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Preliminary study on the biodegradation of adipate/phthalate polyester polyurethanes of commercial-type by *Alicycliphilus* sp. BQ8

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ABSTRACT: Accumulation of polyurethane (PU) waste has increased considerably due to its extensive use. Even though many efforts are being carried out to develop more biodegradable PU, the use of these new materials is far from being commercially available. Here, we analyzed the susceptibility of solid polyester polyurethanes (PS-PU) of commercial-type, to biodegradation by *Alicycliphilus* sp. BQ8, a polyurethanolytic bacterial strain. Four polyester polyols were synthesized from dipropylene glycol (DPG) or diethylene glycol (DEG), and adipic acid (ADA) or phthalic anhydride (PHA), and were combined with either 4,4′- and 4,2′-methylene diphenyldiisocyanate (MDI) or 2,4- and 2,6-toluene diisocyanate (TDI). Synthesized polyols and PUs were characterized. PU biodegradation was assessed by the capacity of the polymers to support bacterial growth, and by scanning electron microscopy (SEM), Fourier transformed infrared (FTIR) spectroscopy, and gas chromatography/mass spectrometry (GC-MS) analyses. Although all the synthesized PUs supported BQ8 growth, SEM analysis showed that PHA-based PU foams were the most affected by bacterial growth. FTIR spectroscopy and GC-MS analyses of bacterial treated PS-PUs showed that they were attacked at ester and urethane groups, suggesting that esterase and amidase activities are involved. Extra-cellular and membrane bound esterase activities were detected during the five days of analysis. Our results suggest that solid PHA-based PUs might be more susceptible than ADA-based PUs to microbial biodegradation in the environment. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 42992.

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INTRODUCTION

Polyurethanes (PUs) rank in the sixth place among the more widely used plastic materials in modern society.[1] They are suitable for a broad range of applications, such as the manufacture of automotive components; hard-plastic parts for electronic instruments; rigid foam insulation panels; nonflexible, high-resilience foam seating; durable elastomeric wheels and tires; surface coatings and sealants; electrical potting compounds; microcellular foam seals and gaskets; high performance adhesives; and synthetic fibers, among others. PUs were invented by Otto Bayer[2,3] searching for polymers that would compete with nylon, and were commercialized in the 50's. They have revolutionized the market of synthetic materials because of their versatility and their physical and chemical resistance. Worldwide PU foam production increased from 8.5 Mt in 2000 to 10.8 Mt in 2004,[4] and in Europe, in 2012, from 57 Mt of plastics produced, 7.4% were PU, and 38% of total plastic waste went to landfill sites (Plastics-Europe, 2015).[5] PU high production and slow degradation after their useful life have generated large accumulation and serious waste management problems. Even though several works have been developed to synthesize more biodegradable PUs by including compounds from renewable sources in its structure, such as triglyceride oils from soybean, canola, and sunflower seeds or even castor oil or liquefied wood,[6–8] PU synthesized from petroleum-derived chemicals is still the most widely used in different industries, and it seems still far that more biodegradable PU can be used in our daily commodities.

For long time, it was assumed that PU cannot be degraded. However, in 1968, it was reported that PU was susceptible to

Additional Supporting Information may be found in the online version of this article.

fungal attack, being polyester-PU (PS-PU) more affected than polyether-PU (PE-PU).[9] Since then, several fungi and bacteria able to degrade PU have been reported[1,10] and some enzymatic activities related to its biodegradation have been identified. We previously reported the isolation of two polyurethanolytic bacterial strains, *Alicycliphilus* sp. BQ1 and BQ8, able to grow in mineral media (MM) containing a waterborne PS-PU varnish (Hydroform®) as the sole carbon source. After incubation with these strains, the surface of PU films appeared degraded and the ester bonds were hydrolyzed, which correlated with an extracellular esterase activity detected in the supernatant (SN) of the culture medium.[11] Impranil™, a PS-PU colloidal dispersion, has been widely used as a model system for the study of PU biodegradation.[12–15] On the other hand, solid PS-PU biodegradation has been less studied.[9,16–18] Changes in mechanical properties of foams and films, weight loss, and increase in the number and size of pores on the surface of solid PUs have been correlated with biodegradation of solid PUs.[19–24] Cleavage of PU hard segments at urethane bonds has been correlated with the release of aromatic amines from PU foams exposed to microbial action,[25,26] whereas cleavage of soft segments has been evidenced by gas chromatography/mass spectrometry (GC-MS) analysis, Fourier transformed infrared (FTIR) spectroscopy, and correlated with esterases and esterase/amidase activities.[16,23,24,27–30]

One of the most important characteristics of PU is the great variety of materials that can be generated by changing the polyol type and the isocyanate ratio during PU synthesis.[31,32] PUs properties and stability are correlated with their chemical composition, topology, and crystallinity. In the search for developing more biodegradable solid PUs, here, we aimed to find the relationship between the chemical composition of different, commercial-type PUs, and their susceptibilities to be attacked by the polyurethanolytic bacterium *Alicycliphilus* sp. BQ8. Commercial-type PUs were synthesized from four different polyols, using diethylene glycol (DEG) or dipropylene glycol (DPG) and adipic acid (ADA) or phthalic anhydride (PHA) as raw materials, combined with two diisocyanates (TDI or MDI). PUs biodegradation by BQ8 strain was evaluated by supporting of bacterial growth, scanning electron microscopy (SEM), FTIR spectroscopy, and GC-MS analyses. All the eight different PUs supported BQ8 growth, but PU synthesized from PHA-based polyols were the most degraded. Detailed analysis showed that PU was mainly affected at ester and urethane groups, indicating the participation of esterase and amidase activities. Indeed, extra-cellular and membrane bound esterase activities were detected during the five days of analysis. Our results suggest that the inclusion of PHA-based polyols in PU synthesis might be favorable for the improvement of PU biodegradation in the environment.

