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MICROBIAL DEGRADATION OF LOW-DENSITY POLYETHYLENE (LDPE) BY FUNGUS ISOLATED FROM LANDFILL SOIL

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ABSTRACT

A low density polyethylene (LDPE) is one of the hazardous polymers accelerates land and water pollution. Standardizing the protocol for degradation of plastics in an aesthetic approach is a big task. Low density polyethylene polymer can be degraded by microbial enzymes by means of cutting down the molecular chains. Two fungal strains *Aspergillus fumigus* and *Xylaria sp*. were showing high degradable activity through the determination of pH variation and carbon di oxide estimation. *Aspergillus fumigus* and Xylaria sp shows pH variation in the culture media containing LDPE strips after 30 days and was recorded as 6.5 to 8.4 and 6.5 to 7.7 respectively. The efficacy of polyethylene degradation was confirmed further by CO_2 estimation of culture filtrate and was recorded as 1.460 g/l in *Aspergillus fumigus* and 1.350 g/l in *Xylaria sp*. Surface erosion and the formation of pits and cavities on the surface of the LDPE strips were also observed using Scanning Electron Microscope and significant disappearance in carbonyl peak with respect to the control band absorbance (2361.61 cm-1) and increase or decrease in peak intensity with respect to control indicates structural changes in chemical bond due to degradation process.

Keywords: Low density polyethylene, Biodegradation, Fungal degradation, Pollution control

INTRODUCTION

Plastic is the most versatile synthetic 'manmade' substance, created out of the fossil fuel resources, composed of petroleum based materials called resins (e.g., polythene and polypropylene) – materials that are resistant to biodegradation that enable most of the industrial and technological revolutions of the 19th and 20th centuries (Kumar *et al.,* 2007). Low density polyethylene is a polymer made of long chains of ethylene monomers, one of the major sources of environmental pollution. As per data each year use of polyethylene rising to 12% and about 140 million tons of synthetic polymers are produced worldwide (Hussein *et al.,* 2015). Substantial quantities of plastic accumulation in the environment affects biotic and abiotic factor. Seabirds, sea turtles, fishes, and marine mammals often consume marine debris (plastics) as food by mistake and cause seriously harm for marine life, lead to starvation or malnutrition. Entanglement of marine life on white pollution leads to suffocation, starvation, drowning, increased vulnerability to predators (Jeftic *et al.,* 2009). For example, the percentage of entangled species that have been increased among various groups of marine organisms, 100 % of marine turtles, 67 % of seals, 31 % of whales and 25 % of seabirds (Bergmann *et al.,* 2015). Plastic bags get buried under soil surface will generate soil infertility and contaminates the water resources by leaching out toxic chemicals from plastics (Balakrishnan and Flora 2017). The chemicals additives on plastic have lethal effects on human such as promote endocrine disruption, damage in nervous system and some genetic diseases cause birth defects, genetic mutations (Talsness *et al.,* 2009). Biodegradation is an eco friendly method of synthetic polymer degradation where microorganisms utilizing carbon as energy source by converting organic complex polymer into simpler one. Recently several microorganisms were reported for plastic degradation such as *Bacillus sp., Staphylococcus sp., Streptococcus sp., Pseudomonas sp. and Moraxella sp, Aspergillus niger, A. cremeus, A. flavus, A. candidus* and *A. glaucus* etc. In most studies, fungi were considered favorable for the degradation of LDPE due to their ability to form hydrophobic proteins that can attach to the polymer surface (Kershaw and Talbot 1998, Seneviratnem 2006), the faster growth of fungal biomass compared to bacteria (Kim 2003), their generation of degrading enzymes that are well matched to the insoluble LDPE (Shah 2008). During enzymatic degradation process microbes attach to the polymeric surface, with the help of enzyme break the complex structure of polymer and utilize it as nutrient source. So the polymer is depolymerized and carbon dioxide (CO_2), water (H_2O) or methane (CH_4) is produced as end products of mineralization process (Das and Kumar 2014). Therefore isolating the efficient low density polyethylene (LDPE) degrading fungal strains from plastic waste and studying their degradation property will open up many routes to create plastic free zone.

MATERIALS AND METHODS

Sample collection

Land fill soil was collected from Salem City plastic dumped site, Tamil Nadu, India and was used for the isolation of LDPE degrading microorganisms.

