

Microbial deterioration of high-density polyethylene by selected microorganisms

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ABSTRACT

Plastics either natural or synthetic represent the class of polymeric substance having excessive use in all the sectors of the industrialization. The accumulation of disposed plastics leads a serious threat to the environment. This environmental threat can be reduced or eliminated by developing and using biodegradable plastics, which can be degraded by microorganisms. High-density polyethylene (HDPE) is one of the polymers that are nearly impossible to be degraded fully till date. The present study is focused on evaluating the degradation ability of HDPE and presented in two phases (i) Isolation and identification of polyethylene degrading microorganisms, and (ii) Biodegradation of polyethylene. Out of 16 fungal isolates, three fungal strains were screened from soil dump and these fungal isolates were identified as *Aspergillus fumigatus*, *A. flavus*, and *Fusarium* sp. Efficiency of these microbes in polymer degradation was analyzed by percent weight reduction during a time period of 90 days at 28°C. *Fusarium* sp. was found to provide the maximum percentage degradation of 2.65% in 60 days. Surface deformities were visualized by scanning electron microscope (SEM). The results of the present study are useful as all the isolates have shown significant biodegradation.

1. INTRODUCTION

In today's lifestyle, plastic has gained its use in almost all of the household products such as toothbrush, kitchen utensils, and water bottles. The huge number of plastic bottles has become an environmental issue that needs to be addressed. They are the major cause of earth pollution. The aggregation of plastic products and their disposal has become a global issue for which every country is concerned now. The world organizations have banned the use of single-use plastics as they are very harmful for the environment [1]. Not only the environment but also the animals and birds are facing harmful effects of plastic aggregation. It is seen that the birds and animals eat the plastic unknowingly which later on becomes the cause of their death. Biodegradable plastics are emerging and taking place of single-use plastics. Plastics (compared to any other segment of solid waste) have gained the focus of media and health organizations because of their durability in the litter. It's durability in the soil affects the plant life which includes plants and vegetables.

Polymers are an extensive collection of materials which are prepared by repeating units of smaller molecules called monomers. Among all the polymers, plastic creates a wide range of waste. Commodity plastics are produced from different polymers such as polyethylene, polypropylene,

polystyrene, polyvinylchloride, polyurethane, polyethylene terephthalate, and nylon [2]. Polyethylene can be divided into numerous diverse forms such as low-density polyethylene (LDPE), linear low-density polyethylene (LLDPE), and high-density polyethylene (HDPE). HDPE has a stronger force between the molecules and strong tensile strength because of low degree of branching. A suitable choice of reaction conditions ensures the low degree of branching. HDPE is widely used as a packaging material in margarine tubs, detergent bottles, milk jugs, garbage containers, etc. Nearly half of all toys and vehicle parts are made up of HDPE.

Under suitable environmental conditions, organic constituents are transformed into simple compounds with the help of microorganisms. This process is known as biodegradation. Microorganisms stimulate the process of biodegradation with the help of enzymes which are released during catabolic reactions. Literature [3-6] has reported that synthetic and natural polymers are capable of hydrolyzing these polymers as a carbon source.

The present study focuses on isolation of prospective fungal isolates from a soil sample which are capable of degrading the HDPE. Soil samples were collected from the polyethylene dumping sites. The amount of polyethylene degradation was determined using analytical methods.

2. MATERIALS AND METHODS

The present topic can be explained in two phases: (i) Isolation and identification of polyethylene degrading organisms and (ii) biodegradation of polyethylene.

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2.1. Isolation and Identification of Polyethylene Degrading Organisms

2.1.1. Soil sample collection

Soil samples were collected from the different dumping sites of Hardwar district. The chosen dumping sites have been used to dump the plastic for a long time. Plastic degrading organisms were expected to be present at these sites. The samples were collected in sampling sterile polythene bags. These samples were then carefully transported to the laboratory and stored at a temperature of 4°C.

