



## Biodegradation of Low-Density Polyethylene using Bacteria and Fungi Isolated from Dumpsite Soil in Kano State, Nigeria

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### ABSTRACT

The most widely available plastic in the world today is polyethylene and the current methods for disposing of plastic wastes mainly include landfilling, incineration and mechanical and chemical recycling. In most countries, especially the developing countries, landfilling is the major method for plastic wastes disposal due to its operability and low cost. In Nigeria, the increase in volume and types of plastic wastes that are indiscriminately dumped continues to cause serious environmental pollution and other undesirable consequences due to slow deterioration rates. This call for new approaches to waste management such as better use of naturally occurring microorganisms with intrinsic ability to use, grow on and biodegrade LDPE waste. The aim of the study is to biodegrade low density polyethylene (LDPE) using bacteria and fungi isolated from dumpsite soil in Kano state, Nigeria. Microorganisms were isolated from a dumpsite soil, collected from Sheka Yarkasuwa, Kumbotso Local Government, Kano State, Nigeria. The mean aerobic mesophilic bacterial and fungal count was found to be  $9.30 \times 10^6 \pm 4.2 \times 10^5$  and  $3.30 \times 10^6 \pm 2.8 \times 10^5$ . The biodegradability of LDPE films was analysed by combination of methods including weight loss method, Scanning Electron Microscopy and Fourier Transform Infrared Radiation. The individual weight loss of bacterial isolates shown by *Lysinibacillus sphaericus* (27.37%) was higher compared to *Bacillus horneckiae* (26.17%), their consortia (10.53%), *Penicillium* sp. (7.6%) *Aspergillus niger* (6.67) and their consortia (12.32%). The biodegradation assay using weight loss, Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Radiation (FTIR) confirmed the ability of these microorganisms to breakdown LDPE, resulting in morphological changes and alterations in chemical structure. These findings suggest that dumpsite soil microorganisms hold promise for developing biotechnological solutions for LDPE waste management.

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### INTRODUCTION

The word "plastic" comes from the Greek word "plastikos," which means "which can be molded into different shapes." Plastics are defined as polymers that begin to move when heated, allowing them to be cast into molds (Elahi *et al.*, 2021). The environmental buildup of plastic garbage is a serious concern. Approximately 60%

of the 400 million metric tons of plastic manufactured each year are polyolefins, including polyethylene (PE) and polypropylene (Gregory *et al.*, 2022).

The amount and variety of plastic garbage that is being carelessly disposed of in Nigeria is increasing, which is having detrimental effects on the environment (Kunlere *et al.*,

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2019). Because of their structure and chemical makeup, the majority of plastics that wind up in the environment do not break down naturally, resulting in slow rates of disintegration (Maiga *et al.*, 2023).

Currently, landfilling, incineration, and mechanical and chemical recycling are the main ways that plastic trash is disposed of. Due to its affordability and ease of use, landfilling is the primary method for disposing of plastic trash in the majority of countries, particularly in developing nations (Ru *et al.*, 2020). Due to the ineffectiveness of conventional plastic disposal methods and the severe negative effects plastics like low density polyethylene (LDPE) have on human health and the environment, new waste management strategies are needed, such as making greater use of naturally occurring microbes that have the innate capacity to consume, thrive on, and biodegrade LDPE waste (Kunlere *et al.*, 2019).

The biological elements that naturally break down plastic are bacteria, fungus and algae (Ayeeni *et al.*, 2022; Kale *et al.*, 2015). In comparable studies, most researchers observed that LDPE was utilized by microorganisms as carbon source (Kunlere *et al.*, 2019). Biodegradation involves a consortium of microorganisms, some of which breakdown the polymer into smaller constituents, while others utilize the monomers and release less toxic and recalcitrant by-products that serve as energy source for other microbial groups (Ayeeni *et al.*, 2022). Microorganisms are important for the biological breakdown of materials (Chizike *et al.*, 2022).

By releasing endo and exo-enzymes to break down substrates into smaller components, microbes demonstrate their high degree of environmental adaptation. These elements serve as a source of energy and carbon (Fachrul *et al.*, 2023). This study was focused on exploring the potentials of microorganisms to degrade polyethylene as an alternative to the conventional methods of disposal.

## MATERIALS AND METHODS

### Sample Site

The soil sample was collected at ShekaYar-kasuwa Dumpsite, which is situated at Kumbotso Local Government Area of Kano State. Kumbotso Local Government Area is 450 meters above sea level, between latitudes 11°53'17"N and longitudes 8°30'10"E and has a land mass of approximately 158 km<sup>2</sup> (Yelwa *et al.*, 2015).

### Collection of Soil Sample from the Dumpsite

The procedure described by Chizike *et al.*, (2022) was adopted. Soil samples were collected from surface soil within 15 cm depth into sterile Ziploc bags using a hand auger from the dumpsite. The samples were transported to the laboratory and were preserved under laboratory conditions for further use.

