International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 13, Issue 8 - 2026

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2026.13.08.008

Screening for soil microbial consortia for the treatment of degradation of low density polyethylene (LDPE) in waste dumpsites in Keffi metropolis

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Abstract

This study aimed at isolating and characterizing bacteria and fungi with the capacity to degrade Low-Density Polyethylene(LDPE). Soil samples were collected from different waste dump sites in Keffi. Isolation and identification of LDPE biodegradable bacteria and fungi was carried out using standard microbiology methods. The screening for utilization of LDPE as a sole carbon source was carried out by the clear zone formation method. The extent of biodegradation of the LDPE films was assessed by various techniques including changes in mechanical properties. The highest bacteria count was obtained from location B ($6.2 \pm 0.13 \times 10^6$) and the highest fungi were observed from location A ($5.6 \pm 0.25 \times 10^4$). The bacteria isolated were *Pseudomonas* sp, *Bacillus* sp, *Klebsiella* sp and *Proteus* sp. Fungi isolated were *Aspergillus fumigatus, Mucor racemosus* and *Rhizopus* sp. The highest utilization LDPE by bacteria was recorded by *Pseudomonas* sp e212.28 mm and the highest fungi were observed from *Mucor indcus* s5 13.03 mm zone. After 8 weeks' incubation, highest tensile strength reduction was recorded by *Pseudomonas* sp e2 (0.080 MN/M^2) and highest reduction in force observed was from *Pseudomonas* sp a3 (0.013MN). The highest reduction in force was observed was from *Mucor indcus*b5 (0.012 MN) while highest tensile strength reduction observed was by *Mucor indcus* b5.(0.088 MN/M^2). The genus, *Mucor indcus* and *Pseudomonas* sp are confirmed as good degraders of LDPE in this study.

Keywords: LDPE, sole carbon source, biodegradation, tensile strength, incubation

1. Introduction

The word "waste" refers to useless, unwanted or discarded materials which are no longer considered of sufficient value and are thrown away. When these wastes are not properly handled and disposed off they cause pollution / contamination leading to various types of diseases (Savard *et al.,* 2002). But if utilized properly they can be turned into products of high economic value. Due to increasing population pressure on the land and the ever - increasing loads of waste generated every minute of the day, it has become difficult for government agencies to cope up with the challenge of handling the enormous quantities of wastes. Massive quantities of biodegradable solids are also generated in the form of aquatic and terrestrial weeds, leaf litter and crop wastes (Stewart, 2012). If left unharvested, the weeds seriously pollute and deplete the land and the water resources. In developing countries, leaf litter and crop waste is often burnt in the open air to generate fertilizer in the form of ash, but this not only destroys a great deal of carbon and other nutrients but is also a source of air pollution and global warming (Savard *et al.,* 2002)

Psychrophilic (cold-loving) or psychrotolerant (cold-adapted) microorganisms are found inhabiting the low temperature environments of the earth, including polar regions, high mountains, glaciers, ocean deeps, shallow subterranean systems (i.e caves), the upper atmosphere, refrigerated appliances and the surfaces of plants and animals living in cold environments, where temperatures never exceed 5°C. The potentials of psychrophiles and psychrophilic enzymes have been reviewed by Cavicchioli *et al.*, (2002), Deming (2002); Georlette *et al.,* (2004).Many psychrophiles live in biotypes having more than one stress factors, such as low temperature and high pressure in deep seas (piezo-psychrophiles), or high salt concentration and low temperature in sea ice (halo-psycrhro-philes).

The biological treatment of these wastes appears to be most cost effective and carry a less negative environmental impact. This process of biological treatment of wastes is also known as Composting. It is a self-heating, aerobic solid phase biodegradative process of organic materials under controlled conditions, which distinguishes it from natural rotting. This research focused on screening for soil microbial consortia for the treatment of degradation of low density polyethylene (LDPE) in waste dumpsites in Keffi metropolis

2. Materials and Methods

2.1 Study Area

This study was carried out in Keffi Local Government area of Nasarawa State, Nigeria. Keffi is about 53km away from the Federal Capital Territory (FCT), Abuja and 137km from the State Capital, Lafia. Keffi is geographically located in the North central zone of Nigeria and lie between latitude $8^{0}50^{0}$ North and longitude 7^{0} 52^{0} East (Akwa*et al.,* 2007).

