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**BOOK OF  
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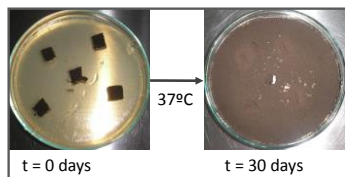
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**NEWALK**  
MATERIAIS - COMPONENTES - TECNOLOGIAS  
PARA CALÇADO DO FUTURO

Nowadays the footwear industry is seeking for more sustainable and bio-based solutions in shoe manufacturing, including the use of biodegradable materials. As a first step towards the proposal of such solutions, in this work different microorganisms were screened for their intrinsic capacity to degrade a base TPU. The biodegradability was tested using the agar plate method against different bacteria, fungi and a consortium of both. The most promising results, corresponding to higher weight-loss percentages of the TPU sample, were obtained using the consortium of *Pseudomonas aeruginosa* and *Aspergillus niger*. These best conditions were thereafter applied to TPU samples compounded with biobased additives.

### Introduction

At present, footwear is considered as one of the most internationalised sectors of the Portuguese industry with a significant importance for the national economy. This sector seeks to affirm itself in market niches with greater technical and scientific requirement, value-added and high quality standards, resulting in research and technological development investment. Moreover, there has been an increasing interest in reducing the environmental impact of this sector by seeking for more sustainable and bio-based solutions.

Among the wide variety of materials employed in shoes' production, different polymers are frequently used as they exhibit a versatile array of properties in terms of strength, lightness, durability and resistance to degradation, with thermoplastic polyurethanes (TPUs) being one of the most widely used [1]. In what concerns footwear industry it is estimated that TPU footwear components represent about 60% of the whole production in Europe. On the other hand, the use of a wide variety of additives in TPUs formulation (e.g. pigments, coatings, and fillers) limits the possibility of recycling. In such scenario, biodegradable polymers could offer an excellent solution to the environment hazard posed by conventional polymers [2]. Therefore, given the widespread use of TPUs and associated waste management problems, it makes sense to invest on the development of more biodegradable and environmental compatible solutions.

As a first step towards the proposal of such solutions, encompassing for example the production of modified TPUs by compounding

with natural additives, in this work different microorganisms, including bacteria, fungi and an association of both (consortium), were screened for their intrinsic capacity in degrading a TPU sample used in the footwear industry.

### Materials and Methods

**Samples:** A polyester-based TPU used in footwear industry was chosen for the screening assays. For testing the best conditions 2 commercial additives and 3 natural substances (lignin, cellulose and starch) have been selected.

**Biodegradation assays:** The degradation assays were performed in agar plates by sample inoculation using bacteria, fungi and different consortia (Table 1). The culture media and incubation conditions used for each assay are also presented in Table 1.

**Table 1.** Microorganisms and culture conditions used in the biodegradation assays in agar plates.

	Microorganisms	Culture media	Incubation
Bacteria	<i>Bacillus subtilis</i> ATCC 1234	Nutrient agar	30 days 37°C
Bacteria	<i>Pseudomonas aeruginosa</i> ATCC 9027	Nutrient agar	30 days 37°C
Fungi	<i>Aspergillus niger</i> ATCC 16404	Sabouraud dextrose agar	30 days 30°C
Fungi	<i>Penicillium chrysogenum</i> ATCC 1234	Sabouraud dextrose agar	30 days 30°C
Consortium	<i>A. niger</i> + <i>P. chrysogenum</i>	Sabouraud dextrose agar	30 days 30°C
Consortium	<i>A. niger</i> + <i>P. aeruginosa</i>	Sabouraud dextrose agar	30 days 37°C

For the assays with bacteria, the inoculum was adjusted to approximately 0.5 McFarland turbidity standard and spread over the agar plates. The TPU sample was aseptically transferred to the agar surface and inoculated with 100  $\mu$ L of the bacterial inoculum (Figure 1A). The degradation assays with fungi were performed as previously described [3]. Briefly, a concentrated spore suspension was prepared by scraping the fungi mycelium with a sterilized loop. The suspension was filtered to remove agar and mycelium residues and centrifuged (15 min, 4000 rpm). The obtained pellet was re-suspended in 5mL sterile distilled water, used for spore count in a Neubauer chamber and diluted to a final concentration of approximately  $1 \times 10^6$  spores/mL. The agar plates were spread with the spore suspension which was also used for sample inoculation (100  $\mu$ L) (Figure 1B). In the assay performed with the consortium of bacteria and fungi, the bacteria inoculum was spread over the agar and square samples (1x1 cm) of *A. niger* mycelium were transferred to the agar surface. The TPU samples were inoculated with both bacteria inoculum and fungi mycelium (Figure 1C). After incubation, the TPU samples were collected, washed with ethanol and dried at ambient conditions before weighing. The biodegradation was estimated based on the weight-loss of the samples. All TPU samples were tested in duplicate.



**Figure 1.** Biodegradation assays of TPU samples after 30 days of incubation (A and B) and at the beginning of the assay (C). Plates were inoculated with *B. subtilis* (A), consortium of *A. niger* and *P. chrysogenum* (B) and consortium of *P. aeruginosa* and *A. niger* (C).

## Results and Discussion

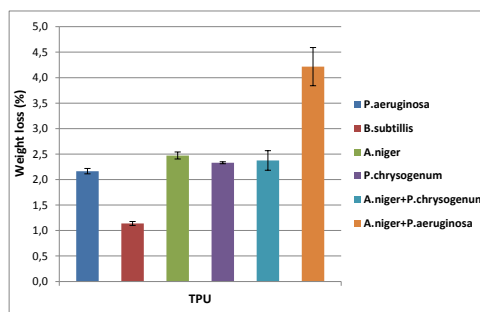
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As expected, and regardless of the tested microorganism, modest weight losses percentages were achieved for the TPU samples under evaluation. In general, the sample degradation was slightly higher in the assays performed with fungi when compared to bacteria, with best results being achieved for *A. niger* (Figure 2). Among the two tested bacteria, the higher weight loss was achieved with *P. aeruginosa*. Similar results to the ones performed with the individual microorganisms were obtained with the fungi consortium. By the contrary, the weight loss % improved significantly when using the consortium *aeruginosa* + *A. niger* (Figure 2). These results evidence the existence of a synergistic activity between fungal and bacterial degradation that could be further explored in this field of TPUs biodegradation. In what concerns the tested additives lignin gave the best results.



**Figure 2.** Results of the TPU samples weight loss tested with different microorganisms.

## Conclusion

The use of a consortium comprised of bacteria and fungi, namely *P. aeruginosa* and *A. niger*, led to better results in what concerns weight loss % of the tested TPU samples. The preparation of TPU samples modified by compounding with biobased additives pointed out for the best results achieved with natural substances such as lignin, starch and cellulose (data not shown).