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High Density Polyethylene (HDPE) Degradation in Aqueous Solution by Fungi Isolated from Garbage Landfills at Thanjavur, Tamil Nadu, India

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A B S T R A C T

High density polyethylene (HDPE) is the most frequently found non-degradable solid waste among the polyethylene which leads to innumerable environmental hazards. In the present study, HDPE degrading diverse fungal strains were isolated from the polyethylene garbage dumped sites of Thanjavur, Tamil Nadu, India and screened in aqueous culture under in vitro condition. The two fungal strains identified as *A. flavus* STR1 and *Oidium Sp.* STR2 by morphological characterization are capable of adhering to the surface of PE pieces, through enrichment technique. A thick network of fungal hyphae was observed on the surface of the plastic pieces under light microscope. Based on weight loss, viscosity and visible increase in growth and Spectrophotometric analysis, they were found to be efficient in HDPE degradation. The study further substantiated that the fungal isolates were considered for their ability to form hydrophobic proteins that can attach to the polymer and the ability of fungal isolates was proved to utilize virgin High Density polyethylene (HDPE) as the sole carbon source without any pre-treatment and pro-oxidant additives.

Introduction

Plastics being a man-made synthetic organic polymer has vast varieties of applications in every human life. Plastics are globally one of the most used and widespread materials and add comfort, convenience, and safety to human life (Alam *et al.*, 2018). They had become the essential commodity to enhance the comfort and quality of life due to their versatile qualities. Based on the density and branching of Plastics are classified into different types. The following properties such as the extent and type of branching, the crystal structure, and the molecular weight involved in the mechanical properties of plastics. HDPE, LLDPE, and LDPE were considered the most important Polyethylene grades. Generally, it was believed that higher the density greater the stability because of shortened bond length and tight packaging. Physically, HDPE is harder, more opaque and can withstand somewhat higher temperatures (Balasubramanian *et al.*, 2010).

Littering and voluminous accumulation of waste plastics in the biosphere has given rise to the problem of severe environmental pollution. Polythene being xenobiotic is the most frequently found non-degradable solid waste that has been recognized as a major threat to land and aquatic life both. The widely used packaging plastic (mainly polythene) constitutes about 10% of the total municipal waste generated around the globe (Barnes *et al.,* 2009). Only a fraction of this polythene waste is recycled whereas most of the wastes enter into the landfills and taken hundreds of years to degrade (Lederberg, 2000; Moore, 2008). Polythene sometimes could cause blockage in fish, birds, mammals and choke to death. An estimate of one million birds and ten thousand mammals die each year as a result of ingestion of or trapping of plastics in oceans (Lee *et al.,* 1991; Palmisano *et al.,* 1992).

The areas of temple city Thanjavur located at the delta of Cauvery river proudly denoted as 'Rice Bowl of Tamil Nadu' are at risk of plastic pollution was primarily because of high rate of anthropogenic activities. These problems have made vast focus on the solid waste management. Therefore, biodegradation of synthetic polymers gained great focus using effective microorganisms. Biodegradation of polyethylene has been studied extensively earlier (Breslin, 1993). Biodegradation resulting from the utilization of polyethylene as a carbon source in aqueous culture may be more efficient if the degrading micro-organism forms a biofilm on the polyethylene surface (Albertsson *et al.,* 1994; Ehara *et al.,* 2000).

Microbial degradation of solid polymers such as HDPE requires the formation of a biofilm on the surface of the polymer, which enable the microorganisms to degrade the non soluble substrate efficiently. Fungi are a rich source of oxidative enzymes and have the ability to survive in harsh environments under low nutrient and moisture conditions. In addition, they have the ability to extend hyphae that can penetrate into cracks and crevices (Sangeethadevi *et al.,* 2015). Most of the studies on the biodegradation of polyethylene are based on the biotic environment, but some studies have used axenic fungal strains amended with polyethylene. This study aims to isolate and identify the potential indigenous polyethylene degrading fungal strains without any pre-treatment and also attempts to understand the degradation ability of two fungal strains, *A. flavus* STR1 and *Oidium Sp.* STR2.

