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Biodegradation of polyethylene by indigenous fungi from waste recycling site, South West, Nigeria

Temitope Oluwatosin Ayeni^{1*} , Daniel Juwon Arotupin¹ and Oisagah Ezekiel Ayo²

Abstract

Background: Indiscriminate disposal of polyethylene materials has become a regular practice among developing nations of Africa, especially in Nigeria. This has resulted in environmental pollution; hence, this study investigates the microbial degradation of polyethylene obtained from a polyethylene dumpsite in South West, Nigeria, under static in vitro condition. Soil samples were analysed for mineral composition and physicochemical characteristics. The fungal isolates were screened for polyethylene degradation using minimal salt medium containing polyethylene as sole source of carbon and nitrogen for their ability to degrade polyethylene. Gravimetric analysis and Fourier-transform infrared spectroscopy (FTIR) were used to monitor the biodegradation of the polyethylene.

Results: *Aspergillus flavus*, *A. nidulans*, *Penicillium chrysogenum*, *Mucor mucedo*, *Eurotium repens*, *A. fumigatus* and *Rhizopus stolonifer* were enumerated. Mean microbial count ranged from 1.37×10^7 to 8.2×10^8 SFU/g. Individual weight loss was observed in the polyethylene strip cultured with *P. chrysogenum* (1%), *E. repens* (1%) and *A. nidulans* (2%). The changes observed in the FTIR spectra especially the polyethylene sample inoculated with *A. nidulans* confirm the significant role of fungi in polyethylene degradation. Hence, its usage in the treatment of polyethylene in the environment is a cheap eco-friendly alternative.

Conclusion: *Aspergillus nidulans*, *E. repens* and *P. notatum* play significant roles in the biodegradation of polyethylene which necessitates incorporating in polyethylene products waste management to foster a cleaner environment.

Keywords: Degradation, Dumpsite, Fungi, Polythene, Soil

Background

Polyethylene (PE) constitutes 64% of the total synthetic plastic as it is being used in huge quantity for the manufacture of bottles, carrier bags, disposable articles, garbage containers, margarine tubs, milk jugs and water pipes (Alabi et al. 2019). Annually, 0.5 to 1 trillion PE bags are used all over the world (Biki et al. 2021). The durability, light weightiness and process ability of these polymers cause them to linger in nature for centuries and

end up in landfills and natural water resources creating a severe threat to the environment and its ecosystems (Dawoud et al. 2021).

Polyethylene littering of the environment is a common problem in most urban centres in Africa, because majority of its wastes are often not recycled (Gwada et al. 2019). PE bags have been reported to cause the death of terrestrial animals such as cow (Venkatesh et al. 2021). The degradation of most synthetic plastics in nature is a very slow process lasting thousands of years and involves synergistic action between environmental factors and microorganisms (Mohan et al. 2020). Microorganisms such as bacteria, fungi and algae are involved in the biodegradation of materials (Sarmah and Rout 2020).

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Microorganisms such as bacteria and fungi have been reported to be associated with biodegradation of PE (Yuan et al. 2020). Biodegradation involves a consortium of microorganisms, some of which breakdown the polymer into smaller constituents, while others utilize the monomers and release less toxic and recalcitrant by-products that serve as energy source for other microbial groups (Moharir and Kumar 2019). Fungi have hydrolytic enzyme system which produces hydrolases that help to degrade polysaccharides (Srivastava et al. 2021). Hence, this study was carried out to isolate and identify fungi from a polyethylene dumpsite in Akure, South West, Nigeria, in an effort to devise an environmentally friendly strategy for safe treatment of PE wastes and mitigation of environmental pollution.

Methods

Study design

This is an experimental study conducted between January and June 2016.

Description of study location

Ondo State lies between longitudes 4°30' and 6° East of the Greenwich meridian 5°45' and 8°15' North of the Equator. The State lies entirely in the tropics. Akure is the capital of Ondo State, and it is located in South-Western Nigeria. Akure lies between latitudes 7.25' N and longitude 5.195' E (Fig. 1). The study location was accessed through experimental design. These areas are moderately populated with moderate standard of living. Residents living in these areas are majorly farmers, teachers, traders, civil servants, legal and medical practitioners.

Collection of samples

Polyethylene and soil samples (depth of approximately 10 cm) were collected from the Ondo State Integrated Wastes Recycling and Treatment Project (OSIWRTP) located along Igbatoro Road, Akure in Ondo State, Nigeria.

