



The biodegradation of low-density polyethylene by *Bacillus* species

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Abstract

Polyethylene (PE) products have short-term applications, resulting in the daily release of enormous quantities of plastic wastes into the environment with their attendant public and environmental health threats of global concern. Developing an eco-friendly management method to abate the menace prompted this research work. Four Bacillus species were isolated from different dumpsites in Owerri Metropolis, and identified using 16S rDNA sequencing, as B. pumilus, B. siamensis, B. coagulans and B. subtilis. The ability of the microbial isolates to degrade polyethylene (table water sachets) was determined using microbial growth measurement in liquid medium (Optical Density (OD600)), disappearance and introduction of functional groups (Fourier Transform Infrared spectroscopy (FTIR) and weight loss analysis measured after sixty (60) days of incubation in mineral salt vitamin media. Microbial density showed a gradual and progressive increase from 0 day to 60 days : B. siamensis (0.12 -0.84), B.pumilus (0.11-.67), B.coagulans (0.12 - 0.61) and B.subtilis (0.11 - 0.60), as against the control medium which remained unchanged (0.11). Similarly, FTIR spectra showed additional functional groups (carboxylic acids, ethers, esters, amines, methylene, alcohols, and alkenes in the polyethylene materials exposed to the test isolates: B.siamensis (17 peaks), B.pumilus (12 peaks), B.coagulans and B.subtilis (13 peaks each), but control (8 peaks). There were subatantial reduction in the residual weights of the PE materials: B.siamensis (52%), B.pumilus (26%), B.coagulans and B.subtilis (18% each). The observations showed that these Bacillus spp. indigenous to Owerri Metropolis have the ability to degrade PE and utilize it as a carbon source, and it was concluded that biodegradation was achieved, and that PE which was regarded to be inert can be biodegraded when exposed to the right microorganisms.

Keywords: polyethylene, biodegradation, Bacillus spp., eco-friendly, dumpsite

Introduction

Increased production and wide range applications of plastic materials in various aspects of modern life, including packaging, fibres and textiles, plastic pipes and fittings, medical devices, electronics and automotive parts have made them staple products and indispen-sable to man. Their versatility arises from their advantageous characteristics such as clarity, light weight, hardness, durability, flexibility, easy processsibility, resistance to chemicals and moisture and low cost (Longo et al.,2011; Yoon et al., 2012). However, synthetic plastics exhibit other qualities such as high molecular weights, 3-dimensional structure, hydrophobicity and biochemically inert nature which make them recalcitrant to microbial actions (Esmaeili et al., 2013; Celina et al., 2019; Chamas et al., 2020). Polyethylene (low-density polyethylene (LDPE), me-

dium-density polyethylene (MDPE), high- density linear polyethylene (HDPE) and low-density polyethylene (LLDPE, polyethylene terephthalate (PET)) is the most widely used plastics, and is mainly employed in packaging. Various institutions, commercial centres, pharmacy and supermarkets have customized and designed polyethylene bags with attractive colours for packaging of their goods, and these innovations have consequently enhanced polyethylene production and usage as well as plastic waste generation (Uwakwe et al., 2023). In most developing countries, Nigeria inclusive, the waste are indiscriminately disposed along the roads, streets, dumpsites and landfills, and at least 80% of these plastics and other wastes end up in rivers, oceans, drainages and waterways, causing serious ecological and human problems (Hajramurni, 2018; Brando et al., 2018). Management of plastic wastes is among the greatest global ecological challenges, especially in developing countries of the world. Conventional management methods such as incineration, land filling, open and ocean dumping and recycling have been employed in handling plastic wastes, but most of these methods were found to be inefficient, and are associated with serious environmental and health problems (Ren et al., 2019; Chen et al., 2020; Ni et al., 2022). Similarly, production of biodegradable plastics has been advocated as an alternate method of handling plastic wastes, however the process has not gained enough ground especially in developing countries including Nigeria due to lack of public awareness, limited availability of industrial composting facilities, economic and technical challenges (Kubowicz and Booth, 2017). Furthermore, in the past few decades, there has been an increased interest in the use of diverse microorganisms, including Brevibacillus borstelensis (Hadad et al., 2005), Pseudomonas spp.(Nanda and Sahu,2010), Bacillus mycoides and B. subtilis (Ibiene et al., 2013), Lysinibacillus xylanilyticus and Aspergillus niger (Azeko et al., 2015), Pseudomonas auriginosa (Mukherjee et al., 2018), Paenibacillus sp. (Bardaji et al., 2019)), Eubacterium sp. D1 (Ren et al., 2019), Microbacterium oxydans (Najafi and Levin, 2020), Proteus, Serratia and Lactobacillus spp. (Uwakwe et al., 2023) and a host of other microorganisms to degrade polyethylene and other plastic wastes, with substantial achievements recorded by different researchers in different countries of the world. The process involves attachment of microorganisms to the polymer surfaces, enzymatic breakdown of the complex structure into short units of low molecular weights

