining with the same solution for 20 min. The formation of clear zones around colonies indicated microbial interaction with the polymer substrate. Equation 1 was used to compute the clear zone index [56].

$$\text{Clear Zone Index} = \frac{\text{Clear Zone Diameter}}{\text{Colony Diameter}} \quad (1)$$

2.6. Bacterial compatibility test and consortium preparation.

Bacterial compatibility was assessed before the consortium was constructed using a modified cross-streak antagonism test [57]. The isolates were selected and streaked in opposite directions on MEA plates and incubated aerobically at 28°C for 7 days. Compatibility was determined qualitatively by the absence of inhibition zones or abnormal growth at the interface of the interaction; isolates showing normal growth patterns were considered compatible and prepared for a consortium.

2.7. PE surface transformation assay in liquid medium.

Before incubation, PE sheets were preconditioned. The samples were washed with 70% ethanol and sterile distilled water, dried at 45 °C, and subjected to ultraviolet radiation for 15 minutes per side to reduce surface contamination [55]. The activation of bacterial cultures in MEB was performed at 28°C with shaking at 150 rpm for 24 h. According to the growth curve, cultures at this time point corresponded to the mid-stationary phase and were used for inoculation. The optical density (OD600) was normalized to 0.5, as per the standard PE incubation procedures [53].

Incubation experiments were conducted in liquid MSM with PE as the only solid-phase carbon source. Bacterial consortia were inoculated at 10% (v/v) and incubated at 30°C with shaking at 150 rpm for 60 days; abiotic controls were also included. All treatments were performed in triplicate. PE samples were collected at 0, 15, 30, and 60 days [33]. After incubation, PE fragments were recovered and washed with 2% (w/v) SDS in the presence of sterile glass beads to detach surface-associated biomass. The washing procedure was repeated three times, followed by rinsing with sterile distilled water and treatment with 70% ethanol. The samples were then dried at 50 °C to constant weight prior to gravimetric analysis [58].

2.8. Polyethylene weight loss determination.

The PE mass changes were determined gravimetrically with an analytical balance (±0.00001 g). Equation 2 [59] was used to determine percentage weight change.

$$\text{Weight loss (\%)} = \frac{W_0 - W}{W_0} \times 100 \qquad (2)$$

where W_0 is the initial dry weight and W is the final dry weight of PE samples.

2.9. Monitoring of pH and salinity.

As supporting parameters that indicate microbial metabolic activity and incubation stability, pH and salinity were monitored [60]. The measurements were made on days 0, 15, 30, and 60. pH was measured with a digital pH meter (Horiba 74G, Japan) and salinity with a handheld refractometer (Atago, Japan).

2.10. Bacterial growth determination.

Optical density at 600nm (OD_{600}) and total plate counts (TPC) were used to measure bacterial growth during PE incubation. A microplate spectrophotometer (Thermo Scientific, Japan) was used to measure OD_{600} with sterile MSM as a blank. The bacterial populations on the PE surfaces associated with biofilms were determined through a modified TPC technique

[61]. The fragments of PE (2 x 2 cm) were placed into sterile tubes with distilled water and glass beads, swirled to remove surface-associated cells, serially diluted, and plated on MEA by the pour-plate technique. The plates were incubated at 30°C between 3 and 5 days, and colony counts were expressed as CFU/mL, taking into consideration 30-300 colonies [62].

2.11. Fourier transform infrared (FTIR) analysis.

The FTIR spectroscopy was used to assess chemical surface modification of polyethylene (PE) after incubation with bacteria [63]. An attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectrometer (Thermo Scientific iD5, Japan) was used to analyze control and treated samples over the wavenumber range of 4000–400 cm^{-1}. The measured spectra were plotted as transmittance (%) vs wavenumber. FTIR data were interpreted by focusing on changes in functional groups related to surface oxidation and polymer transformation at the biofilm–PE interface. To quantify oxidative surface modification, the carbonyl index (CI) was calculated as the ratio of the absorbance of carbonyl bond peaks (1760–1690 cm^{-1}) to the absorbance of the methylene reference peaks (1490–1420 cm^{-1}, methylene scissoring peak) [64]. Changes in absorption bands corresponding to carbonyl, hydroxyl, and C–O groups were interpreted as indicators of possible oxidative modification associated with microbial activity [22]. The data were considered in the context of biointerface chemistry rather than as evidence of complete polymer mineralization.

2.12. Scanning electron microscopy (SEM) analysis.

Surface morphology of polyethylene fragments was examined using Scanning Electron Microscopy (SEM) [65]. Before imaging, non-conductive samples underwent sputter-coating with a thin gold layer. Morphological changes were qualitatively assessed by evaluating surface alterations, including cracks, pores, and erosion features, which serve as important indicators of structural degradation in polyethylene, as observed through SEM analysis [66].

