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Microplastics Biodegradation by *Aspergillus flavus* and *Aspergillus versicolor*

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Abstract: Microplastics (MPs) have indeed raised significant concerns due to their widespread presence and potential adverse effects on both the environment and human health. This study aims to illuminate crucial aspects of MPs, including their origins, migration behavior, and the potential for bioremediation as an effective strategy for their removal. Microplastics can originate from various sources, such as the fragmentation of larger plastics, the presence of microbeads in personal care products, the shedding of fibers from textiles, industrial pellets, and products containing microplastics. These diverse sources contribute to the omnipresence of microplastics in both terrestrial and aquatic ecosystems. This study focuses on observing the biological degradation process of two fungi, *Aspergillus flavus,* and *Aspergillus versicolor* when exposed to three different types of microplastics: Polypropylene (PP), Polyethylene (PE), and Polystyrene (PS). After conducting experiments, removal efficiencies of *A. flavus* and *A. versicolor* were calculated. Based on the data collected during the 10th week of using these fungi, it was observed that *A. flavus* exhibited removal efficiencies of 18.3% for PE, 6.8% for PP, and 1.9% for PS. On the other hand, *A. versicolor* yielded removal efficiencies of 6.7% for PE, 5.1% for PP, and 3.3% for PS. It was determined that *A. flavus* and *A. versicolor* exhibited the highest biodegradation efficiency when targeting microplastic PE, while their effectiveness was relatively lower when dealing with microplastic PS.
Keywords: *Aspergillus flavus, Aspergillus versicolor,* Biodegradation, Polyethylene, Polystyrene, Polypropylene.

1. Introduction

The issue of microplastic (MPs) pollution in aquatic environments has garnered significant attention in recent years due to its detrimental effects on ecosystems and potential implications for human health (Katija et al. 2017; Weithmann et al. 2018). MPs, typically defined as plastic particles with a diameter of less than 5 mm, are of particular concern because of their widespread presence and persistence in the environment (Alimi et al. 2018; Hidalgo-Ruz et al. 2012; Murphy et al. 2016). MPs can be categorized into two main types: primary and secondary. Primary microplastics are intentionally manufactured at small sizes for various purposes, such as microbeads in cosmetic products, while secondary microplastics are formed through the degradation of larger plastic items via physical, chemical, or biological processes (Browne et al. 2010; Gewert et al. 2015; Singh and Sharma, 2008). The scale of plastic pollution in oceans is alarming, with an estimated 150 million tons of plastic already present and approximately 8 million tons being added annually. This continuous influx exacerbates the environmental and ecological challenges associated with plastic pollution (Zhang et al. 2020a).

Among the various types of plastics, polyethylene (PE) and polypropylene (PP) are reported to have the highest demand globally. These plastics are commonly used in a wide range of applications due to their versatility, durability, and low cost. However, their persistence in the environment poses significant challenges for waste management and pollution control efforts. In contrast to PE and PP, other types of plastics such as polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), and polyurethane (PU) also contribute to plastic pollution but may exhibit different degradation behaviors and environmental impacts. Understanding the sources, distribution, and effects of various types of plastics is crucial for developing effective mitigation strategies and policies to address this pressing environmental issue (Browne et al. 2010;; Weinstein et al. 2016; Zhang et al. 2020a).

The degradation of MPs in different environments is a complex process influenced by various physicochemical and microbial factors. Although MPs are generally less susceptible to microbial attack compared to other degradable materials, microbial degradation plays a significant role in their transformation (Ammala et al. 2011).

Microbial degradation of MPs occurs through the activity of microorganisms that colonize and utilize MPs as a carbon source. These microorganisms form a novel ecological niche on the surface of MPs, facilitating their degradation. Researchers have isolated various types of microbes from different environmental samples to investigate their role in MP degradation. These microorganisms can be categorized into pure bacterial cultures, pure fungal cultures, bacterial consortia, and biofilms (Rujnic-Sokele and Pilipovic, 2017). Overall, understanding the diversity and activity of microorganisms involved in MP degradation is essential for developing strategies to mitigate plastic pollution. Harnessing the potential of microbial degradation pathways may offer promising solutions for managing MP contamination in various environments (Yuan et al. 2020).

Fungi, being eukaryotic organisms with a nucleus enclosed by a nuclear membrane, have a distinct advantage over prokaryotes in terms of genetic organization and regulation. This characteristic allows for more complex and precise control of cellular functions, potentially aiding in the efficient degradation of MPs (Sanchez, 2020). Laccase, an important enzyme produced by fungi, in the degradation of microplastics (MPs). Laccase indeed plays a crucial role in breaking down certain types of plastics like HDPE (high-density polyethylene) through oxidative cleavage, enabling the degradation process (Othman et al. 2021).

