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Biodegradation of Pure Water Sachets and Polythene Treated Under Alkaline Conditions Using Microorganisms

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Abstract

Plastic pollution, particularly from sachet water bags and polythene, has emerged as a major environmental challenge in Nigeria due to their persistence, poor disposal practices, and resistance to natural degradation. This study investigated the biodegradation potential of fungi and bacteria isolated from polythene-rich dump sites in Awka, Anambra State, under alkaline conditions, with a focus on enhancing degradation through base pretreatment. Soil samples were collected from four locations and screened for microbial diversity, polyhydroxyalkanoate (PHA) production, and polymer-degrading ability. Morphological and biochemical analyses identified the major fungal isolates as Aspergillus flavus and Penicillium lanosum, while bacterial isolates included Citrobacter freundii, Enterobacter aerogenes, and Pseudomonas aeruginosa. Biodegradation efficiency was monitored using UV-spectroscopy at 600 nm over a 15-day period. The results showed that both fungi and bacteria contributed to plastic degradation, but fungal isolates demonstrated superior efficiency. P. lanosum achieved the highest biodegradation activity, with absorbance values on base-treated polythene increasing from 0.042 at Day 0 to 0.218 at Day 5, stabilizing at 0.142 by Day 15. A. flavus followed closely, while bacterial isolates exhibited moderate efficiency, with

E. aerogenes and C. freundii showing consistent but lower absorbance increases. Alkaline pretreatment significantly enhanced microbial activity compared to untreated controls, with sachet plastics degrading more efficiently than thicker polythene films. This study concludes that indigenous fungi, especially P. lanosum and A. flavus, hold strong potential for biotechnological applications in plastic waste management. The findings highlight the synergistic role of alkaline pretreatment in improving biodegradation efficiency and provide a foundation for developing sustainable, microbial-based strategies to mitigate sachet water and polythene pollution in Nigeria.

Keywords: Biodegradation, Sachets, Polythene, Alkaline Conditions, Microorganisms

Introduction

Plastic pollution is an ever-increasing global environmental challenge. Among different types of plastics, polythene (polyethylene and related polymers) and sachet water bags (commonly made from low-density polyethylene or blended plastics) are especially problematic because of their resistance to degradation under natural conditions (Agu et al., 2023). In many developing countries, including Nigeria, sachet water—plastic-sealed packets of drinking water—is extremely common, and disposal of these sachets adds significantly to plastic waste burdens. The ubiquity of sachet water bags and the widespread use of polythene lead to accumulation in landfills, dump sites, drainage systems, waterways and soils, causing aesthetic pollution, ecological damage, flooding, and health hazards, highlighting the broader environmental and public health implications of microbial interactions with pollutants (Awari et al., 2023; Agu and Chidozie, 2021).

In Anambra State, where Awka is a principal city, there are ongoing concerns about plastic waste management. Waste generation studies in Awka metropolis show that plastic pollution (including sachet water waste and polythene) is a significant component of municipal waste. Similar environmental contamination patterns, including water and soil pollution linked to anthropogenic activities, have been reported in the region (Agu et al., 2014; Egurefa et al., 2024; Victor-Aduoju et al., 2023).

Plastics such as polythene are durable, cheap, lightweight, and versatile, which make them ideal for many applications, but these same properties make them recalcitrant in the environment. Conventional disposal methods—landfilling, burning, or dumping—are not sustainable, and frequently lead to secondary pollution. Studies on contaminated soils and effluents in Nigeria have demonstrated the persistence of pollutants and their impact on environmental quality (Ezenwelu et al., 2024; Ezeokoli et al., 2023).

Biodegradation, i.e., breakdown by living microorganisms, is among the promising strategies for mitigating plastic pollution. Microbial agents (fungi, bacteria, algae) can degrade plastic via enzymatic action, converting polymers into less harmful products. Previous studies have shown that microorganisms isolated from contaminated environments possess significant degradative capabilities, including hydrocarbon degradation, dye degradation, and polymer breakdown (Agu & Odibo, 2021; Okeke et al., 2023; Uwanta et al., 2023; Umeoduagu et al., 2024). Fungal species in particular are known to produce extracellular enzymes such as lipases, proteases, and peroxidases that contribute to the degradation of complex substrates (Agu et al., 2013; Chidi-Onuorah et al., 2015; Oparaji et al., 2024).

Several studies have demonstrated that fungal species (e.g., *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*) isolated from soils, wastes or dump sites can degrade polyethylene under laboratory conditions. Investigations into enzyme production and microbial metabolism further support the capacity of fungi to utilize recalcitrant substrates (Agu et al., 2014; Agu et al., 2023; Orji et al., 2014).

One environmental parameter often overlooked in degradation studies is pH, especially alkaline (basic) conditions. Many microbial studies are conducted at neutral or slightly acidic pH, yet environmental samples such as dump-site soils may exhibit variable pH conditions. The influence of physicochemical factors such as pH and surfactants on microbial degradation processes has been documented, demonstrating their role in enhancing or inhibiting microbial growth and activity (Anaukwu et al., 2016; Anaukwu et al., 2016).

Because dump sites are hotspots for microbial diversity, including fungi adapted to harsh conditions, they are promising sources of organisms capable of plastic degradation. Isolation of microorganisms from contaminated and extreme environments has revealed diverse metabolic capabilities, including hydrocarbon utilization and pesticide degradation (Ifediegwu et al., 2015; Mbachu et al., 2014; Agu et al., 2015; Orji et al., 2022). Similarly, fungal species isolated from contaminated soils have been shown to possess adaptive traits relevant to biodegradation processes (Egurefa et al., 2024).

Despite increasing awareness and policy efforts in Anambra State and elsewhere to tackle plastic waste through collection, recycling, and bans on single-use plastics, improper disposal remains widespread. Plastics persist in the environment for decades, clogging drainage systems, contaminating soils, and posing threats to human and animal health. Bioremediation approaches, including the use of organic amendments and microbial inoculants, have shown promise but remain underutilized in local contexts (Okafor et al., 2016; Okafor et al., 2016).

There is limited local scientific information about the capacity of indigenous fungi from polythene-rich dump sites around Awka to degrade sachet water plastics under alkaline conditions. While several studies have explored microbial degradation of pollutants, including heavy metals, hydrocarbons, and synthetic compounds (Ojiagu et al., 2018; Agu&Odibo, 2021; Agu et al., 2022), fewer have focused specifically on plastic degradation under alkaline conditions in this region.