(oligomers and monomers) and their utilization as carbon and energy sources which result to the growth and multiplication of the microbes (Arutchelvi et al., 2008; Bhardwaj et al., 2012; Kopecka et al., 2022). Utilization of the low-molecular weight compounds results in mineralization with carbon dioxide (CO_2), water (H_2O) and biomass as the ultimate end products, making the process eco-friendly (Ren et al., 2019, Uwakwe et al., 2023). However, not much of such work is seen in Imo State and Nigeria generally, hence this study which was an attempt to degrade low-density polyethylene with indigenous bacteria isolated from waste dumpsites in Imo state, Nigeria as way of contributing towards solving this global plastic waste management challenge.

Materials and Methods

Isolation of low-density polyethylene degrading bacteria

Bacterial isolates used in low-density polyethylene degradation were isolated from soil samples collected from solid waste dumpsites in Owerri Metropolis, using mineral salt medium (1.0g NH₄NO₃, 0.2g MgSO₄.7H₂O, 1.0g K₂HPO₄, 0.1g CaCl₂.2H₂O, 0.15g KCl, 0.1g yeast extract and 1.0mg of each of the following microelements: FeSO₄.6H₂O, ZnSO₄.7H₂O and MnSO₄) with polyethylene powder as the sole source of carbon, according to the methods of Gilan et al., (2004), Azeko et al., (2015) and Bardaji et al., (2019). Incubation was in a rotary shaker at 37° C for 4 weeks. Microbial growth was assessed using optical density (OD600) in UV-visible spectrophotometer. Thereafter, suspensions from each set up were serially diluted, cultured on Nutrient agar with sodium azide which is selective for the growth of Bacillus species and incubated for 24 hours. Only the colonies with higher exponential growth were selected, subcultured on nutrient agar and pure cultures were obtained for identification and biodegradation studies.

Identification of isolated Bacillus species

The isolated *Bacillus* spp. were identified using biochemical and molecular characteristics. Biochemical parameters considered included Gram-staining, oxidase, catalase, motility, citrate utilization, Methyl Red and sugar fermentation tests as described by Bargey's Manual of Determinative Bacteriology (Krieg & Holt, 1984); Cheesbrough, (2006) and American Society for Microbiology (ASM), (2020). Similarly, molecular identification was achieved through geno-

mic DNA extraction of each isolate, according the manufacturer's protocol, using genomic DNA isolation kit (numbers 24700, 24750 and 24770), obtained from Norgen Biotek Corporation, Canada. The 16S rDNA of the isolates were amplified using universal primers, and the resulting nucleotide sequences were compared to sequences obtained from GenBank (http://www.lahey.org/studies/webt.html) using the Basic Local Alignment Search Tool, as proposed by Sambrook and Russel, (2001), Janda and Abbott, (2007), and the modified method of Ogbulie and Nwakamma, (2015).

