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Polyethylene Degradation by Fungal Consortium

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ABSTRACT: Polyethylene is a synthetic polymer which is used in our daily life for different purposes. Increased use of polyethylene causes severe environmental problems. There are different methods to decrease problem caused by polyethylene for example source reduction, incineration and land filling and all of them have their own drawbacks. So, the best way to reduce the problem caused by polyethylene is its biodegradation. In our work we isolated, *Curvularia lunata, Alternaria alternata, Penicillium simplicissimum* and *Fusarium* sp. from local dumpsites of Shivamogga Dist. Degradation experiment was carried out using surface sterilized polyethylene for a period of 3 months and degradation was confirmed by weight loss, Fourier Transform Infrared Spectroscopy and Scanning Electron Microscopy studies. Individual weight loss shown by *Curvularia lunata* (1.2%), *Alternaria alternata* (0.8%), *Penicillium simplicissimum* (7.7%) and *Fusarium* sp. (0.7%) was less compared to their combination (27%). Fourier Transform Infrared Spectroscopy and Scanning Electron Microscopy results confirmed degradation. Enzymes responsible for polyethylene degradation were also screened and were identified as laccase and manganese peroxidase. So, the results confirm the significant role of consortium in polyethylene degradation compared to single microorganisms. Microbial consortium can be used to solve problem caused by polyethylene in the environment and it is also eco friendly method without any side effects.

Key words: Microbial consortium, Polyethylene, Degradation, Fourier Transform Infrared Spectroscopy and Scanning Electron Microscopy

INTRODUCTION

The extensive use of polymeric materials (plastics) 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 (Hadad *et al*., 2005) 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 (Yamada-onodera *et al*., 2001; Gilan *et al*., 2004 and Shah *et al*., 2008). 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 to increase polyethylene degradation.

The present work was undertaken to solve the problem caused by polyethylene in the environment.

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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 Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) studies.

MATERIALS & METHODS

Soil samples were collected from local dumpsites of Shivamogga district and brought to the laboratory, preserved under laboratory conditions for further use.

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 4p C in 2% agar slants of malt and yeast extract medium (Yamada-onodera *et al*., 2001).

The isolated fungi were screened for their capacity to degrade polyethylene using plate assay method. 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).

Degradation experiments were carried out by using surface sterilized 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 polyethylene discs were collected, washed thoroughly using distilled water, dried in hot air oven at 50p C over night and then weighed for final weight (Kathiresan, 2003). Same procedure was followed for degradation using consortium.

Polyethylene degradation was confirmed by using SEM and FTIR Spectroscopy (Shah *et al*., 2008).Enzymes responsible for polyethylene degradation were screened. 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.Screening for laccase and manganese peroxidase was carried out by inoculating the isolated fungi to 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.

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

Enzyme activity was calculated using following method. 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 30p C 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).

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 & DISCUSSION

Curvularia lunata, Alternaria alternata, Penicillium simplicissimum and *Fusarium* sp. 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.

Curvularia lunata, Alternaria alternata, Penicillium simplicissimum and *Fusarium* sp. 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.

Curvularia lunata, Alternaria alternata, Penicillium simplicissimum and *Fusarium* sp. 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 less than individual fungi.

Mahalakshmi *et al*., 2012 have 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 *Curvularia lunata, Alternaria alternata, Penicillium simplicissimum* and *Fusarium* sp. 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 400p C. The weight loss values for poronized and non-poronized sample were same at 400p C (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 400p C 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%.

Surface sterilized polyethylene showed morphological changes when observed through SEM. Formation of holes, disruption of polyethylene structure confirmed degradation capacity of *Curvularia lunata, Alternaria alternata, Penicillium simplicissimum* and *Fusarium* sp. and by consortium. SEM photograph of control polyethylene did not show any disruption of polyethylene structure, individual microorganisms showed formation of less disruption compared to SEM photograph of consortium treated polyethylene (Fig. 1).

