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Biocatalytic hydrolysis of di-urethane model compounds in ionic liquid reaction media

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ABSTRACT

The suitability of different enzymes to carry out the hydrolysis of two-different toluene-based urethane model compounds (*i.e.* bis(2-methoxyethyl) (4-methyl-1,3-phenylene)dicarbamate, and bis(2-methoxyethyl) (2-methyl-1,3-phenylene)dicarbamate) has been demonstrated for the first time by taking advantage of ionic liquid (IL) technologies. Toluene-based urethane compounds were prepared from usual substrates in polyurethane industrial synthesis. Afterwards, their carbamate groups were target of a biocatalytic hydrolysis by means of different commercial hydrolases (*i.e.* lipase, urease and proteases) in either water, hydrophilic organic solvents (*i.e.* ethylene glycol or 1,2-dimethyl-1,3-dioxolane-4-methanol, (solketal)), or hydrophobic ILs (*e.g.* $[C_4mim][NTf_2]$, *etc.*) as reaction media. Because of the insolubility of these compounds in water, most of the enzymes were unable to catalyse the hydrolysis of the di-urethane substrates in pure water, being clearly improved (up to 31.6 mU/mg for the urease case) in solketal:water (90:10, v/v) reaction media. When hydrophobic ILs were added into this reaction medium, the urease activity increased by more than twice (74.1 mU/mg). The most promising results for the hydrolysis of these urethane compounds were obtained by combining lipase and urease biocatalysts in a IL: solketal:H_2O (70:25:5, v/v/v) reaction medium. These results demonstrate a possible biocatalytic approach for the hydrolytic depolymerization of polyurethane foam wastes.

1. Introduction

The huge amount of plastic wastes and the difficulty for developing degradation strategies have led to an environmental crisis around the world.[1] One of the main global challenges is the development of sustainable bio-based approaches for plastic recycling through a circular economy. [2–4] In this sense, plastic wastes can be a new feedstock for the production of value-added materials through the design of green approaches.[4,5] For instance, Luo *et al.*[6] evaluated polyethylene terephthalate (PET) waste depolymerization trough a continuous process by means of two consecutive steps. Firstly, the glycolysis of diethylene glycol at 230–200 °C for 7 h with titanium isopropoxide as catalyst, providing glycolyzed PET oligomers. The obtained polyols showed an increase of the hydroxyl number and could be used as feedstocks for polyurethane foams of higher density and compressive strength. Alternatively, Tournier *et al.*[7] demonstrated a depolymerization strategy for PET using an engineered cutinase leading to up to 90% yield PET degradation in less than 10 h at 72 °C, and demonstrating the reusability of terephthalic acid monomers for synthesizing PET.

Polyurethanes (PUs) are a class of synthetic polymers obtained from the polycondensation reaction of a diisocyanate (*i.e.* toluene diisocyanate, TDI) and polyols, providing the characteristic recalcitrant and stable intra-molecular urethane bond (-NH-COO-).[8] These polymeric materials are characterized by their high resistance and boiling point, and they are exceptionally versatile polymers with a wide range of possible structure and chemical nature (*i.e.* soft and hard foams, coatings, adhesives, sealants, elastomers, *etc.*).[9] Indeed, the global market demand for PUs is expected to grow from about 15 million tons in 2020 to 20 million tons in 2025, with an annual growth rate of 7.5%.[6,10] The high level of cross-linking in thermoset polyurethanes makes these compounds to be highly stable, non-meltable, and insoluble in organic solvents. Nowadays, landfilling or incineration are the main end-of-life processes for polyurethane foam wastes, generating a big impact on the environment.[11] Thus, recycling strategies based in circular

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chemistry criteria are a key tool to achieve their depolymerization for further re-introduction in the industrial PU synthesis, or to obtain valuable secondary materials. In fact, molecular recycling permits the conversion of polymer chains to molecules with lower molecular weights by chemical reactions (*i.e.* glycolysis, hydrolysis, phosphorolysis, *etc.*).[12] For instance, glycolysis consists in the use of a glycol to perform the breakdown of the carbamate bonds in PU through a transesterification reaction at high temperatures (170–250 °C) and with a non-selective catalysts (*e.g.* bases, Lewis acids, organometallic complexes, *etc.*) that cannot fully be recovered and reused. As results, the glycol replaces the polyol in the urethane linkage, providing oligomers of lower molecular weight. [13,14] Simon *et al.* [14] developed a glycolysis process by means of two waste substances such as flexible PU foam scraps and crude glycerol (80% purity), by using tin(II)− 2-ethylhexanoate as catalyst in a PU:catalyst (1.5:1, w/w) ratio during 150 min reaction at 190 ºC, obtaining a biphasic system where the recovered polyol could be separated and purified as valuable recycled products. [11,15] However, the extreme conditions of these strategies of depolymerization and separation are clear drawbacks, promoting the growing need the design of sustainable strategies for PU recycling that allow the obtention of the pure initial starting material for further reuse [4].

