# BIODEGRADABILITY OF POLYETHYLENE PLASTICS BY FUNGI ISOLATES FROM PLASTIC COMPOSTED WASTE SOIL

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**BENIN CITY.**

**NOVEMBER, 2025.**

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**(B.Sc UNIBEN)**

**A PROJECT SUBMITTED TO THE COLLEGE OF POSTGRADUATE STUDIES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (M.Sc) IN ENVIRONMENTAL AND PUBLIC HEALTH MICROBIOLOGY.**

**NOVEMBER, 2025**

## CERTIFICATION

We certify that this work was carried out by **Tolulope Adeola ORIMOLOYE** in the Department of Microbiology, Faculty of Science, University of Benin, Benin City, under my supervision.

**PROF. E. I. ATUANYA** **DATE**
**(PROJECT SUPERVISOR)**

**PROF. E. O. IGBINOSA** **DATE**
**(HEAD OF DEPARTMENT)**

## CERTIFICATION OF THESIS

We the undersigned attest and declare that the thesis of **Tolulope Adeola ORIMOLOYE** titled **BIODEGRADABILITY OF POLYETHYLENE PLASTICS BY FUNGI ISOLATE FROM PLASTIC COMPOSTED WASTE SOIL**, has successfully passed the anti-plagiarism test and does not violate any copyright regulation.

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| **(HEAD OF DEPARTMENT)** | |

## DEDICATION

This work is dedicated to the Almighty God who is also the alpha and omega, the first and the last, the yes and amen, who has given me another higher grace to attain this feat and will never stop opening fervent and effectual doors of his unbelievable grace and marvelous mercy in his kindness unspeakable.

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My eternal thanks go to God Almighty who has made me a benefactor of his declarations in grace and granted me favor to do things beyond my eyes can see, ears, hear or my mind can understand, leading me in triumph daily.

God truly works through men, is the story of my master's degree journey so far. How could it have been possible without God working through the gift of Dr. Omorodion Irowa who was used to ignite and manage the vision?

Words cannot express my gratitude enough sir, may the lord reward you and your home with love and mercy.

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## ABSTRACT

Biodegradation is a complete degradation process of a polymer through the activity of enzymes produced by microorganisms. The biodegradation process of plastic wastes is the breakdown of complex polymers into simpler oligomers and monomers. The study investigated the biodegradation of low-density polyethylene (LDPE) and high-density polyethylene (HDPE) plastics using fungal species isolated from plastic composted waste soil.

Soil samples were collected from Oluku dumpsite and Sawmill sites in Benin City, Nigeria. The samples were subjected to physicochemical analysis to provide baseline environmental characteristics. Fungal isolation was carried out using dilution and pour plate methods on Potato Destrose Agar (PDA). Fungal isolates were identified through cultural and microscopic characterization, followed by PCR sequencing using ITS gene markers. Electrophoresis confirmed successful amplification, and sequencing analysis using NCBI BLAST validated the identity of the fungal strains. The primary fungal isolated included *Fusarium oxysporum*, *Aspergillus flavus* and *Aspergillus niger*. Polyethylene sheets were pretreated by abiotic exposure to sunlight for up to 50 days to simulate natural weathering. The sheets were then cut into fragment and incubated with fungal isolated in mineral salt medium broth at 25$^{o}$C for varying durations (10-50 days). Data on biodegradation potential were generated by monitoring percentage weight loss and optical density (OD 600).

The result of the study revealed that abiotic exposure alone caused minimal weight loss, whereas fungal inoculation significantly enhanced degradation. LDPE exhibited greater degradation than HDPE, consistent with its lower crystallinity and simpler structure. Individual isolates and consortium all had equal degradative activity, meaning any isolate can be used for degradation because there was no significant difference between them. These findings highlight microbial degradation as an eco-friendly strategy for plastic waste management, with scope for further optimization of environmental conditions and fungal consortia to enhance efficiency.

# CHAPTER ONE

## INTRODUCTION

Plastics are materials made by humans that do not break down easily in nature. They are mostly made from petroleum, but some come from plants like corn and sugarcane, as well as natural gas and oil. Around 4% of all the oil produced worldwide is used to make plastic, and another 4% is needed to power the process of making it (Loredo Treviño *et al.,* 2012; Jin *et al.,* 2023). Plastics are mainly made of hydrogen and carbon, along with some other organic and inorganic substances (Okal *et al.,* 2023).

Plastics are used everywhere because they have both good qualities and negative effects. They are very useful but also harmful to the environment (Klusener, 2022). In the last few decades, plastic has been used in almost every area of life. It has replaced other materials like glass, wood, and metal in many applications because of its special features that make it useful in many different areas (Okal *et al.,* 2023).

Plastics are used in many industries (Zeghal *et al.,* 2021), including food, home appliances, construction, transportation, healthcare, and entertainment.

They are so popular because they have certain qualities that make them better than other materials. These include being strong, light, long-lasting, easy to make, cheap, and resistant to damage from microbes (Mathur *et al.,* 2011; Kumar *et al.,* 2013; Jin *et al.,* 2023). Some of the most common types of plastics are low-density polyethylene (LDPE), high-density polyethylene (HDPE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polycarbonate (PC), and polyurethane (PU) (Zehal *et al.,* 2021; Srikanth *et al.,* 2022).

Fungi play a big role in breaking down plastic through biodegradation. Some fungi release pro-oxidant ions and enzymes like proteases, cutinases, and lipases, which help in breaking down plastic. These enzymes can change the structure of plastic by creating new chemical groups through processes like oxidation or hydrolysis. This makes the plastic more water-friendly and helps turn large molecules into smaller ones. Some fungi, such as Aspergillus clavatus, Trichoderma viride, Aspergillus nomius, and Penicillium species, are known to help in breaking down plastic waste. This offers a natural and eco-friendly way to manage plastic waste. It is important to develop new and innovative methods to reduce and break down plastic waste using green approaches because plastic pollution is a global problem that affects the whole planet.

## 1.1 AIMS

The aim of the study is to isolate potent low-density polyethylene (LDPE) and high density polyethylene (HDPE) degrading fungi from plastic compost waste site and to test their plastic degradability capacity.

## 1.2 OBJECTIVES

The objectives of the study were to:

1. conduct a physico-chemical analysis of soil samples;
2. isolate and identify polyethylene degrading fungi;
3. prepare low density polyethylene and high-density polyethylene for degradation studies by cutting and subjecting to UV light for days;
4. measure biodegradation by comparing the performances of isolates on plastic;
5. compare the impact of plastic density on degradation; and
6. check if the exposure times contribute to degradation.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Types of Plastic

Plastics come in many different types and are grouped based on their characteristics and chemical structure.

### 2.1.1 Thermal Properties

When looking at plastics based on how they react to heat, they can be split into two main groups: thermoplastics and thermosetting polymers. This classification has been studied by several researchers over the years. (Chan and Ji 1999; Choi *et al.,* 2005; Albano *et al.,* 2009; Choi *et al.,* 2009; Al-Salem *et al.,* 2010; Bărbută *et al.,* 2010; Mohammadian and Haghi, 2013).

1. **Thermoplastic**

Thermoplastics are a type of plastic that does not change chemically when heated (Asiandu *et al*., 2022). This means, they can be melted and reshaped multiple times. Some common examples of thermoplastics include polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polytetrafluoroethylene (PTFE) (Chan and Ji 1999; Choi *et al.,* 2005; Albano *et al.,* 2009; Choi *et al.,* 2009; Al-Salem *et al.,* 2010; Bărbută *et al.,* 2010; Mohammadian and Haghi, 2013).

2. **Thermosetting polymers**

Thermosetting polymers, such as phenol-formaldehyde and polyurethanes (PUR), are different (Asiandu *et al*., 2022). These plastics do not soften or change when heated because they have a structure that is fixed once formed. This chemical change is permanent, which makes thermosetting plastics difficult to recycle. Unlike thermoplastics, which have a linear structure, thermosetting plastics have a cross-linked structure that gives them their solid form. (Albano *et*

*al.,* 2009; Choi *et al.,* 2009; Al-Salem *et al.*, 2010; Bărbută *et al.*, 2010; Mohammadian and Haghi, 2013).

## 2.2 CURRENT PLASTIC WASTE MANAGEMENT SYSTEMS

A new attempt to track where all the synthetic plastics ever made end up found that most plastics have one of three main fates at the end of their life: being thrown away (either in landfills or the environment, making up 60%), being burned (12%), or being recycled (9%). The rest are still being used (Omoregbe, 2016; Zeghal *et al.,* 2021).

### 2.2.1 Landfilling

Most plastics (75.8% of properly collected waste in the US) end up in landfills (Udochuckwu *et al.,* 2022). If handled properly, this can be a good outcome because the carbon is locked away; however, space is limited, especially in cities where most waste comes from.

### 2.2.2 Incineration

Getting energy from waste plastics is usually done by burning them, which is sometimes called "quaternary or energy-recovery recycling."

This process can be inefficient, create greenhouse gas emissions, and may release harmful chemicals. However, with good engineering, these emissions can be controlled (Asiandu *et al.*, 2022).

### 2.2.3 Recycling

Plastics can be collected and recycled, but they have the lowest recycling rate of any material.

Recycling can happen in different ways:

(a) **Primary/mechanical recycling:** Making new products that are similar or different from the original waste material. When we heat and melt polymers again, it can lead to a process called

downcycling. This means the recycled polymer ends up being lower quality than new material, like having less strength or durability (Klusener, 2022).

(b) **Monomer or feedstock recycling (tertiary recycling):** Creating fuels and raw materials from waste plastic is possible for certain types of plastics, such as polyethylene terephthalate and polypropylene. However, this method isn't widely used yet (Omoregbe, 2016; Brandon, 2020).

## 2.3 THE NEGATIVE EFFECTS OF PLASTICS WASTE

Using a lot of plastic leads to a lot of waste ending up in the environment (Ahsan *et al.,* 2016; Asiandu *et al.,* 2022). Plastic waste causes many health issues for living things, including humans. When plastic is accidentally eaten, it can mess up the immune system, block enzymes, and disrupt the hormonal system, leading to problems in the endocrine system. Some chemicals in plastic are harmful and can affect animals living on land and in water (Pavani and Rajeswari, 2014; Munir *et al.,* 2018).

