




# Biodeterioration of low-density polyethylene microplastics by dark septate endophytic fungi supported by mass loss and Fourier-transform infrared spectroscopy – scanning electron microscopy evidence

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## ABSTRACT

Soil contamination by microplastics is an increasing environmental concern due to the high persistence of low-density polyethylene (LDPE). This study investigated the biodeterioration of LDPE microplastics by dark septate endophytic (DSE) fungi in a minimal salt medium, with LDPE provided as the primary carbon-containing substrate. Five DSE isolates, namely *Pleosporales* sp. (BM2 and Mg3) and *Cladosporium* sp. (EC2, Mg2, and PNS), were incubated with sterile LDPE microplastics (250–425  $\mu$ m) for 60 days at 25–28 °C. Biodeterioration performance was evaluated through mass loss measurements, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM), with an additional dose-dependent analysis (0–2 g LDPE) conducted for the best-performing isolate. All isolates were able to colonize LDPE particles and induce oxidative deterioration of the polymer surface. The highest mass loss was observed for *Cladosporium* sp. strain PNS (35.0  $\pm$  2.0% of the initial mass). FTIR analysis revealed a reduction in C–H stretching bands (2915–2848  $\text{cm}^{-1}$ ) and the emergence of O–H (3300–3500  $\text{cm}^{-1}$ ) and C=O (1700–1715  $\text{cm}^{-1}$ ) functional groups, indicating oxidative modification of the polymer structure. SEM observations further confirmed surface deterioration, including cracks and fragmentation, which intensified with increasing LDPE dose. Collectively, these findings demonstrate the potential of DSE fungi, particularly *Cladosporium* sp. strain PNS, to initiate LDPE microplastic biodeterioration and support their prospective application in sustainable, soil-oriented microplastic remediation strategies.

**Keywords:** plastic pollution, fungal biodeterioration, oxidative surface modification, microplastic transformation, soil-based ecological engineering.

## INTRODUCTION

Plastic pollution has become a global environmental issue that extends beyond aquatic systems to terrestrial ecosystems through the accumulation of microplastics in soil. Microplastics are commonly defined as plastic fragments smaller than 5 mm, originating from the fragmentation of larger plastic debris and from

primary sources such as textile fibers and industrial products (Wu et al., 2024). In soils, microplastics have been reported to alter physical structure and porosity, disturb microbial activity, and affect plant growth, potentially leading to broader ecological and health consequences due to their persistence and mobility (Gorde et al., 2024; Sun et al., 2024). The risk is further amplified because microplastics can interact

with and transport co-contaminants, including heavy metals and pesticides, thereby increasing exposure pathways for soil organisms and enabling transfer into food chains via agricultural products (Wan et al., 2023). Despite increasing recognition of these impacts, practical and environmentally benign strategies to mitigate microplastics in soil remain limited.

Among synthetic polymers, low-density polyethylene (LDPE) is one of the most widely used materials and a major contributor to plastic waste (Nguyen et al., 2024). LDPE is characterized by a high molecular weight and a chemically stable backbone with limited reactive functional groups, which makes it intrinsically resistant to biological degradation (Alami et al., 2025). Consequently, LDPE waste management still largely relies on landfilling and incineration, approaches that may shift environmental burdens rather than resolve them (Lyshtva et al., 2025; Ghatge et al., 2020). Although microbial degradation of polyethylene has been reported, performance is highly variable and often depends on initial oxidative modification that introduces oxygen-containing functional groups and improves enzyme accessibility to the polymer chain (Tang et al., 2024). This suggests that microorganisms with strong oxidative capability and ecological adaptability to soil environments represent promising candidates for the development of biological remediation approaches.

Bioremediation offers an attractive alternative by harnessing microorganisms to transform or mineralize pollutants with a lower environmental footprint. In soil and agroecosystems, plant-associated fungi are particularly relevant because they can persist under environmental stress and interact closely with root systems (Chaudhary et al., 2024). Dark septate endophytic fungi (DSE) are melanized root-associated endophytes known to enhance plant tolerance to abiotic stress and to produce oxidative and ligninolytic enzymes that can modify recalcitrant organic substrates (Mattoo and Nonzom, 2022; Santos et al., 2018). Fungal degradation of synthetic polymers may further be facilitated by surface colonization, biofilm formation, and hyphal penetration into microcracks or micropores, which enhance physical contact and promote surface fragmentation and oxidation, thereby increasing enzymatic access to polymer chains (Cai et al., 2023; Srikanth et al., 2022). However, while a variety of fungi have been explored

for plastic degradation, studies that specifically assess DSE isolates for LDPE microplastic biodegradation and link quantitative performance to chemical and morphological evidence of polymer alteration remain scarce.

To address this knowledge gap, the present study aims to systematically evaluate the biodegradation potential of dark septate endophytic (DSE) fungi toward LDPE microplastics under nutrient-limited conditions relevant to soil environments. This work focuses on whether different DSE isolates exhibit distinct biodeterioration capacity, whether melanized DSE taxa show enhanced performance, and whether increasing LDPE availability strengthens polymer surface alteration. We hypothesize that DSE fungi—particularly *Cladosporium* spp.—promote early-stage LDPE biodeterioration primarily through oxidative surface modification rather than complete mineralization. By linking quantitative mass-loss outcomes with chemical (FTIR) and morphological (SEM) evidence, this study provides new mechanistic insight into DSE-mediated LDPE microplastic transformation and supports the development of soil-oriented ecological engineering strategies for microplastic mitigation.

