



## **A Comparative Study of Biodegradation of Virgin and Weathered Low-density Polyethylene by *Proteus* and *Lactobacillus* spp.**

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### **Abstract**

The environmental and public health burdens arising from plastic wastes, and the need to develop an eco-friendly strategy to manage the wastes prompted this research work. In this study, the role of microorganisms in virgin and weathered low-density polyethylene degradation was investigated. Two (2) bacterial species, *Lactobacillus* and *Proteus* were isolated from a waste dumpsite in Avu, Owerri West LGA, Imo State, Nigeria. Incubation of the polyethylene sachets with the test isolates was for 60 days, and their ability to degrade polyethylene was evaluated by microbial growth measurement (OD<sub>600</sub>), changes in the functional groups of the polyethylene (FTIR) and weight loss analysis. Results revealed the presence of gas bubbles and turbidity of the microbial inoculated culture media. Optical density measurement showed progressive and gradual increase in absorbance, with *Lactobacillus* sp. recording higher concentrations in both virgin (0.115 – 0.550) and weathered (0.116 – 0.790) polyethylene cultures than *Proteus* sp. (0.122 – 0.451) and (0.114 – 0.710). Fourier transform infrared spectroscopy (FTIR) revealed the presence of additional carbonyl, hydroxyl and methylene functional groups in the polyethylene sachets treated with the test isolates (*Lactobacillus* sp: virgin PE – 10 peaks, weathered PE – 13 peaks; *Proteus* sp: virgin PE- 7 peaks, weathered PE – 10 peaks) as against the control (virgin PE – 8 peaks and weathered – 7 peaks). Similarly, weight loss analyses also revealed higher percentage weight loss in weathered PE than virgin PE, with *Lactobacillus* sp also recording greater percentages (44% and 32%) than *Proteus* sp (28% and 22%). These observations were indications of microbial metabolic activities, and the degradation and utilization of the polyethylene by the test organisms as sole source of carbon and energy. It was concluded that *Lactobacillus* sp. and *Proteus* sp. have the ability to degrade polyethylene, with *Lactobacillus* sp. displaying a higher capability, and that weathered polyethylene is more susceptible to microbial attack than virgin.

**Keywords:** polyethylene, *Lactobacillus* sp., *Proteus* sp. biodegradation, microbial isolates

## Introduction

Plastics are synthetic polymer which are hydrophobic and chemically inert, with high molecular weights. These properties make them recalcitrant to microbial degradation, hence their persistence in the environment. Plastic products are widely applied in residential homes, institutions, agricultural, industrial and commercial establishments for different purposes. As a result of the versatility in application, global plastic production has been on the increase, with an estimated 140 million metric tons in the early parts of the 20<sup>th</sup> century, 245 million tons in 2020, 390.7 in 2021, 445.25 in 2022 and a projected 590 million metric tons by 2050 (Nanda et al, 2010; Statistica, 2023).

Polyethylene is among the most commonly used plastics, and is mostly employed in packaging. In recent years, polyethylene bags are designed with attractive colours and customized for supermarkets, fast foods centers, restaurants, pharmacy stores, clinics and hospitals, hotels and various other establishments. These innovations have greatly enhanced the production and utility of polyethylene, and consequently increased plastic waste generation. Majority of plastic waste, especially polyethylene serve single-term purposes, after which they are discarded into the environment, where about 56% are placed in open dumpsites, 24% burnt and only about 9-18% recycled (Chamas et al, 2020, Statistica, 2023). It is projected that by 2050, about 12 million metric tons of plastic waste would be released into the natural environment (Ni et al, 2022). Plastics have been incriminated in flooding, disease outbreak, death of aquatic animals, reduction in soil fertility, adverse health impacts and environmental pollution and degradation (Helen, 2017; Uwakwe and Iwuala, 2012, Alshehrei, 2017; Chamas et al, 2020, Zhang et al, 2021).