Plastic waste on land doesn't break down completely. It can release harmful substances into the environment, like heavy metals, plasticizers, stabilizers, and dyes (Klusener, 2022). These pollutants can pollute the land and then get carried by water to the sea. Around 80% of the plastic pollution in the ocean comes from land (Sheavly, 2005; Alabi *et al.,* 2019).

Plastic waste can float and move to different places. It might carry organisms to new areas, introducing invasive species that can harm native species (Derraik, 2002; Hasnat and Rahman, 2018). Burning plastic waste causes fires, which not only damage property but also release toxic gases like carbon monoxide and hydrogen cyanide into the air (Purwaningrum, 2016).

Even though plastic can take hundreds or thousands of years to break down, it doesn't fully decompose. This incomplete breakdown creates toxic fragments that can hurt the environment.

These harmful substances can build up in living things and cause health problems (Kang *et al.*, 2019). Also, the buildup of plastic waste can make places less attractive, which harms tourism and the economy, especially for countries that rely on tourism (Asiandu *et al.*, 2022). This is because plastic waste makes people uncomfortable and less interested in visiting (UNEP, 2018). To fully and safely deal with plastic waste, better methods are needed. Studies suggest using enzymes from microorganisms, like fungi, to break down plastic waste is a good solution to plastic pollution (Kang *et al.*, 2019; Asiandu *et al.*, 2021).

## 2.4 DEGRADATION OF POLYETHYLENE

Degradation of plastic is either abiotic or biotic. The abiotic degradation representing the physical and chemical alterations of polymer; while the biotic absolutely represent the use of or the activity of microorganisms on these polymers, which is a biological alteration by the help of the enzymes, they secrete (Klusener, 2022).

The breakdown of polymers happens when there's a physical or chemical change that makes them lose or change their original features (Matjačić *et al.*, 2021). Physical changes can affect how strong, clear, or conductive the polymer is, leading to problems like crazing, cracking, wearing away, changing color, separating into layers, or peeling off.

Chemical changes happen when the big molecules in the polymer break down into smaller parts.

This can be caused by things like light, heat, water, chemicals, or living organisms (Shah *et al.*, 2008; Vohlidal, 2021). Even though these methods can break down polymers, they are often costly and can create new problems, such as harmful chemicals that make it harder for natural materials in the soil to break down (Ojha *et al.*, 2017). On the other hand, using living organisms to break down plastics is a more cost-effective option. Polymers can fully break down into simple elements

through four main steps: initial damage, breaking into smaller pieces, being absorbed by living things, and finally turning into minerals (Asiandu *et al.,* 2021; Cowan *et al.,* 2022).

## 2.5 BIODEGRADATION OF POLYETHYLENE

Biodegradation is the full breakdown of a polymer caused by enzymes made by microorganisms (Udochuckwu *et al.,* 2022). The process of breaking down plastic waste involves turning complex plastic polymers into smaller oligomers and monomers (Asiandu *et al.,* 2022). These broken-down parts are then taken in by microbe cells (Gu, 2003, Muhonja *et al.,* 2018). In the biodegradation process, microbes attach to the surface of plastic and form biofilms. The ability of microbes to grow on plastic depends on how compatible the plastic surface is with their properties, especially its hydrophobicity. Biofilms formed on plastic help speed up the breakdown of the polymer (Srikanth *et al.,* 2022). There are two main steps in the biodegradation process. The first step is breaking down high molecular weight molecules into organic monomers and acids using biological and chemical processes called hydrolysis. The second step is breaking down these materials into gases, done by microorganisms (Bikiaris, 2013, Kim *et al.,* 2017). Biodegradable polymers are made with bonds such as amides, esters, or ethers. They can be classified into agro-polymers and bio-polyesters (Tiwari *et al.,* 2018). Biodegradation has four stages: biodeterioration, depolymerization, assimilation, and mineralization (Gu, 2003, Muhonja *et al.,* 2018). Biodeterioration is when microorganisms and non-living factors work together to break down polymers into simpler forms. Depolymerization is when microbes release enzymes and other compounds to help break the polymer chain, creating smaller molecules like oligomers, dimers, and monomers (Asiandu *et al.,* 2022). During biodegradation, microbes release enzymes that break down complex polymers into simpler substances. These substances are then used as food and energy sources by the microbes, called depolymerization (Frazer, 1994, Nandi and Joshi, 2013).

Mineralization is when the breakdown process results in gases like CO2, H2O, or CH4 (Premraj and Doble, 2005; Rani and Singh, 2017). This occurs through many steps in the biological process (Kim *et al.,* 2017). The rate at which plastic waste breaks down depends on the structure of the polymers in the plastic, although the differences in these structures are not very big. Other factors that affect biodegradation are the molecular weight, shape, and crystallinity of the polymer (Premraj and Doble, 2005). Studies on plastic biodegradation show that the main organisms involved are bacteria and fungi. These organisms make different enzymes like hydrolases, peroxidases, oxidases, and oxidoreductases (Martin Clavijo, 2012; Paço *et al.*, 2017). These enzymes can be outside, inside, or on the surface of the microbes and help break down polymers into smaller parts. These parts can enter the microbial cells and be used as food and energy, leading to mineralization (Sangale *et al.,* 2019; Ghatge *et al.,* 2020). In some cases, biodegradation can create other byproducts that may break down easily in nature (Bhardwaj *et al.*, 2012). Most studies show that fungi are very effective in breaking down polymers because they help break down organic matter in soil and have a wide range of metabolic abilities (Kim and Rhee, 2003; Zeghal *et al.,* 2021).

### 2.5.1 Types of Biodegradation

Plastics usually break down in nature through different ways depending on the environment. In places with plenty of oxygen, like in the open air, they break down aerobically. In areas without oxygen, such as in the soil or landfills, they break down anaerobically. Some breakdown also happens in compost and soil, but only partially through aerobic processes.

**a) Aerobic Biodegradation**

Aerobic breakdown, also called aerobic respiration, is a key way that harmful substances in the environment are naturally reduced. Certain microbes use oxygen to help break down organic

materials into smaller compounds, along with carbon dioxide and water as by-products. (Rani and Singh, 2017; Fesseha and Abebe, 2019; Srikanth *et al.,* 2022).

**b) Anaerobic Biodegradation**

Anaerobic breakdown happens when microorganisms break down organic materials without the presence of oxygen. This is also important in the natural removal of harmful substances from dangerous waste sites. Some anaerobic bacteria use other substances like nitrate, sulfate, iron, manganese, and carbon dioxide as alternatives to oxygen to break down organic materials into smaller parts, producing methane, carbon dioxide, water, and some remaining material along with biomass. (Rani and Singh, 2017; Fesseha and Abebe, 2019).

### 2.5.2 Mechanism of Plastic Biodegradation

Microorganisms can't easily move large polymer molecules through their outer cell membranes into the cell where chemical reactions happen. To get around this, microbes produce enzymes and free radicals that help break down the plastics into smaller pieces. (Cowan *et al.*, 2022)

The breakdown of plastics by microbes involves breaking the long polymer chains into shorter chains or smaller molecules, like oligomers, dimers, and monomers.

These smaller molecules are then transported across the cell membranes and used for growth by the microbes. Once inside the cells, the monomers are completely broken down into carbon dioxide, water (under aerobic conditions) or carbon dioxide, water, and methane (under anaerobic conditions), which can be used by the microbes for energy. (Fesseha and Abebe, 2019; Cowan *et al.*, 2022; Klusener, 2022).

To get carbon and strength from certain materials, microbes developed a way to release enzymes outside their cells. These enzymes break down the polymer materials outside the cell. There are two main ways this happens: one with oxygen and one without, as shown in Figure 2.1.

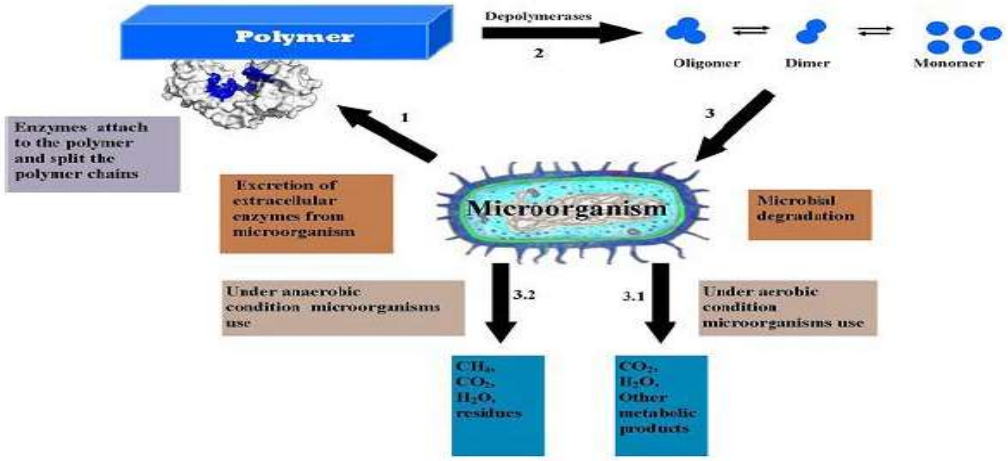



**Fig. 2.1: General Mechanism of Plastic Biodegradation under Aerobic and Anaerobic Conditions**

Source: (Fesseha and Abebe, 2019)

Biodegradation of polymers entails the following steps:

**1. Attachment of the microorganism to the surface of the polymer.**

The process of breaking down polymers happens in several steps. The first step is when microbes start to grow on the surface of the plastic. This is the beginning of the breakdown process, which sets the stage for the enzymes to work.