Figure 1: Optical density of fungal growth at 600nm

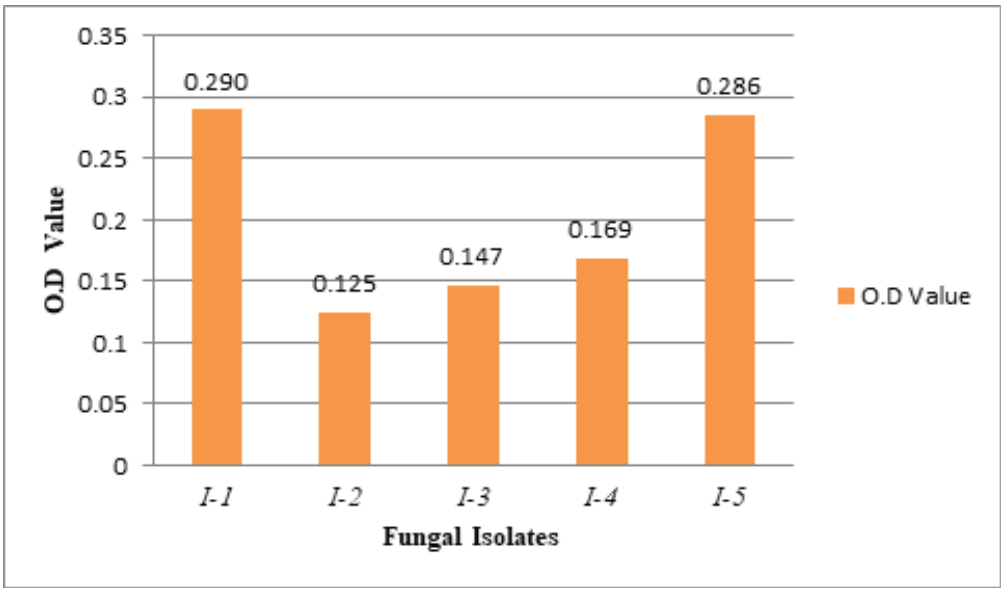
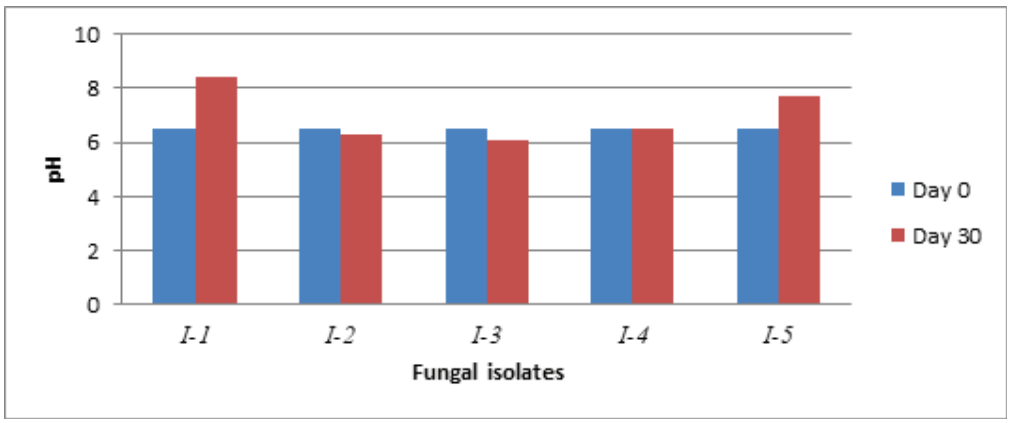


Figure 2: Variation in pH level during biodegradation due to microbial activity



The chemicals used in this present study were purchased from Sigma Aldrich (St. Louis, MO, USA). All other reagents used in this study were of high purity and analytical grade. The LDPE strips used in this study were collected from Salem market, which were 20 micron thick bags. For the biodegradation studies, LDPE strips were cut into small pieces and were sterilized with 70 % ethanol.

A. PRIMARY SCREENING

(i) Isolation and screening of polythene-degrading fungus

The soil sample collected from landfill soil of Salem district was homogenized and passed through a 2-mm sieve to remove gravel. One gram of soil sample was added to enrichment media. Enrichment technique was carried out to select the efficient polythene-degrading fungus. Mineral salt medium (MSM) broth was used to enrich fungus. 100 ml of MSM medium containing 1.0 g NH_4NO_3, 0.2 g $MgSO_4 \cdot 7H_2O$, 1.0 g K_2HPO_4, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.15 g KCl, and 1.0 mg of each of the following microelements: $FeSO_4 \cdot 6H_2O$, $ZnSO_4 \cdot 7H_2O$ and $MnSO_4$ at pH 6.5 were supplemented with 0.5 g of plastic strips (cut into small pieces and weighed) as sole source of carbon energy. One gram of processed soil was inoculated in respective flasks. These flasks were incubated in a shaker at 120 rpm for 15 days. After enrichment process, the solid particles were settle and one ml aliquots of the supernatant were serially diluted and plated on PDA plate for isolation of fungus. For each dilution duplicate potato dextrose plate was made to isolate fungus. Plates were incubated at 30ºC for 2 days. The developed colonies were isolated and sub-cultured repeatedly to obtain the pure culture and stored at 4 ºC.

