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Comparative, polymer-specific degradation of polyethylene, polypropylene and polystyrene microplastics by floc-forming bacteria from mud crab aquaculture systems

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ABSTRACT

This study demonstrates the ability of two floc-forming bacteria, *Bacillus tropicus* SHBF1 and *Cytobacillus firmus* SHBF3, to degrade polyethylene (PE), polypropylene (PP), and polystyrene (PS) microplastics over a 60-day incubation period. A multi-analytical approach confirmed degradation through polymer and strain-specific mechanisms. SHBF1 exhibited higher cell surface hydrophobicity (up to 79.16%) compared to SHBF3 (54.50%), correlating with stronger biofilm-forming potential. Growth kinetic revealed time-dependent MP utilization, with significant increases in viable cell count from Day 6 onwards ($p < 0.001$), peaking at 9.01 log CFU/mL for SHBF1 on PE. Hydrolase secretion was polymer-specific where SHBF1 showed highest activity with PE (2.09 ± 0.05 μg/mL), while SHBF3 was most active with PS (1.74 ± 0.03 μg/mL). Weight loss, influenced significantly by both strain ($H = 8.24$, $p = 0.004$) and polymer type ($H = 6.88$, $p = 0.032$), was highest for PS degraded by SHBF1 (11.08 ± 0.37%). Media pH shifted from neutral to alkaline (up to pH 8.49), indicating active metabolism of breakdown products. FTIR and SEM analyses confirmed polymers' chemical and physical alterations, including pitting and cracking. This comparison identifies *Bacillus tropicus* SHBF1 as a potent microplastic degrader, linking adhesion, enzymatic specificity, and growth dynamics.

1. Introduction

Global plastic pollution has reached a critical threshold, prompting an urgent search for effective remediation strategies. In 2024, global plastic production exceeded 430 million metric tons, and it is projected to continue rising despite increasing public awareness and the implementation of international regulations (Plastics, 2025). Consequently, plastic waste has become an escalating global burden. Although many countries and industries have introduced policies aimed at reducing plastic leakage, current waste-management practices remain heavily dependent on landfilling, incineration, and limited mechanical or chemical recycling. The global recycling rate has stagnated at below 10%, leaving approximately 90% of plastic waste to accumulate in terrestrial and aquatic environments (Jaynes, 2025). In aquatic systems, mismanaged plastics undergo fragmentation and weathering, generating large quantities of microplastics (MPs) plastic particles ranging from 1 to 5000 μm. MPs are now recognized as pervasive contaminants due to their microscopic size, persistence, and substantial surface area,

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which facilitates adsorption of other pollutants including persistent organic pollutants (POPs), heavy metals, antibiotics, and other nano-contaminants (Hale et al., 2020). MPs may originate as primary particles intentionally manufactured at microscopic sizes or as secondary fragments produced from the degradation of larger plastic items (Tursi et al., 2022). Common MPs are composed of polymers such as polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), and polyamide (PA). While MP pollution is universal regardless of chemical composition, the biological impacts and potential removal strategies are polymer-specific (Sheng et al., 2021). Thus, grouping all MPs under a single conceptual category limits our understanding of their organism-specific toxicity and hampers the development of targeted mitigation approaches.

The ecological and health implications of MPs have become a major research focus over the past decade. Numerous laboratory studies have shown that MPs induce cellular stress, oxidative damage, inflammatory responses, impaired growth, reproductive dysfunction, and mortality in aquatic organisms. Emerging clinical and biomonitoring studies indicate that MPs can also accumulate in human tissues, raising concerns about cardiovascular, inflammatory, and endocrine impacts (Yee et al., 2021). Given their persistence and continual input into natural systems, identifying effective strategies to remove or treat MPs is an urgent scientific priority. Because conventional waste-management methods including recycling, landfilling, and incineration cannot adequately address the rising levels of global MP contamination (Shukla et al., 2025), researchers are actively exploring alternative remediation approaches.

Since recycling, landfilling and incineration are not completely capable of controlling the rising number of MP wastes, researcher is evaluating different control measures. Such control measure that has been considered safe and cost-effective is degradation of MP particles. MP degradation can be performed through biodegradation, photodegradation, chemical degradation, and thermal degradation processes. All of these methods have their own limitations and advantages, however when the question is environmentally friendliness and cost-effectiveness, biodegradation of MP particles has been chosen as the most efficient. Biodegradation is the natural process where living organisms, mainly microbes (e.g., bacteria, fungi, algae), breakdown organic materials or pollutants into simpler substances like carbon dioxide (CO_2), water, and biomass, essential for recycling nutrients in ecosystems and managing waste. This process involves stages like biodeterioration (weakening), bio-fragmentation through enzymatic degradation (microbial breakdown), and assimilation (incorporation into new cells) (Wu et al., 2024).

Under controlled laboratory conditions, the biodegradation of MP particles can be enhanced by regulating environmental parameters such as temperature, pH, and nutrient availability, thereby maximizing degradation efficiency. However, biodegradation rates vary considerably depending on the polymer type and the microbial species involved. Different bacterial species exhibit distinct plastic utilization efficiencies. For example, for the same MP type (PE), *Bacillus cereus* achieved a weight loss of 1.78%, whereas *Pseudomonas tuomurensis* resulted in only 0.3% degradation (Kopecká et al., 2022). Similarly, within the same bacterial species (*B. cereus*), degradation efficiency differed across polymer types, with high-density polyethylene (HDPE) showing 8.26% degradation compared to only 1% for polypropylene (PP) (Hooda et al., 2023; Zhong et al., 2024). These findings highlight the need for further research to identify highly efficient bacterial species for effective microplastic bioremediation.

Building on the observed variability in MP biodegradation across polymer types and bacterial species, the present study aims to comparatively evaluate the biodegradation of the three most widely used polymers PE, PP, and PS by floc-forming bacteria. These polymers are among the most extensively produced and predominantly buoyant plastics, leading to their widespread environmental distribution and frequent detection as MP particles (Ziani et al., 2023). Collectively, PE, PP, and PS account for approximately 48% of microplastics identified in global environmental samples (Rani, 2022) and have been reported as the most abundant MP types in aquaculture systems (Bebianno et al., 2025). Recent studies suggest that floc-forming bacteria possess enhanced MP-degrading potential due to their strong aggregation capacity, biofilm formation, and close cell–polymer interactions, positioning them as promising agents for bacterial bioremediation strategies (Hossain et al., 2023). However, comparative assessments of their degradation efficiency across dominant polymer types remain limited. Addressing this gap, the current study seeks to provide systematic insights into polymer-specific biodegradation by floc-forming bacteria, thereby contributing to the development of targeted and effective microplastic remediation approaches.

2. Methodology

2.1. Floc-forming bacteria

The floc forming bacteria were collected from the microbiology lab of the Institute of Tropical Aquaculture and Fisheries (AKUATROP), Universiti Malaysia Terengganu. The bacteria strains, *Bacillus tropicus* SHBF1(OR921381) and *Cytobacillus firmus* SHBF3 (OR921675) were isolated from a commercial mud crab (*Scylla* spp.) farm. The selected bacterial strains were detected with the flocculation activity of 94.99 ± 0.006% for SHBF1 and 88.64 ± 0.067% for SHBF3. The bacterial strains are in use for the biofloc technology (BFT) of the Indoor Multi-Techno Aquaculture System (IMTAS) project at AKUATROP, UMT.

2.2. MPs particles and preparation

PE, PP, and PS microplastics were purchased to make sure that the particles have laboratory standard and are plasticizer free. For the biodegradation assay study, MP particles were purchased from Sigma Aldrich ®. For the biodegradation screening test, MP particles of 300 mesh size (49.67 μm) were purchased from a supplier of China which were confirmed by the Fourier Transform Infrared Spectroscopy (FTIR) analysis before initiating the screening test. Smaller particles were chosen for this phase to maximize surface area and enhance the visibility of degradation halos, allowing a rapid binary (yes or no) assessment of degradation potential.

2.3. Experimental design for the biodegradation assay

The current study was divided into two parts. At first, a screening test was performed to confirm the biodegradability of the selected strains. Following the screening test, 60 days of incubation study of bacterial strains with the MP particles were conducted. The 60 day-incubation study was also divided into three intermittent phases to identify the dynamicity of MP degradation at different time period. So, the periodical changes at 15th, 30th, and 45th over the 60 days were also taken into consideration for the better understanding of the biodegradation events.

