



Microbes Breaking Down Plastic: Insights for Sustainable Waste Management

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ABSTRACT

This research investigates the microbial degradation of low-density polyethylene (LDPE) and high-density polyethylene (HDPE) plastics by *Bacillus* sp., *Proteus* sp., *Pseudomonas* sp., and *Salmonella* sp. The study employs a systematic approach, isolating microorganisms from plastic-contaminated soil and subjecting them to a series of biochemical tests for identification. The research evaluates the weight loss of LDPE and HDPE over two months, revealing varying degrees of degradation among the bacterial strains. Results suggest a potential greater susceptibility of HDPE to microbial degradation. Scanning Electron Microscopy (SEM) analysis provides high-resolution images of the plastic surface, indicating structural changes and biofilm formation during degradation. The findings highlight the unique enzymatic capabilities of each strain and underscore the significance of SEM in elucidating microbial interactions with plastics. The study prompts discussions on optimization, synergistic effects, and the identification of key enzymes in plastic degradation, emphasizing the importance of microbial strategies for waste management. Overall, this research contributes valuable insights into the potential of bacterial strains for addressing plastic pollution challenges.

INTRODUCTION

Plastic is a synthetic polymer derived from petrochemicals, widely used for its durability and versatility. Its persistence in the environment leads to long-lasting pollution, as it does not easily biodegrade. Improper disposal and accumulation in oceans harm marine life, disrupt ecosystems, and contribute to global environmental challenges. Addressing plastic pollution requires sustainable alternatives and responsible waste management (Banerjee & Bhattacharya 2022, Ghatge et al. 2020, Kotova et al. 2021, Montazer et al. 2020). Here are a few examples illustrating the severe consequences of plastic pollution worldwide. The Great Pacific Garbage Patch, an extensive accumulation of plastic debris primarily consisting of microplastics, has formed in the Pacific Ocean, posing a significant threat to marine life through ingestion and entanglement (Ghatge et al. 2020).

Additionally, on Midway Atoll, albatross chicks often meet tragic fates as a result of ingesting plastic fed to them by their parents, mistakenly taken for food (Auman et al. 1997, Sileo et al. 1990). These instances highlight the pervasive and impactful nature of plastic pollution, reaching even the most remote locations and adversely affecting isolated wildlife populations. Efforts to mitigate plastic pollution encompass multidisciplinary strategies rooted in scientific

research and environmental conservation. Initiatives focus on the development of biodegradable polymers, employing advanced microbial degradation processes, and implementing eco-friendly substitutes for traditional plastics (Babaremu et al. 2023, Liu et al. 2020, Song et al. 2009). Technological innovations in waste management, such as advanced sorting and recycling techniques, aim to enhance the efficiency of plastic recovery and reduce reliance on landfill disposal (Byrne et al. 2022, Mohanan et al. 2020, Montazer et al. 2020). Additionally, scientific endeavors emphasize public awareness campaigns, advocating for behavioral shifts to minimize plastic consumption, coupled with policy interventions to regulate the production and disposal of plastics. Collaborative research endeavors are crucial for advancing our understanding of the environmental impact of plastics and developing sustainable solutions that harmonize with ecological systems.

Microbial degradation of plastic involves the enzymatic breakdown of polymer chains by microorganisms, offering a promising avenue for plastic waste remediation (Gajendiran et al. 2016, Kopecká et al. 2022, Kotova et al. 2021, Mohanan et al. 2020). One notable success story involves the discovery of *Ideonella sakaiensis*, a bacterium capable of depolymerizing polyethylene terephthalate (PET), a common

plastic used in beverage bottles. The enzyme PETase, produced by this bacterium, catalyzes the hydrolysis of PET into its monomeric components (Banerjee & Bhattacharya 2022, Kotova et al. 2021, Mohanan et al. 2020, Ojha et al. 2017). Another breakthrough centers on the isolation of a microbial consortium from a plastic-contaminated environment, exhibiting the ability to degrade diverse plastic types. These microbial-driven advancements underscore the potential for harnessing natural processes to address plastic pollution, with ongoing research exploring ways to optimize and scale microbial degradation techniques for practical waste management applications (Gajendiran et al. 2016, Kopecká et al. 2022, Mohanan et al. 2020, Montazer et al. 2020). Here a study is designed to isolate the potential microorganisms capable of degradation of HDPE and PDPE type of plastics. The entire study was planned in a very simple yet effective way, where isolated microorganisms were allowed to degrade the plastic for two months, and the rate of degradation was calculated by measuring the weight loss.