(ii) Identification of polyethylene degrading fungi

The identification of fungi was performed on the basis of macroscopic and microscopic examination. The morphological identification of the fungal isolates was done by lacto phenol cotton blue staining.

B. SECONDARY SCREENING

The most active degrading isolates obtained from primary screening were further screened for LDPE degradation rates by following methods.

(i) Determination of fungal growth

Hundred ml of liquid MSM was dispensed in 250 ml conical flasks and supplemented with LDPE strips (3cm×3cm) as a substrate and sole source of carbon energy. Flasks were sterilized by autoclave at121ºC for 15 minutes, allowed to cool then inoculated with one loop fungal culture. MSM without microbes serve as control (medium inoculated with 1ml distilled water). The flasks were incubated for 7 days at 30°C, 150 rpm. The OD at 600 nm was recorded using UV-VIS spectrophotometer (Hussein *et al.,* 2015).

(ii) Change in pH

The variation in the pH level in the culture media possibly occurred due to the microbial activity was measured at 30th day of the study.

(iii) CO_2 evolution test

Dissolved CO_2 was evolved as a result of LDPE degradation and determined volumetrically by alkalinity method. Sample (25 ml of culture) was taken in a conical flask and 0.05 ml of 0.1 N sodium thiosulphate solutions was added, then 2 drops of methyl orange indicator was added, and

Figure 3: SEM image of untreated LDPE Control (C)

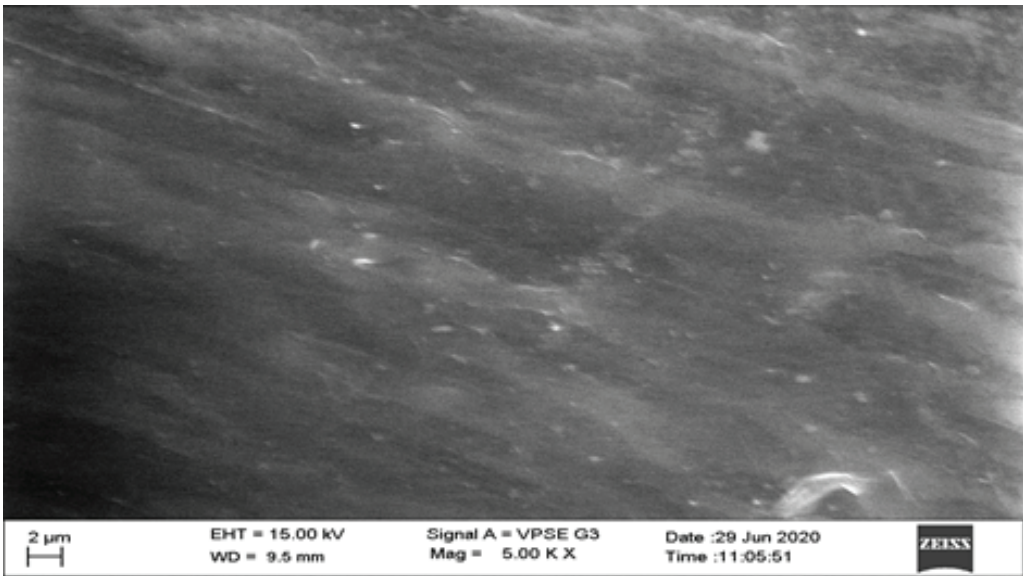


Figure 4: SEM image of LDPE treated with *Aspergillus fumigus*(I-1)

