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Time-Dependent Polyethene Terephthalate (PET) Biodegradation by *Aspergillus niger* and *Aspergillus oryzae* Under Solid State Fermentation

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ABSTRACT

Polyethene terephthalate (PET) is a major plastic pollutant that contributes to environmental problems such as landfill accumulation and microplastic accumulation due to its non-biodegradable nature. This study investigates fungal-mediated PET biodegradation via solid-state fermentation (SSF) as a sustainable, eco-friendly waste management approach. The effects of fermentation time on PET weight reduction were evaluated using *Aspergillus niger* and *Aspergillus oryzae* individually over a 10-week SSF period. Xylanase activity was monitored to assess fungal enzymatic activity during PET treatment, while attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) and field emission scanning electron microscopy (FESEM) were employed to evaluate chemical and surface morphological changes. PET obtained from mineral water bottles was used as the substrate, supplemented with sucrose and yeast extract to promote fungal growth. *A. oryzae* exhibited the highest PET weight loss of 1.91% and xylanase activity of 467.8 U/mL at week 4, indicating effective early-stage PET modification. Meanwhile, *A. niger* achieved a higher peak xylanase activity of 641.9 U/mL at week 2 but showed a lower maximum PET weight loss of 0.76% at week 6. ATR-FTIR and FESEM analyses confirmed structural and surface modifications after fungal treatment,

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with *A. oryzae* showing a greater PET weight reduction than *A. niger*, which demonstrated more pronounced surface roughening and fungal colonisation. These findings highlight the potential of SSF as a low-cost, sustainable strategy to advance fungal-based PET biodegradation.

Keywords: Aspergillus niger, *Aspergillus oryzae*, Polyethene terephthalate, solid state fermentation, xylanase

INTRODUCTION

Plastics have become an essential part of daily life due to their wide applications in packaging, construction, healthcare, automotive, textiles, and electronics. They are generally composed of synthetic or semi-synthetic organic compounds that can be moulded into different shapes and forms. Their structure consists of monomers linked together to form long polymer chains (Noorjahan et al., 2022). The international market demand for plastics has surged fourfold over the past decades due to their versatility, durability, and low cost, with demand projected to double by 2050 (Jaynes, 2025). The most used plastic across many industries is polyethene terephthalate (PET). PET is a thermoplastic polymer derived from the polyester family, and it is produced by polymerising terephthalic acid and ethylene glycol or by transesterification of dimethyl terephthalate and EG (Hiraga et al., 2019). The global PET market was estimated at USD 39.12 billion in 2024 and is expected to grow at a compound annual growth rate (CAGR) of 5.6% from 2025 to 2030, reaching USD 54.47 billion (Grand View Research, 2024). This growth is driven by PET's outstanding phytochemical properties, such as chemical resistance, impermeability to liquids and gases, higher density than water, resistance to acids and bases and good thermal stability (Hiraga et al., 2019; Jovanović et al., 2025). The Asia Pacific PET market, especially China, commanded the international market and accounted for the largest share of turnover, at 38.0% in 2024, in sectors such as packaging, automotive, electronics, and healthcare (Jovanović et al., 2025).

Despite the widespread use and benefits of plastics in industries, plastic waste has become a major environmental concern. For example, single-use plastic bags are often used for only a short period before being discarded, but they can remain in the environment for many years without degrading. Each year, an estimated 14 million tonnes of plastic waste enter the oceans, causing harmful effects on marine organisms and their populations. This amount is comparable to the weight of five blue whales entering the environment every hour (Davison, 2024). PET is one of the top contributors to landfill waste, soil contamination, ocean debris, greenhouse gas emissions, and air pollution (Lionetto & Esposito Corcione, 2021). PET is widely used in food containers and beverage bottles, significantly impacting aquatic and terrestrial ecosystems. These PET wastes are gradually emerging as an environmental hazard (Jovanović et al., 2025). The density of solid PET is 1.27 g/cm^3, and it breaks down more slowly, leading to accumulation on the ocean floor,

whereas empty PET bottles and containers float on the sea surface (Hiraga et al., 2019). Surface fragmentation is a major contributor to microplastic pollution. For example, PET-based textiles that continuously release microfibers during washing will eventually reach the ocean floor and settle there (Maurya et al., 2020). Over 500 billion PET plastic bottles are used annually; however, many are not fully recycled and are instead illegally dumped into the ocean, posing a significant threat to marine species through entanglement and ingestion (Lionetto & Esposito Corcione, 2021).

