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INVITED ARTICLE

Enzymatic transesterification of urethane-bond containing ester

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Abstract

Here we demonstrate the feasibility and successful application of enzymes in polyurethane network synthesis as well as occurring hurdles that have to be addressed when using urethanes synthesis substrates. The enzymatic transesterification of an urethane-bond containing monofunctional ester and a model alcohol carbitol using lipases is discussed. The reaction is optimized in terms of transesterification time and temperature, the reaction solvent, the possibility of a cosolvent and the alcohol amount, the used transesterification environment, and the biocatalyst. Enzymatic cross-linking of polyurethanes can open up a pool of new possibilities for cross-linking and related polyurethane network properties due to the enzymes high enantio-, stereo-, and regioselectivity and broad substrate spectrum.

Keywords Polyurethanes · Transesterification · Biocatalysis · Lipase · Model study

Introduction

Polyurethanes are organic polymers, first synthesized by the polyaddition of di- or polyisocyanates and di- or polyols [1]. Polyurethanes represent one of the most versatile class of polymeric materials, due to the possible high variability of isocyanate and polyol building blocks. This easy variation allows the synthesis of tailor-made polyurethanes for a wide range of applications, e.g., production of all kind of foams for seats, mattresses or thermal insulation, textile fibers and components for coatings, adhesives, and sealant [2]. Due to the increasing awareness of sustainability research, biobased routes for polyurethane synthesis are becoming more interesting in recent years. To develop more sustainable alternatives for the synthesis of polyurethanes, several different pathways have been worked on for phosgene- and isocyanate-free and biobased polyurethane synthesis in the past [3–16].

However, currently reported processes with biobased raw materials and without the use of phosgene and isocyanates are not feasible for industrial large-scale production of polyurethanes, due to their lower efficiency than the conventional synthesis. Therefore, industry is starting to use biobased monomers combined with conventional phosgenation and later polyaddition to generate biobased polyurethanes. The diamines for later phosgenation and derivatization to form the diisocyanates and the polyol components—1,4-butanediol and succinic acid—can in principle be produced biobased by fermentation of glucose. The biobased polyurethane can then be generated by conventional, chemical polyaddition of the biobased diisocyanate and the biobased polyol.

Although enzymes, especially lipases, are successfully used for many reactions in organic chemistry and also for polymerizations [17–29], they are not used so far for industrial polyurethane synthesis, as this method is still not efficient compared with that of classical production processes. However, regarding the demand of new variable polyurethanes with new properties for different applications, the interest of enzymes as biobased and environmentally friendly catalysts steadily increases [30, 31].

Polyurethane variation currently reaches its limit due to the instability of new and demanding building blocks and compounds that are not stable at the elevated temperatures necessary for the conventional polyurethane synthesis. Enzymes have several advantages to overcome this temperature sensitivity of the building blocks, as they are able to catalyze reactions under mild conditions in contrast to chemical catalysts. In addition, enzymes have a broad substrate spectrum and are highly selective and specific, avoiding the sometimes

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necessary use of protective groups for non-specific chemical reactions.

Lipases are a versatile group of biocatalysts. The natural role of lipases is to catalyze the hydrolysis of ester bonds at the oil-water interface. In nonaqueous conditions, they catalyze the reverse reaction, such as esterification, interesterification, and transesterification. The term transesterification refers to the exchange of groups between an ester and an acid (acidolysis), between an ester and an alcohol (alcoholysis), or between two esters (interesterification). The ability of lipases to catalyze these reactions with great efficiency, stability, and versatility renders these enzymes a commercial success.

The characteristic folding pattern of most lipases is the α/β-hydrolase fold [32] consisting of a central β-sheet core, which is surrounded by six α-helices. Lipase active sites consist of a nucleophilic polar serine, an acid aspartic or glutamic acid, and the positively charged histidine [32]. In various lipases, a so-called lid can be observed which consists of an α-helical structure covering the active site [33–35] that can open and close depending on the environment [36].

The catalytic reaction starts with an acylation step resulting in the formation of the acyl-enzyme complex by a nucleophilic attack of the activated serine on the carbonyl C-atom of the substrate ester bond [37–39].

Deacylation regenerates the enzyme in the second step of the reaction, releasing the substrate from the serin and thereby finalizing the hydrolysis reaction.

Here we report and optimize a model reaction that shows the potential of lipases as catalysts for an important step in polyurethane network formation—the lipase-catalyzed ester bond formation for the cross-linking of polyurethanes. Cross-linking in polyurethanes leads to the formation of a three-dimensional network of covalent bonds that improves the mechanical properties.

Enzymatic cross-linking of urethane containing compounds has not yet been reported so far, and therefore, simple transesterification reactions using a model urethane-bond containing ester were performed to fundamentally study the reaction and avoid future problems during the polymer cross-linking. The enzymatic transesterification of a model urethane-bond containing ester is studied using different alcohols and several immobilized lipases. After optimization, more complex substrates are tested that more realistically mimic the later cross-linking purpose.