### Isolation and Identification of Bacteria and Fungi from Soil Samples

Microorganisms were isolated from one gram of soil sample dispensed in 9mL of sterile normal saline followed by serial dilution method (Singh *et al.*, 2016). The Nutrient agar media for bacteria and Potato Dextrose Agar for fungi were prepared and isolation was performed by pour plate technique. For each sample, three replicas were maintained and kept for incubation at 37°C for Bacteria for 24 hours and for fungi at room temperature (30°C) for 3 days. The isolated colonies developed were sub cultured on agar slants and preserved under refrigeration temperature (Singh *et al.*, 2016). The identification of bacteria was performed on the basis of macroscopic and microscopic examination as per Bergey's Manual of Systematic Bacteriology (Krieg *et al.*, 2008). The fungi were identified after staining with cotton blue by following the keys of Al-mohanna (2016).

### Biodegradation of Polyethylene

One gram (1g) of soil sample was added to 50mL of 0.85% autoclaved normal saline solution to prepare the inoculums. The inoculums were kept at 37°C for 2 hours in a shaker incubator before inoculation. Artificial media was prepared containing 0.1% (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>, 0.1% NaNO<sub>3</sub>, 0.1%

K<sub>2</sub>HPO<sub>4</sub>, 0.1% KCl, 0.02% MgSO<sub>4</sub> and 0.001% yeast extract in 1000mL distilled water. Weighed LDPE films were added as the carbon source to each 100 mL of growth medium. One percent (1%) of the prepared inoculums was transferred to 200 mL of synthetic medium to prepare growth culture for the LDPE degrading bacteria and fungi. Films of Polyethylene, weighed, disinfected in 70% ethanol and air-dried for 15 min in an oven was introduced into the synthetic media. All treatments were done in triplicates. Synthetic media with LDPE without inoculum was used as the negative control. All the treatments were incubated in an incubator shaker at 150 rpm for 30 days (Muhonja *et al.*, 2018).

### Determination of Polyethylene Degradation

This was determined according to the method described by Chizike *et al.*, (2022), after the incubation, the LDPE films were recovered, washed with sterile distilled water and air-dried. Weight loss of the LDPE films was measured accordingly using the formula:

$$\text{Weight Loss (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

### LDPE Morphological Change Test

Changes in morphology on LDPE films surfaces were observed after recovery through the following processes such as Fourier Transform Infrared Radiation (FTIR) and Scanning Electron Microscopy (Fachrul *et al.*, 2023; Sowmya *et al.*, 2015).

### Molecular Identification of Bacterial and Fungal Isolates

The genomic DNA extraction was conducted using a hybrid method, which comprises of lyses with commercially available DNA extraction kit and the traditional phenol-chloroform method (Mahuku, 2004) for

purification. Amplification of 16S rRNA gene was carried out in the extracted DNA using Taq Mix (2x) (Master Mix) and universal primers 27F (forward) and 1492R (reverse), targeting the 16S rRNA gene. The PCR reactions were set up individually for each sample. Amplification of 18S rRNA gene was carried out in the extracted DNA using Taq Mix (2x) (Master Mix) and universal primers ITS1 (forward) and ITS4 (reverse), targeting the 18S rRNA gene. To verify the success of PCR amplification and check for the presence of 18S and 16S rRNA amplicons, agarose gel electrophoresis was performed. PCR products with the expected size were purified and initially sent for Sanger sequencing using universal primers at Inqaba Biotechnology. The obtained sequencing data were analysed using bioinformatics tools. Sequences were compared against public databases to identify the bacterial. The results were validated based on sequence similarity and taxonomic classification

### Phylogenetic Analysis

A total of 10 BLAST sequence data were taken for alignment using the Clustal W algorithm, phylogenetic tree was constructed using the Maximum Likelihood method, determination of evolutionary distance was analyzed using the Kimura 2-parameter method and the phylogenetic analysis was done using the MEGA 12 program (Febria *et al.*, 2024).

### Data Analysis

Analysis of Variance (ANOVA) was carried out for this study on all treatments using SPSS Statistics version 20.0.0 package to determine the significant difference in LDPE degradation.

## RESULTS

Table 1: Aerobic Mesophilic Bacterial and Fungal Count

Sample ID	Bacterial Count (Cfu/mL)	Fungal Count (Cfu/mL)
A	6.30×10 <sup>6</sup> ±4.2×10 <sup>5</sup>	3.30×10 <sup>6</sup> ±2.8×10 <sup>5</sup>
B	9.30×10 <sup>6</sup> ±4.2×10 <sup>5</sup>	1.8 ×10 <sup>6</sup> ±2.1×10 <sup>5</sup>
C	8.10×10 <sup>6</sup> ±2.8×10 <sup>5</sup>	2.34×10 <sup>6</sup> ±2.1×10 <sup>5</sup>

Key: Cfu/mL= Colony Forming Units per Millilitre (P<0.001).

