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Polyethylene Degradation Under Controlled Conditions by *Aspergillus* sp. Isolated from a Landfill

Marilín Sánchez-Purihuamán ^{1*}, Ada P. Barturén Quispe ², Segundo A. Vásquez Llanos ², Junior Caro-Castro ³, Lizbeth M. Córdova-Rojas ⁴, Carlos Villanueva Aguilar ⁵, Raúl Siche ⁶, Carmen Carreño-Farfán ^{1, 5}

¹ Academic Department of Microbiology and Parasitology, BlyME: BS-CA, National University Pedro Ruiz Gallo, Lambayeque, 14013, Peru.

² Academic Department of Processes and Unit Operations, CIMAYDS, National University Pedro Ruiz Gallo, Lambayeque, 14013, Peru.

³ Faculty of Biological Sciences, National University of San Marcos, Lima, 15081, Peru.

⁴ National Intercultural University Fabiola Salazar Leguía of Bagua, Bagua, 01721, Peru.

⁵ Faculty of Biological Sciences, National University Pedro Ruiz Gallo, Lambayeque, Peru.

⁶ School of Agroindustrial Engineering, Faculty of Agricultural Sciences, National University of Trujillo, Trujillo 13011, Peru.

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Abstract

Petroleum-derived plastics such as polyethylene are the most widely used polymers globally. However, once used, they are often not properly segregated or disposed of. Because they resist biodegradation, they cause persistent environmental contamination. This study aimed to analyze the degradation of low-density polyethylene (LDPE) by filamentous fungi isolated from a solid waste landfill. To achieve this goal, we isolated and characterized LDPE-degrading filamentous fungi via phenotypic and genotypic methods. We also determined their degradation potential on the basis of the physical, mechanical, and chemical characteristics of the polymer after 150 days of incubation with the fungi. We isolated a total of 77 fungal strains, 46.75% of which demonstrated LDPE degradation capabilities. The weight loss of the polymer after treatment with the degrading fungi ranged from 4.44% to 20.21%, with a reduction rate of 0.000307 to 0.001504 grams per day and a residual half-life of 450.06 to 2257.65 days. Furthermore, 27.78% of the fungi reduced the tensile strength of the LDPE fragments by 5.61% to 41.72%. Compared with the control, the *Aspergillus* sp. 40 CIFOS strain presented the most significant physical and chemical changes in the degraded polymer. In conclusion, fungi isolated from solid waste landfills have a high capacity for LDPE degradation. These results, obtained under laboratory conditions, encourage future studies focused on the application of biotechnological processes and the identification of enzymes involved in the degradation process to optimize the bioremediation of contaminated environments.

Keywords: Biodegradation; Filamentous Fungi; LDPE; Colonization; *Aspergillus* sp.

1. Introduction

Petroleum-derived plastics are low-cost polymers and, as a result, are among the most widely used materials globally. However, after human use, they are mostly discarded and improperly segregated. Their resistance to biodegradation makes them persistent pollutants that contaminate soil [1], water [2], and air [3].

* Corresponding author: msanchezpu@unprg.edu.pe

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Between 1950 and 2017, an estimated 9.2 billion metric tons (Mt) of plastic were produced. Of the discarded plastic, 32% ended up in various ecosystems, 40% in landfills, 14% was incinerated, and 14% recycled. Furthermore, only 2% of these waste plastics are optimally recycled [4].

The plastics with the highest production rates are polypropylene (PP, 16%), low-density polyethylene (LDPE, 12%), polyvinyl chloride (PVC, 11%), high-density polyethylene (HDPE, 10%), and polyethylene terephthalate (PET, 5%) [5]. LDPE is one of the most frequently used plastics, particularly in the production of single-use bags. It is a thermoplastic made of ethylene monomers and lacks functional groups recognizable by microbial enzymatic systems [6]. Its relatively high degree of crystallinity (40–60%) increases its environmental persistence and resistance to degradation, whereas its inherent hydrophobicity limits water interactions, thereby reducing microbial access [7]. In the U.S., an estimated 1 million of these plastic bags are used every minute [4], and they can persist in the environment for more than 100 years [8].

Upon exposure to environmental factors, plastics undergo fragmentation into smaller particles known as microplastics, ranging from large (1–5 mm) to small (0.001–1 mm), and even into nanoplastics (<1 µm). These tiny fragments can act as magnets for a host of harmful substances, including phthalates, bisphenols, pesticides, organohalogens, polycyclic aromatic hydrocarbons, and dioxins [9]. They can also pick up other chemical pollutants, heavy metals, and pathogenic microorganisms [10]. Once ingested, these contaminated microplastics enter the food chain, ultimately making their way into humans [11]. The small size of microplastics makes them easy for animals to swallow. They can become lodged in the gastrointestinal tract, potentially causing perforation or obstruction and often leading to death [12]. Humans are exposed to microplastics primarily through inhalation, ingestion, and skin contact. Estimates suggest an annual intake of 39,000 to 52,000 microplastics per person, with absorption ranging from 74,000 to 121,000 particles per person [13]. The presence of microplastics can disrupt metabolism, alter the immune system, and trigger inflammatory responses. They are also linked to neurotoxicity, carcinogenicity, and indirect harmful effects by acting as vectors for toxic contaminants and disease-causing microorganisms [14].

Biodegradation is the natural process by which matter is broken down through the activities of microorganisms and their enzymes [15]. In nature, several bacteria [16, 17] and fungi [18, 19] have demonstrated the ability to degrade LDPE, using it as a source of carbon and energy. This process typically involves distinct stages: colonization and biodeterioration, biofragmentation and depolymerization, assimilation, and mineralization [20, 21]. Fungi, in particular, are considered promising agents for plastic degradation because of their rapid growth, ability to colonize and metabolize diverse substrates, and major role in organic matter cycling. Moreover, they produce surfactants and extracellular enzymes—such as laccases, manganese peroxidases, lignin peroxidases, and esterases—that promote adhesion to the polymer surface and subsequently facilitate its breakdown [22, 23].

Internationally, filamentous fungi have been isolated from landfills, dumpsites, river and marine sediments, soil, and water, where they are able to grow using LDPE as the sole carbon source over periods ranging from 7 to 180 days [22, 24]. These fungi reduce the weight of LDPE, with reported weight losses of 15.12% by *Cladosporium sphaerospermum* [22], 20.82% by *Fusarium solani* [25], 40.0% by *Aspergillus niger* [26], 29.16–45.83% by *A. terreus* and *Alternaria alternata* [19], and 36.01–45.62% by *A. niger* and *Trichoderma harzianum* [18]. LDPE biodegradation was confirmed through various analytical techniques, including changes in surface morphology (scanning electron microscopy, SEM), surface chemical structure (Fourier transform infrared spectroscopy, FTIR), weight-average and number-average molecular weight (gel permeation chromatography, GPC), crystallinity (X-ray powder diffraction, XRD), and thermal behavior (thermogravimetric analysis, TGA). Additionally, new carbonyl and hydroxyl functional groups (FTIR), key indicators of oxidative degradation, were detected, along with degradation intermediates, predominantly bis(2-ethylhexyl) phthalate, identified via gas chromatography–mass spectrometry (GC–MS). In Peru, filamentous fungi were isolated from plastic waste collected from open dumps. Fungi from the genera *Aspergillus* and *Penicillium* were identified and shown to grow on LDPE as a carbon source for 70–90 days. The degree of polymer weight loss ranged from 2.43–12.98% for individual fungal isolates and 3.59–6.01% for fungal consortia. LDPE degradation was also evidenced by the appearance of new functional groups (FTIR) and SEM observations of fungal mycelia and reproductive structures on the polymer surface [27, 28].

Under controlled conditions, the biodegradation of LDPE has been demonstrated; however, no individual microorganism or microbial consortium has yet been proven effective in achieving complete mineralization of the polymer [20]. In liquid media, LDPE weight loss reached 45.62% after 30 days, whereas in soil the degradation was significantly lower, with a 9.09% weight reduction after 60 days [18]. These findings highlight the slow pace of natural biodegradation, reflecting the generally low efficiency of microbial degradation of plastic pollutants [29, 30]. Therefore, an extensive search is needed in contaminated environments, such as open dumpsites, to identify and select the most efficient candidates for use under both controlled and environmental conditions in bioremediation efforts. Given these challenges, this study aimed to evaluate the degradation of low-density polyethylene by filamentous fungi isolated from a solid waste landfill in Ferreñafe, Lambayeque, Peru.

