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Research Article

BIOLOGICAL DEGRADATION OF POLYURETHANE BY A NEWLY ISOLATED WOOD BACTERIUM

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ABSTRACT

Polyurethane is widely used in many products, such as the manufacture of plastic foams, sponges, paints, coatings and fibers. Conventional polyurethane is mostly produced from polyols and diisocyanate. The ecological problems related to the environmental pollution by synthetic polymers like polyurethane are one of the major concerns of the present days, especially because they are difficult to degrade easily and the entire process is time consuming. Currently research is being undertaken on different aspects of polyurethane biodegradation, such as polyurethane degrading microorganisms, the mechanisms of degradation and on isolation and investigation of polyurethane degrading enzymes. This study focuses on the biodegradation of polyurethane by microorganisms isolated from cedar wood. Microbial degradation of polyurethane is thought to be mainly due to the hydrolysis of carbonyl bonds. Analyses were carried out by Infrared spectroscopy and Scanning electron microscopy.

INTRODUCTION

Polyurethane is a synthetic polymer that is formed by condensation polymerization reaction between polyisocyanate and polyol having intramolecular urethane bonds. Urethanes are derivatives of carbamic acids which exist only in the form of their esters. Polyurethanes have gradually replaced earlier polymers in various areas including marine and aircraft coatings and foams in car seats and furnishings (Howard, 2002). In the medical area, polyurethane is considered as one of the most bio and blood compatible materials known. They have played a major role in the development of many medical devices due to their structural properties, blood and tissue compatibility and resistance to macromolecular oxidation hydrolysis and calcification (Santerre *et al*., 2005). The ecological problems related to the environmental pollution by synthetic polymers like plastics are one of the major concerns of the present days, especially because they are difficult to degrade easily; and the entire process is time consuming. There is a world-wide research effort to develop biodegradable polymers as a waste management option for polymers in the environment. Biodegradation (i.e. biotic degradation) is a chemical degradation of polymers provoked by the action of microorganisms, such as bacteria, fungi and algae. The most common definition of a biodegradable polymer is "*a degradable polymer wherein the primary degradation mechanism is through the action of metabolism by microorganisms*." (Katarzyna *et al*., 2010) Biodegradation is considered a type of degradation involving biological activity. Biodegradation is expected to be the major mechanism of loss for most chemicals released into the environment. Biodegradation is the process in which microorganisms like fungi and bacteria degrade the natural polymers and synthetic polymers (polyurethane) (Gu *et al*., 2000a). Since the microorganisms possess different characteristics, the degradation varies from one microorganism to another. Microorganisms degrade the polymers like polyurethane by using it as a substrate for their growth (Glass *et al*., 1989). Various factors which are responsible for biodegradation are kind of polymers, organism characteristics, and the type of treatment required (Gu, 2000b; Arthan *et al*., 2008). Discoloration, cracking, erosion and delimitation are some of the characteristics which indicate the degradation of polymers. Breakage of bonds, transformation due to chemicals, and synthesis of new functional groups are responsible for the variations (Pospisil *et al*., 1997). In this context, we considered

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studying biodegradation of polyurethane sold under the name Impranil DLN by bacteria isolated from decayed cedar wood.

MATERIALS AND METHODS

Bacterial Strain Isolation and Molecular Identification

The strain was isolated and purified from decaying wood in the old Medina of Fez, Morocco. The strain has been identified by sequence similarity of the 1500 nucleotides of the polymerase chain reaction amplification (PCR) targeting 16s rDNA genes realized using the primers Fd1 and RP2. The extraction of the genomic DNA was performed by the kit of the platform "GenElute Bacterial Genomic DNA kit" SIGMA. The PCR mixture contained, 5 µl of buffer (5x), 0.125 µl of FD1 (100µlM), 0.125 µl of RP2 (100µlM), 0.2 µl of Taq polymerase (5U/ µl (GoTaq Gold; Promega), 100 ng of the DNA template and QSP of H_2O of a final volume of 25 µl. The reaction was amplified in a Thermal Cycler (Verity ABI) using the following program: 95°C, 1min ; (95°C, 15 sec ; 52°C, 20 sec ; 72°C, 15 sec) 35x ; followed by a final extension step of 72°C, for 3 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