Isolation and Identification of Fungi

A measure of 1 g of soil sample, crushed and slightly heated, was diluted in 9 ml of sterile distilled water,

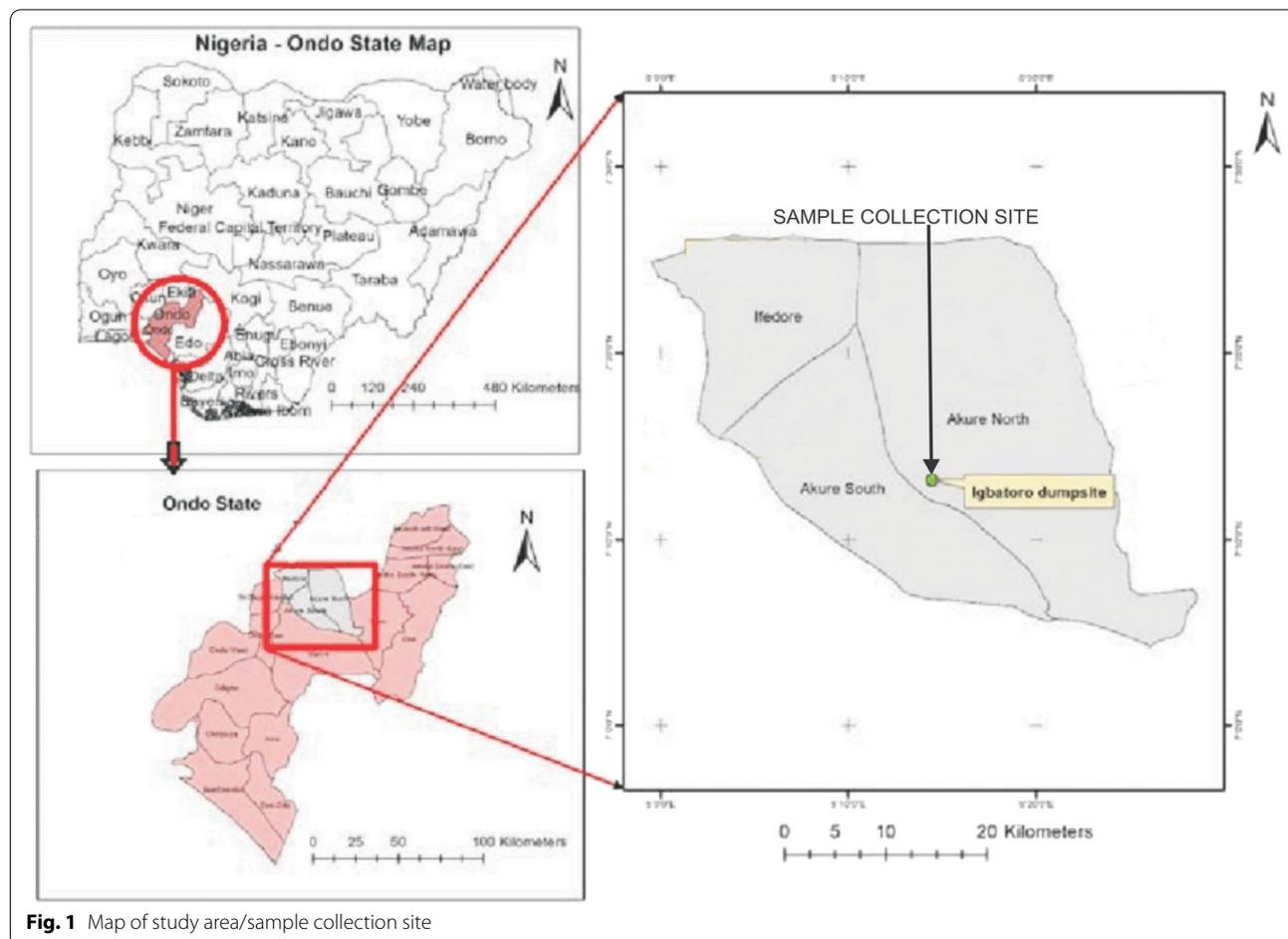


Fig. 1 Map of study area/sample collection site

followed by serial dilution. Serial diluents were aseptically inoculated into different Petri dishes, and sterile medium after cooling to 45 °C was poured unto the inoculums. Sub-culturing was done until distinct colonies were obtained. Microscopic and macroscopic examinations including staining (with lactophenol cotton blue) for morphological characteristics were carried out on fungal isolates, and identification was done based on the characteristics (Adesemoye et al. 2006).