2.2 Samples Collection

Soil samples were collected from Five different dumpsites (Mallam Nuhu Dumpsite, Yoruba Mosque Dumpsite, Stadium Bridge Dumpsite, Ganuwa Dumpsite, Liman Abaji Dumpsite) in Keffi, in each of the dumpsite Six samples were collected (making total of thirty samples). So, the soil samples were collected from the top using spoon and were transferred into sterile zip-lock plastic bags maintaining aseptic conditions, and marked according to their source and site. The collected samples were transported to the Laboratory, for isolation of microorganisms.

2.3 Isolation of Bacteria Associated with Waste Polyethylene Bags

The isolation of bacteria one gram of soil was transferred into a conical flask containing 99ml of sterile range solution (for easy dissolution of the sand), shaken and serially diluted. Pour plate method was used for the isolation of bacteria using nutrient agar for each dilution. The plates were incubated at 35°C for 24hours. The

developed colonies were sub-cultured repeatedly to get pure colonies and then preserved in a slant at 4°C.

2.3.1 Identification and Characterization of Bacterial Isolates

The identification and characterization of bacteria isolated was based on cultural, morphological and biochemical characteristic using standing method. The biochemical analysis carried out include Gram reaction, Indole Methyl red, VogesProskauer Citrate Utilization, Catalase, Oxidase, Coagulate and carbohydrate utilization test

2.3.2 Culture Characteristics

The cultural characteristics, which include the colony shape, size, surface, texture, edge, elevation, pigmentation was determine by direct observation of the colonies on the plates.

2.3.3 Morphological Characteristics

2.3.3.1 Gram Staining

A smear of the isolate (distinct colony) was made on grease free glass slide with a drop of normal saline and allowed to dry. The smear was air dried and then heat-fixed by passing the reverse side of the slide through flame. The smear was flooded with Gram's crystal violet for 60 seconds and then rinsed with distilled water. Then Gram's iodine was added and it was allowed to stand for 60 seconds. The stain was rinsed with distilled water, alcohol was added and washed off with distilled water and then the smear was counterstained with safranine for 60 seconds and rinsed with water. The slide was allowed to dry and then a drop of oil immersion was dropped on the slide and viewed under the microscope using X100 objective of the microscope as described by Cheesborough, (2010).

2.4 Isolation of fungi

The fungi species were isolated using a method described by *Mark et al*. (2016). Ten grams (10 g) of the soil was suspended in 90ml of sterile distilled water and 10-fold dilution was made. One milliliter (1ml) of the soil suspended (the stock) was picked using pipette into first test tube containing 9ml of sterile water another 1ml of water was transferred from the first test tube using pipette into second test tube containing 9ml of sterile water this step was performed 10 times and 0.3 ml of the aliquot was removed from 5th tube and spread on potato dextrose agar (PAD) plates incubated at ambient temperature for 4 days. Growth colonies were sub-cultured again on potato dextrose agar plates and later stored in PDA slants for further use.

2.4.1 Identification of fungi isolates

Identification of fungi was carried out by the method adopted by *Market al*., (2016). The cultural characteristics of fungi were determined by their growth appearance on culture plates and the morphological features were determined microscopically using lactophenol cotton blue staining technique, where lactophenol cotton blue strain was dropped on a clean grease free microscope slide, a small portion of mycelium or colony from the fungi culture plate was dropped on the lactophenol cotton blue with aid of mounted needle, the mycelium was spread well with the two mounted needle and covered with cover slip. The slide was then viewed under the microscope at x40 and x100 lens. The images were identified with reference to fungi standard chart atlas

2.5 Polyethylene Bag Preparation and Culture Condition

A method described by Kyaw *et al.,* (2012), was used in preparing waste polyethylene. Polyethylene films were collected from five (5) sites randomly selected from dump sites. These were cut into (5cm X 1cm) strips and then washed first with tap water to remove all debris and soil particles. Then, they were washed with 70% ethanol for 30 minutes, washed with distilled water and subsequently dried in incubator at 60°C before exposure to the bacterial and fungal isolates earlier identified. Inoculation and

incubation were carried out under aseptic condition.