2.3.1. Screening test for the different MP particles

The clear zone assay was used only as an initial qualitative screening tool to identify candidate strains with potential polymer-degrading activity. A Bushnell Haas agar (BHA) medium was used for the screening analysis. The inclusion of water insoluble MP particles in the BHA media was conducted following the study of Nakei et al. (2022). At first, the MP particles were sterilized using 75% ethanol solution followed by drying in a hot air oven (BINDER ED 23) for 12 h at 60 °C. The sterilized MP powder was kept in a sterile glass container before further analysis. During the preparation of BHA medium, the MP of each type was added at the concentration of 1 g/L. The medium containing MP was autoclaved at 121 °C for 15 min at 15 lb/inch^2. Finally, the agar medium was spread onto the petri dish (Sterilin™ Standard 90 mm) and let it cool down for the solidification. The media were ready to use for the

screening test. The bacterial strains (SHBF1 and SHBF3) were cultured until mid-log phase and then diluted in the sterile saline water (0.9% NaCl). A 100 µl aliquot of the diluted bacterial solution was spread on the BHA (+MP) medium. The petri dishes were incubated for 21 days at 30 °C and then observed for the clear zones and growth of the bacterial strains.

2.3.2. Biodegradation assay

Following the screening test, the biodegradation assay was conducted, following the study of Wang et al. (2020). For the biodegradation of MP particles using the floc-forming bacterial strains, three main preparations were performed: (a) Biodegradation media, (b) Preparation of bacterial strain, and (c) Preparation of MP particles. The biodegradation media used in this study was mineral salt media (MSM). The MSM was prepared by mixing the ingredients: NH_4NO_3 (1.0 g), K_2HPO_4 (0.76 g), KH_2PO_4 (0.7 g), $MgSO_4{\cdot}7H_2O$ (0.7 g), NaCl (0.005 g), $FeSO_4{\cdot}7H_2O$ (0.002 g), $MnSO_4$. H_2O (0.001 g), and $ZnSO_4{\cdot}7H_2O$ (0.002 g) in distilled water (up to 1000 ml). All the prepared MSM media were autoclaved (121 °C, 15 min, 15lb/inch^2) before using for the biodegradation assay. The bacterial strains were cultured until the log phase. At the same time, the PE, PP, and PS MP particles were sterilized (Section 2.3.1.). The biodegradation assay was conducted in 100 mL Erlenmeyer flasks. Each flask contained a total volume of 100 mL of mineral salt medium (MSM) supplemented with microplastics at a concentration of 2 g/L. Bacterial inoculum was added at 10% (v/v) of the total volume, corresponding to 10 mL of bacterial suspension (log phase), and the remaining 90 mL was MSM. Abiotic control flasks were prepared identically but contained no bacterial inoculum; instead, 10 mL of sterile MSM was added to replace the bacterial suspension. All flasks were incubated under the same conditions as described below. The inclusion of abiotic controls allowed assessment of any non-biological degradation (e.g., hydrolysis, leaching, or sterilization-induced changes) over the 60-day incubation period. MP particles greater than 500 µm was considered for the convenience of the analysis such as gravimetric weight loss, SEM, and FTIR. This larger size was necessary to enable reliable retrieval, washing, and weighing of particles after 60 days of incubation without loss during processing.

2.4. Analysis of biodegradation

The biodegradation of MP particles by the floc-forming bacteria was analyzed based on the three events- (a) Onset of bacterial strains on MP particles (Hydrophobicity test, SEM), (b) Utilization of MPs by bacterial strains: bacterial perspective (bacterial growth based on CFU and OD, gravimetric weight loss, polymer reduction rate, half-life of MP, hydrolases activity, pH monitoring, and CO_2 evolution), and (c) Utilization of MP particles by bacterial strains: MP particles perspective (gravimetric weight loss, FTIR, SEM).

2.4.1. Hydrophobicity test

Bacterial adherence to hydrocarbons is a useful technique to measure their ability to attach on a hydrophobic surface such as microplastic surface (Rosenberg, 1984). In this study, bacterial cell surface hydrophobicity was measured following the study of (Tiwari et al., 2024). Four different types of hydrocarbons such as n-hexane (aliphatic apolar), chloroform (aliphatic monopolar), xylene (aromatic apolar), and toluene (aromatic monopolar) were used. At first, the bacterial cells of logarithmic phase were suspended in the MSM after the repeated (3 times) centrifugation at 7000 g for 12min. The suspended cell density was recorded at 600 nm (initial OD) using UV–vis spectrophotometer (Shimadzu UV-1800). 3 ml of suspended cells were taken into a glass vial and 1 ml of hydrocarbon was added (different vials for different hydrocarbons). The glass vials were incubated for 12 min at 37 °C. Then the glass vials were vortexed for 20 s and kept undisturbed for 20 min at 30 °C. Two layers (hydrophobic-hydrophilic) were visible, and the lower aqueous phase was collected and the reading at 600 nm was taken again (Final OD). Cell surface hydrophobicity (%) was taken using the following formula:

$$\textit{Cell surface hydrophobicity}\ (\%) = \frac{(\text{Initial OD} - \text{Final OD})}{\text{Initial OD}} \times 100 \quad \text{(i)}$$

2.4.2. Hydrolases activity

Hydrolase is an enzyme group secreted by microplastics utilizing bacteria during the biodegradation process (Han et al., 2024). The current study measured the hydrolases activity as a confirmation of the secretion of hydrolase enzymes. Fluorescein diacetate (FDA) was used to measure the hydrolases activity (Dzionek et al., 2018). At first, 2 ml of bacterial solution in MSM (30 days of incubation) media was mixed with potassium phosphate buffer (pH 7.6), 0.2 ml FDA solution (1000µg/ml) and incubated at 30 °C for 40 min at 150 rpm in a water bath shaker. Then 20 ml chloroform-methanol solution (2:1 v/v) was added and centrifuged for 3 min at 2000 g. The supernatant was collected and the reading at 490 nm was recorded. The hydrolase activity was calculated from the standard solution (1–20 µg/ml) absorbance value (Adam and Duncan, 2001).

2.4.3. Gravimetric weight loss, polymer reduction rate (k) and half-life of polymer ($t_{1/2}$)

Gravimetric weight loss of MP particles at different days of incubation (DOI: 15th, 30th, 45th, and 60th) was measured to determine the utilization rate of MP by the both bacterial strains (Auta et al., 2017). For this analysis, all the MP particles used in this study were greater than 500 µm. The sterilized MP particles were weighed at first (Initial weight) and then weighed again after the harvesting of the MP particles at different DOI using an analytical balance with precision of 0.0001 g (Sartoriuos CP-224S, 220 g-0.1 mg). The harvested MP particles were dipped into a 2% sodium dodecyl sulfate (SDS) for 6 h and then cleaned with 70% ethanol. The washed MP particles were dried at 50 °C using a hot air oven (BINDER ED 23). The weight loss calculation was conducted using the following equation:

$$\textit{Weight loss}\ (\%) = \frac{\textit{Initial weight of MP particles} - \textit{Final weight of MP particles}}{\textit{Initial weight of MP particles}} \times 100 \quad \text{(ii)}$$

Polymer reduction rate (k) and half-life ($t_{1/2}$) were calculated using the following formula:

$$\textit{MP reduction rate}\ (k) = -\frac{1}{t}\left(ln\frac{w_f}{w_i}\right) \quad \text{(iii)}$$

Here, t = incubation duration (days), w_f = final weight after incubation, w_i = initial weight before incubation

$$\textit{Half-life of MP}\ (t1/2) = \frac{ln2}{k} \quad \text{(iv)}$$

2.4.4. pH

pH change of any bacterial media is an indication of bacterial growth (Sánchez-Clemente et al., 2018). In the current study, the pH changes

