

BIOREMEDIATION OF PLASTICS BY MICROORGANISMS

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Abstract

Soil is an inherent medium that sustains life on our planet. Soil serves as a foundation that is essential for the development and sustenance of life on our planet. It functions as a storage area for air, water, and nutrients that are crucial for the development of living creatures, particularly vegetation and microbes. The nutrient content of the soil is determined by the composition and abundance of microorganisms that reside within it. Plastic pollution is a significant form of pollution that poses a continuing hazard to the entire ecosystem due to its ongoing deposition in the environment. The current research focuses on the degradation of plastic through the method of bioremediation using microorganisms isolated from the garden soil taken from St. Ann's College for Women in Mehdiapatnam, Hyderabad. Fungal extraction has been conducted, and shaker flask studies have been performed to assess the fungi's capacity for degrading plastics. The bioremediation process was evaluated by measuring the weight loss caused by various microorganisms. The bioremediation experiments were conducted over a period of 90 days using a potato dextrose agar medium (PDA). The microbes which degraded the plastics are *Aspergillus* species and *Fusarium* species. The percentage of degradation was found to be 25%.

Keywords: Bioremediation; Degradation; Microorganisms; Plastics

Introduction

Soil serves as a significant reservoir for organic carbon in the Earth's land-based ecosystems (Lal *et al.*, 2021). Practices that lead to a decrease in soil organic matter result in the release of CO₂, as well as harm soil resilience and frequently reduce agricultural yield. (Glanz, 1995)

Soil is a crucial and life-supporting medium for various reasons. It is a fundamental component of the Earth's ecosystem, supporting plant growth, biodiversity, and the multiple benefits provided by

ecosystems are crucial for maintaining environmental conditions and supporting human communities. The capability of soil-based microorganisms is determined not solely by the production of clumps along with enhancement of the mechanical attributes of the soil, additionally by their ecological and cellular features.

Soil microflora, encompassing bacteria, fungi, viruses, and protozoa, constitutes a diverse community of microorganisms in soil. It plays a

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pivotal role in ecosystem functioning and has substantial implications for soil health, plant growth, and environmental sustainability. Soil microbes are essential for the process of nutrient cycling in ecosystems. Microorganisms, specifically bacteria and fungi, have a vital function in the decomposition of organic matter. Specific soil microorganisms have the capacity to inhibit the growth of plant diseases by engaging in activities such as resource competition, synthesis of antimicrobial substances, and triggering systemic resistance in plants. Certain soil microbes establish symbiotic associations with plants, such as mycorrhizal fungi. These interactions facilitate the absorption of nutrients, accelerate the uptake of water, and stimulate overall plant growth.

Microplastic pollution in land is acknowledged as the subsequent most significant research issue in the field of environmental protection and biodiversity (Horton *et al.*, 2017). Relevant research indicates that the quantity of microplastics found on land may be 4-23 times higher than the amount found in the water (Nizzetto *et al.*, 2016). Horton and colleagues (2017) offered a succinct summary of the sources and risks of microplastics in the soil environment. A significant hazard is the potential ingestion of microplastics by both people and creatures through the consumption of food. Microplastics can arise from various places and are easily carried and modified by the earth's surroundings, hence impacting the soil ecology (Yu *et al.*, 2022).

Polymers are synthetic substances composed of interconnected recurring monomers linked through chemical ties. These are widely employed in the container design of a wide range of vital commodities, including dietary supplements, beauty, chemical substances, medications, and surfactants (Ahsan *et al.*, 2016; Mueller, 2006). Plastic packing materials are cost-effective, resilient, portable, efficient in terms of energy and simple to be manufactured. Plastic items have evolved as integral components of our daily lives and enterprises due to their exceptional characteristics (Ahsan *et al.*, 2016; Zahra *et al.*, 2010).

Every year, the worldwide production of plastic materials reaches one trillion tons, increasing at a rate of 12% (Varshney *et al.*, 2023; Caruso, 2015). Plastics are widely utilized due to their inherent stability and long-l nature. Plastics come in many types such as polyethylene (PE), polyethylene terephthalate (PET), nylons, polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyurethane (PUR). (Strong, 2006; Ogunbayo *et al.*, 2019). LDPE, often known as low-density polyethylene, is a plastic composed of elongated

chains of ethylene molecules. It is widely recognized as to be among the most often utilized plastics on a global scale (Roy *et al.*, 2008; Kyaw *et al.*, 2012; Gajendiran *et al.*, 2016). Waste plastic presents a significant peril to the environment. They accumulate in the soil and cause waterlogging, ultimately resulting in soil degradation and hindering agricultural cultivation and plant development (Yoon *et al.*, 2012; Ahsan *et al.*, 2016).