## MATERIALS AND METHODS

### Fungal isolates and study location

The study was conducted at the Laboratory of Agrotechnology, Faculty of Agriculture, Universitas Tadulako, Palu, Central Sulawesi, Indonesia. Five DSE fungal isolates were used, including two isolates affiliated with the order *Pleosporales* (BM2 and Mg3) and three isolates belonging to the genus *Cladosporium* (EC2, Mg2, and PNS). The DSE isolates were originally isolated from plant root tissues, including bamboo (*Bambusa* sp.), mango (*Mangifera indica*), water hyacinth (*Eichhornia crassipes*), and pine (*Pinus* sp.) roots, and were obtained from the culture collection of the Laboratory of Agrotechnology, Universitas Tadulako. Prior to the biodegradation experiments, all isolates were maintained on Potato Dextrose Agar (PDA) under laboratory conditions. Low-density polyethylene (LDPE) microplastics were prepared from commercially available black LDPE carrier bags, commonly produced from polyethylene with a density of 0.915–0.930 g cm<sup>-3</sup> and a melting point of 105–115 °C.

## Preparation of LDPE microplastics

LDPE carrier bags were washed thoroughly with distilled water to remove surface contaminants and air-dried at room temperature. The dried materials were mechanically ground using a laboratory-scale grinder and sieved through 40–60 mesh sieves to obtain LDPE microplastic particles with sizes ranging from 250 to 425  $\mu\text{m}$ . The resulting LDPE microplastics were sterilized by immersion in 70% (v/v) ethanol, followed by drying and subsequent exposure to ultraviolet (UV) irradiation for 15 min to minimize microbial contamination. Sterilized microplastics were stored in sterile sealed containers until use. No chemical or thermal pre-oxidation treatment was applied in order to preserve the original physico-chemical properties of LDPE prior to the biodegradation assays.

## Biodegradation assay and experimental design

Prior to the biodegradation experiments, all fungal isolates were rejuvenated on PDA plates and incubated at 25–28 °C until active mycelial growth was observed. Biodegradation assays were conducted using a minimal salt medium (MSM) adapted from (Ali et al., 2025) with minor modifications. The MSM consisted of ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) as the inorganic nitrogen source, potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) as the phosphate source, and a standard trace element solution. The initial pH of the medium was adjusted to 6.5 prior to sterilization. The MSM was prepared in solid agar form and dispensed into sterile 9 cm diameter Petri dishes.

Sterile LDPE microplastics were added to each plate at a dose of 0.1 g per plate and evenly distributed over the agar surface by gentle manual spreading to ensure uniform contact between fungal mycelia and LDPE particles. The prepared media were incubated for 72 h at room temperature to confirm sterility prior to fungal inoculation. Each DSE isolate was inoculated at the center of MSM plates containing LDPE microplastics and incubated at 25–28 °C for 60 days under static conditions in the dark. All treatments were conducted with three biological replicates. A non-inoculated control consisting of MSM and LDPE microplastics without fungal inoculation was included to account for abiotic mass loss during the incubation period.

## Determination of LDPE mass loss

After 60 days of incubation, LDPE microplastics were collected from the culture media and immersed in 30% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) for 48 h at room temperature to remove fungal biomass and organic residues without affecting the polymer structure (Pfohl et al., 2021). The LDPE particles were subsequently filtered, rinsed thoroughly with sterile distilled water, and dried at 60 °C for 24 h or until a constant weight was achieved. LDPE mass loss was determined under controlled laboratory conditions using a one-factor experimental design, in which the treatment factor was the type of fungal isolate, consisting of six levels: uninoculated control, *Pleosporales* sp. strain Mg3, *Pleosporales* sp. strain BM2, *Cladosporium* sp. strain PNS, *Cladosporium* sp. strain EC2, and *Cladosporium* sp. strain Mg2. Each treatment was performed in three biological replicates ( $n = 3$ ). LDPE mass loss for each replicate ( $L_i$ ) was calculated as the difference between the initial mass ( $M_0$ ) and the final mass after incubation and cleaning ( $M_i$ ), according to:

$$L_i = M_0 - M_i \quad (1)$$

where:  $L_i$  is the LDPE mass loss in the  $i$ -th replicate (g),  $M_0$  is the initial LDPE mass before incubation (g), and  $M_i$  is the final LDPE mass after incubation and cleaning (g).

The mean mass loss ( $\bar{L}$ ) was calculated as:

$$\bar{L} = \frac{1}{n} \sum_{i=1}^n L_i \quad (2)$$

where:  $\bar{L}$  is the mean LDPE mass loss (g),  $L_i$  is the mass loss of the  $i$ -th replicate (g), and  $n$  is the number of biological replicates ( $n = 3$ ).

The standard deviation (SD) of mass loss was calculated as:

$$SDL = \sqrt{\frac{\sum_{i=1}^n (L_i - \bar{L})^2}{n-1}} \quad (3)$$

where:  $SDL$  is the standard deviation of LDPE mass loss (g),  $L_i$  is the LDPE mass loss of the  $i$ -th replicate (g),  $\bar{L}$  is the mean LDPE mass loss (g), and  $n$  is the number of biological replicates. Mass loss values are presented as mean  $\pm$  standard deviation (SD) from three biological replicates.

## Dose-dependent biodegradation assay

The isolate exhibiting the highest LDPE mass loss, *Cladosporium* sp. strain PNS, was selected

for further evaluation under different LDPE microplastic doses (0, 0.5, 1.0, 1.5, and 2.0 g per plate). The dose-dependent biodegradation assay was conducted under the same experimental and incubation conditions described above and incubated for 60 days. Macroscopic fungal growth characteristics, including colony color, surface texture, and mycelial distribution, were recorded throughout the incubation period. LDPE mass loss was determined as described in the determination of LDPE mass loss section. LDPE samples recovered from each treatment were subsequently subjected to FTIR and SEM analyses to evaluate chemical and surface-morphological changes.

