WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 4, 31-40. Conference Article ISSN 2277 – 7105

MICROBIAL DEGRADTION OF POLYESTER POLYUREATHANE

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Article Received on
01 January 2018,

Revised on 22 Jan. 2018,
Accepted on 12 Feb. 2018

DOI: 10.20959/wjpr20184-10762

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ABSTRACT

The present study focuses on isolation of microorganisms viz. bacteria, lower and higher fungi from polluted sites having plastic waste products and checking their ability to degrade polyureathane (PU). Different fungal isolates were screened for degradation of PU for 15 to 30 days. Incubation of PU (1x1 inch and 1mm thickness) with isolated fungi and bacteria resulted in weight reduction of PU film. The preliminary studies showed that one isolate was a *Bacillus sp*, three isolates were lower fungi species viz, *Aspergillus sp, Trichoderma sp.* and *Penicillum sp* and one higher fungi species i.e. *Leucoagaricus sp.* Bacillus sp after 3 months showed complete degradation of the whole PU sheet. The isolates showed weight reduction of PU viz. *Aspergillus* sp41.16%, *Trichoderma* sp 72.31%, *Penicillum* sp 41.68%, *Leucoagaricus* sp 79.69% and Bacillus sp 40.18%. The SEM results showed cell growth, crack and holes on the PU film. The work is concluded with a comparative account of the degradability of the fungal isolates and bacteria.

KEYWORDS: Polyureathane, biodegradation, *Bacillus sp, Aspergillus sp, Trichoderma sp, Penicillum sp, Leucoagaricus sp.*

INTRODUCTION

Degradation of synthetic polymers (polyethylene, polysterne) by microbes is a process termed as biodegradation. Polyurethanes (PU) are a versatile class of man-made synthetic polymers which has been widely used in many applications such as furniture, adhesives, constructional materials, fibers, paddings, paints, elastomers, coatings and synthetic skins.[1,2] Otto Bayer was the first to produce PU in 1937 by a polymerization reaction of di- or

polyisocyanates and polyols.[3,4] By varying the nature of the components as well as the degree of cross-linking, PU materials with almost any desired properties can be synthesized. Solid media containing suspended polymer particles are useful in the isolation of potent degraders.[5]

Plastics are the polymers which are generally made from petroleum products. Commonly plastics are used in many purposes including packaging, disposable diaper backing, agricultural films and fishing nets. Plastics and their use has become a part in all sectors of economy. Infrastructures such as agriculture, telecommunication, construction, packaging, health and medical are all high growth areas that ensure present demand for plastics. Plastic is used in daily activities viz. electrical fittings, plastic furniture, defense materials, automobiles parts pipes and fittings, packages and sanitary wares, flooring, artificial leathers, bottles and jars and other household item. The most widely used plastics used for packaging are polyethylene (LDPE, MDPE, HDPE, LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), polybutylene terephthalate (PBT), nylons. Polyhydroxyalkanoates (PHAs) in an environment such as soil or compost, they are completely degraded to CO2 and water, without the need of any light or chemical treatment, and without leaving any persistent residues.[5,6] Almost invariably, organic polymers mainly comprise plastics. Polythene and polypropylene materials are resistant to biodegradation. Due to this resistance, plastics that are disposed in landfills remain in their original form in perpetuity. Plastics offer a number of advantages over alternative materials–they are lightweight, low cost, extremely durable and relatively unbreakable.[7] The majority of these polymers are based on chains of carbon atoms alone or with sulfur, oxygen, nitrogen hydrogen, silicon, chloride as well. Recently, polyester-PUR-degrading enzymes have been purified and their characteristics reported. Among them, a solid-polyester-PUR-degrading enzyme (PUR esterase) derived from *Comamonas acidovorans* TB-35 had unique characteristics. This enzyme has a hydrophobic PUR-surface-binding domain and a catalytic domain, and the surface-binding domain was considered as being essential for PUR degradation.[8] Polyurethane (PUR) is produced by the diisocyanatepoly addition process.[9] This property of the polymer by repeating unit's molecular structure has allowed plastics to become an indispensable part of the twenty-first century world. Microorganisms can damage the structure and function of synthetic polymers. According to Flemming, the main types of damage include (i) biological coating masking surface properties, (ii) increased leaching of additives and monomers that are used as nutrients, (iii) production of metabolites (e.g., acids),

