JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2014. Vol. **8**(3), p. 2465-2471

Role of Fungi Inhabiting Soil, Cow Dung and Sewage in Degrading Polyethylene Terephthalate and Polystyrene Foam

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(Received: 11 September 2013; accepted: 14 November 2013)

Though several alternate strategies have been devised to degrade plastic, usage of microbes to degrade plastics is a challenging one. In this study, fungi has been isolated from PET (Polyethylene terephthalate) and PS foam (polystyrene foam) waste and its potential to degrade PET and PS foam both under laboratory and field burial (soil,cow dung and sewage) conditions have been evaluated through electron microscopic studies. The observation of the present investigation reveal that fungal species (*Aspergillus* sp., *Penicillium sp. and Fusarium sp*.) were able to successfully colonise PET and PS foam flakes surface and induce micromorphological changes like formation of holes, duckings, surface corrosion and crystals, which was evident in the SEM image. Thus fungal species (*Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp.*)* could be used as biological agents to degrade PET and PS foam.

Key words: Fungi, PET, PS foam, SEM.

Management of synthetic plastic waste is of growing concern in recent time. Polystyrene (PS) is a multipurpose polymer that is used in varied applications in rigid and foamed form. General purpose polystyrene (GPS) is clear and hard, which is used in packaging, laboratory ware and electronics. The excellent physical and processing properties make polystyrene suitable for a lot of applications than any other plastic (Meenakshi *et al*., 2002). Expanded polystyrene (EPS) is used in foam form for packaging as well as insulation in various industrial fields in the world (Kan *et al* ., 2009). EPS is moulded into sheets for thermoforming into trays for packaging of fish, meat and cheeses, egg crates, tubs and cups. Styrene and its metabolites are known to cause serious negative effects on human health (Mooney *et al*., 2006). Styrene causes neurological impairment, toxic effect on liver, central nervous system. Styrene is metabolised by a number of microbes in natural environments. Styrene biotransformation causes the production of styrene oxide that is more toxic to human health. Polyethylene Terephthalate (PET) is a semi crystalline thermoplastic polymer, which is used in the preparation of a variety of products differing widely in their physical characteristics and hence, the end uses. The varieties of prominence are fibres and filaments, sheets and soft drink bottles.

The microbial biodegradation of plastic is widely accepted option and is still underway for its enhanced efficiency. Several microorganisms have been reported to produce polyester degrading enzymes. Several microbial species associated with degrading plastics have been reported (bacteria : *Pseudomonas* spp, *Streptococcus* spp, *Staphylococcus* spp, *Micrococcus* and *Maoraxella* spp*;* fungi: *Aspergillus niger*, *Aspergillus glaucus*, *Actinomycetes* and *Saccharomonospora* genus (Swift,1993). Excellent adherence and colonisation

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properties give advantage to the fungi for bioremediation. Once established on a surface, the fungi cover the whole area by forming mycellial mat. Fungi are able to withstand longer periods of stress conditions and due to saprotrophic nature they are capable of producing a diverse arsenal of enzymes that are able to degrade the recalcitrant compounds(Gu and Gu , 2005). The aim of the present study was to isolate fungal species able to colonise and biodegrade Polystyrene Foam and Polyethylene terephthalate waste and to visualise electron microscopic image.

MATERIALS AND METHODS

Samples collection

The plastic (PET water bottles and PS foam) samples were purchased from local market. Soil and Cow dung was collected from local area and Sewage was collected from TWAD (Tamil Nadu water supply and Drainage Board) in Tiruchirapalli, India.

Preparation of PS Foam and PET Powder

PET bottles and Polystyrene foam (Thermo coal) were cut into small flakes and they were kept in the hot air oven for 30 minutes at 100 ^{0}C and 10 minutes approximately at 100^{0}C for PS foam, respectively. Further, they were crushed and sieved by using 1mm mesh

Preparation of PS Foam and PET flakes

PET bottles and PS foam were cut into 0.5cm× 0.5cm, thereafter both samples were washed with 70% ethanol and distilled water. Each samples was then aseptically transferred to field (Soil, Sewage and Cow dung) and individually placed into sterile minimal salt medium (MSM) in laboratory.

