



## Biodegradation study by *Pseudomonas* sp. of flexible polyurethane foams derived from castor oil



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### ABSTRACT

The synthesis and biodegradation of polyurethane foams obtained from environmentally benign processes were studied.

Flexible polyurethane foams based on castor oil modified with maleic anhydride (MACO) were synthesized. The synthesis involved a single-stage process by mixing castor oil/MACO (weight ratios 75:25 and 25:75) and 2–4 toluene diisocyanate (TDI) in stoichiometric amount of OH:NCO. The biodegradability studies with cultures of a *Pseudomonas* sp. strain (DBFIQ-P36) involved incubation periods of 2 months at 37 °C. Polymers were characterized before and after biodegradation by Fourier Transform Infrared Spectroscopy (FT-IR), INSTRON mechanical tester, and Scanning Electron Microscopy (SEM). The results showed that the addition of MACO produces a considerable increase in the rate of degradation and an important change in the chemical and morphological structures. This is due to the presence of ester groups that are vulnerable to chemical hydrolysis and enzymatic attack. The eco-toxicity after the biodegradation was evaluated. Toxic compounds such as primary amines were identified by Gas Chromatography–Mass Spectrometry (GC–MS) in combination with Nuclear Magnetic Resonance (NMR) as degradation products.

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### 1. Introduction

Due to the environmental impact caused by the growing use of polymers derived from petroleum, there is great interest in the development of new environmental-friendly polymers, with low costs and controlled life span. The materials to be designed must present both good physical properties and biodegradable characteristics. Following this idea, the use of renewable sources like vegetable oils – soy, palm, tung, castor – constitutes an interesting alternative (Guner et al., 2006; Sharma and Kundu, 2008; Aranguren et al., 2012).

Castor oil (CO) exhibits an unusual chemical composition that makes it quite valuable for many applications. It also presents advantages such as: renewability, easy availability in a large quantities, and low cost (Mutlu and Meier, 2010). The reactive hydroxyl functional group contained in its structure can be used as a polyol – polyester to create new and “green” macromolecular architectures

of polyurethanes (PUs) by reaction with different diisocyanates (Hablott et al., 2008; Papadopoulos et al., 2008).

There are several reports related to the elaboration of PUs based on castor oil, such as interpenetrating polymer networks (Quipeng et al., 1990; Xie and Guo, 2002; Niranjana et al., 2009), elastomers (Yeganeh and Mehdizadeh, 2004; Oprea, 2010), coatings (Trevino and Trumbo, 2002), adhesives (Somani et al., 2003), and some semi-rigid PU foams that have potential uses in thermal insulation (Ogunniyi et al., 1996) and other interesting applications. Wang et al. (2008) prepared a polyester-polyol (MACO) by chemical modification of hydroxyl groups with maleic anhydride. Then, biodegradable semi-rigid PU foams were synthesized by copolymerization of MACO with styrene using benzoyl peroxide (BPO) as initiator. The products exhibited mechanical properties comparable to those of a foam derived from commercial polyether. Mazo et al. (2011, 2012a,b) studied the kinetics of transesterification and condensation of castor oil with maleic anhydride using conventional and microwave heating.

PUs based on vegetable oils are polyester-type PUs that exhibit biodegradable characteristics due to the presence of ester groups. As a consequence of their chemical structures, it was reported that the biodegradation mechanism is hydrolysis by water action and by

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enzymatic attack (Gu, 2003, 2007; Wang et al., 2008; Dutta et al., 2009; Oprea, 2010; Aranguren et al., 2012). For example, Wang et al. (2008) studied the degradation rate of PU foams based on CO buried in soil under controlled conditions during 4 months. After the biodegradation the PUs showed a clear weight loss and a decrease of mechanical properties.

The impact of water, fungi, bacteria and enzymes (protease, urease, lipase and esterase) on PUs derived from petroleum has been tested in several opportunities using different techniques under controlled conditions (Darby and Kaplan, 1968; Young and Sung, 1988; Nakajima-Kambe et al., 1995, 1997, 1999; El-Sayed et al., 1996; Skarja and Woodhouse, 1998; Gu and Li, 2005, 2007; Shah et al., 2008a,b; Rodrigues da Silva et al., 2010). It was confirmed that PUs are susceptible to microbial attack, especially by fungi (Toward, 2002). Darby and Kaplan (1968) reported that polyester-type PUs were more susceptible to fungal attack than polyether-type PUs, due mainly to the hydrolysis of ester bonds.

The degradation mechanisms depend on abiotic and biotic (type and quantities of microorganisms and organism) factors. They are also affected by polymer characteristics, such as additives content, morphology, chemical composition, crystallinity degree, hard to soft segment ratios, hydrolysable chain extenders in the hard segment, and diisocyanate types used in the synthesis (Young and Sung, 1988; Toward, 2002, Gu, 2007; Shah et al., 2008a,b; Lucas et al., 2008; Rodrigues Da Silva et al., 2010).

Some authors investigated the degradation of PUs by enzymes secreted by specific bacteria (Nakajima-Kambe et al., 1995; El-Sayed et al., 1996; Nakajima-Kambe et al., 1997, 1999; Akutsu et al., 1998; Howard and Blake, 1998; Howard et al., 1999; Akutsu-Shigeno et al., 2006; Gu and Li, 2006; Shah et al., 2008a,b). El-Sayed et al. (1996) studied the degradation for about 120 h of polyester-type PU coatings by five different bacterial strains isolated from soil and identified as *Acinetobacter calcoaceticus*, *Arthrobacter globiformis*, *Pseudomonas cepacia* and two strains of *Pseudomonas* sp. Other four strains were also investigated: *Pseudomonas aeruginosa*, *Pseudomonas putida*, and two *A. Calcoaceticus* strains provided by the U.S. Navy. All microorganisms exhibited esterase activity. Shah et al. (2008a,b) reported the degradation of polyester-type PU using five types of bacteria isolated from soil identified as *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Arthrobacter* sp. and *Corynebacterium* sp. Also, the presence of esterase enzyme was detected. Gautama et al. (2007) studied the biodegradation of automotive waste polyester-type PU foams using *Pseudomonas chlororaphis* ATCC55729.

In most cases, additional nutrients for microorganisms were incorporated in biodegradation tests. However, Nakajima-Kambe et al. (1995), Nakajima-Kambe et al. (1997) and Akutsu et al. (1988) showed that *Comamonas acidovorans* strain (TB-35) was able to degrade polyester-type PU synthesized from poly (diethylene adipate) (with molecular weight of 2500 and 2690) with TDI, using the PU as sole carbon source. The microorganism exhibited esterase activity. The TB-35 strain produced two different esterases, one of which was secreted to the culture broth and the other was bound to the cell surface. It was detected that the cell surface-bound esterase is vital for PU degradation, hydrolyzing the ester linkage and releasing diethylene glycol and adipic acid.