MATERIALS AND METHODS

Sample Collection

The plastic samples were meticulously gathered from the plastic-laden soil of Valetva, Anand, employing a non-invasive collection method to preserve the integrity of the specimens. A comprehensive survey of the area ensured the representation of various plastic types within the collected samples.

Isolation

The isolation process commenced with a systematic serial dilution technique. In this method, 0.5 g of the plastic soil sample was precisely weighed and introduced into 5 mm of sterile water, initiating a 1:10 dilution. Sequential dilutions followed, with 0.5 mm of each preceding dilution being added to 5 milliliters of sterile water to achieve subsequent dilution factors (e.g., 1:100, 1:1000, and so forth). The last three dilutions underwent plating on Nutrient Agar (NA) medium, enriched with 2% Polyethylene Glycol (PEG), employing the pour plate method (Alshehrei 2017, Nademo et al. 2023). This specific medium was selected for its ability to mimic environments containing crucial sources of plastic. The inoculated plates were then meticulously incubated at 37°C for 48 h, fostering optimal conditions for microbial growth and allowing for the subsequent assessment of microbial colonization and adaptation to plastic-rich environments. The resulting colonies were subjected to further analyses to identify and characterize potential plastic-degrading microorganisms.

Identification

In the microbial identification and characterization process, isolates obtained from plastic-contaminated soil underwent a rigorous set of procedures for thorough analysis. Gram staining involved applying a smear of the bacterial culture onto a clean slide, followed by drying, fixing, and staining with crystal violet, Gram's iodine, Gram's decolorizer, and Safranin. The resulting slides were air-dried, and cell morphologies were meticulously examined under a microscope with an oil immersion objective (100X). Simultaneously, spore staining procedures included preparation of a boiling water bath, staining with malachite green, steaming, counterstaining with Safranin, and microscopic examination of the dried slides. Additionally, colony morphology, encompassing shape, edge, color, and surface characteristics, was assessed for each colony post-purification. The subsequent biochemical tests utilized the Hibacillus identification kit (HIMEDIA) and manual methods for catalase, oxidase, mannitol utilization, malonate utilization, nitrate reduction, citrate utilization, motility, gas production from glucose, carbohydrate utilization, urease, indole, MacConkey agar, and spirit blue agar tests. These tests aimed to unravel key metabolic activities, contributing to a comprehensive identification and characterization of the isolated strains based on their biochemical profiles (Franco-Duarte 2019, Rave et al. 2019).

Determination of Weight Loss

In a controlled experiment, 100 mL of a pre-poured liquid mineral salt medium was dispensed into a 250 mL Erlenmeyer flask, and high-density polyethylene (HDPE) and low-density polyethylene (LDPE) plastics were introduced. The mixture was then subjected to sterilization in an autoclave. Each treatment, including controls on plastic plates in a sterile environment, was contained in separate bottles and placed in a shaker at 150 rpm and room temperature for 2 months. Following one month of continuous shaking, the plastic sheets were carefully collected, thoroughly washed with distilled water, shadow-dried, and subsequently weighed to determine their final weight (Ru et al. 2020, Zeenat et al. 2021). The weight loss data of the plastics were calculated using the dry loss percentage formula

$$\text{Percentage of losses of dry weight} = \frac{W_i - W_f}{W_i}$$

Where: W_i represents the initial dry weight before degradation (g), and W_f represents the dry weight after degradation (g). This methodology ensures a systematic approach to quantifying the impact of environmental

conditions on the degradation of HDPE and LDPE plastics.

Scanning Electron Microscope

In the analysis of plastic degradation by microorganisms using a scanning electron microscope (SEM), the process begins with the collection of plastic samples representing various degradation stages, followed by thorough cleaning and fixation with a suitable fixative. Subsequent steps involve dehydration through a series of ethanol concentrations, critical point drying to preserve sample structure, and mounting onto SEM stubs using conductive adhesive or carbon tabs. Optionally, a thin conductive coating may be applied. Once prepared, the samples are placed in the SEM chamber, and imaging parameters are set to capture high-resolution images of the plastic surface, highlighting features such as cracks, pits, and attached microorganisms. Analysis of SEM images allows for the identification of surface alterations indicative of microbial degradation. Additionally, quantitative analysis using image processing tools can be employed for more detailed measurements. The entire process is meticulously documented, including SEM settings, sample preparation details, and observed surface features, providing a comprehensive record for further interpretation and refinement of the analytical approach based on specific sample characteristics and research objectives.