MATERIALS AND METHODS

Polyester-Polyols Synthesis

Four polyester-polyols: poly(dipropylene glycol adipate) (DPG-ADA), poly(diprophylene glycol phthalate) (DPG-PHA), poly (diethylene glycol adipate) (DEG-ADA), and poly(diethylene glycol phthalate) (DEG-PHA), were synthesized from dipropylene glycol (DPG) (Aldrich) and diethylene glycol (DEG) by esterification reactions with adipic acid (ADA) (J.T. Baker), or phthalic anhydride (PHA) (J.T. Baker), respectively. A 20% molar excess of —OH groups was used for the esterification reactions. The theoretical degree of polymerization (DP) was calculated with the formula $DP=\frac{1+r}{r+1-2rp}$ where $r=\frac{N^o_A}{N^o_B}=$ stoichiometric imbalance factor, p= reaction conversion, and N^O_A and N^O_B= number of A and B functional groups present at the beginning of polymerization.[33] DP was 10 for all the synthesized polyols. Reactions were carried out in batch during 3 h, at vacuum, 90°C, with agitation, and constantly eliminating the condensation water. Sulfuric acid and ter-butyl titanate (TBT) were used as catalysts for reactions with ADA and PHA, respectively. After that time, temperature was increased up to 120°C for 1 h to improve the conversion of glycols to esters, and to eliminate glycols excess. The synthesized polyols were washed twice with water; vacuum dried at 120°C during 10 h, and filtered through diatomaceous earth.

Polyester-PUs Synthesis. PUs were synthesized in batch reactions with one of the two isocyanates in excess[34]: (a) polymeric isomer mixture of 4,4′- and 4,2′-methylene diphenyldiisocyanate (MDI) (Dow Chemical), with a 33% isocyanate content in a 3:1 polyol to isocyanate ratio, or (b) an isomer mix of 2,4- and 2,6-toluene diisocyanate (TDI) (Aldrich), in a 5:1 polyol to isocyanate ratio, and each of the four different polyols. To obtain solid materials and the complete reaction of the hydroxyl groups, PU synthesis required an excess of isocyanates to hydroxyl equivalents. Polymerization was carried out in a glass reactor using 0.01% of the tertiary amine 1,4-diazabicyclo[2,2,2]octane (DABCO) (Goldschmidt Chemical Corp.), as catalyst. Four different types of MDI-containing PU and four different types of TDI-containing PU were synthesized. The PU foams were produced in few minutes and they were left to stabilize for 12 h before grinding. The eight PU were characterized as described below.

Polyester-Polyols and PU Characterization

Molecular weights (*Mw*) and polydispersities (*Mw/Mn*) of polyols were determined by gel permeation chromatography (GPC), with a GPC Alltech chromatographer, using a HP MIXED-B column of 10 μm particle size for a 500 to 1 $\times$ 10^6 Da molecular weights range, and an Alltech ELSD 2000 evaporation and light dispersion detector (Evaporative Light Scattering). Column temperature was 40°C and tetrahydrofurane (THF) was used as eluent at 1 mL/min flux. Polyols were dissolved in THF at 10 mg/mL concentration and 1 μL were used for the analysis. Polyols molecular weight estimation were extrapolated from a universal calibration curve generated with polystyrene standards from 500 to 20,000 Da dissolved in THF.[35] Viscosity of polyols in THF was determined at 40°C in a Brookfield viscometer Mod DV-II+. For estimation of –OH groups, a non-catalyzed technique using acetic anhydride and pyridine in solution (1:3 v:v) was used. The resulting carboxylic acids were determined by titration using a 0.1*N* sodium hydroxide alcoholic solution. Polyols chemical structures were confirmed by Fourier transformed infrared (FTIR) spectroscopy (4000–400 cm^{-1}) in a 1605 Perkin-Elmer spectrometer and by nuclear magnetic resonance spectroscopy (NMR) dissolving 0.1 g each polyol in deuterated chloroform. Analysis of polyols