Although the term “biodegradation” indicates the predominance of biological activity in the phenomenon, in nature biotic and abiotic factors act synergistically to decompose organic matter. The biodegradation of polymeric materials in general includes four steps: biodeterioration, depolymerization or biofragmentation, assimilation and mineralization. These processes generate oligomers, dimer and monomer, and finally CO<sub>2</sub> and H<sub>2</sub>O (Lucas et al., 2008; Shah et al., 2008a,b). However, in the case of PUs, complete

degradation was not reported and the biodegradation mechanism has not been totally elucidated.

In this work, the synthesis, characterization and biodegradation of new flexible PU foams based on CO modified with maleic anhydride are studied. The final properties of the materials are evaluated and compared with traditional PU foams based on polyethers. The degradation of the polymers by enzymatic attack with *Pseudomonas* sp. cultures under controlled aerobic conditions is investigated at laboratory level. The *Pseudomonas* sp. was chosen following previous works (Howard and Blake, 1998; Gu and Li, 2005; Gautama et al., 2007; Shah et al., 2008a,b; Dutta et al., 2009). Also, this bacterium presents advantages such as its **abundance** in the environment and its characteristic property of having plasmids with high ability to degrade a variety of organic compounds which are not used as nutrients by other bacterium species. The weight loss and the tensile strength of the samples are measured in order to determine the degradation degree. Also, Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM) are used to follow molecular and morphological changes along the degradation process. Additionally, the toxic effect after the biodegradation is evaluated by bacterial Microtox® bio-assay and the degradation products are identified by gas chromatography–mass spectrometry (GC–MS) in combination with Nuclear Magnetic Resonance (NMR).

## 2. Materials and methods

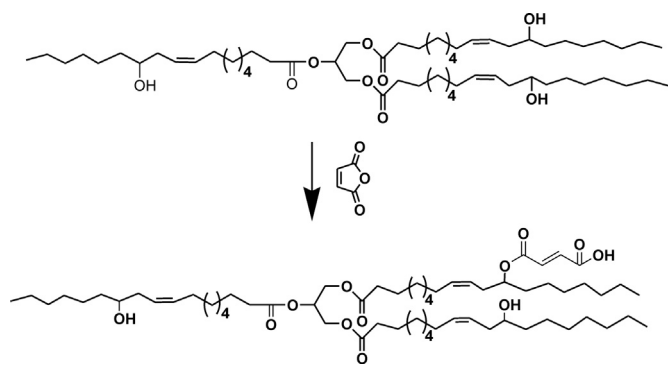
### 2.1. Materials

CO of USB-grade (hydroxyl value 159.5 mg of KOH g<sup>−1</sup>, 930 g mol<sup>−1</sup>) and commercial-grade maleic anhydride (MA) were obtained from Merck (Whitehouse Station, NJ, USA), toluene diisocyanate (TDI, VORANATE™ T-80) from The Dow Chemical Company (California, USA), polyether polyol (Arcol F3040, 3000-molecular weight triol, OH value 56 mg of KOH g<sup>−1</sup>) from Bayer Material Science (Santa Clara Mexico), silicone L-580 (Niax\*) from Momentive (Cotia, Brasil), and triethylenediamine (TEA) and stannum octoate from Sigma–Aldrich. Nutrient Broth, Agar–Agar and Nutrient Agar were provided by Difco (Argentina) and, KOH, KH<sub>2</sub>PO<sub>4</sub>, K<sub>2</sub>HPO<sub>4</sub>, NH<sub>4</sub>NO<sub>3</sub>, Glucose, MgSO<sub>4</sub>·7H<sub>2</sub>O, ZnSO<sub>4</sub>·7H<sub>2</sub>O, CuSO<sub>4</sub>·7H<sub>2</sub>O, FeSO<sub>4</sub>·7H<sub>2</sub>O and MnSO<sub>4</sub>·7H<sub>2</sub>O by Cicarelli (Argentina). For the toxicity tests, the *Vibrio fischeri* Lyophilized bacterium (lot number 8L-1022) from Germany was used.

For the biodegradation study, a bacterial strain belonging to the microorganisms collection of the Biotechnology Department (Facultad de Ingeniería Química, Universidad Nacional del Litoral–Argentina) was employed. It was isolated from soil surface, preserved by freezing at −80 °C (after addition of 15% glycerol) in the nutrient broth, and identified as DBFIQ-P36 strain.

### 2.2. Synthesis of polyol based on the modified castor oil

The esterification reaction of CO (311 g, 0.33 mol) with maleic anhydride (32.70 g, 0.33 mol) was carried out in 250 ml four-necked round-bottom flask equipped with a magnetic stirrer (500 rpm) and thermometer, and under inert conditions. CO and maleic anhydride were loaded using a molar ratio of 1:1. The reaction proceeded with continuous stirring at 100 °C for 2 h. The product was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and extracted three times in order to remove the unreacted anhydride and/or maleic acid. The organic phase was collected, dried over anhydrous MgSO<sub>4</sub> and concentrated under reduced pressure. The reaction was monitored through the determination of the acid number (ASTM American Society for Testing and Materials, 2004f) by titration with 0.1 mol l<sup>−1</sup> KOH ethanol solution. For the modified oil, the hydroxyl value quantified by a standard procedure (ASTM



