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Bioremediation of low-density polyethylene (PE) by Fungi

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ABSTRACT

The necessity of having eco-friendly disposal policies designed for biodegradation of synthetic plastics is of utmost importance and needs attention in the present scenario, considering the serious impact of widely used packaging materials such as low-density polyethylene (PE) on the environment. Our study aimed to explore the degradation of PE by three different fungi species. Alternaria alternata, Aspergillus, Penicillium

The extent of biodegradation was evaluated by weight loss of the PE samples. The weight of the plastic sample before degradation was 1 gr and after the degradation, the sample weight of polyethylene (PE) was (0.15%;0.3%8,0.92%,) gr respectively.

Scanning electron microscope (SEM) analysis revealed that morphological changes in the surface of PE were observed while FTIR images showed functional groups changes after 90 days incubation period. The formation of biofilms on the surface of polyethylene and the adhesion capabilities of fungi are seen as the first step in the biodegradation process. These results. can colonize, modify and utilize PE as the sole carbon source, water. This manuscript also paves the way for future studies on biodegradation to solve the global problem.

Keywords: *Plastic. Biodegradation. Fungi. Polyethylene. Problem.*

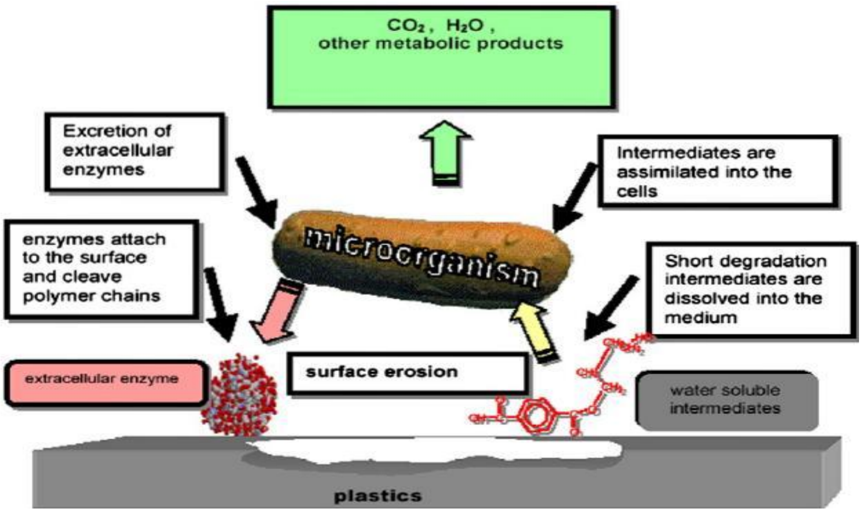
1. INTRODUCTION

Plastic is defined as a polymer of organic components such as cellulose and inorganic components such as carbon, hydrogen, nitrogen, sulfur. plastic is slow to decompose, strong, durable and lightweight made from petroleum derivatives. Plastic has been with use since the beginning of the twentieth century. It is considered an affordable, versatile and durable material. Plastic materials have gained widespread use due to their use in many industries including clothing, food, transportation, construction, medical industries, leather industries and others (**Qassim** *et al.*,2023). Consequently, the global production of plastics is still on the rise. Over the past ten years, global production of plastics has increased by 97 million tons to reach 367 million tons. (Sridharan, 2021).

Plastic plays an important role in many “short live” applications like packaging and these represent the main part of plastic wastes. Because of its propagation in our environment, several communities are now more sensitive to the impact of discarded plastic on the environment, including deleterious effects on wildlife and on the aesthetic qualities of cities and forest (Abbas& Abdulhay, 2024 ; **Qassim** *et al.*,2023).

Bioremediation: is the technique of using microorganism and plants for environmental cleanup of both organic and inorganic xenobiotic (Abatenh et al 2017 ; Qassim et al., 2019). Since bioremediation eliminates, degrades, detoxifies, and immobilizes dangerous wastes and pollutants, microorganisms are crucial. Understanding the whole range of physiological, microbiological, ecological, biochemical, and molecular processes involved in pollutant transformation's essential for successful bioremediation employing microorganism(Nayak and Solanki, 2022). Ex situ or in situ bioremediation can be utilized, depending on a variety of criteria, such as cost, pollutant kinds, and concentration. Bioremediation is cheaper than incineration, and certain contaminants may be handled on site, minimizing exposure hazards for clean-up workers or broader exposure from transportation accidents.

