IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402, p- ISSN: 2319-2399.Volume 18, Issue 12 Ser. 3 (December 2024), PP 12-17
www.iosrjournals.org

Preliminary Study On Biodegradation Of Polyethylene By Pseudomonas Aeruginosa

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Abstract:
Background*: Plastics, particularly polyethylene, have become indispensable due to their versatility and durability, but their persistence poses a significant environmental challenge. Conventional plastic waste management techniques, such as incineration and landfilling, have adverse ecological impacts. Microbial degradation, particularly using Pseudomonas spp., offers an environmentally sustainable solution. Pseudomonas spp. are known for their metabolic versatility, ability to thrive in nutrient-deficient environments, and potential to degrade various plastic polymers. This study aimed to evaluate the biodegradability of polyethylene by Pseudomonas aeruginosa under laboratory conditions.*
Materials and Methods*: Low-density polyethylene (LDPE) films were prepared as circular fragments (18 mm diameter, 20 mg weight) and sterilized. Pseudomonas aeruginosa was cultured in Luria Bertani broth and inoculated into Bushnell Haas (BH) broth supplemented with LDPE as the sole carbon source. The experimental setup included bacterial cultures and control samples without bacterial inoculation. Incubation was carried out at 37°C with constant shaking for 30 days. Biodegradation was assessed by measuring weight loss of LDPE films, bacterial growth (OD600 and CFU/mL.*
Results*: The results demonstrated significant bacterial growth, with an initial adaptation phase followed by active biodegradation and a plateau. LDPE weight loss in the treatment setup reached approximately 6% after 30 days, compared to negligible weight loss in the control. The findings highlighted the ability of Pseudomonas aeruginosa to degrade polyethylene, likely through enzymatic mechanisms.*
Conclusion: *This study confirms the potential of Pseudomonas aeruginosa for polyethylene biodegradation, offering a sustainable alternative to conventional plastic waste management methods. Further optimization of microbial systems is essential for real-world applications in mitigating plastic pollution.*
Key Word*: Plastic waste; Pseudomonas aeruginosa; Biodegradation; LDPE.*

Date of Submission: 17-12-2024 Date of Acceptance: 27-12-2024

I. Introduction

Since the dawn of civilization, humans have sought to create materials with qualities lacking in naturally occurring substances. Among these, plastics stand out as remarkable human-made materials, characterized by their desirable traits such as being lightweight, water-resistant, inexpensive, strong, durable, and flexible[1]. These attributes have made plastics indispensable across diverse sectors, including automotive, packaging, pharmaceuticals, construction, and design. Plastics are often tailored to possess specific properties for specialized applications[2], leading to their widespread use and growing demand over the past 150 years[3]. Global plastic production has surged from 1.5 million tonnes in 1950 to an astonishing 360 million tonnes in 2018[4].

Initially, plastic pollution was only viewed as an aesthetic issue. However, research over the past few years has shed light on the damaging effects of plastic on ecosystems, wildlife, and human health[3]. Many polymers like polyethylene terephthalate (PET), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene (PP) and polystyrene (PS) have been found frequently in water bodies[5]. These plastics accumulate in different environmental compartments, especially water bodies, where their degradation gives meso-, micro-, and nano-plastics—the secondary particles defined by size[6].

The degradation of plastics depends on a combination of intrinsic material properties (e.g., shape, monomer type, molecular weight, crystallinity, hydrophobicity) and external factors, including abiotic conditions (e.g., temperature, moisture, pH, UV radiation, oxygen availability) and biotic influences (e.g., microbial biomass and community composition)[7,8,9]. Plastics degrade in the environment through thermal, mechanical, photochemical, and biochemical mechanisms[10]. Among the mechanisms, photochemical degradation is the most efficient under natural conditions. Thermal oxidation is slow at ambient temperatures but increases with rising temperatures[10]. However, these abiotic processes are often insufficient to address the scale of plastic pollution.

Research efforts are increasingly focusing on developing reliable and sustainable strategies to enhance plastic biodegradation. Material properties, environmental conditions, and microbial communities' interplay needs to be understood for the optimization of degradation processes and mitigation of adverse effects from plastic waste. Microbial degradation is one of the sustainable methods to counter plastic waste through an environmentally friendly approach. Microorganisms utilize their complex enzyme systems to break down plastic polymers into energy source[11]. This biodegradable process provides a more environmentally friendly alternative to conventional ways of managing plastic waste and presents a great opportunity to deal with the global plastic pollution problem.

