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Enhanced Biodegradation of Fenton-treated Polypropylene by *Aspergillus terreus* and *Engyodontium album*

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ABSTRACT

Polypropylene (PP) is a widely used synthetic polymer, but only 1% is currently recycled, leading to significant environmental challenges. To address this, a biological approach using Aspergillus terreus and Engyodontium album fungi was explored to degrade Fenton-treated PP. These fungi were selected for their potential to degrade PP granules (GPP), films (FPP), and metallised films (MFPP). After 90 days of incubation, weight loss was measured, with MFPP degraded by E. album showing the highest weight loss (16.53%) compared to A. terreus (7.97%). Significant differences were observed in FPP and GPP compared to controls ($p<0.05$). E. album produced more biomass (0.80 mg/ml) than A. terreus (0.62 mg/ml) after 90 days, correlating with PP weight loss (0.66 and 0.87 for A. terreus and E. album, respectively). Scanning electron microscopy (SEM) validated PP degradation, showing consistent morphological changes. This study demonstrates the potential of E. album and A. terreus to degrade PP, offering a promising strategy for future plastic waste reduction and disposal.

Keywords: *Aspergillus terreus*, *Engyodontium album*, Fenton-treatment, gravimetric weight loss, polypropylene

1. INTRODUCTION

Global plastic consumption has risen sharply, resulting in a troubling increase in environmental pollution. The majority of plastics, including polyethylene terephthalate (PET), polyethylene (PE), and polypropylene (PP), exhibit high stability and can persist for decades without degrading, thereby becoming prominent pollutants in terrestrial and marine ecosystems (Mollasalehi, 2013). Since 1950, approximately 5.8 trillion kilograms of plastic waste have been produced, with a significant amount generated between 2004 and 2017. This rapid surge in plastic waste production suggests a potential significant buildup in the environment and landfills by 2050 (Sheth et al., 2019). Synthetic polymer materials like PE, PET, and PP are major contributors to this pollution issue.

This study focuses on PP, which is used in various forms such as granules, biaxially oriented films, and metallised films. PP films, in particular, find wide applications in food packaging for items like

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fresh produce, confectionaries, and as a substitute for cellophane in snack and tobacco packaging (Ebnesajjad, 2013; Twede & Goddard, 1998). Nevertheless, the disposal of PP, like other plastics, presents a significant environmental challenge. A biological approach is being explored as a potential solution. While no microbes capable of directly degrading pure PP as their sole carbon source have been reported, several studies have investigated PP blends. For instance, Sheik et al. (Sheik, Chandrashekar, Swaroop, & Somashekarappa, 2015) demonstrated improved PP degradation through biological treatment. Filamentous fungi, such as mycelium, are believed to play a crucial role in polymer degradation (Holt et al., 2012; Jones, Mautner, Luenco, Bismarck, & John, 2020). Some fungal species are capable of generating enzymes that degrade particular polymers or use carbon derived from the polymer chain. These enzymes dismantle complex polymer structures into simpler units that microbial cells can absorb to complete the degradation process (Glaser, 2019).

In prior studies, various pre-treatment methods have been employed on PP before biodegradation, such as the use of Fenton reagent (Samat, Carter, & Abbas, 2023), UV exposure (Jeyakumar, Chirsteen, & Doble, 2013; Morancho et al., 2006), γ-irradiation (Sheik et al., 2015), and thermal processing (Abd El-Rehim, Hegazy, Ali, & Rabie, 2004; Jeyakumar et al., 2013). These techniques reduce the polymer's hydrophobicity, rendering it more vulnerable to microbial attack and thereby enhancing degradation processes *in vivo* or *in vitro*. Additionally, introducing functional groups like C=O or –OH onto the polymer's carbon-carbon backbones increases its susceptibility to degradation (Arutchelvi et al., 2008). Although research in this field is limited, specific fungi and bacteria have shown promise in PP degradation. Previous investigations have utilised *Aspergillus* species such as *Aspergillus* sp., *A. niger*, and *A. terreus* (Oliveira et al., 2020; Sáenz, Borodulina, Diaz, & Banchon, 2019; Sheela & Sangeetha, 2011). These organisms can develop biofilms, allowing them to organise into communal structures and adhere to inert surfaces (Samson & Pitt, 2013). While the degradation of PP by a single strain of *A. terreus* has not been extensively studied, Strömberg and Karlsson (2009) employed a group of fungi and algae, among which was *A. terreus*, to biodegrade both inert and recycled PP. Additionally, *E. album* was chosen based on earlier studies by Jeyakumar et al. (Jeyakumar et al., 2013), where *E. album* MTP09 exhibited potential in degrading pre-treated PP. These fungi were selected for their diverse enzyme profiles and their ability to thrive in challenging environments with minimal nutrients and moisture, highlighting their potential as candidates for PP degradation.