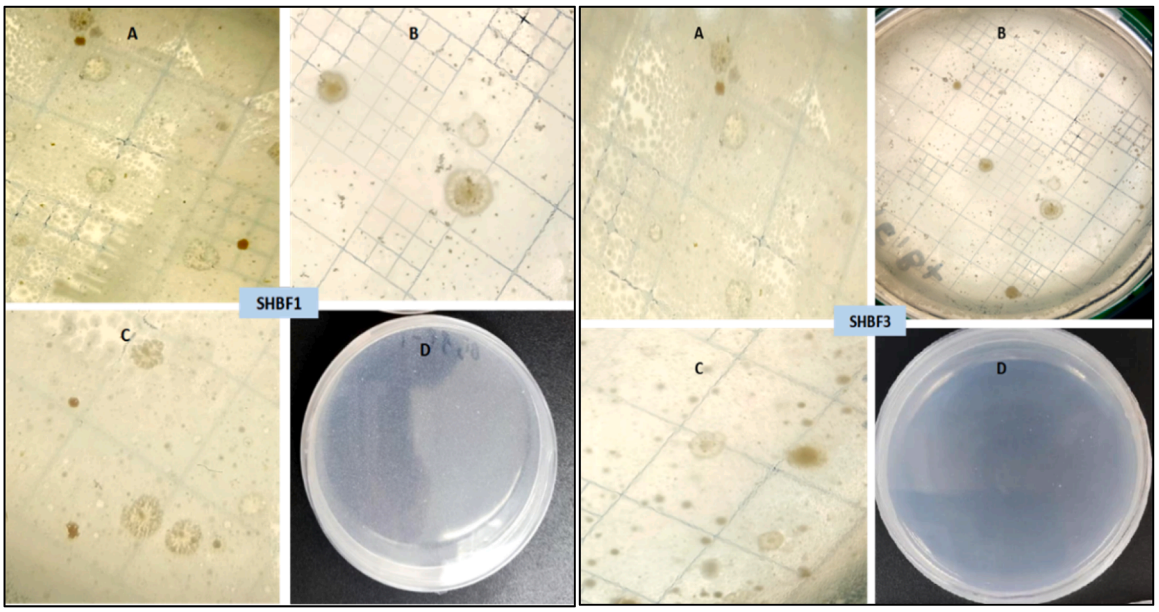

Fig. 1. Bacterial strains (Left: *B. tropicus* SHBF1 and Right: *C. firmus* SHBF3) screening for the biodegradability of (A) PE, (B) PS, (C) PP (control: (D).

were detected over the period of incubation.

2.4.5. Bacterial growth (CFU and OD)

Bacterial growth was measure at regular interval. At first, the bacterial growth at every 2 days was taken. After Day 10, the bacterial growth was taken at the predetermined intervals (DOI: 15th, 30th, 45th, and 60th). For the bacterial growth analysis, 5 ml samples were taken and then 1 ml was diluted to 6 decimals in a sterile solution (0.9% w/v NaCl) and then spread on the marine agar medium (Zobell). The remaining 4 ml was taken to determine the growth at 600 nm wavelength. The following formula was used to calculate the colony forming unit (CFU):

$$CFU = \frac{No.\ of\ colonies\ counted}{dilution\ factor \times volume\ of\ the\ bacterial\ sample} \quad (v)$$

2.4.6. Scanning electron microscopy (SEM)

MP particles were subjected to scanning electron microscopy to observe the changes in the MP surface topography due to the bacterial actions at different incubation time. At different predetermined incubation period, the MP particles were harvested, and a representative number of MP particles ($n = 15$) were randomly selected to observe under a tabletop scanning electron microscope (Hitachi TM-1000). The MP particles were observed at different magnifications (1000x to 7000x). The surface attributes from the microscopic observation were checked with the previous studies (Tareen et al., 2022; Thakur et al., 2023).

2.4.7. FTIR spectral analysis

FTIR analysis is a tool to identify plastic polymers chemically based on their responds towards the infrared lights in the form of spectra. So, a non-degraded MP particle has a unique spectrum which is deviated when the degradation occurs. In this study, the incubated MP particles in the presence and absence of bacterial strains were compared to spot the changes occur inside the plastics spectra due to the bacterial actions. The FTIR analysis was conducted at the wavelength of 400–6000 cm^{-1}, 42 scans and 16cm^{-1} resolution.

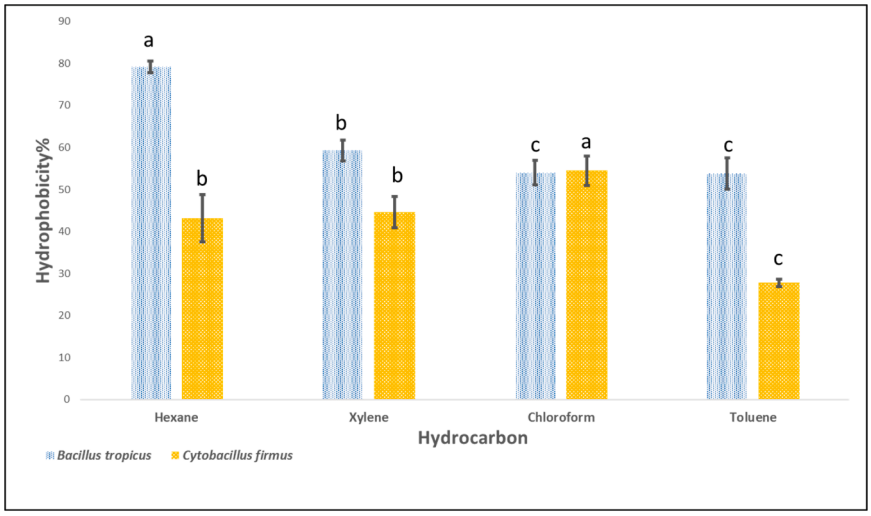

Fig. 2. Hydrophobicity% of *B. tropicus* and *C. firmus* for different types of hydrocarbons.

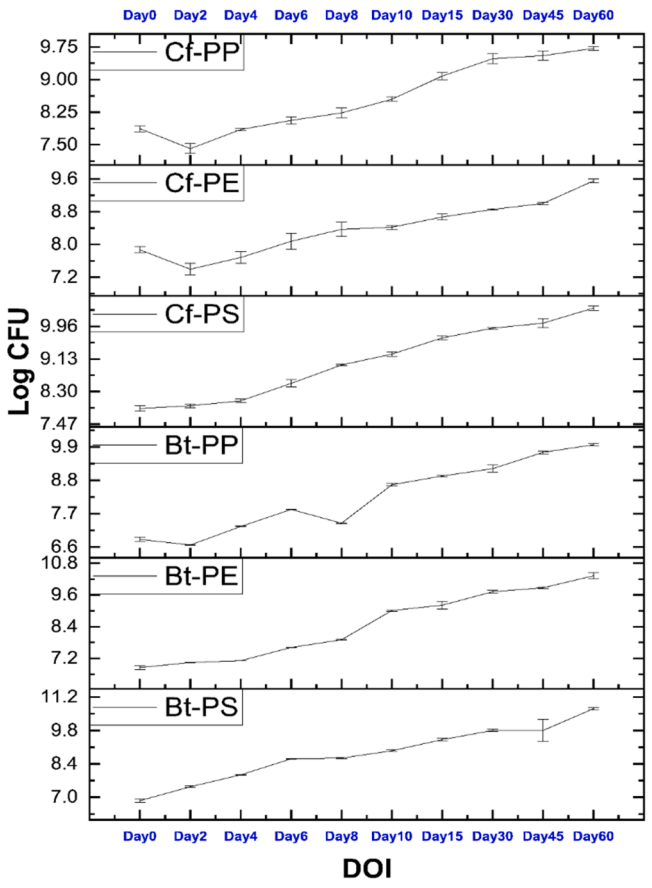

Fig. 3. Viable bacterial cells count in log CFU at different day of incubation (DOI).

2.4.8. *Statistical analysis*

All the data was checked for the normal distribution using the Shapiro-Wilk test or the Kolmogorov-Smirnov test. Based on the normality test, parametric or non-parametric test and related post-hoc test was conducted. Statistical significance was set $p \leq 0.05$. All the analysis was conducted using different packages of R and SPSS v26.0. The results were presented as mean ± standard deviation (SD) for parametric and media for non-parametric data.

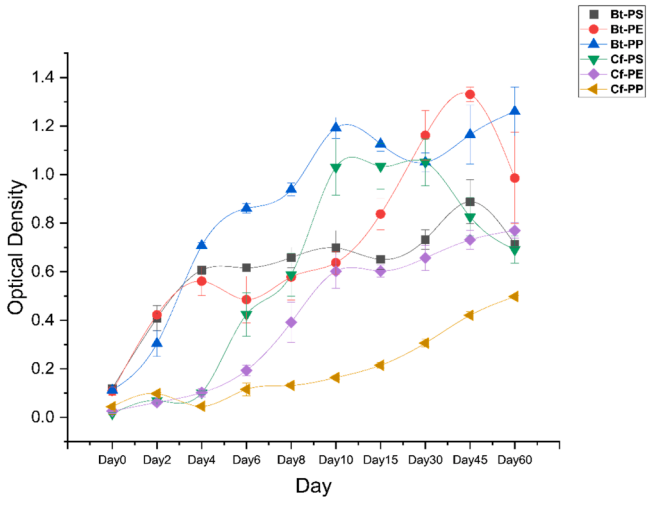

Fig. 4. Bacterial optical density at 600 nm wavelength at different day of incubation (DOI).