Alternatively, the biocatalytic degradation of PU can be considered as a highly interesting approach, because the depolymerization process occurs under milder conditions, avoiding the use of chemical catalysts, which reduces the operational costs and the use of toxic compounds and makes it more sustainable. As results of the enzyme hydrolysis of urethane bonds, amines, polyols, and carbon dioxide are produced. However, the main drawback of this strategy is that these xenobiotic PUs are highly stable and recalcitrant, and have yet to be discovered full active enzymes able to hydrolyse their bonds.[11,16] Although the partial degradation of polyester-polyurethane materials by microorganisms has been reported, [17] there are not references concerning the biocatalytic depolymerization of thermoset polyurethanes. As representative example, the treatment of ultrathin PU samples, obtained from commercial Biomer® (a segmented polyurethane used in blood-contacting devices), with papain and urease for periods of 1–6 months at 37 °C, resulted in a slight modification onto the polymer surface.[18] Moreover, the carbamate and thiocarbamates compounds have been reported to be strong inhibitors of serin hydrolases and proteases, increasing even more the difficulty for developing efficient enzymatic processes for the hydrolysis of polyurethanes.[19] In the same context, a *Trametes versicolor* laccase mediated system was tested on four representative polyester- and polyether-based PU models, revealing a significant reduction in the molar masses after 18 days of incubation at 37 ºC. [9] Alternatively, Branson *et al.*[20] demonstrated the depolymerization of a polyether-polyurethane foam by means of a chemo-enzymatic approach, consisting in a glycolysis reaction using diethylene glycol (DEG) and tin (II)− 2-ethylhexanoate as catalyst at 200 °C (first step), followed by a second enzymatic step catalysed by an urethanase able to perform the hydrolysis of remaining low molecular weight dicarbamates after 48 h at 70 °C.

According to the importance of engineered media and the sustainability of the processes, alternative reaction systems, such as ionic liquids (ILs) may open new opportunities in PU depolymerization. ILs are clean solvents composed entirely by ions that are liquids at temperatures below 100 °C.[21] Their application has led to a green chemical revolution because of their genuine physicochemical properties (*e.g.* low vapour pressure, non-flammable nature, high ionic conductivity, excellent solvent power towards many substrates, *etc.*), being considered excellent alternatives to the Volatile Organic Compounds (VOCs).[22] Typical ILs suitable for biocatalytic processes are based on organic cations (*e.g.* dialkylimidazolium, tetraalkylammonium, *etc.*), paired with anions that have a strongly delocalized charge (*e.g.* tetrafluoroborate [BF_4], bis(trifluoromethylsulfonyl)imide [NTf_2], *etc.*).[22–24] For instance, it has been reported how water-immiscible ILs provide an appropriate microenvironment for enzymes (*e.g.* CALB, chymotrypsin, *etc.*) under low-water concentration due to the preservation of the essential hydration shell.[25–27] Another important property is the high solvent capacity, being able to dissolve recalcitrant polymeric compounds such as cellulose, a linear polymer.[28,29] In this case, homogeneous cellulose solutions in the IL 1-butyl-3-methylimidazolium chloride ([C_4mim][Cl]) were used to pre-treat the cellulose and transform it into a more accessible substrate for the commercial cellulase Celluclast® enzymatic hydrolysis, allowing conversions up to 97.7% after 4 h at 50 °C. In this sense, excellent synergies can be reached by combining the advantages of selective biocatalytic transformations with the genuine properties of ILs, pushing towards the development of clean and sustainable chemical processes [25].

This work shows for the first time the suitability of different commercial biocatalysts (*i.e.* lipases, proteases, ureases, etc) to carry out the hydrolysis of two-different toluene-based di-urethane model substrates, such as bis(2-methoxyethyl) (4-methyl-1,3-phenylene)dicarbamate (2,4-TDC), and bis(2-methoxyethyl) (2-methyl-1,3-phenylene)dicarbamate (2,6-TDC). Both di-urethane compounds were obtained by direct reaction of TDI with ethylene glycol monomethyl ether (EGME), as representative model of carbamate moieties present in polyurethane foam. The enzymatic hydrolysis of these model substates was tested in water, solketal:water or ethylene glycol:water (90:10, v/v) or IL:solketal:water (70:25:5, v/v/v) reaction media for 48 h at 60 ºC (Fig. 1).

2. Materials and methods

2.1. Materials

Toluene diisocyanate TDI 80/20 (mixture of isomers of 2,4- diisocyanatetoluene, and 2,6- diisocyanatetoluene for industrial use) was purchased from Thermo Scientific (USA). Solketal (97% purity), ethylene glycol monomethyl ether (EGME, ≥99.5% purity), 2,6-diaminotoluene (2,6-TDA, 97% purity) and 2,4-diaminotoluene (2,4-TDA, 98% purity) were purchased from Sigma-Aldrich (Spain). Ethylene glycol from Merck (Germany). 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([C_2mim][NTf_2], 98% purity), 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([C_4mim][NTf_2], 98% purity), 1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl)imide ([C_8mim][NTf_2], 98% purity), 1-dodecyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([C_{12}mim][NTf_2], 98% purity) was supplied by Ionic Liquids Technologies (Germany). All other chemicals were purchased from Sigma–Aldrich at highest purity available. Acrylaway® L (EC 3.5.1.1.), Alcalase® Pure 4.0 L (EC 3.4.21.62), Flavourzyme® (EC 3.4.11.1), and *Candida antarctica* lipase B (CALB) (EC 3.1.1.3) enzymes preparations were a gift from Novozymes S.A. (Spain), while Urease (EC 3.5.1.5) was purchased from Sigma-Aldrich (Spain).

