

Value recovery from plastic wastes via biodegradation of low-density polyethylene by *Aspergillus*, *Proteus* and *Serratia* species

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Abstract

Daily production and release of enormous quantities of plastic wastes into the environment are on the increase. Being recalcitrant to degradation, they are known to constitute a waste burden to cities and municipalities as they accumulate in the different environmental media. The search of remedies for plastic accumulation and pollution of our Environment and the possibility of recovering value from plastic wastes via the biodegradation potentials of indigenous microorganisms prompted this research work. Using standard microbiological techniques, 3 microorganisms, *Aspergillus sydowii*, *Proteus vulgaris* and *Serratia marcescens* were isolated from different dumpsites in Owerri West Local Government Areas (LGA), Imo State, Nigeria. Incubation of shredded and ground low-density polyethylene sachets with the test isolates was done in mineral salts vitamin medium (MSVM) for 60 days, and their ability to degrade the polyethylene was evaluated using visible spectrophotometer (OD600), fourier transform infra-red spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS) techniques. Results obtained indicated increase in optical densities (OD600) in the media inoculated with the test isolates: *A. sydowii*, (0.12 – 0.80), *P. vulgaris* (0.11- 0.71) and *S. marcescens* (0.10- 0.69), while the control sample without microbial inoculation remained unchanged. Similarly, the number of functional groups and compounds eluted was low in the control sample (8 and 11), but increased and differed in the three organisms: *A. sydowii* (11 and 44), *P. vulgaris* (14 and 48) and *S. marcescens* (12 and 38) respectively, indicating that the organisms exhibited varying degrees of competence in polyethylene degradation. It was concluded that Indigenous microorganisms were able to degrade low-density polyethylene, and that biodegradation of polyethylene resulted in the release of a variety of chemical compounds with great potentials applicability in various industries. The reactions involved in this biodegradation could be further studied and optimized as a waste-to-wealth strategy towards sustainable environmental management.

Keywords: *biodegradation, polyethylene, industrial chemicals, microorganisms, value recovery*

Introduction

One of the derivatives of the petroleum industry is pla-

stic. Global plastic production and usage are on the increase, and due to short-term use as wraps, bags, gar-

ment coverings, disposables, packaging materials, toys and various other uses, there is equally an increase in the production of plastic wastes, majority of which are discarded in open dumpsites, with only an insignificant amount being recycled (Lungo et al., 2011; Mohan and Suresh, 2015; Chamas et al., 2020; Ni et al., 2022). Of great concern in this regard is the pollution from these petroleum hydrocarbon derivatives, especially polyethylene, which is the most commonly used plastic product, particularly with respect to its recalcitrant nature. Consequently, plastic wastes are found littering every space, causing blockage of gutters/drainages and waterways, flooding, erosion of lands and environmental degradation, thus creating serious wastes management challenges. It is therefore not surprising that mankind has classified plastics as one of worst inventions ever made (Bhardwaj et al., 2012; Yoon et al., 2012; Ebciba and Gnanamani, 2020; Biki et al., 2021). Pollution of the various environmental media gives rise to massive ecosystem effects, ranging from negative changes on nature's climatic system to sundry effects on biota (flora and fauna) and other ecological entities (Monteiro et al., 2018; Ren et al., 2019). Ultimately, man becomes the major recipient of these effects given that his air, water, soil, vegetation, food and raw material sources are terribly impacted upon, and his agriculture and other productive efforts frustrated. Although from the industrial and economic standpoint, man could be said to have met his needs for making plastics, however, he has authored a nuisance of significant magnitude, which requires immediate abatement to save him, his environment and the entire ecosystem. Sundry exposures to plastic waste and its improper management methods have been incriminated in various health problems such as immune disorder, lung diseases, inflammation and heart diseases; thus, they are therefore classified as possible human carcinogens (Monteiro et al., 2018; Kim et al., 2020; Kopecka et al., 2022). Similarly, increasing presence of microplastics, large fragmented pieces of plastics called nurdles and many highly toxic pollutants that accumulate in plastics have been detected in a number of aquatic animals, while lots of them, including fishes and seabirds die of waste plastics; either by being caught in the waste plastic trap or by swallowing the plastic debris. Bye-products of their metabolism can be harmful to man and other animal who get them via food chain by interfering with the body's physiological processes (Helden, 2010; Yoon et al., 2012; Kim et al., 2021). Different solid waste management methods have been adopted in different

countries in handling plastic wastes, however the overall outcomes were found to be inefficient. Thus, the need for remedies for plastic accumulation and pollution of our environments, and the possibility of recovering value from plastic wastes using indigenous microorganisms prompted this research work. The specific objectives of this study were to extract indigenous microorganisms capable of utilizing polyethylene plastics as sole source of organic carbon for energy from local waste dumpsites, using standard microbiological techniques, evaluate the ability of the microbial isolates to degrade polyethylene plastics and evaluate the release of chemical compounds as biodegradation products. Recovering values from waste plastic materials through microbial degradation could serve as parts of waste management and waste to wealth strategies of ridding our environments of plastic wastes.