2.13. Statistical analysis.

Statistical analysis was done in SPSS version 31.0 and GraphPad Prism version 10. Before testing the hypothesis, data normality and homogeneity of variance tests were done. Analysis of Variance (ANOVA) was used to analyze parametric data, and the Kruskal-Wallis test with subsequent post hoc tests was used to analyze nonparametric data [67, 68]. Statistical significance was considered at $p < 0.05$, and exact p-values were reported where applicable.

3. Results and Discussion

3.1. Spatial distribution of culturable bacteria along the Opak River.

Five sampling sites along the Opak River were investigated, including midstream and downstream sections, from which 24 culturable bacterial isolates were recovered. The number of isolates at downstream sites exceeded that at midstream locations, indicating a heterogeneous distribution (Figure 2). This trend is likely driven by cumulative environmental effects, particularly anthropogenic inputs such as domestic and agricultural effluents, which increase the availability of organic matter and provide substrates for heterotrophic microorganisms [69]. Pollutants and organic particulates can also create favorable microhabitats for microbial colonization [70,71]. These findings suggest that downstream

segments act as microbial hotspots, where increased nutrient availability and pollutant-driven environmental heterogeneity promote higher microbial abundance and functional diversity [72]. This pattern is consistent with ecological theory, which predicts that nutrient-enriched environments support higher microbial density and functional diversity [73].

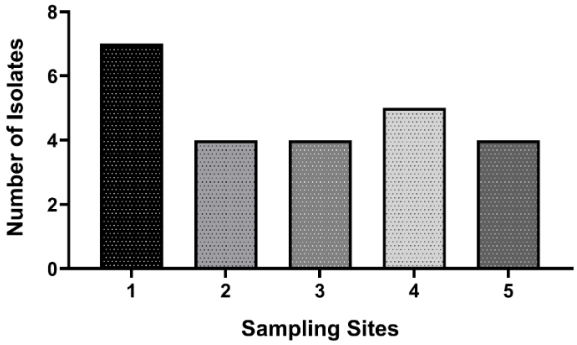

Figure 2. Bacterial isolates from five sampling sites along the Opak River, Yogyakarta, Indonesia.

3.2. Macroscopic and Gram-staining characterization.

All 24 isolates were cultured successfully. The majority of colonies were cream-white to white, with circular, complete margins, moist, and raised elevations (Table 2). Nonetheless, a few isolates had abnormal shapes, lobate or undulate edges, flat heights, or dry textures, suggesting phenotypic heterogeneity. These findings indicate adjustment to changing microhabitats across the river continuum, consistent with earlier research associating environmental complexity with the diversification of colonies [74,75].

Table 2. Macroscopic colony morphology and Gram-staining characteristics of bacterial isolates obtained from the Opak River. Colony morphology was assessed after incubation on Malt Extract Agar (28°C, 24–72 h).

Isolate	Color	Shape	Margin	Elevation	Texture	Opacity	Gram staining
1 SM 1	Cream-White	Circular	Entire	Convex	Moist	Opaque	Gram-positive bacillus
2 SM 1	Cream-White	Circular	Entire	Convex	Moist	Opaque	Gram-positive coccus
3 SM 1	Cream-White	Circular	Entire	Convex	Moist	Opaque	Gram-positive bacillus
4 SM 1	Cream-White	Circular	Entire	Convex	Moist	Opaque	Gram-negative coccus
6 SM 1	Brown	Circular	Entire	Convex	Moist	Opaque	Gram-positive bacillus
7 SM 1	Cream-White	Circular	Entire	Convex	Moist	Opaque	Gram-positive bacillus
8 SM 1	Cream-White	Irregular	Lobate	Flat	Dry	Opaque	Gram-positive coccus
2 SM 2	Cream-White	Circular	Entire	Convex	Moist	Opaque	Gram-positive bacillus
3 SM 2	Cream-White	Irregular	Undulate	Flat	Moist	Opaque	Gram-positive coccus
4 SM 2	Cream-White	Circular	Entire	Convex	Moist	Opaque	Gram-positive coccus
5 SM 2	Cream-White	Circular	Entire	Convex	Moist	Opaque	Gram-positive coccus
1 SM 3	White	Irregular	Undulate	Flat	Dry	Opaque	Gram-positive bacillus
2 SM 3	Cream-White	Irregular	Lobate	Flat	Rough	Opaque	Gram-positive bacillus
3 SM 3	Cream-White	Circular	Entire	Convex	Moist	Opaque	Gram-positive coccus
4 SM 3	Cream-White	Circular	Entire	Convex	Moist	Opaque	Gram-positive bacillus
1 SM 4	Pale Brown	Irregular	Undulate	Convex	Moist	Opaque	Gram-positive bacillus
2 SM 4	Cream-White	Circular	Entire	Convex	Moist	Opaque	Gram-negative coccus
3 SM 4	Cream-White	Circular	Entire	Flat	Moist	Opaque	Gram-positive coccus
4 SM 4	Cream-White	Circular	Entire	Convex	Moist	Opaque	Gram-positive coccus
5 SM 4	Orange	Circular	Entire	Convex	Moist	Opaque	Gram-positive bacillus
1 SM 5	Cream	Irregular	Lobate	Convex	Moist	Opaque	Gram-positive coccus
2 SM 5	Cream-White	Circular	Entire	Convex	Moist	Opaque	Gram-positive coccus
3 SM 5	Cream-White	Circular	Entire	Flat	Moist	Opaque	Gram-positive bacillus
4 SM 5	White	Irregular	Lobate	Convex	Dry	Opaque	Gram-positive bacillus