3. Results

3.1. *Screening test of the bacterial strains*

Clear zone method was conducted to screen the biodegradability of the tested bacterial strains, is a qualitative method use in the microbial degradation study (Folino et al., 2023). The present study found that both of the strains were capable of utilizing MPs when replaced with regular organic carbon sources in the solid mineral salt media. The MPs utilization was captured in the form of circular clear zones as presented in the Fig. 1. Clear zone method is a widely used technique to confirm the MP particles degrading ability of certain bacterial strains. Emulsified MP particles in the agar medium are utilized as nutrient source by the inoculated strains and result in a clear halo zone when the extracellular polymeric substances are diffused through the semi solid agar media (Tokiwa et al., 2009). The present study found many clear zones when the bacterial strains SHBF1, and SHBF3 were inoculated with the MP particles PE, PP, and PS. It confirmed degradability through the depolymerization of the MP particles by both bacterial strains. Similarly, Rafiq et al. (2018) reported clear zone formation as an effective tool to confirm the biodegradability of low-density PE by halophilic bacteria.

3.2. *Hydrophobicity test of the bacterial strains*

In MP biodegradation study, hydrophobicity of bacterial strains has been used as a tool to determine the adhesion and biofilm forming ability on the MPs surface which is the first step of degradation. The present study reported significant differences ($p < 0.001$) in the hydrophobicity among the hydrocarbons and between the bacterial strains (Fig. 2). In SHBF1, hydrophobicity was significantly higher in hexane (79.16%) compared to xylene (59.27%) and toluene (59.99%). For SHBF3, hydrophobicity was the highest in chloroform (54.50%) than in toluene (27.75%). Overall, SHBF1 showed higher hydrophobicity than SHBF3. Cell surface hydrophobicity (CSH) of bacteria is an important attribute in the bioremediation and fermentation industry. CSH causes surface damage of any organic wastes through biofilm formation (Krasowska and Sigler, 2014). At the same time, hydrophobicity of bacterial cells is an important indicator of probiotic properties. Athulya et al. (2024) reported *B. tropicus* ACS1 as a potential probiotic and showed around 64% hydrophobicity in xylene hydrocarbon. The current study used two floc-forming bacteria which are well known for their probiotic properties. In xylene hydrocarbon, *B. tropicus* SHBF1 strain of this study showed 59.27% and *C. firmus* SHBF3 showed 44.6%. Another study by Jayan et al. (2023) detected *B. cereus* NJD1 with >50% hydrophobicity when the weight loss of LDPE was recorded 43% after 120 days of incubation. It is likely an indication that the higher the hydrophobicity the higher the degradation rate as it facilitates the bacterial cells to be attached strongly (Das and Kumar 2013). The higher overall hydrophobicity of SHBF1 compared to SHBF3 correlated with greater viable cell attachment on PE and PP surfaces (Fig. 3) and higher weight loss percentages for those polymers (Fig. 7), supporting that cell surface hydrophobicity facilitates initial adhesion to microplastic surfaces, a critical first step in the degradation process

3.3. *Bacterial growth*

Bacterial growth during the MPs degradation is an effective way to determine the MPs assimilation upon utilization as sole carbon source. The current study observed both the direct and indirect measures of evaluating MPs degradation. The direct measure also known as viable cell count was significantly differed with the progression of time as bacterial strains showed differences in the MPs utilization efficiency. In this study, MP particles have interfered with OD readings due to light scattering. Therefore, CFU counts (Fig. 3) were considered a more reliable metric than OD for assessing bacterial growth in this system. For *B. tropicus* SHBF1 strain, the initial bacterial cell count was 6.85 ±

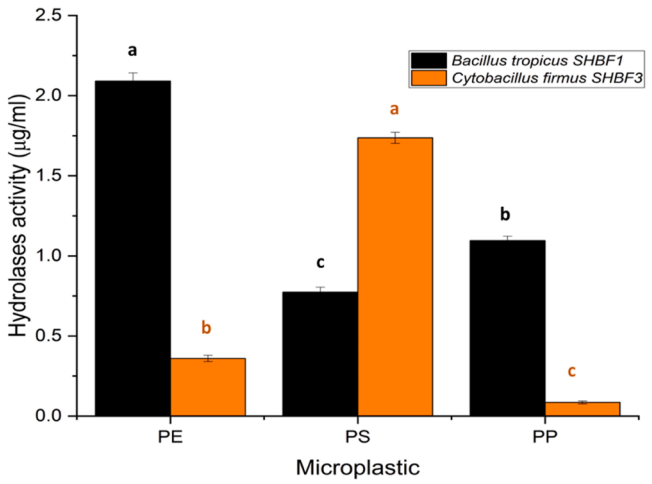

Fig. 5. Hydrolases activity of the bacterial strains SHBF1 and SHBF2 while degrading the MP particles.

0.07logCFU which was then reached to 8.95 ± 0.03logCFU for PS, 9.01 ± 0.02logCFU for PE, and 8.65 ± 0.04logCFU for PP before showing the trend of increasing in a decreasing pattern (Fig. 3). *C. firmus* SHBF3 showed similar pattern with an initial cell count of 7.87 ± 0.07logCFU. The strain showed 9.25 ± 0.06logCFU for PS, 8.42 ± 0.05logCFU for PE, and 8.55 ± 0.05logCFU for PP after day 10. The lowest bacterial count was observed in between 30 and 45 days for all the bacterial incubation. The present study found the bacterial cell count data was non-parametric resulted in conducting Friedman test analysis. Friedman test revealed a statistically significant difference in bacterial growth (log CFU) across the time points ($\chi^2(9) = 155.879, p < 0.001$), indicating that the strain exhibited time-dependent growth when microplastics were used as the sole carbon source. Post hoc pairwise comparisons using Wilcoxon signed-rank tests (Bonferroni-adjusted $\alpha = 0.0056$) revealed that bacterial growth was not significantly different from Day 0 at Days 2 and 4 ($p = 0.616$ and $p = 0.011$, respectively). However, significant increases in bacterial load were observed from Day 6 onwards ($p < 0.001$ for all subsequent time points), indicating the onset of active growth supported by MPs as the sole carbon source. The inter-strain bacterial counts while utilizing different MP types (PE, PP, PS) were significant until day 10. Day 10 onwards, bacterial cells were not growing significantly while utilizing the MP particles as sole carbon source. When the bacterial counts were considered for specific MP types for example, PS, PP, and PE (SHBF1 vs SHBF3), there were no significant differences ($p > 0.05$) for over 60 days. The no significant effect can be understood by the nature of non-significant bacterial growth after 10 days of culture.

Fig. 4 presents the OD value for the respective growth as an indirect way to measure bacterial growth during MP particles utilization. A correlation analysis was conducted to observe the reliability of the media turbidity over time to predict the actual bacterial growth (CFU) in real time. The CFU-OD correlation varied by microplastic type: very strong for PE ($r = 0.800, p < 0.001$), strong for PS ($r = 0.772, p < 0.001$), but only moderate for PP ($r = 0.448, p < 0.001$), suggesting microplastic-dependent effects on the relationship between turbidity and viable cell counts. The polymer-dependent variation in correlation is attributed to differences in MP particle morphology. PE particles were relatively uniform, causing less light scattering, whereas PP particles were irregular, resulting in greater interference.

Bacterial growth serves as a key indicator of MP utilization by the bacterial cells, since the incubation media were supplemented solely with MPs as the hydrocarbon source. It is noteworthy that strains SHBF1 and SHBF3 typically complete a growth cycle in <24 h. However, upon exposure to MP particles, they required a longer adaptation period to adjust to the nutrient-limited conditions. The present study found many growths declining phase when bacterial cells replication was out-numbered by the cell's death. It was due to the cells lysis, presence of inhibitory products, and nutrient-scarce condition (Yuan et al., 2022a). Even though, there were several declining events, the viable cell counts showed that the overall bacterial growth was stable throughout the incubation period (Fig. 3). Similar finding also reported in the study of Denaro et al. (2024) where *Stenotrophomonas rhizophila*-PM6, *S. rhizophila*-PM7, and *Lysinibacillus*-Q2 degraded PP and showed stable growth over 40 days of incubation.

