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Biodegradation of Synthetic Polymers by Fungi

M.R. KHAN[1,*], M. ARSHAD[2] and S.A. RAZA[2]

[1]Center of Policy and Environment, Lahore School of Economics, Lahore, Pakistan
[2]Department of Chemistry, Government College University, Lahore, Pakistan

*Corresponding author: Fax: +92 42 36560905; Tel: +92 42 36560954; E-mail: drrafiq@lahoreschool.edu.pk; rafiq_aquarius@yahoo.com

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The work deals with an important environmental issue *i.e.*, the disposal of plastic wastes. Polyethylene, nylon-6,6 and poly lactic acid were selected as substrates and checked for their biodegradation by fungal species such as *Aspergillus niger*, *Aspergillus orhyzae*, *Rhizopus orhyzae*, *Rhizopus oligosporus* and *Trichoderma viride*. Biodegradation was monitored by following the changes in pH, biomass and weight loss. All species failed to degrade polyethylene and nylon-6,6. Polylactic acid, of course, was consumed by all the fungal species.

Key Words: Biodegradation, Synthetic polymers, Fungi, Pakistan.

INTRODUCTION

Polymers are long chain molecules composed of building blocks termed as monomers. Polymeric materials, such as polyethylene and others are used in almost all areas of human activity, owing to their unique characteristics. Since the onset of the second half of the twentieth century, the worldwide production of synthetic plastics and fibers has grown so much that it now exceeds even that of steel[1]. More than half of polymeric materials based products are currently used in outdoor environment. As a consequence, plastic wastes accumulating in the environment pose a big ecological threat not only to humans but also to terrestrial and marine wildlife. Unfortunately drastic rise in the use of non-biodegradable plastic materials during the past three decades has not been accompanied by a corresponding development of procedures for the safe disposal or degradation of polymers. One of the most problematic plastics, in this regard, is probably polyethylene, which being resistant to microbial attack is the most inert synthetic polymers[2]. That is the reason that the disposal of polyethylene materials is a serious problem. Normally, polyethylene plastics are thrown in water bodies as garbage material or discarded for dumping in landfills to decompose/degrade[3]. It is widely accepted that the resistance of polyethylene to biodegradation stems from its high molecular weight, its three dimensional structure and its hydrophobic nature, all of which interfere with its attack by micro-organisms[4]. Attempts to facilitate colonization of polyethylene by adding nonionic surfactants to the culture medium promoted the biodegradation of polyethylene[5-7] polylactic acid (PA) or polylactide (PLA) is a biodegradable, thermoplastic. It is an aliphatic polyester derived from renewable resources, such as corn starch or sugarcane. Although polylactide has been known for more than a century, it has been of commercial interest in recent years due to its biodegradability. So far, biodegradable plastics have proven too costly and have found limited general use. The critics have pointed out that the only real problem they address is of roadside litter, which is generally regarded as a secondary issue. When such plastic materials are dumped into landfills, they can become "mummified" and persist for decades even if they are supposed to be biodegradable[8].

Pakistan is also facing the problem highlighted above and the challenge before us is how to accomplish the biodegradation of various plastics (biodegradable and non-biodegradable) in context of Pakistan where no work seems to have been undertaken on this important issue.

As degradation of plastics is of huge concern in context of environmental pollution, the work undertaken here was carried to try biodegradation of polyethylene, nylon and polylactic acid using five fungal species, *Aspergillus niger*, *Aspergillus orhyzae*, *Rhizopus orhyzae*, *Rhizopus oligosporus*, *Trichoderma viride*. This work may lead us to innovations which may help in plastic pollution control concern.

EXPERIMENTAL

Fermentation technique was used as the experimental protocol.

Organisms: *Rhyzopus orhyzae*, *Rhyzopus oligosporus*, *Aspergillus niger*, *Aspergillus orhyzae* and *Trichoderma viride* were obtained from Biochemistry Laboratory, Department of Chemistry, GCU, Lahore, Pakistan.

Reproduction of the culture and preparation of inoculum: Selected fungal species were used for the present study and obtained from Biochemistry Laboratory of Government College University Lahore (GCUL). The isolated cultures were maintained on potato dextrose agar slants containing 12 g/L potato extract, 20 g glucose and 15 g of agar. Sporal suspensions were prepared by washing the slants with sterilized water. The spores were centrifuged at 120 rpm for few minutes in 20 cm sterilized centrifuge tube. The suspension was diluted with sterilized water. The optical density of sporal suspension was determined using spectrophotometer at 550 nm. Care was taken that in every transfer of spores, the suspension had the same optical density to keep the sporal population transferred constant. This spore suspension was used for inoculation.