FTIR spectrum of *Curvularia lunata, Alternaria alternata, Penicillium simplicissimum* and *Fusarium* sp. 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. 7).
FTIR spectrum of surface sterilized polyethylene treated with *Curvularia lunata* showed formation of alcohols, phenols (3370,55 cm^{-1}), alkanes (2855,65 cm^{-1}), carboxylic acids, alcohols, esters, ethers (1122,42 cm^{-1}), aromatics (1451,46 and 729,94 cm^{-1}) and alkenes (875,46 cm^{-1}) groups at different frequencies indicating degradation of polyethylene by *Curvularia lunata*.

FTIR spectrum of surface sterilized polyethylene treated with *Alternaria alternata* showed formation of alcohols, phenols (3365,98 cm^{-1}), alkanes (2914,58 cm^{-1}), carboxylic acids, alcohols, esters, ethers (1122,51 cm^{-1}), aromatics (1455,70 and 729,96 cm^{-1}) and alkenes (875,39 cm^{-1}) groups at different frequencies indicating degradation of polyethylene by *Alternaria alternata*.

FTIR spectrum of surface sterilized polyethylene treated with *Penicillium simplicissimum* showed formation of alcohols, phenols (3369,98 cm^{-1}), alkanes (2865,19 cm^{-1}), carboxylic acids, alcohols, esters, ethers (1018,43 cm^{-1}), aromatics (1500-1400 cm^{-1}) and alkenes (875, 38 cm^{-1}) groups at different frequencies indicating degradation of polyethylene by *Penicillium simplicissimum*.
FTIR spectrum of surface sterilized polyethylene treated with *Fusarium* sp. showed formation of alcohols, phenols (3370,65 cm^{-1}), alkanes (2856,61 cm^{-1}), carboxylic acids, alcohols, esters, ethers

Table 1. Weight loss of surface sterilized polyethylene

Name of microorganisms	Initial weight (mg)	Final weight (mg)*	Weight loss (mg)	Weight loss (%)
Curvularia lunata	0.10	0.0988	0.0012 ± 0.00015	1.2
Alternaria alternata	0.10	0.0992	0.0008 ± 0.0001	0.8
Penicillium simplicissimum	0.10	0.0923	0.0077 ± 0.014	7.7
Fusarium sp.	0.10	0.0993	0.0007 ± 0.00022	0.7
Microbial consortium	0.10	0.073	0.027 ± 0.00111	27