Gram-staining analysis revealed a heterogeneous community comprising Gram-positive bacilli and cocci, with a small proportion of Gram-negative cocci. This structural heterogeneity affects cell-wall stability, adhesion to surfaces, and resistance to stress, enabling

bacteria to survive in changing physicochemical environments. In the context of polyethylene (PE) biodegradation, Gram-positive bacteria are commonly associated with initial adhesion to hydrophobic surfaces and biofilm formation via extracellular polymeric substances to optimize enzyme-substrate interactions [76].

3.3. Screening for polyethylene-associated metabolic potential using PEG on solid medium.

The isolates were selected on Mineral Salt Medium (MSM) with polyethylene glycol (PEG) as a model substrate to conduct a preliminary evaluation of polymer-associated metabolic activity. PEG was used as a surrogate molecule due to its partial structural similarity to polyethylene and its higher bioavailability, which enabled rapid screening of microbial utilization capacity [77]. Some of the isolates generated distinct zones after Coomassie Brilliant Blue staining, which is a sign of PEG-utilizing activity (Figure 3). 21 of the isolates had a quantifiable degradation index between 0 and 3.791 mm, and 3 isolates (2 SM 2, 1 SM 4, and 3 SM 4) were inactive.

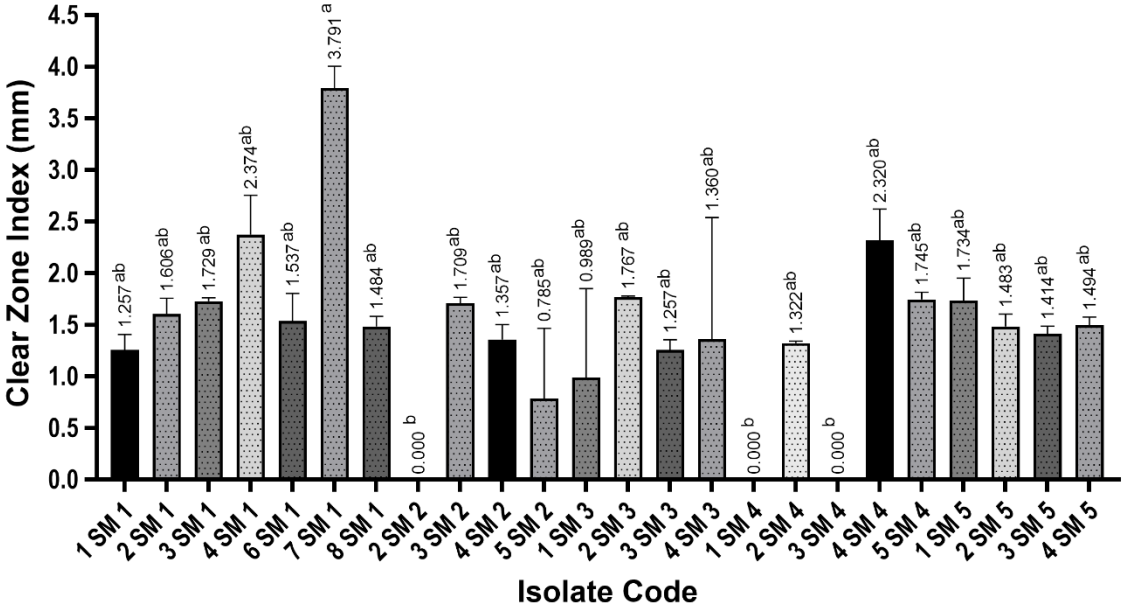

Figure 3. Clear zone index of bacterial isolates during screening for PEG utilization on MSM-PEG medium. Different letters indicate statistically significant differences among isolates based on the Kruskal–Wallis test followed by Dunn's post hoc test at a 0.05 confidence level. Values are presented as mean ± SE (n = 3), with error bars representing the standard error.

Given the small sample size (n = 3) and partial deviation from normality, statistical differences among isolates were evaluated using the non-parametric Kruskal–Wallis test followed by Dunn's post hoc test. The analysis revealed highly significant variation in clear zone indices among isolates ($p < 0.0001$). The heterogeneous distribution of PEG-utilizing phenotypes suggests variability in enzymatic activity and substrate utilization efficiency among isolates [53]. The formation of clear zones is an early indicator of polymer-associated metabolic activity; however, it does not directly indicate polyethylene biodegradation, as PEG is chemically more accessible and less recalcitrant than polyethylene. Therefore, PEG-based screening should be interpreted as a preliminary selection step rather than a direct proxy for polyethylene biodegradation capacity [78].