**2. Growth of the microorganism, using the polymer as a carbon source.**

The next step is called hydrolysis.Here, enzymes from the microbes meet the polymer material and help break it down. These enzymes, called hydrolases, work by breaking the chemical bonds in the polymer (Asiandu *et al*., 2022). The extracellular and intracellular enzymes from microorganisms involve two important processes. Extracellular enzymes,such as depolymerases and hydrolases, induce hydrolytic cleavage to the polymer chain. The polymer chain after being attacked by enzyme enhancement, results in small oligomers or monomers that bacteria can integrate with the cell (Fesseha and Abebe, 2019; Asiandu *et al*., 2022).

**3. Ultimate degradation of the polymer.**

In the first stage of breakdown, the enzymes released from the microbes split the long polymer chains into smaller fragments, like oligomers, dimers, or monomers. (Fesseha and Abebe, 2019). These small molecules can then be used by the microbes as a source of carbon and energy. Some of these small molecules can pass into the microbes and be used inside their cells.

The monomers that enter the cells are then broken down further. Under aerobic conditions, they are turned into carbon dioxide ($CO_{2)}$ and water ($H_2O$, which help the microbes produce energy. Under anaerobic conditions, they turn into carbon dioxide, water, and methane, which also help the microbes make energy, as shown in Figure 2.2 (Rani and Singh, 2017; Srikanth et al., 2022).

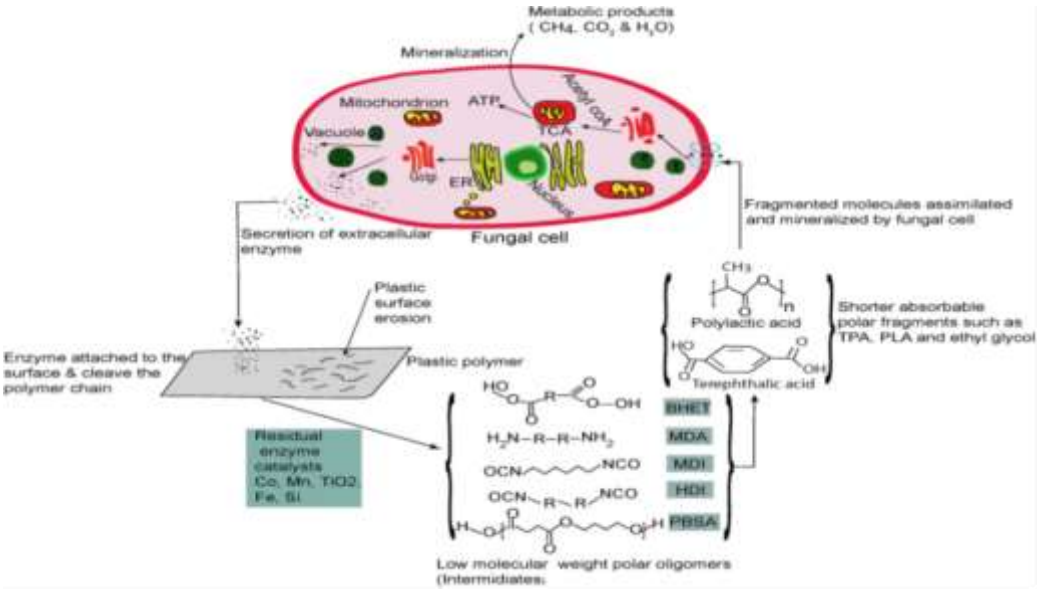



**Fig. 2.2:** Systematic overview of plastic degradation by fungi.

Source: (Okal *et al*., 2023)

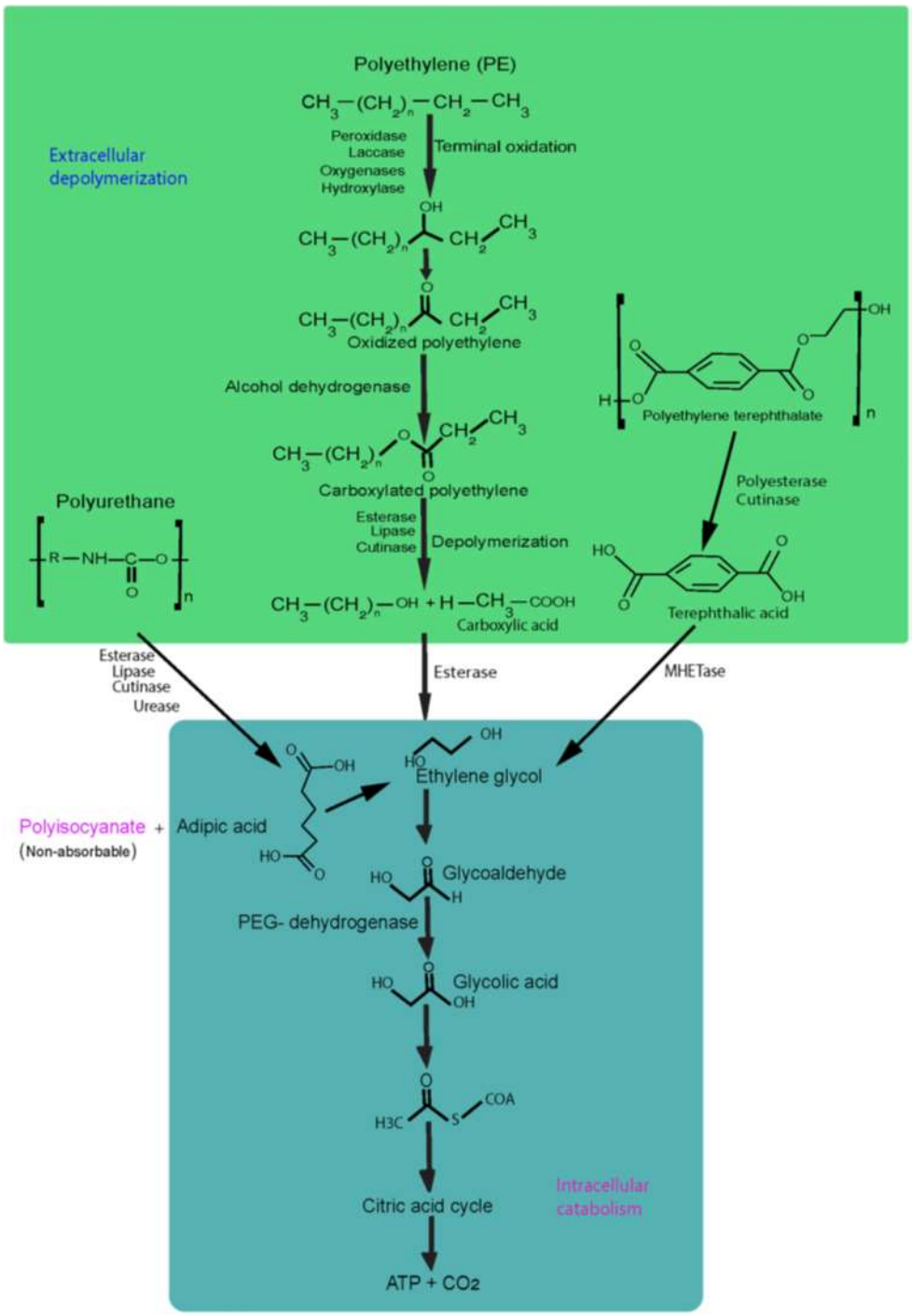



Fig. 2.3: Fungi release enzymes outside their cells that slowly break down plastic polymers into simpler substances. These simpler compounds are then taken into the fungal cells, where they are further broken down through internal processes to form minerals.

Source: (Okal *et al*., 2023)

## 2.6 FACTORS AFFECTING THE BIODEGRADATION PROCESS

The breakdown of plastics by natural processes is a slow process that depends on many factors. Some factors are connected to the plastic itself, like its structure, the type of chemicals it has, how much carbon it contains, how flexible its chains are, how tightly packed its molecules are, how big its molecules are, and whether it has extra ingredients that help it break down. Other factors come from the environment, such as how well microbes can break down the plastic, and how active the enzymes are that help this process. These factors can be influenced by things like how wet it is, how hot or cold it is, how acidic or basic the environment is, how salty it is, whether there is oxygen, sunlight, water, other types of plastics, and what kind of material the plastic is in. By adjusting these conditions, it's possible to make the breakdown of plastics go faster and more completely, eventually turning the plastic into harmless substances like minerals (Muthukumar and Veerappapillai, 2015; Fernandes *et al.,* 2020).

The way a plastic looks and feels, like how tightly its molecules are packed and its shape, along with the kind of chemical structure it has, and the type of fungi used in the process all play a big part in how fast it breaks down (Huang *et al.,* 1992).

Some plastics have chemical links that can be attacked by fungi, like ester groups, while others have only carbon links and don’t break down easily. Plastics like polyethylene, polypropylene, and polystyrene are mostly made of carbon chains without reactive parts, so they don’t break down easily. On the other hand, polyester plastics often have double carbon bonds and reactive groups that fungi can target (Leja and Lewandowicz, 2010). Since fungi are many different types and make different kinds of enzymes, only a few can break down plastics. The specific fungi and the enzymes they produce can greatly influence how well they can break plastics down (Nowak *et al.,* 2011; Gambarini *et al.,* 2021).

Fungi grow best when the temperature and pH are just right for their enzymes to work well.

Also, moisture and the right levels of oxygen are really important for the growth of fungi and the breakdown of plastics (Albertsson and Karlsson, 1990; Magan *et al.,* 2010). During the breaking down process, a lot of carbon dioxide is released as a waste product. If there's too much carbon dioxide, it can slow down the fungi's metabolism, especially in water or submerged conditions (Selke et al., 2015). Too much oxygen and even extra sugar can also stop the fungi from working well because they inhibit the enzymes that help break down the plastic. However, if plastics are first treated with strong chemical agents like UV light, concentrated nitric acid, sulfuric acid, heat, or ozone, this can make the breakdown by fungi much more effective (Khan *et al.,* 2017; Hahladakis *et al.,* 2018; Khan *et al.,* 2020).

Similar results have been seen when certain additives like copper, iron, manganese, cobalt, and surfactants are added to the environment where fungi grow (Liu *et al.,* 2014).

