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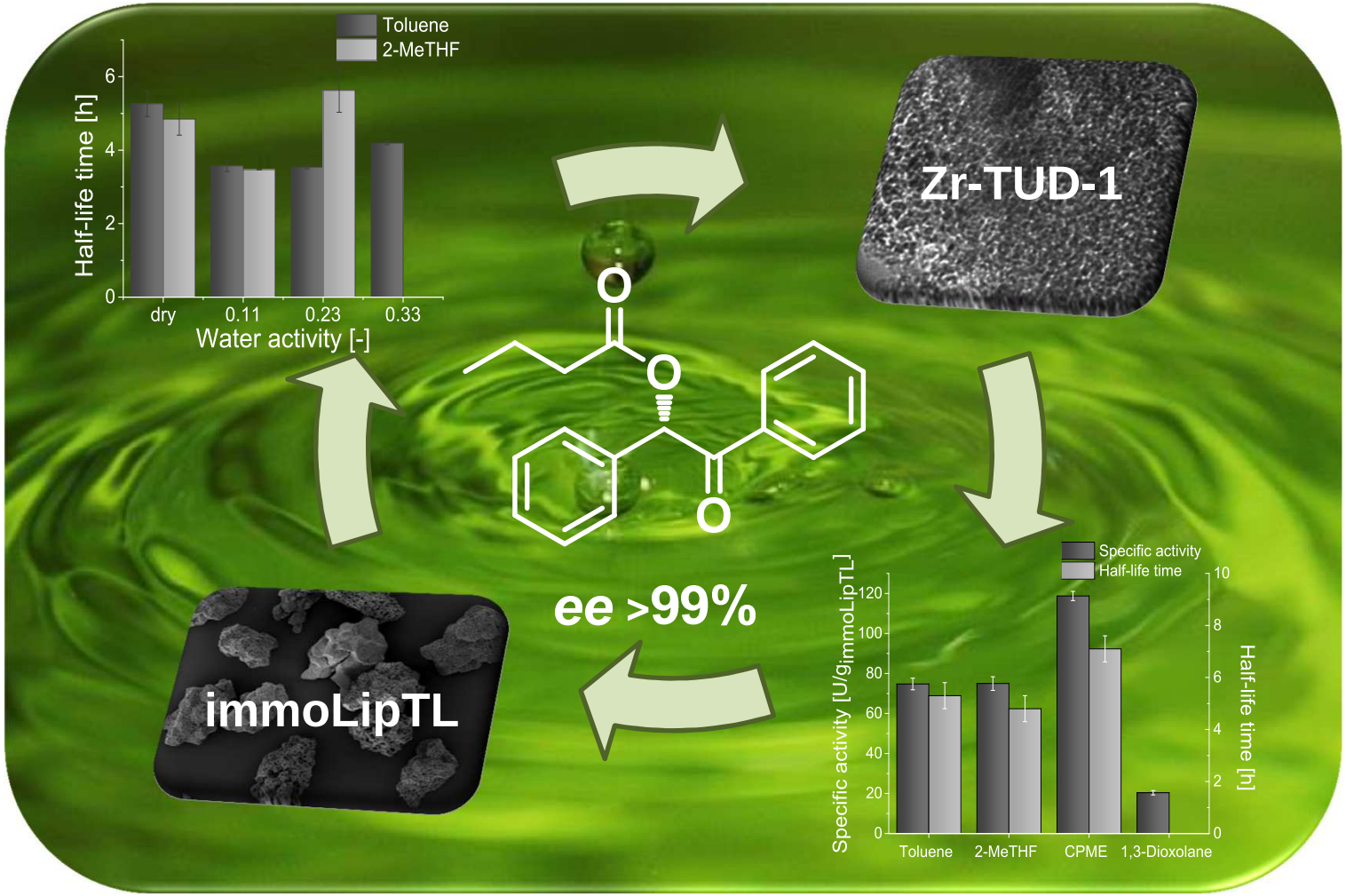
*Graphical Abstract (for review)



HIGHLIGHTS

- Water activity plays an important role for the dynamic kinetic resolution of *rac*-benzoin in chemo-enzymatic cascade. Catalytic performances of both the enzyme (Lipase TL) and the chemo-catalyst (Zr-TUD-1) were higher in dry solvents.