Materials and Methods

Isolation of low-density polyethylene degrading microorganisms

Low-density polyethylene powder used in microbial isolation was obtained from Green Pasture Polyethylene Company, Port Harcourt, Rivers State, Nigeria, while soil samples which served as sources of microorganisms were collected from different dumpsites located in Avu and Obinze mechanic village in Owerri West Local Government Area, Imo State, Nigeria. Isolation of low-density polyethylene degrading microbes was achieved using mineral salt medium (1.0g NH_4NO_3 , 0.2g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0g K_2HPO_4 , 0.1g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.15g KCl, 0.1g yeast extract and 1.0mg of each of the following microelements: $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and MnSO_4) containing 2 g of polyethylene powder, and 2 ml of each soil suspension from the dumpsites. However, another set up containing mineral salt and polyethylene powder was prepared. Incubation was in a rotary shaker at 37°C and 150 rpm for 4 weeks. (Gilan et al., 2004; Azeko et al., 2015; Bardaji et al., 2019). Microbial growth was evaluated using UV visible spectrophotometer at 600nm (OD600). Subculturing was done using Nutrient agar, MacConkey agar and Sabouroud dextrose chloramphenicol agar. Colonies with higher exponential growth were selected and pure cultures were maintained in agar slants

Identification of the Isolates

Biochemical and molecular parameters were employed in the identification of the microbial isolates. The biochemical characteristics considered were Gram-stain-

ning, motility, citrate utilization, catalase, oxidase, Methyl Red and sugar fermentation tests as described by Cheesbrough, (2006) and American Society for Microbiology (ASM), (2020) and Jaina et al., (2023). Similarly, molecular identification was achieved by extracting the genomic DNA of each isolate using genomic DNA isolation kit (Numbers 24700, 24750 and 24770), obtained from Norgen Biotek Corporation, Canada. Amplification of the 16S rDNA of the isolates was achieved using universal primers, and the resulting nucleotide sequences were compared with those of related organisms at GenBank (<http://www.lahey.org/studies/webt.html>) using the Basic Local Alignment Search Tool (BLSAT) as proposed by Ogbulie and Nwakamma, (2015) and Khan et al., (2018).

Low-density polyethylene biodegradation assays

Table (pure) water sachets (low-density polyethylene) obtained from Holy Family table water production factory in Akwakuma, Owerri North LGA, Imo State was used for the biodegradation assays. Incubation of the shredded, ground and sterilized low-density polyethylene sachets with the test isolates in mineral salts vitamin (MSV) medium (1.0g (NH₄)₂ SO₄, 1.0g KH₂PO₄, 8.0g K₂HPO₄, 0.2g MgSO₄ · 7H₂O, 0.1g NaCl, 0.02g CaCl₂ · 2H₂O, 0.01g FeSO₄, 0.5mg Na₂M₁₀O₄ · 2H₂O, 0.5mg MnSO₄, 0.2ml inositol, 0.2mg p-amino benzoic acid, 0.4mg pyridoxine, 2.0µg thiamine, 2.0µg biotine and 120.5µg vitamin B12) was in a rotary shaker at 37°C and 150 rpm for 60 days (Patil and Bagde, 2015). Their ability to degrade the polyethylene and the release of degradation products was evaluated using microbial density measurement, changes in functional groups and chemical compounds in the polyethylene structure.

Measurement of microbial density in liquid medium

Microbial culture of each test isolate was prepared, and 1ml of 10⁻¹ dilution was seeded into different conical flasks containing 100 ml of the mineral salt vitamin media and 0.5g of the polyethylene materials which served as the sole source of carbon. Incubation of the various media was in a rotary shaker at 37°C at 150 rpm for 60 days. Growth of the isolates was monitored by measuring the microbial density (absorbance) in each microbial suspension using visible spectrophotometer at 600nm (OD₆₀₀) at the interval of 10 days (Ren et al., 2019; Beal et al., 2020; Rawat and Dewan, 2022).

Assessment of biodegradation of low-density polyethylene using changes in the functional groups

Changes in the chemical structure of the polyethylene materials following exposure to the test isolates were

investigated using Fourier Transform Infra-Red Spectroscopy (FTIR). At the end of the 60 days of incubation of the polyethylene materials with the test isolates in the mineral salt vitamin medium, the residual polyethylene materials were recovered, dried at room temperature and changes in the chemical structures determined through the disappearance or formation of additional functional groups (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.

Determination of changes in the chemical compounds in the low density polyethylene materials

Further investigation into the ability of the test isolates to degrade low-density polyethylene was done by determining the changes in the chemical compounds present in the polyethylene materials and their concentrations using Gas Chromatography-Mass Spectrometry (GC-MS) (Mohanray et al., 2017; Ren et al., 2019). The GC-MS analysis of compounds in the different polyethylene suspensions was done using Agilent Technologies GC system, Santa Clara, CA, USA (GC-7890A/MS-5975C model) equipped with HP-5MS column. Degradation products (chemical compounds) and their quantities eluted were detected and identified based on GC retention time (Prajapati et al., 2021). Control samples constituted suspensions without microbial inoculation.