These additives seem to help fungi make more of the enzymes and proteins needed to break down plastics (Karlsson *et al.,* 1988; Arkatkar *et al.,* 2009). But more research is needed to understand exactly how these additives help, which parts of the enzymes they affect, and how much each additive speed up the process. Also, researchers are still figuring out if treatments like exposure to light, heat, or using additives to help with chemical reactions can make fungi break down plastics like polyolefins more quickly.Summarily, the biodegradability of a polymer is according to (Fesseha and Abebe, 2019), essentially determined by the following 8 physical and chemical characteristics:

1. The presence of certain groups that make the material less likely to mix with water, meaning it breaks down slower than materials that mix with water easily.
2. The size and how tightly packed the polymer molecules are (smaller and less packed materials break down quicker than larger and more tightly packed ones).
3. The structure of the plastic, which is made up of parts that are arranged in a regular pattern (crystalline) and parts that are not (amorphous), where the parts that are not arranged in a regular pattern break down faster.
4. The way the polymer is built, like whether it is straight or has branches.
5. The presence of bonds that are easier to break, such as ester or amide bonds. The order of how easily these bonds break is ester, then ether, then amide, then urethane.
6. The mix of different materials in the polymer.
7. The type and form of the polymer, such as films, pellets, powder, or fibers.
8. How stiff or soft the polymer is, with softer ones breaking down faster than harder ones.

## 2.7 APPROACHES TO ENHANCE FUNGAL BIODEGRADATION OF PLASTICS

### 2.7.1 Thermochemical and UV light pretreatment

Abiotic pretreatment of polymers using physical and chemical methods greatly speeds up the biodegradation process, especially for polyolefins (Cheng *et al.*, 2022; Okal *et al.*, 2023). When plastics are exposed to UV light, high energy radiation, or heat, they create radicals and oxidized products that start a series of reactions leading to polymer breakdown (Malachová *et al.,* 2020; Okal *et al.*, 2023). Chemical treatments like strong acids, surfactants, hydrogen peroxide, and other oxidants are also used before fungal biodegradation. This helps to cause oxidation, react with stabilizers in the polymer, and prepare specific parts of the polymer that fungal enzymes can break down (Konduri *et al.*, 2011; Yanto and Tachibana, 2014).

### 2.7.2 Blending with simple carbon substrates

Besides making synthetic polymers easier to break down, an abiotic pre-treatment method is also helpful. Another good option is mixing synthetic plastics with organic materials that break down quickly, like starch or other biodegradable substances. This helps speed up the process of breaking down the plastic through hydrolysis. Studies have shown that when these combinations are used, like starch mixed with PE, PLA with starch, or PLA with PP, the breakdown process is much stronger than when using just one type of plastic on its own (Arkatkar *et al.*, 2009). Mixing tough synthetic plastics with common biodegradable materials, especially starch and cellulose, has been studied and shown to help create important enzymes that aid in breaking down plastics (Sameshima-Yamashita *et al.*, 2016; Kaseem and Ko, 2017).

### 2.7.3 Incorporation of biosurfactants to growth media

Adding certain substances such as biosurfactants, cyclodextrins, lecithins, saponins, and phenolic compounds to the environment where microbes grow makes it easier for those microbes to stick to the surfaces of synthetic plastics.

These substances help by making the plastics more soluble and easier to break down, encouraging the production of enzymes that break down the plastic, and increasing the amount of HsBA proteins and EPS (Souza *et al.*, 2014; Okal *et al.*, 2023).

### 2.7.4 Use of microbial consortium

Using a group of different microbes (a microbial consortium) allows for a more complete breakdown of plastics.

This is because each microbe can contribute different enzymes or pathways that are not found in a single strain, helping to break down the plastic more effectively (Jaiswal *et al.,* 2020; Okal *et al.,* 2023).

### 2.7.5 Genetic engineering approach

A genetic engineering approach, which uses techniques like recombinant DNA, gene cloning, and modifying fungal genomes, is expected to play a big role in future research on breaking down hard-to-degrade synthetic plastics (Okal *et al.,* 2023).

## 2.8 BIOTECHNOLOGICAL PROSPECTS AND CURRENT INDUSTRIAL LIMITATIONS OF PLASTIC BIODEGRADATION

Current knowledge about how fungi degrade plastics, how this process can be used on a large scale, and how microbes can break down synthetic polymers depends on biotech techniques such as synthetic and systems biology, genetic engineering, and bioinformatics (Klusener, 2022).

These methods offer ways to speed up the slow breakdown of high molecular weight hydrocarbons and can be used in managing industrial waste from synthetic plastics (Khatua *et al.,* 2023).

Despite valuable findings from studies on microbial degradation of synthetic plastics, many of these studies are done in lab settings and have not been scaled up for real-world use because of challenges (Kubowicz and Booth, 2017; Wierckx *et al.,* 2018).

Most studies test how a single species breaks down one type of plastic, but real environments have a mix of different plastic types. Also, breaking down plastics completely is a multi-step process that needs multiple species working together as a team. The plastics used in lab studies are usually pure, but real-world plastics often have additives like plasticizers and hardeners, which make it harder to use lab results in industry (Hahladakis *et al.,* 2018). Even though studies show fungi can

break down synthetic plastics, the rate of microbial degradation in industry is still slow and not cost-effective compared to methods like burning or landfilling (Roux and Varrone, 2021).

However, with growing interest in biorecycling using systems and synthetic biology, along with improvements in bioplastic production, fungal degradation of synthetic plastic waste in industry might become a reality soon (Defruyt, 2019).

In the future, we imagine large bioreactors where a mix of fungi can be grown to break down different types of plastics. These bioreactors would produce organic matter and plastic monomers as by-products, which could be used in other industries. For example, the fungal biomass could be used as animal feed or fertilizer, while the broken-down plastic monomers could be collected and used to make new plastics (Khatua *et al.*, 2023).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Sample collection

The study area is Oluku dump site and Oluku saw mill point A and B at Ovia North East Local Government, Benin City, Edo State. The soil samples were collected and analyzed. The samples were analyzed for their physicochemical and bacteriological composition (Udochukwu *et al*., 2022).

## 3.2 Physico-chemical analysis of soil

The physicochemical parameters that were tested for the soil morphology include; pH, Temperature, Moisture content, Alkalinity, Chloride, Organic matter, Phosphorus (APHA, 2005; Ugochukwu *et al*., 2022).

### a) Determination of pH

The pH of the soil samples was measured using a handheld pH meter model H198107 (Hanna) in a soil-to-distilled water ratio of 1:5. Before starting, the meter was rinsed in distilled water. After turning the meter on, it was placed in the water sample after 10 minutes, and the reading was taken once the light showed a stable value.

### b) Determination of Available Phosphorus (Brays method)

A brays solution was prepared (0.025N HCl and 0.03N NH4F), One gram (1g) of the soil sample was extracted with 5ml of brays solution and filtered with watchman filter paper.

1ml of the filtrate was added into a test tube, with 1ml of ammonium molybdate solution. The solution was mixed well before 0.5ml of ascorbic acid solution was added and was incubated at

room temperature of 20 – 25$^{o}$C for 15minutes. The absorbance was taken at 882nm in UV visible spectrophotometer. The blank was treated in the same way but without the soil sample.

**c) Determination of Alkalinity**

Alkalinity was measured using a colorimetric method with a HI83200 multi-parameter bench photometer set to a wavelength of 575nm. One gram of soil sample was mixed with 5 parts water in two separate sample bottles. One bottle was used as a blank to set the photometer to zero. The other bottle had a sachet of HI 93755-0 alkalinity indicator reagent added, and it was mixed well. The sample was then placed in the photometer's cell compartment and allowed to sit for 2 minutes. After that, the 'READ' button was pressed to show the alkalinity concentration in milligrams per liter, which was then converted into milliequivalents per 100 grams.

d) **Determination of Temperature**

Water temperature was measured using a mercury-in-glass thermometer. The tip of the thermometer was lowered a few inches below the surface of the soil water (in a 1:5 ratio) and the temperature was read from the scale.

**e) Total Organic Matter (TOC)**

One gram (1g) of the soil sample was preheated in a muffle furnance at 450$^{o}$C for 4hrs. The organic matter is burnt off, hence, loss on ignition (Van Reeuwijk, 2002). Therefore, Organic matter is calculated as follows:

$$\text{Org.M (\%)} = \frac{\text{weight loss}}{\text{Original weight}} \times 100 \qquad \text{---------- Equation 3.1}$$

### f) Determination of Chloride (Mohr's method)

Five milliliters of the digested soil sample were taken, and a few drops of potassium chromate indicator solution were added. The sample was then titrated with standard silver nitrate (AgNO3) until it turned pinkish. A blank value was also determined by titrating 5ml of distilled water with the same silver nitrate and potassium chromate indicator. Chloride concentration was calculated in mg/l as follows:

$$Cl\ (mg/l) = \frac{(A - B) \times conc\ AgNO3 \times molarmass\ Cl}{Volume\ of\ sample\ used} \times 100$$ ---------- **Equation 3.2**

Where A = Titre value for sample

B = Titre value for blank

Therefore, Chloride concentration in mg/l was converted to mg/kg.

### g) Determination of moisture content

Five grams (5g) of the soil sample was weighed into a crucible and dried in an oven at 1105$^{o}$C to a constant weight. The moisture content was then calculated as follows (AOAC, 2005)

$$Moisture\ (\%) = \frac{Wet\ Weight - Dry\ Weight}{Wet\ Weight\ of\ Sample} \times 100$$ ---------- **Equation 3.3**

## 3.3 Fungi culture

### Potato Dextrose Agar (PDA)

This was used for the cultivation of fungi. The medium was prepared from commercially available dehydrated powder according to manufacturer's specification. 39 grams of Potato Dextrose agar powder was dissolved separately in 1 liter of distilled water in a sterile conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and autoclaved at 121$^{o}$C for 15

minutes. The medium was cooled to 45-50$^{o}$C and then, dispensed aseptically into sterile Petri dishes.

