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MICROORGANISMS DEGRADING POLYURETANE FOR FOOT-WEAR WASTE VALORISATION

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Polyurethane (PU) is a widely used polymer in footwear manufacturing, contributing to components such as outsoles, insoles, adhesives, or finishing agents, among others. Currently, 90% of discarded footwear ends up in a landfill. According to the European Directives concerning textile and footwear waste disposal, solutions are required for recovering and valorizing their different materials. Among them, polyurethane is gaining a growing interest. In this sense, strategies involving the action of microorganisms and their enzymes on the structure of polyurethane are being explored to gain insight into its revalorization. One of the key aspects is the discovery of new microorganisms capable of degrading polyurethane, thereby broadening the possibilities of identifying specific enzyme activities or combining them to target different types of polyurethanes. In this study, the isolation of microorganisms (fungi and bacteria) capable of degrading the ester polyurethane model Impranil® DLN was carried out from mature compost samples. Four bacteria and four fungi were identified, and their degradative capacity was subsequently tested using ester-type and ether-type polyurethane foams. The degradative capacity was assessed through Fourier transform infrared spectroscopy and thermogravimetric analysis, in addition to observation via scanning electron microscopy (SEM). Among the identified species, *Gymnasciella dankaliensis* demonstrated the capacity to degrade the ester-type foam. This is the first work that relates this fungus to PU biodegradation.

Keywords: biodegradation, polyurethane, microorganisms.

INTRODUCTION

Polymers are produced massively worldwide, reaching nearly 400 million tons in 2021 (Plastics Europe, 2022), and it is expected to triple by 2060 (Investigate Europe, 2023). Among these, polyurethane (PU) accounts for 5.5% of the total plastic production (Plastics Europe 2022). It is a versatile plastic with applications in numerous areas. In footwear, polyurethane is widely used in components such as soles, insoles, and for adhesives and coatings. PU is synthesized from polyols and isocyanates. Based on the polyols used, PU can be divided into two major types: polyester polyurethane and polyether polyurethane.

Due to its environmental resistance, PU poses a significant pollution challenge, and its biodegradation has become a topic of great interest to help address the problem in an eco-friendly way. In this context, discovery and characterization of highly efficient PU degrading microbes and enzymes is essential for developing green plastic recycling processes. In this regard, microorganisms with degradative capabilities have been identified from various sources, generally from soils (Wu *et al*., 2023). This study aims to isolate and identify PU-degrading microorganisms from mature compost in order to further study its degradative mechanism and consider them as a biological tool for the degradation of PU.

In this work, mature compost was used as a source for isolating bacteria and fungi capable of degrading Impranil® DLN (Impranil), which is a protected anionic aliphatic PU-polyurethane colloidal dispersion, with 40% solid dispersed in 60% water. This dispersion is commonly used in handbags, shoe uppers, and shoe lining materials (Windermuth, 1956, Traeubel, 1988) and has been reported in previous studies as a PU model for an initial isolation (Biffinger *et al*., 2015).

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First, microorganisms that cause the visual dissolution of Impranil (also referred to as "clearing") (Biffinger *et al*., 2015) were isolated on agar plates (Jin *et al*., 2021). After microorganism identification, additional degradation assays with an ester and ether-type PU foams were performed to confirm if these microorganisms effectively degraded PUs different from Impranil. While all eight identified microorganisms efficiently degraded Impranil, only the fungus identified as *Gymnasciella dankaliensis* degraded the ester-type PU foam, according to SEM, FTIR and TGA analysis. None of them exhibited degradative capacity on the ether-type PU foam assayed in this work, which is consistent with the fact that ether PUs are more biologically stable and the ether group has higher hydrolysis resistance than the ester group (Jin *et al*., 2021).

MATERIALS AND METHODS

Polymers studied: The specific polyurethane used in the study was Impranil® DLN (Covestro AG, Germany). The polyester-based foam consisted of a blend of a slightly branched polyester based on adipic acid with a combination of methylene glycol and diethylene glycol, and a polyester prepolymer with its isocyanate part from MDI. The polyether-based foam consisted of a blend of a propoxylated polyether triol with ethylene oxide initiated with glycerin and a polyether prepolymer with its isocyanate part from MDI. Both formulations were prepared without mineral fillers or additives and using an amine catalyst.