Various management procedures such as incineration, land filling and recycling have been employed in handling plastic waste, but the outcome is not impressive due to the high cost and resultant environmental pollution. Furthermore, production of biodegradable plastics

have been advocated, and embraced in developed countries. However, it is not affordable to developing countries, and also does not eliminate environmental pollution completely. Recently, the use of microorganisms to degrade polyethylene is gaining ground, and various species of microorganisms have been employed in polymer degradation with substantial success recorded. Such organisms include *Aspergillus niger* and *Lysinibacillus xylanilyticus* (Esmeali et al, 2013; *Pseudomonas spp* and *Bacillus spp* (Ibiene et al, 2013; Patil and Bagde, 2015; Mukherjee et al, 2018), *Breviibacillus spp* and *Aneurinibacillus spp* (Skariyachan et al, 2015), *Serratia marcescens* (Azeko et al, 2015), *Enterobacter sp.* D1 (Ren et al, 2019), *Proteus* and *Serratia spp* (Uwakwe et al, 2023), *Sterigmatomyces shalophilus* and *meyerozyma spp* (Elsamahy et al, 2023) and various others.

Polyethylene biodegradation can be enhanced by various pretreatment methods such as UV, Irradiation, thermal and chemical treatment, use of mixed microbial consortium, additives and natural weathering (Hadad et al, 2004; Mahalakshmi and Andrew, 2012; Park et al, 2019, Kopecka et al, 2022; Skariyachan et al, 2015, Ni et al, 2022). However, there is paucity of information on polymer biodegradation in Imo State and Nigeria generally. This research work is therefore an attempt to investigate the ability of indigenous microorganisms to degrade low-density polyethylene, and the effect of natural weathering on the biodegradation process.

## Materials and Methods

### Sample Collection and Isolation of Test Microbes

Soil sample used for isolation of test organisms were collected from a dumpsite in Avu, Owerri West LGA, Imo State, at the depth of 15cm, while polyethylene powder was obtained from Green Pastures Polyethylene Company, Port Harcourt, Rivers State. Similarly, sachet water films (pure water sachets) used for biodegradation studies was obtained from Holy Family table water factory, Akwakuma, in Owerri West LGA, in Imo State.

Isolation of test bacteria was achieved using mineral salt enrichment culture which was autoclaved at 121<sup>0</sup>C, for 20 minutes, according to the method of Azeko et al, (2015) and modified method of Ren et al, (2019), and 100mls was dispensed into 5 sterile conical flasks. Thereafter 2g of polyethylene powder was added to each flask, while 2ml from each soil suspension was dispensed into two of the flasks, but no soil suspension was added to the 5<sup>th</sup> flask which served as the control. Incubation was in a rotary shaker for 4 weeks at 150rpm and 30<sup>0</sup>C. Bacterial growth was assessed using optical density in UV-visible spectrophotometer. To confirm bacterial growth, serial dilution of each set up was done accordingly, and 1ml of dilution 10<sup>-4</sup> of each was plated on nutrient agar and incubated for 24hours. Colonies with highest exponential growth were sub-cultured twice to obtain pure cultures. Morphological and biochemical Techniques were employed in the identification of isolates.

### **Biodegradation studies**

The ability of the bacterial test isolates to degrade low density polyethylene was assessed using the polyethylene sachets. Weathered polyethylene was obtained by exposing a part of the sachets to natural environmental condition for nine months (May, 2021-januaray, 2022), after which the sachets were recovered, washed and dried. Both the virgin and weathered sachets were shredded and ground to increase the surface areas for microbial action. Mineral salt vitamin (MSV) medium was prepared and used for the study as proposed by Patil and Bagde (2015). The ability of the isolates to degrade the polyethylene sachets was evaluated by microbial growth measurement using optical density (OD600), changes in functional group of the polyethylene sachet using fourier infrared spectrosctropy (FTIR), viability and weight loss analyses.