Results and Discussion

Isolation and identification of low-density polyethylene degrading microorganisms

Following the 4-week incubation of the different soil samples and the polyethylene powder in mineral salt medium, and sub-culturing in Nutrient agar, MacConkey agar and Sabouroud dextrose chloramphenical agar, three isolates which displayed better growth trends in the media with polyethylene as the sole source of carbon were screened. Based on morphological and biochemical characteristics, and confirmed by molecular analysis based on 16S rDNA sequence alignment of BLAST, the isolates were identified as *Aspergillus sydowii*. (sequence nucleotide: AGCGGGGMATGCCTCCG GCGCG, percentage similarity: 99.23% and GENBank accession number: MZ400589.1), *Proteus vulgaris* (sequence nucleotide: TAATTGAAGAGTTTGATCAT GGC, percentage similarity: 99.87%, GENBank accession number: J01874.1) and *Serratia marscescens* (sequence nucleotide: ACAGACCGTTATGGTATCCAAG, percentage similarity: 100% and GENBank accession

number: CP026383.I). All the isolates grew on the media containing mineral salt, soil and polyethylene powder. However, only *Aspergillus sydowii* grew in the medium containing only mineral salt and polyethylene powder where it showed more profuse growth than in the later. Since the mineral salt medium used in inoculation was sterilized before incubation, it could be inferred that the polyethylene powder was the source of the organism in the medium containing only the mineral salt and polyethylene. *A. sydowii* could therefore be regarded as a contaminant on the polyethylene powder, and was able to survive and multiply by degrading and utilizing it as a carbon source.

Growth dynamics of the microbial isolates in low-density polyethylene suspensions

Exposure of the test isolates to the shredded and ground sachet water materials which served as the sole source of carbon in the MSV media for 60 days showed gradual and steady increase in microbial densities as indicated by the increase in optical density (OD600) of the media. As the period of incubation progressed, the culture media inoculated with the test organisms became turbid, and gas bubbles were observed. However, the control medium without microbial inoculation remained clear without gas bubbles. The highest microbial density was recorded in the medium inoculated with *Aspergillus sydowii* (0.12 - 0.8), followed by *Proteus vulgaris* (0.11 - 0.71) and then *Serratia marcescens* (0.1 - 0.69), (Table 1). The observed air bubbles, increase in turbidity and microbial densities in the media inoculated with the test isolates were indications of microbial metabolic activities, and their ability to adapt to the nutrient conditions of the media, degrade and utilize the polyethylene materials as their sole source of carbon for energy synthesis. These results were in agreement with the findings of Azeko et al., (2013); Ren et al., (2019); Emmanuel-Akerele et al., (2022) and Esamahy et al., (2023) who employed *Serratia marcescens subsp. Marcescens*, *Enterobacter* DI and

