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DEGRADATION OF POLYETHYLENE BY *CHAETOMIUM* SP. AND *ASPERGILLUS FLAVUS*

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ABSTRACT

Chaetomium sp. and *Aspergillus flavus* isolated from the local landfill of Shivamogga district were used in the biodegradation of polyethylene. Soil sample of local landfill was used as source to isolate these two fungi. Degradation was monitored by observing weight loss and changes in physical structure by Scanning Electron Microscopy. Organisms were grown on polyethylene without any treatment and polyethylene, which was irradiated with UV and incubated with nitric acid at 80°C for 06 days before cultivation. Organisms were able to degrade treated polyethylene better than untreated polyethylene. Both of these organisms may act as solution for the problem caused by polyethylene in nature.

INTRODUCTION

Plastic bags are made of polyethylene, which is a polymer of the monomer ethylene. During past three decades, plastic materials are increasingly used in transportation, food –clothing, shelter construction, medical and recreation industries. Plastic are advantageous as they are strong, light weight and durable (Kathiresan, 2003). But, lack of degradability and the closing of landfill sites, as well as growing water and land pollution problems have led to concern about plastics. With the excessive use of plastics and increasing pressure being placed on capacities available for plastic waste disposal, the need for biodegradable plastics and biodegradation of plastic has assumed increasing importance in the last few years. Biodegradation is necessary for water soluble or water immiscible polymers, because they eventually enter water streams which can neither be recycled nor incinerated (Shah *et al*., 2008). The polyethylene is the most commonly found solid waste that has been recently recognized as a major threat to marine life. The polyethylene could sometimes cause blockage in intestine of fish birds and marine mammals (Spear *et al.* 2005; Zarzur, 1999). Degradation of polyethylene is a great challenge as the materials are increasingly used. A very general estimate of worldwide plastic waste generation is annually about 57 million tons (Bollag *et al*., 2000). The solid waste related problems pose threat to mega cities. So attempt has been made in this paper to isolate the potent microorganisms that degrade polyethylene from the soil of Western Ghats.

MATERIALS AND METHODS

Collection of soil sample

Soil sample was collected from a local land fill of Shivamogga dist. and brought to the laboratory, preserved under laboratory conditions for further use.

Isolation and identification of fungi from soil

Enrichment procedure was used for the isolation of microorganisms where polyethylene was used as sole source of carbon. Enrichment medium composed of 0.1g of Polyethylene , 0.3g of NH_4NO_3, 0.5g of K_2HPO_4, 0.1g of NaCl, 0.02g of $MgSO_4.7H_2O$, 0.01g of yeast extract and 100ml distilled water, pH 6 (Medium A). Soil was added to conical flasks containing 100ml of sterilized enrichment medium. Flasks were incubated at 30°C for 4 weeks on rotary shaker at 200rpm. After incubation, 1ml of suspension was added into the 4ml of fresh enrichment medium. After 1 week of shaking, 5µl of the culture was spread on 2% agar plates of medium A and the plates were incubated for several days. Colonies which appeared similar to *Chaetomium* sp. and *Aspergillus flavus* morphology were picked up and inoculated individually on PDA and incubated at room temperature for 4-5 days. Fungi were identified based on their microscopic and macroscopic appearance using standard manuals. The colonies were preserved at 4°C in 2% agar slants of medium B contained 5% malt extract, 0.3% yeast extract and distilled water at pH 5.6 (Yamada-onodera *et al.* 2001).

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Screening of fungi for polyethylene degradation

Plate assay

Both of these isolated fungi were inoculated to medium which contained 0.3g of NH_4NO_3, 0.5g of K_2HPO_4. 0.1g of NaCl, 0.02g of $MgSO_4.7H_2O$, 2g of agar, 0.5g of irradiated or not irradiated polyethylene and 100ml distilled water (Yamada-onodera *et al.* 2001).