(iv) enzymatic attack, (v) physical penetration and disruption, (vi) water accumulation, and (vii) excretion of pigments.[10]

Problem of degradation of plastic is a serious issue since the plastic usage has been increasing over decades and no proper method has been used for ecofriendly disposal of plastic and thus it leads to accumulation which directly or indirectly affects flora and fauna. Increased usage of plastic at dumpsites results in increased plastic accumulation which harms the ecosystem. Biodegradation of plastic by microflora has given best results than other methods of decomposition and degradation.

MATERIAL AND METHODS

Sample source: Soil sample collected from plastic waste collection center from Spicer Aundh road Pune and polluted Mula Mutha River. Higher fungi sample were collected from Abeda Inamdar Senior College campus Pune, India.

Material: PU(Polyureathane)sheet provided from MIDC Pune was used to conduct the research.

Isolation of Polyurethane Degrading Microorganism

Soil sample collected from plastic polluted source was diluted and spread plated on minimal media and Potato Dextrose Agar. A total of 8 fungal isolates and 3 bacteria were obtained.

The isolated fungi was cultured and purified on Potato Dextrose Agar (Hi media)at RT and the isolated bacteria and fungi were spread plated on minimal media containing sterile PU sheet. Plates were incubated at Room temperature for 30 days.

Control contained the sterile PU film placed on minimal media sealed without fungus or bacteria.

All assays for studying degradation of PU were carried out in minimal medium containing (Grams per liter of distilled water): $NaNO_3$ 2.0; KH_2PO_4 0.7; K_2HPO_4 0.3; KCl 0.5; $MgSO_4 \cdot 7H_2O$ 0.5; $FeSO4 \cdot 7H_2O$ 0.1. The pH was adjusted to 6.0–6.5.Sterile pre-weighed PU films were aseptically transferred to all the flasks.

PRETREATMENT OF POLYURETHANE

Plate assay

Polyurethane sheet was cut into films of 1x1 cm and 1 mm thickness. PU film was sterilized with absolute alcohol and kept in desiccator. These sheets were aseptically transferred onto the plates and were sealed with paraffin and kept at R.T. for one day for sterility check. The fungal spores from PDA were aseptically transferred to the plates containing sterile PU film. The PU film was pre weighed before sterility check. For bacteria the minimal media plate containing the purified isolate of KDD5 isolate was spread plated onto plate containing disinfected PU film. For control sterile PU film was maintained on minimal agar plate and sealed with paraffin.

Flask assay

PU sheet was cut into 1x1cm and 1 mm thickness. These sheets were disinfected with absolute alcohol and were transferred aseptically to flask containing 50ml sterile minimal medium broth.

The five isolates *Bacillus sp, Aspergillus sp, Trichoderma sp, Penicillum sp, Leucoagaricus sp* obtained were purified and inoculated into each flask respectively. Each PU film was pre weighed and noted down on each flask. PU film after aseptically transferring into each flask were wrapped with paraffin and kept for sterility check to see if any contamination occurs due to PU sheet. These flasks were kept in shaker at 1500 r.p.m at R.T. After sterility check i.e. after 24 hrs each flask was inoculated with *Bacillus sp, Aspergillus sp, Trichoderma sp, Penicillum sp, Leucoagaricus sp* respectively. *Aspergillus sp, Trichoderma sp, Penicillum sp, Leucoagaricus sp* spores from PDA plates was transferred aseptically, and for *Bacillus sp* loopful of the bacterial suspension was inoculated into the 5^{th} flask containing sterile PU film. The flasks were kept for incubation for 20 days at R.T. 1500 r.p.m. Each flask was sealed with paraffin to avoid carbon dioxide to enter into the flask. Each isolate depended on PU film as it was the sole carbon source. For control, sterile polyurethane films were incubated in culture medium containing 0.05% sodium azide to inhibit microbial contamination. Studies were carried out in triplicates and mean and standard deviation are reported.

