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Morphological Characterization of Polyethylene Biodegradation by Soil-Derived Bacterial Isolate F3 using SEM and FESEM Analysis

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ABSTRACT: Plastic pollution represents a serious environmental concern because synthetic polymers persist for long periods and accumulate in natural ecosystems. Microbial degradation has emerged as a sustainable and environmentally friendly strategy for managing plastic waste, as microorganisms can metabolize polymeric materials through enzymatic activity. In the present investigation, soil-derived microorganisms capable of degrading polyethylene were isolated from plastic-contaminated sites and enriched using polyethylene as the primary carbon source. The biodegradation process was evaluated under controlled laboratory conditions over time using time-dependent weight-loss measurements.

To obtain direct evidence of degradation, surface morphology of polyethylene films before and after microbial exposure was examined using scanning electron microscopy (SEM) and field emission scanning electron microscopy (FESEM). Microscopic observations revealed microbial attachment and biofilm development on polymer surfaces, while treated samples exhibited clear structural damage, including cracks, pores, cavities, and surface erosion. These morphological alterations, together with quantitative weight-loss results, confirm active microbial degradation. Among the isolates evaluated, strain F3 exhibited the highest biodegradation efficiency, with a maximum polyethylene weight reduction of approximately 3.84% after 60 days of incubation. These findings highlight the potential of soil-derived microorganisms for environmentally sustainable plastic waste management.

1. INTRODUCTION

The rapid increase in plastic production and consumption has created a major environmental concern because synthetic polymers persist for prolonged periods in natural ecosystems (1). Plastics are widely used due to their durability, low density, and resistance to chemical and biological degradation; however, these same properties contribute to their environmental persistence after disposal (2). Among synthetic polymers, polyethylene is one of the most extensively produced materials worldwide and is frequently detected in terrestrial and aquatic environments where it accumulates for long periods (3).

Conventional plastic waste management methods such as landfilling, recycling, and incineration present several limitations. Landfills require extensive land area and may lead to soil contamination, while incineration can release harmful gases and toxic residues that affect environmental and human health (4). As a result, research attention has increasingly shifted toward

sustainable alternatives for plastic waste treatment. Biological degradation using microorganisms has emerged as a promising strategy because certain microbes can colonize polymer surfaces and gradually degrade them through enzymatic activity (5).

Microbial degradation of polymers generally begins with microbial attachment to the surface, followed by biofilm formation that enhances enzyme–substrate interaction (6). During degradation, structural changes such as cracks, pits, cavities, and increased surface roughness can appear, indicating progressive deterioration of the polymer matrix (7). Advanced imaging techniques such as scanning electron microscopy (SEM) and field emission scanning electron microscopy (FESEM) are widely used to visualize these microstructural modifications and evaluate degradation patterns at high resolution (8).

Although many investigations have examined biodegradable plastics, comparatively fewer studies focus on highly resistant polymers such as polyethylene. Because of its hydrophobic surface and chemically stable carbon backbone, polyethylene limits microbial adhesion and slows degradation under natural conditions (9). Therefore, detailed investigation of microbial interactions with polyethylene surfaces is necessary to better understand degradation mechanisms and to identify efficient plastic-degrading microorganisms.

The present study was designed to isolate soil microorganisms capable of degrading polyethylene and to evaluate their degradation potential using weight-loss analysis together with morphological characterization by SEM and FESEM, with particular emphasis on the high-performing isolate F3.

2. MATERIALS AND METHODS

2.1 Sample Collection and Enrichment

Soil samples were collected from plastic-contaminated dumping sites for the isolation of microorganisms capable of utilizing polyethylene as a carbon source. Samples were collected in sterile containers, transported to the laboratory, and stored at 4 °C until further processing. For enrichment, aliquots of each sample were inoculated into mineral salt medium supplemented with polyethylene as the sole carbon substrate to selectively enrich plastic-degrading microorganisms. The enrichment cultures were maintained under controlled laboratory conditions to promote microbial adaptation and colonization of the polymer surface. Mineral salt medium consisted of (g/L): KH_2PO_4 (1.0), K_2HPO_4 (1.0), $MgSO_4$ (0.2), NH_4NO_3 (1.0), NaCl (0.5), and trace elements.