Bioremediation carried out in situ, including bioventing, bespangling, and bio augmentation, decontaminates without removing soil from the site while ex situ treatments (such as land farming, biopiling, composting, bioreactors, and electro dialysis) treat the soil that is unearthed at the location (Parween , et al 2018; Qassim et al.,2021).



Bioremediation of Plastics(Elahi, Amina; et al 2021)

2. MATERIAL AND METHODS

2.1 Collection of samples

Samples of soil contaminated with plastic waste were collected in several different areas in Diwaniyah Governorate according to Table1. The method of (Amal ***et al*,.**2018) was followed for the purpose of collecting soil samples. The surface soil was removed to a depth of (5-15cm). 100 g of soil was taken using a spoon and placed in clean plastic bags. It was transferred to the laboratory and stored in the refrigerator at a temperature of 4 °C until laboratory analyses were conducted on it**.**

Table (1) Four different soil samples were collected from different locations within the city of Diwaniyah.

NO	Site of samples
1	Alfrat
2	Algeria
3	Arabism
4	Housing District

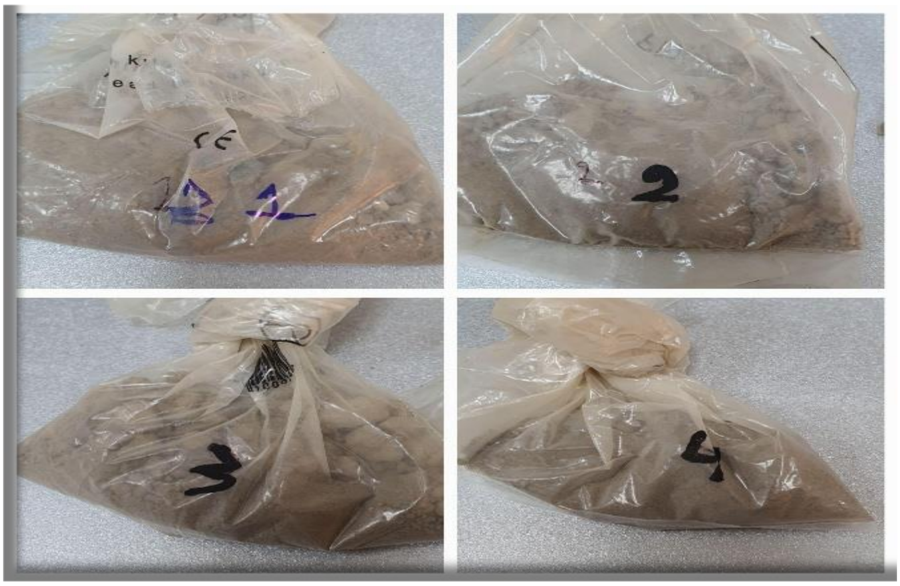


Fig.1contaminated soil sample

2.2:Isolation and identification of Fungi from contaminated soil

We take 1 gm of soil contaminated with microorganisms using a loop, and the petri dishes are numbered according to the number of soil samples, the dishes are planted with soil contaminated with agricultural media, sterile scalpel.

Then incubate for 5 day at 25°C (Sarah;2017). Three types of fungi were identified based on morphological and morphological characteristics and the change in the color of the medium. ***Alternaria alternata, Aspergillus, Penicillium***

2.3 Pretreatment of polyethylene.

The polyethylene was cut into small and fine pieces washed with ethanol chemical to remove soil and dirt then, washed it with distilled water and dried (Khlaif & Abdulhay,2023).

2.4 polyethylene inoculation with Fungi:

The middle of mineral salts (MSB) was disinfected and transferred into the flasks. PE (1g) materials were cut into small parts (5 cm×2 cm), sterilized with 70% ethanol for 30 min and washed with sterile distilled water for 20 min.