The microbes play a vital role in this process, as over 90 species of bacteria, actinomycetes and fungi, have the potential to degrade polymers (Mahdiyah and Mukti, 2013; Ponniah *et al.*, 2014; Sivasankari and Vinotha, 2014; Deepika and Madhuri, 2015). The process by which microorganisms enzymatically degrade polymers is known as biodegradation. It comprises four distinct phases: biological degradation, removal of polymers, incorporation, and weathering.

(Sumathi *et al.*, 2016; Strong, 2006; Olicon-Hernandez *et al.*, 2017). Recently, it has been discovered that microorganisms are capable of breaking down plastic bags using anaerobic procedures in soil and waste. This breakdown leads to the creation of water, carbon dioxide, and methane (Zettler *et al.*, 2013; Bryant *et al.*, 2016). In comparison to bacteria, fungus exhibit a more rapid development rate in soil and possess the ability to extend and infiltrate into other areas through the expansion of mycelial dispersion. This characteristic renders them more well-suited for the breakdown of LDPE (Sanchez, 2020; Gong *et al.*, 2023).

Low-density polyethylene comprises 60% of the overall plastic bag manufacturing, making it the predominant form of solid trash that is polluting the soil (Gajendiran *et al.*, 2016). Showa in Figure 1.

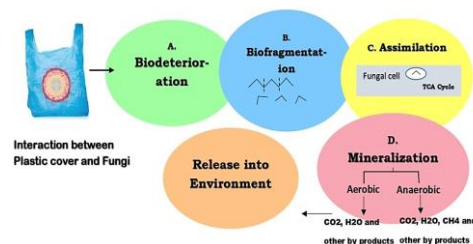


Figure 1. Plastic degradation process

St. Ann's College for Women campus experiences a daily influx of 300 to 400 vehicles. Combustion of fossil fuels releases pollutants like carbon monoxide, nitrogen oxides, and particulate matter, which settle onto the soil. Pollutants alter the nutrient composition of the soil, affecting the

growth of plants and overall soil fertility. Microorganisms present in the Garden soil are able to withstand the fossil fuel pollutants. So, Garden soil has been chosen for the collection of Fungi.

Bordering on a humid semi-arid climate, Hyderabad experiences a tropical wet and dry climate (Norman *et al.*, 1995). The average yearly temperature is 26.6°C (79.9°F), with monthly average temperatures ranging from 21 to 33 °C (70 to 91 °F). The summers, which occur from March to June, are characterized by hot and arid weather. The typical high temperatures range from the mid-to-high 30s Celsius. It is common for the highest temperatures to surpass 40°C (104°F) during the months of April to June (Norman *et al.*, 1995). The most frigid temperatures are experienced in December and January, with occasional drops to as low as 10°C (50°F) (Norman *et al.*, 1995). The month of May experiences the highest temperatures, with daily temperatures ranging from 26-39 °C (79-102 °F) Sridharan and Ji, (2023).

The soil sample was obtained from the garden located by the roadside of St. Ann's College for Women in Hyderabad, which has a Latitude of - 17.3929 and a Longitude of - 78.4374. Shown in Figure 2.

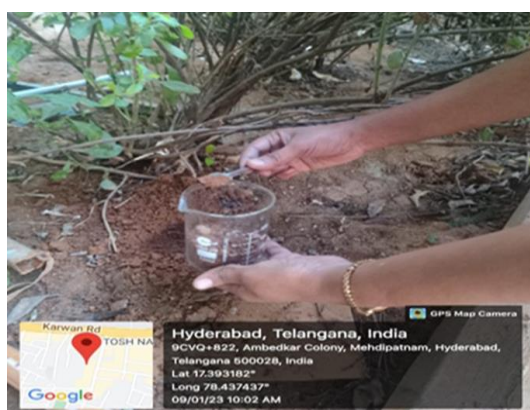


Figure 2. Soil Sample Collection Zone

Materials and Methods

Collection of Sample

The fungal strains included in the present investigation were obtained from the garden of St. Ann's College for Women, located in Mehdiapatnam, Hyderabad, as well as from the surrounding area around the college site. The soil samples were obtained by excavating the soil to a depth of 3 cm. These samples were then utilized to isolate fungi that are capable of

decomposing polythene (Yoon *et al.*, 2012; Ahsan *et al.*, 2016).

Isolation and Identification of Fungi

A soil sample weighing one gram, obtained from the campus garden, was pulverized and combined with 10 ml of sterile distilled water. This was followed by a series of successive dilutions. Using the spread plate method, 0.1ml of the suspension was transferred to petri plates containing sterile Potato Dextrose Agar (PDA) Medium, from dilutions of 10⁻³, 10⁻⁴, and 10⁻⁵. Subsequently, the plates were placed in an environment with ambient temperatures for incubation. Following a 48-hour incubation period, several fungal colonies were observed on the PDA medium and subsequently separated into pure cultures. The fungal isolates were subjected to microscopic and macroscopic observation, which involved staining with Lactophenol cotton blue. Characterization of the fungi was centred on morphological criteria such as the structure of hyphae and the appearance of spores (Adesemoye *et al.*, 2006; Ishii *et al.*, 2007; Ayeni *et al.*, 2022).

