Microbiology Research Journal International

26(1): 1-15, 2018; Article no.MRJI.44851
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Monitoring Fungal Biodegradation of Low-density Polyethylene [LDPE] from Plastic Wastes Dump Sites Using FT-IR Spectra

Babatunde I. Aderiye[1*], Richard O. Akinyeye[2], Adebisi Sulaimon[1], Olusola A. Oluwole[3], Fayokemi J. Kehinde[1], Oluwadamilola E. Ojo[1] and Sunday O. Bamiteko[1]

[1]Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria.
[2]Department of Industrial Chemistry, Ekiti State University, Ado-Ekiti, Nigeria.
[3]Department of Science Laboratory Technology, Ekiti State University, Ado-Ekiti, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2018/44851
Editor(s):
(1) Dr. Zahid Anwar, Department of Biochemistry, University of Gujrat, Pakistan.
Reviewers:
(1) P. Saravana Kumari, Rathinavel Subramaniam College of Arts and Science, India.
(2) Haruna yahaya Ismail, University of Maiduguri, Nigeria.
(3) Muzafar Akbar Rather, Barkatullah University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/28054

Original Research Article

Received 07 September 2018
Accepted 19 November 2018
Published 01 January 2019

ABSTRACT

Introduction: Polyethylene is the most commonly used synthetic plastic and is poorly degraded in natural environments, thus causing serious environmental problems.
Aim of the Study: This study was designed to investigate the polyethylene degrading potentials of fungal strains recovered from several plastic polluted sites.
Place and Duration of Study: Department of Microbiology, Ekiti State University, Ado-Ekiti between January 2016 and October 2017.
Methodology: Soil and buried water sachet samples were analysed for polyethylene degrading bacteria. Their abilities were monitored using dry weight, radial mycelial growth and Fourier transform infrared (FTIR) spectroscopy.
Results: The Ekiti State Waste Management Board site had the highest number of fungi isolates [28.75 x 10^4 and 16 x 10^4 hyphal cells/propagules per ml] obtained from the soil and polyethylene

**Corresponding author: E-mail: jadesolaaderiye@yahoo.com;*

waste samples respectively. Five of the nineteen fungal isolates utilised low density polyethylene [LDPE] and were identified via molecular techniques as *Aspergillus flavus* KMBF 1501*, Penicillium simplicissimum* YK 18*, Alternaria alternata* strain 20UPMNR, *Aspergillus* sp. and an unknown isolate Ps. 10 which could not be identified due to its low amplicon size. The FT-IR spectra revealed a carbonyl absorption band in *Aspergillus flavus* KMBF 1501 and *Penicillium simplicissium* YK 18 degraded polyethylene powder with vibration [V_{fh}] of C=O at 1726.35 cm^{-1} normally observed at the frequency range of 1750 cm^{-1} – 1710 cm^{-1} denoting the formation of ketone or aldehyde group. A new V_{fh} of O-H stretch with H-bonded structure at 3286.81cm^{-1} was formed by the degrading ability of *Aspergillus flavus* KMBF 1501 suggesting the formation of a new functional group usually at frequency range of 3500 and 3200cm^{-1} as alcohol or phenol. A slight decrease in the V_{fh} of O-H bend from 931.65 cm^{-1} to 929.72 cm^{-1}, indicating carboxylic acid was also observed. However, a slight increase in the V_{fh} of C=C stretch from 1635.69 cm^{-1} to 1639.55 cm^{-1} represent an alkene while its O-H stretch gave a carboxylic acid group with no significant change when compared to the control sample.

Conclusion: The FT-IR analyses demonstrated the ability of the fungal isolates to colonise and modify LDPE films.

Keywords: Fungi; low-density polyethylene; biodegradation; weight loss; water sachets; FT-IR spectra.

1. INTRODUCTION

Synthetic plastics, such as polyethylene consist of monomers bounded or connected together by chemical bonds [1,2,3]. They are used extensively in packaging and other industrial and agricultural applications. Amongst the plastic is the Low-Density Polyethylene [LDPE] which is characteristically inert and resistant to microbial attack, leading to their accumulation in the environment [4,5]. Their chemical stability and inertness make them recalcitrant, with improper recycling and waste management systems making them important environmental pollutants [6,7] in many developing countries.

Microorganisms play a significant role in the biological decomposition of materials [8]. However, the high molecular weight, 3-dimensional structure, hydrophobic nature and lack of functional groups in the LDPE interfere with a microbial attack. In most studies, fungi have been investigated for the biodegradation of LDPE because these organisms produce degrading enzymes [8] and extracellular polymers, such as polysaccharides, which can help to colonise the polymer surface [9], and the distribution and penetration ability of the fungal hyphae is an advantage. Fungi are heterotrophic organisms that have shown the capability to breakdown xenobiotic compounds and have been investigated for LDPE biodegradation [10,11,12]. Reports have also shown that fungi species such as *Penicillium simplicissimum*, *Fusarium solani* are used in the degradation of both natural and synthetic polyethylene [13,14,15,16]. Bonhomme et al. [17] reported that with the activities of fungi, polyethylene with a starting molecular weight between 4000 to 28000 was degraded to units with a lower molecular weight of 500 which indicated the biodegradation success. Also, Ojha et al. [18] isolated, identified and reported two fungal strains, *Penicillium oxalicum* NS4 [KU559906] and *Penicillium chrysogenum* NS10 [KU559907] with plastic degrading abilities. Microorganisms especially fungi have thus played important roles in biodegradation of plastics by utilising plastic as the sole carbon source.

The present study was set up to isolate fungal isolates from the soil and water sachet samples collected from plastic waste sites and investigate their LDPE degradation potentials given the vital role of fungi in biodegradation of polymers in soil. The biodegradation of the polymer was estimated by using weight loss of LDPE film, and chemical changes on the polymer surfaces.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Soil and low-density polyethylene bags [water sachet, thickness: 65-80 microns] were scooped from three different sites, including Ekiti State Waste Management Board [EKSWMB], a popular restaurant located in Ekiti State University [EKSU] and a mechanic workshop at

the outskirts of EKSU [0.8 km, to University main gate] at the depth of 2 cm into a sterile container.

2.2 Preparation of Polyethylene Powder

Clean polyethylene sheets [water sachets] were cut into small bits and dissolved in 100 ml of Xylene [Hamburg Chemicals, Germany] by heating at 70°C until a homogeneous solution formed. The solution was allowed to recrystallise and mechanically powdered using a mortar and pestle [19].

2.3 Isolation of Fungi

One gram **[**1g] of dry soil attached to the waste water sachet samples was suspended in a conical flask containing 100 ml of distilled water, agitated and serially diluted [20]. The same procedure was used for the soil samples from these sites. Fungi associated with polyethylene [water sachet] and the soil was isolated by pour plate method using potato dextrose agar. These plates were incubated at 28°C for 3 days. Sub-cultures were made from observed colonies and then preserved in agar slant for further analysis [21].

