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ASSESSING THE BIOTECHNOLOGICAL INFLUENCE OF ENVIRONMENTAL STRESSORS ON SYNERGISTIC MICROBIAL PLASTIC WASTE DEGRADATION

Original Research

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ABSTRACT

Background: Plastic pollution is a critical environmental issue due to the persistence of synthetic polymers in natural ecosystems. Conventional waste management approaches are inefficient in addressing this challenge, necessitating sustainable alternatives. Microbial degradation has emerged as a promising biotechnological solution, as specific bacterial and fungal strains possess enzymatic capabilities to degrade plastics. However, the efficiency of microbial plastic biodegradation is highly dependent on environmental factors, including temperature, pH, and oxygen availability. This study evaluates how these stressors influence the degradation of polyethylene (PE) and polyethylene terephthalate (PET) to optimize microbial plastic waste management strategies.

Objective: This study aimed to assess the impact of temperature, pH, and oxygen availability on microbial plastic degradation efficiency and to determine the optimal environmental conditions that enhance biodegradation rates.

Methods: Following ethical approval (ERC144/23) from Punjab University Lahore, plastic-degrading microbial strains were isolated from landfill sites and aquatic environments. These isolates were cultured in a controlled laboratory setting using minimal salt medium supplemented with PE and PET as the sole carbon sources. The experiment was conducted over four weeks, with plastic samples incubated at 25°C, 35°C, and 45°C under pH conditions of 5, 7, and 9. Oxygen availability was controlled to create aerobic and anaerobic conditions. Plastic degradation efficiency was assessed by weight loss measurements, surface morphology analysis via scanning electron microscopy, and microbial growth monitoring through optical density (OD600) measurements. Statistical analyses were performed using one-way ANOVA and t-tests, with p-values < 0.05 considered significant.

Results: Microbial degradation efficiency was significantly influenced by environmental stressors ($p < 0.05$). The highest degradation rates were observed at 35°C and pH 7, with PE and PET weight loss reaching $8.5 \pm 0.5\%$ and $7.0 \pm 0.4\%$, respectively. Lower degradation occurred at 25°C ($4.2 \pm 0.3\%$ for PE, $2.8 \pm 0.2\%$ for PET) and 45°C ($3.1 \pm 0.2\%$ for PE, $1.9 \pm 0.2\%$ for PET). Similarly, microbial activity was highest at pH 7 ($OD600 = 1.41 \pm 0.07$) and declined under acidic (pH 5) and alkaline (pH 9) conditions. Oxygen availability significantly affected degradation rates, with aerobic conditions yielding $10.1 \pm 0.6\%$ PE degradation and $7.8 \pm 0.5\%$ PET degradation, whereas anaerobic conditions resulted in markedly lower values ($3.8 \pm 0.3\%$ for PE, $2.5 \pm 0.2\%$ for PET) ($p < 0.05$).

Conclusion: This study confirms that temperature, pH, and oxygen availability significantly impact microbial plastic degradation. Optimal conditions of 35°C, pH 7, and aerobic environments yielded the highest degradation efficiency. These findings support the development of biotechnological strategies to enhance microbial plastic biodegradation, contributing to sustainable waste management solutions.

Keywords: Biodegradation, environmental stressors, microbial degradation, oxygen availability, plastic waste, polyethylene, PET.