2.2 Isolation of Microbial Isolates

Following enrichment, culture suspensions were serially diluted and spread onto nutrient agar plates. After incubation, colonies showing distinct morphological characteristics were selected and purified by repeated streaking to obtain pure cultures. The isolates were preserved for further analysis.

2.3 Screening for Polyethylene-Degrading Isolates

Purified isolates were screened for their polyethylene degradation ability by inoculating them into mineral salt medium containing polyethylene films as the sole carbon source. Low-density polyethylene (LDPE) films were used as the test substrate. Incubation was carried out at 37 °C with agitation at 120 rpm for 60 days to promote microbial growth and interaction with the polymer surface.

2.4 Polyethylene Biodegradation Assay

Degradation efficiency was determined by measuring the weight reduction of polyethylene films after incubation. After the experimental period, films were retrieved, washed thoroughly to remove attached biomass, dried to constant weight, and weighed. Percentage weight loss was calculated using Equation (1):

$$\text{Weight loss (\%)} = (Wi - Wf) / Wi \times 100 \quad (1)$$

Where:

Wi = initial weight of polyethylene film

Wf = final weight after incubation

2.5 Microscopic Analysis (SEM and FESEM)

Surface morphology of polyethylene films before and after microbial treatment was analyzed using scanning electron microscopy (SEM) and field emission scanning electron microscopy (FESEM). Samples were mounted on stubs, coated with a thin conductive layer, and examined under appropriate magnifications to observe structural changes. Microscopic analysis was carried out at CSIR–National Chemical Laboratory (NCL), Pune.

3. RESULTS AND DISCUSSION

3.1 Quantitative Assessment of Polyethylene Degradation

Changes in polyethylene mass were monitored to evaluate the degradative performance of the isolated microorganisms. Films incubated with bacterial cultures exhibited a progressive reduction in weight over the experimental period, whereas control samples showed negligible change. The absence of measurable weight loss in control samples confirms that the observed reduction in treated samples resulted from biological activity rather than handling, evaporation, or abiotic factors.

Among the isolates evaluated, strain F3 demonstrated the highest degradation efficiency, exhibiting a maximum polyethylene weight reduction of approximately 3.84% after 60 days of incubation, followed by B13 and F2. The gradual decline in polymer weight indicates continuous microbial metabolism and suggests effective utilization of polyethylene as a carbon source. Similar biodegradation trends have been reported in previous studies (14, 15).

These findings provide quantitative evidence supporting the biodegradation potential of the isolates. The superior performance of strain F3 suggests enhanced enzymatic activity and metabolic capability for polyethylene degradation, making it a promising candidate for further investigation.

3.2 Gram Staining and Morphological Characterization of F3 Isolate

Gram staining analysis was performed to determine the cellular characteristics of isolate F3, identified as the most efficient polyethylene-degrading strain. Microscopic observation revealed that the cells were Gram-positive, appearing purple under light microscopy, and exhibited a rod-shaped morphology. The cells were observed both individually and in clustered arrangements. The Gram-positive nature and bacillus-like morphology of isolate F3 are consistent with characteristics commonly reported for soil microorganisms involved in polymer degradation (9,14). These structural features are known to facilitate surface attachment and biofilm formation, which play a critical role in polyethylene biodegradation.