#### Fourier-transform infrared spectroscopy (FTIR) analysis

LDPE microplastic samples recovered from non-inoculated control and fungal-treated groups were washed with 70% ethanol, rinsed thoroughly with distilled water, and dried completely prior to analysis. FTIR measurements were performed using an attenuated total reflectance (ATR) FTIR spectrometer. Infrared spectra were recorded in the range of 4000–400  $\text{cm}^{-1}$  at a spectral resolution of 4  $\text{cm}^{-1}$ , with 32 scans per sample to improve the signal-to-noise ratio. All spectra were collected at room temperature and background-corrected prior to analysis. Changes in the intensity and position of characteristic absorption bands corresponding to C–H stretching vibrations (2915–2848  $\text{cm}^{-1}$ ), O–H stretching vibrations (3300–3500  $\text{cm}^{-1}$ ), and carbonyl (C=O) stretching vibrations (1700–1715  $\text{cm}^{-1}$ ) were analyzed to evaluate oxidative modification and structural alteration of LDPE microplastics associated with fungal-induced biodeterioration.

#### Scanning electron microscopy (SEM) analysis

Surface morphological changes of LDPE microplastics before and after fungal treatment were examined using scanning electron microscopy (SEM). Prior to observation, LDPE samples were thoroughly dried, mounted on aluminum stubs using double-sided carbon adhesive tape, and sputter-coated with a thin layer of gold (Au) to enhance surface conductivity. SEM observations were performed using a Thermo Scientific Axia ChemiSEM HiVac scanning electron microscope (Serial Number: 9962707) operated under high-vacuum conditions. Micrographs

were acquired at an accelerating voltage of 10–15 kV with magnifications ranging from 1.000 $\times$  to 5.000 $\times$ . SEM images of non-inoculated control and fungal-treated LDPE microplastics were compared to evaluate surface deterioration features, including surface roughening, cracks, pits, peeling, and fragmentation, as indicators of fungal-induced biodeterioration.

#### Data analysis

All experiments were conducted using a one-factor completely randomized design (CRD) with three biological replicates per treatment. The experimental factor was the type of fungal isolate, consisting of six levels. LDPE mass loss was calculated for each replicate as the difference between the initial and final dry mass after incubation and cleaning. Data are presented as mean  $\pm$  standard deviation (SD) from three biological replicates ( $n = 3$ ). Differences among treatments were analyzed using one-way analysis of variance (ANOVA). When significant effects were detected, Tukey's honestly significant difference (HSD) post hoc test was applied to identify pairwise differences between treatments. Statistical analyses were performed using SPSS software, and differences were considered statistically significant at  $p < 0.05$ .

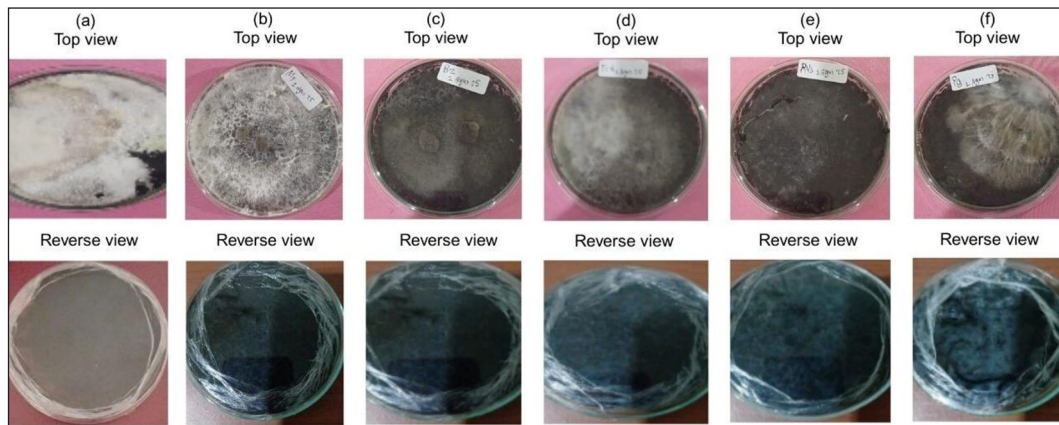
## RESULTS AND DISCUSSION

#### Initial macroscopic morphology of DSE fungi on PDA and MSM media amended with LDPE microplastics

Macroscopic observations revealed clear differences in colony growth patterns among the tested dark septate endophytic (DSE) fungi on PDA and on MSM amended with LDPE microplastics after 60 days of incubation (Figures 1–2). The isolates consisted of two strains affiliated with the order *Pleosporales* (Mg3 and BM2) and three strains of the genus *Cladosporium* (PNS, EC2, and Mg2). The non-inoculated control showed no mycelial growth on either medium, confirming the absence of fungal activity and serving as a baseline for comparison (Figures 1–2).

On PDA (without LDPE) (Figure 1), *Pleosporales* sp. strain Mg3 formed an intensely black colony with a compact surface and strong pigmentation on the reverse side, indicating pronounced





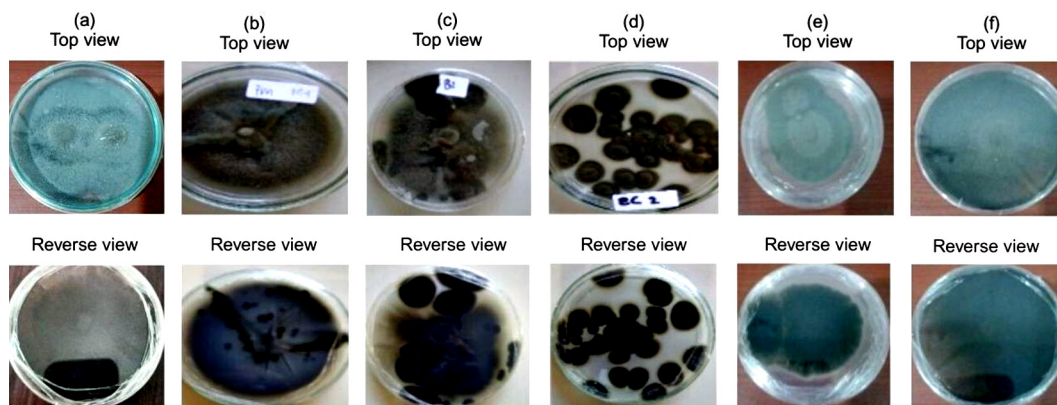
**Figure 1.** Macroscopic colony morphology of DSE fungi grown on PDA medium (without LDPE microplastics) after 60 days of incubation. For each panel, top and reverse views of colonies are presented. (a) Non-inoculated control; (b) *Pleosporales* sp. strain Mg3; (c) *Pleosporales* sp. strain BM2; (d) *Cladosporium* sp. strain PNS; (e) *Cladosporium* sp. strain EC2; (f) *Cladosporium* sp. strain Mg2