3.4. PE surface transformation assay in liquid medium.

The two most promising isolates, 7 SM 1 and 4 SM 1, were tested for compatibility by cross-streak assays. The lack of specific inhibition areas indicated neutral to weakly synergistic interactions, which suggested that the isolates may coexist without antagonistic interactions (Figure 4). Non-antagonistic interactions play a vital role in the formation of stable microbial consortia, as polymer transformation processes do not necessarily rely solely on the metabolic capacity of single strains but also on cooperative interactions, biofilm formation, and the functional complementarity of consortium members [79,80]. Though laboratory tests cannot replicate the complexity of the environment, the observed compatibility provides a starting point for developing a multispecies consortium with possible polyethylene transformation capability.

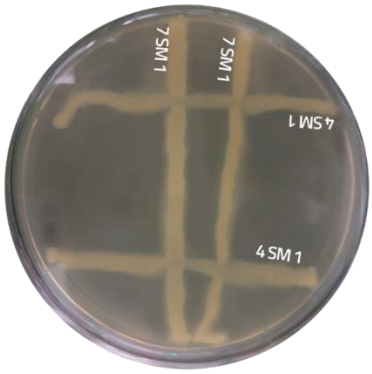

Figure 4. Cross-streak of the selected bacterial isolates. The lack of distinct inhibition spots at contact interfaces is a sign of compatibility, which would justify the formation of a consortium to modify polyethylene surfaces.

Microplastics of polyethylene (PE) were then incubated with the bacterial consortium for 60 days. The abiotic control showed no significant differences in weight, confirming that PE is stable under abiotic conditions. Conversely, the consortium treatment showed a gradual weight loss, which peaked at 2.27 ± 0.05% after 60 days (Figure 5). Before inferential testing, the Shapiro-Wilk analysis was used to verify the normality of both sets of data. Two-way ANOVA revealed that incubation time, treatment, and their interaction ($p < 0.0001$) had a significant effect on PE weight loss. Later post-hoc Šidak comparisons demonstrated that there was no significant difference at day 0, but significant differences were found between treatments at 15, 30, and 60 days ($p < 0.0001$).

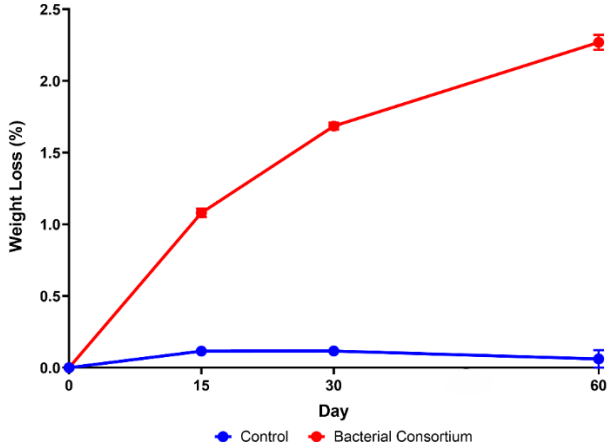

Figure 5. Percentage weight loss of polyethylene (PE) microplastics in liquid medium under abiotic control and bacterial consortium treatments over 60 days of incubation. Two-way ANOVA results indicate that incubation time, treatment type, and their interaction significantly affect PE degradation ($p < 0.0001$). Values are presented as mean ± SE (n = 2), with error bars representing the standard error.

The gradual rise in weight loss indicates a period of microbial adaptation, followed by an increase in surface colonization and metabolic activity at the polymer interface [81–83]. Nevertheless, the relatively low magnitude of weight reduction indicates early-stage surface alteration rather than complete mineralization of polyethylene, which is consistent with previous studies reporting limited mass loss of untreated PE under nutrient-limited conditions [30].

Functional complementarity with consortium members may help to maintain microbial activity at the polymer biofilm interface by stabilizing metabolic activities and lowering the concentration of inhibitory by-products [84]. Similar investigations have reported 1.46–1.68% PE weight reduction in marine bacterial cultures over 90 days, highlighting the slow pace of polyethylene biodegradation [85]. Table 3 provides a comparative summary of PE degradation studies.

Table 3. Biodegradation of PE by bacteria.

Bacterial Strain	Plastic characteristics	Treatment time/d	Weight loss/%	References
Pseudomonas aeruginosa PAO1	LDPE, 5 cm x 1 cm	120 (37°C)	20	[86]
Pseudomonas knackmussii N1-2	LLDPE, 1 cm x 1.5 cm	56 (28°C)	5.95	[87]
Bacillus velezensis C5	LDPE, 4 cm x 4 cm	90 (37°C)	8.01	[88]
Alcaligenes faecalis LNDR-1	PE, 2 cm x 2 cm	70 (37°C)	15.25	[89]
Rhodococcus ruber	PE, 0.92µm	60 (30°C)	NA	[90]
Enterobacter sp. D1	PE, 4 cm x 4 cm	31 (30°C)	NA	[91]
Streptomyces albogriseolus LBX-2	PE, 1 cm x 3 cm	15 (37°C)	NA	[92]
Psychrobacter sp. NJ228	PE	7 (30°C)	13.2	[93]
Consortium	PE, 2 cm x 2 cm	60 (30°C)	2.270	This study

PE = polyethylene; LDPE = low-density polyethylene; LLDPE = linear low-density polyethylene; NA = not available.