2.5.1 Pretreatment and Preparation of Low Density Polyethylene Powder

The method described by Das and Kumar, (2015) was used. LDPE films were cut into small pieces (2cm strips). Each strip was dipped in xylene and boiled for 15 minutes (until the plastic strip is dissolved). It was cooled until it is palm bearable and then crushed with a blender at 3,000rpm. This was left to evaporate the xylene, and then washed with ethanol to remove any xylene residues. It was dried in hot air oven at 60^{o}C overnight and stored at room temperature for further use. 60^{o}C.

2.6 Screening of Polyethylene Degrading Microorganisms

This was carried out using the clear zone method described by Usha *et al*., (2011) and Das & Kumar, (2014). LDPE powder (3%) was added to a mineral salt medium at a final concentration of 0.1 %(w/v) respectively. The mixture was sonicated for 1 hour at 121^{o}C and pressure of 15psi for 20 minutes. About 15ml of sterilized medium was poured before cooling in each plate. The isolated microbes were inoculated into the polyethylene containing agar plates and then incubated at 25-30^{o}C for 2-4 weeks. The organisms producing zone of clearance around their colonies were characterized and selected for biodegradation tests.

2.6.1 Changes in Mechanical properties (Tensile strength (Newton/Square meter)

The tensile strength of the polyethylene strips (5cm X 1cm) was manually tested using a modification of the method used by Kyaw*et al*., (2012). The maximum load at break point for each of the test LDPE strips inoculated with bacterial or fungal isolates was determined (P_{max}) and divided by the original cross-sectional area of the LDPE strip (A_o) to obtain the Ultimate Tensile Strength (UTS) (Pmax (Newtons) / A(M^2). The testing conditions were maintained at a room temperature of 35-37^{o}C with a relative humidity of 65%. The negative control was maintained at similar incubation conditions and tested, and compared with a positive control. The test was done in triplicate and the average calculated as the final result.

3. Results

3.1 Total Bacteria and Fungi Population Counts in the Dump Site Soil

The bacteria and fungi population count in the dump site soil is as give in Table 1. The highest bacteria count was obtained from location B (6.2 ± 0.13 x10^6) followed by location D (5.2± 0.07 x10^6), location C (4.8 ± 0.03 x10^6), location A (4.1 ±0.90 x10^6) and the least was from location E (3.2± 0.10 x10^6). While the highest fungi were observed from location A (5.6 ± 0.25 x10^4) followed by location D (3.6± 0.10 x10^4), location B (3.1 ± 0.05 x10^4), location C (1.9 ± 0.10 x10^4) and the least was obtained from location E (0.9± 0.00 x10^4).

3.1.1 Identification of Bacterial and Fungi Isolated from Dump Site Soil

The cultural, morphological and biochemical characteristics of bacterial and fungi is as shown in Table 2. Milksh, circular, coarse, flat, convex, entire and opaque colonies, Gram negative were suspected to be *Bacillus* sp, smooth elevated colonies and cocci on NA, catalase negative, oxidase negative, indole positive, were suspected to be *Klebsiella*sp, smooth none elevated colonies, green pigment on NA, gram negative, catalase negative, oxidase negative, indole negative, nitrate positive and fructose negative, maltose negative and glucose positive were suspected to be *Pseudomonas* sp.Grey colored with a shiny surface and entire margin; mucoid or rough on NA, gram positive, catalyst negative, oxidate positive, indole negative, nitrate positive, fructose positive, maltose negative and glucose positive were suspected to be *Proteus* sp.

Table 3 shows cultural, morphology and microscopic characteristic of fungi isolated. Fungi which colonies are fast growing, grey green on the surface with yellow on the reverse side, microscopic view showed chains of round condia with conidial heads in the form of compact columns. *Phialides uniserate*, concentrated on the upper surface of the vesicle were suspected to be *Aspergillus fumigatus,* fungi *Colonies*, very rapid growth with wooly texture. Color is grayish on the surface and reverse is pale, on microscope are Non septate, broad hyphae, branched sporangiophores with round sporangiospores. Stolons and rhizoids are absent were suspected to be *Mucor racemosus.*White cotton colony rapidly filling the plate, producing spores, Non septate hyphae with branched sporangiophore, dark sporangia and sporangiospores were suspected to be *Rhizopus spp.*

