Assessment of LDPE degrading potential *Aspergillus* species isolated from municipal landfill sites of Agra

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Abstract

Polythene bags have become an article of common usage in daily life. Huge accumulation of plastic waste cause severe threat to humans, animals and our environment. Recently microbes have become the focus of interest for environment friendly disposal of plastic waste. In present study 18 different types of fungal species were isolated from soil and two fungal species were screened by using polythene powder. The fungal identification was carried out by genomic DNA isolation followed by PCR and ITS rRNA sequencing. BLAST and phylogenetic analysis gave a similarity with the database of *Aspergillus flavus* and *Aspergillus terreus* of 99%. The microbial degradation of LDPE was analyzed by SEM and FTIR analysis. SEM results show disruption of surface texture of LDPE film as compared to control. FTIR analysis gives a close view of CH stretching, CH₂ deformation, CH₂ bending etc. Degradation experiment was done in synthetic media for 4 months and in soil for 9 months. Percentage of weight loss of LDPE film after degradation was 30.6% and 11.4% by *A. flavus* and *A. terreus* respectively in soil after 9 months. In synthetic medium percent weight loss of polythene was 14.3% and 13.1% by *A. flavus* and *A. terreus* respectively after 4 months. There are scanty reports of fungal biodegradation in soil under natural conditions. 30.6% biodegradation in 9 months period with *A. flavus*, a species isolated from dumping sites, give hope of degradation of such LDPE's under environmental conditions without providing any supplement or prior treatment.

Keywords Microbial degradation · LDPE polythene · Synthetic medium · SEM · FTIR

1 Introduction

Polythene is a thermoplastic polymer and it is useful in an extremely large range of application. LDPE is used such as for grocery bags, food packaging films and toys etc. The use of plastic plays a significant role in today's global economy. However the problem arises when these plastics are not properly managed. Accumulation of polythene waste is an ever increasing threat due to its unstoppable usage in daily routine human life. Polythene accumulates in the environment at a rate of 25 million tons per year [1]. Inertness and indiscriminate use of synthetic polythene leading to increased land and water pollution are of great concern [2]. To regulate the plastic waste which

is an ecological threat, an attempt has been made to isolate plastic degrading microorganism from different plastic waste disposal sites. Bioremediation of polythene can be viewed as one of the strategic studies to minimize the plastic waste problem. LDPE is unreactive in nature due to its inert nature, only partial degradation of polythene has been observed after a long time of 32 years inside the soil [3]. To improve the biodegradability of LDPE, it is mixed with pro-oxidants or starch or pretreated with UV radiations, thermal or chemical treatment etc. It was found that thermally and chemically oxidized polythene show more degradation when incubated with *Penicillium simplicissimum* for 3 months as compare to unoxidized polythene [4]. Rate of mineralization of polythene was observed to