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. For biodegradation essay on test tube, Luria-Bertani (LB) medium was prepared by adding 0.5g yeast extract, 10.NaCl, and 1.0g tryptone to 100 ml dH2O. Impranil DLN (Bayer GmbH, Dormagen, Germany) was also added to the medium with the concentration of 0.6%, 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 10 days of incubation and after lyophilization.

Scanning electron microscopy

SEM was used to characterize surface modifications of polyurethane samples. Images of experimental surfaces showed decomposition and penetration of bacterium on the polyurethane film.

RESULTS AND DISCUSSION

The growth of the separated bacterium on polyurethane

The strain identified was *Bacillus safensis* with LN650578.1 access number and percentage similarity of 99%. This bacterium grows in a liquid medium containing 0.6% of Impranil DLN. After 10 days of incubation at 37 ° C, we have noticed a total disappearance of the white color characteristic of Impranil (Fig 1). This result is consistent with other results that have shown degradation Impranil DLN by champignons isolated from soil and plants (Crabbe *et al*., 1994; Cosgrove *et al*., 2007; Darby *et al*., 1968; Pathirana *et al*., 1984). This study showed that the bacterium *Bacillus safensis* isolated from cedar wood has a high activity to degrade polyurethane and *Bacillus safensis* could be Promising sources of biodiversity.

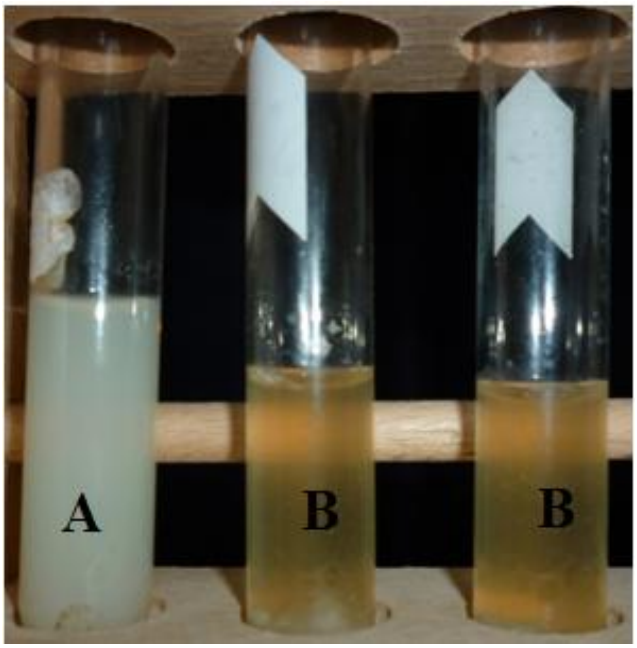

Fig. 1. Tube A: milieu LB + 0,6% Impranil DLN without strain *Bacillus safensis*

Tubes B: milieu LB + 0,6% Impranil DLN after 10 days of incubation with strain

Surface morphology after biodegradation

Surfaces of polyurethanes were observed by scanning microscopy, and are shown in Fig 2. Examination shows that the initial film surface is smooth and continuous. After biodegradation, damage of the polyurethane surfaces was revealed, and having developed a large porous structure, pits and dimples that became more numerous, and we also note a much net growth of the bacterium *Bacillus safensis* on the polyurethane surface.