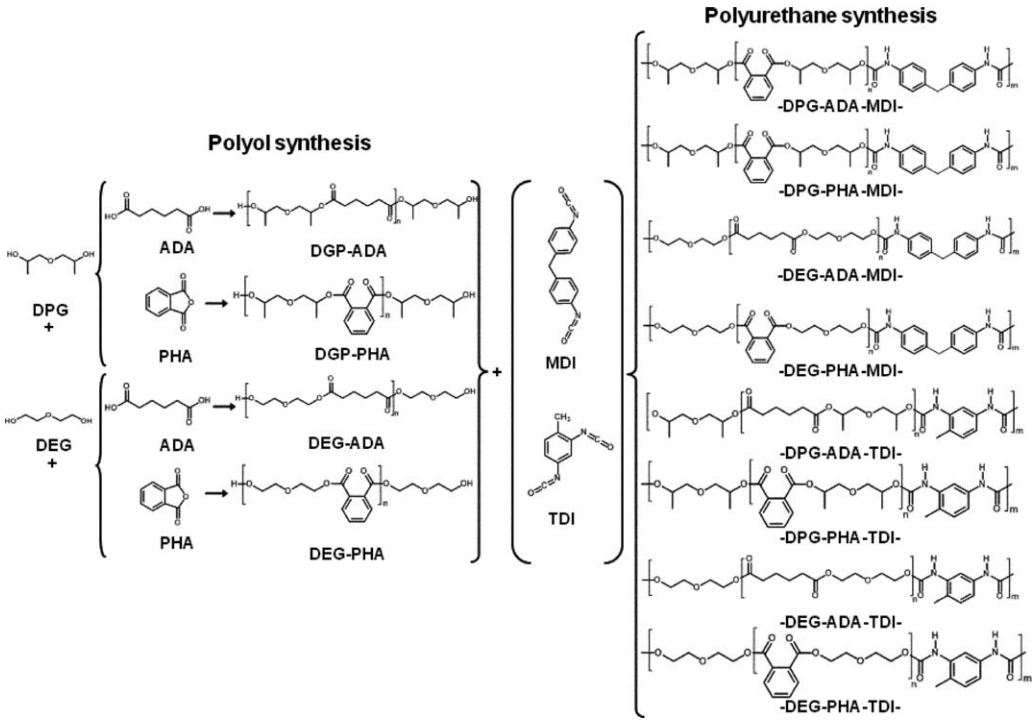


Figure 1. Constitutive repetitive units (CRU) of the polyester polyols and idealized structures of the polyester polyurethanes synthesized in this work.

FTIR spectra was based on previously reported work.[36] Determinations of ^{1}H and ^{13}C spectra were performed in a high resolution Varian NMR spectrometer (Unity Inova) at 400 MHz. PUs chemical structures were confirmed by FTIR spectroscopy. Analysis of PUs FTIR spectra was based on previously reported work.[37,38]

Culture Media and Bacterial Growth Analysis

The salt mineral media (MM) was based on Nakajima Kambe *et al.*[27] It contained K_2HPO_4 (7 g/L), KH_2PO_4 (2 g/L), NH_4NO_3 (1 g/L), $MgSO_4{\cdot}7H_2O$ (0.1 g/L), $FeSO_4{\cdot}7H_2O$ (10 mg/L), $MnSO_4{\cdot}4H_2O$ (2 mg/L), $ZnSO_4{\cdot}7H_2O$ (1 mg/L), $CuSO_4$ (0.1 mg/L), pH 7. The different solid PUs synthesized in our laboratory were used as the sole carbon source in the media at 3% (w/v). For that purpose, solid PUs were ground, producing 1–4 mm diameter particles, and UV sterilized for 6 min at 20 mJ/cm^2 using an Ultraviolet Crosslinker Mod CL-1000 (UVP). Seven flasks (25 mL) with 5 mL of MM containing 0.15 g of UV-sterilized PU were set for each of the different PUs synthesized. Flasks were inoculated with the volume of BQ8 Luria Bertani (LB) overnight culture to reach an OD_{660} of 0.2. All the flasks were incubated at 37°C and 220 rpm. Every day during 6 days, 0.1 mL aliquots were taken from each tube to make dilutions and plate them in LB Petri dishes. Colony forming units (CFU) were counted after 48 h of incubation. Flasks with non-inoculated (NI) MM-PUs were incubated and analyzed at the same conditions, as controls. The specific growth rate (μ) was calculated by using the formula: $\mu = 2.303\ (\lg\ CFU_2 - \lg\ CFU_1)/(t_2 - t_1)$, considering the log phase between 2 and 5 days.

Evaluation of PU Susceptibility to Biodegradation

Biodegradation analyses of the eight PUs were performed after incubation with *Alicycliphilus* sp. BQ8 for 15 days at 37°C. After this time, the solid polymers were collected from the media by filtering through Whatman No. 1 filter paper. The solid PUs recovered were rinsed with distilled water, dried, and analyzed by scanning electron microscopy (SEM) in a JEOL JSM-5900-LV microscope. For FTIR analysis, culture filtrates were centrifuged at 9000 × *g* for 30 min to settle bacteria out; cell-free supernatants were dried and the solids obtained were characterized, as described above. To identify PUs degradation products, gas chromatography/mass spectrometry (GC-MS) was performed. Two 10 mL aliquots from non-inoculated and inoculated-cell-free supernatants were taken from each PU culture. An aliquot of each condition was acidified with 1*N* HCl (pH 1) and the other was alkalinized with 1*N* NaOH (pH 13); after that, they were extracted with ethyl acetate.[16] Ethyl acetate extracts were dehydrated with a small amount of Na_2SO_4. Extracts were methylated with diazomethane, injected in a capillary column HP-5 (26 cm × 0.32 cm) at 230°C, and analyzed in a HP Gas Chromatographer Mod CG6890, with a selective mass detector HP Mod 5973. The running program was: initial temperature 50–80°C with 5°C/min increase, 1 min at 80°C, and from 80 to 230°C with increments of 10°C/min.