Source of microorganisms: Mature compost was provided by the Polytechnic School of Orihuela (EPSO) of the Miguel Hernández University of Elche University of Alicante (Spain).

Microorganism Isolation and Molecular Identification

The isolation medium for all Impranil and PU foam assays degradation was M9 minimal medium with agar (per liter ultrapure [RO] water, in grams): Na_2HPO_4 (33.9), KH_2PO_4 (3.0), NaCl (0.5), NH_4Cl (1.0), and 2.0 mM $MgSO_4$, 0.10 mM $CaCl_2$, adjusted to pH 7.2, and 1% Impranil (v/v) or Impranil discs, which were obtained by allowing a 100 microliter drop of the polymer to dry, as the sole carbon source. Compost was diluted 1:5 in a saline solution and agitated for 2h (120 rpm). Diluted compost suspension was applied onto the surface of the agar plates/Impranil discs (100 µl/plate or /disc) and incubated at 30, 35 or 58ºC. Clearing spots in the agar surface were subsequently developed and microorganisms grew on and around the discs. Microorganisms' samples were taken individually and cultured in new M9-Impranil plates, allowing their isolation. Once isolated, genomic DNA was extracted, and amplified by PCR, using universal primers 8F (Edwards *et al*., 1989) and 1492R (Stackebrandt *et al*., 1993) for bacteria, and primers P-ITS1 and P-ITS4 for fungi (White et al, 1990). Amplicons were then purified and sequenced with SANGER technology, and a BLAST search against the rRNA/ITS database of type material was conducted to determine the closest phylogenetic relatives.

Polyurethane Foam Degradation

Degradation of polyurethane foams was performed by placing samples on top of the agar plates containing M9, thus foam being the sole carbon source. Samples were incubated at 35ºC or 30ºC, depending on the isolated microorganism. The temperature of 58ºC was discarded for these experiments, as we were not able to isolate any microorganisms under these conditions. After the designated incubation period (120 days), samples were sterilized for 15 minutes with a sodium hypochlorite solution, followed by two rinses with deionized water and finally allowed to dry before analysis.

Samples Images

Photographs of the polymer samples (control and biodegraded) were obtained with a Canon EOS M10.

FTIR Analysis of Polyurethane Degradation

Fourier transform infrared (FTIR) spectroscopy was employed to analyze samples (Impranil discs and PU foams) using a Varian 600-IR spectrometer (Varian Australia PYT LTD, Australia) with a diamond prism by attenuated total reflection (ATR). Sixteen scans were averaged at a resolution of 4 cm^{-1}, covering a wavenumber range of 500-4000 cm^{-1}.

SEM

Sample colonization and surface modifications were analyzed using a scanning electron microscope (SEM) Phenom proX, at a 500X magnification. Samples were disinfected prior to the analysis, with 5% sodium hypochlorite followed by two rinses in distilled water.

Thermogravimetric Analysis (TGA)

The thermal stability of the PUs was evaluated using a thermobalance equipment, TGA 2 STARe System thermobalance (Mettler-Toledo AG, Switzerland) with STARe software. Samples (7-10 mg) were heated from 30 to 600°C at a heating rate of 10°C/min under a nitrogen atmosphere.

RESULTS AND DISCUSSION

Impranil Colonization and Degradation

Impranil discs and agar plates inoculated with the compost solution were cultured at 30, 35 and 58ºC, the latter temperature being maintained in temperature-controlled composting processes (although composting in field conditions can reach up to 70ºC) (Vico *et al*., 2024). Microbes generating clarification halos or growing on the discs were isolated and cultured separately. Microbial isolation was successful at 35 and 30°C, while no microorganisms were isolated at 58°C. Among the isolated microorganisms, only the fungus *A. fasciculatus* was isolated at 30°C, while the rest were isolated at 35°C. While the colonization of Impranil discs was observed visually (Figure 1), and under SEM analysis (Figure 2), degradation was confirmed through FTIR analysis of the samples. As is shown in Figure 3, the greater the colonization, the greater the changes in the FTIR spectra. A remarkable decrease of the C=O stretching vibration (1728 cm^{-1}) of the urea/urethane groups is observed as colonization proceeds. The increase of the intensity of N-H stretch (3321 cm^{-1}), N-H bending (1623 cm^{-1}), and C-N stretching (1532 cm^{-1}) vibrations indicates the formation of amines during polyurethane biodegradation.

