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Polymer Recycling

Urethanases for the Enzymatic Hydrolysis of Low Molecular Weight Carbamates and the Recycling of Polyurethanes

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Dedicated to Karl-Erich Jaeger

Abstract: Enzymatic degradation and recycling can reduce the environmental impact of plastics. Despite decades of research, no enzymes for the efficient hydrolysis of polyurethanes have been reported. Whereas the hydrolysis of the ester bonds in polyester-polyurethanes by cutinases is known, the urethane bonds in polyether-polyurethanes have remained inaccessible to biocatalytic hydrolysis. Here we report the discovery of urethanases from a metagenome library constructed from soil that had been exposed to polyurethane waste for many years. We then demonstrate the use of a urethanase in a chemoenzymatic process for polyurethane foam recycling. The urethanase hydrolyses low molecular weight dicarbamates resulting from chemical glycolysis of polyether-polyurethane foam, making this strategy broadly applicable to diverse polyether-polyurethane wastes.

Plastics are indispensable for beverage and food packaging, electrical insulation, construction, textiles, and medical items like masks.[1] Unfortunately, the ease of mass manufacturing and resistance to degradation, two major advantages of plastic, have resulted in the environmental catastrophe of plastic pollution.[2] While we may get rid of plastic waste by incineration, this would release vast amounts of CO_2 and other toxic gases into the atmosphere. Unfortunately, less than 10 % of plastic is currently being recycled. Fortunately, however, there is a rising demand for sustainable plastics[3] and biocatalytic plastic recycling is currently an extremely active field of research.[4] For example, the company Carbios recently used an engineered cutinase to hydrolyse >90 % of amorphised postconsumer PET bottle plastic into monomers within 10 h.[5] After polyesters, polyurethanes make up the second largest class of hydrolysable plastics.[6] This makes polyurethanes an obvious target for enzymatic hydrolysis and recycling. Polyurethanes are used to make soft foams (mattresses, sponges, and upholstery), hard foams (insulation and other building materials), thermoplastics (sports shoes), and coatings (sealants, paints, and adhesives).[7] Because the three-dimensional cross-linking in thermoset polyurethanes (mostly foams) make them un-meltable and insoluble in solvents, these polyurethanes are often mechanically recycled by grinding and adhesive bonding to make secondary materials.[8] While useful for materials like sound proofing and sports mats, this kind of recycling is not capable of producing virgin polyurethane materials. Therefore, almost 50 % of European polyurethane waste is still disposed in landfills.[6,9] Furthermore, due to increasing demand, increasingly larger quantities of polyurethanes are being produced. Therefore, there is a growing need for sustainable recycling methods, which would allow the monomers to be reused.

Urethanases are enzymes that catalyse the cleavage of urethane bonds to release amines, alcohols, and carbon dioxide (Scheme 1).[10] To date, no efficient enzymes that can directly hydrolyse urethane bonds in polymers have been described. While some proteases and amidases have been claimed to hydrolyse these bonds in polyether-polyurethanes, these processes are extremely slow and minimal degradation is achieved even after several months.[11] Furthermore, publications on polyether-polyurethane degradation have mostly focused on filamentous fungi.[12] However, for the recycling of polymers, defined enzymes are needed to obtain defined monomers, not microbes that use complex combinations of hydrolytic and oxidative mechanisms. The "polyurethanases" reported so far are misnamed polyesterhydrolases, like cutinases, acting on the polyester components of polyester-polyurethanes only.[12,13] The polyesters cutin and suberin are very abundant in nature, explaining why many enzymes capable of efficiently hydrolysing synthetic polyesters like polyethylene terephthalate and polyester-polyurethanes have been discovered.[14] Interestingly, degradation of polyester-polyurethanes by polyesterases produces low-molecular weight dicarbamates as degra-

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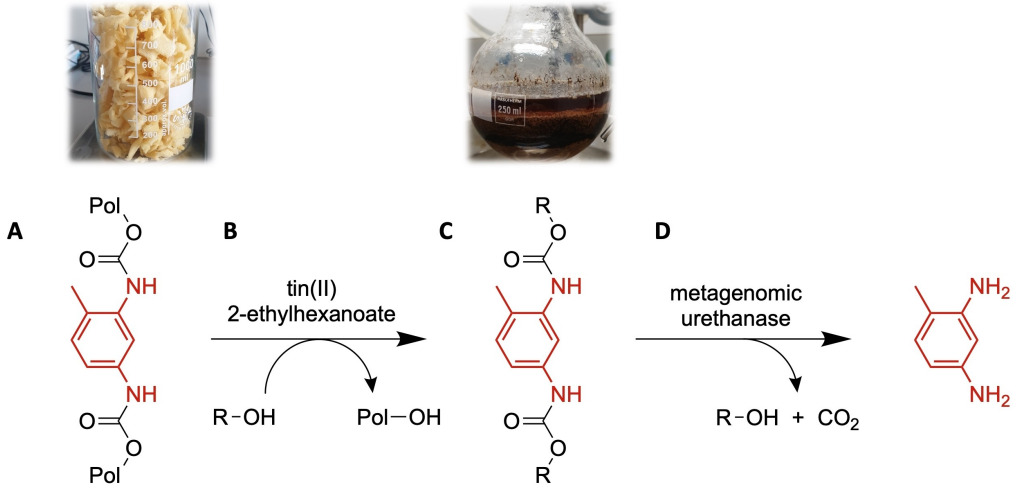