Materials and Methods

Sample Collection

Partially degraded polyethylene along with soil samples, adhering and adjacent to it were collected from eight plastic waste dumped site of Thanjavur, Tamil Nadu, India.

Enrichment of Sample

The enrichment of polyethylene degrading fungi was done by adding 1 g of soil sample and I g of untreated polyethylene films (cut in to pieces) in 100 ml of mineral salt medium (Imam *et al.,* 1999).

Polyethylene Substrate

Commercially available HDPE (50 microns) materials purchased from the local market, Thanjavur, Tamil Nadu, India were used as substrate for the study. These materials act as the grade of environmental pollution rather than pure polyethylene, as the composition of the commercially available HDPE varies from pure one by addition of additives and colourants. The HDPE films were cut into small strips, sterilized with 70% ethanol and dried in sterile condition.

Isolation of HDPE degrading fungi

The isolation was performed according to the method of Sivan *et al.,* (2006). 10 g of the collected soil sample along with the partially degraded polyethylene was inoculated in 100 ml synthetic media (SM) containing (per liter of distilled water): NH4NO3_1.0, MgSO4_7H2O – 0.2, K2HPO4 – 1.0, CaCl2_2H2O – 0.1, KCl – 0.15, yeast extract – 0.1 (g/l) and micronutrients FeSO4_6H2O, ZnSO4_7H2O and MnSO4, 1.0 mg^{-1}/l of each and incubated at 30º C with shaking at 150 rpm min^{-1}. After incubation the heterogeneous fungi were isolated by spread plate technique and purified cultures were maintained in potato dextrose agar (PDA) slants at ambient temperature and frequently revived to sustain their viability.

Morphological identification of fungal isolates

The fungal isolates were identified based on morphological characteristics. The macro-morphology was determined with the aid of the naked eye while the micro morphology was determined microscopically. A drop of lactophenol cotton blue stain was placed on a clean glass slide. A small portion of fungus was removed from a culture plate by bent nichrome wire under sterilized condition. The fungal culture is placed on the lactophenol cotton blue drop and covered with cover slip. The slides were observed under the microscope and the fungal species were identified using the manual of soil fungi (Gillman, 1957).

Screening of HDPE degrading fungi

The isolated fungal strains were screened for HDPE degradation efficiency according to the method followed by Gilan *et al.,* 2004. Each fungal isolate was grown in SM amended with HDPE as sole carbon source. Pre weighed disinfected untreated HDPE films were added aseptically to the flask containing 50 ml of SM. The isolated fungal strains were inoculated and incubated at 30ºC for one month. The media without the fungal inoculum served as control. After 30 days of incubation, the HDPE samples were removed subsequently, washed, dried at 60º C and weighed.

Analysis of HDPE Degradation

The effectiveness of HDPE biodegradation b fungal isolates were determined by the changes in weight loss, viscosity and spectrometric evaluation of biomass.

Determination of weight loss of polyethylene

The Residual polyethylene particles were recovered from the broth cultures (fungal) by passing through a coarse filter paper. To facilitate accurate measurement of the residual polyethylene, the fugal adhering to the polyethylene surface was washed with 2% (v/v) aqueous sodium dodecyl sulphate (SDS) solution for 2-3 hours and then with distilled water (Hadad *et al*., 2005). The washed polyethylene strips was then dried in an oven at $60^0 \pm 2^oC$ to observe a constant weight. The dry weight of recovered polyethylene indicated the rate of biodegradation.

The weight loss was calculated using the following formula:

Percentage of weight loss = [(Final weight - Initial weight) / Original weight] X 100.

Determination of change in Viscosity

The films (0.2±0.02g) were heated to high temperature of 80ºC next with 70ml concentrated naphthalene until complete dissolution. This solution is equally brated to

ambient temperature and filtered into Oswald viscometer. The flow time of each solution in viscometer was compared to pure solvent to calculate the relative viscosity of the thin films. The HDPE thin film was washed with deionised water at 50ºC for 24hrs. Then it was subjected to 50% humidity for 40 hours and tested.