Materials and Methods

All alcohols and solvents were purchased with a purity of 98% or higher. 1-Octanol (CAS number, 111-87-5), 1-propanol (CAS number, 71-23-8), 4-heptanol (CAS number, 589-55-9), 2-ethoxyethanol (CAS number, 110-80-5), 2-methoxyethanol (CAS number, 109-86-4), 2-(2-ethoxyethoxy)ethanol (CAS number, 111-90-0), 2-(methylamino)ethanol (CAS number, 109-83-1) were purchased from TCI Chemicals. Ethyl 2-(hexylcarbamoyloxy)propanoate was synthesized and kindly provided by Covestro Germany. Phosphorus pentoxide, desiccant with moisture indicator (CAS number, 1314-56-3), toluene, anhydrous (CAS number, 108-88-3), diphenyl ether, HPLC grade (CAS number, 101-84-8), *Candida antarctica* lipase B on acrylic resin (CalB, Novozym®435, 5000 + U/g; CAS number, 9001-62-1), and molecular sieves (4 Å; CAS number, 70955-01-0) were purchased from Sigma-Aldrich. Methyl alcohol, anhydrous (CAS number, 67–56-1), and chloroform, ChromAR® (CAS number, 64-66-3), were purchased from Macron Fine Chemicals. The LifeTech™ Lipase immo Kit for immobilized enzyme screening was purchased from Purolite® Life Sciences. Solvesso®100 was purchased from Brenntag Holland—see Table 1. Lewatit beads (Lewatit VP OC 1600) were obtained from Lanxess. Folded filters (grade 15, 65 g/m^2) were purchased from Munktell Ahlstrom.

Ethyl acetate, ChromAR® (CAS number, 141-78-6), and n-hexane, AR® (CAS number, 110-54-3), were purchased in HPLC grade from Macron Fine Chemicals. Silica gel 60/Kieselguhr F254 TLC plates were purchased from Merck, and SiliaFlash® P60 for column chromatography was purchased from SiliCycle.

Chloroform-d (CAS number: 865-49-6) was purchased from Sigma-Aldrich.

Enzyme name, organism name where the enzyme derives from, is mentioned, as well as the hydrolysis or synthesis activity and the material on which the enzyme is immobilized on including the used immobilization technique.

General procedure for CalB-catalyzed transesterification

CalB, Lewatit beads, and molecular sieves were pre-dried for 24 h in the presence of phosphorus pentoxide (P_2O_5) at room temperature under high vacuum. The monofunctional ester, the alcohol, pre-dried CalB or another immobilized lipase (Table 1), pre-dried Lewatit beads (for the negative control reaction), pre-dried molecular sieves, and the solvent and/or cosolvent were added in different amounts into a 10 ml round-bottom flask. The reaction was magnetically stirred at 150 rpm in an oil bath. After flushing out remaining air under reduced pressure (350 mmHg), the reaction was performed either at different temperatures for different times under atmospheric nitrogen environment or under reduced pressure of 200 mmHg.

For all transesterification reactions, corresponding negative control reactions were performed in which the immobilized CalB was replaced by Lewatit beads (the material used for

Table 1 Immobilized enzymes in the LifeTech™ Lipase immo Kit

Name	Organism	Activity (U/g)		Immobilization	
		Hydrolysis	Synthesis	Material	Technique
CalB immo Plus	*Candida antarctica*	–	9270	Styrene/methacrylate	Adsorption
CalA	*Candida antarctica*	2510	–	Epoxy/butyl methacrylate	Covalent
TL	*Thermomyces lanuginosa*	12,000	–	Epoxy/butyl methacrylate	Covalent
RM	*Rhizomucor miehei*	730	–	Epoxy/butyl methacrylate	Covalent
CR	*Candida rugosa*	677	–	Epoxy/butyl methacrylate	Covalent
PC	*Pseudomonas cepacia*	–	690	Styrene-DVB	Adsorption

CalB immobilization). None of these control reactions show transesterification product formation without CalB.

Subsequently 5 ml of chloroform were added to stop the reaction and for solubilization purposes. N435 or Lewatit beads and molecular sieves were filtered out and washed twice with 2 ml of chloroform. The chloroform was removed by evaporation at 40 °C under reduced pressure (356 mmHg).

Thin-layer chromatography was used to verify product formation. The products were purified by column chromatography and analyzed by ^{1}H and ^{13}C measurements.

Thin-layer chromatography (TLC)

Thin-layer chromatography using silica gel 60/Kieselguhr F_{254} TLC plates and an ethyl acetate/n-hexane solvent mixture (ratio 1:3) was used. Ten to twenty milligrams of the sample in 1:200 in the same solvent mixture and 1 μl were applied on the TLC plate. Compound detection using a potassium permanganate solution (10 g/l $KMNO_4$, 67 g/l K_2CO_3, 1.7% (v/v), NaOH solution (5% stock concentration), and subsequent heating to 150 °C was used.

Column chromatography

Column chromatography was performed using silica gel SiliaFlash® P60 and an ethyl acetate/n-hexane solvent mixture (ratio 1:3).

During chromatography 1-ml fractions were taken and analyzed by TLC. Fractions containing the corresponding products were pooled, and the remaining solvent subsequently removed under reduced pressure. The purified products were analyzed by ^{1}H- and ^{13}C-NMR measurement, and the product yield was determined by:

$$\frac{mol\ of\ purified\ product}{mol\ of\ applied\ ester\ for\ reaction} \times 100 = yield\%.$$

^{1}H- and ^{13}C-NMR measurement

^{1}H- and ^{13}C-NMR spectra were recorded on a Varian VXR spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR analysis), using $CDCl_3$-d_1 as the solvent. For NMR-spectra evaluation, the software MestReNova (version: 6.0.2-5475) was used. The chemical shifts reported were referenced to the resonance of $CDCl_3$-d_1.

NMR analysis of the used ester and the obtained products of transesterification

A large variety of different alcohols were tested to achieve complete monofunctional ester conversion during transesterification. For this purpose, qualitative TLC analysis was sufficient to evaluate complete monofunctional ester conversion; therefore, only NMR analysis of the main transesterification (with 2-(2-ethoxyethoxy)ethanol) product is mentioned here.