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be increased by fungus *Penicillium pinophilum* when the polythene were amended with ethanol [5]. Comparative studies between the untreated LDPE, starch blended polythene and those exposed to UV irradiated in soil burial treatment showed more degradation as compared to pure LDPE type polythene [6]. Untreated, thermally treated and starch blended LDPE and HDPE film were subjected to in vitro biodegradation with *Bacillus sphericus* and *Bacillus cereus* for a duration of 1 year. The weight loss of the thermally treated LDPE and HDPE polythene was 19% and 9% respectively and untreated were 10% and 3.5% respectively [7]. The fusion of different starch such as native, adipate, acetylated and cassava starch were investigated in LDPE matrix. An increase of starch into the olefin matrix resulted in decrease of its mechanical properties [8]. Ocanoated starch blends with LDPE were also assessed in soil burial experiment for 6 month, weight loss of OCST during the biodegradation period, found biodegradable, even with such a high degree of substitution [9]. Instead of blended LDPE, pure synthetic LDPE also showed the degradation by the microorganism. Another study rereported that strain of *Staphylococcus epidermis* exhibit growth on LDPE as it only carbon source in inorganic salt minimal nutrient medium, SEM analysis of the film showed pores of almost bacterial dimensions [10]. Evaluation of the degradation of LDPE carrier bags for up to 2 years in natural soil showed changes towards degradation after 17–22 months [11]. *Pseudomonas putida* S3A have ability to degrade polythene in optimum condition such as pH 6.5 containing 0.5% of polythene and incubated at 37 °C with shaking at 180 rpm. This bacterium was also able to survive on up to 0.9% of polythene [12]. Four bacterial species isolated and identified as *Brevibacillus parabrevis* (PL-1), *Acinetobacter baumini* (PL-2, PL-3) and *Pseudomonas citronellolis* (PL-4) have ability to degrade the resistant LDPE [13]. Many microorganisms are known for biodegradation of synthetic polymers, common bacterial and fungal species include *Pseudomonas* and *Aspergillus* species. *Bacillus cereus* was able to degrade autoclaved, UV treated and surface sterilized polythene. Degradation was measured by weight loss, SEM and FTIR analysis [14]. An attempt has been made by [15] to determine the plastic degradation by *Micrococcus luteus* and *Masoniella* species. Biodegradation of LDPE type polythene was studied by fungi isolated from an aerobic aged municipal landfill in Tehran. Isolated fungi were identified as *Aspergillus fumigatus* and *Fusarium solani* [16]. A number of fungal species, *Aspergillus niger*, *Aspergillus japonicus*, *Aspergillus terreus*, *Aspergillus flavus* and *Mucor* species isolated from polythene waste polluted sites exhibit notable degradation on LDPE over a period of 4 weeks [17]. Efficacy of *Fusarium* and *Aspergillus* species for polymer degradation was analyzed by [18] isolated bacteria were identified as *Bacillus subtilis*, *E. coli* and

Klebsiella pneumonia which were able to degrade plastic. Degradation of plastic cup and polythene bag by *Pseudomonas* species, *Bacillus* species, *Staphylococcus* species, *Aspergillus nidulans*, *Aspergillus flavus* and *streptomyces* species was analyzed in liquid medium [19].

2 Materials and methods

2.1 Sample collection and isolation of fungal isolates

The soil samples were randomly collected from landfill sites of Agra dumped with large amount of plastic waste which had been buried for a long time. The sites were selected for increasing the probability of finding microorganism that can degrade polythene under natural condition. Isolation of the fungal colonies was done by sample enrichment method, by adding 50 grams of the soil sample to 25 mL distilled water with 1 g of LDPE powder. The solution was incubated for 1 week in orbital shaker at 30 °C. The enriched sample was serially diluted up to 10^{-6} and 0.1 ml from each dilution was spread on Sabouraud Dextrose Agar plates and these plates were incubated for 4–5 days at 28 °C in the BOD incubator [20]. Procedure was modified.

2.2 Screening of polythene degrading fungi

Polythene degrading microorganism was screened by spread plate technique using mineral salt medium supplemented with 3% polythene powder. The composition of mineral salt media was: (g/l: K_2HPO_4 1.0, KH_2PO_4 0.2, NaCl 1.0, $CaCl_2 \cdot 2H_2O$ 0.002, H_3BO_3 0.005, $NH_4(SO_4)_2$ 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, $CuSO_4 \cdot 5H_2O$ 0.001, $ZnSO_4 \cdot H_2O$ 0.001, $MnSO_4 \cdot H_2O$ 0.001, $Fe_2(SO_4)_3 \cdot 6H_2O$ 0.01, Agar 15) with pH 7.0. Medium was sterilized for 20 min [21].