The structure of this article is as follows: the research methodology is described first, including details on the dataset and processing stages. This is followed by the presentation and discussion of the results, in which filamentous fungi capable of degrading LDPE were identified at the phenotypic level. The selection of strains was carried out indirectly on the basis of biomass formation via the use of powdered LDPE as the sole carbon source over 30 days, and directly through polymer analysis via gravimetric measurements, texture analysis, FTIR, and SEM. In addition, the fungal strain with the highest degradative activity was identified through molecular methods. Finally, the conclusions are presented, along with a concise summary of the key decisions made throughout the study.

2. Material and Methods

2.1. Characteristics of the LDPE and Culture Media Used in the Study

For this study, LDPE served as the sole carbon source and was utilized in both powder and fragmented forms. The LDPE powder (2,000 mesh, Material Science, China) exhibited a melting point range of 104–138 °C and a specific gravity of 0.945 g/cm³. Prior to use, it was sterilized via one hour of exposure to ultraviolet light (254 nm) [31]. Additionally, LDPE bag fragments (10x50 mm, 30 µm thick), acquired from a commercial source, were prepared by weighing, disinfecting with 70% (v/v) ethanol for 30 minutes, rinsing with sterile distilled water for 20 minutes, and drying at 45 °C for 12 hours [32].

The mineral salt medium (MSM), which is essential for cultivating microorganisms, was prepared with the following composition (g/L): K₂HPO₄ (1 g), KH₂PO₄ (0.2 g), NaCl (1 g), CaCl₂·2H₂O (0.002 g), H₃BO₃ (0.005 g), NH₄(SO₄)₂ (1 g), MgSO₄·7H₂O (0.5 g), CuSO₄·5H₂O (0.001 g), ZnSO₄·7H₂O (0.001 g), MnSO₄·7H₂O (0.001 g), FeSO₄·7H₂O (0.01 g) and 1 g of LDPE powder [33]. For solid cultures, MSM agar was prepared by incorporating 15 g of agar powder into 1000 mL of the broth and adjusting the pH to 7.2. Both the liquid and solid media were sterilized by autoclaving at 121 °C and 103.4 kPa for 20 minutes [34]. The methodological workflow is shown in Figure 1.

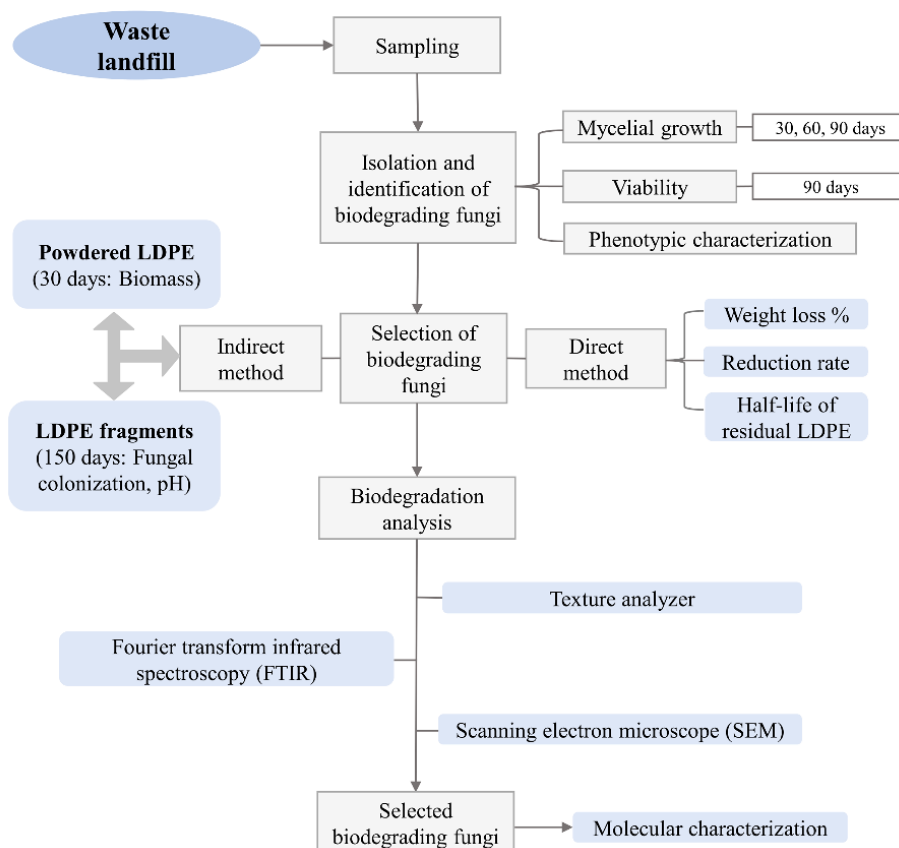


Figure 1. Methodological workflow for the isolation, selection, and characterization of LDPE-degrading filamentous fungi

2.2. Sampling Location

Soil samples were collected from the Ferreñafe solid waste landfill in Lambayeque, Peru, which is located at 6°50'23.4" S latitude and 79°49'30.2" W longitude. This landfill has a storage capacity of 180,176.11 m³ for daily waste [35]. Within the landfill's soil, specifically where deteriorated LDPE bags were present, 20 × 20 cm quadrants were marked. The top 5 cm of surface soil was removed [36], and then 500 g of soil along with fragments of plastic bags were collected for the isolation of filamentous fungi [37].

2.3. Phenotypic Identification of LDPE-Degrading Filamentous Fungi

We identified LDPE-degrading filamentous fungi by observing their growth on agar with powdered polymer as a carbon source, and in broth containing an LDPE fragment, over a 90-day period. Fungal isolation began with an enrichment method: 10 g of soil sample mixed with deteriorated plastics was added to 90 mL of MSM broth supplemented with 0.1% powdered LDPE [34]. After incubating statically at 30 °C for 60 days, 100 µL of the supernatant was spread onto MSM agar containing 0.1% powdered LDPE and incubated at 30 °C for up to 10 days. The selected colonies were subsequently duplicated on potato dextrose agar (PDA) and MSM agar with 1% powdered LDPE. Pure fungal cultures were then inoculated into 10 mL of MSM broth containing a 10x50 mm LDPE fragment [37, 38], and cultivated under aerobic conditions for 90 days. We assessed LDPE utilization every 30 days by visually inspecting the LDPE fragments for fungal mycelial growth and color changes. After 90 days, the filamentous fungi were reisolated on MSM agar with 0.1% LDPE to confirm their viability. Monosporic cultures were prepared for phenotypic genus identification [39], and the fungal-colonized LDPE fragments were examined under a stereoscopic microscope.

The phenotypic identification of fungal genera relies on both macroscopic and microscopic morphological characteristics [40], which are determined from monosporic cultures [39]. Macroscopic observations included colony color (obverse and reverse), surface texture, presence/absence of exudate droplets, radial furrows, and concentric circles. For microscopic examination, mounts of colonies were prepared with a drop of lactophenol blue [41] to differentiate hyphal forms and reproductive structures via established identification keys [42, 43].

2.4. Selection of LDPE-Degrading Fungi

We selected LDPE-degrading filamentous fungi via both indirect and direct methods. Indirect selection involved assessing biomass formation on powdered LDPE as a carbon source over 30 days, fungal colonization of LDPE fragments over 150 days, and the final pH of the culture medium. Direct selection was based on the degradation of LDPE, quantified as the percentage of polymer weight loss, the reduction rate (g/day), and the half-life of residual LDPE (days) after 150 days of incubation.

The 30-day preliminary assay was conducted in 10 mL of MSM broth containing 0.1% powdered LDPE. Each tube was inoculated with 0.5 mL (5%) of a conidial suspension from the fungi under investigation. To prepare the inoculum, conidia were harvested from fungal cultures grown on MSM agar with 0.1% LDPE at 30 °C for 10 days. These conidia were then suspended in 40 mL of distilled water with 1% Tween, and the concentration was adjusted to 10⁵ conidia/mL via a Neubauer chamber. The biodegradation assay was performed in triplicate in tubes containing either 10 mL of MSM broth with 0.1% powdered LDPE or 10 mL of MSM broth with 0.1% glucose (as a positive control). Each tube received 0.5 mL of the fungal inoculum and was incubated at 30 °C for 30 days. Following incubation, the cultures were autoclaved for sterilization, filtered to separate the fungal biomass, dried at 60 °C for 2 hours, and weighed via an analytical balance.