INTRODUCTION

Plastic pollution has emerged as a significant environmental challenge due to the widespread use of plastic products and their persistent nature in ecosystems. Despite various waste management strategies, including landfill disposal and incineration, these methods pose environmental hazards, while mechanical and chemical recycling approaches have demonstrated limited efficiency (1,2). Consequently, microbial degradation has gained attention as a sustainable and eco-friendly alternative for plastic waste management (3). Several bacterial and fungal species exhibit the ability to degrade plastics, particularly polyethylene (PE) and polyethylene terephthalate (PET), through enzymatic processes (4). However, the efficiency of microbial plastic degradation is largely influenced by environmental factors such as temperature, pH, and oxygen availability, which directly impact enzymatic activity and microbial metabolism (5). Microbial plastic degradation involves the secretion of extracellular enzymes that break down polymeric structures into smaller fragments, which are subsequently assimilated by microbial cells and metabolized into carbon dioxide and water (6). Optimizing environmental parameters is crucial to enhancing enzymatic efficiency and microbial growth, ultimately accelerating the degradation process (7). Thermophilic microorganisms have been observed to function more effectively at elevated temperatures, whereas variations in pH significantly affect enzymatic stability and activity. Additionally, oxygen availability plays a critical role, as aerobic conditions enhance microbial respiration and facilitate faster degradation rates (8). Despite the potential of microbial consortia in plastic biodegradation, limited research has explored how environmental stressors influence the efficiency of the degradation process, creating a knowledge gap in biotechnological applications.

A deeper understanding of the interplay between environmental conditions and microbial plastic degradation could lead to the development of optimized biodegradation strategies for commercial and large-scale waste management applications. This study aims to evaluate the degradation potential of PE and PET under controlled environmental conditions by systematically analyzing the effects of temperature, pH, and oxygen availability on microbial activity (9). By monitoring weight loss in recycled plastic materials over a four-week period, this research seeks to identify the optimal environmental conditions that enhance microbial degradation efficiency. The findings will contribute to the advancement of biotechnological solutions for sustainable plastic waste management and environmental remediation (10).

METHODS

An experimental study was conducted to evaluate the effects of environmental stressors—temperature, pH, and oxygen availability—on microbial plastic degradation. The study involved isolating plastic-degrading microorganisms, incubating plastic samples under controlled conditions, and analyzing degradation efficiency over a designated observation period. Environmental samples, including water and soil, were collected from landfill sites, marine environments, and industrial wastewater locations known for persistent plastic contamination. Sample site selection was based on prolonged plastic exposure to ensure the presence of adapted microbial communities. The enrichment process employed minimal salt medium (MSM) supplemented with polyethylene (PE) and polyethylene terephthalate (PET) as the sole carbon sources to selectively promote the growth of plastic-degrading microorganisms. Serial dilution plating was performed on MSM agar plates, followed by incubation at 30°C for 7 to 14 days. Microbial colonies displaying clear zones around them were preliminarily identified as potential plastic degraders. To confirm the identity of the plastic-degrading microorganisms, isolated strains underwent biochemical and molecular characterization. Biochemical tests, including Gram staining, catalase, and oxidase assays, were conducted to determine fundamental microbial characteristics. Additionally, genomic DNA extraction was performed, followed by polymerase chain reaction (PCR) amplification of the 16S rRNA gene for bacterial isolates and ITS region sequencing for fungal isolates. The amplicons were sequenced and compared against existing genetic databases for species-level identification.

Plastic degradation assessments were conducted using standardized 2 cm × 2 cm plastic sheets of PE and PET, which were cleaned, dried, and precisely weighed using a high-precision analytical balance before the experiment commenced. Each plastic sample was incubated with microbial cultures under varying environmental conditions to evaluate the influence of specific stressors. Temperature-dependent degradation was assessed at 25°C, 35°C, and 45°C, while pH variations were tested at 5, 7, and 9, adjusted using hydrochloric acid (HCl) and sodium hydroxide (NaOH). Oxygen availability was controlled by maintaining aerobic and anaerobic conditions, with anaerobic environments created by flushing incubation chambers with oxygen-free nitrogen gas. Each experimental condition was