Screening for polyethylene-degrading fungal isolates from soil samples

Standard dilution plating technique on potato dextrose agar (PDA) (Oxoid, Basingstoke, UK) at 28 ± 2 °C for 72 h was carried out. Purification of fungi was done by sub-culturing distinct colonies on freshly prepared PDA media. Confirmation of fungal isolates for degrading capability was done by growing isolated colonies on plates containing aseptically prepared polyethylene Mineral Salt Medium (PMSM). All the plates were incubated at 30 °C and were observed between 3 and 7 days for growth (Alshehrei, 2017).

Purification of polyethylene-degrading fungi

Isolates that grew on the compounded mineral salt medium with polyethylene powder as the sole carbon source were sub-cultured into already prepared malt extract agar and incubated for 7 days at 30 °C. The pure fungal isolates were preserved in cryo vials aseptically prepared with malt extract agar at ambient temperature.

Biodegradation of polyethylene strips via polymer over-layer method under static condition

This was carried out according to the method of Vimala and Mathew (2016) with slight modification. Prior to medium preparation, polyethylene strips were subjected to thermal ageing in the oven at 70 °C. Then, polyethylene strips were treated with 70% ethanol for 30 min to remove any plasticizers and colouring agents, and then, they were air-dried for 15 min. Mineral salt medium was prepared and aseptically poured into the Petri dish with the polyethylene strip gently placed with the aid of a sterile glass rod on the already solidified agar. The polyethylene mineral salt medium was inoculated with purified fungal isolates and incubated at 30 °C for 72 h and observed for growth.

Spectroscopic method for biodegradation of polyethylene using Fourier-transform infrared (FTIR) spectroscopy

Spectroscopic analysis was carried out to detect degradation which detects changes in the structural units of the polyethylene. Fourier-transform infrared spectroscopy is both quantitative and qualitative analytics

with quantification parameters comprising peak, height, peak area and derivatives. The PE strips recovered after the static biodegradation experiment were thoroughly washed with sterile distilled water, dried for 24 h in the oven at 50 °C and packed for analysis (Dierkes et al. 2021). The FTIR analysis was carried out using Agilent FTIR exoscan 4100 (Texas, Florida, USA). Sample interface was cleaned with methanol. Background spectrum was taken after which the polyethylene sample was placed on the interface and run automatically.

Physical/chemical analysis of soil samples

The physicochemical analyses of the soil samples were determined using the methods the Association of Official Analytical Chemists (AOAC 1990).

Statistical analysis of data

Data obtained were subjected to one-way analysis of variance (ANOVA), and treatment means were separated using Duncan's new multiple range test (DNMRT) at $p \leq 0.05$ level of significance with the aid of Statistical Package for Social Sciences (SPSS) version 23.

Results

Table 1 shows counts from three different dilutions (10^{-5} , 10^{-6} and 10^{-7}). Fungal colonies decrease with increasing dilution. The number of fungal colonies is least in 10^{-7} and highest in 10^{-5} for all plates. Hence, the number of fungal colonies decreases with increasing concentration.

Macroscopic and microscopic characteristics of fungal isolates including their probable identities are shown in Table 2. Pigments/colouration observed include green

Table 1 Fungal count of the polyethylene dumpsite soil

Petri-plate	Dilution	Number of colonies	Fungal count per ml of sample (SFU/ml)
1	10^{-5}	120	1.2×10^7
	10^{-6}	100	1.0×10^8
	10^{-7}	70	7.0×10^8
2	10^{-5}	150	1.5×10^7
	10^{-6}	120	1.2×10^8
	10^{-7}	95	9.5×10^8
3	10^{-5}	140	1.4×10^7
	10^{-6}	150	1.5×10^8
	10^{-7}	80	8.0×10^8
Mean count	10^{-5}	137	1.37×10^7
	10^{-6}	123	1.23×10^8
	10^{-7}	82	8.2×10^8

SFU spore forming unit

Table 2 Macroscopic and microscopic characteristics of fungal isolates from the polyethylene dumpsite soil