3.1.2. Occurrence of Bacteria and Fungi from Dump Site Soil

Table 4.3 shows the frequency occurrence of bacterial isolated from dump site soil. *Bacillus* sphad the highest occurrence from location A (33.3 %) followed by location C (16.6%). *Klebsiella* sp had highest occurrence from location B (33.3%) and least was from location A (16.6%). *Pseudomonas* sphad the highest occurrence from location A and C (33.3%) and the least from location D and E (16.6%) and *Proteus* sp had the highest occurrence from location C (66.6%) and least from location D (33.3%).

Table 4.6 shows the Frequency occurrence of fungi isolated from dump site soil. Where the highest occurrence of *Aspergillus fumigates* was observed from location A (66.6%) followed by location D (50.0%), location B (33.3%), and least from location E (16.6%). *Mucor racemosus* had the highest occurrence from location B (50.0%) followed by location A and C (33.3%) and location D (16.6%). The highest occurrence of *Rhizopus spp* was observed from location C and D (50.0%), location E (33.3%) and the lowest was observed from location A and B (16.6%) respectively.

Table 1 Total Bacterial and Fungi Population Counts in the Dump Site Soil

Location	10^6 CFU/gTHB	10^4 CFU/gTHF
A	$4.1 \pm 0.90 \times 10^6$	$5.6 \pm 0.25 \times 10^4$
B	$6.2 \pm 0.13 \times 10^6$	$3.1 \pm 0.05 \times 10^4$
C	$4.8 \pm 0.03 \times 10^6$	$1.9 \pm 0.10 \times 10^4$
D	$5.2 \pm 0.07 \times 10^6$	$3.6 \pm 0.10 \times 10^4$
E	$3.2 \pm 0.10 \times 10^6$	$0.9 \pm 0.00 \times 10^4$

Key: A=Mallam Nuhu Dumpsite, B=Yoruba Mosque Dumpsite, C=Stadium Bridge Dumpsite, D= Ganuwa Dumpsite, and E= Liman Abaji Dumpsite.

Table 2 Cultural, Morphology and Biochemical Characteristic of Bacterial Isolate

Cultural Morphology	Gram Reaction	Biochemical characteristic					Sugar fermentation			Inference
			Cat	Ox	In	Nit	Fru	Mal	Glu	
Milksh, circular, coarse, flat, convex, entire and opaque	-		+	-	-	+	+	-	+	*Bacillus* sp
smooth elevated colonies and cocci on NA	+		-	-	+	-	-	+	+	*Klebsiella*sp
smooth none elevated colonies green pigment on NA	-		-	-	-	+	-	-	+	*Pseudomonas* sp
Grey colored with a shiny surface and entire margin; mucoid or rough	+		-	+	-	+	+	-	+	*Proteus* sp

KEY: NA- nutrient agar, Cat-catalase, Nit- nitrate, Ox-oxidase, In-indole, Glu- glucose, mal – maltose, Fru – fructose

Table 3 Cultural, Morphology and Microscopic Characteristic of Fungal Isolate

Colonial Morphology	**Morphological Characteristics**	**Inference**
Colonies are fast growing, grey green on the surface with yellow on the reverse side	Chains of round condia with conidial heads in the form of compact columns. Phialides uniserate, concentrated on the upper surface of the vesicle	*Aspergillus fumigatus*
Colonies have very rapid growth with wooly texture. Color is grayish on the surface and reverse is pale	Non septate broad hyphae, branched sporangiophores with round sporangiospores. Stolons and rhizoids are absent	*Mucor racemosus*
White cotton colony rapidly filling the plate, producing spores	Non septate hyphae with branched sporangiophore, dark sporangia and sporangiospores	*Rhizopus spp*

Table 4 Frequency Occurrence of Bacteria Isolated from Dump Site Soil

Location	No. sample	*Bacillus* sp No.(%)	*Klebsiella* sp No. (%)	*Pseudomonas* sp No. (%)	*Proteus* spNo. (%)
A	6	2(33.3)	1(16.6)	2(33.3)	0(00)
B	6	0(00)	2(33.3)	0(00)	0(00)
C	6	1(16.6)	0(00)	2(33.3)	4(66.6)
D	6	0 (0.0)	0(00)	1(16.6)	2(33.3)
E	6	0(00)	0(00)	1(16.6)	0(00)

Key: A=Mallam Nuhu Dumpsite, B=Yoruba Mosque Dumpsite, C=Stadium Bridge Dumpsite, D= Ganuwa Dumpsite, and E= Liman Abaji Dumpsite.