Figure 1. Colonized Impranil discs after incubation with mature compost

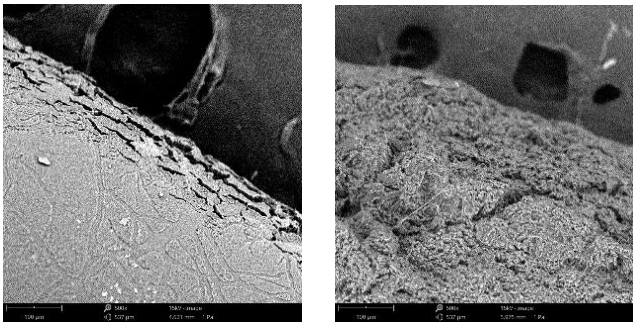

Figure 2. SEM images of the Impranil discs at 500x magnification. Left, control sample. Right, disc incubated for 40 days with the compost extract

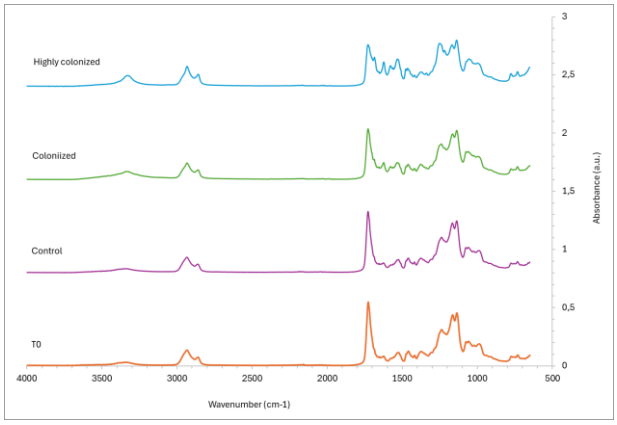

Figure 3. IR images showing degradation of the Impranil discs after 40 days incubation at 35ºC

Microorganisms' Isolation and Identification

The isolates were identified as shown in Table 1. % identity is also displayed, based on 16S rRNA gene sequence for bacteria and ITS for fungi. All the microorganisms were isolated at 35ºC, except for *A. fasciculatus,* which was isolated at 30ºC. Among the isolated microorganisms, only *R. rhodochrous*, generally found in soil, had been previously associated to biodegradation in rubber (Andler *et al*., 2022), low-density polyethylene (Rose, 2020) and plasticizers (Nalli *et al*., 2002). As for the rest of microorganisms isolated from Impranil, none of them had been previously related to PU biodegradation.

Table 1. Identified microorganisms

Type	Name	% identity
Bacteria	*Rhodococcus rhodochorus*	99.99
	Bordetella petrii	99.5
	Limosilactobacillus pontis	99
	Krasinilnikoviella muralis	98.6
Fungi	*Gymnascella dankaliensis*	99.8
	Arachniotus flavoluteus	98.8
	Aspergillus rugulosus	100
	Aspergillus fasciculatus	99.8

Polymer Foam Degradation

Although Impranil has been frequently used as a model for the initial isolation of PU-degrading microorganisms, there are studies that suggest that this capacity should be confirmed

by testing additional PUs, as a positive activity observed for Impranil might not necessarily correlate with a degradative capacity in other PUs (Biffinger *et al*., 2015). While Impranil is a thermoplastic polyurethane, PU foams are thermosetting and therefore more difficult to recycle by conventional mechanical methods. Therefore, the initially isolated microorganisms were cultured in the presence of an ester-type (27 Asker C hardness) and an ether-type PU foam (4 Asker C hardness), both formulations typically used in footwear.

Ester-PU foam samples cultured for 120 days along with some of the isolated microorganisms such as *A. rugulosus* and *A. fasciculatus* showed initial signs of growth, especially samples inoculated with *G. dankaliensis*. However, others (*I. nagnjinensis, A. flavoluteus, L. pontis, R. rhodochrous, B. petrii*) were not able to use the samples as the sole carbon source, despite being isolated as Impranil degrading microorganisms in our previous screening. Ether-PU foam used in this work was not suitable for any of the microorganisms. Considering the variety of PUs than can be synthesized depending on their precursors and additives, this does not necessarily imply that these microorganisms lack the capacity to degrade any other PU formulations, which is an issue that is currently being addressed in additional experiments. The image shows the appearance of the ester-PU foam used in this work incubated exposed to (right) and without (left) *G. dankaliensis* inoculum (Figure 4).

