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SYNERGISTIC DEGRADATION OF POLYETHYLENE BY INDIGENOUS FUNGAL ISOLATES AND THEIR CONSORTIUM

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ABSTRACT

Polyethylene is a widely used synthetic polymer whose extensive consumption has led to serious environmental concerns. Conventional disposal methods such as source reduction, incineration, and landfilling have inherent limitations, highlighting the need for sustainable alternatives. In this study, four fungal species—*Aspergillus niger*, *Chaetomium globosum*, *Aspergillus flavus*, and *Trichoderma harzianum*—were isolated from dumpsites in Shivamogga District and evaluated for their ability to degrade polyethylene. Surface-sterilized polyethylene films were incubated with the isolates for three months. Degradation was assessed through weight-loss measurements, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). The individual fungi caused weight losses of 0.7%, 5.6%, 1.1%, and 13.0%, respectively, whereas their consortium achieved a substantially higher weight loss of 26%. FTIR and SEM analyses further confirmed structural and surface modifications indicative of polyethylene degradation. Enzymatic screening revealed the production of laccase and manganese peroxidase, suggesting their involvement in the biodegradation process. These findings demonstrate the superior efficacy of the fungal consortium and support its potential as an environmentally friendly approach for mitigating polyethylene pollution.

KEYWORDS: Microbial consortium, Polyethylene, Degradation, Fourier Transform Infrared Spectroscopy and Scanning Electron Microscopy.

INTRODUCTION

Plastic is one of the most vital man-made product that has been produced in huge quantity and is used widely for different purposes in our everyday life and gradually, the global demand of this synthetic product is rapidly growing day by day. The word plastic is derived from the Greek word “plastikos” meaning the substance which can be molded into any shape. With the excessive use of plastics and increasing pressure being placed on capacities available for plastic waste disposal, the need for biodegradable plastics and biodegradation of plastic has assumed increasing importance in the last few years. Biodegradation is necessary for water soluble or water immiscible polymers, because they eventually enter water streams which can neither be recycled nor incinerated (Shah *et al*., 2008). The polyethylene is the most commonly found solid waste that has been recently recognized as a major threat to marine life. The polyethylene could sometimes cause blockage in intestine of fish birds and marine mammals (Spear *et al*., 1995; Seechi and Zarur, 1999). The degradation of polyethylene can occur by different molecular mechanisms such as chemical, thermal, photo and biodegradation (Gu, 2003). Biodegradability is evaluated by weight loss, tensile strength loss, changes in percent elongation and changes in polyethylene molecular weight distribution.

Further, the utilization of microbial consortia offers considerable advantages over the use of pure cultures in

the degradation of recalcitrant compounds considering its multifunctional ability and can be more robust to environmental fluctuations (Gilbert *et al.*, 2003 and Roy *et al.*, 2008). Microbial communities or consortium are defined as multispecies assemblages that coexist in an ecological niche. In microbial consortium microorganisms work in multidisciplinary way on a complex substrate and easily degrade it into different simple monomers. Hence, this activity of consortium was used in present work to increase polyethylene degradation.

The present work was undertaken to solve the problem caused by polyethylene in environment. The different organisms were isolated from local dumpsites of Shivamogga District using enrichment method. Further degradation experiments were carried out using surface sterilized polyethylene for a period of three months. Degradation was confirmed by Fourier Transform Infrared (FTIR) Spectroscopy and Scanning Electron Microscopy (SEM) studies.

MATERIALS AND METHODS

1. **Collection of soil sample:** Soil samples were collected from local dumpsites in Shivamogga District, transported to the laboratory, and stored under appropriate laboratory conditions until further analysis and use.
2. **Isolation and identification of fungi from soil:** Fungi were isolated using an enrichment technique in which polyethylene served as the sole carbon source. The isolates were identified on the basis of their macroscopic and microscopic characteristics with the aid of standard taxonomic manuals. (Ellis, 1971 and 1976: Pitt, 1979: Domsch *et al*., 1980: Subramanian, 1983: Ellis and Ellis, 1997: Gilman, 2001 and Nagamani *et al*., 2006). The colonies were preserved at 4ºC in 2% agar slants of malt and yeast extract medium (Yamada-onodera *et al*., 2001).