Screening of fungi to degrade untreated polyethylene disc

The pre-weighed discs of 1cm diameter prepared from polyethylene bags were aseptically transferred to 6 sets of conical flasks containing 50ml of Mineral salt medium. the conical flask containing 50ml of Mineral salt medium. Loop full of organisms was added to this. Control was maintained with polyethylene discs in the microbe free medium. Triplicates were maintained for each organism and left on shaker. After one month of incubation, the plastic discs were collected, washed thoroughly using distilled water, dried in hot air oven at 50^0C overnight and then weighed for final weight (Kathiresan, 2003).). Same method was followed to observe weight loss up to 6 months.

Screening of fungi to degrade treated polyethylene

Polyethylene discs were UV irradiated and incubated with nitric acid at 80^oC for 06 days and these discs were inoculated to Mineral Salt Medium.

Analytical methods to check biodegradation of polyethylene

Observation of colonies grown on medium and organisms grown on polyethylene discs by Stereo binocular Microscopy

The growth of colonies on the medium and over the polyethylene discs was observed under stereo binocular microscope.

Observation of discs using Scanning electron microscopy

Discs from mineral salt and soil were scanned for changes in the physical appearance of polyethylene using Scanning Electron Microscopy. The surface morphology of Low density polyethylene was analyzed through Scanning Electron Microscopy. A drop of sample as dried on a cleaned silicon wafer and electron conductivity created externally to the sample by sputtering with gold nanoparticles using gold sputter (Jeol JFC 1100 E Ion Sputtering device) and analyzed by Field emission scanning electron microscopy (FEI-SIRION, Eindhoven , Netherland).

Analysis for weight loss

Polyethylene discs treated with organisms were taken out from conical flasks and were thoroughly washed with distilled water, these were dried in oven at 50^oC overnight and weight was analyzed.

RESULTS

Isolation and Identification of Fungi

Chaetomium sp. and *Aspergillus flavus* were isolated from soil and identified based on their morphology and microscopic observation after staining with cotton blue, by following the keys of previous observations (Nagamani *et al.,* 2006). The isolated fungi were further confirmed by comparing its growth and morphological characteristics with the pure cultures procured form National Chemical Laboratory, Pune, India (Fig.1).

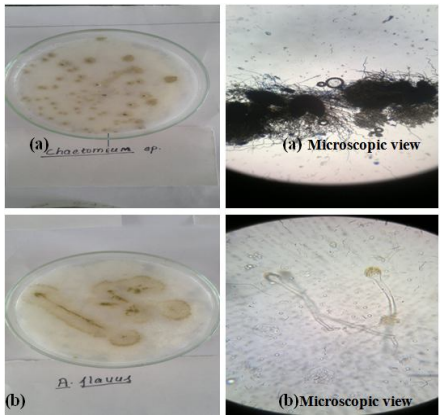


Fig. 1 Moprhololgy and Microscopic view of *Chaetomium* sp. (a) and *Aspergillus flavus* (b).

Screening of fungi for polyethylene degradation

To check ability of fungi to grow on medium containing polyethylene

Colony diameters of both *Chaetomium* sp. And *Aspergillus flavus* on the medium was recorded (Table 1).

Table 1 Ability of fungi to grow on medium containing polyethylene

Sl. no.	Organisms	Colony Diameter (mm)
1.	*Chaetomium* sp.	9
2.	*Aspergillus flavus*	8

Observation of organisms grown on medium and discs using Stereo Binocular Microscope (SBM)

The fungi grown on medium and polyethylene were observed under the stereo binocular microscope which indicate that the *Chaetomium* sp. shows better growth than *Aspergillus flavus* (Fig. 2 & 3).