melanization. In contrast, *Pleosporales* sp. strain BM2 exhibited a more granular colony texture, characterized by distinct sporulation spots concentrated near the colony center. The *Cladosporium* isolates also displayed clear phenotypic differences: *Cladosporium* sp. strain PNS formed an olive-dark colony with dense granular texture and a distinct concentric zonation pattern, whereas *Cladosporium* sp. strain EC2 appeared darker with thinner mycelial coverage and comparatively lower sporulation. Similar isolate-specific growth patterns were observed on MSM amended with LDPE microplastics (Figure 2), suggesting that colony morphology varied with fungal identity under nutrient-limited conditions and in the presence of a hydrophobic polymer substrate.

Macroscopic traits such as colony pigmentation, texture, and sporulation are not merely

descriptive features but may reflect fungal adaptive strategies for persistence and surface colonization under nutrient-limited environments and on hydrophobic substrates such as polyethylene microplastics. Variations in colony color and sporulation intensity have been associated with fungal responses to substrate properties and environmental stress, including adaptation to hydrophobic surfaces (Gadd et al., 2024; Kowalski and Cramer, 2020). In particular, melanized fungi, including many DSE taxa, are frequently linked to higher tolerance to environmental constraints and oxidative conditions, which may support survival during long-term incubation with recalcitrant carbon sources (Baron et al., 2021; Malo et al., 2021).

From a biodeterioration perspective, effective polyethylene transformation typically requires an initial stage of surface attachment and



**Figure 2.** Macroscopic colony morphology of DSE fungi grown on MSM medium amended with LDPE microplastics after 60 days of incubation. For each panel, top and reverse views of colonies are presented. (a) Non-inoculated control; (b) *Pleosporales* sp. strain Mg3; (c) *Pleosporales* sp. strain BM2; (d) *Cladosporium* sp. strain PNS; (e) *Cladosporium* sp. strain EC2; (f) *Cladosporium* sp. strain Mg2

colonization, followed by oxidative modification that increases polymer-chain accessibility to extracellular enzymatic activity (Rong et al., 2024). Consistent with this framework, previous studies have reported that DSE-related taxa, particularly *Pleosporales* and *Cladosporium*, can contribute to polymer deterioration through oxidative enzyme production and hyphal interactions with plastic surfaces (Burov and Turkovskaya, 2022; Sathiyabama et al., 2024). Moreover, *Pleosporales* members have been associated with ligninolytic and redox enzyme systems (e.g., peroxidases and oxidoreductases) potentially involved in early oxidative steps required for polymer-chain scission (Miyauchi et al., 2018), while *Cladosporium* is recognized as an efficient colonizer of hydrophobic materials, including polyethylene (Khatua et al., 2024). Differences in sporulation among isolates may also influence dispersal and colonization efficiency on inert substrates (Hamiot et al., 2023). The macroscopic morphology shown in Figures 1–2 can therefore be positioned as an early indicator of fungal colonization. Accordingly, these observations are interpreted as supportive evidence that complements quantitative mass-loss measurements and the chemical and surface-morphological characterization of LDPE microplastics (FTIR and SEM) presented in subsequent sections (Philip et al., 2020; Santos et al., 2021).

#### LDPE microplastic mass loss after incubation with DSE isolates

LDPE microplastic mass loss was quantified after 60 days of incubation to evaluate the biodegradation potential of each DSE isolate (Figure 3). When expressed as a percentage of the initial LDPE mass, the non-inoculated control exhibited only negligible mass loss ( $0.8 \pm 0.25\%$ ), which is likely attributable to non-biological losses during recovery and handling rather than microbial activity. In contrast, all fungal treatments resulted in significantly higher LDPE mass loss than the control ( $p < 0.05$ ), indicating isolate-dependent effects on LDPE biodegradation. Among the tested fungi, *Cladosporium* sp. strain PNS showed the highest LDPE mass loss ( $35.0 \pm 2.0\%$ ), followed by *Cladosporium* sp. strain Mg2 ( $28.0 \pm 1.5\%$ ) and *Cladosporium* sp. strain EC2 ( $22.0 \pm 2.5\%$ ). The *Pleosporales* isolates also induced measurable LDPE mass loss, with *Pleosporales* sp.

strain BM2 ( $19.0 \pm 1.5\%$ ) significantly exceeding *Pleosporales* sp. strain Mg3 ( $16.5 \pm 2.0\%$ ).