- Specific activity and half-life time of Lipase TL for kinetic resolution of *rac*-benzoin was higher in cyclopentyl methyl ether (CPME) in comparison to toluene, 2-methyltetrahydrofuran (2-MeTHF) and 1,3-dioxolane.

- Racemization activity of Zr-TUD-1 was the best in CPME and in toluene compared to 2-MeTHF and 1,3-dioxolane.

- Specific activity of Lipase TL in deep eutectic solvents was significantly lower than in toluene, 2-MeTHF, CPME, and 1,3-dioxolane, but product solubility in these alternative media hold promises for efficient downstream processing.

- Continuous dynamic kinetic resolution for synthesis of enantiopure (*S*)-benzoin ester was successfully established in stirred tank under optimized conditions (dry CPME).

Medium and reaction engineering for the establishment of a chemo-enzymatic dynamic kinetic resolution of *rac*-benzoin in batch and continuous mode

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1. Introduction

Enzyme catalysis represents an increasingly important research field, where many practical applications for (enantioselective) organic synthesis can be derived, and have been already implemented at industrial scale [1]. The use of non-aqueous media for enzymatic reactions offers several advantages such as high solubility of water-insoluble substrates, less waste water formation and more straightforward downstream processing. In the field of biocatalysis in non-aqueous media lipases have proven to be extremely useful biocatalysts, based on their outstanding resilience to these media, together with the fact that no cofactors are needed for their catalytic performance [2].

To further provide stronger arguments for (industrial) biocatalysis, the use of more environmentally-benign solvents as well as the set-up of continuous processes is presently an area of high interest. Thus, solvents like biomass-derived 2-methyltetrahydrofuran (2-MeTHF) have been reported as a promising alternative to conventional solvents and hence attracted great attention in organic synthesis [3]. Likewise, cyclopentyl methyl ether (CPME) [4] and 1,3-dioxolane [5] have been assessed as environmentally benign alternatives to conventional organic solvents. CPME has been commercially available since 2005 [4b] and has preferable characteristics, *e.g.* a high boiling point, relative stability under acidic and basic conditions, low peroxide formation, high hydrophobicity (ease of drying), and a narrow explosion range [4b]. Similarly, 1,3-dioxolane is considered as a solvent as well as a reagent [5a] owing to its preferable physical, chemical and toxicological properties [5b,c]. Furthermore, deep eutectic solvents (DESs) have been evaluated, which are also environmentally friendly and cost-effective alternatives to many conventional solvents [6]. DESs – eutectic mixtures of an ammonium salt (*e.g.* choline chloride, ethylammonium chloride) and a hydrogen bond donor (HBD) (*e.g.* alcohols, amines, amides and carboxylic acids) – have melting points below room temperature, low volatility, high thermal stability, and tailored characteristics similar to ionic liquids, but largely offer diminished toxicity, better biodegradability, large availability at acceptable costs, and simplified preparation [6]. Applicability of DESs in biocatalyzed reactions has been demonstrated for hydrolases [7a–h], and recently for whole-cell redox biocatalysis [7i], for lyases [7j].

Recently, also tandem catalysis of a hydrolase and organocatalysts was demonstrated [7k]. Thus, DESs are currently also receiving great attention as eco-friendly solvents [8]. Following these important emerging fields – biocatalytic continuous processes and the employment of environmentally-benign non-conventional solvents – the present work focuses on the set-up of a chemo-enzymatic dynamic kinetic resolution process (DKR) for racemic benzoin (aromatic α-hydroxyketone) in a continuous fashion, while assessing different environmentally-friendly non-aqueous solvents at the same time. Chiral α-hydroxyketones are important building blocks with a broad range of applications in the synthesis of biologically active compounds such as pharmaceuticals, agrochemicals, and pheromones [9]. On this basis, various chemical and biocatalytic approaches for their synthesis have been developed [10]. Among them, enzymatic routes show great potential delivering lower amounts of waste and higher stereoselectivities and overall yields compared to the chemical methods [11]. Biocatalytic syntheses of chiral α-hydroxyketones are possible either by (i) reduction of α-diketones using oxidoreductases (EC 1) [12], (ii) acyloin condensation of aldehydes [13], (iii) kinetic resolution of racemic mixtures *via* C–C bond cleavage both catalyzed by thiamine diphosphate (ThDP) dependent lyases (EC 4) [14], or by (iv) dynamic kinetic resolution (DKR) catalyzed by hydrolases (EC 3) combined with a chemo-catalyst [15]. With regard to lipases, as stated above, their high stability in and good compatibility with organic solvents provide special synthetic advantages.