Monofunctional ester

(ethyl 2-(hexylcarbamoyloxy)propanoate)

^{1}H-NMR (400 MHz, $CDCl_3$-d_1, ppm): 7.260 $CDCl_3$-d_1 5.04 (q, 1H), 4.21 (q, 2H), 3.18 (t, 2H), 1.52–1.43 (m, 5H), 1.31–1.25 (m, 9H), 0.88 (t, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$-d_1, ppm): 77.36 $CDCl_3$-d_1 C11: 172.21 (s), C8: 155.71, C10: 68.95 (s), C13: 61.42 (s), C6: 41.29 (s), C3: 31.64 (s), C4 + C5: 26.57 (s), C2: 22.75 (s), C15: 17.47 (s), C1 + C17: 14.32 (s).

Transesterification product of monofunctional ester and 2-(2-ethoxyethoxy)ethanol

(2-(2-ethoxyethoxy) ethyl 2-(hexylcarbamoyloxy)propanoate)

^{1}H-NMR (400 MHz, $CDCl_3$-d_1, ppm): 7.260 $CDCl_3$-d_1

5.07 (q, 1H), 4.30 (t, 2H), 3.70 (t, 2H), 3.63 (t, 2H), 3.57 (t, 2H), 3.51 (q, 2H), 3.16 (t, 2H), 1.47 (dd, 5H), 1.29–1.26 (m, 6H), 1.20 (t, 3H), 0.87 (t, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$-d_1, ppm): 77.36 $CDCl_3$-d_1

C10: 171.71 (s), C7: 155.41 (s), C16 + C19: 70.49 (m), C18: 69.94 (s), C9: 68.95 (s), C17: 68.74 (s), C21: 64.21 (s), C6: 41.13 (s), C3: 31.50 (s), C4 + C5: 26.45 (s), C2: 22.56 (s), C13: 17.36 (s), C22: 15.20 (s), C1: 14.01 (s).

Results and discussion

Simple lipase-catalyzed transesterification using a rather non-complex model urethane-bond containing ester together with different alcohols was successfully conducted—see Fig. 1a. These model reactions will be an important first step to study the enzymatic cross-linking of polyurethane networks.

The model urethane-bond containing ester—ethyl 2-(hexylcarbamoyloxy)propanoate—in the following referred to as monofunctional ester could be easily transesterified by carbitol as suitable model alcohol. Carbitol was chosen as it allows easy product identification and purification. The initial experimental setup and reaction parameters are listed in Fig. 1b.

In this initial reaction, the occurrence of high amounts of non-converted educt (monofunctional ester) required optimization of the procedure. The highest amount of transesterification product that could be achieved so far, based on the experimental setup in Fig. 1, is 25% accompanied by 36% of monofunctional ester.

The catalytic activity of lipases usually follows a general ping-pong model. Transesterification reactions can however not be explained by the general mechanism as (1) lipases synthesize esters by direct alcoholysis of triacylglycerols in a single step and (2) involve hydrolysis of triacylglycerols and subsequent esterification of the resulting fatty acids. In all cases, these are equilibrium reactions, which explain the observed lower yield.

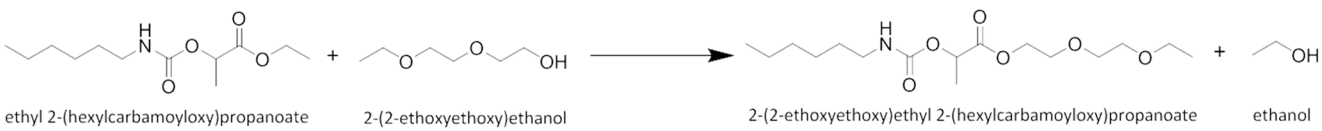
To optimize the reaction and to circumvent this high amount of non-converted monofunctional ester, increased reaction temperatures and increased reaction times were tested. These are known optimization steps for lipase-catalyzed transesterifications.

All experimental results that will be discussed in the following section were gained using the established transesterification model reaction and setup shown in Fig. 1. The shown non-converted monofunctional ester amounts (educt amounts) and product yields were calculated based on the purified products and educts after transesterification. Due to a general material loss (40–50%) during purification via column chromatography, the sum of the purified products yields and educt amounts will be usually around 50 to 60%.

Figure 2 shows an overview of the amount of non-converted monofunctional ester and the product yield of the performed experiments for the transesterification of the monofunctional ester with carbitol using different reaction temperatures (Fig. 2a) and times (Fig. 2b). While changing the reaction temperature the reaction time was kept at 24 h and for the different reaction times, the temperature was kept at 65 °C. From these experiments, it can be clearly observed that no transesterification product is formed when the reaction is performed under 65 °C for 24 h or with a reaction time shorter than 24 h at 65 °C.

Above these reaction parameters—already identified as promising (Fig. 1b)—the amount of non-converted monofunctional ester indeed decreases (33% at 80 °C, 24%

a



b

parameters	
Monomer amount	60 % of total reaction volume
Monomer ratio	1:1
Enzyme amount/Lewatit beads amount	10 wt%
Solvent	toluene
Solvent amount	150 wt %
Temperature	65 °C
Time	24 h
Surrounding (N_2,vacuum)	N_2

Fig. 1 **a** Transesterification model reaction of the monofunctional ester with carbitol and **b** experimental setup of transesterification reaction before optimization