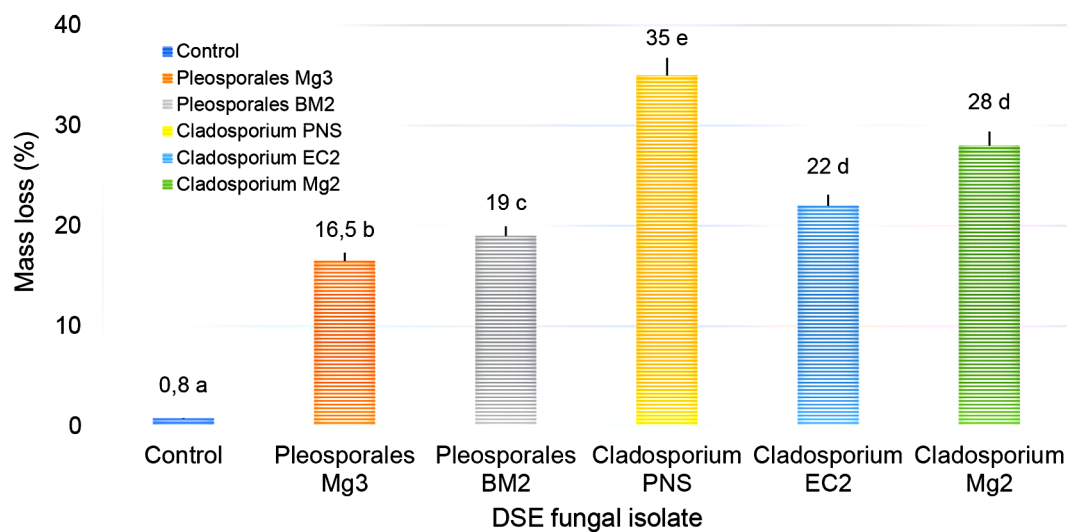
The statistically significant differences in LDPE mass loss among fungal isolates (Figure 3) highlight the superior biodegradation capability of *Cladosporium* spp., particularly strain PNS. The superior performance of the *Cladosporium* isolates is consistent with previous reports describing *Cladosporium* spp. as efficient colonizers of hydrophobic substrates and as fungi capable of initiating oxidative modification of LDPE surfaces. Puliga et al., 2023 reported that *Cladosporium* sp. CPEF-6 isolated from landfill environments could initiate LDPE degradation, evidenced by surface porosity and cracking as well as FTIR-detected functional group changes indicative of polyethylene oxidation. Such oxidation-driven surface modification is widely considered a prerequisite step that increases polymer susceptibility to further chain scission and mass reduction (Gong et al., 2023). In addition, *Cladosporium* spp. have been reported to produce oxidative enzymes and depolymerizing activities associated with deterioration of polyethylene materials (Stepnov et al., 2024), potentially accelerating fragmentation and measurable mass loss. Although the *Pleosporales* isolates showed lower mass loss than the best-performing *Cladosporium* strain, their effects remain mechanistically plausible. Many *Pleosporales* taxa are melanized DSE fungi and have been linked to ligninolytic and redox enzyme systems that may contribute to oxidation of hydrophobic and recalcitrant substrates, including synthetic polymers (Schlosser, 2020). LDPE mass loss data obtained from three biological replicates are summarized in Table 1, while comparative differences among fungal isolates are illustrated in Figure 3.

Importantly, mass loss alone does not confirm “ultimate biodegradation” (i.e., complete mineralization to  $\text{CO}_2$  and biomass); rather, it provides quantitative evidence of polymer deterioration and/or fragmentation under the applied conditions (Cho and Lee, 2025). Therefore, the mass-loss patterns observed in Figure 3 should be interpreted in conjunction with chemical FTIR and surface-morphological SEM evidence to support the occurrence of oxidative modification and structural disruption of LDPE microplastics by DSE fungi, particularly *Cladosporium* sp. strain PNS, which was selected for dose-dependent testing in subsequent analyses.

**Table 1.** LDPE microplastic mass loss after 60 days of incubation with DSE fungal isolates

Fungal isolate	Mass loss replicate 1 (g)	Mass loss replicate 2 (g)	Mass loss replicate 3 (g)	Mean $\pm$ SD (g)
Control (no inoculation)	0.00105	0.00080	0.00055	0.00080 $\pm$ 0.00025 <sup>a</sup>
<i>Pleosporeales</i> sp. strain Mg3	0.0185	0.0165	0.0145	0.0165 $\pm$ 0.0020 <sup>b</sup>
<i>Pleosporeales</i> sp. strain BM2	0.0205	0.0190	0.0175	0.0190 $\pm$ 0.0015 <sup>c</sup>
<i>Cladosporium</i> sp. strain PNS	0.0370	0.0350	0.0330	0.0350 $\pm$ 0.0020 <sup>e</sup>
<i>Cladosporium</i> sp. strain EC2	0.0245	0.0220	0.0195	0.0220 $\pm$ 0.0025 <sup>d</sup>
<i>Cladosporium</i> sp. strain Mg2	0.0295	0.0280	0.0265	0.0280 $\pm$ 0.0015 <sup>d</sup>

**Note:** values represent LDPE mass loss calculated from an initial mass of 0.100 g per replicate. Data are presented as mean  $\pm$  standard deviation (SD) from three biological replicates (n = 3). Different superscript letters indicate significant differences among treatments based on one-way ANOVA followed by Tukey's HSD test ( $p < 0.05$ ).



**Figure 3.** LDPE mass loss (g) after 60 days of incubation with different DSE fungal isolates, expressed as mean  $\pm$  SD from three biological replicates (n = 3). Different letters above bars indicate significant differences (one-way ANOVA followed by Tukey HSD,  $p < 0.05$ ). Bars sharing the same letter are not significantly different. The uninoculated control represents abiotic mass loss

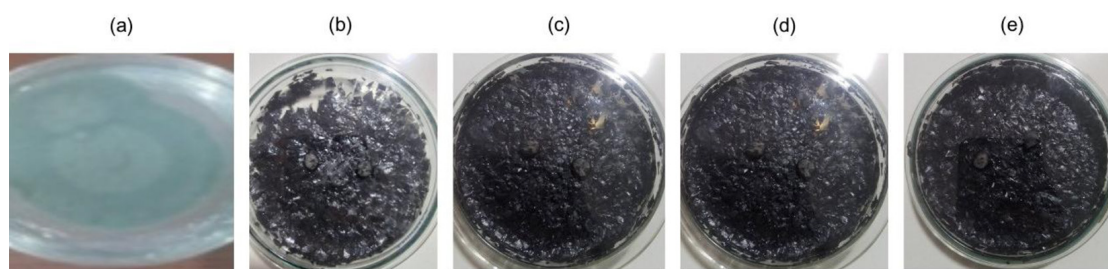
### Dose-dependent macroscopic response of the selected isolate (*Cladosporium* sp. strain PNS) under increasing LDPE microplastic loads

