SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 9 | September 2024 - Peer Reviewed Journal

BIODEGRADATION OF SYNTHETIC PLASTICS BY FUNGAL SPECIES ISOLATED FROM SOIL

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Article DOI: https://doi.org/10.36713/epra18333
DOI No: 10.36713/epra18333

ABSTRACT
Excessive plastic use endangers both the environment and human life on Earth. Plastic accumulation on land and sea has sparked interest in degrading these polymers. To reduce the environmental impact of plastics, appropriate biodegradable methods must be used. Understanding the interaction of microbes and polymers is critical for overcoming the environmental problems associated with plastics. Many living organisms, primarily microorganisms, have developed strategies to survive and degrade plastics. The current review focusses on the various types of fungal species in our environment that are capable of degrading plastic, as well as the time it takes for fungal species to degrade plastic polymers. We have showed the degradation of thin plastic polymer with the isolated fungal species when we kept it for incubation at 27˚C for 44 days the degradation of plastic was found to be 4.85% with the biomass of 2.91g.
KEYWORDS: *Polymer, Biodegradable, Fungal species, Biomass.*

INTRODUCTION
Global plastic production is increasing year after year. In 2018, global plastic production was 359 million tonnes, with 368 million tonnes in 2019. China is the world's leader in plastic production, accounting for 31% of global production in 2019. Although the production of new plastic increases on an annual basis, the rate of plastic waste management and recycling has yet to reach precise levels. In 2018, Europe produced a total of 61.8 million tonnes of plastic. In 2018, Europe collected only 9.4 million tonnes of plastic post-consumer waste [1].

The estimated global total for virgin plastic production in 2017 was 8300 million tonnes. In 2015, 6300 million tonnes of plastic waste were accumulated; only 9% were recycled, 12% were incinerated, and 79% were disposed of in landfills or released into the natural environment [2]. Every year, 25 million tonnes of synthetic plastics accumulate along coastlines and in terrestrial environments [3]. In 2019, 6.1 million tonnes (Mt) of plastic waste were released into aquatic environments, with 1.7 Mt flowing into the ocean. Currently, there has been a significant increase. At least eight million tonnes of plastic end up in the ocean, where it has the potential to degrade into tiny microplastics that could enter our food chains and cause unknown consequences [4].

Plastics play an important role in every sector of the global economy because they are widely used in agriculture, building and construction, health care, and consumer goods. They are the foundation of many industries because they are used to make a variety of products such as defence materials, sanitary wares, tiles, plastic bottles, synthetic leather, and other household items. Plastics are also used to package food items, pharmaceuticals, detergents, and cosmetics [5]. Synthetic plastics production is one of the world's fastest growing industries. Plastics have distinct advantages over other materials. These properties have led to a 20-fold increase in plastic production since 1964, with production exceeding 300 million tonnes per year in 2015, reaching 335 million tonnes [6].

Excessive plastic use endangers both the environment and human life on Earth. Plastic accumulation on land and sea has sparked interest in degrading these polymers. To reduce the environmental impact of plastics, appropriate biodegradable methods must be used. Understanding the interaction between microbes and polymers is critical for overcoming the environmental problems associated with plastics [7].

Plastics can be classified as either degradable or nondegradable based on their chemical properties. Plastics made from renewable resources are biodegradable. Microorganisms also produce these polymers. Non-degradable plastics, also known as synthetic plastics, are made from petrochemicals and have a higher molecular weight due to the repetition of small monomer units [8]. Most fungi are abundant worldwide, but they are inconspicuous due to their small structures and cryptic lifestyles in soil or on dead matter. Fungi are symbionts of plants, animals, and other fungi, as well as parasites. They may become visible when fruiting, either as mushrooms or moulds. Fungi play critical roles in organic matter decomposition, as well as nutrient cycling and exchange in the environment. They have long been used as a direct source of human food, such as mushrooms and truffles; as a leavening agent for

