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Growth of *Pseudomonas* in polyurethane medium

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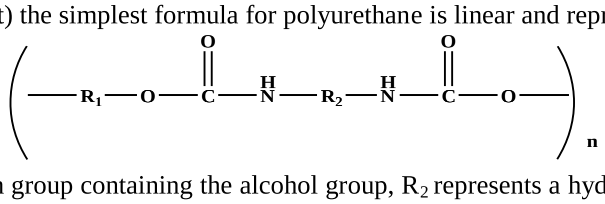
Abstract: - Polyurethane is a material that can be found in many products that we use in our daily lives. They represent a class of polymers that have found a widespread use in the medical, automotive and industrial fields. Polyurethane is a general term used for a class of polymers derived from the condensation of polyisocyanates and polyalcohols. Polyurethanes are favorably characterized by their substantial tensile strength and high melting points which enables them to be extremely durable. They have proven to be excellent replacements of plastics because of their resistance to degradation by water, oils, and solvents. After years of production of polyurethane, manufacturers found them susceptible to degradation. There are many reports on the degradation of polyurethane by microorganisms, especially by fungi. Microbial degradation of polyurethane is thought to be mainly due to the hydrolysis of carbonyl bonds. The main objectives of this research are to investigate polyurethane (PUR) degradation by the bacterium Pseudomonas stutzeri. In order to confirm that Pseudomonas stutzeri persist in the medium despite the high concentration of polymer, and the degradation process continues until the total disappearance of the polymer. Results were justified by Infrared spectroscopy.

Keywords: *biodegradation, Pseudomonas stutzeri, polyurethane, infrared, Impranil DLN.*

I. INTRODUCTION

Since the discovery of the urethane function (carbamate) by the chemist Charles Adolphe Wurtz in 1849, the Polyurethanes have been applied in various fields. For instance, they have been used in the medical industry (automotive, manufacturing glue paints, varnishes), in textiles (sportswear), construction of buildings (thermal insulation, waterproofing). Their resistance to aggressors, such as temperature, water, oils, solvents, is thought to be excellent alternatives to plastic. Polyurethanes are a broad class of materials utilized in a wide variety of applications. Products in this family are chemically complex and may contain several different types of bonds, yet all have the polyurethane linkage in common. This linkage is formed from the reaction between the isocyanate functionality of one component with the alcohol group of another component. By controlling the composition of each component, two solid state phases frequently result. This two-phase morphology provides the key to controlling performance of the final product and gives the manufacturer versatility in tuning properties as desired by varying the composition or content of one or the other phases. They can take various forms (soft to hard) according to the chemical structures of polyisocyanates and polyols (functional group number or molecular weight) the simplest formula for polyurethane is linear and represented by:



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

In general, synthetic plastics (e.g., polyethylene or polystyrene) are not biodegradable, PUR, in particular, polyester PUR, is known to be vulnerable to microbial attack [1, 2]. For this reason, polyester PUR has not been considered as being very useful. With more and more plastics being utilized, environmental problems caused by their non-biodegradable characteristics have become a major concern. Today we are witnessing a massive production of polyurethanes (millions of tons per year). Observation has prompted the scientific community to consider limiting the adverse negative effects even if these non-degradable polymers on the quality of human and animal life.

A fundamental comprehension of the mechanisms of polyurethane degradation should enable researchers to develop a more efficient technique for the biodegradation of polyurethane. As the production and use of

polyurethane increases annually, there is a need for efficient and environmentally friendly techniques designed for its disposal.
As a matters of fact, in our research we studied the biodegradation of polyurethane by bacteria. Several strains have expressed degrading polyurethane power; the highest activity was attributed according to our results the Pseudemonas bacterium

II. MATERIALS AND METHODS

Polymer studied: The specific polyurethane used in this work was Impranil DLN (Bayer GmbH,Dormagen, Germany). Impranil DLN is polyurethane that has been made from a poly hexane/neopentyl adipate polyester and hexamethylene diisocyanate)
Organism: *Pseudomonas stutzeri* was isolated, identified and obtained from the microbiology laboratory of the School of Biology of Seville by its ability to degrade polyurethane.
Degradation of Polyurethanes test, media and culture conditions: 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 0.3%,0.6%, 1%,2%,3%, and 4% and incubated 7 days at 37 ° C.
IR spectra of the medium were analyzed using an IR spectrometer (FT/IR-4100 type A), after 7 days of incubation and after lyophilization

III. RESULT AND DISCUSSION

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.
Of the organisms screened, organisms produced a halo of clearance such as that shown in (Fig. 1).

