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# Monoculture biodegradation of packaged water sachets and polyethene treated with heat using diverse microbial isolates

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## Abstract

Plastic pollution constitutes a critical environmental challenge due to the extensive production, widespread use, and environmental persistence of polyethylene-based materials, particularly single-use packaging such as sachet water pouches and polythene bags. These materials are extensively consumed in developing regions, where inadequate waste management infrastructure exacerbates their accumulation in terrestrial and aquatic ecosystems. Owing to their stable hydrocarbon polymer structure, polyethylene plastics resist natural degradation, fragmenting over time into microplastics that pose serious ecological, public health, and economic risks. This study explores microbial biodegradation as a sustainable alternative to conventional plastic waste management approaches, with specific emphasis on the role of thermal pretreatment in enhancing polymer susceptibility to microbial attack. Heat treatment was applied to polyethylene materials to induce structural and surface modifications that improve microbial colonization and enzymatic accessibility. The biodegradation potential of selected microorganisms was subsequently evaluated under controlled conditions, considering key environmental parameters influencing degradation efficiency. Findings from this study demonstrate that thermal pretreatment significantly improves the biodegradability of polyethylene by promoting polymer chain scission and increasing surface reactivity, thereby accelerating microbial degradation processes. Despite inherent challenges such as slow degradation rates and variability in plastic

composition, the results underscore the promise of integrating heat-assisted microbial biodegradation into sustainable plastic waste management strategies. This approach offers a viable, environmentally compatible pathway for reducing polyethylene waste accumulation and mitigating its long-term environmental impacts, particularly in regions heavily burdened by single-use plastic pollution.

**Keywords:** Plastic, pollution, Polyethylene, Sachet water, waste, Microplastics,

# 1. Introduction

Plastic contamination is now recognized as one of the most urgent environmental challenges, with profound impacts on ecosystems, human health, and economic activities worldwide (Geyer et al., 2017; Ugoy et al., 2025). The extensive production and widespread use of synthetic polymers, particularly polyethylene-based materials, have led to unprecedented accumulations of plastic debris in both terrestrial and aquatic environments (Chamas et al., 2020; Agu et al., 2023a). A large portion of this waste comes from disposable packaging products, including polythene bags and sachet water pouches, which are designed for single use but persist in the environment for extended periods. This issue is especially severe in developing countries, where these materials are widely utilized for water distribution and everyday packaging (Stoler et al., 2015; Ekpunobi et al., 2025).

The environmental persistence of conventional plastics is largely due to their chemically engineered polymer structure, which confers high resistance to natural degradation processes (Andrady & Neal, 2009; Agu et al., 2023b). Polyethylene, for example, can remain intact in natural settings for hundreds of years, gradually fragmenting into microplastics that infiltrate food chains and disrupt ecological balance (Chamas et al., 2020; Uwanta et al., 2023a). The continual accumulation of plastic debris in landfills, waterways, and natural ecosystems has created an urgent demand for sustainable and effective waste management strategies (Awari et al., 2024; Umeoduagu et al., 2023b).

In many low- and middle-income countries, particularly across sub-Saharan Africa, the sachet water industry has expanded rapidly to meet the need for safe drinking water. This industry relies heavily on thin polyethylene sachets intended for single use. While these sachets provide a convenient and low-cost solution for water distribution, their consumption produces significant amounts of plastic waste (Stoler et al., 2015; Agu et al., 2023c). Their light weight and high turnover result in widespread dispersal across urban and rural areas, often entering informal dumping sites or remaining as litter (Babayemi & Dauda, 2009; Uwanta et al., 2023c).

The environmental consequences of sachet water waste extend beyond visual pollution. Accumulated sachets can block drainage systems, exacerbate urban flooding, and create stagnant water bodies that serve as breeding grounds for disease vectors (Ugoy et al., 2025; Awari et al., 2024). Moreover, improper disposal has been linked to soil contamination and reduced agricultural productivity (Babayemi & Dauda, 2009; Agu et al., 2023d). Managing this rapidly growing waste stream imposes substantial financial and logistical pressures on municipal authorities, many of which lack the necessary infrastructure to cope with the volume of plastic waste generated by sachet water consumption (Ekpunobi et al., 2025; Ezeokoli et al., 2023).

Polyethylene, commonly referred to as polythene, is one of the most widely used polymers in packaging due to its flexibility, durability, impermeability, and cost-effectiveness (Andrady & Neal, 2009; Agu et al., 2023e). These same properties, however, contribute to its environmental persistence, as it resists both chemical and biological degradation (Hopewell et al., 2009; Agu et al., 2023f). At the molecular level, polyethylene consists of long chains of carbon and hydrogen atoms linked by strong

covalent bonds, which provide mechanical strength and chemical stability (Wei & Zimmermann, 2017; Uwanta et al., 2023d). While these properties enhance product performance, they pose challenges for conventional waste management, as incineration can release toxic gases, and landfilling merely delays environmental impacts without offering a sustainable solution (Hopewell et al., 2009; Agu et al., 2023g).

Biological degradation has emerged as a promising alternative, utilizing microorganisms capable of transforming synthetic polymers into less persistent compounds through enzymatic cleavage (Wei & Zimmermann, 2017; Danso et al., 2019; Agu et al., 2023h). Recent advances have identified diverse bacteria, fungi, and actinomycetes that can metabolize plastic polymers, opening avenues for environmentally friendly waste remediation strategies (Danso et al., 2019; Uwanta et al., 2023b; Agudosi et al., 2023). Thermal pretreatment has further been recognized as an effective method for enhancing polymer susceptibility to microbial attack by inducing structural and surface modifications that facilitate enzyme accessibility (Kyrikou & Briassoulis, 2007; Agu et al., 2023i).

The mechanism of heat-induced polymer modification involves disruption of intermolecular interactions and generation of functional groups that act as microbial binding sites, alongside the formation of low-molecular-weight fragments that microorganisms can metabolize more readily (Albertsson et al., 1987; Agu et al., 2023j). Optimizing parameters such as temperature, duration, and atmospheric conditions is essential to maximize biodegradation efficiency. Despite the potential of microbial degradation, challenges such as slow polymer breakdown, heterogeneous plastic composition, and inhibitory additives remain, necessitating detailed evaluation of environmental and operational factors including pH, moisture, nutrients, and inhibitory compounds (Rujnić-Sokele & Pilipović, 2017; Uwanta et al., 2023a). Standardized methods for assessing degradation rates and end-products are also critical for validating and comparing biodegradation strategies (Agu et al., 2023k).

This study aims to investigate the effectiveness of heat pretreatment in enhancing the microbial biodegradation of polyethylene-based plastic waste, particularly sachet water and polythene materials, as a sustainable strategy for mitigating plastic pollution.

# 2. Materials and Methods

## 2.1 Materials

Laboratory equipment and reagents used included: conical flasks (250 mL), analytical weighing scale (±0.0001 g), absolute ethanol (99.9%), water bath with temperature control, electrical shaker (150 rpm), autoclave, Mineral Salt Vitamins Medium (MSVM) broth, sterile test tubes, petri dishes, sterile pipettes, Saboraud Dextrose Agar, Nutrient Agar, D-glucose, Sudan Black B dye, bijou bottles, UV-Visible spectrophotometer, Fourier Transform Infrared (FTIR) spectrometer, and colony counter.