Species of the genus *Pseudomonas*, are widely distributed across aquatic and terrestrial environments and are known for their application in the bioremediation of naphthalene, toluene, crude oil, hydrocarbons and other hydrophobic polymers[12,13,14]. The high metabolic versatility and genetic adaptability make *Pseudomonas* species highly promising candidates for synthetic biology applications[15]. Among the microorganisms that have been documented as capable of degrading a large number of different types of plastic polymers, *Pseudomonas* species hold a comparatively higher position. The complete biological breakdown of plastic polymers involves an initial depolymerization into smaller oligomers, followed by further degradation into monomers. These monomers are then transported into the cell, where they undergo assimilation and subsequent intracellular metabolism[16,17,18].

Research indicates that several bacterial species, including *Pseudomonas fluorescens* and *P. aeruginosa*, exhibit a strong ability to degrade polyethylene in aquatic environments, particularly under neutral pH (7) and temperatures ranging from 30°C to 37°C. Acidic conditions, however, significantly hinder their degradation activity compared to alkaline environments[19]. A bacterial consortium comprising *Pseudomonas putida*, *Pseudomonas fluorescens*, *Vibrio alginolyticus*, *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Flavobacterium species*, and *Anabaena* species has also been shown to degrade polyethylene effectively under slightly alkaline conditions (pH 7.5) at 26°C[20]. Further investigations by Tamnou et al explored the biodegradation of polyethylene by *Pseudomonas aeruginosa* in an acidic aquatic microcosm, examining various temperatures (7°C to 44°C) and incubation periods (10 to 30 days)[21]. Their findings revealed significant variations in degradation rates, electrical conductivity, pH, and bacterial cell abundance. Maximum degradation, observed as a 6.25% weight loss, occurred at 44°C after 30 days of incubation.

This study investigates the biodegradability of synthetic polyethylene by *Pseudomonas aeruginosa* under laboratory conditions. The extent of biodegradation was assessed by comparing the initial and final dry weights of polyethylene before and after incubation in the respective culture media. *Pseudomonas* spp. were selected for this study based on their ecological prevalence and metabolic versatility. These bacteria are commonly found in nutrient-deficient environments, enabling them to utilize polyethylene incorporated in the nutrient medium as a carbon and energy source once the basal nutrients in the medium are depleted.

II. Material And Methods

Preparation of polyethylene fragments: Low-density polyethylene (LDPE) films with a thickness of 60 µm were prepared as circular fragments, each measuring 18 mm in diameter and weighing 20 mg. The fragments were thoroughly rinsed with autoclaved distilled water, immersed in 70% ethanol, washed again with distilled water, and heat-dried at 40°C using a hot air oven. The dried LDPE samples were then stored in glass desiccators until further use.

Bacterial culture: *Pseudomonas aeruginosa* was procured from the Department of Biotechnology, Barkatullah University, Bhopal, M.P., India. It was selected for its ability to utilize diverse carbon substrates as the sole source of carbon and energy in a simple mineral medium, its adaptability to a wide range of growth temperatures, its ubiquitous nature, and its potential to form biofilms in the environment. Colonies of *P. aeruginosa* were initially observed on King's B medium and subsequently confirmed through conventional biochemical tests[22]. The bacterial cells were cultured in Luria Bertani (LB) broth, comprising sodium chloride (10 g/L), tryptone (10 g/L), and yeast extract (5 g/L), Ph 7.4.

***P. aeruginosa* inoculum preparation:** For inoculum preparation, 10 mL of overnight *P. aeruginosa* culture was harvested by centrifugation at 12,000 rpm for 2 minutes at 4°C. The cells were washed twice with NaCl (0.85%) solution and resuspended in the same, a process repeated twice to remove residual LB medium completely.

LDPE biodegradation experiment: To assess the ability of *P. aeruginosa* to degrade polyethylene (PE), the bacteria was cultured in 250 mL flasks containing 100 mL of BH broth (K_2HPO_4 (1 g/L), KH_2PO_4 (1 g/L), NH_4NO_3 (1 g/L), $CaCl_2$ (0.02 g/L), $MgSO_4$ (0.20 g/L), and $FeCl_3$ (0.05 g/L), pH 7) and LDPE film as carbon source[23].The prepared cells were then inoculated into 100 mL BH broth and incubated at 37°C with constant

shaking at 135 rpm. LDPE film incubated in BH broth without bacterial inoculation served as the negative control under identical conditions. As a blank, untreated LDPE film was incubated without bacterial culture. All experiments were conducted independently in triplicate.

