

EXPLORING MICROBIAL-MEDIATED BIODEGRADATION PATHWAY OF POLYMERS IN ENVIRONMENTAL SYSTEMS.

ABSTRACT

Context: The global increase in environmental plastic has been a topic of increasing discussion and concern over the past decades. Ease of manufacturing, durability, inexpensiveness, and resistance to corrosion are desirable characteristics for consumer products but the resulting environmental longevity is causing a massive proliferation of plastic pollution.

Aim: To find out various microbial-mediated biodegradation pathway of polymers.

Settings and design: The research was carried out by obtaining dump soil from a mechanic dump site and a hospital dump site in Ibadan, Oyo state.

Materials and Methodology: Dump soil were obtained, diluted (serial) and cultured and morphological and microscopic observation were carried out while polythene bags which are black nylon and white nylon were also degraded at Day 5, 10, 15 and 20.

Result: Biodegradation of polymers is influenced by both polymer characteristics and the presence of specific microorganisms in the environment. Fungi, with species like *A. niger*, *A. flavus*, and *C. fungus* were identified in a dump site. Weight analysis of polythene bags (black nylon and white nylon) over days revealed slight changes, indicating some degradation. PCR analysis confirmed genetic material from fungal isolates, highlighting the specificity and sensitivity of the chosen primers.

Conclusion: The presence of *Aspergillus* species in the dump sites suggests their potential for bioremediation efforts in challenging environments.

Keywords: Fungi, polymers, dumpsite, biodegradation, soil.

INTRODUCTION

The amount of mismanaged plastic waste in the environment was estimated to be as much as 60–99 million metric tonnes in 2015 (Lebreton & Andrady, 2019). Plastic is a ubiquitous pollutant (Eriksen, M., *et al.*, 2014; Xanthos & Walker, 2017), and its persistence in the environment, and the potential harm that it may cause to organisms and ecosystems is an emotive modern day reality.

The quantifying the amount of plastic waste in the environment is challenging, and global estimates of plastic waste vary. For example, Lebreton, L. C., *et al.*, (2017) propose an annual global input of plastic waste from rivers to the marine environment of 1.15–2.41 million tons, while Schmidt, Krauth, and Wagner (2017) put this figure at 0.41–4 million tons. There are many different plastic polymers that make up plastic pollution: polystyrene (PS), polyethylene (PE), and polypropylene can all be found as contributing environmental pollutants. Despite being a recyclable material and being environmentally toxic, plastics in the United States have a recycling rate of just 8.8% as of 2016 (Gu & Ozbakkaloglu, 2016).

Unconstrained plastic debris is transported through and between environments. Environmental modeling can estimate plastic concentrations and abundances in the environment. Geyer, Jambeck, and Law (2017) estimate that 79% of the 6,300 metric tons of plastic waste generated up to 2015 are either in landfill or in the natural environment. However, quantifying the amount of plastic waste in the environment is challenging, and global estimates of plastic waste vary. Plastics may also act as vectors for other pollutants. The ingestion of plastics to which chemicals are sorbed is a known pathway by which organisms are exposed to chemical pollution (Gallo, F., *et al.*, 2018).

Biodegradable polymers are materials that can work for a limited time before degrading into readily discarded products through a regulated procedure (Peng, X., *et al.*, 2021). They might be made from a variety of wastes or/and bioresources, such as wastes of food, animal, agro-waste as well as other sources such as starch, and cellulose. Bioplastics made from renewable resources are often less expensive than those made from microbial resources prompting producers to concentrate on making bioplastics from renewable resources (Taha, T. H., *et al.*, 2022). The use of biodegradable polymers has environmental benefits such as regeneration of raw materials, biodegradation and reduction of carbon dioxide emissions that are led to global warming (Zhu, J. and Wang, C. 2020).

Microorganisms such as bacteria and fungus may consume biodegradable polymers and convert them to H₂O, CO₂, and methane. The biodegradation process depends on the material's composition (Künkel, A., *et al.*, 2016). The polymer morphology, polymer structure, chemical and radiation treatments, and polymer molecular weight are all parameters that influence the biodegradation process (Ghanbarzadeh, B. & Hadi A. 2013). Biodegradable polymers are also called biopolymers (Zhang, L., Jing, Z., & Xiaofeng, R. 2017). There are two reasons to use the polymers from renewable resources; (i) environmental concerns in terms of increasing plastic waste and global warming as a result of releasing of carbon dioxide when burning waste, and (ii) petroleum resources are limited and ended (Ghanbarzadeh, B. & Hadi A., 2013).