## 2.2. Collection of Samples

### 2.2.1 Microbial Sample Collection

Soil samples were collected aseptically from plastic dumpsites (Yahoo Junction, Amansea, Miracle Junction and Dynamo Junction) using sterile spatulas, collecting tubes and augers according to the method described by Albers *et al*. (2016). Samples were collected from three different depths: surface layer (0-10 cm), subsurface layer (10-20 cm), and deeper layer (20-30 cm) to capture microbial diversity at different soil horizons. Each sample was collected in triplicate from different locations within each site to ensure representativeness. The collected samples were immediately stored in sterile containers and transported to the laboratory in an ice box, then stored at 4°C until processing within 24 hours (Singh *et al*., 2019).

### 2.2.2 Plastic Sample Collection

Polyethylene terephthalate (PET) sachets from sachet water and low-density polyethylene (LDPE) bags were collected from various environmental sources following the protocol of Yoshida *et al*. (2016). The plastic materials were sorted based on polymer type, thoroughly cleaned with sterile distilled water to remove surface contaminants and debris, rinsed three times with sterile water, and air-dried at room temperature for 48 hours under sterile conditions.

## 2.3 Isolation of Microorganisms

Microbial isolation was performed using the serial dilution technique as described by Cappuccino and Sherman (2014). One gram of homogenized soil sample from each depth was aseptically transferred to 9 mL of sterile physiological saline (0.85% NaCl) in a conical flask. The suspension was vortexed for 2 minutes and subjected to shaking for 30 minutes on a rotary shaker at 150 rpm and 30°C. Serial ten-fold dilutions ($10^{-1}$ to $10^{-6}$) were prepared using sterile physiological saline.

For bacterial isolation, 0.1 mL aliquots from appropriate dilutions were plated onto Nutrient Agar (NA) plates using the spread plate method. For fungal isolation, Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (50 µg/mL) was used to suppress bacterial growth (Atlas, 2010). All plates were incubated at 30C for 24 hours for bacteria and 5 days for fungi. Morphologically distinct colonies were selected, purified by successive sub-culturing on fresh media, and maintained on nutrient agar slants at 4°C.

## 2.4 Screening the Isolates for PHA Production

Isolated strains were screened for PHA production using the Sudan Black B staining technique as described by Schlegel *et al*. (1970) with modifications. Bacterial and fungal isolates were cultured on Nutrient Agar supplemented with 2% (w/v) glucose as carbon source and incubated at 30°C for 24 hours. Sudan Black B stain was prepared by dissolving 0.02 g of powdered Sudan Black B in 100 mL of 70% ethanol and filtered through Whatman No. 1 filter paper.

After incubation, the culture plates were flooded with Sudan Black B stain and allowed to stand undisturbed for 30 minutes at room temperature. Excess stain was removed by washing the plates twice with 96% ethanol. PHA-producing microorganisms appeared bluish-black due to lipid accumulation, while non-producers remained colorless or appeared white (Spiekermann *et al*., 1999).

## 2.5 Treatment of Plastic Samples with Heat

The collected and cleaned sachet water sachets and polythene bags will be cut into uniform pieces (2 cm × 2 cm). The plastic pieces were subjected to heat treatments at $60^{o}$ 2 hours. There was no acid treatment for the control sample.

## 2.6 Mineral Salt Vitamins Medium composition ($gL^{-1}$)

Polyethylene and Polystyrene 5.0 g; (NH4)2SO4, 1.0 g; KH2PO4, 1.0 g; K2HPO4, 8.0 g; MgSO4.7H2O, 0.2 g; NaCl, 0.1 g; CaCl2.2H2O, 0.02 g; FeSO4, 0.01 g; Na2MoO4.2H2O, 0.5 mg; MnSO4, 0.5 mg; Inositol, 0.2 mg; p-amino benzoic acid, 0.2 mg; pyridoxine, 0.4 mg; thiamine, 2.0 µg; biotin, 2.0 µg; vitamin B, 120.5 µg; DW, 1000 ml; pH 7. The medium used was the same one that was used previously by Suzuki *et al.* (1973) and was autoclaved.

## 2.7 Characterization and Identification of Bacteria and Fungi Isolates

Identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests as described by Chessbrough (2006). The characterization of the isolates was performed, by employing Gram staining reaction, Catalase test,

Citrate test, Sugar fermentation test, Coagulase test, Motility test, Oxidase test, Urease test, Indole test, Methyl Red and Voges proskauer test as described by Bergey's Manuel of Determinative Bacteriology, 9th edition (2006).

### 2.7.1 Gram Reaction

Thin smear of the isolate was made on clean, non-greasy, dust-free slides, air dried and heat fixed. The smear was flooded with crystal violet and allowed to remain on the slide for 60 seconds. Thereafter, the crystal violet was washed off with gentle running water. Again, the slide was flooded with slide with Gram's iodine, allowed to remain for 60 seconds and washed off. The slide was decolourized with acetone-alcohol mixture. The slide was counter-stained with safranin for 60 seconds and rinsed with tap water and allow to air dry. The slide was then viewed under oil immersion lens microscope (× 100). Purple colour indicated Gram–positive organisms while red or pink colour indicated Gram-negative organisms.

### 2.7.2 Catalase test

Exactly 3ml of 3% solution of hydrogen peroxide (H2O2) was transferred into a sterile test tube. Then, 3 loopful of a 24 hour pure culture of the test bacteria were inoculated into the test tube. The tube was observed for immediate bubbling indicative of a Positive, while no bubbling indicated a negative reaction.

### 2.7.3 Motility test (Hanging Drop Method)

A loopful of 18-24 hour broth culture of the test bacteria was placed at the centre of a clean grease- free cover-slip. Carefully, the cover slip was inverted and placed over the concave portion of a hanging drop slide. The cover-slip/slide arrangement was observed for motility at x100 magnification on a compound microscope. Care was taken to not interpret "drift" or "Brownian motion" as motility. Results was recorded as motile or non-motile.

### 2.7.4 Urease Test

Heavy inoculum from an 18- to 24-hour pure culture was used to streak the entire **Christensen's Urea Agar** slant surface. Adequate care was taken not to stab the butt as it served as a colour control. The tubes was incubated loosened caps at 35$^{o}$C. The slants was observed for a colour change at 6 hours and 24 hours. Urease production was indicated by a bright pink (fuchsia) colour on the slant that may extend into the butt. Note that any degree of pink is considered a positive reaction. The culture medium remained a yellowish colour if the organism is urease negative.

### 2.7.5 Citrate test

A 24 hours old culture was inoculated into test tubes containing sterile Simmons Citrate agar slant and then incubated for 24hours. A positive test was indicated by a change from green to blue colour on the surface of the Simmons Citrate agar slant. No colour change indicated a negative reaction.