Currently, PET waste is treated using various methods, including incineration, chemical and mechanical processing, burning, oxidative degradation, thermal degradation, and photodegradation (Ekanayaka et al., 2022; Zeng et al., 2023). Among these methods, biodegradation is considered a promising approach because of its pollution-free, environmentally friendly nature (Srikanth et al., 2022). Biodegradation of plastics is referred to as microbial plastic degradation, as it involves microorganisms that break them down into organic byproducts such as water and carbon dioxide (Bahl et al., 2021). One of the most effective biodegradation methods is solid-state fermentation (SSF), using filamentous fungi. Fungi can grow on insoluble substrates or solid surfaces with little to no free water (Sadh et al., 2018). SSF substrates are primarily lignocellulosic materials that promote the production of lignocellulolytic enzymes, including cellulase, xylanase, cutinase, and β-glucosidase, which are crucial for plastic degradation. The related microorganisms will release enzymes and pro-oxidant ions to facilitate the breakdown of plastic (Chandren et al., 2025; Safdar et al., 2024). The related microorganisms will release enzymes and some pro-oxidant ions, which will degrade the plastics (Srikanth et al., 2022). Biodegradation is a cost-effective and efficient process for plastic disposal compared to traditional methods like incineration or landfilling. Additionally, the secondary products generated by SSF biodegradation are often more economically valuable than those from incineration or landfilling. Biodegradation is a complex process involving several mechanisms, including depolymerisation, mineralisation, and biodeterioration. During this process, microorganisms utilise synthetic polymers as a carbon source, resulting in microbial biomass formation (Sanniyasi et al., 2021; Webb et al., 2013).

Although numerous studies have investigated bacterial PET degradation, comparatively fewer studies have examined fungal-mediated PET degradation under solid-state fermentation (SSF). Furthermore, although fungal extracellular enzyme production has been frequently reported, the temporal relationship between enzyme activity and PET surface modification during prolonged SSF remains poorly understood. Limited information is available on how changes in extracellular enzymatic activity correspond with physicochemical alterations of PET throughout the fermentation process. PET, due to its strong hydrophobicity and long carbon chain, is highly resistant to biodegradation. Fungal species such as Aspergillus, Acremonium, Fusarium, Penicillium, and Phanerochaete

have shown potential for PET degradation due to their enzymatic versatility (Zhang et al., 2020). The biodegradation of PET involves PET breakdown into mono-(2-hydroxyethyl) terephthalate (MHET), bis- (2- hydroxyethyl) terephthalate (BHET), and ethylene glycol (EG) by PETase. Then, MHETase hydrolyses the MHET into terephthalic acid (TPA) and EG, which are transported into cells and converted into protocatechuic acids (PCAs). These compounds are subsequently integrated into cellular metabolism via the tricarboxylic acid (TCA) cycle (Tegariyanto et al., 2024). *Aspergillus niger* and *Aspergillus oryzae* have the potential to degrade PET because they can grow freely in soil and at garbage sites. *Aspergillus* species produce hydrophobin protein and hydrolase enzymes that can decrease the hydrophobic properties of plastics by hydrolysing C-C and C-O bonds, which act as catalysts for the degradation of high molecular weight plastics into smaller fragments (D'Souza et al., 2021; Zhang et al., 2020). Therefore, this study investigated the time-dependent biodegradation potential of PET by *Aspergillus niger* and *Aspergillus oryzae* under solid-state fermentation. PET surface modification was evaluated over a 10-week fermentation period using weight-loss measurements, xylanase activity as an indicator of extracellular enzymatic activity, ATR-FTIR spectroscopy, and FESEM analysis to provide complementary evidence of fungal-induced changes to the PET surface.

MATERIALS AND METHODS

Preparation of PET strips

Polyethene terephthalate (PET) samples were obtained from post-consumer mineral water bottles collected within the Universiti Selangor (UNISEL) campus. The bottles underwent cleaning, such as washing with tap water to remove visible contaminants, and the labels and caps were removed. The PET bottles were cut into strips measuring approximately 2 × 2 cm. The strips were cleansed by washing with tap water, followed by distilled water, and air-dried at room temperature. For surface sterilisation, the PET strips were immersed in 70% ethanol for 30 min, rinsed with sterile distilled water, and exposed to ultraviolet (UV) light for 30 min. The sterilised strips were then dried in a hot-air oven at 40 °C for 1 h before being transferred to a desiccator containing silica gel. The initial weight of each PET strip was recorded prior to use in the biodegradation experiment (Chandren et al., 2025; Nag et al., 2021).

Preparation of Media and Fungi

Potato dextrose agar (PDA) was prepared by dissolving 7.8 g of PDA powder (TM MEDIA) in 200 mL of distilled water. The medium was sterilised by autoclaving at 121 °C for 15 min and then poured aseptically into sterile Petri dishes. After solidification, *Aspergillus niger* and *Aspergillus oryzae* were subcultured onto separate PDA plates.

The *A. niger* cultures were incubated at 30 °C for 5 days. *oryzae* cultures were incubated at 35 °C for 7 days to obtain sufficient fungal growth for inoculum preparation (Chandren et al., 2025; Ma et al., 2021; Shinkawa & Mitsuzawa, 2020). Following the incubation period, fungal spore suspensions were prepared from fully grown cultures of *Aspergillus niger* and *Aspergillus oryzae*. After sufficient sporulation was observed, the fungal spores were collected by adding 10 mL of sterile distilled water to each petri dish. The spores were gently dislodged from the culture surface with a sterile loop, and the suspension was adjusted to the required volume with sterile distilled water before use in subsequent experiments (Ahmed et al., 2025). The resulting spore suspension was filtered through a sterile filter funnel lined with sterile filter paper to remove mycelial fragments and obtain a homogeneous spore suspension. The filtered suspension was collected in a sterile conical flask and used immediately as the fungal inoculum for subsequent solid-state fermentation experiments (Latif et al., 2025).