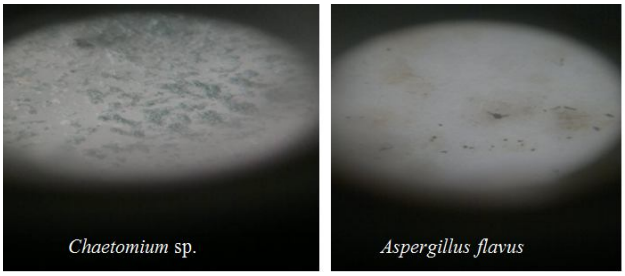


Fig. 2 Stereo binocular microscopic observation of *Chaetomium* sp. (a) and *Aspergillus flavus* (b) grown on medium

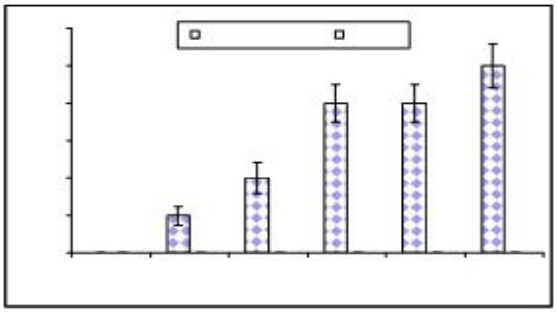


Fig. 3 Growth of *Chaetomium* sp. (a) and *Aspergillus flavus* (b) on polyethylene disc

Observation of discs using Scanning Electron Microscopy

Degradation was further confirmed by SEM observation where we can make out modifications in the surface of polyethylene. SEM photograph of control, untreated and treated polyethylene disc with *Chaetomium* sp. (Fig. 4) and *Aspergillus flavus* (Fig. 5) showed distinct characteristics.

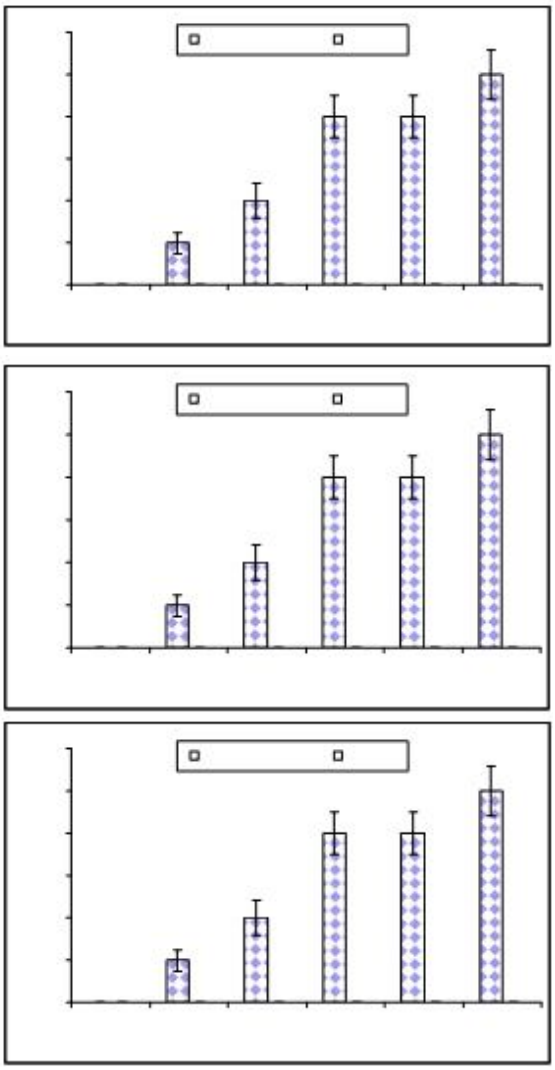


Fig. 4 SEM photograph of control (a), untreated (b) and treated (c) polyethylene with *Chaetomium sp*.