3. Screening of fungi for polyethylene degradation

3.1. Plate assay

The isolated fungi were inoculated to medium which contained 0.3g of NH_4NO_3, 0.5g of K_2HPO_4. 0.1g of NaCl, 0.02g of $MgSO_4.7H_2O$, 2g of agar, 0.5g of polyethylene and 100ml distilled water (Yamada-onodera *et al*., 2001). This agar plate test is also a simple semi- quantitative method to know depolymerization of polymer by the organism. After inoculation with fungi into the medium containing fine particles of polyethylene, the formation of a clear hallow around the colony indicates the first step of fungal biodegradation (Nishida and Tokiwa, 1993).

3.2. Degradation of Polyethylene

Pre-weighed, surface-sterilized polyethylene discs (1 cm in diameter) prepared from polyethylene bags were aseptically transferred to conical flasks containing 50 mL of mineral salt medium. A loopful of fungal spores was inoculated into each flask. Control flasks containing polyethylene discs in uninoculated medium were maintained simultaneously. All treatments were performed in triplicate and incubated on a rotary shaker. After three months, the polyethylene discs were recovered, thoroughly washed with distilled water, dried overnight in a hot-air oven at 50 °C, and reweighed to determine weight loss following the method of Kathiresan (2003). The same procedure was employed to evaluate polyethylene degradation by the fungal consortium.

4. Confirmation of Polyethylene degradation.

Polyethylene degradation was confirmed by using SEM and FTIR Spectroscopy (Shah *et al*., 2008).

5. Screening of enzymes responsible for polyethylene degradation

Previous studies showed involvement of laccase and manganese peroxidase in polyethylene degradation. Hence, screening, mass production and enzyme activity of these enzymes was also calculated.

5.1. Screening of laccase and manganese peroxidase enzyme

The isolated fungi were screened for the laccase production using laccase screening medium (LSM). Fungi were inoculated in LSM agar plate and the plate was incubated for 7 days in dark condition. The substrate utilized reddish brown color in screening medium indicated the positive strain for laccase (Viswanth *et al*., 2008). For manganese peroxidase, H_2O_2 was used to the same medium.

5.2. Mass production by sub-merged fermentation

The mass level production of the enzyme was carried out in mineral salt medium under suitable environmental conditions (Shradda *et al*., 2011).

5.3. Enzyme assay

Laccase activity was assayed by mixing 1 mL of culture supernatant with 1 mL of 2 mM guaiacol and 3 mL of 10 mM sodium acetate buffer (pH 4.6). The reaction mixture was incubated at 30 °C for 15 min, and the increase in absorbance due to color development was measured spectrophotometrically at 450 nm. One unit of laccase activity was defined as the amount of enzyme required to oxidize guaiacol under the assay conditions during the incubation period. Manganese peroxidase activity was determined using the same procedure, with the addition of 1 mL of H_2O_2 to the reaction mixture before incubation. (Papinutti *et al*., 2006).

5.4. Protein estimation

Protein estimation was done to calculate specific activity of enzymes. The protein concentration was determined by the Lowry's method, as described by Lowry's (1951) using Bovine Serum Albumin (BSA) as a standard.

RESULTS

1. Isolation and Identification of Fungi

Aspergillus niger, *Chaetomium globosum*, *Aspergillus flavus* and *Trichoderma harzianum* were isolated and identified based on their morphological characters. These microorganisms were selected for the study, because of their predominant presence in soil contaminated with waste polyethylene plastic bags.

2. Screening of fungi for polyethylene degradation

2.1. To check ability of fungi to grow on medium containing polyethylene

The growth of *Aspergillus niger*, *Chaetomium globosum*, *Aspergillus flavus*, and *Trichoderma harzianum* on agar medium containing polyethylene as the sole carbon source demonstrated their ability to utilize and potentially degrade polyethylene.

2.2. Degradation of surface sterilized polyethylene

Aspergillus niger, *Chaetomium globosum*, *Aspergillus flavus* and *Trichoderma harzianum* were able to degrade surface sterilized polyethylene. This method confirmed that these organisms can utilize polyethylene without any pre-treatment like, heat, UV light and acid. The weight loss for surface sterilized polyethylene by isolated microorganisms and microbial consortium is shown in following table (Table 1). Weight loss shown by consortium was more compared to single fungi.

Table 1: Weight loss of surface sterilized polyethylene.