3.5. Environmental dynamics during polyethylene transformation.

The environmental conditions were maintained at the best levels of microbial activity. The pH remained close to neutral to slightly basic, with slight changes, whereas salinity varied moderately (Figures 6 and 7) [94,95]. The small initial pH decrease is probably an indication of early metabolic activity, such as organic acid synthesis and release of metabolites during biofilm formation. Following stabilization, buffering and metabolic changes aid quorum sensing, exopolysaccharide synthesis, and biofilm development, thereby enabling continued surface modification of PE [96, 97]. Moderate salinity promoted microbial persistence and biofilm structural stability, but not osmotic stress, which indirectly facilitated polymer depolymerization [98].

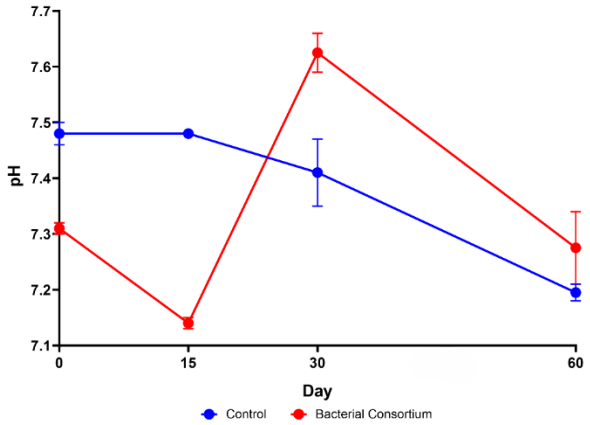

Figure 6. pH monitoring of polyethylene (PE) microplastic transformation in liquid medium. The pH level is shown as the mean value with the standard error of the mean on the 0, 15, 30, and 60 days of the control and bacterial consortium. Error bars represent the standard error (SE).

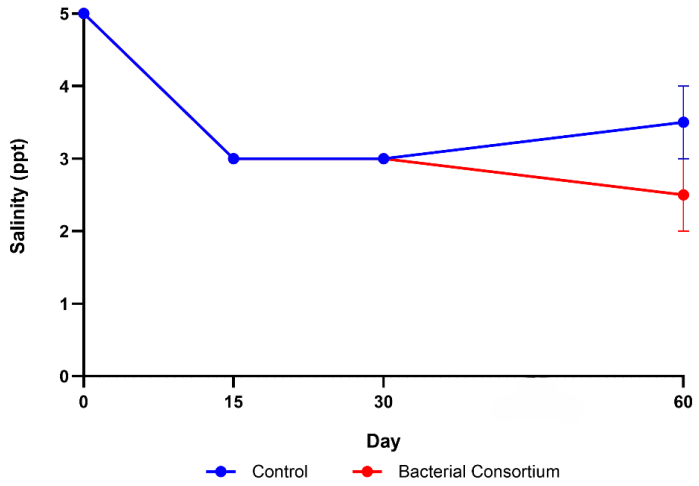

Figure 7. Observation of salinity (ppt) during polyethylene (PE) microplastic transformation in liquid medium. Values are presented as mean ± SE on days 0, 15, 30, and 60 for the control and bacterial consortium.

Bacterial population dynamics during polyethylene transformation were quantified using OD_{600} and total plate count (TPC). The consortium exhibited dynamic changes, with an initial decline in OD_{600} followed by recovery, whereas the control remained relatively stable (Figure 8). This trend indicates that planktonic growth was replaced by surface-bound growth under carbon-limited conditions [99,100]. The TPC analysis showed progressive biofilm growth, from $(2.86 \pm 0.13) \times 10^3$ CFU/mL at day 0 to $(1.95 \pm 0.15) \times 10^8$ CFU/mL at day 60. The observed negative relationship between OD_{600} stabilization and increasing TPC indicates a shift from planktonic to surface-attached growth, which maximizes enzyme–substrate interactions. Control samples confirmed that the observed dynamics were biologically driven and associated with PE surface interactions [97, 101].