2.4 Mineral Salts Media [MSM]

Polyethylene powder was added in mineral salt media to serve as carbon source. The media contained 0.1g of NH_4NO_3, 0.2 g of $MgSO_4$. $7H_2O$, 1.0 g K_2HPO_4, 0.1 g of $CaCl_2$. $2H_2O$, 0.15 g of KCl, and 0.1 g yeast extract; and 0.1 mg/l of each of these micro-elements: $FeSO_4$. $6H_2O$, $ZnSO_4$. $7H_2O$ and $MnSO_4$ in 1L of distilled water, pH wss adjusted to 6.0 [22]. Polyethylene powder was put into mineral salt medium at a final concentration of 1.0% [w/v]. For preparation of mineral salts agar [MSA], 15 g/L agar-agar was added to the broth medium prior to autoclaving.

2.5 Screening of Polyethylene Degrading Fungi

The fungi isolates were inoculated onto mineral salt agar [MSA] containing polyethylene as sole carbon source, plated and incubated at 28°C. The growth rate was monitored for 11days [23]. Radial mycelial growth was estimated as the area [πr^2, where r = radius in mm] covered by the growth of the fungi isolates on the plate [24]. Growth on Potato dextrose agar was used for comparison and as a standard for measuring the growth of the fungal isolates. The organisms that showed prominent growth on the MSM were selected for further analysis.

2.6 Molecular Identification of the Isolates

Molecular characterisation of the fungal isolates was identified by 18S rRNA sequence analysis. The fungal genomic DNA was isolated as described by Aderiye and Oluwole [25].

2.6.1 Fungal genomic DNA extraction

The genomic DNA was extracted from five to seven days old fungal cultures grown on culture broth [26]. The fungal mass from the culture broth was obtained by filtering the culture broth through a 10 mL syringes containing glass wool that will allow the broth to pass through, while retaining the fungal mass and was placed in a 2 ml tube containing a ceramic pestle, 60–80 mg sterile glass beads [425–600 µM, Sigma] and lysis buffer [100 mM Tris HCl [pH8.0], 50 mM EDTA, 3% SDS]. Homogenisation of fungal mass was done twice in a FastPrep®-24 tissue homogeniser [MP Biomedicals, USA] [27].

The resulting fungal tissue homogenate was centrifuged at 13,000 rpm for 10 min and the supernatant was transferred to a fresh microcentrifuge tube. To the supernatant, 2ml of RNase A [10 mg/ml] was added and incubated at 37°C for 15 min. After the RNase A treatment, phenol: chloroform: Isoamyl alcohol in the proportion [25:24:1] was added and mixed well, followed by centrifugation at 13,000 rpm for 10 min. The upper aqueous layer was taken in a fresh micro centrifuge tube and then an equal volume of 100% ethanol was added. Following precipitation at -20°C for 30 min, the whole content was centrifuged at 12,000 rpm for 10 min to pellet down the DNA [27]. The DNA pellet was washed with 70% ethanol and centrifuged at 12,000 rpm for 5 min. The DNA pellets were air dried and dissolved in 1× TE buffer [28].

2.6.2 PCR amplification

The PCR reactions were performed in a 25 µl reaction volume containing 16 µl PCR grade water [Sigma], 100 ng of genomic DNA, 2.5 µl of 10 × reaction buffers, 2.5 µl of 10mM dNTPs mix [Sigma-Aldrich], 2 µl [10 pmol/ µl] of random decamer oligonucleotide primer OPA–1 and 1 µl [5 U/ µl] of Taq DNA polymerase [Sigma-Aldrich]. Amplification was performed in an Eppendorf Master Cycler [Eppendorf, Hamburg]. The PCR cycling conditions consisted of an initial

denaturation step at 94°C for 2 min and subjected to 40 cycles of the following program, 94°C for 30 s, 37°C for 1 min, 72°C for 2 min and final extension at 72°C for 10 min [29].

2.6.3 Sequencing of amplified ITS gene

The PCR products were purified using Montage PCR Clean up kit [Millipore]. The purified PCR products of approximately 1,500 bp and the fungal sequencing and identification were performed as described by Liu et al. [29] using two primers ITS4 [TCCTCCGCTTATTGATATGS and ITS5 [GGAAGTAAAAGTCGTAACAAGG]. The sequences of PCR products were analysed using standard protocols with a dideoxy nucleotide dye terminator [Big Dye vs. 3.1— Applied Biosystems, CA, USA] and Genetic Analyzer 3130 [Applied Biosystems, CA, USA]. All 23S rRNA gene sequences were checked for quality, aligned, and analysed with Codon-Code Aligner v.3.7.1 [CodonCode Corp.; Centerville, MA, USA]

2.7 Biodegradation Studies

The pre-weighed LDPE films was aseptically transferred into the conical flask containing 300ml of mineral salt medium and then inoculated with the spore suspension of identified polythene degrading fungi. Control was maintained with LDPE films in the microbe free medium and left in a rotary shaker [120 rpm] at 33.3°C, for 21 days [30]. After incubation, the films were collected, washed thoroughly using distilled water; shade dried and further analysed the biodegradation.

2.7.1 Preparation of fungal spore suspension

Following the identification of the fungal isolates using 18S rRNA sequencing, the fungal spore suspension of *Penicillium simplicissium* KC 503976.1, *Aspergillus flavus* JX025733.1, and *Alternaria alternata* KJ779602.1 was prepared as described by Aderiye and Ogundana [31] for biodegradation of polyethylene strips monitored by FT-IR spectroscopy. Screening for polyethylene utilisation by these fungi was carried out with one milliliter culture suspension of each fungus added to 100 ml of mineral salt medium which contained 0.5 g of polyethylene strips as the sole carbon source and incubated at 30±2°C for 30 days [32].

2.7.2 Dry weight determination

Dry weight determination was done by the shake flask method [33]. Identified fungi species were inoculated into 50 ml minimal salt medium containing water sachet films cut in 5cm x 5cm as the only carbon source. All these flasks were put into a shaker incubator at 28°C for 28 days, maintained at 180rev/min. The LDPE films were recovered after 7, 14, 21 and 28 days incubation from the culture media, washed thoroughly with distilled water and dried over night at 45°C. The final weight was determined after each successive incubation period. The control was maintained with the LDPE films in the microbe-free medium. From the data collected, the weight loss of the plastic films was calculated.

2.7.3 Fourier-transform infrared spectroscopy [FT-IR]

FT-IR was used to confirm biodegradation by determining the formation of new functional groups or disappearance of groups in the polymer. Changes in the polyethylene structure following natural weathering and subsequent incubation with the fungal isolates were analysed by FTIR spectrophotometer [8400 Shimadzu, Japan, with Hyper IR-1.7 software for Windows] [34]. The LDPE film exposed to the isolates was analysed after 28 days of incubation period which was recorded from frequency of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} at room temperature with a helium–neon laser lamp as a source of IR radiation.