Prior to use, enzyme preparations were ultrafiltered to eliminate all the low molecular weight additives, as follows: 25 mL of each enzyme preparation were diluted in 225 mL of water, and the resulting solutions were concentrated 10-fold by ultrafiltration at 8 ºC, using a Vivaflow 50 (Sartorious®) system equipped with polysulphone membranes (10 kDa. cut-off). For each enzyme, the process was repeated three-times, leading to Acrylaway L (13.9 mg protein/mL), Alcalase (76.1 mg protein/mL), Flavourzyme (48.7 mg protein/mL), and CALB (11.0 mg protein/mL), and Urease (5.0 mg protein/mL) solutions, respectively. Moreover, a stock solution of Urease (5.0 mg protein/mL) was prepared.

2.2. Synthesis of the 2,4-TDC and 2,6-TDC di-urethane model compounds

Into a 250 mL total volume flask, 62.5 mL tetrahydrofuran (THF), 12.5 g of TDI 80/20 (71.8 mmol), and 22.6 mL EGME (286.6 mmol) were added (see Fig. 2). The reaction mixture was incubated under stirring for 12 h at 60 °C. Finally, the reaction mixture was concentrated by evaporation of THF and the unreacted EGME under reduced pressure

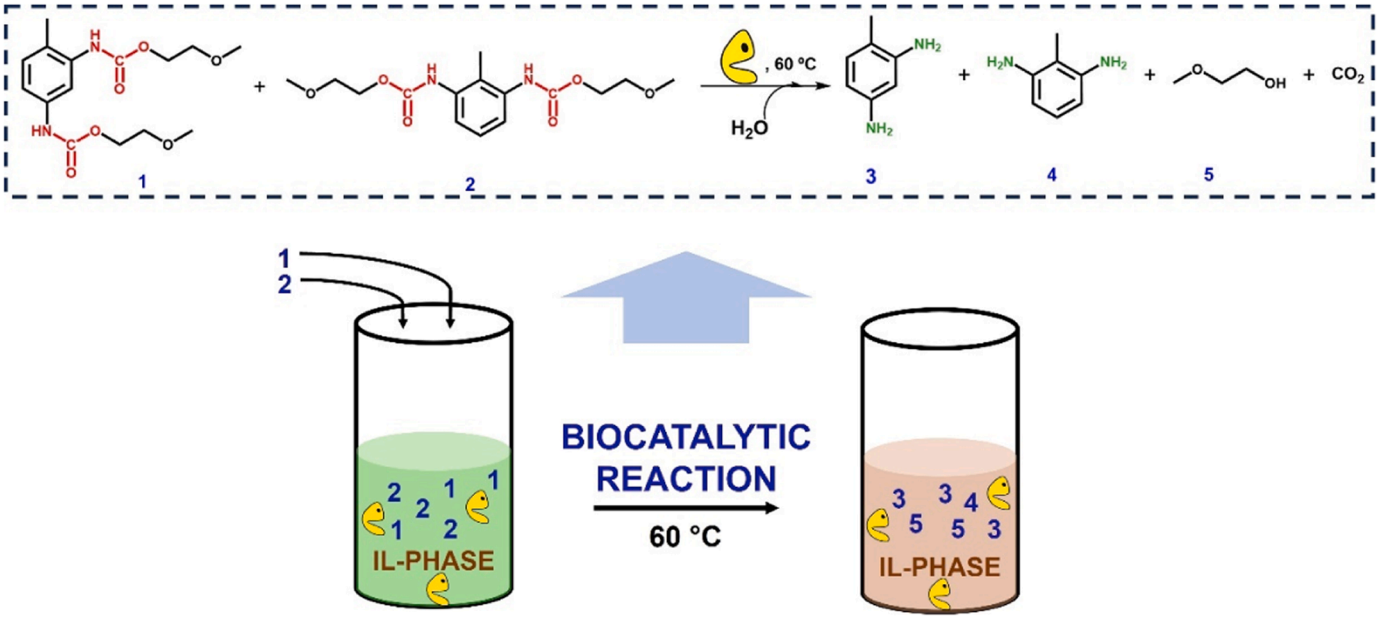

Fig. 1. Schema of the enzymatic hydrolysis of bis(2-methoxyethyl) (4-methyl-1,3-phenylene)dicarbamate (2,4-TDC, **1**), and bis(2-methoxyethyl) (2-methyl-1,3-phenylene)dicarbamate (2,6-TDC, **2**) in several reaction media, obtaining the corresponding 2,4-toluene (**3**) and 2,6-toluene diamine (**4**), and EGME (**5**) products.