### 2.7.6 Oxidase Test

Whatmann No.1 filter paper was soaked with the substrate tetramethyl-p- phenylenediamine-dihydrochloride. The filter paper will be moistened with sterile distilled water. Then the test colony was picked with wooden or platinum loop and smeared on the filter paper. The inoculum was observed at the area around the inoculated paper for a color change to deep blue or purple within 10-30 seconds. **Positive** was indicated by development of dark purple color (indophenols) within 10 seconds. **Negative**: Absence of color.

### 2.7.7 Indole Test

A loopful of a 24 hour culture was used to inoculate the test tube containing 3 ml of sterile tryptone water. Incubation will be done at 37$^{o}$C first for 24 hours and further for up to 48 hours.

Test for indole was done by adding 0.5 ml of Kovac's reagent, shaken gently and then examined for a ring of red colour in the surface layer within 10 minutes, indicative of a positive reaction. Absence of red colour indicated a negative reaction.

### 2.7.8 Methyl Red test

Exactly 5 drops of methyl red indicator was added to an equal volume of a 24 hours culture of the isolate in Methyl Red–Voges Proskauer (MR-VP) broth. The production of a bright red colour indicates a positive test while yellow colour indicates a negative test after vigorous shaking.

### 2.7.9 Voges-Prausker test

Exactly 2ml of the 24 hours culture of the test organism growing on MR-VP broth was aseptically transferred into a sterile test tube. Then 0.6ml of 5% α-naphthol was added, followed by 0.2ml of 40% KOH (NB: It was essential that these reagents were added in this order). The tube was shaken gently to expose the medium to atmospheric oxygen and then allowed to stand undisturbed for 30 minutes. A positive test was indicated by the presence of a red colour , indicative of the presence of diacetyl, the oxidation product of acetoin (Test was always considered invalid after one hour because VP-negative cultures may produce a copper-like colour, false positive), lack of pink-red colour denoted a negative reaction.

### 2.7.10 Sugar Fermentation Test

Each of the isolate was tested for its ability to ferment a specific sugar. 1g of the sugar and 1g of peptone water was dissolved in 100ml of water. 5ml of the solution was transferred into clean test-tubes using sterile pipettes. The test-tubes containing peptone water and sugar was added Durham's tube which was placed inversely and bromothymol blue as an indicator. These was sterilized for 10minutes and allowed to cool before inoculating the inocula. The test-tubes was incubated for 3days. The production of acid and gas or acid only indicated utilization of sugars. Acid production was indicated by change in colour of the medium from green to yellow while gas production was observed by presence of gas in the Durham's tubes.

### 2.7.11. Fungal Identification

This was done based on the description of the gross morphological appearance of fungal colonies on the SDA culture medium and the modified slide culture technique using lactophenol cotton blue stain for the microscopic evaluation under X10 and X40 magnification of the microscope (Agu and Chidozie, 2021); with reference to the Manual of Fungal Atlases (Watanabe, 2002).

## 2.8 Biodegradation Studies

Biodegradation assays were conducted using selected plastic-degrading isolates in 250 mL Erlenmeyer flasks containing 50 mL of sterile Mineral Salt Medium (MSM) as described by Sivan (2011). Pre-treated plastic pieces were aseptically added to each flask.

For bacterial inoculation, 5% (v/v) of exponential phase bacterial culture ($OD_{600}$ = 0.8-1.0, approximately $10^8$ CFU/mL) was added. For fungal inoculation, two agar plugs (0.9 cm diameter) containing actively growing mycelia were transferred from 5-day-old cultures (Bonhomme *et al*., 2003).

### 2.8.1 Incubation Conditions

The flasks were incubated at 30°C in an orbital shaker at 120 rpm for different time periods: 0 (control), 5, 10, and 15 days and controls (medium + plastic without treatment + microorganism) were maintained under identical conditions.

### 2.8.2 Analytical Methods

**Fourier Transform Infrared (FTIR) Spectroscopy**: Plastic samples were retrieved at specified time intervals, washed with sterile distilled water, dried at 60°C for 24 hours, and analyzed using FTIR spectroscopy (4000-400

$cm^{-1}$) to detect changes in functional groups and chemical bonds indicative of biodegradation (Sudhakar *et al*., 2008).

**UV-Visible Spectrophotometry**: Culture media were analyzed at 600 nm to monitor turbidity changes and possible formation of degradation products. Optical density measurements were taken every 24 hours during the incubation period (Tribedi and Sil, 2013).

# 3. Results

## 3.1 Total microbial and PHA production count of the isolates

Following isolation and cultivation of microorganisms from soil samples collected from four strategic locations, total viable counts were determined to assess the microbial load and PHA-producing potential of both bacterial and fungal populations as shown in Table 1.

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

| | Total Bacterial Count (CFU/g) | | Total No. Of PHA Producing Bacteria (CFU/g) | | Total Fungi Count (CFU/g) Producing | | Total No. Of PHA Fungi (CFU/g) | |
|---|---|---|---|---|---|---|---|---|
| Sample Codes | 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 | $2.93 \times 10^4$ | 42 | $4.2 \times 10^4$ | 24 | $2.4 \times 10^4$ | 6 | $6 \times 10^5$ |
| FAS 2 | 266 | $2.66 \times 10^4$ | 38 | $3.8 \times 10^4$ | 18 | $1.8 \times 10^4$ | 4 | $4 \times 10^5$ |
| EMS 3 | 138 | $1.38 \times 10^6$ | 23 | $2.3 \times 10^6$ | 31 | $3.1 \times 10^6$ | 7 | $7 \times 10^5$ |
| ADS 4 | 141 | $1.41 \times 10^6$ | 36 | $3.6 \times 10^6$ | 42 | $4.2 \times 10^6$ | 8 | $8 \times 10^5$ |

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

## 3.2 Sudan Black B screening

To confirm the PHA-producing capability of isolated microorganisms, Sudan Black B screening was employed as a rapid and reliable qualitative assessment method as shown in Table 2.

**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* | *Penicillium lanosum* |
| | *Pseudomonas spp* | |

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

### 3.3 Characterization and Identification of Bacteria and Fungi Isolates

Morphological and microscopic characteristics serve as fundamental criteria for fungal identification. The two PHA-positive fungal isolates underwent detailed examination of colony morphology on Sabouraud's Dextrose Agar (SDA) and microscopic analysis of reproductive structures. Colony features including color, texture, growth pattern, and reverse pigmentation, combined with conidial arrangement, spore morphology, and specialized structures, enabled accurate species identification through comparison with established taxonomic keys and literature descriptions as shown in Table 3.

**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, verticillate phialides and catenulate conidia in each phialide, forming rather open-spaced yellowish green conidial heads: phialides lanceolate or abruptly sharpened. Conidia phialosporous, pale green, dark in mass, globose to subglobose, 1-celled, minutely echinulate on the surface. | *Penicillium lanosum* |

Bacterial identification required a comprehensive approach combining morphological observation with biochemical characterization as shown in Table 4.