Our 150-day degradation assay was conducted in 100 mL of standard MSM broth, each containing two LDPE fragments (10x50 mm). These fragments had initial weights ranging from 0.0172-0.0189 g and were sterilized prior to inoculation [37, 38], without receiving any pretreatment to enhance biodegradation [23, 44]. We inoculated each setup with 5 mL of a fungal conidial suspension (10⁵ conidia/mL) [32] and incubated them statically at 30 °C. After 150 days [40], the LDPE fragments were placed in Petri dishes to assess fungal colonization. We visually observed mycelial growth and estimated the percentage of the fragments' edges and surfaces that were colonized [45]. Colonization was categorized via a conventional scale: excellent (76–100%), good (51–75%), regular (26–50%), and scarce (1–25%). Additionally, we examined the fragments under a stereoscopic microscope and measured the final pH of the filtered culture broth via a HANNA HJ5221 pH meter.

Following incubation, the LDPE fragments were recovered and washed with 2% (v/v) sodium dodecyl sulfate (SDS) for 4 hours at 50 °C. The samples were then rinsed with distilled water and dried at 60 °C until a constant weight was achieved. This allowed us to determine the weight loss of the polymer by calculating the difference between the initial and final weights [46] and expressing it as a percentage [40]. The percentage of weight loss was then used to calculate the reduction rate via the following formula [38] (Equation 1):

$$K = -\frac{1}{t} \ln \frac{W}{W_0} \quad (1)$$

where K represents the polymer reduction rate in grams per day, t represents the incubation time in days, W represents the final (residual) weight of the polymer in grams, and W₀ represents the initial weight of the polymer in grams.

On the basis of the polymer's reduction rate (g/day), we calculated the half-life of the residual LDPE in days, which corresponds to the time needed for the polymer's weight to decrease by half. The following formula was used for this calculation [22] (Equation 2):

$$t_{1/2} = \frac{\ln(2)}{K} \quad (2)$$

where K denotes the polymer's reduction rate per day and t signifies the time in days.

2.5. Analysis of Biodegraded LDPE Characteristics

After washing and drying, the 10x50 mm LDPE fragments were analyzed via a texture analyzer (Stable Microsystems model T. AHD Plus, UK) to determine their tensile strength (TS, MPa) and elongation at break (EAB, mm). We followed the ASTM D882-02 standard method. The fragments were conditioned with an initial grip separation of 30 mm and stretched at a crosshead speed of 10 mm/min [47]. The TS and EAB were then compared to those of uninoculated LDPE fragments, which served as control [48].

The fragments that presented the greatest percentage of weight loss and the lowest tensile strength due to fungal degradation were further analyzed via Fourier transform infrared (FTIR) spectroscopy. The infrared spectrum of functional groups was obtained using a Nicolet iS50 FTIR spectrometer (Thermo Scientific, Germany) equipped with a three-bounce diamond ATR Miracle accessory. Scans were performed in the 1,000-4,000 cm^{-1} range, with a resolution of 4 cm^{-1} over 32 scans [40]. To observe the cellular morphology, the fragments were sputter-coated with gold using an SPI-Module Tm, supplied at 18 mA and 8 mbar, and then visualized under a scanning electron microscope (Inspect TM S50, FEI).

2.6. Molecular Characterization of the Selected Fungus

We extracted fungal DNA via the Wizard Genomic DNA Purification Kit (Promega). The internal transcribed spacer (ITS) region of the most effective LDPE-degrading fungal strain was amplified by PCR with the universal primers ITS1-F and ITS4. The resulting PCR products were then sequenced via the Sanger method by the "Marcel Gutiérrez Correa" Mycology and Biotechnology Laboratory at the National Agrarian University La Molina (UNALM). Preliminary identification of the assembled sequences was conducted via BLASTN, and these sequences were then aligned with related ITS sequences retrieved from the NCBI database [49]. Finally, phylogenetic relationships were inferred via MEGA11 software [50], which employs the neighbor-joining method with 1000 bootstrap replicates.

2.7. Data Processing and Analysis

The percentage weight loss values of the polymers were subjected to tests for normality and homogeneity of variance. One-way analysis of variance (ANOVA) was then applied, followed by Tukey's multiple comparison test to determine significant differences between treatments ($p < 0.05$). All the statistical analyses were carried out via SPSS v 27.0 software.

3. Results and Discussion

3.1. Identification of LDPE-Degrading Filamentous Fungi

From all the cultivation methods used, we isolated 77 filamentous fungal strains. After 30 days of incubation, 40 fungal strains displayed dark mycelia directly on the LDPE fragment, whereas 37 developed white mycelia adhering to the test tube walls and above the LDPE fragment. After 60 days of incubation, the growth of the 40 strains with dark mycelia on the LDPE fragment was maintained. However, only 29 strains still presented white mycelia adhering to the tube walls, and 8 fungi presented no mycelial growth. At the 90-day mark, 39 fungi continued to show dark mycelia on the LDPE fragment, 4 displayed white mycelia, and 34 fungi had no visible mycelia. Fungal mycelium development between 30 and 90 days of incubation is shown in Figure 2. Furthermore, microscopic observations consistently revealed the presence of hyphae and conidia adhered to the surface, edges, and corners of the LDPE fragments across all experimental setups, with the exception of the control, as illustrated in Figure 3. After 90 days of cultivation in MSM broth, we successfully reisolated 36 filamentous fungi from the LDPE fragments. This represents 46.75% of the initially isolated strains, demonstrating their viability and survival by utilizing LDPE as their sole carbon and energy source. These 36 strains were thus classified as LDPE degraders.

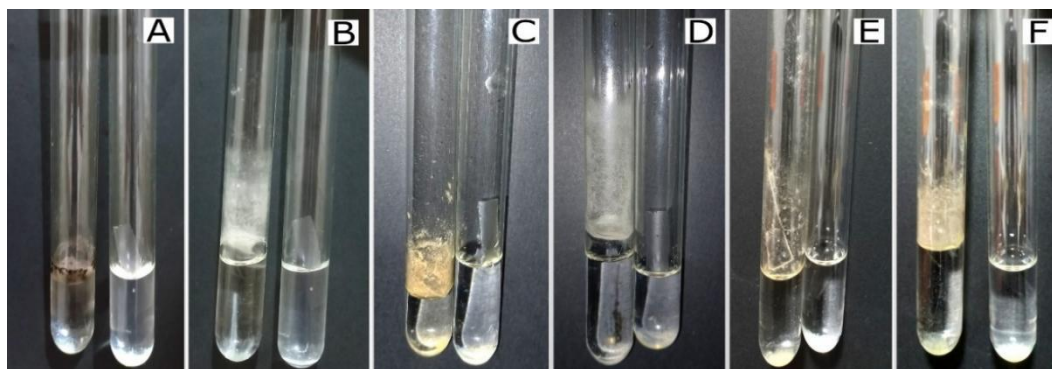


Figure 2. Development of dark and white mycelia from filamentous fungi grown in mineral salts medium with LDPE fragments. A-B) Initial mycelial development after 30 days of incubation. C-D) Continued growth and colonization at 60 days. E-F) Advanced stages of mycelial coverage on LDPE fragments after 90 days.

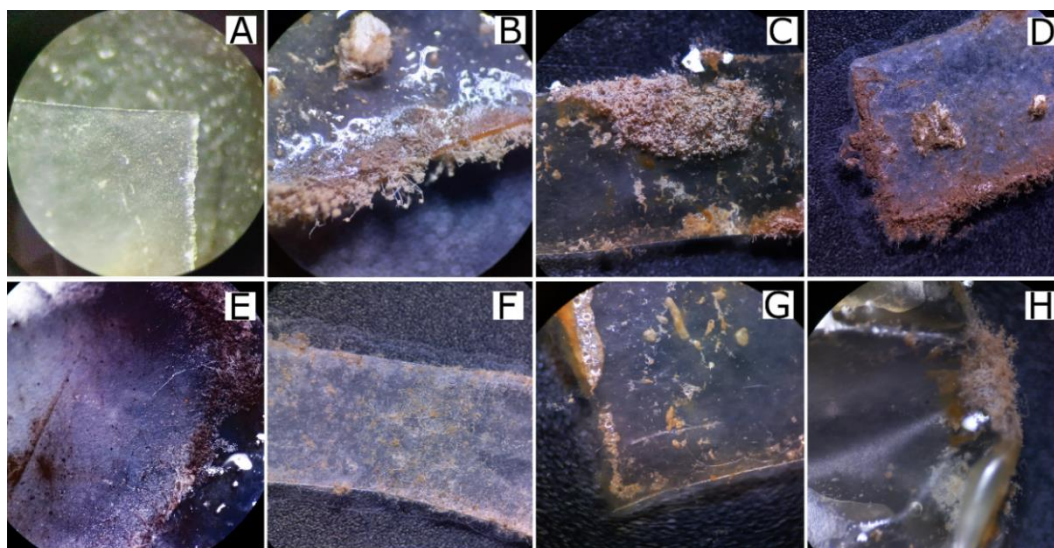


Figure 3. Comparison between uncolonized LDPE control fragments (A) and LDPE fragments (B-H) colonized by filamentous fungi over a 90-day period