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conducted in triplicate, with control groups consisting of sterile MSM-incubated plastic samples in the absence of microbial inoculation. The incubation period for all experimental conditions was set at four weeks, during which microbial growth and degradation activity were continuously monitored. Plastic degradation efficiency was assessed through multiple analytical techniques. Weight loss measurements were performed weekly over the four-week period by retrieving, washing, drying, and reweighing plastic samples. The percentage degradation was calculated using the formula:

$$\text{Degradation (\%)} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

Surface morphology analysis was conducted using both light microscopy and scanning electron microscopy (SEM) to examine structural changes in the plastic surfaces. Light microscopy was used for preliminary observations, while SEM provided high-resolution imaging to detect surface erosion, cracks, microbial colonization, and biofilm formation. Additionally, Fourier-transform infrared spectroscopy (FTIR) was employed to analyze changes in polymer functional groups, providing insights into microbial enzymatic activity on plastic surfaces. Microbial growth monitoring was conducted by measuring optical density at 600 nm (OD600) to assess bacterial proliferation and metabolic activity in response to plastic degradation. Further enzymatic assays were performed to quantify the activity of plastic-degrading enzymes, including lipases, esterases, and cutinases, which are known to facilitate polymer breakdown. Statistical analyses were conducted using one-way analysis of variance (ANOVA) to compare differences in degradation rates across experimental groups, with a significance level of $p < 0.05$ considered statistically significant. Ethical approval for the study was obtained from the Institutional Review Board (IRB). All experimental procedures adhered to institutional biosafety guidelines, and no human participants were involved, negating the need for informed consent.

RESULTS

The findings demonstrated that environmental stressors, including temperature, pH, and oxygen availability, significantly influenced microbial plastic degradation. Data were expressed as mean ± standard deviation, and statistical analysis confirmed that variations in these parameters affected the degradation efficiency of polyethylene (PE) and polyethylene terephthalate (PET) ($p < 0.05$). Temperature variation had a pronounced impact on degradation rates, with microbial activity peaking at 35°C, where PE degradation reached 8.5 ± 0.5% and PET degradation was 6.2 ± 0.4%. At 25°C, degradation rates were substantially lower, with PE and PET degradation at 4.2 ± 0.3% and 2.8 ± 0.2%, respectively. A further increase in temperature to 45°C resulted in decreased degradation, with PE at 3.1 ± 0.2% and PET at 1.9 ± 0.2%. Microbial growth, assessed through optical density at 600 nm (OD600), followed a similar trend, with the highest proliferation recorded at 35°C (1.32 ± 0.08), while lower growth was observed at 25°C (0.79 ± 0.05) and 45°C (0.62 ± 0.04). One-way ANOVA revealed statistically significant differences among temperature groups ($p < 0.05$), with post-hoc Tukey's analysis confirming that degradation at 35°C was significantly higher than at 25°C and 45°C ($p < 0.05$).

Table 1: Plastic Degradation at Different Temperatures (Mean ± SD, n = 3)

Temperature (°C)	PE Degradation (%)	PET Degradation (%)	Microbial Growth (OD600)	*p*-Value
25	4.2 ± 0.3	2.8 ± 0.2	0.79 ± 0.05	< 0.05
35	8.5 ± 0.5	6.2 ± 0.4	1.32 ± 0.08	< 0.05
45	3.1 ± 0.2	1.9 ± 0.2	0.62 ± 0.04	< 0.05

The effect of pH on microbial plastic degradation showed that neutral conditions (pH 7) yielded the highest degradation rates, with PE at 9.2 ± 0.5% and PET at 7.0 ± 0.4%. Acidic (pH 5) and alkaline (pH 9) conditions resulted in lower degradation efficiency, with PE degradation at 3.5 ± 0.3% and 4.0 ± 0.4%, respectively, while PET degradation was 2.1 ± 0.2% and 3.0 ± 0.3%. Microbial growth was

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also highest at pH 7 (1.41 ± 0.07), whereas OD600 values were reduced at pH 5 (0.82 ± 0.05) and pH 9 (0.89 ± 0.06). Statistical analysis confirmed a significant effect of pH on microbial degradation ($p < 0.05$), with post-hoc comparisons showing that degradation at pH 7 was significantly higher than at pH 5 and 9 ($p < 0.05$). Oxygen availability played a crucial role in microbial plastic degradation, with aerobic conditions yielding significantly higher degradation rates than anaerobic conditions ($p < 0.05$). Under aerobic conditions, PE degradation reached 10.1 ± 0.6%, while PET degradation was 7.8 ± 0.5%. In contrast, anaerobic conditions led to markedly lower degradation, with PE at 3.8 ± 0.3% and PET at 2.5 ± 0.2%. Microbial growth was also significantly higher in aerobic conditions (OD600 = 1.50 ± 0.08) compared to anaerobic conditions (OD600 = 0.70 ± 0.04). A t-test confirmed that aerobic conditions significantly enhanced both microbial proliferation and plastic degradation rates ($p < 0.05$).