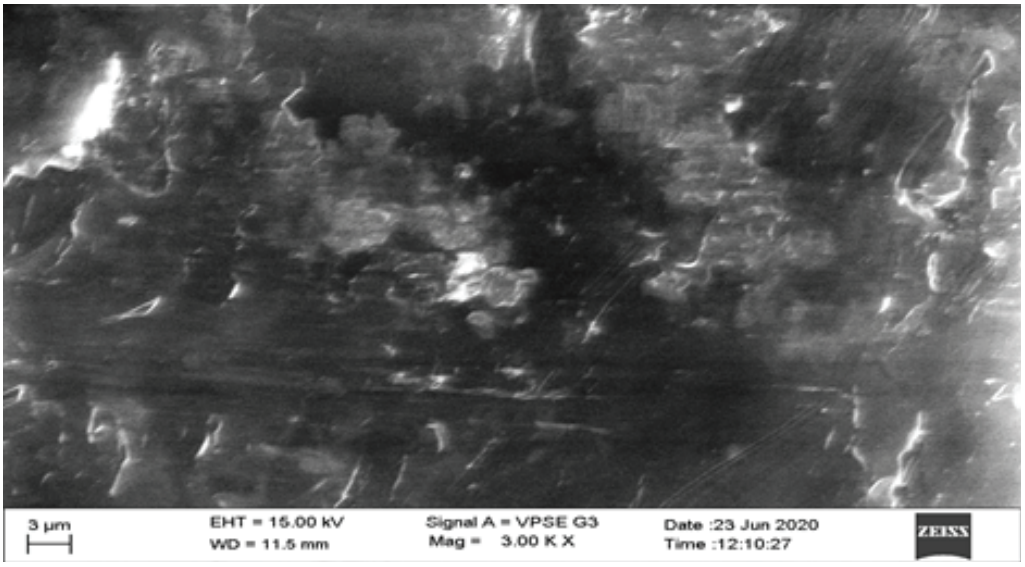


Figure 5: SEM image of LDPE treated with *Xylaria sp*(I-5)

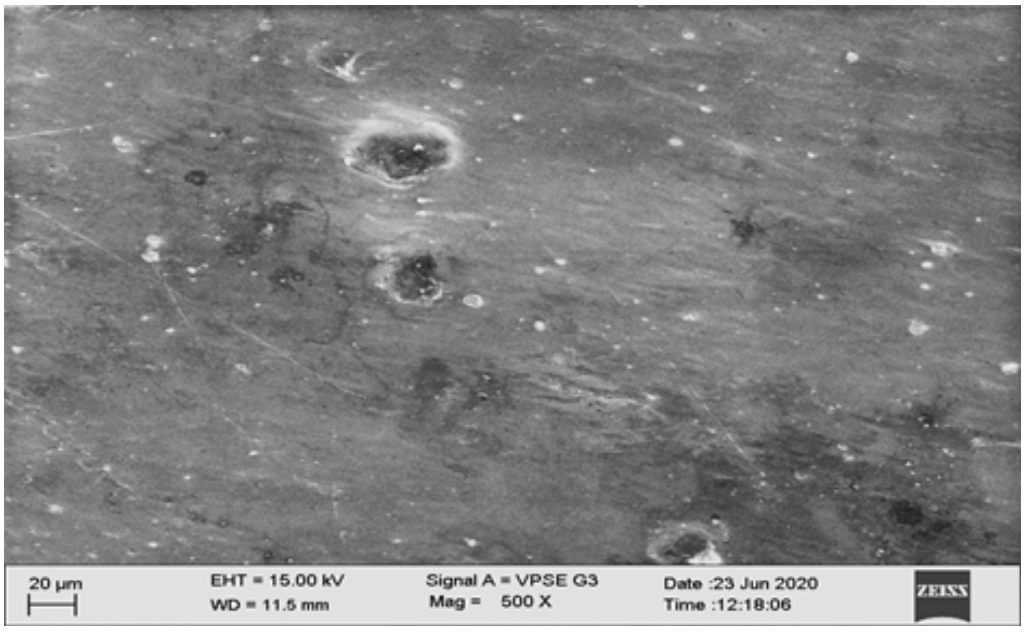


Figure (3, 4 & 5): Scanning electron micrographs revealing the surface changes (after washing the biofilm layer with 2% SDS) after incubation with *Aspergillus fumigus*(I-1), *Xylaria sp*(I-5) and Control(C) after 30days of incubation.

then titrated against 0.02N sodium hydroxide solution until the colour was changed from orange red to yellow. Two drops of phenolphthalein indicator was added and titration continued till a pink color developed (Hussein *et al.,* 2014). Volumes of the titrant used were recorded and the amount of CO_2 calculated according to the equation:

Dissolve CO_2 (mg/l) =

$$\frac{A \times B \times 50 \times 1000}{V}$$

Where;

A = ml of NaOH titrant;

B = normality of NaOH;

V = ml of the sample

C. BIODEGRADATION ANALYSIS

The pre-weighted strip of LDPE films (3×3cm) aseptically transferred into the conical flask containing 150 ml of mineral salt medium and then inoculated with identified polythene degrading microorganisms. Control maintained in the microbe free medium with LDPE strips and incubated at 30ºC for 30 days. After the period of incubation the strips were collected, washed thoroughly using 70% ethanol and distilled water, air dried and further analyzed for degradation activity (Botre *et al.,* 2015)