We successfully isolated LDPE-degrading fungi from soil and deteriorated plastics sourced from a landfill, which aligns with findings of Sutkar et al. [26], Chigwada et al. [34] and Saira et al. [41]. Landfills are potential habitats for the exploration of biological agents with applications in the biodegradation of biopollutants [51]. In these environments, organic waste undergoes uncontrolled decomposition, leading to increased temperatures, the release of toxic compounds, and the survival of microorganisms with versatile metabolisms. These microbes are capable of producing biosurfactants, resistance structures, and biofilms. Species from genera such as *Aspergillus*, *Cladosporium*, *Fusarium*, *Alternaria*, and *Penicillium*, among others, persist in landfills [22, 25, 41], where they tolerate harsh conditions of humidity, temperature, and limited nutrients. As a result, they have adapted their enzymatic systems to metabolize a wide range of poorly oxidizable substrates, such as LDPE [15]. Given their highly heterogeneous nature, landfills and dumpsites harbor a rich microbial diversity with the potential to degrade various wastes, including LDPE [34]. However, fungi capable of degrading this polymer have also been isolated from diverse environments such as soil [26], plastic-contaminated soil and water bodies [22], buried plastics [40], compost, activated sludge, and river sediment [52], marine sediment [19], contaminated water [53], the rhizosphere [46], and plastic waste itself [54].

The observed fungal mycelial growth on LDPE fragments over 90 days provided clear evidence of the utilization of the polymer as a carbon source, which is consistent with previous reports by Sutkar et al. [26], Garcia Moreno et al. [55] and Gao et al. [54]. The time required for this development varies, with fungal colonization of the polymer being reported in as little as 7 days [22], 15 days [56], 21 days [53], 30 days [23], and 45 days [25]. The biodegradation of polyethylene typically proceeds through four distinct stages: colonization-corrosion, depolymerization, assimilation, and mineralization [54], or colonization-biodeterioration, biofragmentation-depolymerization, assimilation, and mineralization [21]. Fungi facilitate their attachment to the hydrophobic surface of plastics by producing hydrophobins, which are specialized proteins [57]. During this process, the hyphae colonize the polymer and secrete extracellular components—primarily carbohydrates—that form a matrix embedding the fungal structures, leading to the formation of a biofilm [58]. The mycelium that develops on LDPE signifies colonization by microorganisms that, either individually or in consortia, form a biofilm adhered to the deteriorating surface of the polymer. This process decreases in the hydrophobicity of plastic due to the action of microbial extracellular enzymes [54].

A total of 46.75% of the fungi remained viable after 90 days of cultivation with LDPE as the sole carbon source. In contrast, the 53.25% that did not retain viability might have suffered from a lack of organic nitrogen sources. Mohy Eldin et al. [46] similarly noted the necessity of periodically adding a nitrogen source to prevent N starvation, which can reduce the hydrophobic nature of microorganisms and negatively affect their adhesion to LDPE. The LDPE-degrading filamentous fungi were identified as belonging to several genera: *Aspergillus* (19 strains), *Fusarium* (6), *Penicillium* (9), *Memmoniella* (3), *Alternaria*, *Chaetomium*, *Cladosporium*, and *Rhizopus* (1 each). We also differentiated six distinct morphotypes among the *Aspergillus* species. All identified genera have been previously reported as LDPE degraders, including *Aspergillus* [26], *Fusarium* [25], *Penicillium* [59], *Alternaria* [23], *Cladosporium* [22], *Rhizopus* [15], *Memmoniella* and *Chaetomium* [56]. Spina et al. [60] further emphasized that microbial communities are not always easily isolated and identified from contaminated environments, suggesting enrichment through burying plastic fragments in soil for six months. This method allowed the isolation of 95 fungi from 14 genera and 27 species, with Ascomycota, particularly *Fusarium*, *Aspergillus*, and *Purpureocillium*, predominating and collectively accounting for 70% of the isolated fungi.

The predominance of *Aspergillus* species in our study aligns with research by Aguiar et al. [59], Kuswytasari et al. [40] and Mostajo-Zavaleta and Ambur-Soncco [61]. Various *Aspergillus* species are known for their efficient LDPE degradation capabilities and high activity of key enzymes such as laccases [32], manganese peroxidase [46], lignin peroxidase [62], esterases, cellulases [63], lipases, and alkane-hydroxylases [40]. Laccases and peroxidases are considered the primary enzymes responsible for LDPE biodegradation [23, 32]. Fungi attached to plastic release these enzymes, which, in turn, facilitate the hydrolytic breakdown of the polymer [46].

3.2. Selection of LDPE-Degrading Filamentous Fungi

We assessed the ability of filamentous fungi to degrade low-density polyethylene (LDPE) through a series of experiments. For 30 days, the biomass formed by filamentous fungi using LDPE as a carbon source ranged from 0.150 to 0.535 g, whereas it ranged from 0.477–0.580 g in the glucose control. The biomass produced with LDPE corresponded to 42.44–98.39% of the control biomass, with the highest values achieved by *Aspergillus* sp. 21 (98.39%), *Fusarium* sp. 4 (98.15%), and *Aspergillus* sp. 40 (96.75%). The fungi grown on powdered LDPE formed visible mycelia, which is consistent with Spina et al. [60], who reported that 97% of the fungi grew on 5 and 10 g/L LDPE. The highest biomass values (0.500–0.538 g) were achieved with *Aspergillus* spp. and *Fusarium* sp., partially aligning with El-Sayed et al. [32], who reported high biomass for *Aspergillus* spp. but low biomass for *Fusarium* sp. The latter showed a prolonged lag phase (over 40 days) and only 0.01 g/L of biomass after 112 days. In contrast, Ortega and Acosta [24] reported 0.280–0.400 g of biomass for *F. solani* cultured for 180 days with LDPE as the sole carbon source. In the 150-day assay, 16.67% of the fungi exhibited excellent colonization of LDPE fragments, 25% showed good colonization, 27.78% displayed regular colonization, and 30.55% had scant colonization, as illustrated in Figure 4-A. Furthermore, the pH of the broth cultured for 150 days ranged from 5.896–6.618 for fungi with excellent, good, and regular colonization. This range was lower than the range of 6.303–6.607 reported, with fungi showing little colonization and the range of 6.998 reported for the uninoculated control.

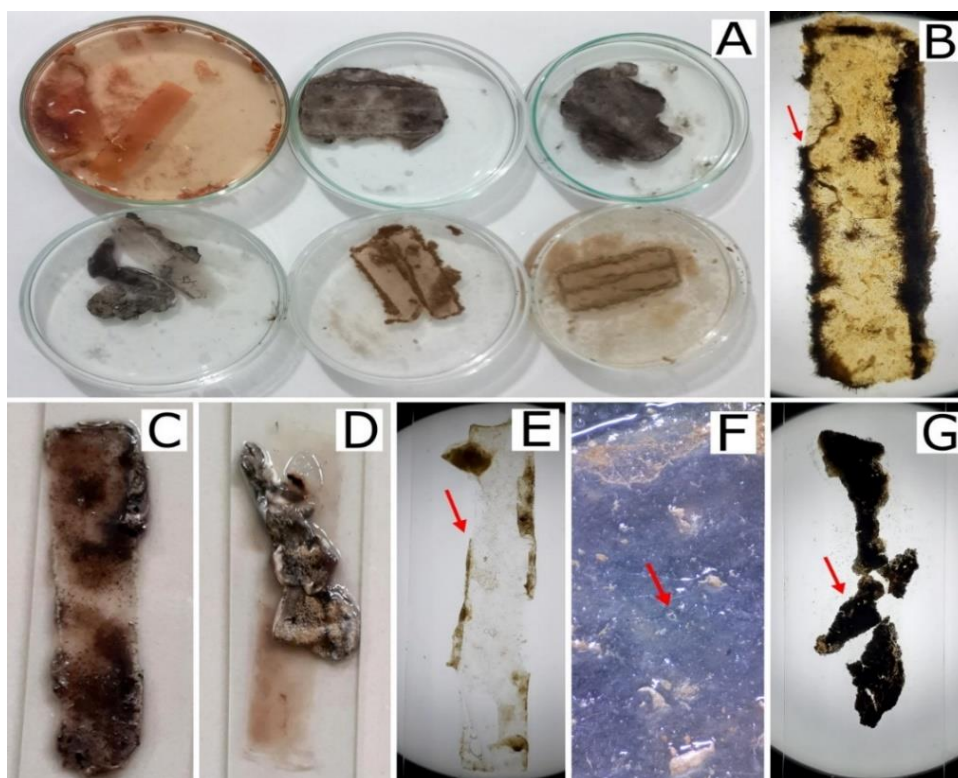
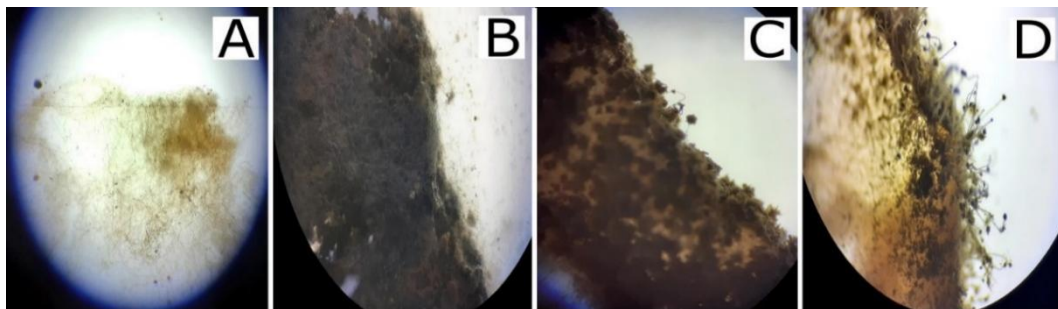