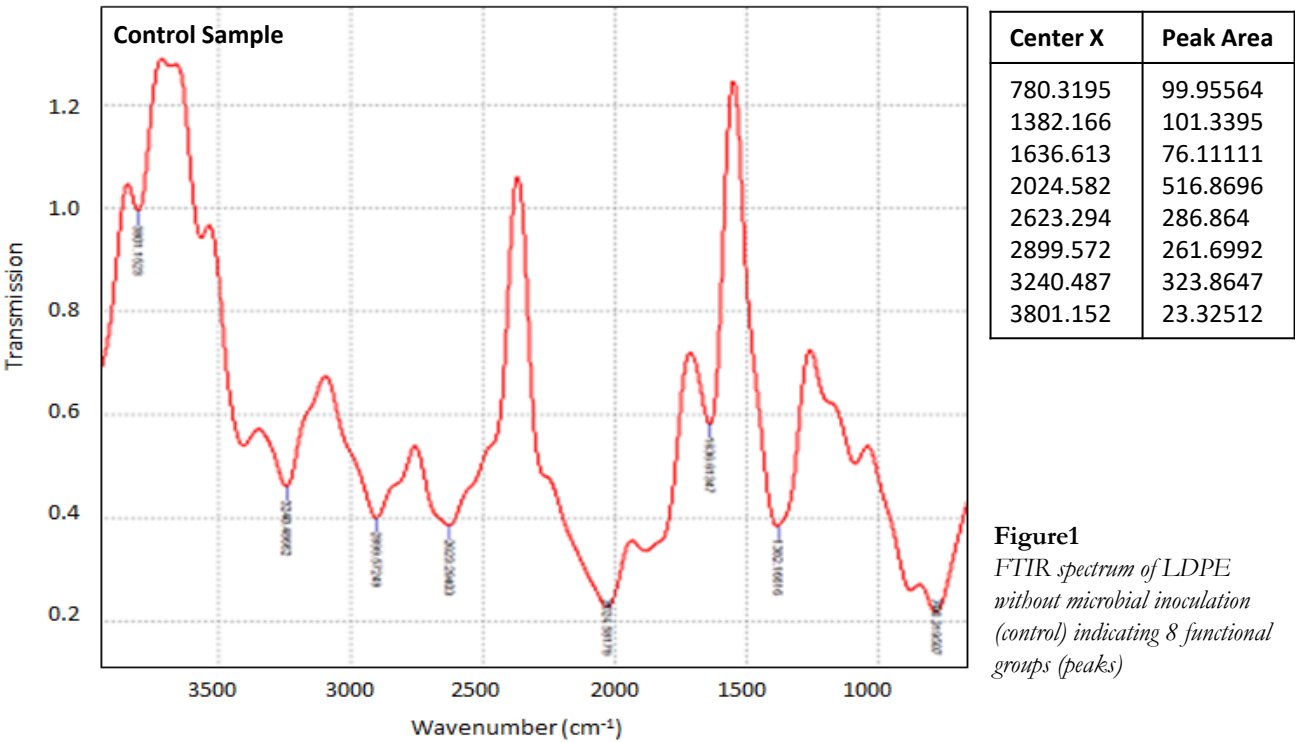
yeast consortium in polyethylene degradation. It could therefore be inferred that the test organisms were able to adapt to the culture media as their new environment, switched over to its nutrient contents, grew and multiplied, hence adaptability is one of the key factors affecting biodegradation process (Uwakwe et al., 2025). The adaptive capabilities of the isolates accounted for their survival and the differences in their microbial densities in the culture media (MSV) with polyethylene as the only source of carbon, maintained in similar conditions for 60 days. There was no significant increase in the microbial densities between zero day and 20 days, showing that the isolates experienced a lag phase resulting from adjustments to changes in temperature, pH, nutrient composition, and oxidative stress in their media, as well as repair of DNA damaged by environmental stressors, and the need to synthesize protein to adapt to the new environment and grow. 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. Achieving these adjustments was therefore responsible for the significant increase in the microbial densities from 30 days to 60 days of incubation. From the results, it could be inferred that *Aspergillus sydowii* exhibited the highest level of adaptation, hence the highest microbial density (0.80) at the end of the incubation period.

Changes in the chemical structure and compounds in the low-density polyethylene due to exposure to the test organisms

FTIR spectra of the control sample not exposed the test isolates revealed 8 peaks (function groups) comprising ethane (1362.166 cm⁻¹), amine (1636.613 cm⁻¹), carboxylic acid (2024.583 cm⁻¹), methylene (2623.294 cm⁻¹ and 2899.527cm⁻¹) and alcohols (3240.487cm⁻¹ and 3801.152 cm⁻¹). However, in the polyethylene materials exposed to the test isolates, increased numbers of peaks were recorded, suggesting introduction of new

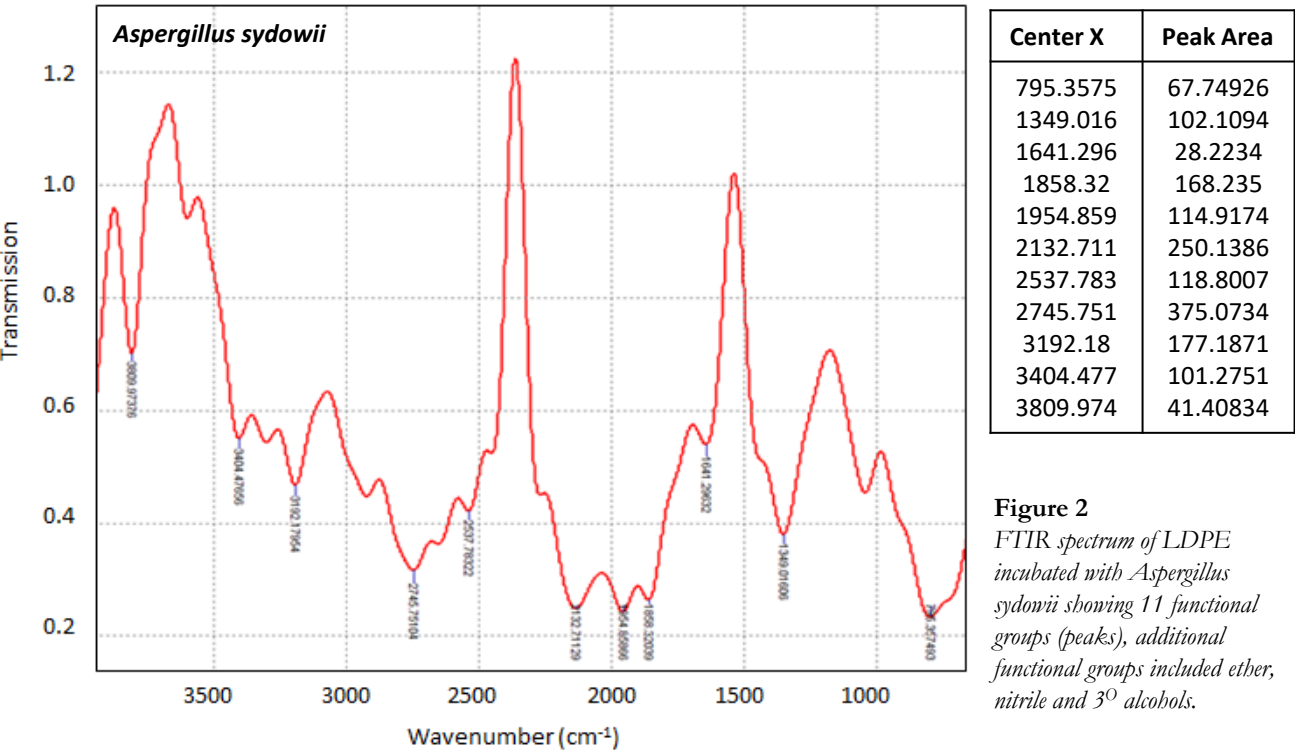
Table 1. Mean growth of microbial isolates (Absorbance atOD600) following exposure to low-density polyethylene for 0- 60 days

