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Biodegradation of Poly(ester urethane)s by *Bacillus subtilis*

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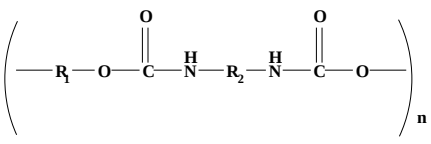
ABSTRACT: Polyurethanes (PURs) are polymers that can be generated by a step-growth polyaddition reaction of diisocyanates with polyols. The number of well-developed chemical procedures applicable to their manufacture, and the diversity of chemical structures in which polyurethanes can be built, account for the widespread use of these materials in the last few decades for both domestic and technical applications due to its excellent mechanical and thermal properties and their high resistance to the climatic changes. Nevertheless, its high resistance to degradation in aqueous media or by microorganism constitutes not only a great disadvantage for its use as material in medical applications (vascular grafts, artificial heart diaphragms, valves, catheters…) but also as one of the sources of the pollution of the environment. In this work, we report the degradation of a commercial poly (ester-urethane) by microorganisms isolated from cedar wood. This is the first study that demonstrates the degradation of polyurethane by isolated microorganisms from wood. Analyses were carried out by Infrared spectroscopy.

Key words: Biodegradation, *Bacillus subtilis,* Polyurethane, Infrarouge, Impranil DLN

INTRODUCTION

Polyurethanes were first produced and investigated by Dr. Otto Bayer in 1937. Polyurethane is a polymer in which the repeating unit contains a urethane moiety. Although PUR may contain urethane groups, other moieties, such as urea, ester, ether or an aromatic may also be included (Bayer, 1947). The addition of these functional groups may result in fewer urethane moieties in the polymer than functional groups. The urethane linkage results most readily through the reaction of an isocyanate with an alcohol (Dombrow, 1957; Kaplan *et al.*, 1968). The hydrogen atom of the hydroxyl group is transferred to the nitrogen atom of the isocyanate (Saunders and Frisch, 1964). The major advantage of PU is that the chain is not composed exclusively of carbon atoms, but rather of heteroatoms, oxygen, carbon and nitrogen (Saunders and Frisch, 1964). The simplest formula for PUR is linear and represented by:



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R_1 represents a hydrocarbon containing the alcohol group, R_2 is a hydrocarbon chain and n is the number of repetitions.

PUR has been in use since the 1940s and is now widely used as a base material in various industries. PUR can adopt various forms (from soft to hard) depending on the chemical structures of the polyisocyanates and polyols (functional group number or molecular mass), the raw materials of PUR. Polyurethanes are found just about everywhere in modern life. Some of the applications of this versatile polymer include foams, elastomers, poromerics, paints, bers, fabric coatings, adhesives, and sealants. The persistence of synthetic polymers introduced into the environment by human industry poses a major threat to natural ecological systems. The sheer volume of plastics produced each year presents a problem for waste disposal systems. Recently, environmental pollution by plastic wastes has become a serious issue; and polyester PUR had attracted attention

because of its biodegradability. Enzymatic degradation of PUR by fungi (Cosgrove *et al.*,2007; Crabbe *et al.*, 1994; Darby and Kaplan, 1968; Pathirana and Seal, 1984) and bacteria (Howard and Blake,1998; Howard and Hilliard, 1999; Howard*et al.*, 2001; Kay *et al.*, 1991; Nakajima-Kambe *et al.*, 1999; Oceguera-Cervantes *et al.*, 2007; Rowe and Howard, 2002.) has been demonstrated. Soil fungi comprise the majority of organisms screened for PUR degradation activity (Cosgrove *et al.*, 2007; Crabbe *et al.*, 1994). In this context, we considered studying biodegradation of polyurethane sold under the name Impranil DLN by bacteria isolated from decayed cedar wood.

MATERIALS & METHODS

Polymer studied: The specific polyurethane used in the study was Impranil DLN (Bayer GmbH,Dormagen, Germany). Impranil DLN is used for outwear, bag/luggage, fashion shoe uppers, and shoe lining materials. It is 40% solid polymer dispersed in 60% water. The solid part of Impranil DLN is a high molecular weight linear polyurethane polymer consisting of a linear aliphatic diisocyanate and aliphatic polyester.