The biodegradable plastics industry is highly promising. However, they need to be developed in tandem through examination of end-of-life processes of treatment and a worldwide integration with organic management of waste as selective biowaste collection expands. Biodegradable plastics have the benefit of being able to be handled biologically at the end of their lives (composting or anaerobic digestion).

MATERIALS AND METHODOLOGY MATERIALS

Apparatus: Test tubes and test tube rack, Conical flasks, Beakers, Weighing balance, Forcep, Measuring cylinder, Masking tape, Microscope, Slide and slide cover, Marker, Wireloop and Flames, Soil sample, Polythene bags, Pipette.

Reagents: Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose broth, Ethanol, Mercury and Lactophenol

SAMPLE COLLECTION FROM DIFFERENT SITES FOR MICROBIAL DEGRADATION

The soil samples and Polythene bags were collected from two different dump sites and a mechanic workshop in Ibadan, Oyo state. These samples were separated and labeled according to their location. The soil samples were collected from the plow layer (0–15 cm in depth) of the soil at different places. About 100 g of the soil were taken and packed into labeled sterilized bottles.

SURFACE STERILIZATION OF POLYTHENE BAG

The collected polythene bags were cut into small pieces and cleaned with tap water and surface sterilized with ethanol. Then washed with distilled water, 0.1% mercuric chloride and again washed with distilled water.

METHODOLOGY

ISOLATION OF SOIL FUNGI

One gram of the soil sample was suspended in 10 ml of sterile distilled water to make serial dilutions (10^{-1} to 10^{-5}). One mL of each dilution was placed on Potato Dextrose Agar (PDA) containing 1 % streptomycin. The plates were incubated at 28°C in the dark. The plates were observed for one week. The cultures were incubated at room temperature in an incubator for three to five days.

MEDIA PREPARATION

The soil fungi were isolated by the soil dilution method. 4g of Sabouraud Dextrose Agar (SDA) was weighed and dissolved in 60ml of distilled water and autoclave at 121°C for 15 minutes. The media was allowed to cool. The media was then poured aseptically into the petri-dishes and allowed to solidify.

SERIAL DILUTION

9 test tubes were arranged in a test tube rack, 3 test tubes for each of the 3 samples. 9ml of distilled water was pipette into each of the test tubes. 1g of the soil sample was added into the first test tube containing the distilled water and shakes vigorously to have a homogeneous mixture. 1ml of the solution in the first test tube was pipette into the second test tube and the second test tube to the third test tube. The process is then repeated for the rest of the samples. 1ml of the solution was spread on the freshly prepared media for culture and stored at room temperature for one week.

SUBCULTURE AND PURE CULTURE

After the incubation, the fungal colonies were observed, and the pure cultures were maintained. Subculture of each of the different colonies was done on to a Sabouraud Dextrose Agar (SDA) plate and stored at room temperature for 7 days.

MICROSCOPIC OBSERVATION

Fungal morphology was studied macroscopically by observing the colony features (color, shape, size and hyphae), and microscopically by a compound microscope with a digital camera using a lactophenol cotton blue stained slide mounted with a small portion of the mycelium.

DEGRADATION OF POLYTHENE BAG

Nutrient broth was prepared and autoclaved at 121°C for 15 minutes. 200 ml of cooled nutrient broth was poured into eight 250 ml sterile conical flasks. The sterile pre weighed polythene bag pieces were aseptically transferred into the nutrient broth. A loop full of fungal culture was inoculated into nutrient broth. The flasks were incubated at 37°C for 5, 10, 15 and 20 days. The polythene bags were carefully removed from the culture by using forceps after different days of incubation. The collected pieces were washed thoroughly with tap water, ethanol and then distilled water. The pieces were shade dried and weighed for final weight. The data were recorded. The same procedure was repeated for all the treated samples.

DETERMINATION OF DEGRADATION PERCENTAGE OF POLYTHENE BAG

The percentage of degradation of polythene bag pieces by *Aspergillus niger* and *Candida fungus* was determined by calculating the percentage of weight loss of plastics. The percentage of weight loss was calculated by the following formula.

Percentage of weight loss = $\frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$.

MOLECULAR IDENTIFICATION OF FUNGAL SPECIES (*Candida fungus*) DNA

EXTRACTION AND PCR AMPLIFICATION

The DNA extraction of genomic DNA from the fungi was conducted from a one-week-old SDA culture using Zr Fungal/Bacterial Dna Miniprep (Manufactured By Zymo Research). PCR products were purified using the QIA quick PCR purification kit.

