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Microbial Approach To Minimise The Polythene Waste

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ABSTRACT
Polythene bags consist of long chain of ethylene monomers. Ethylene is derived from natural gas and petroleum. Polythenes are stable, light weight, imperviousness to water, durable and low cost product. These properties make this synthetic polymer valuable in domestic, industrial, commercial and environmental applications. These endless applications of polythene have subsequently resulted in the generation of large quantities of waste, leading to their dumping in the environment. The problems caused by polythene waste are mainly due to its persistent nature. Now polythene waste pollution has become a hassle to the human beings and environment. A number of physical and chemical methods are used to reduce the polythene waste, but both physical and chemical methods release toxic gases and secondary pollutant. Biodegradation of polythene carry bags is eco friendly technology that uses microorganism to degrade the plastic and polythene. Biodegradation is an attractive option for efficient disposal of plastic. In present work fungal species were isolated from municipal landfill soil of Agra, and screened by using polythene powder to confirm the degradation of HDPE. Single fungal strain was screened. The microbial degradation of HDPE polythene was analyzed by weight loss, SEM and FTIR. Degradation of polythene was investigated by soil burial and shake flask method in laboratory. After biodegradation surface of plastic material turned from smooth to rough and the weight of polythene strip was also reduced due to the fungal activity. Microbial approach is a promising alternative to current practices for waste disposal and minimizing polythene waste, as it is a low cost process.
Keywords*: Strains, Biodegradation, Shake flask, Synthetic medium*

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INTRODUCTION

Every year, increased manufacturing of polythene and polyethylene, has made it world's most important plastic. Uses of plastic include film, packaging and container from bottles to bucket. Polythene are produce in 3 main forms that is low density polythene (LDPE), linear low density polythene (LLDPE), high density polythene (HDPE). In this study an attempt has been made to degrade the HDPE type carry bag by fungi isolated from polythene waste dumping sites of Agra. HDPE is mainly used in food packing, shopping bags, dustbins, detergent bottle, water pipe etc. HDPE is produced at relatively low pressure (10-80 atm) in the presence of a Ziegler- Natta (organic metallic catalyst) and temperature ranges between 350-420K. Plastics are chemically inert in nature and remain in the environment without any change for many years[1]. To prevent the accumulation of plastic waste in environment, researchers have presented studies to biodegrade the plastic, for its proper disposal. Several studies have reported on biodegradation of polythene by bacteria and fungi. Recent studies revealed that two fungal strains *Penicillium oxalicum* NS4 (KU559906) and *Penicillium chrysogenum* NS10 (KU559907) degrade LDPE and HDPE. Degradation was analysed by FE-SEM, AFM and FTIR[2].A thermophilic bacterium *Brevibacillus borstelensis* which degraded the LDPE polythene and revealed reduction in molecular weight of polythene by

30% was isolated in 2005[3]. Studies have shown that some bacterial strains which are capable of degrading polythene are *Streptomyces* KU5, *Streptomyces* KU1, *Streptomyces* KU6, *Streptomyces* KU8[4]. Bacterial strain *Ideonella sakaiensis 201-F6* degrade polyethylene tetraphthalate(PET) rapidly and secreted a protein identified as ISF6-4381 isolated in Japan[5].Plastic degrading fungi was isolated from soil and identified as *Fusarium solani.*This was used in degradation of both natural and synthetic polythene as a potential carbon source[6] . Some potential fungal species which have capability of polythene degradation are *Aspergillus niger. Aspergillus glaucus, Cladosporium, Fusarium, Mucor,Penicillium Phanerochaete* and *Trichoderma[7, 8]* [9, 10]

MATERIAL AND METHODS

Collection of polythene Sample: HDPE sheets of 20 micron thickness were collected from local market of Agra.

Soil sample collection, isolation and screening of polythene degrading fungi: The soil samples were collected from plastic waste dumping sites of Agra. Isolation of the fungal colonies was done by sample enrichment method[11]. Isolated fungal cultures were screened for polythene degradation on mineral salt medium. Composition of mineral salt medium is given in table1.

