Degradation of polymers by fungi isolated from dumpsites

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Research Article

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Abstract

The present study aims at identifying the ability of nine fungal species, which were isolated from garbage of different sites to degrade commonly used polymers, viz. polyethylene, polystyrene, and polyurethane when treated with four methods separately, viz., sterilized and unsterilized drench methods, sterilized and unsterilized mulching methods for four months. All the species considerably degraded the polymers by the above-mentioned methods. However, polystyrene demonstrated the greatest degradation compared to the other two polymers, particularly by sterilized and unsterilized drench methods. Seven fungal species caused greater than 50% weight loss of polystyrene when treated with the above-mentioned methods. *Aspergillus flaus* instigated the greatest weight loss (74.78 ± 2.85%) by the unsterilized drench method. Of nine, three species caused more than 50% weight loss of polyurethane by the unsterilized drench method. *A. niger* divulged greater than 50% weight loss of the polymer by sterilized drench method. In this study, polyethylene was found least degraded compared to polystyrene and polyurethane by the selected fungal species. Of nine, only two species, viz. *Aspergillus flaus* and *A. niger* caused a higher than 50% weight loss of polyethylene only by sterilized drench method. Scanning Electron Microscopy (SEM) images of six elevated degraded polymer samples were taken to reveal the formation of spores and hyphae on the surface of the plastic. The images demonstrated the formation of cracks and crevices on the surface of different polymers by spores and the fungal hyphae.

Introduction

Nonbiodegradable plastic waste is a global environmental concern due to its adverse consequences on the entire biosphere (Thompson, 2017) since the majority of bacterial and fungal species do not generally decompose them. On the contrary, worldwide plastic waste has been continuously increasing since the last few decades due to the wide-ranging consumption of plastic products. The first synthetic plastic, namely Bakelit, was produced in 1907. Before World War II, the commercial production of plastic was sluggish. However, worldwide production of plastics has skyrocketed after the 1950s particularly for the packaging of food, agricultural and industrial products due to its great demand. Over the subsequent 65 years, the annual global production of plastics swelled to almost 200-folds, that is, 381 million tons in 2015 (Ritchie and Roser, 2018). According to an estimate, 60 to 99 million tons of mismanaged plastic trash were added to the living planet during 2015 (Lebreton and Andrady, 2019).

Consequently, there is increasing attention among the researchers to discover both the bacterial and fungal species that can decompose the ever-increasing plastic waste, besides their photo-oxidation and thermal decomposition (Ali, et al., 2021). Studies divulge that the plastic degradation by fungal species is more encouraging compared to that of bacterial species (Muhonja, et al., 2018; Barratt, et al., 2003). Therefore, many researchers have studied and identified the fungal species that can cause the degradation of various types of plastics by using different methods.

*Aspergillus niger* decomposes thermally oxidized high-density polyethylene (Mathur, et al., 2011). This fungus reveals great potential for the degradation of low-density polyethylene under natural conditions (Esmaeili, et al., 2013). *A. clavatus* degrades low-density polyethylene when incubated in an aqueous medium for 90 days (Gajendiran et al., 2016). *A. oryzae* reduces substantially the weight of low-density polyethylene in a shaker incubator (Muhonja, et al., 2018). *A. flaus* and *A. tubingensis* consume high-density polyethylene as a carbon source without pre-oxidant and pretreatment (Devi, et al., 2015). *Rhizopus oryzae* decreases the weight of thermally treated high-density polyethylene and substantially reduces its tensile strength in a sustainable manner (Awasthi, et al., 2017). *Zalerion maritimum*, a marine fungus, has the potential to utilize polyethylene as a carbon source in a minimal growth medium (Paço, et al., 2017). The endophytic fungi degrade the gamma-irradiated low-density polyethylene strips (Sheik, et al., 2015).