Isolates	Time in days						
	0	10	20	30	40	50	60
Aspergillus sydowii	0.12 ±0.22	0.12 ±0.03	0.21 ±0.11	0.45 ±0.16	0.66 +0.06	0.71 ±0.05	0.80±0.01
Proteus vulgaris	0.10 ±0.13	0.12 ±0.15	0.24 ±0.03	0.38 ±0.07	0.59 +1.02	0.69 ±0.11	0.71±0.03
Serratia . marcescens	0.11 ±0.06	0.12 ±0.11	0.19 ±0.04	0.38 ±0.06	0.57 +0.01	0.59 ±0.15	0.69±0.06
Control	0.11 ±0.04	0.11 ±0.01	0.12 ±0.11	0.11 ±0.05	0.11 +1.01	0.11 ±0.00	0.11±0.01



functional groups or increase in the number of existing groups due to the action of the microbial isolates. There were additional chlorine (884.2887 cm⁻¹), carbonyl groups which included, esters, ethers, carboxylic acids and amines (1600 cm⁻¹-2199 cm⁻¹), nitriles (2467 cm⁻¹ - 2597cm⁻¹), alcohols which included primary, secondary and tertiary alcohols (3000cm⁻¹ - 3500cm⁻¹),

alkene (1200 cm⁻¹-1599 cm⁻¹), methylene (2600 cm⁻¹-2999 cm⁻¹) and thiocyanate (1915.205 cm⁻¹).The highest number functional groups was recorded in the polyethylene materials exposed to *Proteus vulgaris* (14), followed by *Serratia marcescens* (12) and *Aspergillus sydowii* (11), (Fig. 2, 3 and 4).