± = Standard Deviation, * = Mean

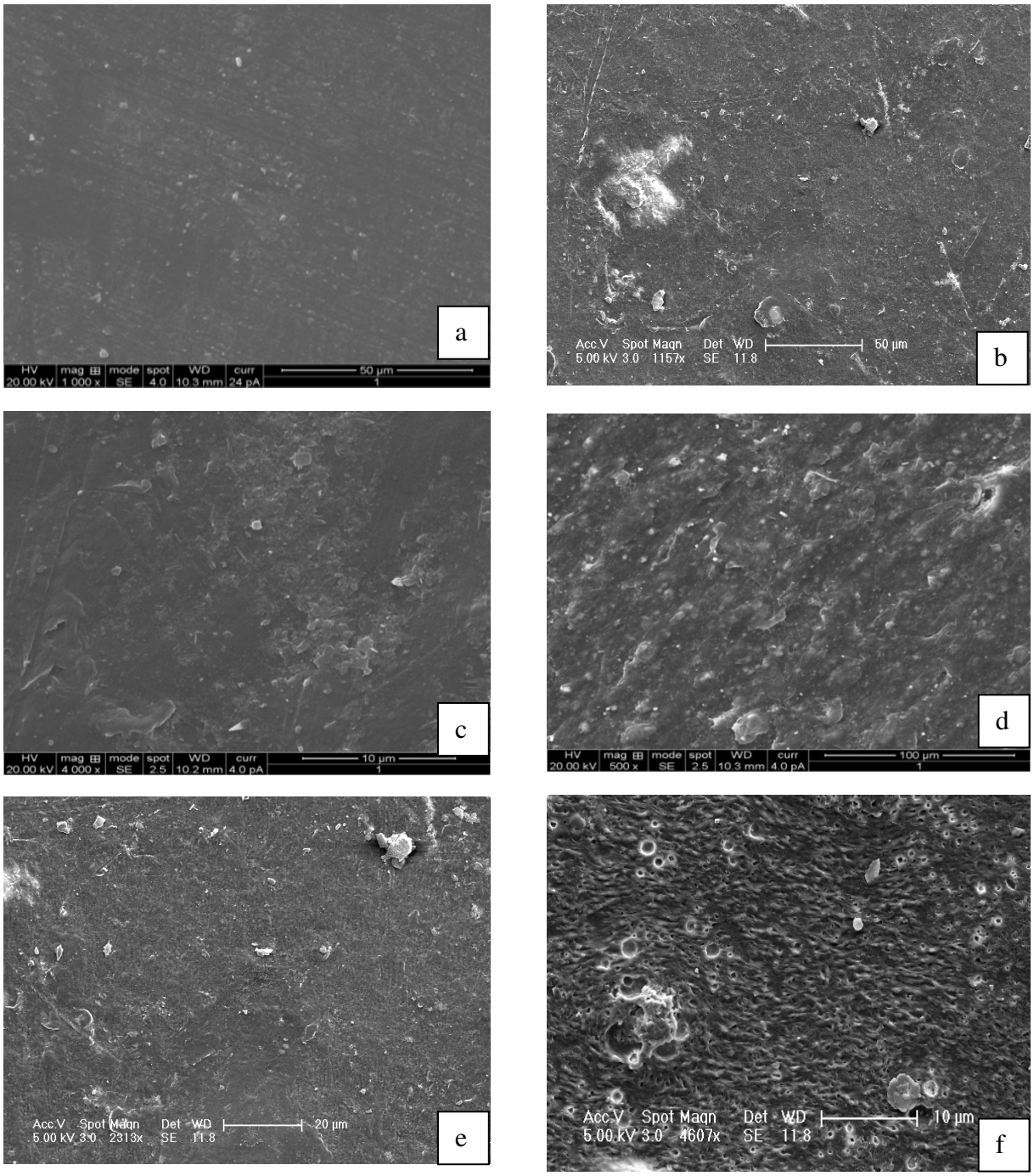

Fig. 1. SEM photograph of (a) control polyethylene and polyethylene treated with (b) *Curvularia lunata* (c) *Alternaria alternata* (d) *Penicillium simplicissimum* (e) *Fusarium* sp. and (f) consortium

(1018,50 cm^{-1}), aromatics (1459,17 and 729,96 cm^{-1}) and alkenes (848,22 cm^{-1}) groups at different frequencies indicating degradation of polyethylene by *Fusarium* sp.

FTIR spectrum of polyethylene treated with consortium (*C. lunata, A. alternata,P. simplicissimum* and *Fusarium* sp.) showed formation of carboxylic acids (3194, 52 cm^{-1}), alkanes (2893, 15 cm^{-1}), aldehydes (2717,81 cm^{-1}), aromatics (1452, 05 and 898, 63 cm^{-1}), alcohols, esters, ethers (1298, 63 cm^{-1}), alkyl halides (1167, 12 cm^{-1}) and alkenes (997, 26 cm^{-1}) groups.