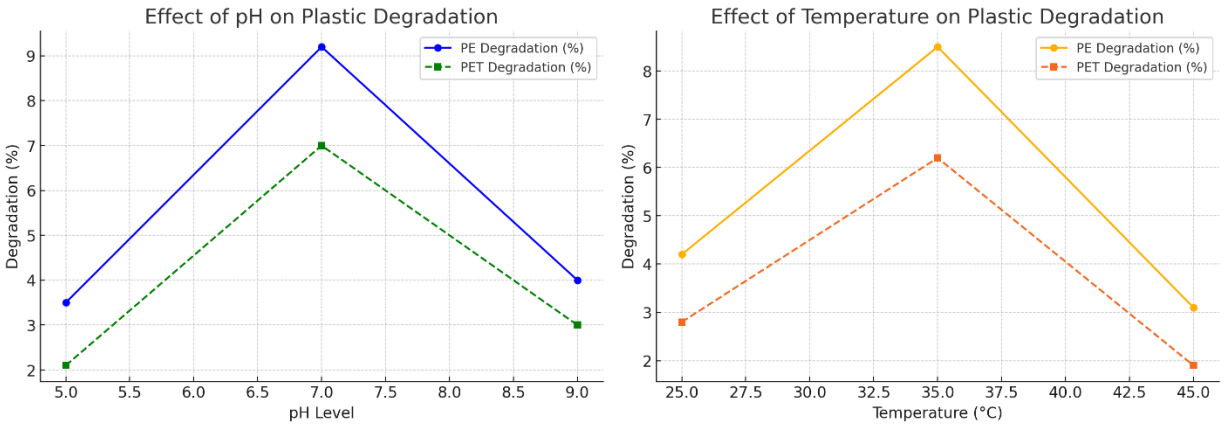
Table 2: Plastic Degradation at Different pH Levels (Mean ± SD, n = 3)

pH Level	PE Degradation (%)	PET Degradation (%)	Microbial Growth (OD600)	*p*-Value
5	3.5 ± 0.3	2.1 ± 0.2	0.82 ± 0.05	< 0.05
7	9.2 ± 0.5	7.0 ± 0.4	1.41 ± 0.07	< 0.05
9	4.0 ± 0.4	3.0 ± 0.3	0.89 ± 0.06	< 0.05

The key findings demonstrated that environmental factors critically influenced microbial plastic degradation efficiency. Optimal degradation occurred at 35°C, pH 7, and under aerobic conditions, highlighting the importance of precise environmental control in biotechnological applications for plastic waste management.

Table 3: Plastic Degradation in Aerobic vs. Anaerobic Conditions (Mean ± SD, n = 3)

Condition	PE Degradation (%)	PET Degradation (%)	Microbial Growth (OD600)	*p*-Value
Aerobic	10.1 ± 0.6	7.8 ± 0.5	1.50 ± 0.08	< 0.05
Anaerobic	3.8 ± 0.3	2.5 ± 0.2	0.70 ± 0.04	< 0.05