Isolate code	Macroscopic/microscopic characteristics	Probable identity
F1	Yellow–green spores, septate hyphae, rough and colourless conidia	<i>Aspergillus flavus</i>
F2	Green spores, septate hyphae, woolly colony, white mycelia. Conidia are glubose, rough	<i>Aspergillus nidulans</i>
F3	Light–green with whitish edges, brush-shaped conidia. Multinucleated septate and colourless hyphae	<i>Penicillium chrysogenum</i>
F4	Whitish colonies, raised and branched apical and globular spores	<i>Mucor mucedo</i>
F5	Yellow–green colonies with dendritic white edges. Conidia are ellipsoidal	<i>Eurotium repens</i>
F6	Blue–green spores. Columnar, uniseriate conidial heads. Hyphae are septate. Conidia are round, smooth to finely roughen	<i>Aspergillus fumigates</i>
F7	Greyish fluffy mass. Visible elevated black spore. Non-septate hyphae	<i>Rhizopus stolonifer</i>

and whitish spores with septate and non-septate hypha was observed in fungal isolates in Fig. 2a–d.

Table 3 shows the occurrence and percentage occurrence of the fungal isolates. Spore counts are shown with varying degree of percentage occurrence. Yellow–greenish fungal spores have the highest percentage occurrence (25%), while the light–greenish have the least (6%).

The fungal isolates obtained were screened for their ability to utilize PE as carbon and nitrogen sources. All the fungal isolates were able to utilize PE as both carbon and nitrogen source, although the growth pattern differed. Four out of the seven fungal isolates had rapid growth with sporulation on the minimal salt medium (MSM) in which PE was added as both carbon and nitrogen source, while the remaining three showed slow growth with no sporulation and hence were not used for the biodegradation study. The four fungal isolates, with rapid sporulation, were further purified and used for the biodegradation study.

Weight loss during biodegradation process by fungal isolates of test polythene strip is shown in Table 4. The table revealed percentage weight loss from the first week (week 1) through the last week (week 6) when biodegradation was terminated. There was no percentage weight loss in polyethylene (PE) sample inoculated with *Aspergillus flavus*. PE sample inoculated with *Aspergillus nidulans* showed a 1% and 2% weight loss in weeks 5 and 6, respectively, while the *Penicillium chrysogenum* and *Eurotium repens* samples showed a 1% weight loss in week 6.

The Fourier-transform infrared spectroscopy (FTIR) analysis was used to monitor changes in the functional groups of the polyethylene strips during the biodegradation experiment. The FTIR analysis carried out on the experimental and control PE sample strips showed little/no changes in wavenumbers in Figs. 3, 4, and 5. This implied that there was an insignificant level of degradation of polyethylene samples. Figure 6 shows significant changes in the functional group of the PE strip as there was formation of new peaks in the experimental sample.

A comparative physical/chemical analyses and mineral composition of polyethylene and non-polyethylene dumpsite soils are shown in Tables 5 and 6, respectively. Table 5 shows the physical properties of the sampled soils. These included soil colour, soil texture, particle size, consistency and soil odour. The table shows a significant difference in soil properties such as colour, particle size and odour, while properties such as texture and consistency show little difference for both sampled soils (polyethylene and non-polyethylene dump soils). Table 6 shows the chemical properties and mineral composition of the sampled soils. These include pH, moisture content, available phosphorus, organic carbon, organic matter, total organic nitrogen, cation exchange capacity, sodium, potassium, calcium, magnesium, copper, iron, manganese, nickel, cobalt, lead, cadmium and chromium. The table shows differences in the chemical characteristics and mineral composition of both the polyethylene dump soil and non-polyethylene dump soil samples. All parameters analysed for both polyethylene and non-polythene dump soils were significantly different from each other with the exception of chromium.