Determination of cell counts during LDPE biodegradation: The absorbance of the culture broth was measured at 600 nm (OD_{600}) every two days to monitor bacterial growth. Additionally, viable cell counts were determined using the colony-counting method. For this, 100 µL of culture broth was spread onto LB agar plates, which were then incubated at 37°C for 24 hours. The viable cell count was expressed as CFU/ml.

Determination of weight loss: Every 10 days, the LDPE film was removed from the cultures and subjected to a sequential washing process: 30 minutes in 2% sodium dodecyl sulfate (SDS) solution, followed by 30 minutes in 70% ethanol solution, and finally 30 minutes in distilled water to remove bacterial biomass. The film was then dried at 60°C for 1 hour and weighed. The weight loss of the LDPE film was calculated using Eq:
Weight loss (%) = (initial weight -final weight)/(initial weight) ×100

III. Result

In this study, *Pseudomonas aeruginosa* was selected as a potential candidate for the degradation of polyethylene (PE). The bacterium was cultured in a BH medium supplemented with PE film fragments and incubated at 37 °C for 30 days as part of degradation assessment. Notably, significant bacterial growth was observed, as indicated by the absorbance measurement at 600 nm after the incubation period (Fig.1).

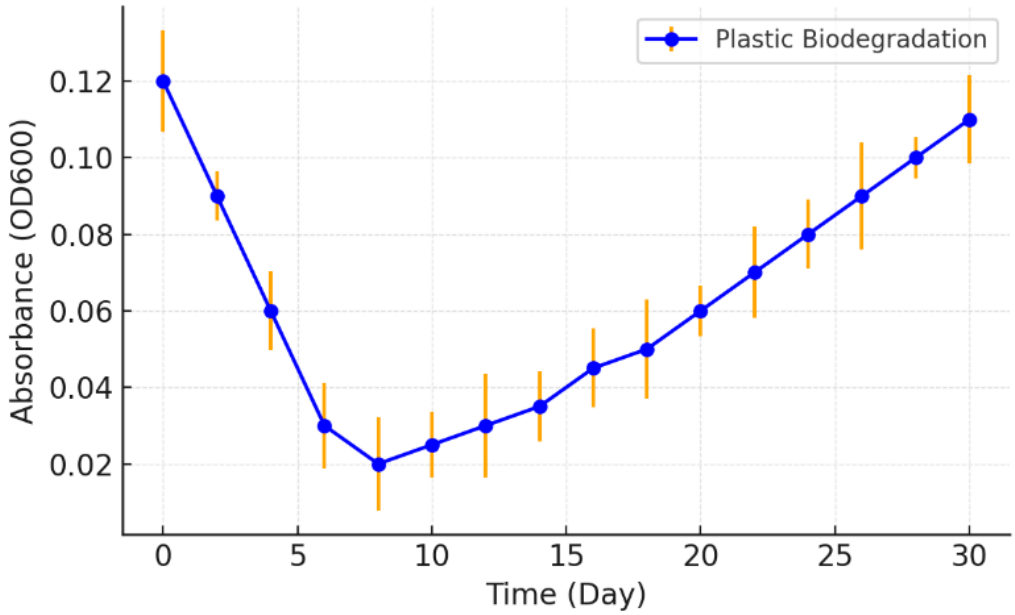

Fig.1 Growth profiles of *Pseudomonas aeruginosa* in a culture medium supplemented with PE as a sole carbon source.

The graph illustrates the biodegradation of plastic by Pseudomonas aeruginosa over 30 days, showing distinct phases of bacterial activity. Initially, there is a sharp decline in absorbance (OD600) from 0.12 to 0.03 within the first 4 days, representing a lag phase where bacteria adapt to the plastic environment, likely metabolizing surface additives or easily degradable compounds. Between Days 4 and 8, absorbance reaches its lowest point, indicating the depletion of readily available nutrients and the onset of more challenging polymer degradation. From Days 8 to 18, absorbance gradually increases as the bacteria adapt and actively degrade plastic, likely facilitated by the release of plastic-degrading enzymes, leading to an increase in bacterial biomass. Beyond Day 18, the absorbance stabilizes, suggesting a stationary phase where bacterial growth balances with nutrient availability, and degradation slows due to the remaining recalcitrant plastic polymers. The variability indicated by error bars, particularly during the active and stationary phases, reflects differences in degradation rates and environmental conditions. Overall, the results highlight the *P. aeruginosa* potential for plastic degradation but also reveal the challenges posed by persistent plastic polymers.