3. RESULTS

3.1 Soil Type and Mycoflora of Polyethylene Strips from Sampling Sites

The soil type collected from the sampling sites varied from the humus (EKSWMB), loamy (restaurant) to the loose sandy soil found in the mechanic workshop. The colour of the soil was deep brown, beige to black and brown respectively (Tables 1 and 2). The average number of propagules/hyphal strands of the fungi isolates obtained from the different sampling sites is shown in Table 1. After 3 days of incubation, about 2.08 and 2.1 x 10^5 hypha cell/propagules per ml respectively were estimated on potato dextrose agar (PDA) from soil and polyethylene bags collected from the restaurant dump site while about 1.9 x 10^5 hyphal cells/ml and 1.53 x 10^5 hyphal cell/ propagules per ml respectively were obtained from the soil and polyethylene bags collected from the mechanic workshop. Soil and the polythene waste samples obtained from EKWMB

site had the highest number of fungi isolates (2.88 x 10^5 hyphal cell/propagules per ml and 1.6 x 10^5 hyphal cells/propagules per ml) respectively. Nineteen fungal isolates were eventually recovered and screened for LDPE utilisation.

3.2 Comparison of the Growth of Fungal Isolates on PDA and MSM Plates

Nineteen (19) fungal isolates were obtained from different soil and LDPE samples at the dumpsites and later subjected to growth on potato dextrose agar. The growth of 19 fungal isolates from the three different dump sites was monitored for five days on PDA by measuring their radial mycelial growth. It was observed that there was increase in the mycelia area as the incubation period increased. All the isolates had their optimal growth on day 5 of incubation (Table 2). Table 2 reveals the growth pattern of these fungal isolates in 5 days. There was no evidence of growth after day 2 in any of the fungal isolates except in Ps 15 where there was tremendous growth (380.30 sq.mm). After 4 days the mycelia had increased about three times (1134.62 sq.mm) its size in 48h. By the 5th day, the organism had overgrown the culture medium. Similarly, isolates Ps. 1, Ps. 11, Ps. 12, Ps. 14 and Ps. 19 exhibited tremendous growth with mycelia mass of 380.3, 962.5, 962.54, 616.03 and 707.18 sq.mm within 4 days.

However, when grown on Mineral salts agar plates, only 5 isolates (Ps. 2, Ps. 4, Ps. 10, Ps. 13 and Ps. 19) grew (Table 4). Ps. 10 exhibited the greatest degrading ability, evident from its mycelia size (1134.62sq.mm) after 11 days. Unfortunately, isolate Ps 10 was not identified. Fungal growth on supplemented MSM was very poor in all the isolates except Ps 10 after 5days incubation, where mycelia growth was 154.01 sq.mm. On the 11th day, the fungal growth was tremendous (1134.62 sq.mm). The rate of growth of this isolate was 140.94 sq.mm/day. Mycelia growth of Ps. 13 was the least (7.07 sq.mm) even after 11days of experimentation while the size of mycelia of Ps. 2 (113.15sq.mm) and Ps. 4 (63.65sq.mm) was about one-tenth of those of Ps. 10 (1134.62sq.mm) and Ps. 19 (616.03 sq.mm) respectively. It was observed that isolate Ps10 and *Aspergillus* sp. grew best (113.62 and 616.03 sq.mm respectively). The five fungal isolates grew well on PDA and on supplemented mineral salt media which revealed their ability to utilise LDPE.

Table 1. The properties of the soil from the polyethylene waste sites

Sampling (Dump) site	Soil colour	Soil type
I. EKSWMB		
i. Soil	Deep brown	Humus
ii. Polyethylene		
II. Restaurant		
i. Soil	Beige to black	Loamy
ii. Polyethylene		
III. Mechanic workshop		
i. Soil	Brown	Sandy
ii. Polyethylene		

Table 2. Mycoflora of the soil and polyethylene samples from the sampling sites

Sampling (Dump) site	Fungal count (log_{10} x10^5 hyphal cells)
I. EKSWMB	
i. Soil	2.88 (5.46)
ii. Polyethylene	1.6 (5.2)
II. Restaurant	
i. Soil	2.08 (5.32)
ii. Polyethylene	2.1 (5.32)
III. Mechanic workshop	
i. Soil	1.9 (5.28)
ii. Polyethylene	1.53 (5.18)

Table 3. Growth (sq.mm) of fungi isolates on potato dextrose agar

Isolate code	Day 2	Day 3	Day 4	Day 5
Ps 1	0.00	28.29	380.30	660.82
Ps 2	0.00	38.50	95.08	176.58
Ps 3	0.00	28.29	113.15	176.79
Ps 4	0.00	3.14	38.50	78.58
Ps 5	0.00	28.29	95.08	154.01
Ps 6	0.00	0.00	3.14	12.57
Ps 7	0.00	7.07	19.25	63.65
Ps 8	0.00	28.29	154.01	283.66
Ps 9	0.00	28.29	154.01	283.66
Ps 10	0.00	50.29	176.79	314.3
Ps 11	0.00	154.01	962.5.	Over grown
Ps 12	0.00	314.3	962.54	Over grown
Ps 13	0.00	7.07	132.79	283.66
Ps 14	0.00	63.65	616.03	Over grown
Ps 15	380.30	962.94	1134.62	Over grown
Ps 16	0.00	0.00	12.57	50.29
Ps 17	0.00	78.58	95.08	176.79
Ps 18	0.00	28.29	154.01	254.58
Ps 19	0.00	660.82	707.18	38.50

Key: < 50 sq.mm=poor growth, 101-200 sq.mm=little growth
51-100 sq.mm=moderate growth 201-350 sq.mm=high growth
>350 sq.mm = tremendous growth

Table 4. Growth (sq.mm.) of the fungi isolates on mineral salts (MSM) agar supplemented with LDPE powder

Isolate Code	Day 3	Day 5	Day 7	Day 9	Day 11
Ps. 2	0.00	0.00	12.57	50.29	113.15
Ps. 4	0.00	7.07	7.86	38.50	63.65
Ps. 10	7.07	154.01	314.3	908.33	1134.62
Ps. 13	0.00	0.00	0.00	3.14	7.07
Ps. 19	7.07	63.65	254.58	380.30	616.03

Key: < 50 sqmm= poor growth, 51-100 sq.mm = slow growth,
101-200 sqmm = moderate growth, 201-350 sq.mm = high growth,
> 350 sqmm =tremendous growth,

3.3 Molecular Characterisation and Identification of Fungi Isolates Associated with LDPE Degradation

Molecular techniques were employed for the characterisation and identification of the fungi isolates. The DNAs were extracted (Fig. 1) and the PCR carried out with genomic DNA (Fig. 2) were checked for their amplicon sizes. Some amplicons (i. e. those of Ps. 2, Ps. 4, Ps. 13 and Ps. 19) resulted in strong amplification of an expected 1.5 kb fragment while the fifth isolate (Ps.10) revealed below 0.65 kb fragment. The names of the isolates initially written as Ps. 2, Ps. 4, Ps. 13 and Ps. 19 were revealed as *Aspergillus flavus* KMBF 1501 (accession number JX025733.1), *Penicillium simplicissimum* strain YK 18 (accession number KC503976.1), *Alternaria alternata* HM 543460.1 (and *Aspergillus* species enrichment culture clone RS 4 (accession number KT 211865.1) respectively (Fig. 3; Table 5). Isolate Ps. 10 did not reveal any sequence identity because of its small amplicon size.