Discussion

Three hundred and forty-two fungi (Table 1) were isolated from the sampled soils using standard plate count technique. This fungal population may be as a result of the high organic carbon and organic content of the polyethylene dump site soils. This may also be as a result of the increased availability of biodegradable organic and inorganic substrates from the variety of municipal wastes continuously being dumped at these sites. This is similar to the findings of Tanunchai et al. (2021) who reported the presence, in large number, of fungi in soils harbouring plastic films. The high fungal count of 8.2×10^8 SFU/ml (Table 1) is also similar to the finding of Haider et al. (2018) who reported that a high number of microbial populations in an environment are in correlation with the signs of disintegration of mechanical properties of natural polymer films present, indicating the role of

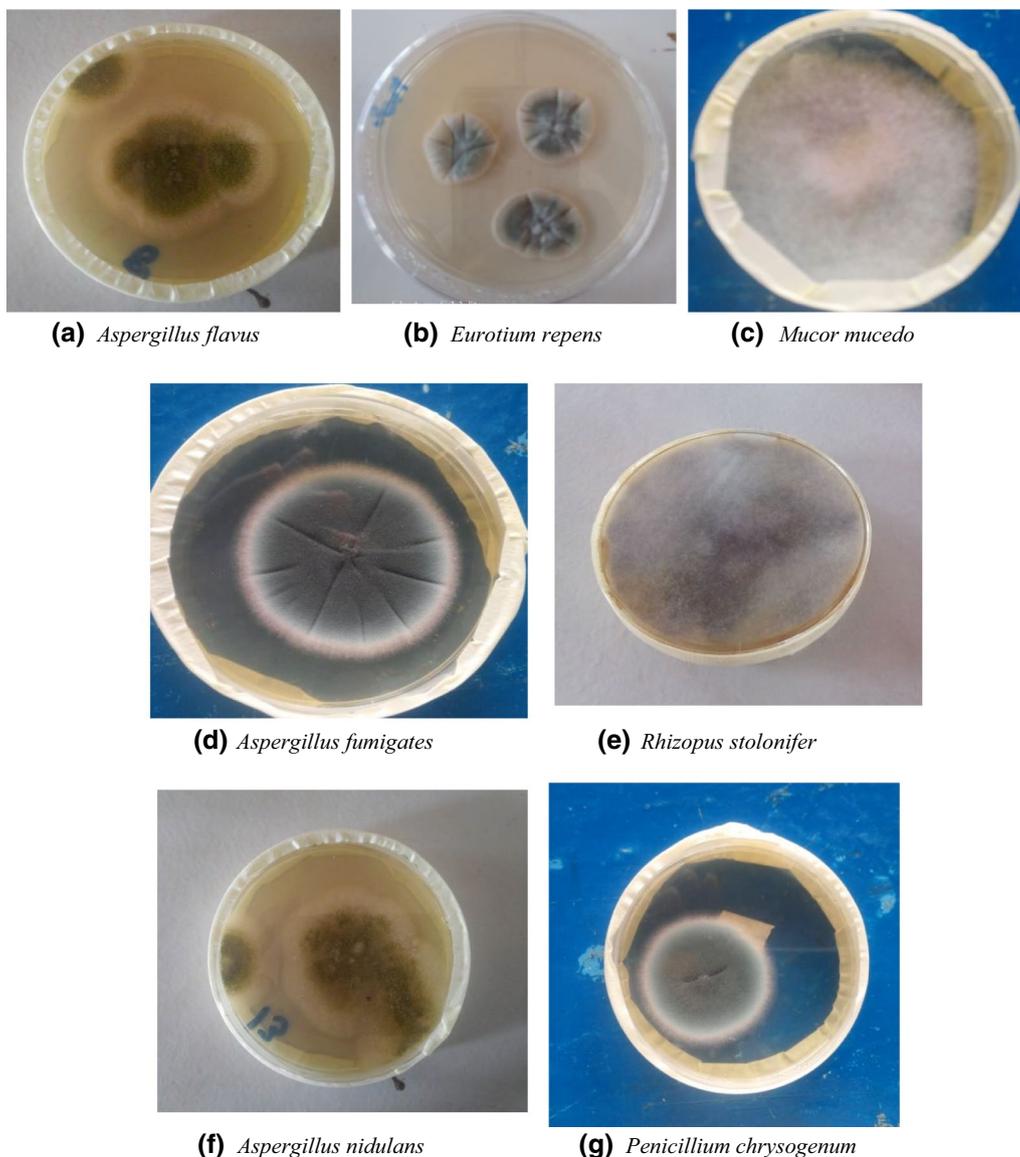


Fig. 2 a *Aspergillus flavus*, b *Eurotium repens*, c *Mucor mucedo*, d *Aspergillus fumigatus*, e *Rhizopus stolonifer*, f *Aspergillus nidulans*, g *Penicillium chrysogenum*