Materials of PE were placed in flasks containing the MSB (100ml) then they were inoculated with 1ml of activated isolated by ***Alternaria alternata, Aspergillus. Penicillium.***

The flasks control and flask contain plastic were incubated in the incubator on a rotary shaker at 37°C. Results were observed after Three month(Tarafdar; et al 2017)

Fig 2: Polyethylene inoculation with Fungi.

2.5Weight loss measurement of PE after Fungi Growth:

PE were recovered after three months of incubation of culture, washed with methanol and finally washed with distilled water. The weight loss was measured according to the following equation (Gauri,2016).

$$\textbf{\textit{weight}}^{1}\ \textbf{\textit{loos}} = \frac{(\textbf{\textit{Initial weight}} - \textbf{\textit{final weight}} \times \textbf{100}\%)}{\textbf{\textit{Initial weight}}}$$

Table3: Polyethylene weight loss after three months with isolated fungi ***Alternaria alternata, Aspergillus, Penicillium***

	Weight of polyethylene (g)		
Isolated fungi	Initial weight	Final weight	Percentage%
Penicillium	1	0.85	0.15%
Alternaria alternat	1	0.62	0.38%
Aspergillus	1	0.08	0.92%

2-6. Fourier transform infrared spectroscopy (FT-IR) analysis.

FTIR spectrum of PE (without fungi inoculation) treatments had peaks of C–H stretching and C–H blending (in the range of 2800–3000, 1400–1550, and 650–750 cm−1) which indicates functional groups of alkanes in the PE backbone (Fig 4-a) .

The control samples of PE treatment exhibited the range of 3200–3400 cm−1which relates to the peak for OH group, whereas the samples of inoculated fungi exhibited a reduction in the peak intensity in the same range of 3200–3400 cm−1. The fungi treatment generated new functional groups in the nitro compound (–N=O stretch) range of 1550–1500 cm−1 and the alcohol, carboxylic acid, esters, ethers compound (–C–O stretch) range of 1320–1000 cm−1 which was not observed in the control treatment**.**

The ground polyethylene sample treated with ***Penicillium*** fungus demonstrates noticeable oxidative changes and modifications due to fungal activity on the FTIR spectrum. The broad peak at 3186.40 cm^{-1} variety peaks in the O-H region, indicating oxidative changes and possibly also interactions related to moisture content. Peaks at 2858.51 cm^{-1} and 2675.27 cm^{-1} were obtained by C-H stretching vibrations partially retaining the hydrocarbon chain in polyethylene. The peak at 1627.92 cm^{-1} represents C=O stretching, which means an ox datively degraded form of carbon along with the formation of carbonyl groups. Peaks at 1465.90 cm^{-1} and 1373.32 cm^{-1} resulting from C-H bending vibrations are minor structural changes in the polymer backbone. At 1303.88 cm^{-1}, we see C-O stretching, and peaks at 1026.34 cm^{-1} and 1038.63 cm^{-1} are due to the structure of the polymer and oxidative modifications it has undergone. Peaks at 923.90 cm^{-1} and 719.45 cm^{-1} are about C-H out of plane bending, reflecting some partial retention of this shape characteristic retained on our polymer. Lastly, the peaks at 682.80 cm^{-1}, 615.29 cm^{-1}, and 565.14 cm^{-1} lines reflect interactions with changes in chain structure from fungal activity (Mishurov *et al.*,2020).

The FTIR spectra of the treated ground polyethylene sample infected with ***Aspergillus niger*** showed significant oxidative changes and structural transformations. A broad band at 3433.20 cm^{-1} is for the O-H stretching vibration, reflecting oxidative changes and moisture absorption. 2926.01 and 2848.86 cm^{-1} are C-H stretching vibrations peaks, representing corn retention in the polyethylene hydrocarbon skeleton. With 1658.78 cm-1 for the peak, C=O stretching is seen, which indicates the oxidative decomposition of C-H bonds to give carbonyl groups. 1606.70 cm^{-1} is a peak of aromatic C=C stretches, which means there is some structure change under the influence of fungus infection in the aromatic ring. 1465.90 cm^{-1} and 136949 cm-1 are peaks of C-H bending vibrations, indicating only minor modifications to the polymer structure. At 1078.21 cm^{-1}, which is for the C-O stretching vibration, it is reflected that some parts of the polyethylene molecule's backbone are being oxidized. 842.89 and 719.45 cm-1 are aromatic C-H out-of-plane bending peaks, showing that the aromatic structure has retained somewhat. Finally, 626.87 and 553.21 cm^{-1} peaks are for ring deformation and effects of fungus infection, respectively. (Mwangi, & Okoth, 2021).