### **Microbial growth measurement**

Growth of the test bacteria in mineral salt vitamin media amended with 0.5g of ground virgin and weathered polyethylene sachet in different conical flasks was evaluated. Microbial broth culture of

each of the test isolates was prepared and 1ml of the dilution 10<sup>-1</sup> was seeded into appropriate conical flasks and labeled accordingly. Control was maintained without microbial inoculation. Incubation was in a rotary shaker at 150rpm and 30<sup>0</sup>C for 60 days. Measurement of bacterial growth was assessed using a UV-spectrophotometer (Apel 300UV) at optical density 600nm (OD600) taken at 10 days intervals. Survival of the isolates was also assayed by colony forming unit count (cfu/ml) at the end of the incubation period.

### **Changes in the functional groups of the polyethylene sachets**

Changes in the polyethylene functional groups following incubation with the test isolates were determined using FTIR spectroscopy (Bucks scientific infra-red spectrophotometer M530, USA). Virgin, weathered and untreated (control) polyethylene samples were analyzed at the end of the incubation period.

### **Weight loss analysis**

Incubation of 0.5g of both virgin and weathered sachets was as described above. At the end of the 60 days incubation period, residual sachets were recovered, washed, dried and reweighed to calculate the mean and percentage weight loss.

## **Results**

### **Isolation and identification of test organisms**

The polyethylene powder was the only carbon source in the enrichment culture used for incubation. A few microbial colonies were isolated and after sub-culturing, two colonies showed a better growth trend, and selected for biodegradation essays. Using morphological and biochemical characteristics, the isolates were identified as *Proteus sp* (AVD(NA)A), a gram-negative, motile short rod bacterium and *Lactobacillus sp* (AVD(NA)B), a gram-positive non-motile straight rod bacterium.

**Biodegradation assays**

The degradation capabilities of the test isolates were determined by UV- spectrophotometer, FTIR, percentage weight loss and colony-forming unit count (cfu/ml).

**Microbial growth measurement**

Measurement of bacterial concentration during the 60 days of incubation was taken at 10 days

intervals. Results obtained showed a progressive and gradual increase in bacterial concentration, with *lactobacillus sp.* indicating higher concentrations (growth) in both virgin and weathered polyethylene cultures. As the period of incubation progressed, gas bubbles were observed in the media which became turbid. However the control sample remain clear with no gas bubbles and no significant change in absorbance (medium concentration) as shown in table 1 and 2

Table 1: Mean Microbial Growth Measurement in Virgin Polyethylene Using OD<sub>(600)</sub> in Days

Sample code	Isolate	0	10	20	30	40	50	60
AVD(NA)A	<i>Proteus sp.</i>	0.122	0.124	0.226	0.351	0.394	0.420	0.451
AVD(NA)B	<i>Lactobacillus sp.</i>	0.115	0.112	0.259	0.350	0.396	0.439	0.550
Control		0.102	0.108	0.108	0.108	0.108	0.108	0.109

Table 2: Mean Microbial Growth Measurement in Weathered Polyethylene Using OD<sub>(600)</sub> in Days

Sample code	Isolate	0	10	20	30	40	50	60
AVD(NA)A	<i>Proteus sp.</i>	0.114	0.118	0.239	0.381	0.459	0.520	0.710
AVD(NA)B	<i>Lactobacillus sp.</i>	0.116	0.103	0.220	0.350	0.592	0.699	0.790
Control		0.118	0.118	0.119	0.119	0.119	0.119	0.119

The organisms showed similar growth trend in both virgin and weathered polyethylene cultures, with significant increase in bacterial concentrations recorded from day 20.

Similarly, both *Lactobacillus sp.* and *Proteus sp.* recorded higher rate of growth in weathered polyethylene cultures (0.790 and 0.710) than in virgin polythene cultures (0.550 and 0.451) as indicated in fig.1 below.

