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BIODEGRADABILITY OF POLYETHYLENE BY *ASPERGILLUS NIGER*

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ABSTRACT

The work presented here for the degradation of LDPE and HDPE by the fungal isolate. Isolation was done by the serial dilution and pour plate method. Four soil samples were collected from different polyethylene dumping sites. Fungus were isolated on Czapek Dox Agar (CDA) medium and screen on minimal salt medium (MSM) supplemented with polyethylene powder by colony diameter method that was 39 mm in diameter. Main dominating fungus was identified as *Aspergillus niger*. Degradation was performed by shake flask method. Polyethylene weight was measured in every 15 days which is increase with time. Degradation in HDPE was minimum (0.217±09) within 15 days and maximum (1.116±54) within 60 days whereas for LDPE it was minimum (1.013±47) within 15 days and maximum (3.389±05) within 60 days respectively. Therefore it can be speculated that *Aspergillus niger* has enough potential to degrade polyethylene with due course of time.

KEYWORDS: *Aspergillus niger*, HDPE, LDPE, biodegradation.

INTRODUCTION

Polyethylene is one of the most valuable synthetic non biodegradable polymers made up of elements that is a thermoplastic polymer made from repeating units of ethylene. Degradation of polyethylene is of great challenge as the materials are increasingly used. Microorganisms biologically transform organic complex polymer to simpler one and utilized it as a carbon source. The microorganisms secrete several enzymes in different quantities, which expressed its degradation efficiency of the microorganism.[1] Polyethylene contains the chemical elements carbon and hydrogen. In terms of degrading

ability of polyester polyurethanes a number of microorganisms, principally fungi, have been characterized.[2] Microorganisms play a significant role in the decomposition of material. In most studies, fungi have been investigated for the biodegradation of PE because these organisms produce degrading enzymes[3] and, extracellular polymers (such as polysaccharides) facilitate to colonise the polymer surface,[4] and the ability of distribution and penetration of the fungal hyphae is an advantage. Some studies have investigated the PE biodegradation process using fungal isolates, such as *Phanerochaete chrysosporium*[5] *Aspergillus niger*[4,6] and other strains of the *Aspergillus* genus including *A. flavus*, *A. terreus,* [7] and *A. fumigatus*.[8]

Some of the currently available laboratory studies are rather optimistic and suggest that under optimal conditions; previously highly oxidized PE can be transformed to CO from more than 50% during about one year.[9] Other published experiments suggest that the process could be slower, but even the biodegradation of some fraction of the material is evidenced.[10] Methods used in PE biodegradation studies span from ones concerning the testing of mechanical properties, as well as those employed in the field of solid phase physics and analytical chemistry, to microbiology and biochemistry methods. The degradation was considered to be initiated by hydrolysis of ester bonds by some hydrolytic enzymes, such as esterases.[11] Production of plastics has grown up significantly in the last 30 years averaging an annual growth rate of 10%. A general estimate of worldwide plastic waste generation is annually about 57 million tons.[12]

In this study, selected fungus was isolated from polyethylene dump site and main dominating fungus was identified as *Aspergillus niger*. The ability of these isolate to degrade polyethylene strip in soil was investigated.

MATERIAL AND METHOD

Collection of test sample

Commonly used polythene bag and plastic bottles were collected from local market in Haridwar, Uttarakhand, India and used for degradation studies.

Soil Sample Collection

The soil sample was collected from polythene accumulated, domestic waste dumping site. The soil samples were collected at a depth of 3-5cm, in a sterile container.

Isolation and Identification of Polyethylene Degrading Fungus

1 mg of soil sample was taken into a test tube containing 10 ml of sterile distilled water. This content was vortexed and serially diluted up to[13] and pour in sterile CDA plated to isolate heterotrophic fungi. The plates were incubated at 28 ± 2°C for 5days. Fungus was identified on the basis of colony characteristic, microscopic view Lacto-phenol cotton blue was used to stain the fungi for microscopic view and for the spore's arrangement.

Preparation of Polyethylene powder

Polyethylene sheets were cut in to small bits and immersed in xylene and boiled for 15 min. xylene dissolve the polyethylene and the residue was crushed while it was warm with the help of pestle and mortar. The polyethylene powder so obtained was washed with ethanol to remove residual xylene and allowed to evaporate (approx 2-3 hr) to remove ethanol. The powder was dried at room temperature and stored in closed containers in room temperature.[14,15]

Minimal salt media (MSM)

Polyethylene powder was added in mineral salt medium (MSM) containing (g/l of distilled water): NH_4NO_3, 1.0; $MgSO_4.7H_2O$, 0.2; K_2HPO_4, 1.0; $CaCl_2.2H_2O$, 0.1; KCl, 0.15; and yeast extract, 0.1; and 1.0 mg/l of each of the following micro-elements: $FeSO_4.6H_2O$, $ZnSO_4.7H_2O$ and MnSO4; Polyethylene powder was added in mineral salt medium at a final concentration of 0.1% (w/v) respectively.[16] Medium was sterilized at 121°C and pressure for 15 lbs for 20 minutes. About 15 ml sterilized medium was poured before cooling in each plate.

Screening of Polyethylene degrading fungus

The isolated organisms were inoculated on polymer containing agar (MSM) plates and then incubated at 28 ± 2°C for 2-4 weeks.[17] The organisms, showing growth on the MSM supplement with polyethylene were selected for further analysis. Measure the colony diameter of organism on MSM.