2. METHODS

2.1 Polypropylene Samples

The commercial PP samples used in this study included granules (GPP), films (FPP), and metallised films (MFPP). GPP denotes isotactic polypropylene in white pellet form, with an average molecular weight of approximately 250,000 and an average Mn of about 67,000. It exhibits 0.9 g/ml of density at 25 °C. FPP is a transparent, biaxially oriented film, measuring 0.05 mm in thickness and 600 × 600 mm in dimensions. MFPP denotes metallised film with an aluminium-plated surface. It is 0.008 mm thick and measures 0.5 m × 75 mm. Both FPP and MFPP was purchased from Sigma-Aldrich®.

2.2 Pre-treatment of PP Samples

PP underwent disinfection using 70% ethanol and subsequent weighing. They were designated as Fenton-treated polypropylene (FR-GPP, FR-FPP, and FR-MFPP for granules, film, and metallised film, respectively). GPP, FPP and MFPP underwent pre-treatment using Fenton's reagent (pH 5.5 with the gradual addition of 30% H_2O_2) for 7 days (Arkatkar, Juwarkar, Bhaduri, Uppara, & Doble, 2010). 20 mg of either untreated or pre-treated PP samples were then placed into Erlenmeyer flasks containing 200 ml of minimal salt medium (MSM), comprising 1 g/l KH_2PO_4, 0.2 g/l NaH_2PO_4, 0.5 g/l

$MgSO_4.7H_2O$, 0.1 g/l $CaCl_2$, 0.169 g/l $MnSO_4.H_2O$, 1 g/l yeast extract, and 1 ml of vitamin solution. Additionally, 2 ml of fungal culture [10] were introduced into each flask. All chemicals used in the experiments are sourced from Sigma-Aldrich®.

2.3 Growth and Preparation of *Engyodontium album* (BRIP 61534a) and *Aspergillus terreus* (ATCC 20542)

According to the supplier's recommendations, the *A. terreus* strain was restored (ATCC Product Sheet *Aspergillus terreus* ATCC® 20542™). The *E. album* strain was supplied by the Department of Agriculture and Fisheries (DAF), Queensland, Australia. These fungi were subcultured and maintained in fresh Potato Dextrose Agar (PDA) medium at 25°C. Unless otherwise specified, all chemicals used were provided by Sigma-Aldrich®.

2.4 Fungal Inoculation and Incubation Condition

Fungal inoculation was carried out at 30°C (200 rpm) using an incubator shaker (Thermoline TU-400). After 30, 60, and 90 days, the PP samples were retrieved, washed with sterile water, and then dried at 50°C in an oven before further analysis. Both pre-treated and untreated PP samples without any microorganisms served as the abiotic control. Flasks containing only the pure culture without PP samples were designated as the biotic control. These control samples underwent the same incubation conditions as the test samples (PP with microorganisms). Each experiment was carried out in triplicate.

2.5 Biomass of *E. album* and *A. terreus*

A 1 mL aliquot of the fungal culture was placed into a 1.5 mL microcentrifuge tube and centrifuged at 12,000 rpm for 25 minutes with an Eppendorf® Centrifuge 5424. The subsequent pellet was dried at 50 °C until a constant weight was achieved, which was then recorded. Fresh fungal mycelium was suspended in sterile water, filtered, dried, and recorded as the initial reading (Paço et al., 2017). This method was adapted from the protocol described by Artham and Doble (Artham & Doble, 2010).

2.6 Polymer Characterisation

2.6.1 Gravimetric weight loss (GWL)

The GWL of the residual PP samples was determined by recovering them from the mineral salt media through sterile filtration (Auta, Emenike, Barasarathi, & Fauziah, 2017). The samples were weighed using a Mettler Toledo ME204E analytical balance, which has a sensitivity of 0.001 g (Mohan, Sekhar, Bhaskar, & Nampoothiri, 2016). The fungi colonising the PP samples were removed by a four-step washing process, each step lasting 2 minutes, using 70% ethanol. Subsequently, the samples were dried overnight in an oven at 50 °C. The weight of the residual PP samples was then measured to determine the extent of biodegradation. The percentage weight loss was calculated using Equation (1):

$$\% \, GWL = \frac{W0 - W}{W0} \times 100 \quad (1)$$

Where W0 is the initial weight (g), and W is the residual weight of the PP samples (g).