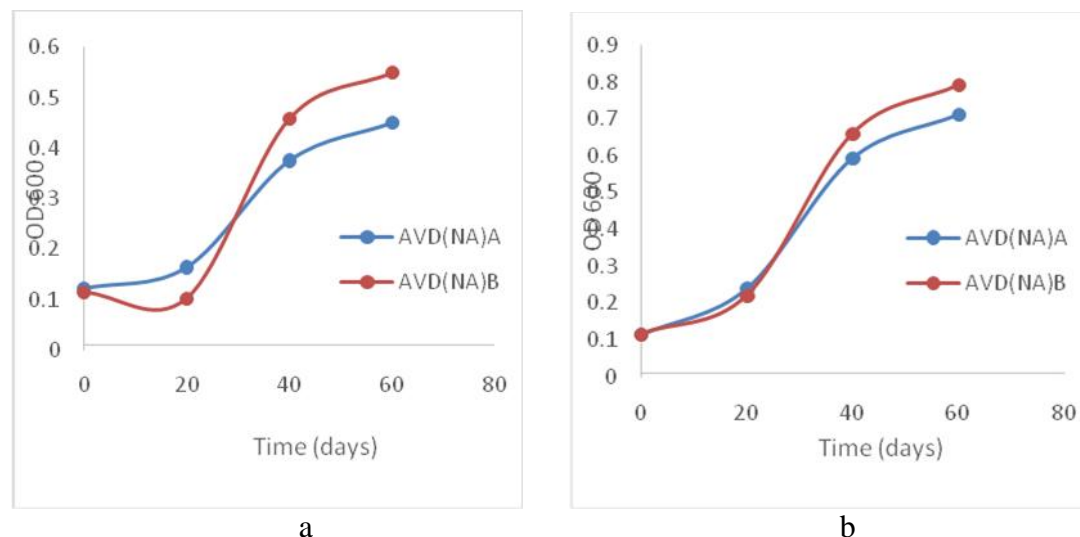


Fig.1: microbial growth measurement (OD600) a: in virgin polyethylene culture: *Proteus sp.* (AVD(NA)A) - 0.451, and *Lactobacillus sp.* (AVD(NA)B) - 0.550; b: in weathered culture: *Proteus sp.* - 0.710 and *Lactobacillus sp.* - 0.790.

### Viability Assay of Microbial Isolates

The survival of the test isolates was determined by colony-forming unit count at the end of the 60 days of incubation. Results obtained showed that the isolates adapted to the new environment by utilizing the polyethylene sachets as their sole

source of carbon, hence their survival in the liquid media. *Lactobacillus sp.* showed higher numbers of colonies than *proteus sp.*, and also the two isolates indicated higher survival rates in the cultures with weathered polyethylene. Generally, there were reductions in the number of viable colonies as shown in table 3.

Table 3: Mean and standard deviations of total bacterial count of test isolate before and after fermentation

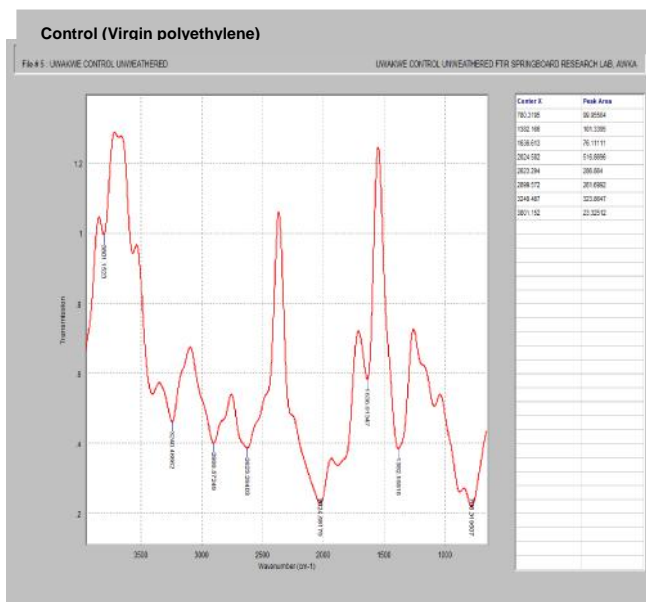
Isolates	Initial count before fermentation	Final count (Virgin)	Final count (Weathered)
<i>Proteus sp.</i>	$2.04 \times 10^3 \pm 3.00$	$4.83 \times 10^2 \pm 1.53$	$7.43 \times 10^2 \pm 2.52$
<i>Lactobacillus sp.</i>	$2.77 \times 10^3 \pm 3.51$	$1.36 \times 10^3 \pm 3.06$	$1.61 \times 10^3 \pm 1.58$