RESULTS AND DISCUSSION

Based on the results of primary isolation, four different colonies were identified for further study. These four bacterial isolates (Isolate 1, Isolate 2, Isolate 3, and Isolate 4) were subjected to a series of biochemical tests to characterize their metabolic activities. All isolates exhibited positive results for the catalase and oxidase tests, indicating the presence of catalase enzyme and cytochrome c oxidase, respectively. In terms of motility, Isolate 3 and Isolate 4 demonstrated motility, while Isolate 1 and Isolate 2 were non-motile. The nitrate reduction test revealed positive results for Isolate 1, Isolate 2, and Isolate 4 but negative for Isolate 3. Isolate 3 was the only one to utilize citrate, as indicated by a positive citrate utilization test. Malonate utilization was not observed in any of the isolates. Isolate 2 and Isolate 4 exhibited positive results for the phenylalanine agar test, while Isolate 1 and Isolate 3 were negative. All isolates showed positive results for starch hydrolysis. The indole test yielded negative results for all isolates, and only Isolate 2 and Isolate 4 were positive for the urease test. Lysine decarboxylation was not observed in any isolate. Isolate 2 and Isolate 3 showed positive results in the triple sugar iron test, while Isolate 1 and Isolate 4 were negative.

Isolate 2 and Isolate 4 tested positive for the methyl red test. The MacConkey agar test indicated positive results only for Isolate 1. None of the isolates demonstrated lipase activity, as observed in the negative results for the spirit blue agar test. The carbohydrate utilization profiles of four bacterial isolates (Isolate 1, Isolate 2, Isolate 3, and Isolate 4) were determined through a series of tests for different sugars. All isolates exhibited positive reactions for the utilization of Dextrose, Sucrose, Mannitol, Fructose, and Maltose. Isolate 2, Isolate 3, and Isolate 4 displayed positive results for Galactose and Lactose, while Isolate 1 did not utilize these sugars. Based on the results of the biochemical assay, the isolates were identified as Isolate 1 in *Bacillus* sp., Isolate 2 in *Proteus* sp., Isolate 3 in *Pseudomonas* sp., and Isolate 4 in *Salmonella* sp.

Results of Degradation of LDPE and HDPE by isolates

In the investigation of plastic degradation by bacterial strains, namely *Bacillus* sp., *Proteus* sp., *Pseudomonas* sp., and *Salmonella* sp., on low-density polyethylene (LDPE) and high-density polyethylene (HDPE), the initial and final weights of each plastic sample were meticulously recorded to assess the extent of degradation. *Bacillus* sp. exhibited a 20% weight loss for LDPE, with a difference of 0.004 grams, and a 22.36% weight loss for HDPE, with a difference of 0.1154 grams, indicating its notable capability to degrade both plastic types. *Proteus* sp. demonstrated a 15% weight loss for LDPE, with a difference of 0.003 grams, and a 17.28% weight loss for HDPE, with a difference of 0.0864 grams, showcasing its efficiency in plastic degradation as well. *Pseudomonas* sp. exhibited a 20% weight loss for LDPE, with a difference of 0.004 grams, suggesting a comparable performance to *Bacillus* sp., while demonstrating a remarkable 23.08% weight loss for HDPE, with a difference of 0.1118 grams. *Salmonella* sp. displayed a substantial 22.5% weight loss for LDPE, with a difference of 0.0045 grams, and an even more significant 24.36% weight loss for HDPE, with a difference of 0.1218 grams, indicating its effectiveness in degrading both plastic types (Table 1 & Fig. 1).

The varying degrees of plastic degradation observed among the bacterial strains underscore their unique enzymatic capabilities and metabolic pathways involved in plastic biodegradation. The higher weight loss percentages for HDPE compared to LDPE in most cases suggest a potentially greater susceptibility of HDPE to microbial degradation. These findings contribute to the ongoing exploration of microbial strategies for plastic waste management, emphasizing the importance of understanding the specific bacterial strains that can effectively degrade different types of plastics (Gajendiran et al. 2016, Islami et

al. 2019, Kyaw et al. 2012, Midhun et al. 2015, Nademo et al. 2023, Ojha et al. 2017, Sanniyasi et al. 2021).

The results prompt further discussions on the optimization of conditions for enhanced plastic degradation, potential synergistic effects when combining different bacterial strains, and the identification of key enzymes involved in the degradation process (Cai et al. 2023, Crystal Thew et al. 2023, Pischedda et al. 2019, Rani et al. 2021). Additionally, exploring the genetic and biochemical mechanisms underlying the observed variations among the bacterial strains can provide valuable insights for the development of targeted approaches to address plastic pollution challenges (Gilani et al. 2023, Kumari et al. 2021, Purohit et al. 2020, Urbanek et al. 2021).