2.6.2 Scanning electron microscopy (SEM) analysis of PP samples

After 90 days, the surface structure of the degraded PP samples was examined using a Phenom XL scanning electron microscope (SEM). Prior to imaging, the samples underwent sputter-coating with a mixture of palladium and gold at 18 mA in an argon (Ar) atmosphere at 150 kPa. The samples were then visualised at a maximum magnification of 3800× (Sekhar et al., 2016).

2.6.3 Statistical analysis

The gravimetric weight loss percentage and biomass dry weight data were statistically analysed with analysis of variance (ANOVA). The correlation matrices were analysed using Pearson correlation coefficients. GraphPad Prism 9 version 9.1.0 was used to conduct these analyses, applying the LSD post-hoc test at a significance level of $P = 0.05$.

3. RESULTS AND DISCUSSIONS

3.1 Gravimetric Weight Loss (GWL)

We quantified the degradative effectiveness by measuring the GWL of Fenton-treated GPP, FPP, and MFPP over a 90-day incubation period. The GWL of the pre-treated PP samples consistently exceeded that of the control samples across all microbial treatments. Specifically, the results indicated that MFPP incubated with *E. album* showed the highest weight loss of 16.53%, followed by *A. terreus* at 7.97%, compared to the control-FR-MFPP at 3.59% after 90 days of incubation (Figure 1). Significant differences in percentage weight loss were observed between FR-MFPP and both treated and untreated controls ($p < 0.005$) after 90 days of incubation with *E. album* and *A. terreus*.

However, no significant GWL was observed for the Fenton-treated FPP and GPP compared to the controls. This indicates that these fungi can metabolise the carbon source or produce particular enzymes capable of breaking down Fenton-treated MFPP, leading to partial PP degradation (Auta et al., 2017). Moreover, the elevated GWL percentage of MFPP incubated with *E. album* suggests that this fungus may degrade and attack MFPP more effectively than others, resulting in partial PP degradation. In addition, MFPP demonstrated the highest GWL compared to GPP and FPP, indicating that pre-treatment and metal additives have a pronounced impact on PP biodegradation by both *A. terreus* and *E. album* fungi (Jeyakumar et al., 2013). This increased degradation potential may be due to the production of specific enzymes, like laccase, by *E. album* (Artham & Doble, 2010).