The survival of the isolates is an indication of their ability to degrade the polythene sachet, and utilize it as a sole source of carbon and energy, and *Lactobacillus sp* displayed higher survival rate than *Proteus sp.*

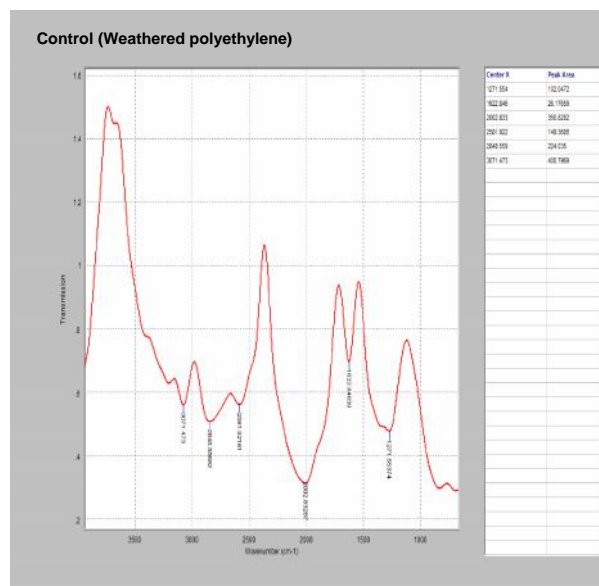
### Changes in functional groups of the polyethylene sachets

Further investigation into the ability of the test isolates to degrade polyethylene was carried out using FTIR to determine any changes in the

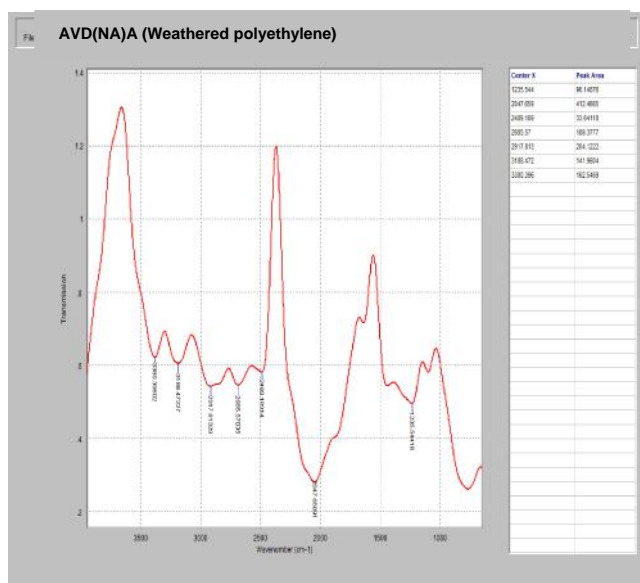
functional groups of the polyethylene sachets after incubation with the test isolates. Results obtained revealed higher number of peaks than in the control, with *lactobacillus sp* recording more peaks than *proteus sp.* Similarly, weathered polythene contained more functional groups than the Virgin. The presence of more carbonyl groups, methylene and alcohols are strong indications of oxidative degradation of the polyethylene sachets by microbial isolates as against the control as shown in figure 2a - f