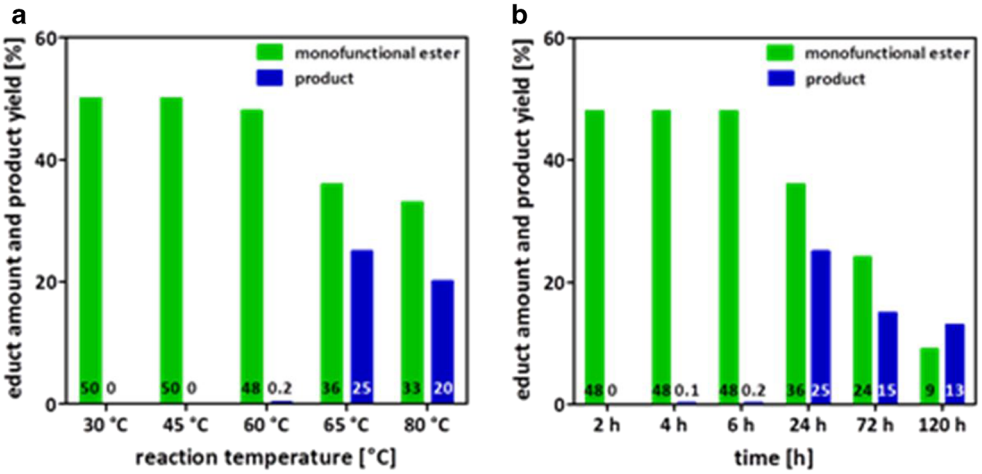


Fig. 2 Changes in temperature and time: overview of educt amount and product yield of all performed transesterification reactions

after 72 h, 9% after 120 h), but also the yield of the transesterification product is reduced (20% at 80 °C, 15% after 72 h, 13% after 120 h). From these results, it can be concluded that the transesterification reaction (regarding the extrinsic parameters: temperature and time) is working optimally at 65 °C for 24 h. This means that the limitation in product formation and complete conversion is based on intrinsic parameters such as the chosen model alcohol (carbitol), the reaction solvent (toluene), and the enzymatic catalyst (CalB). As already mentioned, this can be explained by the fact that the lipase-catalyzed transesterification is an equilibrium reaction.

In the following optimization step, we substituted the primary heteroatom alcohol carbitol with more simple primary alcohols as 1-octanol and 1-propanol keeping the previously selected parameters (Fig. 1b) to achieve higher monofunctional ester conversion. However, these experiments did also not result in a complete monofunctional ester conversion (data not shown).

Therefore, further experiments were performed to analyze the influence of the reaction solvent, the application of a cosolvent, the carbitol itself, the reaction byproducts, and the enzymatic catalyst on the reaction efficiency. Enzymes, in general, are dynamic structures that are surrounded and protected by a protective shell of water molecules. Lipases within their protective shell are always in movement and are changing their conformation steadily between a more closed, native state and a more open, native state. This dynamic behavior is in general dependent on the surrounding temperature and in the specific case of using enzymes as biocatalysts for chemical reactions also influenced by the solvent system used. Enzymes show high activity in non-polar, hydrophobic solvents (log $P > 2$) [40–43] and only in a few polar, hydrophilic solvents (log $P < 2$) [44–46]. The reason for this is the polar solvent interaction with the water molecules of the protective shell: polar, hydrophilic solvents are stripping off the surrounding water molecules [47] and thereby destroying the protective hydrate shell of the enzyme. This leads to the enzyme denaturation and deactivation because it is losing its native, active structure. Of course, this is not an "all or nothing" process but varies with the polarity and concentration of the applied hydrophilic solvent. This polarity effect on the enzyme's structural behavior could be the reason for the limitation in transesterification product formation and monofunctional ester conversion.

So far the relatively high non-polar solvent toluene (log P = 2.5) was used as a reaction solvent and should allow high activity of immobilized CalB due to no interference with the surrounding water molecules that keep CalB in a more closed, native state. But this more closed state may limit the structural ability of CalB to bind the rather complex monofunctional ester.

Therefore, the addition of a polar, hydrophilic solvent in a suitable concentration was studied to promote the disruption of the protective hydrate shell in a balanced way maintaining the enzyme's activity but allowing the more complex monofunctional ester to get in sterical proximity to the active center due to the more open structure of the enzyme. Therefore, a cosolvent system was applied for transesterification of the monofunctional ester with carbitol including the already used non-polar, hydrophobic toluene and the polar, hydrophilic solvent methanol. In a suitable concentration (5–15%), the methanol should disrupt the hydrate shell in a balanced way and mediate a more open structure of CalB and in this way a more efficient binding of the monofunctional ester.

Despite the addition of methanol as a cosolvent, the so far best experimental setup was maintained. Figure 3 shows the result of the cosolvent system with different amounts of methanol applied (5–15%) in comparison with a pure toluene system (0%). The addition of the polar, hydrophilic methanol does not result in the desired reaction shift towards large amounts of transesterification product and lesser amounts of non-converted monofunctional ester. Due to the fact that with the addition of any methanol concentration the product amount slightly decreases from 25% in the toluene system to 11–15%

could indicate an almost negative effect on CalB activity. As previously mentioned, the solvent polarity influence and the solvent interaction with the enzyme hydrate shell is not an absolute but always varying effect; depending on the selected solvent polarity, it is possible that methanol is not the solvent of choice for this purpose. The very low log P of − 0.320 is rendering methanol a too high non-polar solvent, that is—unlike expected—destabilizing CalB. To further investigate the effect of the cosolvent system on transesterification of the monofunctional ester with carbitol and in order to find an appropriate cosolvent, it is possible to test different polar, hydrophilic solvents that are less polar than methanol, e.g., 1-propanol (log $P = 0.559$) or isopropanol (log $P = 0.420$). However, these mentioned polar solvents react with the monofunctional ester as an alcohol to form a transesterification product. Such a side product of the monofunctional ester with the cosolvent was observed within the methanol cosolvent system. Therefore, it was decided to not further investigate a non-polar/polar solvent system but to change the solvent system completely.