The research findings provide a fascinating insight into the potential of bacterial strains in degrading plastic materials, particularly LDPE and HDPE. The varying degrees of degradation observed among *Bacillus* sp., *Proteus* sp., *Pseudomonas* sp., and *Salmonella* sp. highlight the unique enzymatic capabilities of each strain. Interestingly, the higher weight loss percentages for HDPE compared to LDPE

suggest that HDPE might be more susceptible to microbial degradation. This could be due to the structural differences between LDPE and HDPE. HDPE has a more crystalline structure which might make it easier for the bacteria to access and degrade the polymer chains (Kyaw et al. 2012, Midhun et al. 2015, Ojha et al. 2017). The results also open up several avenues for further research. For instance, it would be interesting to explore the optimization of conditions for enhanced plastic degradation. Factors such as temperature, pH, and nutrient availability could potentially influence the efficiency of plastic degradation. Another intriguing aspect is the potential synergistic effects when combining different bacterial strains (Ojha et al. 2017, Rani et al. 2021).

Furthermore, identifying the key enzymes involved in the degradation process could provide valuable insights. Understanding the biochemical mechanisms underlying the observed variations among the bacterial strains can lead to the development of targeted approaches to address plastic pollution challenges. Overall, this research underscores the importance of exploring microbial strategies for plastic waste management. It emphasizes the need for a deeper

Table 1: Result of degradation of plastic LDPE and HDPE by Bacteria.

Bacterial strain	Type of plastic	Initial weight [g]	Final weight [g]	Difference	Weight loss [%]
<i>Bacillus</i> sp.	LDPE	0.02	0.016±0.010	0.004	20%
<i>Bacillus</i> sp.	HDPE	0.5	0.3846±0.08	0.1154	22.36%
<i>Proteus</i> sp.	LDPE	0.02	0.017±0.007	0.003	15%
<i>Proteus</i> sp.	HDPE	0.5	0.4136±0.07	0.0864	17.28%
<i>Pseudomonas</i> sp.	LDPE	0.02	0.016±0.0011	0.004	20%
<i>Pseudomonas</i> sp.	HDPE	0.5	0.3882±0.08	0.1118	23.08%
<i>Salmonella</i> sp.	LDPE	0.02	0.0155±0.007	0.0045	22.5%
<i>Salmonella</i> sp.	HDPE	0.5	0.3782±0.06	0.1218	24.36%

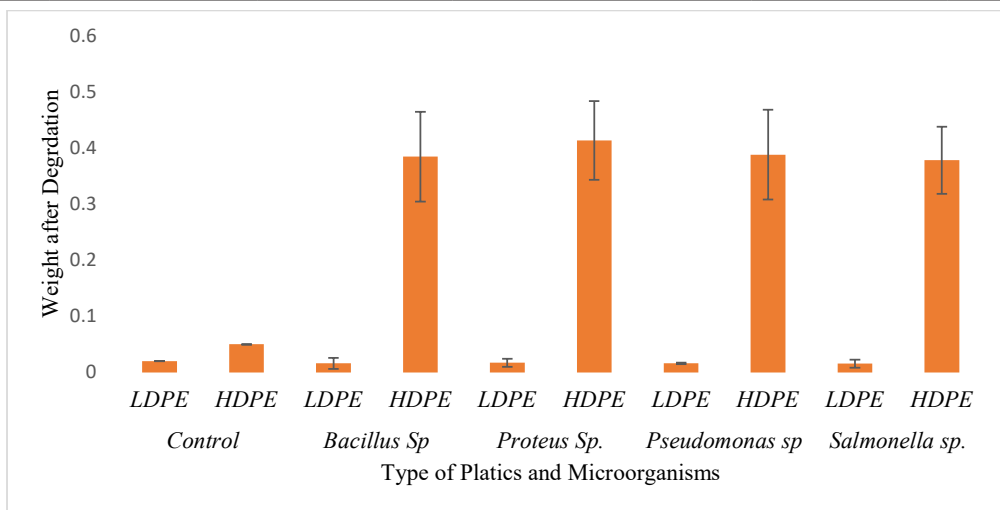


Fig. 1: Result of degradation of plastic LDPE and HDPE by Selected Microorganisms.