Table 3 Microbial occurrence of PE degrading fungal isolates

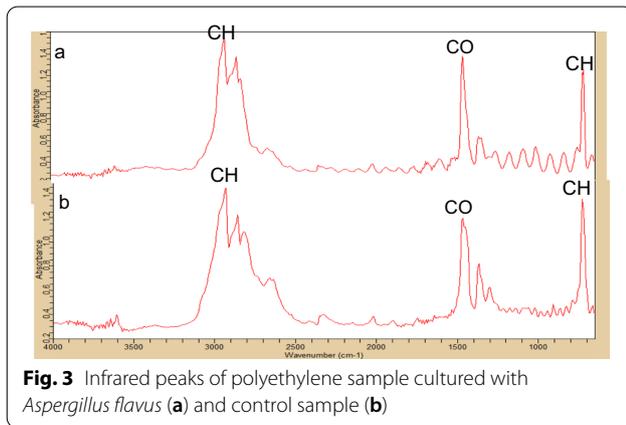
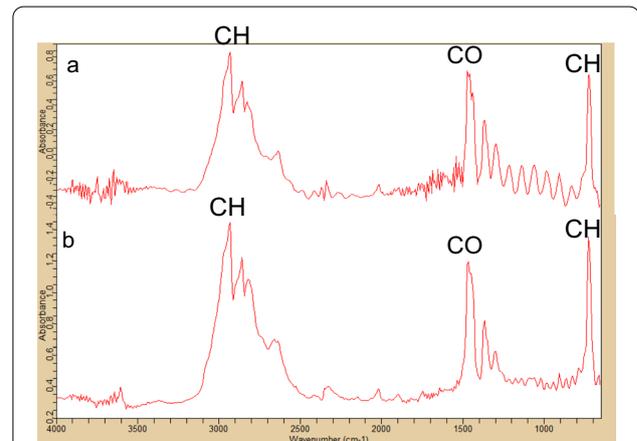
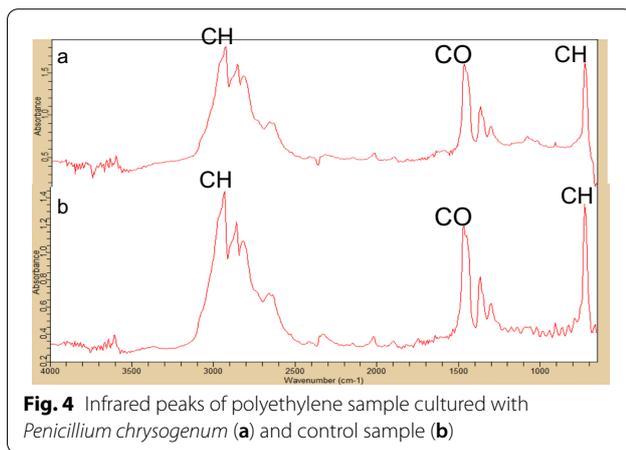
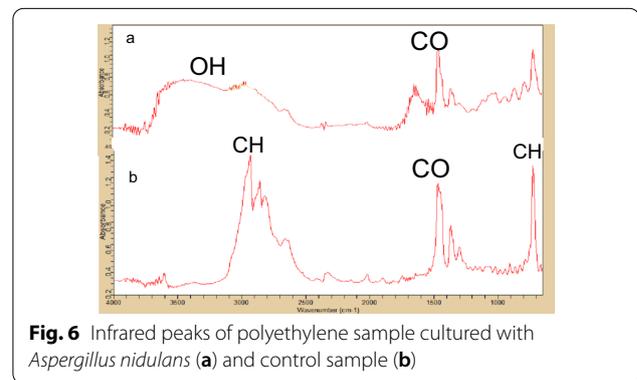
Isolate	Occurrence	Percentage occurrence (%)
Yellow–Green spores	12	25
Green spores	5	10
Light–green spores	3	6
Whitish spores	5	10
Yellow–green with white edges	10	21
Blue–green spores	6	13
Greyish fluffy Mycelia	7	15

biotic component in degradation process. *Aspergillus*, *Mucor*, *Penicillium*, *Rhizopus* and a variety of yeasts have been reported to be associated with waste biodegradation (Douglas et al. 2020). The fungal isolates’ polyethylene degrading ability could be as a result of the ability of these fungi to survive better in static–solid medium; this is similar to the findings of Khruengchai et al. (2021).