The rate of change in cell abundance generally increased with rising incubation temperatures. The graph demonstrates the bacterial colony-forming units (CFU/ml) in log scale over the same period as plastic biodegradation (Fig.2).

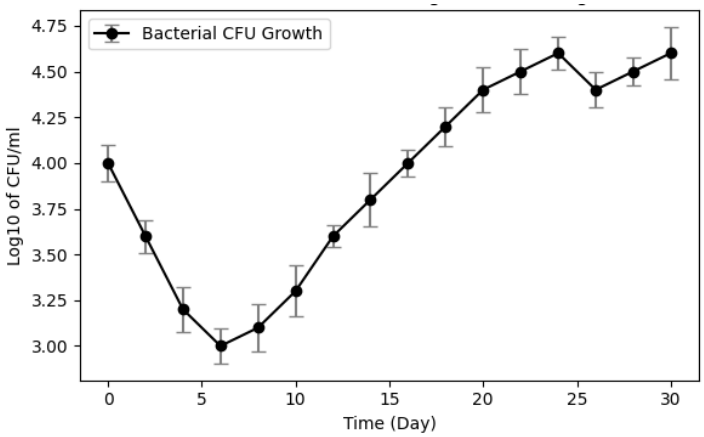

Fig.2 Bacterial growth as CFU/ml during plastic biodegradation

Initially, there is a decline in bacterial population from Log10 4.0 to 3.0 within the first 4 days, corresponding to the lag phase observed in the absorbance graph. This decline likely indicates metabolic adaptation to the plastic environment. Between Days 4 and 8, the bacterial population stabilizes and begins to recover, indicating the onset of plastic degradation and bacterial proliferation. From Days 8 to 18, there is a steady increase in CFU/ml, correlating with the active phase of degradation, where bacterial enzymes efficiently hydrolyse plastic polymers, supporting growth. Beyond Day 18, the CFU/ml levels plateau, suggesting a stationary phase where bacterial growth is balanced by nutrient limitations or the accumulation of metabolic byproducts. The slight decline around Day 24 may indicate nutrient depletion or stress, followed by a minor recovery. Error bars reflect variability, likely due to heterogeneity in growth conditions or metabolic activity. This graph complements the absorbance data by confirming bacterial growth dynamics during plastic biodegradation.

The analysis of plastic degradation by *Pseudomonas* was conducted by monitoring the percentage weight loss over a 30-day incubation period (Fig.3).

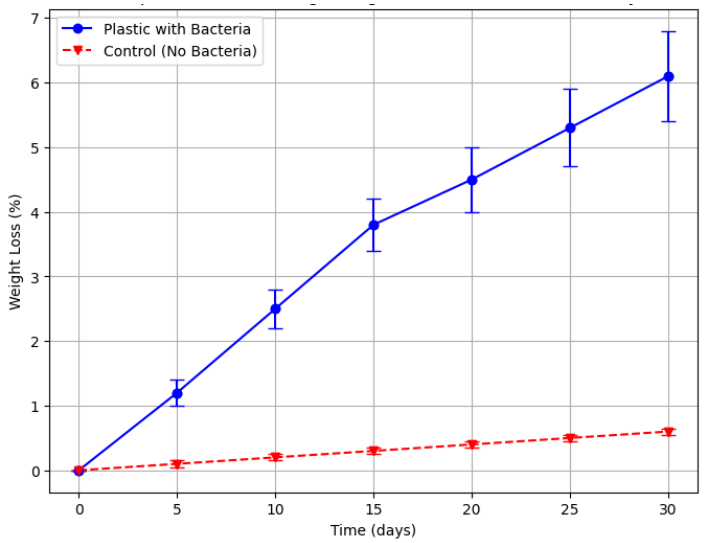

Fig.3 Percentage weight loss of PE by *P. aeruginosa* over 30 days of incubation.