Khan, et al., (2017) stated that *Aspergillus tubingensis* decomposes the polyester polyurethane film into smaller pieces in a liquid medium after two months. Taghavi, et al., (2021) reported greater surface degradation of plastics under mixed microbial system. They found the greatest weight loss of polyethylene when incubated with *A. flaus* in unstimulated mix condition. Barratt et al., (2003) reported that *Geomyces pannorum*, *Nectria gilocladioides*, and *Penicillium ochrochloron* degrade polyester polyurethane and reduce its tensile strength up to 60% under a varied range of soil water holding capacity. Álvarez-Barragán et al., (2016) stated that Cladosporium cladosporioides greatly reduces the weight of polyurethane after 14 days of incubation. Urzo, et al., (2017) found that *Lasiodiplodia theobromae*, *Penicillium janthinellum*, *Fusarium verticillioides*, and *Paeclomyces puntonii* trim down the weight of polyurethane and consume it as the sole nitrogen and carbon.

Studies on the degradation of polystyrene by fungal species are insufficient compared to that of polyethylene and polyurethane. However, few studies have demonstrated the degradation of polystyrene by fungal species. For instance, *Cephalosporium* sp. and *Mucor* sp. reduce 2.17 ± 0.16% and 1.81 ± 0.13%
weight of polystyrene respectively when incubated with them separately for eight weeks (Chaudhary and Vijayakumar, 2019). Tsujiyama and Takada (2004) reported a slow degradation of polystyrene by *Phanerochaete chrysosporium*, a white-rot fungus.

In the present study, different colonizing fungal species and strains were collected from the surface of polyethylene, polystyrene, and polyurethane, which were dumped in different localities of the University of Karachi. These polymers are the most widely exploited disposable plastics, which are largely used as household packaging materials in the city. Polyethylene and polystyrene are thermoplastic while polyurethane is a thermosetting plastic. The investigation will help to discover the role of the selected fungal species in degrading different types of plastics on the topsoil and to determine new approaches for their degradation.

**Materials And Methods**

The samples of polyethylene, polystyrene, and polyurethane were collected along with the soil from six garbage dumping sites of Karachi University during March and April 2019. Their coordinates are presented in Table 1. Three replicates of each type of plastic and soil were taken from each site for the study. Two different sets of used polystyrene food boxes were collected from the garbage of different locations to reveal any difference in its degradation by varied fungal species. Five different samples of *Aspergillus flavus* were collected from the garbage of different locations to divulge any difference in the degradation characteristics of its varied collections. In each case, four replicates were taken to avoid an error.

| Site # | Coordinates                      |
|-------|---------------------------------|
| 1     | 24°56'26.14"N, 67°7'10.44"E     |
| 2     | 24°56'28.94"N, 67°7'10.11"E     |
| 3     | 24°56'29.94"N, 67°7'14.38"E     |
| 4     | 24°56'40.72"N, 67°7'15.17"E     |
| 5     | 24°56'23.60"N, 67°7'11.30"E     |
| 6     | 24°56'20.54"N, 67°7'16.46"E     |

The samples were taken to the laboratory and were kept separately for 24 hours at room temperature. Each plastic sample was washed with distilled water and then cut into small pieces with sterilized scissors. The washed pieces of the plastic samples were then weighed and placed separately on Potato Dextrose Agar (PDA) plates, which were supplemented with streptomycin and penicillin. All the plates were incubated at 28°C for five to six days to identify the fungal species using the references (Barnett and Hunter 1998; Raper and Thom 1949; Visagie et al. 2014).

The fungi were isolated from the soil samples by soil dilution technique (Nash and Snyder, 1962; Urooj et al., 2018). Soil samples were collected from 10 cm depth at each site. Each soil sample was transferred in separate test tubes with 10mL sterilized water, which was diluted up to a 1: 10 ratio. 0.1 mL of each diluted sample was hosted on different petri-dishes that contain PDA. All the plates were stored at 28°C for 5 days for the growth of fungal species. The PDA plates were augmented with streptomycin and penicillin. The fungal species were identified by using the above-mentioned references.

Test fungal species were grown in 250mL conical flasks that contained 100mL Czapek’s Dox broth. Each flask was immunized with a 5mm disc of test fungi, cut from the briskly growing culture. The flasks were protected at room temperature (25-30°C). Three different types of un-used plastic, namely, polyethylene, polystyrene, and polyurethane were procured to run the pot experiment.