3.4. Hydrolases activity

The present study demonstrated hydrolase enzyme activity for all MP particles, suggesting the secretion of depolymerase enzymes by the bacterial strains to degrade and utilize the long-chain hydrocarbons of MPs (Fig. 5). Strain SHBF1 exhibited significantly higher ($p < 0.05$) hydrolase activity toward PE (2.09 ± 0.05 µg/ml) compared to PS (0.77 ± 0.03 µg/ml) and PP (1.10 ± 0.03 µg/ml). In contrast, SHBF3 produced significantly higher ($p < 0.05$) hydrolase activity against PS (1.74 ± 0.03 µg/ml) than against PE (0.36 ± 0.02 µg/ml) or PP (0.09 ± 0.01 µg/

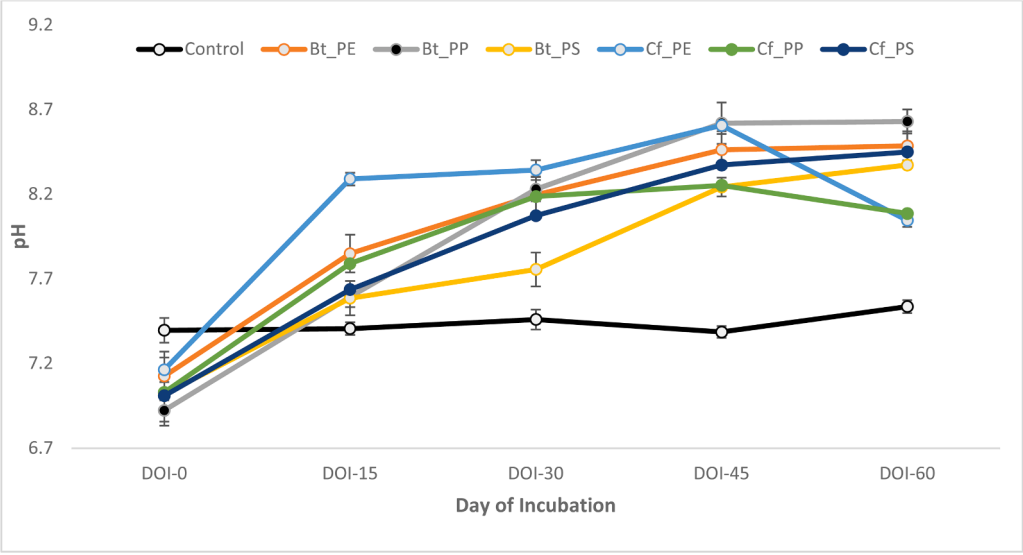

Fig. 6. pH changes of the incubation media at different day of incubation (DOI) with Bt = *B. tropicus* SHBF1, and Cf = *C. firmus* SHBF3.

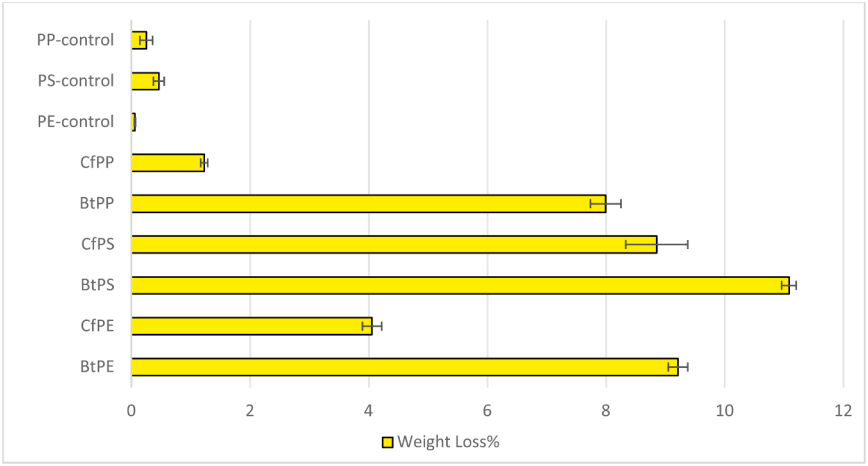

Fig. 7. Weight Loss% of PE, PS and PP MP particles. Here Bt stands for *B. tropicus* SHBF1 and Cf = *C. firmus* SHBF3.

ml), with the lowest activity observed for PP MPs. Thus, the two strains showed polymer-specific hydrolase profiles where SHBF1 was more effective against PE and PP, whereas SHBF3 was more effective against PS.

Prior to attachment on MP surfaces, the first step for bacteria to thrive in nutrient-limited, MP-rich environments is the secretion of extracellular and intracellular enzymes. Hydrolases are one of the major enzymes that secrete during MPs degradation process (Hou and Majumder, 2021). Hydrolases include proteases, lipases, cutinases, carboxylesterases, and esterases that are found linked with the degradation of MP particles (Hou et al., 2021). The present study recorded *B. tropicus* SHBF1 and *C. firmus* SHBF3 as a potential PE, PP, and PS degrader as they secreted a significant hydrolases activity to utilize the particles once they showed their adhesive ability on MPs surface. Similarly, Jeyavani et al. (2024) reported the secretion of protease, lipase, and esterase enzyme for the degradation of PP MP particles. This study was designed to demonstrate the secretion of hydrolase enzymes by floc-forming bacteria during MP degradation, rather than to elucidate the detailed enzymatic mechanisms. While FDA hydrolysis confirmed total hydrolase activity, it does not identify specific MP-degrading enzymes such as cutinases, alkane hydroxylases, or styrene monooxygenases. Future studies should focus on mechanistic investigations using polymer-specific enzyme assays.

3.5. pH alteration

Fig. 6 presents the pH changes over 60 days of incubation for different incubation treatments. All the treatments showed significantly different pH alterations with the progression of the incubation periods. The highest pH fluctuation was recorded from 7.13 to 8.49 for the PP MP particles when were utilized by the SHBF1 strain. The lowest was recorded for the control treatment from 7.39 to 7.43 indicated no bacterial activity. The remaining treatments showed similar pattern of pH change from neutral to alkaline condition. Previous studies also noticed the changes in pH during the incubation of bacterial degradation of MP particles. For instance, pH turned alkaline (7 to 8.3) in the incubation of LDPE with *Bacillus amyloliquefaciens* (Das and Kumar 2015) Contrarily, reduction in pH from 7.12 to 7.03 was recorded in the degradation of PE by *Bacillus* sp. strain SM1. The mixed culture of *Lysinibacillus xylanilyticus* and *Aspergillus niger* with LDPE in soil showed a similar pattern of pH changes with the present study. The study recorded pH fluctuations (decreasing and increasing) over 20 weeks of incubation (Esmaeili et al., 2013).

3.6. Weight loss, polymer reduction rate and half-life

In this study, SHBF1 showed the highest weight loss (%) for PS MP particles with 11.08 ± 0.37% after 60 DOI. SHBF3 showed the lowest MPs weight loss while incubated with PP (1.23 ± 0.10%) (Fig. 7). The remaining weight loss% were 9.21 ± 0.165% for PE by SHBF1, 8.854 ± 0.523% for PS by SHBF3, 7.99 ± 0.26% for PP by SHBF1, and 4.06 ± 0.16% for PE by SHBF3. In abiotic control flasks (MP particles in sterile MSM without bacteria), weight loss after 60 days was negligible for all polymers: PE control (0.06 ± 0.009%), PS control (0.46 ± 0.09%), and PP control (0.25 ± 0.11%). These values confirm that non-biological processes such as hydrolysis, leaching, or photodegradation did not significantly contribute to the observed weight loss. Therefore, the weight loss recorded for SHBF1 and SHBF3 is attributable to bacterial degradation activity.

Weight loss of microplastic particles was significantly influenced by both bacterial strain and polymer type. Scheirer Ray Hare analysis showed a significant effect of bacterial strain ($H = 8.24$, $p = 0.004$), indicating that SHBF1 and SHBF3 differ in their overall degradation capacity. Microplastic type also had a significant effect ($H = 6.88$, $p = 0.032$), with PS exhibiting higher weight loss than PP and PE. No significant interaction was detected between bacterial strain and microplastic type ($H = 0.062$, $p = 0.969$), suggesting that the relative differences among MP types were consistent across both bacteria. The absence of a significant strain × polymer interaction ($p = 0.969$) indicates that the relative degradation performance of SHBF1 and SHBF3 is consistent across PE, PP, and PS. Biologically, this suggests that SHBF1 possesses a generally higher intrinsic degradation capacity for all three polymer types compared to SHBF3, rather than having polymer-specific adaptations. This overall superiority of SHBF1 may be attributed to its higher cell surface hydrophobicity (68.2% vs. 52.4% for SHBF3), greater hydrolase enzyme secretion, and enhanced biofilm formation ability on MP surfaces. Post-hoc pairwise comparisons using Dunn's test (Bonferroni correction) revealed that, overall, PS showed significantly higher weight loss than PP (adjusted $p = 0.028$), while differences between PE and the other polymers were not significant.