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bread; and in the fermentation of a variety of food products, including wine, beer, and soy sauce. Since the 1940s, fungi have been used for the production of antibiotics. More recently, various enzymes produced by fungi are used industrially and in detergents [9]. Fungal species from the *Aspergillus* genus, specifically *Aspergillus niger, Aspergillus flavus, and Aspergillus oryzae*, are commonly used in low density polythene biodegradation due to their ability to grow freely and abundantly in soil and garbage sites, as well as their longer incubation time compared to other fungal species [10]. Studies show that using a consortium of fungal species gives better results than using individual fungi in a variety of research areas such as degradation of textile dyes, production of nanoparticles for targeting breast cancer, and treatment of dairy wastewater [11]. This observation has been proved to be consistent in the biodegradation of polyethylene as well, where a consortium of fungi showed superior degradation rates when compared to the use of individual fungi [12]. Polymers can be degraded through various processes, including photo-oxidative degradation, thermal degradation, ozone degradation, mechanochemical degradation, and catalytic degradation. Photo-oxidative degradation involves light absorption, producing ester, aldehyde, propyl, and format groups at soft segments of polymers. Thermal degradation occurs by accidental or depolymerization reactions, with reactions occurring on the entire polymer surface. Ozone degradation, present in the atmosphere, causes polymers to last longer when oxidative processes are not active. Mechanochemical degradation involves polymer chains breaking under mechanical stress and ultrasonic irradiations, producing macro radicals used in polymerization reactions. Catalytic degradation transforms waste polymers into hydrocarbons, improving the quality of products obtained after pyrolysis of plastics [5].

Biodegradation can occur through aerobic or anaerobic mechanisms. In aerobic biodegradation, microorganisms use oxygen as an electron acceptor to break down large organic compounds like plastics, producing carbon dioxide, water, and residual carbon as by-products [13]. Conversely, in anaerobic biodegradation, oxygen is not required. Instead, microorganisms utilize alternatives such as nitrate, iron, sulphate, manganese, or carbon dioxide as electron acceptors, resulting in the formation of methane, carbon dioxide, water, and residual carbon. Both processes contribute to the natural breakdown of plastics, albeit under different environmental conditions [14]. This study revolves around the following crucial objectives:

- Isolation of Fungal species from Black Soil and Red Soil.
- Identification of Fungal species.
- Degradation of Plastic material by Isolated Fungal species.

MATERIALS AND METHODOLOGY

Materials Required

The materials used in this biodegradation study included two types of soil, black and red, which were collected from the MGM University Campus in Chh. Sambhajinagar, Maharashtra, India. Thin plastic samples were gathered from the surrounding environment for testing. The instruments employed in the study comprised an autoclave, incubator, laminar air flow, pan balance, and hot plate. Various glassware items, including conical flasks, beakers, test tubes, petri plates, and measuring cylinders, were also used. The chemical components essential for the experiments included dextrose, agar powder, sucrose, sodium nitrate, dipotassium phosphate, magnesium sulphate, potassium chloride, ferrous sulphate, peptone, hydrogen phosphate, monopotassium phosphate, epsomite, ammonium nitrate, calcium chloride, and other related compounds. These materials supported the isolation, growth, and biodegradation experiments conducted with the fungal species.

Isolation of Fungi from Soil

Different Soil sample were collected and diluted with 1gm soil in 10 ml sterile distilled water, serial dilution was performed in the laminar air flow from 10^{-1} to 10^{-5}. 1ml of sample of each dilution was inoculated to different sterile petri plates with the help of micropipette containing sterile Potato Dextrose Agar, Sabouraud Dextrose Agar and Czepek-dox agar. Then the plates were incubated at 30˚C for 3 days in incubator.

Morphological Characterization by Lactophenol Cotton Blue staining

Isolated fungi were morphologically characterised using lactophenol cotton blue staining. Place a drop of 70% ethanol onto a microscope slide. Immerse the specimen or material in a drop of alcohol. Add one or two drops of lactophenol/cotton blue mount stain before the alcohol dries. Holding the coverslip between your forefinger and thumb, touch one edge of the drop of mount to the coverslip edge and gently lower, avoiding air bubbles. The preparation is now ready for the examination.

Biodegradation of plastic using isolated and identified fungal species

Samples of thin plastic polymers were cut in small fragments and were placed in a glass flask containing paper towel fragments and mineral medium, 100 ml mineral medium was prepared and divided into 4 conical flasks with the quantity of 25ml each. After that the identified fungus was inoculated in a glass flask.

