CODEN: WJAPAC Impact Factor: 5.48 ISSN: 3049-3013

World Journal of Advance Pharmaceutical Sciences

Volume 3, Issue 3, Page: 106-113 Research Article www.wjaps.com

BIODEGRADATION OF POLYETHYLENE BY FUNGAL ISOLATES AND THEIR CONSORTIUM FROM DUMPSITES OF SHIVAMOGGA DISTRICT

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How to cite this Article: Sowmya H. V., Nayanashree G. and *Thippeswamy B. (2026). BIODEGRADATION OF POLYETHYLENE BY FUNGAL ISOLATES AND THEIR CONSORTIUM FROM DUMPSITES OF SHIVAMOGGA DISTRICT. World Journal of Advance Pharmaceutical Sciences, 3(3), 106-113.

Article Info

Article Received: 07 January 2026,

Article Revised: 27 January 2026,

Article Accepted: 17 February 2026.

DOI: https://doi.org/10.5281/zenodo.18817533

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ABSTRACT

Polyethylene is a widely used synthetic polymer that has become a major environmental concern due to its non-biodegradable nature. Conventional waste management methods such as landfilling and incineration pose environmental risks, highlighting the need for sustainable alternatives. Biodegradation using microorganisms offers an eco-friendly solution for polyethylene waste management. In the present study, *Trichodermaharzianum*, *Aspergillus candidus*, *Aspergillus fumigatus*, and *Chaetomiumglobosum* were isolated from local dumpsites of Shivamogga District and evaluated for their ability to degrade polyethylene. Surface-sterilized polyethylene sheets were subjected to degradation for three months. The extent of degradation was assessed by weight loss analysis, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). Among the individual isolates, *T. harzianum* showed the highest degradation (13%), followed by *C. globosum* (5.6%), *A. candidus* (1.7%), and *A. fumigatus* (1.6%). Notably, the fungal consortium demonstrated enhanced degradation with 25% weight loss. FTIR and SEM analyses confirmed structural and surface changes in polyethylene, indicating biodegradation. Enzyme screening revealed the production of laccase and manganese peroxidase, suggesting their involvement in the degradation process. These findings demonstrate that fungal consortia play a significant role in polyethylene biodegradation and offer a promising, eco-friendly strategy for managing plastic waste.

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

INTRODUCTION

The escalating accumulation of plastic waste has become a pervasive global environmental crisis, driven by the massive production and extensive use of polymeric materials such as polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET). Among these, polyethylene—particularly low-density polyethylene (LDPE)—is one of the most prevalent and persistent polymers in terrestrial and marine ecosystems due to its chemical stability, hydrophobic nature, high molecular weight, and resistance to biodegradation under natural conditions (Gorish *et.al* 2024).

Traditional waste management strategies such as mechanical recycling and incineration are limited by economic inefficiency, incomplete degradation, and secondary environmental impacts. Consequently, biotechnological approaches targeting the biological breakdown of plastics via microorganisms have emerged as promising alternatives(Salinas *et.al* 2024).

Recent research has highlighted the potential of microbial consortia—complex communities of interacting microorganisms—to degrade recalcitrant polymers more effectively than individual strains due to synergistic metabolic pathways and multifunctional enzymatic activities. Studies have demonstrated that consortia enriched on plastic substrates show enhanced degradation efficiencies for LDPE and other plastics including PET and LLDPE, with evidence of polymer chain oxidation and structural disruption confirmed by analytical techniques such as Fourier Transform Infrared (FTIR) spectroscopy(Salinas *et.al* 2024).

Fungal communities are gaining attention due to their biofilm formation, secretion of oxidative and hydrolytic enzymes**,** and ability to penetrate three-dimensional polymer matrices, thereby facilitating biodegradation of LDPE and other plastics. Mixed fungal cultures, such as *Alternaria* and *Trametes* species, have been reported to degrade LDPE under laboratory conditions, with observable surface erosion and chemical modification of the polymer (Yang et. al., 2024).