In this study, DKR of racemic benzoin with a lipase from *Pseudomonas stutzeri* (under the trade name Lipase TL) and the chemo-catalyst Zr-TUD-1 (Si/Zr = 25) [16] was investigated (Scheme 1) for solvent needs and continuous operation. Several TUD-1 catalysts with aluminum (Al), zirconium (Zr) and tungsten (W) as incorporated metals had been evaluated for this reaction in a previous study [17]. Among these, Zr-TUD-1 (Si/Zr = 25) excelled by its high racemization rate and hence was chosen for further study. The combination of both catalysts in a heterogeneous formulation leads to a yield and enantioselectivity of >99%, and offers high recyclability in batch reaction systems [17].

[Please insert Scheme 1]

For achieving high productivities process parameters such as water activity (a_w) [18] and solvent [19] are crucial. Hence, the effect of these parameters on the activity of the catalysts and the stability of the enzyme (*i.e.* half-life time) were in-depth evaluated. The optimized reaction conditions identified for the DKR of *rac*-benzoin were combined and then demonstrated in a continuous stirred tank reactor (CSTR, see SI) under temperature and water activity controlled conditions.

2. Experimental

2.1 Materials

All chemicals were purchased from Sigma-Aldrich (Schnelldorf, Germany), Carl Roth GmbH (Karlsruhe, Germany) or VWR (Dresden, Germany) and were used as received with a purity ≥98%. Lipase from *P. stutzeri* (trade name Lipase TL) was kindly provided by Meito Sangyo Co., Ltd. (Tokyo, Japan). Accurel MP1001 carrier was obtained from Membrana GmbH (Obernburg, Germany). The humidity sensor HMT337 was purchased from Vaisala GmbH (Bonn, Germany).

2.2 Immobilization of Lipase TL

Lipase TL solution at 30 g/L concentration was prepared in a potassium phosphate buffer (200 mM, pH 6.2) and stirred (at 1000 rpm) for at least 1 h at room temperature. Insoluble components were removed by centrifugation (2600 g) for 10 minutes. Accurel MP1001 carrier was mixed with absolute ethanol (7.6 $mL_{ethanol}/g_{carrier}$) and incubated for 30 minutes. The lipase solution was added to the pre-treated carrier (20 mL lipase solution/$g_{carrier}$) and the suspension was shaken (at 340 rpm) for 4 h at 40 °C. After incubation, the particles were washed two times with buffer (20 mL buffer/$g_{carrier}$) and filtered afterwards. The immobilized enzyme preparation was dried over silica gel under reduced pressure for at least five days before further application. For the fragmented immobilized lipase the formulation was then crushed in a mortar.

2.3 Analysis of protein loading

Protein loading was determined by measurement of the protein concentration before and after immobilization in the supernatant fraction using Bradford reagent (Roti®-Nanoquant, Carl Roth GmbH & Co. KG.) according to the manufacturer's instructions and bovine serum albumin (BSA) as a standard.

2.4 Synthesis of deep eutectic solvents (DESs)

DES forming components (*i.e.* ammonium salts and hydrogen bond donors) were weighed in a glass vessel at a defined molar ratio (*e.g.* 1:2 or 1:1.5). The mixture was stirred at 100 °C until a clear liquid was obtained (at least 1 h up to 24 hours). Water was removed using a rotary evaporator and the DESs were stored for 72 hours over silica gel under reduced pressure.

2.5 Adjustment of water activity

Saturated salt solutions were prepared by mixing a large excess of each salt and distilled water and stored at 25°C in a desiccator for several days before using. Nine reagent grade salts with defined water activities were used: $LiCl_2$, KAc, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, NaBr, NaCl, KCl, K_2SO_4 (Table 1).

For initial experiments, all reaction components (*i.e.* substrates, solvent, and chemo-catalyst) were stored separately in a desiccator over saturated salt solutions for three days at 50 °C. Only in case of the enzyme, the a_w values were adjusted at 25 °C as a decrease in the enzyme specific activity (51% (in toluene) and 54% (in 2-MeTHF) within 72 h) was observed when incubation was performed at 50 °C. Later, all reaction components were incubated with saturated salt solutions at 25 °C as the a_w values at 25 °C and at 50 °C were not significantly different (Table 1). The time for reaching equilibrium a_w (72 hours) was previously determined using a Humidity Sensor HMT337.