SEQUENCING AND ANALYSIS

The PCR products were sent for sequencing to Bioformatics services, Mokola Ibadan, Nigeria. The obtained sequences were compared with the other related sequences.

RESULT

Morphological and Microscopic identification of fungi.

Table 1 shows the microscopic and morphological characteristics of three (3) fungal species isolated from dump site soils. The macroscopic and microscopic characteristics of the fungal species showed the organisms to be *A. niger*, *A. flavus*, and *C. fungus* respectively. Fungi were capable of growing in the dumped site soil.

Table 1: Morphological and Microscopic identification of fungi isolated from 2 different dump sites (hospital and refuse) and mechanic workshop in Ibadan, Oyo state.

S/NO	Morphological	Microscopic characteristics	Organism
Isolate A1	White yellow colony covered by a dark brown layer.	The conidial heads are radial and they split into columns, the conidiophores are smooth and coloured	<i>Aspergillus niger</i>
Isolate A2	White yellow colony covered by a dark brown layer.	The conidial heads are radial and they split into columns, the conidiophores are smooth and coloured	<i>Aspergillus niger</i>
Isolate B1	A greenish, flat dry colony	Short, slender tube like structure, the conidiophores are short and inflated	<i>Candida fungus</i>
Isolate B2	Whitish colony then turns to yellow-green.	The conidial are characteristically green, the conidiophores are colorless and rough textured	<i>Aspergillus flavus</i>
Isolate C1	White yellow colony covered by a dark brown layer to black	The conidial heads are radial and they split into columns, the conidiophores are smooth and colored.	<i>Aspergillus niger</i>
Isolate C2	A greenish, flat dry colony.	Short, slender tube like structure, the conidiophores are short and inflated	<i>Candida fungus</i>

Biodegradation of polythene bag by *Candida fungus* (black nylon).

Table 2 indicates to have uniform initial weight of the polythene bag (Black nylon) which is 0.06g for all the days of the treatment. There was slight changes in the final weight of the polythene bag (black nylon) with Day 5 and 10 having 0.05g as the final weight of the polythene bag (black nylon) and Day 15 and 20 had 0.06g as the final weight of the polythene bag (Black nylon).

Table 2: Biodegradation of polythene bag by *Candida fungus* (black nylon)

S.NO	Days of Treatment	Initial weight of polythene bag (g)	Final weight of polythene bag (g)	% of weight loss
1	5	0.06	0.05	1.67%
2	10	0.06	0.05	1.67%
3	15	0.06	0.06	0%
4	20	0.06	0.06	0%

Biodegradation of polythene bag by *Aspergillus Niger* (white nylon).

Table 3 indicates to have uniform initial weight of the polythene bag (white nylon) which is 0.06g for all the days of the treatment. There was slight changes in the final weight of the polythene bag (white nylon) with Day 5 having 0.06g as the final weight of the polythene bag (white nylon) while Day 10 and 15 had 0.04g as the final weight of the polythene bag (white nylon) and Day 20 had 0.05g as the final weight of the polythene bag (white nylon).

Table 3: Biodegradation of polythene bag by *Aspergillus Niger* (white nylon)

S.NO	Days of treatment	Initial weight of polythene bag (g)	Final weight of polythene bag (g)	% of weight loss
1	5	0.06	0.06	0%
2	10	0.06	0.04	33.3%
3	15	0.06	0.04	33.3%
4	20	0.06	0.05	1.67%

MicroscopicObservationOfFungisolate

IsolatedOrganism

IsolatedOrganism

(Undermicroscope)