Scheme 1. Chemoenzymatic recycling of TDI-based polyether-polyurethane foam. A) The waste TDI-based polyether-polyurethane foam used for glycolysis. The polyether polyols and further oligomer units are represented by "Pol". B) The polymer is broken down by glycolysis at 200 °C using an excess of diethylene glycol (DEG) containing 1 % (w/w) of tin(II)-2-ethylhexanoate as catalyst. The alcohol is represented by "R-OH" and the released polyether polyol by "Pol-OH". C) Products of the glycolysis reaction with polyether-polyols separating into an upper phase and low molecular weight TDA-dicarbamates (and excess DEG) forming the lower phase. D) The low molecular weight dicarbamate TDA-DEG is then hydrolysed by a metagenome-derived urethanase discovered in our study, releasing the glycol (DEG), carbon dioxide, and the aromatic diamine (TDA).

dation products that, in contrast to the insoluble high molecular weight polyurethanes, are enzymatically hydrolysable.[11b,17] Therefore, we envisioned a chemoenzymatic approach where polyether-polyurethanes are first converted to low molecular weight dicarbamates by chemical glycolysis, followed by enzymatic hydrolysis of the low molecular weight dicarbamates (Scheme 1).

Polyurethanes can be chemically recycled by hydrolysis or aminolysis under conditions of high temperature (up to 450 °C) and pressure (up to 8 MPa).[6,7e,18] Alternatively, alcoholysis, a trans-urethanisation between the hydroxyl groups of an alcohol and the urethane groups of a polyether-polyurethane, can convert the polyurethane into low molecular weight dicarbamates and polyether-polyols in the presence of a catalyst. Suitable alcohols include methanol, ethanol, ethoxyethanol, and ethylene glycol (Figure S1).[7e,8b,19] In the case of diols like diethylene glycol (DEG) with high boiling points, reactions can safely proceed at atmospheric pressure. Transurethanisation is then usually carried out at temperatures around 200 °C and an excess of the glycol is used in the presence of, e.g., organometallic salts as catalysts.[7e,18a,19a] This process releases the polyether-polyols that make up the bulk of the polyurethane, which can then be reused to produce new polyurethanes.[20] To date, the highly pure polyol moiety and the significantly milder reaction conditions make glycolysis the more effective and cost-efficient method in comparison to chemical hydrolysis.[7e,18] However, while the polyether-polyols can be reused, the aromatic diamines are trapped as dicarbamate side products and their chemical hydrolysis to aromatic diamines would require high temperature, pressure, and pH, making many of the advantages of chemical glycolysis over chemical hydrolysis untenable.[6,18] For example, the glycolysis of toluene diisocyanate (TDI)-based polyurethanes with diethylene glycol results in TDA-DEG, the dicarbamate of TDA with DEG (Figure S1).[7e,19a] To further increase the potential of glycolytic recycling, enzymes capable of hydrolysing the low molecular weight dicarbamates, for recovery of the aromatic diamines, are of high interest, because this reaction proceeds under mild conditions at room temperature.[21]

Some enzymes hydrolysing low molecular weight urethanes have been reported.[21,22] Pesticides and herbicides like carbaryl and carbofuran are reactive *O*-aryl carbamates. The broad application of these labile compounds explains why many enzymes capable of hydrolysing them have been identified.[23] In contrast, *N*-aryl *O*-alkyl carbamates are significantly more stable[24] and few enzymes (mostly esterases and amidases) capable of hydrolysing them have been identified.[21,23f,25] These rare enzymes are most relevant to enzymatic polyurethane recycling since glycolysis of the most common polyurethanes results in dicarbamates of aromatic diamines (TDA and 4,4'-methylendianiline (MDA)).[10,17,21,26] Thus, there is a need for more urethanases with broad and diverse substrate specificities to deal with the range of oligourethanes resulting from alcoholysis or glycolysis of diverse polyurethane wastes. Therefore, we isolated DNA from soil collected from a site that had been exposed to polyurethanes and produced a metagenome library (see Supporting Information). This library was screened for urethanase activity, leading to the discovery of three new urethanases (UMG-SP-1 to UMG-SP-3, GenBank accession codes: OP972509, OP972510, and OP972511). The sequence of UMG-SP-1 is 52.44 % and 52.67 % identical to that of UMG-SP-2 and UMG-SP-3, respectively. The closest homologue (75.06 % identity) is an amidase signature family protein from *Sphingomonas* sp. AX6. The sequences of UMG-SP-2 and UMG-SP-3 are 83.07 % identical. The