The selected isolate, *Cladosporium* sp. strain PNS, was further evaluated under increasing LDPE microplastic doses (0, 0.5, 1.0, 1.5, and 2.0 g per plate) following 60 days of incubation. Representative colony morphologies at each dose are shown in Figure 4. Overall, colony pigmentation intensity, surface coverage, and macroscopic texture changed progressively with increasing LDPE dose. In the absence of LDPE (0 g), colonies appeared brownish-black with darker pigmentation concentrated at the center (Figure 4a), reflecting baseline growth on the medium. At 0.5 g LDPE, colonies became intensely black with an uneven surface and visible cracking/dry appearance (Figure 4b). At 1.0

g LDPE, colonies remained intensely black but appeared denser and expanded to cover nearly the entire plate surface (Figure 4c). Further increases to 1.5 g resulted in very dense growth with diffuse colony margins (Figure 4d), while at 2.0 g colonies exhibited the most extensive coverage, pronounced surface cracking, a thicker central region with thinner peripheral margins, and very dark coloration consistent with abundant conidiation and/or pigmentation (Figure 4e).

A progressive increase in dark pigmentation and colony coverage indicates that *Cladosporium* sp. strain PNS tolerated elevated LDPE loads and maintained active surface colonization under polymer-rich conditions. Enhanced melanization and sporulation are commonly interpreted as adaptive responses that improve fungal persistence under stressful or recalcitrant





**Figure 4.** Macroscopic colony morphology of *Cladosporium* sp. strain PNS grown on MSM medium under increasing LDPE microplastic doses after 60 days of incubation. For each panel, top and reverse views of colonies are presented: (a) 0 g (no LDPE), (b) 0.5 g, (c) 1.0 g, (d) 1.5 g, and (e) 2.0 g LDPE per plate

substrate conditions (Perera et al., 2023). In addition, *Cladosporium* spp. have been reported as efficient colonizers of hydrophobic materials and have been associated with oxidative enzyme systems implicated in polymer surface modification (Albayrak Turgut and Örtücü, 2023). Therefore, the progressive changes in colony density and surface texture across LDPE doses may reflect strengthened colonization and physiological responses to LDPE microplastics.

Nevertheless, macroscopic morphology alone does not demonstrate polymer utilization or “ultimate biodegradation”; it primarily provides phenotypic evidence of tolerance and colonization potential (Ibrahim et al., 2024). Accordingly, the dose-dependent patterns shown in Figure 4 should be interpreted as supportive observations that complement quantitative mass loss and polymer alteration evidence (FTIR and SEM) presented in subsequent sections, consistent with reports that endophytic and DSE fungi can participate in microplastic transformation via extracellular oxidative and related enzymatic activities (Arif et al., 2024; Varshney et al., 2022).

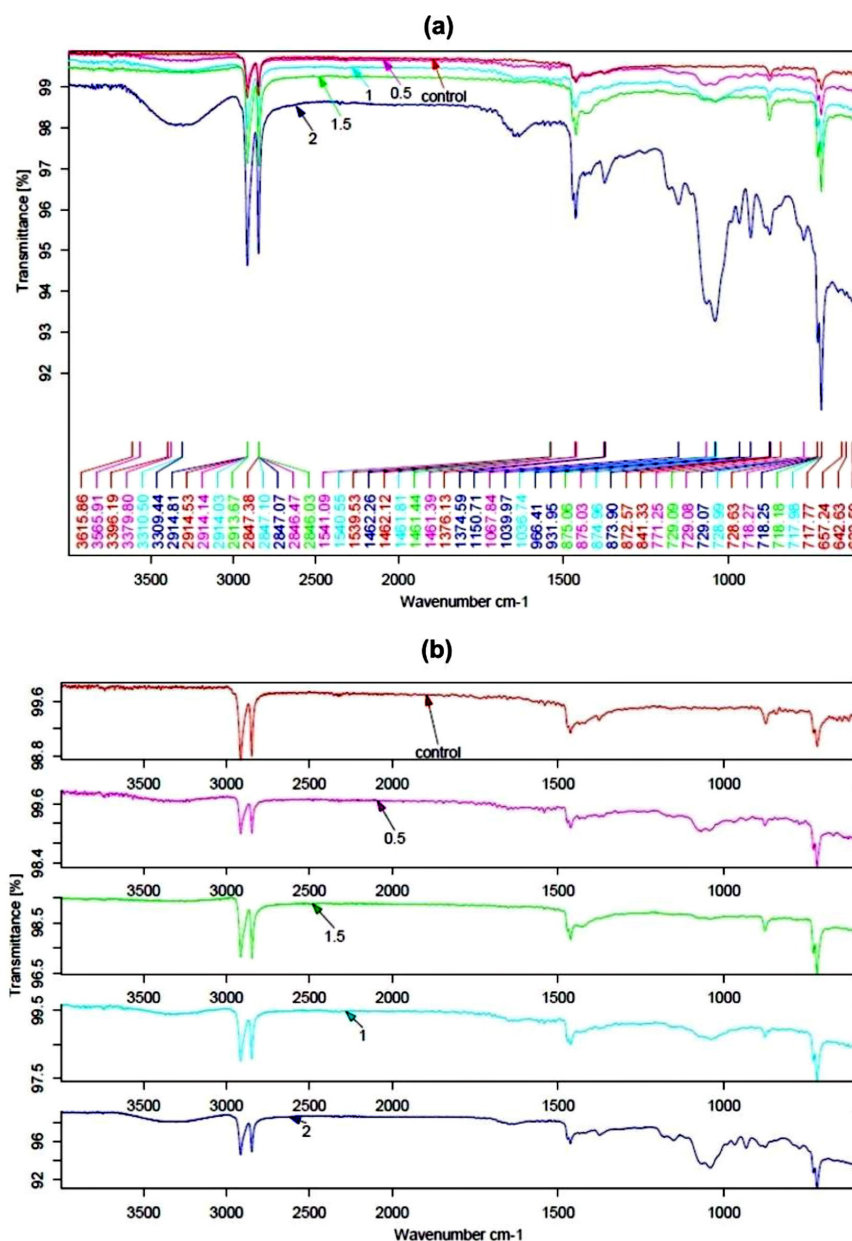
#### FTIR analysis of LDPE microplastics after incubation with *Cladosporium* sp. strain PNS