Name of microorganisms	Initial weight (mg)	Final weight (mg)*	Weight loss (mg)	Weight loss (%)
Aspergillus niger	0.10	0.0993	0.0007 ± 0.00015	0.7
Chaetomium globosum	0.10	0.0944	0.0056 ± 0.00020	5.6
Aspergillus flavus	0.10	0.0989	0.0011 ± 0.00026	1.1
Trichoderma harzianum	0.10	0.087	0.013 ± 0.0013	13
Microbial consortium	0.10	0.074	0.026 ± 0.00111	26

± = Standard Deviation, * = Mean

3. Confirmation of polyethylene degradation

3.1. Observation of discs using Scanning Electron Microscopy

Surface sterilized polyethylene showed morphological changes when observed through SEM. Formation of holes, disruption of polyethylene structure confirmed degradation capacity of *Aspergillus niger*, *Chaetomium globosum*, *Aspergillus flavus* and *Trichoderma harzianum* and by consortium. SEM photograph of *Aspergillus niger*, *Chaetomium globosum*, *Aspergillus flavus* and *Trichoderma harzianum* showed formation of less disruption compared to SEM photograph of consortium treated polyethylene (Fig. 1).

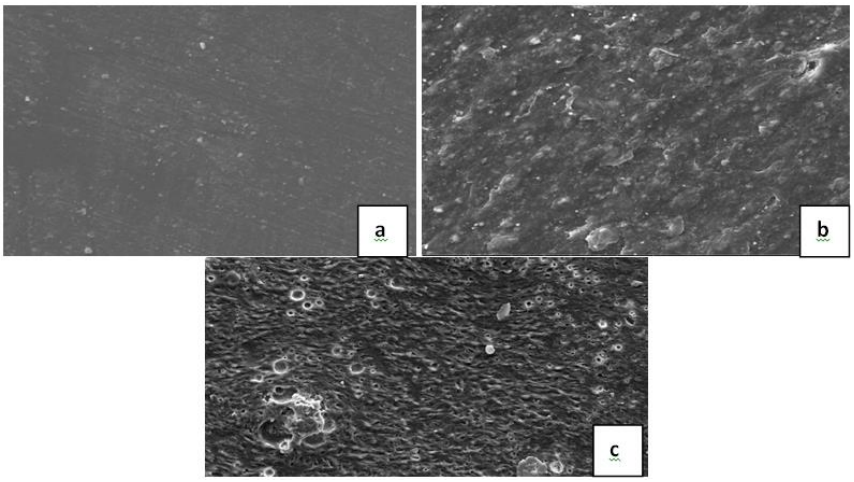

Fig. 1: SEM photograph of (a) control polyethylene, treated with (b) *Trichoderma harzianum* and (c) consortium.

3.2. Observation of discs using Fourier Transform Infrared Spectroscopy

FTIR spectrum of *Aspergillus niger*, *Chaetomium globosum*, *Aspergillus flavus* and *Trichoderma harzianum* and combination of all these microorganisms showed formation ethers, aldehydes, esters and carboxylic acids groups indicating polyethylene degradation. Degradation products were not found in FTIR spectrum of control polyethylene (Fig. 2). Following are the figures showing FTIR spectrum of surface sterilized polyethylene treated with different microorganisms and consortium (Fig. 3 and Fig. 4).

