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Screening and Identification of Soil Fungi with Potential of Plastic Degrading Ability

KEYWORDS: Plastic, Soil Fungi, Biodegradation..

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ABSTRACT *This article reveals the biodegradation of polyethylene and Polyvinyl Chloride with the help of fungi isolated from soil. The degrading ability of the fungal strains was evaluated by performing FTIR spectra, SEM and CO_2 production. With the excessive use of plastics and increasing pressure being placed on capacities available for plastic waste disposal, awareness of the waste problem and its impact on the environment has created interest among research workers about polymers biodegradation. Fungi, isolated from plastic buried in soil, were subjected to growth in a medium containing plastic as the sole carbon source. The increase in fresh weight of the fungi and weight loss of plastic material in the medium after regular time intervals is evident that the fungi are utilizing plastic as the carbon source. SEM image reveals reduction in particle size of PVC*

Introduction
Plastic materials are strong, light-weight, and durable and thus are widely used in food, clothing, shelter, transportation, construction, medical, and recreation industries (Orhan and Buyukgungor, 2000)[1*]. More than 40 million tons of plastics are produced every year (Yang et al., 2007)[2*]. However, because of its xenobiotic origin and recalcitrant nature, its biodegradation is problematic and it accumulates at a rate of 25 million tons per year (Orhan and Buyukgungor, 2000)[1*]. Plastic is a common term used to include all sorts of polythene (polyethylene), polyvinyl chloride (PVC) and many other related polymeric materials. Plastics possess a number of key characteristics including inertness, flexibility and low production costs that have led to their application in many areas of human life. But the problem that neutralizes all these attributes in their recalcitrance, i.e., they cannot be degraded easily by nature.. Some fungal members are shown their activity on plastic material[*3]. The speed at which the decomposition occurs called the "rate of decomposition", depends on the temperature, moisture and chemical composition of the organic matter. The oxygen level is another important factor, since fungi require oxygen for growth. In low oxygen environments, fungal growth is slow resulting in decrease in the decomposition process.

Recent research works have shown that most of the constituents of plastics can be degraded by microbes and the film plastics can be treated by microbial systems. Acrylonitrile fibres are attacked by species of *Aspergillus, Penicillium, Stachybotrys, and Nigrespora*. *Pullularia pullulans* can degrade polycaprolactone and other aliphatic polyesters. N-alkenes, alkenes and other aliphatic hydrocarbons are readily utilized by yeasts and fungi. Since a wide variety of fungi grow and degrade plastics and their polymers, only they have to be upgraded**[*4]**.

It has been recently shown that the members of order Xylariales belonging to class Ascomycetae such as *Xylaria* also grow on the plastic strips (as a source of carbon)[*5] Microorganism for biological decomposition of polythene and plastics are isolated and tested for their ability in in-vivo and in-vitro condition by P. Nayak et.al.**[*6].**

Materials and Methods
Different types of plastic bags were cut in to pieces. Each piece was buried for about two months. During the period moisture was maintained in soil to ensure fungal growth**[*7].** After two moths these plastic sheets were removed. Each plastic type was clean separately for removal of excess of soil artifact.

Serial dilution:
After removal of excess soil material each plastic type were taken saperatly and 1gm of this sample was cut into pieces and added to 9 ml of sterile water to make 1:10 dilution, adding 1ml of the 1:10 dilution of 9ml of sterile water makes a 1:100 dilution and so on. In this way each sample were prepare with six dilution i.e. 10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6}.. Each dilution inoculated in separate sterilized Petri plates containing synthetic medium and kept for inoculation for 3 to 7 days.

Preparation of Medium (SM) Constitutions of medium in 1000 ml distilled water (K_2HPO_4, 1 g; KH_2PO_4, 0.2 g; NaCl, 1g; $CaCl_2.2H_2O$, 0.002 g; $(NH_4)_2SO$, 1 g; $MgSO_4.7H_2O$, 0.5 g; $CuSO_4.5H_2O$, 0.001 g; $ZnSO_4.7H_2O$,0.001 g; $MnSO_4.H_2O$, 0.001 g and $FeSO_4.7H_2O$, 0.01 g. and 100 mg of polymer source[*8].