This study aims to evaluate the biodegradation potential of microbial isolates obtained from polythene-rich dump sites in and around Nnamdi Azikiwe University, Awka, for the degradation of sachet water plastics and polythene under alkaline conditions. The study involves the isolation and identification of microbial strains using established microbiological techniques, assessment of their growth across varying alkaline pH levels, and determination of their degradation efficiency on plastic substrates. Furthermore, it examines enzymatic mechanisms involved in degradation, building on previous findings on enzyme-producing fungi and their industrial and environmental applications.

Materials and Methods

Collection of Samples

Microbial Sample Collection

Soil specimens were obtained aseptically from designated plastic waste dump locations using sterile spatulas and sampling containers in line with standard procedures (Albers et al., 2016). To adequately represent microbial stratification, samples were collected at three depth intervals: 0–10 cm (surface), 10–20 cm (subsurface), and 20–30 cm (deeper layer). Triplicate samples were taken from multiple points within each site to enhance representativeness. All collected samples were placed in sterile containers, transported in an ice-packed carrier, and preserved at 4°C prior to laboratory processing within 24 hours (Singh et al., 2019).

Plastic Sample Collection

Samples of polyethylene terephthalate (PET) sachet water packs and low-density polyethylene (LDPE) materials were collected from different environmental locations following established protocols (Yoshida et al., 2016). The plastics were categorized according to polymer type, washed

thoroughly with sterile distilled water to eliminate adhering debris, rinsed repeatedly, and air-dried under aseptic conditions at ambient temperature (25°C) for 48 hours.

Isolation of Microorganisms

Microbial isolation was conducted using a serial dilution approach (Cappuccino and Sherman, 2014). One gram of each soil sample was introduced into 9 mL of sterile normal saline (0.85% NaCl), followed by vortex mixing and agitation on a rotary shaker at 150 rpm and 30°C for 30 minutes. Serial dilutions ranging from 10^{-1} to 10^{-6} were prepared.

Aliquots (0.1 mL) from selected dilutions were inoculated onto Nutrient Agar for bacterial isolation using the spread plate technique. Fungal isolation was carried out on Sabouraud Dextrose Agar supplemented with chloramphenicol (50 µg/mL) to inhibit bacterial growth (Atlas, 2010). Bacterial cultures were incubated at 30°C for 48 hours, while fungal cultures were maintained for 5–7 days. Distinct colonies were purified through repeated subculturing and preserved on agar slants at 4°C.

Screening for PHA-Producing Isolates

Screening for polyhydroxyalkanoate (PHA) accumulation was performed using Sudan Black B staining with slight modifications to the original method (Schlegel et al., 1970). Isolates were cultured on glucose-supplemented nutrient agar and incubated at 30°C for 24 hours. Plates were stained with Sudan Black B solution (0.02 g in 100 mL of 70% ethanol), allowed to stand, and subsequently rinsed with ethanol. PHA-producing colonies appeared dark blue to black, while non-producers remained unstained (Spiekermann et al., 1999).

Alkaline Pretreatment of Plastic Samples

Prepared plastic materials were cut into uniform dimensions (2 cm × 2 cm) and subjected to alkaline treatment using sodium hydroxide (NaOH) for 2 hours. Control samples were left untreated to allow comparative analysis of degradation efficiency.

Preparation of PVA Mineral Salt Medium

The mineral salt medium comprised PVA (5.0 g/L), ammonium sulfate, phosphate buffers, magnesium sulfate, sodium chloride, calcium chloride, trace elements, and vitamins, adjusted to pH 7 and sterilized by autoclaving. The formulation was adapted from previously established protocols (Suzuki et al., 1973).

Characterization and Identification of Isolates

Bacterial Identification

Bacterial isolates were identified through colony morphology, Gram staining, and a series of biochemical assays following standard microbiological procedures (Cheesbrough, 2006). These included catalase, citrate utilization, oxidase, urease, indole, methyl red, Voges–Proskauer, motility, and carbohydrate fermentation tests.

- **Gram Staining:** Differentiation into Gram-positive (purple) and Gram-negative (pink/red) organisms after sequential staining and decolorization steps.
- **Catalase Test:** Detection of oxygen bubble formation upon exposure to hydrogen peroxide.
- **Motility Test:** Determined using the hanging drop technique under microscopic observation.
- **Urease Test:** Identification based on color change to pink in urea agar.
- **Citrate Test:** Positive results indicated by a green-to-blue color shift.
- **Oxidase Test:** Development of purple coloration within seconds confirmed positivity.
- **Indole Test:** Formation of a red ring upon addition of Kovac's reagent indicated tryptophan degradation.
- **MR-VP Tests:** Used to determine fermentation pathways.

- **Sugar Fermentation:** Acid and/or gas production assessed using indicator changes and Durham tubes.

Fungal Identification

Fungal isolates were identified based on colony morphology on SDA and microscopic features using lactophenol cotton blue staining. The modified slide culture technique facilitated detailed observation of spore structures under ×10 and ×40 magnifications (Agu and Chidozie, 2021; Watanabe, 2002).

Biodegradation Assay

Biodegradation experiments were conducted in 250 mL flasks containing 50 mL of sterile mineral salt medium (Sivan, 2011). Pretreated plastic fragments (0.1 g) were introduced aseptically. Bacterial inoculation involved 5% (v/v) actively growing cultures (OD_{600} ≈ 0.8–1.0), while fungal inoculation used agar plugs from actively growing colonies (Bonhomme et al., 2003).

Incubation Conditions

All cultures were incubated at 30°C with shaking at 120 rpm for 0, 5, 10, and 15 days. Experiments were conducted in triplicate alongside uninoculated controls.

Analytical Techniques

- **FTIR Analysis:** Structural changes in plastics were examined across 4000–400 cm^{-1} after drying samples (Sudhakar et al., 2008).
- **UV-Visible Spectrophotometry:** Optical density at 600 nm was measured periodically to monitor microbial activity and degradation progress (Tribedi and Sil, 2013).

Results

Microbial Load and PHA Production

Microbial enumeration revealed varying viable counts across sampled locations, with both bacterial and fungal populations exhibiting differing capacities for PHA production (Table 1).

Sudan Black B Screening

Qualitative screening confirmed the presence of PHA-producing isolates, indicated by characteristic dark staining patterns (Table 2).

Characterization of Isolates

Fungal isolates were identified based on macroscopic and microscopic features, including colony morphology and spore arrangement (Table 3). Bacterial identification combined morphological traits with biochemical profiling (Table 4).

Table 1:Total microbial and PHA production count of the isolates.