closest homologues are amidase signature family proteins from *Sphingomonas alpina* (60.23 % identical to UMG-SP-2) and *Sphingomonas* sp. ERG5 (61.61 % identical to UMG-SP-3). Sequences are provided in the Supporting Information. The specific activities (Table 1) of the three urethanases were determined at 30 °C using 100 μM 7-carbethoxy-4-methylcoumarin in 50 mM sodium phosphate buffer (pH 8.0). The enzymes had similar activities and UMG-SP-1 was the most active (0.749 ± 0.012 μmol min^{-1} mg^{-1}). In the range between pH 4 and pH 11, all enzymes were most active at pH 10 (Table 1). UMG-SP-1 and UMG-SP-2 were most active at 70 °C, the highest temperature we studied. In contrast, UMG-SP-3 was maximally active at 35 °C, one of the lowest temperatures investigated. The optimal reaction temperatures were significantly higher than the maximum temperatures at which the enzymes were stable over a 12 h period (Table 1 and Table S1). UMG-SP-1 retained 48 % of its activity after incubation at 37 °C for 12 h. UMG-SP-2 retained 44 % of its activity after incubation at 29 °C for 12 h. In contrast, UMG-SP-3 lost 100 % of its activity after incubation at 20 °C (the lowest temperature tested) for 12 h. UMG-SP-1 was most active in the absence of DMSO, while UMG-SP-2 and UMG-SP-3 were both most active in the presence of 10 % DMSO. Therefore, subsequent reactions were performed at room temperature, with up to 10 % DMSO (detailed protocols can be found in the Supporting Information).

The urethanases were subsequently studied for the hydrolysis of a broad range of low molecular weight dicarbamates. In preliminary experiments, UMG-SP-1, UMG-SP-2, and UMG-SP-3 could all hydrolyse all of the low molecular weight dicarbamates we tested (Figure S1 and Table S1). These substrates were MDA-methanol, MDA-benzyl alcohol, MDA-ethanol, TDA-ethoxyethanol, and TDA-diethylene glycol (TDA-DEG). In particular, UMG-SP-2 could hydrolyse >90 % of TDA-DEG (20 mM) within 24 h (Figures S2 and S3). Therefore, we investigated the suitability of this urethanase for the chemo-enzymatic recycling of aromatic diamines from TDI-based polyurethane foam (Scheme 1). For a realistic case demonstration, we took post-consumer soft foam from an old pillow and added it to an equal mass of preheated (200 °C) DEG containing 1 % (w/w) of tin(II)-2-ethylhexanoate as a catalyst. All kinds of soft mattresses are usually made from TDI-based polyether-polyurethanes.[7e] The comparison of the glycolysate from our post-consumer sample (Figure 2) with the synthetic TDA-DEG sample (Figure S2) by uHPLC proves the post-consumer foam sample to consist mainly of TDI-based polyurethane. Under these conditions, the foam dissolved in the DEG and was incubated at 200 °C for an additional 2 h.[20] After cooling, the reaction mixture separated into two layers (Scheme 1C). The upper layer contains the polyether polyols. The lower phase contains excess DEG and a mixture of transurethanisation products, mostly the DEG-dicarbamates of 2,4-TDA and 2,6-TDA (Scheme 1 and Figures 2 and S2). The lower phase of the glycolysate (1 % w/v) was used in reactions containing UMG-SP-2 lysate (20 % v/v, prepared as described in the Supporting Information). The conversion of TDA-DEG to 2,4-TDA and 2,6-TDA was measured after 0.5, 1, 2, 6, and 24 h using uHPLC and quantified by reference to 2,4-TDA and 2,6-TDA standards. Approximately 65 % conversion to TDA was achieved within 24 h (Figure 1). Adding more enzyme led to full conversion after 48 h (Figure 2). We could therefore show that UMG-SP-2 has potential for the chemo-enzymatic recycling of polyurethanes.

The versatility of polyurethanes results from the various possible combinations of polyols and polyisocyanates that can be used to synthesize them, which allows physical properties to be tailored to a broad range of applications.[6,7f] Alcoholysis or glycolysis can reduce this chemical diversity to a small number of low molecular weight dicarbamates. The low molecular weight TDA-DEG dicarbamates resulting from glycolysis are a potential source of aromatic diamines for the synthesis of isocyanates that can be used to produce new polyurethanes. While chemical hydrolysis requires high temperature, pressure, and pH, enzymatic hydrolysis using the metagenome-derived urethanases proceeds under mild conditions at room temperature.