Biodegradation Assays

Low-density polyethylene (table (pure) water sachets) which was shredded, ground and sterilized was used in assessing the ability of the test isolates to degrade low-density polyethylene. Mineral salt vitamin (MSV) medium (1.0g (NH₄)₂SO₄, 1.0g KH₂PO₄, 8.0g K₂HPO₄, 0.2g MgSO₄.7H2O, 0.1g NaCl, 0.02g CaCl₂.2H₂O, 0.01g FeSO₄, 0.5mg Na₂MoO₄.2H₂O, 0.5mg MnSO₄, 0.2ml inositol, 0.2mg p-amino benzoid acid, 0.4mg pyridoxine, 2.0 µg thiamine, 2.0µg biotine and 120.5µg vitamin B12), dissolved in 1 litre of distilled water and autoclaved at 121°C for 20 minutes) was used for incubation as proposed by Patil and Bagde (2015).

Microbial growth measurement in liquid medium

Microbial broth culture of each test isolate was prepared, and 1ml of 10⁻¹ dilution was seeded into different conical flasks containing 100 ml of mineral salt vitamin media and 0.5g of the polyethylene material. Incubation was in a rotary shaker at 37°C and 150 rpm for 60 days. Growth of the isolates was monitored by measuring the absorbance using visible spectrophotometer at 600nm (OD600) of each medium at the interval of 10 days. The polyethylene served as the sole source of carbon in the medium.

Assessment of changes in the functional groups in the polyethylene structure

Changes in the chemical structure in the polyethylene materials following exposure to the test isolates were investigated using Fourier Transform Infra-Red Spectroscopy (FTIR). At the end of of the 60 days incubation period, the residual polyethylene materials were recovered, dried at room temperature and chemical changes investigated. (Azeko et al., 2015; Ren et al., 2019). The absorbance (wavelength) ranged from 4000 cm⁻¹ to 650 cm⁻¹. Control sample was without microbial inoculation.

Weight loss analysis

Incubation of 0.5g of the polyethylene materials in mineral salt vitamin medium with the test isolates was also for 60 days. At the end of the incubation, the residual polyethylene materials were recovered and washed thoroughly with 2% aqueous dodecyl sulphate solution to remove the nutrient medium and microbial biofilms. They were further washed with 70% alcohol, rinsed with distilled water, dried and reweighed to determine the average weight loses (Nanda et al., 2010; Skariyachan et al., 2018). Percentage weight loss was calculated.

Results and Discussion

Isolation and identification of low-density polyethylene degrading bacteria

Following the cultivation of the soil samples in mineral salt medium and sub-culturing in nutrient agar with sodium azide, four Bacillus species with the potential of degrading low-density polyethylene were isolated. The colonies appeared white or creamy or light grey with either smooth or rough surfaces. Analysis of the bio-chemical characteristics of the isolates indicated that they are gram-positive, motile, oxidase and catalase positive, but citrate, indole and methyl red negative. Similarly all the species are glucose, sucrose, maltose, galactose and mannitol fermenters, with acid production, while none of the isolates fermented lactose and sorbitol. However, only one of the isolates could ferment inositol. The similarities found in the morphological and biochemical characteristics of the isolates are strong indications of their being closely related, and these findings corroborated the American Society of Microbiology (2020) methods of identifying bacteria. Furthermore, based on the 16S rDNA sequencing, identities of the species were confirmed with their percentage similarities to similar Bacillus species at GenBank (http://www.lahey.org/studies/webt.html) as: Bacillus siamensis (with sequence nucleotide: GATGTTAGCGGCGGACGGGTGA, 100% simimila-rity and GenBank accession number OR763561); Bacillus subtilis (sequence nucleotide: TGGTAGTGTT AGGGGGTTTCCGC, 99.7% si- milarity and GenBank accession number KU740250); Bacillus coagulans (sequence nucleotide: GAATGCATTTATG CAACATGGGC, 100% similarity and MA820466 GenBank accession number and Bacillus pumilus with sequence nucleotide: GCAGTCGAGCGGACAGAA

GGGA, 99.86% similarity and OR243874 GenBank accession number). The mole-cular identification results gave credence to the morphological and biochemical results, and therefore confirmed that the isolates belong to the same genus.