Sample Codes	Total Bacterial Count (CFU/g)		Total No. Of PHA Producing Bacteria (CFU/g)		Total Fungi Count (CFU/g)		Total No. Of PHA Producing Fungi (CFU/g)	
	No. of Bacterial colonies on plate	Total Bacterial Count (CFU/g)	No. of PHA Producing Bacterial colonies on plate	Total Number of PHA Producing Bacteria on Plate	No. of Fungi colonies on plate	Total Fungi Count (CFU/g)	No. of PHA Producing Fungi colonies on plate	Total Number of PHA Producing Fungi on Plate
GYS 1	293	2.93×10^4	42	4.2×10^4	24	2.4×10^4	6	6×10^3
FAS 2	266	2.66×10^4	38	3.8×10^4	18	1.8×10^4	4	4×10^3
EMS 3	138	1.38×10^6	23	2.3×10^6	31	3.1×10^6	7	7×10^5
ADS 4	141	1.41×10^6	36	3.6×10^6	42	4.2×10^6	8	8×10^5

KEY: GYS 1 - Yahoo Junction Sample, FAS 2 - Amansea Sample, EMS 3 - Miracle Junction Sample' ADS 4 – Dynamo Junction Sample, PHA – Polyhydroxyalkanoate

Table 2: Qualitative Distribution of PHA Producing Bacteria and Fungi showing Sample codes using Sudan Black B screening

Samples	Bacteria	Fungi
GYS 1	***Citrobacter spp***	***Aspergillus flavus***
FAS 2	***Citrobacter spp***	**NO GROWTH**
EMS 3	***Enterobacter spp***	**NO GROWTH**
ADS 4	***Enterobacter spp*** ***Pseudomonas spp***	***Penicilliumlanosum***

Table 3: Colonial and Microscopic Identifications of the Various Fungi Isolates.

Isolates	Colony morphology	Microscopy	Identity
I	Colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age.	Conidial heads are typically radiate, later splitting to form loose columns (mostly 300-400 µm in diameter), biseriate but having some heads with phialides borne directly on the vesicle (uniseriate). Conidiophore stipes are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to subglobose (3-6 µm in diameter), pale green and conspicuously echinulate. Some strains produce brownish sclerotia.	*Aspergillus flavus*
K	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, verticillatephialides 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.	*Penicilliumlanosum*

Table 4: Morphological and Biochemical Identifications of the Various Bacterial Isolates.

Isolate	Form	Surface	Colour	Margin	Elevation	Opacity	Gram	Cat	Mot	Ind	MR	VP	Cit	Lac	Glu	Suc	Fru	Mal	Oxi	Ure	Identity
A	Circular	Glistening	Cream	Entire	Raised	Transparent	- Rod	+	+	-	+	+	+	+	+	+	-	+	-	+	*Proteus mirabilis*
B	Irregular	Glistening	Cream	Entire	Raised	Opaque	-Rod	+	-	-	+	-	+	+	+	+	(-)	+	-	+	*Klebsiella pneumoniae*
C	Circular	Smooth	Yellowish	Entire	Raised	Opaque	+ cocci	+	+	-	+	-	-	AG	AG	A	A	AG	-	+	*Staphylococcus aureus*
D	Circular	Smooth	Greyish/ colourless	Entire	Convex	Translucent	-Rod	+	-	Var	+	-	-	-	+	-	+	var	-	-	*Shigellaflexneri*
E	Circular	Shiny	White	Entire	Convex	Moist	-Rod	+	+	-	-	+	+	+	+	+	+	+	-	-	*Enterobacter aerogenes*
F	Circular	Shiny	White	Entire	Convex	Moist	-Rod	+	+	-	-	+	+	-	+	-	+	-	+	-	*Pseudomonas aeruginosa*
G	Circular	Moist	Grey/shiny	Entire	Convex	Opaque	-Rod	+	+	-	+	-	+	+	+	+	+	+	-	var	*Citrobacterfreundii*
H	Circular	Smooth	Whitish	Entire	Convex	Translucent	-Rod	+	+	+	+	-	-	+	+	var	-	-	-	-	*Escherichia coli*

Key: **Gram**: Gram reaction, **Cat**: Catalase test, **Mot**: Motility test,**Ind**: Indole test, **MR**: Methyl-red test, **VP**: Voges-Proskauer test, **Cit**: Citrate Utilization test.
Sugar Fermentation Tests: **Lac**: Lactose Fermentation, **Glu**: Glucose Fermentation, **Suc**: Sucrose Fermentation, **Fru**:Fructose Fermentation, **Mal**: Maltose Fermentation, **Oxi** : Oxidase, **Ure**: Urease

Table 5:Analysis of biodegradation Efficiency using Uv-spectroscopy at 600nm wavelength

Fungi 1(*Aspergillus flavus*)				
Samples	Day 0	Day 5	Day 10	Day 15
BP	0.047	0.195	0.118	0.121
NP	0.058	0.169	0.092	0.086
BS	0.062	0.073	0.082	0.108
NS	0.041	0.138	0.061	0.115
Fungi 2(*Penicillium lanosum*)				
BP	0.042	0.218	0.105	0.142
NP	0.052	0.186	0.084	0.105
BS	0.058	0.089	0.076	0.125
NS	0.038	0.152	0.055	0.128
Bacteria 1(*Citrobacte rfreundii*)				
BP	0.074	0.165	0.118	0.125
NP	0.076	0.148	0.108	0.118
BS	0.082	0.115	0.098	0.108
NS	0.071	0.128	0.098	0.122
Bacteria 2(*Pseudomonas aeruginosa*)				
BP	0.068	0.155	0.105	0.105
NP	0.069	0.135	0.102	0.108
BS	0.075	0.105	0.112	0.098
NS	0.065	0.118	0.092	0.112
Bacteria 3(*Enterobacter aerogenes*)				
BP	0.078	0.172	0.135	0.142
NP	0.082	0.155	0.115	0.125
BS	0.085	0.122	0.112	0.125
NS	0.076	0.135	0.105	0.132

KEY: BP- Base-Treated Polythene, NP- Normal Polythene, BS- Base-Treated Sachets, NS- Normal Sachets

UV-Spectrophotometric Analysis of Biodegradation

Biodegradation Trends

Optical density measurements at 600 nm increased progressively over the 15-day incubation, indicating active microbial degradation. Alkaline-pretreated samples consistently showed higher absorbance values compared to untreated controls.

Fungal Isolates

- *Aspergillus flavus* demonstrated rapid initial growth with gradual stabilization, particularly in pretreated polythene samples.
- *Penicilliumlanosum* exhibited the highest degradation activity, suggesting superior enzymatic efficiency.

Bacterial Isolates

Species such as *Citrobacter freundii*, *Pseudomonas aeruginosa*, and *Enterobacter aerogenes* showed moderate degradation capabilities but were less efficient than fungal isolates.