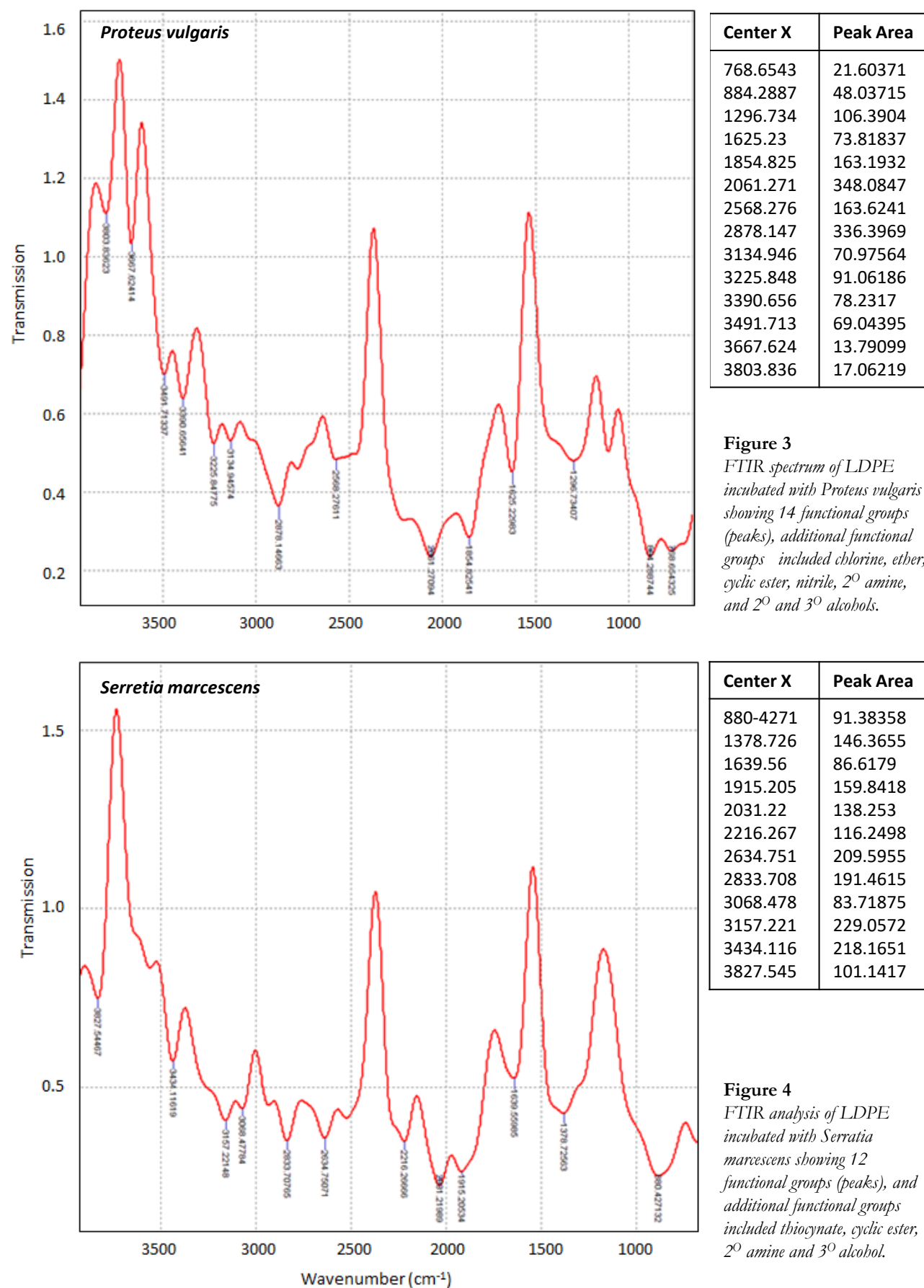


Figure 3
FTIR spectrum of LDPE incubated with *Proteus vulgaris* showing 14 functional groups (peaks), additional functional groups included chlorine, ether, cyclic ester, nitrile, 2° amine, and 2° and 3° alcohols.

Figure 4
FTIR analysis of LDPE incubated with *Serratia marcescens* showing 12 functional groups (peaks), and additional functional groups included thiocyanate, cyclic ester, 2° amine and 3° alcohol.

Similarly, analysis of the chemical compounds in the low-density polyethylene after exposure to the test organisms using GC-MS revealed the release of varying compounds of different compositions. The largest amount of compounds was eluted from the sample exposed to *Proteus vulgaris* (48), followed by *Aspergillus sydowii* (44) and *Serratia marcescens* (38) (Fig. 6, 7 and 8). However, only 11 compounds were eluted from the control sample which was not exposed to the microbial isolates (Fig. 5). All the compounds eluted from the

control sample were also present in the microbial exposed samples at higher amounts, except heptafluorobutyric acid, supraene and sarcosine. Eluted compounds common to all the samples exposed to the different isolates included dodecane and dodecanoic acids, hexadecane, octadecane and octadecanoic acids, eicosene, dibutyl phthalate, tetradecane, squalene, benzene compounds, docosene, oleic acids, phenol compounds, succinic acids, carbonic acids and esters. However, they differed in abundance, composition and retention times.