3.3.1 Phylogenetic tree

The sequence of the organism was compared with the Gene Bank on National Centre for Biotechnology (NCBI) and the phylogenetic tree was re-covered from Mole BLAST. The percentage similarities of the organisms with the sequence revealed *Aspergillus flavus* KMBF 1501 (99.25%), *Aspergillus* species enrichment culture clone RS 4 (99.25%), *Penicillium simplicissimum* strain YK 18 (94%) and *Alternaria alternata* 20PMNR (86%) (Table 5).

The alignment statistics and the identities of the organisms showed that the relatedness was due to the sequence similarities, related information from the 18S ribosomal RNA gene and the number of matches from the sequence. *Aspergillus flavus* KMBF 1501 had about 99.25% relatedness to *Aspergillus* sp. clone RS 4 due to some sequence similarity while *Penicillium simplicissimum* strain YK18 gave a slight sequence deviation from the number of matches from the sequence length (94%). Approximately 84% similarity was noticed from *Alternaria alternata* 20UPMNR sequence in comparison with the sequence of other related organisms on the phylogenetic tree (Fig. 3).

3.4 Dry Weight Determination of Degraded Polyethylene Films

The effectiveness of degrading fungi strains was studied over a period of 28 days and the percentage weight loss of the LDPE treated with the strains is represented in Fig 4. These fungal isolates were found to be responsible for the decrease in the weight of polyethylene films by adhering on its inert surface. It was observed that *Penicillium simplicissimum* strain YK 18 had the least degrading ability (0.038%), while *Aspergillus* species enrichment culture clone RS 4 degraded 0.084% of the slips after 28days. *Alternaria alternata* strain 20UPMNR degraded the polymer by 0.087% whereas *Aspergillus flavus* KMBF 1501 exhibited the highest degrading ability by reducing the polymer weight by 0.143%.

3.5 Monitoring LDPE Degradation by FT-IR

Comparing the control sample with the other LDPE samples treated with fungi in this study, it was observed that some of the vibrational frequencies obtained from the control sample (uninoculated synthetic medium) indicated particular one functional groups or the other in the chemical structure of the sample (polyethylene synthetic medium). Any alteration of these functional groups either by band shift, appearance or disappearance of characteristic bands due to any of these functional groups in the remaining samples treated with microorganisms indicate degradation in the polymer structure. In Fig. 5, there were some functional groups denoted by vibrational frequencies (V_{fh}) present in the control sample at 3441, 2918 and 2851, 1636 and 932cm^{-1}, with vibrational bands for an O-H stretch H-bond, C-H stretch, N-H bend and O-H bend respectively. These bands are indicative of the presence of the following functional groups; alcohols/phenols, alkanes, primary amines and carboxylic acids with intensities of 63%, 71%, 73%, 81% and 91% respectively (Table 6). The characteristic bands from the FT-IR spectra on LDPE degraded by *A. flavus, P. simplicissimum, A. alternata* and the control sample is presented in Table 6. The observed trend in each treatment is hereby compared with that of the control sample. From the data in Table 6, characteristic -C=C- stretching vibration band were observed in the control and the three fungi strain LDPE treatment. This featured at 1635cm^{-1}in control, 1640 cm^{-1} in *A. flavus*, 1632 cm^{-1} in *P. simplicissimum* and 1638 cm^{-1} in *A. alternaria* respectively. The control samples had no carbonyl (C=O) (stretch) absorption band around 1725 cm^{-1}. However, new vibrational bands (peak) at 1726 cm^{-1}, 1724 cm^{-1} in the three different fungal isolates were due to the microbial oxidation of the LDPE polymer leading to the formation of carbonyl (C=O) stretch bonds with characteristic absorption at 1740-1720 (s) cm^{-1} due to the formation of aldehyde or ketone groups.

Apart from the general oxidation trend shown by the microorganisms, specificity in behavior of each fungi isolate was observed. A new vibrational peak at 3287 cm^{-1} was observed in the *A. flavus* degraded LDPE but not in the other treatments with *P. simplicissimum* and *A. alternata*. This was traced to the formation of O-H stretch with H-bonded structure formed by the microorganisms on the substrate. This was not observed on the *P. simplicissimum* and *Alternaria alternata* degraded sample. There was disappearance of the peak at 932 cm^{-1} in the *P. simplicissimum* and *Alternaria alternata* treated LDPE samples. This was due to the activity of the microorganisms on the OH- band vibration, but was not observed in the *A. flavus* degraded LDPE

There were slight differences in the intensity of the characteristics peaks due to influence of microbial activity on the identified chemical bonds. The specificity of enzymes from the microorganisms is particularly a determinant in the degree of degradation. *A. flavus* degraded better than the other two strains based on its ability to form two new bands at 1726 cm^{-1} and 3286 cm^{-1} as against only one band in the other two isolates. A new V_{fh} of O-H stretch with H-bonded structure at 3287 cm^{-1} was formed by *A.*

flavus and this denotes the formation of a new functional group usually at frequency range of 3500 and 3200 cm^{-1} which is indicative of the presence of alcohol or phenol. The characteristic band O-H bond at 950-910 (m) cm^{-1} was observed in the control sample at 932 cm^{-1} and in *A. flavus* at 929 cm^{-1}. The isolates of *P. simplicissimum and A. Alternaria* do not show the bands due to the fact that it had been completely degraded. It was also observed that the V_{fh} of O-H bend and O=H stretch with H-bonded structure which denotes the carboxylic acid group and the alcohol or phenol group respectively which disappeared among the vibrational frequencies as a result of the polymer oxidation. In addition to the formation and disappearance of vibrational frequencies (functional groups), there was slight decrease in the V_{fh} of O-H stretch (i.e. hydroxyl group) from 3441.12 cm^{-1} to 3439.19 cm^{-1} and C=C stretch from 1635.69 cm^{-1} to 1631.83 cm^{-1} (i.e. the alkene group) when compared to the control sample.

