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Microbial Biodegradation of Household Microplastics using Mineral salt Medium

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Abstract

Microplastic (MP) pollution has become a pressing environmental issue, with particles smaller than 5000 μm now pervasive in both aquatic and terrestrial ecosystems. Addressing this challenge, our study investigates microbial biodegradation as a sustainable and eco-friendly solution for MP remediation. Soil samples collected from household waste dump yards in Tirupattur district, Tamil Nadu, India, were used to isolate microorganisms capable of degrading MPs. The MPs were extracted using density separation and introduced into a mineral salt medium (MSM) as the sole carbon source to simulate nutrient-limited conditions. Through biochemical and microscopic analyses, we identified three bacterial isolates—*Pseudomonas aeruginosa, Pseudomonas alcaligenes,* and *Bacillus subtilis*—as potential candidates for MP degradation. Biodegradation experiments revealed that *Pseudomonas aeruginosa* outperformed the others, achieving a degradation efficiency of 40.0 ± 1.1%, followed by *Bacillus subtilis* (30.0 ± 1.0%) and *Pseudomonas alcaligenes* (20.0 ± 1.0%). Growth kinetics, monitored via optical density (OD) at 600 nm, demonstrated that *Pseudomonas aeruginosa* not only degraded MPs effectively but also exhibited the highest growth rate, highlighting its ability to utilize MPs as a carbon source. Scanning electron microscopy (SEM) provided compelling visual evidence of MP surface degradation, with *Pseudomonas aeruginosa* treated samples showing extensive physical changes, including pits, cracks, and biofilm colonization. These findings underscore the remarkable potential of *Pseudomonas aeruginosa* in MP bioremediation, likely due to its robust enzymatic machinery and biofilm-forming capabilities. This study emphasizes the importance of harnessing microbial power to combat MP pollution and offers a promising foundation for future research. By optimizing these biodegradation processes, we can develop innovative, sustainable strategies to mitigate one of the most significant environmental challenges of our time.

Key words : bioremediation, growth kinetics, Mineral salt medium.

Microplastics (MPs), defined as plastic particles smaller than 5000 μm, were first identified in the late 1990s and early 2000s[3]. These tiny particles, composed of carbon and hydrogen polymers, have since become a pervasive environmental concern, infiltrating aquatic ecosystems, the atmosphere, and even remote regions far from human activity[7,9]. MPs are secondary pollutants, formed through the gradual breakdown of larger plastic items, and their sources are as diverse as they are widespread. Industrial processes, such as raw plastic production, textile manufacturing, and laundry, contribute significantly to MP pollution, as do everyday household practices and agricultural activities[4,17]. Alarmingly, MPs have been detected in some of the most remote corners of the planet, from coral islands to the icy waters of the Chukchi Sea, where concentrations can reach up to 17,813 pieces per square kilometer[6,13]. Even personal care products, such as toothpaste and face cleansers, are significant sources, with some toothpaste brands containing up to 96% MPs and face cleansers around 21% [11]. The environmental impact of MPs is profound and multifaceted. They disrupt microbial communities, alter ecosystems, and act as carriers for harmful organic contaminants, posing risks to both environmental and human health[1,19]. As MPs continue to accumulate in our environment, understanding their sources, distribution, and ecological effects has never been more urgent. This study seeks to contribute to this growing body of knowledge by exploring microbial biodegradation as a sustainable solution to mitigate the pervasive threat of MP pollution.

Microplastic collection and isolation of microorganisms :

Soil samples were collected from household waste dump yards in Tirupattur district, Tamil Nadu, India. Microplastics (MPs) were extracted using density separation with a saturated Sodium chloride (NaCl) solution, filtered through a 0.45 μm membrane, and rinsed with distilled water. MPs were added to a mineral salt medium (MSM) containing KH_2PO_4, $(NH_4)_2SO_3$, $MgSO_4 \cdot 7H_2O$, $CoCl_2$, $CaCl_2$, $Fe_2(SO_4)_3$, Na_2HPO_4, $MnSO_4 \cdot 7H_2O$, and $(NH_4)_6Mo_7O_{24}$ as the sole carbon source. The medium was inoculated with soil microorganisms and incubated at 180 rpm for 60 days. Biofilm formation on MPs was observed. Bacterial colonies were isolated using Nutrient Agar (NA), *Bacillus* differentiation agar and Pseudomonas Isolation Agar. Isolates were identified as *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Pseudomonas alcaligenes* through Gram staining, motility tests, and endospore staining.