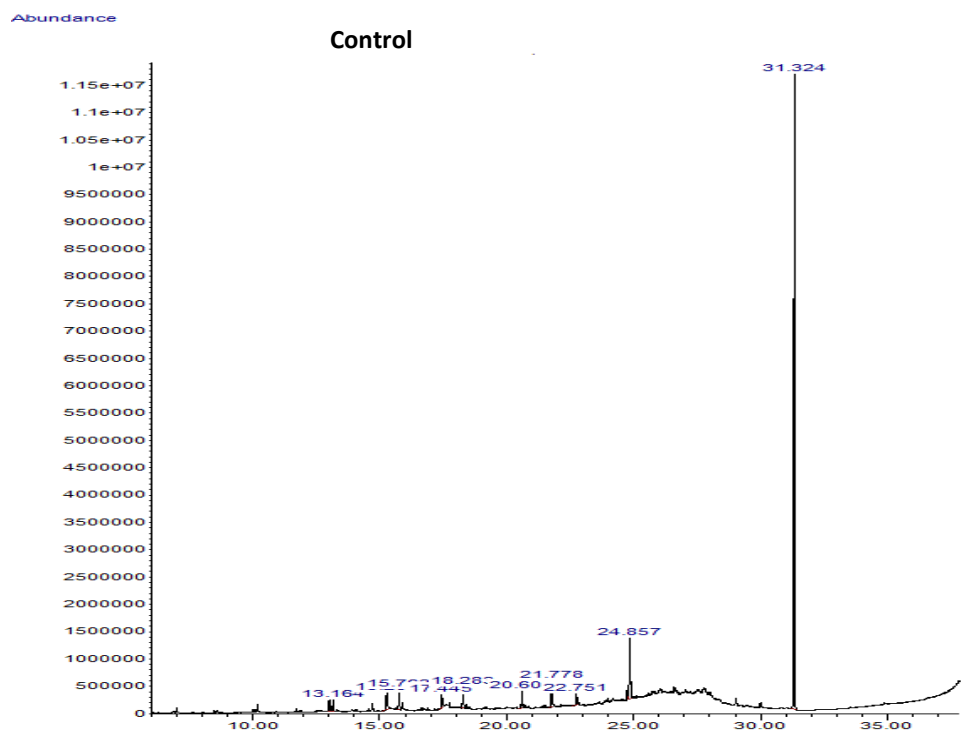
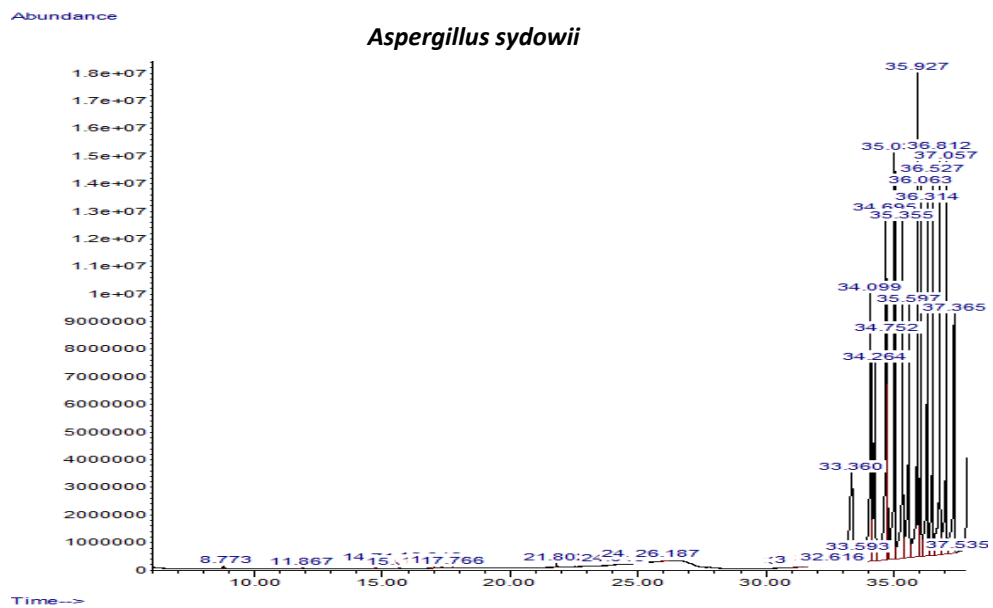


Figure 5
Chromatograph of the compounds in control sample indicating 11 compounds (peaks).



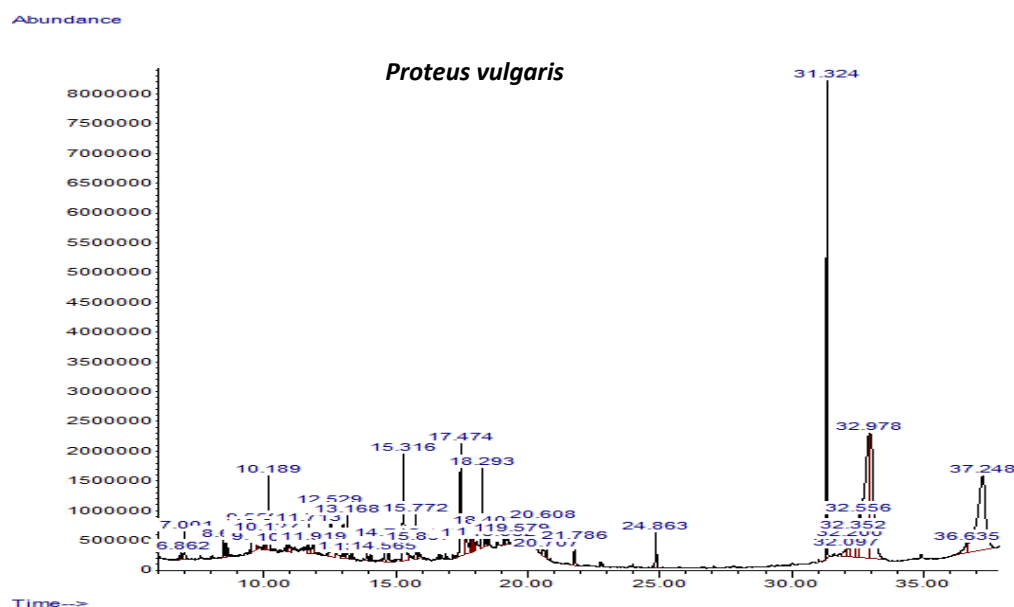


Figure 7
Chromatograph of LDPE exposed to *Proteus vulgaris* showing 48 peaks (48 compounds).