**Scheme 1.** Simplified representation of the MACO synthesis.

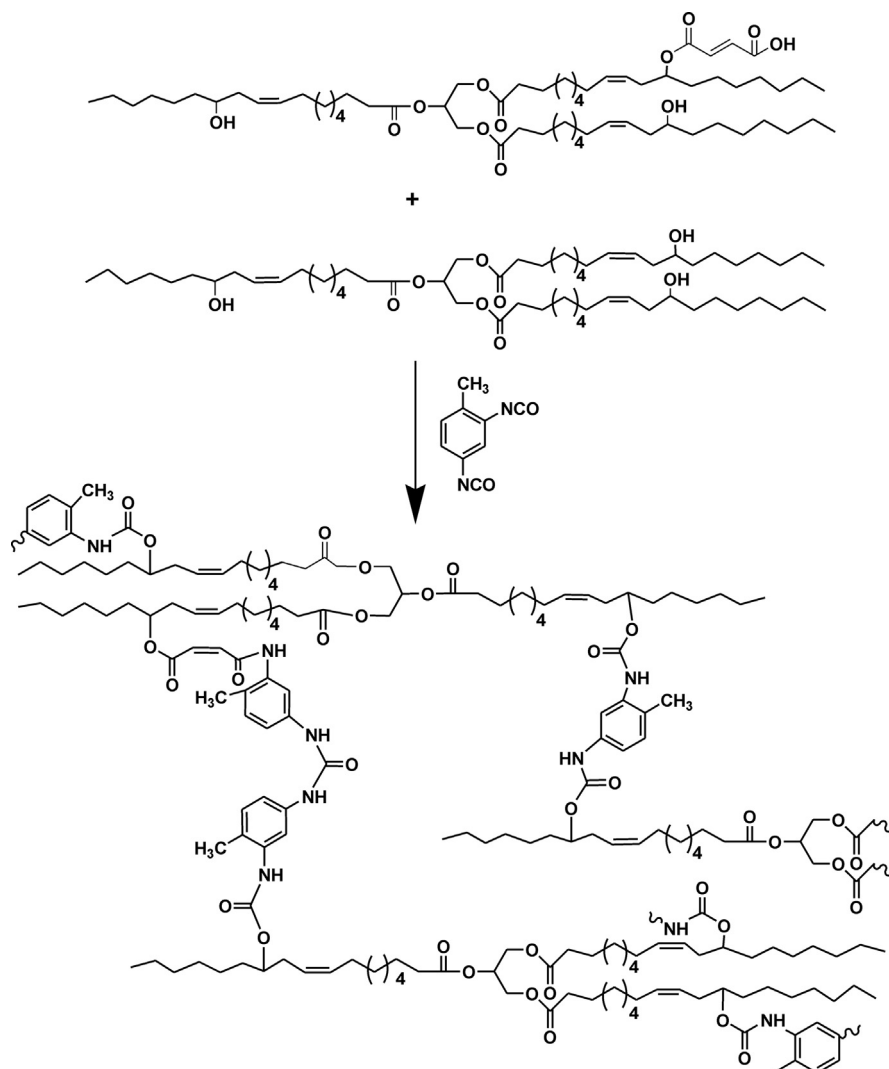
American Society for Testing and Materials, 2004e) was  $89.9 \text{ mg KOH g}^{-1}$  which indicates a conversion of hydroxyl groups of ca. 32%. Scheme 1 shows the MACO synthesis. This is a simplified representation of the reaction since statistically there could be triglyceride molecules reacted with zero, one, two or three maleic anhydride molecules. However, GPC analyses (not shown) indicated the presence of one peak corresponding to the addition of one molecule of maleic acid to one molecule of triglyceride.

### 2.3. Synthesis of polyurethanes

Two PU foams from MACO/CO (PU-1, PU-2) were prepared using a single-stage process (Scheme 2) and the reaction conditions are shown in Table 1. The following reagents were added: CO/MACO, silicone (25–35% of siloxane and molecular weight of 6000), distilled water, TEA, and stannum octoate. A homogenous mixture of reagents was obtained by using a mechanical stirrer at 1200 rpm. Then, TDI was added into the reactor and the system was stirred for 10 s. The resultant mixture was immediately introduced into an open mold to produce free-rise foam. The amount of TDI required for the reaction with the MACO and distilled water was calculated from their equivalent weights. To complete the reaction, an excess of TDI ( $\text{NCO/OH} = 1.1$ ) was used. After preparation, the foams were placed in an oven at  $30^\circ\text{C}$  for 24 h. Finally, the cured foams were cut ( $100 \text{ mm} \times 3 \text{ mm} \times 1 \text{ mm}$ ). In addition, conventional PU foam (PU-3) was prepared for comparison purpose from a commercial polyether and TDI using similar experimental conditions.

### 2.4. Degradability tests

The composition of the basal mineral medium used for degradation assays was described by Nakajima-Kambe et al. (1995). The



**Scheme 2.** Possible structure of the polyurethanes prepared from MACO/CO.

**Table 1**  
Synthesis of Polyurethane Foams. Reaction Conditions.

Sample	PU-1	PU-2	PU-3
CO/MAC <sup>o</sup>	25/75	75/25	—
Polyether polyol (g)	—	—	100
Silicone (g)	0.50	0.50	0.50
H <sub>2</sub> O (g)	1.50	1.50	1.50
TEA (g)	0.25	0.25	0.25
Stannum octoate (g)	0.20	0.20	0.20
Stirring rate (rpm)	1200	1200	1200

<sup>a</sup>Molar ratio. Weights are reported considering 100 g of the total mass.

glucose and ammonium nitrate were omitted when PU foams were supplied as the sole carbon and nitrogen source. The composition of basal medium was defined as follows: KH<sub>2</sub>PO<sub>4</sub> (2000 mg l<sup>-1</sup>), K<sub>2</sub>HPO<sub>4</sub> (7000 mg l<sup>-1</sup>), NH<sub>4</sub>NO<sub>3</sub> (1000 mg l<sup>-1</sup>), Glucose (3000 mg l<sup>-1</sup>), MgSO<sub>4</sub>·7H<sub>2</sub>O (100 mg l<sup>-1</sup>), ZnSO<sub>4</sub>·7H<sub>2</sub>O (1 mg l<sup>-1</sup>), CuSO<sub>4</sub>·7H<sub>2</sub>O (0.1 mg l<sup>-1</sup>), FeSO<sub>4</sub>·7H<sub>2</sub>O (10 mg l<sup>-1</sup>), MnSO<sub>4</sub>·7H<sub>2</sub>O (2 mg l<sup>-1</sup>) and the pH was 7. For each test, 20 ml of this liquid culture media was poured into 100 ml flasks and sterilized by autoclaving at 121 °C for 15 min.

#### 2.4.1. Bacterial strain

Cultures used for the biodegradation assays were obtained activating the preserved DBFIQ-P36 strain by two consecutive inoculations in test tubes. These cultures were incubated at 37 °C during 24 h to obtain a cell concentration between 10<sup>8</sup> and 10<sup>9</sup> colony forming units (CFU) ml<sup>-1</sup>.

#### 2.4.2. Preliminary biodegradation studies using the Petri dishes technique

The biodegradation of foams was first observed by the method in Petri dishes (Clear Zone method) using different carbon and nitrogen sources. The method was previously employed for polyesters PUs (Shah et al., 2008a,b) and allows visualizing the formation of clear zones around of the colony, indicating the activity of enzymes secreted on the polymer particles.