Biodegradation of PS foam and PET powder under laboratory condition

250 mg of prepared plastic samples (PET and PS foam powder) separately were directly inoculated into 250 ml of minimal salt medium (Soil + Minimal Salt medium, Sewage + Minimal salt medium and cow dung + Minimal salt medium) . The culture was carried out for a month on a rotary shaker at 120 rpm. Fungal population was counted every week by pure plate method using Rose Bengal Chlormophenicol Agar. Further plates were incubated at 28 ^{o}C for 7 days and developed colonies were isolated and sub-cultured to get pure colonies and stored in refrigerator for further studies. Further, Fungal isolates were identified by using Lacto-phenol cotton blue stain and observed under the Light Microscope (Nigam,1965; War cup,1950).

Biodegradation of PS foam and PET powder under field condition

Soil, Cow dung and sewage were transferred to plastic tray and inoculated with PS and PET flakes separately for a period of 70 days. Soil, cow dung and sewage not inoculated with PS foam and PET was maintained as control simultaneously .At end of the 70th day, the PS foam and PET inoculated in soil, cow dung and sewage were collected and sonicated in MSM. Fungi in the MSM were isolated using Rose Bengal Chlormophenicol Agar by pour plate methods. Further, colonies were identified by adopting Lacto-phenol cotton blue stain and observed under the Light Microscope (Nigam,1965; War cup,1950).

Scanning Electron Microscopy (SEM)

The scanning electron microscopic analysis of surface of PET and PS foam flakes were carried out using Scanning electron microscope (VEGA3 TESCAN). The surface of the treated PET samples was coated with conductive heavy metals such as gold/ palladium.

Statistical analysis

The mean and standard error for fungal populations and treatments were compared using analysis of variance (Two way-ANOVA) and Duncan new multiple range test (DMRT) was applied to test the significance of means by SPSS version 16.0.

RESULTS

Statistically significant variation between the fungal population of soil (control: 29.4167 cfu mL^{-1}) and treated groups ($P<0.001$; $F=105.896$) (Sewage with PET : 23.0833 cfu mL^{-1}: soil with PET : 32.6667 cfu mL^{-1}, Cow dung with PET : 43.9167 cfu mL^{-1}) was evident (Table 2). This could be attributed to the adherence of fungi to the PET surface and in turn utilise it as a carbon source. Among the treated groups, cow dung with PET harboured significantly higher population of fungi when compared to the other treated groups and control. Significantly higher fungal population was registered after third (39.0833 cfu mL^{-1}) and fourth

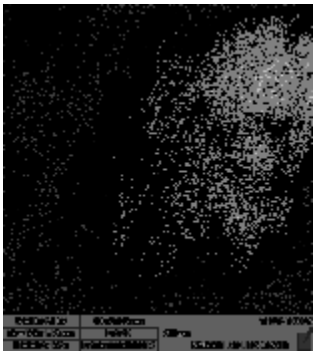


Fig 1. SEM image of untreated PET flakes

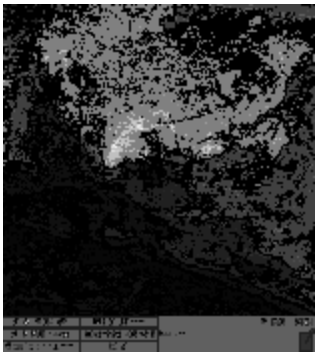


Fig 3. SEM image of PET flakes buried in sewage for a period of 70 days

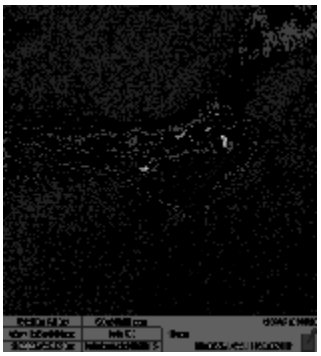


Fig 2. SEM image of PET flakes buried in the soil for a period of 70 days

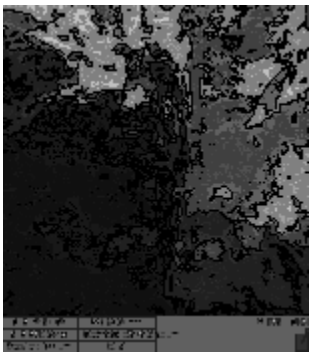


Fig 4. SEM image of PET flakes buried in cowdung for a period of 70 days

Table 1. Experimental design showing inoculation of PET and PS in soil, cowdung and sewage

