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Research Article

Biodegradation of Polyethylene Terephthalate (PET) Bottle by Mold Isolated from a 30 Days Vermicompost

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Abstract

Polyethylene terephthalate (PET) bottles are composed of many polymerized styrene monomers that are generally considered to be recalcitrant and are resistant to biodegradation. In this study, the ability of fungi to degrade Styrofoam were investigated using fungi isolated from a 30-day vermicompost prepared with soil gotten from Ifite, Awka in Anambra State, South-east, Nigeria. The fungi isolated were *Aspergillus niger and Penicillium chrysogenum*. The ability of these fungi to degrade polyethylene terephthalate was studied using a Mineral Salt Vitamin Medium and the polyethylene terephthalate as a carbon source. The level of degradation was assessed through spectrophotometric analysis at 680 nm, all of which were considered at 5-day intervals for 20 days. The results showed maximal increase in optical densities for *Aspergillus niger* and a decrease on certain days for *Penicillium chrysogenum*. The increase in optical density indicates increase in degradation and corresponds with the principle of spectrophotometry which states that the higher the optical density, the lower the transmittance. Decrease in optical density could be as a result of decrease in nutrients present in the medium which is the decline phase. The results showed that *penicillium chrysogenum* has a higher ability to degrade PET when compared with *Aspergillus niger* as seen in the graphical presentation. This study showed that fungi have the potential to be used in biodegradation of PET bottles. Hence, the use of fungi for PET degradation has gained importance recently and can be applied in other to control the pollution that evolves from the use of PET bottles.

Keywords: Biodegradation; Polyethylene Terephthalate; Bottle; Mold; Vermicompost

Introduction

Biodegradation is a natural process in which organic materials are broken down into simpler compounds through the metabolic activities of microorganisms such as bacteria and fungi [1]. These microorganisms secrete extracellular enzymes that catalyze the decomposition of complex substrates into simpler molecules that can be assimilated into the environment [2,3]. This process plays a significant role in nutrient recycling, soil fertility improvement, and environmental sustainability by reducing the accumulation of organic pollutants [4,5]. The efficiency of biodegradation is influenced by several environmental factors, including temperature, pH, moisture, and the composition of microbial communities present in a given habitat [6]. Consequently, biodegradation remains a critical ecological mechanism for maintaining environmental balance and mitigating pollution.

Microorganisms, particularly bacteria and fungi, are central to the breakdown of complex organic materials such as plant residues, animal waste, and synthetic compounds [7]. Their enzymatic systems facilitate the transformation of complex polymers into simpler end products such as carbon dioxide, water, and biomass [5,8]. In addition to their ecological roles, these microorganisms have been widely studied for their potential in bioremediation of contaminated environments, including hydrocarbon-polluted soils and industrial waste sites [9-11]. Their metabolic versatility enables them to adapt to diverse and often harsh environmental conditions, making them suitable candidates for biodegradation studies.

The biodegradation of polyethylene terephthalate (PET) plastics involves the breakdown of polymer chains by microbial enzymatic activity. Although PET is inherently resistant to degradation due to its stable chemical structure, certain microorganisms have demonstrated the ability to utilize plastic polymers as carbon sources [12,13]. Enzymes such as hydrolases, lipases, and oxidases play key roles in initiating the depolymerization process, leading to structural modification and eventual mineralization of plastic materials [2,14]. However, the natural degradation rate of PET remains slow, necessitating the exploration of more efficient biological systems and environmental conditions to enhance degradation.

Recent studies have highlighted the potential of fungi isolated from enriched environments such as compost and vermicompost systems in plastic biodegradation. These environments support diverse microbial populations capable of producing enzymes adapted for the breakdown of complex organic and synthetic materials [15,16]. Fungal isolates, particularly species of *Aspergillus* and *Penicillium*, have demonstrated significant biodegradation capabilities due to their ability to secrete a wide range of extracellular enzymes [2,8]. Additionally, environmental conditions such as nutrient availability and substrate composition can enhance microbial adaptation and degradative efficiency.

Vermicomposting, which involves the use of earthworms to decompose organic waste, creates a biologically active environment enriched with microorganisms capable of degrading complex compounds. Microbial isolates obtained from such systems may exhibit enhanced enzymatic activity and metabolic diversity, enabling them to act on recalcitrant substrates such as PET [17,18]. Furthermore, microorganisms isolated from contaminated environments have been shown to possess adaptive traits that improve their efficiency in degrading pollutants, including plastics and hydrocarbons [16,19].