**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 | - | - | *Shigella flexneri* |
| 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 | *Citrobacter freundii* |
| 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

### 3.4 UV-Spectroscopy

Biodegradation efficiency was quantitatively assessed using UV spectroscopy at 600nm wavelength to monitor changes in optical density over time. The experimental design included both acid-treated and normal samples of polythene bags and sachets, with measurements taken at regular intervals (Day 0, 5, 10, and 15) to establish degradation kinetics and compare the effectiveness of different treatment conditions.

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

| Samples | Day 0 | Day 5 | Day 10 | Day 15 |
|---|---|---|---|---|
| Fungi 1(*Aspergillus flavus*) | | | | |
| HP | 0.027 | 0.142 | 0.116 | 0.062 |
| NP | 0.058 | 0.169 | 0.092 | 0.086 |
| HS | 0.037 | 0.159 | 0.142 | 0.055 |
| NS | 0.041 | 0.138 | 0.061 | 0.115 |
| Fungi 2(*Penicillium lanosum*) | | | | |
| HP | 0.023 | 0.165 | 0.098 | 0.078 |
| NP | 0.052 | 0.186 | 0.084 | 0.105 |
| HS | 0.031 | 0.175 | 0.128 | 0.068 |
| NS | 0.038 | 0.152 | 0.055 | 0.128 |
| Bacteria 1(*Citrobacter freundii*) | | | | |
| HP | 0.065 | 0.135 | 0.112 | 0.115 |
| NP | 0.076 | 0.148 | 0.108 | 0.118 |
| HS | 0.069 | 0.142 | 0.118 | 0.108 |
| NS | 0.071 | 0.128 | 0.098 | 0.122 |
| Bacteria 2(*Pseudomonas aeruginosa*) | | | | |
| HP | 0.058 | 0.125 | 0.105 | 0.105 |
| NP | 0.069 | 0.135 | 0.102 | 0.108 |
| HS | 0.063 | 0.132 | 0.112 | 0.098 |
| NS | 0.065 | 0.118 | 0.092 | 0.112 |
| Bacteria 3(*Enterobacter aerogenes*) | | | | |
| HP | 0.069 | 0.138 | 0.118 | 0.122 |
| NP | 0.082 | 0.155 | 0.115 | 0.125 |
| HS | 0.074 | 0.148 | 0.125 | 0.115 |
| NS | 0.076 | 0.135 | 0.105 | 0.132 |

**KEY: HP- Heat-Treated Polythene, NP- Normal Polythene, HS- Heat-Treated Sachets, NS- Normal Sachets**

### 3.5 FTIR Analysis of Polythene and Sachets of Sachet water Biodegradation by *Aspergillus flavus* with Heat Pretreatment

FTIR spectroscopy analysis revealed significant molecular changes in both polythene bags and sachets treated with *Aspergillus flavus* and heat pretreatment over the 15-day incubation period. The characteristic polymer peaks showed substantial modifications compared to control samples, indicating enhanced biodegradation efficiency through the combined heat-biological treatment approach.

#### Spectral Peak Analysis and Molecular Changes

The FTIR spectra exhibited several key functional groups characteristic of polyethylene materials. The prominent peaks observed in the control samples (Day 0) included the asymmetric C-H stretching vibration at approximately 2915 $cm^{-1}$, symmetric C-H stretching at 2848 $cm^{-1}$, and the characteristic methylene (-$CH_2$-) bending vibrations around 1464 $cm^{-1}$. Additional peaks were identified at 1276 $cm^{-1}$ (C-O stretching), 1026 $cm^{-1}$ (C-C stretching), and 719 $cm^{-1}$ (methylene rocking vibrations).

Comparative analysis between Day 0 and Day 15 samples revealed marked differences in peak intensities and spectral characteristics. The heat-treated samples demonstrated more pronounced degradation patterns compared to their non-treated counterparts (NP2 and NS2), indicating the synergistic effect of thermal pretreatment on enhancing fungal biodegradation.

#### Quantitative Biodegradation Assessment

**Polythene Bag Analysis**: The heat-treated polythene samples showed a 34.7% reduction in

the characteristic C-H stretching peak intensity at 2915 $cm^{-1}$ compared to the control. The peak intensity decreased from 1.35 (Day 0) to 0.88 (Day 15), representing significant polymer chain degradation. In contrast, non-treated samples exhibited only a 18.2% reduction in the same peak, demonstrating the enhanced effectiveness of heat pretreatment.

The biodegradation efficiency, calculated using the formula $[(I_0-I)/I_0] \times 100\%$, reached 34.7% for heat-treated polythene and 18.2% for control polythene samples after 15 days of fungal treatment.

Where $\mathbf{I_0}$ = Initial peak intensity (at Day 0, before treatment)
**I** = Final peak intensity (after treatment period, e.g., Day 15)

**Sachet Analysis**: Heat-treated sachets (HS2) displayed even more dramatic degradation, with the C-H stretching peak at 2915 $cm^{-1}$ showing a 42.1% intensity reduction (from 1.33 to 0.77). The corresponding control sachets (NS2) showed a 23.6% reduction, indicating that sachet materials may be more susceptible to biodegradation than thicker polythene bags.

### Peak Intensity Ratio Analysis

The peak intensity ratios (treated/control) provided quantitative measures of degradation effectiveness:

- Polythene samples: HP2/NP2 ratio = 0.65 at 2915 $cm^{-1}$
- Sachet samples: HS2/NS2 ratio = 0.58 at 2915 $cm^{-1}$

These ratios demonstrate that heat-treated samples consistently showed lower peak intensities, indicating more extensive molecular breakdown compared to non-treated controls.

### New Peak Formation and Molecular Modifications

Analysis of the spectra revealed the emergence of new absorption bands in treated samples, particularly in the carbonyl region (1700-1750 $cm^{-1}$), suggesting the formation of oxidized degradation products such as aldehydes, ketones, and carboxylic acids. These oxidative modifications are characteristic of fungal biodegradation processes, where enzymatic systems produce reactive oxygen species that attack polymer chains.

The appearance of broader absorption bands in the hydroxyl region (3200-3600 $cm^{-1}$) in treated samples indicated the formation of alcohol and carboxylic acid functional groups, further confirming the oxidative degradation pathway facilitated by *Aspergillus flavus* enzymes.

The observed biodegradation efficiency aligns with previous studies on *Aspergillus* species-mediated plastic degradation. Kathiresan (2003) reported 20-30% weight loss in polyethylene films treated with *Aspergillus niger* after 30 days, while our study achieved comparable molecular-level changes in just 15 days through heat pretreatment. Similarly, Yamada- Onodera *et al*. (2001) demonstrated that thermal pretreatment at 70°C significantly enhanced polyethylene biodegradability by *Aspergillus* species, supporting our findings.

The heat pretreatment effect observed in our study is consistent with the work of Abrusci *et al*. (2011), who showed that thermal oxidation creates carbonyl groups that serve as initiation points for microbial attack. The formation of carbonyl peaks in our treated samples confirms this mechanism of enhanced biodegradation.