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

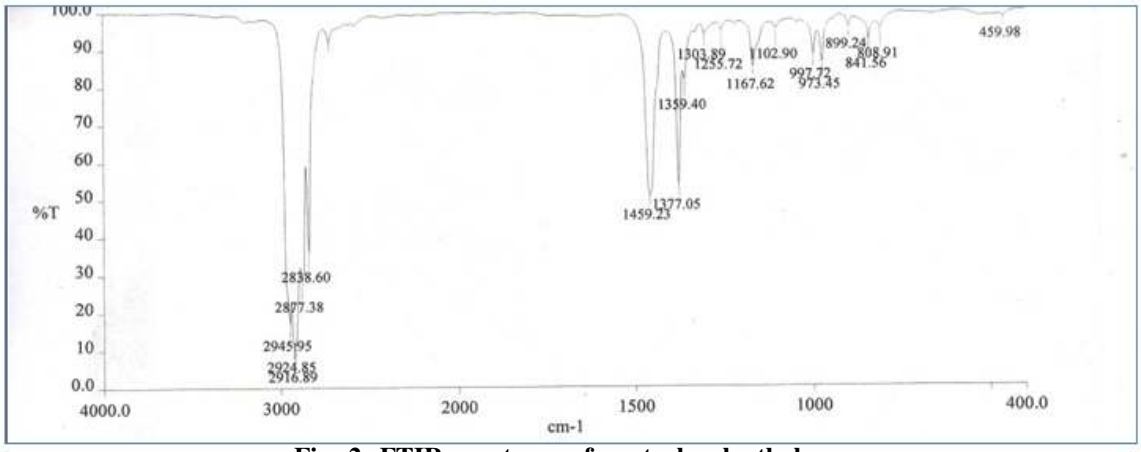

Fig. 2. FTIR spectrum of control polyethylene

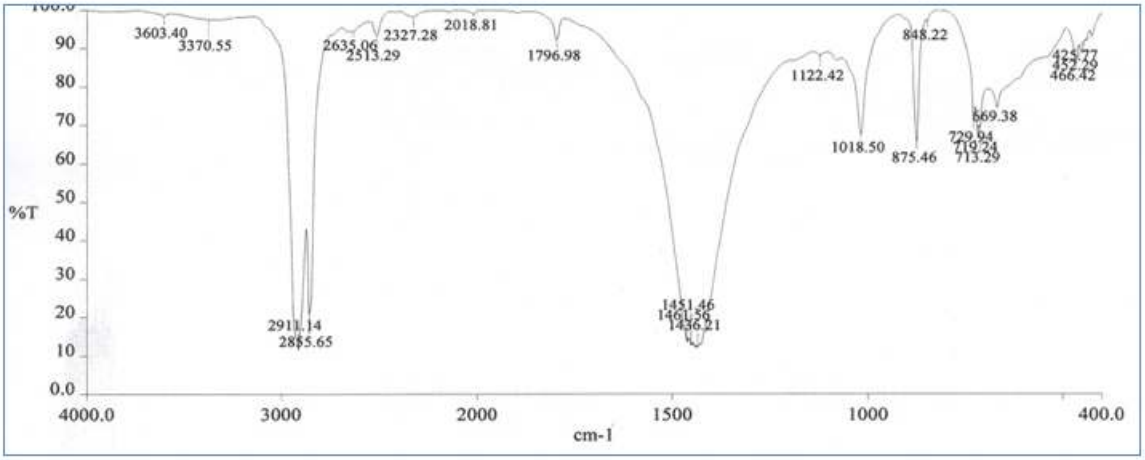

Fig. 3. FTIR spectrum of polyethylene treated with *Curvularia lunata*

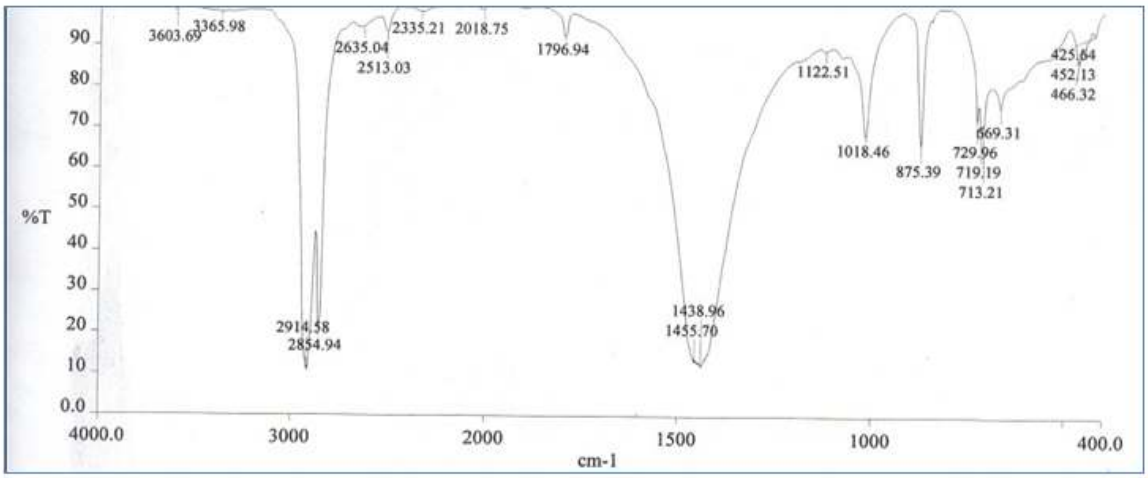

Fig. 4. FTIR spectrum of surface sterilized polyethylene treated with *Alternaria alternate*

