Malaysian Applied Biology (2024) 53(2): 93-100
https://doi.org/10.55230/mabjournal.v53i2.2819

Research

Degradation of Polypropylene Using Fungal Enzyme As A Sustainable Approach To Management Plastic Waste

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ABSTRACT

Polypropylene (PP) is a major environmental problem in Malaysia because it has been ranked the 28th highest plastic polluter in the world (at 56kg per capita per year) in 2021. Landfilling is one of the most common ways of dealing with plastic because leachate may cause increased probability of cancer and neurological impairment in humans. The use of fungi in mycoremediation makes the process eco-friendly. In addition, fungi have a vast hyphal network and broader metabolic competence. The objective of this study was to investigate fungi remediation of PP via the detection of manganese peroxidase and laccase activity in Bushnell Haas Broth (BHB). PP degradation activity was measured via the activity of laccase and manganese peroxidase at a wavelength of 450nm and 610nm, respectively. Of the 17 species of fungi isolated from the Jeram landfill, 12 species of fungi showed growth in BHB with PP as the sole carbon source. *Penicillium* sp. 1, *Aspergillus* sp., *Penicillium levitum*, *Talaromyces louisianensis*, *Aspergillus tamarii*, *Cunninghamella bertholletiae*, *Penicillium* sp. 2 and *Aspergillus niger* demonstrated high and longer laccase activity, and these fungi could be considered as potential fungi. *P. levitum*, *P. janthinellum*, *Penicillium* sp, and *T. louisianensis* have high and longer MnP activity. In summary, *P. levitum* and *T. louisianensis* have a high and long duration of MnP and laccase activity in degrading PP, which can be developed and integrated into plastic waste management.

Key words: Fungi, laccase, manganese peroxidase, polypropylene, sustainable land management

Article History
Accepted: 11 May 2024
First version online: 30 June 2024

Cite This Article:
Hock, O.G., Kee, W.K., Ahmed, R.D.A.H., Ying, J.K.Z., Rui, W.R., Er, L.K. & Tanee, T. 2024. Degradation of polypropylene using fungal enzyme as a sustainable approach to management plastic waste. Malaysian Applied Biology, 53(2): 93-100. https://doi.org/10.55230/mabjournal.v53i2.2819

INTRODUCTION

Plastic pollution is becoming a global issue. Geyer (2020) estimated that 6300 million metric tonnes of plastic waste were generated in 2015. Malaysia is no exception, ranking 28th in the world in terms of plastic pollution in 2021 (at 56 kg per capita annually) (Fauziah *et al*., 2021). Polypropylene (PP) is responsible for 16% of all plastics produced (Alsabri *et al*., 2022). PP is one of the most versatile plastics, but its decomposition is hindered because of its structural stability. When its molecules are arranged in an atactic conformation, they are soft and elastic. When the molecules are arranged in an isotactic conformation, it is hard and strong (Omnexus, 2022). It has been used to create packaging, medical devices such as syringes, clothing, car parts, tapes (Alsabri *et al*., 2022; Nikolovska, 2022), and single-use plastics (Million Marker, 2020). PP is similar to polyethylene (PE), but it resists stress cracking far better. This stability is critical to its slow biodegradability in natural environments (Jeon *et al*., 2021).

According to Huang *et al*. (2022), the current methods of dealing with plastics are landfilling, recycling, pyrolysis, liquefaction, road construction and tar manufacture, and concrete production, with landfilling being the worst. Unfortunately, landfilling is the most common method for

disposing of plastic waste due to its ease of use (Chen *et al*., 2021). Plastic pollution is a problem because when plastics degrade, they cause problems such as the leaching of endocrine-disrupting chemicals (EDC) (El Moukhtari, *et al*., 2023). It has been discovered that PP affects androgen hormones and causes toxic or stress responses in cells (Healthline, 2020).

