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Assessment of polyethylene degradation by *Aspergillus niger* using submerged cultivation and soil burial method

Ingavale R.R., Patil C.K. and Raut P.D.*
Department of Environmental Science, Shivaji University, Kolhapur, MS, India
drpdraut@yahoo.co.in

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Abstract

Polyethylene (PE) has occupied a vital role in human life but the real problem has emerged due to post-use of plastic. Waste plastic lays enormous burden on the environment, because their recalcitrance to degradation accelerates its accumulation in nature. In the present study, the feasibility of a fungus, Aspergillus niger for active degradation of Low Density Polyethylene (LDPE) and High Density Polyethylene (HDPE) was examined by submerged cultivation and soil burial method. Test flasks were subjected for incubation on a rotary shaker at laboratory condition for 30 days where PE was only carbon source. PE degradation was confirmed by 3.97% weight loss in submerged cultivation method and 1.89% in soil burial method. Major change in functional group or formation of side chain was not observed on FTIR Spectroscopy analysis but the reduction in percentage transmittance after 60 days on incubation with fungus Aspergillus niger reveals the microbial activity on PE and thus confirms the degradation of PE. Hence, Aspergillus niger proves more efficient in submerged cultivation method rather than soil burial method.

Keywords: Polyethylene (HDPE, LDPE), plastic, degradation, *Aspergillus niger.*

Introduction

The demand of plastic products has been increased tremendously in current years as the upgradation of society and increased living standards. More than 35% plastic that is manufactured all over the world is mostly exploited for packing purpose and annually the rate of it is growing by about 10% and more. Annually, 57 million tons of plastic waste is generated worldwide while in India, more than 59,000 and 61,000 tons of plastic wastes have been generated in year 1999 and 2000, respectively[1]. Durable nature of the plastic and its cost are the prime reasons for alarming demand worldwide[2]. Due to their high demand, production of plastic has increased massively. However, the problems associated with the plastic post-use have revealed the devastating impact on aquatic ecosystem, thus making it as a most prominent issue[2].

The demand of plastic material has not been accompanied by a proportionate disposal measures of plastic waste generated. Polymerization of ethylene yields a synthetic resin Polyethylene (PE) which is light and versatile material. It is a synthetic type of polymer obtained from a hydrocarbon source. Polyethylene resin contains this material as a base block[3]. These plastics are significantly inert and thus makes its availability difficult for microorganisms[4,5]. Under natural environment rate of degradation of polyethylene is very slow and hence it would take longer period to biodegrade[6]. However, biodegradation can prove more efficient if the PE itself is utilized as a carbon source by microorganisms and develops the biofilm on PE sample[5,7].

Fungi have been proved to biodegrade PE by secreting enzymes[5]. Some studies have investigated biodegradation of PE by fungal isolate viz., *Phanerochaete chrysosporium*[6], *Mucorcircenollides, Pseudomonas citronellolis*[8], *Aspergillus niger, A. flavus, A. terreus, A. fumigatus, Penicillium sp*[9], *Penicillium oxalicum*, *Penicillium chrysogenum*[10], *A. japonicus*[11] etc. Esmaeili *et al.,* have demonstrated the ability of *Aspergillus* spp and *Lysinibacillus* spp to modify and colonise PE treated with UV and without UV-treatment as a carbon source and thus confirmed its ability in PE biodegradation in soil[12]. PE when exposed to microbial consortia of soil for 10-32 years, only 1% carbon mineralization was recorded thus showing very low biodegradation rate[13,14].

The present study aims to evaluate the potential of *Aspergillus niger* in degradation of PE in submerged cultivation method and soil burial method without any prior treatment to LDPE and HDPE. The capability of *A. niger* to utilize PE as a carbon source was determined by weight loss and chemical changes occurred were detected by using Fourier Transform Infrared Spectroscopy (FTIR).

Materials and methods

In the present study, LDPE and HDPE pellets were kindly provided by Laxman Udyog Samuh, Plastics and Allied Industries, MIDC, Gokul Shirgaon, Kolhapur. At laboratory these pellets were converted into fine sheets with uniform dimensions. For further use, the prepared sheets were disinfected by exposing to alcohol for few minutes and later air dried. A fungus, *Aspergillus niger* has remarkable ability to

degrade PE[8,9]. Hence, species of *Aspergillus niger* was selected for further studies.