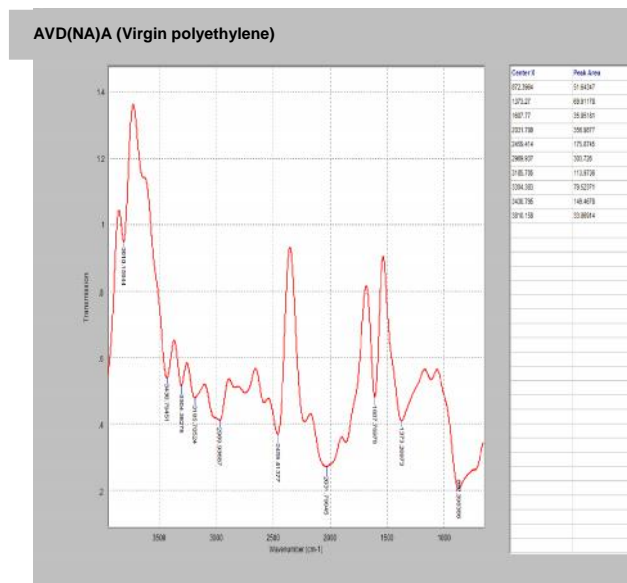
a



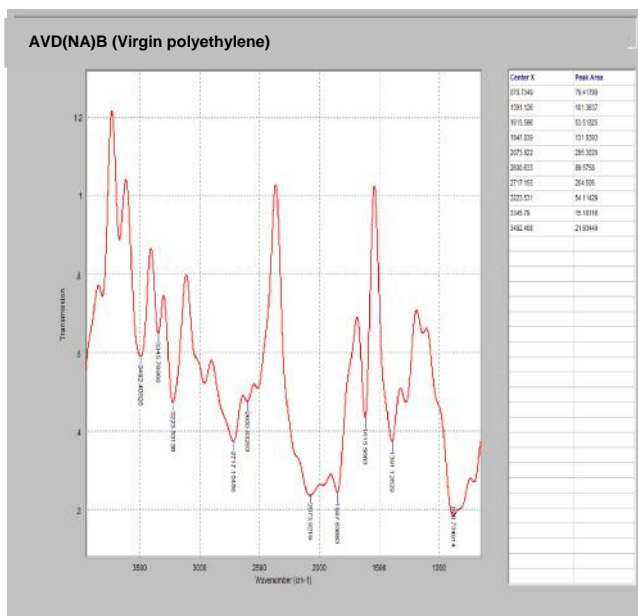
b



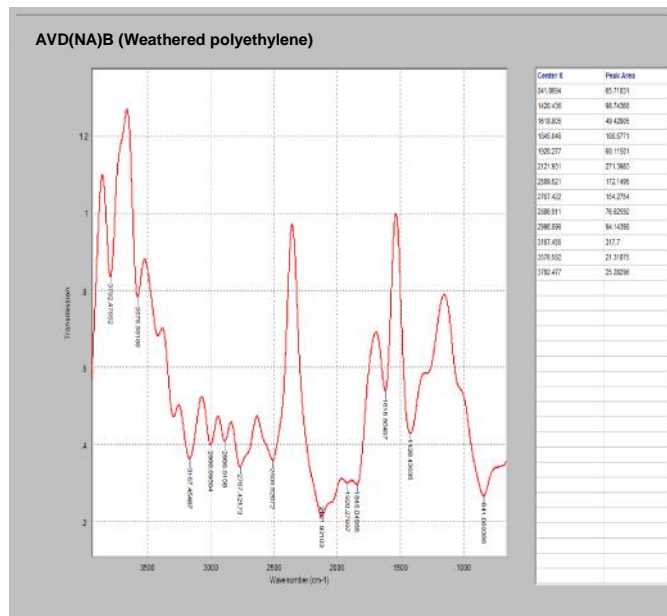
c



d



e



f

Fig 2: FTIR results: a: control for virgin polyethylene showing 8 functional groups; b: control for weathered polyethylene with 6 functional groups; c: *Proteus sp.* in virgin polyethylene culture showing 7 functional groups; d: *Proteus sp.* in weathered culture with 10 functional groups; e: *Lactobacillus sp.* in virgin polyethylene culture showing 10 functional groups and f: *Lactobacillus sp.* in weathered polyethylene culture showing 13 functional groups.