As a result, mycoremediation was proposed to reduce the environmental impact of PP pollution. Fungi are naturally decomposing organisms with long life cycles, a vast hyphal network, extensive biomass growth, and a broader metabolic competence (Kapahi & Sachdeva, 2017; Akhtar & Mannan, 2020; Ong *et al*., 2021). They are known to secrete their enzymes extracellularly and can secrete a wide range of enzymes, including manganese peroxidase and laccases (Kumar & Chandra, 2020). Fungal laccases and peroxidases, which are commonly utilized by fungi to break down lignin, also degrade polyethylene (PE) and polyvinyl chloride (PVC) (Temporiti *et al*., 2022). As a result, both enzymes are being studied in this study. Manganese peroxidase is an extracellular hemeprotein that catalyzes the H_2O_2-dependent oxidation of polymers based on lignin derivatives (E.C. 1.11.1.13. Mn2+: H_2O_2 oxidoreductases). It belongs to the oxidoreductase family, more specifically those that act on peroxide as an acceptor (peroxidases) (Xu *et al*., 2017). Manganese peroxidase is a ligninolytic enzyme that primarily degrades lignin but has also been found to degrade dyes and organic pollutants (Kumar & Arora, 2022). Laccases (EC 1.10.3.2, benzenediol: oxygen oxidoreductase) are multicopper oxidases that are usually secreted by organisms as extracellular monomeric glycoproteins (Solomon *et al*., 1996). Laccases can degrade phenolic compounds using oxygen and produce only water as a byproduct (Mayolo-Deloisa *et al*., 2020). Fungi provide a wide array of enzymes specialized in the degradation of recalcitrant substances and are very promising candidates in the field of plastic degradation (Temporiti *et al*., 2022) As a result, using fungi in mycoremediation of plastic has the advantage of being cost-effective, eco-friendly, and effective because it uses a biological system to degrade and remediate plastic (Akhtar & Mannan, 2020).

Although some studies have reported the involvement of fungi in polypropylene biodegradation, no studies have yet investigated the use of fungal enzymes in PP biodegradation (Ragaert *et al*., 2017; Pires *et al*., 2019; Mohanan *et al*., 2020; Anjana *et al*., 2020; Othman *et al*., 2021; Temporiti *et al*., 2022). The indigenous fungi growing on polluted sites should be considered for further studies as they are adapted to the high concentration of various pollutants in harsh environmental conditions (Akhtar & Manna, 2020). Therefore, to find an alternative solution to Malaysia's growing PP waste problem, local fungal species were screened to determine their ability to deal with PP waste. The purpose of this study is to investigate fungi remediation of PP in Bushnell Haas Broth (BHB) using manganese peroxidase and laccase activity detection.

MATERIALS AND METHODS

Sampling and fungi identification

Plastic bags purchased from vendors were sent to the INBIOSIS, Universiti Kebangsaan Malaysia (UKM), Malaysia for FTIR analysis.

The surface soil samples (5 to 10 cm depth) were obtained from three locations within the Jeram Sanitary Landfill, Malaysia (geo- geo-coordinates: 3.1890889,101.3625547). The soil samples were then diluted to 10^{-3} and 10^{-5} using sterilized water before mixing into the Rose Bengal agar (RBA) (OXOID, UK) of a 1:9 ratio. The RBA was then incubated at room temperature for 2 to 3 days. Single fungal isolate was picked and subcultured onto Potato Dextrose Agar (PDA) to obtain young and pure culture. Lastly, the culture was cultured in Potato Dextrose broth (PDB) for DNA extraction. DNA extractions were performed using the FavorPrepTM Plant Genomic DNA Extraction Mini Kit, following the protocol provided by Favorgen Biotech Corr (Favorgen Biotech Corr, n.d.). The extracted DNA was then subjected to the polymerase chain reaction (PCR) amplification using a pair of primers targeting the ITS1 (5'TCC GTA GGT GAA CCT TGC GG 3') and ITS4 (5'TCC TCC GCT TAT TGA TAT GC 3') region (Fujita *et al*., 2001), following the Biotaq Polymerase protocol obtained from Bioline. The PCR product was then purified and sequenced by Genomics BioSci & Tech, Malaysia. The obtained DNA sequence was examined using the Basic Local Alignment Search Tool (BLAST).

Screening for fungi species metabolizing PP

A pure fungal was inoculated into a flask containing autoclaved 50 mL of Bushnell Haas Broth (BHB) (OXOID, UK) mixed with 0.04 g of shredded PP (Braun *et al*., 2021). The flasks were shaken at 150 rpm for seven days at room temperature (Khruengsai *et al*., 2021). The experiment was carried out in triplicates. At the end of the incubation period, fungi that can metabolize PP as the sole carbon source growth was determined by the turbidity of the culture with the naked eye. The BHB with PP in

the absence of fungi culture was used as a control.