Table1. Synthetic medium proposed by[12].

Chemicals	gm/l
NH_4NO_3	1.0gm/l
$MgSO_4.7H_2O$	0.2g/l
K_2HPO_4	1.0g/l
KCl	0.15g/l
Agar	20g
Polythene Powder	1%g/l
Tween20	0.1%g/l

Identification of isolated fungi: Isolated fungi were identified on the basis of morphological characters like colour of septate hyphae, nature of hyphae, shape presence of special structure sporangiophore or conidiophores etc.

Preparation of fungal suspension: Fungi were cultured on SDA slants and incubated at 28˚C. After 4 to 5 days spore of fungi on slants were washed with 6ml physiological saline solution. The spore mixture was placed in a test tube and vortex for 3-4 minutes aseptically. Add 3ml of this spore mixture in known volume of distilled water. Fungal spore were counted by haemocytometer.

Experiment setup for polythene degradation:-

Soil burial treatment: Sterile soil was taken in pots and the pre-weighted polythene carry bag (HDPE) strips (4cm×3cm), disinfected with 70% ethanol for 30 minutes were placed in it. The fungal culture suspension was supplied to the soil. Care was taken to ensure that the samples were completely covered with soil. The pot was then kept at room temperature. All the experiment were run simultaneously in triplicates.

Shake flask test (in liquid synthetic medium): In this biodegradation experiment 2ml of fungal suspension was added to flask containing 150ml of liquid synthetic medium. Disinfected film (4cm×3cm) was added in flask and incubated at 30 ^{o}C in orbital shaker at 120 rpm for two month. All the experiments were run simultaneously in triplicates. Composition of Synthetic medium is shown in table 2 given by [12] with some modification.

Table: 2 Composition of liquid synthetic medium

Chemicals	gm/l
NH_4NO_3	1.0gm/l
$MgSO_4.7H_2O$	0.2g/l
K_2HPO_4	1.0g/l
KCl	0.15g/l
$FeCl_3.6H_2O$	5.0g/l
$ZnCl_2$	0.0084g/l
H_3BO_3	0.0001g/l
$CaCl_2.2H_2O$	0.001g/l
$MnCl_2.4H_2O$	0.0016g/l
Malt extract	1%

Film Collection: After incubation period of nine months in soil and two months in liquid synthetic medium HDPE polythene strips (treated and untreated) were collected and washed with running tap water. Later 70% ethanol was used to remove as much as cell mass from the residual film as possible. The washed polymer pieces were placed on a filter paper and dried overnight at room temperature before weighing. The film was dried 24 hours in oven[13].

Assessment of degradation of polythene: -

Weight Loss Method-Weight loss of polythene strip was determined by an analytical balance (SHIMADZU CORPORATION TYPE AY220). The weight difference between initial and final weight indicate the extent of polythene utilization by the fungi. Percentage weight loss was determined using the formula -

Weight Loss%=$\frac{Initial\ Weight - Final\ Weight}{Initial\ Weight}$ * 100

FTIR-Infrared spectra of polythene film were recorded on Cary 630 FTIR (Agilen technologies) over a range of 4000cm^{-1}-800cm^{-1}.

SEM- The surface morphology and microstructure of the polyethylene strip due to biodegradation were analyzed through scanning electron microscopy. The polythene film were examined by JSM 6490 LV (JEOL JAPAN)

RESULTS AND DISCUSSION

Soil sample collection ,isolation and screening of polythene degrading fungi: Sample was collected in sterile container from different waste dumping site of Agra 18 different types of fungi were isolated from the soil of different plastic waste dumping sites. Medium containing polythene powder was used to screen the polythene degrading fungi. The plates were incubated at 30^0 C for 7 to 10 days with isolated fungi. Out of 18 fungi only 1 fungi showed growth on screening medium which was selected for further degradation experiment.