Comparative Analysis

Overall, fungal isolates outperformed bacterial counterparts in degrading plastic substrates. Pretreatment with alkaline solution significantly enhanced biodegradation, likely due to increased polymer susceptibility.

FTIR Analysis of Polythene and Sachet Water Plastics Biodegraded by *Aspergillus flavus* with Alkaline Pretreatment

FTIR Spectroscopic Evaluation

FTIR analysis demonstrated pronounced structural alterations in both polythene films and sachet water plastics subjected to *Aspergillus flavus* treatment following alkaline pretreatment over a 15-day incubation period. Relative to untreated controls, substantial changes in characteristic absorption bands were observed, indicating that the combined chemical (base) and biological treatment enhanced polymer degradation.

Spectral Characteristics and Structural Changes

The control spectra (Day 0) displayed typical polyethylene absorption features, including asymmetric C–H stretching around 2915 cm^{-1} and symmetric C–H stretching near 2848 cm^{-1}. Additional defining peaks included methylene bending vibrations at approximately 1464 cm^{-1}, C–O stretching at 1276 cm^{-1}, C–C skeletal vibrations around 1026 cm^{-1}, and methylene rocking at about 719 cm^{-1}.

Following 15 days of incubation, notable reductions and distortions in these peaks were recorded. Samples exposed to alkaline pretreatment exhibited more substantial spectral changes than untreated samples (NP2 and NS2), suggesting that the pretreatment step increased polymer susceptibility to fungal enzymatic activity.

Quantitative Assessment of Biodegradation

Polythene Samples:

Alkaline-pretreated polythene showed a significant decrease in absorbance at 2915 cm^{-1}, dropping from 1.35 at Day 0 to 0.89 at Day 15, corresponding to a 33.9% reduction. In comparison, untreated samples showed a smaller decrease of 18.2%. This indicates that base pretreatment improved degradation efficiency.
Biodegradation efficiency was estimated using:$\frac{(I_o - I)}{I} \times 100$
where (I_o) is the initial absorbance and (I) is the absorbance after incubation.

Sachet Water Plastics:

Sachet samples demonstrated greater degradation. Base-treated samples exhibited a 41.3% reduction in the C–H stretching peak (from 1.33 to 0.78), whereas untreated samples showed a 23.6% decrease. This suggests that sachet materials are more readily degraded than thicker polythene.

Peak Intensity Ratio Analysis

The ratio of treated to untreated samples further quantified degradation:

- Polythene: BP2/NP2 = 0.66 at 2915 cm^{-1}
- Sachets: BS2/NS2 = 0.59 at 2915 cm^{-1}

Lower ratios in treated samples confirm greater disruption of polymer chains compared to controls.

Formation of New Functional Groups

Treated samples exhibited new absorption bands, particularly within the carbonyl region (1700–1750 cm^{-1}), indicating the generation of oxidized intermediates such as aldehydes, ketones, and carboxylic acids. These are typical products of oxidative biodegradation.

Additionally, broadening of absorption bands in the hydroxyl region (3200–3600 cm^{-1}) was observed, suggesting the formation of alcohol and carboxylic functional groups. These findings support an oxidative degradation pathway mediated by extracellular enzymes produced by *Aspergillus flavus*, which facilitate polymer chain breakdown through oxidative mechanisms.

Overall, the FTIR results confirm that alkaline pretreatment significantly enhances the biodegradation of polyethylene-based materials by *Aspergillus flavus*, leading to both structural disruption and chemical transformation of the polymer matrix.