The pot experiment was conducted by taking 1 kg soil per pot. Sterilized and non-sterilized sets of pots were taken for the experiments of plastic degrading properties of fungi. Three different types of plastics were selected to investigate fungal activities. In the sterilized pot experiment, the soil and plastic were sterilized, while in the unsterilized pot experiment, the soil and selected plastic samples were used without sterilization. The plastic samples were sterilized with 1% bleach for 3 minutes, then with 70% alcohol for 3 minutes, and finally washed with distilled water.

In both the sterilized and unsterilized pot experiments, two different methods namely, Drench Method and the Mulching method were used for the study. In Drench Method, the fungal broth is directly drained over the plastic while in Mulching Method, the fungal broth is drained over the soil. The unused plastic samples were buried in the soil of pots. The buried samples were taken out from the soil with a one-month interval to observe visually the degradation of plastic. We found very little degradation of different types of plastics during the first three months. However, after four months, noticeable cracks and roughness appeared on the surface of plastics. Therefore, after four months, all the buried plastics were taken out from the soil and washed with water to observe them through dissecting microscope for further verification of the degrading plastics before their analysis. The plastic samples were found eroded and decolorized. Prominent deterioration and unevenness were observed on the surface of plastics under the microscope. Each plastic sample was washed
with distilled water and then dried at room temperature. They were weighed separately to calculate the reduction in the weight due to the application of different fungal species. Weight loss is extensively used as a measure of biodegradation of polymer (Taghavi, et al., 2021; Chaudhary and Vijayakumar, 2019; Muhonja, et al., 2018; Álvarez-Barragán et al., 2016)

Six plastic samples that demonstrated elevated degradation were selected for a detailed investigation like the interaction of fungal hyphae with plastic through Scanning Electron Microscopy (SEM).

Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) were performed by using SPSS 16 for the analysis.

Results

Figure 1 demonstrates the weight loss of polyethylene when treated with nine different fungal species by employing four different designated methods. The analysis of variance reveals a highly significant variation in reducing the weight of the plastic by different fungal species (p < 0.001) when treated with varied methods (p < 0.004). *Aspergillus flavus* showed the greatest degradation of polyethylene when treated with sterilized drench and unsterilized mulching methods while *Penicillium rubrum* divulged its greatest degradation when treated with unsterilized drench and sterilized mulching methods. As a whole, the sterilized drench method was found to be the most effective method for the degradation of polyethylene by different fungal species.

Figure 2 exhibits the weight loss of polystyrene when treated with nine different fungal species by applying four different methods. The statistical analysis discloses a highly significant variation in reducing the weight of the plastic by different fungal species (p < 0.001) when treated with varied methods (p < 0.001). *Aspergillus flavus* showed the greatest degradation of polyethylene when treated with unsterilized drench and unsterilized mulching methods while *A. niger* divulged the greatest degradation when treated with sterilized drench and sterilized mulching methods. Overall, the unsterilized drench method was found to be the most worthwhile method for the degradation of polystyrene by different fungal species and *A. flavus* demonstrated its highest degradation properties by different designated methods.

Figure 3 discloses the degradation of polyurethane when treated with nine different fungal species by using four different methods. The statistical analysis demonstrated a significant variation in the weight loss of the plastic by different fungal species (p < 0.02) when treated with varied methods (p < 0.001). *Aspergillus flavus* showed the greatest degradation of polyethylene when treated with unsterilized drench and unsterilized mulching methods while *A. niger* divulged the greatest degradation when treated with sterilized drench and sterilized mulching methods. Overall, the unsterilized drench method was found to be the most useful method for the degradation of polyurethane by different fungal species and *A. flavus* revealed its highest degradation properties by different designated methods.

Figure 4 demonstrates the degradation of three different types of polymers by five different samples of *Aspergillus flavus*, which were collected from different sources and locations. Analysis of variance shows a non-significant difference in the degradation of all three types of polymers, viz. polyethylene, polystyrene, and polyurethane when treated with different samples of *A. flavus*. However, a highly significant difference (p < 0.001) was found in the weight loss of different plastics when treated with four different designated methods.

Fig 5 shows the treatment of two different samples of polystyrene with nine different fungal species using four designated methods. The samples were collected from two different sites. Statistically, a non-significant difference was found among the weight losses of two polystyrene samples when treated with nine different fungal species. However, the weight losses of the polystyrene samples differ significantly due to different fungal species when treated with sterilized drench (p < 0.002), unsterilized drench (p < 0.001), and sterilized mulching (p > 0.001) methods. Yet, a non-statistical difference was found between the weight losses of polystyrene samples due to different fungal species when treated with unsterilized mulching.