For the sample of polyethylene treated with Alternaria alternata. The FTIR spectrum shows noticeable oxidative and structural changes brought about by the interaction between fungi and polymers. The broad peak at 3433.29 cm^{-1} is O-H stretching, suggesting that these substances undergo oxidative modification and absorb humidity. Peaks at 2918.30 cm^{-1} and 2848.86 cm^{-1} represent C-H stretching vibrations, representing an almost unchanged polyethene hydrocarbon base. The peak at 1658.78 cm^{-1} due to C=O stretching, meaning oxidative degradation and formation of carbonyl groups. The peak at 1463.97 cm^{-1} is C-H bending vibrations, representing minor structural changes in the polymer. Further changes at 1369.46 cm^{-1} represent chain breakage and structural alteration. The peak at 1178.51 cm^{-1} reflects C-O stretching vibrations, representing an oxidative change in the polymer chain. Peaks at 1020.34 cm^{-1} and 875.68 cm^{-1} are bending vibrations and out-of-plane deformations, showing structural modification resulting from fungal activity. The final two peaks (at 719.45 cm^{-1} and 619.15 cm^{-1}) are aromatic ring breakage and small changes in the polymer backbone. In summary, the spectrum shows that due to fungal interaction, both oxidative degradation and structural modifications are present in polyethylene. However, some basics of the polymer 's internal makeup remain unchanged(Yadav, & Prasad,2024).

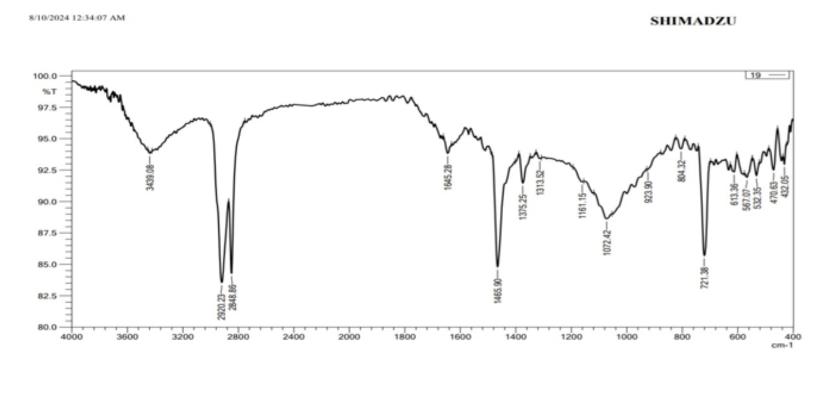


Figure (4-a): FTIR spectra of polyethylene (PE) before consumption by fungi.

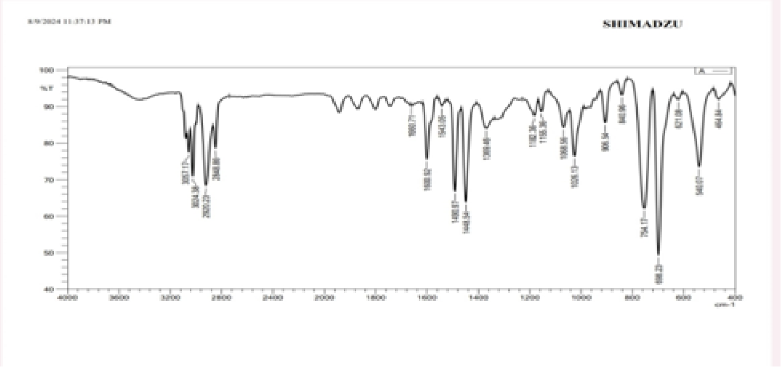


Figure (4-b) FTIR spectra of polyethylene (PE)by fungi *Penicillium*.