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Table 2: Morphological and Biochemical Characteristics of Bacterial Isolates

Isolates	YKS 2	YKS 7	YKS 11	YKS 13	YKS 14	YKS 16	YKS 18
Colony shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Colony Surface	Flat	Flat	Rough	Smooth	Smooth	Flat	Convex
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Gram's	+	-	+	-	-	+	+
Reaction							
Arrangement	Cluster	Chain	Pairs	Single	Cluster	Chain	Cluster
Spore	-	-	+	-	-	+	+
Acid Fast	-	-	-	-	-	-	-
Motility	+	-		+	+	+	+
Starch hydrolysis	-	-	+	-	-	-	-
Oxidase test	+	-	-	+	+	+	+
Catalase test	+	+	+	+	+	+	+
Nitrate reduction	-	-	+	-	-	-	-
Citrate	+	+	+	-	+	+	+
Suspected Organism	<i>Corynebacterium xerosis</i>	<i>Klebsiella pneumoniae</i>	<i>Bacillus homeckiae</i>	<i>Campylobacter</i> spp	<i>Pseudomonas</i> spp	<i>Bacillus cereus</i>	<i>Lysinibacillus sphaerius</i>

Table 3: Morphological and Microscopic Characteristics of Fungi Isolated from Dumpsite Soil

Isolate	Morphological characteristics	Microscopic characteristics	Isolated organisms
YKS 6	Green colonies with spreading yellow reverse pigmentation	Colorless, thick-walled and roughened conidiospore, with vesicles that are globose	<i>Aspergillus flavus</i>
YKS 7	White colonies with white bottom and reddish-brown reverse pigmentation	Septate and hyaline hyphae that are thread like structures	<i>Penicillium</i> spp
YKS 8	Brown dusty colonies	Small yellow subglobose smooth and rough conidia	<i>Aspergillus</i> spp
YKS 9	Structure with numerous black dots and yellow reverse pigmentation	Globose conidia with hyaline septate hyphae	<i>Aspergillus niger</i>
YKS 14	Fill the plates with dense cottony aerial mycelium, at first white then becoming grey	Non-septate mycelium with branched hyphae	<i>Rhizopus stolonifer</i>

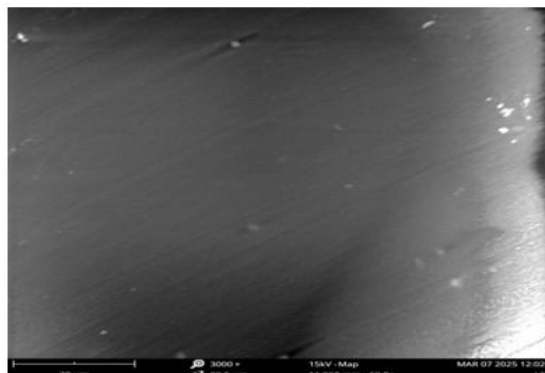
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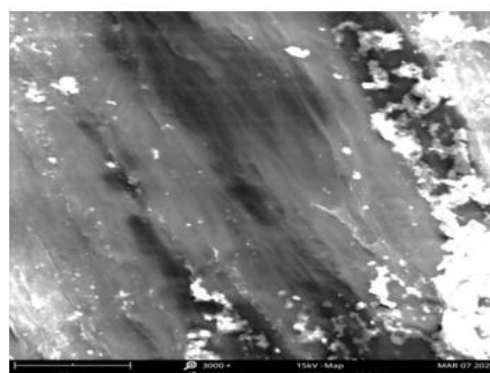
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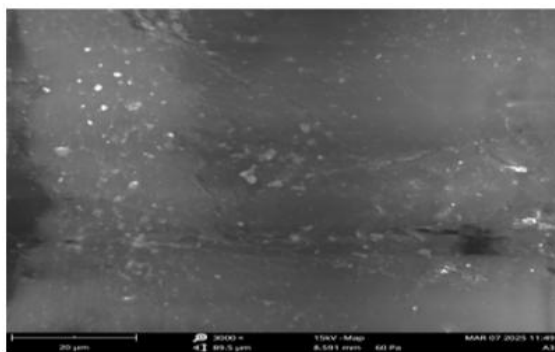
Isolate	Morphological characteristics	Microscopic characteristics	Isolated organisms
YKS 18	Dark green spreading colonies with yellow reverse pigmentation	Globose conidia with rough thick walls which are short and unbranched.	<i>Aspergillus parasiticus</i>