Solid State Fermentation (SSF)

Solid-state fermentation (SSF) was performed independently with *Aspergillus niger* and *Aspergillus oryzae* as separate cultures, with each fermentation flask containing a total mixture of 10 g, including 3 g of solid components and 7 mL of liquid. The solid components consisted of 2.8 g of PET strips supplemented with 0.1 g each of sucrose and yeast extract as supplementary nutrients. Because sucrose and yeast extract were supplied in the fermentation medium, the present experimental design does not establish PET as the sole or primary carbon source for fungal growth. SSF was conducted at an initial moisture content of 70%. To achieve 70% moisture content, 6 ml of sterilised distilled water will be added to the fermentation medium, followed by inoculation of 1 mL spore suspension. The sucrose, yeast extract, and distilled water were prepared as a nutrient solution and sterilised separately by autoclaving at 121 °C for 20 min. The PET strips were not autoclaved and were instead surface treated with 70% ethanol, followed by UV irradiation, as described in Section 2.1. The use of ethanol-treated PET samples in fungal biodegradation experiments has also been reported by Safdar et al. (2024). After sterilisation and cooling of the nutrient solution, the sterile nutrient solution was aseptically combined with the prepared PET strips, followed by the addition of 1 mL of the respective fungal spore suspension. The contents were mixed aseptically to distribute the moisture and inoculum throughout the substrate.

Separate fermentation experiments were performed for *A. niger* and *A. oryzae*. The *A. niger*-inoculated flasks were incubated at 30 °C, whereas the *A. oryzae*-inoculated flasks were incubated at 35 °C. For each fungal species, 18 fermentation flasks were prepared, consisting of three independent biological replicates for each incubation period of 0, 2, 4, 6, 8, and 10 weeks (Chandren et al., 2025). PET samples were recovered at each sampling interval for the determination of PET weight loss, extracellular xylanase activity,

ATR-FTIR characterisation, and FESEM analysis. Similar approaches using fungal incubation followed by weight loss, FTIR, and SEM-based characterisation of PET degradation have been reported previously (Maheswaran et al., 2023; Safdar et al., 2024). A non-inoculated sample containing PET and the corresponding fermentation components was used as the week 0 reference to establish the initial condition. Since this sample was assessed only in week 0, it served as a baseline reference rather than an abiotic control throughout the fermentation period.

Recovery and Preparation of PET Strips After Fermentation

After each incubation period, the PET strips were carefully recovered from the fermentation flasks and separated from the fungal biomass. The recovered PET strips were first washed with 70% ethanol to remove fungal cells and lose biomass, then thoroughly rinsed with tap water to eliminate residual ethanol and other adherent materials. The washing procedure was applied consistently to all recovered PET samples (Ahmed et al., 2025; Rojas-Villacorta et al., 2025). Following washing, the PET strips were dried in a hot-air oven at 40 °C until a constant weight was achieved. The dried samples were subsequently transferred to a desiccator containing silica gel to minimise moisture absorption before weighing. The final dry weight of each recovered PET strip was recorded to determine the gravimetric PET weight loss (Zulkafli et al., 2024).

Determination of the Weight Loss of the PET Strips

The initial and final weights of PET strips from each fermentation flask were measured using an analytical balance before and after the solid-state fermentation process, respectively. The initial weight (W_i) was recorded prior to fungal inoculation, while the final weight (W_f) was obtained after recovery, washing, and drying of the PET strips following the fermentation period (D'Souza et al., 2021). The percentage of PET weight loss was calculated for each individual replicate using Equation 1:

$$Loss\ of\ weight\ (\%) = \frac{[(Wi - Wf) \times 100]}{Wi} \quad [1]$$

where Wi represents the initial dry weight of PET strips, and W_f represents the final dry weight of PET strips after fermentation. Three independent fermentation flasks were analysed for each incubation period, and the average percentage of weight loss was calculated from the triplicate measurements. The results were expressed as mean ± standard deviation. The gravimetric method was used to quantify changes in the mass of recovered PET following fungal treatment. Because gravimetric mass loss alone cannot distinguish polymer degradation from other potential sources of mass change, such as incomplete

biomass removal, washing-related variation, or the physical loss of small PET fragments, the measured parameter was interpreted as PET weight loss rather than as definitive evidence of PET biodegradation.

Xylanase Assay

Extracellular xylanase activity during SSF was measured using the 3,5-dinitrosalicylic acid (DNS) method as described by Miller (1959), with xylan serving as the substrate. The activity was monitored in accordance with established protocols (Bailey et al., 1992) as an indicator of extracellular enzyme production by the fungi. Since the assay quantified reducing sugars released from xylan hydrolysis rather than PET breakdown, the xylanase activity was not used as a measure of PET depolymerisation and was analysed separately from PET weight loss.