The seven fungal isolates were subjected to identification after their macroscopic and microscopic characterization. Four out of the seven fungal isolates were selected and used for the biodegradation process (Table 2). The four fungal isolates used for the

Table 4 Percentage weight loss of the polyethylene strips during six weeks of degradation in static condition

Sample	Week 1 (%)	Week 2 (%)	Week 3 (%)	Week 4 (%)	Week 5 (%)	Week 6 (%)
Control	0	0	0	0	0	0
<i>Aspergillus flavus</i>	0	0	0	0	0	0
<i>Aspergillus nidulans</i>	0	0	0	0	1	2
<i>Penicillium chrysogenum</i>	0	0	0	0	0	1
<i>Eurotium repens</i>	0	0	0	0	0	1

**Fig. 3** Infrared peaks of polyethylene sample cultured with *Aspergillus flavus* (a) and control sample (b)**Fig. 5** Infrared peaks of polyethylene sample cultured with *Eurotium repens* (a) and control sample (b)**Fig. 4** Infrared peaks of polyethylene sample cultured with *Penicillium chrysogenum* (a) and control sample (b)**Fig. 6** Infrared peaks of polyethylene sample cultured with *Aspergillus nidulans* (a) and control sample (b)

biodegradation study were *Aspergillus flavus*, *A. nidulans*, *Eurotium repens* and *Penicillium notatum*. This was similar to those reported by Mishurov et al. (2020) who isolated *Penicillium* and *Aspergillus* from polymer while working on using polymeric materials as substrates for micromycetes. Gravimetric method used to determine the percentage weight loss of polyethylene sample (Table 4) was similar to the method used by Yu et al. (2019) in their findings of degradation of polyethylene by fungal consortium.

Table 5 Physical properties of sampled dumpsite soil

Soil property	Polythene dump soil	Non-polythene dump soil
Colour	Blackish	Slightly brownish
Texture	Gritty	Slightly coarse
Particle size (mm)	0.04	0.20
Consistency	Sticky	Slightly sticky
Odour	Rotten	None

Table 6 Chemical characteristics/mineral composition of the sampled soils in ppm (parts per million)

Parameters	Polythene dump soil	Non-polythene dumpsite soil
Ph	7.12 ± 0.06 ^a	7.01 ± 0.01 ^b
Moisture content (%)	0.79 ± 0.01 ^a	0.52 ± 0.01 ^b
Phosphorus (mg/kg)	40.81 ± 0.01 ^a	33.52 ± 0.01 ^b
Organic carbon (g/kg)	24.94 ± 0.01 ^a	16.35 ± 0.01 ^b
Organic matter (g/kg)	42.97 ± 0.01 ^a	28.19 ± 0.01 ^b
Total organic nitrogen (g/kg)	1.24 ± 0.01 ^a	0.81 ± 0.01 ^b
Cation exchange capacity	34.84 ± 0.34 ^a	28.21 ± 0.01 ^b
Sodium (ppm)	11.23 ± 0.03 ^a	10.20 ± 0.06 ^b
Potassium (ppm)	18.04 ± 0.32 ^a	10.63 ± 0.01 ^b
Calcium (ppm)	141.07 ± 0.07 ^a	91.10 ± 0.06 ^b
Magnesium (ppm)	6.62 ± 0.01 ^a	5.62 ± 0.01 ^b
Copper (ppm)	0.61 ± 0.01 ^a	0.53 ± 0.01 ^b
Iron (ppm)	163.07 ± 0.07 ^a	149.07 ± 0.07 ^b
Manganese (ppm)	0.96 ± 0.01 ^a	0.60 ± 0.01 ^b
Nickel (ppm)	0.36 ± 0.01 ^a	0.23 ± 0.01 ^b
Cobalt (ppm)	0.73 ± 0.01 ^a	0.65 ± 0.01 ^b
Lead (ppm)	0.50 ± 0.01 ^a	0.42 ± 0.01 ^b
Cadmium (ppm)	0.04 ± 0.01 ^a	0.02 ± 0.01 ^b
Chromium (ppm)	0.77 ± 0.09 ^b	0.74 ± 0.01 ^b

Key Parameters with different alphabetical labels ('a' and 'b') along the same row are significantly different from each other, while the parameter with the same alphabetical label is not significantly different

Polymer breakdown by fungi prior to biodegradation is aided by the action of pre-treatment with high temperature. Fungi such as *Penicillium chrysogenum*, *Eurotium repens* and *Aspergillus nidulans* showed ability to degrade the polyethylene samples and were subjected to thermal pre-treatment. This is similar to the findings of Peterson et al. (2019) who reported that temperature is a crucial factor in determining the rate of thermo-oxidation. It was noted that *Penicillium pinophilum*, *Aspergillus niger* and *Phanerochaete chrysosporium* showed biodegradative capability on thermally treated low density polyethylene.