The present work was undertaken to solve the problem caused by polyethylene in environment. The different organisms were isolated from local dumpsites soil 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 of Shivamogga district and brought to the laboratory, preserved under laboratory conditions for further use.
2. **Isolation and identification of fungi from soil:** Enrichment procedure was used for the isolation of fungi where polyethylene was used as sole source of carbon. Isolated fungi were identified based on their microscopic and macroscopic appearance using standard 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 4℃ 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

The pre-weighed discs of Surface sterilized polyethylene of 1cm diameter prepared from polyethylene bags were aseptically transferred to the conical flask containing 50ml of Mineral Salt Medium. Loop full of organisms was added to medium. Control was maintained with polyethylene discs in the microbe free medium. Triplicates were maintained for each type of fungi and left on shaker. After three months of incubation, the plastic discs were collected, washed thoroughly using distilled water, dried in hot air oven at 50℃ overnight and then weighed for final weight (Kathiresan, 2003). Same procedure was followed for degradation using 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

Earlier studies revealed that, laccase and manganese peroxidase are responsible for 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

One ml of the culture supernatant was added with one ml of 2mM guaiacol and 3ml 10mM Sodium acetate buffer (pH 4.6). The reaction mixture was incubated at 30℃ for 15 mins. The color change was measured using spectroscope at 450 nm. One unit of laccase activity was defined as amount of enzyme required to hydrolyze guaiacol during incubation period**.** For the enzyme activity calculation of manganese peroxidase same procedure was used but for the reaction mixture 1 ml of H_2O_2 was added and incubated (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

Trichodermaharzianum, Aspergillus candidus, Aspergillus fumigatus and *Chaetomiumglobosum* 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

Trichodermaharzianum, Aspergillus candidus, Aspergillus fumigatus and *Chaetomiumglobosum* were able to grow on agar medium containing polyethylene as sole carbon source. This showed their capacity to utilize polyethylene as carbon source and their capacity to degrade polyethylene.

2.2. Degradation of surface sterilized polyethylene

Trichodermaharzianum, Aspergillus candidus, Aspergillus fumigatus and *Chaetomiumglobosum* 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 fungal consortium (25) was more compared to *Trichodermaharzianum* (13%), *Aspergillus candidus*(1.7%), *Aspergillus fumigatus*(1.6%) and *Chaetomiumglobosum*(5.6%).

Table 1: Weight loss of surface sterilized polyethylene.

Name of microorganisms	Initial weight (mg)	Final weight (mg)*	Weight loss (mg)	Weight loss (%)
Trichodermaharzianum	0.10	0.087	0.013 ± 0.0013	13
Aspergillus candidus	0.10	0.0983	0.0017 ± 0.00015	1.7
Aspergillus fumigates	0.10	0.0984	0.0016 ± 0.00015	1.6
Chaetomiumglobosum	0.10	0.0944	0.0056 ± 0.00020	5.6
Microbial consortium	0.10	0.075	0.025 ± 0.00111	25

± = 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. SEM photograph of control did not show any disruption of polyethylene structure (Fig. 1) Formation of holes, disruption of polyethylene structure confirmed degradation capacity of *Trichodermaharzianum, Aspergillus candidus, Aspergillus fumigatus* and *Chaetomiumglobosum* and by consortium. SEM photograph of *Trichodermaharzianum, Aspergillus candidus, Aspergillus fumigatus* and *Chaetomiumglobosum* showed formation of less disruption when compared to SEM photograph of consortium treated polyethylene (Fig. 1).