(i) Scanning electron microscopy (SEM) analysis

The samples after 30 days of incubation with fungal isolates, subjected to SEM analysis. The sample were expose to pre treatment by washing with 2 % SDS and distilled water for few minutes and finally sterilized with 70 % ethanol to remove the bioflim attached on the surface of LDPE strips. The treated and untreated LDPE sample was pasted onto the

Figure 6: FTIR spectra of untreated LDPE Control(C) (I-5)

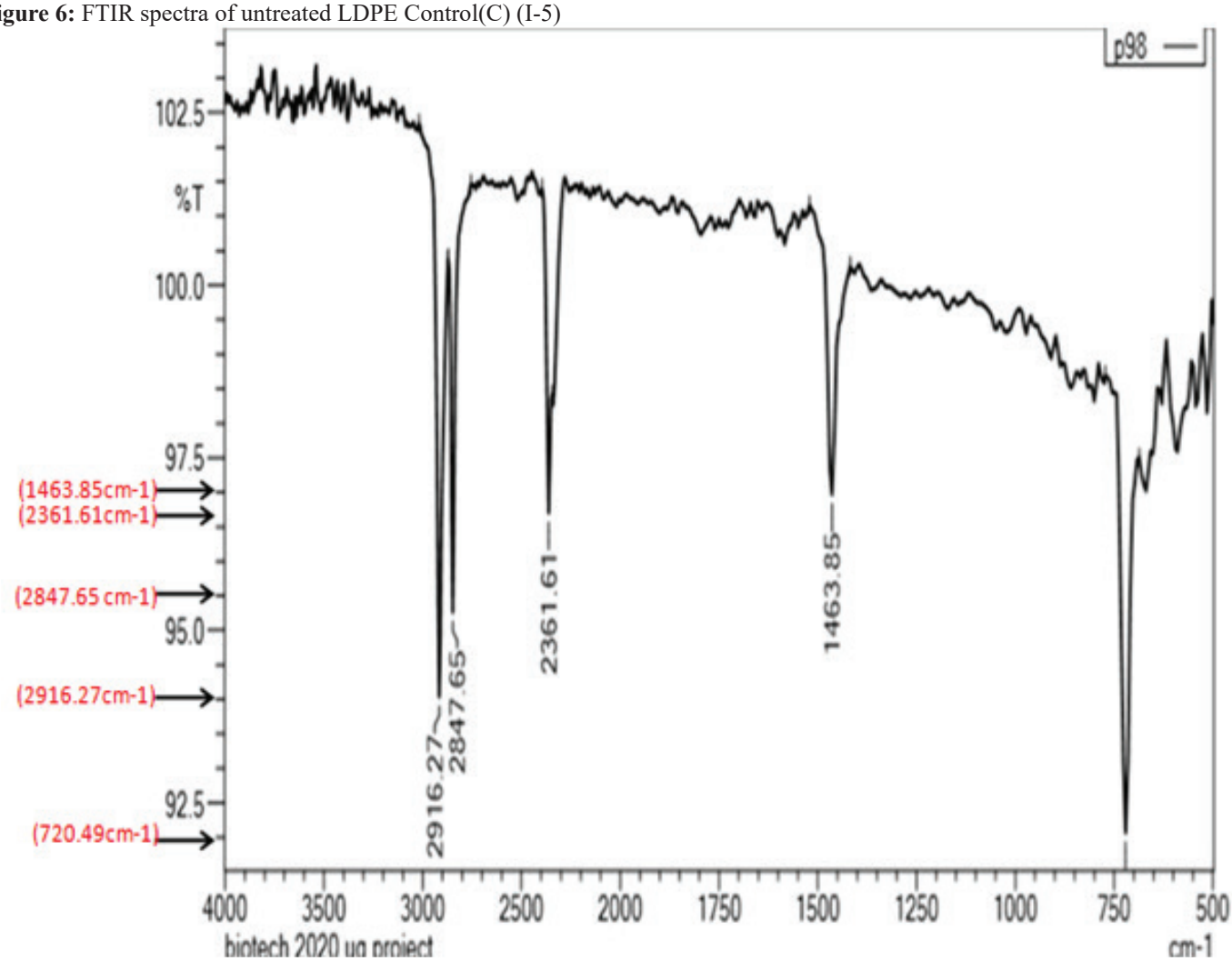


Figure 7: FTIR spectra of LDPE treated with *Aspergillus fumigus*(I-1)