Submerged cultivation procedure: *Aspergillus niger* assayed for its capability of utilizing PE as the sole source of carbon was performed in Synthetic Media (SM) containing (gm per litre of distilled water): $MgSO_4.7H_2$-0.2; $CaCl_2.2$-H_2O - 0.1; KCl - 0.15; K_2HPO_4 - 1; NH_4NO_3 - 1; and yeast extract - 0.1; and 1.0mg/l of each of the following micro-elements: $FeSO_4.6H_2O$, $ZnSO_4.7H_2O$ and $MnSO_4$.Preweighted and disinfected PE sheets were added to 100 ml sterilised SM in Erlenmeyer flask. Suspension of *Aspergillus niger* was inoculated to the test flasks and were kept for incubation on a rotary shaker (110rpm) at room temperature for next 60 days. Simultaneously, a blank sample was run with PE samples and SM but without fungal culture. The set was maintained in triplicates.

Soil burial method: Degradation of LDPE and HDPE was also studied under soil burial method at Laboratory scalein Borosil glass tray. Soil required was collected from such area of Kolhapur city where plastic waste is dumped since few years. Soil and manure was added in a proportion of 3:1. It was inoculated with 30ml suspension of *Aspergillus niger* and left for degradation for 60 days. Moisture was maintained during this period by sprinkling water.

After completion of 60 days, the sheets were taken out from flask and borosil tray (submerged cultivation method and soil burial method respectively) and sheets were subjected for reduction of weight, diameter and thickness. Also, the sheets were analysed by FTIR technique for changes in functional groups and determine the degradation of polyethylene.

Results and discussion

Microbes release various enzymes into the soil and water, which initiates the disintegration of the polymers into small fragments. Couple of enzymes called intracellular and extracellular depolymerases is engaged in the degradation of polymer. Exoenzymes from the microorganisms initially disintegrate the complex polymers into monomers. The formed monomers can be sufficiently passed through the cell wall and can be consumed as carbon and energy source. The procedure is called as depolymerization. When the final product is carbon dioxide, water or methane, the method is called as mineralization[6]. Degradation of LDPE and HDPE sheets in submerged cultivation and soil burial method using *Aspergillus niger* was carried out for 60 days. Findings elaborated in the following sections are the results observed from two diverse sets of degradation experiments.

Reduction in weight, diameter and thickness of PE sheets: Bonhomme *et al.,* analyzed that polyethylene with a significantly high molecular weight was degraded to lower molecular weight after three months of treatment with fungal culture in liquid conditions, which indicated the degradation of polyethylene by biotic organisms[15]. The reduction in weight can be considered as an indicator of biodegradation of PE. Other parameters like diameter and thickness were also recorded. The LDPE and HDPE sheets under study were removed after 60 days from medium, washed with distilled water and dried. The amount of degradation was determined by studying reduction in weight, diameter and thickness of sheets. After 60 days of incubation of LDPE and HDPE sheets with *Aspergillus niger* maximum reduction of all the parameters was observed in submerged cultivation method than composting method which is depicted in Figure-1 and Figure-2. LDPE incubated at submerged cultivation method showed weight loss of 3.97% while 1.89% weight loss was recorded for soil burial method. Kathires an carried out degradation of plastic and polyethylene using microorganisms isolated from mangrove soil in liquid medium. Among fungi *Aspergillusglaucus* degraded 28.80% of polyethylene and 7.26% of plastic and *Aspergillus niger* degraded 17.35% of polyethylene and 5.54% of plastic[16]. Orhan *et al.,* observed 1.3% and 2.1% weight loss for LDPE and HDPE respectively after 9 months in composting method[17]. Vijaya and Reddy observed 11.01% weight loss for LDPE and 3.68% for HDPE in composting method[18]. Das and Kumar have observed 16% weight loss of PE on exposure with *Bacillus amyloliquefaciens* in liquid culture media after 60 days[19]. Degradation of PE sheets treated with selected fungi was observed by measuring reduction in thickness, diameter and weight of sheets.