Analysis of Esterase Activity

BQ8 cell cultures were centrifuged at 10,600 × *g* during 10 min in an Eppendorf microcentrifuge 5417R. Cell free supernatant (SN) served as the source for extracellular enzymatic activity. Cell pellet was resuspended in phosphate buffer 0.2 *M* pH 7.0 and sonicated at 150 W for six 15 sec pulses on ice. The suspension was centrifuged as indicated above. The pellet, corresponding to cell membrane fraction (CMF), was resuspended in phosphate buffer 0.2 *M* pH 7.0. Esterase activity in SN and CMF was measured at inoculation and at days 1, 3, and 5, by

Table I. Characterization of Polyester-Polyols

Polyol	*Mw* (Da)	*Mw/Mn*	$[\eta]$	DP_{calc}	Mg OH/g polyol
DPG-ADA	1370	1.30	3.2	9.8	31.9
DPG-PHA	1210	1.02	1.8	8.1	28.6
DEG-ADA	1410	1.35	3.7	11.2	34.6
DEG-PHA	1190	1.03	2.3	8.8	29.5

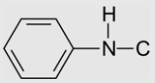
Mw, weight average molecular weight.
Mw/Mn, polydispersity.
$[\eta]$, intrinsic viscosity.
DP_{calc}, calculated degree of polymerization.

following the hydrolysis of *p*-nitrophenyl acetate measured spectrophotometrically by changes in absorbance at 405 nm, as reported previously.[11]

RESULTS AND DISCUSSION

Synthesis reactions of the four polyester-polyols: DPG-ADA, DPG-PHA, DEG-ADA, and DEG-PHA are shown in Figure 1 (left). They were liquids, with viscosities between 2500 and 4500 cp, Mw from 1200 to 1400 Da (Table I). GPC analysis showed that polyester chains had a degree of polymerization closed to 10, with two OH's per molecule (data not shown). Polyols FTIR spectroscopy analysis showed that no carboxy-terminal groups were present (Supporting Information Figure S1 and Supporting Information Table SI) and the ^{1}H and ^{13}C NMR analysis confirmed the expected chemical structure (Supporting Information Figure S2). Formulas of the synthesized PUs structures are shown in Figure 1 (right). MDI-PU synthesized from aliphatic

Table II. FTIR Assignments of Synthesized PUs.[37]

Wavenumber	Assignment
~3300 cm^{-1}	
~2730-2900 cm^{-1}	CH_2 and CH_3
~1720 cm^{-1}	C=O
~1535 cm^{-1}	Urethane N-H bending plus C—N stretch
~1450 cm^{-1}	Aliphatic CH_2 bending
~1230 cm^{-1}	Urethane C—N stretching
~1100 cm^{-1}	C—O—C stretch and terminal OH[38]
~900-700 cm^{-1}	

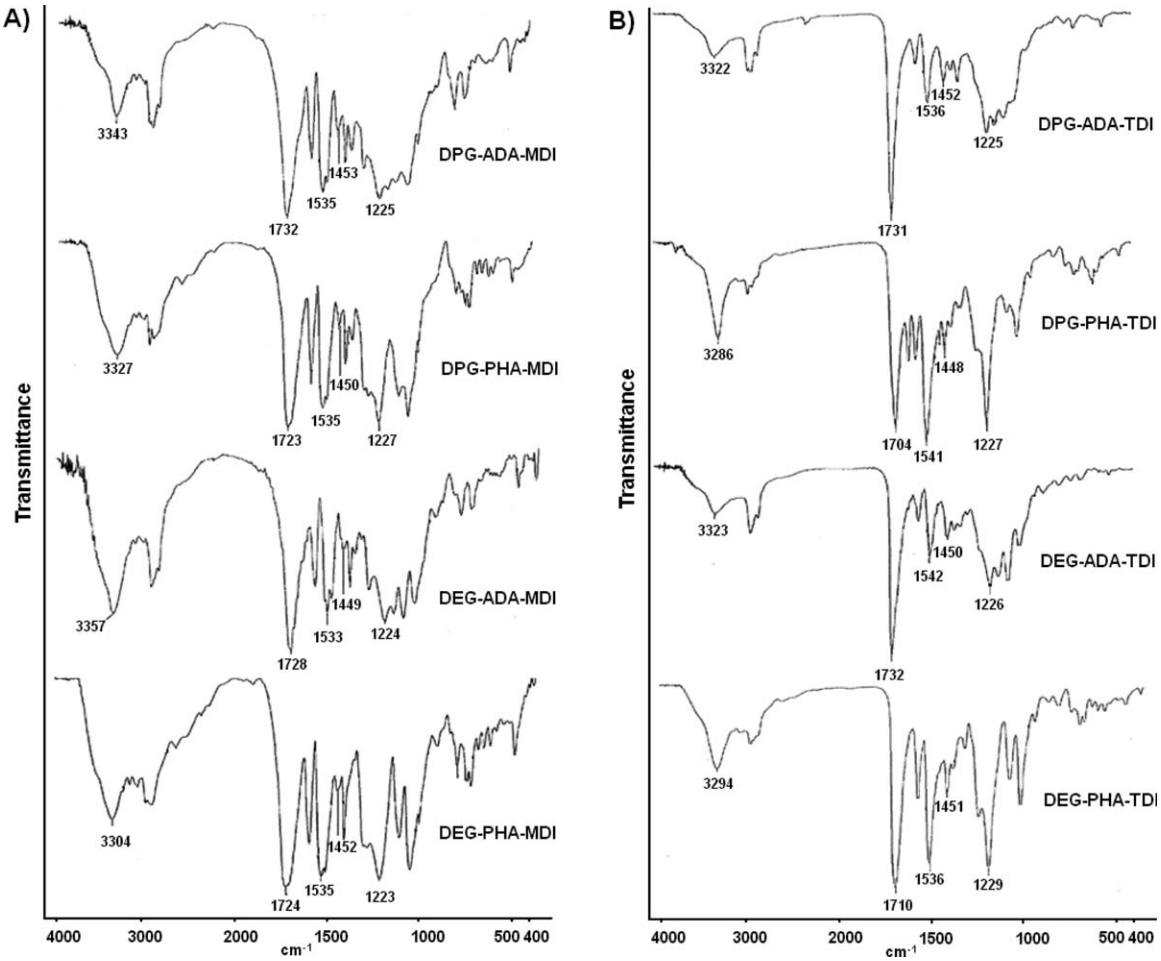


Figure 2. FTIR spectra of the synthesized polyurethanes.