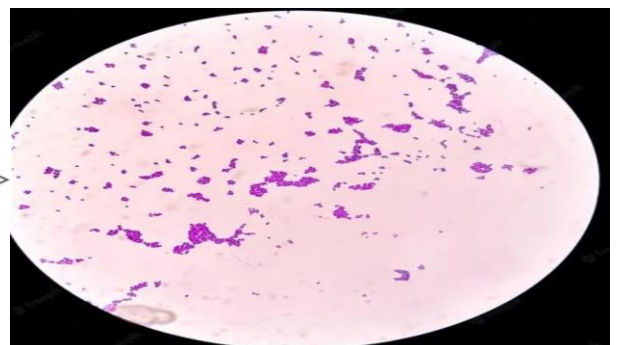
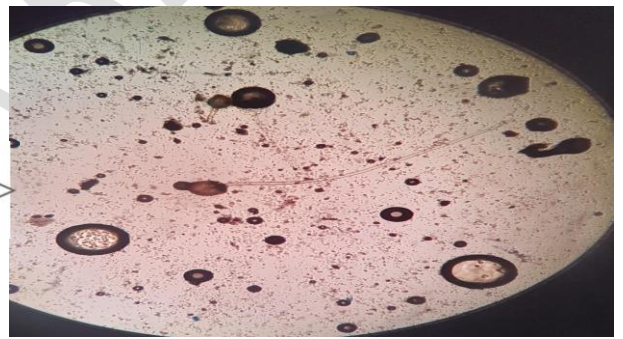
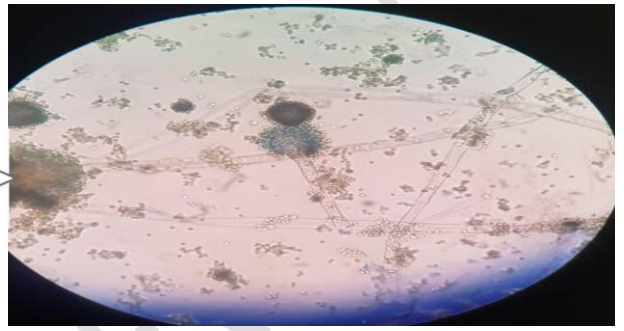
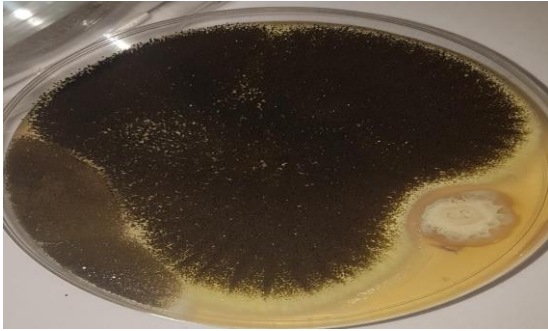


Plate 1. Microscopic Observation Of Fungus Isolate

Gel images showing high molecular weight DNA extracted from the isolates

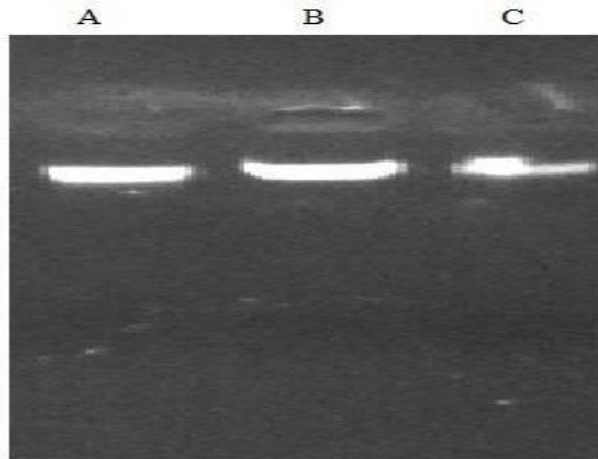


Plate 2. Gel images showing high molecular weight DNA extracted from the isolates

PCR products obtained from fungus isolates using ITS primer pair.

PCR (Polymerase Chain Reaction) to amplify specific DNA regions from fungi isolates. In the gel image, Lane M contains molecular weight markers for reference, Lane C is the negative control, and Lanes A and B show PCR products from isolates A and B.

In Panel A, the PCR of the fungal universal region was performed. Lane M in this panel represents a molecular weight marker. The bands labeled A, B, and C represent different isolates of *Candida guilliermondii*. It's noteworthy that Isolate 19, identified as *Candida guilliermondii*, exhibits a high pairwise similarity of 98.97% with *Candida intermedia* strain JYC551. The PCR-RFLP (Restriction Fragment Length Polymorphism) technique was employed to characterize and differentiate various fungal isolates, providing valuable insights into their genetic similarities and differences.