Phenotypic characterization of isolated fungal species: Fungi was identified as *Aspergillus fumigatus* by their growth characteristics and colony morphology. (Table3 and fig1.)

Table3. Morphological Characteristics and Identification of Fungi isolated from the soil sample

Color of aerial hyphae	Colour of septate hyphae	Nature of hyphae	Shape	Presence of special structure	Sporangiophore or conidiophore	Characteristics of spore head Probable	Organism
Green	Brown	Septate	Oval greenish	Foot cell Present	Long erect non-Septate	Multinucleate green vesicles	*Aspergillus fumigatus*

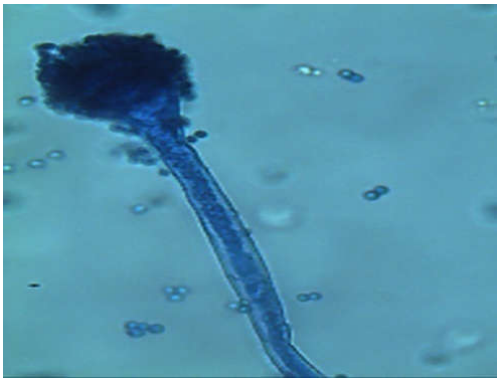


Fig1.microscopic image of A.fumigatus

Weight loss: The degradation of polythene was determined by calculating the percentage of weight loss of polythene. Film was weighed, with an accurate four digit balance before and after incubation in soil as well as in liquid synthetic medium. The percentage of weight loss is shown in table 4 and 5.

Table4. Weight reduction of polythene in soil

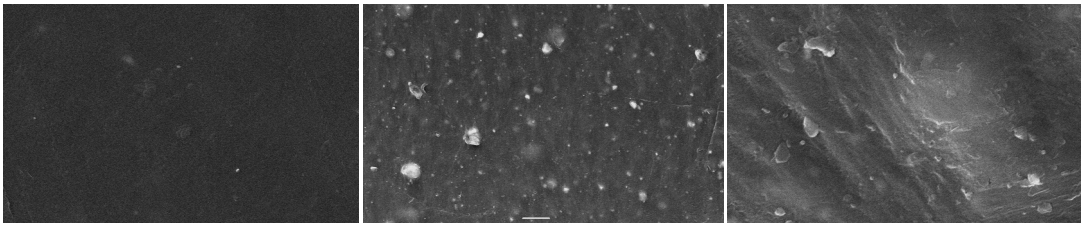
Isolated Organism	Initial weight(in mg)	Final weight(in mg)	Weight loss(in mg)	% of weight loss
A.fumigatus	60.4	33.6	26.8±0.35	59%
Control	60.4	60.4	0	0

Table5. Weight reduction of polythene in synthetic medium

Isolated Organism	Initial weight(in mg)	Final weight(in mg)	Weight loss(in mg)	% of weight loss
A.fumigatus	60.4	42.8	17.6±0.37	29.1%
Control	60.4	60.4	0	0

Weight loss of polythene was measured after 9 months in soil. The weight of HDPE was reduced from 60.4mg to 33.6mg after 9 months. Whereas total weight loss of HDPE in synthetic medium after 2 months of incubation on orbital shaker was 60.4mg to 42.8 mgby fungal isolate *A.fumigatus*. Control sample of HDPE strip did not show any reduction. The weight difference in polythene carry bag showed that the fungi was able to degrade HDPE in soil better as compared to degradation in liquid synthetic medium.