Bacterial Strain Isolation and Molecular Identification:The strain used throughout this work was isolated from diver's sites of the former "Derblamté" in the old Medina of the Fez city, Morocco. For molecular identification, the genomic DNA was extracted using thermal shock. The 16S rRNA gene was PCR amplified using the primers, fD1 (5'AGAGTTTGATCCTGG-CTCAG3') and Rs16 (5'TACGGCTACCTTGTTACGAC TT3') (Mostakim *et al.*,2011). The PCR mixture contained 1.5 mM $MgCl_2$, 200 µM of each dNTPs (Promega, Madison, USA), 1 µM of each primer (Metabion, Bangalore, India), 4 µL of Taq buffer (5X) and 1 unit of Taq polymerase (GoTaq Gold; Promega). To this mixture, 2 µL of the DNA template was added. In the control tube, 2 µL of ultrapure water was added instead of DNA. The total reaction volume was 20 µL. The reaction was amplified in a Thermal Cycler (TECHNE, UK) using the following program: 94 °C for 5min; 35 cycles of 94 °C for 1min, 50 °C for 1 min, 72 °C for 1 min followed by a final extension step of 72 °C for 5 min. DNA sequencing was performed using ABI 3130 (Applied Biosystems) according to the manufacturer's instructions. Comparative sequence analysis was performed by comparing sequences with those available in the online databases provided by the National Centre for Biotechnology Information (NCBI) using the BLAST search program.

Degradation of Polyurethanes test, media and culture conditions: For biodegradation essay on plate dish, Luria-Bertani (LB) medium was prepared by adding 0.5g yeast extract, 10.NaCl, and 1.0g tryptone to 100 ml dH_2O. Impranil DLN (Bayer GmbH, Dormagen, Germany) was also added to the medium with the concentration of 0.3% and 0.6%. Then, to confirm these results the tests 0.3% and 0.6% were repeated in a liquid medium and incubated 7 days at 37 ° C.

IR analysis of polyurethane degradation: IR spectra of the medium were analyzed using an IR spectrometer VERTEX 70, after 7 days of incubation and after lyophilization

RESULTS & DISCUSSION

Identification of bacteria: The comparison with a BLASTN database of the 16S rRNA gene sequence of this bacterial isolate indicated that the latter could be identified as *Bacillus subtilis* with a JN700079.1 access number and percentage similarity of 99%. Initial test in a solid medium: Impranil DLN, polyester polyurethane (PUR), is an opaque milky suspension that becomes transparent upon degradation. Organisms capable of degrading this polymer display a zone of clearance around the growing culture. A collection of six strains isolated from cedar wood taken from an old house in the Old Medina of Fez were analyzed to see their ability to grow and degrade the polyurethane using the test of clear halo around the colony. Of the organisms screened, organisms produced a halo of clearance such as that shown in (Fig. 1).

Fig.1. growth of bacillus subtilus on a LB plate supplemented with 0.6% impranil with a zone of clearing around the edges of colonies showing polyurethane degradation

Test in a liquid medium: To confirm these results we repeated the tests 0.3% and 0.6% in a liquid medium. After 7 days of incubation at 37°C. We noticed the disappearance of the characteristic white color of impranil DLN (Fig. 2). Media were centrifuged to remove the bacteria, and the remaining liquid was lyophilized to IR analysis.

IR analysis of the degradation of PUR: The mechanism of PUR degradation was initially investigated by infrared spectroscopy (Shah et al., 2008; Kay et al.,1993). PUR samples of Impranil DLN display a large absorption peak at 1735/cm corresponding to the C(O)-O ester linkage in the polyurethane polymer (Fig. 3). The bacterium bacillus subtilis was added to the media 0.3% and 0.6% of polyurethane. The media were analyzed by IR spectroscopy for the duration of the degradation experiment. A progressive reduction in the relative intensity of the peak at 1730/ cm was observed and was accompanied by more subtle changes at another wave number (Fig. 4). By the time the culture has become visually transparent, there was a complete loss of the absorbance peak at 1735/cm (Fig. 5). The loss of this peak is consistent with hydrolysis of the ester bond in the urethane linkage.