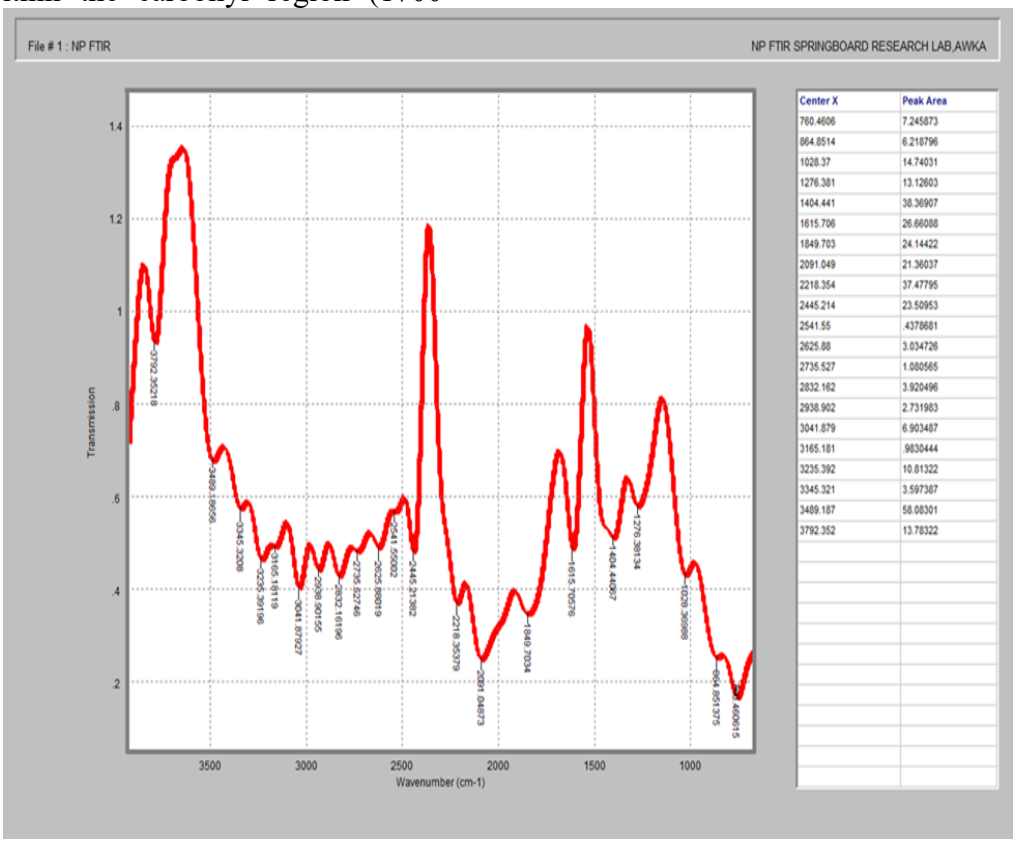

Figure 1: Normal Polythene Day Zero *(Aspergillus flavus)*

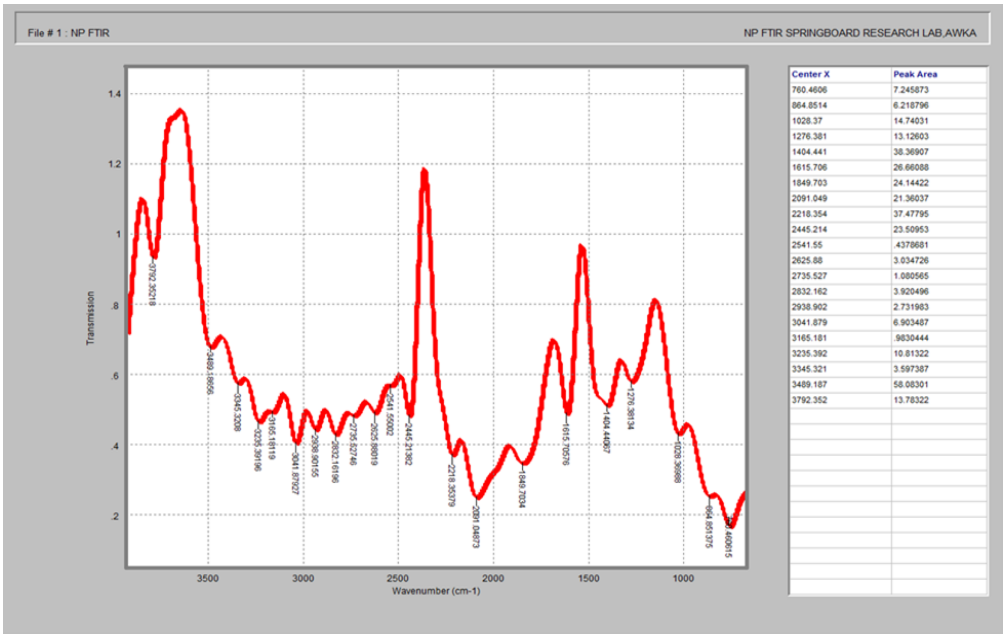

Figure 2: Heat-Treated Polythene Day Zero *(Aspergillus flavus)*

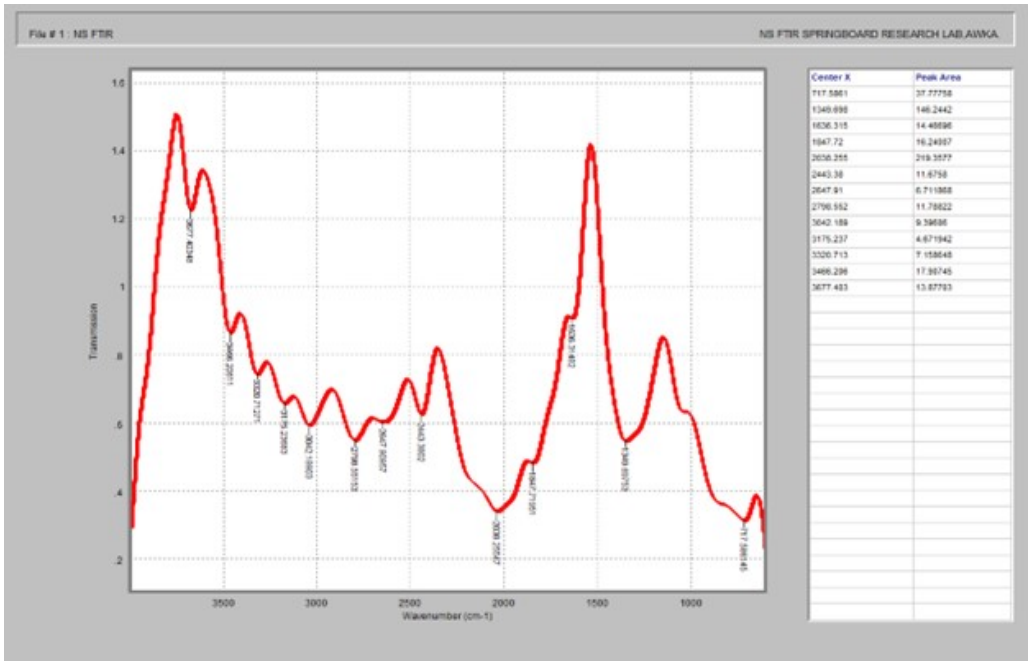

Figure 3: Normal Sachets Day Zero *(Aspergillus flavus)*

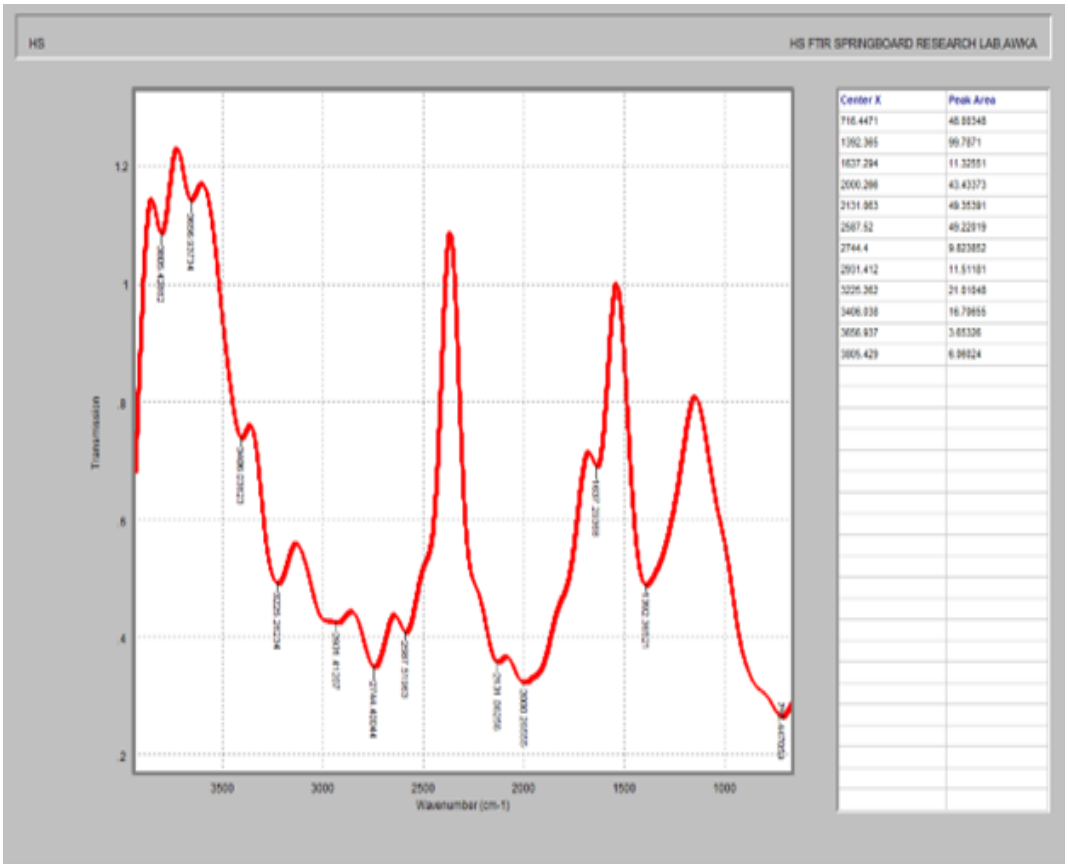

Figure 4: Heat-Treated Sachets Day Zero *(Aspergillus flavus)*

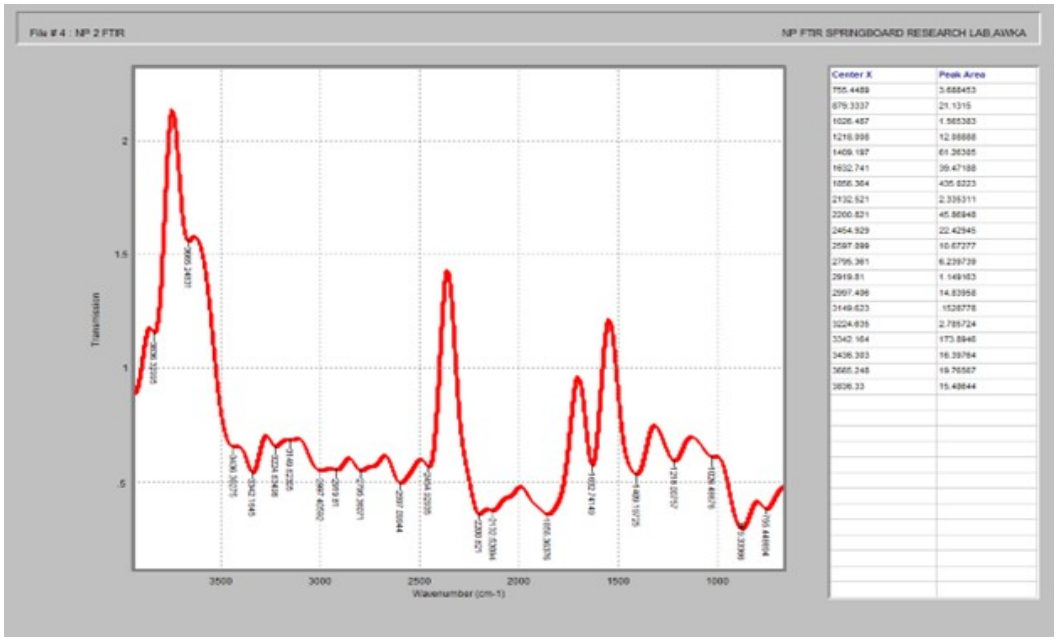

Figure 5: Normal Polythene Day 15*(Aspergillus flavus)*

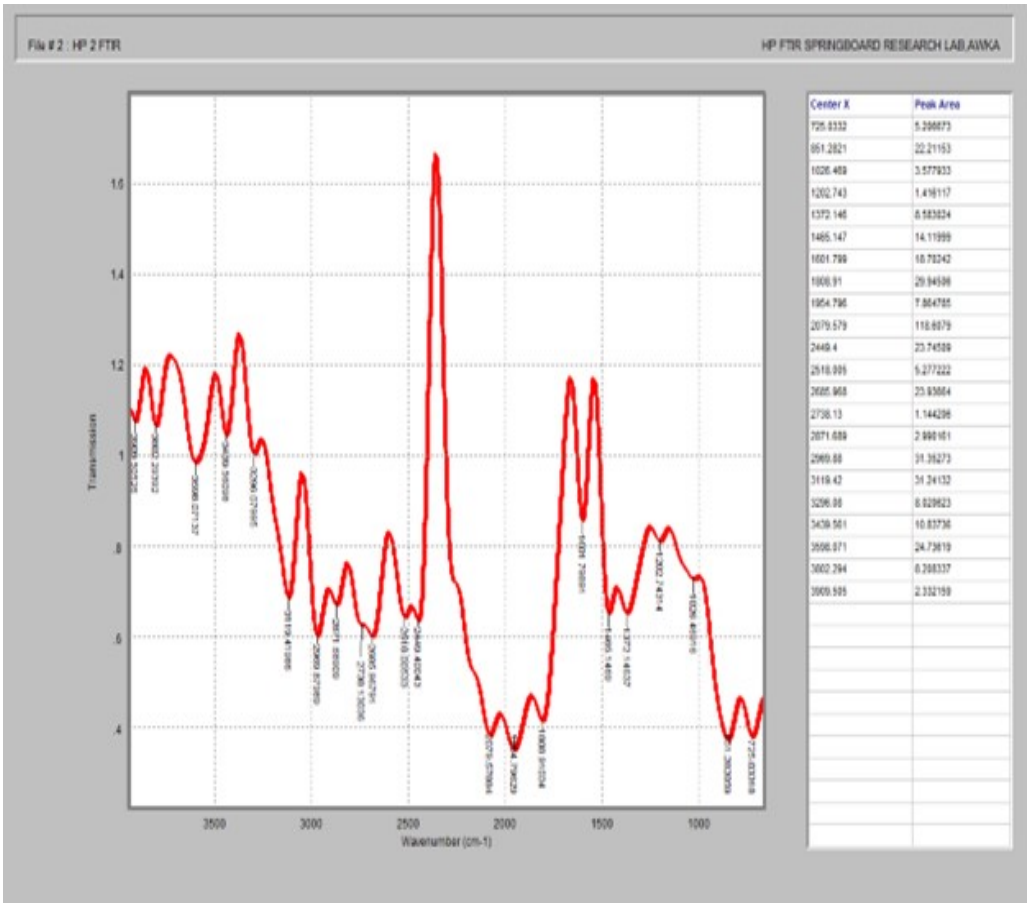

Figure 6: Heat-Treated Polythene Day 15 *(Aspergillus flavus)*

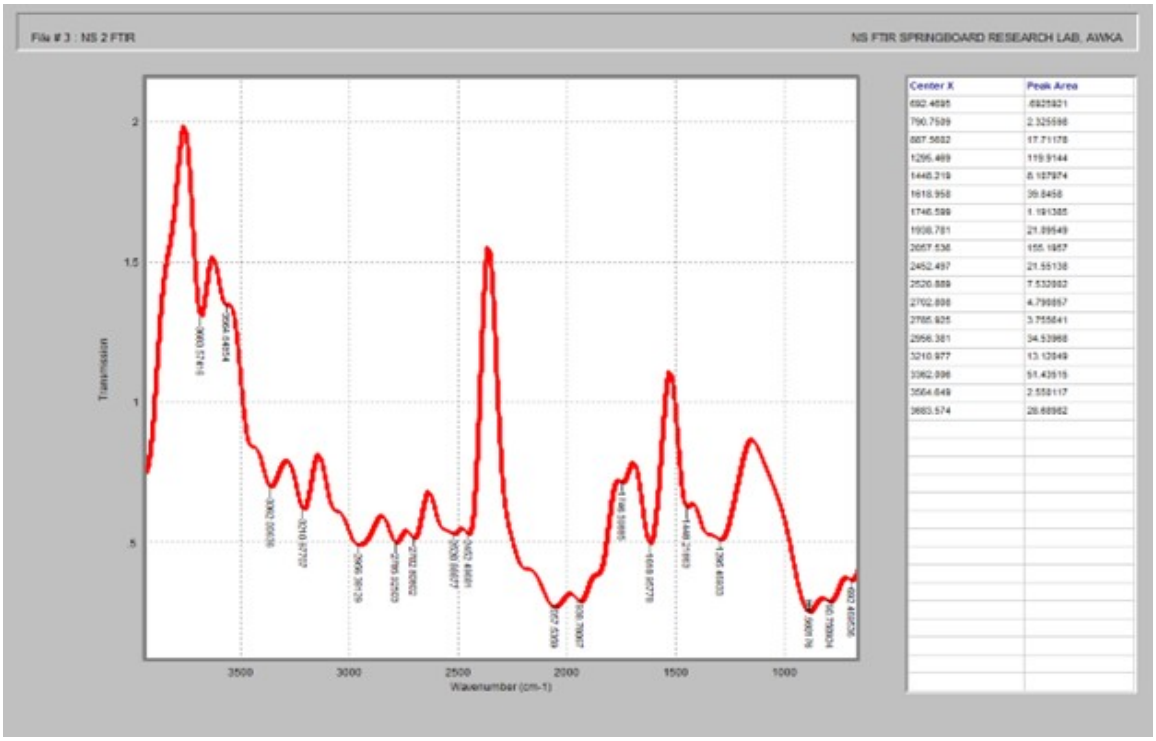

Figure 7: Normal Sachets Day 15 *(Aspergillus flavus)*

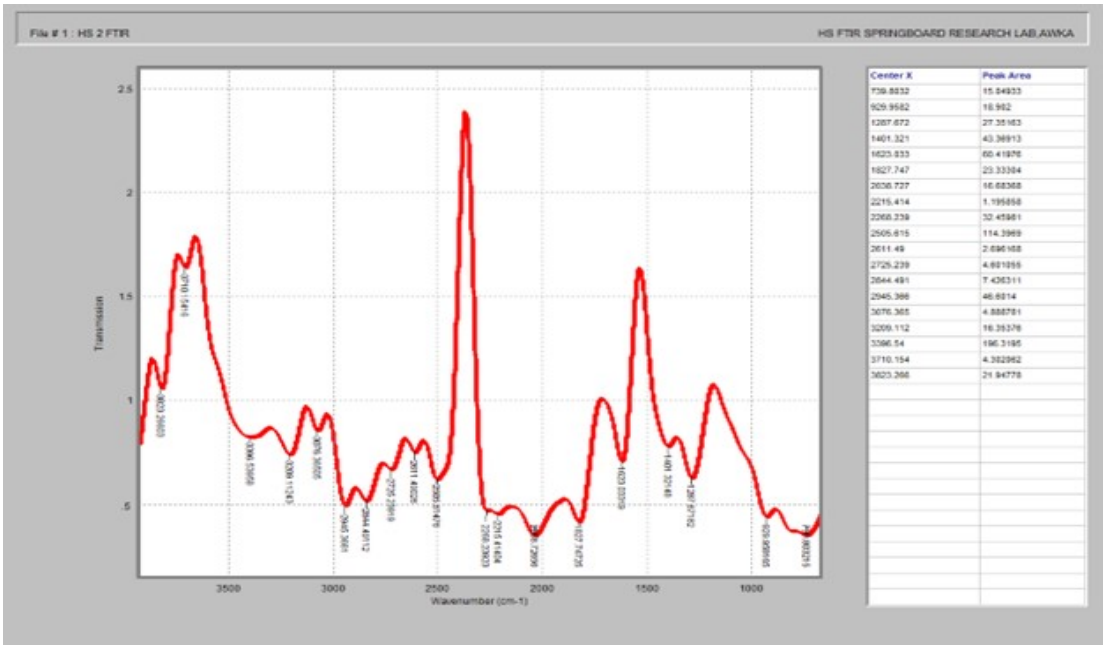

Figure 8: Heat-Treated Sachets Day 15 *(Aspergillus flavus)*

Discussion of Results