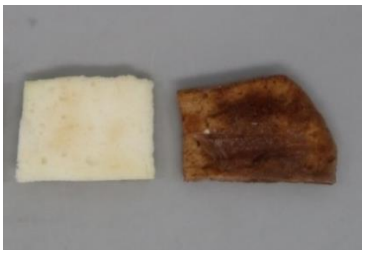

Figure 4. Colonisation of *G. dankaliensis* of the ester-PU sample after 120 days (right), compared to the sample incubated without any microorganism (left)

As SEM images show (Figure 5), the ester-PU foam that was exposed to *G. dankaliensis* revealed breakage between the foam's pores, along with a high number of cracks. PU foam incubated with the rest of microorganisms and ether-PU foam showed no relevant modifications in the same period (images not shown). This correlates to previous works stating that ether PU is more biologically stable, and the ether group presents higher hydrolysis resistance than the ester group (Jin *et al*., 2021).

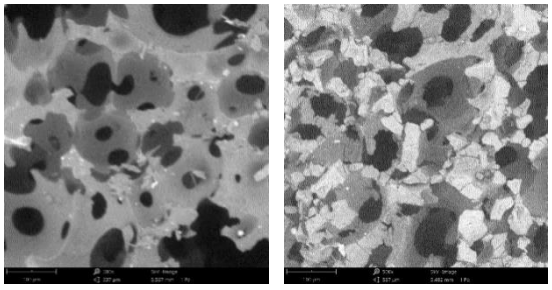

Figure 5. SEM images of the ester PU incubated with *G. dankaliensis*. Control (left). Biodegraded (right)

FTIR spectra comparisons between the control sample and the *G. dankaliensis* biodegraded sample are shown in Figure 5. Both spectra were baseline corrected and normalised based on the peak at 2950 cm^{-1}, which corresponds to C–H stretching vibrations. According to Bhavsar *et al*. (2024), the proportional change in these components is minimal due to the comparative abundance of C–H bonds in the polymer molecules and, therefore, can be used for normalisation

and comparison of the spectra of the blank and the biodegraded sample. There is an increase in the intensity of the peaks at 3311 cm^{-1}, corresponding to N-H stretching, and 1595 and 1531 cm^{-1}, corresponding to N-H bending/C-N stretching, for the biodegraded sample. These increases can be associated with amine formation after polyurethane hydrolysis. The peak at 1726 cm^{-1} is associated with the C=O of the free carbonyl of the urethane decreases in the biodegraded sample. This also indicates the decomposition of the polyurethane by hydrolysis.