Enzyme assay

Culture samples were centrifuged at 4000 r.p.m for 10 min at 4 °C (Klimek-Ochab *et al*., 2011) to obtain cell-free supernatant. The supernatant was then used as a source for laccase and manganese peroxidase enzyme assay.

The laccase enzyme activity was measured using the method described by Zhang *et al*. (2015). After mixing 1 mL of the supernatant with 1 mL of guaiacol and 3 mL of sodium acetate buffer (pH 6.7), the mixture was allowed to incubate at room temperature for 10 min. The reaction mixture was then transferred to a glass cuvette and measured at 450 nm wavelengths on a spectrophotometer. The laccase activity was calculated using the formula:

$$\text{Enzyme activity } \left(\frac{U}{L}\right) = \frac{A_{450} \times 10^6}{0.5\ \text{mL(enzyme)} \times 0.5\ \text{min} \times e(4460\text{L},\text{m}^{-1}\cdot\text{cm}^{-1})}$$

(Zhang *et al*., 2015)

The manganese peroxidase (MnP) activity was measured using the method described in Silva *et al*. (2014). Two sets of reaction media were made for each sample by mixing 500 µL of supernatant, 50 µL of manganese sulfate (2.0 mM), 200 µL of bovine albumin (0.5% w/v), 50 µL of hydrogen peroxide (2.0 mM) in sodium succinate buffer (0.2 M, pH 4.5), 100 µL sodium lactate (0.25 M) and 100 µL of phenol red (0.01% w/v) into a test tube. One set of reaction tubes was subjected to boiling temperatures for 10 min to ensure there were no reactions within that sample. This media was used to blank the spectrophotometer at 610 nm. The other set was left at room temperature for 30 sec to allow the reaction to occur before terminating the reaction using 40 µL sodium hydroxide (2.0 M). After that, 1 mL of the terminated reaction media was transferred to a cuvette read at 610 nm with a UV spectrophotometer. The manganese peroxidase activity was calculated using the formula:

$$\text{Enzyme activity } \left(\frac{U}{L}\right) = \frac{A_{610} \times 10^6}{0.5\ \text{mL(enzyme)} \times 0.5\ \text{min} \times e(4460\text{L},\text{m}^{-1}\cdot\text{cm}^{-1})}$$

(Silva *et al*. (2014)

RESULTS AND DISCUSSION

Results of the FTIR analysis performed using wavelength between 780 nm to 1 mm (Infratec, 2022) confirmed the plastic is made of polypropylene (PP) with the indicative peaks (Jung *et al*., 2018) as shown in Figure 1. The PP plastic bags were manually shredded and used in the subsequent experiment.

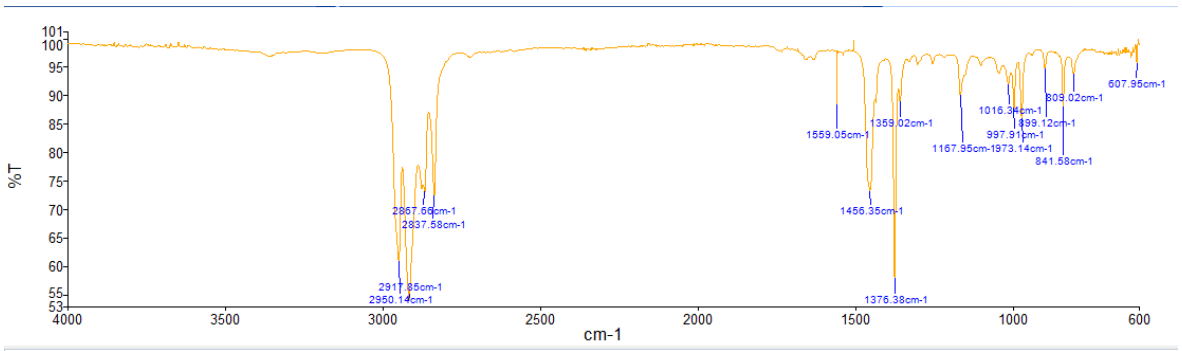

Fig. 1. FTIR analysis for PP identification.