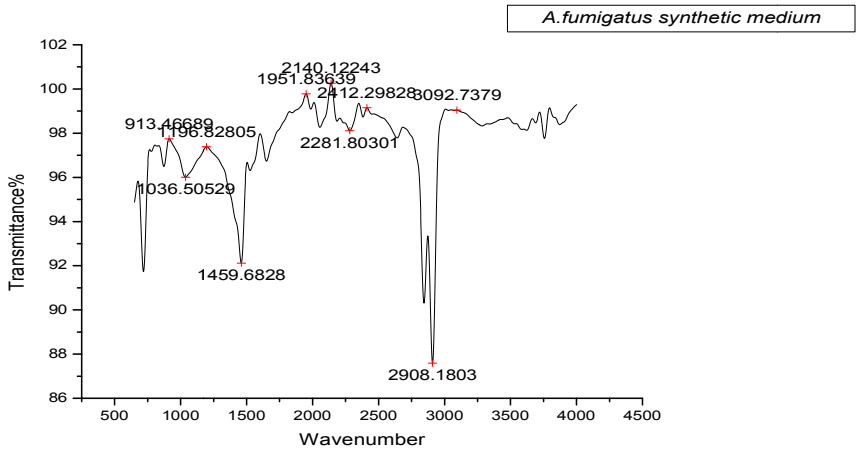
4.4 SEM Results: SEM images revealed changes in the surface morphology of polythene strip. The film treated with *A. fumigatus* in soil show more cracks and holes on polythene film than in liquid synthetic medium. Microscopic images of the HDPE samples are shown in Fig.2 Under scanning electron microscopy, the control sample appeared as a uniform homogenous sheet. After 9 months of degradation in soil, peeling, holes and exfoliation in the film surface were observed. After 2 months in synthetic medium, fractures and cracks are seen on the surface HDPE film. Longer soil exposure of polythene sample would lead to its deterioration and degradation. Loss of integrity of HDPE film network resulted after degradation. As compared to control, microbial colonies on the film appeared. SEM micrographs also showed that after biological attack, the surface was physically weak and readily disintegrated under mild pressure. In this study SEM images exhibit that, upon exposure in soil and in liquid synthetic medium, surface erosion took place and the matrix of polythene strip became perforated.

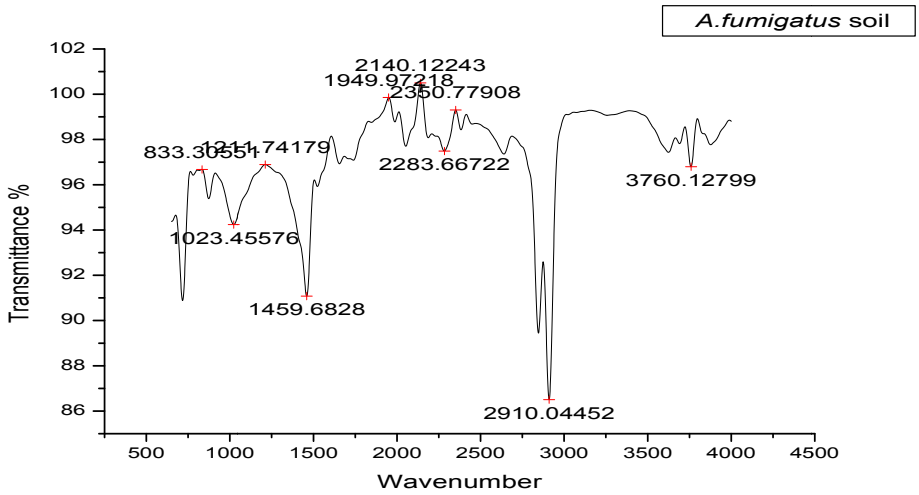


(a) (b) (c)

Fig2. Scanning Electron Micrograph of untreated and treated polythene HDPE strip by *A. fumigatus* a. control, b. synthetic medium , c. Soil (Scale 8kv X10000 1µm)

FTIR results: FTIR is an important tool for analysis of structural changes due to induced degradation in polythene. FTIR analysis gives a close view of stretching, deformation, bending and new bond formation of functional group. There are two important regions 4000-1300cm-1 and 900-650cm-1for examination of spectrum in FTIR. The high frequency portion of the spectrum is called functional group region.