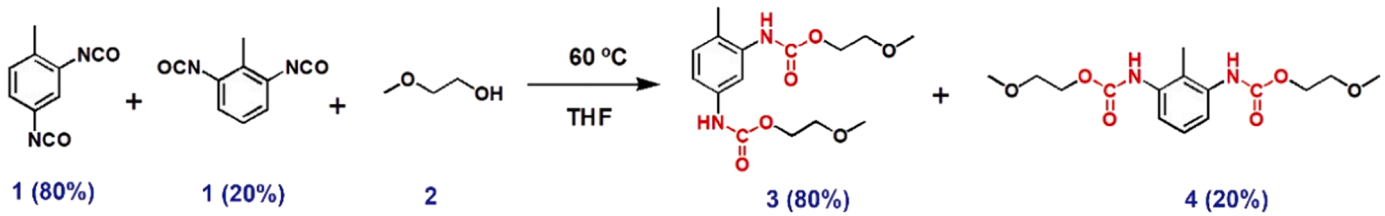

Fig. 2. Scheme of the synthesis of 2,4-TDC (**3**), and 2,6-TDC (**4**) urethane model substrates from TDI 80/20 (**1**) and EGME (**2**) in THF for 12 h at 60 °C.

(100 mbar) for 1 h at 45 °C, which resulted in the precipitation of the urethane compounds mixture.

2.3. Enzymatic hydrolysis of 2,4-TDC and 2,6-TDC di-urethane model in organic solvents or ILs medium

Reactions were set up in 4 mL glass vials containing the 2,4-TDC:2,6-TDC (80:20, mol/mol) mixture (50 mg, 0.2 mmol) and 1 mL of water, solketal:water (90:10, v/v), or ethylene glycol:water (90:10, v/v), respectively. Then, 25 μL of the corresponding enzyme solution were added (Flavourzyme, 1.22 mg, Alcalase, 1.90 mg, CALB, 0.27 mg, Acrylaway, 0.35 mg, or Urease, 0.13 mg), and the reaction mixtures were incubated for 48 h at 60 °C and 200 rpm.

Alternatively, the 2,4-TDC:2,6-TDC (80:20, mol/mol) substrate mixture (50 mg, 0.2 mmol) was previously dissolved in 525 μL of an hydrophobic IL (*i.e.* $[C_2mim][NTf_2]$, $[C_4mim][NTf_2]$, $[C_8mim][NTf_2]$, or $[C_{12}mim][NTf_2]$), and the resulting solution was mixed with 0.225 mL of a solketal:water (90:10, v/v) solution. Then, the reaction was started by the addition of one, or several, enzyme solution (25 μL of each one), being then incubated under magnetic stirring for 48 h at 60 °C. At different reaction times, aliquots from the reaction mixture (0.1 mL) were taken and dissolved in 0.5 mL of MeOH:H_2O (90:10, v/v), then vigorously shaken and centrifuged (15,000 rpm, 5 min, RT). Finally, 300 μL of the resulting upper phase were added diluted with 200 μL MeOH:H_2O (90:10, v/v) for HPLC analysis. One unit of hydrolytic activity was defined as the amount of enzyme that produced 1 μmol of 2,4-TDA (or 2,6-TDA) per minute at 60 ºC.

2.4. FT-IR and HPLC analysis

Fourier-transform infrared spectra (FT-IR) were recorded using a Fourier transform infrared spectrometer FT/IR-4700 Jasco (Madrid, Spain), in ATR mode in a spectral range of 400–3500 cm^{-1} with a spectral resolution of 4 cm^{-1}. An atmospheric background was collected before each sample analysis (32 scans, resolution 4 cm^{-1}).

The separation and identification of the carbamate substrates and diamine products were performed on a Shimadzu LC-20 HPLC (Shimadzu Europe, Germany) equipped with a photodiode array detector (SPD-M20A, Shimadzu) and a RP-C18 column LiChroCART-LiChrospher (250 mm × 0.25 mm × 5 μm size particle, Merck, USA). Both substrates and products were analysed under the following conditions: column temperature at 50 °C and 1 mL min^{-1} flow rate of a mixture of the solvents: A, water; B, methanol. The following elution gradient was used: 0.01 min (70:30, v/v), 20–25 min (10:90, v/v), and 26–35 min (70:30, v/v), and the peaks were identified by UV detection at 280 nm. Peak retention times (min) were as follows: 2,4-TDC and 2,6-TDC (11.8); 2,4-TDA and 2,6-TDA (4.3).

3. Results and Discussion

3.1. Characterization of 2,4-TDC and 2,6-TDC di-urethane model compounds by FT-IR

The versatility of PU materials results from the wide range of possible combinations of polyols and (poly)isocyanates used for their preparation.[30,31] In this work, a low molecular weight di-urethane (2,4-TDC

and 2,6-TDC) mixture was synthetized as model to analyse the suitability of different enzymes to carry out its further hydrolysis. The urethane bond was formed through a condensation reaction between the isocyanate group of TDI 80/20 and the alcohol group from EGME using a 1:4 (mol/mol, respectively) ratio in THF at 60 °C. Since an isocyanate mixture of two isoforms was used for the synthesis, a mixture of both compounds would be expected in the resulting polymer (see Fig. 2).