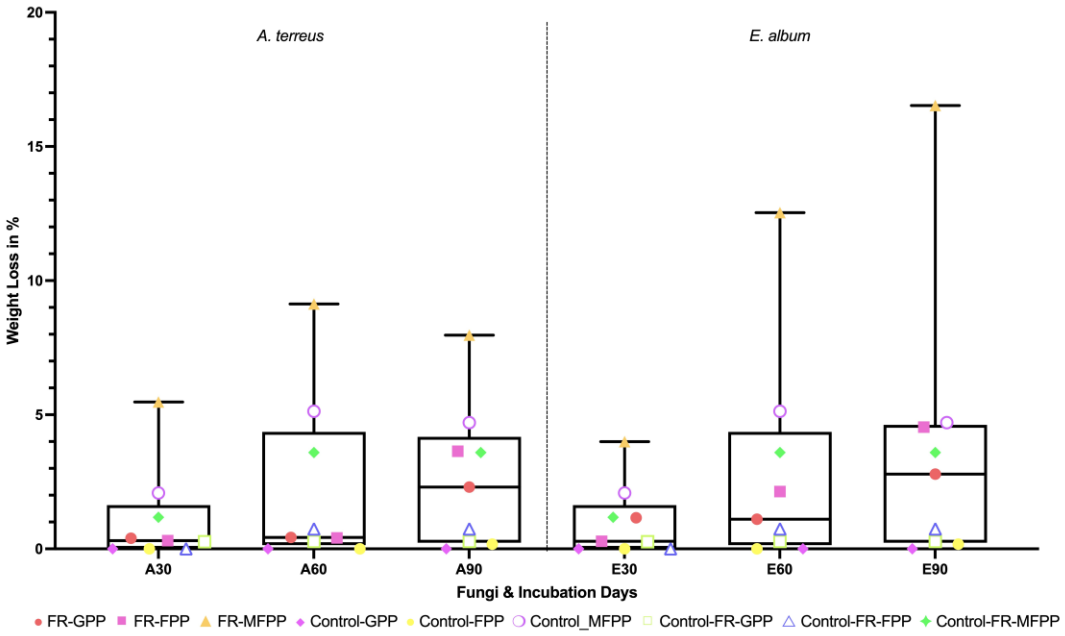

Figure 1. Gravimetric weight loss percentage after 30, 60, and 90 days of incubation of *A. terreus* and *E. album* with Fenton-treated GPP, FPP and MFPP and treated and untreated controls (MSM + treated/WPC samples). A30: *A. terreus* after 30 days; A60: *A. terreus* after 60 days; A90: *A. terreus* after 90 days; E30: *E. album* after 30 days; E60: *E. album* after 60 days; E90: *E. album* after 90 days.

In an earlier investigation with PP films, Jeyakumar et al. (Jeyakumar et al., 2013) reported an 18.8% reduction in weight for UV-treated, catalyst-blended PP exposed to *E. album*, and a 9.42% weight loss when incubated with *P. chrysosporium* over a 12-month period. These findings emphasise the impact of metal ion additives and UV pre-treatment on the degradation of PP. Sudhakar et al. (Sudhakar et al., 2007) observed a 0.5% weight decrease in untreated PP exposed to marine water for 6 months, whereas Auta et al. (Auta et al., 2017) reported a 6.4% weight loss in PP film degradation when incubated with *Rhodococcus* sp. strain 36 and a 4% weight decrease with *Bacillus* sp. strain 27 on UV-treated PP microplastics.

3.2 Biomass Production and the Correlation Between GWL of PP, Biomass, and Laccase Production of *A. terreus* and *E. album*

Based on previous findings, we proceeded to evaluate fungal growth by analysing the biomass generated during incubation with Fenton-treated PP samples (Auta et al., 2017). Overall, both *E. album* and *A. terreus* and exhibited higher biomass production when incubated with Fenton-treated samples compared to the control (without PP). *E. album* showed the highest biomass production after 90 days of incubation with Fenton-treated MFPP (0.80 mg/ml) (Figure 2a), followed by *A. terreus* (0.62 mg/ml) (Figure 2b) under the same conditions. ANOVA was used to further analyse the p-values of biomass produced by both fungi across different PP samples.

A significant difference in biomass production was noted for *A. terreus* in Fenton-treated MFPP compared to its control ($p<0.0001$). Generally, both *E. album* and *A. terreus* exhibited significantly higher biomass production than the control, except for instances such as GPP after 30 days with *A. terreus* and FPP after 90 days with *E. album*. This variation could stem from the fungi's limited acclimatisation to the culture conditions, potentially influenced by the brief incubation period or

unfavourable conditions within the PP samples hindering fungal growth. Additionally, the presence of certain metabolites may have made the culture medium unsuitable for fungal proliferation (Auta et al., 2017). These results indicate that *E. album* and *A. terreus* exhibited varied responses to the different PP samples when compared to the control, with *E. album* showing better adaptation to the culture conditions when exposed to MFPP, possibly utilising the PP samples as a carbon source for its growth.

Then, the correlations among GWL, biomass, and laccase production for both fungi were evaluated. Figures 2c and 2d present the correlation matrices illustrating the relationship between weight loss, biomass production, and laccase activity for *A. terreus* and *E. album*. These findings underscore how each fungus responds differently to the substrate used. As mentioned earlier, the substrate consumed influences fungal biomass, reflecting strain growth in the medium (Jeyakumar et al., 2013). Analysis of the matrices (Figures 2c-d) revealed a strong correlation between GWL and biomass production, along with laccase levels. This indicates that biomass production significantly influences laccase production, thereby contributing to PP weight loss.