Figure 4. Stages and forms of fungal interaction with and degradation of LDPE fragments after 150 days of incubation. A) Good colonization, B-D) fungal mycelium, E) edge breakage, F) hole formation, and G) fragmentation

The percentage of LDPE weight loss for fungi with excellent, good, and regular colonization ranged from 4.44–20.21%, whereas no weight reduction was observed for fungi exhibiting scant colonization. These characteristics of LDPE-degrading fungi are presented in Table 1. The LDPE reduction rate ranged from 0.000307–0.001504 g/day, and the half-life of the residual polymer ranged from 2,257.65–450.06 days. We also observed fungal colonization on the edges and surface of the fragments, abundant mycelial growth, edge breakage, surface holes, and fragmentation of the LDPE (as shown in Figure 4-B to 4-G), along with biofilms displaying pink, black, orange, and brown pigmentation. Additionally, we successfully differentiated hyphae, mycelia, and reproductive structures on the degraded LDPE fragments, as shown in Figure 5.

Table 1. Fungal colonization, culture broth pH, weight loss percentage, reduction rate, and residual half-life of LDPE during 150-day biodegradation assays

Strain	Fungal colonization	Culture broth pH	Weight loss percentage* (%)	Reduction rate (g/days)	Residual Half Life (days)
<i>Aspergillus</i> sp. 40	Excellent	5.896	20.21±0.23 a	0.001504	450.06
<i>Aspergillus</i> sp. 7	Excellent	6.239	12.29±0.45 cd	0.000875	721.14
<i>Aspergillus</i> sp. 10	Excellent	6.397	11.67±0.29 d	0.000830	835.06
<i>Aspergillus</i> sp. 6	Excellent	6.298	14.09±0.76 c	0.001005	689.65
<i>Aspergillus</i> sp. 2	Excellent	6.334	11.43±0.19 d	0.000807	858.86
<i>Fusarium</i> sp. 4	Excellent	6.320	13.36±0.45 c	0.000807	858.86
<i>Aspergillus</i> sp. 23	Good	6.284	8.72±0.23 e	0.000607	1,141.85
<i>Aspergillus</i> sp. 21	Good	6.309	17.46±0.34 ab	0.001282	540.64
<i>Memmoniella</i> sp. 17	Good	6.084	8.14±0.56 e	0.000563	1,231.08
<i>Memmoniella</i> sp. 14	Good	6.102	9.68±0.21 e	0.000680	1,019.26
<i>Aspergillus</i> sp. 16	Good	6.400	15.87±0.26 b	0.001154	600.61
<i>Aspergillus</i> sp. 11	Good	6.536	8.89±0.21 e	0.000621	1,116.10
<i>Penicillium</i> sp. 1	Good	6.511	9.55±0.98 e	0.000673	1,029.87
<i>Aspergillus</i> sp. 12	Good	6.334	7.18±0.55 f	0.000498	1,391.77
<i>Penicillium</i> sp. 5	Good	6.386	7.82±0.23 f	0.000541	1,281.15
<i>Aspergillus</i> sp. 22	Regular	6.526	9.44±0.56 e	0.000665	1,042.26
<i>Memmoniella</i> sp. 28	Regular	6.430	7.39±0.19 f	0.000513	1,351.07
<i>Penicillium</i> sp. 35	Regular	6.387	8.33±0.26 e	0.000578	1,199.13
<i>Fusarium</i> sp. 3	Regular	6.558	5.62±0.19 g	0.000384	1,804.95
<i>Penicillium</i> sp. 30	Regular	6.305	6.15±0.34 g	0.000420	1,650.23
<i>Aspergillus</i> sp. 8	Regular	6.519	4.44±0.34 g	0.000307	2,257.65
<i>Penicillium</i> sp. 34	Regular	6.618	5.78±0.34 g	0.000398	1,741.46
<i>Alternaria</i> sp. 48	Regular	6.590	5.23±0.89 g	0.000363	1,909.37
<i>Aspergillus</i> sp. 60	Regular	6.480	6.17±0.89 f	0.000427	1,623.19
<i>Chaetomium</i> sp. 9	Regular	6.553	5.56±0.55 g	0.000384	1,804.95

*Average of three repetitions; $p < 0.05$ **Figure 5. Detailed microscopic views of various fungal structures found on the surface of LDPE fragments degraded by filamentous fungi: A) Hyphae, B) Mycelium and C-D) Reproductive structures**

The observed decrease in pH within the culture medium containing LDPE fragments aligns with the findings of Khan et al. [58], who reported a pH reduction from 7.0 ± 0.2 to 6.0 ± 0.3 after 90 days of incubation with *P. citrinum*. These researchers concluded that biochemical reactions driven by enzymatic activity during fungal growth are linked to LDPE degradation and its byproducts. The resulting pH variation confirms the polymer's use as a sole carbon source and indicates the presence of various monomers as degradation products. Similarly, Gong et al. [64] reported a decrease in culture medium pH from 6.55 ± 0.03 to 6.19 ± 0.02 after 30 days when LDPE was degraded by *Cladosporium* sp., which was attributed to enzymatic catabolism and the production of organic acids during the degradation process.

When LDPE was degraded by *A. niger* for 30 days, the pH decreased from 6.5 to 5.8. The variations in pH and degradation rate are interdependent. Acidification is due to organic acids secreted by the fungus and byproducts released from polymer breakdown. However, the pH must remain within an appropriate range (5–7), which is optimal for fungal enzymatic activity [18].

The weight loss of LDPE incubated with filamentous fungi for 150 days ranged from 4.44% to 20.21%, which falls within the previously reported range of 3.8–47.0% as shown in Table 2 [32, 59]. However, it exceeds 0.4–3.8% weight loss reported by El-Sayed et al. [32] for polymers cultured with landfill-isolated fungi over 112 days. This difference can likely be attributed to the longer incubation period used in our study. The variation in LDPE weight reduction is attributed to the diverse metabolic activities among fungal species. Furthermore, weight loss directly correlates with the deteriorated surface area caused by the fungal consumption of the polymer [32]. In this context, the highest percentage of weight loss (20.21%) was achieved with *Aspergillus* sp. 40, which exhibited excellent colonization of the polymer. This maximum value surpassed the 3.80% achieved with *A. carbonarius* [32] and the 12.5% achieved with *A. terreus* [40]. However, it was lower than the 22.90–45.62% weight loss observed with *A. niger* [18, 26, 41], *A. terreus* [46] and *Aspergillus* sp. [59]. This greater loss in LDPE degradation in other studies can be attributed to the addition of nitrogen [46], constant agitated incubation (100–150 rpm) [18, 26, 41], the use of smaller-sized microplastics [59], and higher incubation temperatures [46].