2.6 Analysis of specific activity for kinetic resolution of *rac*-benzoin

Benzoin was prepared in the organic solvent typically at a concentration of 20 g/L (94 mM). The immobilized lipase was pre-incubated with the benzoin solution for 10 minutes at 50 °C at 20 g/L. The reaction was started by the addition of vinyl butyrate

(6 molar equiv.) and the reaction mixture was shaken at 1100 rpm and 50 °C. Aliquots were taken (30 µL) at definite time intervals and mixed with 1000 µL isopropyl alcohol followed by HPLC analysis (see 2.11). Experiments were performed at least in duplicates, all shown results are averages.

2.7 Set-up for continuous operation and analysis of process stability

Continuous experiments were performed in a 6 mL glass vessel using a HPLC pump (K-501, Knauer GmbH). The reaction vessel was equipped with two tubes made of Teflon. Substrate solution was pumped continuously through one tube and samples were taken at the outlet of the second tube (see SI). The reaction mixture was rigorously mixed *via* a magnetic stirrer at 500 rpm. The flow rate was chosen according to the specific activity of the immobilized lipase in the respective solvent (typically 0.5–1.0 mL/min). For stability measurements, the reaction vessel was loaded with 50 mg of immobilized Lipase TL and the substrate solution (47 mM benzoin was chosen to avoid crystallization of benzoin in toluene or CPME within the pump or pipes due to the low solubility in these solvents) with 3 equiv. vinyl butyrate was pumped through the reactor with the chosen flow rate at 50 °C. Aliquots were taken (30 µL) at definite time intervals, mixed with 500 µL isopropyl alcohol and conversion was analyzed *via* HPLC. Process stability was determined from the decrease of conversion over time (*i.e.* half-life time).

2.8 Analysis of (*R*)-benzoin racemization

The chemo-catalyst Zr-TUD-1 (Si/Zr = 25) at 40 g/L was loaded in a Schlenk flask under nitrogen atmosphere. The solution of (*R*)-benzoin (typically 20 g/L, 94 mM) was added and the flask was placed in an oil bath at 50 °C. The reaction mixture was magnetically stirred at 500 rpm and samples were taken at definite time intervals. The *ee* values were measured *via* HPLC.

2.9 Analysis of dynamic kinetic resolution (DKR)

The immobilized Lipase TL at 20 g/L and the chemo-catalyst Zr-TUD-1 (Si/Zr = 25) at 40 g/L were loaded in a Schlenk flask under nitrogen atmosphere. Substrate solution (typically 20 g/L benzoin, 94 mM) was added and the mixture was incubated for 10 min.

The reaction was started by the addition of 6 equiv. vinyl butyrate and the flask was placed in an oil bath at 50 °C. Samples were taken at definite time intervals, the conversion and enantiomeric excess (*ee*) values were followed by HPLC analysis.

2.10 Continuous synthesis of (*S*)-benzoin butyrate

For continuous synthesis of (*S*)-benzoin butyrate the previously described CSTR set-up (see 2.7) was used. The reaction vessel was loaded with 100 mg of immobilized Lipase TL and 200 mg Zr-TUD-1 (Si/Zr = 25). The substrate solution containing 10 g/L *rac*-benzoin (47 mM) and 3 equiv. vinyl butyrate in dry CPME was pumped through the reactor with a flow rate of 0.05 mL/min. The reaction mixture was rigorously mixed *via* a magnetic stirrer at 900 rpm (for better dispersion of the chemo-catalyst in the reactor volume) at 50 °C. Samples were taken at definite time intervals and conversion and *ee* values were measured *via* HPLC.

2.11 HPLC analytics

HPLC measurements were performed using PLATINblue uHPLC (Knauer GmbH) equipped with a Daicel Chiralpak IA column. The flow rate of n-hexane/isopropyl alcohol (90:10) was 2 mL/min and the column temperature was 40 °C. Typical retention times were: (*R*)-benzoin butyrate 2.9 min, (*S*)-benzoin butyrate 3.3 min, (*R*)-benzoin 5.8 min, (*S*)-benzoin 6.6 min.