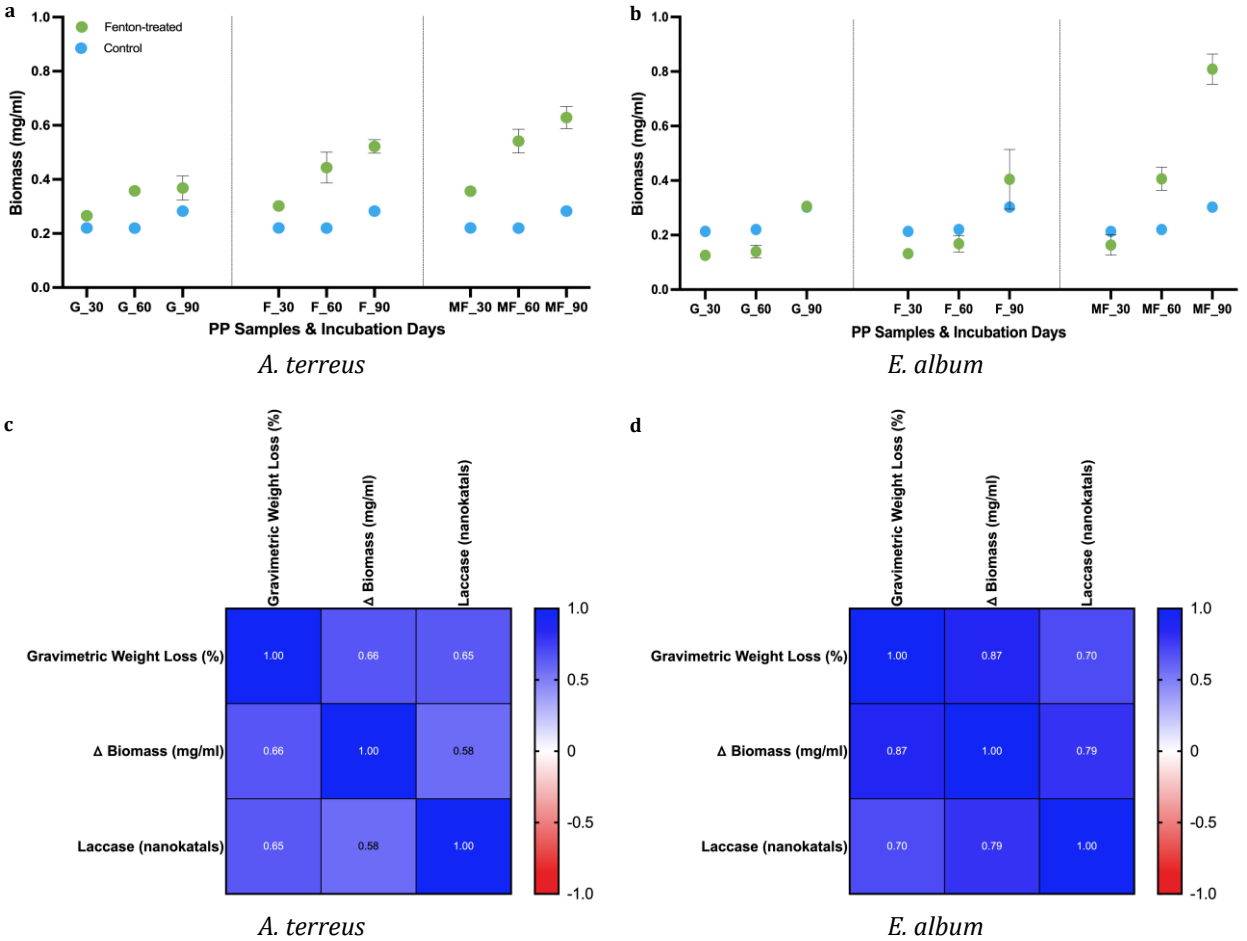

Figure 2. Biomass production of fungi and its correlation with gravimetric weight loss, biomass and laccase production of *A. terreus* and *E. album.* **a-b**, Biomass weight (mg.ml) of *A. terreus* and *E. album* incubated with Fenton-treated GPP, FPP and MFPP, and control (MSM + *A. terreus*/*E. album* only). **c-d,** the correlation matrix of the relationship between gravimetric weight loss, biomass, and laccase production of *A. terreus,* and *E. album* after 90 days of incubation.

3.3 Surface morphological changes

The SEM analysis revealed the extent of PP degradation through surface morphological changes. The Fenton-treated controls exhibited slightly rough surfaces (Figure 3a-c) due to the Fenton reagent's action. In contrast, Fenton-treated GPP, FPP, and MFPP incubated with both *A. terreus* and *E. album* showed significant surface alterations, including large cracks, grooves, and irregularities (Figure 3d-i). These irregularities were primarily caused by the abiotic pre-treatment strategy, as few were observed in the controls (Figure 3a-c). This demonstrates that pre-treatment can modify the surface morphology, and the formation of cracks indicates the increased brittleness of pre-treated PP (Jeyakumar et al., 2013).