Biodegradation experiments :

Biodegradation was assessed in 200-mL flasks containing MSM, bacterial suspension, and MPs (25–100 μm, 500 mg L^{-1}). Controls without MPs were included. Flasks were incubated at 25°C and 130 rpm for 30 days. Colony-forming units (CFU) were monitored, and MPs were recovered by vacuum filtration for analysis. MPs were analyzed using scanning electron microscopy (SEM) and X-ray diffraction (XRD). SEM imaging was performed at 10 kV in secondary electron (SE) and backscattered electron (BSE) modes to confirm surface degradation.

Isolation and Identification of Microorganisms :

Three isolates were identified from the mineral salt medium (MSM) and selective media. Based on colonial morphology and biochemical tests, the isolates were identified as: *Pseudomonas aeruginosa* (CC1): Gram-negative, motile, catalase-positive, oxidase-positive, citrate-positive, and nitrate reduction-positive. *Pseudomonas alcaligenes* (CC2): Gram-negative, motile, catalase-positive, oxidase-positive, citrate-positive, and nitrate reduction-negative. *Bacillus subtilis* (CC3): Gram-positive, motile, catalase-positive, Voges-Proskauer-positive, and positive for gelatin, casein, and starch hydrolysis (Table-1).

The selected strains were tested for antagonism to determine if they inhibited each other. For this purpose, a horizontal line was drawn in the centre of a Petri dish with MSM from pure cultures of each of the strains, and equidistant vertical lines were drawn on the horizontal line with the strains to be contrasted. In addition, three negative controls were performed with the strains individually seeded in a horizontal line. Petri dishes were incubated at 30 ºC for five days. Mixed cultures were prepared by incubating pure cultures in LB broth for 48 hours at 30 ºC and 140 rpm. These cultures were centrifuged at 4000 rpm for 10

Table-1. Biochemical identification for the isolated bacteria

Characters studied	CC 1	CC2	CC3
Gram staining	Gram negative	Gram negative	Gram positive
Motility	Motile	Motile	Motile
Catalase	Positive	Positive	Positive
Oxidase	Positive	Positive	Variable
Urease	Negative	Negative	Negative
Indole	Negative	Negative	Negative
Methyl red	Negative	Negative	Negative
Voges proskauer	Negative	Negative	Positive
Citrate utilization test	Positive	Positive	Positive
TSI test	Alkaline slant, Alkaline butt, no gas, No H_2S	Alkaline slant, Alkaline butt, no gas, no H_2 S	Alkaline slant, Acidic butt, no gas, no H_2 S
Nitrate reduction	**Positive**	**Negative**	Positive
Gelatin hydrolysis	**Positive**	**Negative**	Positive
Casein hydrolysis	Negative	Negative	Positive
Starch hydrolysis	Negative	Negative	Positive
Resulting Organism	*Pseudomonas aeruginosa*	*P. alcaligenes*	*Bacillus subtilis*

Formation of mixed cultures

min, washed twice with 10 mL of MSM, and the resulting pellets were resuspended in 5 mL of MSM [10]. The pellets were carefully weighed to determine the wet weight of the biomass, which was then used to calculate the concentration of the inoculum (g/mL). To ensure consistency across all experiments, varying volumes of the mineral salt medium (MSM) were added, allowing each bacterial strain to start at the same concentration. Once prepared, the strains were combined in equal proportions to create mixed cultures, setting the stage for the biodegradation experiments.

Microplastic Biodegradation assay :

To assess the biodegradation potential of the isolated bacteria, 1 mL of either pure or mixed culture was added to Erlenmeyer flasks containing 9 mL of Mineral Salt Medium (MSM). A control flask was prepared with 10 mL of MSM and 0.1 mL of 1% (w/v) sodium azide (NaN_3) to inhibit microbial growth and ensure accurate comparisons[12]. Each flask was then supplemented with 10 mg of microplastics, carefully weighed using an analytical balance. All assays were conducted in replicates and incubated at 30 ºC with continuous shaking at 80 rpm for 60 days to simulate natural environmental conditions.

Determination of Biodegradation Rate :

The biodegradation efficiency of *Pseudomonas aeruginosa, Pseudomonas alcaligenes*, and *Bacillus subtilis* was evaluated by monitoring the weight loss of microplastics (MPs) over the 60-day incubation period. The initial weight of MPs (P_0) was standardized at 100 mg for all experiments. After incubation, the final weight (P) of the MPs was measured, and the percentage of degradation was calculated to quantify the effectiveness of each bacterial strain in breaking down the MPs

Table-2. Biodegradation Percentage of Microplastics by Isolated Bacteria.