The non-polar, hydrophobic solvent diphenyl ether (log $P = 4.05$) is commonly used in CalB-catalyzed polymerization reactions [19, 21]. Due to this proven efficient compatibility of immobilized CalB with diphenyl ether, the previously used toluene system was substituted with diphenyl ether. Based on similar polarities of toluene (log $P = 2.5$) and diphenyl ether (log $P = 4.05$), no structural change of CalB leading to a more open conformation was expected (Fig. 4a). Although it was proven that the monofunctional ester is not soluble in diphenyl ether at room temperature, it completely dissolves when the mixture is heated to the reaction temperature of 65 °C. Figure 4 b shows the non-converted monofunctional ester amount and the product yield of the transesterification in the diphenyl ether system compared with the toluene system. Also, the change of the reaction solvent did not result in higher

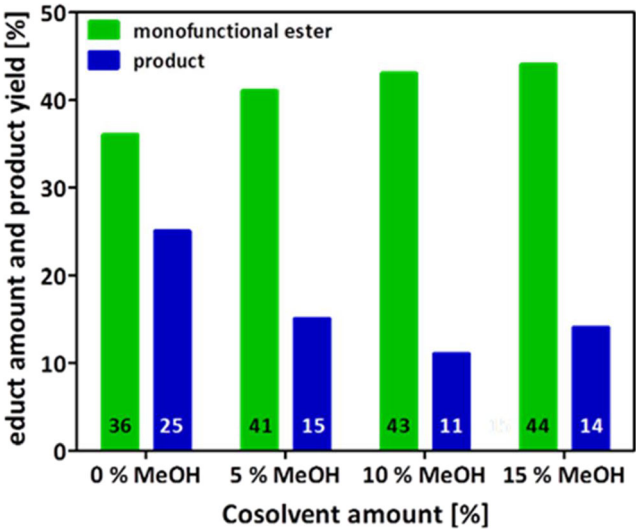


Fig. 3 Cosolvent system: educt amounts and product yields

transesterification product formation and monofunctional ester conversion.

Quite contrary it seems that transesterification is less efficient in the diphenyl ether system as indicated by the slight decrease of product yield to 15% and a higher amount of non-converted monofunctional ester (61%). Mindful of the previously stated hypothesis of the solvent polarity effect on CalB structure, it is possible that the slightly higher non-polarity of diphenyl ether causes, other than expected, an even more closed CalB conformation impeding substrate binding and conversion. But it is also possible that the assumed better solubility and miscibility of the mixture of compounds is not given in this system and therefore decreases conversion efficiency.

Since neither the addition of a cosolvent nor the change of the solvent system under the chosen conditions resulted in an improved transesterification efficiency towards higher product amount and nearly complete conversion of the monofunctional ester, the next experimental steps were focused on carbitol as an optimization target. Carbitol as an alcohol for conventional transesterification reactions is usually applied in higher amounts compared with the applied ester leading to product yields of up to 90% [48]. Therefore, the selected promising experimental setup of the transesterification model reaction was used as shown in Fig. 1b and adapted regarding different monomer ratios.

Four different monomer ratios, additionally to the established 1:1 ratio, 1:2, 1:4, 1:6, and 1:8 (ester/alcohol) were applied. No significant beneficial difference in educt amount and product yield was observed when applying a two-, for-, or sixfold increased amount of carbitol for transesterification with the monofunctional ester (Fig. 5). Gratifyingly, an eightfold increase in carbitol amount resulted in a product yield of nearly 50%, and the remaining monofunctional ester amount reduced to 11%. Also, a second transesterification with a monomer ratio of 1:8 confirmed this high conversion rate (data not shown). This means that the carbitol itself, in these high amounts, has an impact on transesterification efficiency. Two hypotheses could explain this beneficial influence of the carbitol. (1) The carbitol in these high amounts is acting as a polar, hydrophilic solvent leading to a more open conformation of CalB and thereby improving substrate binding, or (2) that carbitol itself is acting as a basic catalyst due to the basic toluene environment this way promoting basic transesterification. If the high transesterification rate of nearly 50% (Fig. 5) is dependent on the alcohol that in high concentration acts as a polar, hydrophilic solvent (log $P = 0.030$), stripping off the water from CalB and thereby opening up its structure for better substrate accessibility, then this should be the case for a different alcohol with the same polarity applied in high concentrations as well. This hypothesis was tested by performing two additional transesterification reactions based on the established experimental set up applying two different

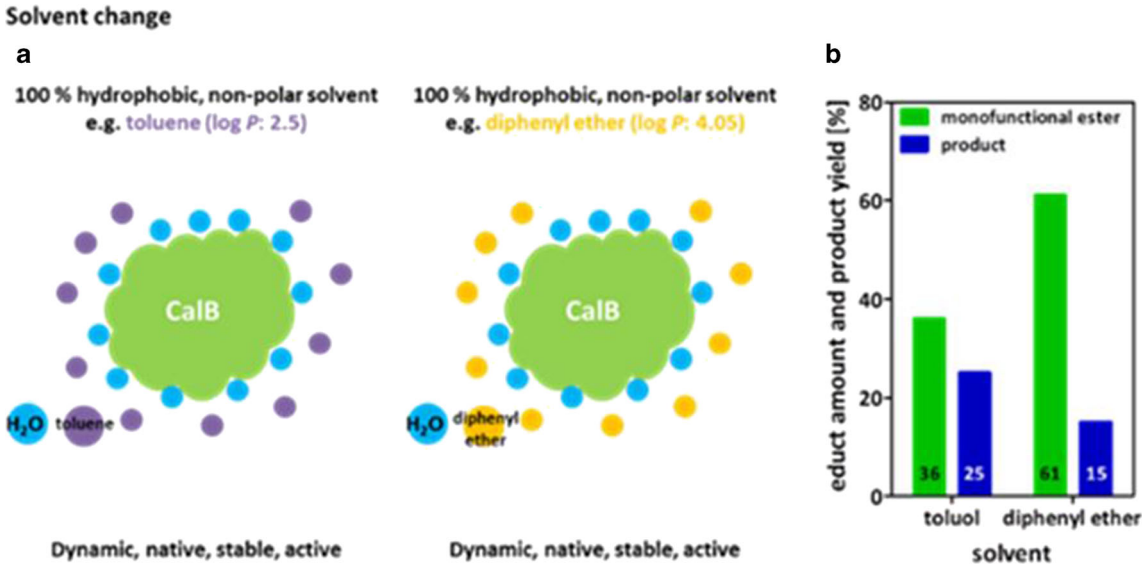


Fig. 4 Solvent change: effects of the reaction solvent change on transesterification efficiency. **a** Effects of hydrophobic solvent systems on CalB structure and **b** educt amounts and product yields in the cosolvent system