3. Results and discussion

3.1 Effect of water activity on enzyme activity and stability and on racemization rate

In a first set of experiments, the solvents toluene and 2-MeTHF were assessed with a focus on water activity, to evaluate the activity and stability of the biocatalyst as well as the racemization rate of the chemo-catalyst. For a proper understanding of the effects of reaction parameters (a_w and solvent), the catalytic performance of the enzyme and chemo-catalyst were investigated separately.

First, kinetic resolution of *rac*-benzoin catalyzed by Lipase TL was investigated in toluene and in 2-MeTHF at definite a_w values at 50 °C (Table 1). These were adjusted as described in the experimental section (see 2.5). For dry reaction conditions all

compounds were dried over molecular sieve or in a desiccator over silica gel for several days resulting in an a_w of ≤0.02.

[Please insert Table 1 here]

The effect of a_w on the specific activity of immobilized Lipase TL for transesterification of benzoin in toluene and in 2-MeTHF is shown in Figure 1. In case of toluene a sharp decrease in the enzyme activity was observed which was significant up to an a_w value of 0.43. At a_w of 0.96 only 6.5% residual activity was measured. A similar decrease in Lipase TL activity was also observed in 2-MeTHF between the a_w values of ≤0.02 and 0.74 and no activity was detected at an a_w value of 0.96. The results indicate that Lipase TL requires only a very small amount of water for optimal transesterification activity. This was advantageous as it has been reported in literature that free water can hydrolyse the ester substrate [21a] or be in a competition with the alcohol [21b]. Hence the reaction system could be as dry as required for optimal performance.

[Please insert Figure 1 here]

Half-life time ($t_{1/2}$) of the immobilized Lipase TL in toluene and in 2-MeTHF at definite a_w was evaluated (Figure 2). Herein, values between ≤0.02 and 0.43 for toluene and between ≤0.02 and 0.33 for 2-MeTHF were chosen, as the highest residual enzymatic activities (>70%) were detected in these ranges (see Figure 2). It was observed that $t_{1/2}$ of Lipase TL in toluene decreased initially with increasing a_w, but slightly increased at an a_w value of 0.33. In 2-MeTHF the half-life time is lower at 0.11, but increased by 16% at 0.23. The reason for the high $t_{1/2}$ at 0.23 in case of 2-MeTHF is not clear yet. We attributed this observation to the different hydration levels of Lipase TL in the investigated solvents and a_w values, which can also influence the activity and/or stability of an enzyme [21b]. Nevertheless, for highest stability of the immobilized Lipase TL the a_w value in toluene and in 2-MeTHF should be ≤0.02. In case of 2-MeTHF, the a_w could be increased to 0.23, if required.

[Please insert Figure 2 here]

The catalytic performance of the chemo-catalyst Zr-TUD-1 for racemization of (*R*)-benzoin (*ee* ≥99%) at similar a_w values was evaluated in toluene and 2-MeTHF (Figure 3). The results showed no significant change in the racemization rate in toluene with increasing a_w values which means the dynamic kinetic resolution could be run, if needed, in toluene up to a water activity of 0.33 without any change in the activity of Zr-TUD-1. In 2-MeTHF the racemization rate was identical at the a_w values ≤0.02 and 0.11. At 0.23 a sharp decrease in the racemization rate was observed. Based on these results 2-MeTHF can be used as dry form (a_w of ≤0.02) or at an a_w value of 0.11 without a loss in the Zr-TUD-1 activity. For deeper understanding the exact effect of the water on the Zr-TUD-1 has to be further investigated at catalytic level, and surely novel options for (further) catalyst design would appear.

[Please insert Figure 3 here]

3.2 Effect of alternative organic solvents on enzyme and chemo-catalyst performance

Apart from (petroleum-based) toluene and the biomass-derived 2-MeTHF, alternative, environmentally more benign organic solvents were evaluated, namely CPME and 1,3-dioxolane. As enzymatic activity had been demonstrated to decrease with increasing a_w values (Figure 2), the solvents were used only in dry form. It was observed that in CPME 10 g/L of benzoin could be dissolved at 25 °C and 20 g/L at 50 °C. In case of 1,3-dioxolane, 20 g/L benzoin could already be dissolved at 25 °C. Analysis of the activity of immobilized Lipase TL showed that the lipase activity in CPME at equal starting conditions is 1.6-fold and 6-fold higher than in toluene (or 2-MeTHF) and in 1,3-dioxolane, respectively (Figure 4). Furthermore, in stability analyses Lipase TL showed the best performance in CPME. Half-life time was determined to be 1.5-fold higher than in 2-MeTHF and 1.3-fold higher than in toluene. Due to the very low activity of Lipase TL in 1,3-dioxolane, no stability was determined. Thus, considering activity of immobilized Lipase TL, CPME seems to be the most suitable solvent among the screened

ones, and would hold promising potential for establishing continuous biocatalytic processes. Interestingly, this observation is consistent with improved enzyme activity and enantioselectivity reported recently for the transesterification of *rac*-solketal in CPME with lipase from *Pseudomonas cepacia* [4a].