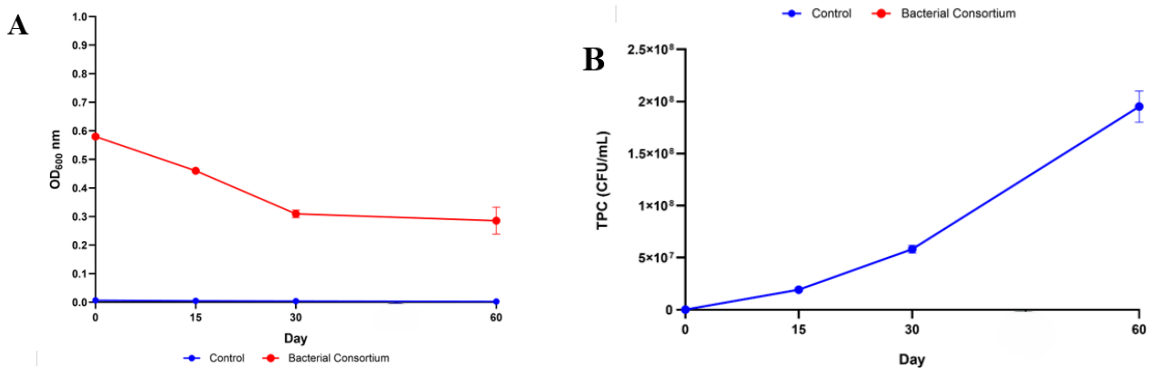

Figure 8. Dynamics of bacterial growth during polyethylene (PE) microplastic transformation. **(A)** Optical density at 600 nm (OD_{600}); **(B)** total plate count (TPC) expressed as CFU/mL. Values are presented as mean ± SE on days 0, 15, 30, and 60.

3.6. Fourier transform infrared (FTIR) analysis of treated polyethylene microplastic.

FTIR spectroscopy was used to qualitatively assess chemical changes on the surface of polyethylene (PE) microplastics following incubation with a bacterial consortium. Transmittance is the proportion of infrared radiation passing through the sample and is used to infer the absorption characteristics of specific chemical bonds within the polymer's structure [102]. In the current study, FTIR analysis was applied to identify changes in functional groups associated with oxidative surface modification of polyethylene, rather than quantifying the extent of polymer degradation. The differences in transmittance at given wavenumbers are associated with the difference in infrared absorption due to vibrational modes of chemical bonds in the polymer matrix [103,104]. The FTIR analysis of PE microplastic particles treated

with the bacterial consortium showed noticeable changes in functional groups compared to the abiotic control (Figure 9). The increased transmittance at 2956.64 cm^{-1} (–C–H stretching) and 995.83 cm^{-1} (=C–H bending) suggests possible alterations in hydrocarbon bonding and structural organization, whereas slight changes at 1666.58 cm^{-1} (C=O) indicate a small number of carbonyl groups formed [64,105,106]. Oxidized polyethylene residues often contain hydroxyl groups after microbial treatment [107], which could indicate oxidative surface modification reactions associated with microbial activity at the polymer interface. The presence of more signals at 1218.04 cm^{-1} (C–O stretching) also supports the development of oxygen-containing functional groups on the polymer surface.

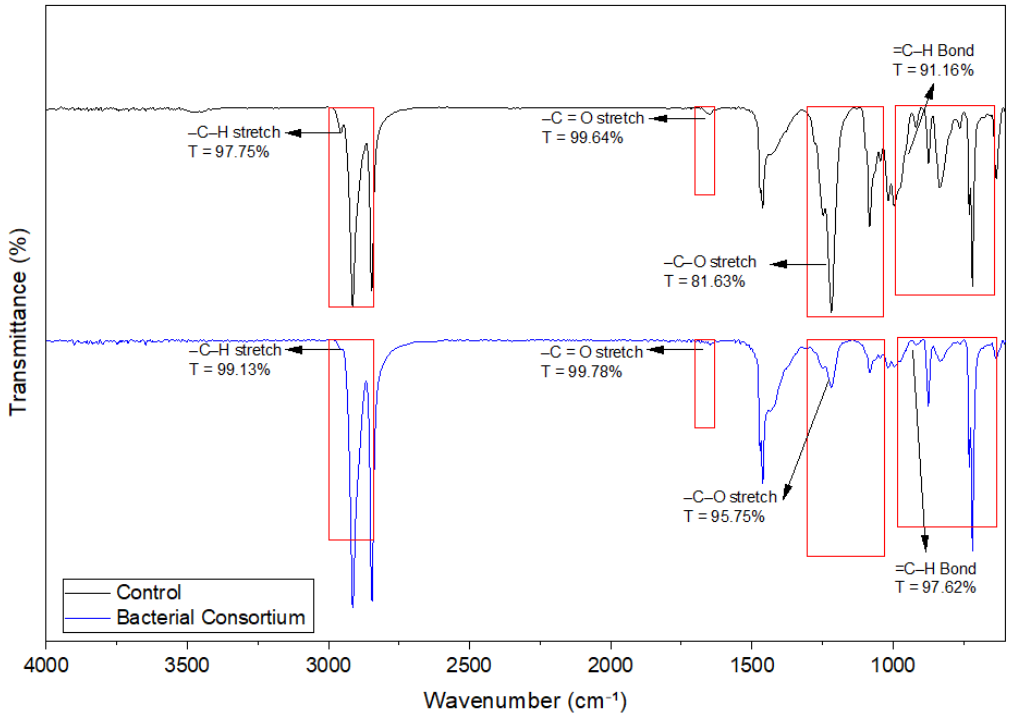

Figure 9. ATR-FTIR spectra of polyethylene (PE) microplastics after 60 days of incubation under abiotic control and bacterial consortium treatment, showing changes in characteristic absorption bands (3000–2850, 2830–2695, 1710–1665, 1470–1450, 1320–1000, and 1000–650 cm^{-1}), indicating chemical modification of the PE surface.