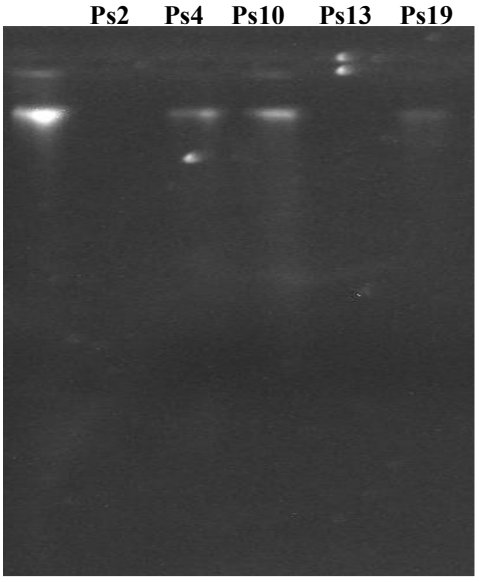

Fig. 1. Gel electrophoresis of extracted DNA from the isolates

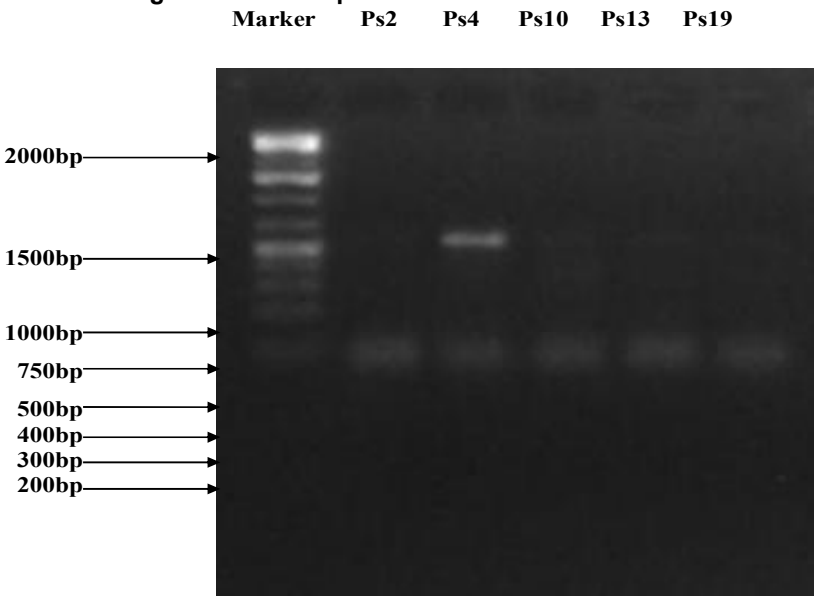

Fig. 2. Polymerase chain reaction amplification on agarose gel

Table 5. Molecular characterisation of fungal isolates

Isolate code	Names	% Similarity	Accession number
Ps. 2	*Aspergillus flavus* KMBF 1501	99.3	JX025733.1
Ps. 4	*Penicillium simplicissimum* strain YK 18	94	KC503976.1
Ps. 13	*Aspergillus* sp. enrichment culture clone RS 4	-	KT211865.1
Ps. 19	*Alternaria alternata* strain 20UPMNR	86	HM543460.1

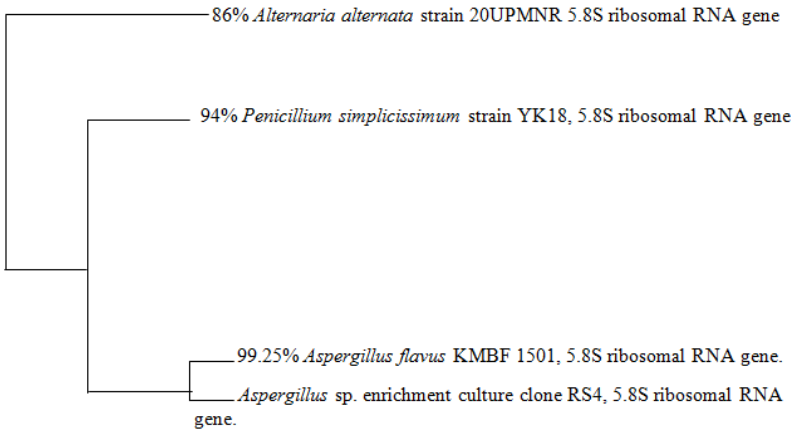

Fig. 3. Phylogenetic tree showing the relatedness of the organisms from their percentage similarity

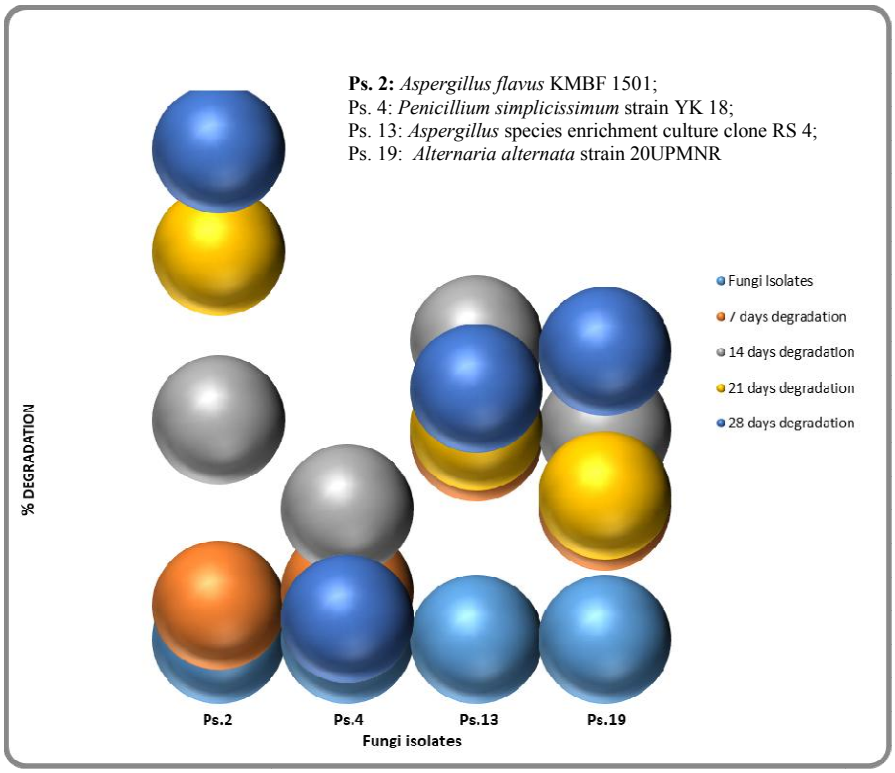

Fig. 4. Percentage degradation of LDPE by fungal isolates after 28 days

Similar to the one observed in LDPE treated with *A. flavus* KMBF 1501, the carbonyl absorption band with vibrational frequency of C=O at 1726.35 cm^{-1} was observed when the polyethylene powder was degraded by *A. alternata* strain 20UPMNR at the frequency range of 1750 cm^{-1} to 1710 cm^{-1}, indicating the formation of ketone or aldehyde group. There was a slight shift in V_{fh} of O-H stretch (i.e. hydroxyl group) from 3441.12cm^{-1} to 3423.76cm^{-1} and also shift increase in V_{fh} of C=C stretch (i.e. alkene group) from 1635.69cm^{-1} to 1637.62cm^{-1} when compared to the control sample. In addition, there was disappearance of V_{fh} of O-H bend and O-H stretch with H-bonded structure indicating complete oxidation of carboxylic acid and alcohol or phenol respectively.

4. DISCUSSION

The soil colour depicts the nature of the activity of each of the organisations where the samples were sourced. For example, the disposal of kitchen wastes and run-offs has encouraged biological life, resulting into black loamy soil found in the dumpsite [35].

The microbial degradation process of polymers is initiated by the secretion of enzymes which cause a chain cleavage of the polymer into monomers [13,8,36,37,38]. Metabolism of the split portions leads to progressive enzymatic dissimilation of the macromolecules from the chain-ends; eventually, the chain fragments become short enough to be consumed by microorganisms [39]. Ps. 1 and Ps. 13 showed exceptional growth capability of over 1344% and 1878% respectively in 24h (i.e. between day 3 and day 4). The mycelia size of these fungi increased by 2335% and 4012% respectively after 5days.