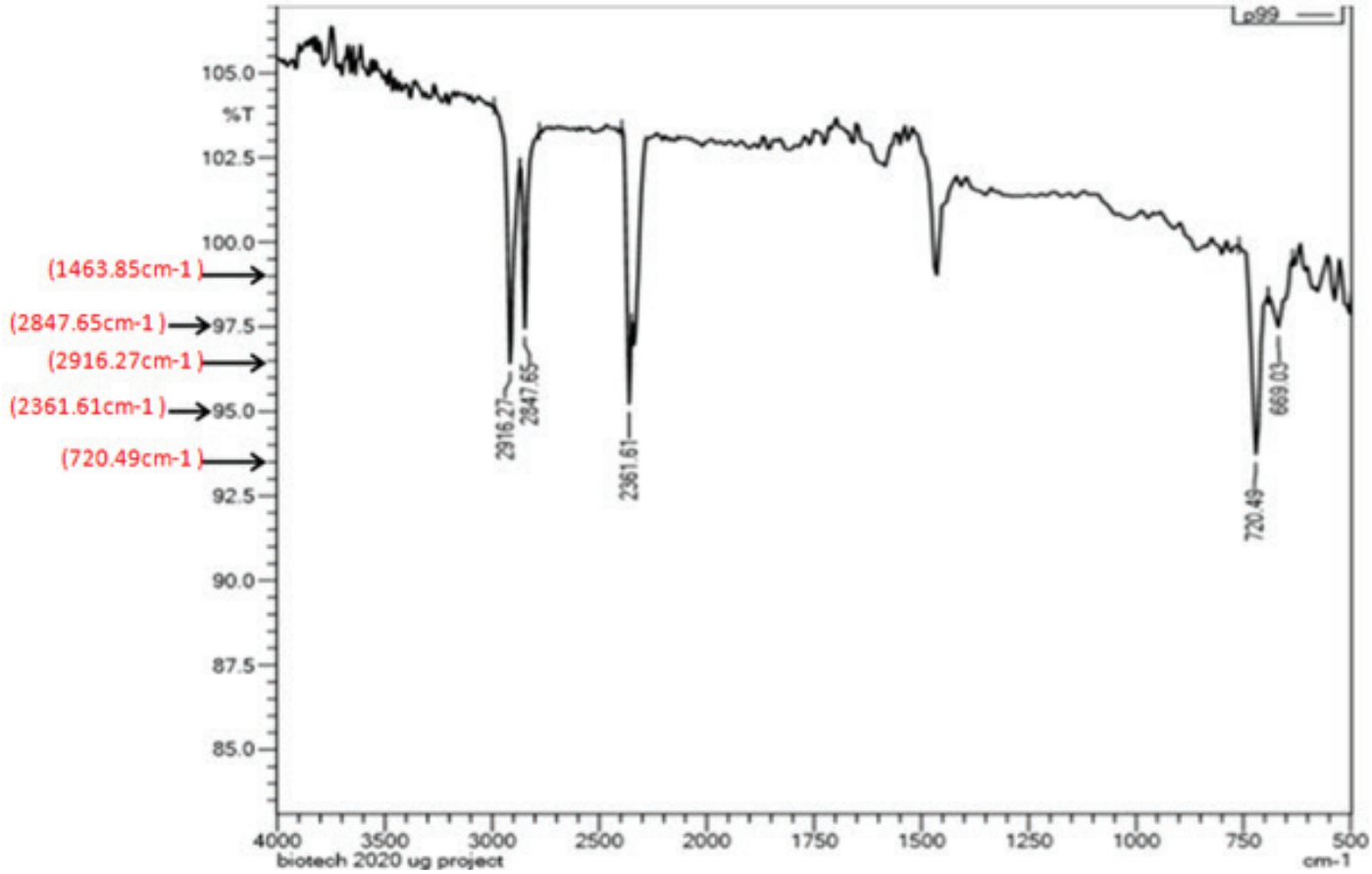


Figure 8: FTIR spectra of LDPE treated with Xylaria sp(I-5)