Degradation of plastic initiates from the edges of the sheets. Hence, reduction in diameter can also be considered as an important parameter to understand degradation of PE. In the current study, diameter loss for LDPE in submerged cultivation method was 3.22% and in soil burial method it was 2.91%. Chonde *et al.,* have recorded reduction in thickness for Nylon 6 and Nylon 6, 6 as 46% and 49% respectively on exposing it with *Pseudomonas aeruginosa* NCIM 2242 for 6 months[20]. Chonde *et al.,* have observed similar thickness loss for Nylon 6 on treating it with fungus *Trametes versicolor* NCIM 1086 after 75 days[21]. In the present study, thickness reduction recorded for LDPE was 3.47% and 2.91% for submerged cultivation method and soil burial method respectively. Similarly, HDPE showed maximum reduction in thickness in submerged cultivation method i.e. 4.38% than soil burial method i.e. 1.71%.

Vijaya and Reddy concluded that in natural environment i.e. under composting conditions rate of biodegradation of plastic is very slow[18]. Kumari *et al.,* also studied degradation of PE strips under compost conditions and observed degradation of PE to 4.50% in terms of weight loss[3]. The number of micro-organisms present in the compost depends entirely on environmental conditions and the source of compost. Hence, the tests required for the process of composting are not reproducible though the method is most practically favourable for waste treatment[22]. By using compost treatment, varied kind of plastics can be degraded at different rate of degradation and obtained product too varies in the quality.

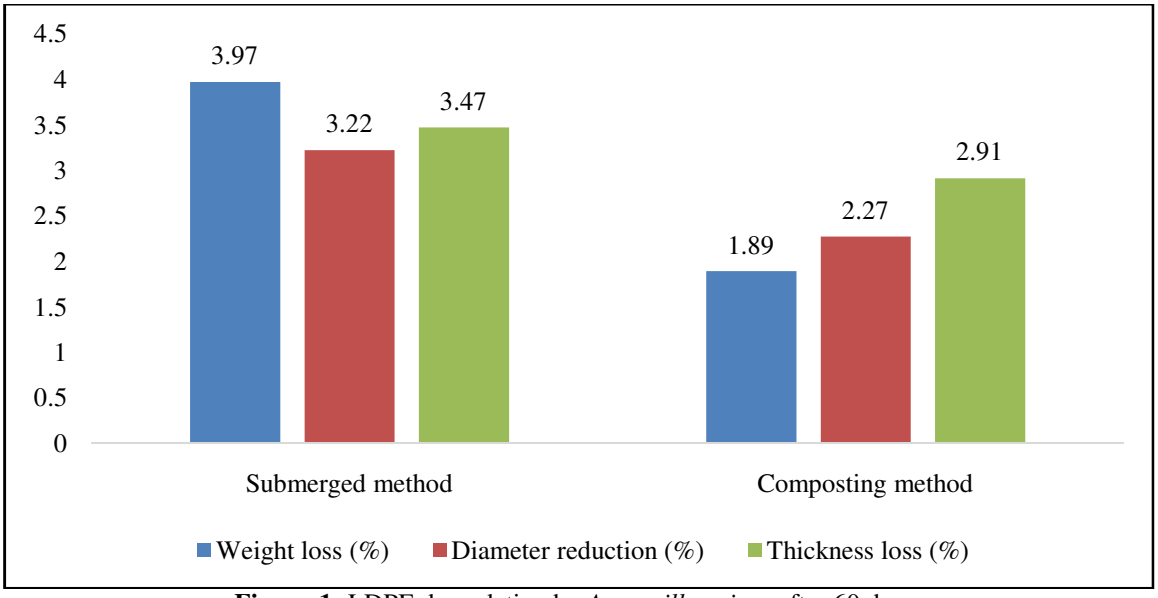

Figure-1: LDPE degradation by *Aspergillus niger* after 60 days.