Only five of the nineteen isolates were able to utilise LDPE on MSM agar. The five (5) of the isolates were characterised using 18S rRNA sequencing as *Aspergillus flavus, Penicillium simplicissimum, Alternaria alternata*, *Aspergillus* sp.; an unknown isolate Ps10 were able to utilise LDPE as sole carbon source within 11days. These isolates have been reported to be capable of utilising LDPE as sole carbon source [13,36,37,38,40,41] with *Aspergillus niger* [9,10], and other strains of the *Aspergillus* genus including *A. terreus*, *A. fumigates* [11] and *A. flavus* [42] being chiefly implicated.

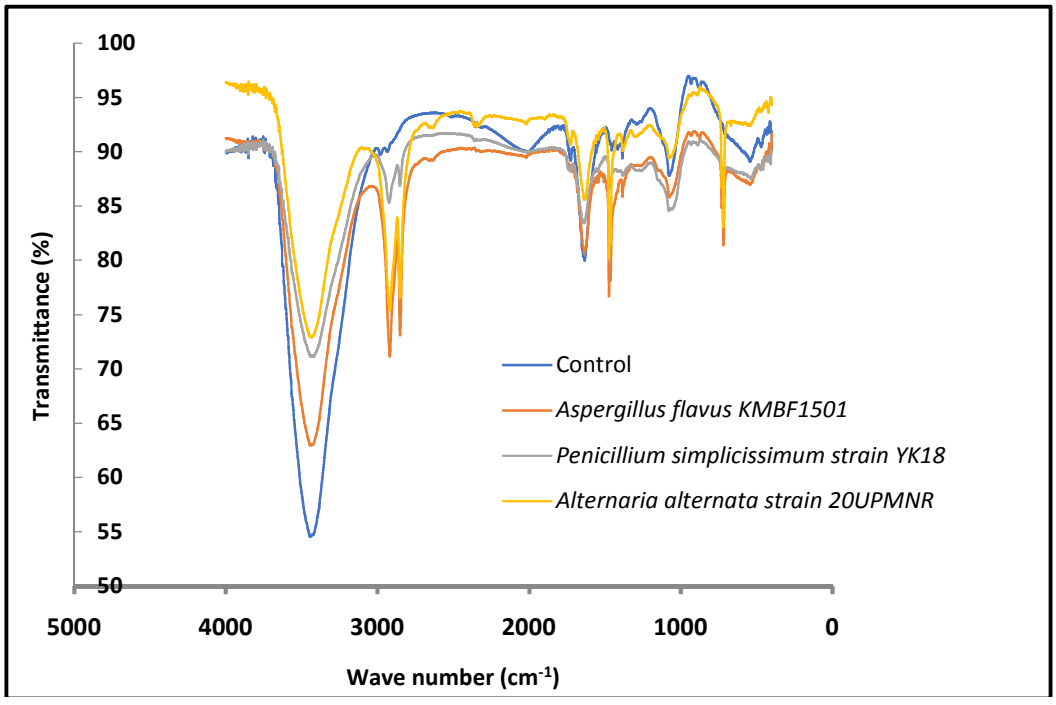

Fig. 5. FT-IR spectra on LDPE degraded by different fungi strains compared with the control sample without treatment

Table 6. Summary of the FT-IR results of the degraded LDPE samples by the three fungal isolates in comparison with the control sample

Vibrational Freq. (V_{fh}) cm^{-1}	Control sample (cm^{-1})	% intensity	*Aspergillus flavus* (cm^{-1}) KMBF 1501	% intensity	*Penicillium simplicissimum* (cm^{-1}) strain YK 18	% intensity	*Alternaria alternata* (cm^{-1}) strain 20UPMNR	% intensity	Functional groups/ Bands
3500-3200 (s,b)	3441	63	3441	53	3439	73	3424	49	O-H stretch
1740-1720 (s)	Nil	-	1726	93	1724	91	1726	84	C=O stretch
1680-1640 (m)	1636	81	1640	82	1632	86	1638	72	-C=C- stretch
950-910 (m)	932	91	930	100	Nil	-	Nil	-	O-H bend
3000 -2850 (m)	2918 & 2851	71 & 73	2922 & 2851	76 &78	2918 & 2851	76&77	2922 & 2851	69&72	C-H stretch
1360-1290 (m)	1385	86	1383	95	1383	90	1316	83	NO_2 stretch
3300-2500 (m)	Nil	-	3287	67	Nil	-	Nil	-	O-H stretch with H bonded structure

Control: Uninoculated Polyethylene Powder

Total genomic DNAs extracted from the isolates were used as template for PCR amplification using the primers (Forward and Reverse). In the present study, the genomic DNA amplified had the same transition weight (1.5 kb) probably due to the fact that the isolates maybe of the same family (Fig. 1), this agrees with Liao et al. [43] who reported that isolates with holotype DNA nucleotide sequence may be amplified on the same DNA molecular weight unless there is large variance in the sequence and the family, resulting into new variant organism. Schloss and Handelsman [44] reported that currently 99% of the microbes present in many natural environments such as the soil may have nucleic acids that may possess the same DNA molecular weight and thus amplified at the same region. The PCR carried out with these genomic DNAs from the organisms using the set of degenerate oligonucleotide primers for highly conserved regions among the various fungi gene sequences resulted in strong amplification of an expected 1.5 kb fragment. The arrows point to the specific gene fragments (1.5 kb) on the DNA ladder which showed that amplification occurred at 1.5 kb DNA transition weight. The amplified fragments in the PCR procedure were purified and sequenced (Fig. 2). The clear and abundant DNA fragments amplified during the PCR process were probably due to the fact that the fungi isolates possess the same DNA sequence and weights, some of which had 99% identity with sequence from the GenBank, an observation similar to the findings of Rajendhran and Gunasekaran [45] on *Rhizobium leguminosarum* bv. *viciae* 3841 (accession no. CAK07882)

In this study, the degradability potential of *Aspergillus flavus* KMBF 1501 was about four times (3.76) greater than that of *Penicillium simplicissimum* strain YK 18. Sowmya *et al*. [36] also isolated A*lternaria alternata* and *Penicillium simplicissimum*, and found that the fungi were able to degrade surface sterilised polyethylene bags with evident weight loss of 0.8% and 7.7% respectively after 3 months of incubation. Unlike the findings of Sowmya et al. [36], the results obtained in this study confirmed that these organisms can utilise polyethylene without any pre-treatment like heat, UV light and acid. Abraham et al. [37] also identified an *Aspergillus* species which was able to degrade LDPE incubated in soil over a period of 90 days resulting in 4.9% weight reduction. Yamada-Onodera et al. [13] investigated a strain *P. simplicissimum* YK and observed that the isolate was able to grow on LDPE plate at 0.5 w/v concentration although they noticed that there was a need to irradiate the films before the isolate was able to utilise it. However, Rani and Singh [46] reported degradation of PE by *A. flavus* with up to 12% degradation ability.