In this study the degradation of polyurethane by *Bacillus subtilis* has been chemically demonstrated by infrared spectroscopy, which shows the

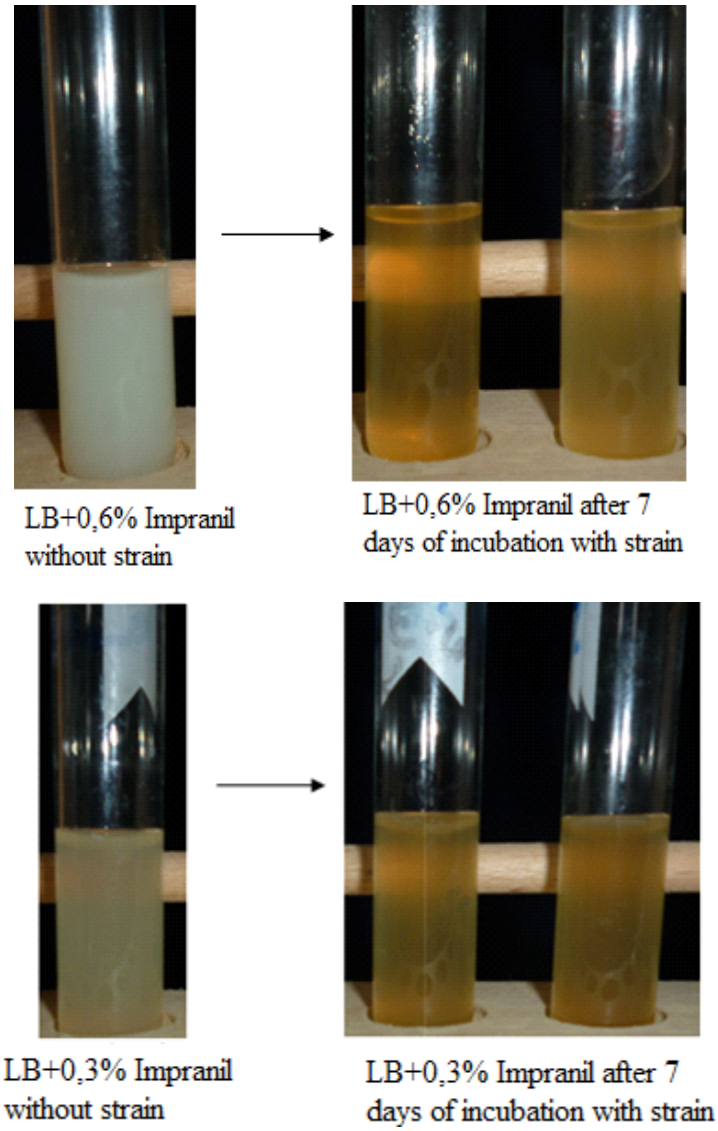

Fig. 2. Impranil DLN degradation in liquid LB medium with the concentration 0,6% and 0,3% after 7 days of incubation

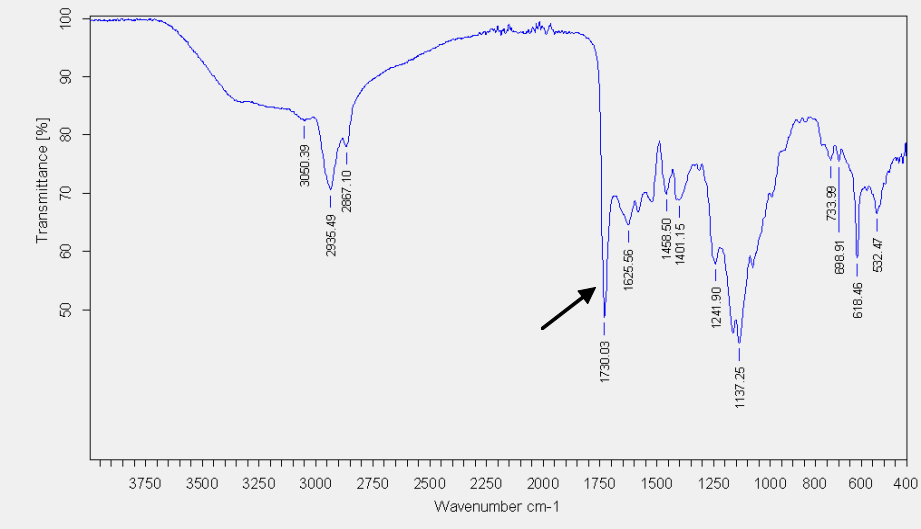

Fig. 3. Infrared spectra of PUR liquid medium containing 0.6% of Impranil DLN without strain *Bacillus subtilis*

