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Microbial Deterioration of Low Density Polyethylene by *Aspergillus* and *Fusarium* sp.

Merina Paul Das*, Santosh Kumar

Department of Industrial Biotechnology, Bharath University, Chennai, India.

***Corres.author: merinadas@gmail.com**

Abstract: Plastics either natural or synthetic represent the class of polymeric substance having variant and excessive use in all the sectors of the industrialization. The high accumulation of disposed plastics leads a serious threat to environment. Hence some biodegradable plastics have been developed in response to demands for waste-free plastic products. Biodegradable plastics can be degraded by microorganism or enzymes by means of cutting down the molecular chains. Low density polyethylene (LDPE) is one of the polymers that are up till date nearly impossible to be degraded fully in faster way. In present findings, five fungal strains were isolated from soil buried LDPE films and showed attachment with it. Out of five, four were identified as *Aspergillus* sp. and the rest one was *Fusarium* sp. Efficacy of the microbes in polymer degradation was analyzed by weight reduction, change in pH, CO_2 evolution test over a period of 60 d at 33.3 °C. The fungal colonization was visualized by scanning electron microscope (SEM) whereas the surface chemical changes were confirmed by Fourier transform infrared spectroscopy (FTIR). As all the isolated fungi showed promising biodegradation results, these strains can be used for bioremediation in this line.
Keywords: LDPE, Biodeterioration, *Aspergillus* sp., *Fusarium* sp., Biodegradation analysis.

Introduction and Experimental

Plastic is a macromolecule, comprised of smaller, repetitive smaller units. Such polymer molecules may be linear, branched, have a network structure, etc. These different molecular geometries influence the properties of the given polymer very significantly[1]. Plastics due to their versatility become the essential ingredients to provide a quality to life. These are now rival metals in breadth of use and in severity of applications because of their flexibility, toughness, excellent barrier and physical properties and ease of fabrication[2-6]. The worldwide utility of polyethylene is expanding at a rate of 12% per annum and approximately 140 million tonnes of synthetic polymers are produced worldwide each year [7]. Plastics are composed of petroleum based materials called resins (e.g., polythene and polypropylene) materials that are resistant to biodegradation. Due to this resistance, plastics that are disposed in landfills remain in their original form in perpetuity[8]. As a result, accumulation of plastics in the environment has been recognized as a big issue. To enhance the environmental degradation of polyethylene a number of different approaches are used, such as copolymerization or compounding with additives susceptible to the environmental factors[9].

Polyethylene (PE) is a thermoplastic polymer consisting of long chains produced by combining the ingredient monomer ethylene. The most important polyethylene grades are HDPE, LLDPE and LDPE. LDPE is defined by a density range of 0.910-0.940 g/cm^3. LDPE has low density because it has more branching[10]. LDPE accounts for 60 % of the total plastic production and the most commonly found solid waste is the non-degradable polythene bags[8]. They are extremely hazardous particularly the thin color ones. They release toxic chemicals which contaminate food items[11]. The limitations of this polythene can be overcome by taking some alternative approaches, such as biodegradable plastic, bioremediation of plastic waste.

Biodegradation of polythene is a natural process where microorganisms utilizing this organic complex polymer as carbon and energy source and biologically transforming to simpler one. As the microorganisms possess different characteristics, so the degradation varies from one microorganism to another [12]. This microbial degradation is most widely accepted one because of its efficiency. Recently several microorganisms have been reported for degradation of plastics. The bacterial species identified from the polythene bags tested were *Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Diplococcus* sp., *Micrococcus* sp., *Pseudomonas* sp. and *Moraxella* sp. Among the fungal species identified, *Aspergillus niger*, *A. ornatus*, *A. nidulans*, *A. cremeus*, *A. flavus*, *A. candidus* and *A. glaucus* were the predominant species[13]. 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[14,15], the faster growth of fungal biomass compared to bacteria[16], their generation of degrading enzymes that are well matched to the insoluble LDPE[17]. Enzymatic degradation process is most simple process where microbes will secrete the extracellular enzymes. The microbes will attach to the inert surface by enzymes and grow on this film by utilizing it as nutrient source. So the polymer is depolymerized and the degradation is done by mineralization processes where carbon dioxide (CO_2), water (H_2O) or methane (CH_4) is produced as end products[18]. Figure 1 shows the mechanism of enzymatic degradation of polymer [17].

The aim of this study was to isolate potent low density polyethylene (LDPE) degrading fungal strains from plastic waste, and subsequently study the degradation with various supporting parameters.