Weight loss analysis is a direct way to understand the MPs degradation in terms of mass loss due to the bacterial utilization of hydrocarbons for their growth. The MP particles degradation varies among the bacterial strains due to enzymatic capability, metabolic pathways, biofilm formation ability, genetic variations, chemical composition of MPs, additives in MPs, MPs molecular weight, and environmental factors. The present study reported differences between the bacterial strains SHBF1 and SHBF3 in degrading PE, PP, and PS. Previous studies on these three MP particles also found varied weight loss for different bacterial strains. Studies found PE degradation of 17.3 to 19.13% by *Proteus* spp. (Dey

Table 1
Polymer reduction rate (PRR) and Half-life of the different MP particles.

Microplastic	Strain	Polymer reduction rate ($gday^{-1}$)	Half-life (days)
PE	*B. tropicus* SHBF1	0.0016 ± 0.000056^{b}	431.16 ± 15.014^{b}
	C. firmus SHBF3	0.00071 ± 0.000039^{c}	982.60 ± 52.55^{b}
PS	*B. tropicus* SHBF1	0.0019 ± 0.000039^{a}	353.27 ± 7.158^{b}
	C. firmus SHBF3	0.0014 ± 0.00016^{b}	488.13 ± 47.99^{b}
PP	*B. tropicus* SHBF1	0.0013 ± 0.000076^{b}	533.67 ± 31.94^{b}
	C. firmus SHBF3	0.00021 ± 0.000019^{d}	3420.59 ± 296.30^{a}

et al., 2026), of 2.58 to 19.44% by *Bacillus* spp. (Pereira et al., 2025), and of 10.15% by *B. tropicus* MK318648 (Samanta et al., 2020). In this study, SHBF1 and SHBF3 significantly degraded PE MP particles, with weight losses of 9.21 ± 0.165% and 4.06 ± 0.16%, respectively. This indicates that SHBF1 showed significantly higher PE degradation efficiency than SHBF3 ($p < 0.05$). However, the degradation rate was not the highest if compared with the previous studies as mentioned above. The potential reason might be not using any pre-treatment that trigger degradation or the strain-specific MPs degrading ability. Current study also found that PS degradation was non-significantly different between SHBF1(11.08 ± 0.37%) and SHBF3 (8.85± 0.91%). Previous study reported PS degradation of 4.5–5.4% by *Pseudoalteromonas* spp. (Lv et al., 2024) whereas Kim et al. (2021) reported 1.45 – 1.52% in 30 days by *Pseudomonas lini* JNU01 and *Acinetobacter johnsonii* JNU01. On the other hand. *B. cereus* CH6 showed 10.7% degradation (Yuan et al., 2022b) which is almost nearer to the current study. For the PP MPs, SHBF3 showed the lowest degradation rate of 1.23 ± 0.10% which was non-significantly different to the control treatments indicating very little efficiency of the strain. SHBF1 showed comparatively better degradation efficiency ($p < 0.05$) and was almost 6-fold higher than the efficiency of SHBF3. Although the study of Helen et al. (2017) reported 12% degradation of PP by *B. cereus* and 11% by *Sporosarcina globispora*.

Several factors are related with the bacterial-degradation of organic pollutants including different microbial strains, MPs properties, available nutrients, temperature, pH, Moisture, and available oxygen (Mbachu et al., 2021). The current study maintained these factors carefully so that the environmental influence can be omitted throughout the MPs-bacteria incubation time. However, the differences in the degradation efficiency between the SHBF1 and SHBF3 might be noticed due to the production of certain metabolites inhibited the process (Liu et al., 2025). Additionally, the surface properties specificity of enzymes secreted by the strains might have a role that needs to be investigated during the further continuation of this study. This study examined the changes in microplastic (MPs) utilization over time at different intervals. As shown in Table 1, the Polymer reduction rate (PRR) was significantly higher ($p < 0.05$) for all the MP types used in this study.

However, the half-life of the MP types was found to be significant only for PP MP particles. Half-life values were calculated assuming first-order degradation kinetics based on weight loss data from the 60-day experimental period. These extrapolations assume that degradation rates remain constant over time, which may not hold over longer periods due to factors such as biofilm maturation, nutrient limitation, enzyme inhibition, or changes in polymer crystallinity. Therefore, the reported half-lives should be interpreted only as relative comparisons between strains and polymer types within this study, not as absolute predictions of environmental degradation timelines. The extremely long half-life calculated for PP degradation by SHBF3 (3420 days) reflects the very low degradation rate observed, which was statistically indistinguishable from the abiotic control.

Correlation analysis between weight loss, pH alteration, and bacterial growth revealed distinct patterns between the two bacterial isolates (Fig. 8). For *B. tropicus* SHBF1, a strong positive correlation was observed between weight loss and bacterial growth ($\rho = 0.70$, $p < 0.05$), suggesting SHBF1 employs a more efficient, biomass-dependent degradation mechanism. In contrast, for *C. firmus* SHBF3, all correlations were non-significant. The relationship between weight loss and pH alteration showed opposing directions, with SHBF1 exhibiting a weak positive correlation ($\rho = 0.29$) and SHBF3 showing a weak negative correlation ($\rho = -0.38$).

3.7. SEM analysis of the MP particles

SEM imaging is an effective way to capture different MPs degradation events at different stages of biodegradation. The MPs degradation efficiency of the SHBF1 and SHBF3 strains were determined by observing various surface changes of the incubated MP particles. SEM images were captured before (control) and after the incubation with the bacterial strains. SEM captured different distinct events that can explain the overall biodegradation process. Fig. 9 shows the SEM images of MP particles where A showed the incubation for SHBF1 and B showed the incubation for SHBF3 strain. The present study observed bacterial cells attachment (Fig. 9Ai), surface fragmentation (Fig. 9*Av*), biofilm formation (Fig. 9Aiii), grooves (Fig. 9vi), pits (Fig. 9Aiv), produced metabolites (Fig. 9Aii) for SHBF1 strain. On the other hand, attached cells (Fig. 9Bi), bacterial colony (Fig. 9Bii), metabolites (Fig. 9Biii), holes (Fig. 9Bv, vi), swelling (Fig. 9Biv) were observed for SHBF3 strain. All

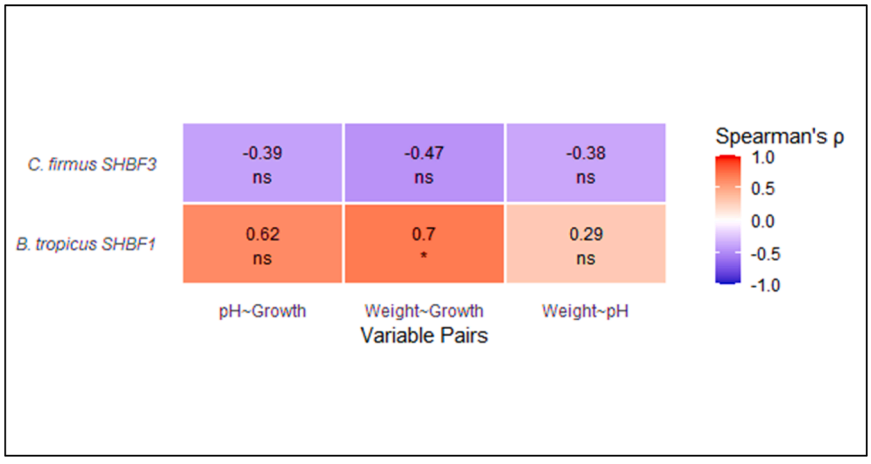

Fig. 8. Spearman correlation coefficients (ρ) between weight loss, pH alteration, and bacterial growth for *B. tropicus* SHBF1 and *C. firmus* SHBF3. Asterisks indicate statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), ns = non-significant.