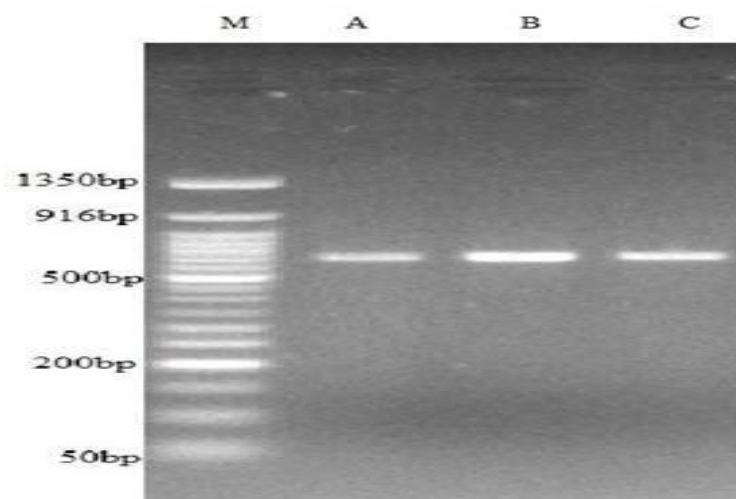


Plate 3. PCR products obtained from fungi isolates using ITS primer pair

DISCUSSION

The extent of biodegradation is affected by polymer characteristics and the micro-organisms present in certain environment. Fungi are capable of growing in the dumped site soil. The isolated fungi are inhabitants of soil and plant decayed matter organisms. Biodegradable polymers are designed to degrade quickly by the microbes due to their ability to degrade organic and inorganic materials, such as lignin, starch, cellulose, and hemi-cellulose (Kumar., *et al.*, 2013). The mechanism of biodegradation involves the action of microbial enzymes on the surface of the plastics Montazer., *et al.*, 2019)

Different strains of fungi from dumped site soil were isolated and identified by morphological and microscopic characteristics and are presented in Table 1. Three (3) fungi were isolated from dump site soil. Their macroscopic and microscopic characteristics confirmed the species as *A. niger*, *A. flavus* and *C. fungus* respectively (Zhang, K., *et al.*, 2022, Álvarez-Barragán, J., *et al.*, 2016)

Table 2 indicates to have uniform initial weight of the polythene bag (Black nylon) which is 0.06g for all the days of the treatment. There was slight changes in the final weight of the polythene bag (black nylon) with Day 5 and 10 having 0.05g as the final weight of the polythene bag (black nylon) which indicates a slight weight loss and also in correspondence with weight loss measurement is a global analysis, which constitutes the first global approach for studying the biodegradation of polymer by Magnin, A., *et al.*, 2018 and Day 15 and 20 had 0.06g as the final weight of the polythene bag (Black nylon) which was same with the initial weight.

Table 3 indicates to have uniform initial weight of the polythene bag (white nylon) which is 0.06g for all the days of the treatment. There was slight changes in the final weight of the polythene bag (white nylon) with Day 5 having 0.06g as the final weight of the polythene bag (white nylon) while Day 10 and 15 had 0.04g as the final weight of the polythene bag (white nylon) which indicates a decrease in the weight of the polythene bag which is in line with Magnin, A., *et al.*, 2018 findings and Day 20 had 0.05g as the final weight of the polythene bag (white nylon) which is also in line with the findings of Magnin, A., *et al.*, 2018.

PCR was used to amplify genetic material from fungi isolates using specific primers. In the gel, Lane M

represents molecular weight markers, Lane C is the negative control, and Lanes A and B show PCR products from isolates A and B. In Panel A, the PCR results for the fungal universal region are displayed. M represents a 50 base pair molecular weight marker and it's indicate the rate of biodegradation being proportional to the molecular weight of the plastic targeted which is inline with Muthukumar A, Veerappapillai S. 2015 study. The samples labeled Iso18, Iso19, and Iso20 are all identified as *Candida guilliermondii*.

Additionally, it's noted that Isolate 19 shares a high similarity of 98.97% with a *Candida intermedia* strain called JYC551. This suggests a close genetic relationship between Isolate 19 and this particular *Candida intermedia* strain. These results showed that the primers are specific, highly sensitive and targeted fungal rDNA. They were reported to be on the conserved region that identified ITS/rDNA region for *Aspergillus*

UNDER PEER REVIEW

species. The presence of these three *Aspergillus* species on the dumped site soil environment shows their ability to produce spores capable of resisting and spreading in harsh conditions such as tropical bed bugs in peninsular Malaysia and this potential suggests they are good for bioremediation study.

CONCLUSION

The rise in plastic pollution has a significant impact on living organisms. Fungal biodegradation of plastics offers a promising solution to mitigate this issue. This area of research has garnered substantial attention in addressing plastic pollution. Fungi, specifically *Aspergillus flavanus*, *Aspergillus Niger*, and *Candida fungus*, were isolated from soil at a dump site and a mechanic workshop. The findings revealed that the microbe tested was identified as *Candida fungus* through molecular analysis. This discovery holds significant potential for effectively degrading and detoxifying dump site soil. This review offers valuable insights into the diverse range of fungi that play a role in breaking down various types of plastic polymers.

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