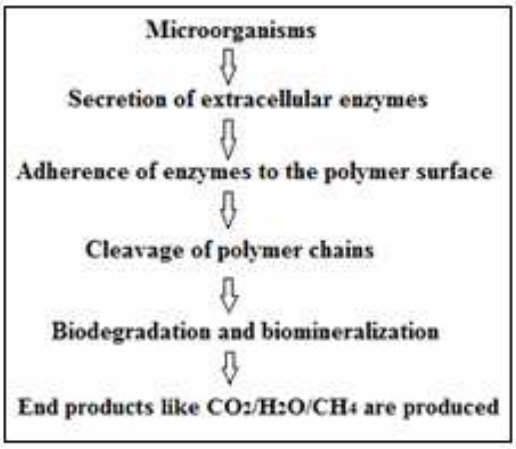

Figure 1. Mechanism of enzymatic degradation of plastic

Preparation of LDPE powder

The LDPE films were cut into small pieces and were dipped in xylene and heated, when the plastic gets dissolved it was cooled to palm bearable heat and was crushed to fine particles. Later it was kept to evaporate the xylene and was washed with ethanol to remove xylene residues. Then it was dried in hot air oven at 50 °C for overnight.

Isolation and screening of LDPE utilizing fungi

The soil samples were collected from municipal solid waste as it is rich in plastics and serially diluted. The LDPE degrading fungi were screened by spread plate technique using mineral salt medium supplemented with 3 % LDPE powder. The composition of mineral salt media was as follows: (g/l: K_2HPO_4 1.0, KH_2PO_4 0.2, NaCl

1.0, $CaCl_2.2H_2O$ 0.002, H_3BO_3 0.005, $NH_4(SO_4)_2$ 1.0, $MgSO_4.7H_2O$ 0.5, $CuSO_4.5H_2O$ 0.001, $ZnSO_4.H_2O$ 0.001, $MnSO_4.H_2O$ 0.001, $Fe_2(SO_4)_3.6H_2O$ 0.01, Agar 15) with pH 7.0. The plates were incubated at 37 °C for 7 d. The developed fungal mats were subcultured on Saboraud's dextrose agar to get pure culture and preserved in slant at 4 °C.

Identification of polyethylene degrading fungi

The identification of fungi was performed on the basis of macroscopic and microscopic examination. The fungi were identified after staining them with cotton blue by following the keys of Raper and Fennell [19].

Biodegradation analysis

The pre-weighted strip of LDPE films (1.5×1.5 cm) aseptically transferred into the conical flask containing 300 ml of mineral salt medium and then inoculated with identified polythene degrading microorganisms. Control was maintained with LDPE films in the microbe free medium and left in a rotary shaker at 33.3°C, 130 rpm for 60 d. After the period of shaking the strips were collected, washed thoroughly using distilled water, shade dried and further analyzed the biodeterioration. Each test was consisted of three replicates.

Measurement of biodegradation

Weight reduction: A simple way to measure the biodegradation of polymers is by determining the weight loss. To facilitate accurate measurement of the dry weight of the residual polyethylene, the fungal colonization was washed off from the polymer surface by dipping the fungi-treated films with 2 % (v/v) aqueous sodium dodecyl sulphate solution for 4 h and then with distilled water. The washed polymer film was placed on a filter paper and dried overnight at 60 °C before weighing.

Change in pH: The variation in the pH level in the culture media possibly occurred due to the microbial activity was measured at an interval of 10 d during the study.

CO_2 evolution test: Biodegradation of polymer chain leads to production of carbon dioxide. This CO_2 evolved due to LDPE biodegradation was determined by Sturm test [20] which was self modified.

SEM analysis: The fungal attachment of surface films and different changes on surfaces such as micro-cracks, pits on LDPE film by the growing fungi were visualized by SEM.

FTIR analysis: Fourier transform infrared (FTIR) measurements were carried out for identification of surface structural changes on polymer.

Results And Discussion

To determine the efficiency of different fungal isolates for degrading the low density polyethylene, LDPE film was used as only carbon and energy source and different parameters were checked, which supports biodegradation of LDPE.

Screening and identification of LDPE degrading fungi

After inoculation of soil samples in the mineral salt medium supplemented with 3 % LDPE powder, five different fungal isolates were screened which can utilize the LDPE as carbon source. All the fungi were pure cultured (Figure 2) and identified. Out of five isolates four were identified as *Aspergillus* sp. (FSM-3, 5, 6, 8) and the rest one was *Fusarium* sp. (FSM-10) based on the microscopic examination and morphological characteristics.