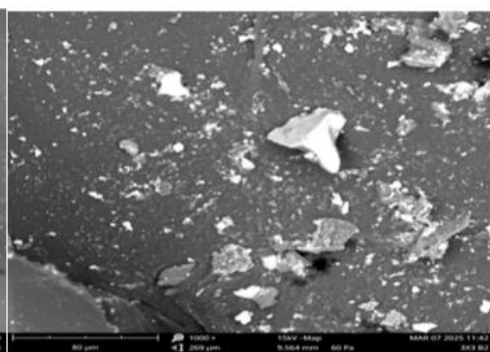
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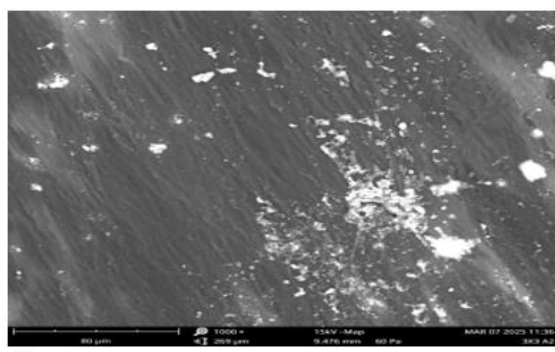
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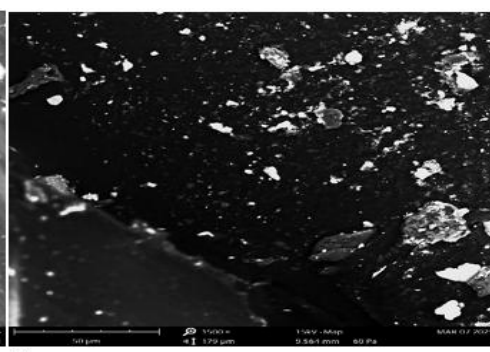
c)



d)



e)



f)

**Plate 1:** SEM morphology image of LDPE plastic biodegradation: a) Control (before biodegradation), b) *Aspergillus niger* c) *Bacillus horneckiae* d) Fungal consortium e) *Lysinibacillus sphaericus* f) *Penicillium* sp. (after biodegradation).

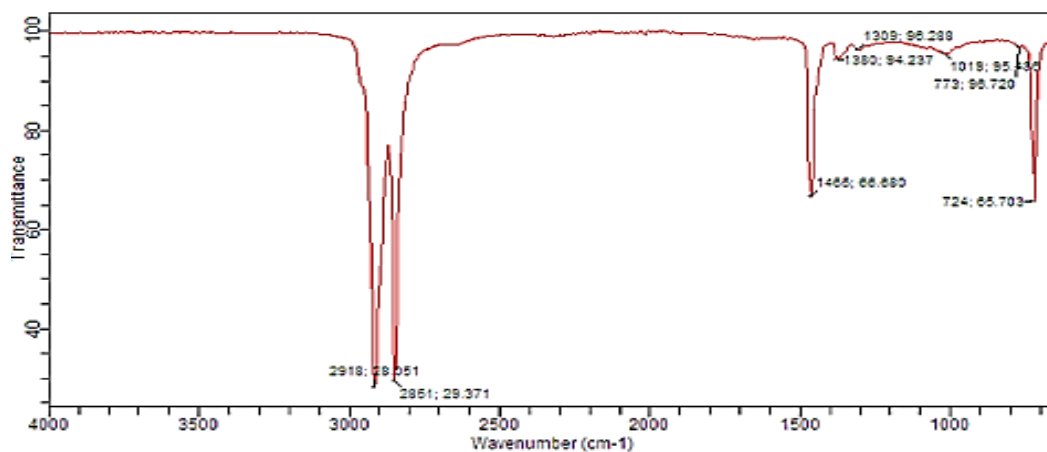
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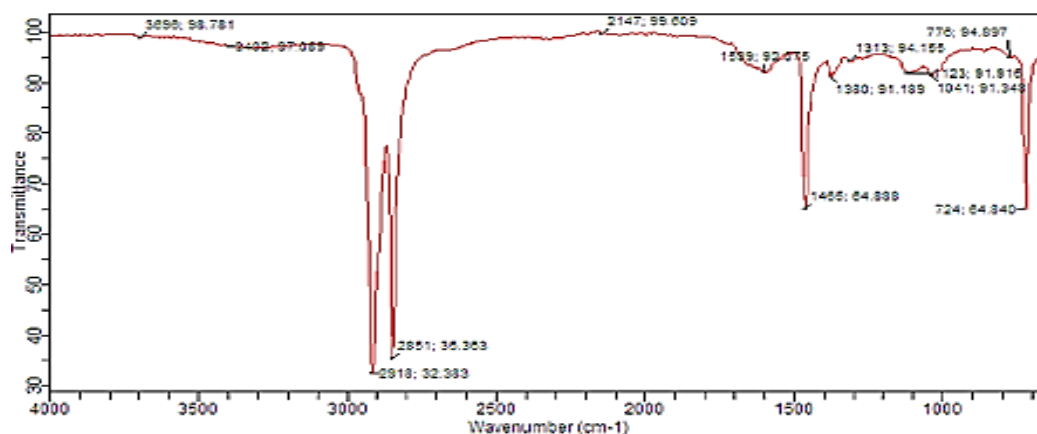
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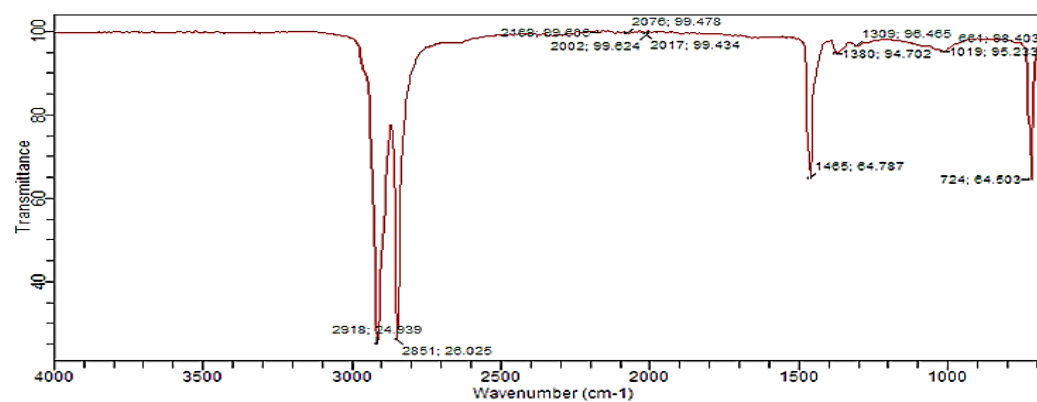