Preparation of growth medium for nylon and fermentation: The submerged culture fermentation was carried in conical flasks. The fermentation medium was prepared dissolving, per liter of distilled water: 10 g glucose, 1 g KH_2PO_4, 0.2 g NaH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2$ and 0.169 g (1 mM) $MnSO_4 \cdot H_2O$. The liquid medium thus formed was distributed into 250 mL Erlenmeyer flasks (100 mL each) fibers of commercial grade nylon-6 were cut into *ca.* 2 cm long fragments and their 200 mg were added to 100 mL medium in each flask. The pH of the medium was noted, the flasks are autoclaved at 121 ºC for 20 min and subsequently cooled to room temperature. Each flask was inoculated with 5 mL inoculum prepared above and transferred to an orbital shaking incubator. The fermentation was carried out at 30 ºC. Changes in nylon-6 degradation were monitored on the basis of weight loss analysis. It was carried out on monthly basis[9]. An abiotic flask was used as a blank. It contained fermentation medium but no inoculum and thus no biological species.

Growth medium for polyethylene and fermentation: The procedure followed for inoculum preparation and fermentation was the same as in case of nylon except the medium for polyethylene contained 3.0 % (wt/vol) malt extract in deionized water (pH 4.5) for fungal strains. 100 mL of culture medium in 250 mL Erlenmeyer flasks was incubated with shaking for 24 h before inoculation to ensure asepsis. Culture medium was inoculated with spores from specific microorganism and was incubated with shaking at 120 rpm for 6 weeks at 28 ºC for the fungus to cultivate. Control samples were also stored for reference studies[9]. Weight losses were measured for every fungus to monitor polyethylene degradation after every 15 days. Analysis was carried out on bi-monthly basis in duplicate.

Growth medium preparation for polylactic acid and fermentation: Culture medium was composed of $(NH_4)_2SO_4$ (0.6 g/L), KH_2PO_4 (1.3 g/L), $Na_2HPO_4 \cdot 2H_2O$ (0.12 g/L), $MgSO_4 \cdot 7H_2O$ (0.3 g/L) and KCl (0.3 g/L). Each mineral salt was sterilized separately followed by the addition of vitamins. Two grams of polylactic acid oligomers were added to each sample containing 50 mL of culture medium in 250 mL Erlenmeyer flasks. Duplicate storage was used to monitor the biodegradation of polylactic acid. Control was also stored under same storage conditions[10]. The fermentation was carried as above and fugal effects on degradation of plastics were studied by monitoring the changes in pH and biomass. All species were subjected to further analysis to determine polylactic acid degradation. Oligomers of polylactic acid were subjected to shake-flask culture for the measurement of PLA-Oligomer degradation.

RESULTS AND DISCUSSION

The weight losses during fermentations are reported in Table-1. The results indicate that there was no weight loss in case of control sample. After fifteen days of fermentation weight losses of 0.0004, 0.0002, 0.0004, 0.0002 and 0.0003 were observed for *Rhyzopus orhyzae*, *Rhyzopus oligosporus*, *Aspergillus niger*, *Aspergillus orhyzae* and *Trichoderma viride*, respectively. After fifteen days no further loss in weight could be observed up to two months. Fig. 1 shows the percentage degradation of polyethylene.

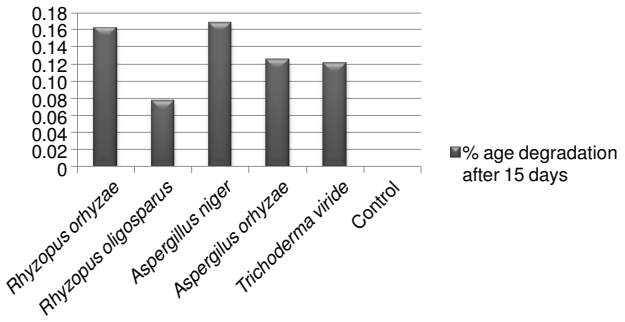

Fig. 1. Polyethylene degradation

Initial losses of weight were probably due to the removal of fillers present in commercial polyethylene. All mentioned species failed to degrade polyethylene in two month experimental procedure and the experiments were extended up to six months but no degradation was observed. Five fungi were tested for their ability to degrade synthetic polymer polyamide-6,6 generally known as nylon-6,6 in shake flask culture (Table-2). No degradation was observed for any fungal species after 60 days shake flask cultivation. After 60 days the cultivation was continued for another 4 months period but still no degradation was observed. All five fungi exhibited negative results in nylon degradation as no weight loss was observed in any culture. Similar findings were observed by some other workers also[11].