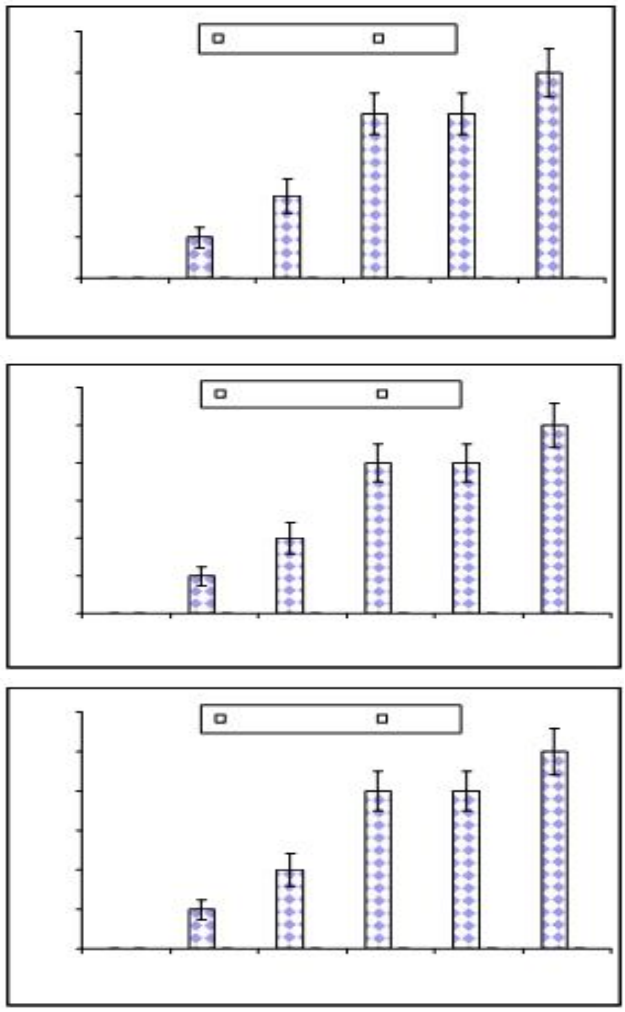
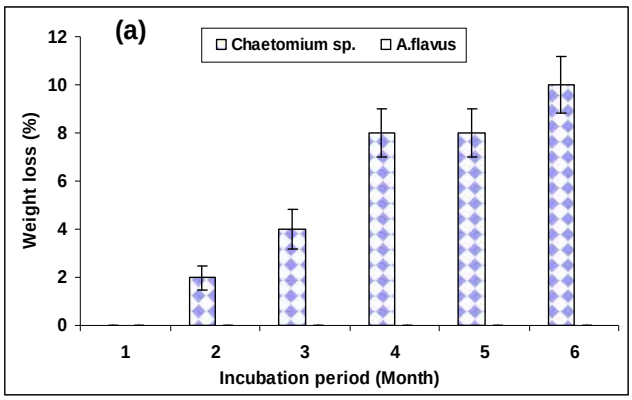


Fig. 5 SEM photograph of control (a), untreated (b) and treated (c) polyethylene with *Aspergillus flavus.*

Screening of fungi to degrade treated polyethylene

Both organisms were able to degrade treated polyethylene more efficiently than untreated polyethylene (Table 3). In one month of time interval, the UV untreated and UV treated polyethylene biodegradation ability by *Chaetomium* sp. and *Aspergillus flavus* was checked for six times (Fig. 6).



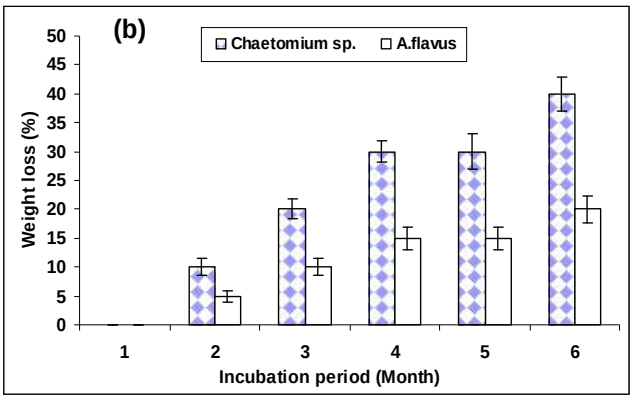


Fig. 6 Percentage weight loss of UV untreated (a) and UV treated (b) polyethylene inoculated with *Chaetomium* sp. and *Aspergillus flavus*. Error bars are standard error of the mean.

polyethylene as carbon source for its growth.