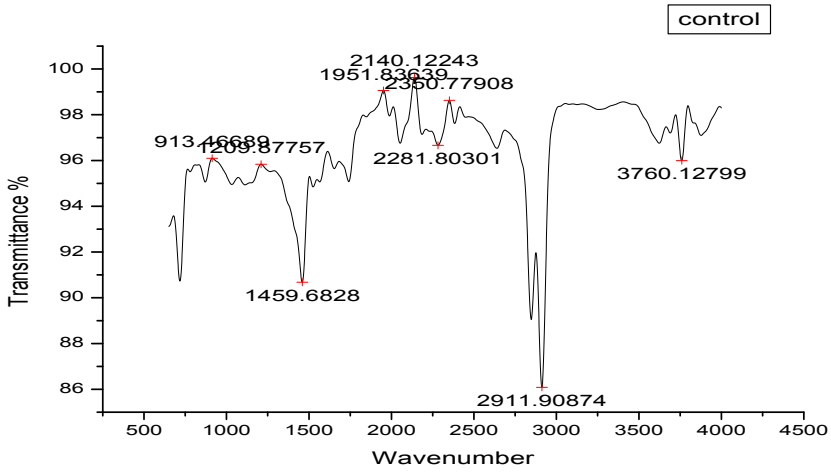


Fig3.FTIR spectra of HDPE degraded by isolated fungi in synthetic medium, soil and control

Control sample gave a peak at 1459.68 cm^{-1} which was increased after treatment with *A.fumigatus* in soil as well as in synthetic medium which show methylene C-H bend. A shift in native peak from 2919.9 cm^{-1} to 2910.04 cm^{-1} and 2908.18 cm^{-1} in soil and synthetic medium treatment respectively was observed due to stretching in asymmetric CH_2 bond. (Fig.3) Several new peaks of different functional groups were observed such as ketone, aldehyde, carboxylic acid and alcohols etc. after biodegradation of HDPE strip. New absorption band at 833.30cm^{-1} appeared after treatment with fungi in soil as compared to control at 913cm^{-1}. This may be due to formation of (=C-H) alkenes functional group. In addition the range between 1320-1000cm^{-1} bands at 1036cm^{-1},1196.82cm^{-1}, 1211.74cm^{-1} in both the degrading medium (soil and liquid synthetic medium) due to –C-O stretch. Between the range of wavenumber 2700-2200 cm^{-1} control show only 2 peaks at 2281 cm^{-1} and 2360 cm^{-1} which decreased as well as increased at 2282 cm^{-1}, 2283 cm showed formation of carbonyl addition with ammonium group. Characterization of FTIR peaks are given in table 5

Table5. Assignment of IR absorption peaks for HDPE [14]

Wave numbers before and after treatment with *A.fumigatus* (cm-1)			**Band**	**Functional Group**
Control	Soil	Synthetic medium		
913	833	913	=C-H Bend	Alkenes
1209.87	1011.74	1036.50	-C-O stretch	Carboxylic acid,esters and ether
1459.68	1023.45	1196.82	-C-H bend	Alkanes
1951.83	1459.68	1459.68	$C=C=CH_2$	Cumulative double bond stretch in allenes
2140.12	1949.97	1951.83	-SCN	Aliphatic cynide
2281.8 2360.71	2140.12	2140.12	C=O	Carbonyl in addition of ammonium
	2283.66	2281.80	-C-H stretch	Alkanes
2911.9	2910.04	2908	-OH stretch	Alcohol
3760.12	3760.12	3092	-OH stretch, C=O	Alcohol, Ketone