	Plastic samples	Control	Soil	Cowdung	Sewage
Laboratory	PET powder	—	+	+	+
conditions	PS powder	—	+	+	+
Field	PET flakes	—	+	+	+
condition	PS foam	—	+	+	+

- untreated samples, + treated samples

Table 2. Variation in the Fungal population on PET and PS foam inoculated in sewage, soil and cowdung

Weeks	PET	PS Foam
1st week	22.5833c	16.1667d
2nd week	28.6667b	35.5000c
3rd week	39.0833a	45.6667 b
4th week	38.7500a	1.0183E2a
F value	90.540***	3.560E3***

*** Significant at p<0.001.
In a column, figures having dissimilar letters differ significantly according toDuncan New Multiple Range Test (DMRT)

Table 3. Variation of fungal population on PET and PS foam during experimental period

Treatments	PET	PS foam
Control	29.4167 c	29.4167 d
Sewage	23.0833 d	49.7500 c
Soil	32.6667 b	67.0833a
Cow dung	43.9167 a	52.916 b
F Value	105.896***	634.661***

*** Significant at p<0.001.
In a column, figures having dissimilar letters differ significantly according toDuncan New Multiple Range Test (DMRT)

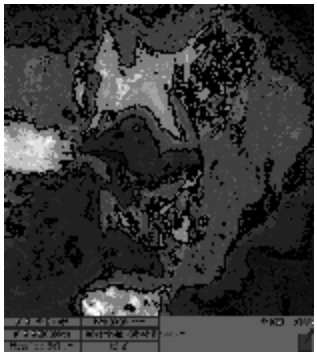


Fig 4a. SEM image of PET flakes buried in cowdung for a period of 70days

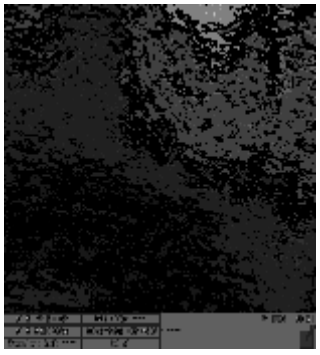
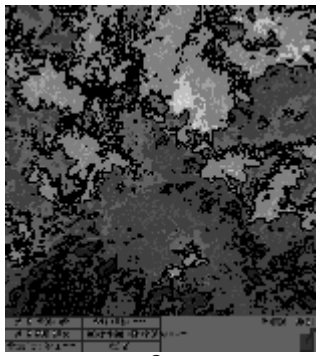


Fig 5. SEM image of untreated PS foam flakes



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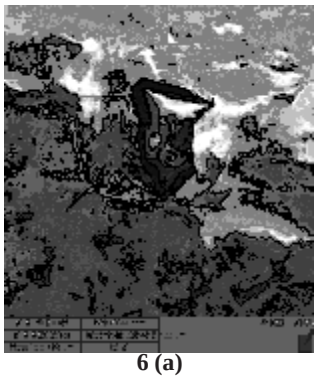
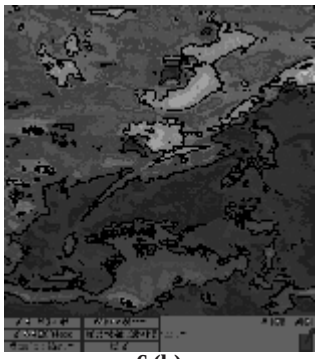


6 (a)



6 (b)

Fig 6, 6a, 6b. SEM image of PS foam flakes buried in soil for a period of 70 days

week (38.7500 cfu mL^{-1}) of incubation of PET with sewage, soil and cow dung when compared to the first (22.5833 cfu mL^{-1}) and second week (28.667 cfu mL^{-1}) (P< 0.001 ; F = 90.540) (Table -3). Compared to control (29.4167 cfu mL^{-1}), inoculation of sewage (49.7500 cfu mL^{-1}), soil (67.0833 cfu mL^{-1}) and cow dung (52.9167 cfu mL^{-1}) with PS foam significantly enhanced the fungal growth (Table 3) (P<0.001 ; F = 634 .661). Significantly increase in the fungal population was evident after inoculation of PS foam with soil, cowdung and sewage during the course of the experiment (First week : 16.1667 cfu mL^{-1} ; second week : 35.5000 cfu mL^{-1} ; third week ; 45.6667 cfu mL^{-1}; fourth week : 1.0183E2 cfu mL^{-1}) (P< 0.001 F= 3.560E3) (Table -3). The fungal species adhering to the surface of PET and PS foam flakes were *Fusarium* sp., *Penicillium* sp and *Aspergillus* sp., (Fig. 1,2,3)