Film Harvest from flask

Each PU film from flask were removed aseptically and washed with sterile D.W several times to remove cell mass from surface of film. The film was than washed with 70% ethanol

to remove cells and mycelia growth from surface of PU film. The PU film was washed several times alternately in D.W and ethanol to remove possible cell debris.

Each film was washed in aseptic condition and one at a time to avoid confusion the film were placed in sterile petri plate with labeled pre weight of PU film.(as on flask were noted). Each plate was kept in laminar air flow for drying up for 20-25 mins.

Film Harvest from Plate

Each PU film was removed with sterile forceps and placed in beaker containing D.W.

The same process for harvesting was applied as that for flask. Alternate wash with D.W and 70% ethanol several times was done to remove maximum mycelia growth and cell mass grown on PU film. Each film was placed in sterile petri plate with labeled initial weight of each film respectively.

Analysis of degradation by quantitative and qualitative assay

Weight loss measurement (Quantitative assay)

Degradation analysis for weight loss measurement was considered as a quantitative assay as PU films would show reduction in weight when compared with initial weight.

Weight of each PU film was noted before incubation with the isolates and after incubation of 20 days. The weight of each film was taken and noted down to check if there was any reduction of weight of PU film comparing it with initial weight. Weight of control was checked after harvesting the film with the same process to check any reduction in weight due to external factors.

The percent weight reduction was computed with the formula:

% weight reduction= (W1−W2/W1) ×100, where *W1 is the pre-incubation weight and* W2 is the post-incubation weight of the polyurethane films. Each value represents mean ± SD of triplicates.

FESEM analysis (Qualitative assay)

FESEM analysis was considered qualitative assay as it would show spots, cracks, holes and submerged mycelia in PU film. Pieces of fungal incubated polyurethane films and bacterial incubated polyurethane film were fixed overnight at 4 °C in 4% glutaraldehyde in 0.05M phosphate buffer (pH 7.3) and washed three times (10 min each) in 0.05Mphosphate buffer.

Samples were then dehydrated through 70%, 80%, 90%, and 100%ethanol (5 min in each stage) and three changes in 100% ethanol at room temperature. Samples were then dried under vacuum. They were then mounted, sputter-coated with gold and examined.

The PU films 3 from flask assay and 2 film from plate assay was selected for FESEM analysis. FESEM analysis was considered as a qualitative assay. The films were analyzed from Savitribai Phule Pune University (SPPU) Central Instrumentation Centre, Pune, India.

RESULTS

Biodegradation is the most ecofriendly method to resolve the plastic waste problem. 4 fungi, 1 bacterium and 1 higher fungus showed adherence to PU film utilizing PU film as sole carbon source.

Polyurethane Weight Loss

A comparative account of reduction of weight by plate and flask assay has been listed in Table 1 and 2. PU degradation was monitored by measuring weight of polyureathane films before and after incubation with *Trichoderma sp*, *Aspergillus sp*, *Leucoagaricus sp*, *Penicllium sp*, and *Bacillus sp*.

Table 1: Results for Plate Assay.

Isolates	Initial weight	Final weight	% Reduction
Aspergillus sp	0.186	0.095	48.92%
Trichoderma sp	0.185	0.084	54.59%
Penicillum sp	0.183	0.103	43.71%
Leucoagaricus sp	0.181	0.059	67.40%
Bacillus sp	0.187	0.119	36.36%
Control	0.185	0.185	0%

Table 2: Results for Flask Assay.

Isolates	Initial weight	Final weight	% Reduction
Aspergillus sp	0.184	0.101	45.10%
Trichoderma sp	0.181	0.099	45.30%
Peicillum sp	0.186	0.094	49.46%
Leucoagaricus sp	0.187	0.078	58.28%
Bacillus sp	0.182	0.128	29.67%
Control	0.183	0.183	0%

FLUROSCENT EMISSION SCANNING ELECTRON MICROSCOPE

FESEM has better resolution and greater magnification than SEM and was used for the research to check the effect of growth on the PU film. FESEM images with different magnification showed holes, pinholes, cracks and submerged mycelia growth on PU film. The attachment of the spores and hyphae to the surface suggests that the development of a biofilm may be an important step in the biodegradation of polyurethane.[1] Spots showed spores and hyphae which were dispersed over the PU film. FESEM studies were carried out to study the effect of the growth of *Aspergillus sp, Penicillum sp, Trichoderma sp, Leucoagaricus sp and Bacillus sp* on PU film. Figure a, b, c, d, e and f respectively represents FESEM photomicrographs showing submerged mycelial growth and pits.