After each fermentation period, 70 mL of distilled water was added directly into each fermentation flask containing the fermented substrate. The flask contents were manually shaken to facilitate the extraction of extracellular enzymes into the aqueous phase. The extract was then filtered to separate the PET strips and fungal biomass from the liquid fraction. The collected liquid extract was centrifuged at 15,000 rpm for 10 min to remove suspended particles. The resulting supernatant was further filtered using a vacuum filtration system with Whatman No. 1 qualitative filter paper (90 mm diameter; Cat. No. 1001-090) to obtain a clarified enzyme extract used for xylanase analysis. The xylanase assay was performed by mixing 0.5 mL of crude enzyme extract with 1.0 mL of 1% corn Xylan prepared in 0.05 M citrate buffer. The reaction mixture was incubated in a water bath at 50 °C for 20 min. Subsequently, 3 mL of dinitrosalicylic acid (DNS) reagent was added to terminate the enzymatic reaction, and the mixture was heated at 100 °C for 5 min to develop colour. After cooling, the absorbance was measured at 540 nm using a spectrophotometer. Xylanase activity was calculated based on the amount of reducing sugar released. One unit (U) of xylanase activity was defined as the amount of enzyme required to release 1 µmol of D-xylose per minute under the assay conditions. The enzyme activity was expressed as U/mL (Chandren et al., 2025). The enzyme activity for each sample was calculated utilising the formula given in Equation 2.

$$\text{Xylanase activity} = \frac{\mu\,\text{mol}}{\text{mL}} = \frac{\text{U}}{\text{ml}} = \left(\text{xylose concentration}\,\frac{\text{g}}{\text{mL}}\right) \times \frac{1}{0.5\,\text{mL}} \times \frac{1}{20\,\text{min}} \times \frac{1\,\text{mol}}{150.13\,\text{g}} \qquad [2]$$

Statistical Analysis

All experiments were conducted using three independent biological replicates for each fermentation period. Data on PET weight loss and extracellular xylanase activity were

analysed using IBM SPSS Statistics. Differences in PET weight loss and xylanase activity among fermentation periods were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test where appropriate. Statistical significance was considered at $p < 0.05$. Pearson correlation analysis was performed as an exploratory analysis to assess the linear association between PET weight loss and xylanase activity during the fermentation period. Because xylanase is not a PET-specific depolymerising enzyme, any observed correlation was interpreted only as a statistical association between the two measured variables and was not considered evidence of a causal relationship between xylanase activity and PET degradation (Agbangba et al., 2024).

Attenuated Total Reflectance – Fourier Transformed (ATR-FTIR) Analysis

The structural changes of PET samples before and after solid-state fermentation (SSF) were analysed using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy. Following the fermentation period, PET samples were collected, washed with distilled water to remove attached fungal biomass and residual fermentation components, and dried prior to analysis. The dried PET samples were placed directly onto the ATR crystal of a PerkinElmer FTIR spectrometer for spectral analysis. The FTIR spectra were recorded over a wavenumber range of 4000–400 cm^{-1}, and the resulting spectra were presented as transmittance (%) against wavenumber (cm^{-1}). Changes in the characteristic absorption bands associated with PET functional groups were compared between untreated PET samples and PET samples subjected to SSF. The obtained spectra were used to evaluate alterations in the chemical structure of PET following fungal treatment (D'Souza et al., 2021).

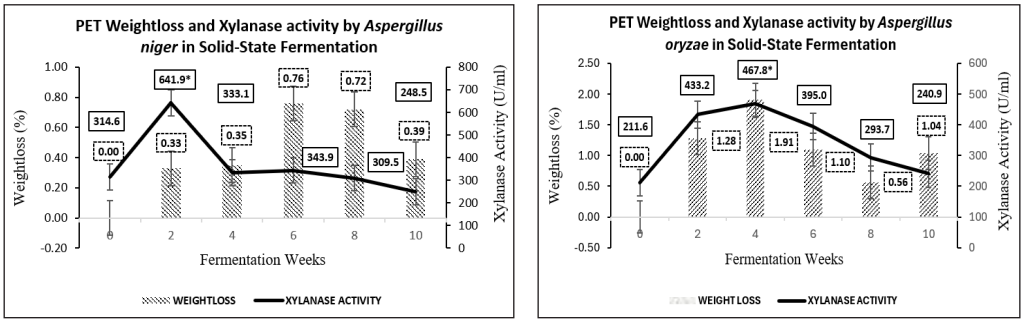
Field Emission Scanning Electron Microscopy (FESEM) Analysis

The surface morphology of untreated PET and PET samples recovered after solid-state fermentation (SSF) was examined using field emission scanning electron microscopy (FESEM). Following the fermentation period, the PET strips were carefully removed from the fermentation flasks, washed thoroughly with distilled water to remove loosely attached fungal biomass and residual fermentation medium, and dried in a hot-air oven at 40 °C until a constant weight was achieved. The dried PET samples were mounted onto aluminium stubs using conductive carbon adhesive tape and sputter-coated with a thin layer of gold to improve surface conductivity before imaging. The surface morphology of the samples was then observed using a field-emission scanning electron microscope at an appropriate acceleration voltage. Micrographs of untreated PET and fungal-treated PET were compared to evaluate changes in surface morphology, including fungal attachment, surface roughness, cracks, pits, and erosion patterns following SSF (Pandey et al., 2021).