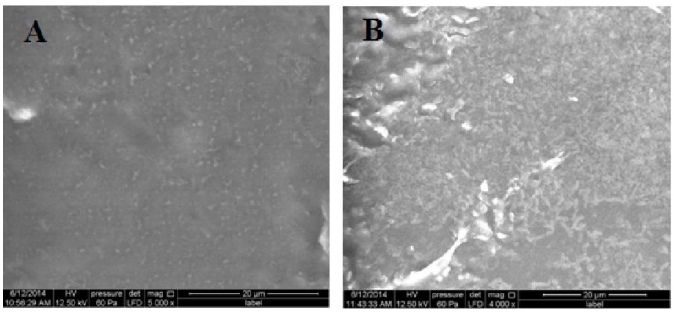

Fig. 2. Surface A : befor contact without strain *Bacillus safensis*

surface B: After contact with stain *Bacillus safensis.*

The growth of the *Bacillus safensis* on a liquid medium resulted in the visual disappearance of the polyurethane. polyurethane possesses carbonyl bonds that are vulnerable to hydrolysis. It is therefore thought that degradation of polyurethane is mainly due to the hydrolysis of carbonyls bonds. IR analysis of the degradation of PUR confirms this hydrolyze, PUR tests of Impranil DLN before contact with bacillus safensis display a large absorption peak at 1730 cm^{-1} corresponding to the C (O)-O ester linkage in the polyurethane polymer (Fig. 3). Bacterium was added to the media of polyurethane after 10 days of incubation at 37°C, the media was analyzed by IR, and results show a complete loss of the absorbance peak at 1730 cm^{-1} and more subtle changes at another wave number (Fig. 4). The loss of this peak is consistent with hydrolysis of the ester bond in the urethane linkage. These results are the same given by bacillus subtilis (Nakkabi *et al*., 2015).

In this study the degradation of polyurethane by *Bacillus safensis* has been chemically demonstrated by infrared spectroscopy, which shows the disappearance of the 1730cm-1 peak of the characteristic function carbonyl. The polymer used in this study is a polyester polyurethane, the ester function is Located along the polyurethane chain. A possible biodegradative pathway of PU is shown schematically (Fig. 5). Several investigators have suggested microbial attack on PUs could be through enzymatic action of hydrolyses such as ureases, proteases and esterases (Evans 1968; Fililip *et al* 1978; Hole 1972; Griffin 1980). Hydrolysis of this function causes the degradation of polyurethane. Indeed, the front contact surface with the bacteria was opaque and homogeneous, after contact with the bacteria the surface showed cracks with a clear growth of the bacteria on the surface. This examination technique of the surface by the scanning electron microscope