2.1.2. Test sample preparation

The commercially used plastic water bottles (HDPE) were selected to be the test samples for the study as they are dumped large in numbers. The water bottles were purchased from the local market and cut into square pieces of 2 cm length. These HDPE strips were washed manually with absolute ethanol using cotton wool and rinsed severally with distilled water to remove dirt and other organic particles adhering to the surface and then allowed to dry in air for 30 min. The stripes were then dipped into the solvent (xylene) which is heated at a temperature of 140°C and stirred continuously. The resulted mushy mixture was crushed and filtered with the help of a white cloth and then rinsed severally with acetone to remove the solvent. The acetone got evaporated and the leftover was the powdered HDPE. The HDPE powder was stored in closed containers at room temperature [4-5].

2.1.3. Isolation and enrichment

From the soil samples (Section 2.1.1.), fungal colonies were isolated on Czapek-Dox Agar (CDA) using pour plate method and serial dilution method. The plates obtained were incubated at 28°C for 3–4 days. Carefully chosen fungi from CDA plates were subcultured for storage and further characterization.

2.1.4. Screening of fungus

Polyethylene powder was contained in a minimal salt medium and acts as a sole carbon source. Isolated fungus was inoculated on minimal salt medium at 28°C for 4 weeks, thereafter, the colony diameter was measured [5-6].

2.1.5. Identification of selected fungi

Selected fungi were identified by colony morphology and staining. Morph taxonomical identification was performed at NFCCI, Agharkar Research Institute, Pune, India.

2.2. Biodegradation of Polyethylene

2.2.1. Treatment of HDPE

The following three pretreatment strategies were employed for the present study.

- (i) HDPE films were treated at 80°C thermally in a hot air oven for 120 h to persuade oxidation.
- (ii) HDPE films were exposed to UV light (UVC > 300 nm wavelength) for 10 days.
- (iii) HDPE films were suspended in concentrated nitric acid (HNO₃) for 10 days to enhance percent elongation.

The treated as well as non-treated samples of HDPE films were used for isolation of HDPE degrading strains [7,8].

2.2.2. Biodegradation study using shake flask method

The pre-weighted treated as well as untreated HDPE strips were aseptically shifted into the conical flask containing 150 ml of minimal salt medium and then inoculated with selected fungus. Control was maintained with HDPE strips in the sterile medium.

Experiments were performed with three sets of flasks and with three isolates, as shown in Table 1. The three sets of flasks were maintained at a temperature of 28°C and shaken at 120 rpm. After every 30 days of incubation, the plastic discs were collected, washed thoroughly using distilled water, dried in hot air oven at 50°C overnight, and then weighed for residual polyethylene (Swomya *et al.*, 2012). The percentage degradation of polyethylene was calculated by the following formula:

$$\text{Percentage degradation} = (W_0 - W_t) / W_0 \times 100 \quad (1)$$

Where

W_0 = Initial weight of polyethylene

W_t = Final weight of residual polyethylene

2.2.3. Field emission scanning electron microscopic (FE-SEM)

The pieces of HDPE (after incubation with fungal cultures) were taken out from the culture and repeatedly rinsed with distilled water. The fungal mycelium was removed carefully and the polymer film was dried at room temperature. FESEM was carried out to study the surface morphology and structural changes in HDPE film before and after biotic exposure, following the biodegradation in shake flask method. The images of treated as well as untreated test samples were captured and compared. FESEM provides evidence of physical deterioration of the polymer surface caused by the microorganisms.