### Weight loss analysis

After the 60 days of incubation with the bacterial isolates, the weights of the residual virgin and weathered polyethylene sachets were found to

decrease. However, the percentage weight loss was higher in the weathered polyethylene than virgin, while, *Lactobacillus sp* recorded higher degradation ability as depicted in higher percentage weight loss as shown in table 3.

Table3: Mean and percentage weight loss in virgin and weathered polyethylene sachets, after 60 days of incubation with test organisms.

Isolates	Initial wt of polyethylene	Virgin Sachet	Polyethylene	Weathered Polyethylene Sachet	
		Final mean wt	% wt. loss	Final mean wt	% wt. loss
<i>Proteus sp.</i>	0.5	0.39	22%	0.36	28%
<i>Lactobacillus sp.</i>	0.5	0.34	32%	0.29	42%

## Discussion

The growth dynamics of the microbial species from the different culture media containing either virgin or weathered polyethylene as a sole source of carbon were evaluated in terms of optical density at 600nm using UV-visible spectrophotometer. As the period of incubation progressed, gas bubbles appeared in the culture media, which also became turbid. The turbidity of the media with microbial inoculation and the appearance of gas bubbles were indications of microbial metabolic activities, and a suggestion of their ability to utilize the polyethylene sachets. Similar observations were recorded by Azeko *et al.*, (2015); Ren *et al.*, (2019) and Uwakwe *et al.*, (2023) who employed *Serratia marcescenes* and its cell free extracts, *Enterobacter sp.*, and *proteus* and *serratia* spp. respectively in polyethylene degradation. Similarly, progressive and gradual increase in microbial concentrations in the test media were recorded ( $OD_{600}$ ) as the period of incubation progressed, but the change between 0 and 10 days was quite insignificant. This could be attributed to the initial struggle of the microbes to adapt to the new environment with polyethylene as the sole source of carbon, and the resultant lag phase in the microbial growth. However, the rate appreciated steadily from 20 days till the end of the incubation period of 60days, while the control samples remained unchanged (tables 1 and 2). The increase indicated the degradation and utilization of the polyethylene sachets by the isolates as their carbon and energy source, hence resumption of growth and their increased concentrations. These findings agree with those of Lalit (2013): Ren *et*

*al.*, (2019) and Uwakwe *et al.*, (2023) who recorded similar trends in  $OD_{600}$  measurement during polyethylene microbial biodegradation assays with different species of bacteria.

Furthermore, higher bacterial concentrations (growth) were observed in cultures with weathered polyethylene than virgin, with *Lactobacillus sp.* (0.790 and 0.550) recording higher values than *Proteus sp.* (0.710 and 0.451). This result is consistent with works carried by Hadad *et al.*, (2005), Nanda *et al.*, (2010), Longo *et al.*, (2011), Mahalakshmi and Andrew (2012) and Ibenie *et al.*, (2013) where biodegradation of virgin polyethylene was compared with pretreated ones. Weathering subjected the polyethylene to solar and high energy radiation, heat, humidity, air and other environmental factors which affect its physical and chemical properties, causing mechanical damage, thereby making it easier for the breakdown of the complex structure to simple units that could be degraded by microorganisms (Kopecka *et al.*, 2022). Therefore, the presence of gas bubbles, turbidity of the culture media and increase in microbial concentrations proved the utilization of the polyethylene sachets as the sole source of carbon energy, hence their ability to degrade it.