Soil samples collected from the Jeram Sanitary Landfill, Malaysia yielded a total of 14 fungi species, identified using molecular markers ITS1 and ITS4 region. A total of 14 isolates were successfully identified up to species level, namely *Cunninghamella bertholletiae*, *Cunninghamella polymorpha*, *Paecilomyces variotii*, *Trichoderma asperellum*, *Penicillium janthinellum*, *Penicilluim glaucoroseum*, *Penicillium simplicissimum*, *Penicillium cremeogriseum*, *Talaromyces flavus*, *Penicillium levitum*, *Aspergillus niger*, *Talaromyces louisianensis*, *Aspergillus tamarii,* and *Trichosporon asahii.* Out of the 14 isolates, 9 fungi species were found able to grow in BHB media using PP as the sole carbon source (Table 1). As a result, these nine fungal species were analyzed for the detection of laccase and manganese peroxidase activities.

Table 1. Fungal species isolated from the landfill with demonstrated ability to grow in BHB media with PP as the sole carbon source.

Growth	No Growth
C. bertholletiae	*C. polymorpha*
P. janthinellum	*P. variotii*
P. glaucoroseum	*T. asperellum*
P. simplicissimum	*T. flavus*
P. cremeogriseum	*T. asahii*
P. levitum	
A. niger	
T. louisianensis	
A. tamarii	
Growth	No Growth
Cunninghamella bertholletiae	*Cunninghamella polymorpha*
Penicillium janthinellum	*Paecilomyces variotii*
Penicillium glaucoroseum	*Trichoderma asperellum*
Penicillium simplicissimum	*Talaromyces flavus*
Penicillium cremeogriseum	*Trichosporon asahii*
Penicillium levitum	
Aspergillus niger	
Talaromyces louisianensis	
Aspergillus tamarii	

Manganese peroxidase (MnP) activity

Figure 2 shows *P. levitum* consistently showed high MnP activity (>100 IU) from week 1 to week 4, indicating that this is the best fungus species for consistently releasing MnP over a long period. However, no information on *P. levitum* remediation has previously been reported. *P. levitum* was identified as one of the most often isolated species in soil (Vukicevich *et al*., 2018), indicating that this species is a common fungus species but that more research on its potential or application is needed.

P. glaucoroseum and *T. louisianensis* showed similar trends with high MnP activity (>50 IU) from week 1 to week 4. *P. glaucoroseum* demonstrated an increasing trend from week 1 to week 3 with high MnP activity (>100 IU) but decreased sharply on week 4 (65.47 IU). *P*. *glaucoroseum* was found as one of the most plastic-degrading microorganisms (Taghavi *et al*., 2021), where it can survive on plastic even after a lengthy period (270 days) by colonizing and consuming it as a carbon source with the maximum weight loss (1.8%) was reported in polystyrene. *T. louisianensis* demonstrated a consistently high MnP activity (>100IU) from week 1 to week 3 but decreased sharply on week 4 (54.11 IU). *However, because no information on T. louisianensis remediation has previously been reported, a more extensive study to gain a better understanding of the mechanism of PP remediation could be conducted.*

In conclusion, because of the high (>100 IU) and consistent MnP activity (from week 1 to week 4) shown by *P. levitum*, *P. glaucoroseum*, and *T. louisianensis*, these fungi could be considered as potential fungi to biodegrade PP. A more comprehensive study could be conducted to gain a better understanding of the mechanism of PP remediation. The current study has certain limitations because many fungal species isolated from local landfills have never been reported before, making it difficult to make significant comparisons or establish a benchmark as a standard guideline.