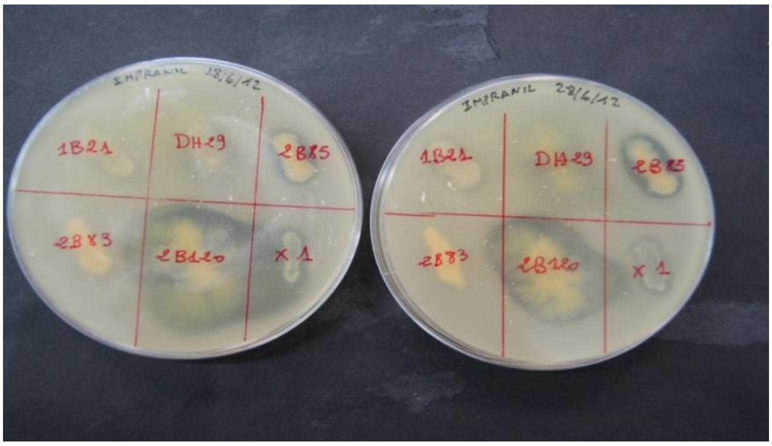
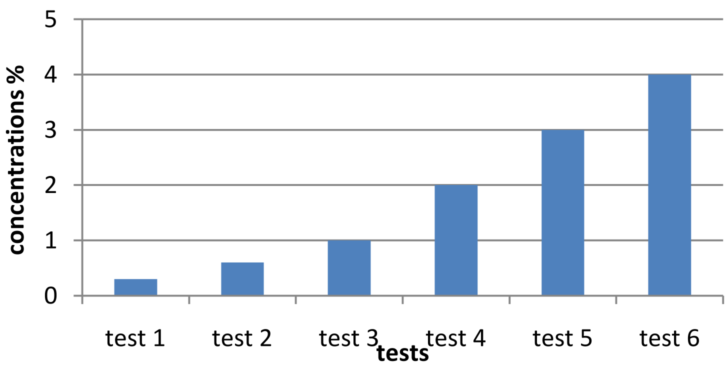
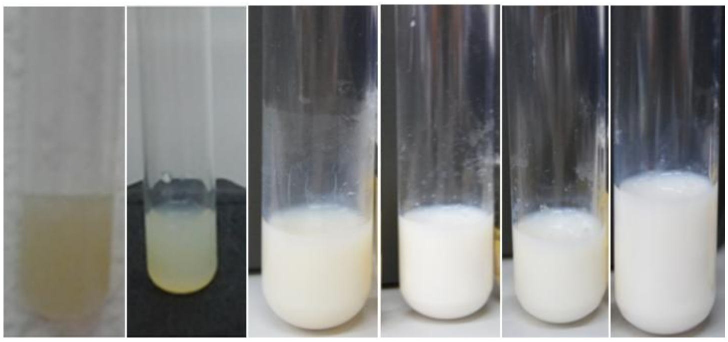


Fig.1. growth of *Pseudomonas stutzeri* on a LB plate supplemented with 0.6% impranil with a zone of clearing around the edges of colonies showing polyurethane degradation

Test in a liquid medium: to test the action of the bacteria isolated on the polymer to degrade, we conducted several tests with different concentrations of polymers 0.3%, 0.6%, 1%, 2%, 3%, and 4% (Fig 2)



(A)



(B)

Fig 2: A: medium with different concentration of Impranil DLN from 0,3% to 4%.

B: medium without *Pseudomonas stutzeri*

We find for the two tests 0.3% and 0.6% that the polymer is completely degraded after 7 days of incubation at 37°C, we noticed the disappearance of the characteristic white color of impranil.

For tests in which the concentration of polymer in the medium is from 1% to 4%, we noticed a degradation from the 20th day (Fig 3). These tests showed that the bacteria *Pseudomonas stutzeri* persist in the medium despite the high concentration of polymer, and the degradation process continues until the total disappearance of the polymer. Media were centrifuged to remove the bacteria, and the remaining liquid was lyophilized to IR analysis