Micromorphological changes on PET and PS foam flakes observed under SEM

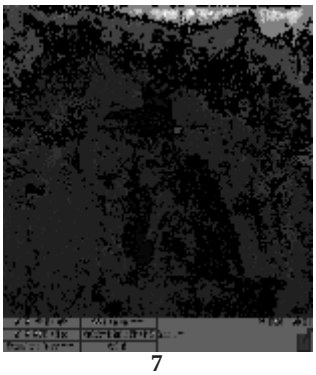
The SEM images of untreated PET flake are depicted in Fig. 1. It has a smooth continuous surface. After 70 days the soil burial, PET flakes were recovered, washed and observed under SEM. Damage to the surface of PET flake could be seen as cracks, surface corrosion and duckings (Fig. 2) . Extensive surface corrosion and cracks could be detected on PET surface inoculated in sewage. However, after sewage burial, fungal colonization was observed. Extensive hyphae, organized into colonies were observed on PET flakes buried in sewage (Fig. 3). Degradation of PET flakes in the form of crystals and extensive surface damage was detected in SEM image of PET buried in cow dung (Fig.4,4a).

The surface of the untreated PS foam flakes has a continuous surface (Fig. 5). SEM image of PS buried in soil resulted in formation of many holes on their surface (Fig. 6). Further, crystals of PS foam embedded in the holes could be seen under SEM microscope (Fig. 6a). Damage to the surface of soil buried PS foam could be seen under the fungal hyphae, which is evident in the SEM images (Fig. 6). The growth of fungal mycelium on the entire surface of PS foam buried in sewage, resulted in the formation of small holes and duckings (Fig. 7). A single fungal hyphae was observed in the SEM image of sewage PS foam

surface (Fig. 7a). Extensive damage in the form of fracture, surface corrosion is evident in Fig.7b and Fig.7c. SEM image of PS foam buried in cow dung reveals formation of cracks (Fig. 8).

DISCUSSION

Fungi like *Fusarium sp.*, *Penicillium* sp. and *Aspergillus* sp., were able to colonise and grow on the PET and PS foam plastic surfaces. Further, Colonisation of fungi on the surface of PET and PS foam buried in soil, sewage and cow dung was evident in SEM images. This finding lies in parallel with the observations of Barratt *et al*., (2003) who have found microbial colonization on the surface of polyester PU (Poly urethane). Further, extensive hyphae organized into colonies , were observed on all samples buried in soil (Dale and Squirrel, 1990). SEM images of PS foam flakes buried under soil exhibited extensive coverage of hyphae on



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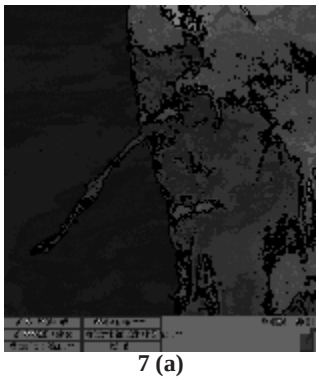
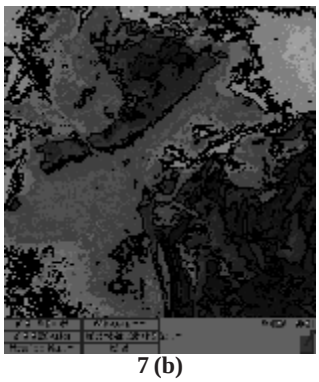
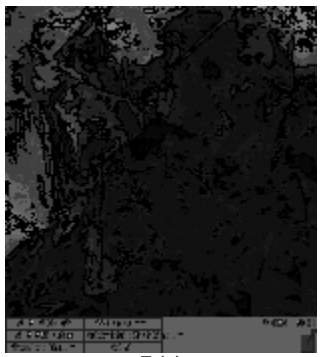