To further support these observations, the carbonyl index (CI) was calculated using the absorbance ratio of the carbonyl band at 1697 cm^{-1} to the methylene reference band at 1464 cm^{-1}. As shown in Table 4, the CI increased from 0.0093 in the abiotic control to 0.0157 in the bacterial consortium treatment, indicating a higher degree of oxidative modification in the presence of the bacterial consortium. Nevertheless, the overall CI values remained low, suggesting that oxidation was limited to early-stage surface modification rather than extensive polymer degradation [108,109]. Other peaks in the range of 1300–1100 cm^{-1} (C–O/C–O–C stretching) and 900–750 cm^{-1} (CH_2 rocking or deformation) show an increase in chain mobility and a decrease in structural order of the polymer matrix, which is also in line with selective surface oxidation and not extensive cleavage of the methylene backbone [110, 111]. Such chemical modifications can increase the hydrophilicity of the polymer surface and enhance microbial attachment and interaction at the polymer-biofilm interface. These observations therefore support the conclusion that microbial activity was involved in the early surface modification of polyethylene, thereby providing the environment in which more extensive transformational processes could occur with extended incubation.

Table 4. Carbonyl index (CI) of polyethylene (PE) for abiotic control and bacterial consortium treatments.

Sample	Carbonyl band (1697 cm^{-1})	Methylene band (1464 cm^{-1})	Carbonyl index (CI)
Abiotic control	0.00034	0.03639	0.00934
Bacterial consortium	0.00076	0.04825	0.01575

3.7. Scanning electron microscopy (SEM) analysis.

SEM images (Figure 10) revealed progressive morphological changes on the surface of polyethylene (PE) microplastics after 60 days of incubation. The abiotic control (Figure 10A–B) remained smooth and homogeneous without any apparent cracks or pores, suggesting that no significant physical changes were observed under abiotic conditions. This supports the interpretation that the observed surface modifications in treated samples were primarily associated with microbial presence. Conversely, the bacterial consortium treatment exhibited noticeable surface changes. Figure 10C shows a relatively intact surface, but after 60 days (Figure 10D), the surface had developed significant characteristics of pits, holes, microcracks, and delamination. These structural defects imply the localized surface erosion and polymer matrix weakening. Morphological characteristics of a similar nature have been widely described as evidence of polymer surface deterioration linked to microbial colonization [66,112]. The appearance of these characteristics is consistent with the surface-level oxidative modification processes, which may increase the surface roughness and promote further interactions between microorganisms and the polymer interface [65,113]. However, no evidence of bulk fragmentation or complete structural disintegration was observed, indicating that the transformation remained at an early stage. This suggests that the process is dominated by biodeterioration and surface oxidation rather than complete polyethylene biodegradation.