**Figure 1** FTIR spectrum of LDPE film after 30 days incubated with *Penicillium* sp. in liquid medium (MSM).



**Figure 2:** FTIR spectrum of LDPE film after 30 days incubated with *Aspergillus niger* in liquid medium (MSM).



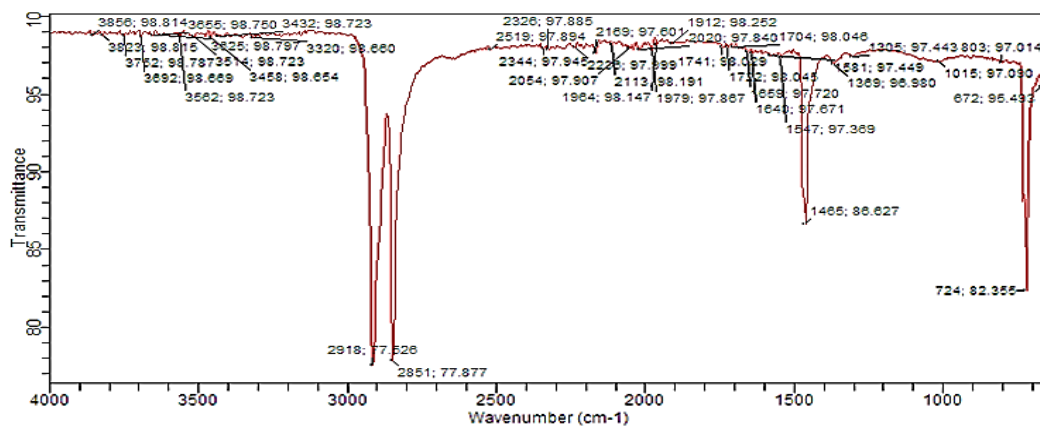
**Figure 3:** FTIR spectrum of LDPE film after 30 days incubated with *Bacillus horneckiae* in liquid medium (MSM).