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.

Curvularia lunata, Alternaria alternata, Penicillium simplicissimum and *Fusarium* sp. showed positive result for both laccase and manganese peroxidase enzymes. Laccase and manganese

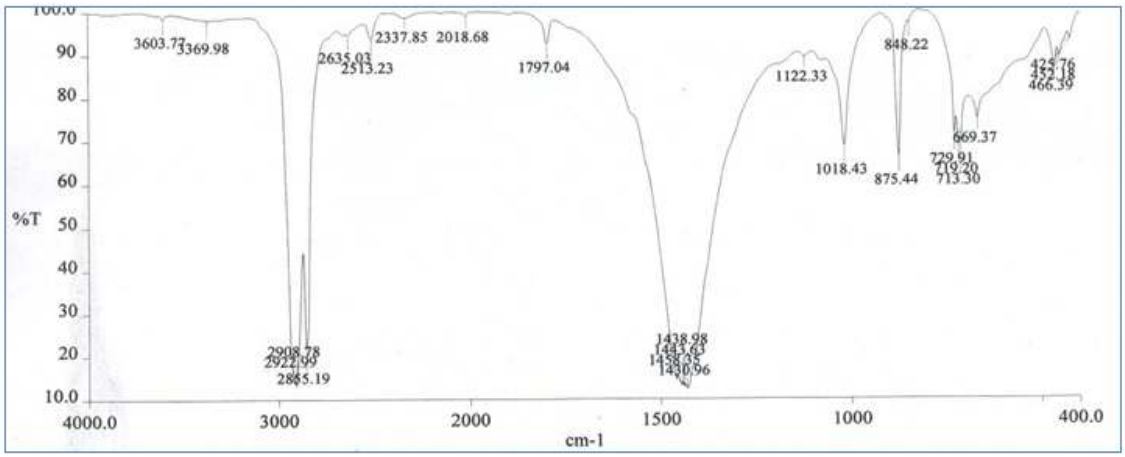

Fig. 5. FTIR spectrum of surface sterilized polyethylene treated with *Penicillium simplicissimum*