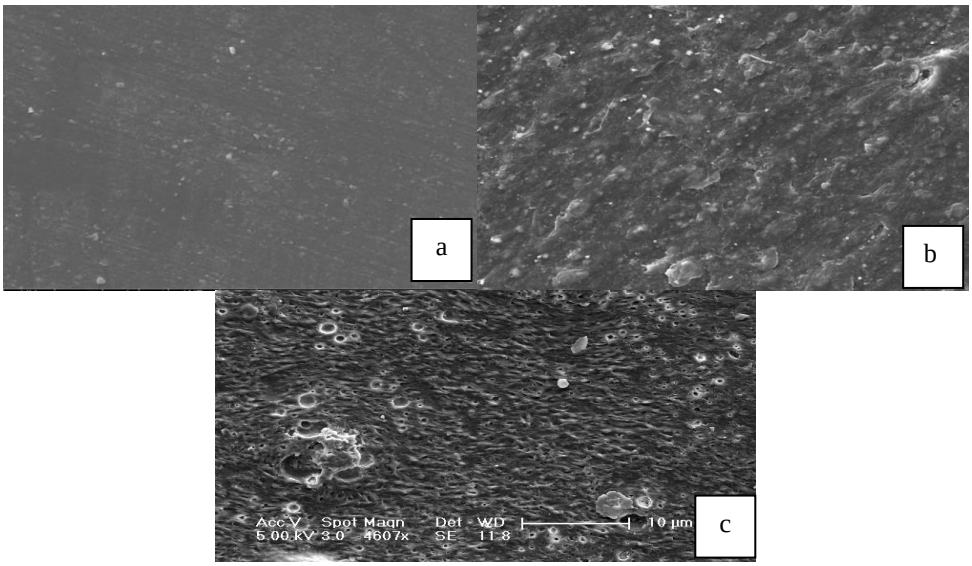

Fig. 1: SEM photograph of (a) control polyethylene and treated with (b) *Trichodermaharzianum* and (c) consortium.

3.2. Observation of discs using Fourier Transform Infrared Spectroscopy

FTIR spectrum of *Trichodermaharzianum, Aspergillus candidus, Aspergillus fumigatus, Chaetomiumglobosum* 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.2 and Fig. 3).