2.3 Identification of fungi

Isolated fungi were identified on the basis of morphological identification like shape, colour and structure under light microscope and through molecular characterization. Genotypic characterization was done by DNA extraction and ITS sequencing. The obtained sequence was compared against the sequences available in the NCBI database using BLASTN. DNA was extracted by using spin column kit (HiMedia) [22]. Fungal ITSrRNA gene (600 bp) was amplified using polymerase chain reaction in a thermal cycler and were purified using Exonuclease I—Shrimp Alkaline Phosphatase (Exo-SAP) [23]. Purified amplicons were sequenced by Sanger method in ABI 3500xl genetic analyzer (Life Technologies, USA). Genomic DNA was

checked for its integrity using agarose gel electrophoresis and concentrations were checked using Nano drop Lite Spectrophotometer (Thermo Scientific). Further, 50 ng DNA was used for amplification of ITS rRNA gene using universal fungal primers ITS1 (5'TCCGTAGGTGAACCTGCG 3') and ITS4 (5'TCCTCCGCTTATTGATATGC 3'). The PCR reaction was carried out in 50 µl reaction mixture containing 3.5 pM primers (Eurofins), 5 mM deoxynucleotide triphosphate (dNTPS-GeNei), 1U Taq polymerase (GeNei), PCR reaction buffer (with 15 Mm MgCl₂), DNA template and PCR water. The PCR product was run in agarose gel.

2.4 Preparation of fungal suspension

Fungi were cultured on SDA slants during the month of March and incubated at 30 °C for 48 h after completion of sporulation. Spore of fungi on slants were washed with 6 ml physiological saline solution. Spore mixture was placed in a small beaker on stirrer and stirred for 10 min aseptically. 3 ml of this spore mixture was added in known volume of distilled water. Fungal spores were counted by haemocytometer.

2.5 Biodegradation experiment

2.5.1 Soil burial treatment

Plastic containers of 500 ml capacity were filled with sterile soil. Pre-weighted LDPE strips (4cmx3 cm) were first disinfected with 70% ethanol for 30 min, and then transferred to benzene for another 30 min to remove the plasticizer. Later it was placed in distilled water for 10 min and dried for 15 min in laminar air flow chamber. These strips were then placed in the container at a depth of about 5 cm and inoculated with fungal suspension. The soil was kept moist by sprinkling distilled water, at regular time interval to maintain 40–45% humidity. The experiment was set in the month of February up to 9 months at room temperature ranging from 25 to 38 °C. The degradation of samples was studied at regular interval of 3 months by removing the samples carefully from the soil and washing gently with distilled water to remove the soil adhering on the surface. Experimental setup was maintained under normal condition of temperature.

2.5.2 Shake flask test (in liquid synthetic medium)

In this biodegradation experiment 2 ml of fungal suspension was added to flask containing 150 ml of liquid synthetic medium (composition of synthetic medium is same as screening medium except agar). Added disinfected film (4 cm × 3 cm) in flask and incubated at 30 °C in orbital shaker at 120 rpm for 4 months.

2.5.3 Film harvest

After incubation period of 9 months in soil and 4 months in liquid synthetic medium, polythene strips were harvested and washed with running water. After this 70% ethanol was used to remove as much as cell mass from the residual film as possible. The film was dried for 24 h in oven.

2.6 Assessment of degradation of polythene

2.6.1 Weight loss method

Sample weight loss was determined by an analytical balance (SHIMADZU CORPORATION TYPE AY220). Percentage weight loss was determined using the formula

$$\text{Weight loss\%} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} * 100$$

2.6.2 FTIR

Infrared spectra of polythene film were recorded on Cary 630 FTIR (Agilen technologies) over a range of 4000–800 cm⁻¹. Samples were powdered and analyzed (Table 1).

2.6.3 SEM

Changes in surface morphology and microstructure of the polyethylene strip due to biodegradation were analyzed through scanning electron microscopy. The polythene films were examined by JSM 6490 LV (JEOL JAPAN).

3 Results and discussion

3.1 Isolation and screening of fungi

Samples were collected in sterile containers and stored at room temperature. 18 different types of fungi were isolated from different plastic waste dumping sites of Agra. Medium containing LDPE powder was used to screen the polythene degrading fungi. The plates were incubated at

Table 1 Degradation of polythene in soil within 9 months

Isolated organisms	Initial weight (mg)	Final weight (mg)	Weight loss (mg)	% of weight loss
<i>A. terreus</i>	70.9	61.6	9.3 ± 0.46	13.1
<i>A. flavus</i>	70.9	49.2	21.7 ± 2.2	30.6
Control	70.9	70.9	0	0