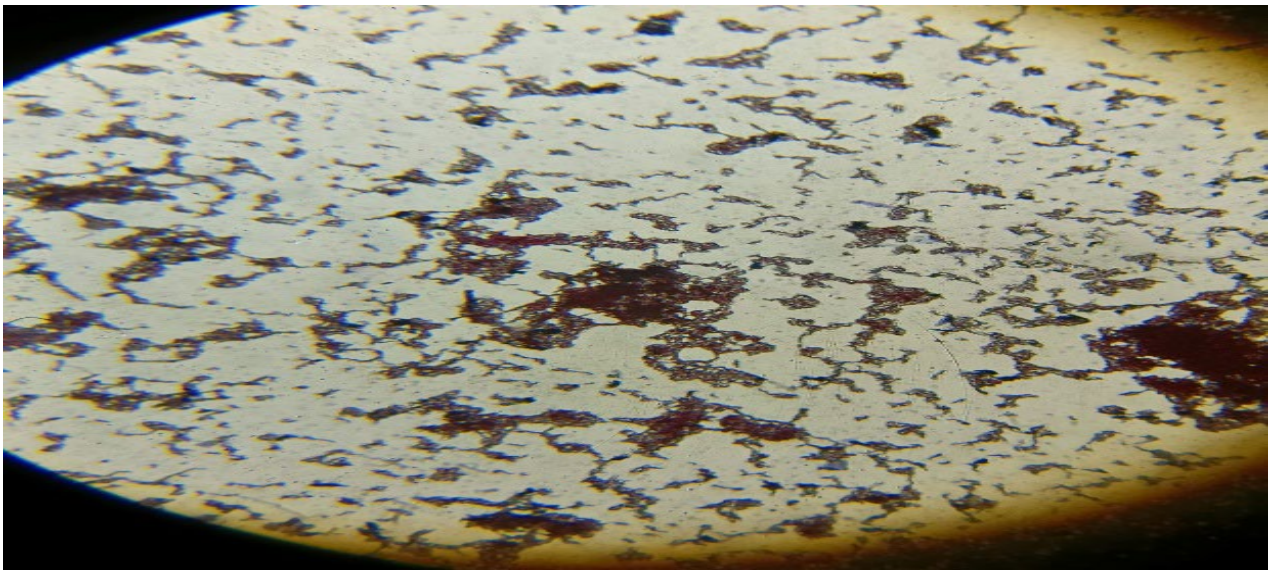


Figure 1. Gram staining of isolate F3 showing Gram-positive rod-shaped bacterial cells under oil immersion (1000× magnification).

3.3 SEM Analysis of F3 Isolate

Scanning electron microscopy (SEM) was employed to examine the cellular morphology and surface characteristics of isolate F3. The SEM micrographs revealed a predominantly rod-shaped structure, which is characteristic of many polymer-degrading soil bacteria.

At higher magnification (20,000×), well-defined rod-shaped cells with intact morphology and smooth to slightly textured surfaces were observed (Figure 2a). At intermediate magnification (10,000×), the cells formed dense clusters as well as individual arrangements (Figure 2b), indicating strong cell-to-cell interactions. At lower magnification (5,000×), uniform distribution of cells was observed (Figure 2c), suggesting effective surface colonization. These observations confirm the structural integrity and active colonization ability of isolate F3, supporting its significant role in polyethylene biodegradation.