Das and Kumar [47] observed in their investigation that the loss in weight of LDPE films was as a result of fungal adherence to the films' inert surface prior to utilising it as the only carbon and energy source which resulted in an increase in the fungal growth. Kavitha et al. [48] also reported that the percentage of weight reduction of incubated Low density polyethylene films was not as a result of chemicals in the mineral salt medium, but because of a biological process. The initial breakage of PE chains is the longest and most difficult step in LDPE degradation and with long incubation periods, significant quantities of carbonyl groups are produced which will initiate further decomposition process [49].

FT-IR was used to confirm biodegradation of the LDPE strips by determining the formation of new functional groups or disappearance of groups in the polymer. The formation and disappearance of carbonyl and double vibrational bond bands and other groups using the FT-IR was posited for elucidating the mechanism of the biodegradation of LDPE [48]. Carbonyl absorption band of V_{fh} of C=O observed in *P. simplicissimum* strain YK 18 degraded polyethylene powder at 1724.42cm^{-1} was in the frequency range of 1750cm^{-1}–1710cm^{-1} similar to that observed in *A. flavus* [KMBF 1501] degraded sample, denoting the formation of ketone or aldehyde. A slight shift in the vibrational frequency of ketone or aldehyde of polyethylene treated with *P. simplicissimum* strain YK 18 was as a result of degradation of the polyethylene by the fungus [10]. The formation of these bands indicates the oxidation of LDPE by the fungi or their enyzmes [50,51].

5. CONCLUSION

Soil contain microorganism that are able to bring about degradation of synthetic polymers. Degradation by the fungal strains in this study viz *Aspergillus flavus, Penicillium simplicissium, Alternaria alternata and Aspergillus* sp. was monitored using weight loss and FTIR analysis. These strains were able to grow on the polymer containing medium as well as performing degradation of the LDPE without any additives or pretreatment of the polymer hence showing potential for large scale polymer waste bioremediation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gnanavel G, Valli VMJ, Thirumarimurugan M. Degradation of plastics using micro-organisms. Int. J. Pharm. Chem. Sci. 2012; 1:691-694.
2. Sangale MK, Shahnawaz M, Ade AB. A review on biodegradation of polythene: The microbial approach. J. Bio. Biodegrad. 2012;3:164.
   DOI: 104172/2155 – 61991000164
3. Vatseldutt S, Anbuselvi S. Isolation and characterization of polythene degrading bacteria from polyethyene dumped garbage. Int. J. Pharm. Sci. 2014;25(2): 205-206.
4. Duddu MK, Tripura KL, Guntuku G, Divya DS. Biodegradation of low density poly-ethylene (LDPE) by a new biosurfactant-producing thermophilic *Streptomyces coelicoflavus* NBRC 15399T. African Journal of Biotechnology. 2015;14:327–340.
5. Veethahavya KS, Rajath BS, Noobia S, Kumar BM. Biodegradation of low density polyethylene in aqueous media. Procedia. Env. Sci. 2016;35:709–713.
6. Jayasiri HB, Purushothman CS, Vennila A. Plastic litter accumulation on high-water strandline of urban beaches in Mumbai India, Env. Mon. Assess. 2013;185:7709-7719.
7. Kulkarni A, Dasari H. Current status of methods used in degradation of polymers: A review. MATEC Web of Conferences. 2018;144:02023.
8. Shah AA, Hasan F, Hameed A, Ahmed S. Biological degradation of plastics: A comprehensive review. Biotech Adv. 2008; 26:246-265.
   Available:https://doi.org/10.1016/j.biotechadv.2007.12.005
9. Volke-Sepulveda T, Saucedo-Castanede G, Gutierrez-Rojas M, Manzur A, Favela-Torres E. Thermally treated low density polyethylene biodegradation by *Penicillium* pinoohilum and *Aspergillus niger*. J. Appl. Polym. Sci. 2002;83:305–314.
   DOI: 101002/app2245
10. Manzur A, Limon-Gonzalez M, Favela-Torres E. Biodegradation of physio-chemically treated LDPE by a consortium of filamentous fungi J. Appl. Polymer Sci. 2004;92:265–271.
    Available:https://doi.org/10.1002/app.13644
11. Sahebnazar Z, Shojaosadati SA, Mohammad-Taheri M, Nosrati M. Biode-gradation of low-density polyethylene (LDPE) by isolated fungi in solid waste medium Waste Manag. 2010;30:396–401.
12. Rani A, Singh P. Biodegradability of polyethylene by *Aspergillus niger*. World J. Pharm. Res. 2015;4(3):1621-1626.
13. Yamada-Onodera K, Mukumoto H, Katsuyaya Y, Saiganji A, Tani Y. Degradation of polyethylene by a fungus *Penicillium simplicissimum* YK. Polym. Degrad. Stab. 2001;72,323-327.
    Available:https://doi.org/10.1016/S0141-3910[01]00027-1
14. Shah AA. Degradation of poly [ε-caprolactone] by a thermophilic bacterium *Ralstonia* sp strain MRL-TL isolated from hot spring. Int Biodeter Biodegrad. 2015; 98:35–42.
    Available:https://doi.org/10.1016/j.ibiod.2014.11.017
15. Devi RS, Kannan VR, Natarajan K, Nivas D, Kannan K, Chandru S, Antony AR. The role of microbes in plastic degradation. In: Environmental Waste Management, CRC Press, United States. 2016;341-370.
16. Dineshraj DP, Ganesh P. Screening and characterization of isolated fungi from plastic waste dump yard sites. Int. J. Sci. Res. 2016;5:1.
17. Bonhomme S, Cuer A, Delort AM, Lemaire J, Sancelme M, Scott G. Environmental biodegradation of polyethylene. Polym. Degrad. Stab. 2003;81:441–452.
    Available:https://doi.org/10.1016/S0141-3910[03]00129-0
18. Ojha N, Pradhan N, Singh S, Barla A, Shrivastava A, Khatua P, Rai V, Bose S. Evaluation of HDPE and LDPE degradation by fungus implemented by statistical optimization Sci. Rep. 2017;7: 39515.
    DOI: 10.1038/srep39515
19. Rajandas H, Parimannan S, Sathasivam K, Ravichandran M, Yin LS. Novel FTIR-ATR spectroscopy-based technique for the estimation of low density polyethylene biodegradation. Polym. Test. 2012;31: 1094-1099.
20. Aderiye BI, Ogundana SK. Survival of botryodiplodia theobromae in yam tissues. Nova Hedwigia. 1984;40:171-178

(ISSN: 0029-5035)