3. RESULTS AND DISCUSSION

From the serial dilution technique, 16 fungi were isolated and out of these, three fungi were screened (on the basis of colony diameter) as polyethylene degrader and have shown growth on minimal salt medium. The three fungi screened were *Fusarium* sp. (91.44 mm colony diameter), *Aspergillus fumigatus* (78.74 mm colony diameter), and *A. flavus* (80 mm colony diameter). The isolated strains identified were *Aspergillus fumigatus*, *A. flavus*, and *Fusarium* sp. [Figure 1]. These fungal isolates were subjected to degradation of polyethylene by shake flask method. Dry weight of leftover polyethylene was calculated after 30, 60, and 90 days. The amount of degradation performed by three fungal isolates is shown in Table 1. It was observed that maximum degradation is taking place in case of treated HDPE. The literature on degradation of *Aspergillus* spp. [5,6,8-13] like *Aspergillus terreus*, *A. niger*, *Aspergillus cremeus*, *Aspergillus oryzae*, *A. flavus*, *Aspergillus nidulans*, *Aspergillus candidus*, *Aspergillus glaucus*, and *Aspergillus ornatus* has suggested the pretreatment of HDPE. The present study has also observed a significant amount of degradation of treated HDPE compared to non-treated HDPE. Maximum degradation in case of treated HDPE was found by *Fusarium solani*, that is, 2.65% in 60 days and 2.58% in 90 days.

The degraded HDPE strips were examined under scanning electron microscope (SEM) to analyze surface deformities [Figure 2]. It can be observed from Figure 2 that surface deformities and cracks are present

Table 1: Percentage degradation of treated and non-treated HDPE by dry weight of polyethylene

Isolates	30 days		60 days		90 days	
	TP*	NTP*	TP	NTP	TP	NTP
<i>A. flavus</i>	0.19	0.56	0.61	1.47	2.12	1.43
<i>A. fumigatus</i>	0.55	0.43	1.29	0.57	1.38	1.31
<i>Fusarium solani</i>	1.43	0.91	2.65	1.69	2.58	1.84
Control	0.002	0.001	0.003	0.001	0.002	0.001

*TP: Treated polyethylene, NTP: Non-treated polyethylene

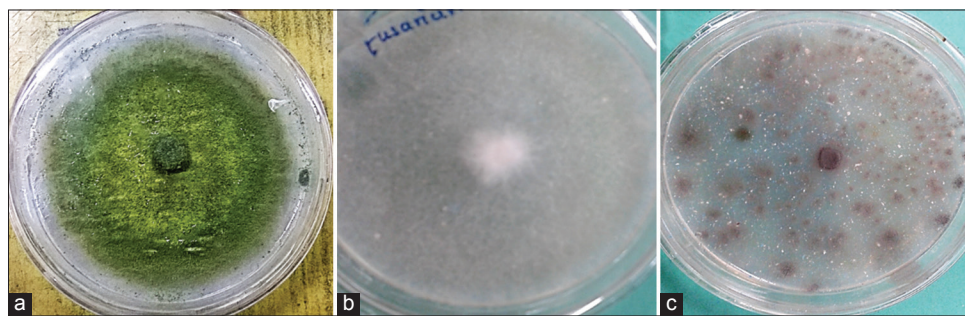


Figure 1: Pure culture of fungal isolates (a) *A. flavus*, (b) *Fusarium* sp., (c) *A. fumigatus*

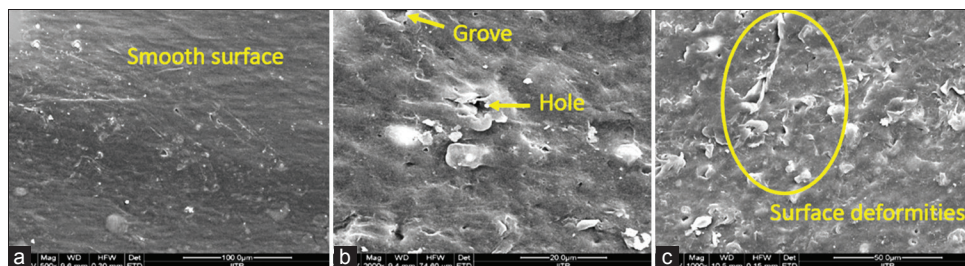


Figure 2: SEM analysis of HDPE surface deformities (a) Control, (b) treated HDPE, and (c) non-treated polyethylene

on the surface of degraded polyethylene. The surface deformities and cracks are due to enzyme activities that take place on the surface of the polyethylene under degradation. It is already proven [3-6] that extracellular enzymes are secreted by the fungal strains and degrade the HDPE film. However, the complete description of extracellular enzymes is still unknown and yet to be carried out.