CONCLUSION

It has been widely known that polyethylene plastics are resistant to degradation and it takes very long time to degrade completely in the environment. The problem of plastic

pollution has now become a mess for mankind. There is no part of the world untouched from its worse impact. In the present period of globalization there is stress on environment for safe disposal of polythene waste for making it commercial. An ecofriendly way to get rid of polythene waste is to exploit microorganism to degrade polythene. The current study demonstrates the isolation, characterization and biodegradation of HDPE film by fungi. Result of degradation of HDPE by fungi indicated that these fungi were able to grow in soil as well as synthetic medium and use polymer as carbon source. There was no reduction of weight in control. Percentage weight loss of HDPE was 59% in soil after 9 months and 29.1% in synthetic medium after 2 months. SEM result revealed morphological changes on the sheet caused due to degradation by the fungal isolates. Images of SEM show microbial colonization on the film surface that is evident of fungal degradation. FTIR results exhibit changes or either new peak formation or disappearance of a peak or else change in the peak range as accounted as monitoring parameter and regarded as the change occurred on the surface of polythene due to the action of fungi. The isolated fungi were able to degrade polythene without any additive and prior treatment. It was assumed that the isolate produced enzymes which are capable of degrading polythene, exact mechanism of enzymatic degradation has not been fully known. Thus the fungal strain can be used as a valuable application to solve the plastic waste problem.

REFERENCES

1. Orhan, Y. and H. Büyükgüngör, *Enhancement of biodegradability of disposable polyethylene in controlled biological soil.* International biodeterioration & biodegradation, 2000. **45**(1-2): p. 49-55.
2. Ojha, N., et al., *Evaluation of HDPE and LDPE degradation by fungus, implemented by statistical optimization.* Scientific reports, 2017. **7**: p. 39515.
3. Hadad, D., S. Geresh, and A. Sivan, *Biodegradation of polyethylene by the thermophilic bacterium Brevibacillus borstelensis.* Journal of applied microbiology, 2005. **98**(5): p. 1093-1100.
4. Usha, R., T. Sangeetha, and M. Palaniswamy, *Screening of polyethylene degrading microorganisms from garbage soil.* Libyan agriculture research center journal international, 2011. **2**(4): p. 200-204.
5. Yoshida, S., et al., *A bacterium that degrades and assimilates poly (ethylene terephthalate).* Science, 2016. **351**(6278): p. 1196-1199.
6. Gu, J.D., *Biodegradation of Synthetic and Biological Polymers Used in Engineering and Construction.* Biopolymers Online: Biology• Chemistry• Biotechnology• Applications, 2005. **10**.
7. Kathiresan, K., *Polythene and plastics-degrading microbes from the mangrove soil.* Revista de biologia tropical, 2003. **51**(3-4): p. 629-633.
8. Koutny, M., et al., *Acquired biodegradability of polyethylenes containing pro-oxidant additives.* Polymer degradation and stability, 2006. **91**(7): p. 1495-1503.
9. Upreti, M. and R. Srivastava, *A potential Aspergillus species for biodegradation of polymeric materials.* Current Science, 2003. **84**(11): p. 1399-1402.
10. Yamada-Onodera, K., et al., *Degradation of polyethylene by a fungus, Penicillium simplicissimum YK.* Polymer degradation and stability, 2001. **72**(2): p. 323-327.
11. Bolo, N.R., et al., *Isolation, Identification, and Evaluation of Polyethylene Glycol and Low-Density Polyethylene-Degrading Bacteria from Payatas Dumpsite, Quezon City, Philippines.* Philippine Journal of Health Research and Development, 2015. **19**(1): p. 10.
12. Esmaeili, A., et al., *Colonization and Biodegradation of Photo-Oxidized Low-Density Polyethylene (LDPE) by New Strains of Aspergillus sp. and Lysinibacillus sp.* Bioremediation Journal, 2014. **18**(3): p. 213-226.
13. Singh, J. and K. Gupta, *Screening and identification of low density polyethylene (LDPE) degrading soil fungi isolated from polythene polluted sites around Gwalior City (MP).* International Journal of Current Microbiology and Applied Sciences, 2014. **3**(6): p. 443-448.
14. Coates, J., *Interpretation of infrared spectra, a practical approach.* Encyclopedia of analytical chemistry, 2000. **12**: p. 10815-10837.