Albertsson *et al.* (1987) concluded that carbonyl groups are produced by UV light or oxidizing agents and that these groups are the main factors at the beginning of the degradation, being attacked by microorganisms that degrade the shorter segments of polyethylene chains. Ohtake *et al.* (1994) also observed biodegradtion of polyethylene buried in the soil for 32-37 years, which was promoted by UV irradiation. Cornell *et al.* (1984) concluded that photo-oxidative degradation of polymers does not always facilitate progressive attack by microorganisms, because the oligomer fractions produced during photo-oxidation may support microbial growth, but polymers with a high molecular weight resulted in little or no growth.

Table 2 Screening of fungi to degrade UV untreated polyethylene discs

Sl. No.	Organism	Initial weight (mg)	Final weight (mg)	weight loss (mg)	Weight loss (%)
1.	*Chaetomium* sp.	0.10±0.006	0.09±0.003	0.01	10
2.	*Aspergillus flavus*	0.10±0.005	0.10±0.004	0.00	0

All results are expressed as Mean ± Standard Error of Mean; n=6

Table 3 Screening of fungi to degrade UV treated polyethylene discs

Sl. No.	Organism	Initial weight (mg)	Final weight (mg)	Weight loss (mg)	Weight loss (%)
1.	*Chaetomium* sp.	0.10±0.005	0.06±0.004	0.04	40
2.	*Aspergillus flavus*	0.10±0.004	0.08±0.005	0.02	20

All results are expressed as Mean ± Standard Error of Mean; n=6

Nearly 50% polyethylene reduction was observed in each test. Both organisms were able to degrade treated polyethylene more efficiently than untreated polyethylene. These results showed that both *Chaetomium* sp. and *Aspergillus flavus* were able to utilize polyethylene as carbon source for their growth. But, *Chaetomium* sp. was able to utilize polyethylene more efficiently than *Aspergillus flavus.* is more efficient in using polyethylene as carbon source than *Aspergillus flavus.*

DISCUSSION

Chaetomium sp. and *Aspergillus flavus* were isolated from local landfill of Shivamogga district. Organisms were identified based on both Macroscopic and microscopic observations. These organisms were grown on medium containing polyethylene and agar. *Chaetomium* sp. showed better growth than *Aspergillus flavus.* When these organisms were inoculated to Mineral Salt Broth, containing untreated and treated polyethylene, and incubated for 6 month on rotary shaker at 150rpm only *Chaetomium* sp. was able to degrade 10% untreated polyethylene and. *Aspergillus flavus* was able to degrade 20% of treated polyethylene and *Chaetomium* sp. degraded 40% of treated polyethylene. These results showed that *Chaetomium* sp. was able to use both untreated and treated polyethylene as carbon source more efficiently and *Aspergillus flavus* was unable to utilize untreated polyethylene, but it was able to utilize treated

They suggested that these observations could explain some of the contradictions in the literature about microbial degradation of polymers. Albertsson and Karlsson (1988) concluded that the biodegradation of inert material such as polyethylene takes more than 10 years and that of degradable material containing a UV sesnsitizer takes 2 years or less. We observed little if any reduction of untreated polyethylene during 1 month of cultivation with fungi. While in treated polyethylene weight loss was more. Degradation was further confirmed by SEM, where we can make out modifications in the surface of polyethylene.

CONCLUSION

Degradation of polyethylene was carried out with *Chaetomium* sp. and *Aspergillus flavus.* These organisms were isolated from landfill soil. Both organisms were able to degrade polyethylene. Efficiency of *Chaetomium* sp. to use polyethylene as sole source of carbon was much better than *Aspergillus flavus.* Both of these were able to degrade treated polyethylene more efficiently but *Chaetomium* sp. was able to degrade untreated polyethylene also, whereas *Aspergillus flavus* were unable to degrade the untreated. Surface modification was observed using Scanning Electron Microscopy. Pores were formed on surface of polyethylene which was treated with fungi. There was not any change in the surface of control polyethylene and untreated. As these organisms were isolated from landfill soil, it appears that polyethylene can be used by these organisms for their

growth as main carbon source. Hence, we can conclude that these fungi can biodegrade polyethylene.

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