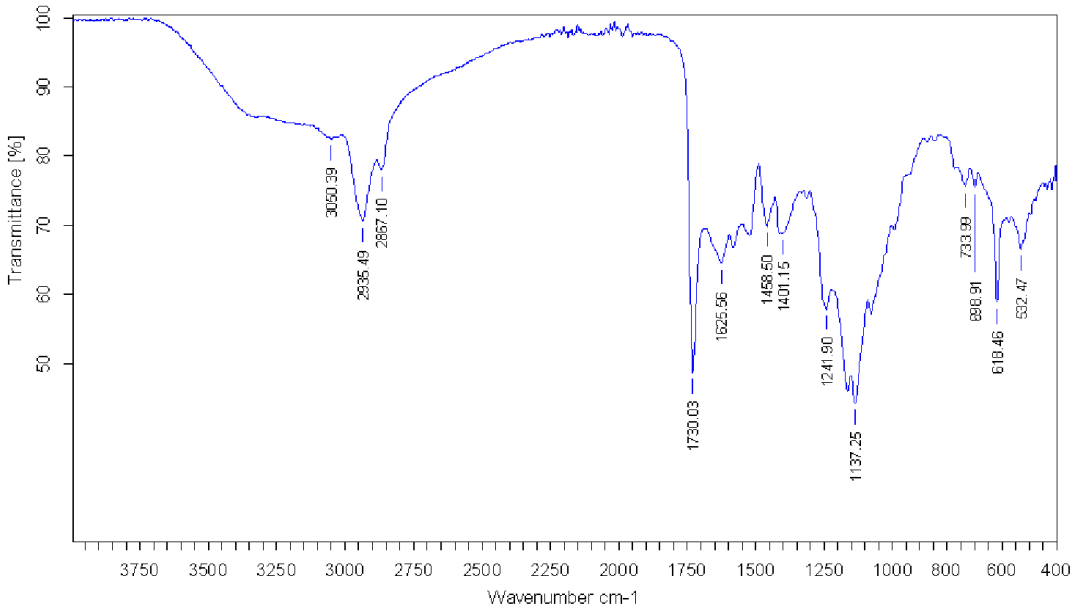

Fig. 3. Control; infrared spectrum of the medium LB + 0.6% Impranil DLN before contact with the bacteria *Bacillus safensis*

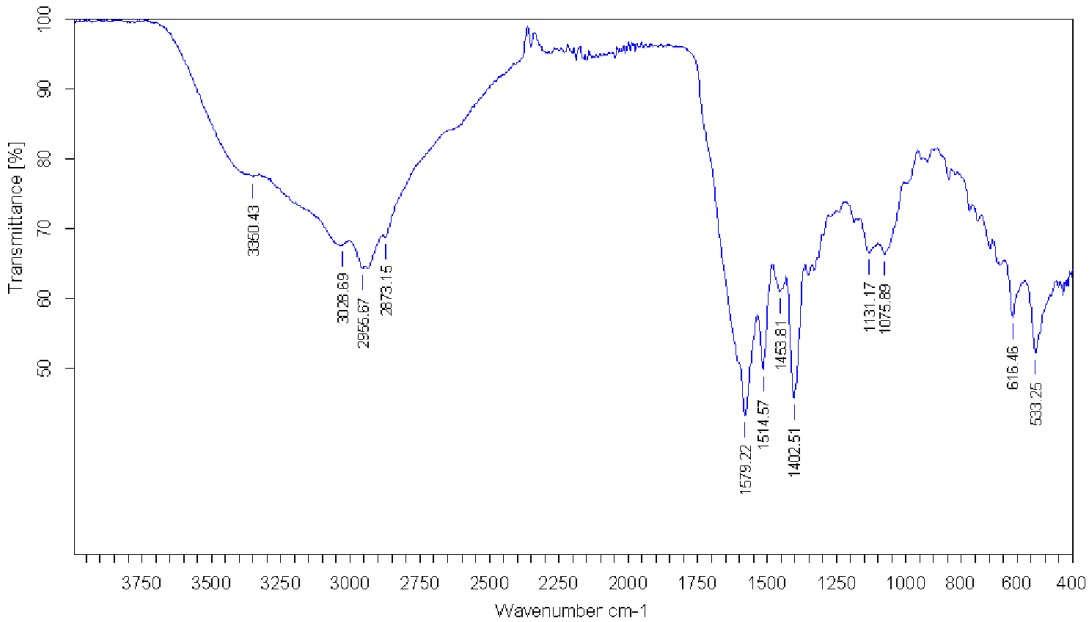

Fig. 4. Infrared spectra of PUR liquid medium containing 0,6% Impranil DLN taken after 10 days of incubation with the strain *Bacillus safensis*

has been used in other reports that showed polyurethane degradation by fungi and bacteria (Stefan 2010).

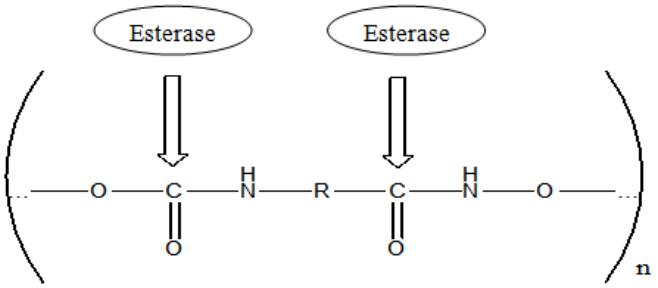

Fig. 5. Theoretical degradative pathway of polyester-polyurethane by esterase activity of Bacillus safensis

Conclusion

In conclusion, the bacterium *Bacillus safensis* isolated from cedar wood could degrade polyurethane. In a first step, test on a liquid medium showed the complete disappearance of the white color of the product Impranil. The second step of this study examined the surface of the product and the corresponding images confirmed the degradation of the polyurethane and the growth of the bacteria on the surface. The last step is infrared analysis showed the disappearance of the carbonyl.