Dry weight determination

Dry weight determination was done by shake flask method. Identified fungal sp was inoculated in 50 ml minimal salt medium containing LDPE and HDPE (collected from local market of Haridwar) as only carbon source. All flasks were incubated at 28^{o}C at 110 rpm for two months. The LDPE and HDPE pieces were recovered after 15, 30, 45 and 60 days of

incubation from culture medium and washed with distilled water and dried it at room temperature. Degradation was monitored by measuring the weight of LDPE and HDPE before and after incubation.

Weight loss=initial weight- final weight

%Weight loss = initial weight- final weight/initial weight ×100

RESULT

Four soil samples were collected from different dump sites different fungal isolates were isolated. Out of total 15 isolated fungi *Aspergillus niger* identified as most dominating fungus. This fungus was screened on the basis of colony diameter that was 39 mm on the minimal salt medium supplemented by polyethylene powder. Degradation was observed on the basis of weight loss of the HDPE and LDPE which was for HDPE 0.217±09, 0.718±27, 0.691±22 and 1.116±54 and for LDPE 1.013±47, 2.198±31, 3.215±86 and 3.389±05 in 15, 30,45and 60 days respectively.

Table 1: weight loss of LDPE and HDPE strips used as a sole carbon source in shaking liquid culture, indicating the activity of the *Aspergillus niger*. (average of triplicates ± SD)

Days	PE type	Initial weight (gm)	Final weight (gm)	Weight loss (gm)	%degradation	Dry weight of fungus (gm)
15	HDPE	0.459	0.458	0.001±0.00	0.217±0.09	0.212±0.32
	LDPE	0.296	0.284	0.003±0.001	1.013±0.47	0.292±0.29
	Control	0.245	0.245	0.000	0.000	0.00
30	HDPE	0.418	0.415	0.003±0.001	0.718±0.27	0.221±0.11
	LDPE	0.273	0.267	0.007±0.001	2.198±0.31	0.227±0.22
	Control	0.282	0.282	0.000	0.000	0.00
45	HDPE	0.434	0.431	0.003±0.001	0.691±0.22	0.170±0.23
	LDPE	0.311	0.301	0.010±0.001	3.215±0.86	0.262±0.41
	Control	0.339	0.339	0.000	0.000	0.00
60	HDPE	0.448	0.443	0.005±0.001	1.116±0.54	0.459±0.21
	LDPE	0.413	0.399	0.013±0.004	3.389±0.05	0.301±0.26
	control	0.332	0.332	0.00	0.00	0.00

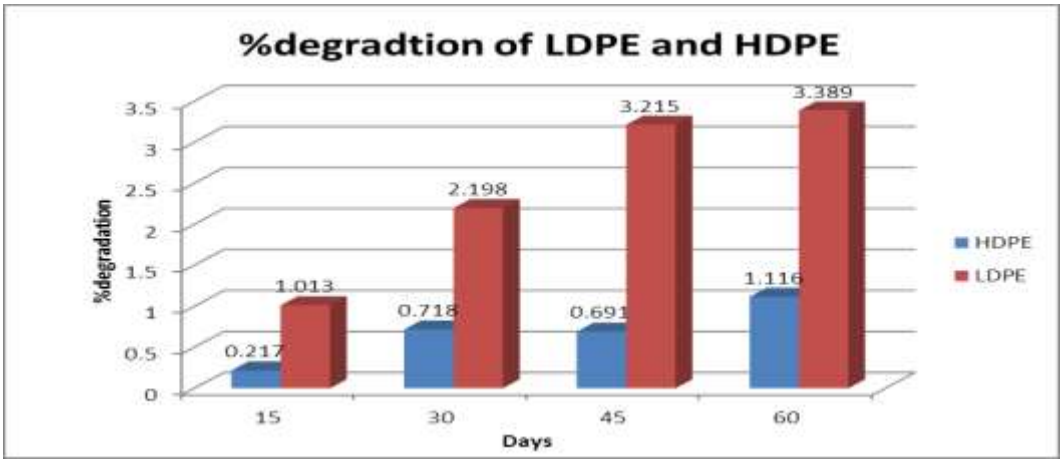

Fig: Showing % degradation of LDPE and HDPE by *Aspergillus niger*.

DISCUSSION

The present study deals with the isolation of polyethylene degrading fungi obtained from the different dumping soil and to test their ability for polyethylene degradation in laboratory condition. The findings of present investigation showed that 4% degradation occurred by *Aspergillus niger* in 60 days similar work were performed by several scientist among which Singh and Gupta (2014) isolated and identified the *Aspergillus niger* from the polyethylene polluted site and found 20% degradation by the *Aspergillus niger*. The carbon dioxide measurements showed that the biodegradation in the uninoculated treatments were slow and were about 7.6% of mineralisation for the non-UV-irradiated LDPE after 126 days. Kannahi and Sudha (2013) find 11.01±0.51 µg weight loss in 55 days by *Aspergillus niger*. According to Volke-sepulveda (2002), Mineralization of LDPE 0.57% for *A. niger* was obtained, for samples without ethanol, respectively. A model to explain morphological and structural changes on biologically treated LDPE is also proposed.

CONCLUSION

Soil contain microorganism that are able to bring about degradation of synthetic polymers. The fungal isolate showing growth on the polymer containg medium indicated their ability to utilize polyethylene as a source of nutrient (carbon). In the present study the isolation, screening and identification of *Aspergillus niger*, as LDPE and HDPE degradative fungi, from different polluted sites were focused. The fungal isolate was responsible for the decreasing weight of LDPE and HDPE by adhering on their surface and utilizing it as the only carbon and energy source which was evident by increasing in the fungal growth. This knowledge can be used as a valuable application to solve the polyethylene waste problems using a microbial tool.

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