The results, depicted in the graph, clearly illustrate the significant role of bacterial activity in promoting plastic degradation compared to control setup. The plastic incubated with *P. aeruginosa* displayed a progressive increase in weight loss, starting from negligible levels at Day 0 and reaching approximately 6% by Day 30. This steady trend highlights the biodegradation potential of *P. aeruginosa*, which likely involves enzymatic mechanisms capable of breaking down complex polymer chains. In stark contrast, the control condition, consisting of plastic without bacterial inoculation, exhibited minimal weight loss, remaining below 0.6% throughout the experimental period. This marginal reduction in the control can be attributed to abiotic factors such as environmental exposure or leaching of additives, which are insignificant compared to the microbial activity observed in the treatment setup.

Error bars on the graph indicate moderate variability in the bacterial treatment, which may result from fluctuations in bacterial metabolism, nutrient availability, or experimental conditions across replicates. Meanwhile, the control condition demonstrates consistent and minimal variability, reflecting the absence of significant biological processes. The stark divergence between the bacterial treatment and control conditions underscores the dominant role of *P. aeruginosa* in plastic degradation. The widening gap over time between the two conditions confirms that biodegradation, rather than abiotic mechanisms, is the primary driver of weight loss. These findings highlight the enzymatic activity of *P. aeruginosa* as a critical factor in breaking down plastic polymers, offering valuable insights into its application for bioremediation.

IV. Discussion

Pseudomonas species, widely recognized for their ability to degrade oil contaminants, also possess significant potential for breaking down plastic waste. The present study highlights the efficacy of *Pseudomonas aeruginosa* in degrading polyethylene (PE) under controlled laboratory conditions. The ability of this bacterium to utilize PE as a sole carbon source aligns with prior findings, showcasing the versatility of Pseudomonas species in addressing plastic pollution challenges through microbial biodegradation.

When plastic is supplied as the sole carbon source, bacteria have to obtain energy from plastic biodegradative byproducts to generate cellular components and maintain cellular activity and reproduction via cell division[14,24]. Fragments produced from PE depolymerization could be easily converted to fatty acids via enzyme-mediated oxidation steps, and then subsequently converted to acetyl-CoA for energy production via beta-oxidation and the TCA cycle[25]. The supply of sufficient energy and other nutrients resulting from rapid PE depolymerization allowed steep population increases of *P. aeruginosa*. A previous study showed that pyrolysis of PE by *P. aeruginosa* PAO-1 can produce polyhydroxylalkanoate (PHA)[26]. Thus, PE biodegradation is an effective process by which *P. aeruginosa* can obtain energy under nutrient-limiting conditions. The efficiency of plastic biodegradation is highly determined by the amount of expressed enzyme and the catalytic ability of the secreted enzymes[27,28] and through hydrolysis can change the surface property from hydrophobic to hydrophilic, thus reducing the plastic's mechanical strength[29,16,30]. Recently, several enzymes that participate in plastic biodegradation have been reported, including serine hydrolase (SH) secreted from *P. aeruginosa*[31,32,33]. Thus, the more efficient biodegradation of PE might be because depolymerases secreted from *P. aeruginosa* can mediate the depolymerization of PE better than the other kinds of plastics due to the strong binding affinity of the enzyme for PE, or because certain specific PE-degrading enzymes are more highly expressed than others[17,34].

Several studies have reported on the biodegradation potential of *Pseudomonas aeruginosa* across various polymer types. Gupta and Devi demonstrated that *Pseudomonas aeruginosa* strain ISJ14 not only adhered effectively to polyethylene surfaces but also induced significant morphological changes, including fissures and surface erosion, after 60 days of incubation in minimal salt media[35]. Similarly, Lee et al highlighted the remarkable capacity of *P. aeruginosa* to depolymerize polyethylene more effectively compared to other plastics, such as polystyrene and polypropylene, corroborating the high metabolic adaptability of this strain. The findings underscore the potential for scaling microbial approaches to mitigate plastic waste in aquatic and terrestrial environments[36].

V. Conclusion

The degradation of PE by *Pseudomonas aeruginosa* represents a sustainable alternative to conventional plastic waste management methods, such as incineration and landfilling, which pose environmental and health hazards. This study supports the feasibility of using microbial systems to address plastic pollution, emphasizing the need for further research to optimize these processes for real-world applications.

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