Despite growing interest in microbial plastic degradation, there remains limited information on the biodegradation potential of mold species isolated from vermicompost systems, particularly within the Nigerian context. Most existing studies have focused on bacterial degradation or fungi from other environments, leaving a gap in knowledge regarding locally adapted fungal strains and their enzymatic mechanisms [15,20]. Understanding these mechanisms is essential for developing sustainable and environmentally friendly approaches to plastic waste management.

Aim of the Study

The aim of this study is to investigate the biodegradation potential of polyethylene terephthalate (PET) by mold species isolated from a 30-day vermicompost system. Specifically, the study focuses on isolating and identifying mold species using standard microbiological techniques evaluating their capacity to degrade PET under controlled conditions, and elucidating the underlying mechanisms involved in the biodegradation process, including enzymatic activity and physicochemical changes in the polymer structure.

Materials and Methods

Study area

Vermicompost was prepared using soil gotten from Ifite, Awka in Anambra State, South-east Nigeria. Ifite is a developing community mostly populated by students of Nnamdi Azikwe University, and other traders.

PET polymer blend material

Before use, polymer of PET was extract by blending several bottles in to a experientable volume and surface area. About 25g of the sample was collected after blending using a hand blender. PET polymer was acquired from commercial, non-carbonated mineral water bottles and was used as nanoparticles, prepared from 1 × 1 cm pieces of PET bottle.

Serial dilution and culturing of the organism

One gram (1g) of each soil sample was aseptically weighed and transferred into 9 mL of sterile distilled water, followed by thorough shaking to achieve proper homogenization. A ten-fold serial dilution of each sample was subsequently carried out using sterile distilled water as the diluent. An aliquot of 0.1 mL from the appropriate dilution (10^{-2}) was inoculated onto sterile Sabouraud Dextrose Agar plates using the pour plate technique for fungal isolation. The inoculated plates were incubated aerobically at 25°C for 48 - 72 hours. After incubation, developing colonies were counted to obtain the total fungal count, expressed as colony-forming units per milliliter (CFU/mL). Discrete fungal colonies were purified by subculturing onto fresh SDA plates and subsequently identified using standard microbiological methods.

The total fungal count (TFC) was calculated using the formula:

TFC (CFU/mL) = (Number of colonies × dilution factor)/volume plated.

PET mineral salt vitamin medium agar composition (g L^{-1})

The mineral salt vitamin (MSV) medium used for biodegradation studies comprised the following components (g L^{-1}): PET, 5.0; $(NH_4)_2SO_4$, 1.0; KH_2PO_4, 1.0; K_2HPO_4, 8.0; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.1; $CaCl_2 \cdot 2H_2O$, 0.02; $FeSO_4$, 0.01; $Na_2MoO_4 \cdot 2H_2O$, 0.5 mg; $MnSO_4$, 0.5 mg; inositol, 0.2 mg; p-aminobenzoic acid, 0.2 mg; pyridoxine, 0.4 mg; thiamine, 2.0 µg; biotin, 2.0 µg; vitamin B_{12}, 0.5 µg; distilled water, 1000 mL, with the pH adjusted to 7.0. The medium formulation was adapted from [21].

Identification of fungi (Fungal teasing technique)

Fungal identification was carried out based on the macroscopic characteristics of colonies on Sabouraud Dextrose Agar and microscopic features observed using the modified slide culture technique. Lactophenol cotton blue staining was employed for microscopic examination under ×10 and ×40 objective lenses, following the method described by Agu and Chidozie (2021). Identification was further supported using standard fungal taxonomic atlases, including Illustrated Genera of Imperfect Fungi and Pictorial Atlas of Soil and Seed Fungi.

For microscopic preparation, a drop of lactophenol cotton blue stain was placed on a clean glass slide, and a small portion of fungal mycelium with spore-bearing structures was aseptically transferred into the stain using a sterile inoculating loop. The preparation was gently teased and covered with a grease-free coverslip, ensuring that no air bubbles were trapped, and then examined under the microscope at low and high magnifications.

Preparation of mineral salt vitamin medium

One thousand milliliters (1000 mL) of distilled water was measured into a sterile beaker, after which the required salts and vitamins were added. The mixture was stirred using a magnetic stirrer until complete dissolution was achieved. The pH of the medium was adjusted to 7.0 using a calibrated pH meter. Thereafter, 5g of the carbon source (PET or Styrofoam, as applicable) and 20g of agar were added to the medium. The prepared medium was then sterilized by autoclaving at 121°C for 15 minutes.

Results

On completion of the practical below are the total results obtain from the isolation, identification and degradation ability of polyethylene terephthalate by a named fungal species isolated from a thirty days vermicompost.

Discussion

Due to the wide range of environmental pollution and inconveniences caused by polyethylene terephthalate waste bottles, a polymer with low degradability, a search for more suitable solution has urgently been requested. Conventional disposal methods obviously contribute to more pollution, necessitating the exploration of more revolutionary approaches.