Table 2 Degradation of polythene in synthetic medium within 4 months

Isolated organisms	Initial weight (mg)	Final weight (mg)	Weight loss (mg)	% of weight loss
<i>A. terreus</i>	70.9	62.8	2.3 ± 1.27	11.4
<i>A. flavus</i>	70.9	60.7	10.2 ± 0.35	14.3
Control	70.9	70.9	0	0

30 °C for 7–10 days with isolated fungi. Out of 18 fungi only 2 types of fungi showed growth on screening medium and were able to utilize polyethylene as carbon source, thereby degrading polyethylene. Therefore these two species were selected for further degradation of LDPE (Table 2).

3.2 Phenotypic and genotypic characterization of isolated fungal species

Fungi were identified as *Aspergillus* species by their growth characteristics and colony morphology. Molecular

characterization showed that Sample 1 and 2 gave 99% similarity with *A. flavus* and *A. terreus*. Potentially closely related type strain sequences were found using the BLASTN program [24]. Phylogenetic analysis was done by the neighbor-joining method (software MEGA 6 was used to constructing neighbor joining phylogenetic tree) [25–28], the phylogenetic tree is shown in Figs. 1 and 2. The obtained product size was 700 bp. Figures 3 and 4 depict the gel pictures of genomic DNA isolation and PCR amplification.

3.3 Determination of % weight loss of LDPE film

The degradation of polythene was determined by calculating the percentage of weight loss of polythene by isolated fungi. Weight loss of polythene by measured after 3 months of interval up to 9 months. The percent weight of LDPE reduced from 11.4 to 30.6% in soil after 9 months. Whereas total weight loss of LDPE in synthetic medium after 4 months of incubation on orbital shaker was 13.1% and 14.3% by ht fungal isolates *A. terreus* and *A. flavus*. The

Fig. 1 Phylogenetic relationship of *A. flavus* against other species **Fig. 2** Phylogenetic relation of *A. terreus* against other species

Fig. 3 Genomic DNA gel image

Fig. 4 PCR product gel image

Table 3 Characteristics of soil used in soil experiment

Parameters	Results
pH	6.5
Oxidizable organic carbon	0.505–0.750 (medium)
Ammonical nitrogen	Less than 12 (low)
Available potassium	112 kg/ha (medium)
Humidity	40–45%

percentage of weight loss of polythene strips in soil and synthetic medium is shown in Tables 3 and 4. The weight difference between initial and final weight indicate the extent of polythene utilization by the fungi. Result indicated that both the fungal isolate are capable of degrading LDPE in soil as well as in liquid synthetic medium. These

2 fungal species were separately allowed to degrade the polythene under the soil burial method and in liquid synthetic medium. Among the fungal species *A. flavus* was found to be most active degrading 30.6% in soil and 14% in liquid synthetic medium.

Soil quality parameter was tested by SOIL TESTING KIT KO54 by HIMEDIA given in Table 5.

3.4 SEM results

SEM results exhibit the surface of the polythene film becomes pitted and eroded. SEM pictures of polythene film revealed that the film treated with *A. flavus* show more cracks and holes than with *A. terreus* treated in soil and in liquid synthetic medium. Microscopic images of the LDPE samples are shown in Figs. 5, 6a–c shows the changes in the surface morphology of polythene strip. Under scanning electron microscopy, the control sample appeared as a uniform homogenous sheet (Fig. 5a). After 9 months degradation in soil; peeling, holes and exfoliation in the film surface were observed. After 4 months in synthetic medium, fractures and cracks were seen on the surface of LDPE film. Polythene strip treated in soil showed more cavities, crack and delamination of the LDPE film as compared to strips in synthetic medium kept for 4 months. Prolonged exposure of LDPE in soil with fungal strain resulted in deterioration and degradation of polythene strips. Fungal degradation resulted in physical weakening and disintegration of the strips, making surface rough and fragile. Depilation and perforations were clearly seen in scanning electron micrographs.