FT-IR analyses were performed to confirm the synthesis of the di-urethane model through the identification of the urethane bond. Fig. 3 depicts a comparison of the IR spectra of both the starting materials (TDI 80/20 and EGME), and the mixture containing 2,4-TDC and 2,6-TDC di-urethane compounds. As can be seen in Fig. 3 (spectra b), the characteristic isocyanate band (2236.1 cm^{-1}) [32] is not present in the urethane-model spectra (spectra c). The EGME spectra (spectra a) shows an absorption peak near 1063.6 cm^{-1}, which is the characteristic band of the ether bond (C–O) [15]. Instead, a band at 1720.2 cm^{-1} identifying a carbamate group (NH-COO-) appears, demonstrating the formation of the urethane bond after 6 h. It has been reported that the 1533.2 cm^{-1} band was assigned to the N-H bond, and the 1066.4 cm^{-1} band corresponds to the aliphatic and aromatic stretching of the C–O–C bond [33, 34].

3.2. Enzymatic hydrolysis of 2,4-TDC and 2,6-TDC di-urethane model compounds in water and organic solvents

Table 1 shows the results of the specific activity of all the enzymes tested in the hydrolysis of the 2,4-TDC and 2,6-TDC mixture. As can be seen, the hydrolytic activity was not observed for any of the assayed enzymes when the reaction was carried out in water as reaction media (entries 1–5, Table 1), which was attributed to the insolubility of these substrates in water. To overcome the problem of solubility, reaction media based on two different hydrophilic organic solvents (*i.e.* solketal and ethylene glycol) were tested in order to improve the enzymatic efficiency for this hydrolytic reaction. The use of ethylene glycol as cosolvent for dissolving the substrate (*i.e.* ethylene glycol:water, 90:10, v/v) did not improve the solubilization of the substrate resulting in heterogeneous and biphasic mixtures (entries 6–10, Table 1) and the absence of hydrolytic activity. However, when reaction mixtures were based on solketal:water (90:10, v/v) mixtures, full solubilization of the TDC di-urethane mixture was observed, allowing enzymes to carry out the reactions (entries 11–15, Table 1). For this reaction medium, all the assayed enzymes were able to hydrolyse the di-urethane model, though

Table 1
Specific activities of different enzymes for the hydrolysis of 2,4-TDC and 2,6-TDC di-urethane mixture (0.2 mmol) in different hydrophilic reaction media (1 mL total volume).

Entry	Enzyme	Commercial Name (mg of total Prot assayed)	Reaction medium (v/v)	Specific Activity (mU/mg)
1	Protease	Flavourzyme (1.22)	Water	n.d.
2	Protease	Alcalase (1.90)	Water	n.d.
3	Lipase	*Candida antarctica* lipase B (0.27)	Water	n.d.
4	Protease	Acrylaway (0.35)	Water	n.d.
5	Urease	Urease (0.13)	Water	n.d.
6	Protease	Flavourzyme (1.22)	EG:water (90:10)	n.d.
7	Protease	Alcalase (1.90)	EG:water (90:10)	n.d.
8	Lipase	*Candida antarctica* lipase B (0.27)	EG:water (90:10)	n.d.
9	Protease	Acrylaway (0.35)	EG:water (90:10)	n.d.
10	Urease	Urease (0.13)	EG:water (90:10)	n.d.
11	Protease	Flavourzyme (1.22)	Solketal:water (90:10)	5.4
12	Protease	Alcalase (1.90)	Solketal:water (90:10)	1.5
13	Lipase	*Candida antarctica* lipase B (0.27)	Solketal:water (90:10)	12.3
14	Protease	Acrylaway (0.35)	Solketal:water (90:10)	3.8
15	Urease	Urease (0.13)	Solketal:water (90:10)	31.6

n.d.: non detected.

with different efficiency, highlighting the performance of urease (31.6 mU/mg), closely followed by CALB (12.3 mU/mg) and Flavourzyme, (5.4 mU/mg). However, the proteolytic enzymes Acrylaway and Alcalase showed lower activities, which could be related to a more powerful inhibition of these enzymes by carbamates and thiocarbamates.[19] Additionally to the full solubilization of substrates in this medium, these results may be attributed to the differences in the active centre of enzymes and their catalytic mechanism. Although the carbamate groups present a structural resemblance to the amide, ester or urea groups that are specific targets of proteases, lipases, or ureases, respectively, these results suggest a greater suitability of the active centre of the urease for

