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RESEARCH ARTICLE

DEGRADATION OF POLYETHYLENE BY *TRICHODEMA* SP. AND ASPERGILLUS NIDULANS

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ABSTRACT

The *Trichoderma* sp. and *Aspergillus nidulans* were isolated from soil sample of local landfill in Shivamogga district for check the efficiency of polyethylene degradation. 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 UV irradiated and incubated with nitric acid at 80°C for 06 days before cultivation. Organisms were able to degrade treated polyethylene more efficiently than untreated polyethylene. Both of these organisms may act as solution for control the problem caused due to polyethylene contamination in nature.

INTRODUCTION

Plastic bags are made of polyethylene (PE), 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 increasing pressure being placed on capacities available for plastic waste disposal and 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.,* 1995). The degradation of polyethylene can occur by different molecular mechanisms such as chemical, thermal, photo and biodegradation (Gu, 2003). Biodegradability is evaluated by weight loss, tensile strength loss, changes in percent elongation, and changes in polyethylene molecular weight distribution. Physicochemical degradation of plastic is initiated by treatment with acid at 70^0C and 365nm UV irradiation of the polyethylene film. These pretreatment favors the microbial degradation of polyethylene. 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). Polyethylene is one of the synthetic polymers of high hydrophobic level and high molecular weight. It is not biodegradable in nature, thus their use causes dangerous environmental problems (Kwpp and Jewell, 1992). 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.

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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 0.3g 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 plate medium A and the plates were incubated for several days for fungal growth. Colonies which appeared similar to *Trichoderma* sp. and *Aspergillus nidulans* morphology were picked up and inoculated individually on PDA and incubated at room temperature for 4-5 days. Isolated fungi were identified based on their microscopic and macroscopic appearance using standard manuals and by compared with the standard pure cultures procured from National Chemical Laboratory, Pune, India. The pure cultures were preserved at 4^0C in 2% agar slants of medium B (5% malt extract, 0.3% yeast extract and distilled water; pH 5-6) (Yamada-onodera *et al*., 2001).

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 and 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 the conical flask containing 50ml of Mineral salt medium and loop full of organisms was inoculated. 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).

Screening of fungi to degrade treated polyethylene

Polyethylene discs were UV irradiated and incubated with nitric acid at 80°C for 06 days. After incubation these discs were added into the Mineral Salt Medium and test fungi were inoculated. The fungal inoculated medium was incubated with the control for PE degradation.

Analytical methods to check biodegradation of polyethylene

Observation of fungal colonies growth on medium and polyethylene discs using Stereo binocular Microscopy

The fungal growth in the medium containing PE was observed and formation of fungal colony in hypal growth form was confirmed with the PE disc stereo binocular microscopic observation.

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°C overnight and weight was analyzed.

Observation of discs using Scanning electron microscopy

Discs from minimal broth medium 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 water 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).

RESULTS AND DISCUSSION

Isolation and Identification of Fungi

Trichoderma sp. and *Aspergillus nidulans* were isolated from local landfill of Shivamogga district. Organisms were identified based on Morphological and microscopic observations after staining them with cotton blue, by following the keys of previous observation (Nagamani *et al*., 2006) (Fig.1). These organisms were selected for study because of their common and predominant presence in their native region of soil contaminated with waste polyethylene plastic bags.

Screening of fungi for polyethylene degradation

To check ability of fungi to grow on medium containing polyethylene

These isolated organisms were grown on medium containing polyethylene and agar. Colony diameter of both the fungi was determined after their growth on their specific growth medium containing polyethylene (Table 1). Degradation of polyethylene has been monitored in terms of the growth of microorganisms including fungi. Biodegradation of polyethylene was done with the microbes isolated from soil environment. This agar plate test is also a simple semi-quantitative method to know depolymerize of poymer by the organism. After inoculation with fungi into the medium containing fine particles of PE, the formation of a clear halo around the colony indicates the first step of fungal biodegradation (Nishida and Tokiwa, 1993). Fungi isolated from soil enriched with organic carbon results in increased PE uptake.