Growth dynamics of the *Bacillus* spp. in lowdensity polyethylene suspensions

Results obtained from the incubation of the Bacillus spp. in MSV media with the sachet water materials as the sole source of carbon for 60 days showed gradual and steady increase in microbial density as indicated by the increase in absorbance values at OD600. As the period of incubation progressed, gas bubbles were observed in the culture media, while the turbidity increased. However, the control media remained clear without gas bubbles. These results corroborate the the findings of Azeko et al., (2013) and Ren et al., (2019). The highest absorbance (microbial density) was recorded in the medium with Bacillus siamensis (0.12 - 0.84), followed by B.pumilus (0.11 - 0.67), B.coagulans (0.12 - 0.61) and B.subtilis (0.11 -0.60) (Ta ble 1). The observed air bubbles, turbidity and increase in microbial densities in the media inoculated with the test isolates were indications of microbial ac-

tivities, and their ability to adapt to the nutrient conditions of the media, degrade and utilize the polyethylene materials as their sole source of carbon. It could therefore be inferred that the isolates adapted to, and switched over to the nutrient contents of the culture media, hence adaptability is one of the key factors for biodegradation. These re-sults corroborateed the findings of Azeko et al., (2013); Ren et al., (2019); Emmanuel-Akereleet et al., (2022) and Esamahy et al., (2023) who employed different microorganisms in polyethylene degradation. However the varying absorbance rates showed that the different Bacillus spp. possess different capabilities of degrading polyethylene, and this could be attributed to the sources of isolation, environmental factors and the genetic make-up of the species. The organisms were isolated from different dumpsites with differences in their coordinates and components: B.siamensis and B.subtilis (Nekede Mechanic village), B.pumilus (Orji) and B.coagulans (Obinze) in Owerri Metropolis. Similar observations and assertions were made by Skariyachan et al., (2017); Biki et al., (2021) and Kopecka et al., (2022) in their separate works on microbial degradation of polymers.

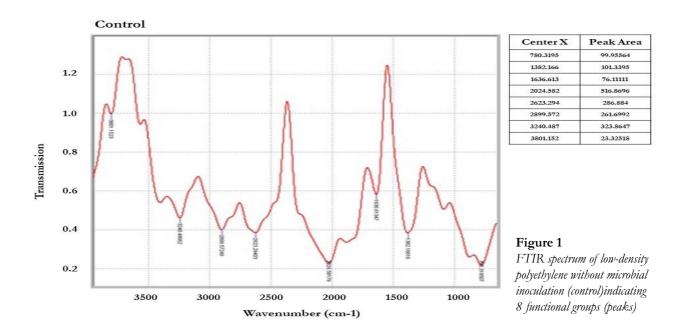
Isolates	Dates Time in days						
	0	10	20	30	40	50	60
B. pumilus	0.11 <u>+</u> 0.00	0.12 <u>+</u> 0.09	0.18 <u>+</u> 0.37	0.32 <u>+</u> 0.23	0.56 <u>+</u> 0.07	0.62 <u>+</u> 0.28	0.67 <u>+</u> 0.0
B.subtilis	0.11 <u>+</u> 0.00	0.11 <u>+</u> 0.07	0.27 <u>+</u> 0.11	0.34 <u>+</u> 0.90	0.50 <u>+</u> 0.09	0.57 <u>+</u> 0.00	0.60 <u>+</u> 0.3
B. siamensis	0.12 <u>+</u> 0.00	0.12 <u>+</u> 0.00	0.27 <u>+</u> 1.05	0.45 <u>+</u> 0.33	0.70 <u>+</u> 0.33	0.77 <u>+</u> 0.08	0.84 <u>+</u> 0.0
B. coagulans	0.12 <u>+</u> 0.00	0.12 <u>+</u> 0.06	0.23 <u>+</u> 0.16	0.38 <u>+</u> 0.11	0.51 <u>+</u> 0.29	0.53 <u>+</u> 0.09	0.61 <u>+</u> 0.2
Control	0.11 <u>+ 0</u> .00	0.11 <u>+</u> 0.01	0.12 <u>+</u> 0.11	0.11 <u>+</u> 0.05	0.11 <u>+</u> 0.21	0.11 <u>+</u> 0.00	0.11 <u>+ 0.0</u>

Table 1. Mean growth of Bacillus spp. (Absorbance atOD600) following exposure to low-density polyethylene for 0 - 60 days