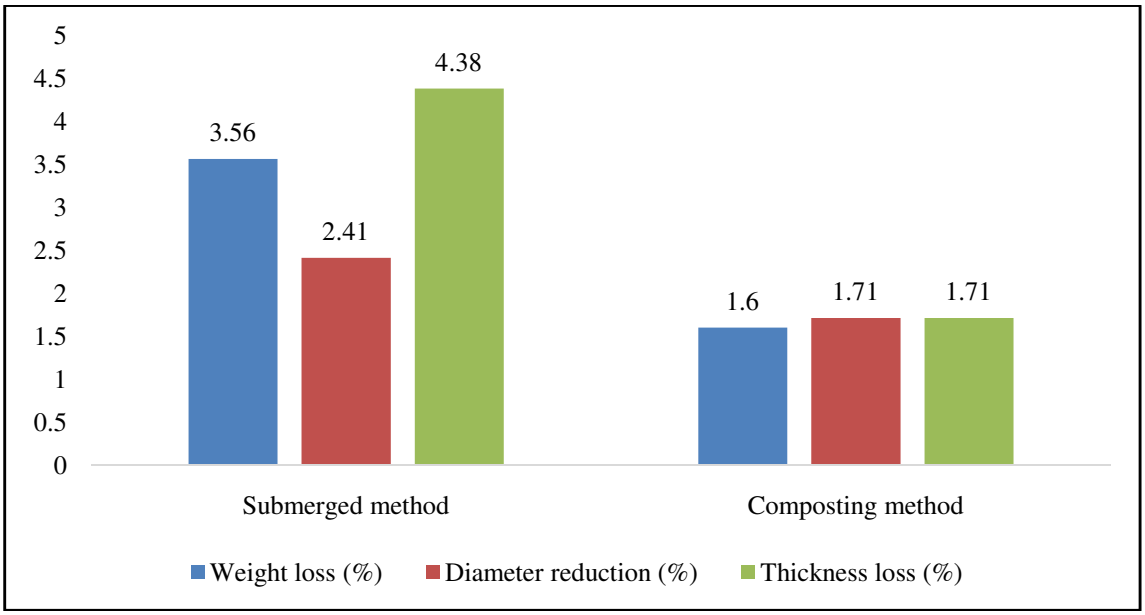

Figure-2: HDPE degradation by *Aspergillus niger* after 60 days.

Fourier Transform Infrared Spectroscopy (FTIR) of Polyethylene sheets incubated with *Aspergillus niger:* Newly formed or disappearance of existing functional groups can be examined by significant device called as Fourier transform infrared spectroscopy (FTIR). Hence, degradation products, chemical moieties assimilated into the polymer molecules such as branches, co-monomers, unsaturation and presence of additives (antioxidants) can be determined by this technique[23]. Previously, the formation of carbonyl groups on abiotic degradation of PE were determined by FTIR-ATR technique and. Along with it, the fragmentation of polymer chains into shorter chains observed in the FTIR-ATR spectrum reflects the reduction of native bonds[24].

LDPE and HDPE are made of the elements carbon (C) and hydrogen (H), which forms chains of repeating $–CH_2–$units. Gulmine *et al.,* observed the distinct peaks at wave numbers 2919, 2851, 1473, 1377 and 720cm^{-1} on FTIR analysis of pure LDPE. These peaks are assigned to the native bonds of polymer[25]. In the current study, FTIR analysis of undegraded pure LDPE and HDPE (control) showed absorbance at 2913cm^{-1}, 2846cm^{-1}, 1462cm^{-1}, 1376cm^{-1} and 719cm^{-1} (Figure-3 and Figure-6). Absorbance at 2850 to 3000cm^{-1} region corresponds to the alkyl groups (CH_3CH_2, CH). Band at 2914cm^{-1} and 2847cm^{-1} corresponds to CH_2 group with strong intensity and possess asymmetric and symmetric stretching respectively. The strong band at about 1462cm^{-1} in polyethylene is due mainly to

bending mode of the CH_2 group[25]. Absorbance at 1376cm^{-1} corresponds to CH_3 symmetric deformation with weak intensity[26]. 719cm^{-1} is assigned to rocking deformation mode of CH_2 group with medium intensity[25,26].

PE incubated with *Aspergillus niger* was subjected for FTIR for analyzing the chemical changes after 60 days of incubation. It is proved that the percentage of PE and percentage of transmittance are closely related to each other. If more percentage of PE is present in the standards then the percentage of transmittance would also be more and vice versa. In the LDPE and HDPE incubated with *Aspergillus niger* reduction in the percentage transmittance was observed with intact native bonds. LDPE sheets incubated in submerged cultivation showed more reduction in transmittance percentage than in soil burial method (Figure-4 and Figure-5). Similar results were noticed for FTIR of HDPE incubated with *Aspergillus niger* for 60 days. Rate of percentage transmittance in submerged cultivation method is reduced noticeably as compared to soil burial method (Figure-7 and Figure-8).