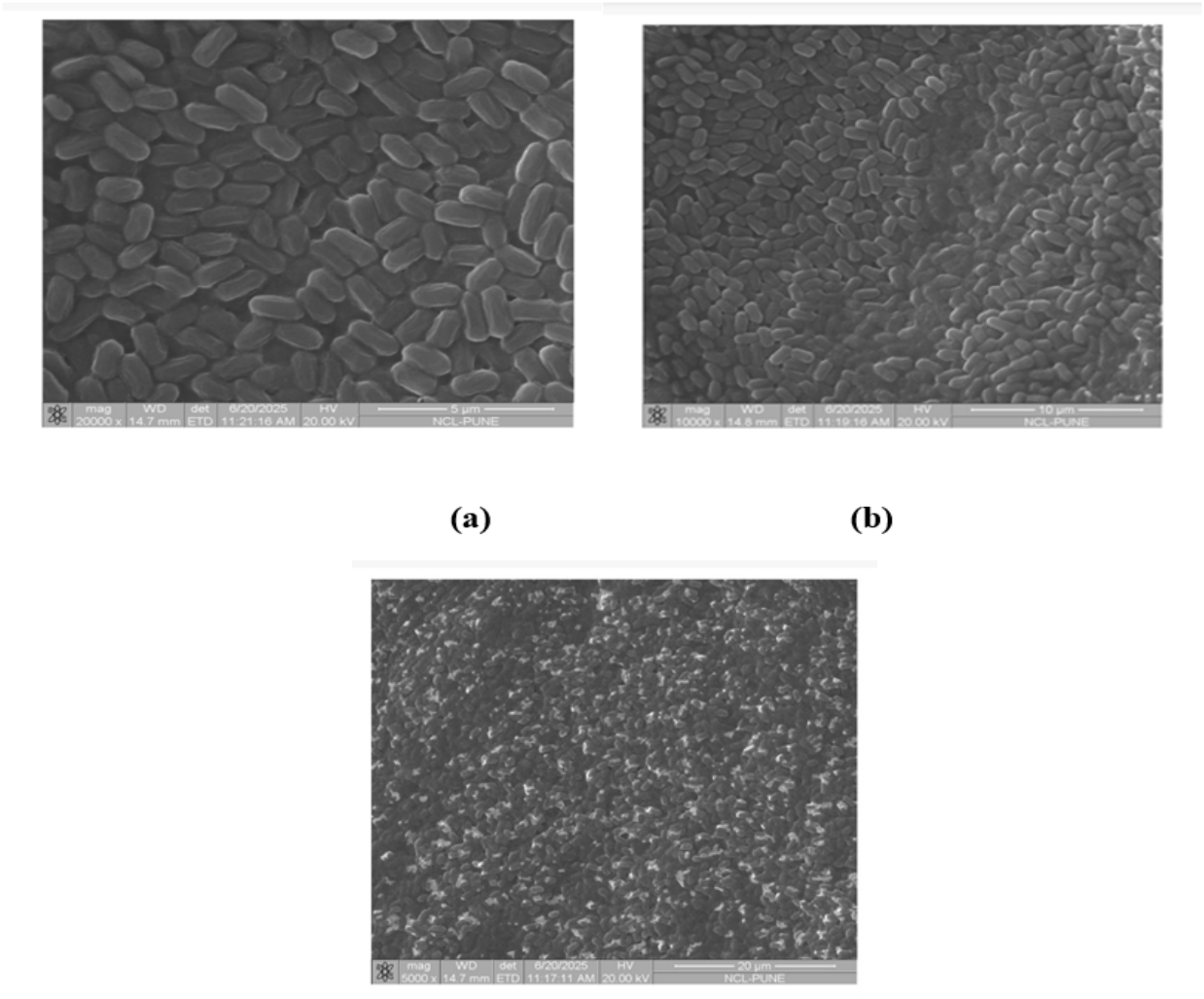


Figure 2. SEM analysis of isolate F3 showing cellular morphology and surface characteristics.

(a) SEM micrograph at 20,000× magnification (scale bar: 5 µm) showing rod-shaped cells.

(b) SEM image at 10,000× magnification (scale bar: 10 µm) showing cellular aggregation.

(c) SEM micrograph at 5,000× magnification (scale bar: 20 µm) showing surface distribution of cells.

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3.4 SEM Analysis of Polyethylene Film after Microbial Treatment

Scanning electron microscopy was used to evaluate structural modifications on polyethylene films following incubation with isolate F3. Compared with untreated control samples, the treated films exhibited significant surface irregularities, indicating biologically mediated degradation of the polymer.

The treated polyethylene films showed clear signs of erosion, cracks, pits, cavities, and increased surface roughness. The presence of depressions and openings of varying sizes suggests localized disruption of the polymer matrix, most likely caused by enzymatic activity associated with microbial biofilm formation. These observations are consistent with previously reported studies on polyethylene biodegradation (7, 14).

Furthermore, the loss of surface smoothness and the appearance of fractured regions confirm progressive structural deterioration of the polymer. These features were absent in control samples, confirming that the observed changes were due to microbial activity rather than abiotic factors.