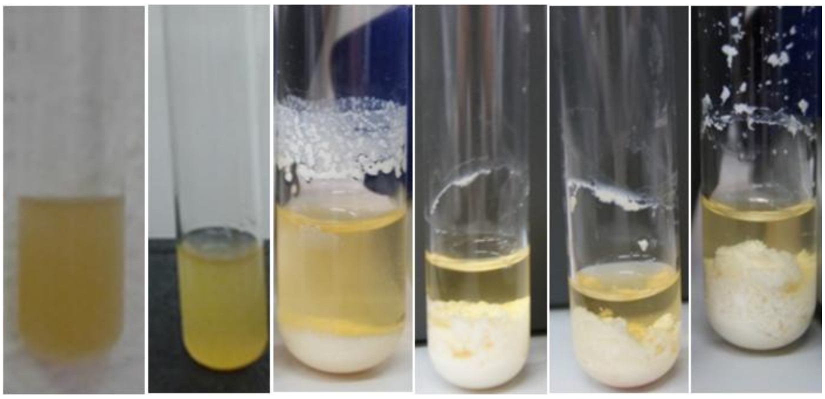
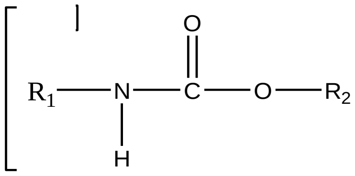


Fig 3: Medium after incubation with *Pseudomonas stutzeri*

IR analysis of the degradation of PUR: The mechanism of PUR degradation was initially investigated by infrared spectroscopy [3, 4]. PUR samples of Impranil DLN display a large absorption peak at 1731 cm^{-1} corresponding to the C (O)-O ester linkage in the polyurethane polymer (Fig. 4). The bacterium *Pseudomonas stutzeri* was added to the media 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^{-1} was observed and was accompanied by more subtle changes at another wave number (Fig. 5). By the time the culture has become visually transparent, there was a complete loss of the absorbance peak at 1735 cm^{-1} (Fig. 6). The loss of this peak is consistent with hydrolysis of the ester bond in the urethane linkage.



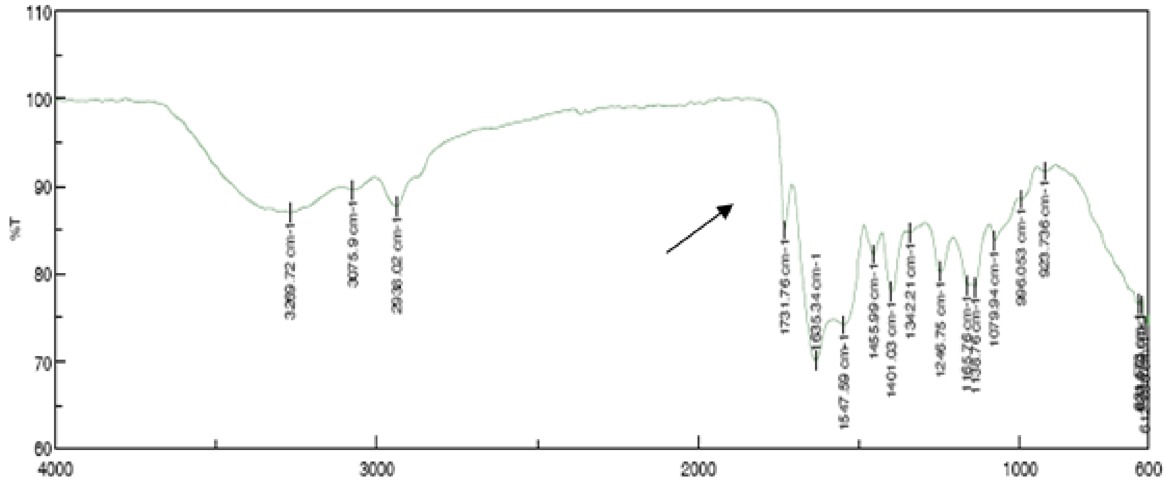


Fig.4. Infrared spectra of PUR liquid medium containing 0.6% of Impranil DLN without strain *Pseudomonas stutzeri*