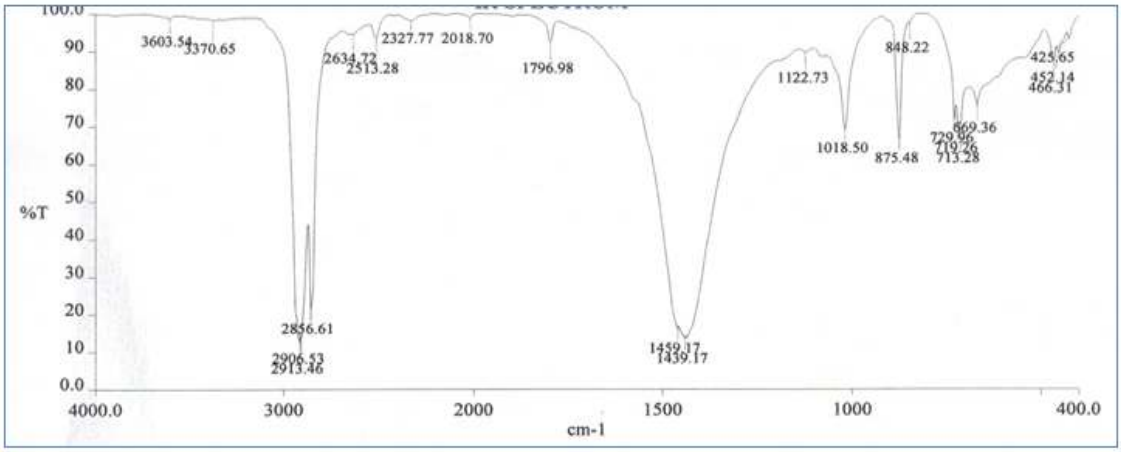

Fig. 6. FTIR spectrum of surface sterilized polyethylene treated with *Fusarium* sp.

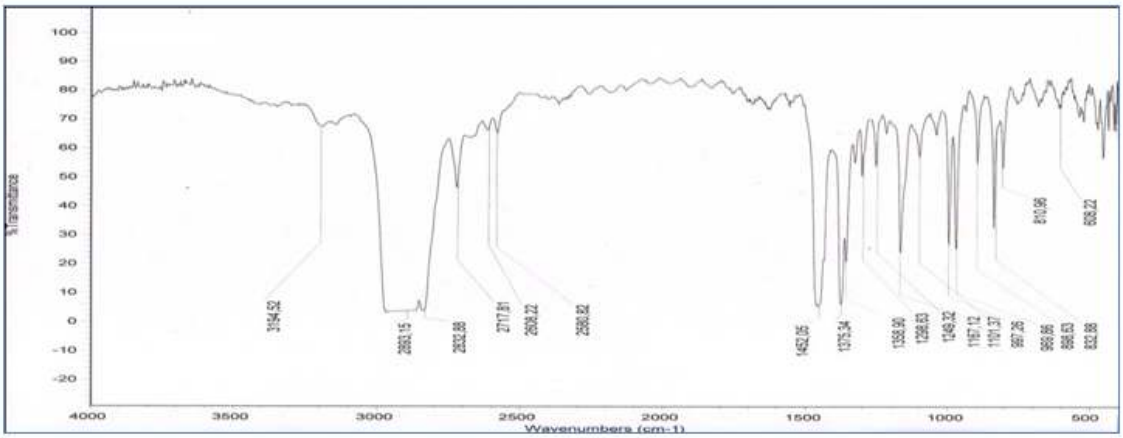

Fig.7. FTIR spectrum of polyethylene treated with consortium (*C. lunata, A. alternata, P. simplicissimum* and *Fusarium* sp.)

peroxidase enzymes were produced in large amount using submerged fermentation.

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. *Penicillium simplicissimum* 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).

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 2. Enzyme activity of Laccase