Figure 2. Pure culture of identified fungal isolates

Microbial degradation of LDPE by identified fungi

In the laboratory conditions, LDPE was degraded by the most potent five fungal strains using broth culture method for a period of 60 d. After the incubation period various parameters were determined.

Determination of weight reduction: The most convenient method to determine the degradation is to measure the weight loss. During the degradation time, the systems were maintained as undisturbed with no addition and removal of medium which indicates that the microorganisms used the LDPE film as carbon source. The microbial enzymes catalyzed the depolymerization and thus there was weight reduction of polyethylene. Among the fungi strains, FSM-10 (9 %) and FSM-3 showed maximum (8 %), both FSM-6, 8 exhibited moderate (7 %) and FSM-5 showed less (5 %) LDPE weight reduction and thus degradation (Figure 3).

Change in pH: As the polymer was degraded, there will be change of pH of the media due to the presence of different monomer products. The pH of individual culture broth with LDPE film was measured before, during and after degradation (Figure 4). For all fungal broth culture, initial pH was 7.0. But after the incubation period, pH values were lower than the initial one that means the media became acidic for all the cases. The culture broth containing FSM-5,6,8 were more acidic than FSM-3, 10 after the degradation.

Evolution of CO_2 during degradation: The most common end products of polyethylene degradation were CO_2, CH_4, and/or H_2O. Mineralization is the evolution of CO_2 during depolymerization. Thus the level of CO_2 was calculated from the control and reaction chamber after 60 d study. Theoretical carbon dioxide for 3 % LDPE was calculated to be 11 g and the percentage of mineralization level of LDPE through evolved carbon dioxide from reaction chambers was determined (Table 1). As degradation of LDPE films by FSM-10,3 were maximum thus they produced large amount of CO_2 compared to other strains.

SEM analysis of LDPE films: SEM analysis is used for the analysis of LDPE films after the degradation period. These microorganisms utilize polythene film as a sole source of carbon by colonizing on the surface of the polyethylene films. These results the formation of biofilm. Cell surface hydrophobicity of these organisms was found to be an important factor in the formation of biofilm on the polythene surface, which consequently enhanced biodegradation of the polymers [21]. The microbial colonization of a polymer surface is the first requirement for its biodegradation [22]. Scanning electron micrograph showed the attachment of fungi on LDPE surface and formation of various holes and irregularities whereas the control film was appeared with smooth surface having no any pits, cracks or any particles attached on its surface (Figure 5).

FTIR analysis: Polymer degradation has been reflected in changes of bond scission, chemical transformation and formation of new functional groups [23]. The FTIR spectroscopy analysis exhibited several changes on the surfaces after the degradation of 60 d with different fungal isolates (Figure 6). The increase in 1079 cm^{-1} and 2418 cm^{-1} band due to formation of C=O and O–H stretch were observed. The peak at 2920 cm^{-1} was distorted after the treatment with fungal isolates. The films were more effected by FSM-10,3,6 than the others.