Spectrometric determination of fungal biomass

The growth of the fungal strains in the liquid media due to the presence of HDPE film was determined according to the method adopted by Banerjee *et al.,* (1993). During degradation study, the liquid cultures were withdrawn at weekly intervals during one month of incubation. The turbidity of the liquid medium was measured by detecting its absorbance in UV–Visible spectrophotometer at 450 nm against a blank of uninoculated sterile medium.

Results and Discussion

High Density Polyethylene (HDPE) degrading fungal strains were isolated from the partially degraded polyethylene wastes along with adhered soil samples collected from the garbage dumping sites of Thanjavur by enrichment technique. The Fig. 1 points up the fungal colonies in plate culture and total fungal colony forming units (CFU/ml) from the enrichment technique. The enrichment technique gave rise to a mixture of heterogenous fungus capable of growing in liquid synthetic medium (SM) containing HDPE as a sole carbon source.

Among a number of fungal populations obtained from the fungal mixtures, two isolates designated as STR1 and STR2 exhibited the good growth and showed increased biofilm formation over the HDPE surface. The fungal isolates were screened for HDPE degradation efficiency in SM supplemented with HDPE as sole carbon. During degradation study, the fungal isolates were colonized over the HDPE surface within a few days.

The fungal isolates were identified macroscopically and microscopically and the fungal species were identified as *Aspergillus flavus* and *Oidium sp.* respectively (Fig. 2). The two potential strains were subjected to degradation study in an interval of 60 days, 90 days and 120 days of incubation. The HDPE degradation was monitored by weight loss. Our isolates showed better HDPE biodegradation ability than the previously reported work. The weight loss observed by the fungal isolates STR1 and STR2 was depicted in Fig. 3.

The percentage of weight loss on long time exposure to fungal isolates STR1 and STR2 was shown in Fig. 4. El-Shafei *et al.,* (1998) investigated the ability of fungi and Streptomyces strains to attack disposed polyethylene bags, and isolated eight different Streptomyces strains and two fungi *M. rouxii* NRRL 1835 and A. flavus from sewage sludge. Seneviratne *et al.,* (2006) reported the degradation ability of HDPE by *Penicillium frequentans* and *Bacillus mycoides* through biofilm formation. *A. flavus,* isolated from sanitary landfills was also found to be capable of degrading polyethylene (Mendez *et al.,* 2007). Most of the previously reported work in Aspergillus spp. like *Aspergillus terreus* (Balasubramanian *et al.,* 2014), A. niger (Volke *et al.,* 2001), *Aspergillus cremeus, Aspergillus ornatus, Aspergillus glaucus, Aspergillus candidus, Aspergillus nidulans* (Konduri *et al.,* 2010) and *Aspergillus oryzae* (Indumathi *et al.,* 2016) suggested the pretreatment of HDPE. Hence, the potential HDPE degraders, *A. flavus* STR1 and *Oidium Sp.* STR2 had shown up highest degradation rate without any pre-treatment (Fig. 3 & 4).

Fig.1 Fungal isolates in plate culture isolated from different soil samples

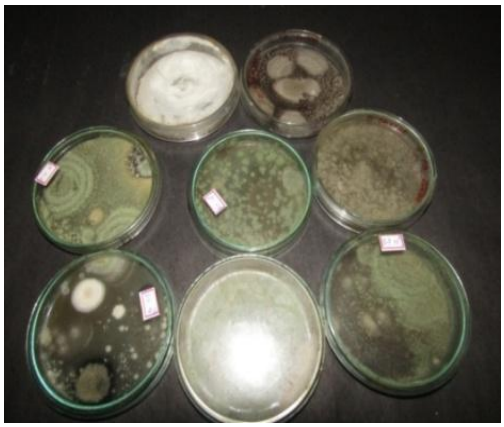
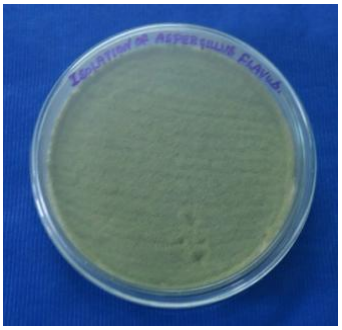