FTIR analysis was conducted to evaluate chemical modifications of LDPE microplastics after 60 days of incubation with *Cladosporium* sp. strain PNS at different LDPE doses (Figure 5). FTIR analysis was conducted to evaluate chemical modifications of LDPE microplastics after 60 days of incubation with *Cladosporium* sp. strain PNS at different LDPE doses (0.5, 1.0, 1.5, and 2.0 g). An abiotic LDPE control incubated without fungal inoculation was included for comparison. The FTIR spectrum of the abiotic LDPE control (incubated without fungal inoculation)

exhibited the characteristic absorption bands of intact LDPE, including strong aliphatic C–H stretching vibrations at 2915–2848  $\text{cm}^{-1}$ , C–H bending vibrations at 1470–1460  $\text{cm}^{-1}$ , and  $\text{CH}_2$  rocking vibrations at 720–730  $\text{cm}^{-1}$ , indicating preservation of the polyethylene backbone. Compared with the abiotic LDPE control, LDPE samples incubated with *Cladosporium* sp. strain PNS at increasing doses (0.5–2.0 g) showed progressive spectral changes. A decrease in transmittance intensity was observed at the characteristic C–H stretching bands ( $\sim 2920$  and  $\sim 2850$   $\text{cm}^{-1}$ ), suggesting alteration and partial disruption of hydrocarbon chains as a result of fungal activity. In addition, samples treated with 1.0–2.0 g LDPE exhibited band broadening in the region of 3300–3500  $\text{cm}^{-1}$ , which is indicative of O–H stretching vibrations associated with hydroxyl group formation. This observation implies the occurrence of oxidative processes on the LDPE surface. Subtle but discernible changes were also detected in the carbonyl region around 1700–1715  $\text{cm}^{-1}$ , particularly at higher LDPE doses (1.5 and 2.0 g), indicating the possible formation of carbonyl-containing functional groups such as ketones or aldehydes. These spectral changes were more pronounced at higher LDPE doses, suggesting a dose-dependent enhancement of oxidative modification due to increased contact between fungal biomass and polymer surfaces.

The FTIR spectral changes observed in this study indicate that *Cladosporium* sp. strain PNS induced oxidative modification of LDPE microplastics during incubation. The reduction in C–H band intensity, together with the appearance of O–H and C=O functional groups, is widely recognized as an early indicator of polyethylene oxidation and surface degradation. Such oxidative transformations increase polymer hydrophilicity and facilitate subsequent fragmentation processes.





**Figure 5.** ATR-FTIR spectra of LDPE microplastics after 60 days of incubation with *Cladosporium* sp. strain PNS under different LDPE doses (0.5–2.0 g): (a) overlay spectra showing dose-dependent changes, and (b) individual spectra for each treatment. An abiotic LDPE control incubated without fungal inoculation was used as the reference

Previous studies have demonstrated that fungal-mediated degradation of polyethylene commonly involves oxidative enzymes, including peroxidases and laccase-like oxidases, which initiate surface oxidation prior to polymer chain scission. Shah et al. (2008) reported that the emergence of carbonyl absorption bands is a key marker of microbial-induced polyethylene oxidation. Similarly, Ojha et al. (2017) observed decreased C–H band intensity and increased hydroxyl group formation during LDPE biodegradation by *Aspergillus* and *Penicillium*. The spectral patterns observed in the

present study are also consistent with the mechanisms described by Ghatge et al. (2020), who reported that fungi producing oxidative enzymes can induce O–H and C=O functional groups as critical precursors to polymer fragmentation.

The more pronounced FTIR changes observed at higher LDPE doses (1.5–2.0 g) suggest a dose-dependent response, in which greater substrate availability enhances fungal–polymer interactions and accelerates oxidative reactions at the polymer surface. Importantly, while FTIR provides strong chemical evidence of LDPE surface

modification, it does not alone confirm complete mineralization. Instead, these results should be interpreted as evidence of oxidative and structural transformation of LDPE microplastics, which complements the observed mass loss and supports subsequent SEM-based observations of surface deterioration. Overall, the FTIR analysis confirms that *Cladosporium* sp. strain PNS is capable of inducing oxidative modification of LDPE microplastics, reinforcing its potential role in fungal-driven transformation of polyethylene under controlled incubation conditions.

### SEM observations of LDPE microplastic surface deterioration after incubation with *Cladosporium* sp. strain PNS

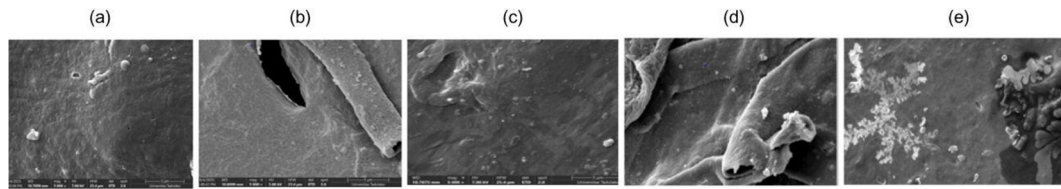
SEM was used to examine surface morphological changes of LDPE microplastics after 60 days of incubation with *Cladosporium* sp. strain PNS under increasing LDPE doses (0.5, 1.0, 1.5, and 2.0 g) (Figure 6). An abiotic LDPE control incubated under identical conditions without fungal inoculation was included for comparison. Clear dose-dependent surface deterioration was observed in the fungus-treated samples relative to the abiotic LDPE control. The abiotic control LDPE microplastics exhibited a relatively smooth and homogeneous surface without visible cracks, pits, or erosion features (Figure 6a). Compared with the abiotic LDPE control, early signs of surface alteration were apparent at 0.5 g LDPE, including fine scratches/striations and micro-cracks, accompanied by a slight increase in surface roughness (Figure 6b). At 1.0 g LDPE, cracks became more distinct and localized pitting features were observed, indicating progressive surface weakening relative to the lower dose (Figure 6c). More extensive deterioration was evident at 1.5 g LDPE, where wider and longer cracks, peeling/flaking areas, and pronounced erosion zones were present (Figure 6d). At 2.0 g LDPE, the most severe surface disruption was observed, characterized by large/deep cracks, cavity-like structures, and fragmentation/collapse features consistent with advanced biodeterioration (Figure 6e).