The result of the FTIR analyses showed the control samples (virgin and weathered polyethylene) recording 8 and 6 peaks, with 2 and 1 carbonyl and alcohol groups respectively (Fig 2a and b). However, in the samples inoculated with the microbial isolates, there were pronounced increase in the number and intensity of different functional groups. Similarly, higher

numbers of functional groups were generally recorded in cultures with weathered polyethylene than virgin, with *Lactobacillus* sp. also recording more (13 and 10) than *Proteus* sp. (10 and 7). These results give credence to the higher absorbance rates recorded by *Lactobacillus* sp in OD600 measurement. There were also additional carbonyl groups which include esters, ethers, carboxylic acids and amines (16000-21990), hydroxyl groups comprising primary, secondary and tertiary alcohols (3000-3500), alkene (1200cm<sup>-1</sup>-1599cm<sup>-1</sup>) and methylene (2600cm<sup>-1</sup>-2999cm<sup>-1</sup>). The carbonyl compounds are typical products of oxidative degradation, and are therefore points of cleavage in PE degradation. These results are consistent with the findings of Hadad *et al.*, (2015), and Ren *et al.*, (2019) who recorded higher carbonyl index, additional absorption peaks and carbonyl groups in PE cultures inoculated with *Brevibacillus borstelensis* and *Enterobacter* sp. D1 than in the un-inoculated cultures. The presence of more ether, carbonyl, alkanes and methylene in the microbial inoculated cultures is an indication of the oxidative breakdown of the complex structure of polyethylene to smaller units by the test organisms, and hence their ability to utilize it as their source of carbon and energy. The result corroborates the findings of Esmaili *et al.*, (2013); Azeko *et al.*, (2015) and Ren *et al.*, (2019) who identified new functional groups which include alkene, alcohol and carboxylic acid on PE structure after treatment with *Lysinibacillus xylanilyticus* and *Aspergillus niger*; *Serratia marcescens*, and *Enterobacter* sp. D1 respectively. Further evidence of PE degradation by the isolates were presence of stretching, asymmetrical, weak and bending vibrations attributed to the actions of the microbial isolates as also reported by Ibiene *et al.*, (2013).

The ability of the test organisms to degrade polyethylene was confirmed by weight loss analysis after the 60 days of incubation of the virgin and weathered PE sachets with the bacterial isolates. Results arising from the analysis indicated reduction in net weight of the polyethylene sachets (virgin and weathered) incubated with the microbial isolates, while the

control samples showed no decrease in weight (table 3). However, higher weight loss percentages were obtained from weathered polyethylene, and *Lactobacillus* sp. recorded higher values in both virgin and weathered polyethylene cultures (32% and 42%) than *Proteus* sp (22% and 28%). This confirms that exposing plastics to natural environment reduces their hydrophobicity, and damages the mechanical structure, thereby increasing the likelihood of microbial attack. The decrease in weight signifies the degradation of the polyethylene and its utilization by the microorganisms as their sole carbon source. It also confirms the results obtained from the other analyses carried out in this current work which indicated increased microbial concentrations (growth) and formation of additional functional groups, and also those recorded by Nanda *et al.*, (2010), Skariyachan *et al.*, (2018), Park and Kim (2019) and Chamas *et al* (2020) who investigated polyethylene biodegradation by different microbial isolates with significant reduction in the net weight of the polymer materials.

## Conclusion

In this study, indigenous microbes, *Lactobacillus* sp. and *Proteus* sp. were isolated from a dumpsite in Imo State and screened for polyethylene biodegradation. The results obtained show that the organisms exhibited great potentials for low-density polyethylene biodegradation. However, their capabilities differ, and the rate of biodegradation is found to be faster in the naturally weathered polyethylene than in the virgin. From the findings, it is therefore concluded that indigenous microorganisms are capable of degrading polyethylene, and that *Lactobacillus* sp. showed greater capability than *Proteus* sp. Natural weathering of polyethylene and other pretreatment methods can enhance the degradation process and could be adopted for improved results since plastic degradation process is generally slow. It is further concluded that biodegradation could be adopted for industrial treatment of plastics since it is eco-friendly.



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