Bacteria	Initial weight (P_0, mg)	Final weight (P, mg)	Degradation percentage (%)
Pseudomonas aeruginosa	100	30 ± 5.5	40.0 ± 1.1
Pseudomonas alcaligenes	100	40 ± 4.8	20.0 ± 1.0
Bacillus subtilis	100	35 ± 5.2	30.0 ± 1.0

Among the tested bacterial strains, *Pseudomonas aeruginosa* exhibited as the most efficient in degrading microplastics (MPs), reducing their weight to 35 ± 5.2 mg, which corresponds to a degradation efficiency of 40.0 ± 1.1%. *Bacillus subtilis* showed moderate performance, with the final weight of MPs decreasing to 30 ± 5.5 mg, achieving a degradation rate of 30.0 ± 1.0%. In contrast, *Pseudomonas alcaligenes* exhibited the lowest efficiency, leaving the MPs at 40 ± 4.8 mg, equivalent to a degradation percentage of 20.0±1.0%. These results clearly demonstrate that *Pseudomonas aeruginosa* is the most effective strain for MP degradation under the given experimental conditions, followed by *Bacillus subtilis* and *Pseudomonas alcaligenes*. This highlights the promising

potential of these bacteria, particularly *Pseudomonas aeruginosa*, in addressing the growing challenge of microplastic pollution through bioremediation (Table-2).

Growth Kinetics of microorganisms :

The growth of *Pseudomonas aeruginosa, Pseudomonas alcaligenes*, and *Bacillus subtilis* was monitored over 60 days in a mineral salt medium (MSM) supplemented with MPs as the sole carbon source. Optical density (OD) measurements at 600 nm, recorded at 10-day intervals (Table-3), provided insights into the growth patterns and adaptability of these strains to MP-based nutrient conditions.

Table-3. Optical Density (OD) at 600 nm for *Pseudomonas aeruginosa*, *Pseudomonas alcaligenes*, and *Bacillus subtilis* over 60 days.

OD values at different Time interval (days)	*Pseudomonas aeruginosa*	*Pseudomonas alcaligenes*	*Bacillus subtilis*
0	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
10	0.15 ± 0.02	0.10 ± 0.02	0.12 ± 0.02
20	0.30 ± 0.03	0.20 ± 0.03	0.25 ± 0.03
30	0.55 ± 0.04	0.35 ± 0.04	0.45 ± 0.04
40	0.75 ± 0.05	0.50 ± 0.05	0.60 ± 0.05
50	0.85 ± 0.06	0.60 ± 0.06	0.70 ± 0.06
60	0.90 ± 0.07	0.65 ± 0.07	0.75 ± 0.07

Pseudomonas aeruginosa demonstrated the highest growth rate, achieving an OD of 0.90 ± 0.07 by the 60th day. *Bacillus subtilis s*howed steady growth, reaching an OD of 0.75 ± 0.07 by the 60th day. *Pseudomonas alcaligenes* exhibited slower growth compared to *P. aeruginosa*, with an OD of 0.65 ± 0.07 by the 60th day. The growth curves indicate that all three organisms utilized microplastics as a carbon source, with *Bacillus subtilis* showing the most efficient growth under the experimental conditions (Table-3).

SEM analysis :

Scanning electron microscopy (SEM) was employed to analyze surface changes in microplastics (MPs) before and after biodegradation by *Pseudomonas aeruginosa*. The SEM images revealed significant surface degradation of MPs after 60 days of incubation. Untreated MPs exhibited a smooth and intact surface morphology, with no visible signs of degradation. In contrast, MPs treated with *Pseudomonas aeruginosa* showed extensive surface erosion, including the formation of pits, cracks, due to enzymatic activity and biofilm colonization. Additionally, fragmentation and roughening of the MP surface were observed with structural collapse and deep cavities after 60 days. These morphological changes confirm the ability of *Pseudomonas aeruginosa* to degrade microplastics, as evidenced by the physical alterations observed in the SEM images.