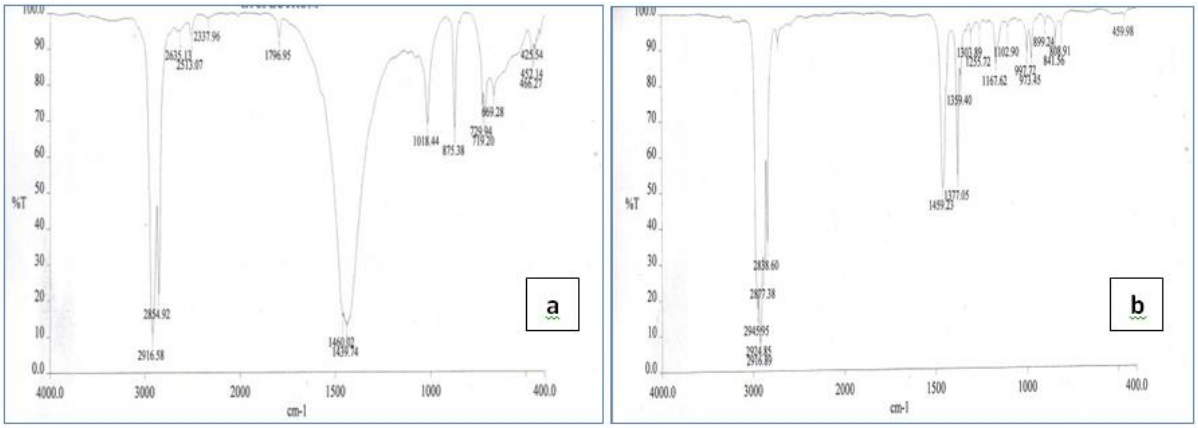

Fig.2. FTIR spectrum of (a) control polyethylene and treated with (b) *Aspergillus niger.*

FTIR spectrum of surface sterilized polyethylene treated with *Aspergillus niger* showed formation of alkanes (2850-3000 cm^{-1}), aldehydes (2635,13 cm^{-1}), carboxylic acids, alcohols, esters, ethers (1018,44 cm^{-1}), aromatics (1439,74cm^{-1}) and carbonyl (1796,95 cm^{-1}) groups at different frequencies indicating degradation of polyethylene by *Aspergillus niger*.

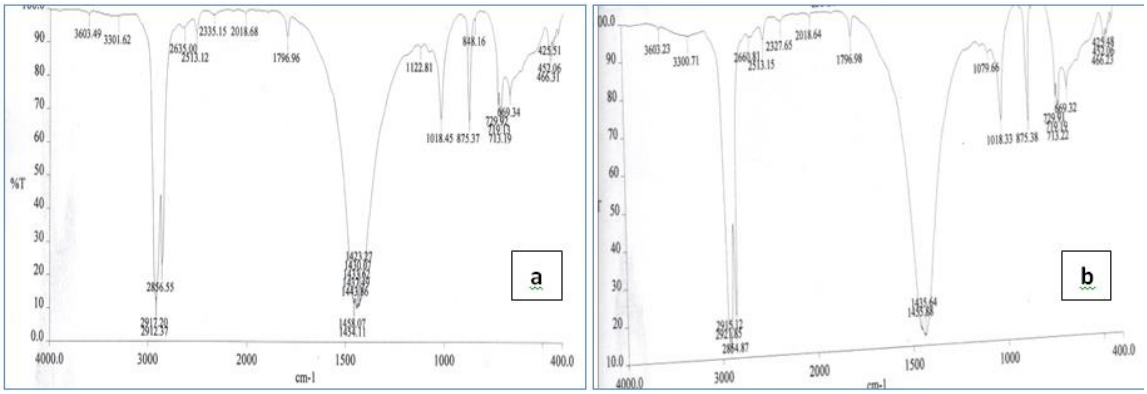

Fig.3. FTIR spectrum of polyethylene treated with (a) *Chaetomium globosum* and (b) *Trichoderma harzianum*.

FTIR spectrum of surface sterilized polyethylene treated with *Chaetomium globosum* showed formation of alcohols, phenols (3603,49 cm^{-1}), alkanes (2856,55 cm^{-1}), carboxylic acids, alcohols, esters, ethers (1018,45 cm^{-1}), aromatics (1454,11 cm^{-1}) and alkenes (875, 37 cm^{-1}) groups at different frequencies indicating degradation of polyethylene by *Chaetomium globosum*.

FTIR spectrum of surface sterilized polyethylene treated with *Trichoderma harzianum* showed formation of phenols (3300, 71 cm^{-1}), aldehydes (2660,81 cm^{-1}), carboxylic acids, esters, ethers (1079,66-1018,33 cm^{-1}), aromatics (910-675 cm^{-1}), alkyl halides (1167, 12 cm^{-1}) and alkenes (875, 38 cm^{-1}) groups at different frequencies indicating degradation of polyethylene by *Trichoderma harzianum*.