Screening and Identification
After aseptically inoculation SM was incubated at 37 c temperature for 1week. From third day mycelium grows on SM in plates. In first set about 15 fungal forms were observed.

All these 15 strain were tested repeatedly for their plastic degrading ability by using two different polymer viz. polyethylenes, Polyvinylchloride. Out of these 15 forms five forms are found more active. All these six forms with extensive network of fungal hyphae were observed under light microscope. On the basis of microscopic examination and morphologic characteristics, the fungal strain was identified with the help of "Manual of soil Fungi"[*9]. Further this taxonomic identification is confirmed to Agharkar Research Institute, Pune. These forms are species of genus *Chrysonelia Aspergillus, Penicillium*..

During second set of experiment more fungal forms are isolated by using same synthetic medium where plastic is sole carbon source instead of glucose. About 13 different forms are found growing on powder of PVC and granules of LDPE and HDPE. Out of thirteen forms four fungal strains were use for further studies. The taxonomic identification was later confirmed by Agharkar Research Institute, Pune. These forms are species of genus, Aspergillus, Penicillium.. Fusarium, and Chaetomium

Measurement of Plastic Degradation
a) Weight difference

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The degrading ability of fungi is tested in laboratory by different methods. The tested samples were checked by calculating weight difference between before and after treatment of sample. These weight differences noted separately for different fungal species which reveal activity differences of different species

b) Physical test-
Particle size and surface changed were analyzed and tested by SEM technique from SAIF IIT, Pawai, Mumbai.

c) FTIR Test
Fourier Transform Infrared Spectroscopy analysis was used for detecting the formation of new functional groups or changes in the amount of existing functional groups

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Result and Discussion
The present study deals with the isolation of polyethylene degrading fungi and to test their ability for plastic degradation in laboratory condition. Polyethylene and PVC is used as plastic material. Fungal organisms with the ability to degrade plastic were isolated in synthetic medium supplemented with plastic powder/grannuels and these organisms were used for degradation study. Colonization study with the fungi showed a result of visible decrease in the polymer weight of fungus after 04 to 12 week incubation. The difference in before and after treatment weight of polymers by respective fungi species are given in table no.1.1

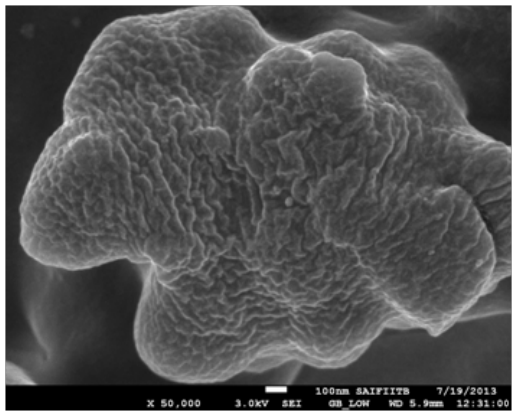
Fungal strains found colonized on the surface of plastic material causing some physical changes that will be evident in our study by image of Scanning electron microscope. The particle size of PVC material is 100 nanometer, which brought in to more fine particles by breakage in polymer molecules.

The spectral data of original PVC molecule compared with PVC degraded by fungal for different time interval (04 weeks and 08 weeks) reveals a visible change in molecule In PVCF peak at 3444^{cm-1} is again disappeared and very small peaks observed at 2150^{cm-1} and 2023 $^{cm-1}$, these peaks absent in both PVCO and PVC4 spectra. Peaks between 1370 $^{cm-1}$ to 710 $^{cm-1}$ are almost disappeared and becomes very small which indicates drastic change in the polymeric molecule towards monomeric unit or less molecular weight compound.