Samples	Total fungal count (Cfuml-1)		Organism distribution
	No. of fungi colonies on plate	Total fungi Count (cfuml-1)	
Sample A	TFTC	TFTC	*Penicillium chrysogenum* and *Aspergillus niger*

Table 1: Total fungal count (Cfuml-1) and fungal distribution.

Key: (TFTC): Too few to count.

Colony morphology	Microscopy	Identity
Colonies were compact with white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads.	Conidial heads are large (upto 3 mm by 15 to 20 µm in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophore stipes are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (3.5-5 µm in diameter), dark brown to black and rough-walled	*Aspergillus niger*
Cultures on SDA are fluffy, bright yellowish green with bluish green tint, funiculose with bundles of hyphae, reverse yellowish pink with reddish purple tint. Rather good in growth.	Conidiophores hyaline, erect, developed from aerial hyphae, branched penicillately at the apexes with primary and secondary metula, verticillate phialides and catenulate conidia in each phialide, forming rather open-spaced yellowish green conidial heads: phialides lanceolate or abruptly sharpened. Conidia phialosporous, pale green, dark in mass, globose to subglobose, 1-celled, minutely echinulate on the surface.	*Penicillium chrysogenum*

Table 2: Colonial and microscopic identifications of the various fungi isolates.

Days	Values
0	0.537
5	0.723
10	0.743
15	0.979
20	1.287

Table 3: Optical density reading for *Aspergillus niger*.

Days	Values
0	0.169
5	0.154
10	1.560
15	1.630
20	1.514

Table 4: Optical density reading for *Penicillium chrysogenum.*

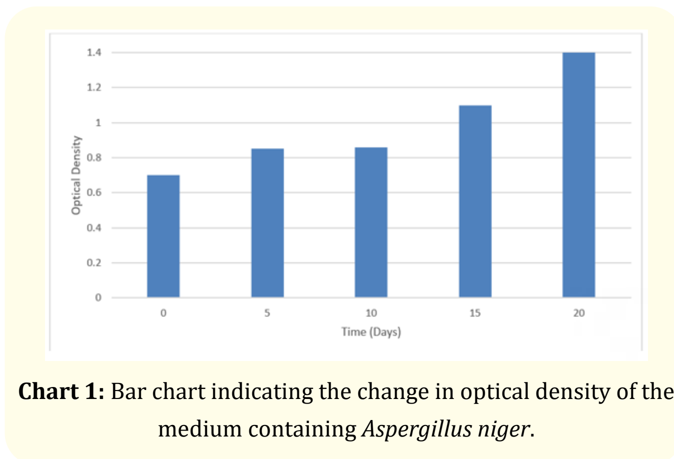

Chart 1: Bar chart indicating the change in optical density of the medium containing *Aspergillus niger*.

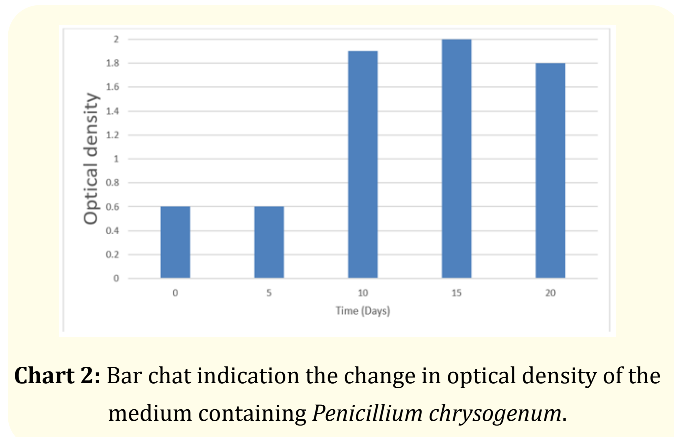

Chart 2: Bar chat indication the change in optical density of the medium containing *Penicillium chrysogenum*.

An approved method capitalized on the use of microbial enzymatic activity, providing a promising avenue for environmentally friendly waste management. I carried out this study to investigate the biodegradation of polyethylene terephthalate using genetically modified molds isolated from a 30-days vermicompost prepared during the course of this study.