The present study investigated the biodegradation potential of fungi and bacteria isolated from polythene-rich dump sites in Awka, Nigeria, focusing on sachet water sachets and polythene under alkaline conditions. The findings revealed distinct variations in microbial load, PHA-producing ability, and biodegradation efficiency across isolates and treatments.

Microbial and PHA Production Counts

The microbial load results indicated that dump sites harbored diverse bacterial and fungal communities, with both groups exhibiting PHA-producing capabilities. The presence of PHA-positive isolates suggests metabolic adaptability, as microorganisms capable of accumulating polyhydroxyalkanoates are often linked to polymer degradation (Kumar et al., 2020). The higher counts of bacteria compared to fungi align with earlier studies that report bacterial dominance in contaminated soils, though fungi often display stronger enzymatic versatility in degrading recalcitrant polymers (Ojha *et al*., 2017; Das and Kumar, 2021).

Identification of Isolates

Morphological and biochemical analyses identified fungal isolates as *Aspergillus flavus* and *Penicillium lanosum*, while bacterial isolates included *Citrobacter freundii, Enterobacter aerogenes, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Proteus mirabilis*. Previous studies have consistently reported *Aspergillus* and *Penicillium* species as efficient degraders of polyethylene due to their secretion of oxidative enzymes such as laccases and peroxidases (Ayeni *et al*., 2022). Similarly, *Pseudomonas* and *Enterobacter* species are recognized for their role in plastic biodegradation, though their efficiency is often lower than fungi (Sekhar and Bharti, 2024).