### 3.4 Isolation of Polyethylene Degrading Fungi

One gram of soil sample was transferred into a test tube containing 9ml of sterile distilled water. This content was shaken and serially diluted. Potato Dextrose Agar was used to culture the sample using pour plate method. The plates were incubated at 28$^{o}$C for 72hours and total heterotrophic fungi count would be noted (Rani and Singh, 2017).

### 3.5 Enumeration of Fungi

The fungi counts were used to enumerate the total viable counts of the isolates. The discrete colonies on the potato dextrose agar (PDA) plates were selected and counted. The mean colony count on the PDA plates were used to estimate the total viable counts for the samples in colony forming units per gram (cfu/g) (Margesin *et al*., 2005). Fungi culture was also identified by cultural and microscopic features.

### 3.6 Preparation of low- and high-density polyethylene (LDPE) for degradation studies

Polyethylene sheets of low density and high density was exposed to an abiotic degradation process through exposure to sunlight at different time of 10, 20, 30, 40 and 50days. Thereafter, the low density and high-density polyethylene sheets were cut into fragments of 2cm and mixed separately with 10ml of mineral salt medium broth and their degrading fungus will be inoculated into the mixture and incubated at 25°C for different time of, 10, 20, 30, 40 and 50days. Measure the weight of LDPE and HDPE before and after incubation and monitor the rate of degradation (Rani and Singh, 2017; Hyder *et al*., 2021).

### 3.7 Preparation of Mineral salt medium

Mineral salt medium broth containing 1litre of distilled water was prepared by adding the following compounds: $NH_4NO_3$ 1g, $MgSO_4.7H_2O$ 0.2g, $K_2HPO_4$ 1g, $CaCl_2.2H_2O$ 0.1g, KCl 0.15g and yeast extract 0.1g and 1mg/l of each of the following micro-elements: $FeSO_4.6H_2O$, $ZnSO_4.7H_2O$ and $MnSO_4$. The solution was sonicated for 1hour at 120 rpm in a shaker and sterilized at 121°C for 15minutes. Ten milliliters (10ml) sterilized medium was used as a substrate (Rani and Singh, 2017).

### 3.8 Identification of degrading fungus

Fungus was identified by looking at it under a microscope and with the naked eye. To see the fungi clearly under a microscope, a special stain called Lacto-phenol cotton blue was used. This helped to see how the spores were arranged (Rani and Singh, 2017).

### Molecular Characterization of plastic degrading bacterial isolates

#### a) Fungi DNA Extraction protocol

About 100 mg of the fungus mycelium was ground up using a special buffer called Dellaporta extraction buffer.

This buffer has a mix of Tris, EDTA, sodium chloride, and beta-mercaptoethanol. DNA was extracted using a standard procedure. Each sample was ground in 1000 µl of the buffer inside a sterile bag. The mixture was then put into a sterile eppendorf tube, and 40 µl of 20% SDS was added. The mixture was briefly shaken and then heated to $65^0$C for 10 minutes.

At room temperature, 160 µl of 5 M potassium acetate was added, and the mixture was shaken again. It was then spun in a centrifuge at 10,000 g for 10 minutes. The liquid on top was collected

into another eppendorf tube and 400 µl of cold isopropyl alcohol was added. The mixture was gently mixed and cooled at – $20^0$C for 60 minutes.

The mixture was then spun again at 13,000 g for 10 minutes to help the DNA settle at the bottom.

The liquid was carefully poured off, making sure not to disturb the DNA pellet. The pellet was then washed with 500 µl of 70% ethanol, and spun again at 10,000 g for 10 minutes. The ethanol was poured off and the DNA was allowed to air dry at room temperature until all the ethanol had evaporated.

The DNA pellet was then placed back into 50 µl of Tris EDTA buffers to keep it safe and ready for use (White *et al.*, 1990; Schoch *et al.*, 2012).

**b) PCR (Polymerase Chain Reaction) Analysis**

To identify the fungus using the ITS gene, a set of universal primers that target the ITS1, 5.8S, and ITS2 regions was used. The PCR preparation included 10 microliters of 5x GoTaq colourless reaction mix, 3 µl of 25 mM MgCl2, 1 µl of 10 mM dNTPs mix, 1 µl of each 10 pmol ITS 1 and ITS 4 primers, 0.3 units of Taq DNA polymerase, and the DNA sample. The total volume was made up to 42 µl with sterile distilled water.

PCR was conducted using a GeneAmp 9700 PCR System Thermalcycler. The cycle started with an initial denaturation at $94^0$C for 5 minutes. This was followed by 35 cycles of denaturation at $94^0$C for 30 seconds, annealing at $55^0$C for 30 seconds, and extension at $72^0$C for 1.5 minutes. The cycle ended with a final extension at $72^0$C for 7 minutes (White *et al.*, 1990; Gardes and Bruns, 1993; Nilsson *et al.*, 2019).

### c) INTEGRITY (Agarose Gel Electrophoresis)

The quality of the amplified gene fragment, which is about 1.5 Mb in size, was tested using a 1% agarose gel. First, the buffer, called 1XTAE buffer, was made and used to prepare a 1.5% agarose gel. The sample was boiled in a microwave for 5 minutes. The melted agarose was cooled to 60°C and then mixed with 3 µl of ethidium bromide, a dye that can light up under UV light. A comb was placed in the gel tray, and the melted agarose was poured into the tray. The gel was left to harden for 20 minutes to form the wells. The 1XTAE buffer was then added to the gel tank so that it just covered the gel. A small amount, 2 µl, of blue loading dye was mixed with each 4 µl of PCR product and loaded into the wells after the 100bp DNA ladder was put into the first well. The gel was run at 120 volts for 45 minutes and then viewed under UV light. A photo was taken. The size of each PCR product was estimated by comparing it to the movement of the 100bp DNA ladder that was run with the samples (Nilsson *et al.,* 2008; Usyk *et al.,* 2017).

## PURIFICATION OF AMPLIFIED PRODUCT

After checking the gel, the amplified fragments were purified using ethanol to remove any extra PCR materials. The procedure started by adding 7.6 µl of 3M sodium acetate and 240 µl of 95% ethanol to about 40 µl of the PCR product in a new sterile tube. The mixture was shaken well and kept at -20°C for at least 30 minutes. The mixture was then spun in a centrifuge for 10 minutes at 13,000 g and 4°C. The liquid on top was poured off, and the tube was placed on trash. The pellet was washed by adding 150 µl of 70% ethanol, shaken well, and spun again for 15 minutes at 7,500 g and 4°C. Again, the liquid was removed and the tube was placed on paper tissue. It was left to dry in a fume hood at room temperature for 10 to 15 minutes. The pellet was then mixed with 20 µl of sterile water and stored at -20°C before sequencing. The purified fragment was checked again on a 1.5% agarose gel run at 110 volts for about an hour, as before, to make sure the purified

product was present. It was also measured using a Nanodrop 2000 spectrophotometer (Toju *et al.,* 2012; Schoch *et al.,* 2012).

**d) SEQUENCING (sequencing of the 16s rRNA gene)**

The amplified pieces were then analyzed using a Genetic Analyzer 3130xl from Applied Biosystems, following the instructions provided by the manufacturer. The sequencing was done with the BigDye Terminator v3.1 cycle sequencing kit. For all the genetic analysis, the Bio-Edit software and MEGA 6 were used (Schoch *et al.,* 2009; Begerow *et al.,* 2010).

### 3.9 Determination of LDPE and HDPE degradation Potential

The percentage degradation of LDPE and HDPE by *Aspergillus niger* and *Aspergillus flavus*, *Fusarium oxysporum* and the consortium of both *Aspergillus flavus* and *Fusarium oxysporum* was determined by calculating the percentage weight loss of LDPE and HDPE polyethylene. The percentage weight loss was calculated by the following formula.

Weight loss=initial weight- final weight ---------- **Equation 3.4**

%Weight loss = initial weight- final weight/initial weight ×100 ---------- **Equation 3.5**

### 3.10 Optical density determination

The low density and high density polyethylene fragments after abiotic exposure were mixed separately with 10ml of mineral salt medium broth and their degrading fungi, *Fusarium oxysporum, Aspergillus flavus*, *Fusarium oxysporum* and *Aspergillus flavus* and *Aspergillus niger* were inoculated into the mixture of the experimental test groups with a negative control and incubated at 25℃ at different time of, 10, 20, 30, 40 and 50days.The optical density (OD) of LDPE and HDPE at initial and final incubation was noted (Abraham *et al*., 2017).

$$\text{Weight loss} = \frac{\text{initial optical density (OD) - final optical density (OD)}}{\text{Initial optical density (OD)}} \quad \textbf{-------- Equation 3.6}$$

$$\%\text{Weight loss} = \frac{\text{initial optical density (OD) - final optical density (OD)}}{\text{Initial optical density (OD)}} \times 100 \quad \textbf{--- Equation 3.7}$$

The most common wavelength used to measure bacterial/fungal growth in a broth is 600nm, which is known as optical density (OD600) measurement.