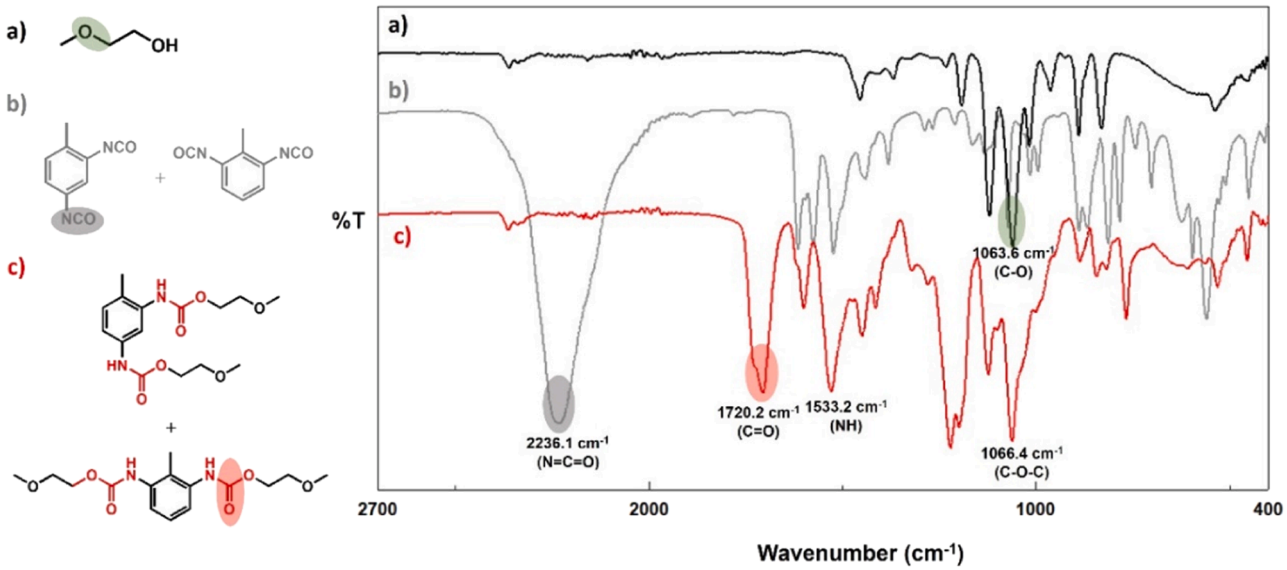

Fig. 3. FT-IR spectra with band main assignment of (**a**) EGME, (**b**) TDI, and (**c**) 2,4-TDC and 2,6-TDC.

the hydrolysis of these toluene di-carbamate compounds.

3.3. Enzymatic hydrolysis of 2,4-TDC and 2,6-TDC di-urethane model compounds in ionic liquids

Hydrophobic ionic liquids (*e.g.* [C_4mim][NTf_2], etc) have been widely reported as exceptional reaction media for enzymes, because of the clear enhancements in activity, selectivity and stability displayed by them, even under harsh conditions (*i.e.* 150 ºC, 120 bar).[11,22]. Firstly, it was observed the excellent suitability of several hydrophobic ILs (*i.e.* [C_2mim][NTf_2], [C_4mim][NTf_2], [C_8mim][NTf_2] and [C_{12}mim][NTf_2]) for dissolving the 2,4-TDC and 2,6-TDC substrates. Then, the hydrolytic activity of the selected commercial enzyme preparations (*i.e.* Flavourzyme, Alcalase, CALB, Acrylaway and urease) was assayed, by using reaction media based on IL:solketal:water (70:25:5, v/v/v), that provided monophasic reaction mixtures. Reaction media based on IL/water mixtures resulted in biphasic systems, and enzyme hydrolytic activity was not observed. As can be seen in Fig. 4, all the assayed biocatalysts preparation improved their specific activity for urethane degradation in reaction media containing the IL-[C_4mim][NTf_2] with respect to corresponding IL-free reaction medium (see Table 1). These results can be explained by the excellent synergy between enzymes and ILs [35], whereas any possible deactivation effect of solketal, as polar organic solvent that may favour water-stripping on enzymes, resulted diminished in the presence of this neoteric solvent. Onge again, it is demonstrated how the use of water-immiscible ILs medium for biocatalytic transformations offers significant advantages for the enzyme activity. It should be underlined how the specific activity of urease and CALB was increased by more than twice (74.2 and 22.7 mU/mg, respectively) in the IL:solketal:water medium, compared to the enzymatic activity observed in solketal:water (90:10, v/v) medium (*i.e.* 31.6 and 12.3 mU/mg, respectively). Indeed, Urease and CALB provided the most promising hydrolytic results for all reaction systems, while the poor results obtained by proteases (*i.e.* Flavourzyme, Acrylaway and Alcalase) could be related to an increase in autolysis phenomena, which may be enhanced in these hydrophobic environments with low water content. Consequently, this excellent enzymatic performance point out the enzyme suitability for further depolymerization of polyurethane foam wastes.[36].

In this context, it was reported a possible mechanism of PU hydrolysis catalysed by ureases that consisted in the breakdown of urethane bonds, providing oligomers of lower molecular weight, which are repetitively attacked by enzymes until full degradation. [17,37] On the other hand, biocatalytic degradation of polymers with different nature (*e.g.* PET and PU) by serine-hydrolases (*e.g.* lipases) having a consensus GXSXG sequence have been reported as closely related with (poly) urethanase activity.[7] For instance, it has been demonstrated how an esterase from *Pseudomonas fluorescens* containing this GXSXG motif was able to hydrolyse a polyester-polyurethane dispersion [38].