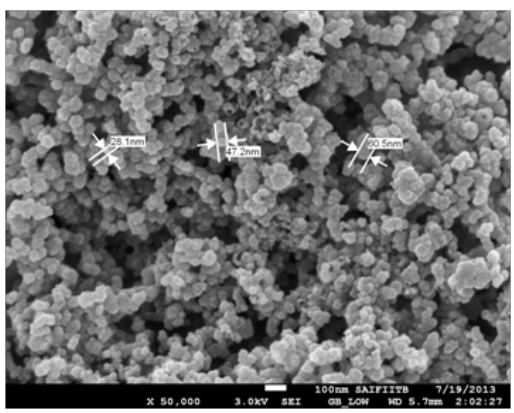
The intermediate portion of the spectrum 1300^{cm-1} to 900 $^{cm-1}$ is usually referred as fingerprint region. the absorption pattern in this region is frequently complex. This portion of the spectrum is extremely valuable when examined in reference to the other region. Absorption in this intermediate region is probably unique for every molecular species. The drastic change in the appearance of peak in this region in PVC4 and PVCF indicates change in polymeric molecule towards monomeric unit or low molecular weight compound. Biodegradation of PVC brought some structural changes in the FTIR spectra of the polymer*[10].

Conclusion
Soil contains microorganisms (fungi) that are able to bring about some degradation of synthetic polymers. All the fungal isolates showing adherence and growth on the polymer surface indicated their ability to utilize plastic as a source of nutrient (Carbon). The changes in the peaks of the FTIR spectra of the test samples as compared to control, is an indication of breakdown of plastics as a result of fungal treatment. Also breaking of particle size in to fine size visible in SEM image of PVC reveal the activity of fungi.

Scanning Electron Image Compared

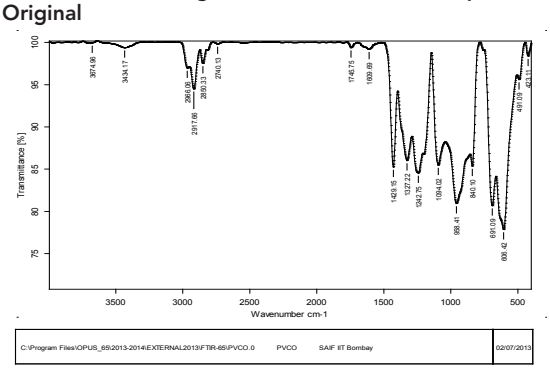


Original



After Treatment

FTIR Results for Original and Treated PVC Sample
Original



After Treatment

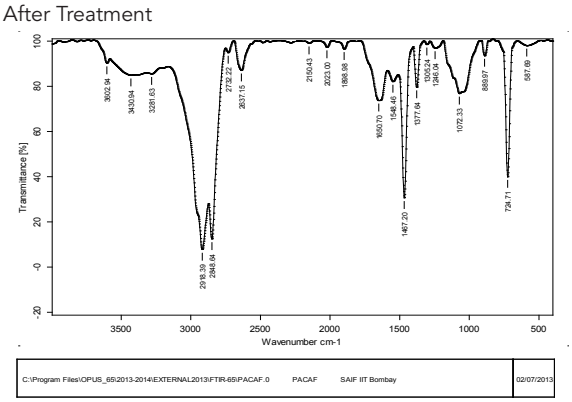


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Table no. 1.1
List of isolated fungal species with plastic degrading potential.

Sr. No.	Name of fungus	Weight loss of polymer In gms.		
		PVC	HDPE	LDPE
01	*Mucor hiemalis* Wehmer	0.064	0.002	0.128
02	Aspergillus versicolor gr.	0.300	0.289	0.412
03	Aspergillus niger gr.	0.341	0.245	0.412
04	Aspergillus flavus Link.	0.619	0.587	0.700
05	Penicillium sp.	0.082	0.001	0.012
06	*Chaetomium globosum* Kunze and Schmidt	0.240	0.494	0.209
07	*Fusarium oxysporum* (Martius) Appel and wollenweber	0.330	0.410	0.541
08	*Fusarium solani* (Martius) Saccardo	0.240	0.494	0.209
09	Phoma sps.	0.364	0.414	0.468
10	Chrysonilia setophila (Mont) Arx.	0.145	0.130	0.220

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