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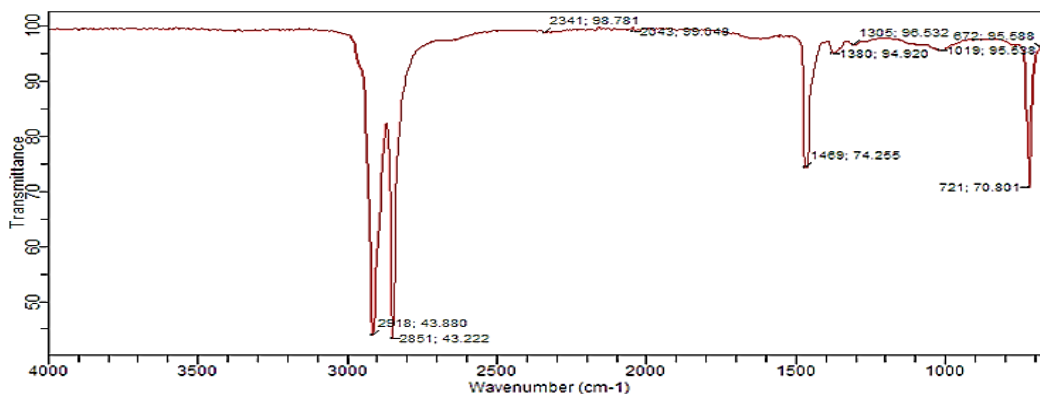
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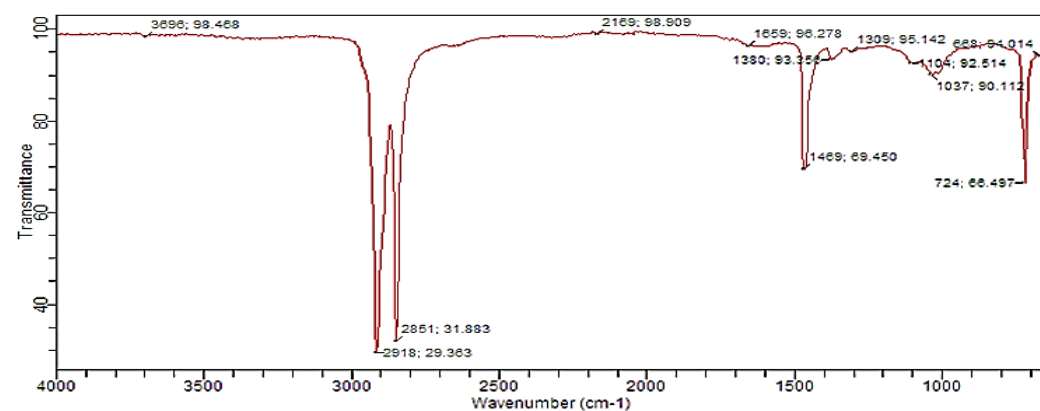
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**Figure 4:** FTIR spectrum of LDPE film after 30 days incubated with *Lysinibacillus sphaericus* in liquid medium (MSM).



**Figure 5:** FTIR spectrum of LDPE film after 30 days incubated with fungal consortium (YKS 7 & 9) in liquid medium (MSM).



**Figure 6:** FTIR spectrum of LDPE film after 30 days incubated with Bacterial consortium (YKS 11& 18) in liquid medium (MSM).

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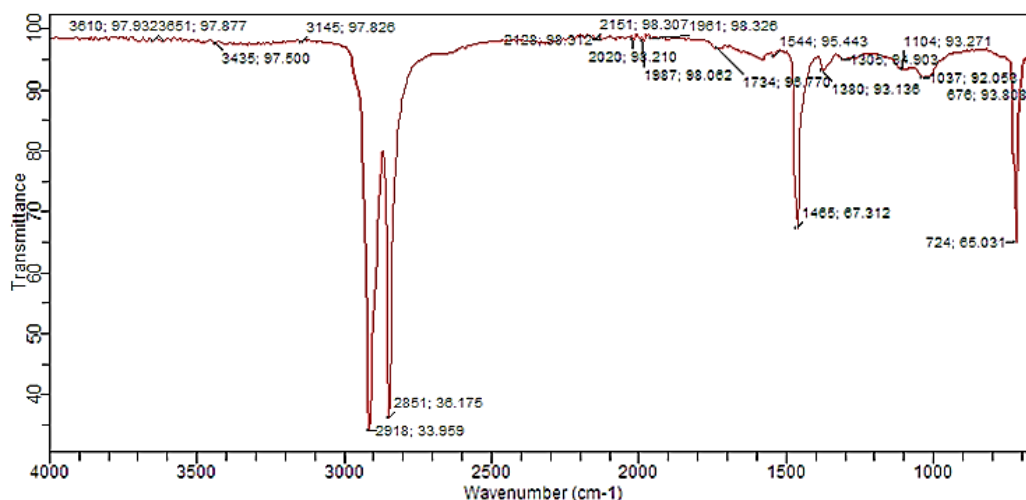


Figure 7: FTIR spectrum for LDPE film (Control)

Table 4: Percentage weight loss of surface-sterilized LDPE samples biodegraded by selected bacterial and fungal isolates.