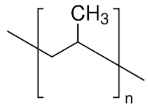
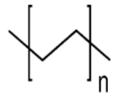
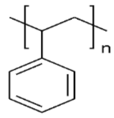
Fungal species, such as *Zalerion maritimum*, *Aspergillus niger*, and *Aspergillus fumigatus*, have demonstrated the ability to degrade MPs and utilize them as a sole carbon source. *Aspergillus* sp. demonstrates the ability to degrade polyurethane, which is a widely used synthetic polymer. The observed 20% degradation within 28 days highlights the effectiveness of this fungal species in breaking down polyurethane (PU) (Osman et al. 2017). Fungal MP degradation was demonstrated by Osahon and Williams (2021) using isolated fungi from a river. *A. niger* achieved a degradation rate of 71.1% for PP-MPs under the given conditions (1 g/L NaCl and ambient temperature) within 90 days. *A. fumigatus* exhibited a degradation rate of 53.1% for PP-MPs under the same conditions over the same 90-day duration. Adding carbon sources such as glucose and malt extract to the growth medium can significantly enhance the enzyme activity of the marine fungus *Zalerion maritimum*. These carbon sources likely provide the necessary nutrients and energy for the fungi to produce enzymes capable of breaking down polyethylene microplastics. The use of optimized growth conditions, including the pre-incubation process, led to a removal efficiency of 56% (Paco et al. 2017).

This study aims to investigate whether *A. versicolor* and *A. flavus* can degrade PP, PS, and PE by using the measurement of dry weight. The investigation will help to discover the role of the selected fungal species in degrading different types of MPs in the environment and to determine new approaches for their degradation.

2. Materials and Method

PP, PS, and PE were used as microplastics. PP, PS, and PE granules were obtained from Sigma Aldrich Chemical Co. (Product of USA) with densities of 0.9, 0.918, and 1.04 g/mL at 25°C, respectively. The granules were spherical in shape and white in color. The sizes of microplastics used in the study are PP and PE 5 mm, PS 3 mm. Some other properties are given in Table 1.

Table 1. Types and properties of used microplastics

	(PP)	(PE)	(PS)
Structure formula			
T_m (°C)[a]	160-165	100-125	95
Melt index	4.0 g/10 min (230 °C/2.16kg)	1.0 g/10 min (230 °C/2.16kg)	2.0-4.0 g/10 min (200 °C/5 kg)
Form	beads	pellets	pellets

[a]T_m, melting temperature.

MPs were steeped in 70% ethanol for sterilization at room temperature for 24 hours. Subsequently, they were transferred from the conical flasks into sterile beakers using sterile forceps. Approximately 0.5 grams of each microplastic type was weighed and placed in petri dishes. The microplastics in the petri dishes were left to stand under UV light for 24 hours.

A. flavus and A. versicolor were obtained from the Environmental Microbiology Laboratory, Eskişehir Technical University, Turkey. Fungi were cultivated in Potato Dextrose Agar (PDA) media, incubated at 28°C with a pH of 7 for 7 days.

The mineral salts medium (MSM) used for the biodegradation experiment contained the following composition in gram per liter (g/l): $MgSO_4.7H_2O$, 0.02 g; $FeSO_4.7H_2O$, 0.01 g; $ZnSO_4.7H_2O$, 0.001 g; $MnSO_4.H_2O$, 0.001 g; $CuSO_4.5H_2O$, 0.001 g; $(NH_4)_2SO_4$, 0.2g; KCl, 0.15g; $CaCl_2.2H_2O$, 0.002 g; NaCl, 0.15g; $CoCl_2.6H_2O$ 0.001 g; and KH_2PO_4 0.001 g; which was prepared by dissolving the salts in distilled water. The medium was sterilized by autoclaving at 121°C for 20 minutes. In a 100 mL conical flask, 50 mL of MSM was taken along with MPs positive (mineral salt media + MPs + fungus) and negative (mineral salt media + MPS) controls, respectively. The flasks were then preserved in a shaking incubator at a shaking rate of 100 rpm.

After exposing fungal isolates for five, seven, and ten weeks at 28°C MPs pieces were harvested, washed in 70% ethanol to remove as much biomass as possible, dried at 45°C, and equilibrated and weights were determined with each of the MPs pieces with and without treatment.

The weight reduction was determined after the degradation by using Eq. (1),

$$\% \, weight \; loss = \frac{(Mi - Mf)}{Mi} x100 \qquad (1)$$

where M_i is the initial weight of MPs and M_f is the weight of MPs after the incubation period.