[Please insert Figure 4 here]

Motivated from such promising activity and stability of Lipase TL in CPME, the catalytic performance of Zr-TUD-1 in dry CPME was evaluated for comparison with toluene and 2-MeTHF, and with 1,3-dioxolane despite the low activity of Lipase TL in this solvent (Figure 5). The highest Zr-TUD-1 activity was observed in toluene, but the racemization rate in CPME was only slightly lower. (*R*)-Benzoin (*ee* ≥99%) was completely racemized after about 10–12 hours both in CPME and in 2-MeTHF; however, with a lower racemization rate in 2-MeTHF than in CPME or in toluene. Conversely, a much slower racemization rate was observed in 1,3-dioxolane. Hence, 1,3-dioxolane appeared to be not suitable for Zr-TUD-1. As this was consistent with the effect on Lipase TL, this solvent was excluded for further investigation.

[Please insert Figure 5 here]

3.3 Applicability of deep eutectic solvents

Apart from the above-assessed conventional organic solvents, the applicability of deep eutectic solvents (DESs) for DKR of benzoin – and eventually continuous processes –, was evaluated. So far several studies have been devoted to the application of DESs for different transesterification reactions catalyzed by various lipases [7]. Recently, novel DESs from renewable resources (e.g. choline chloride:isosorbide (ChCl:Iso) and choline chloride:levulinic acid (ChCl:LA), etc.) were introduced [6f]. Hence, in this study a wide variety of DESs such as ChCl:Gly, ChCl:U, EAC:Gly, ChCl:Iso, choline chloride:oxalic acid (ChCl:Ox) and ChCl:LA were applied. At limited solubility of benzoin, isopropyl alcohol (*i*PrOH) was investigated as cosolvent in some of these DESs.

The solubility of benzoin at chosen concentrations (standard 20 g/L, minimum 5 g/L) in the aforementioned DESs (with or without *i*PrOH (10% (v/v) as cosolvent) was investigated (Table 2). For the DESs ChCl:Gly and EAC:Gly with *i*PrOH, 5 g/L of benzoin were not soluble. When the suspension was heated up to 50 °C (by shaking at 1200 rpm for 24 hours) the benzoin concentration in the soluble fraction was detected to be at least 2.5 g/L, which was confirmed by HPLC analysis. The DES ChCl:U formed a two-phase system with *i*PrOH. Due to these given limitations these DESs were excluded from further investigation.

[Please insert Table 2 here]

Subsequently, the activity of Lipase TL (free and immobilized form) in the above described DESs for kinetic resolution of *rac*-benzoin was evaluated (Table 3). Despite the highest benzoin solubility observed, even in ChCl:LA no product formation could be detected with free enzyme. In the case of the novel DES ChCl:Iso (without *i*PrOH), however, an activity of 28.5 $U/g_{protein}$ was found. The use of immobilized enzyme exhibited 2.5-fold lower activity in the same DES. For increasing enzyme activity, immobilized enzyme was fragmented since Durand *et al.* [7c] had demonstrated that this can improve lipase activity in DESs. Accordingly, with this treatment the highest activity of 77.3 $U/g_{protein}$ (1.94 $U/g_{immoLipTL}$) was obtained. However, fragmented immobilized enzymes showed no activity in other DESs (Table 3). By adding *i*PrOH the viscosity could be reduced and the benzoin solubility could be increased, but no activity improvement was observed.

It is worth mentioning that free enzymes applied in organic media are suspensions as they are insoluble in non-aqueous solvents [22]. Hence, the reason why free Lipase TL exhibited low activities in DESs could be explained by the diffusion limitation resulting from aggregation of the enzyme. When Lipase TL was immobilized, enzyme activity increased 3-fold in ChCl:Iso, presumably due to two effects: reduced mass transfer limitation (*i.e.* increased accessibility of enzymes) by spreading on a solid support and hyper-activation of lipase by hydrophobic interactions with the Accurel carrier [17]. Overall, lipase activity was significantly lower in DES ChCl:Iso in comparison to the

previously investigated organic solvents (see Figure 4). One problem could probably be the high viscosity (even with *i*PrOH) of DESs which may result in mass transfer limitations [7b].