RESULTS AND DISCUSSION

PET Weight Reduction and Xylanase Activity by *Aspergillus niger* and *Aspergillus oryzae* During Solid-State Fermentation

The changes in PET weight reduction and extracellular xylanase activity during solid-state fermentation (SSF) using *Aspergillus niger* and *Aspergillus oryzae* for a 10-week incubation period are illustrated in Figure 1a and Figure 1 b, respectively. The corresponding mean values and standard deviations (SD) obtained from triplicate experiments are summarised in Table 1. PET weight reduction was monitored to evaluate the effect of fungal treatment on PET samples, whereas xylanase activity was measured to assess extracellular enzyme production throughout the fermentation period.



(a) (b)

Figure 1. PET weight loss and xylanase activity: (a) PET weight loss and xylanase activity by *Aspergillus niger*; (b) PET weight loss and xylanase activity by *Aspergillus oryzae*

Figure 1a shows that the mean PET weight loss for *A. niger* increased from 0.00 ± 0.00% at week 0 to 0.76 ± 0.84% at week 6, followed by a slight decrease to 0.72 ± 0.32% and 0.39 ± 0.27% at weeks 8 and 10, respectively. Although an increasing trend was observed during the early stages of fermentation, one-way ANOVA showed that the fermentation period did not significantly affect PET weight loss ($F(5, 12) = 1.583$, $p = 0.238$). Similarly, *A. oryzae* showed an increase in mean PET weight loss from 0.00 ± 0.00% at week 0 to a maximum of 1.91 ± 1.43% at week 4, followed by decreases to 1.07 ± 0.54%, 0.56 ± 0.49%, and 1.04 ± 0.93% at weeks 6, 8, and 10, respectively (Figure 1b). The fermentation period also did not significantly affect PET weight loss in *A. oryzae* ($F(5, 12) = 1.497$, $p = 0.262$). Nevertheless, the numerically higher PET weight loss observed for *A. oryzae*, particularly at week 4, may indicate greater interaction or colonisation of the PET surface under the conditions tested. Previous studies have reported that filamentous fungi can interact with polymer surfaces through fungal colonisation, extracellular enzyme secretion, and biofilm formation, potentially altering

polymer surface characteristics (Safdar et al., 2024). *A. oryzae* has also been reported to exhibit active extracellular hydrolase production during cultivation, which may contribute to its interaction with complex substrates (Maurya et al., 2020; Srikanth et al., 2021). PET weight loss alone does not provide definitive evidence of PET biodegradation. ATR-FTIR and FESEM were therefore used as complementary techniques to characterise chemical and morphological changes associated with fungal treatment; however, these techniques also cannot independently confirm PET depolymerisation.

Xylanase activity showed a different temporal pattern from PET weight loss. In *A. niger*, xylanase activity increased from 314.63 ± 86.14 U mL^{-1} at week 0 to a maximum of 641.90 ± 100.12 U mL^{-1} at week 2, followed by decreases to 334.13 ± 5.10, 343.90 ± 20.22, 309.53 ± 13.11, and 248.47 ± 23.31 U mL^{-1} at weeks 4, 6, 8, and 10, respectively. One-way ANOVA indicated a significant effect of fermentation period on xylanase activity ($F(5, 12) = 18.830$, $p < 0.001$), with Tukey's HSD showing that xylanase activity at week 2 was significantly higher than that observed at the other fermentation periods ($p < 0.05$). In *A. oryzae*, xylanase activity increased from 211.63 ± 0.75 U mL^{-1} at week 0 to a maximum of 470.50 ± 17.23 U mL^{-1} at week 4, followed by decreases to 394.97 ± 60.00, 293.73 ± 4.69, and 240.90 ± 30.24 U mL^{-1} at weeks 6, 8, and 10, respectively. Fermentation period significantly affected xylanase activity in *A. oryzae* ($F(5, 12) = 42.086$, $p < 0.001$), with Tukey's HSD indicating significant differences between several fermentation periods ($p < 0.05$). The higher enzyme activity observed during the early fermentation stages may reflect active extracellular enzyme production, whereas the subsequent decline may be associated with nutrient depletion or changes in fungal metabolic activity during prolonged fermentation (Maurya et al., 2020; Srikanth et al., 2021).

Xylanase activity exhibited temporal patterns that differed from those observed for PET weight loss. In *A. niger*, maximum xylanase activity occurred at week 2, whereas the highest numerical PET weight loss occurred at week 6. In *A. oryzae*, maximum xylanase activity and the highest numerical PET weight loss were both observed at week 4. However, because xylanase is not a PET-specific depolymerising enzyme, these temporal patterns were not interpreted as evidence of a direct relationship between xylanase production and PET degradation. Xylanase activity was therefore considered an indicator of extracellular enzyme production during SSF rather than a measure of PET hydrolysis. The differences between xylanase activity and PET weight-loss patterns may reflect the involvement of multiple factors, including fungal colonisation, other extracellular enzymes, physicochemical changes at the PET surface, and changes in substrate accessibility during SSF. Therefore, the observed PET weight reduction should be interpreted as evidence of PET mass change associated with fungal treatment rather than definitive proof of biodegradation. Complementary analyses such as FTIR and FESEM may provide additional evidence of

changes in the chemical characteristics and surface morphology of PET following fungal treatment (Safdar et al., 2024). Overall, the results demonstrate that fermentation time significantly influenced xylanase production in both fungal species, whereas its effect on PET weight loss was not statistically significant under the conditions tested.