Two assays were carried out in Petri dishes. In the first one, the PUs were directly mixed with agar–agar and constituted the sole source of carbon and nitrogen. In the second test, the basal medium indicated above was also incorporated that contains glucose and ammonium nitrate as carbon and nitrogen source. In both cases, to maintain the dispersion of polymeric particles, the autoclaved substrate suspension was gently agitated while the mixture was poured into Petri dishes.

Then, DBFIQ-P36 strain was inoculated in the Petri dishes, and incubated at 30 °C for 10 days under aerobic conditions.

#### 2.4.3. Biodegradation studies in liquid medium

Sterile PUs were immersed in autoclaved individual flasks containing 20 ml of the basal medium. A cell population of 10<sup>5</sup> CFU ml<sup>-1</sup> of tDBFIQ-P36 strain was inoculated into each flask and incubated at 37 °C in aerobic conditions. The degradation/deterioration of the PU samples was periodically monitored for 2 months.

### 2.5. Analysis

The biodegradability and long-term integrity of tested PUs were investigated under aerobic conditions on the basis of bibliographical references and ASTM methods (ASTM American Society for Testing and Materials, 2004a,b,c,d). From samples taken along the degradation process, the following analyses were carried out: i) mass loss by gravimetry, ii) bacterial growth by cell counts, iii) morphological and molecular structures by FT-IR and SEM, and iv)

by INSTRON mechanical tester. Also, toxicity tests were performed to the final samples.

#### 2.5.1. PU hydrophilicity

The PUs hydrophilicity was indirectly quantified by the measurement of the water absorbed at 37 °C. The sterilized PU samples were immersed in sterile basal medium and kept at 37 °C. Then, they were removed from water at predetermined time intervals, gently wiped with filter paper, and weighted. After the samples were dried under vacuum at 60 °C, and the weight of the dry samples was determined. To estimate water uptake percent (wt%) the following formula was used:

$$\text{wt\%} = [(W_w - W_d)/W_d] \times 100 \quad (1)$$

where  $W_w$  represents the weight of the wet sample after immersion and  $W_d$  represents the weight of the sample after drying.

#### 2.5.2. Weight loss

To measure the weight loss, the samples were washed with aqueous solution of 50% ethanol and dried under vacuum at 50 °C for 2 days. All determinations were made by triplicate. The weight loss percent was calculated as follow:

$$\text{Weight loss \%} = [(W_i - W_f)/W_i] \times 100 \quad (2)$$

where  $W_i$  and  $W_f$  are the weights of the samples before and after degradation respectively.

#### 2.5.3. Bacterial growth by viable cell counts

The kinetics of bacterial growth was followed by the number of viable cells. For each determination, 1 ml of liquid medium was added to 9 ml of peptone water (0.1% w/w) and several dilutions were prepared. Then, 1 ml of each dilution was loaded in the Petri dish and nutrient-agar was also incorporated under gently agitation. The incubation was carried out at 37 °C for 24–48 h and the colonies were counted by using a colony counter.

#### 2.5.4. Molecular and morphological changes

The molecular structures before and after degradation were analyzed by FTIR. A spectrometer Perkin Elmer, model Spectrum One with accessories of transmission, wavenumber range 400–4000 cm<sup>-1</sup> and 4 cm<sup>-1</sup> resolution was used. All the samples were prepared with KBr. A fine suspension of the samples was dispersed in KBr, and then was pressed to form transparent KBr pellets.

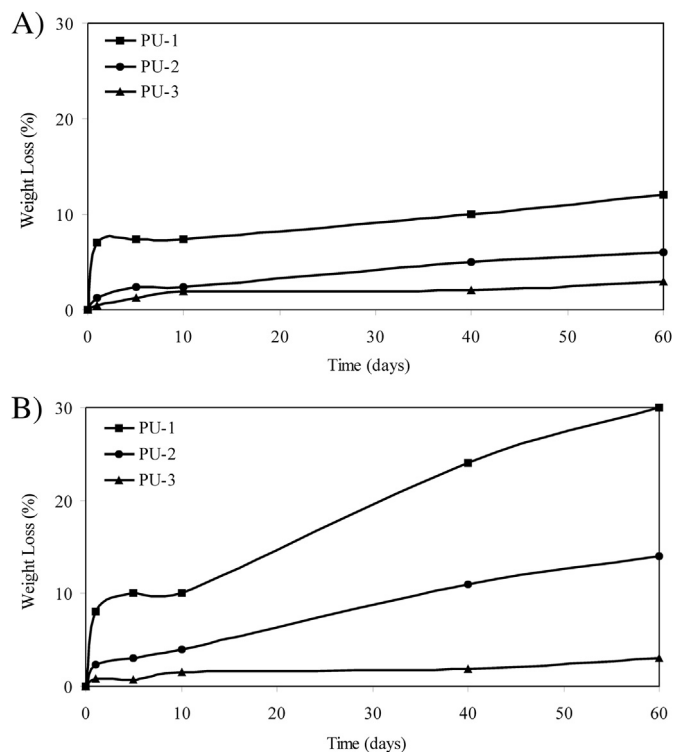
Morphological modifications of the sample surface were evaluated by SEM (JEOL, model JSM-35C) with digital imaging system at an activation voltage of 20 kV.

#### 2.5.5. Mechanical properties

Stress-strain measurements were performed at room temperature (23 °C) in a Universal Testing Machine INSTRON (Model 3344 Q 1469) equipped with a 1 KN load cell, using sample dimensions 100 mm × 10 mm × (0.25–0.35) mm. The strain rate was of 10 mm min<sup>-1</sup>.

#### 2.5.6. Toxicity test

The toxicity of the liquid medium after the degradation was determined using the bacterial Microtox<sup>®</sup> bio-assay. This method allows monitoring changes in natural light emissions of the luminescent bacteria *V. fischeri* (*Photobacterium phosphoreum*) as result of its normal metabolic process. A 500 Microtox<sup>®</sup> analyzer (Azur Environmental, USA) was used. The toxicities of the liquid medium after 5, 15 and 30 min of exposure were measured. The volumes of

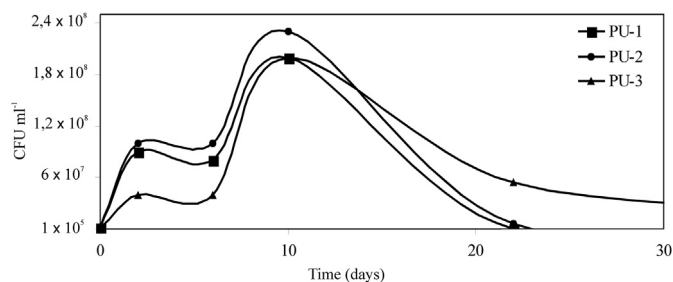


**Fig. 1.** Mass loss profiles of PU samples in absence of microorganisms (A) and in presence of DBFIQ-P36 (B).

sample (in percentage) that produce 20% and 50% decrease of luminescence are reported as the inhibition concentration  $IC_{20}$  and  $IC_{50}$ , respectively, by using Microtox<sup>®</sup> software. The toxicity of phenol standards was measured as a quality reference before analyzing the samples.