Table 1: Ability of fungi to grow on medium containing polyethylene

Sl. No.	Organism	Colony Diameter (mm)
1.	*Trichoderma* sp.	09
2.	*Aspergillus nidulans*	09

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

The fungi grown on medium and observed under the stereo binocular microscope indicate that the *Trichoderma* sp. show better growth than *Aspergillus nidulan* (Fig. 2). The increased hypha formation was observed on the surface of PE disc. The

hyphal growth indicates initiation of PE degradation. Microbial degradation of plastics is due to the enzymatic chain cleavage of the polymer into oligomers and monomers. Aerobic metabolism results in carbon and water as the end product (Starnecker and Menner, 1996).

Screening of fungi to degrade untreated polyethylene disc

When these organisms were inoculated to Minimal Salt Broth, after 1 month of incubation on rotary shaker at 150rpm *Trichoderma* sp. degraded 20% of untreated polyethylene and *Aspergillus nidulans* degraded only 10% of untreated polyethylene (Table 2). Both organisms were able to degrade untreated polyethylene in small amount. With the fungal activity PE with a starting weight of 0.1mg was degraded to 0.08mg after one month of liquid cultivation, which indicated the biodegradation of that PE.

nidulans was checked for six times and in each test period nearly 50% of polyethylene reduction was found (Fig.3)**.** No reduction was observed during the initial one month of incubation. The results showed that both *Trichoderma* sp. and *Aspergillus nidulans* were able to utilize polyethylene as carbon source for their growth. But, *Trichoderma* sp. was able to utilize polyethylene more efficiently than *Aspergillus nidulans.* UV light is a known as initiator of polyethylene oxidation and enhances the fungal degradation when compared with its corresponding UV untreated control (Le *et al*., 1991). Both UV and acid treatment causes pro-oxidant and photo-oxidant to produce free radicals on the long chain, causing the material to lose some of its physical properties, to become oxidized and more accessible to microbial biodegradation (Cornell *et al*., 1984). The fungal attachment was found on the surface of the plastic and it indicates possible utilization of plastic as a source of nutrient.

Table 2: Screening of fungi to degrade untreated polyethylene disc

Sl. No.	Organism	Initial weight (mg)	Final weight (mg)	Weight loss (mg)	Weight loss (%)
1.	*Trichoderma* sp.	0.10±0.002	0.08±0.004	0.02	20%
2.	*Aspergillus nidulans*	0.10±0.003	0.09±0.005	0.01	10%

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

Table 3: Screening of fungi to degrade treated polyethylene disc

Sl. No.	Organism	Initial weight (mg)	Final weight (mg)	Weight loss (mg)	Weight loss (%)
1.	*Trichoderma* sp.	0.10±0.003	0.06±0.005	0.04	40%
2.	*Aspergillus nidulans*	0.10±0.004	0.07±0.004	0.03	30%

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

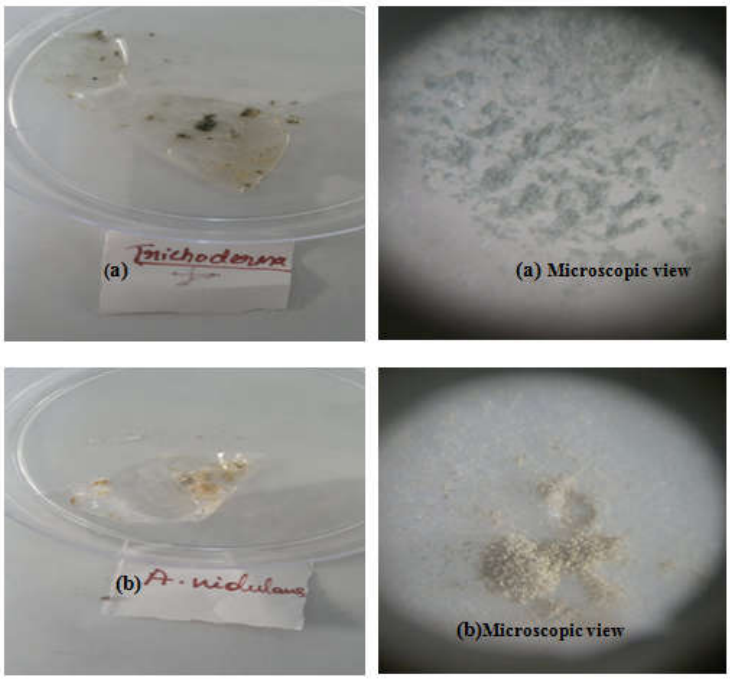

Fig. 2: Stereo binocular microscopic observation of *Trichoderma* sp. (a) *Aspergillus nidulance* (b) grown on medium and polyethylene discs