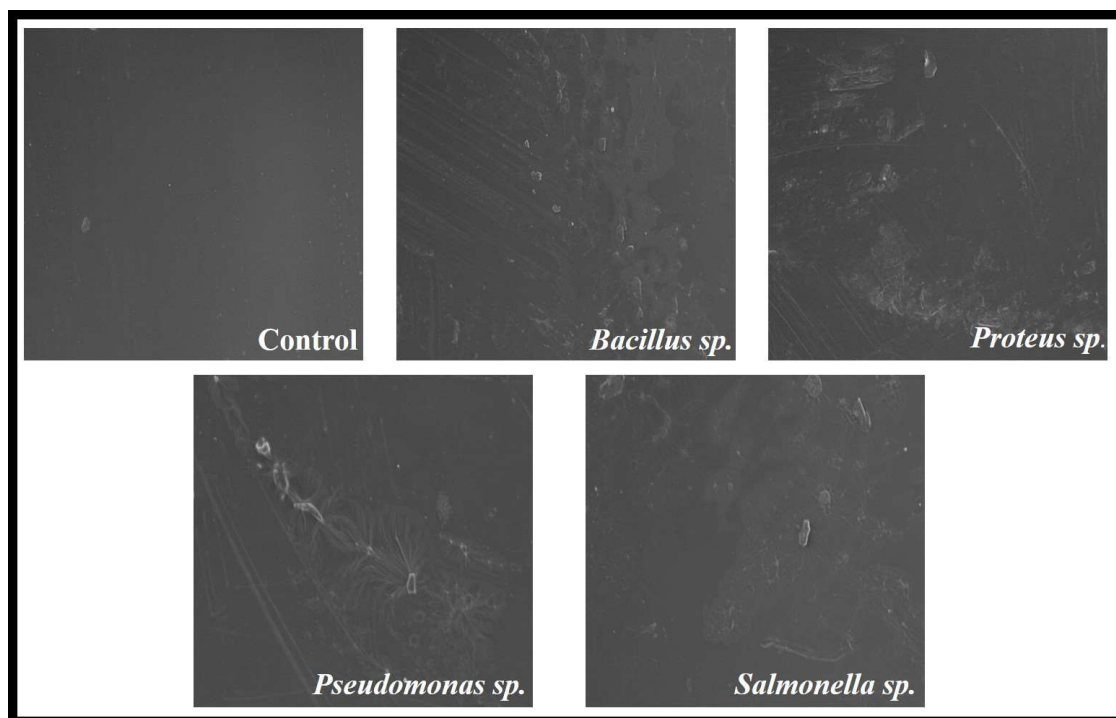


Fig. 2: SEM analysis of LDPE by selected microorganisms.

understanding of the specific bacterial strains that can effectively degrade different types of plastics. This could potentially lead to innovative solutions to tackle the global issue of plastic pollution.

SEM Analysis

The Scanning Electron Microscopy (SEM) analysis plays a significant role in the study of microbial degradation of plastics. It provides high-resolution imaging of the surface morphology, which allows for the observation of microbial attachment, biofilm formation, and structural modifications on the plastic surface. In this study, SEM analysis revealed that the surface of LDPE became rough upon exposure to microorganisms, indicating the action of microbial enzymes on the plastic (Fig. 2). This observation suggests that LDPE can be efficiently digested by microbial enzymes, leading to its degradation. SEM analysis not only facilitates the identification of the microorganisms involved in the degradation process but also enables quantitative analysis of surface features and the evaluation of degradation quality. This is crucial for monitoring time-course studies and understanding degradation kinetics, which can inform the development of biodegradable plastics (Cai et al. 2023, Crystal Thew et al. 2023, Gilani et al. 2023). The findings from this study underscore the importance of SEM analysis in plastic degradation

research. It provides valuable insights into the interactions between microorganisms and plastics and the subsequent changes in the plastic's surface morphology. These insights can guide future research and development efforts in the field of plastic waste management, particularly in the design of biodegradable plastics and the optimization of microbial degradation processes.

CONCLUSION

In conclusion, this study elucidates the diverse enzymatic capabilities of *Bacillus* sp., *Proteus* sp., *Pseudomonas* sp., and *Salmonella* sp. in the degradation of HDPE and LDPE plastics. The observed variations in plastic degradation underscore the significance of considering polymer-specific characteristics in microbial waste management. Additionally, SEM analysis provides crucial visual insights into structural modifications on LDPE surfaces during microbial action, informing our understanding of degradation kinetics. The findings emphasize the need for further research on optimization conditions, potential synergistic effects, and the identification of key enzymes involved in the degradation process. Overall, this study contributes valuable knowledge to the multidisciplinary efforts addressing plastic pollution, highlighting the potential of microbial strategies in sustainable plastic waste management.

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