Table 2. Reported weight loss of untreated LDPE during fungal degradation

<i>Aspergillus</i> species	Time (days)	Temperature (°C)	Weight loss (%)	Reference
<i>Aspergillus</i> sp. 40 CIFOS	150	30	20.21	This study
<i>A. carbonarius</i>	112	30	3.80	El Sayed et al. [32]
<i>A. terreus</i>	105	35	24.00	Mohy Eldin et al. [46]
<i>A. niger</i>	30	28	22.90	Saira et al. [41]
<i>A. flavus</i>	90	25	12.98	Ambur Soncco & Mostajo Zavaleta [28]
<i>A. terreus</i>	30	30	12.50	Kuswytasari et al. [40]
<i>A. brasiliensis</i>	30	30	1.89	Garcia Moreno et al. [55]
<i>Aspergillus</i> sp.	28	30	47.00	Aguiar et al. [59]
<i>A. niger</i>	30	30	45.62	Ahmed et al. [18]
<i>A. niger</i>	20	30	40.00	Sutkar et al. [26]
<i>A. niger</i>	56	37	19.40	Ogu et al. [15]

LDPE degradation depends not only on the type of microorganism [32] but also on various other factors. These include the culture medium, agitation speed, temperature, pH, and incubation time [26, 53, 63], as well as any pretreatment of the polymer [65]. Furthermore, inherent polymer properties such as thickness, molecular mass, crystallinity, and constituent plasticizers also play a role [21, 66]. The 150-day incubation period was considered appropriate to demonstrate the viability of fungi when LDPE was used as the sole carbon source, thereby eliminating the influence of any other organic contaminants [24]. The impact of plastic biodegradation largely depends on the incubation period, with longer durations generally enhancing degradative activity [19]. However, the reported timeframe for evaluating LDPE degradation is broad, ranging from as little as 7 days [22] to as long as 180 days [24]. The significance of native degrading microorganisms lies in their potential for enhanced efficiency when used in consortia. Chigwada et al. [34] reported a 22.2–55.23% weight reduction in LDPE fragments during a 90-day enrichment of landfill soil microorganisms. Molecular analysis of their consortia revealed 2,908 bacterial species and 187 fungal species. While polymer weight loss is the primary direct parameter for evaluating biodegradation [66, 67], this value can be influenced by residual microbial biomass on the sample or by weight loss due to the volatilization of intermediary components and soluble impurities [68].

The weight loss of LDPE reported during fungal degradation shows sometimes contradictory range, from 3.80% with *A. carbonarius* after 112 days under constant agitation at 120 rpm [32], to 47.0% with *Aspergillus* sp. after only 28 days with agitation at 150 rpm [59]. In contrast, other studies reported much lower weight loss, such as 0.30% with *Cladosporium* sp. cultivated on UV-irradiated LDPE [64], 0.37% with *Trametes hirsuta* on untreated LDPE under constant agitation (150 rpm) [23], and 45.62% with *A. niger* under agitation on untreated LDPE [18], all over a 30-day incubation period. These results do not consistently support the commonly held assumption that polymer pretreatment, constant agitation, and longer incubation times enhance microbial degradation of LDPE [15, 19, 44]. Therefore, comprehensive verification of LDPE degradation involves assessing changes in mechanical properties such as tensile strength [41], alterations in surface morphology, and modifications in functional groups [53], as well as changes in crystallinity [26], thermal behavior [18], molecular weight [22], and the identification of degradation intermediates [32].

3.3. Characteristics of Biodegraded LDPE

Changes in mechanical properties are direct evidence of LDPE degradation. The maximum tensile strength (TS), or the maximum stress a material can withstand before breaking, and the percentage reduction in these properties are crucial for determining polyethylene degradation [69]. After 150 days of incubation, 27.78% of the fungi reduced the TS of LDPE fragments by 5.61–41.72%. This change in mechanical properties indicates that the polymer is becoming brittle [62]. Various studies have reported variable reduction values for different fungal species, such as 12.5% with *Phlebiopsis flavidoalba* [69], 23.21% with *Colletotrichum fructicola* [62], 40–58% with *Trichoderma viride* and *A. nomius* [70], and 76.04% with *A. awamori* [71].

In terms of elongation at break (EAB), while 40% of the fungi caused a decrease of 3.24-36.03%, the remaining 60% led to an increase, as illustrated in Figure 6. Reductions in both tensile strength and elongation at break are generally linked to a decrease in molecular weight. However, inconsistencies between weight loss and elongation at break can occur, as demonstrated by Soleimani et al. [48]. They reported that LDPE degraded by *Streptomyces granticus* (174.11 mm), *Nocardia* sp. (157.29 mm), and *Rhodococcus ruber* (170.23 mm) exhibited increased elongation at break compared with the control (140.35 mm).

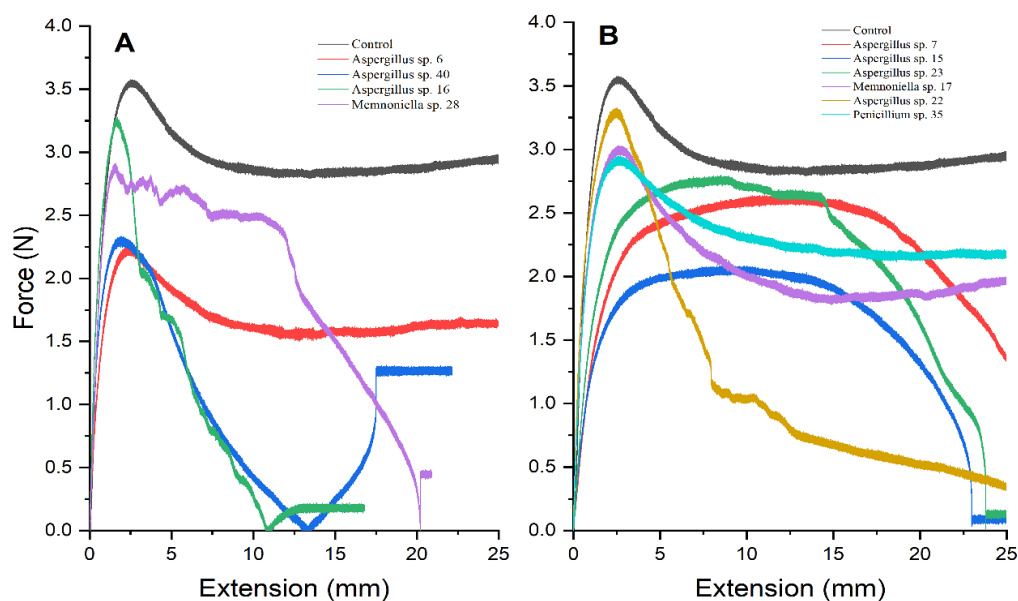


Figure 6. Tensile strength (TS) of LDPE fragments degraded by filamentous fungi over 150 days. A) Reduction of TS and EAB, and B) reduction of TS

The FTIR spectra of both the control LDPE and the LDPE treated with *Aspergillus* sp. 40 CIFOS revealed similarities in their core spectral features. Both showed two strong bands at approximately 2914 cm^{-1} and 2847 cm^{-1} , which are characteristic of $-\text{CH}_2$ asymmetric and symmetric stretching vibrations, respectively. Additionally, bands at 1471 cm^{-1} and 718 cm^{-1} corresponded to the $-\text{CH}_2$ scissoring and rocking motions, respectively [57], as shown in Figure 7. However, unlike the control, the FTIR spectrum of LDPE degraded by *Aspergillus* sp. 40 CIFOS showed distinct modifications. A new band was identified at 3277.46 cm^{-1} , and noticeable changes occurred at 1628.14 cm^{-1} and 1049.27 cm^{-1} . These chemical modifications provide clear evidence of the deterioration of the polymer chain [72] or oxidation of the LDPE surface [73]. Similarly, in LDPE degraded by *C. sphaerospermum*, changes were observed at 3362 cm^{-1} , 1640 cm^{-1} , and 1042 cm^{-1} , corresponding to hydroxyl ($-\text{OH}$), amide ($\text{C}=\text{O}$), and anhydride ($\text{C}-\text{O}$) groups, respectively [22]. The 1680 cm^{-1} to 1600 cm^{-1} range, indicative of double bonds, frequently appears alongside hydroxyl groups (3550 cm^{-1} to 3200 cm^{-1}) in residual plastic, indicating that hydroxylation and oxidation are driven by microbial enzymes [74].