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REFERENCES

Artham, T. and Doble, M. 2008. Biodegradation of Aliphatic and Aromatic Polycarbonates. *Macromol Biosci*; 8: 14-24

Cosgrove, L., P. L. McGeechan, G. D. Robson and P. S. Handley, 2007. Fungal communities associated with degradation of polyester polyurethane in soil. *Appl. Environ. Microbiol*; 73: 5817–5824.

Crabbe, J. R. J. R. Campbell, L. Thompson, S. L. Walz and W. W. Schultz 1994. Biodegradation of a colloidal ester-based polyurethane by soil fungi. *Int. Biodeterior. Biodegrad*; 33: 103–113.

Darby, R. T. and Kaplan, A. T 1968. Fungal susceptibility of polyurethanes. *Appl. Microbiol*; 16: 900-905.

Evans, D.M. and Levisohn, I 1968. Biodeterioration of polyester-based polyurethane. *International Biodeterioration Bulletin*; 4: 89–92.

Flilip, Z. 1978. Decomposition of polyurethane in a garbage landfill leakage water and bysoil microorganisms. *European Journal of Applied MicrobiologyBiotechnology*; 5, 225–231.

Glass, JE and Swift, G. 1989. Agricultural and Synthetic Polymers, Biodegradation and Utilization, ACS Symposium. *American Chemical Society Washington* DC; Series 433

Griffin, G.J.L. 1980. Synthetic polymers and the living environment. *Pure and Applied Chemistry*; 52: 389–407.

Gu, JD., Ford, TE., Mitton, DB. and Mitchell, R. 2000. Microbial corrosion of metals. In: Uhlig corrosion handbook; (2nd Edn) Wiley, New York

Gu, JD., Ford, TE., Mitton, DB and Mitchell, R. 2000. Microbial degradation and deterioration of polymeric materials. Revie RW (eds) In: Uhlig corrosion Handbook; (2ndedn) John Wiley and Sons, New York

Hole, L.G. 2002. Artificial leathers. Reports on the Progress of Applied Chemistry 1972; 57: 181–206. Howard, G. T. Biodegradation of polyurethane: a review. *Int. Biodeterior. Biodegrad*; 49:245–252.

Katarzyna Leja and Grażyna Lewandowicz, 2010. Polymer Biodegradation and Biodegradable Polymers *Polish J. of Environ. Stud;* Vol. 19, No. 2 , 255-266

Nakkabi, A. *et al*. 2015. Biodegradation of Poly(ester urethane)s by Bacillus subtilis *Int. J. Environ. Res*; 9(1):157-162, ISSN: 1735-6865

Pathirana, R. A. and Seal, K. J. 1984. Studies on polyurethane deteriorating fungi. *Int.Biodeterior. Biodegrad*; 20: 163–168

Pospisil, J. and Nespurek, S. 1997. Highlights in chemistry and physics of polymer stabilization. Macromol Symp; 115:143-63

Santerre, J.P., Woodhouse, K., Laroche, G. and Labow, R.S. 2005. Understanding the biodegradation of polyurethanes: from classical implants to tissue engineering materials. *Biomaterials*; 26: 7457–7470.

Stefan, 2010. Oprea Dependence of fungal biodegradation of PEG/castor oil-based polyurethane elastomers on the hard-segment structure polymer degradation and stability; 95: 2396-2404.

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