Attenuated Total Reflectance – Fourier Transformed (ATR-FTIR) Analysis of PET Samples

A comparative analysis of the ATR-FTIR spectra of untreated PET and PET samples recovered after solid-state fermentation is presented in Figures 2, 3, and 4.

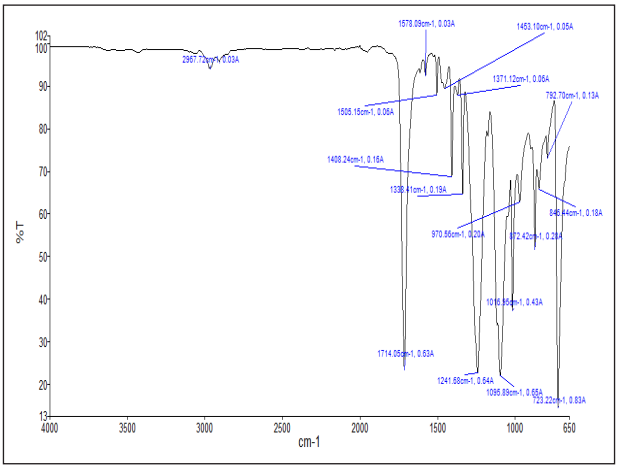


Figure 2. ATR-FTIR image of PET samples before solid-state fermentation

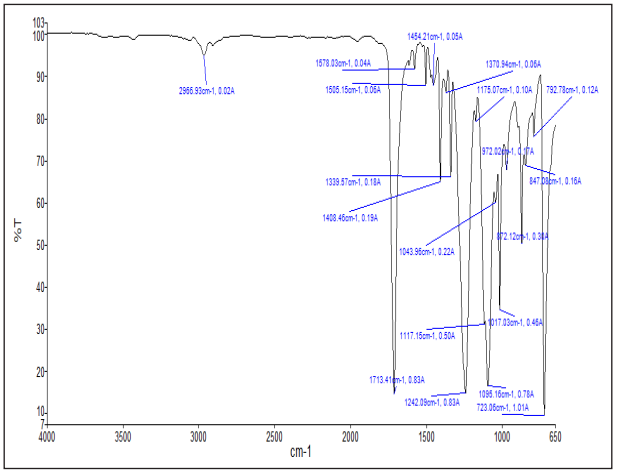


Figure 3. ATR-FTIR image of PET samples treated with *Aspergillus niger* after solid-state fermentation

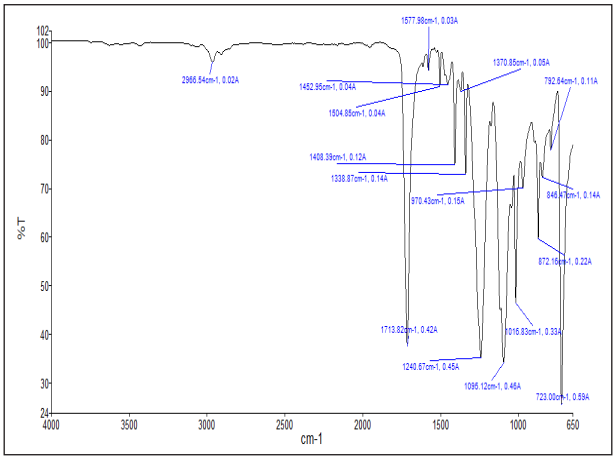


Figure 4. ATR-FTIR image of PET samples treated with *Aspergillus oryzae* after solid-state fermentation

The ATR-FTIR spectrum of the untreated PET sample showed characteristic absorption bands associated with the chemical structure of polyethene terephthalate (PET), including the ester carbonyl (C=O) stretching vibration at approximately 1710–1715 cm^{-1}, C–O stretching vibrations within the 1300–1100 cm^{-1} region, and characteristic aromatic ring vibrations. These absorption bands are consistent with the chemical structure of PET reported in previous studies (D'Souza et al., 2021; Sivan, 2011). Following fungal treatment, slight changes in the ATR-FTIR spectra were observed for PET exposed to both *A. niger* and *A. oryzae*. The observed changes in the intensity and/or position of some absorption bands, particularly those associated with ester functional groups, may indicate alterations in the chemical environment of the PET surface following fungal exposure. Such changes may result from localised interactions between the fungal biomass, extracellular metabolites, and the polymer surface.