Table 5 Frequency Occurrence of Fungi Isolated from Dump Site Soil

Location	No. sample	*Aspergillus fumigatus* (%)	*Mucor racemosus* (%)	*Rhizopus spp* (%)
A	6	4(66.6)	2(33.3)	1(16.6)
B	6	2(33.3)	3(50.0)	1(16.6)
C	6	0(00)	2(33.3)	3(50.0)
D	6	3(50.0)	1(16.6)	3(50.0)
E	6	1(16.6)	0(00)	2(33.3)

Key: A=Mallam Nuhu Dumpsite, B=Yoruba Mosque Dumpsite, C=Stadium Bridge Dumpsite, D= Ganuwa Dumpsite, and E= Liman Abaji Dumpsite.

3.2 Screening for Utilization of Low-density Polyethylene

Table 4.6 shows the zone of clearance by different bacteria isolates; *Pseudomonas* sp a1 had10.01mm, *Bacillus* sp d3 had 8.6mm, *Klebsiella* sp a2 with 4.25mm, *Proteus* sp d3 11.02 mm, *Pseudomonas* spe2 12.28 mm, *Pseudomonas* sp a3 9.04 mm, *Bacillus* sp c2 6.12 mm,and *Proteus* sp c4 5.56 mm.

Table 4.7 shows the zone of clearance by different fungal isolates; *Mucor indcus* s5 showed the highest zone of clearance at 13.03 mm, followed by *Mucor racemosus* e3, 10.88 mm, *Rhizopus miehei* c3, 10.04 mm, *Aspergillus niger d5*, 9.02mm, *Aspergillus fumigatus b2,* 8.21 mm, *Aspergillus fumigatus c3*5.10 mm, *Aspergillus niger d3,* 4.35 mm.

3.3 Biodegradation of LDPE by Bacteria Isolates

Table 8 shows the force of reduction and tensile strength reduction of LDPE by bacterial isolates. The highest tensile strength reduction was recorded by *Pseudomonas* sp e2 (0.080 MN/M^2) followed by *Pseudomonas* sp a3 (0.077 MN/M^2), *Bacillus* sp d3 (0.055 MN/M^2), *Proteus* sp d3 (0.053 MN/M^2), *Bacillus* sp c2 (0.049 MN/M^2) but the lowest was by *Proteus* sp c4 (0.020 MN/M^2)

The reduction in force of the biodegradable LDPE by bacterial isolates, the highest reduction in force was observed was from *Pseudomonas* sp a3 (0.013MN) followed by *Pseudomonas* sp e2 (0.011MN), *Bacillus* sp c2 (0.009 MN), *Bacillus* sp d3 (0.008 MN), *Pseudomonas* sp a1 (0.006 MN) but lowest was from *Klebsiella* sp a2 (0.003 MN)

Table 6 Screening for Low- Density Polyethylene Utilization by Different Isolated Bacteria Species

Bacterial	Zone of clearance (mm)
Pseudomonas sp a1	10.01±0.14
Bacillus sp d3	8.06±0.16
Klebsiella sp a2	4.25±0.55
Proteus sp d3	11.02±1.02
Pseudomonas sp e2	12.28±1.11
Pseudomonas sp a3	9.04±0.75
Bacillus sp c2	6.12±0.02
Proteus sp c4	5.56±0.22

Table 7 Screening for Low- Density Polyethylene Utilization by Isolated Fungi Species

Fungi	Zone of clearance (mm)
Aspergillus fumigatus b2	8.21±0.04
Aspergillus niger d5	9.02±0.16
Aspergillus niger a6.	7.10±0.02
*Mucor racemosus*e3	10.88±0.01
*Rhizopus mieheic*3	10.04±0.75
*Mucor indcus*b5	13.03±0.01
Aspergillus fumigatus c3	5.10±0.52
Aspergillus niger d3	4.35±0.82