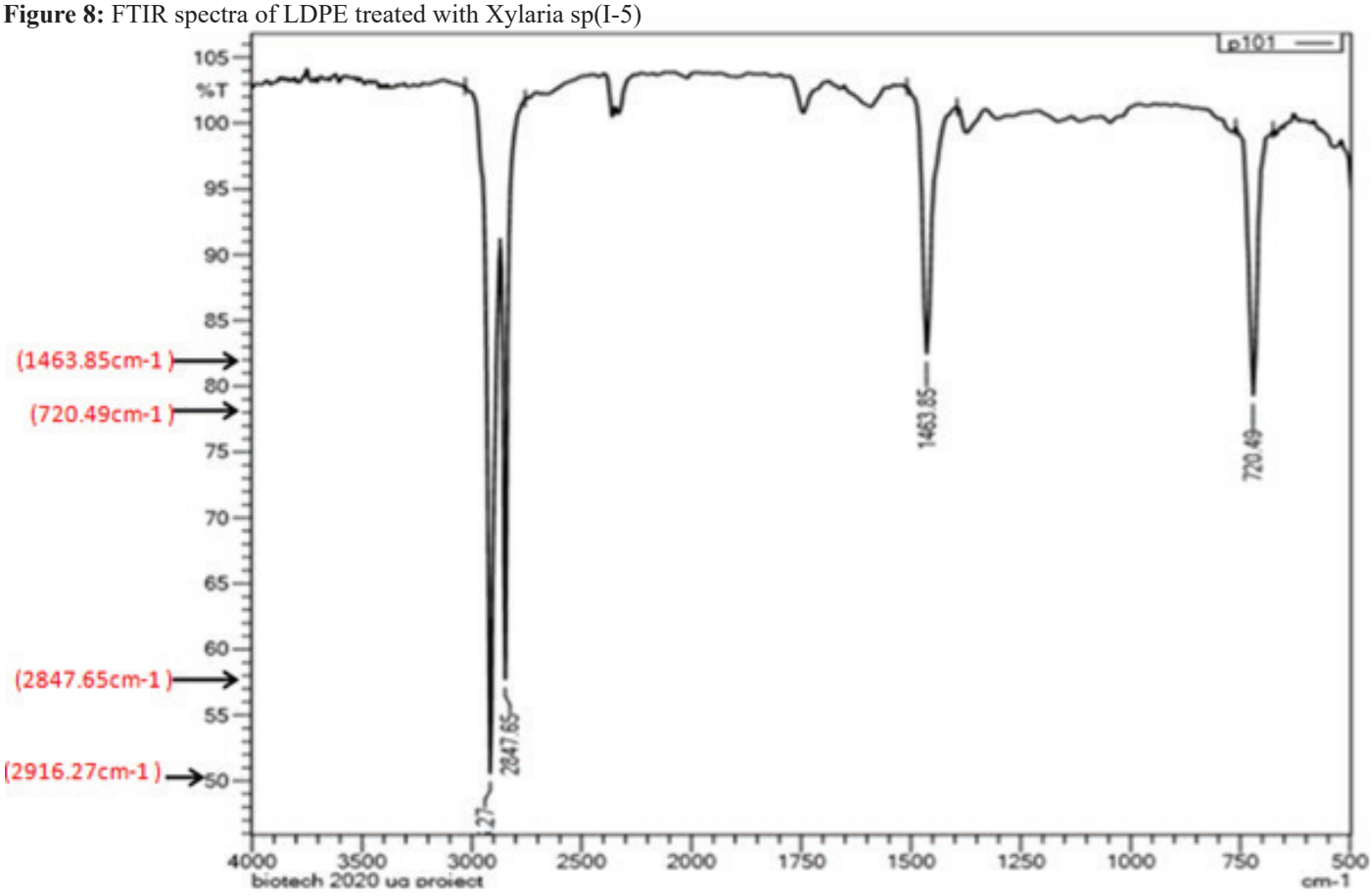


Figure (6,7 & 8): FTIR spectrum of biodegradation of LDPE strip after 30 days of incubation. The presence of functional groups supporting the conformational change on the polymer surface of *Aspergillus fumigus*(I-1), *Xylaria sp*(I-5) and Control(C)

Table 1: Amount of CO_2 evolution (30 days)

Isolated organisms	Evolution of CO_2 (g l-1) after 30 days
Aspergillus fumigus	1.460
Aspergillus flavus	0.020
Nodulisporium gregarium	0.760
Aspergillus terreus	0.180
Xylaria sp.	1.350

SEM analysis stub using a carbon tube and the sample was coated with the gold for 40 s and analyzed under high-resolution scanning electron microscope to detect the changes on LDPE surface (Gajendiran *et al.,* 2016).

(ii) Fourier transforms infrared (FTIR) analysis

Fourier transform infrared (FTIR) measurements were carried out for identification of surface structural changes (formation and disappearance of functional group) on polymer (Das and Kumar 2014).

RESULTS AND DISCUSSION

All the fungal isolates from landfill soil area were screened for their potency to degrade LDPE, after 15 days of incubation at 30ºC in enrichment mineral salt medium. Based on screening 5 isolates such as *Aspergillus fumigus (I-1), Aspergillus flavus (I-2), Nodulisporium gregarium (I-3), Aspergillus terreus (I-4) and Xylaria sp (I-5)* identified based on microbial and biochemical characterization.