FTIR spectra from this research showed little alteration in the structural composition of the sampled polyethylene (Figs. 3, 4, 5, 6). This is similar to the findings of Janczak et al. (2020) who studied degradation of polyethylene using organisms isolated from compost soil. They studied degradation by inoculating isolated microorganisms into mineral salt medium containing one gram of polyethylene film and reported that carbonyl band corresponds to the ketone and ester functional groups, which is a typical product of thermal-oxidation degradation of polyethylene. A significant structural change was observed in the 3000 cm⁻¹ band of the asymmetric stretching in the FTIR spectra

of the sample polythene strip cultured with *Aspergillus nidulans* (Fig. 6), which indicates bending deformations around bands 1500–2500 cm⁻¹ and 1600–1300 cm⁻¹. This is similar to the findings of Kelkar et al. (2019) who observed structural changes after investigating the effect of thermal ageing in sulphuric acid solution of high-density polythene (HDPE).

The result from the FTIR analysis of polyethylene subjected to microbial attack indicated little or no significant alteration in functional group bonding of the polythene strip (Figs. 3, 4, 5), but the displacement of the CH group by OH at band 3000 cm⁻¹ and a slight decrease in the carbonyl group at band 1600–1300 cm⁻¹ showed considerable alteration (Fig. 6). Some *Aspergillus* species have been reported to produce aflatoxin, a toxic metabolite with OH as one of its functional group. This functional group was observed after six weeks of the biodegradation process. The formation of intermediate product such as polymeric OH bond can easily be converted to water and degraded by the fungus. This is similar to the findings of Zhang et al. (2020) who reported that some new intermediate products were observed after polymer breakdown by *Aspergillus flavus*.

From the study, soil sampled was both gritty and slightly coarse (sandy in nature) (Table 5). This observation agrees with the findings of Akinbile et al. (2021), who observed the sandy nature of top soils sourced from several municipal dump sites in Akure, Western Nigeria. However, this trend is in contrast with the findings of Daniel et al. (2021), who observed that majority of soil samples collected from waste dump sites in Port Harcourt, Nigeria, were silty in nature. This contrasting observation about soils textural class could be as a result of differences in geographical locations of soil sites and is plagued by different weather conditions.

Minerals and the physicochemical parameters of both sampled soils have the same compositions with little variations in quantity (Table 6). This accounts for the similarity in the microbial type present in both soils. Heavy metals were in trace amount, while the pH of test sampled soils was alkaline with a substantial amount of total organic carbon (TOC) and small particle size. This is in agreement with the findings of Khadhar et al. (2020) who observed that pH, total organic carbon and particle size distribution are among several components of soil that affect its availability, retention and mobility of metals. Also, the alkaline nature and presence of trace amount of heavy metals in sampled soils agreed with the findings of Yuan et al. (2021) who demonstrated that soils with acidic pH levels tend to have an increased heavy metal concentration and vice versa.

Conclusions

In conclusion after six weeks of biodegradation, the alkylene group (monomers of polythene) was broken down by *Aspergillus nidulans*, *Eurotium repens* and *Penicillium chrysogenum* with respect to percentage weight loss with highest being 2% by *A. nidulans*. There was reduction of peaks such as methylene stretch of alkylene group, CH bend and the formation of new bonds polymeric OH stretch as shown in FTIR spectra of *A. nidulans* polyethylene sample. Hence, polyethylene showed an appreciable level of degradation by three out of the four fungal isolates used in this study.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42269-022-00871-4>.

Additional file 1. Appendix.

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Author contributions

DJA designed the study. TOA developed the methodology and acquired, analysed and interpreted the data obtained. TOA wrote the first draft. DJA and OEA previewed the final draft. All authors read and approved the final manuscript.

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Availability of data and materials

There is availability of data and materials in supplementary data files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

Authors declare that no competing of financial interests or personal relationship exists.

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