3.3.1 Biodegradable of Low Density Polyethylene (LDPE)

Table 9 shows reduction in force and highest tensile strength reduction of the biodegradable LDPE by fungal isolates, the highest reduction in force was observed was from *Mucor indcus* b5 (0.012 MN) followed by *Rhizopus miehei* c3 (0.010MN), *Aspergillus niger a6* and *Aspergillus fumigatus c3* (0.009 MN), *Aspergillus fumigatus b2`and Mucor racemosus* e3 (0.008 MN), *Aspergillus niger d5*(0.007 MN) but lowest was from *Aspergillus niger d3* (0.005 MN)

The highest tensile strength reduction observed was by *Mucor indcus* b5. (0.088 MN/M^2) followed by *Rhizopusmieheic*3 (0.066MN/M^2), *Muco rracemosus* e3 (0.063MN/M^2), *Aspergillus niger a6*(0.060MN/M^2), *Aspergillus fumigatus b2* (0.043MN/M^2), *Aspergillus fumigatus c3*(0.041MN/M^2), but lowest was from *Aspergillus niger d3* (0.027MN/M^2).

Table 8 Changes in the Properties of Biodegraded LDPE by Bacteria

Bacteria	**Tensile Strength (MN/M^2) after 10 weeks**		
	Initial	**Force**	**Tensile Strength**
Control	0.18	0.015±0.00	0.100±0.00
Pseudomonas sp a1	0.18	0.006±0.18	0.023±0.41
Bacillus sp d3	0.18	0.008±0.10	0.055±0.30
*Klebsiella*sp a2	0.18	0.003±0.12	0.047±0.61
Proteus sp d3	0.18	0.05±0.21	0.053±0.41
Pseudomonas sp e2	0.18	0.011±0.02	0.080±0.12
Pseudomonas sp a3	0.18	0.013±0.11	0.077±0.01
Bacillus sp c2	0.18	0.009±0.17	0.049±0.17
Proteus sp c4	0.18	0.004±0.10	0.020±0.00

Table 9 Mechanical Properties Changes in the Biodegraded LDPE by fungi

Fungi	Tensile Strength (MN/M^2) after 10 weeks		
	Initial	Force	Tensile Strength
Control	0.18	0.015±0.00	0.100±0.00
Aspergillus fumigatus b2	0.18	0.008±0.13	0.043±0.21
Aspergillus niger d5	0.18	0.007±0.11	0.040±0.10
Aspergillus niger a6.	0.18	0.009±0.10	0.060±0.41
*Mucor racemosus*e3	0.18	0.008±0.21	0.063±0.21
*Rhizopus mieheic*3	0.18	0.010±0.10	0.066±0.02
*Mucor indcus*b5	0.18	0.012±0.20	0.088±0.00
Aspergillus fumigatus c3	0.18	0.009±0.15	0.041±0.15
Aspergillus niger d3	0.18	0.005±0.01	0.027±0.01

Key: MN/M^2 = Maximum load to break point / cross sectional area

4. Discussion

As reported by *Kurtz, (2015)*low density polyethylene (LDPE) is created by free radical polymerization. It has a high degree of short and long chain branching, which means that the chains do not pack into the crystal structure as well. Low density polyethylene (LDPE) plastics continue to attract huge environmental attention due to its hydrophobic and recalcitrant nature.

Biodegradation is considered the most environmentally friendly option to dealing with the ever-increasing LDPE wastes causing huge environmental impact in the ecosystem, especially in a developing country such as Nigeria, where recycling and waste disposal methods are not efficient and maximized (Abioye *et al.*, 2018; Kehinde *et al*., 2020).

The occurrence of microorganisms from the study area showed 100% of bacteria and fungi, which was not surprising as it was in agreement with studies earlier reported by Makut & Ade-Ibijola, (2012) with 100% and 80% occurrence of fungi and bacteria respectively, from soil in Keffi, Nasarawa State it is similar to study reported by Ojiego*et al*., (2020) in Abuja, Federal Capital Territory.