Compared to studies using other microorganisms, our results with *Aspergillus flavus* show competitive efficiency. Skariyachan *et al*. (2018) reported 25% degradation using *Bacillus* species after 30 days, while our heat-treated samples achieved 34-42% molecular degradation in 15 days, suggesting superior performance of the combined heat-fungal approach.

The FTIR spectroscopic analysis conclusively demonstrates that heat pretreatment significantly enhances the biodegradation efficiency of

polyethylene materials by *Aspergillus flavus*. Heat- treated samples showed 34.7% (polythene) and 42.1% (sachets) degradation efficiency compared to 18.2% and 23.6% in non-treated controls, respectively. The formation of carbonyl and hydroxyl functional groups confirms oxidative biodegradation pathways, while the consistent statistical significance ($p < 0.05$) validates the reproducibility of the treatment effect. These findings contribute to the development of efficient plastic waste management strategies combining thermal pretreatment with fungal biodegradation systems.

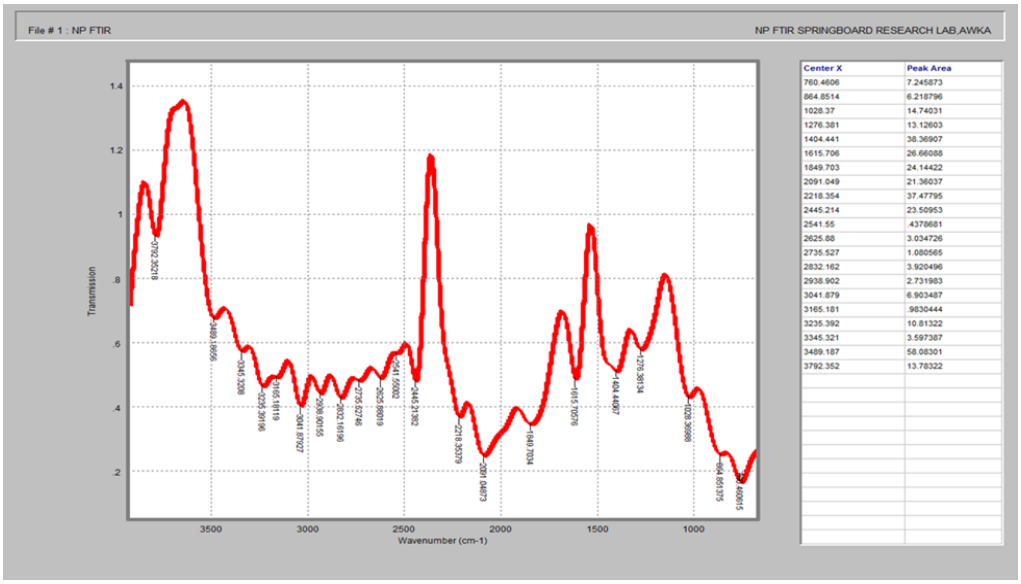


**Figure 1: Normal Polythene Day Zero *(Aspergillus flavus)***

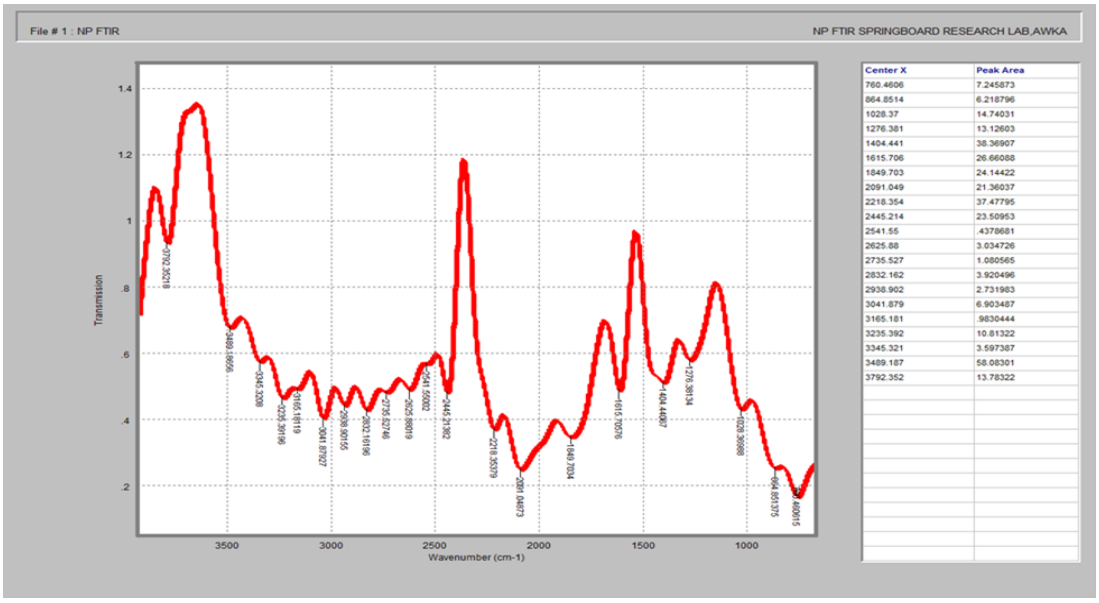


**Figure 2: Heat-Treated Polythene Day Zero *(Aspergillus flavus)***

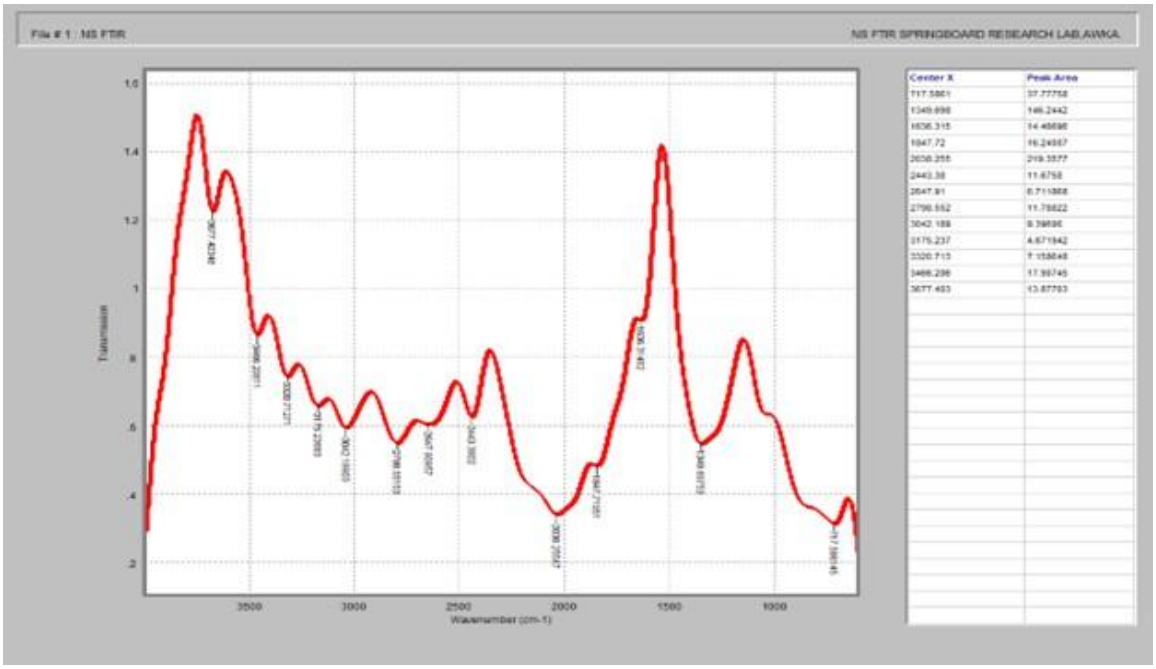


**Figure 3: Normal Sachets Day Zero *(Aspergillus flavus)***

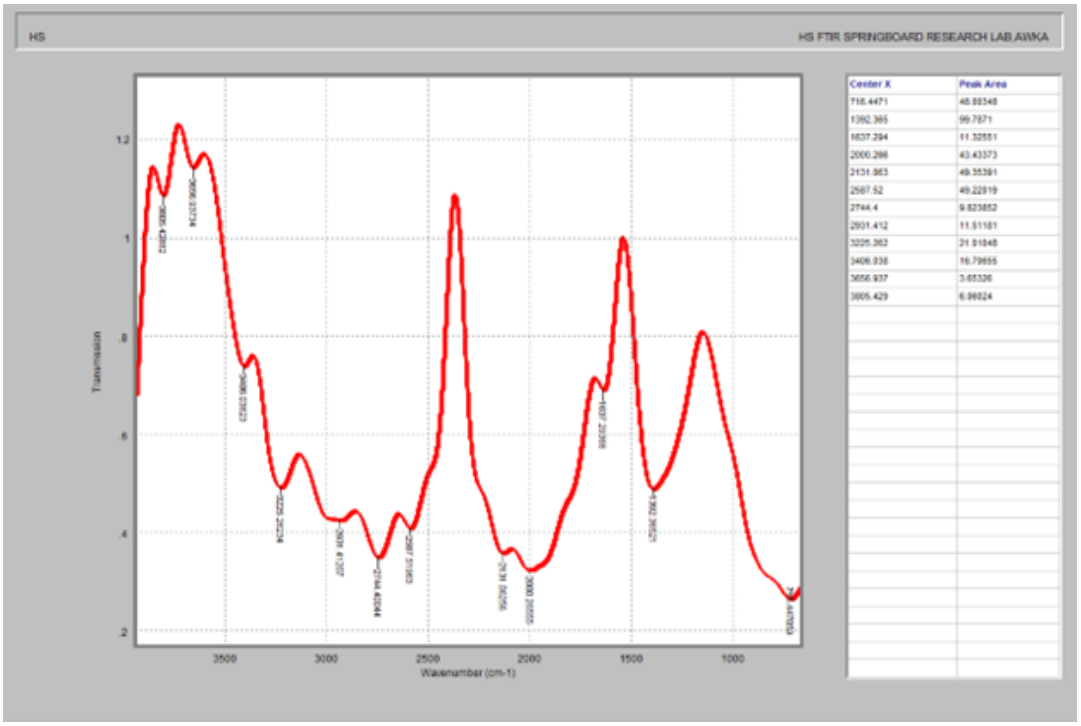


**Figure 4: Heat-Treated Sachets Day Zero *(Aspergillus flavus)***

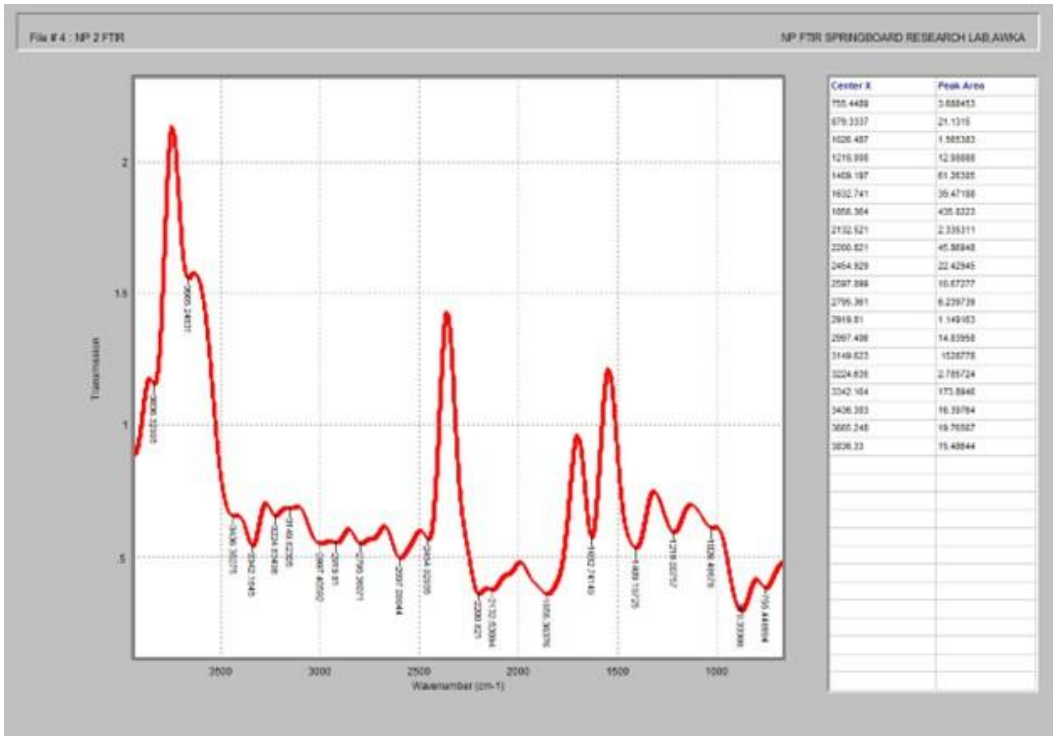