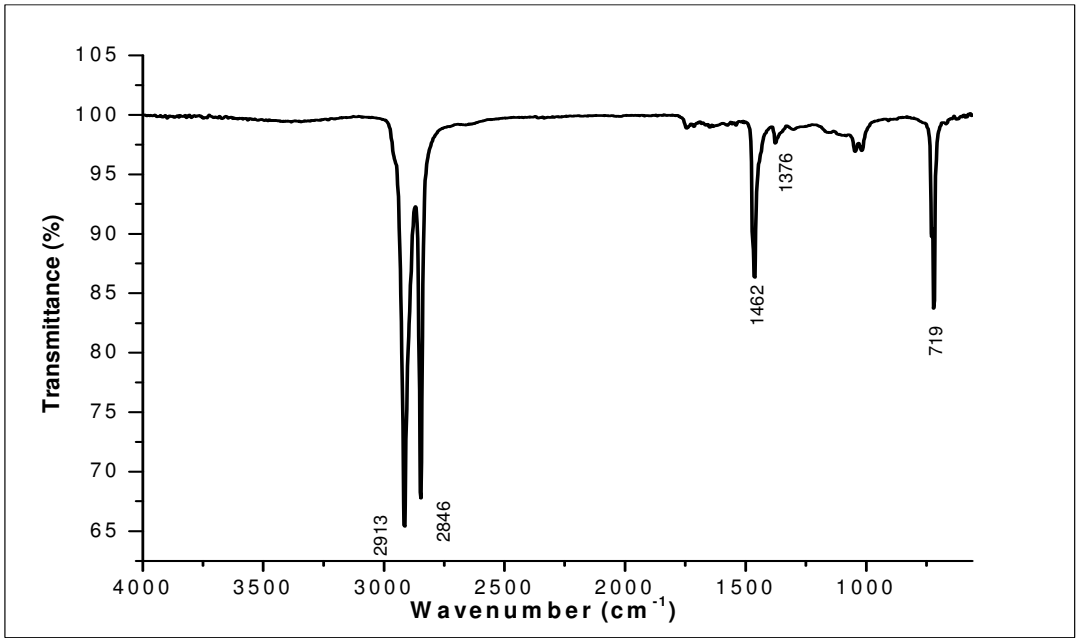

Figure-3: FTIR spectrum of control LDPE for the range 4000–560 cm^{-1}.

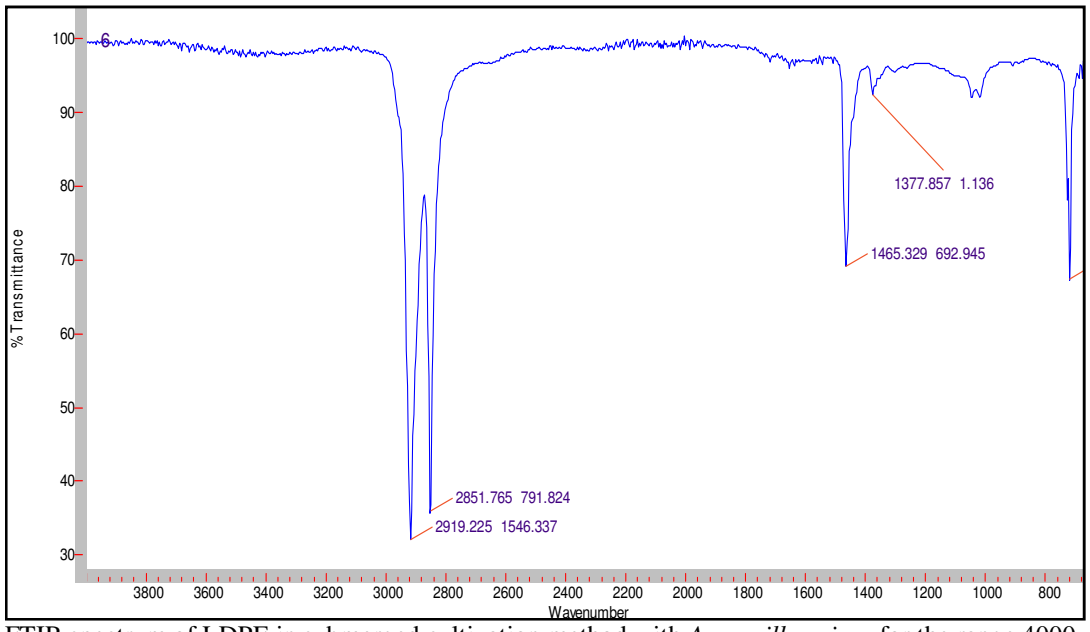

Figure-4: FTIR spectrum of LDPE in submerged cultivation method with *Aspergillus niger* for the range 4000–560 cm^{-1}.