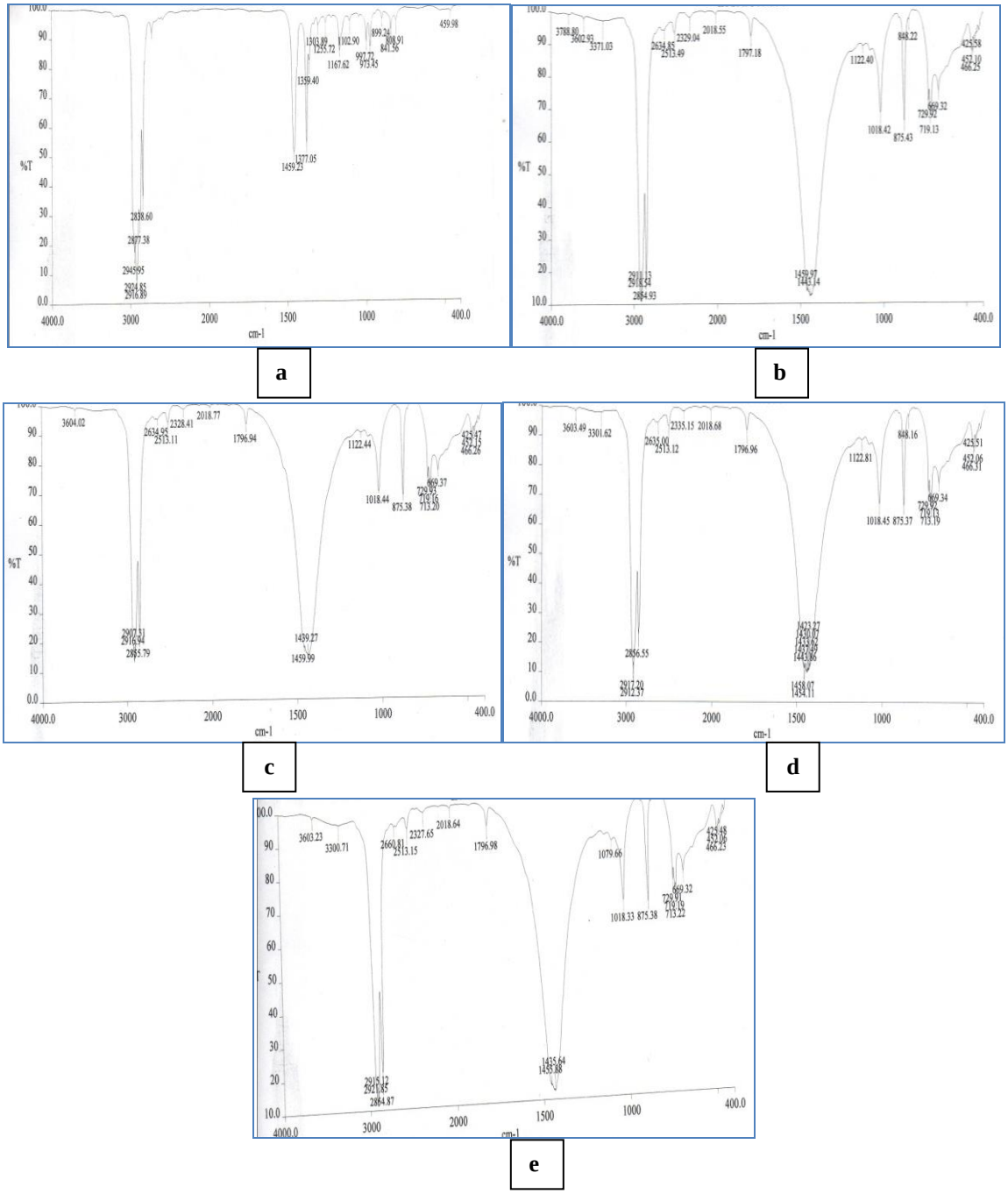

Fig. 2: FTIR spectrum of (a) control polyethylene and (b) *Aspergillus fumigatus* (C) *Aspergillus candidus* (d) *Chaetomiumglobosum* (e) *Trichodermaharzianum.*

FTIR spectrum of polyethylene treated with *Aspergillus fumigatus* showed formation of, alcohols, phenols (3371, 03 cm^{-1}), alkanes (2854, 93 and 1459, 97cm^{-1}), aromatics (875, 43 cm^{-1}) and alcohols, esters, ethers, carboxylic acids (1018, 42 cm^{-1}) groups.

FTIR spectrum of surface sterilized polyethylene treated with *Aspergillus candidus* showed formation of carboxylic acids (3300-2500 cm^{-1}), alkynes (2260-2100 cm^{-1}), alkanes (1459,99 cm^{-1}), aldehydes (2634,95 cm^{-1}), alcohols, esters, ethers (1018,44 cm^{-1}), aromatics (1459,99cm^{-1}) and alkenes (1000-650 cm^{-1}) groups at

different frequencies indicating degradation of polyethylene by *Aspergillus candidus*.

FTIR spectrum of surface sterilized polyethylene treated with *Chaetomiumglobosum* showed formation of alcohols, phenols (3603,49 cm^{-1}), alkanes (2856,55cm^{-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 *Chaetomiumglobosum*.

FTIR spectrum of surface sterilized polyethylene treated with *Trichodermaharzianum* 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 *Trichodermaharzianum*.

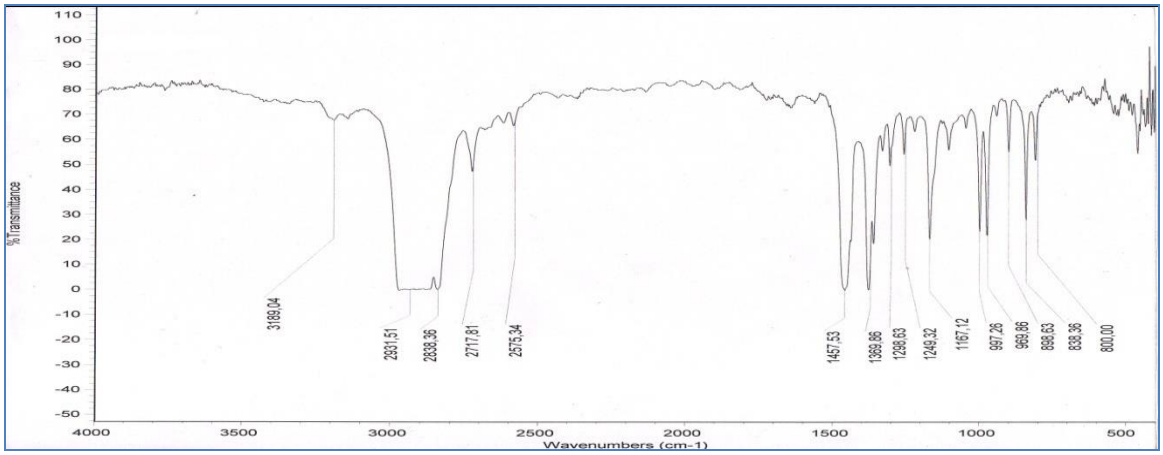

Fig. 3. FTIR spectrum of polyethylene treated with consortium (*A. candidus, T.harzianum, A.fumigatus*and *C. globosum*).