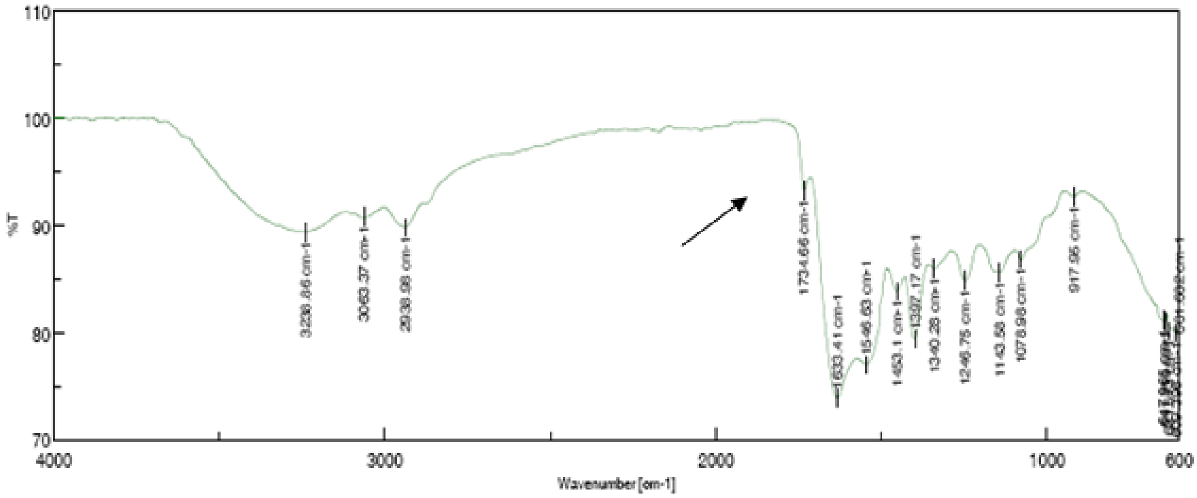


Fig.5. Infrared spectra of PUR liquid medium containing 0.6% of Impranil DLN taken after 2 days of incubation with the strain *Pseudomonas stutzeri*

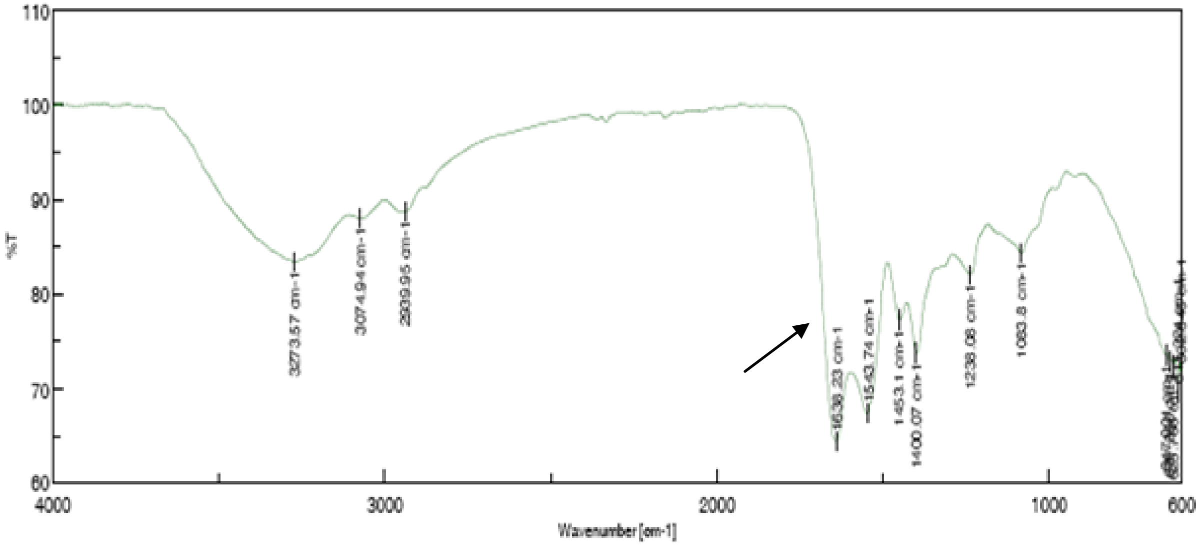


Fig.6. Infrared spectra of PUR liquid medium containing 0,6% Impranil DLN taken after 7days of incubation with the strain *Pseudomonas stutzeri*.

Polyester PUR possesses many ester bonds that are vulnerable to hydrolysis. It is therefore thought that degradation of polyester PUR is mainly due to the hydrolysis of ester bonds. In this study the degradation of polyurethane by *Pseudomonas stutzeri* has been chemically demonstrated by infrared spectroscopy, which shows the disappearance of the 1730cm-1 peak of the characteristic function urethane. There are three reported mechanisms of PUR biodegradation: fungal biodegradation [5-8], bacterial biodegradation [9-15] and degradation by polyurethanase enzymes [16].

However, in order to make PUR waste treatment realistic in practice, the treatment conditions, such as the growth conditions of microorganisms and composition of the medium components need to be investigated. The objective of this paper is therefore to investigate the effects of different concentrations of PUR on bacterial growth. The results showed that the bacteria persist in the medium despite the high concentration of polymer, and the degradation process continues until the total disappearance of the polymer. The long-term goal is to design a process for plastic waste biological treatment.

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