TABLE-1
PERIODIC WEIGHT MEASUREMENTS (g) IN CASE OF POLYETHYLENE DEGRADATION

Strains	Initial weight	After 15 days	After 30 days	After 45 days	After 60 days
Rhyzopus orhyzae	0.2454	0.2450	0.2450	0.2450	0.2450
Rhyzopus oligosporus	0.2572	0.2570	0.2570	0.2570	0.2570
Aspergillus niger	0.2356	0.2352	0.2352	0.2352	0.2352
Aspergillus orhyzae	0.2381	0.2378	0.2378	0.2378	0.2378
Trichoderma viride	0.2469	0.2466	0.2466	0.2466	0.2466
Control	0.2388	0.2388	0.2388	0.2388	0.2388

TABLE-2
PERIODIC WEIGHT LOSSES (g) IN CASE OF NYLON DEGRADATION

Strains	Initial weight	After 15 days	After 30 days	After 45 days	After 60 days
Rhyzopus orhyzae	0.2014	0.2014	0.2014	0.2014	0.2014
Rhyzopus oligosporus	0.2010	0.2010	0.2010	0.2010	0.2010
Aspergillus niger	0.2013	0.2013	0.2013	0.2013	0.2013
Aspergillus orhyzae	0.2002	0.2002	0.2002	0.2002	0.2002
Trichoderma viride	0.2014	0.2014	0.2014	0.2014	0.2014
Control	0.2008	0.2008	0.2008	0.2008	0.2008

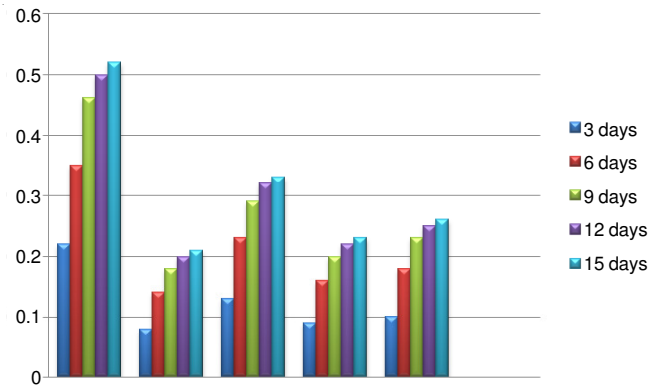

Fig. 2. Bimass production in polyethylene degradation (order of species as in Fig. 1

Five fungal species were applied to monitor polylactic acid degradation and utilization for the initial screening of fungal species. All the five fungal species exhibited positive results as shown in Table-3.

TABLE-3
CONSUMPTION OF DL-LACTIC ACID BY FUNGI AFTER SEVEN DAYS

Strains	pH	Biomass (g/L)
Rhyzopus orhyzae	4.9	0.5
Rhyzopus oligosporus	4.7	0.2
Aspergillus niger	7.1	0.7
Aspergillus orhyzae	4.6	0.2
Trichoderma viride	7.0	0.8
Control	3.6	0.0

Lactic acid consumption was observed by monitoring increase in pH and biomass. Increase in pH was due to decrease in amount of lactic acid which was a clear indication of lactic acid utilization by fungi. Increase in biomass was due to growth of fugal species which is another indicator of lactic acid consumption.

The changes in pH and biomass during polylactic acid-oligomer degradation by fungi after 7 days are reported in Table-4. Maximum consumption was found in case of *Aspergilus niger*. pH value for Aspergilus niger was found as 7.1 and for *Trichoderma viride* it was 7.0. Control sample pH value was 3.6. This degradation resulted in decrease of pH in beginning then it increased due to lactic acid consumption. Maximum lactic acid consumption was shown by *Aspergillus niger*. Tables 3 and 4 show the results after 7 days of incubation.

The results indicate that in case of the medium supplied in DL-lactic acid, final lactic acid concentration, biomass dry weight, weight degradation and pH varied depending on the fungal strain. Strain dependence was also observed when the oligomers were the sole carbon and energy source (Table-4).