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

Trichodermaharzianum, Aspergillus candidus, Aspergillus fumigatus and *Chaetomiumglobosum* 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. *Trichodermaharzianum* 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
Trichodermaharzianum	0.0010± 0.0003	0.0019± 0.0004	0.0033± 0.0001	0.0052± 0.0003	0.00705± 0.0011	0.0086± 0.0004	0.0107± 0.0002	0.0088± .0004	0.0071 ± 0.0003
Aspergillus candidus	0.0002 ± 0.0001	0.0006 ± 0.0003	0.0009 ± 0.0002	0.0011 ± 0.0004	0.0012± 0.0001	0.0018± 0.0003	0.0040 ± 0.0011	0.00222± 0.0003	0.0013± 0 .0004
Aspergillus fumigatus	0.0002± 0.0002	0.0006± 0.0001	0.0010± 0.0004	0.0020± 0.0011	0.0030± 0.0002	0.0050± 0.0004	0.0068 ± 0.0001	0.0050± 0.0011	0.0033± 0.0001
Chaetomiumglobosum	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±0 .0004
Consortium	0.00122 ± 0.0001	0.00224 ± 0.0004	0.00364 ±0.0003	0.00548 ± 0.0001	0.00724± 0.0004	0.00898± 0.0003	0.001097 ± 0.0001	0.00984± 0.0003	0.00746±0 .0003

± = Standard Deviation, * = Mean

Table 3: Enzyme activity of Manganese peroxidase.

Enzyme/Weeks	4	5	6	7	8	9	10	11	12
Trichodermaharzianum	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
Aspergillus candidus	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 fumigatus	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
Chaetomiumglobosum	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
Consortium	0.00132 ± 0.0001	0.00237 ± 0.0004	0.00378 ±0.0003	0.00564 ± 0.0001	0.00738 ± 0.0004	0.00901 ±0.0003	0.001120 ± 0.0001	0.00997 ±0.0003	0.00786 ±0.0003

± = 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 candidus*	0.0094 ± 0.002	0.0100 ± 0.114
2	*Aspergillus fumigatus*	0.0062 ± 0.006	0.0166 ±0.006
3	*Trichodermaharzianum*	0.0510 ± 0.114	0.0540 ± 0.001
4	*Chaetomiumglobosum*	0.0079 ± 0.114	0.0082 ± 0.002
5	Consortium	0.0660± 0.006	0.00680± 0.002

± = Standard Deviation, * = Mean

DISCUSSION

The present investigation was carried out to degrade polyethylene using different fungi and microbial consortium. *Aspergillus candidus, Aspergillus fumigatus, Trichodermaharzianum* and *Chaetomiumglobosum* 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.

Mahalakshmi *et al*., (2012) studied degradation of polyethylene using microorganisms isolated from compost soil. They studied degradation by inoculating isolated organisms into mineral salt medium containing 1 gram of polyethylene films as sole carbon source. Degradation was studied using SEM and FTIR. They analyzed degraded products by Gas Chromatography. SEM studies showed formation of cavities and erosion. SEM and FTIR were also used in our study to evaluate biodegradation. In our work also polyethylene treated with *Aspergillus candidus, Aspergillus fumigatus, Trichodermaharzianum* and *Chaetomiumglobosum* showed formation of cavities and erosions.

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 400^0C. The weight loss values for poronized and non-poronized sample were same at 400^0C (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 400^0C 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.

ACKNOWLEDGMENT

This work was carried out with the help of financial support provided by Rajiv Gandhi Fellowship, UGC New Delhi. Author is also grateful to Chairman, Department of Microbiology, Kuvempu University, Shankaraghatta for giving a good support and laboratory facility for this research work.

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