BIODEGRADATION OF POLYESTHERS BY FILAMENTOUS FUNGI

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INTRODUCTION

Biodegradable polymers have gained increasing importance in the last decades and their production is constantly growing. However, biodegradation is a complex process, involving a large number of variables, and the study of biodegradation under different environmental conditions is still a debated research topic. Polyesters, such as poly(butylene succinate) (PBS), poly(butylene sebacate) (PBSE) and poly(butylene adipate co-terephthalate) (PBAT) are among the most diffused biodegradable polymers and are now used in a variety of applications. Although they meet the conditions for biodegradation in industrial composting, concerns remain about their effective biodegradability in other environments, i.e. at lower temperature and with less varied microbial populations [1,2]; furthermore, the actual fate of the degradation products has been poorly investigated. The present study aimed at investigating the role of filamentous fungi in the biodegradation of PBS, PBSE and PBAT, at low temperature and in simple cultural media, to extend the existing investigations to less favorable conditions and to gain information on the ability of microorganisms to proliferate in the presence of the polymer as sole carbon source.

EXPERIMENTAL

Materials: PBS, PBSE and PBAT pellets were obtained from commercial producers. Films were prepared by compression molding of the granules, using a laboratory press, while powders were obtained by grinding the granules in an ultracentrifugal mill (Retsch ZM 200). Ninety-nine fungal strains preserved at Mycotheca Universitatis Taurinensis (MUT, University of Turin) were selected for the biodegradation tests. The tested fungi had previously been isolated from plastic materials collected from landfill soil, from tanning industry-treated wastewater and from different environments at 15 °C.

Characterizations: the polymers were characterized by Gel Permeation Chromatography (GPC), Differential Scanning Calorimetry (DSC) and contact angle measurements, before and after the biodegradation tests.

With the aim of maximizing the surface area of the tested materials, the first biotic screening was performed with PBS electrospun membranes. The membranes were cut into small pieces of about 3 cm per side and UV-sterilized. Every sample was placed on the surface of Petri dishes with autoclaved agarized mineral medium (MM), using sterile tweezers and sleeved needle. Fungal strains from well-grown colonies were inoculated in the middle of the plates. Negative (MM without any carbon source) and positive (MM with 0.1% w/v glucose) controls, as well as an abiotic control (MM and polymer membrane without fungus) were also prepared. The colony diameters and, if present, the clear zone of degradation were measured after 5 and 7 days. For the secondary screening, bioplastics were used in form of films, approx. 40 μm thick and 4 cm in diameter. Following UV sterilization, films were placed on the plates and fungi were inoculated as previously described. Plates were incubated both at 24 °C and 15 °C for 2 months. The colony diameters and an eventual clear zone of degradation around the fungal colony were periodically measured. At the end of the experiment, each film was removed with a sterile tweezers and characterized.

Liquid trials on polymeric powders and monomers (succinic, sebacic, adipic and terephthalic acid and 1,4-butanediol) were carried out in two different culture media: a minimal MM and a rich medium (MEA). Polymers and monomers were weighed in Eppendorf tubes and powders were washed with ethanol 70% and sterilized by tyndalization (60°C, 1 h for 5 days). A conidia suspension of two fungal strains selected from previous screenings was prepared and inoculated in each flask. To stimulate the fungal growth, a pre-growing phase was set up: fungi were preliminarily incubated in a rotatory shaker at 24 °C at 110 rpm. After 2 days, polymers (1% w/v) or monomers (500, 1000 or 2000 ppm each) were added to the flasks. The trials lasted for 14 days on the monomers and 24 days on the polymers. Each test was performed in triplicate.

At the end of the experiment, the biomass and the residual polymers were separated from the liquid medium that was furtherly analyzed by HPLC-RI/UV-Vis, to quantify the monomers and/or oligomers eventually present in the solution.

RESULTS AND DISCUSSION

The primary biodegradation screening was carried out to select fungal strains capable of growing in the presence of the polymer membranes as sole carbon source. In total, 48 fungal genera and 83 species were tested. 14% of fungi showed a significantly ($p \leq 0.05$) higher growth in the presence of the polymer, than in positive controls, 28% did grow comparably, whereas 58% of them grew less than the controls. 37 fungi selected from the preliminary screening were then incubated with each polyester film (e.g. PBS, PBSE, PBAT) as the sole carbon source, at 24 and 15°C, in order to assess their degradative capacity in extreme conditions. The results of the characterization of the polymer films are summarized in Table 1. The tested polymer was shown to play an important role, with 22 strains being able to degrade PBS in at least one condition, while only 8 strains affecting PBSE, the most recalcitrant polymer. PBSE's recalcitrance to biodegradation was mainly attributed to its hydrophobicity and high degree of crystallinity, which slow down the biodeterioration process. Temperature also played a major role, with stronger effects usually observed at 24°C, although with some exceptions. Fungi that gave the best results, in particular at low temperature, belong to two specific genera and, among these, two strains, PL75 and PL81, appeared more promising and were selected for the monomer assimilation capacity tests. Succinic and sebacic acid were fully assimilated in both mediums, at each concentration tested. Adipic acid was almost completely assimilated at all three concentrations and in both culture media by PL75, while an inhibitory concentration effect was observed with PL81 at 2000 ppm in MM. 1,4-Butanediol was transformed more slowly than the diacids, and its transformation was not complete, for both strains in both media. The assimilation rate in MEA was higher than in MM, due to the presence of simple sugars which may have helped the strain growth and its metabolism. Terephthalic acid was not assimilated by pL81 and its assimilation occurred only partially by pL75. The same behavior was observed following the biodegradation tests of the polymers: although we did observe weight losses for all polymers, HPLC analysis of the degradation media did not detect succinic, adipic and sebacic acids, whose fast assimilation does not allow their accumulation in the liquid medium. Terephthalic acid on the contrary steadily accumulated in the culture medium. An initial accumulation of 1,4-Butanediol due to depolymerization was observed for all polyesters and with both fungal strains, followed by slow assimilation in the rich medium only. Overall, PBS undergoes depolymerization to monomers in both culture media, albeit with different rates. PBSE should be weakly depolymerized in MM, since neither 1,4-butanediol nor sebacic acid were detected, while the depolymerization of PBAT in MM was confirmed by the presence of terephthalic acid in solution, whose concentration increased over time.

Sample	Contact angle (°)	Crystallinity (%)	M_w (kDa)
PBS	75 ± 2.4	35 ± 0.5	67.2 ± 4.4
PBSE	86 ± 0.9	51 ± 1.1	64.3 ± 6.1
PBAT	76 ± 5	13 ± 0,5	63.7 ± 4.4

Table 1

CONCLUSION

The results of this study allowed to gain knowledge on the biodegradation process of aliphatic and aliphatic-aromatic polyesters mediated by fungal microorganisms, under controlled conditions of low temperature and lack of additional nutrients. We have shown that the chemical and physical characteristics of the polymers play a role both on the degradation efficiency by the microorganisms and on their ability to assimilate the degradation products.

References

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