Laccase activity

Figure 3 shows the activities of laccase activity of the nine fungal species grown on BHB media using PP as the sole carbon source. *P. levitum*, *T. louisianensis*, and *A. tamarii* expressed relatively higher laccase activity (>1 IU) from the beginning of the incubation period until the end. This observation suggests that all five isolates have the potential to perform better in degrading PP plastic. To date, no information on *P. levitum*, *T. louisianensis,* and *A. tamarii* on any pollutant remediation has been reported. *A more extensive study on how these three isolates degrade PP plastic can be conducted to gain a better understanding of the mechanism involved.*

The laccase activity of *C. bertholletiae* demonstrated low activity (<0.5 IU) in weeks 1 and 2 but peaked (>2 IU) in weeks 3 and 4, whereas *A. niger* peaked (>1 IU) from week 2 to week 4. These fungal species could be candidates for long-term degradation because they have high laccase activity for a long duration. *C. bertholletiae* demonstrates the potential for degradation in lowering petroleum hydrocarbons in soil environments (Agbor *et al*., 2018). This supports the potential of *C. bertholletiae*

in PP degradation as PP is derived from petroleum hydrocarbons. *C. bertholletiae* (MUT 2231, MUT 2861 & MUT 4924) demonstrated reduced C=C double bond and carboxylic acid reductase activity as biocatalysts for fine chemical transformations (Romagnolo *et al*., 2016). The enzyme laccase was found to be more abundant in *A. niger* grown with low-density polyethylene (LDPE) film, most likely due to a higher demand for polymer breakdown reaction (Khruengsai *et al*., 2021), and *A. niger* van Tieghem F-1119 can degrade polyvinyl chloride (PVC) plastic effectively (Boyle, *et al*., 2020), implying that this species may react similarly in the presence of PP.

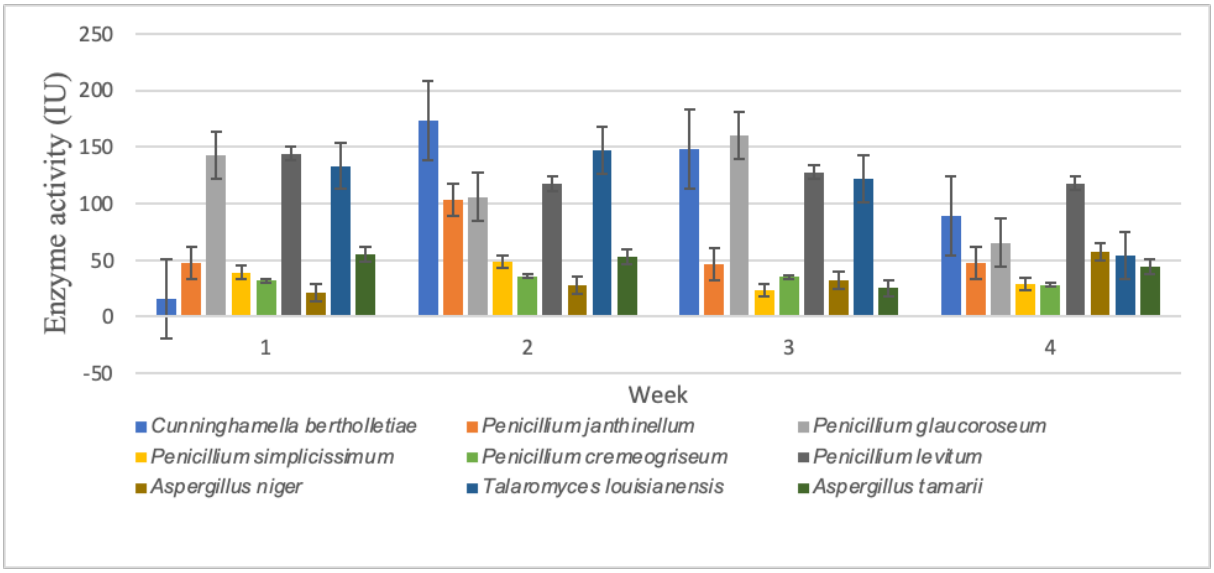

Fig. 2. The manganese peroxidase activity (IU) of the fungal species metabolizing PP.