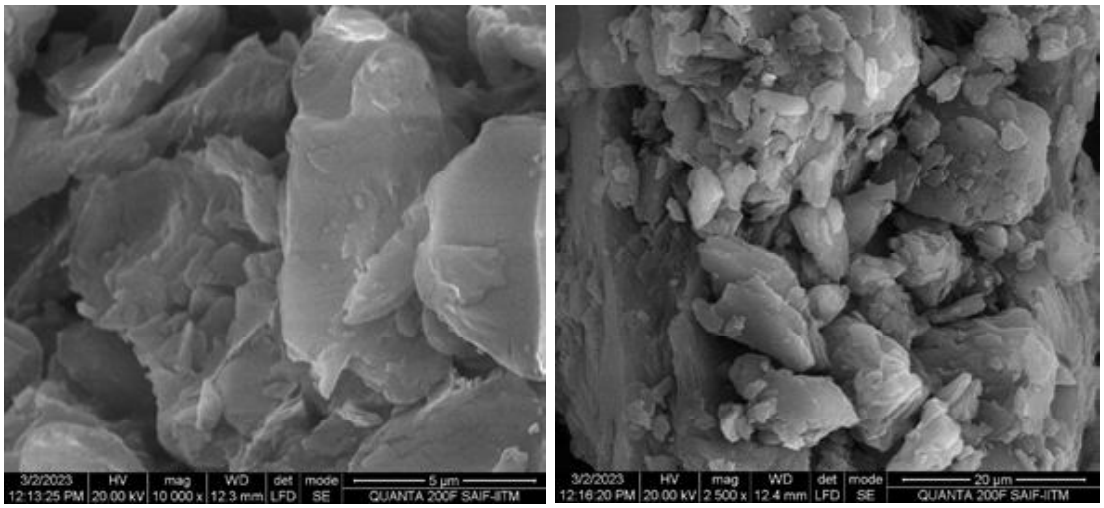

Figure 1. SEM images showing the biodegradation of MPs by *Pseudomonas aeruginosa*

The exceptional biodegradation efficiency of *Pseudomonas aeruginosa* can be attributed to its ability to form biofilms on microplastic (MP) surfaces, as observed in this study. Biofilms enhance microbial attachment and create a microenvironment that facilitates enzymatic degradation of MPs. This aligns with findings by Auta *et al.*[2], who reported that biofilm-forming bacteria, such as *Pseudomonas* spp., significantly enhance polyethylene degradation by producing extracellular enzymes like lipases and esterases. The SEM analysis in this study further supports this, showing surface erosion and fragmentation of MPs due to biofilm activity. The enzymatic machinery of *Pseudomonas aeruginosa* and *Bacillus subtilis* plays a critical role in breaking down MP polymer chains into smaller, metabolizable units. Enzymes such as esterases, lipases, and hydrolases are key to this process, as evidenced by the pits and cracks observed on MP surfaces. In contrast, *Pseudomonas alcaligenes* showed lower efficiency, likely due to its limited enzymatic activity, emphasizing the importance of strain-specific capabilities in MP degradation. Growth kinetics revealed that MPs can serve as a viable carbon source, particularly for *Bacillus subtilis*, which exhibited efficient growth despite moderate degradation efficiency. This suggests a potential trade-off between biomass production and polymer breakdown, as noted in other studies[8,18]. The formation of mixed cultures without antagonism opens possibilities for synergistic biodegradation strategies, combining the biofilm-forming ability of *Pseudomonas aeruginosa* with the carbon utilization efficiency of Bacillus subtilis to enhance degradation rates, as demonstrated by Skariyachan *et al.*[16]. The variability in biodegradation efficiency among strains underscores the need for strain-specific studies. For instance, *Ideonella sakaiensis* uniquely evolved to degrade PET, highlighting the importance of identifying and engineering strains with optimal enzymatic and metabolic traits[21]. These

findings have significant implications for bioremediation strategies, suggesting the potential use of *Pseudomonas aeruginosa* and *Bacillus subtilis* in wastewater treatment or bioreactors for MP removal. However, further research is needed to optimize large-scale applications and assess ecological impacts[5].

This study highlights the potential of *Pseudomonas aeruginosa, Pseudomonas alcaligenes,* and *Bacillus subtilis* in the biodegradation of microplastics. *Pseudomonas aeruginosa* emerged as the most effective strain, demonstrating significant surface degradation and biofilm formation on MPs. The growth kinetics and SEM analyses provided valuable insights into the mechanisms of MP degradation, confirming the ability of these bacteria to utilize MPs as a carbon source. The findings underscore the importance of microbial bioremediation as a sustainable approach to addressing MP pollution. Future research should focus on optimizing biodegradation conditions, exploring mixed-culture synergies, and scaling up these processes for real-world applications. By harnessing the capabilities of these microorganisms, we can develop innovative solutions to mitigate the environmental impact of microplastics and contribute to a cleaner, healthier ecosystem.

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