Visual comparison of the spectra suggested somewhat greater variation in selected absorption bands in *A. oryzae*; however, these differences were not quantitatively evaluated and should therefore be interpreted with caution. The untreated PET corresponds with the higher numerical PET weight loss observed for *A. oryzae* during the earlier stages of fermentation. These observations may indicate greater interaction between *A. oryzae* and the PET surface under experimental conditions. Filamentous fungi may interact with polymeric materials through surface colonisation and the secretion of extracellular enzymes and metabolites, potentially resulting in localised changes in polymer surface chemistry (Danso et al., 2019). However, the observed FTIR changes were relatively small. Therefore, they should be interpreted as evidence of surface chemical modification rather than definitive evidence of extensive PET depolymerisation. In addition, changes

in FTIR peak intensity can be influenced by sample preparation, surface heterogeneity, contact between the sample and ATR crystal, and changes in surface morphology.

The relatively modest spectral changes observed in the present study may also be associated with the chemical stability and crystallinity of PET, which limit its susceptibility to microbial attack. Previous studies have demonstrated that substantial enzymatic hydrolysis of PET requires effective PET-hydrolysing enzymes, such as PETase, cutinase, or related esterases, that can attack the ester bonds within the polymer structure (Austin et al., 2018; Yoshida et al., 2016). Therefore, the ATR-FTIR findings in the present study suggest that fungal attachment may facilitate close contact between the fungal biomass and the PET surface and may contribute to localised surface modification of the PET surface following fungal treatment, but they do not independently confirm complete polymer biodegradation. The FTIR results should therefore be considered alongside PET weight-loss measurements and FESEM observations to provide a more comprehensive assessment of fungal interaction with PET.

FESEM Analysis of PET Surface Morphology

The ATR-FTIR observations are further supported by FESEM analysis, which demonstrated fungal attachment and localised surface erosion after SSF. The combination of chemical and morphological evidence strengthens the interpretation that fungal colonisation resulted in surface modification of PET rather than merely physical contamination. The surface morphology of untreated PET and PET samples after solid-state fermentation (SSF) with *Aspergillus niger* and *Aspergillus oryzae* was examined using field-emission scanning electron microscopy (FESEM). The FESEM micrographs are presented in Figures 5-7.

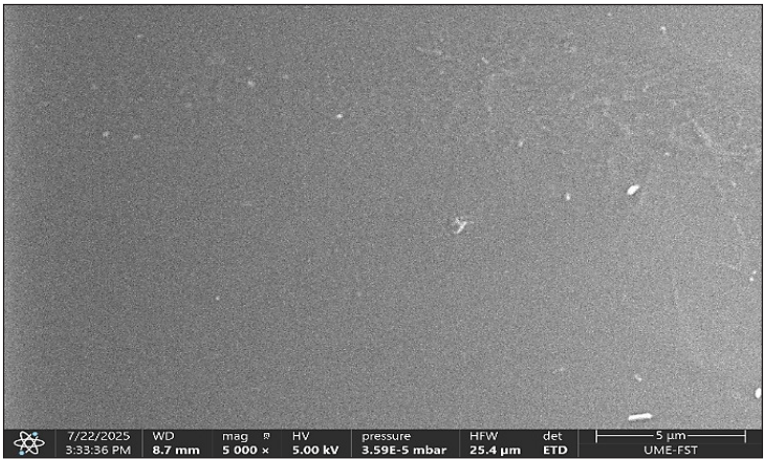


Figure 5. FESEM image of PET samples before solid-state fermentation

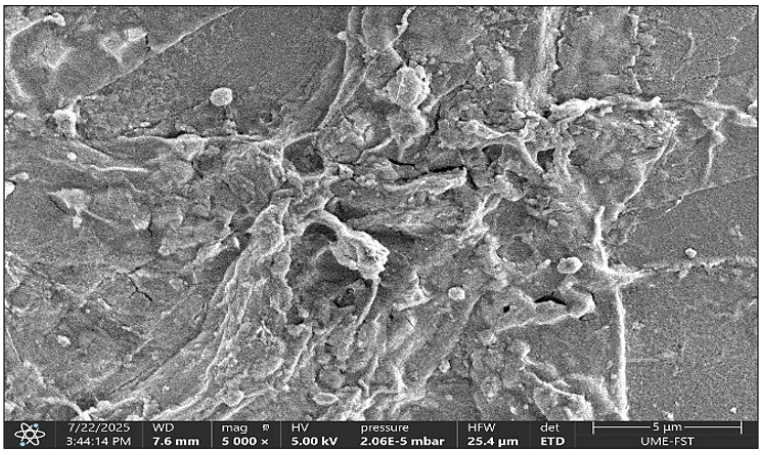


Figure 6. FESEM image of PET samples treated with *Aspergillus niger* after solid-state fermentation