TABLE-4
POLYLACTIC ACID-OLIGOMER DEGRADATION BY FUNGI AFTER SEVEN DAYS

Strains	pH	Biomass (g/L)
Rhyzopus orhyzae	4.6	0.6
Rhyzopus oligosporus	3.9	0.4
Aspergillus niger	5.7	1.8
Aspergillus orhyzae	4.9	1.5
Trichoderma viride	5.2	1.2
Control	4.5	0.0

It can be seen from controls that after 7 days of incubation, a high fraction of oligomers was transformed into soluble lactic acid. All the species applied were able to degrade oligomers partially within the selected aging period. Moreover, some fungi assimilated lactic acid itself more easily than the residual oligomers. Biomass production and final pH values show the ability to assimiliate DL-lactic acid and oligomers (Table-4). In case of *A. niger* strain, the final biomass production was the highest (2.0-2.4 g of dry weight mass per liter) and for the other fungi, values were very poor (from 0-1.8 g/L). Furthermore, final pH values were high with microorganisms able to assimilate lactic acid type compounds to some extent. For the strain mentioned before, pH values were above 7.0 in case of lactic acid consumption and were lower in oligomer-containing media.

For controls, the final pH was lower in lactic acid-based medium, confirming that not all the oligomers were transformed in lactic acid. From these results, *A. niger* strain can be considered to have more important metabolic activity.

From the foregoing discussion, it may be concluded that no significant biodegradation of polyethylene was indicated by five fungal species. Weight losses in the beginning were probably due to degradation of fillers present in the polyethylene. *Rhizopus orhyzae* and *Aspergillus niger* exhibited same values of weight loss which is 0.0004 g and is greater than all other fungal strains applied for the degradation of polyethylene. Biomass production for *Rhizopus orhyzae* was higher than *Aspergillus niger* and all other fungal species. There was no further weight loss of polyethylene for any fungal specie up to 6 months trial.

Initial screening of fungal species was carried out by measuring the consumption of lactic acid as carbon source. All fungal species were found positive in terms of lactic acid consumption. Biomass production for *Aspergillus niger* was maximum among all fungal strains which clearly indicates the lactic acid consumption after degradation of poly-lactic acid oligomers. Increase in pH was also a clear indication of lactic acid consumption from oligomers[12].

In spite of the transparent fact that no biodegradation was accomplished in the experiments carried as part of this research

study, it does not mean that the attempts on this frontier should be stopped. The following recommendations may be made to extend project in future: More studies with strains of different species should be carried out patiently as that is the demand of the importance of the research work. Further studies with the selected microorganisms will help to better understand their actual potential to biodegrade polylactic acid derivatives. Plastic may be primarily treated with some chemical reagents such as alkalis, acids, oxidizing agents in hope that some sights may be uncovered for action of some enzymes produced by the organisms.

REFERENCES

1. J. Guillet, Macromolecular Symposia 123/37th Microsymposium on Macromolecules, Biodegradable Polymers, pp. 206-224 (1997).
2. F. Kawai, *Adv. Biochem. Eng. Biotechnol*., **52**, 151 (1995).
3. A.C. Albertsson and B. Ranby, In Proc. 3rd Int. Biodegradation Symposium, Applied Sciences Publ. Ltd., London (1975).
4. A.C. Albertsson and S. Karlsson, *Prog. Polym. Sci.*, **15**, 177 (1990).
5. A.C. Albertsson, B. Erlandsson, M. Hakkarainen and S. Karlsson, *J. Environ. Polym. Degrad.*, **6**, 187 (1998).
6. J.H. Cornell, A.M. Kaplan and M.R. Rogers, *J. Appl. Polym. Sci.*, **29**, 2581 (1984).
7. K. Ehara, Y. Lioyshi, Y. Tsutsumi and T. Nishida, *J. Wood Sci.*, **46**, 180 (2000).
8. J.E. Glass and G. Swift, ACS Symposium Series 433, American Chemical Society, Washington DC, pp. 9-64 (1989).
9. J. Friedrich, P. Zalar, M. Mohorcic, U. Klun and A. Krzan, *Chemosphere*, **67**, 2089 (2007).
10. A. Torres, S.M. Li, S. Roussos and M. Vert, *Appl. Environ. Microbiol*., **62**, 2393 (1996).
11. V. Vichaibun and M. Chulavatnatol, *Sci. Asia*, **29**, 297 (2003).
12. Y. Zheng, E. Yanful and A. Bassi, *Crit. Rev. Biotechnol*., **25**, 243 (2005).