alcohols in an eightfold excess. 2-Ethoxyethanol (log P = 0.020) and 2-methoxyethanol (log $P = -0.370$) both are, as well as carbitol, primary heteroatom alcohols. While for the 2-ethoxyethanol (log $P = 0.020$), similar results are expected as observed for carbitol due to the nearly same log P value, no or less product formation is expected when using 2-methoxyethanol (log $P = -0.370$). The low log P value of 2-methoxyethanol makes it a strong polar, hydrophilic solvent that as previously described will strip off the water molecules from CalB and in this way destroy its native structure making it inactive for catalyzing transesterification.

In contrast to the previous experimental results, the products from this experiment were not purified, but their amount

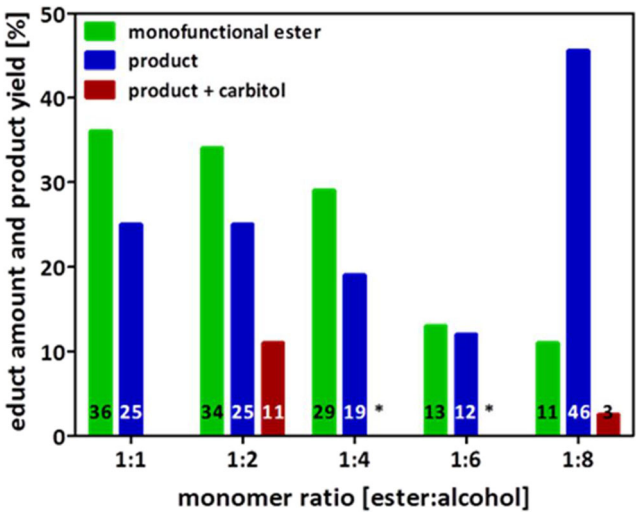


Fig. 5 Alcohol effect: educt amounts and product yields of transesterification with increased carbitol amount. The asterisk indicates a non-recovery of the product/carbitol mixture during purification

was visually quantified by thin-layer chromatography. This technique is sufficient to judge differences when comparing the amount of non-converted monofunctional ester and product yields of the transesterification with the different alcohols. The transesterification with an eightfold input of 2-ethoxyethanol (log $P = 0.020$) did not result in similar amounts of remaining educt and product as previously described for carbitol (Fig. 5), but showed similar less product amount as the applied polar, hydrophilic 2-methoxyethanol (log $P = -0.370$; Table 2). This result led to the conclusion that the high transesterification efficiency based on the eightfold input of carbitol is not due to a carbitol polarity effect.

What always has to be considered during such optimization trials is the general interaction and dependency of all components that are applied and are necessary for efficient transesterification. This means specifically in the case for the advantage of high carbitol amounts that these high product yields and decreased educt amounts are based on two beneficial properties of carbitol that support CalB as the catalyst. Firstly, its eight carbon/heteroatom chain length, because a chain length of about eight atoms is known for highest conversion activity of CalB, and secondly, its feature of being a moderate polar, hydrophilic alcohol. When subtracting one of these two properties, the transesterification output will not be the same, as was proven with 2-ethoxyethanol. This alcohol indeed showed nearly the same log P value but is shorter than carbitol. Another option would be to go back to another alcohol already tested for transesterification, 1-octanol, which was chosen because of its eight carbon atom chain length but was later refused due to a similar polarity to the transesterification product this way impeding sufficient product purification. The disadvantage of using 1-octanol again is its property of being a

Table 2 Expectations and results of different alcohols effects on transesterification efficiency

Alcohol	log *P*	Expectation	Result
2-Ethoxyethanol	0.020	High product yield	Similar product amount*
2-Methoxyethanol	− 0.370	Less product or no product	

*Refers to the visual quantification of remaining educt amount and transesterification product by thin-layer chromatography

non-polar, hydrophobic alcohol; this would then again be in disagreement with the previously mentioned necessary alcohol properties.

Nevertheless, we also tested the second hypothesis, namely, that carbitol itself is acting as a basic catalyst due to the basic toluene environment this way promoting basic transesterification. If so the addition of a simple basic catalyst, e.g., sodium carbonate, should be sufficient to shift the transesterification reaction towards high product yields. This way it would also be possible to avoid the addition of such high alcohol concentrations. As described for conventional chemical transesterifications [48], 20 wt% of sodium carbonate were applied for transesterification additionally to the established experimental setup. However, no transesterification product could be detected at all (data not shown). The amount of added sodium carbonate was calculated based on a reaction setup for a conventional chemical reaction [48], so this 20 wt% (based on the total monomer amount) may be too high for this CalB-catalyzed reaction. Maybe here the already mentioned interaction between the several compounds comes again into account, which means that the sodium carbonate as a base catalyst is counteracting the enzymatic catalysis.

Nonetheless, it was possible to optimize the transesterification of the monofunctional ester with carbitol concerning a high product yield of about 50% and a relatively low amount of non-converted monofunctional ester (11%).