Preparation of the polymer

Low density polyethylene covers were collected from the garbage in the street close by to the college. The covers were cut into strips weighing 40mg each. All the films were cleansed with 70% ethanol for one hour and then rinsed in sterile water and dried in the hot air oven. The sterilized strips were preserved for further use.

The petri dishes prepared with potato dextrose agar medium are inoculated with fungal species. After the growth of fungi for 72 h, sterilized low-density polyethylene strips weighing 40mg were placed on the agar medium. The plates were placed in an incubator at a temperature of 30 degrees Celsius for a duration of 45 days. Shown in Figure 3-6.

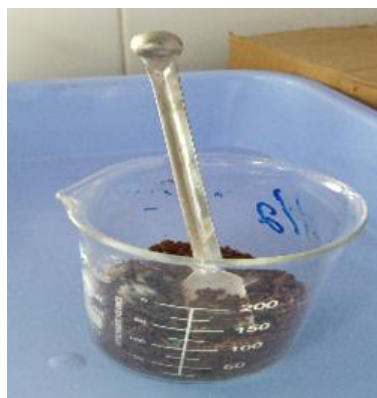


Figure 3. Collection of soil samples



Figure 4. Serial Dilutions



Figure 5. Preparation of PDA plates



Figure 6. Incubation of Plates with plastic

Following a 45-day incubation period, LDPE strips were extracted from the fungal culture. They were subsequently washed in a 70% ethanol solution for twenty-four hours, followed by a rinse in sterile water for one hour. Finally, the films were dried in a hot air oven (Orr *et al.*, 2004; Khruengsai *et al.*, 2021). These strips were weighed after 24 h. of washing.

Results and Discussion

Fungi were isolated from garden soil samples that is exposed to the roadside pollution. A total of 10 fungal strains were isolated from the sample. All the ten fungal strains were separately inoculated in petri dishes containing PDA medium. After three days of incubation sterile LDPE strips each weighing 40mg were placed and allowed for 45 days of incubation. Among the ten isolates, two strains of fungi have shown the changes in the weight.

The weight was calculated with the formula:

$$\text{Weight loss (\%)} = \frac{\text{Weight (initial)} - \text{Weight (resident)}}{\text{Weight (initial)}} \times 100$$

The isolates have shown 25% of weight loss after 45 days of incubation. The two fungal isolates where degradation was seen are identified as *Aspergillus niger* and *Fusarium oxysporum*. Further characterization is yet to be done. Shown in Figures 7-10. Figures: Incubation of plates inoculated with fungi and plastic (LDPE) for 45 days at room temperature.



Figure 7. Isolate 1

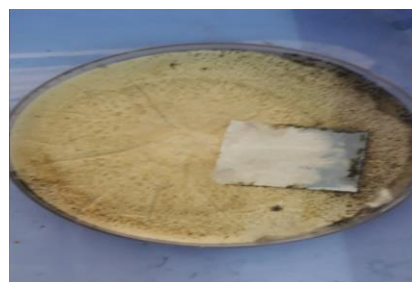


Figure 8. Isolate 2



Figure 9. Isolate 3



Figure 10. Isolate 4

Biodegradation is the most efficient approach for managing plastic pollution. (Das and Adholeya, 2012). Fungi release extracellular enzymes through their mycelia, which degrade large organic compounds into simpler chemicals. Microorganisms utilize these molecules as nutrients and eventually metabolize them into carbon dioxide (CO₂) and water (H₂O). (Sanchez, 2020; Gong *et al.*, 2023). Examining the attachment of mycelia to the LDPE sheets also demonstrates the degradation capacity. In this study, we conducted the first tests to determine the biodegradable capability of the fungal strains *Aspergillus niger* and *Fusarium oxysporum* on LDPE strips. We evaluated their growth and the degree of attachment to LDPE sheets.

Conclusions and Recommendations

The experiments are carried out in bench scale by taking soil samples from St. Ann's College which can be extended to other sites. The studies can be further extended to garbage sites, lake/River sediments, eustrine/Mangrove regions, Ocean areas and Varmi composts by motivating the student community to take up such activities. In the present study the microbes *Aspergillus niger* and *Fusarium oxysporum* have actively participated in LDPE degradation in Lab conditions. The study can be extended by optimizing different physico-chemical conditions such as PH, temperatures, and other type of microbes available in different sources. The growth kinetic studies and biochemical tools will be employed for further studies. The study can be improvised by taking a consortium of microbes as the degradation of plastics is a burning problem. The potential usage of plastic as carbon source and metabolism studies will improvise the environmental pollution caused by plastics. The present study offers a natural solution for controlling the plastic pollution.

Future Studies

The isolated microorganism can be / will be studied in detail. The novel technology can be shared with scientific community for further suggestions for study and reports has to be communicated to common people and policy makers.

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