# CHAPTER FOUR

## RESULT

**Table 4.1: Physicochemical properties of the soil sample collected from polyethylene compost dumping site**

| Samples | pH | Temp. ($0^{O}C$) | Moisture content (%) | Alkalinity (Meq/100g) | Chloride (mg/kg) | Org.M (%) | P(mg/kg) |
|---|---|---|---|---|---|---|---|
| A | 7.4 | 23 | 13.8 | 3.0 | 31.95 | 14.6 | 520 |
| B | 6.8 | 25 | 10.2 | 2.6 | 56.8 | 8.1 | 230 |
| C | 6.4 | 26 | 9.9 | 2.0 | 106.5 | 6.5 | 170 |

Sample A = Oluku dump site

B = Oluku saw mill point A

C = Oluku saw mill point B

**Table 4.2: Total heterotrophic fungi count (Df $10^4$)**

| **Samples** | **THFC (X$10^4$ cfu/g)** |
|---|---|
| Homogenized soil sample(A,B & C) | $8.0x10^4$ |

**Table 4.3: Characterization of Fungi Isolates from the Homogenized Soil Samples**

| | Cultural | Microscopic | Isolates |
|---|---|---|---|
| A | Green and flat colony with reverse side white | Brush-like conidia septate with branching. Conidiophore with smooth or rough walled | *Penicillium sp.* |
| B | White and cottony hyphae with reverse side pinkish. | Non-septate hyphae with sporangiosphore | *Fusarium sp.* |
| C | Light green colony with reverse side pale yellow. | Simple septate and branched conidia in chain. | *Aspergillus sp.* |
| D | Dark greenish colony with reverse side black. | Septate and branched, conidia rough-walled. | *Aspergillus sp.* |

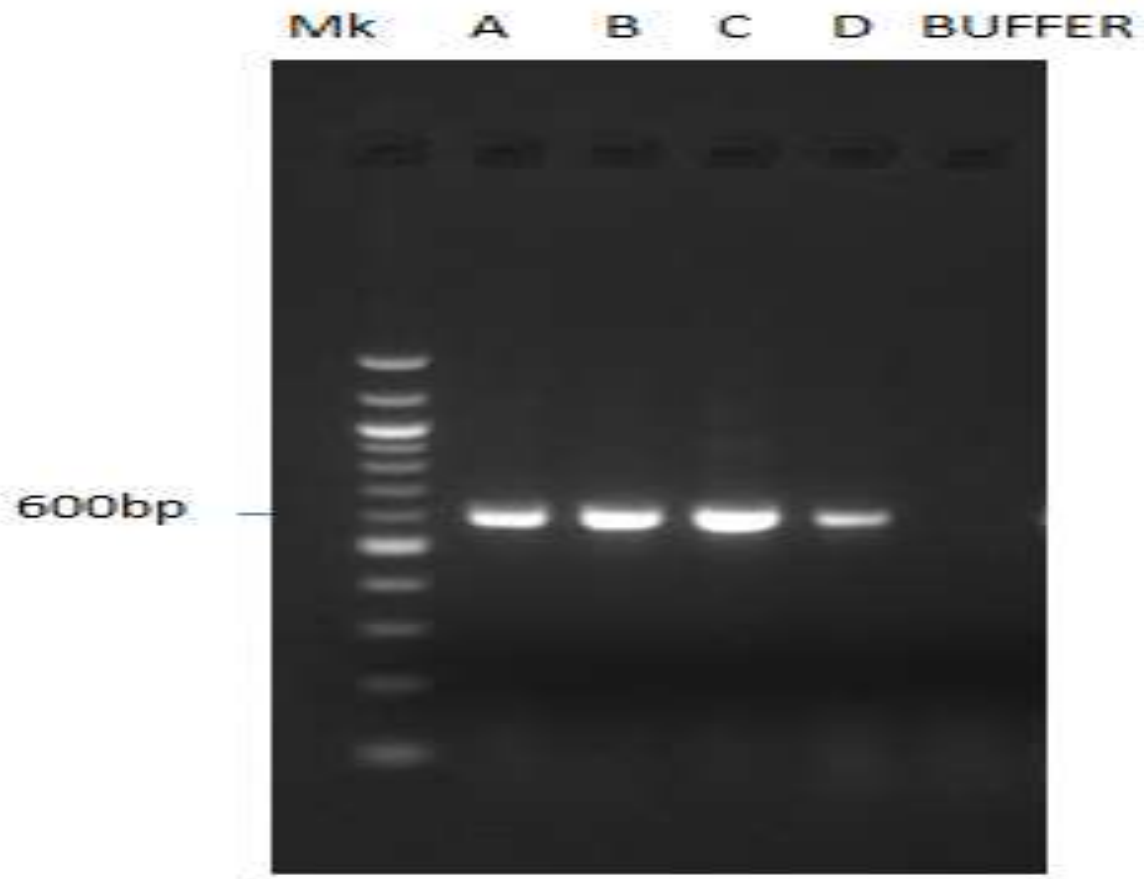
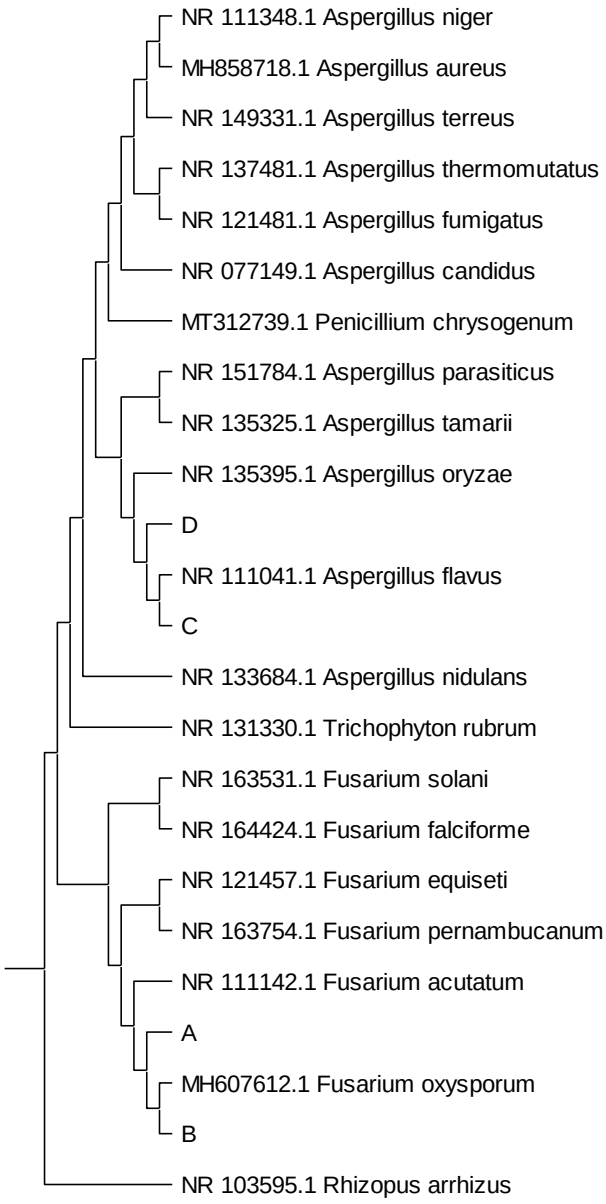



**Plate 4.1:** Agarose gel electrophoresis of the PCR products ITS REGION amplified from selected fungi isolates. (Band size approximately 600bp). Gel image indicates a positive amplification in all samples

**Table 4.4:** NCBI Blast showing the sequence identity of the isolate

| SAMPLE ID | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Accession |
|---|---|---|---|---|---|---|---|
| A | *Fusarium oxysporum* | 1092 | 1092 | 99% | 0 | 99.67% | OQ352044 |
| B | *Fusarium oxysporum* | 990 | 990 | 99% | 0 | 100.00% | OQ352041 |
| C | *Aspergillus flavus* | 1083 | 1083 | 100% | 0 | 99.66% | OQ352042 |
| D | *Aspergillus flavus* | 1083 | 1083 | 100% | 0 | 99.50% | OQ352043 |
| E | *Aspergillus niger* | 1090 | 1090 | 100% | 0 | 100% | MW332264.1 |


NR 111348.1 Aspergillus niger
MH858718.1 Aspergillus aureus
NR 149331.1 Aspergillus terreus
NR 137481.1 Aspergillus thermomutatus
NR 121481.1 Aspergillus fumigatus
NR 077149.1 Aspergillus candidus
MT312739.1 Penicillium chrysogenum
NR 151784.1 Aspergillus parasiticus
NR 135325.1 Aspergillus tamarii
NR 135395.1 Aspergillus oryzae
D
NR 111041.1 Aspergillus flavus
C
NR 133684.1 Aspergillus nidulans
NR 131330.1 Trichophyton rubrum
NR 163531.1 Fusarium solani
NR 164424.1 Fusarium falciforme
NR 121457.1 Fusarium equiseti
NR 163754.1 Fusarium pernambucanum
NR 111142.1 Fusarium acutatum
A
MH607612.1 Fusarium oxysporum
B
NR 103595.1 Rhizopus arrhizus