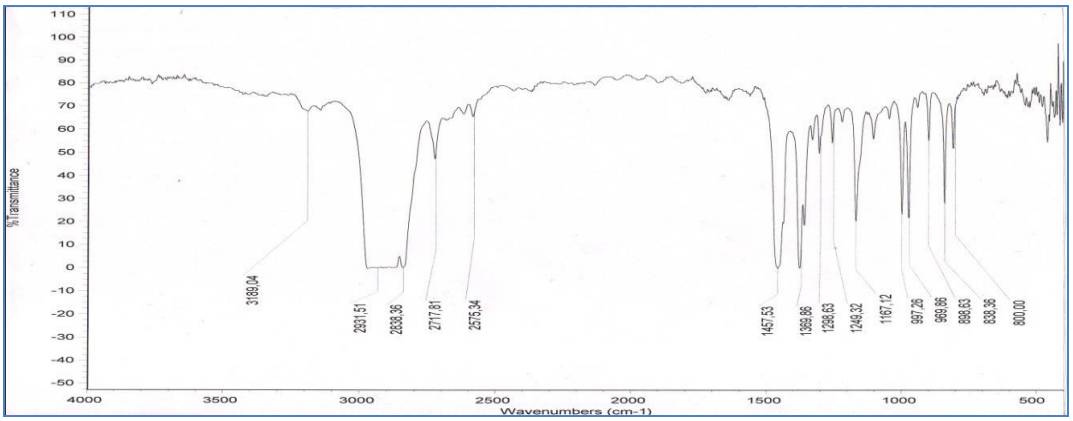

Fig.4. FTIR spectrum of polyethylene treated with consortium (*A. candidus, T. harzianum, A. fumigatus* and *C. globosum*).

FTIR spectrum of polyethylene treated with consortium (*Aspergillus niger, Chaetomium globosum, Aspergillus flavus* and *Trichoderma harzianum*) showed formation of carboxylic acids (3189, 04 cm^{-1}), alkanes (2931, 51 cm^{-1}), aldehydes (2717, 81 cm^{-1}), aromatics (1457, 53 cm^{-1}), esters, ethers (1298, 63 cm^{-1}), alkyl halides (1167, 12 cm^{-1}) and alkenes (969, 86 cm^{-1}) groups at different frequencies.

4. Screening and characterization of polyethylene degrading enzymes

Aspergillus niger, Chaetomium globosum, Aspergillus flavus and *Trichoderma harzianum* showed positive result for both laccase and manganese peroxidase enzymes.

4.1. Mass production of enzymes

Laccase and manganese peroxidase enzymes were produced in large amount using submerged fermentation.

4.2. Enzyme assay

All the isolated microorganisms did not show any enzyme activity for first 3 weeks. Activity of manganese peroxidase was more in all organisms compared to laccase. *Trichoderma harzianum* showed more activity compared to other organisms. Laccase and manganese peroxidase activity of all the organisms is shown in following table (Table 2 and Table 3).

Table 2: Enzyme activity of Laccase.

Enzyme/Weeks	4	5	6	7	8	9	10	11	12
Aspergillus niger	0.00019± 0.0003	0.00044± 0.0002	0.00064± 0.0004	0.00082± 0.0001	0.00104± 0.0003	0.00130± 0 .0002	0.00235± 0.0004	0.00117± 0.0003	0.00078± 0.0001
Chaetomium globosum	0.00026± 0.0001	0.00052± 0.0004	0.00115± 0.0002	0.00209± 0.0001	0.00326± 0.0003	0.00509± 0.0011	0.00705± 0.0002	0.00535± 0.0001	0.00300± .0004
Aspergillus flavus	0.00025± 0.0002	0.00064± 0.0001	0.00101± 0.0004	0.00203± .0011	0.00309± .0002	0.00501± 0.0004	0.00683± 0.0001	0.00505± 0.0011	0.00331± 0.0001
Trichoderma harzianum	0.00026± 0.0001	0.00052± 0.0004	0.00115± 0.0002	0.00209± 0.0001	0.00326± 0.0003	0.00509± 0.0011	0.00705± 0.0002	0.00535± 0.0001	0.00300± .0004

± = Standard Deviation, * = Mean

Table 3: Enzyme activity of Manganese peroxidase.