Although increasing the alcohol amount applied for transesterification to eightfold resulted in the best and promising product yields and non-converted monofunctional ester amounts so far, further optimization approaches were performed. As already mentioned, the high input of carbitol for transesterification is not a desired feature and should, therefore, be avoided. During transesterification of the monofunctional ester with carbitol, ethanol is produced as a byproduct (Fig. 1a). This byproduct could disturb the equilibrium of the ongoing transesterification that should be shifted towards product formation.

To analyze the effectiveness of byproduct removal on transesterification propagation and this way producing higher product amounts, two experiments were performed in parallel. The usual transesterification so far was performed under nitrogen atmosphere, due to the high vapor pressure of the used toluene (≈ 58 mmHg at 40 °C). Now the same experimental setup was chosen, but additionally, the pressure was reduced to 350 mmHg every 2 h for 5 min to remove the released ethanol (≈ 400 mmHg at 65 °C). In addition, an experiment was set up using again diphenyl ether as the reaction solvent. The low vapor pressure of diphenyl ether (0.06 mmHg at 40 °C) allows the reaction to be performed completely under vacuum (2 mmHg), and the released ethanol can in this way be directly evaporated. Results of both experiments under vacuum atmosphere were compared with the ones performed within a nitrogen environment (Fig. 6). By comparison of both solvent systems regarding the educt amounts and product yields under nitrogen and vacuum atmosphere, it is striking that the product yields are quite the same. In the toluene system, the product yields are about 25% for transesterification under a nitrogen atmosphere and for those with regularly reduced pressure of 350 mmHg (Fig. 6 left hand side). A similar result could be observed for the diphenyl ether system; the product yield for both transesterifications performed under different atmospheres is nearly the same (about 1%) (Fig. 6 right hand side). This means that the removal of the ethanol byproduct from the reaction has no beneficial effect on product yields.

In contrast to this, the amount of non-converted monofunctional ester is in both solvent systems indeed higher when the pressure is reduced. About 50% in the toluene system (Fig. 6 left hand side) and 20% in the diphenyl ether system (Fig. 6 right hand side). This is a discrepancy when taking all results together: similar amounts of products but 20/50% less or more non-converted monofunctional ester. This

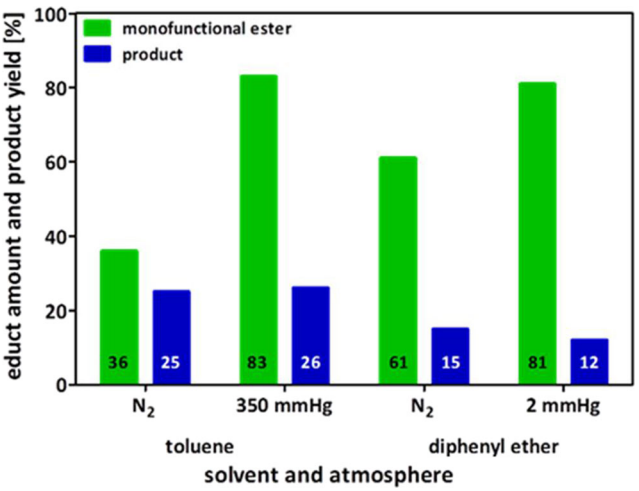


Fig. 6 Atmosphere effect: educt amounts and product yields after transesterification under nitrogen and vacuum atmosphere

possibly indicates some kind of instability of the monofunctional ester during transesterification under nitrogen atmosphere. To exclude such instability of the monofunctional ester or a possible degradation by the enzyme, negative control reactions were performed. These reactions were done with the same setup but without the enzyme, to test the monofunctional ester stability, and also with the enzyme but without the alcohol, to test the degradability of the monofunctional ester by the enzyme. These controls showed no degradation or instability of the monofunctional ester. We conclude that this phenomenon could be explained with the already mentioned interaction or cross-reaction of the different compounds in the reaction mixture. In these control reactions, not all components for the "real" reactions were added and therefore do not allow the assumption that no degradation or instability is given when all compounds are present.

Previously mentioned optimization approaches targeted each parameter applied for the efficient transesterification of the monofunctional ester with carbitol. The reaction time and temperature were extended and increased, a reaction cosolvent system and a completely different solvent system were tested, the used alcohol carbitol was analyzed regarding its effects on transesterification efficiency, and shifting the transesterification reaction towards product formation by changing the reaction atmosphere was tried. Despite the use of an eightfold increase of carbitol for transesterification that resulted in the highest product yield and the lowest amount of non-converted monofunctional ester so far, all other attempts failed to achieve these results.

Based on the fact that a high alcohol input for transesterification is not a suitable option, a further optimization approach was performed by changing the enzymatic catalyst. As already mentioned, the immobilized lipase CalB so far used is the most commonly and successfully used lipase in chemical reaction systems, but of course, this is not the only lipase that is able to catalyze transesterification reactions.

Table 1 in the materials section shows a list of the tested immobilized lipases and their properties together with the standard used immobilized CalB (CalB). These commercially available and used lipases from different organisms show different hydrolysis or synthesis activities and are immobilized on different materials via different immobilization techniques. These facts are necessary to consider when later interpreting the results. The different immobilized lipases were tested according to the standard experimental setup (Fig. 1b). The results of the transesterifications catalyzed by the different immobilized lipases were compared with the product yields and the amount of non-converted monofunctional ester after transesterification with standard used immobilized CalB (Fig. 7). Despite the immobilized lipase from *Psedomonas cepacia* (PC) (Table 1), none of the tested lipases was able to catalyze transesterification of the monofunctional ester with carbitol.