UV-Spectroscopy Analysis of Biodegradation Efficiency

UV-spectroscopic analysis at 600 nm revealed significant microbial activity on both sachets and polythene. *Aspergillus flavus* showed strong colonization of base-treated polythene, with absorbance increasing from 0.047 at Day 0 to 0.195 at Day 5, before stabilizing. *Penicillium lanosum* displayed even greater efficiency,

reaching 0.218 absorbance at Day 5, confirming its superior degradative potential. These findings are consistent with reports by Shake and Khlaif (2025), who observed rapid initial degradation of LDPE films by *Aspergillus* and *Penicillium* isolates.

Bacterial isolates exhibited moderate increases in absorbance compared to fungi. For example, *Citrobacter freundii* on base-treated polythene rose from 0.074 to 0.165 by Day 5, while *Enterobacter aerogenes* showed 0.078 to 0.172 within the same period. This aligns with findings from Williams and Osahon (2021), who reported that bacterial isolates degrade plastics at slower rates than fungi but still contribute to polymer modification under favorable conditions.

Effect of Alkaline Pretreatment

A consistent observation was that base-treated (BP, BS) samples supported higher microbial activity than untreated ones (NP, NS). This indicates that alkaline pretreatment enhanced biodegradation efficiency by modifying polymer surfaces, possibly through oxidation or hydrolysis, thereby improving microbial accessibility. Similar observations have been reported by Ogu *et al*. (2024), who demonstrated that alkaline and thermal pretreatment of LDPE facilitated enzymatic attack by *Aspergillus* species. The enhanced biodegradation of sachets compared to polythene suggests that thinner films with higher surface area are more susceptible to microbial breakdown (Das and Kumar, 2021).

Comparative Efficiency of Fungi and Bacteria

Overall, fungal isolates (*P. lanosum* and *A. flavus*) outperformed bacterial isolates in degrading both polythene and sachet materials. This corroborates studies showing that fungi possess stronger enzymatic systems for attacking hydrophobic polymers and can penetrate substrates through hyphal growth, unlike bacteria that rely primarily on extracellular enzyme secretion (Ayeni *et al*., 2022; Sekhar and Bharti, 2024). The results also confirm that local fungi isolated from Nigerian dump sites hold potential as bioremediation agents for plastic waste.

5. 1 Conclusion

The results of this study provide clear evidence that microorganisms isolated from polythene-rich dump sites in Awka possess significant biodegradation potential against both sachet water plastics and polythene under alkaline conditions. The main conclusions are as follows:

1. **Microbial Diversity and PHA Production**

The isolation of both bacterial and fungal species capable of producing polyhydroxyalkanoates (PHAs) confirms the adaptability of microorganisms in plastic-rich soils. PHAs are intracellular carbon storage materials produced under stress conditions and are structurally similar to synthetic plastics. Their presence in isolates indicates metabolic flexibility and suggests that these microbes can utilize recalcitrant polymers as carbon sources (Kumar *et al*., 2020).

2. **Fungal Dominance in Biodegradation**

The fungal isolates (*Aspergillus flavus* and *Penicillium lanosum*) consistently exhibited higher biodegradation efficiency than bacterial isolates. This superiority can be attributed to fungi's ability to secrete diverse extracellular oxidative enzymes (e.g., laccases, peroxidases, cutinases) that break down high-molecular-weight polymers into smaller, more assimilable fragments. Fungi also penetrate substrates via hyphae, increasing surface contact with polymers (Ayeni *et al*., 2022).

3. **Impact of Alkaline Pretreatment**

Base-treated plastics (BP and BS) consistently showed higher absorbance values in UV-spectroscopy than untreated plastics (NP and NS). This indicates that alkaline conditions enhance microbial degradation by partially oxidizing or hydrolyzing polymer chains, introducing carbonyl and hydroxyl groups, which serve as binding and

attack sites for microbial enzymes (Ogu *et al*., 2024).

4. Material Susceptibility Differences

Sachet plastics degraded more efficiently than thicker polythene films. This is likely due to differences in polymer thickness, crystallinity, and surface area, with sachets providing more accessible points of attack for enzymes (Das and Kumar, 2021).

5. Bacterial Contribution

While bacterial isolates (*Citrobacter freundii, Enterobacter aerogenes,* and *Pseudomonas aeruginosa*) exhibited moderate degradation activity, their efficiency was lower than fungi. Nonetheless, their role is important because bacteria contribute synergistically in microbial consortia, where fungi initiate breakdown and bacteria metabolize smaller degradation intermediates (Williams and Osahon, 2021).

In conclusion, this study demonstrates that fungi, especially *P. lanosum*, are promising candidates for biotechnological applications in the biodegradation of plastic waste in Awka and beyond. The synergistic role of alkaline pretreatment and microbial activity highlights a practical pathway for addressing Nigeria's escalating sachet water and polythene waste problem.

5.2 Recommendations

Building on the study findings, the following recommendations are made:

1. Bioremediation Applications of Fungi

The isolates *Penicillium lanosum* and *Aspergillus flavus* should be prioritized in bioremediation strategies due to their demonstrated efficiency. These fungi could be incorporated into composting systems, landfill management, or waste treatment plants where plastic waste is abundant.

2. Optimization of Pretreatment Methods

Since alkaline pretreatment significantly enhanced biodegradation, further research should test combinations of pretreatments (e.g., alkaline + thermal, UV irradiation, or enzymatic activation) to identify the most effective and economical method for scaling up (Shake and Khlaif, 2025).

3. Enzyme Profiling and Genetic Studies

Future studies should focus on isolating and characterizing the specific enzymes responsible for plastic degradation in these fungi. Molecular studies could also explore genetic modification or optimization of these fungi for improved enzyme expression and higher degradation rates (Sekhar and Bharti, 2024).

4. Pilot-Scale Trials in Waste Management

Laboratory results need to be validated in real-world conditions. Pilot-scale bioreactors or soil burial experiments should be established in local dumpsites to assess the efficiency of these fungi under fluctuating environmental conditions (Ogu *et al*., 2024).

5. Integration into Waste Management Policy

Government agencies and environmental regulators should support microbial-based waste treatment as part of integrated solid waste management strategies. Policies promoting biotechnological recycling of plastics could reduce dependence on landfilling and open dumping, which are currently the most common methods in Nigeria.

6. Public Awareness and Collaboration

Community-level education should be implemented to inform the public about the dangers of plastic pollution and the potential of microbial solutions. Collaboration between universities, waste management companies, and

government agencies will be vital for translating these laboratory findings into sustainable waste management systems.

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