3.5 FTIR results

FTIR is an important tool for analysis of structural changes due to induced degradation in polythene. FTIR analysis gives a close view of stretching, deformation, bending and new bond formation of functional group. There are two important regions $4000\text{--}1300\text{ cm}^{-1}$ and $900\text{--}650\text{ cm}^{-1}$ for examination of spectrum in FTIR. The high frequency portion of the spectrum is called functional group region.

Control sample gave a peak at 1459.68 cm^{-1} which was decreased after treated with *A. terreus* and *A. flavus* in soil which show methylene C–H bend. A shift in peak was observed when treated by *A. terreus* and *A. flavus* in liquid synthetic medium and in soil peak is decreased and shifted to 3443.30 that show vibrational stretch in hydroxylated and carboxylated compounds. Several new peaks of different functional groups are observed such as ketone, aldehyde, carboxylic acid and alcohols etc. after biodegradation of LDPE (Tables 4, 5, 6). The FTIR spectra of untreated LDPE film incubated with *A. flavus* and *A. terreus* for 4 months in liquid synthetic

Table 4 Characterization of FTIR peaks in control [29, 30]

Wave numbers after treatment cm^{-1}	Wave numbers cm^{-1}	Bond	Functional group
913	1000–650	=C–H bend	Alkenes
1209.87	1320–1000	–C–O stretch	Carboxylic acid, esters and ether
1459.68	1470–1450	–C–H bend	Alkanes
1951.83	2000–1900	C=C=CH ₂	Cumulative double bond stretch in allenes
2140.12	2280–2000	–SCN	Aliphatic cynide
2281.8	2700–2200	C=O	Carbonyl in addition of ammonium
2360.71			
2911.9	3000–2850	–C–H stretch	Alkanes
3760.12	3760	–OH stretch	Alcohol

Table 5 Characterization of peaks in FTIR after degradation of LDPE

Wave numbers after treatment with <i>A. flavus</i> in soil cm^{-1} and in synthetic medium		Bond		Functional group	
Soil	Synthetic medium	Soil	Synthetic medium	Soil	Synthetic medium
853.8	1015	C=C	–C–O	Conjugation of Alkenes	Carboxylic acid, ester and ether
10,327.77	1237.84	-do-	-do-	Carboxylic acid, ester and in ether	
1239.70	1534.25	-do-	NH ₃	-do-	Stretch in symmetric and asymmetric amide
1459.68	2141.98	–C–H bend	C≡C	Alkane	Alkynes
1667.28	2285.53	–C=O stretch	C=O	Ketone and Aldehyde	Carbonyl in addition of ammonium
1948.10	2348.91	C=C=CH ₂	-do-	Cumulative double bond stretch in allenes	-do-
2134.52	2917.50	-do-	–C–H stretch	-do-	Alkanes
2272.48	3005.11	-do-	–C–H vibration	-do-	Epoxy ring
2346.18	3622	-do-	C=O	-do-	Hydroxylated and carboxylated compound
2908.18	3756.39	-do-	-do-	-do-	-do-
3005.11	3795	-do-	-do-	-do-	-do-
379.95		-do-	-do-	-do-	-do-

Fig. 5 Scanning electron micrograph of untreated and treated polythene strip in soil **a** control, **b** *A. flavus*, **c** *A. terreus*

medium and 9 months in soil containing polythene as sole carbon source are shown in Figs. 7, 8 and table. In range 1000–650 cm^{-1} new absorption band at 840 cm^{-1} ,

853 cm^{-1} and 947 cm^{-1} of the spectra were observed in LDPE polythene film treated with fungi as compare to control at 913 cm^{-1} this is possibly due to formation of

Fig. 6 Scanning electron micrograph of untreated and treated polythene strip in liquid synthetic medium. **a** control, **b** *A. flavus*, **c** *A. terreus*