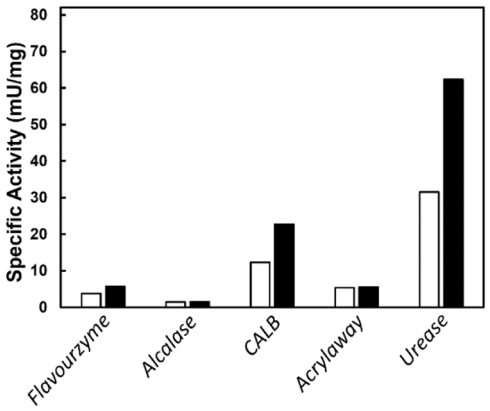

Fig. 4. Enzyme-catalysed hydrolysis of 2,4-TDC and 2,6-TDC di-urethane compounds in IL-[C_4mim][NTf_2]:solketal:water (70:25:5, v/v/v) (black bars), or solketal:water (90:10, v/v) (white bars) for 48 h at 60 ºC.

In another example, Fig. 5 depicts the time-course profiles of urease and CALB-catalyzed hydrolysis of the carbamate bond in 2,4-TDC and 2,6-TDC di-urethane model substrates in both IL/solketal/water (hydrophobic), or solketal/water (hydrophilic) monophasic reaction media. As can be seen, both enzymes provided the better results in IL-[C_4mim][NTf_2] medium, being Urease enzyme the most suitable catalysts to carry out the hydrolysis of the TDC di-urethane model.

In this context, the influence of the hydrophobicity of the IL-based media on the urease-catalysed hydrolysis of carbamate bonds was also studied by using reaction media based on alkyl imidazolium ILs with different alkyl side chain length in the cation (*i.e.* [C_2mim][NTf_2], [C_4mim][NTf_2], [C_8mim][NTf_2] or [C_{12}mim][NTf_2]) (see Fig. 6). Beside the different hydrophobic character, monophasic systems were obtained because of the miscibility and high solubility of solketal in these water-immiscible ILs media. In all cases, Urease was able to hydrolyse the TDC di-urethane model, being the specific activity improved by 1.25-times when the IL hydrophobicity increased from [C_2mim][NTf_2] to [C_4mim][NTf_2], resulting in the best enzymatic activity (up to 74.1 mU/mg). The additional increase in the alkyl chain length of imidazolium cation (*i.e.* [C_8mim][NTf_2] and [C_{12}mim][NTf_2]) did not improve the enzymatic activity (*i.e.* 60.5 and 62.8 mU/mg, respectively) with respect to the [C_2mim][NTf_2] initial case. According to these results, all evaluated IL systems showed better results compared to those obtained in hydrophilic solvents (*i.e.* solketal), demonstrating once again the suitability of the IL-containing medium. To explain these results, the observed improvement in activity promoted by ILs can be attributed to the combination of their hydrophobic character that have a protective effect on the biocatalyst, whereas too long alkyl chain lengths increase the viscosity which may affect the mass transfer phenomena during the biocatalytic reaction.

In an attempt to improve the efficiency of the biocatalytic hydrolysis of the TDC di-urethane model compounds, enzymatic "cocktails" were assayed in the [C_4mim][NTf_2]:solketal:water (70:25:5, v/v/v) reaction medium. Fig. 7 shows the amount of TDA released as a function of the enzymatic cocktail assayed after 48 h reaction at 60 ºC. The combination of urease and CALB showed the best product hydrolysis values, being slightly better than those observed for both enzymes separately assayed (see Fig. 7). The combination of the aminopeptidase Flavourzyme with either urease, or the urease-CALB mixture, in this reaction medium with low water content did not improve the hydrolysis of the TDC di-urethane model mixture. This fact could be related with a proteolytic side-effect of this aminopeptidase on the urease and/or CALB proteins, promoting the loss in activity. In this context, it has been reported synergistic effects between esterases and amidases on polycaprolactone polyol-based thermoplastic PU degradation in aqueous medium with high water content.[39] However, it should be underlined the positive effect on the biocatalytic hydrolysis of these TDC urethane models that results by the combination of CALB (lipase) and urease, improving the overall urethane-cleaving activities, which should be further investigated. These results could open a possible experimental approach for the biocatalytic degradation of polyurethane foam wastes through the combination of lipases and ureases of different sources, based on the proper design of the reaction media by using ionic liquid with recognized enzyme stabilizing power [21,35].