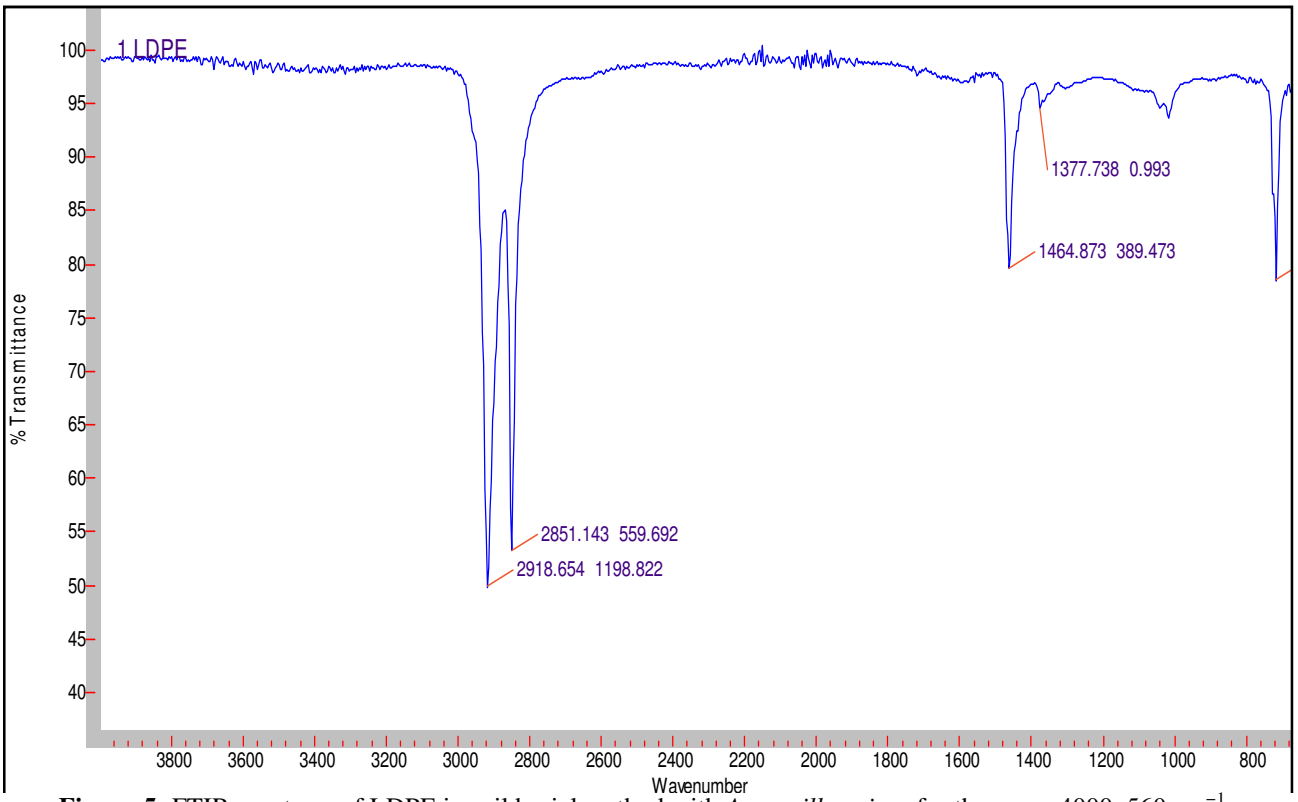

Figure-5: FTIR spectrum of LDPE in soil burial method with *Aspergillus niger* for the range 4000–560 cm^{-1}.