7 (a)



7 (b)



7 (c)

SEM image of PS foam flakes buried in sewage for a period of 70 days

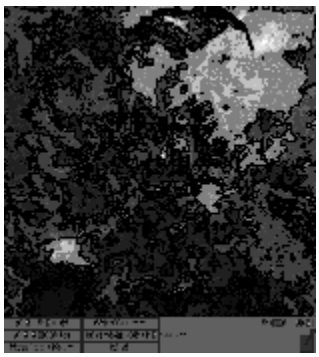


Fig 8. SEM image of PS foam flakes buried in cow dung for a period of 70 days

their surface. This observation in well supported by Barratt *et al*.,(2003) who have observed a high coverage of the PU buried in soil at 20- 80 % water holding capacity for 44 days by fungal hyphae and spores. The cracking observed for PET flakes buried in soil , sewage and PS buried in cowdung in this study in typical of the effects of degradation of PET and PS as a result of soil, sewage and cowdung burial. This result is in good accord with the findings of Dale and Squrell (1990) and Barratt *et al*., (2003) who have reported PU degradation as a result soil burial. Griffin (1980) has suggested that cracking of PU after soil burial could cause

damage to PU, which allows penetration of fungal mycelium into the plastic leading to increased mechanical damage of the plastic (Dale and Squirrel, 1990). This explanation could be extended to the present observation that damage to PET and PS foam occurred after soil and sewage burial after 70 days. Crystal formation is evident on the PET flakes surface buried in cow dung and PS foam buried in soil. There have been few studies that have established a link between plastic degradability and the degree of crystallization of the polymer (Griffin *et al*., 1980 ; Bonhomme *et al* ., 2003 ; Muller, 2005). Nowak *et al*., (2011) have observed that *Penicillium funiculosum* is able to degrade PET inoculated Binolle **.**

Soil, sewage and cow dung burial for longer than 70 days reveal that other organisms are also involved in the degradation process. Colonization are known to occur on other plastic *in situ*. For example, the filamentous fungus *Aureo basidium pullulans* (primary colonies) is followed by *Rhodotorula aurantiaca, Kluyveromyces spp.*, and other yeast and yeast –like fungi (secondary colonizers) on environmentally exposed plasticized polyvinyl chloride (Nowak *et al* ., 2011). Both fungi and bacteria have been isolated from the surface of soil buried polyester PU (Webb *et al*.,2000; Pathirana & Sea, 1984). Bentham *et al*., (1987) have isolated a number of fungi belonging to the genera *Aspergillus sp., Emericella sp., Fusarium sp., Penicillium*, *Trichoderma sp.* and *Gliocladium sp.*, from the surface of polyester PU foam buried for 28 days in soil (Nakajima-Kambe *et al*., 1995). The presence of *Penicillium* sp., on the surface of PET and PS foam agrees with that of Barratt *et al*., (2003) who have identified three major colony types of polyester PU degrading fungi *Nectria gliocladioides* (white colony type), *Penicillium ochrochloron* (green colony types) and *Geomyces pannorum* (peach colony type). Soil burial shows the effect of soil microbes on the plastic and is widely used for assessing the extent of degradation of plastic by microbial communities in soil (Nakajima-Kambe *et al*., 1995; Barratt *et al.*, 2003). Two isolates of *Pestalotiopsis microspora* with the ability to efficiently degrade and utilize PUR as the sole carbon source when grown anaerobically, a unique observation among reported PUR biodegradation activities (Bentham *et al*.,1987).

CONCLUSION

Accumulation of excessive use of plastic waste in day to day life has been of environmental concern as it could have adverse impact on the biota. Further, their degradation process is a time consuming one. Hence the present investigation was initiated to utilize indigenous fungi prevalent in the PS and PET waste to degrade plastics under soil, sewage and cow dung burial conditions. Comparatively, *Penicillum sp*., is able to degrade efficiently PET and PS foam flakes buried in sewage, soil and cow dung. From the results of the present study it is evident that fungi could be used as a bioagent to degrade PET waste and PS foam.

ACKNOWLEDGEMENT

The authors register their thanks to the University Grants Commission New Delhi, India for providing financial assistance.

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