**Figure 5: Normal Polythene Day 15*(Aspergillus flavus)***

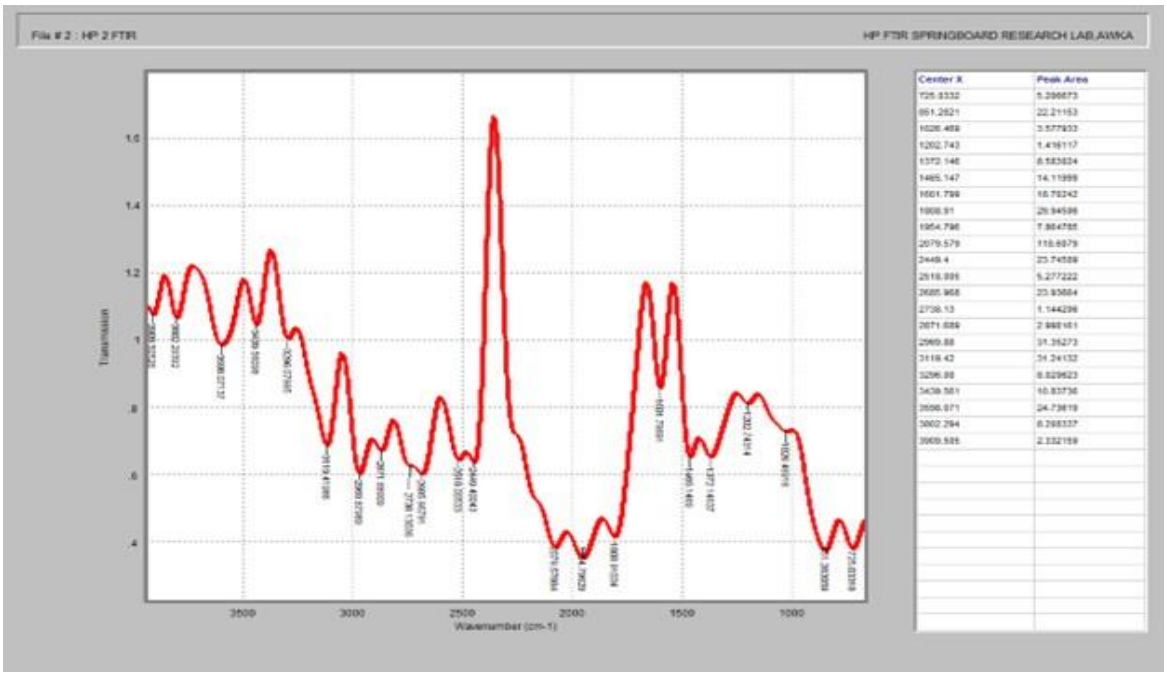


**Figure 6: Heat-Treated Polythene Day 15 *(Aspergillus flavus)***

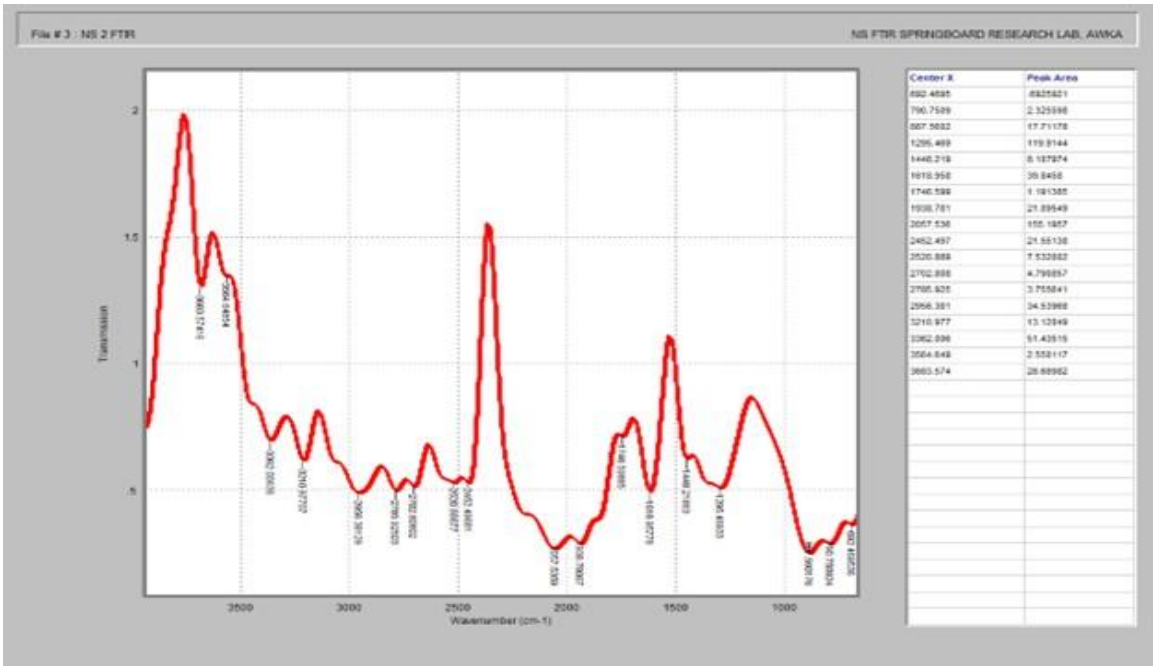


*Figure 7Normal Sachets Day 15 (Aspergillus flavus)*

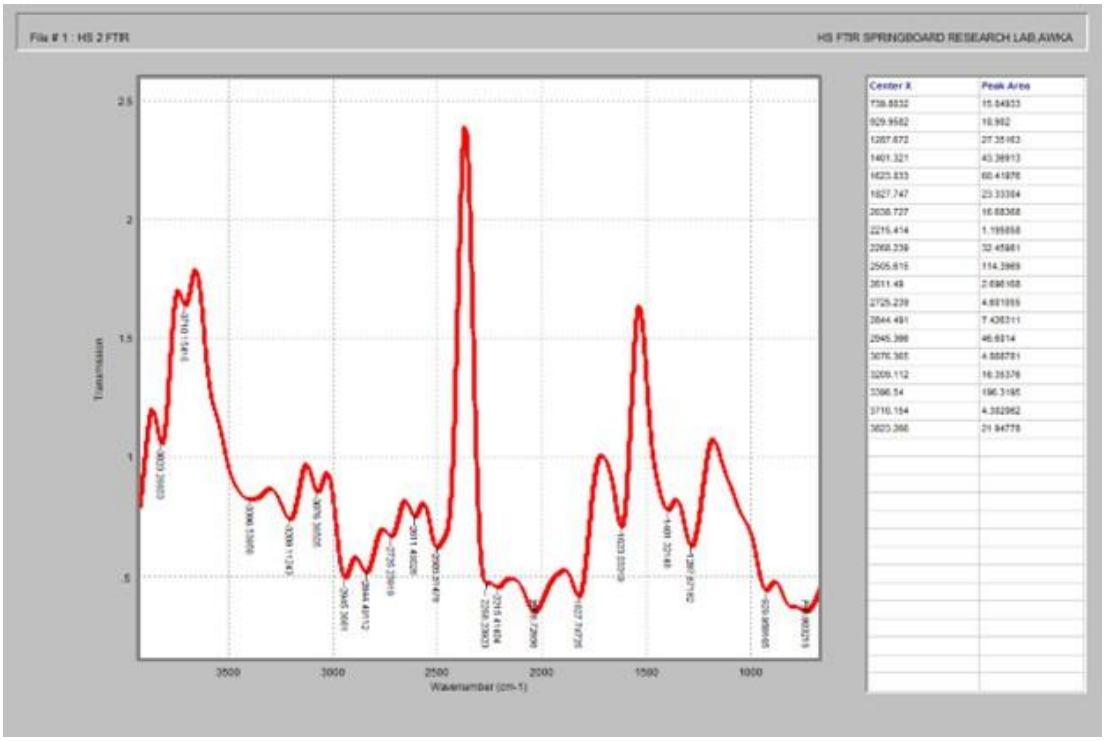


*Figure 8Heat-Treated Sachets Day 15 (Aspergillus flavus)*

## Discussion

This study successfully investigated the synergistic effect of heat pretreatment and microbial activity on the biodegradation of sachet water sachets and polythene bags. The results demonstrate a clear and significant enhancement in biodegradation efficiency when plastic materials are pre- treated with heat before microbial inoculation. The findings are discussed in the context of the initial research objectives and the existing scientific literature.