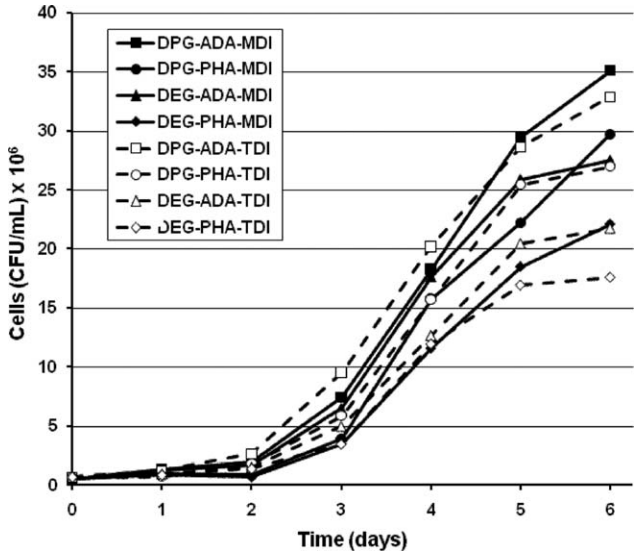


Figure 3. Bacterial growth of *Alicycliphilus* sp. BQ8 strain on mineral medium with the different synthesized PUs as the sole carbon source.

diols (DPG-ADA-MDI and DEG-ADA-MDI) could not be dissolved in any of the tested solvents, indicating a high crosslinking degree. FTIR spectroscopy analyses of the synthesized PU were carried out to confirm the formation of the urethane bond (Figure 2). The characteristic signals corresponding to PU structure were observed in FTIR spectra and are described in Table II. FTIR assignments confirmed the expected chemical structures of synthesized PUs in which the presence of the urethane bond is detected by the signals around 1535 cm^{-1} corresponding to urethane N—H bending plus C—N stretch, and around 1230 cm^{-1} which corresponds to urethane C—N stretching.

Preliminary analysis of *Alicycliphilus* sp. BQ8 growth in MM, using the different raw materials for PU synthesis, glycols and diisocyanates, as carbon sources showed that BQ8 was able to grow in glycols, but not in diisocyanates. It was also able to use all the different synthesized polyester polyols (3% w/v) as carbon sources when growing in liquid media, demonstrating the capacity to attack ester bonds (data not shown). BQ8 grew in all the synthesized PUs, exhibiting a typical curve with a lag phase of 2 days and a log phase from 2 to 5 days. In general, PU with aliphatic esters and branched chains, such as MDI-PUs, supported slightly greater bacterial growth, and showed slightly higher μ values (DPG-ADA-MDI, 0.037 h^{-1}; DPG-PHA-MDI, 0.045 h^{-1}; DEG-ADA-MDI, 0.038 h^{-1}; and DEG-PHA-MDI, 0.045 h^{-1}) than TDI-PUs (DPG-ADA-TDI, 0.033 h^{-1}; DPG-PHA-TDI, 0.037 h^{-1}; DEG-ADA-TDI, 0.036 h^{-1}; and DEG-PHA-TDI, 0.035 h^{-1}) (Figure 3). Bacterial growth in media with PU synthesized from aliphatic polyols (DPG-ADA), combined with either TDI or MDI (DPG-ADA-MDI and DPG-ADA-TDI) was the highest, whereas growth in PUs synthesized from aromatic polyols, DEG-PHA (DEG-PHA-MDI and DEG-PHA-TDI) was the lowest. The other PUs supported intermediate growth.

Solid powdered PUs samples were exposed to *Alicycliphilus* sp. BQ8 during 15 days of incubation. After this time, all the materials presented significant changes in morphology, and looked yellowish, compared to non-inoculated polymers, incubated for