Isolates	Dimension (cm)	Initial weight (g)	Final weight (g)	Weight Loss (g)	% Weight Loss (%)
Control	3×3	0.010067 ± 5.77E-05	0.0100 ± 1E-04	0.0000667±0.00011	0.66±0.05 <sup>e</sup>
YKS 7	2×2	0.002633 ± 5.77E-05	0.002433 ± 0.000643	0.0002±0.0007	7.6±0.456 <sup>cd</sup>
YKS 9	2×2	0.003 ± 0.0001	0.0028± 1E-04	0.0002±0.0002	6.67±0.40 <sup>d</sup>
YKS 7 and 9	3×3	0.0111± 0.00099	0.009733± 0.000208	0.001367±0.0009	12.32±0.65 <sup>b</sup>
YKS 11	2×2	0.002167± 0.000153	0.0016± 0.000173	0.000567±0.00011	26.17±2.511 <sup>a</sup>
YKS 18	2×2	0.002433± 0.000231	0.001767± 0.000252	0.000667±0.0003	27.37±2.50 <sup>a</sup>
YKS 11 and 18	2×2	0.0019± 0.0001	0.002333± 0.000306	0.0002±0.0001	10.53±0.20 <sup>bc</sup>

Key: ± Standard Deviation(p<0.001)

Table 5: Summary of 18S and 16S rRNA sequencing results of the fungal and bacterial isolates to identify the closest homologs

Sample ID	Sequence bp	Identity of the closest homologs of the sequence	% identity	Accession No of the closest homolog
YKS 9	525	<i>Aspergillus niger</i> strain LJJ19-HLJO47	99.81%	ON231642.1
YKS18	713	<i>Lysinibacillusphaericus</i> strain ARD1	99.30%	KX023224.1
YSK11	602	<i>Bacillus horneckia</i> strain JA-1	99.67%	KU297171.1

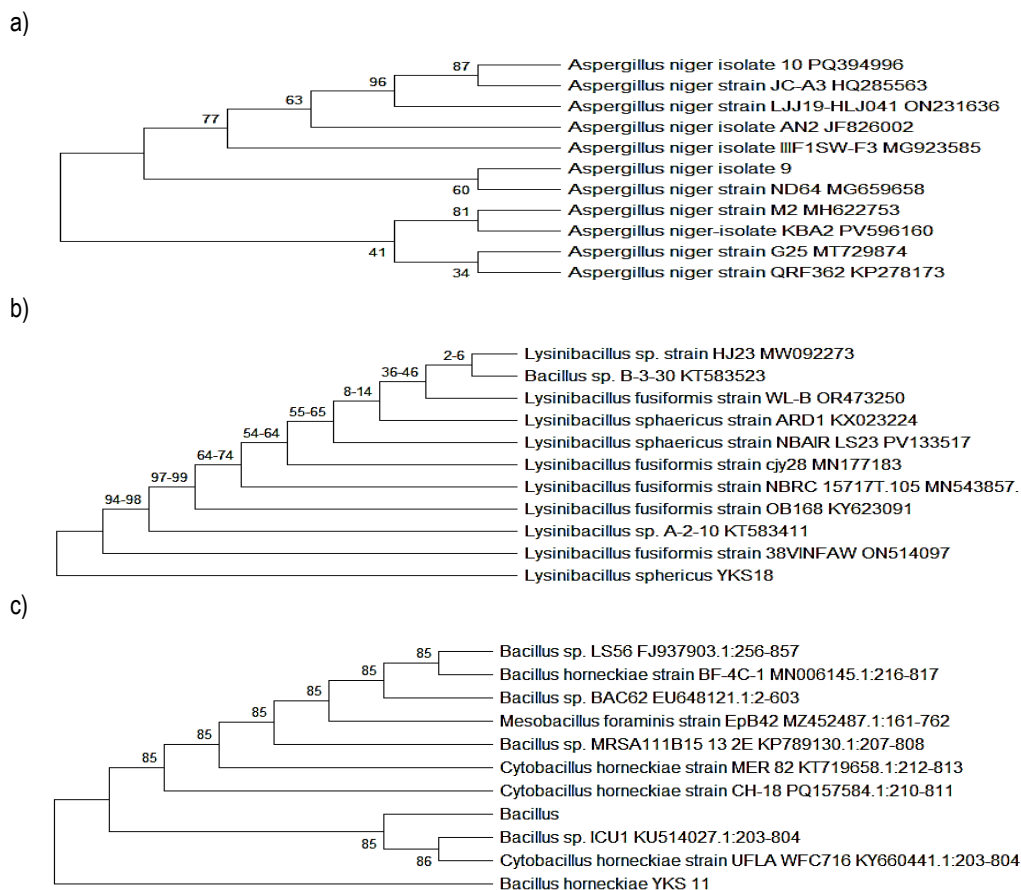
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**Figure 8:** Phylogenetic tree of a) *Aspergillus niger* b) *Lysinibacillus sphaericus* and c) *Bacillus honeckiae* based on 18s and 16s rRNA sequencing. The newly sequenced isolates were tagged with their codes

## DISCUSSION

Microbial enumeration revealed that sample B had the highest count of bacterial and Sample A had the highest load for fungal count in CFU/mL, possibly due to high carbon content on which fungi typically thrive on (Table 1). These differences reflect the influence of physicochemical parameters, particularly organic carbon and texture, on microbial abundance. The results support the finding of Nakei *et al.*, (2022) and vary slightly from the work of Ayeni *et al.*, (2022) and Deepika and Jaya Madhuri, (2015) where higher bacterial counts were reported and this might possibly be due to differences in dump composition and age.