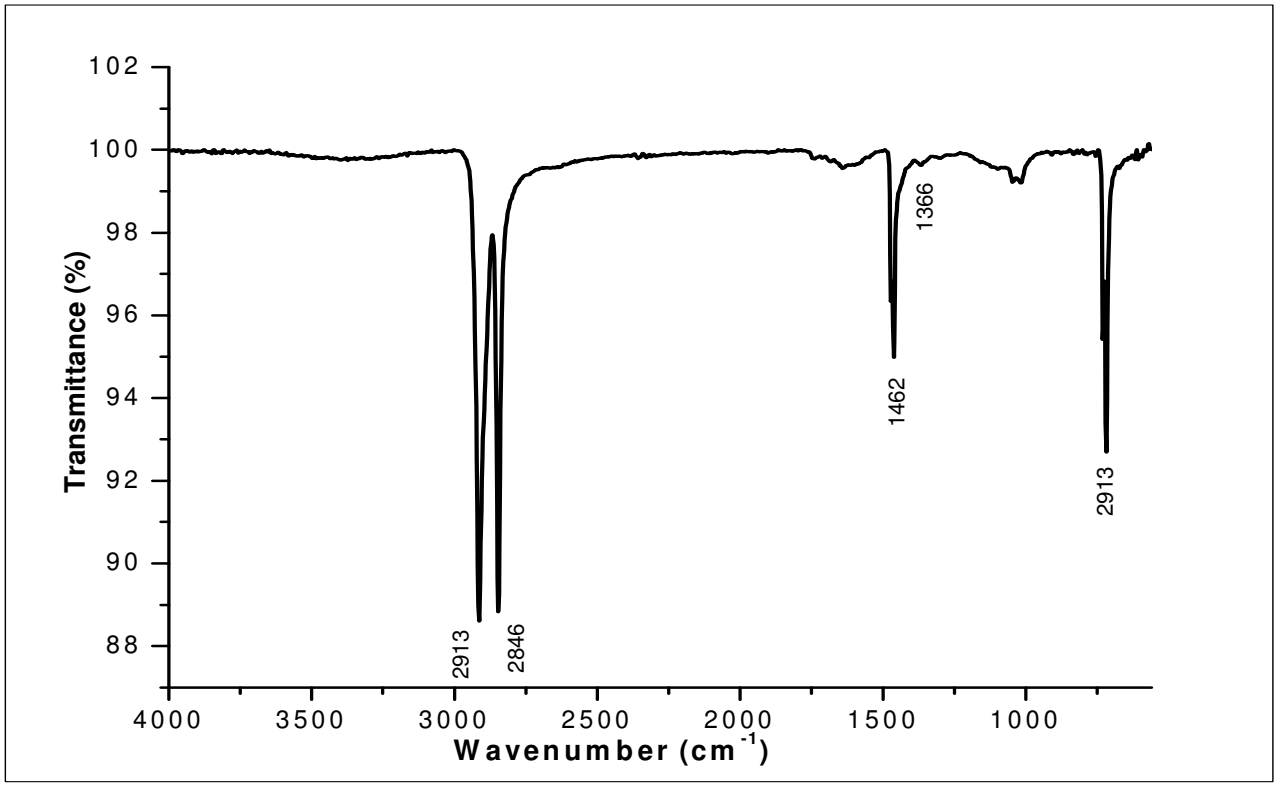

Figure-6: FTIR spectrum of control HDPE for the range 4000–560 cm^{-1}.