Screening of fungi to degrade treated polyethylene

Both organisms were able to degrade treated polyethylene more efficiently than untreated polyethylene. *Trichoderma* sp. was able to degrade 40% of treated polyethylene and *Aspergillus nidulans* degraded 30% of treated polyethylene (Table 3). In one month of intervals the polyethylene biodegradation ability of *Trichoderma* sp. and *Aspergillus*

The ability of fungal strains form a biofilm on PE was attributed to gradual decrease in hydrophobicity for its surface (Gilan *et al*., 2004). In this study, the hot nitric acid treated polyethylene having molecular weight higher than 100,000 was degraded to low molecular weight during inoculated with fungal culture. The results of HT-GPC and FT-IR analysis showed that some of the double carbon bonds of polyethylene

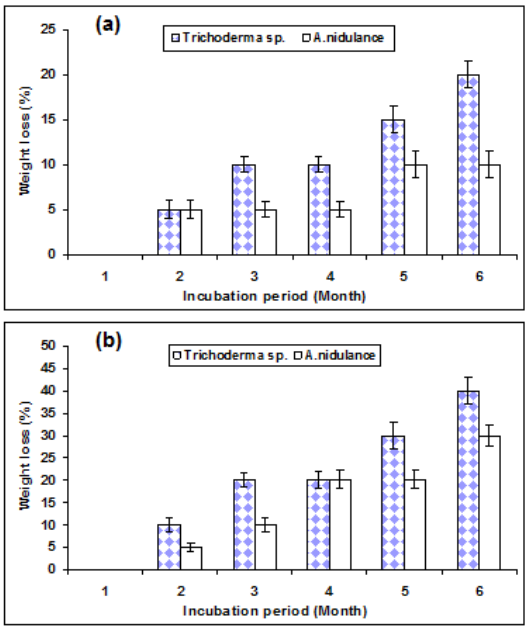

Fig. 3: Percentage weight loss of Treated (a) and Untreated (b) polyethylene inoculated with *Trichoderma* sp. and *A.nidulance*. Error bars are standard error of the mean

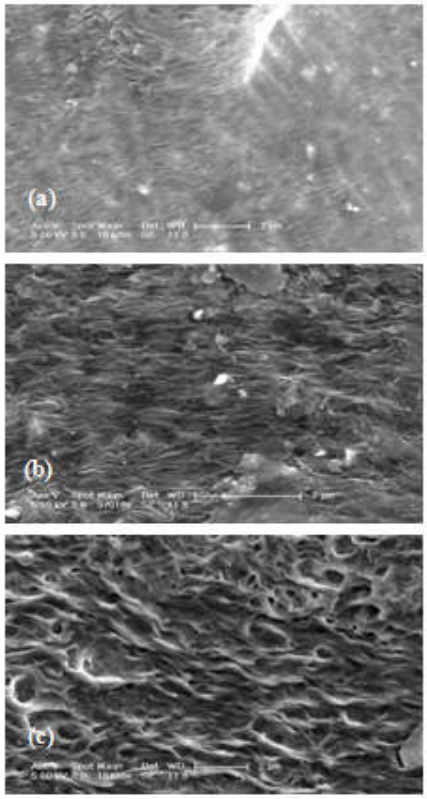

Fig. 4: SEM photograph of control (a), untreated (b) and treated (c) polyethylene with *Trichoderma* sp.

were cut by fungal hyphae (Onodera *et al.,* 2001). The study confirmed that to make PE biodegradable requires modifying its crystalline level, molecular weight and mechanical properties that are responsible for PE resistance towards degradation. Acid and Uv pretreatment improves PE