> A

CCTTGGTCATTTAAAAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGC

GGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCAATTGTTG

CCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTA

AACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAA

CAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAA

TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCA

GTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCCCGGGTTT

GGTGTTGGGGATCGGCGAGCCTCACGGCAAGCCGGCCCCGAAATACAGTGGCGGTC

TCGCTGCAGCTTCCATTGCGTAGTAGTAAAACCCTCGCAACTGGTACGCGGCGCGGC

CAAGCCGTTAAACCCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCG

CTGAACTTAAGCATATCAATAGGCCGGGAGGA

> B

AAAATCTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACC

AGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCAATTG

TTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCC

CTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTT

CAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAG

TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCG

CCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCCCGGG

TTTGGTGTTGGGGATCGGCGAGCCTCACGGCAAGCCGGCCCCGAAATACAGTGGCG

GTCTCGCTGCAGCTTCCATTGCGTAGTAGTAAAACCCTCGCAACTGGTACGCGGCGC

GGCCAAGCCGTTAAACCCCCAACTTCTG

>C

TCCGAGGTGAACCAGCGGAAGGATCATTACCGAGTGTAGGGTTCCTAGCGAGCCCA

ACCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCC

GCCGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCT

GATCTAGTGAANTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGA

TCTCTTGGTTCAGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATT

GCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCG

GGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGG

TCGTCGTCCCCTCTCCGGGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCC

GATCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCC

GAACGCAAATCAATCTTTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGA

ACTTAAGCATATCAATAAGCGGAGGA

>D

CCTAGGTGAACCTGCGGAAGGATAATTACCGAGTGTAGGGTTCCTAGCGAGCCCAA

CCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCG

CCGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTG

ATCTAGTGAANTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGAT

CTCTTGGTTCAGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATT

GCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCG

GGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGG

TCGTCGTCCCCTCTCCGGGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCC

GATCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCC

GAACGCAAATCAATCTTTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGA

ACTTAAGCATATCAATAAGCGGAGGA

>E

AGTTTCTCTAGAGGAGGAGGGAGCTTTGGTGCGTAACAAGGTCTCGGTTGGTGAACC

AGCGGAGGATCATTAACCGAGTTACAACCTCCAAACCCCTGTGAACATACCAATTGT

TGCCTCGGCGGATCAGCGCTCCCGGTAAAACGGGACGGCCCGCCAGGAGACCCCTA

AACTCTGTTTCTATATGTAACTCTGAGTAAAACCATAAATAAATCAAACTTTCAA

CAACGGATCTCTTGGTTCTGGCATCGATGAAGACGCAGCAAATGGGATAAGTAA

TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTCGCCCGCCA

GTATTCGGCGGCATGCCTGTTCGAGCTCATTTCAACCCTCAAGCCCCGGGTTT

GGTGTGGGGATCGGCAGCCTCACGGCAAGCGGCCCCGAAATACAGTGGCGGTCTCG

CTGCAGCTTCCATTGCGTAGTAGTAAAACCTCGCAACTGGTACGCGGCGGCGGCAA

GCGGTTAAACCCCAACTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAA

CTTAAGCATATCAATAGGCCGGGAGGA

**Table 4.5: Changes in weight after Abiotic exposure for LDPE**

| Samples | Exposure days | Initial weight (g) | Final weight (g) | Weight loss (g) |
|---|---|---|---|---|
| $A_{10}$ | 10 | 0.24 | 0.24 | 0.0 |
| $A_{20}$ | 20 | 0.24 | 0.24 | 0.0 |
| $A_{30}$ | 30 | 0.24 | 0.24 | 0.0 |
| $A_{40}$ | 40 | 0.24 | 0.23 | 0.1 |
| $A_{50}$ | 50 | 0.24 | 0.23 | 0.1 |

**Table 4.6: Changes in weight after Abiotic exposure for HDPE**

| Samples | Exposure days | Initial weight (g) | Final weight (g) | Weight loss (g) |
|---|---|---|---|---|
| $A_{10}$ | 10 | 1.0 | 0.99 | 0.1 |
| $A_{20}$ | 20 | 1.0 | 0.98 | 0.2 |
| $A_{30}$ | 30 | 1.0 | 0.98 | 0.2 |
| $A_{40}$ | 40 | 1.0 | 0.98 | 0.2 |
| $A_{50}$ | 50 | 1.0 | 0.98 | 0.2 |

**Table 4.7: Mean effect of Fungi Isolates on the Optical density and Weight loss of plastics.**

| Isolates | Optical density | Weight loss(g) |
|---|---|---|
| *Fusarium oxysporum* | 0.1407±0.213[a] | 0.4340±0.426[a] |
| *Aspergillus flavus* | 0.1652±0.226[a] | 0.4393±0.420[a] |
| Consortium | 0.1702±0.210[a] | 0.4290±0.430[a] |
| *Aspergillus niger* | 0.1860±0.219[a] | 0.4305±0.427[a] |
| P-value | 0.485 | 0.909 |
| Level of significance | Not significant | Not significant |

Means that follow the same letter(s) within a column is not statistically significant at 5% level of probability or P>0.05

**Table 4.8: Mean effect of Plastic Density on the Optical density and Weight loss of plastics**

| Density | Optical density | Weight loss(g) |
|---|---|---|
| LDPE | 0.1013±0.054[a] | 0.1684±0.083[a] |
| HDPE | 0.0902±0.062[a] | 0.9555±0.030[b] |
| P-value | 0.850 | 0.000 |
| Level of significance | Not significant | Significant |

**Table 4.9: Mean effect of plastic Time of exposure on the Optical density and Weight loss of plastics**

| Time of exposure (day) | Optical density | Wight loss (g) |
|---|---|---|
| A10 | $0.0665 \pm 0.057^{b}$ | $0.4430 \pm 0.431^{a}$ |
| A20 | $0.1134 \pm 0.137^{ab}$ | $0.4354 \pm 0.426^{a}$ |
| A30 | $0.1742 \pm 0.207^{ab}$ | $0.4299 \pm 0.428^{a}$ |
| A40 | $0.2263 \pm 0.263^{a}$ | $0.4334 \pm 0.424^{a}$ |
| A50 | $0.2472 \pm 0.284^{a}$ | $0.4242 \pm 0.429^{a}$ |
| P-value | 0.035 | 1.000 |
| Level of significance | Significant | Not significant |

# CHAPTER FIVE

# DISCUSSION

This study investigated the degradation potential of various fungal species (*Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger* and the consortium of both *Fusarium oxysporum* and *Aspergillus flavus*) on Low-Density Polyethylene (LDPE) and High-Density Polyethylene (HDPE). The findings provide insights into the biodegradation efficiency of these fungi, which were isolated from plastic-contaminated soil following the method of Rani and Singh, (2017) who using the macro and microscopic method only in their research, identified *A. niger*, *A. flavus* like this study, and *A. fumigatous*, *A. terrus* and *Fusarium solani* unlike this study.

The soil sample collected from polyethylene compost dumping sites using the method of previous researchers (Rani and Singh, 2017; Udochukwu *et al*., 2022) exhibited pH values ranging from 6.4 to 7.4, indicating a slightly acidic to neutral environment. Moisture content varied between 9.9% and 13.8%, which suggests favorable conditions for microbial activity. The presence of organic matter (6.5-14.6%) and phosphorus (170-520 mg/kg) indicates the availability of nutrients that support fungal growth. The total heterotrophic fungal count (THFC) was $8.0 \times 10^4$ cfu/g, confirming a high fungal presence in plastic contaminated soil, which implies microbial adaptation to these environments.

Four fungal isolates were identified based on cultural and microscopic characteristics: *Penicillium sp*., *Fusarium sp*., and two *Aspergillus sp*. Molecular characterization using ITS sequencing and BLAST analysis confirmed that the dominant plastic-associated fungi were: *Fusarium oxysporum* and *Aspergillus flavus*. These fungi are known for their enzyme-producing capabilities, making them potential candidate for plastic degradation. This is very similar to the work of Udochukwu *et al*., ( 2022), who employing the same molecular method of characterization in their study of

biodegradability of polystyrene plastics by bacterial isolates from plastic composted waste soil identified *Bacillus subtilis* strain BS3902, *Pseudomonas aeruginosa* strain KAVKOI and *Bacillus subtilis* strain AER111-2.

The study successfully extracted and analyzed fungal DNA, identifying fungal species through PCR amplification and sequencing of the ITS region. The study employed robust molecular techniques, including sequencing using the Genetic Analyzer 3130xl and bioinformatics tools (BioEdit and MEGA 6). The high sequence identity and strong PCR amplification confirmed the reliability of the methodology used. The DNA extraction method effectively isolated fungal DNA, as confirmed by gel electrophoresis. The use of Dellaporta extraction buffer ensured the breakdown of fungal cell walls, while isopropanol precipitation helped recover high-quality DNA. The ethanol washed further removed impurities, ensuring a clean DNA template for PCR amplification. The PCR result showed successful amplification of the ITS region, with clear bands of approximately 600bp observed in all samples. This indicates that the ITS primers effectively amplified the target regions across different fungal isolates, validating the use of ITS markers for fungal identification. BLAST analysis of the sequence identified two major species: *Fusarium oxysporum* (Samples A and B) with sequence identity of 99.67% and 100%; *Aspergillus flavus* (Sample C and D) with sequence identity of 99.66% and 99.50%. This high sequence identity values confirm accurate identification of the fungal isolates.

In this study, Sample A was initially identified as *Penicillium sp*. Based on morphological characteristics but later confirmed as *Fusarium oxysporum* through molecular characterization (ITS sequencing and Blast analysis) which provides higher accuracy. The ITS region (ITS1, 5.8S, and ITS2) is highly variable among fungal species, making it an excellent marker for distinguishing between closely related fungi. This discrepancy is a common occurrence in fungal

taxonomy and can be explained by several factors: morphological identification relies on colony appearance, color, hyphae structure, and conidial arrangement. However, fungal species can exhibit high phenotypic plasticity, meaning: different environmental conditions e.g temperature, pH, and nutrient availability can alter colony morphology; *Fusarium oxysporum* and *Penicillium sp.* Share some microscopic features, such as septate hyphae and conidial chains, which may lead to misidentification; some fungal species can exhibit atypical morphologies when grown in culture, making it difficult to distinguish them visually. The possible misindentification in Sample A (the green flat colony with a white reverse side) seen resembles *Penicillium,* but some *Fusarium* strains can also exhibit similar characteristics under specific conditions. Microscopic examination showed brush-like conidia, which are common in *Penicillium,* but some *Fusarium oxysporum* strains may exhibit overlapping structures (Pitt and Hocking, 2009; Leslie and Summerell, 2006; Klich, 2002). This case highlights why morphological identification alone is insufficient and should always be validated with molecular techniques, especially for closely related fungal species with similar morphological traits; environmental fungi that may exhibit atypical growth in artificial culture conditions; fungi with high genetic diversity where different strains may appear morphologically different.

Before testing for biological degradation of polyethylene by exposure of plastic to isolates, LDPE and HDPE plastics were exposed to sunlight for 50 days to access abiotic degradation. LDPE exhibited minimal weight loss (0.1g) after 50 days, indicating high resistance to environmental breakdown. HDPE showed slightly higher degradation (0.2g weight loss) but was still largely resistant to abiotic factors. This confirms that natural environmental conditions alone are insufficient to degrade plastics efficiently, necessitating microbial intervention.