A total of 23 bacterial isolates and 18 fungal isolates were isolated and identified using morphological, microscopic and physiological (biochemical) methods (Table 2). The identified bacterial isolates include *Corynebacterium xerosis*, *Klebsiella pneumonia*, *Bacillus honeckiae*, *Campylobacter* spp, *Pseudomonas* spp, *Bacillus cereus*, and *Lysinibacillus sphaericus*. While the fungal isolates identified are *Aspergillus flavus*, *Aspergillus* spp, *Aspergillus niger*, *Aspergillus parasiticus*, *Penicillium* spp and *Rhizopus stolonifer*. The genus of these organisms were previously reported to possess hydrocarbon degrading capabilities.

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Scanning Electron Microscopy (SEM) micrographs (Plate 1) were carried out and it showed changes in the morphology of the LDPE film which is evident of biodegradation by both bacteria and fungi. The Scanning electron micrograph showed various holes, cracks, pits and irregularities on the LDPE film, this confirms structural disruption. The control film appeared with a smooth surface. In agreement with this study, Nademo *et al.*, (2023) reported several cracks developed on the surface of the LDPE film treated with *Methylobacterium radiotolerans*.

Further confirmation was obtained via FTIR analysis and the spectrum of LDPE films, incubated with the selected bacterial and fungal isolates, showed the appearance of new bands and the disappearance of existing band due to bacterial and fungal activity. Analysis of the polyethylene spectral characteristics indicates the formation of new peaks (Figure 1-6). In the current study, FTIR analysis of virgin/ untreated LDPE (control) showed transmittance at 2918 cm<sup>-1</sup>, 2851 cm<sup>-1</sup>, 1465 cm<sup>-1</sup>, 1380 cm<sup>-1</sup>, 1045 cm<sup>-1</sup>, 776cm<sup>-1</sup> and 724 cm<sup>-1</sup>. These peaks correspond to the native bonds present in polyethylene. In the observations made by Ingavale and Raut, (2018) and Martinez *et al.*, (2021), similar peaks were observed on FTIR analysis of pure LDPE.

The changes observed in the FTIR spectrum of the LDPE film treated with the bacterial and fungal isolates as well as their respective consortia suggests that the treatment may have caused some changes in the chemical structure of the LDPE film. These changes may be due to the degradation of the LDPE film by the bacterial and fungal isolates. Similar results were reported by Rong *et al.*, (2024). The decrease in intensity of the peaks corresponding to the methylene (-CH<sub>2</sub>-) group and the C-C bond suggests that the treatment may have caused a breakdown of the polyethylene chains. The shift in the peak corresponding to the methyl (-CH<sub>3</sub>) group suggests that the treatment may have caused a change in the conformation of the methyl group. The FTIR spectra suggest that the LDPE film treated with bacterial and fungal isolates as well as their consortia has undergone some chemical

changes which may be indicative of biodegradation.

Selected isolates were subjected to 30 days degradation studies in a mineral salt medium (MSM) (Table 3). Within 5-7 days, the organisms began attaching to the LDPE surface and by day 15 visible biofilm covered the entire surface, indicating active colonization. This biofilm is crucial as it enhances the secretion of extracellular enzymes necessary for polymer breakdown. Among the isolates, *Lysinibacillus sphaericus* had the highest degradation efficiency, followed by *Bacillus horneckiae*, Fungal consortium, Bacterial consortium, *Penicillium* spp and *Aspergillus niger*. This results align with studies conducted by Gong *et al.*, (2023) and Kunlere *et al.*, (2019), confirming the biofilm-forming capacity and degradation potential of both bacteria and fungi.

## CONCLUSION

A diverse range of bacteria and fungi were successfully isolated and identified, including potential plastic degrading species. The combination of SEM and FTIR analysis revealed detectable chemical changes in LDPE post treatment, particularly the shift of functional groups indicating which indicate oxidative breakdown and bond scission and observed surface roughness which confirms degradation at physical and molecular level. These findings highlight the potential of harnessing native microbial communities for the bioremediation of plastic contaminated environments and underscore the critical need for further research to optimize conditions that can accelerate the biodegradation of persistent polymer like LDPE.

## RECOMMENDATIONS

The microorganisms identified should be utilized for in-situ bioremediation of LDPE contaminated sites and the enzymes/genes involved in LDPE biodegradation in *Bacillus horneckiae* should be further investigated. So also, the end products of low density polyethylene degradation should be monitored to confirm mineralization or to detect potentially harmful intermediates.



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