21. Singh J, Gupta K. Screening and identification of low density polyethylene [LDPE] degrading soil fungi isolated from polyethylene polluted sites around Gwalior city (MP) Int. Curr. Microbiol. Appl. Sci. 2014;3:443-448.
22. Gilan I, Hadar Y, Sivan A. Colonization biofilm formation and biodegradation of Polyethylene by a strain of *Rhodococcus ruber.* Appl. Microbiol. Biotech. 2004;65: 97–104.
23. Usha R, Sangeetha T, Palaniswamy M. Screening of polyethylene degrading microorganisms from garbage soil. Libyan. Agric. Res. Center J. Int. 2011;2(4):200-204.
24. Aderiye BI, David OM. *In vitro* antibacterial activity of aqueous extracts of cashew [*Anacardium occidentale* L.] fruit peels using bioautography method. Eu. J. Med. P. 2014;4(3):284-291.
25. Aderiye BI, Oluwole OA. How to obtain the organelles of prokaryotic and microbial eukaryotic cells. J. Cell Anim. Biol. 2014; 8(6):95-109.
26. Griffin DW, Kellogg CA, Peak KK, Shinn EA. A rapid and efficient assay for extracting DNA from fungi. Lett. Appl. Microbiol. 2002;34:210–214.
Available:https://doi.org/10.1046/j.1472-765x.2002.01071.x
27. Faggi E, Pini G, Campisi E. Use of magnetic beads to extract fungal DNA. Mycoses. 2005;48:3–7.
28. Liu D, Coloe S, Baird R, Pedersen J. Molecular determination of dermatophyte fungi using the arbitrarily primed poly-merase chain reaction Brit. J. Dermatol. 1997;137:351–355.
Available:https://doi.org/10.1111/j.1365-2133.1997.tb03737.x
29. Liu D, Coloe S, Baird R, Pedersen J. Rapid mini-preparation of fungal DNA for PCR J. Clin. Microbiol. 2000;38(1):471.
30. Munir EF, Sipayung C, Priyani N, Suryanto D. Potential of bacteria isolated from landfill soil in low density polyethylene plastic IOP conference series. Earth Env. Sci; 2018.
DOI: 101088/1755-1315/126/1/012144
31. Aderiye BI, Ogundana SK, Adesanya SA, Roberts M. The effect of beta-sitosterol on spore germination and germ-tube elongation of Aspergillus flavus and Botryodiplodia theobromae. Int. J. Food Microbiol. 1989;8(1):73-78.
Available:https://doi.org/10.1016/0168-1605[89]90082-2
32. Immanuel OM, Ibiene AA, Stanley HOJ. Enhanced biodegradation of polyethylene by fungus isolated from the koluama mangrove swamp in the Niger Delta. J. Microbiol. and Biotech. Res. 2014;4(2):1-9.
33. Singh MJ, Sedhuraman P. Biosurfactant polythene plastic and diesel biode-gradation activity of endophytic Nocardiopsis sp mrinalini isolated from Hibiscus rosasinensis leaves. Bioresour Bioprocess. 2015;2(1):2.
Available:https://doi.org/10.1186/s40643-014-0034-4
34. Milstein O, Huttermann A, Frund R, Ludemann Hans D. Enzymatic co-polymerization of lignin with low molecular mass compounds Appl. Microbiol. Biotech. 1994;40(5):760-767.
Available:https://doi.org/10.1007/BF00173342
35. ASTMD 5988. Standard test method for determining aerobic biodegradation in soil of plastic material or residual plastic materials after composting; 2003.
36. Sowmya HV, Ramalingappa B, Nayanashree G, Thippeswamy B, Krishnappa M. Polyethylene degradation by fungal consortium Int. J. Env. Res. 2015;9(3):823–830.
37. Abraham KJ, Chan JN, Salvi JS, Ho B, Hall A, Vidya E, Guo R, Killackey SA, Liu N, Lee JE, Brown GW, Mekhail K. Intersection of calorie restriction and magnesium in the suppression of genome-destabilizing RNA-DNA hybrids. N. A. Res. 2016;44(18):8870-8884.
Available:https://doi.org/10.1093/nar/gkw752
38. Gajendiran A, Subramani S, Abraham J. Effect of *Aspergillus versicolor* strain JASS1 on low density polyethylene degradation. IOP. Conf. Ser. Mater. Sci. Eng. 2017;26:022-038.
39. Lau AK, Cheuk WW, Lo KV. Degradation of greenhouse twines derived from natural fibers and biodegradable polymer during composting. J. Env. Man. 2009;90:668–671.
Available:https://doi.org/10.1016/j.jenvman.2008.03.001
40. Hunter BB, Bamett HL. Deuteromycetes [Fungi Imperfecti] In: Handbook of microbiology; Laskin AI, Lechevalier HA Eds. 4th Edition CRC Press Boca Raton FL. 2000;1-234.

41. Aderiye BI, Fagbohun ED, Adebayo CO. Compendium of fungi. Gospel Publishing and Printing Press, Ado-Ekiti, Nigeria. 2017;461.
42. El-Shafei HA, Nadia H, El-Nasser A, Kanosh AL, Ali AM. Biodegradation of disposable polyethylene by fungi and Streptomyces species. Poly. Degrad. Stab. 1998;62:361–365.
Available:https://doi.org/10.1016/S0141-3910[98]00019-6
43. Liao C, Heckel DG, Arkhust R. Toxicity of *Bacillus thuringiensis* insecticidal protein for *Helicoverpa armigera* and *H. punctigera* major pests of cotton: J. Invert. Pathol. 2002;80:55-63.
Available:https://doi.org/10.1016/S0022-2011[02]00035-6
44. Schloss PD, Handelsman J. Biotechnological prospects from metagenomics. Curr Opin Biotech. 2003;14:303-310.
45. Rajendhran J, Gunasekaran P. Strategies for accessing soil metagenome for desired applications J. Biotech. Adv. 2008;26:576-590.
Available:https://doi.org/10.1016/j.biotechadv.2008.08.002
46. Rani A, Singh P. Screening of polyethylene degrading fungi from polyethylene dump site. Int. J. Chem. Tech. Res. 2017;10(3): 699-704.
47. Das MP, Kumar S. An approach to low density polyethylene biodegradation by *Bacillus amyloliquefaciens*. Biotech. 2014; 5:81–86.
DOI: 101007/s13205-014-0205-1
48. Kavitha R, Mohanan AK, Bhuvaneswari V. Biodegradation of low density polyethylene by bacteria isolated from oil contaminated soil. Int. J. Plant Anim. Env. Sci. 2014;601: 2231-4490.
49. Nowak B, Pajak J, Drozd-Btarkowicz M, Rymarz G. Microorganisms participating the biodegradation of modified polyethylene films in different soils under laboratory conditions Int. Biodeter. Biod. 2011;30:1–11.
Available:https://doi.org/10.1016/j.ibiod.2011.04.007
50. Das MP, Livingstone JR, Veluswamy P, Das J. Exploration of *Wedelia chinensis* leaf-assisted silver nanoparticles for antioxidant, antibacterial and in vitro cytotoxic applications. J. Food Drug Anal.; 2017.
Available:https://doi.org/10.1016/j.jfda.2017.07.014
51. Das MP, Kumar S, Das J. Fungal-mediated deterioration and biodegradation study of low-density polyethylene (LDPE) isolated from municipal dump yard in Chennai, India Energ. Ecol. Environ; 2018.
Available:https://doi.org/10.1007/s40974-018-0085-z

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/28054