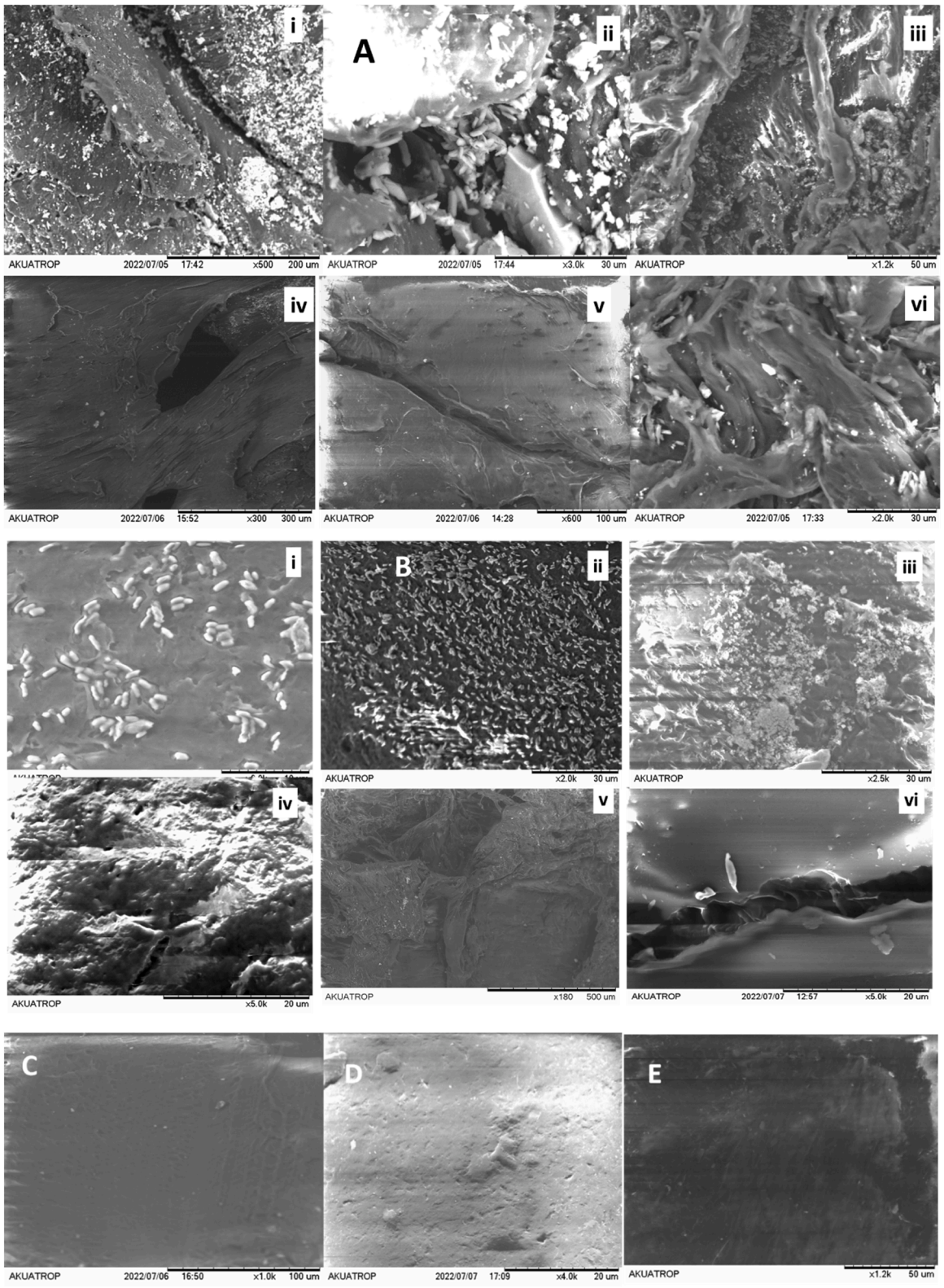

Fig. 9. SEM imaging of MP particles: (A) *B. tropicus* SHBF1 showing attached bacterial cells (Ai), produced metabolites (Aii), biofilm formation (Aiii), pits (Aiv), surface fragmentation (*Av*), and grooves (Avi); (B) *C. firmus* SHBF3 showing attached cells (Bi), bacterial colony (Bii), metabolites (Biii), swelling (Biv), holes (Bv, Bvi); (C) PE control; (D) PS control; (E) PP control.

the observed changes have been shown to occur as sequential events in the biodegradation of plastic polymers, consistent with findings from previous studies. Biodegradation begins with the attachment of bacterial cells to the surface of the microplastics (MPs), followed by the secretion of extracellular enzymes and the formation of a biofilm. Through this biofilm, bacteria utilize the carbon in the polymer to survive in the nutrient-limited environment, gradually altering the initially smooth surface. As bacteria colonize and proliferate, the surface develops visible

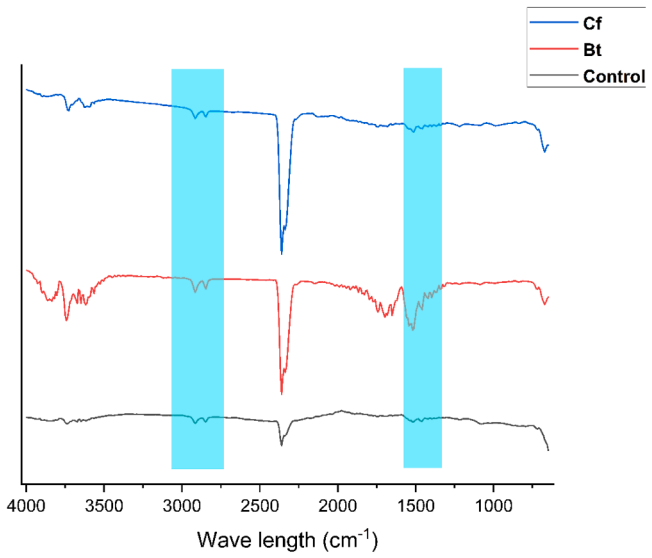

Fig. 10. FTIR spectrum of polyethylene (PE) MP particles after 60DOI with *B. tropicus* SHBF1 (Bt) and *C. firmus* SHBF3 (Cf).

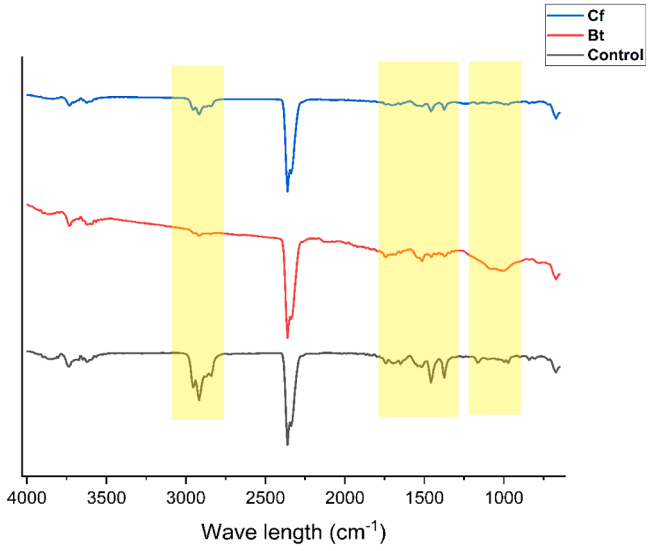

Fig. 11. FTIR spectrum of polypropylene (PP) MP particles after 60DOI with *B. tropicus* SHBF1 (Bt) and *C. firmus* SHBF3 (Cf).

degradation features such as pits, holes, grooves, and increased roughness (Hossain et al., 2024). The ultimate impact of these changes on the surface results in the mass of MPs whether it is gravimetric or molecular. The present study considered the gravimetric mass of the MPs particles. These events are further supported by additional findings from the present study. Bacterial attachment to the microplastic surface is evidenced by the measured cell surface hydrophobicity. The secretion of extracellular enzymes is confirmed by the detection of hydrolase activity. Bacterial growth and reproduction are demonstrated by increases in viable cell counts and supported by optical density measurements. SEM images worked here as microscopic visible evidence of these events.