Table 1 divulges the weight losses in terms of the percentage of three different types of plastics when treated with nine different fungal species using four different methods. Duncan Multiple Range Test (DMRT) was applied to determine the statistical difference of the mean values, which are presented in this table. The mean values with different alphabets differ significantly (P < 0.05).

Images 1 – 9 represent the Scanning Electron Microscopic (SEM) images of the degradation of different types of plastic. Images 1 and 4 demonstrate the untreated surfaces of the two polystyrene samples, viz. Polystyrene-1 and Polystyrene-2 respectively. Image 7 exhibits the untreated surface of polyurethane. The surfaces of all three samples were found intact. The untreated plastics were not degraded even after the four months of the experiment.
Image 2 reveals the degraded surfaces of Polystyrene-1 when treated with Aspergillus flavus. The fungal species penetrated into the surface of the plastic appeared after four months. Image 3 demonstrates the spread of the spores of Penicillium expansum on the surface of the plastic. The arrows identify the fungal spores and the penetration of hyphae on the surface of the plastic. Images 5 and 6 divulge the degraded surfaces of Polystyrene-2 when treated with A. flavus and Macrophomina phaseolina respectively. The spores of M. phaseolina on the surface of plastic are shown by arrows.

Images 8 and 9 divulge the degraded surfaces of Polyurethane when treated with Aspergillus niger and A. flavus respectively. The spread of spores and the penetration of hyphae on the entire surface of the plastic appeared after four months, which are shown by arrows.

Discussing

Polystyrene

The present study demonstrates that many fungal species substantially degraded polystyrene when treated with sterilized and unsterilized drench methods. Greater than 50% weight loss of polystyrene is found when treated with seven different species, viz. Aspergillus flavus, A. niger, Penicillium decumbens, P. rubrum, P. expansum, Macrophomina phaseolina, and Fusarium solani by employing sterilized drench and unsterilized drench methods for four months duration. A. flavus demonstrated the greatest weight loss (74.78 ± 2.855 %) of polystyrene when treated with an unsterilized drench method. Furthermore, A. flavus, A. niger, and Macrophomina phaseolina caused a weight loss of greater than 50% when treated with the unsterilized mulching method. Moreover, A. niger resulted in 50.14 ±15.17% weight loss of polystyrene when treated with the sterilized mulching method. All the nine species demonstrated greater than 30% weight loss of polystyrene when treated with all the four designated methods separately.

Polyurethane

Of nine fungal species, only three species, viz. Aspergillus flavus, Penicillium rubrum, and P. expansum caused more than 50% weight loss of polyurethane when treated with an unsterilized drench method. Moreover, only A. niger resulted in greater than 50% weight loss of the polymer by employing the sterilized drench method. Mathur and Prasad (2012) reported a 60.6% weight loss of polyurethane when incubated with a shaking culture of A. flavus having 4.8 x 10^6 spores ml for 30 days. Oviedo-Anchundia, et al., (2021) reported a 28.34% weight loss of this polymer by Penicillium spp.

Not a single fungal species caused greater than 50% weight loss of polyurethane by sterilized mulching and unsterilized mulching methods. Less than a 20% reduction in weight of polyurethane is found when treated with Aspergillus oryzae, Penicillium decumbens, and P. frequentans by employing sterilized mulching and unsterilized mulching methods. Furthermore, Macrophomina phaseolina by sterilized mulching method and Penicillium rubrum and Fusarium solani by sterilized mulching method reduced less than 20% of the weight of polyurethane.

Polyethylene

The present study reveals that polyethylene is least affected by the selected fungal species when treated with the designated drench and mulching methods compared to polystyrene and polyurethane. Of nine species, only two species, viz. Aspergillus flavus and A. niger caused a higher than 50% weight loss of the polymer when treated with the sterilized drench method. These two species have not reduced the weight of polyethylene to fifty percent by the other three designated methods.