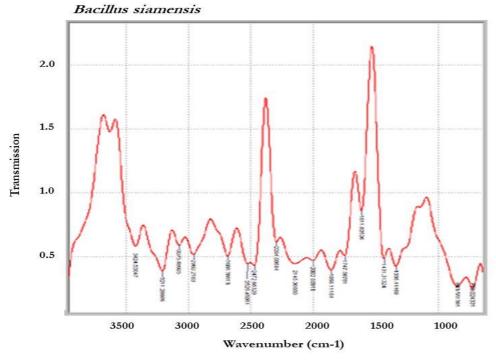
Structural changes in the low-density polyethylene due to exposure to the *Bacillus* spp

At the end of the sixty (60) days of exposure of the low-density polyethylene, to the test isolates, analysis of the chemical structure of the polyethylene with FTIR spectroscopy revealed that in the control sample which was not exposed to microbial inoculation, there were 8 peaks (function groups) comprising ethane (1362.166 cm⁻¹), amine (1636.613 cm⁻¹), carboxylic acid (2024.583cm⁻¹), methylene (2623.294cm⁻¹ and 2899.527cm⁻¹) and alcohols (3240.487cm⁻¹ and 3801.152 cm⁻¹), as shown in Figure 1. However, in the

polyethylene materials exposed to the different *Bacillus* species, increased number of peaks were recorded, suggesting introduction of new functional groups or increase in the number of existing groups due to microbial actions. The FTIR spectrum of the polyethylene surface inoculated with *Bacillus siamensis* recorded 17 peaks (functional groups), *B. subtilis* and *B. coagulans*, 13 each and *B. pumilus* 12. There were additional carbonyl groups which included esters, ethers, carboxylic acids and amines (1600cm⁻¹-2199cm⁻¹), nitriles (2467cm⁻¹ - 2597cm⁻¹), alcohols comprising primary, secondary and tertiary alcohols (3000cm⁻¹ -



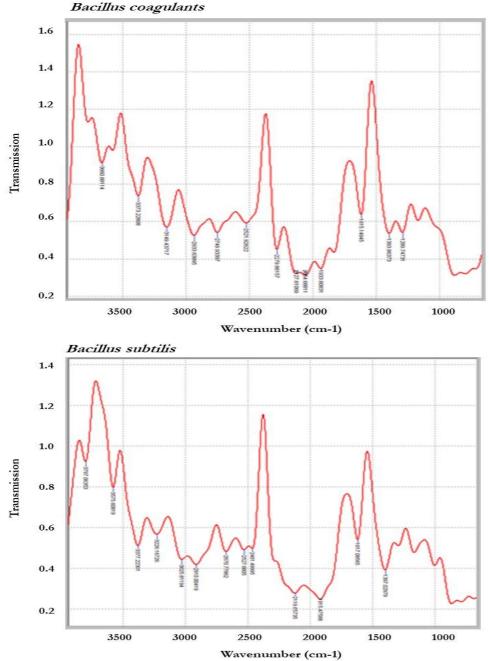
 3500 cm^{-1}), alkene ($1200 \text{ cm}^{-1} - 1599 \text{ cm}^{-1}$) and methylene ($2600 \text{ cm}^{-1} - 2999 \text{ cm}^{-1}$) as presented in Figures. 2 – 5. The additional functional groups are byproducts of oxidative degradation of the lowdensity polyethylene materials by the *Bacillus* species, with the carbonyl compounds being points of cleavage in PE degradation. Similarly the increased numbers of ethenes and methylene were an indication of the breaking down of the complex structure of the polyethylene to smaller units, resulting in the formation of smaller molecules of short-chained oligomers, dimmers and monomers of low molecular weights, which can pass through the semi-permeable outer membrane of microbial cells, and then be utilized as energy and carbon sources. These results agreed with the findings of Hadad et al., (2015) and Ren et al., (2019); Srikant et al., (2022) and Ugueri et al., (2022) who recorded the formation of new