Compared with the used standard immobilized CalB (CalB), transesterification catalyzed by the PC lipases only resulted in 6% of transesterification product. These low amounts of product formation could be explained with the sevenfold lower synthesis activity of the PC lipase (690 U/g) compared with CalB (5000 U/g). Of course, to achieve similar activity of the PC lipase for transesterification, the wt% input could be adapted to 70 wt%, but this way, the whole reaction equilibrium would be different. For a successful transesterification reaction, the total amount of monomers and the amount of enzyme has to be set up in a balanced way to facilitate the formation of a mixture in which the monomers are able to get in proximity to the lipase. Therefore, the enzyme input is always calculated based on the monomer input. It is quite striking that the CalB immo Plus lipase that is in origin the same lipase as the used standard one (CalB) was not able to catalyze the transesterification, especially based on the documented higher synthesis activity (9270 U/g) (Table 1). Based on the fact that both CalB lipases are also immobilized on the same material via the same immobilization technique allows only one explanation for the inactivity of CalB immo Plus towards the monofunctional ester and the carbitol. CalB immo Plus is most likely a modified variant of the standard CalB. This means that amino acids within CalB have been exchanged to achieve a higher activity towards a specific substrate. This causes indeed a higher activity of CalB towards this substrate but can lead to complete inactivity for other substrates, such as the monofunctional ester and the carbitol substrates used here.

For the other tested lipases from *Candida antarctica* [49–53], *Thermomyces lanuginosa* [54–57], *Rhizomucor*

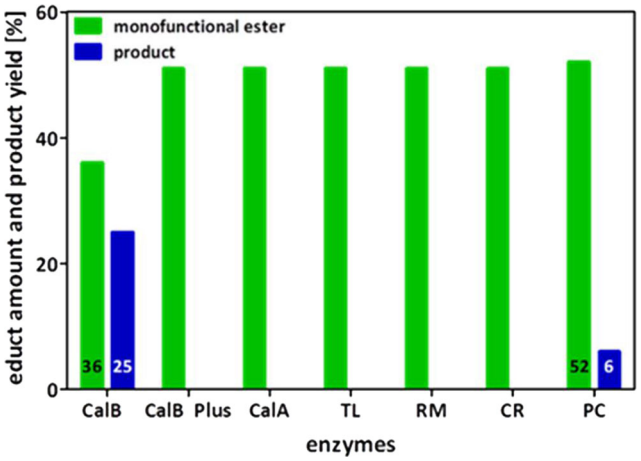


Fig. 7 Biocatalyst effect: educt amounts and product yields after transesterification catalyzed by different immobilized lipases. The monofunctional ester reacts with carbitol in a 1:1 ratio, with 10 wt% of the different lipases and 150 wt% of toluene at 65 °C, for 24 h under nitrogen atmosphere with pressure reduction to 350 mmHg every 2 h for 5 min or under nearly complete vacuum leading the transesterification product 2-(2-ethoxyethoxy)ethyl 2-(hexylcarbamoyloxy)propanoate in blue, remaining non-converted monofunctional ester in green

miehei [58, 59], and *Candida rugosa* [60–65], only hydrolysis activity is reported and they are immobilized on a different material in a completely different way. Therefore, it is not possible to state whether these lipases are in general unable to catalyze the transesterification of the monofunctional ester with carbitol or their activity is hampered based on the used immobilization technique. The lipid-water interface is very important for the catalytic activity, and lipases usually reveal a so-called interfacial activation; the presence of a hydrophobic phase—a lipid droplet dispersed in water or an organic solvent—increases the catalytic activity. This effect is also very important when immobilizing enzymes—CALB in N435 is for instance immobilized with interfacial activation rendering N435 such an efficient catalyst formulation. To really compare these enzymes, it would be necessary to immobilize them on the same material as N435 via the same immobilization technique. Also, other CalB variants are available and also additional enzymes such as cutinases that are known to perform transesterifications, but here it was initially decided to start with an analysis of commercially available and commonly used immobilized lipases for their potential to catalyze this specific transesterification. In conclusion, it has to be said that no other tested immobilized lipase so far is able to catalyze the transesterification of the monofunctional ester with carbitol.

Conclusions

Immobilized CalB is able to accept an urethane-bond containing monofunctional ester and is suitable to catalyze the ester bond formation with the model alcohol carbitol. The major drawback of this transesterification is the low conversion accompanied with rather high amounts of non-converted monofunctional ester. This is unavoidable, as the lipase-catalyzed transesterification is an equilibrium reaction. To increase the conversion rate and the transesterification product yield, every parameter in the experimental setup was optimized—transesterification time and temperature, the reaction solvent, the possibility of a cosolvent and the alcohol amount, the used transesterification environment, and the biocatalyst. Just the application of an eightfold excess of carbitol compared with the monofunctional ester showed an distinct increase in conversion. Here it was possible to achieve a reproducible high yield of transesterification product (~ 50%) and relatively low amounts of non-converted monofunctional ester (~ 10%). However, such a high alcohol input for the transesterification is not favored regarding the later larger scale syntheses in concerns of cost and time efforts for the subsequent reprocessing.

The observed conversion is however good enough to achieve high enough enzymatic cross-linking of industrially viable polyurethanes via transesterification, and we are currently studying this in our follow-up studies.

Code availability Not applicable

Authors' contributions Conceptualization: P.S., D.M., and K.L.Methodology: P.S. and D.M.Validation: P.S. and D.MFormal analysis: P.S., D.M., and M.K.E.C.Investigation: P.S., D.M., and M.K.E.C.Data curation: P.S. and D.M.Writing (original draft preparation): P.S.Writing (review and editing): P.S., D.M., M.K.E.C., and K.LVisualization: P.S. and D.M.Supervision: K.L.

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Data availability All data generated or analyzed during this study are included in this published article.

Compliance with ethical standards

Conflict of 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.

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