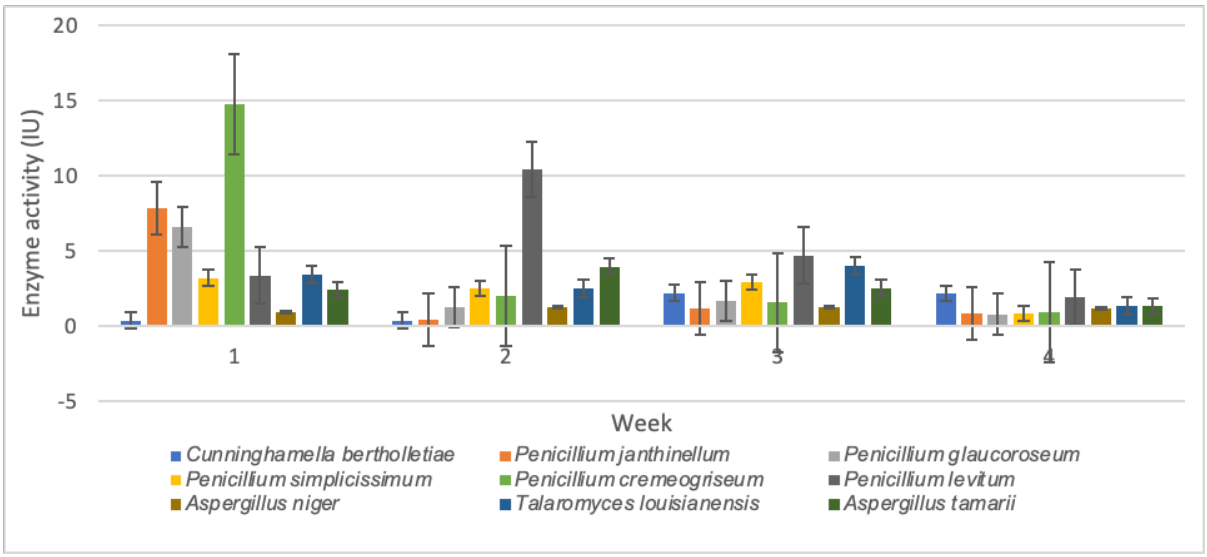

Fig. 3. The laccase activity (IU) of the fungal species metabolizing PP.

The laccase activities monitored throughout the 4 weeks were found to be inconsistent in *P. simplicissimum* and *P. cremeogriseum*. However, laccase activities peaked (>1 IU) between week 1 to week 3 but dropped on week 4 (1 IU). As a result, these two species may be suitable if rapid degradation of PP plastic is desired. *P. simplicissimum* isolated from a local dumpsite in Thailand was also reported to express laccase and manganese peroxidase that metabolized plastic made of polyethylene (Sowmya *et al*., 2015). *P. simplicissimum* degraded LDPE and reduced molecular weight from 190,000 to 20,000 (Ghosh & Pal, 2021), as evidenced by surface morphological degradation in SEM analysis and carbonyl group and C=C group production in the FTIR study. *P. simplicissimum* LAR 13 produced the highest polyhydroxybutyrate degradation rates (Sang *et al*., 2002; Viel *et al*., 2023). *According to FTIR*

analysis, P. simplicissimum YK utilizes intact polyethylene as a carbon source and can cut some of the double carbon bonds of polyethylene in 3 weeks and is used in the degradation of both natural and synthetic polyethylene *(Yamada-Onodera et al., 2001;* Ghatge *et al*., 2020*)*. In a similar study, it was discovered that *P. simplicissimum* secretes high laccase activity during the primary metabolism phase, and possesses an active ligninolytic system involving laccase activities (Zeng *et al*., 2006). *However, because no previous information on P. cremeogriseum remediation has been reported, further study to gain a better understanding of the mechanism of PP remediation can be explored.*

Finally, due to the high and longer laccase activity demonstrated by *P. levitum*, *T. louisianensis*, *A. tamarii*, *C. bertholletiae*, and *A. niger*, these fungi could be considered potential fungi. To gain a better understanding of the mechanism of PP remediation, a more comprehensive study could be conducted.

CONCLUSION

Most of the fungi found to have grown in BHB with PP as the sole carbon source are from the Eurotiomycetes class, particularly, the *Aspergillus* and *Penicillium* genera. In conclusion, *Penicillium levitum* and *Talaromyces louisianensis* have the potential to degrade PP due to their high and long-lasting MnP and laccase activities*. However, because no information on both fungi species in remediation has been previously reported, a more extensive study to gain a better understanding of the mechanism of PP remediation could be conducted.*

ACKNOWLEDGEMENTS

This project was supported by the INTI International University research grant scheme (INTI-FHLs-04-03-2021). We are grateful to Jeram Sanitary Landfill, Malaysia for allowing us to collect soil samples.

ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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