Increase in the OD values represents the increase in concentration of inoculated organisms on the culture media (Mohanasrinivasan *et al.,* 2013). In this context the present investigation reveals the high adsorbent value for *Aspergillus fumigus* (*I-1*) as 0.290 and *Xylaria sp* (*I-5*) as 0.286 (Figure 1).

Study of pH variation in the culture is to ensure the variation in metabolic activity of the fungal isolates and healthy progressive growth of microbial cells reveals the positive approach of the study. The variation in pH of the medium during this biodegradation study was recorded (Figure 2). Among five isolates, *Aspergillus fumigus(I-1) and Xylaria sp(I-5)* shows fluctuation in the pH of culture by increasing the value of 6.5 to 8.4 in *Aspergillus fumigus (I-1)* and to 7.7 in *Xylaria sp(I-5).*

The amount of dissolved CO_2 evolved was depicted in Table 1. The results explaining significant differences in dissolved CO_2 evolution among the fungal isolate (Hussein *et al.,* 2015). The isolates *Aspergillus fumigus (I-1)* and

Xylaria sp (I-5) showed highest amount of dissolved CO_2 generation during 30 days of incubation, reached 1.460g l-1 and 1.350g l-1 due to the utilization of LDPE film as the carbon source. Similar results were observed in *Fusarium sp* (Shah *et al.,* 2009) as 1.85 g l-1 evolution of CO_2 after a 30 day period of incubation. The *Fusarium* strain releases CO_2, CH_4 and H_2O as end products of polyethylene degradation were also recorded by Das and Kumar 2014.

The SEM analysis was performed to monitor the changes in the surface of the strips. The adhesion of the microorganisms to the polymeric surface is fundamental activity of biodegradation mechanism (Volke-Sepulveda *et al.,* 2002). The changes in surface morphology of the LDPE strips were investigated by SEM after 30 days of biotic exposure (after removal of biofilm). The surface changes on the polythene strips indicates the act of degradation as cracks in the case of *Aspergillus fumigus(I-1)* (Figure 4) and pits in the case of *Xylaria sp(I-5)* (Figure 5) and there were no pits or cracks appeared in the control film and it resume smooth as in the beginning (Figure 3).

The variation in band intensity of microbial treated sample *Aspergillus fumigus(I-1)* and *Xylaria sp (I-5)* has been studied by comparing with control sample. For control sample, the characteristic absorption bands were at 2916.27cm-1 and 2847.65cm-1(both shares C-H stretch), 2361.61cm-1 (CHO stretch), 1463.85 cm-1 (C=C stretch) and 720.49cm-1 (C–H bend). Significant changes were found in both fungal isolates treated LDPE strips. The disappearance in carbonyl peak with respect to the control band absorbance (2361.61 cm-1) assignable to CHO stretch has been observed in case of *Xylaria sp (I-5)* whereas *Aspergillus fumigus (I-1)* showed reduction in peak intensity. The strong absorption peaks at 720.49 cm-1 became weaker due to microbial treatment. In addition, intensity of the bands at 1463.85 cm-1 showed highest peak reduction in *Aspergillus fumigus (I-1)* than the *Xylaria sp (I-5)* isolates. Similar pattern was also observed by Ibiene *et al.,* 2013 and recorded as, band around 1461–1466 cm-1 revealing a bending deformation, and another band at 720–724 cm-1 which indicates a rocking deformation. The further reduction in intensity of peaks were observed in case of *Xylaria sp (I-5)* in contrast, peaks at absorbents value 2916.27 cm-1 and 2847.65 cm-1 become increased in the treated sample *Aspergillus fumigus (I-1)* than the control. Hence all the variation confirms depolymerisation activity of the microbial isolates. The change in the peak values of almost all functional groups supporting the conformational change on polymer surface (Das and Kumar 2015).

CONCLUTION

The current study aimed to develop an approach on microbial degradation of long-chain hydrocarbon by fungus which isolated from municipal waste dumped landfill of Salem City, Tamil Nadu. To identify efficient LDPE degrading fungus, various preliminary parameters were conducted for isolation and screening the fungi. The best two plastic-degrading isolates *Aspergillus fumigus* (*I-1*) and *Xylaria sp* (*I-5*) were identified for their growth on LDPE by utilizing carbon energy source. SEM analysis shows the surface changes on the above fungal species treated polythene strips. Further conformation done by FTIR resulting in formation and deformation at chemical structure of LDPE. Thus, the present study suggests that non harmful beneficiary microbes screened from polluted areas can be used as cost-effective, eco-friendly, safe and effective method for plastic waste management. However, in the present study, the conclusions were drawn based on a short-term study with limited observations. As biodegradation by microorganisms is a slow process, this study need to be extended further for long periods and boost the degradation ability by developing microbial consortium.

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