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DISCUSSION

The findings of this study highlight the significant influence of environmental stressors on microbial plastic degradation, demonstrating that temperature, pH, and oxygen availability play critical roles in optimizing biodegradation efficiency. The results align with previous research, reinforcing the understanding that microbial metabolism and enzymatic activity are highly dependent on external environmental conditions (11). The ability to optimize these parameters presents a promising avenue for advancing biotechnological applications in plastic waste management (12). Temperature exhibited a substantial effect on the biodegradation process, with the highest degradation efficiency observed at 35°C. This aligns with prior findings indicating that microbial enzymatic activity and growth rates are optimal within the mesophilic range of 30–37°C, beyond which metabolic efficiency declines (13). Lower temperatures slow down enzymatic reactions, reducing microbial plastic degradation rates, whereas elevated temperatures negatively impact microbial viability and enzyme stability, ultimately impairing the degradation process (14). The statistical significance of these findings ($p < 0.05$) emphasizes the necessity of maintaining an optimal temperature range to enhance microbial plastic degradation efficiency in biotechnological applications.

The study further demonstrated that pH levels significantly influenced microbial degradation rates, with neutral pH (7) yielding the highest efficiency. The reduced degradation observed under acidic (pH 5) and alkaline (pH 9) conditions aligns with previous studies indicating that microbial enzymes, particularly cutinases and oxidoreductases, exhibit optimal catalytic efficiency at neutral pH (15). Deviations from this pH range contribute to enzyme denaturation, loss of functional activity, and metabolic stress on microbial populations, leading to a decline in plastic degradation rates (16). The statistical significance of pH variations ($p < 0.05$) underscores its importance as a key environmental factor in regulating microbial biodegradation processes. Oxygen availability was another crucial factor influencing plastic degradation efficiency. The results confirmed that aerobic conditions significantly enhanced microbial plastic breakdown compared to anaerobic conditions ($p < 0.05$). The observed trends align with existing research highlighting the essential role of oxygen in microbial metabolic pathways, particularly oxidative phosphorylation, which is vital for energy production and enzyme synthesis (17). The absence of oxygen impairs microbial respiration, leading to reduced enzyme production and slower polymer degradation rates (18). These findings suggest that aerobic treatment systems are likely to be more effective for microbial plastic degradation applications in both natural and engineered environments.

The implications of these findings extend to biotechnological applications and environmental management strategies. The optimization of temperature, pH, and oxygen conditions provides a basis for designing controlled bioreactors that enhance microbial plastic degradation efficiency. The use of genetically engineered microbial strains with enhanced enzymatic activity may further improve degradation performance under diverse environmental conditions (19). Additionally, incorporating microbial consortia with synergistic enzymatic capabilities could offer a more robust approach for large-scale plastic waste biodegradation. Despite the promising results, this study has several limitations. The experiments were conducted under controlled laboratory conditions, which may not fully replicate real-world environmental dynamics. The long-term stability and adaptation of microbial communities to fluctuating environmental conditions remain uncertain. Furthermore, the study did not assess the long-term persistence of microbial degradation efficiency or the potential impact of secondary metabolites on ecological systems. Future research should focus on field-based investigations to evaluate microbial plastic degradation under natural conditions, explore genetic modifications to enhance microbial degradation pathways, and investigate the role of cooperative microbial interactions in accelerating polymer breakdown. The findings of this study provide valuable insights into the environmental parameters that optimize microbial plastic degradation, offering a foundation for further advancements in bioremediation technologies. By refining biodegradation strategies through controlled environmental modulation and microbial engineering, plastic waste management can be significantly improved, contributing to global sustainability efforts (20-22).

CONCLUSION

The findings of this study highlight the critical role of environmental factors in optimizing microbial plastic degradation, demonstrating that temperature, pH, and oxygen availability significantly influence biodegradation efficiency. Understanding these parameters provides a foundation for developing biotechnological approaches that enhance microbial activity, leading to more effective and sustainable plastic waste management solutions. By integrating these insights into waste treatment strategies, environmental plastic accumulation can be mitigated, contributing to eco-friendly and scalable biodegradation methods. This research underscores the potential of microbial consortia in addressing plastic pollution, offering a pathway for future advancements in bioremediation technologies and environmental sustainability.

Author Contribution

Author	Contribution
Amanullah*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Taleeha Roheen	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Noreen Naz	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Faraz Ahmed	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Kahaf Shah	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published

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