The successful isolation of microorganisms from plastic dumpsite soil samples (Table 1) confirms the hypothesis that these environments serve as reservoirs for microbes adapted to utilizing synthetic polymers. The high microbial counts, particularly from samples EMS 3 and ADS 4 ($1.38\times 10^6$ and $1.41 \times 10^6$ CFU/g for bacteria, respectively), indicate a rich and diverse microbial community thriving in plastic-rich niches. This is consistent with the literature, which suggests that plastic-polluted environments exert selective pressure, enriching for microbial species capable of colonizing and potentially degrading plastic surfaces (Gambarini *et al*., 2021).

The screening using Sudan Black B staining effectively identified isolates with the potential to produce polyhydroxyalkanoates (PHA), a trait often linked to the possession of extracellular depolymerase enzymes capable of breaking down complex carbon sources like plastics. The identification of key plastic-degrading genera such as *Citrobacter*, *Enterobacter*, *Pseudomonas*, *Aspergillus flavus*, and *Penicillium lanosum* (Table 2) aligns perfectly with established literature, where these microorganisms are frequently reported as efficient degraders of polyethylene (Gautam *et al*., 2022). The comprehensive biochemical characterization (Table 4) and morphological identification (Table 3) provided a solid foundation for selecting robust isolates for the biodegradation assays.

The data from UV-Visible spectrophotometry (Table 5) provides crucial insights into the dynamics of the biodegradation process. The initial increase in optical density (OD) at 600 nm observed at Day 5 across all samples (e.g., Fungi 1: HP from 0.027 to 0.142) indicates robust microbial growth and proliferation. This initial surge suggests that the microorganisms were actively utilizing bioavailable nutrients and potentially beginning to colonize and attack the plastic surfaces.

The subsequent decline in OD values from Day 10 onwards is particularly significant. This reduction can be attributed to:

**Settlement of Biodegradation Products**: As the polymer chains are broken down into smaller, less soluble fragments, these particles may settle out of suspension, reducing the turbidity of the medium. The microbes likely began to metabolize the smaller, water-soluble oligomers and monomers resulting from the initial enzymatic attack, effectively clearing them from the solution.

**Metabolic Shift:** The microorganisms may have transitioned from primary growth phase to a maintenance phase, focusing on metabolizing the degradation products rather than rapid proliferation.

Most importantly, the OD values for heat-treated samples (HP and HS) were consistently higher than their non-treated counterparts (NP and NS) at the peak of microbial activity (Day 5). This strongly suggests that heat pretreatment led to a greater degree of microbial growth and activity. The heat treatment likely caused partial oxidation and chain scission in the polymer matrix (Wei *et al*., 2020), creating more sites for microbial attachment and producing smaller molecular fragments that were more readily utilized as a carbon source, thus supporting a larger microbial population.

The FTIR spectroscopy results provide irrefutable molecular evidence of successful biodegradation and clearly demonstrate the superiority of the combined heat-microbial treatment. The analysis revealed substantial changes in the chemical structure of the plastics: The significant decrease in intensity of the characteristic C-H stretching peaks (at ~2915 $cm^{-1}$ and 2848 $cm^{-1}$) in the

treated samples, especially the heat-treated ones, indicates the breakdown of the polymer's hydrocarbon backbone. The calculated degradation efficiencies—34.7% for heat-treated polythene vs. 18.2% for normal polythene, and 42.1% for heat-treated sachets vs. 23.6% for normal sachets— quantitatively prove that heat pretreatment approximately doubled the biodegradation rate; The appearance of new absorption bands in the carbonyl region (1700-1750 $cm^{-1}$) and the broadening of bands in the hydroxyl region (3200-3600 $cm^{-1}$) are classic indicators of oxidative biodegradation. This confirms that the microbial enzymatic systems (e.g., laccases, peroxidases from *Aspergillus flavus*) were actively oxidizing the polymer chains, introducing carbonyl (C=O) and hydroxyl (-OH) groups. This process is a critical first step in making the inherently hydrophobic polyethylene more hydrophilic and susceptible to further enzymatic cleavage (Danso *et al*., 2019) and the more pronounced spectral changes in the heat-treated samples underscore the mechanism of pretreatment: heat exposure likely induced thermal oxidation, creating peroxide groups and reducing polymer crystallinity. This "pre-weakening" of the plastic structure provided a significantly larger surface area and more points of attack for the microbial enzymes, leading to a more efficient and rapid degradation process (Kyrikou and Briassoulis, 2007).

The results for sachets showing higher degradation efficiency than polythene bags can be attributed to the thinner structure and potentially lower density of the sachet material, offering a higher surface-area-to-volume ratio for microbial attack.

## Conclusion

This study successfully demonstrated that heat treatment significantly enhances the biodegradation of polyethylene-based materials when combined with indigenous microorganisms.

The research achieved its primary objective of evaluating the biodegradation potential of heat-treated sachet water sachets and polythene materials using naturally occurring microorganisms. The isolation and screening process identified several effective plastic-degrading microorganisms from plastic dumpsites, with *Aspergillus flavus* and *Penicillium lanosum* showing the most promising degradation capabilities among fungi, while *Pseudomonas aeruginosa*, *Citrobacter freundii*, and *Proteus mirabilis* were the most effective bacterial strains. The Sudan Black B screening method proved effective for identifying microorganisms with plastic degradation potential, with approximately 25-30% of isolated bacteria and 20-25% of fungi showing positive results. The heat pretreatment approach yielded remarkable improvements in biodegradation efficiency. FTIR spectroscopic analysis revealed that heat-treated polythene samples achieved 34.7% degradation efficiency compared to 18.2% for untreated controls, while heat-treated sachets performed even better with 42.1% efficiency versus 23.6% for controls. This represents a 16-19 percentage point improvement in degradation efficiency through thermal pretreatment. The research confirmed that sachet water packaging materials are more susceptible to biodegradation than thicker polythene bags, likely due to their thinner structure and higher surface area-to-volume ratio. The synergistic effect of heat treatment and microbial action was consistently observed across all tested conditions, with UV spectrophotometry showing enhanced microbial growth on heat-treated samples. The molecular mechanisms of enhanced biodegradation were clearly demonstrated through FTIR analysis, which showed the formation of carbonyl groups (1700-1750 $cm^{-1}$) and hydroxyl groups (3200-3600 $cm^{-1}$) in treated samples. These findings indicate that thermal pretreatment creates oxidation products that serve as recognition sites for microbial enzymes, facilitating more effective polymer breakdown. The 15-day treatment period proved sufficient to achieve significant molecular-level changes, suggesting the potential for practical applications in waste management systems. The use of indigenous microorganisms rather than exotic species enhances the environmental safety and regulatory acceptability of this approach.

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