Enzyme/Weeks	4	5	6	7	8	9	10	11	12
Curvularia lunata	0.00015 ± 0.0001	0.00026 ± 0.0003	0.00040 ± 0.0004	0.00052 ± 0.0003	0.00073 ±0.0002	0.00094 ±0.0004	0.00118 ±0.0002	0.00099 ± .0003	0.00078 ± 0.0002
Alternaria alternata	0.00013 ± 0.0001	0.00023 ± 0.0003	0.00037 ± 0.0004	0.00052 ± 0.0002	0.00066 ±0.0003	0.00081 ± 0.0002	0.00116 ± 0.0001	0.00083 ± 0.0011	0.00065 ±0 .0003
Penicillium simplicissimum	0.00078 ± 0.0001	0.00113 ± 0.0004	0.00124 ± 0.0002	0.00287 ± 0.0003	0.00509 ±0.0001	0.00679 ±0 .0004	0.00888 ±0 .0011	0.00549 ± .0004	0.00379 ±0 .0002
Fusarium sp.	0.00014 ± 0.0002	0.00027 ± 0.0001	0.00041 ± 0.0003	0.00057 ± 0.0002	0.00077 ±0.0003	0.00092 ± 0.0011	0.00115 ±0.0002	0.00086 ± 0.0011	0.00052 ± 0.0002
Microbial Consortium	0.00096 ± 0.0002	0.00134 ± 0.0003	0.00148 ± 0.0002	0.00325 ± 0.0001	0.00541 ± 0.0002	0.00698 ± 0.0003	0.00711 ± 0.0002	0.00661 ± 0.0003	0.00441 ± 0.0002

± = Standard Deviation, * = Mean

Table 3. Enzyme activity of Manganese peroxidase

Enzyme/Weeks	4	5	6	7	8	9	10	11	12
Curvularia lunata	0.00018 ±0.0003	0.00036 ±0.0001	0.00052 ±0.0002	0.00072 ± 0.0003	0.00083 ±0.0001	0.00094 ±0.0002	0.00118 ±0.0011	0.00101± 0.0004	0.00088 ±0.0002
Alternaria alternata	0.00019 ±0.0001	0.00033 ±0.0011	0.00047 ±0.0001	0.00064 ± 0.0003	0.00080 ±0.0002	0.00101 ±0.0003	0.00126 ±0.0001	0.00089± 0.0004	0.00070 ±0.0004
Penicillium simplicissimum	0.00084 ±0.0003	0.00120 ±0.0001	0.00133 ±0.0004	0.00294± 0.0002	0.00515 ±0.0003	0.00685± 0.0001	0.00896± 0.0011	0.00553± 0.0001	0.00384 ±0.001
Fusarium sp.	0.00020 ±0.0011	0.00035 ±0.0001	0.00051 ± 0.0002	0.00067 ± 0.0003	0.00087 ±0.0004	0.00106 ±0.0001	0.00135 ±0.0003	0.00090± 0.0004	0.00072 ±0.0001
Microbial consortium	0.00102 ± 0.0002	0.00154 ± 0.0003	0.00189 ± 0.0001	0.00345 ± 0.0002	0.00562 ± 0.0001	0.00715 ± 0.0003	0.00734 ± 0.0001	0.00672 ± 0.0001	0.00451 ± 0.0003

± = Standard Deviation, * = Mean

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	*Curvularia lunata*	0.0019 ± 0.002	0.0021 ± 0.002
2	*Alternaria alternata*	0.0024 ± 0.001	0.0026 ± 0.006
3	*Penicillium simplicissimum*	0.0350 ± 0.002	0.0389 ± 0.114
4	*Fusarium* sp.	0.0018 ± 0.002	0.0019 ± 0.001
5	Microbial consortium	0.0490 ± 0.002	0.0499 ± 0.001

± = Standard Deviation, * = Mean

CONCLUSIONS

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. 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..

REFERENCES

Domsch, K.H., Gams, W. and Anderson, T.H. (1980). Compendium of soil fungi. (New York: Academic Press Inc)

Ellis, M.B. (1976). More Dematiaceous hyphomycetes. (England: Kew: Common wealth mycological institute)

Ellis, M.B. and Ellis, J.P. (1997). Microfungi on Land plants: An Identification Handbook. (London: Richmond Publishers)

Ellis, M.B. (1971). Dematiaceous hyphomycetes. (England: Kew: Common wealth mycological institute)

Gilan, I., Hadar, Y. and Sivan, A. (2004). Colonization, biofilm formation and biodegradation of polyethylene by a strain of Rhodococcus ruber. Applied Microbial Biotechnology, **(65),** 97-105.