Center X	Peak Area 22.5602	
719.0243		
843.5614	82.41958	
1335.445	2111.2549	
1431.313	125.7181	
1611.825	111.4555	
1747.588	15.98983	
1856.111	390.3767	
2002.609	179.7694	
2145.381	401.8579	
2294.006	25.23037	
2472.683	128.5582	
2525.451	149.1887	
2686.286	7251.924	
2960.218	239.238	
3075.486	118.3603	
3211.287	270.6248	
3424.538	189.3183	

Figure 2

FTIR spectrum of LDPE incubated with Bacillus siamensis showing 17 peaks (functional groups)



Center X	Peak Area	
1289.747	25.91511	
1393.864	70.16718	
1615.149	81.07222	
1933.608	225.8559	
2054.688	122.1303	
2127.611	127.1059	
2279.862	104.0252	
2521.926	172.4828	
2749.934	177.2707	
2933.639	209.0282	
3149.437	187.0556	
3373.228	129.4428	
3660.881	139.4439	

Figure 3

FTIR spectrum of LDPE exposed to Bacillius coagulans with 13 peaks (functional groups)

······	Center X	Peak Area
	1397.03	65.02322
	1617.086	45.36264
······	1915.476	226.662
	2119.657	226.8666
	2461.500	51.12382
<u> </u>	2527.681	69.03948
0	2670.779	102.45
Λ	2910.004	170.3024
	3025.812	113.6746
	3226.167	109.9265
	3377.223	132.315
	3575.608	51.89232
A	3797.064	14.02707
4000 100-	Figure 4 FTIR spectra inoculated with	h Bacillus
1500 1000	substilis indice (functional gre	ating 13 peaks oups)

functional group in polymer structures after exposure to different microbial isolates for varying periods of time. The degradation processes are enzymatic, involving extracellular enzymes which depolymerize synthetic polymers (cleavage) to give rise to the formation of additional functional groups, and intracellular enzymes (depolymerases) which breakdown the smaller units into absorbable forms which are utilized by the microorganisms for energy release. This assertion corroborates the findings of Novotny et al., (2018); Nag et al., (2021); Prajapati et al., (2021)., Zhang et al., (2022) and Devi et al., (2023)

who identified such enzymes as lipases, hydrolases, carboxylmethyl cellulase (CMCase), xylanases, proteases, oxidases and peroxidases in their separate works on biodegradation of plastics. Further evidences of PE degradation by the isolates were the presence of more stretching and asymmetrical vibrations observed in carboxylic acid, ether, nitrle, amines, ethane and ester bands, as well as weak methylene bands in all the PE samples inoculated with the Bacillus spp. Similar observations were also recorded by Ibiene et al., (2013); Khandare et al., (2021) and Lin et al., (2022). The vibrations and bending could be attributed to

Peak Area

76.03855 61.9371

40.0118

19.11066 175.75

364.6644

174.6521

119.3712

226.6266

136.791 151.4937

25.52436

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the weakening of the chemical structure of the PE due to microbial activities, suggesting their ability to degrade and utilize the PE as their sole source of carbon and energy. Furthermore, exposure of polyethylene to the test isolates resulted in the release of various chemical compounds including alcohols, carboxyl compounds, esters, benzene, methylene, paraffins, xylene, ethane, toluene and various others of medical, pharmaceutical, agricultural and industrial uses, as also recorded by Prajapati et al, (2021); Lin et al., (2022); Ni et al., (2022).

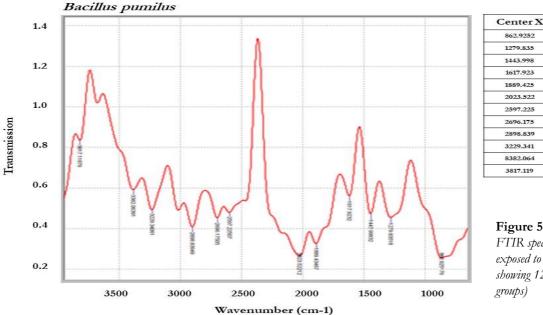


Figure 5 FTIR spectrum of LDPE exposed to Bacillus pumilus showing 12 peaks (functional