Table 1 shows data derived from the isolation of fungal mold from the vermicompost. The study reports the fungal load in the Vermicompost sample as "too few to count" which suggests a limited fungal count in the SDA culture plate. However, despite the challenges associated with low fungal count, I identified growth of *Aspergillus niger* and *Penicillium chrysogenum*, which is coherent with the study of [22] who isolated *Aspergillus niger* from compost and Vermicompost in Italy, also [23] identified both organism from Vermicompost collected in India. Results for polyethylene terephthalate degradation for both *Aspergillus niger* and *Penicillium chrysogenum* is present in table 3 and 4. Table 3 evaluates the optical density values measuring the degradation of the P.E.T sample over a 20-days period (at five days interval) for *Aspergillus niger.* The results indicates that the organism has a perceptible impact on the degradability efficiency of the PET sample as observed by the change in optical density values signifying degradation kinetics and also the potential of *Aspergillus niger* having the ability to degrade more quantity of the sample provided the source of nutrient isn't limited. This findings is coherent with that of [24] who reported efficacy of *Aspergillus niger* in the degradation of different plastics including polyethylene glycol which is the precursor of polyethylene terephthalate. The strain of the *Aspergillus niger* shows an increase in optical density over the 20 days period. On the day 0, the strain exhibited the a lower optical density of about 0.537. This was as a result of the organisms striving to adapt to a new habitat having a different source of nutrient. However, at day 5 to day 20, their was perceptible acceleration in the optical density indicating that the organism were growing in the medium utilizing the nutrients acquired from the carbon source (PET), thereby indicating degradation.

Similarly to *Penicillium chrysogenum*, table 4 indicates an accelerating change in optical density from day 0 to day 15, this is consistent with findings of [25]. It was also observed that there was a deceleration in the optical density by day 20 and it is suggested to have been as a result of heavy growth of the organisms, there was a strive for nutrients amongst the strains and at that point growth rate was altered and some of the organisms began to die off while the degradation was also halted. Although the degradation using *Penicillium chrysogenum* was altered during the process due to lack of nutrients, results still show that degradation was more discernible in *Penicillium chrysogenum* than that of *Aspergillus niger.*

Conclusion

In view of the investigation carried out on biodegradation of polyethylene terephthalate using molds isolated from a 30 days vermicompost, the study evaluated an enormous potential that fungi show more effective ability to control polyethylene terephthalate waste in the environment however, the *Aspergillus niger* strain showed a slower biodegradability potential on the sampled carbon source than that of the *penicillium* strain as shown from the Spectrophotometry table.

Hence more genetic analysis should be done to help in understanding the specific modifications responsible for the degradation efficiency observed in the mold strains. The knowledge will aid in maximizing the genetic engineering approaches for improved environmental remediation

Long-term studies: Extension of the duration of the study will help in assessing a long term performance and sustainability of mold, this would provide more valuable insights into the longevity and dependability of these strains in world waste management science.

Appendix

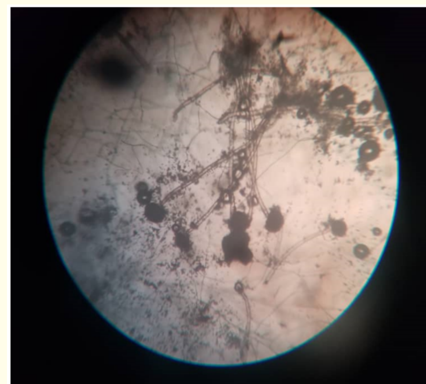

Figure 1: Isolated fungal culture (*Aspergillus niger*) under microscopy view.

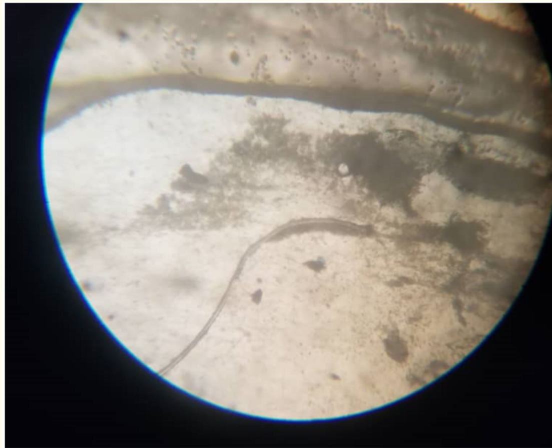

Figure 2: Isolated fungal culture (*Penicillium chrysogenum*) under microscopy view.

Figure 3: Petri dish containing colonies of genetically modified *Aspergillus niger*.

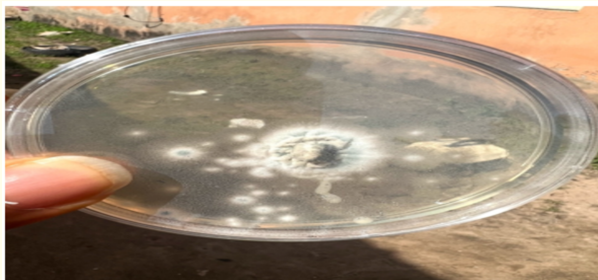

Figure 4: Petri dish containing colonies of *penicillium chrysogenum*.

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