Fig.2 Macromorphology of HDPE-degrading fungal isolates



Aspergillus flavus

Oidium Sp. STR 2

Fig.3 Weight loss of HDPE films on exposure to fungus

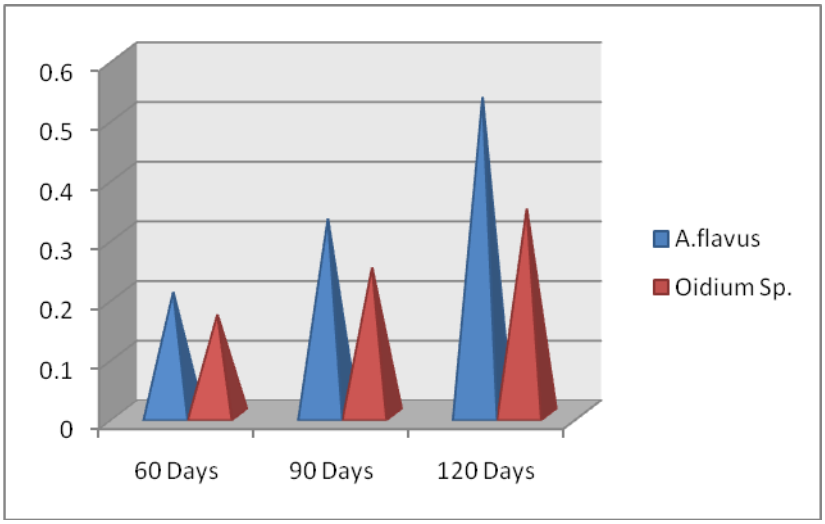


Fig.4 Percentage weight loss of HDPE films due to degradation

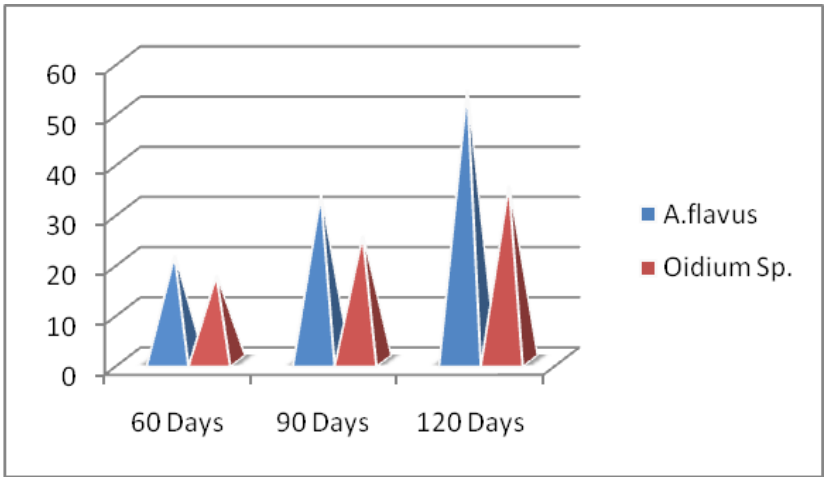


Fig.5 Change in viscosity during degradation of HDPE films

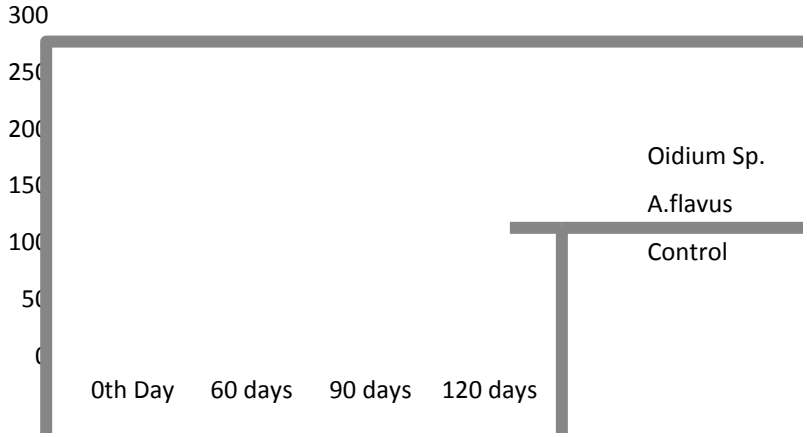
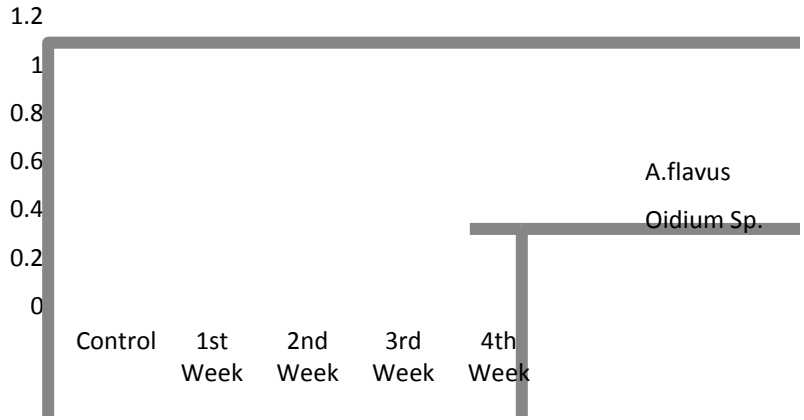


Fig.6 Determination of turbidity due to fungal biomass



The viscosity of the media after being inoculated with fungal strains was measured during the degradation. The change in viscosity was depicted in Fig. 5. The turbidity of the SM increased with time on being inoculated with the fungal strains was measured at 450 nm (Fig. 6). The fungal isolates that survived in the SM supplemented with HDPE as the only sole carbon source should possess some mechanism in degrading the HDPE to access its carbon content. The increase in turbidity and the displayed colloidal suspensions were observed within one week of incubation. But in control set up, the SM supplemented with HDPE did not show any turbidity. The slow increase of the turbidity could be due to the formation of biofilms and the residues occurred during the biodegradation process (Chatterjee *et al.*, 2010). When compared to *Oidium Sp.* STR2, the turbidity was observed higher in *A. flavus* STR1. The increase in turbidity in the consecutive weeks indicates that the enhanced growth and biofilm formation by the fungal strains. The study further substantiated that the fungal isolates *Aspergillus flavus* STR1 and *Oidium* Sp. STR2 were considered for their ability to form hydrophobic proteins that can attach to the polymer.

In conclusion the HDPE have become as a key material of basic needs and an integral part in our daily life. Random littering of unskilled recycling and non-biodegradability of HDPE pose several environmental issues. In general, biodegradation of HDPE by microorganisms and enzymes seems to be ecofriendly and the most effective process. The current study demonstrates that the degradation of HDPE film by two fungi namely, *A. flavus* STR1 and *Oidium Sp.* STR2 had shown up remarkable degradation rate without any pre-treatment. Among these two strains, the colonization, biofilm formation and biodegradation of HDPE film by A. flavus STR1 was higher than *Oidium Sp.* STR2. It is evident that both the fungal strains release the extracellular enzymes to degrade the HDPE film, but the detailed characterization of these enzymes is still needed to be carried out. The experimental results demonstrated the degradation ability of the fungal strains under in vitro condition and also provide a feasible solution to the environmental threat created by the littering and dumping of HDPE polymer. Hence, further study will be focused in the field of functional group analysis due to degradation, genomics and proteomics, which could speed up the rate of degradation.

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