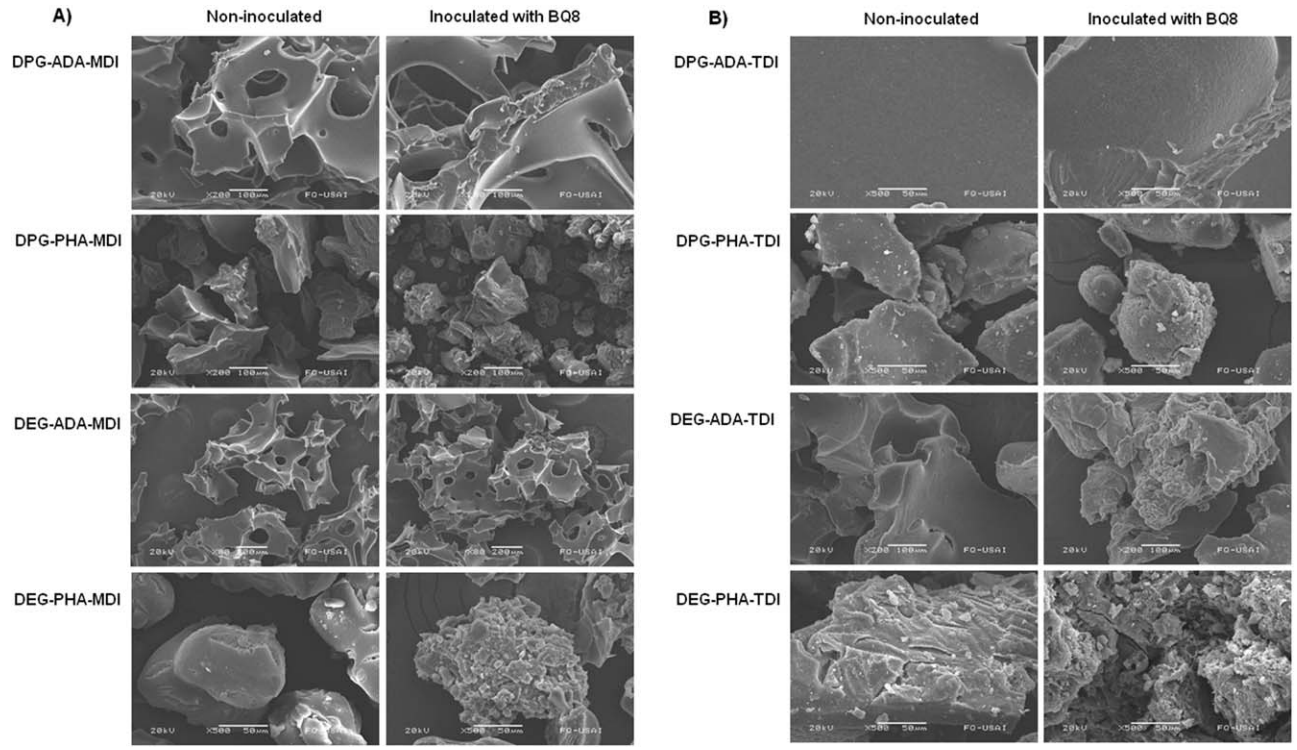


Figure 4. Photomicrographs of the different PUs incubated without (left) or with (right) *Alicycliphilus* sp. BQ8, during 15 days. MDI-PUs (A) and TDI-PUs (B).

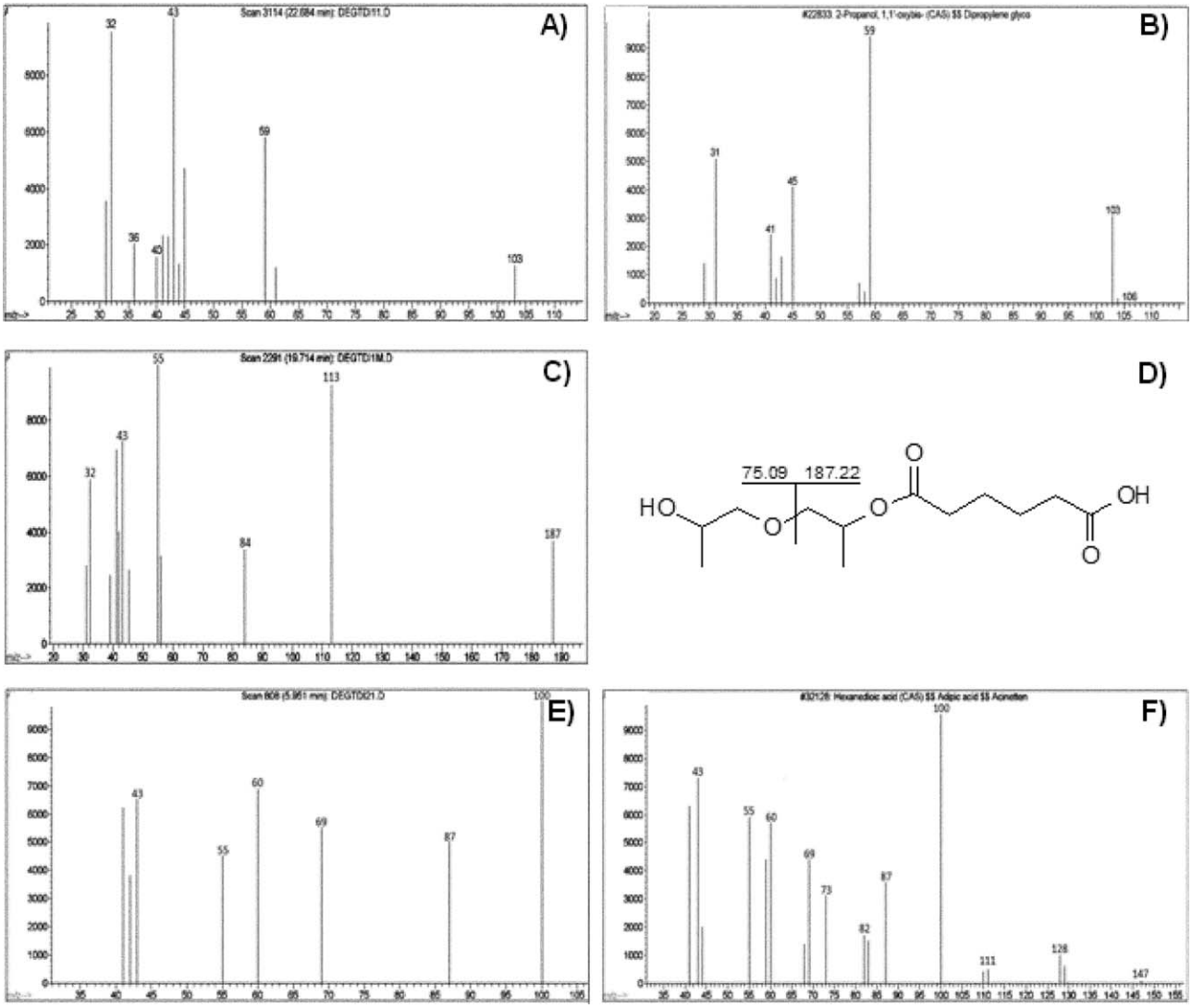


Figure 5. GC-MS analysis of the compounds present in the supernatant of MM-DPG-ADA-TDI inoculated with BQ8 (A,C,E). (B) Wiley 275 Databank spectrum for DPG; (D) Proposed structure for one of the degradation products, DPG-ADA, showing the position for proposed hydrolysis; and (F) Wiley 275 Databank spectrum for ADA.