Enzyme/Weeks	4	5	6	7	8	9	10	11	12
Aspergillus niger	0.00110 ±0.0001	0.00200± 0.0003	0.00345 ±0.0011	0.00530 ±0.0001	0.00712± 0.0002	0.00870± 0.0004	0.01080± 0.0001	0.00890± 0.0004	0.00726± 0.0003
Chaetomium globosum	0.00033± 0.0004	0.00069 ±0.0001	0.00102± 0.0002	0.00120± 0.0003	0.00136± 0.0001	0.00192± 0.0011	0.00409± 0.0004	0.00226± 0.0003	0.00136± 0.0001
Aspergillus flavus	0.00030± 0.0003	0.00072 ±0.0004	0.00110± 0.0001	0.00213± 0.0003	0.00320± 0.0004	0.00521± 0.0001	0.00712± 0.0011	0.00539± 0.0001	0.00331± 0.0003
Trichoderma harzianum	0.00030 ±0.0001	0.00059 ±0.0003	0.00120± 0.0004	0.00215± 0.0001	0.00330± 0.0003	0.00515± 0.0004	0.00710± 0.0011	0.00540± 0.0002	0.00338± 0.0001

± = Standard Deviation, * = Mean

5.3. Protein estimation

Specific activity of manganese peroxidase enzyme was more compared to that of laccase. Specific activity of both laccase and manganese peroxidase enzymes is shown in following table (Table 4).

Table 4: Specific activity of laccase and manganese peroxidase enzyme.

Sl. No.	Name of the organisms	Specific activity of Laccase	Specific activity of Manganese peroxidase
1	*Aspergillus niger*	0.0094 ± 0.002	0.0100 ± 0.114
2	*Chaetomium globosum*	0.0062 ± 0.006	0.0166 ±0.006
3	*Aspergillus flavus*	0.051 ± 0.114	0.0540 ± 0.001
4	*Trichoderma harzianum*	0.0079 ± 0.114	0.0082 ± 0.002

± = Standard Deviation, * = Mean

DISCUSSION

The present investigation was carried out to degrade polyethylene using different microbial consortia. *Aspergillus niger, Chaetomium globosum, Aspergillus flavus* and *Trichoderma harzianum* were isolated from dumpsite soil by enrichment method. Degradation experiment was carried out using single microorganisms and using combination of all organisms. Compared to treatment of polyethylene with single organism, use of consortium showed much better result in same span of time. These results confirmed that consortium can be used as a better method for biodegradation of polyethylene.

Negi *et al*., (2011) studied the biodegradation of LDPE film in the presence of potential bacterial consortia enriched soil. FTIR and SEM studies revealed significant surface degradation of LDPE. Even in our work FTIR and SEM studies revealed structural changes in the structure of polyethylene. As they carried out their work in soil, they concluded that, environmental factors like sun-light, temperature and rain fall may enhance the rate of biodegradation of polymer in nature. In our work, we have also used microbial consortia to degrade polyethylene. We confirmed polyethylene degradation by SEM and FTIR studies.

Soni *et al*., (2009) have compared biodegradation of poronized and non-poronized LDPE using indigenous microbial consortium. They carried out biodegradation of both kind of polyethylene at 400ºC. The weight loss values for poronized and non-poronized sample were same at 400ºC (24.12% and 24.48%, respectively) as compared to their controls (4% and 4.5% respectively).

Satlewal *et al.*, (2008) made use of consortium for biodegradation of HDPE and LDPE for first time. HDPE treated with consortium at 400ºC was degraded to a greater extent than LDPE, which showed weight loss up to 22.41% and LDPE showed 21.70% of weight loss. Without bacterial consortia the weight loss values for HDPE was 2.5% and for LDPE it was 4.5%.

CONCLUSION

The extensive use of polyethylene during past decade in all the sectors of life has created serious problems with plastic waste due to its accumulation in the environment. Further, thermoplastics are inert materials and resistant to biodegradation because of its high molecular weight, long carbon chain backbone, three dimensional structure, hydrophobic nature and lack of functional groups recognizable by existing microbial enzyme systems. However, several attempts were made earlier to investigate the microorganisms capable to utilize the thermoplastics. Further, the utilization of microbial consortia offers considerable advantages over the use of pure cultures in the degradation of recalcitrant compounds considering its multifunctional ability and can be more robust to environmental fluctuations. Degradation of polyethylene by individual microorganisms and microbial consortium resulted in better degradation of polyethylene. FTIR, SEM and weight loss results confirmed biodegradation. The organisms in the consortium combined together their activities to show better degradation experiments. FTIR results showed formation of alcohol, phenol, carboxylic acids, ketones, aldehydes and ether groups. SEM photographs revealed morphological changes in polyethylene structure. By observing all these results we can conclude that consortium can be used as better solution for biodegradation of polyethylene than individual microorganisms.

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