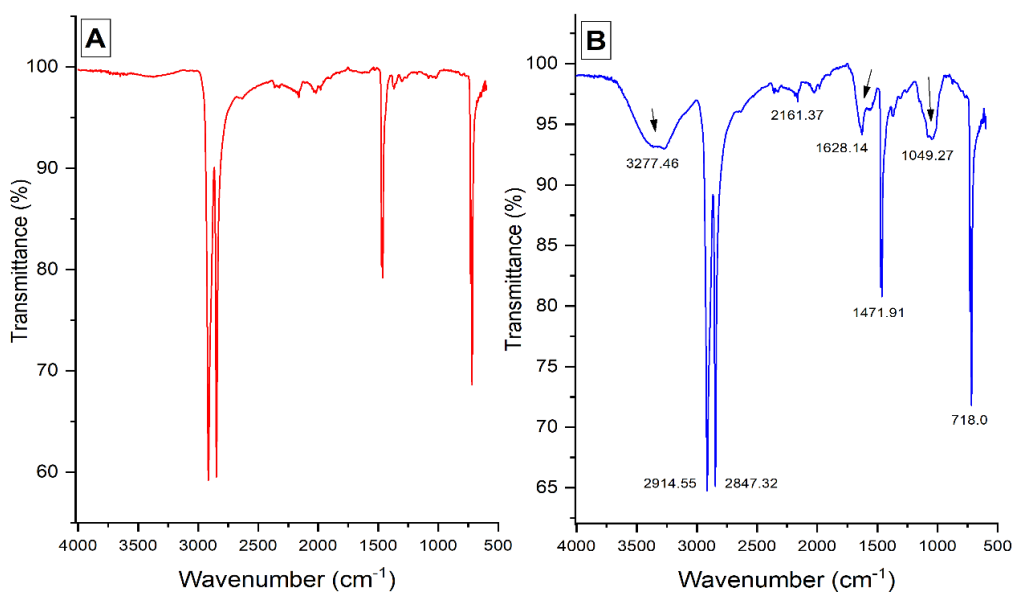


Figure 7. FTIR spectra of biodegraded LDPE after 60 days of incubation. A) Control sample and B) LDPE sample treated with *Aspergillus* sp. 40 CIFOS

Specifically, the band at 3277 cm^{-1} in our study indicates changes in the hydroxyl groups. The incorporation of these groups, often facilitated by microbial hydroxylases, aids in the breakdown of LDPE. This finding is consistent with other FTIR analyses where changes in the hydroxyl region were observed at $3500\text{--}3250\text{ cm}^{-1}$ with *Aspergillus* sp. [59], 3263 cm^{-1} with *C. cladosporioides* [57], 3362 cm^{-1} with *C. sphaerospermum* [22], and 3440 cm^{-1} with *A. niger* [41]. The shift in the FTIR spectrum at 1628 cm^{-1} suggests the formation of C=C bonds from amide groups. These products are considered intermediate products of biodeterioration and biofragmentation, resulting from the oxidative and hydrolytic action of microbial enzymes [32, 46]. Similar changes in the FTIR spectrum were observed at $1750\text{--}1500\text{ cm}^{-1}$ for *Aspergillus* sp. [59], 1610 cm^{-1} for *A. niger* [41], 1644 cm^{-1} for *C. cladosporioides* [46], and 1648 cm^{-1} for *Phanerodontia chrysosporium* [69]. Furthermore, the change in the peak at 1049 cm^{-1} indicates the presence of C-O groups (anhydrides) in the degraded LDPE [22]. It has also been reported that changes in the $1100\text{--}1030\text{ cm}^{-1}$ region, corresponding to the C-O-C, C-C, and C-OH stretching groups of the pyranose ring, might be related to fungal mycelia adhering to the LDPE film surface [57].

After 150 days, scanning electron microscopy (SEM) revealed notable differences between the control and treated LDPE fragments. The control LDPE fragments showed an unaltered surface with no attached biological structures. In contrast, the polymers degraded by *Aspergillus* sp. 40 CIFOS exhibited significant changes, including increased roughness, colonization by hyphae and mycelia, and the presence of fungal fruiting bodies, features and structures that are distinguished in Figure 8. This visual evidence clearly demonstrates the extent of microbial activity and physical deterioration on the LDPE surface.

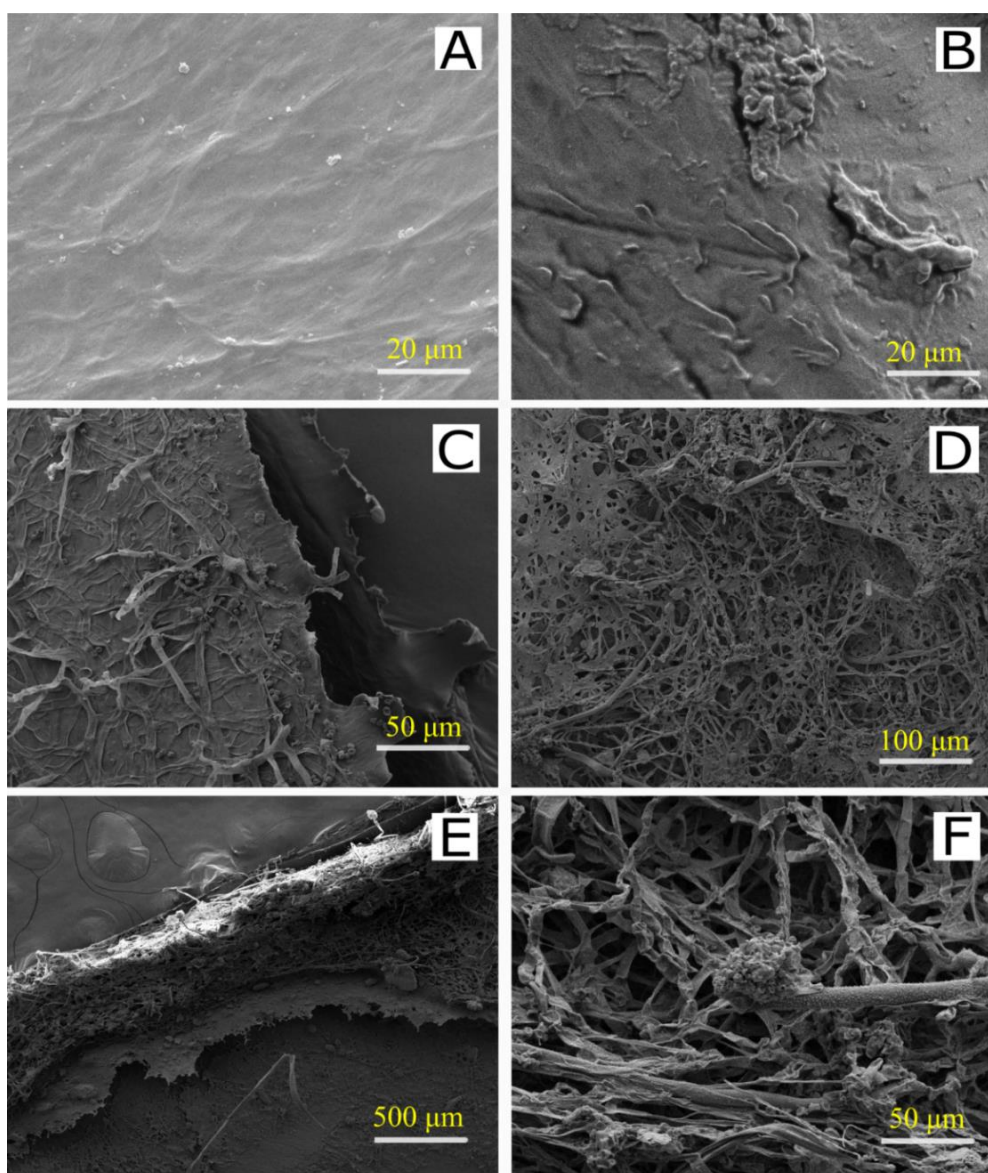


Figure 8. SEM images of LDPE before and after *Aspergillus* sp. 40 incubation. A) Control, B) rough surface, C) hyphae, D) mycelia, and E-F) reproductive structures

The presence of a biofilm and reproductive structures on the LDPE surface clearly demonstrates the fungus's adhesion to the polymer [18]. This attachment is crucial for establishing microbial contact and secreting the extracellular enzymes vital for initiating biodegradation [75]. The hyphae and spores adhering to LDPE provide direct evidence of biofilm formation, which progressively reduces surface hydrophobicity, thereby promoting degradation [58]. Surface physical changes are attributed to the utilization of LDPE as a carbon source [26], and are associated with microbial adhesion, biofilm formation, and the secretion of enzymes such as laccases, manganese peroxidases, and alkane hydroxylases [48]. The hydrolytic action of these enzymes leads to fragmentation of the polymer chain, resulting in visible alterations on the LDPE surface, including streaks [62], roughness, erosion, fractures [34], flaking [46], grooves, cracks, and holes [56].