In summary, we report the first true urethanases discovered from a metagenome library for this purpose. We further demonstrated how these urethanases could be

Table 1: Summary of the characteristics of the metagenome-derived urethanases.

Urethanase	UMG-SP-1	UMG-SP-2	UMG-SP-3
Specific activity[a] [μmol min^{-1} mg^{-1}]	0.749 ± 0.012	0.377 ± 0.020	0.651 ± 0.064
Optimal pH	10	10	10
Optimal temp. [°C]	70	70	35
Temp. stability[b] [°C], residual activity [%]	37 °C, 48 %	39 °C, 44 %	4 °C, 100 %
Optimal DMSO conc. [% v/v]	0	10	10

[a] Using 7-carbethoxy-4-methylcoumarin as substrate. [b] Highest temperature at which residual activity could be determined after a 12 h incubation.

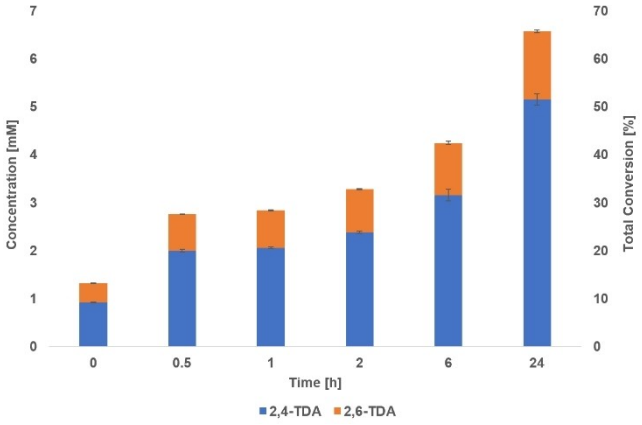

Figure 1. Conversion of the lower phase of the TDI-based polyurethane foam glycolysate to 2,4-TDA and 2,6-TDA by UMG-SP-2. Hydrolysis of 1 % (w/v) glycolysate by 20 % (v/v) crude lysate resulted in 65 % conversion after 24 h. Data plotted are the means and standard deviations calculated from three independent measurements.

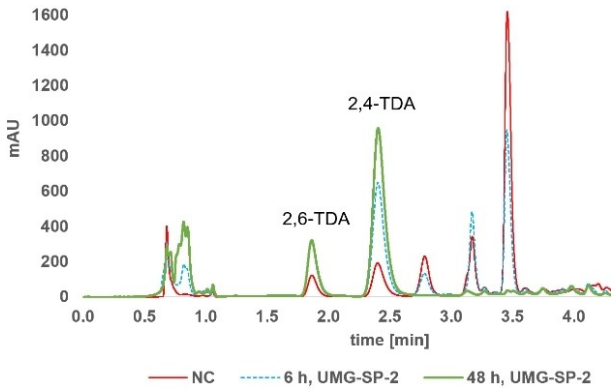

Figure 2. uHPLC analysis of the hydrolysis of the foam glycolysate (mostly TDA-DEG) by the urethanase UMG-SP-2. The reactions contained 1% (w/v) of the lower phase of the glycolysate and 20% (v/v) UMG-SP-2 crude lysate. Samples were analysed after 6 h and 48 h. A negative control (NC) without enzyme is shown for comparison and was measured as shown both in the beginning and the end of the reaction with identical peaks. The glycolysis product consisted of five major peaks representing 2,4-TDA, 2,6-TDA, the dicarbamates of these diamines with diethylene glycol, and a monocarbamate (peak 4 transiently increases and then decreases, suggesting that this is a monocarbamate intermediate of hydrolysis). Next to the dicarbamates, amines were formed from the reaction of urea groups during glycolysis.[7e] After 48 h, all major peaks except those of the TDA monomers disappeared completely, indicating full conversion. All samples were measured in triplicate. The identities of the peaks for the reaction products 2,4-TDA and 2,6-TDA were validated by reference to commercial standards.

applied in a chemo-enzymatic process for polyether-polyurethane foam recycling. Importantly, this process can recover not only the polyether-polyols (from chemocatalytic glycolysis), as has been shown before, but also the aromatic diamines (by enzymatic hydrolysis of low molecular weight carbamates), which subsequently can be isolated as described in literature.[27] Future work will focus on the optimization of UMG-SP-2, identification of more urethanases, and recovery of the diethylene glycol and the tin(II)-2-ethylhexanoate used as catalyst for glycolysis.

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Conflict of Interest

The authors SS, LS, LR and GJ are employees of Covestro. Patent applications have been filed.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Keywords: Aromatic Diamine · Glycolysis · Polyether Polyol · Polyurethane · Urethanase

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