1. *Aspergillus* sp shows submerged mycelia growth and pits.
2. *Penicillum* sp shows cracks, dark spots and submerged mycelial growth.
3. *Trichoderma* sp shows submerged mycelial growth, spots indicating spores.
4. *Leucoagaricus* sp shows erosion, and a deep pit surrounded by submerged hyphae.
5. *Bacillus* sp showing hole at 10 000x.

In case of control sample (Fig. f), surface was smooth. Large number of fungal spores and hyphae can be seen dispersed over the polyurethane surface. The attachment of the spores and hyphae to the surface suggests that the development of a biofilm may be an important step in the biodegradation of polyurethane. The ability of *Leucoagaricus sp*. to degrade PU was demonstrated by numerous holes produced in solid PU observed by FESEM The surface of materials has turned from smooth to rough with cracking. This may be due to the compounds secreted extracellularly by the microbes that may break the complex molecular structure of plastics.

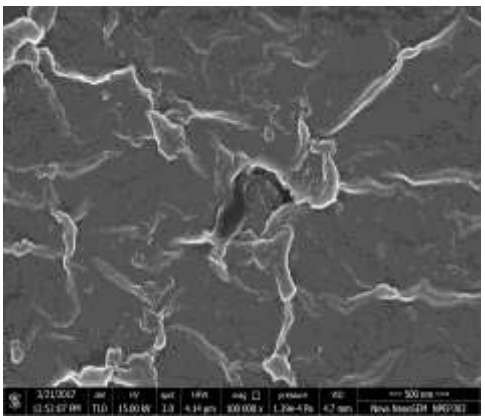

Fig. 1(a).

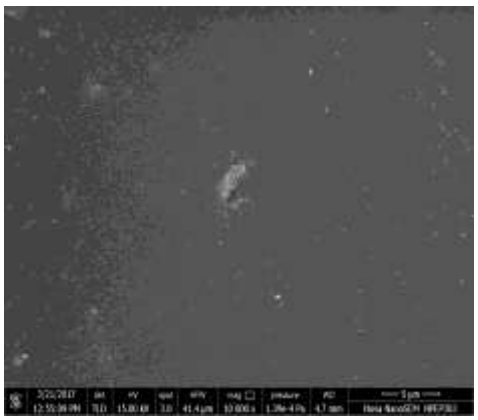

Fig. 1(b).

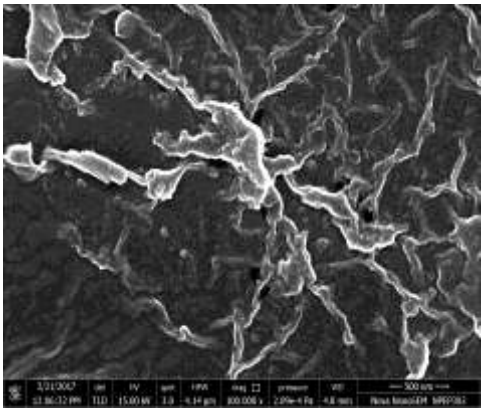

Fig. 1(c)

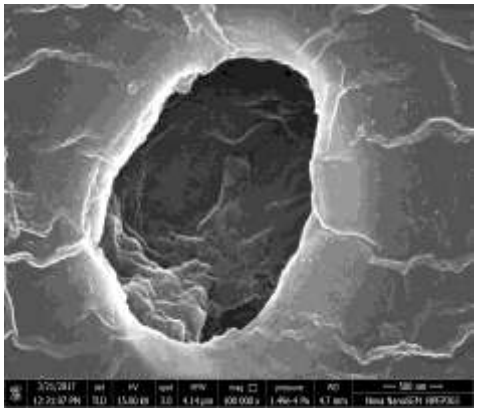

Fig. 1(d)