Biodegradation of polyethylene was measured by using weight loss and the result showed that there was no significant difference among the isolates, suggesting, all isolates have similar effects on weight. Considering the density of the plastics, the result revealed that the result showed that HDPE had the higher mean weight loss (0.9555±0.030); LDPE had lower weight loss (0.1684±0.083) showing there is significant difference and that the density significantly affect weight loss, with HDPE retaining the more, and LDPE losing the more. HDPE is more resistant to weight loss, while LDPE degrades the more (Table 4.9). The results indicate that LDPE was more susceptible to fungal biodegradation than HDPE. This is expected because LDPE has a more branched and less crystalline structure, making it more accessible to microbial enzymes. In contrast, HDPE has a higher degree of crystallinity (higher molecular weight and crystallinity) making it more resistant to microbial attack. The impact of time of exposure on weight loss was also measured and it showed that time of exposure of plastic does not significantly influence weight loss, shockingly the reverse was the case for optical density and the possible reasons are discussed below under the results of optical density.

Optical density was also used as a parameter to access biodegradation of polyethylene. The result reads that among the isolates, there is no significant effect on the optical density of the isolates; all isolates have similar optical density values (Table 4.7). It was also observed that there was no significant difference among the density of the plastics used either LDPE or HDPE. It was however observed that there was significant difference in the time of exposure. A50 which is exposure of plastic to fungal biodegradation for fifty days gave the highest mean effect (0.2472±) at the fiftieth day; while A10 gave the lowest (Table 4.9). Sample A40 and A50 with the higher values had higher Optical density, meaning time of exposure impacts optical density. In contrast to weight lost while looking at the impact of time of exposure on degradation, Optical density result shows

that value rise across all samples suggest that the fungi were metabolizing available carbon sources in the medium, however, higher optical density value does not always correlate with higher plastic degradation, as fungi could be growing on alternative nutrients in the mineral salt broth (Zeghal *et al*., (2021); Cernosa *et al*., 2024).

## CONCLUSION

The finding of this research showed that abiotic degradation is too weak a method, and is highly independable in the considerations of quicker solution to degradation of polymers like plastic in particular and there will always be need for biodegrdation. This study provides strong evidence that fungi, particularly *Fusarium oxysporum*, *Aspergillus flavus*, and *Aspergillus niger*, can biodegrade polyethelene plastics. The findings highlight the potential of fungal-based bioremediation as a sustainable approach to plastic waste management. The results indicated that fungal consortia are very effective in breaking down plastics. Future research should focus on optimizing fungal growth conditions and enzyme production to enhance plastic degradation efficiency. The density of the plastic (LDPE and HDPE) obviously affected the degradation activities of the isolates leading to significant weight loss; LDPE having the higher mean value as expected and reported the in literature that it is easier for microbial community to biodegrade low-density polyethylene than high-density polyethylene due to its simplicity in structure and polymeric nature. The time of exposure had no significant impact on the weight loss during degradation; it was noticed that the optical density was highly significant. It therefore means that although the optical density values were significant, showing that there was utilization of carbon source due to increase in fungal biomass or fungal proliferation, this growth does not necessarily mean the plastic was the only carbon source.

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## APPENDIX

```
UNIANOVA WGT BY ISL DEN SPL
  /METHOD=SSTYPE(3)
  /INTERCEPT=INCLUDE
  /POSTHOC=ISL DEN SPL(DUNCAN)
  /EMMEANS=TABLES(OVERALL)
  /PRINT=DESCRIPTIVE
  /CRITERIA=ALPHA(.05)
  /DESIGN=ISL DEN SPL ISL*DEN ISL*SPL DEN*SPL ISL*DEN*SPL.
```

### Tests of Between-Subjects Effects

Dependent Variable: WGT

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 17.874[a] | 79 | .226 | 1.731 | .008 |
| Intercept | 30.027 | 1 | 30.027 | 229.691 | .000 |
| ISL | .003 | 3 | .001 | .006 | .999 |
| DEN | 17.824 | 3 | 5.941 | 45.449 | .000 |
| SPL | .006 | 4 | .002 | .012 | 1.000 |
| ISL * DEN | .009 | 9 | .001 | .008 | 1.000 |
| ISL * SPL | .007 | 12 | .001 | .004 | 1.000 |
| DEN * SPL | .006 | 12 | .001 | .004 | 1.000 |
| ISL * DEN * SPL | .019 | 36 | .001 | .004 | 1.000 |
| Error | 10.458 | 80 | .131 | | |
| Total | 58.359 | 160 | | | |
| Corrected Total | 28.333 | 159 | | | |

a. R Squared = .631 (Adjusted R Squared = .266)

**Estimated Marginal Means**

**Grand Mean**

Dependent Variable: WGT

| Mean | Std. Error | 95% Confidence Interval | |
|---|---|---|---|
| | | Lower Bound | Upper Bound |
| .433 | .029 | .376 | .490 |

**Post Hoc Tests**

**Isolates**

**Homogeneous Subsets**

**WGT**

Duncan[a,b]

| Isolates | N | Subset |
|---|---|---|
| | | 1 |
| Consortium | 40 | .4290 |
| Aspergillus niger | 40 | .4305 |
| Fusarium oxysporum | 40 | .4340 |
| Aspergillus flavus | 40 | .4393 |
| Sig. | | .909 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .131.

a. Uses Harmonic Mean Sample Size = 40.000.

b. Alpha = .05.

**Density**

**Homogeneous Subsets**

**WGT**

Duncan[a,b]

| Density | N | Subset | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| Control (LDPE) | 40 | .1180 | | |
| LDPE | 40 | .1684 | | |
| Control (HDPE) | 40 | | .4910 | |
| HDPE | 40 | | | .9555 |
| Sig. | | .535 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .131.

a. Uses Harmonic Mean Sample Size = 40.000.

b. Alpha = .05.

**Samples**

**Homogeneous Subsets**

**WGT**

Duncan[a,b]

| Samples | N | Subset 1 |
|---|---|---|
| A50 | 32 | .4242 |
| A30 | 32 | .4299 |
| A40 | 32 | .4334 |
| A20 | 32 | .4354 |
| A10 | 32 | .4430 |
| Sig. | | .857 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .131.

a. Uses Harmonic Mean Sample Size = 32.000.

b. Alpha = .05.

```
UNIANOVA OPD BY ISL DEN SPL
  /METHOD=SSTYPE(3)
  /INTERCEPT=INCLUDE
  /POSTHOC=ISL DEN SPL(DUNCAN)
  /EMMEANS=TABLES(OVERALL)
  /PRINT=DESCRIPTIVE
  /CRITERIA=ALPHA(.05)
  /DESIGN=ISL DEN SPL ISL*DEN ISL*SPL DEN*SPL ISL*DEN*SPL.
```

**Tests of Between-Subjects Effects**

Dependent Variable: Optical Density

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 2.000[a] | 79 | .025 | .376 | 1.000 |
| Intercept | 4.383 | 1 | 4.383 | 65.041 | .000 |
| ISL | .042 | 3 | .014 | .209 | .890 |
| DEN | .802 | 3 | .267 | 3.964 | .011 |
| SPL | .735 | 4 | .184 | 2.726 | .035 |
| ISL * DEN | .064 | 9 | .007 | .105 | .999 |
| ISL * SPL | .032 | 12 | .003 | .040 | 1.000 |
| DEN * SPL | .244 | 12 | .020 | .302 | .987 |
| ISL * DEN * SPL | .082 | 36 | .002 | .034 | 1.000 |
| Error | 5.392 | 80 | .067 | | |
| Total | 11.775 | 160 | | | |
| Corrected Total | 7.392 | 159 | | | |

a. R Squared = .271 (Adjusted R Squared = -.450)

**Estimated Marginal Means**

**Grand Mean**

Dependent Variable: Optical Density

| Mean | Std. Error | 95% Confidence Interval | |
|---|---|---|---|
| | | Lower Bound | Upper Bound |
| .166 | .021 | .125 | .206 |

**Post Hoc Tests**

**Isolates**

**Homogeneous Subsets**

**Optical Density**

Duncan[a,b]

| Isolates | N | Subset |
|---|---|---|
| | | 1 |
| Fusarium oxysporum | 40 | .1407 |
| Aspergillus flavus | 40 | .1652 |
| Consortium | 40 | .1702 |
| Aspergillus niger | 40 | .1860 |
| Sig. | | .485 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .067.

a. Uses Harmonic Mean Sample Size = 40.000.

b. Alpha = .05.

**Density**

**Homogeneous Subsets**

**Optical Density**

Duncan[a,b]

| Density | N | Subset 1 | Subset 2 |
|---|---|---|---|
| HDPE | 40 | .0902 | |
| LDPE | 40 | .1013 | |
| Control (LDPE) | 40 | | .2194 |
| Control (HDPE) | 40 | | .2512 |
| Sig. | | .850 | .585 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .067.

a. Uses Harmonic Mean Sample Size = 40.000.

b. Alpha = .05.

**Samples**

**Homogeneous Subsets**

**Optical Density**

Duncan[a,b]

| Samples | N | Subset 1 | Subset 2 |
|---|---|---|---|
| A10 | 32 | .0665 | |
| A20 | 32 | .1134 | .1134 |
| A30 | 32 | .1742 | .1742 |
| A40 | 32 | | .2263 |
| A50 | 32 | | .2472 |
| Sig. | | .121 | .062 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .067.

a. Uses Harmonic Mean Sample Size = 32.000.

b. Alpha = .05.

## AGAR COMPOSITION AND BIOCHEMICAL REAGENTS

### Bray's No 1 Method Reagents

Bray No 1 (0.025N HCl + 0.03N $NH_4F$)

1ml of Ammonium molybdate solution

0.5ml of Ascorbic acid solution

### Mohr's Reagents

Silver Nitrate ($AgNO_3$) solution

Sodium Chloride (NaCl)

Potassium Chromate ($K_2CrO_4$) indicator solution

Distilled water

Nitric acid ($HNO_3$)

### Potato Dextrose Agar

Potato infusion- 200gm

Dextrose- 20gm

Agar- 20gm

Distilled water- 1 litre

### Mineral Salt Medium

NH4NO3 1g,

MgSO4.7H2O 0.2g,

$K_2HPO_4$ 1g,

$CaCl_2.2H_2O$ 0.1g,

KCl 0.15g

Yeast extract 0.1g and

1mg/l of each of the following micro-elements: $FeSO_4.6H_2O$, $ZnSO_4.7H_2O$ and $MnSO_4$.