Percentage weight loss in LDPE due to microbial actions

After the 60 days incubation of LDPE with the *Bacillus* species, results obtained showed reduction in net weight of the polyethylene materials, with *Bacillus siamensis* recording highest value (52%), followed by *B.pumilus* (26%) and *B.coagulans* and *B.subtilis* (18%) respectively, while no change was observed in the control sample not exposed to microbial attack, as shown in table 3. The decrease in the net weight of the residual polyethylene materials confirmed the results obtained from microbial growth and FTIR analyses, and the ability of the *Bacillus* species to utilize the polyethylene as their sole source of carbon and energy. The organisms exhibited different degrees

.in their ability to degrade polyethylene. . Similar results were recorded by Nanda et al., (2010) who used 3 strains of *Pseudomonas* P1 (31.4% and 16.3%), P2 (39.7% and 19.6%) and P3 (46.2% and 29.1%) to degrade natural and synthetic polyethylene, Ibiene et al., (2013) using *Bacillius subtilis* (17.72% and 23.155) and *Bacillius mycoides* (10.50% and 11.35%) to degrade HDPE and LDPE, Jaina et al., (2023) who degraded polyethylene with different bacterial species coded P1 (50%), P2 (-) and P3 (33.3%) and Aziza et al., (2024) who recorded varying degrees of net weight reduction in residual plastic material after exposure to various microorganisms which include *Vibrio alginolyticus* (0.4%), *Pseudoalteromonas* (4.9%), *Microbulbifer pacificus* (4.2%), *Pseudomonas marincola* (6.3%), and *Bacillus*

	Initial weight before	Final weight after 60 da	-	
Isolates	incubation	Mean Value $(n = 3)$	% wt Loss	
B. pumilus	0.5	0.37±0.05	26%	•
B. subtilis	0.5	0.41 ± 1.08	18%	
B. siamensis.	0.5	0.24 ± 0.09	52%	Table 2
B. coagulans	0.5	0.41 ± 0.00	18%	Mean and 1 Loss in LDP1
Control	0.5	0.50 ± 0.00		Loss in LDP1 Incubation with

Mean and Percentage Weight Loss in LDPE after 60 Days of Incubation with Bacillus Spp subtilis (5.5%) The differences recorded in the polyethylene-degradation capabilities of the microbial isolates could be attributed to the variations in enzyme and biofilm syntheses, species-specific characteristics, environmental factors prevalent in their sources of isolation, biosulfactants and reactive oxygen species production, period of incubation and genetic constitution and adaptability (Prajapaty et al., 2021; Lin et al., 2022; Ni et al., 2022., Uwakwe et al., 2023; Aziza et al., 2024). In this present study, Bacillus siamensis was found to possess higher level of competence in polyethylene degradation, and this is a pointer to its greater rate of adaptation, through genetic mutation, to the changes in its environment and nutrition from the dumpsite to mineral salt vitamin medium used for incubation

Conclusions

Bacillus species indigenous to Owerri Metropolis and isolated from dumpsites were able to degrade lowdensity polyethylene, hence their ability to utilize it as a sole source of carbon, as depicted by the increased microbial densities, functional groups and decrease in the net weight of the residual LDPE materials after the 60 days of incubation. Based on these findings, It was concluded that biodegradation of low-density polyethylene was achieved, disabusing the generally accepted impression in Imo State that polyethylene and other plastic products were non-biodegradable. Bacillus species exhibited varying degrees of biodegradative competence, with Bacillus siamensis being identified as the most effective for degrading low-density polyethylene. The organisms exhibited the same pattern of growth with minimal increase in bacterial densities within the first 20 days of incubation, and significant increase subsequently, indicating their adaptation to changes in their environment and nutrition. It was further concluded that biodegradation of polyethylene resulted in the release of chemical compounds such as alcohols, acids, amines, esters, ethane, methylene, nitriles, ethers, benzene and various other chemical compounds which could be utilized in the medical, pharmaceutical and various industrial establishments for variable uses, making the process a waste-towealth scheme that could be embarked upon for economic growth and development. It was therefore recommended that indigenous microbial species be exploited and employed in polymer waste manage-.

ment, and the strategies be optimized for maximal outcome and to minimize environmental pollution and health hazards associated with polymer disposal and improper management.

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