Table 6 Characterization of peaks in FTIR after degradation of LDPE

Wave numbers after treatment with <i>A. terreus</i> in soil cm^{-1} and in synthetic medium		Bond		Functional group	
Soil	Synthetic medium	Soil	Synthetic medium	Soil	Synthetic medium
947.0002	840	$-\text{CH}=\text{CH}_2$	$\text{C}=\text{C}$	Formation of new vinyl groups	Conjugation of Alkenes
1036.50	11,017.86	$-\text{C}-\text{O}$ stretch	-do-	Carboxylic acid, ester and ether	-do-
1233.34	1237.84	-do-	-do-	-do-	-do-
1459.68	1658.42	$-\text{C}-\text{H}$ bend	$-\text{C}=\text{O}$ stretch	Alkanes	Ketone and aldehyde
1661.69	2143.85	-do-	$\text{C}\equiv\text{C}$	-do-	Alkynes
1946	2283.66	$\text{C}=\text{C}=\text{CH}_2$	$\text{C}=\text{O}$	Cumulative double bond stretch in allenes	Carbonyl in addition of ammonium
2046.91	2348.91	-do-	-do-	-do-	-do-
2175.54	2913.77	-do-	$-\text{C}-\text{H}$ stretch	-do-	Alkanes
2908.18	3012.57	-do-	$-\text{C}-\text{H}$ vibration	-do-	Epoxy ring
3003.25	3602.31	$\text{C}=\text{O}$		Hydroxylated and carboxylated compound	
3448.8	3795.54	-do-	-do-	-do-	-do-

($=\text{C}-\text{H}$) alkenes functional group and $\text{C}=\text{C}$ conjugation, it is also reported by [31] assigned a peak of $\text{C}=\text{C}$ conjugation band at 862 cm^{-1} . In addition the range between 1320 and 1000 cm^{-1} bands at 1032 cm^{-1} , 1036 cm^{-1} , 1233 cm^{-1} , 1237 cm^{-1} and 1239 cm^{-1} also appeared by the action of *A. flavus* and *A. terreus* in both the degrading medium (soil and liquid synthetic medium) due to $-\text{C}-\text{O}$ stretch and only one absorption band was seen in control at 1209.87 cm^{-1} . New absorption band between 1000 and 1700 cm^{-1} (1227 and 1372 cm^{-1}) of spectrum were observed due to oxidized moieties containing $-\text{OH}$ group [32]. New peak at 1534 cm^{-1} was seen which show symmetric and asymmetric amide group after treated by *A. flavus* in synthetic medium. Control sample do not show any peak in range 1710 – 1605 cm^{-1} , 3 new absorption band appeared at 1658 cm^{-1} , 1661 cm^{-1} , 1667 cm^{-1} which show formation of ketone and aldehyde. Strong absorbance in the region 1650 – 1860 cm^{-1} is due to presence of carbonyl compounds, including

esters, aldehyde and carboxylic acid as reported by [33]. Between the range of wave number 2700 – 2200 cm^{-1} control show 2 peaks only at 2281 cm^{-1} and 2260 cm^{-1} which is decreased as well as increased at 2272 cm^{-1} , 2285 cm^{-1} , 2283 cm^{-1} , 2346 cm^{-1} and 2348 cm^{-1} showed formation of carbonyl addition with ammonium group. New absorption bands formed at 3003 , 3005 , 3012 show $-\text{C}-\text{H}$ vibration in epoxy ring. The native band 2911 cm^{-1} is decreased to 2908 cm^{-1} in soil medium by *A. flavus* and increased to 2917 cm^{-1} in synthetic medium by *A. flavus*. Additionally new absorption bands between 3800 and 3100 cm^{-1} of the spectra were observed at 3448.8 cm^{-1} , 3602 cm^{-1} , 3795 cm^{-1} as compared to control at 3760 cm^{-1} due to formation of carboxylated compounds. A similar shift in peak was observed by [34] when LDPE and HDPE sheets were treated with *Penicillium chrysogenum* NS10 at 3436.85 cm^{-1} and 343.45 cm^{-1} . These peaks were observed due to the vibration in the stretching of the $\text{O}-\text{H}$ bond in alcohols.