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Table 1. Weight of Conical Flasks

Conical Flask	Plastic Polymer (wt.)	Paper Towel (wt.)
1.	0.350g	0.035g
2.	0.350g	0.070g
3.	0.350g	0.105g
4.	0.350g	0.140g

After the complete immersion of the plastic polymer and the paper towel. The conical flask was kept for incubation at 27°C for 44 days. After incubation the results were analyzed.

RESULTS

Isolated fungal species: Various types of 17 fungal species were isolated on the petri plates, some of the fungal species were found to be in single growth form and some were found to be in mixed growth form. The fungal species were firstly identified by their morphological structure. The mixed form species were later sub cultured for further objectives.

Fig. 1(a)

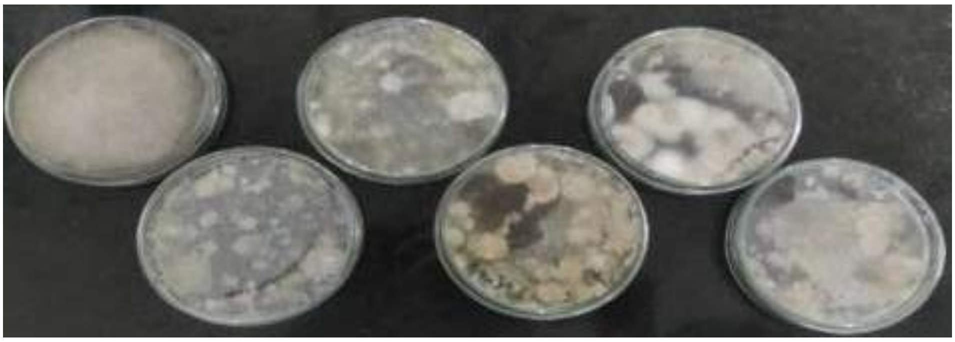

Fig. 1(b)

Fig.1. 17 Isolated Fungal Species

Lactophenol cotton blue was performed for the isolated fungal species: The fungus was identified at 40X objective under microscope, the structure was having sporangium and hyphae (Fig. 2(a) and Fig. 2(b)). The conical flask containing plastic polymer and paper towels kept for incubation at 27˚C for 44 days was removed and the last objective to measure the amount of plastic degraded was measured.

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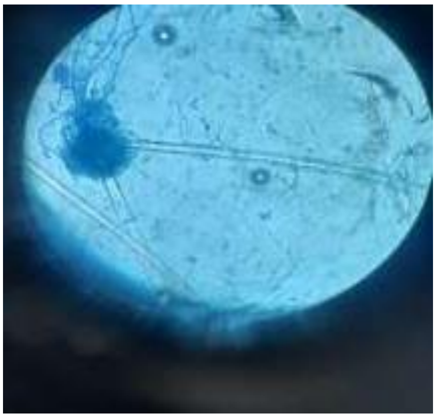

Fig. 2(a)

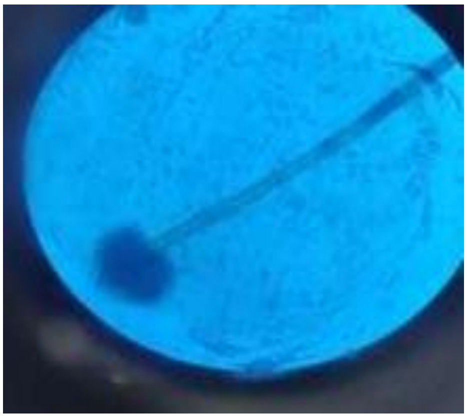

Fig. 2(b)

Fig. 2. Microscopic Image of Fungal species

Weight of plastic polymer and dry mass was measured with the pan balance and the results were as follows: Among 4 conical flask the results of 2 conical flask were found to be positive as the weight of plastic polymer in those conical flasks was reduced. The amount of degradation was found to be 4.85% and 0.8% respectively (Fig. 4. (a) & Fig. 4. (b)). The amount of dry mass obtained it was also measured it was found to be 2.85g and 2.91g respectively (Fig. 4. (c) Fig. 4. (d)).