3.4. Phylogenetic Analysis

The filamentous fungal strain 40 CIFOS demonstrated the highest LDPE degradation activity. Through BLASTN analysis, it was identified as *Aspergillus* sp. This identification was further corroborated by phylogenetic analysis (GenBank accession number: PQ685988.1), as shown in Figure 9.

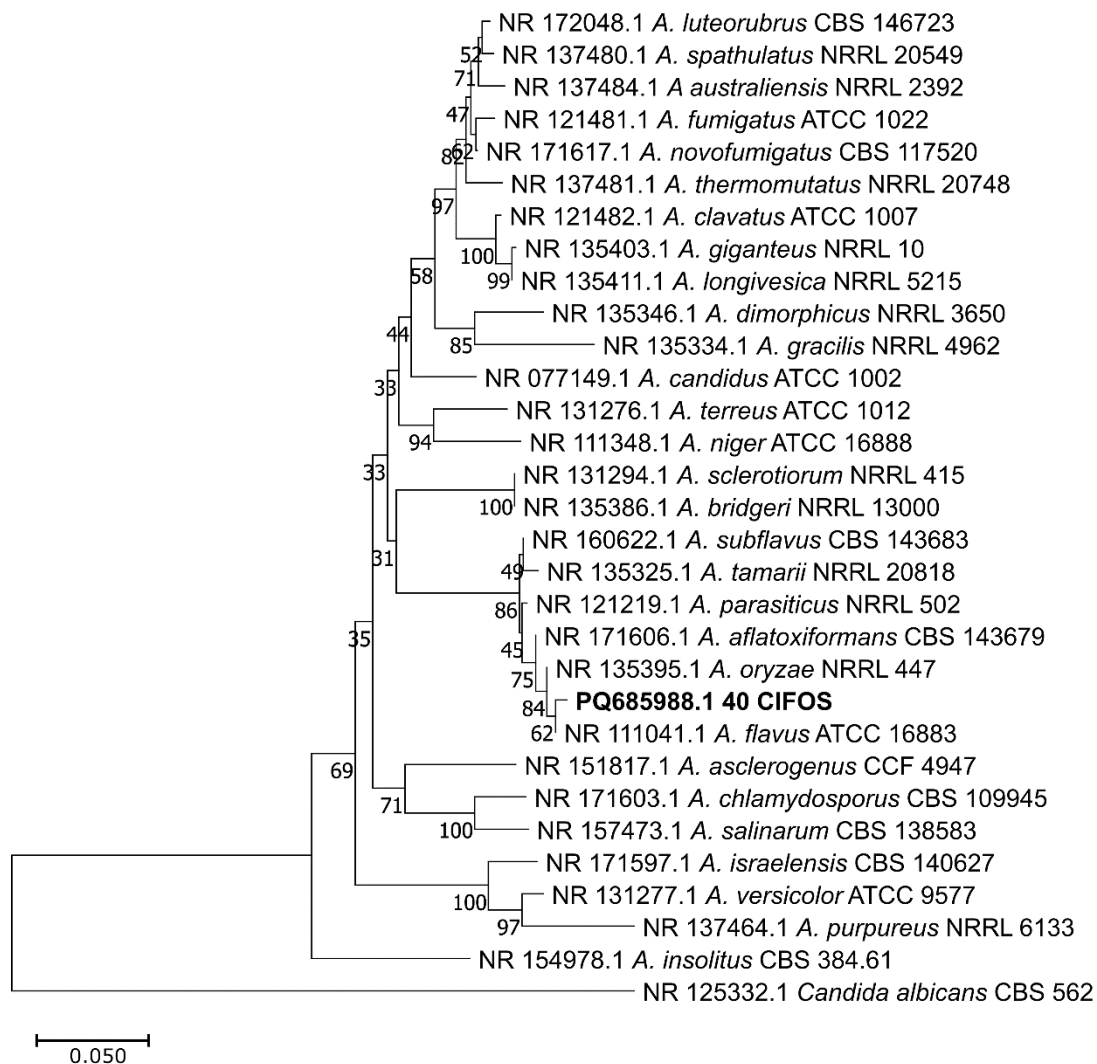


Figure 9. Phylogenetic tree inferred from the internal transcribed spacer (ITS) sequences of strain 40 CIFOS and other *Aspergillus* sp. sequences. This tree was inferred via the neighbor-joining method with 1000 bootstrap replicates. An ITS sequence from *Candida albicans* was selected as the outgroup.

Various *Aspergillus* species have demonstrated the ability to degrade LDPE and hold potential for mitigating this pollutant due to their capacity to generate substantial biomass when LDPE is used as the sole carbon source [59]. Weight loss in residual LDPE has been documented in *A. brasiliensis* [55], *A. fumigatus*, and *A. flavus* [66]. In addition, *A. niger* has been shown to induce changes in surface morphology and chemical structure, as well as to alter the thermal stability and crystallinity of the polymer [18, 26]. Degradation intermediates have been identified during LDPE breakdown by *A. terreus* [19] and *A. carbonarius* [32]. Microbial communities can degrade plastics via

cometabolism, a process in which certain species utilize easily oxidizable substrates for growth, while the enzymes they release promote the breakdown of more recalcitrant compounds that are otherwise difficult to use as carbon and energy sources [23]. Fungal degradation mechanisms rely on both direct growth and cometabolic activity [41]. In this context, further investigation into LDPE degradation by consortia of filamentous fungi is warranted, especially those strains that exhibited extensive colonization and achieved 11.43–20.21% polymer weight loss after 150 days. The synergism observed in microbial consortia is attributed to the combined enzymatic activities required for effective polymer degradation, which individual strains may lack on their own [17, 32, 76].

4. Conclusion

Fungi isolated from a municipal solid waste dumpsite demonstrated the ability to adhere to and colonize LDPE without any pretreatment, under sugar-free conditions, using the polymer as the sole carbon source, and without continuous agitation over a prolonged period of 150 days. Fungal viability was maintained throughout the incubation period, and the residual polymer showed weight loss (gravimetric analysis) as well as significant alterations in mechanical properties, specifically TS and EAB. Among the tested strains, *Aspergillus* sp. 40 CIFOS achieved a 20.21% weight reduction, a degradation rate of 0.001504 g/day, a residual half-life of 450.06 days, a 41.72% decrease in TS, and a 36.03% reduction in EAB. The degradation of LDPE was further supported by SEM, which revealed morphological surface alterations, and FTIR, which confirmed significant changes in surface functional groups.

Owing to technical limitations, this study did not assess the evolution of dissolved organic carbon associated with fungal colonization, nor did it evaluate oxygen consumption or CO₂ production, which are key indicators of polymer utilization. Future research should focus on evaluating the enzymatic activity of *Aspergillus* sp. strains such as 40 CIFOS, both individually and in fungal consortia. It is also essential to characterize the genes involved in the biosynthesis of these enzymes to optimize LDPE degradation as a promising, environmentally friendly, and cost-effective strategy. In this context, the complete genome sequencing of *Aspergillus* sp. 40 CIFOS could provide valuable insights into its degradation potential, particularly when combined with transcriptomic and proteomic analyses to identify key genes and proteins involved in the process. Functional studies—such as gene cloning, heterologous expression, and enzymatic assays—are necessary to confirm their role in LDPE degradation at the molecular level. Moreover, future investigations should explore synergistic effects within fungal consortia, characterize specific enzymes involved in polyethylene breakdown, and apply genetic engineering to increase the degradation rate. Ultimately, such efforts may lead to the validation of this process as a viable and eco-friendly solution for mitigating plastic pollution.

5. Declarations

5.1. Author Contributions

Conceptualization, C.F.; methodology, S.P., V.L.L., C.R., V.A., and C.F.; formal analysis, S.P., B.Q., V.L.L., C.R., and R.S.; investigation, C.F., S.P., B.Q., and V.L.L.; writing—original draft preparation, S.P., B. Q., C.C., R.S., and C.F.; writing—review and editing, S.P., C.C., and C.F. All authors have read and agreed to the published version of the manuscript.

5.2. Data Availability Statement

All the data are available in the manuscript file. However, if some supplementary data are needed, they will be made available from the corresponding author upon reasonable request.

5.3. Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

5.4. Institutional Review Board Statement

Not applicable.

5.5. Informed Consent Statement

Not applicable.

5.6. Declaration of Competing Interest

The authors declare that there are no conflicts of interest concerning the publication of this manuscript. Furthermore, all ethical considerations, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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