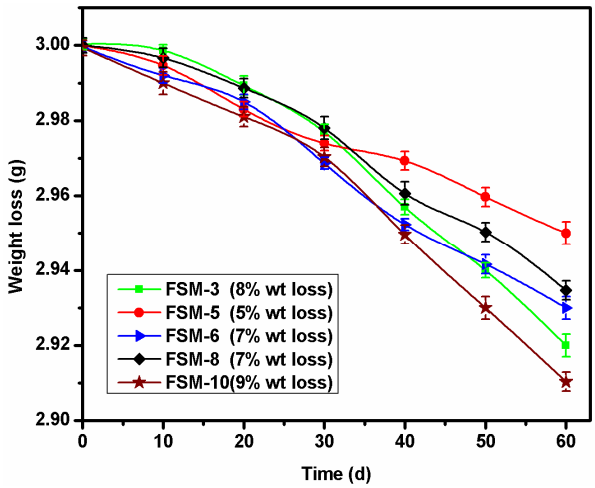

Figure 3. Weight reduction of LDPE films by fungal isolates

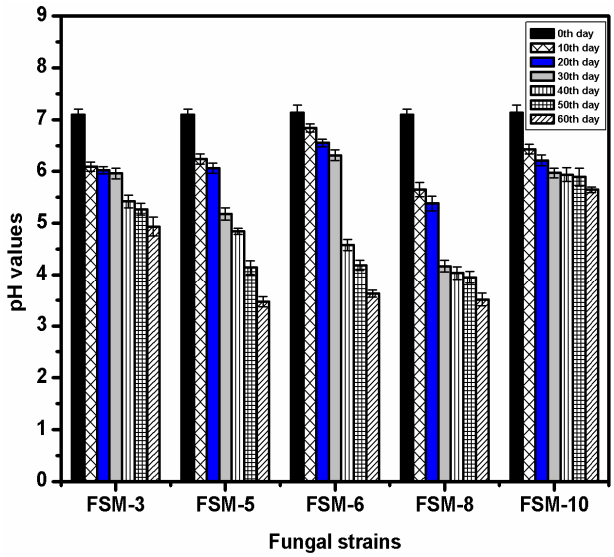

Figure 4.Change in pH of the different fungal culture medium

Table 1. CO_2 evolved level during biodegradation of LDPE using fungi

Fungal isolates	Carbon dioxide evolution (g/L)		Mineralization level (%)
	Control	Test	
FSM-3	19.13	20.26	10.3
FSM-5	17.53	18.47	8.6
FSM-6	16.87	17.93	9.7
FSM-8	16.83	17.84	8.96
FSM-10	18.17	19.38	13.02

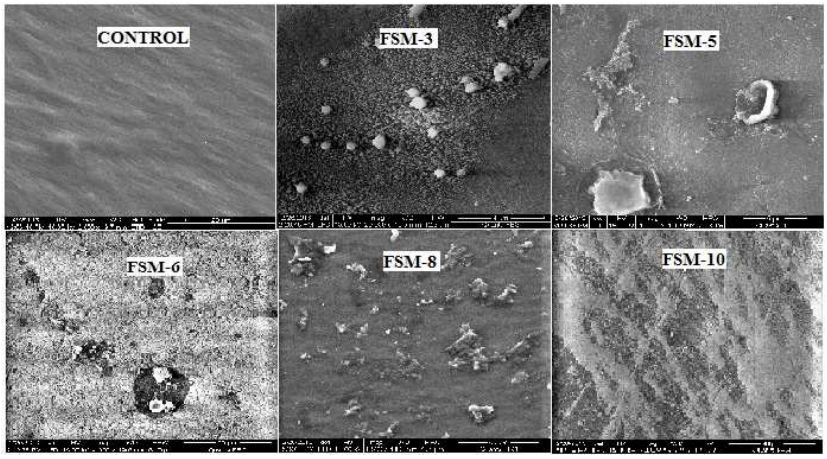

Figure 5. Scanning electron micrograph of untreated and treated sample of polymer sheet

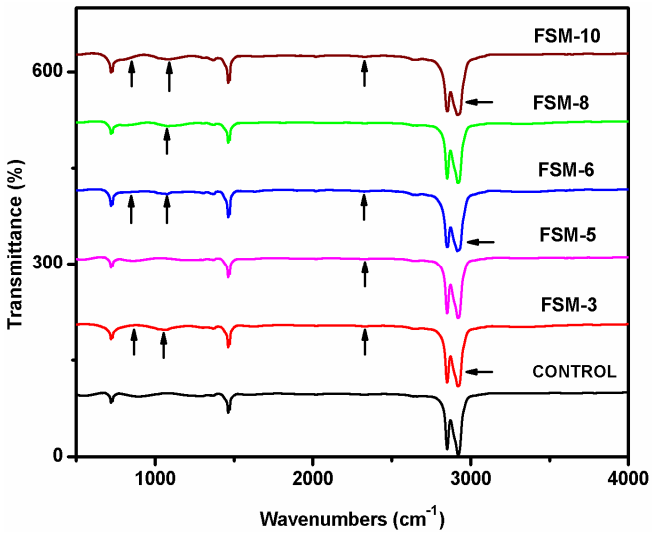

Figure 6. FTIR spectra of LDPE film after 60 d of incubation with (FSM-3,5,6,8,10) and without (Control) fungal strains

Conclusion

This article reveals that the biodeterioration of low density polyethylene by fungal strains isolated from municipal solid waste. These microbial isolates were responsible for the decreasing weight of LDPE films by adhering on this inert surface and also utilizing it as the only carbon and energy source which was evident by increase in the fungal growth. This knowledge can be used as a valuable application to solve the plastic waste problems using a microbial tool.

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