Fig. 3. Degradation of fragments of plastic polymer in conical flask[V]

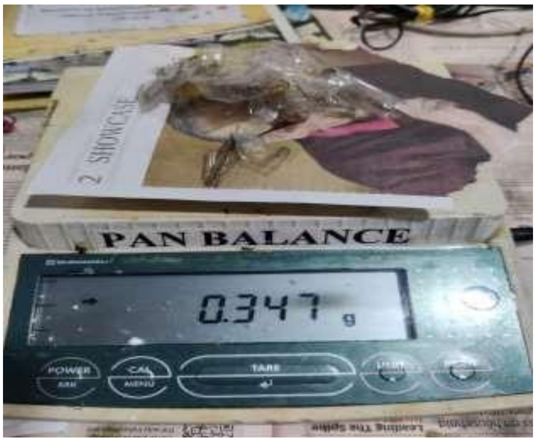

Fig. 4. (a)

Fig. 4. (b)

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Fig. 4. (c)

Fig. 4. (d)

Fig. 4. Weight of plastic polymer and dry mass

The Lactophenol cotton blue staining was performed on the dry mass to observe the fungal mycelium structure. Under a microscope at 40X magnification, the mycelium structure was clearly visible, confirming the presence and characteristics of the fungal growth. The observed structures were documented in Fig. 5(a) & Fig. 5(b), showcasing the effectiveness of the isolated fungal species in the degradation process. This staining technique helped in identifying the morphology of the fungi involved in plastic biodegradation.

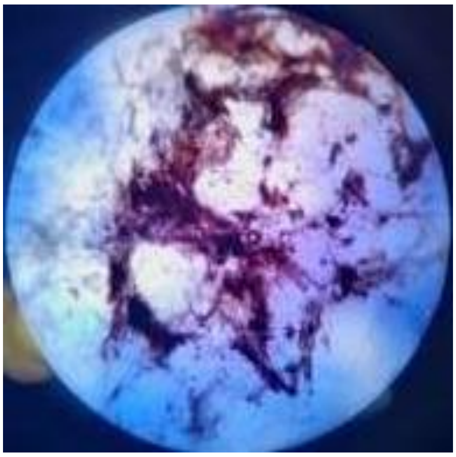

Fig. 5. (a)

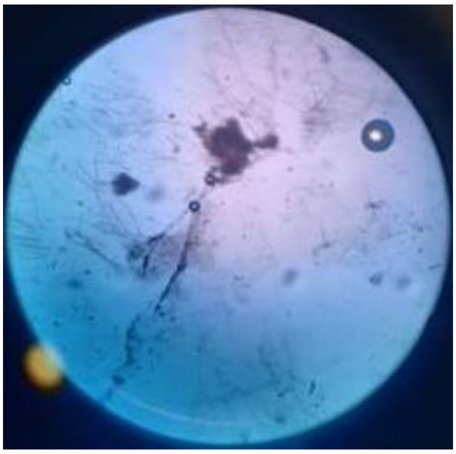

Fig. 5. (b)

Fig. 5. Plates Showing Mycelium Growth of The Biomass

DISCUSSION AND CONCLUSION

The primary aim of this study was to explore the potential of fungal species isolated from black and red soil in degrading plastic polymers. Plastic pollution is a significant environmental concern, as synthetic plastics are resistant to natural degradation. This study demonstrated that certain fungal species, particularly from the genus *Aspergillus*, can degrade thin plastic polymers. After 44 days of incubation at 27°C, a degradation rate of 4.85% was achieved, accompanied by a dry biomass accumulation of 2.91g. This suggests that fungi possess the ability to utilize plastic as a carbon source, breaking down complex polymers into smaller molecules. The research aligns with previous studies indicating that fungi play an essential role in organic matter decomposition and may also contribute to plastic degradation. The use of a consortium of fungal species for biodegradation could further enhance the degradation rates, as supported by other studies in microbial degradation fields. The use of fungi in biodegradation processes presents an eco-friendly and promising solution for reducing plastic waste in the environment, though the overall rate of degradation needs to be optimized for real-world applications.

This project successfully demonstrated the biodegradation of plastic polymers using fungal species isolated from black and red soil. The study confirmed that fungi, particularly those from the genus *Aspergillus*, are capable of breaking down plastic polymers under controlled conditions. The findings support the potential of using fungal species as part of a sustainable solution to plastic pollution. However, further research is necessary to optimize the degradation process, including the exploration of different plastic types,

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fungal species, and environmental conditions. In conclusion, fungal biodegradation offers a promising eco-friendly approach to mitigate plastic waste and its environmental impact.

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