4. CONCLUSION

The most effective process of HDPE degradation is through microorganisms and enzymes. The experimental results of the current study confirm the degrading ability of the polyethylene under *in vitro* conditions and suggest a possible clarification to the ecological hazard posed by the polyethylene. The following conclusions can be drawn from the present study.

- (i) *A. fumigatus*, *A. flavus*, and *Fusarium solani* are identified as the most significant isolates for degrading the HDPE.
- (ii) Treated HDPE degrades faster than the non-treated HDPE.
- (iii) *Fusarium solani* degrades the HDPE faster than any other identified isolates.
- (iv) Due to enzyme activities, surface deterioration and cracking take place on the surface of HDPE under degradation. These deteriorations increase with time and degrade the polymer.

5. CONFLICT OF INTEREST

Authors declared that they do not have any conflicts of interest.

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None.

REFERENCES

1. Vinhas GM, Almeida YM, Lima MA, Santos AS. Study of the properties and biodegradability of polyester/starch blends subjected to microbial attack. *New Chem* 2007;30:1584-8.
2. Bonhomme S, Cuer A, Delort AM, Lemmaire J, Sanceime M. Environmental biodegradation of polyethylene. *Polym Degrad Stab*

- 2003;81:441-52.
3. Gu JD. Microbiological deterioration and degradation of synthetic polymeric materials: Recent research advances. *Int Biodeterior Biodegradation* 2003;52:69-91.
4. Das MP, Kumar S. An approach to low-density polyethylene biodegradation by *Bacillus amyloliquefaciens*. *Biotech* 2015;5:81-6.
5. Rani A, Singh P. Screening of polyethylene degrading fungi from polyethylene dump site. *Int J Chem Tech Res* 2017;10:699-704.
6. Sowmya HV, Ramalingappa M, Krishnappa M. Degradation of polyethylene by *Chaetomium* sp. and *Aspergillus flavus*. *Int J Rec Sci Res* 2012;3:513-7.
7. Artham T, Sudhakar M, Doble M, Umadevi VR, Viduthalai RR, Kumar KS, et al. Effect of biofouling on stability of polycarbonate in tropical seawater. *Open Macromol J* 2008;2:43-53.
8. Mahalakshmi V, Andrew SN. Assessment of physicochemically treated plastic by fungi. *Ann Biol Res* 2012;3:4374-81.
9. El-Shafei HA, El-Nasser NH, Kansoh AL, Ali AM. Biodegradation of disposable polyethylene by fungi and *Streptomyces* species. *Polym Degrad Stab* 1998;62:361-5.
10. Mendez MF, Shapira JS, McMurtry A, Licht E, Miller BL. Accuracy of the clinical evaluation for frontotemporal dementia. *Arch Neurol* 2007;64:830-5.
11. Yamada-Onodera K, Mukumoto H, Katsuyaya Y, Saiganji A, Tani Y. Degradation of polyethylene by a fungus, *Penicillium simplicissimum* YK. *Polym Degrad Stab* 2001;72:323-7.
12. Balasubramanian V, Natarajan K, Rajeshkannan V, Perumal P. Enhancement of *in vitro* high-density polyethylene (HDPE) degradation by physical, chemical, and biological treatments. *Environ Sci Poll Res* 2014;21:12549-62.
13. Volke-Sepulveda T, Saucedo-Castaneda G, Gutierrez-Rojas M, Manzur A, Favela-Torres E. Thermally treated low density polyethylene biodegradation by *Penicillium pinophilum* and *Aspergillus niger*. *J Appl Polym Sci* 2002;83:305-14.

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