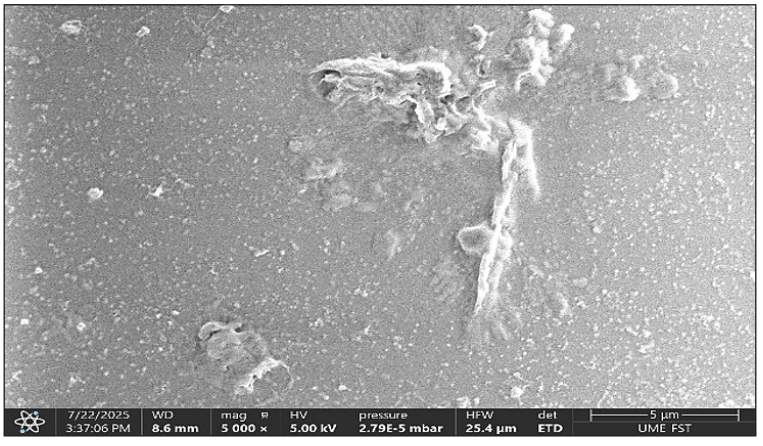


Figure 7. FESEM image of PET samples treated with *Aspergillus oryzae* after solid-state fermentation

As shown in Figure 5, the untreated PET exhibited a relatively smooth, compact, and homogeneous surface, with no prominent cracks, pits, or extensive erosion features. The relatively intact morphology observed before fermentation indicates that the PET surface had not undergone noticeable physical alteration prior to fungal treatment. A similar, relatively smooth surface morphology has been reported for untreated PET samples in studies investigating microbial interactions with PET (Danso et al., 2019). Following incubation with *A. niger*, noticeable morphological changes were observed on the PET surface (Figure 6), including increased surface roughness, irregular erosion patterns, shallow fissures, and fungal hyphae attached to the polymer surface. The presence of fungal hyphae indicates that *A. niger* was able to colonise and establish close contact with the PET surface during SSF. Such fungal attachment may increase the interaction between

extracellular enzymes or metabolites and the polymer surface, potentially contributing to localised surface modification. Similar changes, including surface roughening, cracks, and erosion, have been associated with microbial attachment and biodeterioration of polymer surfaces (Danso et al., 2019). However, morphological observations alone cannot establish that the polymer chains were extensively depolymerised.

The PET treated with *A. oryzae* (Figure 7) also showed evidence of fungal attachment and localised surface irregularities compared with the untreated PET. However, the visible surface alteration appeared less pronounced than that observed for *A. niger*. Interestingly, this observation does not directly correspond to the higher numerical PET weight loss recorded for *A. oryzae*. This difference may be explained by the fact that FESEM provides a localised visualisation of the polymer surface, whereas weight-loss measurements represent the overall change in PET mass. Consequently, localised surface roughening observed by FESEM may not necessarily correspond proportionally to the total mass reduction of the PET sample. In addition, fungal interactions may involve chemical modifications or more uniformly distributed surface changes that are not readily distinguishable in FESEM images.

Overall, the FESEM observations provide supporting evidence of fungal colonisation and localised physical changes on the PET surface following SSF. The more apparent surface roughening and erosion observed following *A. niger* treatment indicates more pronounced localised surface alteration, whereas *A. oryzae* showed greater numerical PET weight loss despite less pronounced visible surface erosion. This discrepancy suggests that PET mass loss and surface morphology may reflect distinct aspects of fungal polymer interaction. Therefore, FESEM results should be interpreted alongside PET weight loss and ATR-FTIR analysis rather than treated as definitive evidence of PET biodegradation. More conclusive evidence would require additional analyses, particularly the identification and quantification of PET degradation products such as terephthalic acid (TPA), mono-(2-hydroxyethyl) terephthalate (MHET), bis-(2-hydroxyethyl) terephthalate (BHET), and ethylene glycol (EG), as demonstrated in studies of enzymatic PET degradation (Austin et al., 2018; Yoshida et al., 2016).

CONCLUSION

The study evaluated the time-dependent interaction of *Aspergillus niger* and *Aspergillus oryzae* with PET under solid-state fermentation. *A. niger* showed the highest numerical PET weight loss at week 6 (0.76%), with maximum xylanase activity at week 2 (641.9 U/mL), whereas *A. oryzae* exhibited the highest numerical PET weight loss and xylanase activity at week 4 (1.91% and 467.8 U/mL, respectively). Notably, PET weight loss was not significantly affected by fermentation period for either fungal species, nor did the temporal patterns of xylanase activity show a consistent correspondence with PET weight loss. Therefore, xylanase activity alone cannot be used as a direct indicator of PET degradation

and should be regarded as an indicator of extracellular enzyme production during SSF. FESEM observations revealed fungal attachment and localised surface morphological alterations on PET, while ATR-FTIR analysis indicated relatively minor changes in the chemical characteristics of the treated PET. Collectively, these findings provide preliminary evidence of fungal-mediated interaction and surface modification of PET rather than definitive proof of PET biodegradation or depolymerisation. The observed PET mass changes should therefore be interpreted with caution, given the limitations of gravimetric assessment and the absence of PET-specific degradation-product analysis. Further studies incorporating PET-hydrolysing enzymes, such as cutinases or esterases, together with the detection and quantification of degradation products, including terephthalic acid, MHET, BHET, and ethylene glycol, are recommended to provide stronger evidence of PET biodegradation. Kinetic modelling and controlled abiotic experiments should also be considered in future studies to better characterise PET degradation behaviour and distinguish biological degradation from non-biological mass changes.

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