Furthermore, the other seven fungal species have not reduced the weight of polyethylene to fifty percent by any of the four designated methods. Penicillium decumbens and P. frequentans reduced less than 20% of the weight of polyethylene by unsterilized drench and sterilized mulching methods. Fusarium solani dropped less than 20% of the weight of polymer by sterilized and unsterilized mulching methods. Penicillium rubrum plummeted less than 20% of the weight of the polymer by an unsterilized mulching method.

Muhonja, et al., (2018) reported that A. oryzae brought about 36.4 ± 5.53% weight loss of polyethylene in four months, which is comparable to the present study. A. oryzae resulted in a weight loss of 40.71 ± 2.73% (unsterilized mulching), 29.99 ± 2.33% (sterilized drench), 28.57 ± 22.99% (unsterilized drench), 22.14 ± 2.73% (sterilized mulching).

Declarations

Conflict of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.
Ethical approval and consent to participate: Not applicable since humans and/or animals are not involved in this study.

Consent for publication: Not applicable since individual person’s data are not used in any form.

Availability of data and materials: The authors have shared the data and wish to public them.

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Tables
Table 1: Weight losses (%) of three different types of plastics when treated with nine different fungal species using four different methods. According to Duncan Multiple Range Test, mean values with the same alphabet do not differ significantly.

| Fungal species      | Sterilized Drench | Sterilized Mulching | Un-Sterilized Drench |
|---------------------|-------------------|---------------------|----------------------|
|                     | Polyethylene     | Polystyrene        | Polyurethane        | Polyethylene     | Polystyrene        | Polyurethane        |
| Control             | a1.42 ±1.64      | a3.75 ±2.33        | a1.81 ±2.33         | a1.42 ±1.64      | a3.75 ±2.33        | a1.10 ±1.42         |
| Aspergillus flavius | b56.13 ±3.21     | c57.62 ±1.61       | d36.95 ±11.76       | b23.99 ±2.29     | a49.86 ±10.52      | d34.00 ±3.23        |
| A. oryzae           | c39.99 ±2.33     | c41.25 ±1.54       | d22.14 ±2.73        | b48.42 ±12.84    | b18.75 ±11.08      | b28.57 ±2.29        |
| A. niger            | d70.74 ±2.33     | e71.88 ±9.65       | e57.5 ±2.88         | b22.85 ±4.03     | c50.14 ±15.17      | d29.28 ±4.87        |
| Penicillum decumbens| ef50.71 ±2.73    | f71.88 ±9.65       | f57.5 ±2.88         | b22.85 ±4.03     | c50.14 ±15.17      | e50.67 ±8.60        |
| P. frequentans      | g33.56 ±2.73     | h49.78 ±9.45       | h28.75 ±4.78        | b18.56 ±3.68     | d33.53 ±18.91      | g12.5 ±2.88         |
| P. rubrum           | d22.85 ±4.03     | c51.49 ±1.53       | c49.78 ±9.45        | b18.56 ±3.68     | d33.53 ±18.91      | h12.5 ±2.88         |
| P. expansum         | e39.28 ±14.82    | b66.39 ±8.56       | f42.5 ±2.88         | b22.85 ±2.33     | f44.89 ±1.60       | e35.00 ±9.12        |
| Macrophomina phaseolina | f64.42 ±1.43 | g64.96 ±20.31     | d38.75 ±4.78        | b31.42 ±2.33     | f44.89 ±1.60       | e35.00 ±9.12        |
| Fusarium solani     | cd31.42 ±2.33    | f57.71 ±2.01       | d31.75 ±11.08       | b23.14 ±4.08     | f36.14 ±14.20      | d20.00 ±4.08        |

Figures
Figure 1

Treatment of Polyethylene by different species of fungi using varied methods. The control represents untreated plastic.
Figure 2

Treatment of Polystyrene by different species of fungi using varied methods. The control represents untreated plastic.
Figure 3

Treatment of Polyurethane by different species of fungi using varied methods. The control represents untreated plastic.
Figure 4

Treatment of plastic by Aspergillus flavus using different methods. The fungus was collected from plastic and soil that was found at varied locations (suffixes p and s, followed the fungal name, represent plastic and soil while the number indicates the site).
Figure 5

Treatment of Polystyrene collected from 2 different sites with different fungal species using varied methods