the same time (controls). SEM analysis showed that all the PU surfaces presented degradation signals compared to controls, but that PU containing aromatic polyols (DEG-PHA-TDI/MDI and DPG-PHA-TDI/MDI) looked more degraded than PUs containing aliphatic polyols (Figure 4). Interestingly, these polymers supported lower bacterial growth than their aliphatic counterparts (Figure 3), suggesting that bacteria might be attached to the polymer surfaces, instead of being growing planctonically in the supernatant, from which samples were taken to quantify bacterial growth. Biofilm formation during PU biodegradation has been reported for *Acinetobacter* sp. Tol 5 and *A. gerneri* P7, which were able to array over solid PU surfaces forming multilayer or particulate cell clusters,[39] or films.[40] BQ8 attachment to the surface of aromatic-polyols-based-PUs might take place via hydrophobic PU surface-binding domains of membrane proteins, as proposed for a polyester polyurethane esterase from *Comamonas acidovorans* TB-35.[41] This would allow the bacteria to attack the hydrophobic polymers more efficiently.

GC-MS analyses of the supernatants extracted with ethyl acetate from BQ8 cultures growing on the different PUs, were not possible for all the polymers, probably because of the low amount of degraded molecules. From the experiment where DPG-ADA-TDI was incubated with BQ8, three compounds were identified by MS analysis, based on the Wiley 275 databank (Figure 5). One of the molecules was identified as DPG [Figure 5(A,B)]. A second molecule was identified as an adipic acid ester with characteristic signals at 55 and 84 [Figure 5(C)]. This molecule could be produced by DPG-ADA hydrolysis at the position indicated in Figure 5(D), generating two molecules, an adipate-base with signal at 187, observed in the spectrum, and another molecule with signal at 75, that is not observed in the spectrum, but that might have suffered loss of an –OH and a CH_3, generating a propyl alcohol with a signal at 43, indeed observed [Figure 5(C)]. The third compound was identified as adipic acid [Figure 5(E,F)]. From these analyses we concluded that the identified molecules were produced by hydrolysis of ester bonds, by an esterase activity, generating DPG, and hydrolysis of amide bonds, by an amidase activity generating DPG-adipate and/or

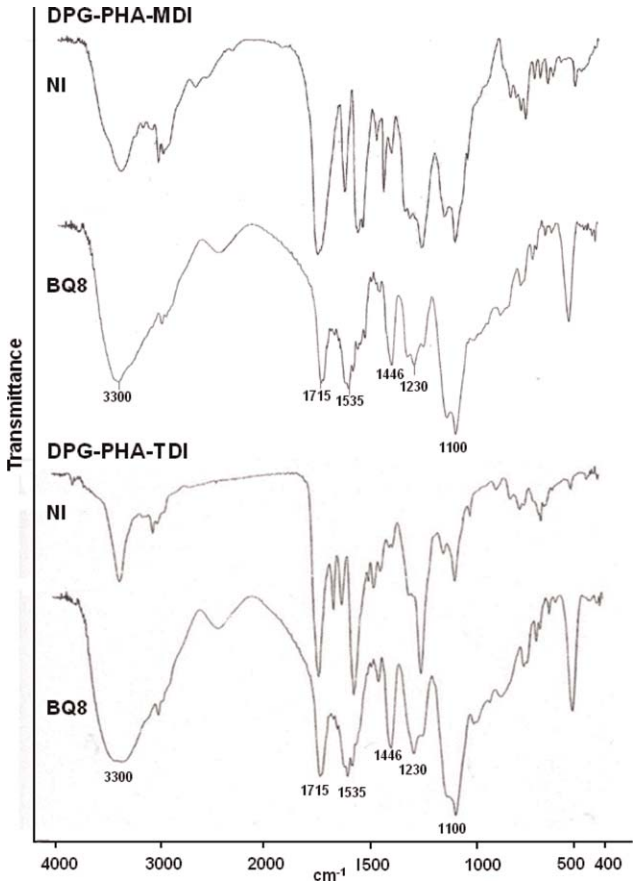


Figure 6. FTIR spectra of the supernatant obtained from cultures with DPG-PHA-MDI and DPG-PHA-TDI, non-inoculated (NI) and inoculated (BQ8) with *Alicycliphilus* sp. BQ8.

adipic acid. Ester hydrolysis products generated by bacterial and fungal action over PUs have been previously reported.[16,41,42] On the other hand, an enzyme that hydrolyzes urethane bonds in low molecular weight substrates has been reported[29]; however, evidence for the activity of this enzyme over polymerized urethane bonds is still missing.