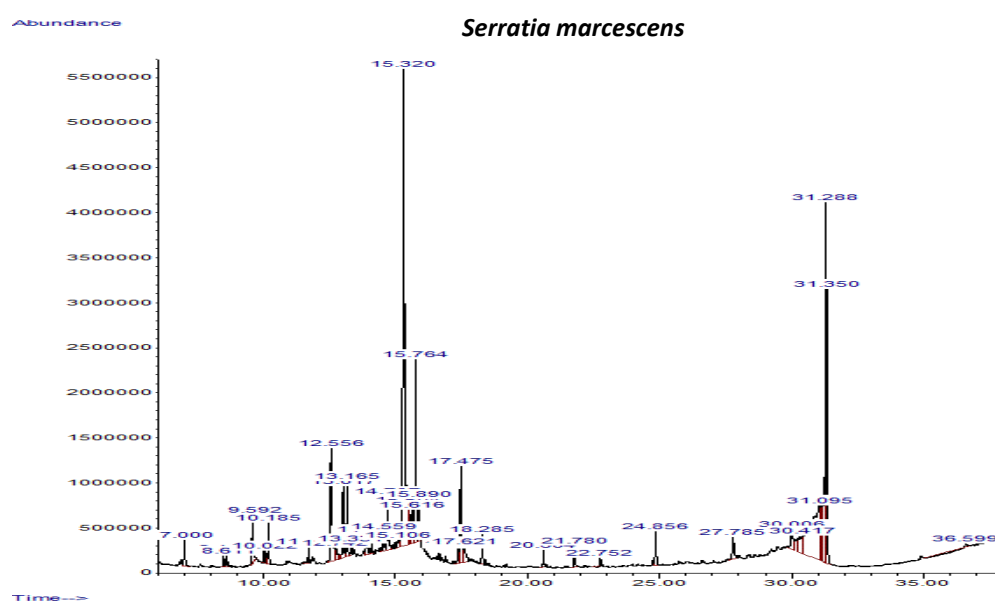


Figure 8
Chromatograph with 38 peaks indicating by-products (38 compounds) of degradation of LDPE by *Serratia marcescens*

Interaction of the indigenous microbial isolates with plastic entities resulted in the release of various functional groups indicative of various chemical compounds, which included different types of acids, alcohols, amines, cyclic esters, nitrile, methylene, ethane and benzene, phenol compounds, hexadecane, eicosene, dibutyl phthalate, dodecane and various others. These results agreed with the findings of Hadad et al., (2005); Ren et al., (2019); Srikant et al., (2022) and Ugueri et al., (2022), who recorded the formation of new functional groups and eluted compounds in polymer structures following exposure to different microbial isolates for varying periods of time. The release of the compounds was attributed to the degradation of

the polyethylene by the microbial isolates. The number of functional groups and compounds eluted differed in the three organisms: *Aspergillus sydowii* (11 and 44), *Proteus vulgaris* (14 and 48) and *Serratia marcescens* (12 and 38) respectively, indicating that the organisms exhibited varying degrees of competence in polyethylene degradation. The differences recorded in the polyethylene-degradation capabilities of the microbial isolates could be attributed to the variations in enzymes, species-specific characteristics, environmental factors prevalent in their sources of isolation, biosurfactants and reactive oxygen species production, and genetic constitution and adaptability (Prajapati et al., 2021; Lin et al., 2022; Ni et al., 2022., Uwakwe et al., 2023; Aziza et al., 2024).

That the control (without any deliberate microbial inoculation) recorded the lowest functional groups and eluted compounds (6 and 11 respectively), was a further proof of the ability of the microbial isolates to degrade and utilize low-density polyethylene as a source of carbon and energy. The additional compounds are therefore oxidative degradation by products of the low-density polyethylene materials by the microbial isolates 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 smaller molecules of short-chained oligomers, dimers 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 (Ren et al., 2019; Chen et al., 2020; Brandson et al., 2021; Uwakwe et al., 2025). The degradation processes are enzymatic, involving extracellular enzymes which depolymerize synthetic polymers 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, carboxymethyl cellulase (CMCase), xylanases, proteases, oxidases and peroxidases in their separate works on mechanism of plastic biodegradation. These chemical compounds are of variable utilities in medical and pharmaceutical industries as cleaning agents, antiseptics, wound dressing agents and preservatives; agricultural and food establishments for production of fertilizers, insecticides, additives and preservatives; cosmetic Industry for the manufacture of cosmetics, solvents, dyes, inks, paint remover as well as in petrochemical, biotechnological and other industrial establishments for the synthesis of lubricants, transformer oil, anticorrosion agents, emulsions, fragrance, surfactants, textiles, paper, leather and several other products, making polymer biodegradation a value recovery and waste to wealth strategy.

Conclusion and Recommendation

Indigenous microorganisms were able to degrade low-density polyethylene, hence their ability to utilize it as a sole source of carbon for energy, and the degrees of biodegradation competence varied based on microbial

densities, number of functional groups and eluted compounds. Biodegradation of polyethylene resulted in the release of a variety of chemical compounds with great potentials as industrial chemicals with versatile applicability in various sectors of the economy. It was therefore recommended that the reactions involved in this biodegradation be further studied to validate the optimization conditions, so that sourcing of industrial chemical feed stocks becomes coupled to biodegradation of plastic, thus serving as part of the strategies for management of plastic wastes and the likes, mopping up these from our environments-waste-to-wealth scheme towards sustainable environmental management for economic growth and development goals.

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