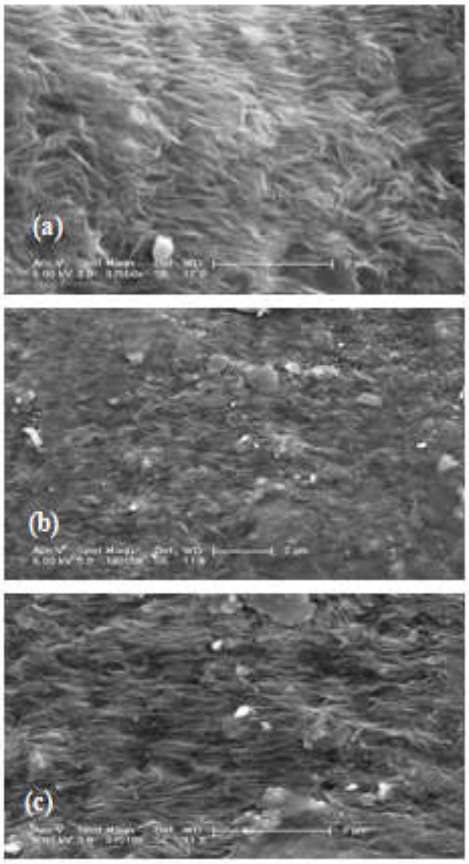

Fig. 5: SEM photograph of control (a), untreated (b) and treated (c) polyethylene with *Aspergillus nidulance*

hydrophilic level and reducing its polymer chain length by oxidation for easy accessible of PE for microbial degradation (Bikiaris *et al*., 1999). The results suggest that the biodegradation of PE depends upon polymer characteristics, organism type and nature of pretreatment. The degradation of synthetic PE requires complex enzymatic reactions with high metabolic activities. After the mineral nutrients from the medium were completely utilized by the fungal strain, polyethylene served as the carbon and energy source. The fungal hyphae adhere to the polyethylene surface rather than being in the liquid media indicates the PE as carbon source for fungi and intern leads to its degradation (Nanda *et al*., 2010). The carbonyl groups are produced by UV light or oxidizing agents and these groups are the main factors for the beginning of degradation, being attacked by microorganisms that degrade the shorter segments of polyethylene chains (Albertsson *et al*., 1987). Biodegradation of polyethylene buried in soil for 32-37 years, which was promoted for degradation by UV irradiation (Ohtake *et al*., 1994). The 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 of organism (Cornell *et al*., 1984). The biodegradation of inert material such as polyethylene takes more than 10 years and that of degradable material containing a UV sensitizer takes 2 years or less for maximum degradation (Secchi and Zarzur, 1999). Hence the

results showed the significance of UV and acid treatment of PE before inoculated with fungi for degradation.

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 polyethylene disc treated with *Trichoderma* sp. and *Aspergillus nidulans* shown distinct characteristics in the Fig. 4 & 5. The structural changes in the form of pits and crosions observed under scanning electron microscopy indicated surface damage of PE incubated with *Trichoderma* sp. and *Aspergillus nidulans*. This study suggested that the fungal strain was able to adhere to the surface of PE and cause surface damage by brake down of polymer chain (Shah *et al*., 2007). SEM also indicates that the surface of plastic materials has turned from smooth to rough with creaking. This may be due to the compounds secreted extra cellular by the fungi that may break the complex molecular structure of plastic.

CONCLUSION

The significant research in biodegradation of polyethylene has increased the interest in screening of novel microorganisms, their enzymes and also the selection of polymer for easy biodegradation. Degradation of polyethylene was carried out with *Trichoderma* sp. and *Aspergillus nidulans* isolated from landfill soil. Both organisms were able to degrade polyethylene. *Trichoderma* sp. has an efficiency to use polyethylene as sole source of carbon and shown much better results when compared to *Aspergillus nidulans.* Both of these were able to degrade treated polyethylene more efficiently than untreated. Surface modification was observed using Scanning Electron Microscopy. Pores were formed on the surface of polyethylene which was treated with fungi. These pores indicate the degradation activity of fungal organisms. There was not any change on the surface of control polyethylene which was not treated with fungi. As these organisms were isolated from landfill soil, it appears that polyethylene can be used as main carbon source for fungal growth. The use of single strain for degradation study helps in easier manipulation of culture conditions and gene technology. Hence, we can conclude that these fungi can biodegrade polyethylene and further its application is required in field study.

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