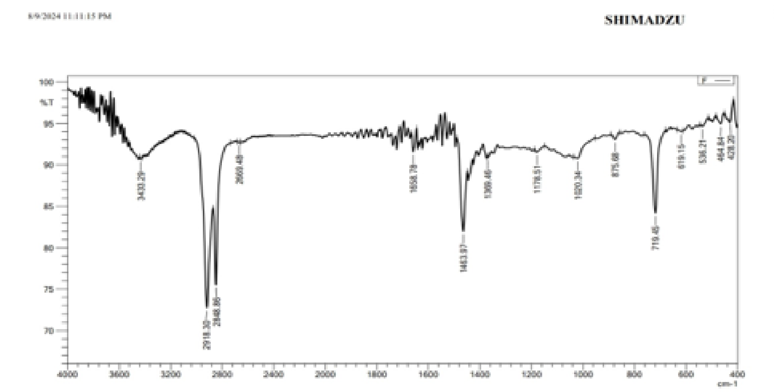


Figure (4-c): FTIR spectra of polyethylene (PE)by fungi *Aspergillus niger*.

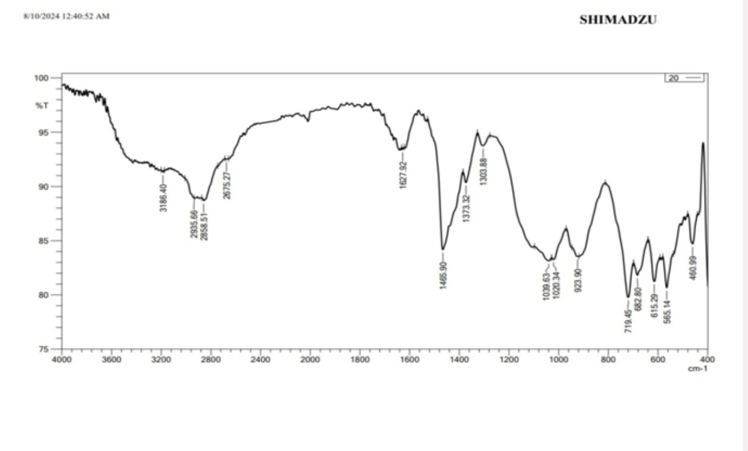
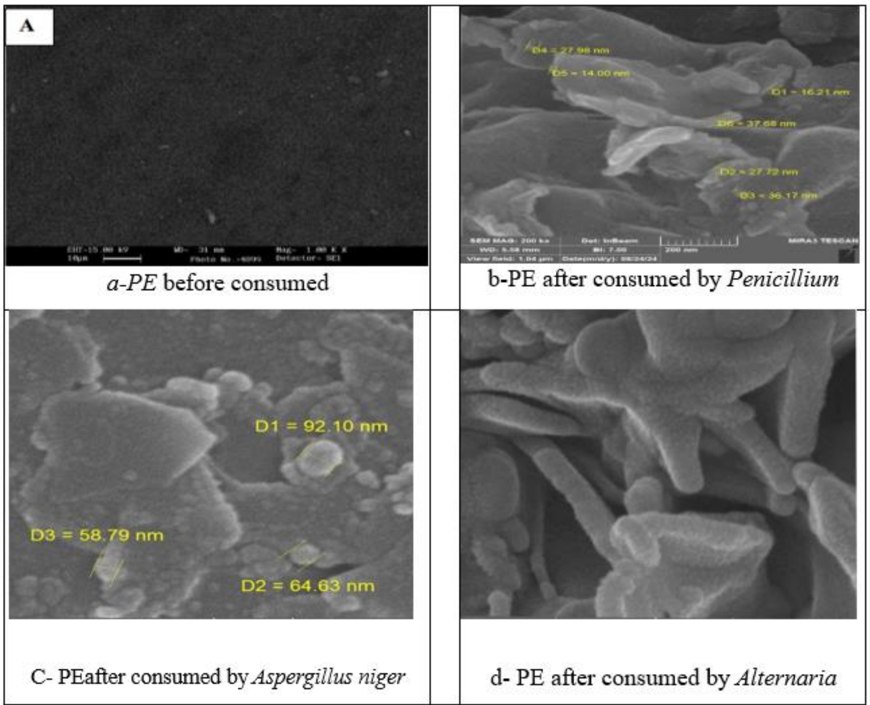