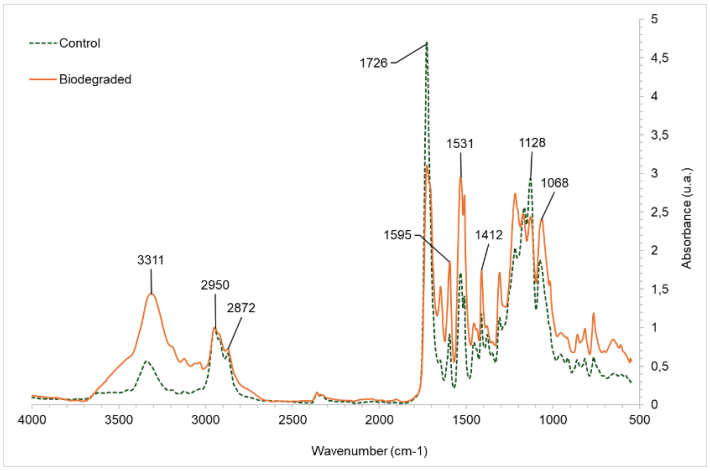

Figure 6. FTIR spectra comparison of the blank and the biodegraded samples

Thermal stability was assessed by thermogravimetric analysis (TGA). Polyurethanes exhibit a segmented structure comprising soft (S) and hard segments (H). The soft segments, typically derived from polyols, impart flexibility, while the hard segments, formed from diisocyanates, provide rigidity and thermal stability. Thermal stability of polyurethane depends on the type and structure of the H-S to S-S ratio (i.e. NCO/OH ratio). As the NCO/OH ratio increases, the thermal stability and crystallinity increases due to increase in the hydrogen bond interaction in the H-S (Szycher, 1999). As shown in Figure 6, the thermal stability of the biodegraded sample is lower than the control sample. If the derivatives of the TGA curves are analyzed (Figure 7), in both cases, a peak above 356-370°C is observed, which may be attributed to the decomposition of the hard segments. A peak above 395-405°C is also noted, which may be due to the decomposition of the soft segments. The area under the curve of the derivative of weight loss as a function of temperature, in the range of 220 to 380°C, can be correlated with the amount of hard segments present in the sample, whereas the area under the curve between 380 and 480°C can be associated with the quantity of soft segments. Since soft segments are more prone to biodegradation than hard segments (Pfohl, 2022), the lower content of soft segments in the biodegraded sample compared to the control sample indicates a higher biodegradation in the former.

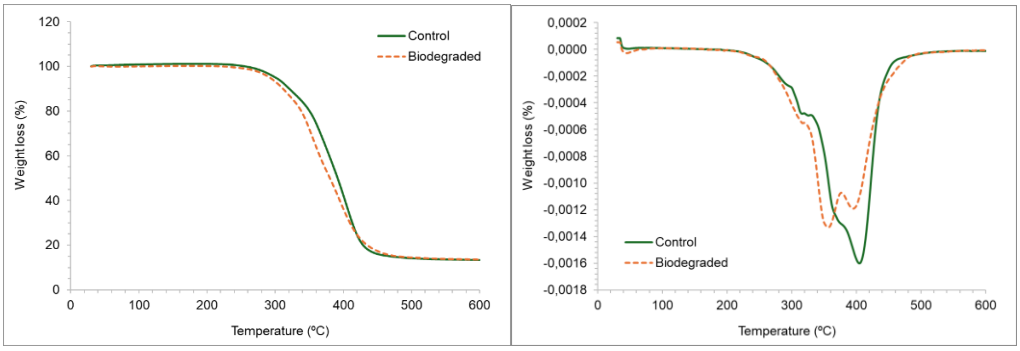

Figure 7. Left. TGA curve comparison of the control and biodegraded samples. Right. DTGA curve comparison of the control and biodegraded samples

In the present study, *G. dankaliensis* (NCBI:txid166389), isolated from mature compost, demonstrated the capacity to degrade an ester-PU foam. This fungus had not been previously reported as polyester PU-degrading fungi in the literature. Degradation of PU by *G. dankaliensis* can be attributed to rupture of the ester bond, as evidenced by the IR spectra. Thermogravimetric analysis showed a predominant degradation of the soft segments of the polymer, which is consistent with previous findings for other fungi (Jin *et al*., 2021). This finding contributes to the catalog of species described as PU-degrading microorganisms (Liu *et al*., 2021). However, further research is needed to study the mechanism of ester-PU foam degradation by *G. dankaliensis*, identifying the different by-products obtained and the specific enzymes involved in this activity. This will support future strategies for PU waste valorization, applicable not only for the polyurethanes used in the footwear industry, but also extensible to all types of polyurethanes.

CONCLUSIONS

The study presented here successfully isolated and identified a variety of microorganisms, including both bacteria and fungi, from mature compost samples, capable of degrading the ester-type polyurethane model Impranil® DLN. Among the microorganisms tested, *Gymnasciella dankaliensis* demonstrated capacity to degrade an ester-type polyurethane foam, marking the first instance of this species being linked to PU biodegradation. The results highlight the varying degradative capacities of microorganisms depending on the chemical structure of the polyurethane, with ester-type PU being more susceptible to microbial attack than ether-type PU due to the inherent chemical differences, particularly the greater hydrolysis resistance of ether groups, but also for the distribution and arrangement of these bonds in the different polyurethanes (Rajan *et al*., 2024; Su *et al*., 2023).

This research adds valuable knowledge to the growing field of biological polyurethane degradation, suggesting that specific microorganisms, such as *G. dankaliensis*, could serve as potential tools for the valorization of footwear waste containing polyurethane. The ability to target and degrade specific types of polyurethanes presents a sustainable alternative to traditional chemical and mechanical recycling methods. However, additional studies are required to fully understand the mechanisms of PU degradation, including identifying the enzymes involved and exploring the potential for upscaling this process for industrial applications. Further research will be essential to enhance the efficiency of microbial PU degradation, not only for footwear waste but for broader applications in polyurethane recycling and valorization.

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