3.8. FTIR analysis

FTIR analysis of the degraded MP particles was conducted to detect changes in the chemical composition of the incubated plastics. Evidence of bacterial degradation was obtained by comparing the spectra of the bacteria-incubated particles with those of the non-incubated control

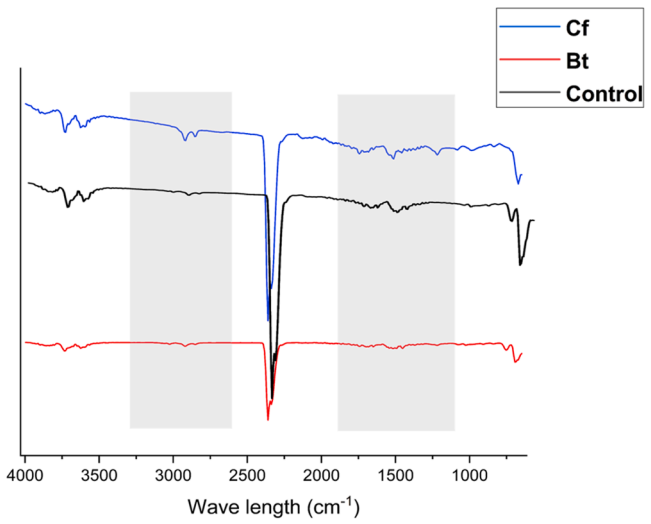

Fig. 12. FTIR spectrum of polystyrene (PS) MP particles after 60DOI with *B. tropicus* SHBF1 (Bt) and *C. firmus* SHBF3 (Cf).

particles. For PE MP particles (Fig. 10), the characteristic peaks were observed at 2914 cm^{-1} for CH_2 asymmetric stretching, 2847 cm^{-1} for CH_2 symmetric stretching, 1470 cm^{-1} bending deformation, and 718 cm^{-1} for rocking deformation (Amelia et al., 2016). After 60 DOI, the bacterially incubated PE particles were compared with the control non-inoculated particles. The bacterially incubated particles showed many differences in the peaks such as, formation of new peaks from 1250 to 2000 cm^{-1} regions that cover a portion of fingerprint region. Additionally, the peak intensity differences at 1470 cm^{-1} were also observed. The changes in the 2914 and 2847 cm^{-1} intensities in the SHBF1 and SHBF3 incubated particles were also noticed. The spectra changes align with the previous studies conducted on PE MPs for different bacterial strains. Nadeem et al. (2021) reported the changes in the PE chemical composition through the increased transmittance at 1459.7 cm^{-1} indicated the reduction in CH_2 bends. The appearance of new peaks near 1650–1730 cm^{-1} in the FTIR spectra of SHBF1-incubated MP particles indicates the formation of carbonyl groups, reflecting oxidation of the degraded PE polymer chains (Kowalczyk et al., 2016). This observation also supports the higher degradation efficiency of PE by SHBF1 compared to SHBF3, which is further corroborated by the greater percentage of weight loss (9.21%), increased enzyme secretion, higher cell surface hydrophobicity leading to stronger biofilm formation (SEM), and enhanced bacterial growth.

FTIR analysis of the control PP microplastic particles in the present study showed characteristic peaks at 2952, 2916, 2869, 1457, 1374, 1163, 1000, 976, 844, and 809 cm^{-1} (Fig. 11), confirming that the polymer structure remained unchanged during incubation. In the present study, FTIR analysis revealed alterations in the characteristic PP functional groups following incubation with the SHBF1 and SHBF3 strains, particularly in the C—H stretching region (2952–2869 cm^{-1}) and CH_3 bending region (1457–1374 cm^{-1}), where SHBF1 induced stronger peak reductions, splitting, and the formation of a broad unresolved band, indicating extensive modification of the PP backbone. These observations closely correspond with patterns reported in previous studies by Helen et al. (2017) and Wróbel et al. (2023), which documented clear changes in methyl (1700−1800 cm^{-1}) and carbonyl (1600 −1850 cm^{-1}) regions, as well as shifts within 2000–1500 cm^{-1} and 1000–400 cm^{-1} during microbial degradation of PP. Similar to our findings, the other study also detected altered CH_3 bending peaks (1462 and 1377–1381 cm^{-1}) and multiple changes in the fingerprint region, reflecting polymer structural disruption. Although the previous study additionally reported the formation of new peaks at 2372, 2304, and 2042 cm^{-1} associated with C—H stretching both sets of results consistently highlight the

degradation-induced modification of C—H and CH_3 vibrations and the disruption of isotactic PP signatures. Together, the alignment of spectral changes across studies provides strong evidence that the transformations observed in our FTIR spectra represent genuine biodegradation of PP microplastics, with SHBF1 demonstrating a higher degradation capacity than SHBF3.

The characteristic peaks for the PS MP particles were noticed at 3028 cm^{-1}, 2922 cm^{-1}, 2854 cm^{-1}, 1656–1456 cm^{-1}, 756 cm^{-1}, and 697cm^{-1} in the control treatment (Fig. 12). After 60 days of incubation, notable changes were observed in the transmittance of the FTIR peaks for PS microplastics treated with SHBF1 and SHBF3. In addition, new peaks appeared in the spectra, including a distinct peak at 1227 cm^{-1}, corresponding to C—O stretching in the PS chain. The appearance of this peak provides clear evidence of bacterial-induced degradation of the polymer. The previous study reported that bacterial treatment of PS microplastics caused slight increases in -OH (1372 cm^{-1}) and -CH, CH_3 and CHO (3024, 2920, 2849 cm^{-1}) peaks after 30 days, with further increases and the appearance of new C=O peaks (2360 cm^{-1}) after 60 days, indicating oxidative modification and microbial degradation.

Previous studies on bacterial degradation of PS microplastics consistently show chemical modifications indicative of oxidative degradation. Xiang et al. (2023) reported a significant decrease in carbon content and increase in oxygen content, confirming oxidation reactions during microbial treatment. FTIR analyses in this study revealed that while the characteristic benzene ring peaks remained largely intact, new or altered peaks appeared in regions corresponding to O–H stretching bending, and carbonyl C=O stretching, indicating bacterial-induced oxidation. Wang et al. (2024) similarly observed the development of new functional groups at 2360 cm^{-1} (C=O) and changes in -CH, -CH_3, and -CHO stretching bands, with these modifications becoming more pronounced over 30–60 days of incubation. Collectively, these findings align with the present study, where PS microplastics incubated with SHBF1 and SHBF3 exhibited new C—O stretching at 1227 cm^{-1} and changes in the intensity of existing peaks, confirming progressive oxidative modification and microbial degradation of the polymer.

4. Conclusion

To the best of our knowledge, this study is the first to comparatively evaluate the microplastic-degrading efficiency of two taxonomically distinct floc-forming bacterial genera (*Bacillus* and *Cytobacillus*) against three major polymer types (PE, PP, PS) under identical conditions. Across all analyses, SHBF1 consistently demonstrated superior performance over SHBF3. The highest degradation level was observed for PS microplastics, where SHBF1 achieved approximately 11% gravimetric weight loss. Supporting parameters including cell surface hydrophobicity, pH changes, enzyme secretion, bacterial growth patterns, SEM imaging, and FTIR spectral alterations collectively confirmed the stronger degradation potential of the SHBF1 strain. These findings highlight the need for future research focusing on the molecular mechanisms underlying microplastic degradation by such bacteria. A deeper understanding of the cellular and enzymatic pathways involved will be essential for bioengineering improved strains, particularly to enhance the degradation rate beyond the current maximum of 11%.

Ethics statement

No human participants or vertebrate animals were used in this study. Bacterial strains were isolated from environmental aquaculture samples, and no specific ethical approval was required under institutional or national guidelines.

CRediT authorship contribution statement

Shahadat Hossain: Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. **Tashrif Mahmud Minhaz:** Writing – review & editing. **Benedict Terkula Iber:** Writing – review & editing. **Zuhayra Nasrin Ahmad Shukri:** Methodology, Data curation. **Che Engku Noramalina Che Engku Chik:** Writing – review & editing. **Norhafiza Ilyana Yatim:** Software, Resources, Methodology, Formal analysis. **Ahasan Habib:** Writing – review & editing. **Mohd Shamzi Mohamed:** Resources, Data curation. **Mohd Ihwan Zakariah:** Resources, Methodology, Data curation. **Azmie Ghazali:** Software, Resources, Methodology, Formal analysis. **Nor Azman Kasan:** Writing – review & editing, Validation, Supervision, Resources, Project administration.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Nor Azman Kasan reports financial support was provided by Higher Institution centers of Excellence (HICOE), Ministry of Higher Education, Malaysia. Nor Azman Kasan reports a relationship with Institute of Tropical Aquaculture and Fisheries Research, University Malaysia Terengganu that includes: non-financial support. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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