After 90 days of fungal incubation, the surface irregularities became more pronounced, indicating the presence of fungal biomass. Additionally, noticeable biomass and microbial cells were observed on the PP samples' surfaces incubated with *A. terreus* and *E. album*, particularly on MFPP (Auta et al., 2017; Jeyakumar et al., 2013). This suggests microbial proliferation within these fissures, crucial for PP biodeterioration. Moreover, various crevices and pits on the surface were attributed to microbial activity (Auta et al., 2017). However, the observed damage was less severe compared to studies involving UV and heat-treated PP, where significant cracks and grooves were evident due to the UV and heat treatment methods (Samat et al., 2023).

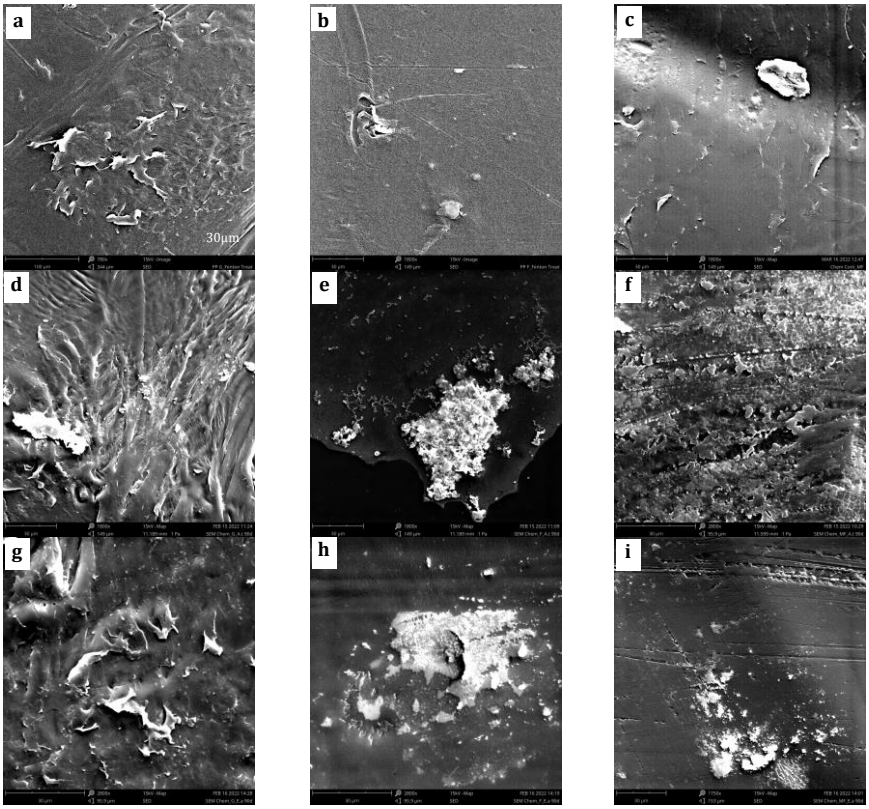

Figure 3. SEM analyses. **a**, SEM micrographs of Fenton-treated control GPP. **b**, Fenton-treated control FPP. **c**, Fenton-treated control MFPP. **d**, GPP incubated with *A. terreus*. **e**, FPP incubated with *A. terreus*. **f**, MFPP incubated with A*. terreus*. **g**, GPP incubated with *E. album*. **h**, FPP incubated with *E. album*. **i**, MFPP incubated with *E. album*.

4. CONCLUSION

This study explored the combined impact of Fenton pre-treatment and fungal biodeterioration on various type of PP (GPP, FPP, and MFPP). The most substantial degradation occurred in Fenton-treated MFPP exposed to *E. album*. The significant gravimetric weight loss and changes in surface morphology observed indicate successful biodegradation. The increased biomass production in PP-infused media suggests enhanced microbial colonisation, thereby accelerating PP degradation. The confirmed synergistic effects of pre-treatments alongside fungal degradation are crucial for developing effective biological strategies to address PP waste and other synthetic polymers on a broader scale.

4.1 Author Contributions

A.A and A.F.S conceptualised the study. A.F.S analysed the data, conducted the experiments, and drafted the manuscript, with input from A.A, G.F.W and D.C. A.A and D.C contributed to the study's planning, supervision, and manuscript review. All authors participated in results and discussions and provided comments on the manuscript.

4.2 Competing Interests Statement

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

5. ACKNOWLEDGMENTS

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