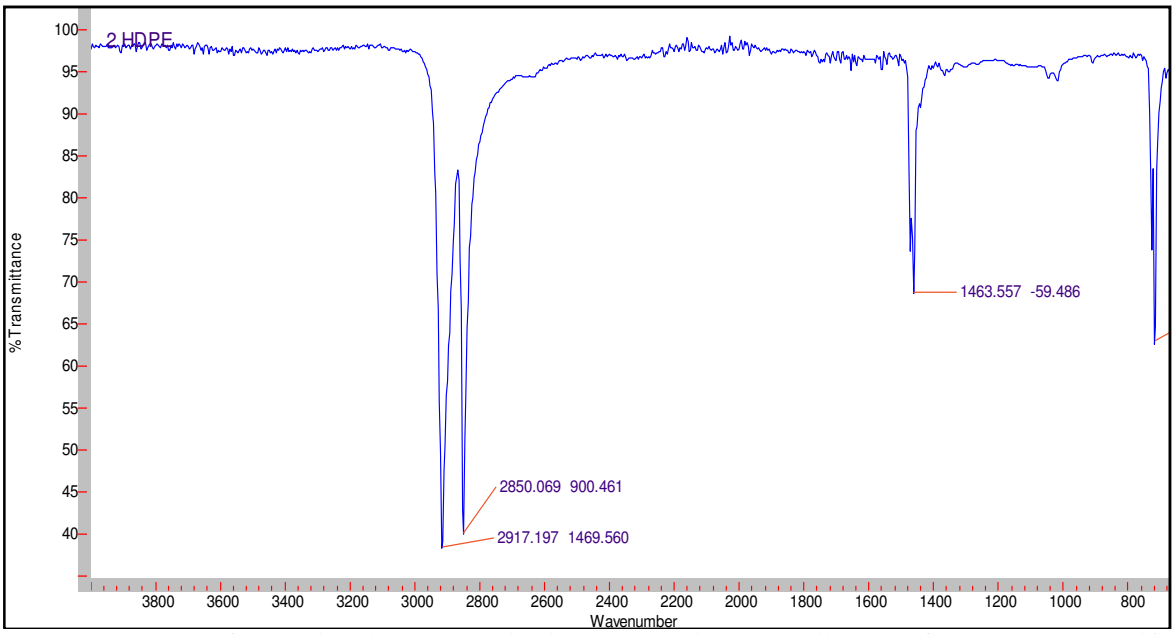

Figure-7: FTIR spectrum of HDPE in submerged cultivation method with *Aspergillus niger* for the range 4000–560 cm^{-1}.

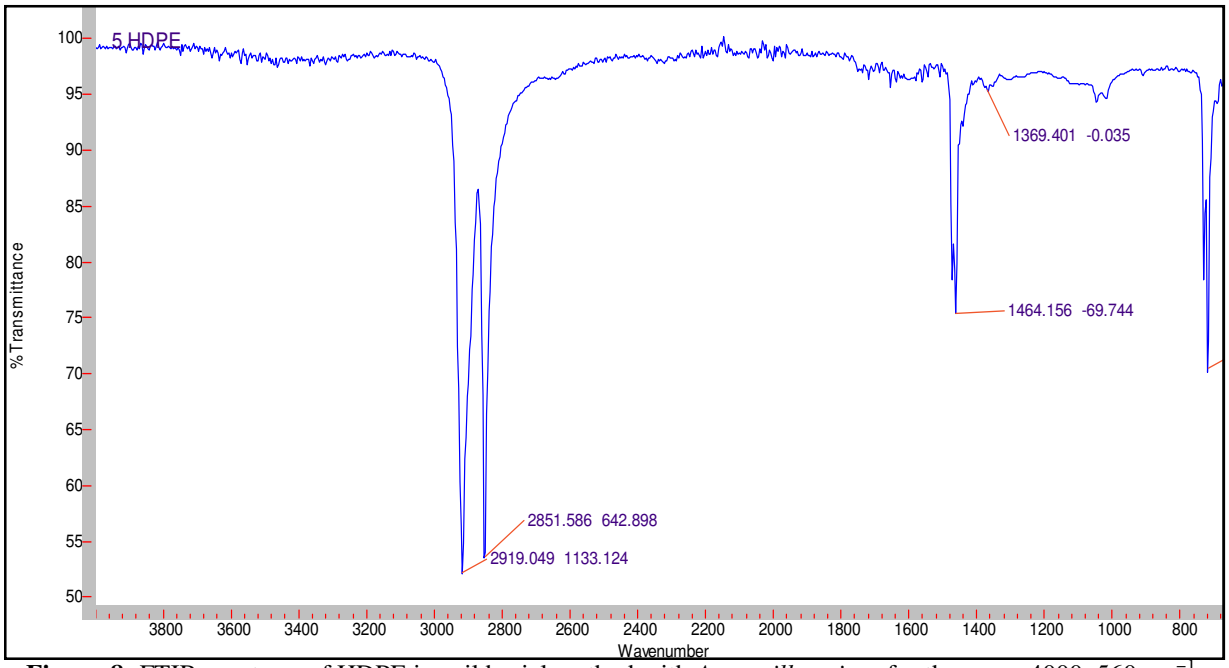

Figure-8: FTIR spectrum of HDPE in soil burial method with *Aspergillus niger* for the range 4000–560 cm^{-1}.

Conclusion

Fungus *Aspergillus niger* mediated biodegradation of polyethylene is reported in this paper. Degradation of polyethylene was observed efficient in submerged condition as compared to soil burial method in the presence of fungus *Aspergillus niger.* Further, the degradation of polymer was confirmed by reduction in weight, decrease in thickness and decrease in intensity of functional groups. The colour of sheets also changed and developed the cracks at edges when exposed to fungal strain. This shows consequence of enzymatic action suggesting degradation of polyethylene.

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