3. Results

Fungal degradation of MPs was assessed over various durations, specifically five, seven, and ten weeks, utilizing *Aspergillus versicolor* and *Aspergillus flavus*. Physical modifications and degradation rate of MPS after fungal degradation were determined by mean weight loss. The results obtained from the fungal degradation of MPs pieces by *A. versicolor, and A. flavus*, are as shown in Figures 1 and 2.

Biodegradation efficiencies observed for *Aspergillus versicolor and Aspergillus flavus* on different types of plastic pieces for various durations:

For PS; *A. versicolor* has efficiencies of 1.8% (5 weeks), 2.118% (7 weeks), 3.3% (10 weeks), and *A. flavus* has; 0.2% (5 weeks), 0.3% (7 weeks), 1.9% (10 weeks). For PP; *A. versicolor* has efficiencies of 4.2% (5 weeks), 4.5% (7 weeks), and 5.1% (10 weeks); *A. flavus* has 3.0% (5 weeks), 3.7% (7 weeks), 6.8% (10 weeks). For PE; *A. versicolor* has efficiencies *of* 4.9% (5 weeks), 5.1% (7 weeks), 6.7% (10 weeks), and *A.flavus* has10.1% (5 weeks), 12.1% (7 weeks), 18.3% (10 weeks). These results show the biodegradation efficiencies achieved by each fungus on different plastic types over different periods.

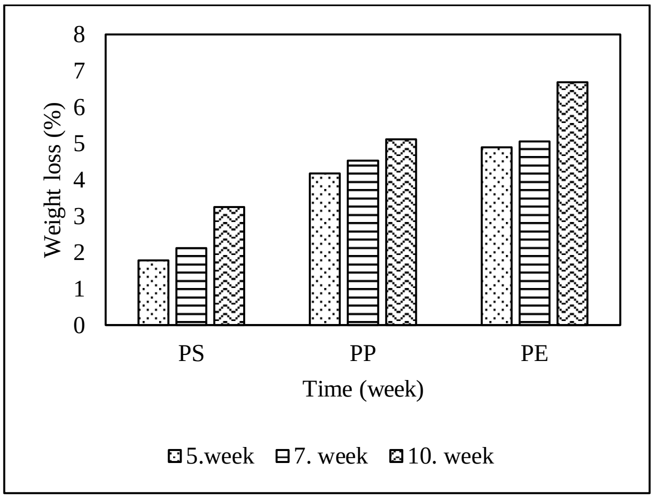


Fig. 1 Fungal biodegradation of MPs pieces of *A. versicolor*

4. Discussion

A. flavus reduced the weight of the PE strip up to 3.9% after 28 days of incubation (Zhang et al. 2020b). Taghavi et al. (2021) observed maximum weight loss was measured by incubation of PE with *Aspergillus flavus* (5.5%) in an unstimulated mix condition. Biodegradation of disposable PE using *Aspergillus* species was carried out by Singh et al. (2012). The strongest ability was observed by *A. terreus* with 7.6% biodegradability followed by *A. versicolor* with 3.8% of biodegradability.

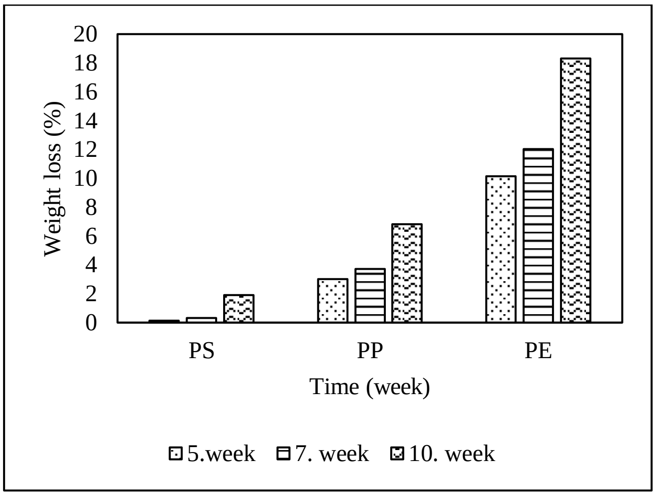


Fig. 2 Fungal biodegradation of MPs pieces of *A. flavus*

Williams and Osahon (2021) studied the PE degradation ability of *Aspergillus niger,* and *Aspergillus fumigatus* after the exposure for 45 days. They observed that the degradation potential of *Aspergillus niger* (71.1%) was more when compared to the *Aspergillus fumigatus* (53.1). *Aspergillus* consortium showed the highest percentage of reduction of PE weight with (26.2%) (Dsouza et al. 2021). Four species of fungi *Aspergillus* sp. were isolated by Alshehrei (2017). PE degradation ability of *A. flavus* 16.2%, *A. niger* 19.5%, *A. terreus* 21.8%, and *A. fumigatus* 20.5% were weighted.