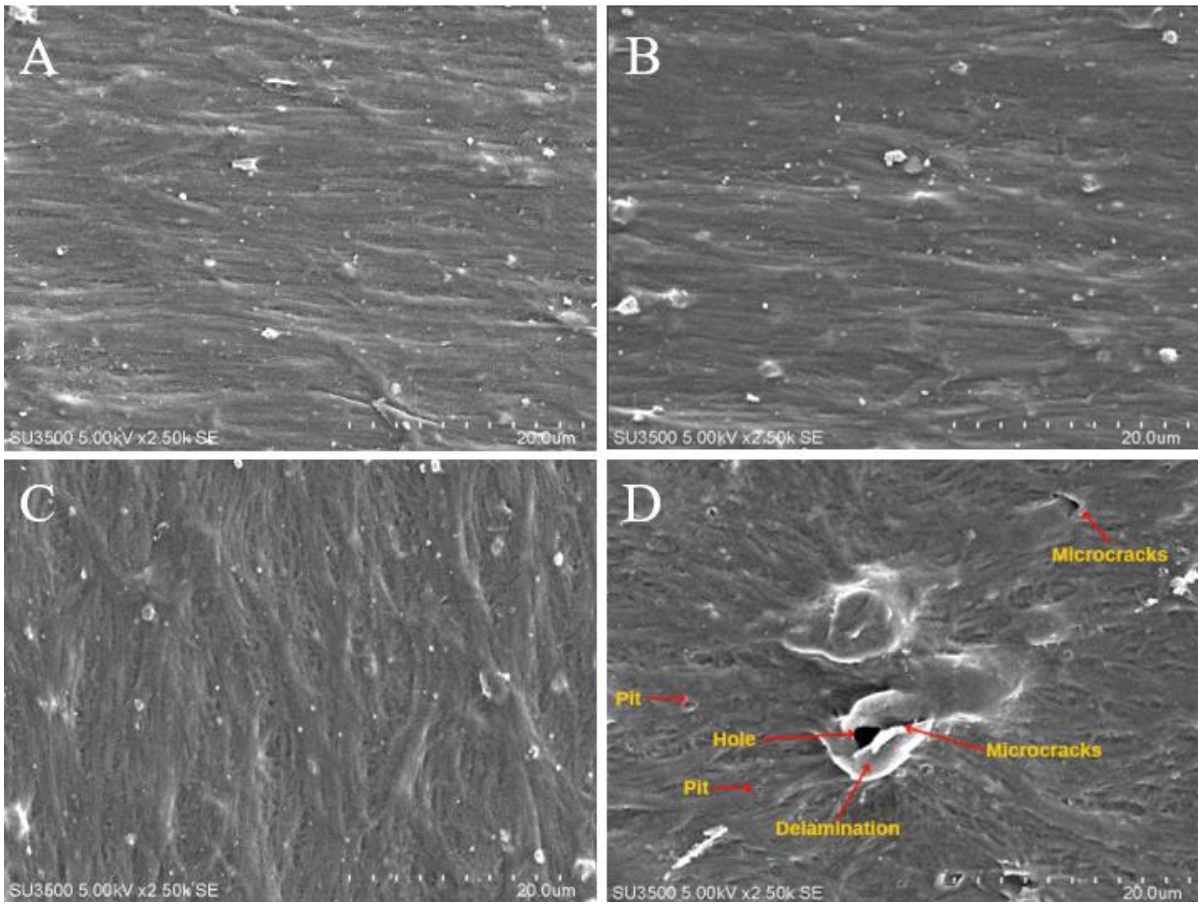

Figure 10. SEM images of PE microplastic surfaces before and after 60 days of incubation. **(A)** Abiotic control (day 0); **(B)** abiotic control (day 60); **(C)** bacterial consortium (day 0); **(D)** bacterial consortium (day 60), showing surface degradation (pits, holes, microcracks, and delamination). Images were taken at 2,500× (SE mode, 5 kV; scale bar = 20 µm).

4. Conclusions and Future Perspectives

This study isolated and characterized 24 culturable indigenous bacterial strains from the Opak River in Yogyakarta, showing spatial heterogeneity, with higher abundance in downstream sections, likely due to nutrient enrichment and anthropogenic sources. Macroscopic and microscopic analyses revealed that Gram-positive bacilli and cocci were predominant, promoting biofilm formation and early adhesion to polyethylene surfaces. The screening of MSM-PEG medium identified 21 isolates that utilized substrates associated with polymers; two compatible isolates were selected to form a bacterial consortium. The consortium showed gradual polyethylene (PE) microplastic weight loss during 60 days of incubation, with a maximum weight loss of 2.270 ± 0.052 %. The environmental conditions that favored microbial activity and biofilm stability were neutral pH and moderate salinity. The population dynamics of the bacterial community showed a strategic shift from planktonic to surface-attached growth, thereby facilitating microbial interactions with the polymer surface. Chemical surface alterations of PE were verified using Fourier-transform infrared (FTIR) analysis, which showed partial weakening of hydrocarbon bonds and the presence of oxygenated functions, indicating that the biofilm–polymer interface is subject to microbial transformation of the polymer surface. SEM analysis further supported these findings by revealing surface morphological changes, including pits, microcracks, and erosion features, confirming localized degradation on the PE surface.

These findings highlight the potential of indigenous riverine bacteria to initiate and alter the transformation of PE microplastics, providing insights into natural attenuation and guiding approaches to microplastic bioremediation. Future research must examine the long-term kinetics of degradation, clarify the metabolic mechanisms of PE depolymerization, and test the behavior of multi-strain consortia under environmentally relevant conditions. In addition, combining molecular identification with enzyme activity profiling would enhance understanding of bacterial mechanisms that transform polyethylene.

Author Contributions

Conceptualization, R.A.K.; methodology, G.N.K., Y.J. and R.A.K.; investigation, G.N.K. and Y.J.; formal analysis, G.N.K. and R.A.K.; data curation, G.N.K. and R.A.K.; visualization, G.N.K.; resources, S. and R.A.K.; validation, S. and R.A.K.; writing—original draft preparation, G.N.K. and R.A.K.; writing—review and editing, G.N.K. and R.A.K.; supervision, S. and R.A.K.; project administration, R.A.K.; funding acquisition, R.A.K. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

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

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Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Definition
ANOVA	Analysis of Variance
BRIN	National Research and Innovation Agency (Indonesia)
CFU	Colony-Forming Unit
FTIR	Fourier Transform Infrared Spectroscopy
MEA	Malt Extract Agar
MEB	Malt Extract Broth
MSM	Mineral Salt Medium
OD_{600}	Optical Density at 600 nm
PE	Polyethylene
PEG	Polyethylene Glycol
SDS	Sodium Dodecyl Sulfate
SE	Standard Error
SEM	Scanning Electron Microscope
SPSS	Statistical Package for the Social Sciences
TPC	Total Plate Count

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