Gilbert E. S., Walker A. W. and Keasling J. D. (2003). A constructed microbial consortium for biodegradation of the organo phosphorus insecticide parathion. Applied Microbiology and Biotechnology, **(61),** 77–81.

Gilman, J.C. (2001). A manual of soil fungi. 2nd edition. (New Delhi: Biotech Books)

Hadad, D. Geresh, S. and Sivan, A. (2005). Biodegradation of polyethylene by the thermophilic bacterium Brevilbacillus borstelensis. Journal of Applied Microbiology, **(98),**1093-1100.

Kathiresan, K. (2003). Polyethylene and plastic degrading microbes from the mangrove soil. Revista de Biologia Tropical, **(51),** 629-634.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., (1951). Protein measurement with the folin phenol reagent, Journal of general Microbiology, **(31),**3017-3027.

Mahalakshmi, V. Siddiq, A. and Andrew, S.N. (2012). Analysis of polyethylene degrading microorganisms isolated from compost soil. International journal of pharmaceutical and biological archives: 1190-1196.

Nagamani, A., Kunwar, I.K. and Manoharachary, C. (2006). Hand book of soil fungi. (New Delhi: I. K. International Pvt. Ltd).

Negi, H., Gupta, S., Zaidi, M.G.H. and Goel, R. (2011). Studies on biodegradation of LDPE film in the presence of potential bacterial consortia enriched soil. Biologia, **(57),** 141-147.

Nishida, H. and Tokiwa, Y. (1993). Distribution of poly (β-Hydroxybutyrate) and poly (€- caprolactone) aerobic degrading microorganisms in different environments, Journal of Environmental Polymer Degradation, **(1),** 227- 233.

Papinutti, L., and Martinez, J. M., (2006). Production and Characterization of Laccase and manganese peroxidase from the ligninolytic fungus Fomes sclerodermeus, Journal of technology and biotechnology, **(81),**1064-1070.

Pitt, J.I. (1979). The genus Penicillium and its teleomorphic states Eupenicillium and Talaromyces. Academic Press Inc, Ltd. London:1-634.

Roy P. K., Titus S., Surekha P., Tulsi E., Deshmukh C., Rajagopal C. (2008). Degradation of abiotically aged LDPE films containing pro-oxidant by bacterial consortium. Polymer Degradation and Stability, **(93),** 1917–1922.

Satlewal, A., Soni, R., Zaidi, M.G.H., Shouche, Y. and Goel, R. (2008). Comparative biodegradation of HDPE and LDPE using an indigenously developed microbial consortium. Journal of Microbiology and Biotechnology, **(18),** 477-482.

Shah, A.A., Hasan, F., Hameed, A. and Ahmed, S. (2008). Biological degradation of plastics: A comprehensive review. Biotechnology Advances, **(26),** 246-265.

Shraddha, Shekher, R., Sehgal, S., Kamtania, M., and Kumar, A. (2011). Laccase microbial source Production, Purification, Potential biotechnological application, Enzyme Research, 1--11.

Soni, R., Kapri, A., Zaidi. and Goel, R. (2009). Comparative biodegradation studies of non-poronized and poronized LDPE using indigenous microbial consortium. Journal of Polymers and Environment, **(17),** 233-239.

Subramanian, C.V. (1983). Hyphomycetes, taxonomy and biology. London. (New York: Academic Press)

Viswanath, B., Chandra, M.S., Pallavi,H. and Reddy, B.R. (2008). Screening and assessment of laccase producing from different environmental samples. African Journal of Biotechnology, **(7),**1129-1133.

Yamada-onodera, K., Mukumoto, H., Katsuyaya, Y., Saiganji, A., Tani, Y. (2001). Degradation of polyethylene by a fungus Penicillium simplicissium YK. Polymer Degradation and Stability, **(72),** 323-327.