#### 2.5.7. Degradation compounds by gas chromatography–mass spectrometry studies (GC–MS) in combination with Nuclear Magnetic Resonance (NMR)

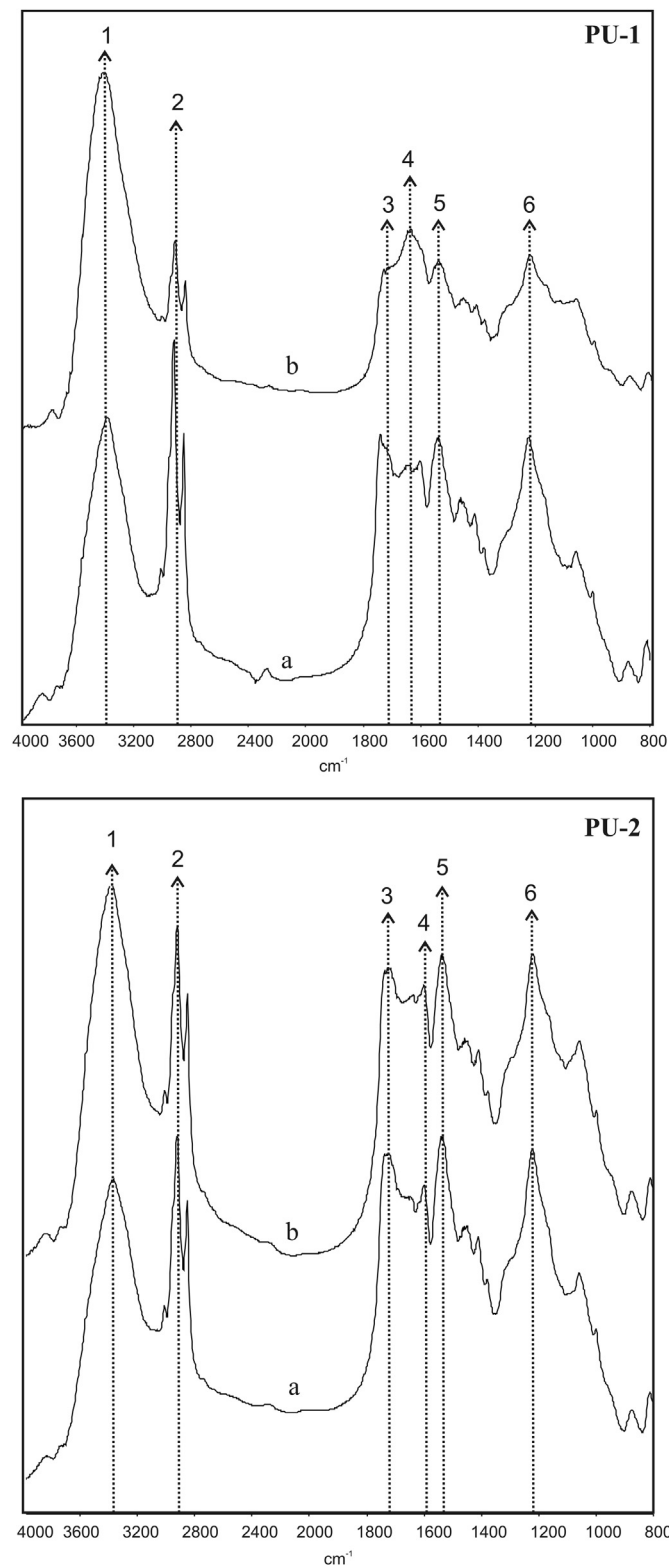
The liquid media that contained degraded PU samples during 60 days was sterilized by filtration through Sartorius membrane of 0.45  $\mu\text{m}$  pore diameter, extracted with ethyl ether, and analyzed by GC–MS and NMR. For GC–MS measurements, a Shimadzu Chromatograph GC-17A coupled to a Shimadzu spectrometer QP-5000 (ionization: 70eV) was used. The equipment characteristics and conditions were: i) column: HP-5, 30 m  $\times$  0.32 mm, 0.25  $\mu\text{m}$  (thickness of film); ii) temperature program: 70  $^{\circ}\text{C}$  (5 min) – 10  $^{\circ}\text{C min}^{-1}$  – 280  $^{\circ}\text{C}$  (25 min), injector: 280  $^{\circ}\text{C}$ , detector: 290  $^{\circ}\text{C}$ ; iii) flow rate: 2.3 ml  $\text{min}^{-1}$ , split: 20, injection volume: 1  $\mu\text{l}$ .  $^1\text{H}$  300 MHz NMR analysis were obtained using a Bruker 300 spectrometer with Fourier transform,  $\text{CDCl}_3$  as solvent and TMS as internal standard.



**Fig. 2.** Bacterial growth determined by cell viable count.

### 3. Results and discussions

First, a preliminary study in Petri dishes was carried out to verify the ability of the bacterial strain to degrade the polymers. After 10 days of incubation, the formation of clear zones (by hydrolysis)



**Fig. 3.** FT-IR spectra of the foams before (a) and after 60 days of biodegradation (b) by DBFIQ-P36.

around of the microorganism colonies was not observed for PU foams in basal medium with agar–agar as C and N source. In contrast for PU-1 and PU-2, the experiments carried out with basal medium containing agar, glucose and ammonium nitrate as the sole C and N sources showed the formation of clear zones around of the colonies indicating the activity of the enzymes on the polymer particles. Biodegradation of PU-3 was not observed through this method.