Figure (4-d) FTIR spectra of polyethylene (PE) by *fungiAlternaria alternata*

2-7.Fungi growth scanning by SEM.

All types of isolated fungi showed the formation of biofilms on the surface of the plastic samples (PE) but in different percentages of formation and this showed by SEM. The result of SEM showedThe extent of biodegradation was examined by detecting changes in surface morphology through scanning electron microscopy analysis. Several changes in the physical appearance of the fungi-treated PE film were observed after 90 days of incubation. However, the untreated PE sample showed a smooth surface (Fig5). The microbially treated sample confirms the surface deformation by 2showing various cracks and irregularities formed as a result of microbial activity The change displayed in the surface. (Khan *et al.,* 2022 ; Mohy Eldin *et al.*, 2022).



a-PE before consumed

b-PE after consumed by *Penicillium*

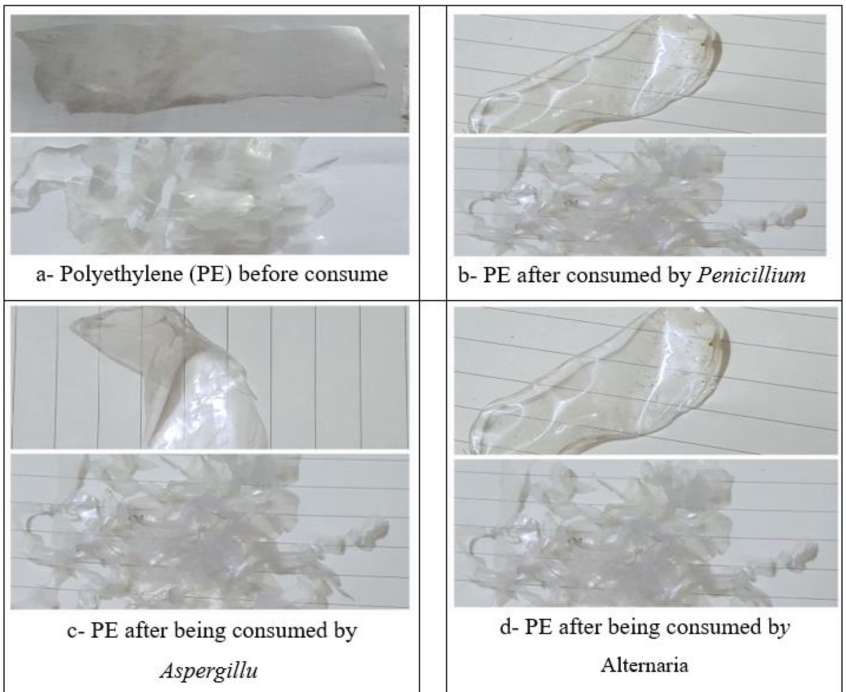
C- PEafter consumed by *Aspergillus niger*

d- PE after consumed by *Alternaria*

Figure 5: Scanning electron microscopy shows polyethylene before (a) and after consumption by isolated fungi (b, c and d) by *Penicillium*; *Aspergillus niger* . ;*Alternaria alternata*.

2-8. Physiological changes of fungi isolated from plastic

We noticed a difference in the plastic Before and after treatment with the isolated fungi. There is a difference in the shape, size and thickness of the plastic **and** this ensure the degradation of polyethylene by fungi (Abbas and Abdulhay,2024).



a- Polyethylene (PE) before consume

b- PE after consumed by *Penicillium*

c- PE after being consumed by *Aspergillu*

d- PE after being consumed by Alternaria

Figure 6: Physiological changes of fungi isolated from plastic

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