FTIR spectroscopy analysis of the supernatants obtained from the culture media containing either DPG-PHA-MDI or DPG-PHA-TDI, incubated during 15 days, presented PU molecules, which were released from solid PUs. These molecules were observed in both, non-inoculated and BQ8-inoculated conditions (Figure 6), indicating that abiotic chemical hydrolysis occurred as result of the incubation process. Nevertheless, important changes related to biological degradation of PU by hydrolytic enzymatic activities were observed in the bacterial treated condition: (1) The increase in the intensity at 3300 cm^{-1}, which corresponds to N—H stretch, indicates the hydrolysis of urethane groups from the polymeric structure, generating amines; (2) The C—N stretching signal from the urethane group (1230 cm^{-1}) considerably decreased, indicating that the urethane group is disappearing; (3) The large decrease in the 1535 cm^{-1} signal, which corresponds to N—H bending plus C—N stretch from the urethane, implies the loss of the C—NH from the urethane group. These three changes are complementary and co-related indicating that the hydrolysis of the urethane bond is mediated by an amidase activity. The action of an amidase enzyme over urethane groups would imply that, after the hydrolysis, free amines and carboxylic groups had to be released. The carboxylic groups must be detected in the carbonyl signal, approximately at 1715 cm^{-1}. However, the observed decrease in the carbonyl signal indicates loss of this group, very probably by the action of an esterase activity acting on the ester bond side of the molecule, releasing CO_2 and generating alcohols. (4) The increase in the signal at 1100 cm^{-1} corresponding to —OH is consistent with the previous observation regarding an esterase reaction. (5) The signal at 1446 cm^{-1}, representing aliphatic —CH_2— increased in the bacterial treated PU, indicating the release of aliphatic chains, originated by breaking of the PU molecule at different points, probably in the intersection between the soft and the hard segment of PU. These results indicate that *Alicycliphilus* sp. BQ8 was able to hydrolyze ester and urethane groups present in PUs by the action of esterase and amidase activities. Because neither urethane nor amines or ammonium were detected by GC-MS in the supernatants of the culture media, it is likely that these groups were consumed by the bacteria as soon as they were generated. Evidences supporting the action of esterase activities on PS-PU biodegradation have been reported previously.[11,13,16,30,43,44] As far as we know, evidence for the action of an amidase activity hydrolyzing the urethane group in a polymeric material has not been reported yet. The data presented here are the first evidences supporting that an amidase activity is involved in PS-PU biodegradation.

Based on these analyses, degradation of ester bonds in the solid PUs synthesized in this work should have occurred by the action of extracellular and/or membrane-bound esterase activities. To address these possibilities, SN and CMF of BQ8 cultures growing in MM DPG-PHA-MDI or MM DPG-PHA-TDI were analyzed. Esterase activity was detected during the 5 days incubation period, both in SN as in CMF of cultures containing

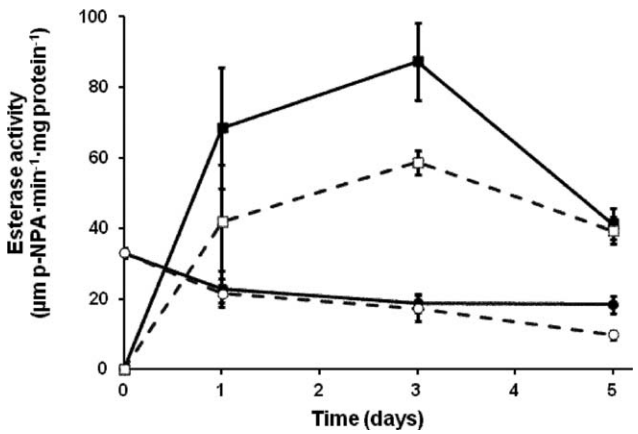


Figure 7. Extracellular and cell membrane esterase activity detected in *Alicycliphilus* sp. BQ8 cultures growing in MM DPG-PHA-PUs. Cell-free SNs (■, ●) and cell membranes fractions (□, ○) of BQ8 cultures grown in DPG-PHA-MDI (■, □) and DPG-PHA-TDI (●, ○) were analyzed for esterase activity using *p*-nitrophenyl acetate as substrate. Experiments were performed in triplicate. Bars indicate standard deviations.

the tested PUs. Extracellular esterase activity was relatively high (42–69 μm *p*-NPA/min/mg protein) at day 1, then it increased at day 3, and it decreased at levels lower than initial by day 5. This activity was higher in cultures with DPG-PHA-MDI than with DPG-PHA-TDI (Figure 7). In contrast, membrane-bound esterase activity was detected at lower levels (34 μm *p*-NPA/min/mg protein) since the beginning of the incubation period and then it gradually decreased up to values of 18 and 9 μm *p*-NPA/min/mg protein in cultures with DPG-PHA-MDI and DPG-PHA-TDI, respectively (Figure 7). Evidences of extracellular and membrane-bound esterases able to attack PUs at different extent have been reported previously. An extracellular esterase of *Pseudomonas chlororaphis* was responsible of Impranil degradation whereas a cell-surface bound esterase of *Comamonas acidovorans* TB35 catalyzed the degradation of solid PS-PU.[13,17] Whether the extracellular esterase activity, the membrane-bound one, or both, are relevant for PU biodegradation by *Alicycliphilus* sp. is still an unsolved question.

Our results suggest and provide evidence that, to degrade solid PS-PUs, membrane-bound esterases of *Alicycliphilus* sp. BQ8 adhere to the polymer surface via their surface binding-domains, and along with extracellular esterases, and most likely amidases, hydrolyze ester and urethane groups, respectively, generating small molecules (glycols, diacids, ester acids, and amines), which are assimilated by the bacteria to sustain their growth.

CONCLUSIONS

Preliminary biodegradability analysis of commercial-type PUs, carried out with the polyurethanolytic bacteria *Alicycliphilus* sp. BQ8, showed that degradation was more effective in PU containing PHA aromatic polyols combined with either MDI or TDI, than in PU containing ADA aliphatic polyols. Therefore, incorporating PHA aromatic polyols in polyester-PU synthesis might generate PU polymers more susceptible to microbial biodegradation.

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