The subsequent biodegradation study was carried out in the liquid culture medium incubated at 37 °C for 60 days. In Fig. 1 the mass loss profiles of the samples degraded by hydrolysis in absence of microorganisms (Fig. 1A) and in presence of DBFIQ-P36 (Fig. 1B) are represented. During the first 10 days there were no significant differences between the mass loss profiles of samples with and without the bacteria. After this period, the differences were significant in some cases, indicating the strong influence of bacteria enzymatic activity that generates increments of the degradation rate. The biodegradation rate of the PU-1 sample immersed in culture medium with DBFIQ-P36 exhibited a significant increase after 10 days and at 60 days the mass loss was 30% (Fig. 1B), while the same foam exposed in culture-medium without microorganisms (MOs) had a mass loss of 12% (Fig. 1A). For PU-2 exposed in culture medium with bacteria at 60 days the mass loss was 12% and in culture medium without MOs the mass loss was only 5%. In the PU polyether (PU-3) taken as reference, important differences of mass loss were not detected. The degradation rate variations of the different samples are associated to the hydrophilicity of the polymeric structures due to the presence of ester groups and to the effect of amide groups incorporated by the presence of MACO on the hard segment. Therefore, PU-1 with a higher content of ester groups exhibited an increase of the hydrolysis and the diffusion of water molecules. In contrast, the weight loss percent of the reference PU (PU-3) is lower than the PUs derived from the CO (PU-1 and PU-2) due to lower ester group content.

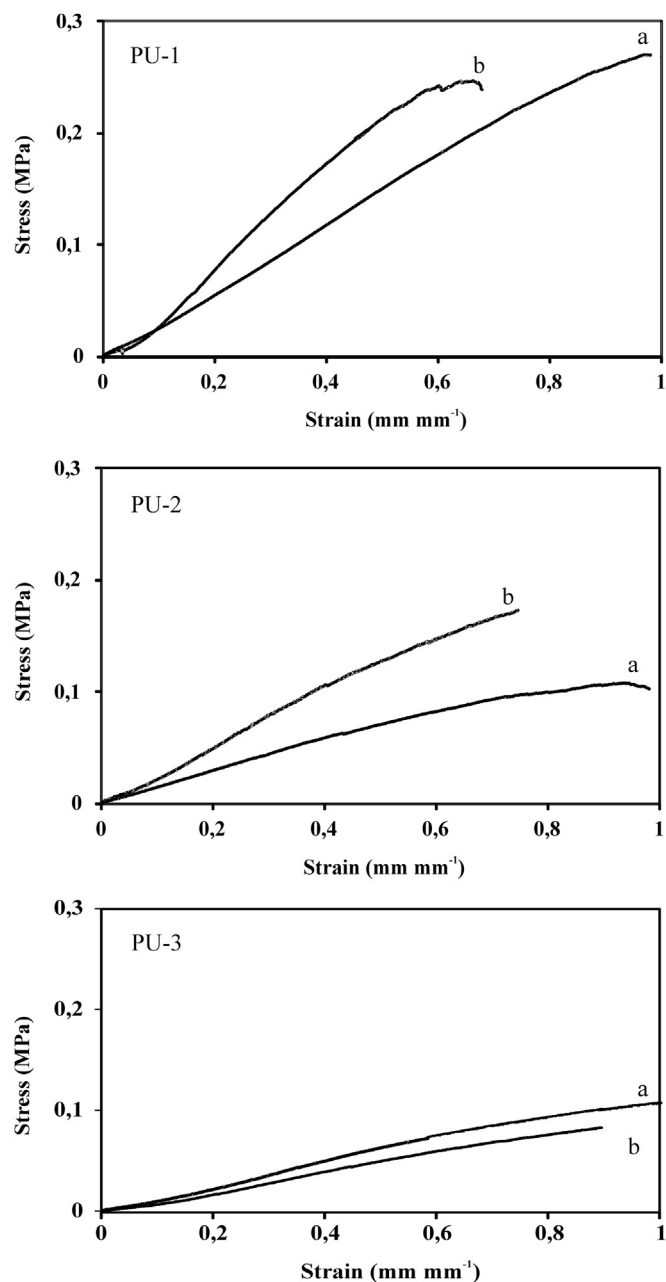
The growth of viable cells [colony-forming units (CFU ml<sup>-1</sup>)] is represented in Fig. 2. During the first 10 days, the cell population grew from about 10<sup>5</sup> to 10<sup>8</sup> CFU ml<sup>-1</sup>. Then, between 10 and 20–30 days of culture, the bacterial populations decreased to 10<sup>5</sup> CFU ml<sup>-1</sup> approximately. This is coincident with the significant increase of the weight loss for PU-1 and PU-2 after 10 days of biodegradation due to the bacterial enzymatic activity (Fig. 1B). These results indicate that the enzymes involved in biodegradation process are probably endo-cellular enzymes secreted into the culture medium during the bacterial cell lysis stage.

Fig. 3 shows FTIR spectra of the different foams before and after 60 days of biodegradation. This technique allows to follow the evolution of the functional groups involved in the process by

**Table 2**  
Absorption Band Assignments of FT-IR Spectroscopy

Bands	Wavenumber (cm <sup>-1</sup> )	Assignment
1 N–H O–H	3400	N–H hydrogen bond and OH stretching vibration (str., vib.) band
2 –CH <sub>2</sub>	2999	Methylene str. vib. asym. modes
–CH <sub>2</sub>	2880	Methylene str. vib. sym. modes
3 C=O	1630	Urea hydrogen bond str. vib.
	1660	Free urea str. vib.
	1700	Urethane hydrogen bonded str.vib
	1720	Free urethane str. vib.
	1750–1725	Ester and amide str. vib.
4 –C=O–NH–	1540	N–H bond vib. (in secondary amide)
5 –CH <sub>2</sub>	1470–1430	Methylene groups. str. vib. asym. modes
6 C–N	1292–1226	C–N str. vib. modes
7 C–O–C	1192	Ether str. vib. modes

variations of the corresponding absorptions. The FTIR bands of interest are described in Table 2 and they are indicated with numbers in Fig. 3 (Wu and Charles, 2005). The difference observed in Fig. 3 between the spectrum of polymer with high MACO content (PU-1) after 60 days of biodegradation and the spectrum of the initial sample shows important changes of chemical structure due to the action of the bacteria. For the degraded PU-1, the absorbance intensity in spectral region of 3450–3300 cm<sup>-1</sup> attributed to N–H hydrogen bonds and O–H vibration exhibits an increase respect to the initial sample due to the increment of O–H groups produced by the rupture of ester bridges. Also, it can be observed a decrease of absorbance intensities at 3000 and 2900 cm<sup>-1</sup> which indicates the cleavage of CH<sub>2</sub> bonds and subsequent formation of double bonds at 1614 cm<sup>-1</sup> approximately by oxidation reactions. The bands