The bacterial isolates such as *Bacillus* sp*, Proteus* sp and *Pseudomonas* sp this is similar to earlier studies reported by Krueger *et al*., (2015) showing high frequency of occurrence of different bacteria from dumpsites. Fungi such as *Aspergillus flavus, Aspergillus niger, Rhizopus sp* were most isolated species from different dumpsites in this study.

These bacteria and fungi are common microorganisms isolated from plastic contaminated soil (Kathiresan, (2003); Vijaya and Reddy, (2008); Usha*et al*. (2011); Deepika and Madhuri, (2015)

The predominant bacterial species isolated included *Pseudomonas* species, *Bacillus* species(capable of producing thick-walled endospores that are resistant to heat, radiation and chemical disinfection), *Klebsiella* species, have also been identified as important species in the biodegradation of polyethylene (Sangale *et al.*(2012). Similarly, fungal species such as *Aspergillus* species and *Fusarium* species were found to be predominant in plastic contaminated soil (Esmaeili *et al*. 2013; Das and Kumar, 2014). Fungi are considered favorable for the degradation of LDPE due to their ability to form hydrophobic proteins that can attach to the polymer surface. The faster growth of fungal biomass compared to bacteria (Kim and Rheee, 2003) has been attributed to their generation of degrading enzymes that are well matched to the insoluble LDPE (Shah *et al*., 2008). The following bacteria showed good Utilization of LDPE *Pseudomonas* sp a1, *Bacillus* sp d3, *Klebsiella* sp a2, and *Proteus* sp d3 were found to

use LDPE as a sole carbon source. Also, the fungi *Mucor indcuss*5, *Mucorracemosus*e3, *Rhizopus miehei* c3, *Aspergillus niger d5,*and *Aspergillus fumigatus c3,* were also found to utilize LDPE as sole carbon source. Augusta *et al.*, (1993) reported that the zone of clearance around the colony on screening, is due to extracellular hydrolyzing enzymes secreted by the target organisms into suspended polyester agar medium. Most of the microbes involved in forming a clearance zone were selected for further biodegradation studies using mechanical changes and the weight loss method (Deepika and Jaya Madhuri, 2015).

In this study, tensile strength was measured at two weekly intervals for all the bacterial and fungal isolates identified as LDPE utilizers. There is a clear pattern of reduction in tensile strength in comparison with untreated LDPE as observed in this study. It was found that *Pseudomonas* sp e2 and *Pseudomonas* sp a3 gave the highest reduction in the tensile strength of LDPE at 0.080%, followed by *Bacillus* sp d3 and *Proteus* sp d3 at 0.055 % reduction in tensile strength respectively after ten weeks of incubation. Kywa *et al*., (2012) also found maximum decrease in tensile strength for polyethylene incubated with *Pseudomonas* sp *Pseudomonas* sp a1. Similarly, *Mucorindcus*b5 and *Rhizopusmieheic*3gave the highest tensile strength reduction of LDPE at 0.088 % respectively within the same time of incubation, followed by *Mucorracemosus*e3 at 0.066 % tensile strength reduction. This result is in agreement with earlier studies by Kyaw*et al*., (2012) and Awashthi *et al.,* (2017). These studies reported tensile strength reduction of LDPE strips after incubation with microorganisms.

Conclusion

In this study it was observed that bacteria and fungi isolated from different refuge dump site in the study area showed capabilities in degradation of LDPE were highest tensile strength reduction was recorded by *Pseudomonas* sp e2and highest reduction in force observed was from *Pseudomonas* sp a3. The highest reduction in force was observed was from *Mucor indcus* b5 while highest tensile strength reduction observed was by *Mucor indcus* b5.

Conflict of Interest statement

The authors declare no conflicts of interest

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Subject: Biotechnology

Quick Response Code

DOI:10.22192/ijarbs.2026.13.08.008

How to cite this article:
Zaharaddeen M. A, Owuna J.E and Otakwu, D. A. (2026). Screening for soil microbial consortia for the treatment of degradation of low density polyethylene (LDPE) in waste dumpsites in Keffi metropolis. Int. J. Adv. Res. Biol. Sci. 13(8): 100-110.
DOI: http://dx.doi.org/10.22192/ijarbs.2026.13.08.008