4. Conclusions

Polyurethane foam wastes are recalcitrant polymers which lack of an efficient and sustainable approach for their depolymerization. The suitability of several commercial hydrolytic enzymes (esterases, amidases, proteases, *etc.*) to hydrolyse a mixture of two-different toluene-based urethane model compounds (*i.e.* bis(2-methoxyethyl) (4-methyl-1,3-phenylene)dicarbamate, and bis(2-methoxyethyl) (2-methyl-1,3-

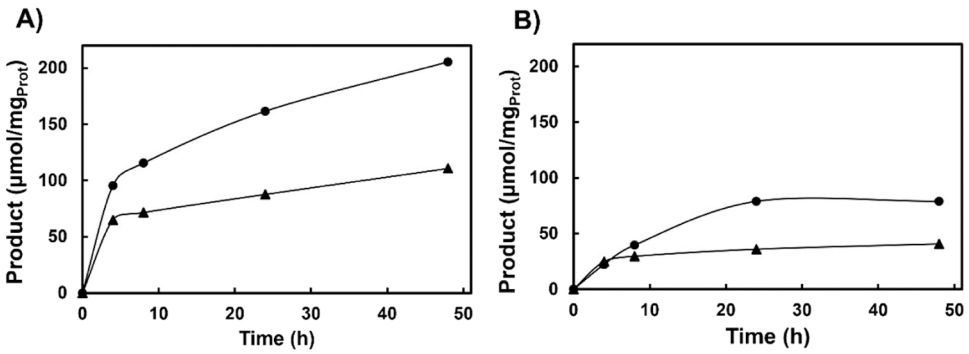

Fig. 5. Time-course profiles for Urease (**A**) and CALB (**B**) catalysed hydrolysis of the carbamate bond of the 2,4-TDC and 2,6-TDC di-urethane model (0.2 mmol) in IL-[C_4mim][NTf_2]:solketal:water (70:25:5, v/v/v) (●) or solketal:water (90:10, v/v) (▲) medium for 48 h at 60 °C.

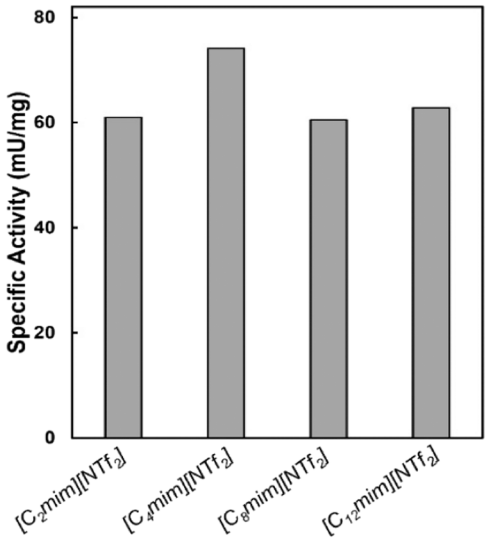

Fig. 6. Influence of the alkyl-side chain of the ILs on the urease specific activity for the hydrolysis of the 2,4-TDC and 2,6-TDC di-urethane model (0.2 mmol) in IL-[$C_n$mim][NTf_2]:solketal:water (70:25:5, v/v/v) medium for 48 h at 60 °C.

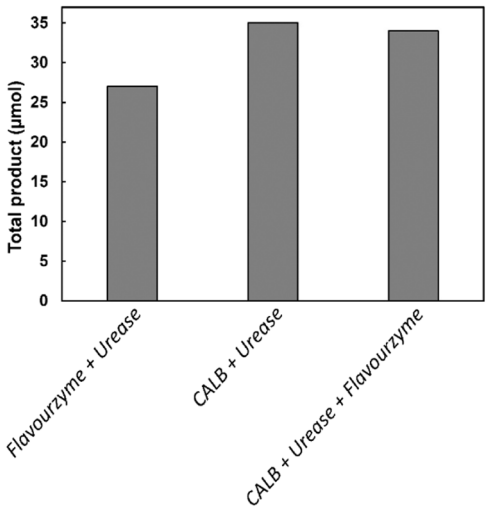

Fig. 7. Total 2,4-TDA and 2,6-TDA products (μmol) resulted by the enzymatic hydrolysis of the 2,4-TDC and 2,6-TDC di-urethane model compounds in [C_4mim][NTf_2]:solketal:water (70:25:5, v/v/v) reaction medium after 48 h reaction at 60 ºC.

phenylene)dicarbamate) has been demonstrated. The ILs technology has improved the reaction media providing full solubilization of substrates, and favouring the enzymatic hydrolysis of urethane bonds by means of a mixture of urease and lipase under restricted water content (up to 5% v/v). The presence of proteases did not provide significant improvements in the overall hydrolysis of the di-urethane model. Despite the recalcitrant behaviour of this kind of polymeric compounds not available in Nature, these preliminary results demonstrate the potential of natural catalyst to afford their depolymerization in combination with the medium engineering and open the door towards new sustainable approaches aimed for the recycling of valuable monomers and wastes reduction.

CRediT authorship contribution statement

Villa Rocio: Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Salas Rebeca:** Data curation, Formal analysis, Investigation, Methodology. **García-Verdugo Eduardo:** Conceptualization, Funding acquisition, Investigation, Writing – review & editing. **Lozano Pedro:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Cano Sergio:** Formal analysis, Investigation, Methodology. **Nieto Susana:** Data curation, Formal analysis, Investigation, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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