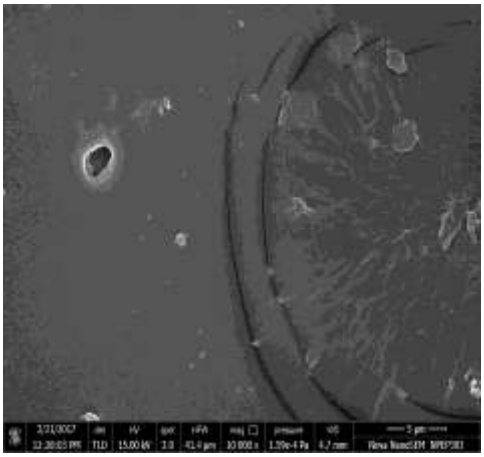

Fig. 1(e)

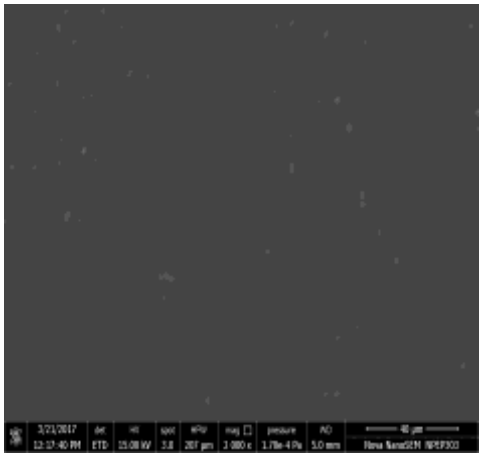

Fig. 1(f)

Fig. 1: Scanning electron micrographs of polyurethane with *Aspergillus sp, Penicillum sp, Trichoderma sp* (a, c, d) Leucoagricus sp (e) and *Bacillus* sp (b) and Control (f).

DISCUSSION

Microorganisms play a significant role in biological decomposition of materials, including synthetic polymers in natural environments. High-density and low-density polyethylenes are the most commonly used synthetic plastics and they are slow in degradability in natural environments, causing serious environmental problems. There is no report as far on Biodegradation of Polyester Polyurethane by *Leucoagaricus sp* i.e. higher fungi that showed highest % weight reduction of PU film on plate assay 67.40% and on flask assay 58.28%.[1,2,]

Aspergillus sp showed lesser degradation activity on plate assay 48.92% on comparing with flask assay it showed 45.10% degradation activity[1] *Bacillus* sp showed complete degradation of Polyester Polyurethane film 1x1cm and 1mm in thickness on plate assay in 3 months.[3] FESEM for 5 isolates showed better resolution than SEM depicting cracks, hole and mycelia growth on PU film. Plate assay has been performed concluding that it provides natural

environment for degradation on comparing with flask assay where specific temperature and r.p.m. is provided to the growth of microorganism.[5] Recent work on plate assay with different Technique has been performed.[5] On comparing with previous research *Leucoagaricus sp* was found to degrade synthetic polymer i.e. a higher density plastic.[10]

All 5 isolates utilized polyester polyurethane as the sole source of carbon.[11,12] Comparative account of 5 isolates showed degradation ability of fungi and bacteria Fungi are predominant in degrading polyester polyurethane than bacteria.[13,14] Fungi degrade plastic at a faster rate when compared with degradation activity of bacteria. Various soil samples were screened for the presence of microorganisms specially fungi both higher and lower which showed the ability to degrade polyurethane sheet rather than polyurethane compound.[15]

CONCLUSION

Plastic biodegradation reduces environmental pollution and will give better sustainable environment. Further studies could be carried out to find enzymes that would be responsible for degradation of PU, carry out Soil burial experiment with same isolates on low density PU and to carry out the study at field trial and use coloured PU sheet to check whether these isolates could degrade them.

ACKNOWLEDGEMENTS

We would like to thank Dr. E. M. Khan, Principal, and Abeda Inamdar Senior College for providing us with the necessary infrastructure conducive for research.

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