**Fig. 4.** Mechanical properties of the foams before (a) and after 60 days of biodegradation (b).

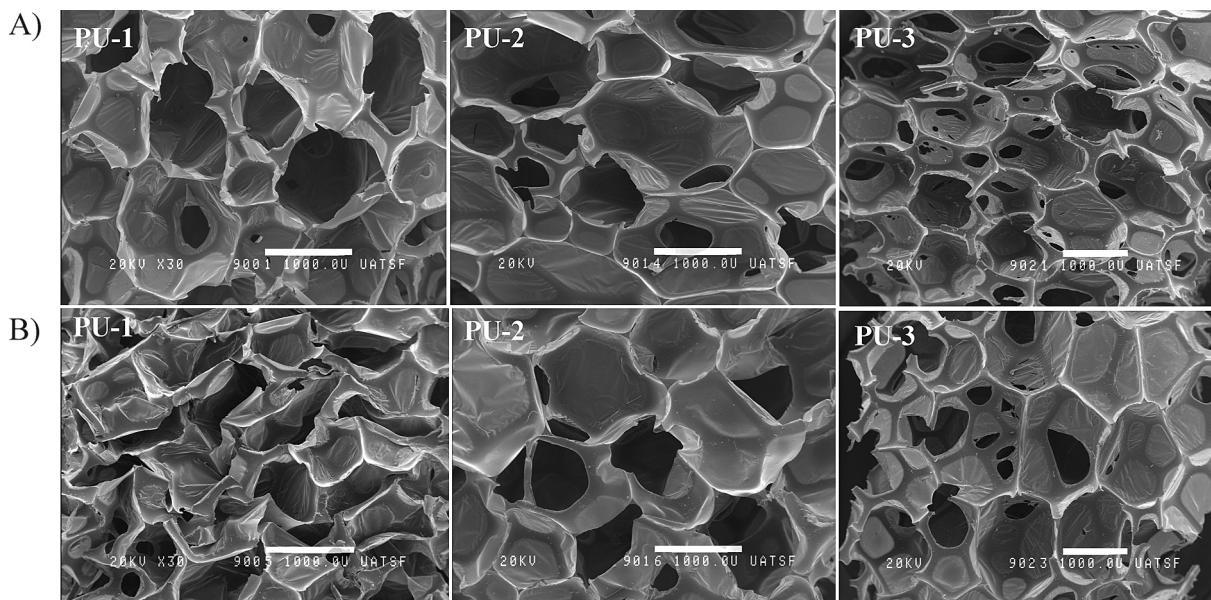


Fig. 5. SEM images of PUs: before (A) and after 60 days of degradation by DBFIQ-P36 (B).

between  $1740$  and  $1720\text{ cm}^{-1}$  assigned to the  $\text{C}=\text{O}$  groups of ester bridge, amide and free urethane respectively, disappeared after the degradation by chain scission of  $\text{C}=\text{O}$  groups. Also, at  $1720$ – $1714\text{ cm}^{-1}$  and  $1700$ – $1668\text{ cm}^{-1}$  (as a shoulder) bands attributed hydrogen-bonds of urethane and amide groups are observed. After degradation the bands between  $1715$  and  $1600\text{ cm}^{-1}$  were broadened due to H-bonds of carbonyl with hydroxyl groups, and double bonds formed by oxidation reactions. The absorbance intensities at  $1660$ ,  $1540$  and  $1260\text{ cm}^{-1}$  assigned to  $\text{C}=\text{O}$  urea,  $\text{N}-\text{H}$  and  $\text{C}-\text{N}$  bonds respectively, decreased after the degradation process. The results suggest that urethane, amide and urea bonds would be vulnerable to hydrolysis by the bacterial enzymatic attack. However, other reactions such as oxidation could have also occurred, since the characteristic bands of oxidation are in the same range. In addition, it was observed a color change in the degraded sample.

For PU-2 (Fig. 3) the intensity changes in absorbance at  $3450$  and  $3000$ – $2900\text{ cm}^{-1}$  of the degraded sample were lower. The absorbance intensity between  $1740$  and  $1720\text{ cm}^{-1}$  attributed to  $\text{C}=\text{O}$

groups decreased respect to the initial sample. The band at  $1714$  and  $1700\text{ cm}^{-1}$  assigned to H-bond carbonyl groups decreased. FT-IR analysis revealed that the PU-2 is less degraded than the PU-1 and it is consistent with the mass loss profiles showed above. The differences are mainly due to the lower content of ester groups in PU-2 structure.

The spectrum corresponding to PU-3 after 60 days of biodegradation presented a decrease in the absorbance intensity at  $1730\text{ cm}^{-1}$  and an increase at  $1715\text{ cm}^{-1}$ . However, noticeable structure changes were not observed.

In Fig. 4 the mechanical analysis of the samples before and after degradation is presented. It can be observed that PU-1 and PU-2 exhibited enhancements of mechanical properties respect to the traditional PU-3 due to the addition of MACO. After 60 days of exposure to the bacteria, PU-1 and PU-2 showed an increase in tensile strength and a decrease in elongation at break. The changes of the mechanical properties can be associated both to chemical and physical modifications. The chemical changes are mainly due to

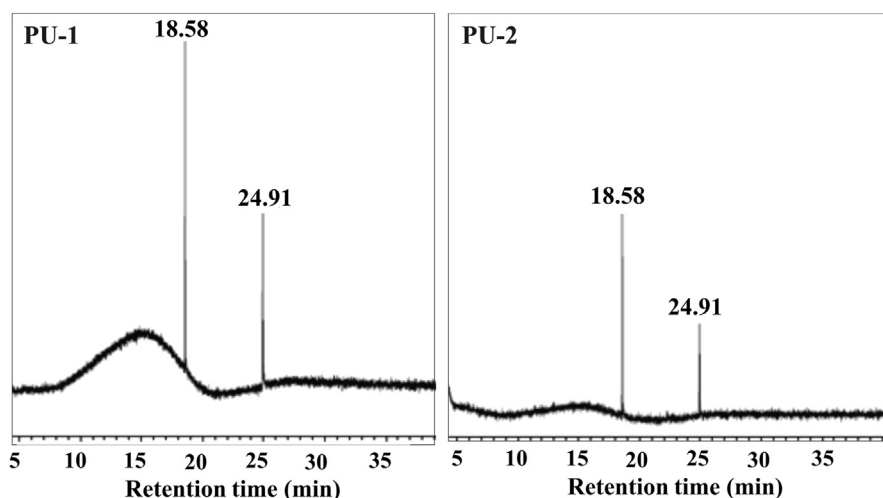


Fig. 6. GC chromatograms of degradation products for: PU-1, and PU-2.