Compared to the literature information, it has been determined that *A. versicolor* and *A. flavus* are effective in the biodegradation of PP, PE, and PS microplastics (Table 2). In particular, *A. flavus* showed the highest percentage of reduction of PE weight with (18.8%).

It has been observed in studies relying on literature data that the efficiency of degradation can vary depending on the fungal species used and the type of microplastic. Different fungal species and types of microplastics may exhibit various enzymatic activities and substrate properties, leading to varying degrees of degradation or biodegradation by different fungal species.

For example, certain fungal species may be more effective on specific plastic polymers, while others may excel in degrading different types of plastics. Additionally, the enzymatic activities and adaptation capabilities of fungi can contribute to the degradation of microplastics to varying degrees under different environmental conditions and parameters.

Therefore, studies related to microplastic biodegradation often test various combinations to evaluate the varying efficiencies between different fungal species and types of microplastics. The results of these studies play an important role in determining which fungal species can effectively degrade which types of microplastics.

In research related to microplastic biodegradation, it is important to assess the effects of fungal species and microplastic types on efficiency. This information can contribute to the development of more effective biological methods and help reduce plastic pollution.

Table 2. Characteristics of biodegradation of microplastics

Strain	MP	I.T., day	W.L., %	T., °C	References
A. flavus	PE	28	3.9	28	(Zhang et al. 2020b)
A. flavus	PE	100	5.5	30	(Taghavi et al. 2021)
A. terreus	PE	30	7.6	28	(Singh et al. 2012)
A. versicolor	PE	30	3.8	28	(Singh et al. 2012)
A. niger	PE	45	71.1	28	(Williams and Osahon, 2021)
A. fumigatus	PE	45	53.1	28	(Williams and Osahon, 2021)
A. consortium	PE	55	26.2	28	(Dsouza et al. 2021)
A. flavus	PE	30	16.2	28	(Alshehrei, 2017)
A. niger	PE	30	19.5	28	(Alshehrei, 2017)
A. terreus	PE	30	21.8	28	(Alshehrei, 2017)
A. fumigatus	PE	30	20.5	28	(Alshehrei, 2017)
A. versicolor	PS	70	3.3	28	Our study
A. versicolor	PP	70	5.1	28	Our study
A. versicolor	PE	70	6.7	28	Our study
A. flavus	PS	70	1.9	28	Our study
A. flavus	PP	70	6.8	28	Our study
A. flavus	PE	70	18.8	28	Our study

I.T.: Inkubation Time
W.L.: Weight loss
T: Temperature

5. Conclusion

The pervasive use of plastic has become a defining aspect of modern life, finding applications across various sectors owing to its durability and easy availability. However, this widespread plastic consumption has given rise to a notable surge in plastic pollution. Plastics have become an integral part of our daily lives, thanks to their resilient nature and widespread availability. Unfortunately, this extensive use has resulted in a considerable upswing in plastic pollution, with microplastics emerging as a critical environmental concern. Due to the chemical composition of microplastics, their biological removal is quite challenging. Microplastic pollution poses substantial harm to living organisms, the environment, and ecosystems.

In this study, the biodegradation of PP, PS, and PE microplastics by *A. versicolor* and *A. flavus* was observed. Biodegradation efficiencies were calculated at the end of a 5, 7, and 10-week incubation period at 28°C.

For PS; *A. versicolor* has efficiencies of 1.8% (5 weeks), 2.118% (7 weeks), 3.3% (10 weeks), and *A. flavus* has; 0.2% (5 weeks), 0.3% (7 weeks), 1.9% (10 weeks). For PP; *A. versicolor* has efficiencies of 4.2% (5 weeks), 4.5% (7 weeks), and 5.1% (10 weeks); *A. flavus* has 3.0% (5 weeks), 3.7% (7 weeks), 6.8% (10 weeks). For PE; *A. versicolor* has efficiencies *of* 4.9% (5 weeks), 5.1% (7 weeks), 6.7% (10 weeks), and *A.flavus* has10.1% (5 weeks), 12.1% (7 weeks), 18.3% (10 weeks).

It appears that all results demonstrate a significant level of microbial activity in the degradation and breakdown of PE pieces. Additionally, these results indicate that the rate of degradation of PE pieces is dependent on the duration of microbial activity.

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Authors' contributions: SM: obtaining data, editing, and writing; BSU, editing and writing.

Conflict of interest disclosure:

The authors declare that there were no conflicts of interest in the realization of this research.

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