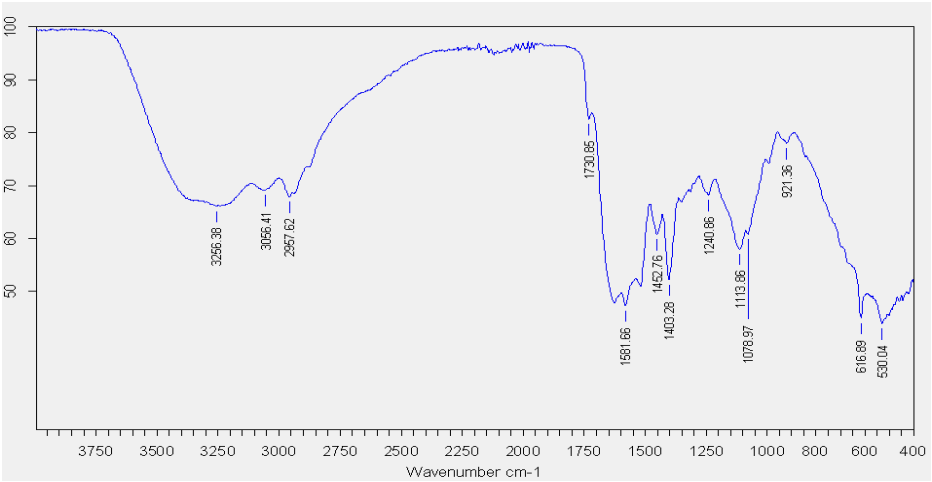

Fig. 4. Infrared spectra of PUR liquid medium containing 0.6% of Impranil DLN taken after 2 days of incubation with the strain *Bacillus subtilis*

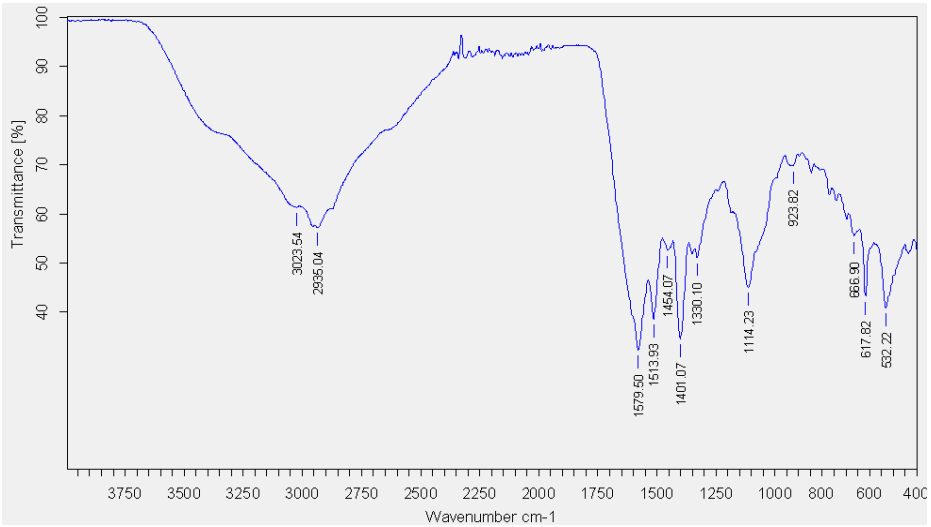

Fig. 5. Infrared spectra of PUR liquid medium containing 0,6% Impranil DLN taken after 7days of incubation with the strain *Bacillus subtilis*

disappearance of the 1730/cm peak of the characteristic function urethane. Polyurethane degradation is the result of synergistic activity between endopolyurethanases and exopolyurethanases. Endoenzymes hydrolyze the PU molecule at random locations throughout the polymer chain leading to loss of tensile strength. Exoenzymes remove successive monomer units from the chain ends, however, show little loss of tensile strength (Wales and Sagar, 1988).

CONCLUSION

Several reports have appeared in the literature on the susceptibility of PUs to fungal attack (Darby and Kaplan,1968; Kaplan et al.,1968; Ossefort and Testroet, 1966). These studies revealed that polyester-type PUs are more susceptible to fungal attack than other forms. In addition, polyethers PUs were noted to be moderately too highly resistant. The current literature reports some fungi having the ability to degrade polyurethane (Cosgrove et al.,2007; Darby and Kaplan, 1968; Gautam et al., 2007), although these studies have focused primarily on microorganisms isolated from samples of soil and plant (Jonathan et al .,2011). This is the first study that demonstrates the degradation of polyurethane by isolated microorganisms from wood. The wide distribution of activity suggests that microorganisms isolated from cedar wood could be promising sources of biodiversity for testing important activities for bioremediation.

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