The progressive increase in surface roughness, cracking, pitting, and fragmentation across LDPE doses is consistent with a dose-dependent intensification of LDPE surface deterioration during incubation with *Cladosporium* sp. strain PNS. In fungal–polyethylene interaction studies,

such surface features are commonly interpreted as physical manifestations of biodeterioration that can arise from sustained surface colonization, localized oxidative reactions, and subsequent weakening of the polymer matrix (Sathiyabama et al., 2024). Importantly, SEM provides morphological evidence of LDPE surface disruption but does not, by itself, confirm polymer mineralization; therefore, these observations must be interpreted together with LDPE mass loss and FTIR-detected oxidative functional group changes, which collectively support oxidative modification and structural transformation of LDPE microplastics. These SEM findings should be interpreted together with the observed LDPE mass loss and FTIR-detected functional group changes (appearance of O–H and carbonyl-related bands), which collectively support oxidative modification and structural transformation of LDPE microplastics under the tested conditions (Dailianis et al., 2024; Ghatge et al., 2020). Taken together, the concordance among mass loss, FTIR oxidation signatures, and SEM-observed surface deterioration strengthens the evidence that *Cladosporium* sp. strain PNS promotes dose-dependent LDPE microplastic biodeterioration over the 60-day incubation period (Bajo et al., 2024).

### Integrated interpretation and ecological engineering relevance

The combined evidence from LDPE mass loss measurements, FTIR analysis, and SEM observations demonstrates a coherent and dose-dependent pattern of LDPE microplastic biodeterioration mediated by *Cladosporium* sp. strain PNS. Quantitative mass loss data revealed a clear increase in LDPE weight reduction relative to the abiotic control, indicating that fungal activity contributed substantially to polymer deterioration rather than non-biological handling losses (Morales Ramos et al., 2024). This quantitative trend was further supported by FTIR spectra showing progressive attenuation of characteristic aliphatic C–H bands, accompanied by the emergence of O–H and carbonyl-related functional groups, which are widely recognized as indicators of oxidative modification of polyethylene surfaces. SEM micrographs provided complementary morphological evidence, revealing progressive surface roughening, cracking, pitting, and fragmentation with increasing LDPE dose,



**Figure 6.** SEM micrographs of LDPE microplastics after 60 days of incubation: (a) abiotic LDPE control (incubated without fungal inoculation); (b–e) LDPE treated with *Cladosporium* sp. strain PNS under increasing LDPE doses, showing progressive surface roughening, cracking, pitting, and fragmentation. Images were acquired at 5.000× magnification

consistent with physical manifestations of oxidative surface weakening (Benmiloud et al., 2020; Chabira et al., 2019).

Taken together, these results support a mechanistic framework in which initial fungal colonization and surface attachment facilitate localized oxidative reactions at the LDPE interface, leading to chemical modification (increased hydrophilicity and functional group diversity) and subsequent structural deterioration of the polymer matrix. While the present study does not assess ultimate mineralization, the concordance among mass loss, FTIR-detected oxidation signatures, and SEM-observed surface disruption provides robust evidence of LDPE microplastic biodeterioration under controlled incubation conditions.

From an ecological engineering perspective, these findings highlight the potential of dark septate endophytic fungi as biological agents for initiating microplastic transformation in soil-related systems, in line with recent studies that emphasize the need for environmentally compatible and sustainable approaches to mitigate microplastic contamination (Ramadani et al., 2025). DSE fungi are naturally adapted to nutrient-limited environments and plant-associated niches, suggesting that their oxidative and colonization capabilities could be harnessed within engineered soil–plant–microbe systems to accelerate the early stages of microplastic breakdown (Chabira et al., 2019; Ermis et al., 2024). Such an approach aligns with ecological engineering principles that emphasize the use of native or ecologically compatible organisms to enhance ecosystem functions, including pollutant transformation and soil health restoration (Nanlohy et al., 2024). Consequently, *Cladosporium* sp. strain PNS represents a promising candidate for further evaluation in plant-associated or soil-based bioremediation frameworks targeting LDPE microplastic contamination.

## CONCLUSIONS

This study demonstrated that DSE fungi are capable of inducing biodeterioration of LDPE microplastics under controlled incubation conditions. The integrated evaluation based on mass loss measurements, FTIR, and SEM provided consistent and mechanistic evidence of LDPE surface deterioration, including oxidative modification and structural disruption. Among the tested isolates, *Cladosporium* sp. strain PNS exhibited the highest biodeterioration performance, which was further supported by dose-dependent changes in colony morphology, chemical functional groups, and surface damage features. Although the present study did not assess ultimate mineralization of LDPE, the concordance among quantitative mass loss, FTIR-detected oxidation signatures, and SEM-observed surface deterioration clearly indicates fungal-mediated transformation of LDPE microplastics. From an ecological engineering and environmental technology perspective, these findings highlight the potential of DSE fungi as environmentally compatible biological agents for initiating early-stage microplastic transformation in soil-related systems and provide a foundation for future development of sustainable, soil-oriented strategies for LDPE microplastic remediation.

## Acknowledgements

The authors gratefully acknowledge the Institute for Research and Community Service (LPPM), Universitas Tadulako, for financial support provided for this research through the DIPA BLU budget of Universitas Tadulako, in accordance with the Rector's Decree of Universitas Tadulako No. 4620/HK.02/2025, dated 2 June 2025.



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