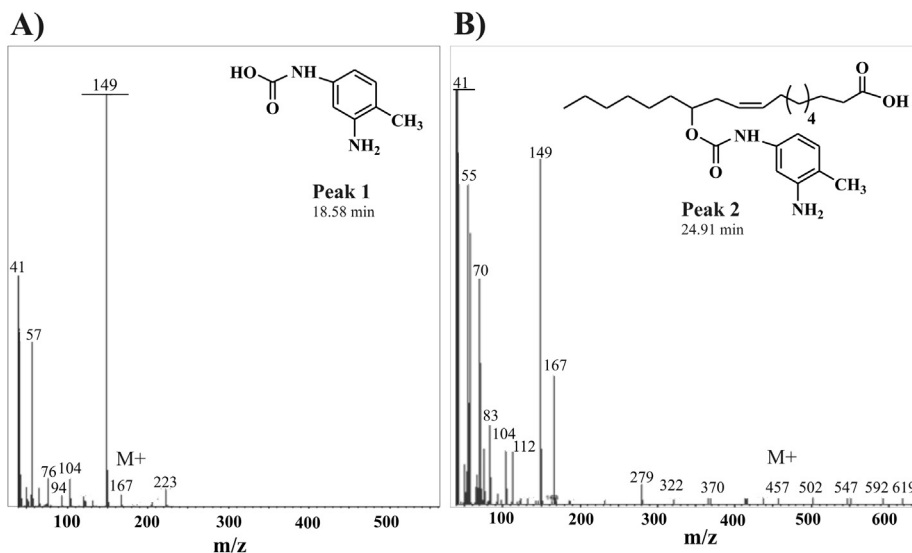


Fig. 7. MS spectra of degradation products for PU-1 and PU-2.

the rupture of the chains by hydrolysis and oxidation. The physical changes could be produced by hydrophilic nature of the material that promotes the collapse of pore structures. The measurements suggest a higher effect of the physical changes generating more rigid and closed structures. The PU-3 did not exhibited differences in the mechanical properties along the process.

Fig. 5 shows the micrographs obtained by SEM. It can be observed the surface morphology of the PUs before and after 60 days of biodegradation. The structure of PU-1 exhibited greater damage than the PU-2. In contrast, PU-3 almost shows no changes on its surface. These observations are consistent with the above mentioned changes in mechanical properties and FT-IR spectra.

For the ecotoxicity test, the bacterial Microtox<sup>®</sup> bio-assay was used. The liquid media that contained degraded PU samples during 60 days were found to be toxic to *V. fischeri*. In the test, the luminescence inhibition of the bacterium was evaluated for exposure period of 5, 15 and 30 min. For degraded samples of PU-1, the values of  $IC_{50}$  were 13.29, 8.99 and 6.47% (V/V) at 5, 15 and 30 min, respectively, indicating the presence of toxic organic compound released during the degradation.

Finally, the products released to the liquid media during the degradation of PU-1 and PU-2 were analyzed and identified by GC–MS in combination NMR spectra. The results are presented in Fig. 6 y 7. Two peaks corresponding to degradation products were detected by GC (Fig. 6). It can be noted that equal retention times were measured for PU-1 and PU-2. The peak 1, corresponding to retention time of 18.58 min, was identified by MS as a primary amine derived from TDI (Fig. 7A). The peak 2, corresponding to retention time of 24.91 min, was identified as an amine derived from CO (Fig. 7B). To validate the proposed structures from GC–MS, the samples were analyzed by NMR. The obtained results (not shown in the paper) allowed to corroborate the compounds identified previously. Other possible compounds of degradation such as oligomers and polyamines could not be separated by GC. In contrast, no degradation products released to the liquid media after degradation for the PU-3 were detected by GC–MS. These results are in agreement with the previous analysis of mass loss profiles, FT-IR spectra and mechanical properties.

From the results by FT-IR, mass loss profiles and GC–MS, it can be suggested that the main degradation mechanism of PU foams is

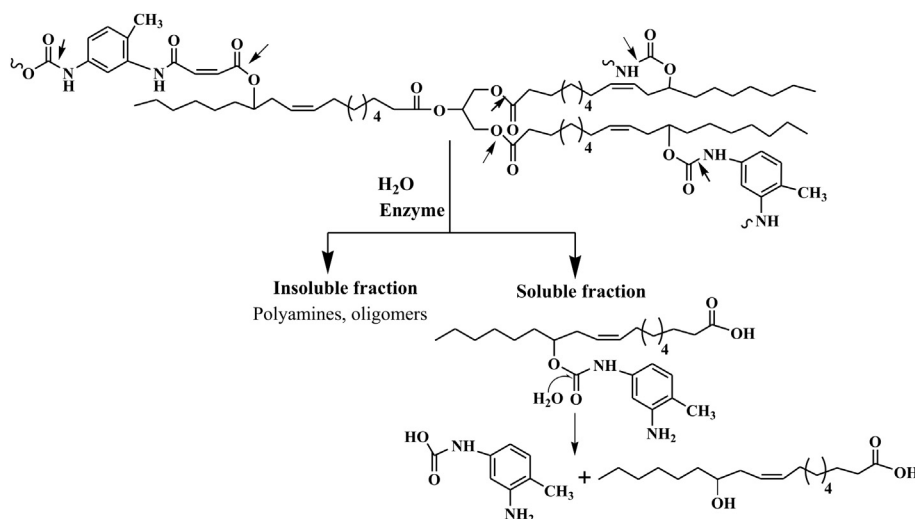


Fig. 8. Proposed mechanism for degradation.

by hydrolysis of the ester bonds catalyzed by hydrolytic enzymes (Loredo-Trevino et al., 2012). The rupture of ester bonds generates compounds such as oligomers, polyamines and amines as it is shown in Fig. 8.

#### 4. Conclusions

The biodegradation by *Pseudomonas* sp. strain of flexible PU foams based on castor oil modified with maleic anhydride was studied by different techniques.

The bacterial growth and the mass loss were monitored along the degradation process, and the corresponding changes in the chemical structure and morphology were followed by FTIR, SEM and mechanical test.

The materials with high MACO content exhibited a considerable increase of the degradation rate associated to the hydrophilicity of the polymeric structures due to the presence of ester groups and to the effect of amide groups on the hard segment. In addition, the results indicate that the enzymes involved in biodegradation process are endo-cellular enzymes.

SEM and mechanical measurements revealed that the presence of MACO generates more collapsed and closed structures and an increase of the tensile modulus after degradation.

Toxicity tests indicated the presence of toxic compounds released during the degradation that were identified as amines of different molecular weight from GC-Mass analysis in combination with NMR. A degradation mechanism that considers the generation of amines and oligomers was proposed. More detailed studies along the process can be carried out to elucidate the complete degradation mechanism. This will be the subject of a future communication.

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