Fig. 7 FTIR spectra of LDPE degraded by isolated fungi in synthetic medium

Fig. 8 FTIR spectra of LDPE degraded by isolated fungi in soil

4 Conclusion

Polythene is probably the polymer used frequently in the world, because it is easily processed, stable and low cost product. An ecofriendly way to get rid of polythene waste is to exploit microorganism to degrade polythene. The current study demonstrates biodegradation of LDPE film by *Aspergillus* species isolated from landfill sites of Agra. Out of 18 fungi isolated, only 2 isolates were found to show considerable higher growth rate as compared to rest. After genotypic characterization isolates were identified as *A. flavus* and *A. terreus*. These fungi were able to grow in soil as well as in synthetic medium, using polymer, as carbon source. Weight of polythene strip decreased from 70.9 to 62.8 mg in synthetic medium and 61.6 mg in soil by *A. terreus*. With *A. flavus*, weight of polythene strip decreased from 70.9 to 49.2 mg in soil and 60.7 mg in synthetic medium respectively. *Aspergillus flavus* showed more weight loss in soil as well as in synthetic medium. There was no reduction of weight in control. SEM result revealed morphological changes on the sheet caused due to degradation by the fungal isolates. Images of SEM show microbial colonization on the film surface that is evident of fungal degradation. FTIR results exhibit changes or either new peak formation or disappearance of a peak or else change in the peak range as accounted as monitoring parameter and regarded as the change occurred on the surface of polythene due to the action of fungi. FTIR analysis of degraded LDPE showed the presence of alcohols, alkanes, amines, and alkenes after degradation in soil and in synthetic medium, indicating that degradation was carried out successfully. The isolated fungi were able to degrade polythene. It is likely that these isolates produce enzymes which are capable of degrading polythene; exact mechanism of enzymatic degradation has not been fully known. Thus the microbial strain can be used as a valuable application to solve the plastic waste problem. In most of the reports, microbial degradation is studied under laboratory condition i.e. in synthetic medium. There are scanty reports of fungal biodegradation in soil or natural conditions. Effort was made to degrade LDPE waste in dumping sites where it scatter. 30.6% biodegradation in 9 months period with *A. flavus*, a species isolated from dumping sites, give hope of degradation of such LDPE's under natural condition without providing any supplement or prior treatment. Biodegradation rate of LDPE in soil is higher than the rates reported previously. Degradation of LDPE by fungi in synthetic medium showed more or less degradation of polythene strips. There are some reports on present status of LDPE degradation. [21], 2014 measured biodegradation in terms of weight loss,

which was nearly 16 to 36% after a period of 4 weeks in synthetic medium. Fungal strain *Aspergillus japonicus* F3 (36%), *Fusarium* sp F6 (32%), *A. flavus* F1 (30%) showed effective degradation results in 4 weeks as compared to *Penicillium* sp F5 (24%), *A. niger* F2 (20%), *Mucor* sp F4 (16%). In 2016 35% LDPE degradation within 90 days reported by *Aspergillus clavatus* strain JAK1 in aqueous medium [35]. In 60 days LDPE degradation. *Aspergillus flavus*, *A. niger*, *Aspergillus foetidus* was reported to be 31.2%, 38% and 26.1% respectively in synthetic medium by [36]. Biodegradation of LDPE under laboratory condition in liquid synthetic medium was studied by [16] fungi *A. japonicus* and *A. niger* during 1 month degraded 11.11% and 5.8% respectively. In 2018 [37] reported 36.4%, 24%, 18% degradation of LDPE in 4 months by *Aspergillus oryzae*, *A. fumigatus* and *A. nidulans* respectively. To check the efficiency of biodegradation, weight loss method was performed under laboratory conditions for 2, 4 and 6 months. Experimental data revealed that *Streptomyces* sps have highest plastic degradation capacity and it degrades LDPE polymer granule up to 46.7%, this degradation was followed by the *A. niger* (26.17%), bacterial species *Pseudomonas* sps (24.22%) and *A. flavus* (16.45%) for the period of 6 months [38]. In all earlier report and in the present study *A. flavus* strain was found to be common. Percent biodegradation in synthetic medium of earlier report was more or less identical to biodegradation by *A. flavus* in soil, under natural conditions, in the current study.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this paper.

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