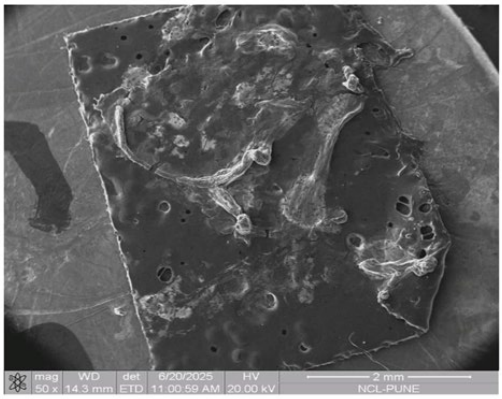
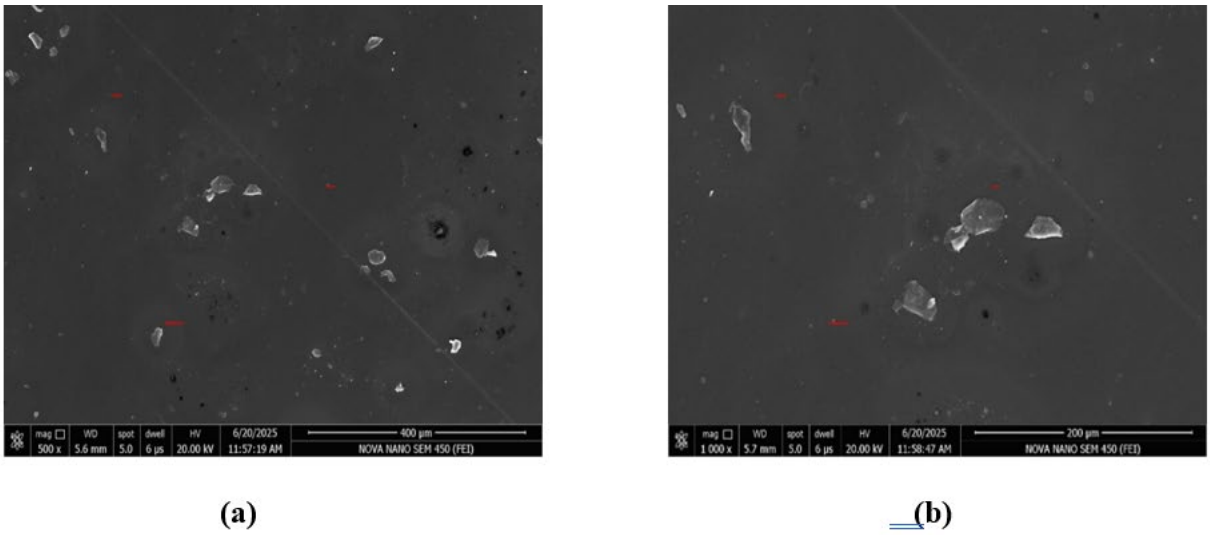


Figure 3. SEM micrograph of polyethylene film after treatment with isolate F3 showing surface erosion, cracks, pits, and cavities indicating microbial degradation.

3.5 FESEM Analysis of Polyethylene Film after Microbial Treatment



(a) **(b)**

Figure 4. FESEM micrographs of polyethylene film after treatment with isolate F3 showing structural degradation.

(a) 500× magnification (scale bar: 400 µm) showing surface roughness and cracks.

(b) 1000× magnification (scale bar: 200 µm) showing microcracks, pits, and cavities.

4. OVERALL INTERPRETATION

Overall, observations obtained from FESEM analysis provide convincing evidence that isolate F3 actively contributes to polyethylene degradation. The observed morphological changes are consistent with the quantitative weight-loss results, which showed a maximum polyethylene degradation of approximately 3.84% after 50 days of incubation.

5. CONCLUSION

The present study demonstrates the biodegradation potential of polyethylene by soil-derived microorganisms through a combination of quantitative weight-loss analysis and detailed morphological characterization. A consistent reduction in polyethylene weight in treated samples, compared to negligible change in controls, confirms the active role of microbial processes in polymer degradation.

SEM and FESEM analyses revealed significant surface deterioration of polyethylene films, including cracks, pits, cavities, and erosion, providing direct visual evidence of microbial-mediated degradation. These structural modifications indicate progressive weakening of the polymer matrix during microbial activity.

Among the isolates, strain F3 exhibited the highest degradation efficiency, achieving a maximum polyethylene weight reduction of approximately 3.84% after 60 days of incubation, supported by both quantitative and microscopic observations. Its Gram-positive, rod-shaped morphology and ability to form biofilms suggest enhanced surface colonization and enzymatic activity, contributing to effective polyethylene degradation.

Overall, the findings highlight the potential of soil-derived microorganisms, particularly isolate F3, for application in environmentally sustainable plastic waste management. Further studies focusing on molecular identification, enzymatic mechanisms, and optimization of degradation conditions may enhance its practical applicability.

6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

7. FUNDING

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