[Please insert Table 3 here]

Finally, the solubility of the product (*S*)-benzoin butyrate in DES ChCl:Iso without *i*PrOH (which showed the highest activity) and the kinetic resolution of benzoin were investigated. The maximum solubility of (*S*)-benzoin butyrate was determined to be 0.3 g/L. Due to the large difference to benzoin solubility (5 g/L), this offers a great potential for downstream processing by simplifying the product separation. For transesterification a conversion of 10% could be achieved after 48 h (see Figure SI 4), but no further increase occurred. Nevertheless, dry ChCl:Iso without *i*PrOH is certainly a candidate for further investigation. One possibility to improve the lipase activity could be combining two different hydrogen bond donors (*e.g.* isosorbide and levulinic acid) for an increased benzoin solubility.

3.4 Continuous synthesis of (*S*)-benzoin butyrate via DKR

Among all tested solvents CPME (dry form) turned out to be the best candidate for DKR of *rac*-benzoin. DKR was first established in batch mode. Data given in Table 4 compares the results in CPME with the published conversion and *ee* values [17] in toluene and in 2-MeTHF. With regard to conversion, DKR in CPME yielded similar result as in toluene, but achieved a slightly higher *ee* value. Compared to the results in 2-MeTHF, conversion and *ee* value in CPME was improved. Considering the lower toxicity of CPME compared to toluene (see SI) and its lower boiling point (see SI), which could simplify downstream processing of products, exchange of toluene for CPME in the process thus appears beneficial.

[Please insert Table 4 here]

DKR in CPME (dry form) was finally investigated in a continuous process (Figure 6) using the same reactor system as used for stability measurements. In the simple set-up, a maximum conversion of 40% was achieved after 2.5 h. Conversion then decreased steadily to 11% in 76 h. The *ee* value was ≥98% (*S*) during the whole run-time. Thus, it was demonstrated that continuous synthesis of (*S*)-benzoin butyrate can successfully be performed, but requires further optimization.

[Please insert Figure 6 here]

4. Conclusion

In this study, the effect of water activity and reaction solvent on the dynamic kinetic resolution of *rac*-benzoin *via* a chemo-enzymatic cascade was investigated. Herein special attention was devoted to environmentally-benign solvents. Activities of both the enzyme and chemo-catalyst were higher under dry conditions. Among the solvents evaluated, CPME was determined to be a promising solvent for the enzyme as well as chemo-catalyst.

The application of DESs for the model chemo-enzymatic cascade was explored for the first time. The enzyme performs to a lesser extent in DESs than in CPME; nevertheless, further improvements (alternative hydrogen bond donors by combining such as isosorbide and levulinic acid) could result in higher lipase activities. The very limited solubility of the ester product in DESs on the other hand offers great potential for downstream processing.

Dry CPME was chosen as solvent for the DKR based on the high performance of enzyme and chemo-catalyst. DKR was demonstrated in a continuous fashion and enantiopure (*S*)-benzoin ester was synthesized over 76 h with conversions between 40 to 11%. Optimization will be performed in future research.

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Table Captions

Table 1. Water activity values of selected saturated salt solutions at 25 °C and 50 °C.

Table 2. Evaluation of the solvents for the solubility of *rac*-benzoin at 50 °C (by shaking at 1100 rpm for 24 hours).

Table 3. Activity of immobilized Lipase TL in different dry DESs.

Table 4. Result of conversion and *ee* value of the dynamic kinetic resolution of *rac*-benzoin in CPME compared to toluene and 2-MeTHF after 5 h.

Table 1

Salt	a_w [25°C][a]	a_w [50°C][a]
LiCl	0.11	0.11
KAc	0.23	0.19[b]
$MgCl_2$	0.33	0.31
KCO_3	0.43	0.43
$Mg(NO_3)_2$	0.53	0.45
NaBr	0.58	0.51
NaCl	0.75	0.74
KCl	0.84	0.81
K_2SO_4	0.97	0.96

[a]L. Greenspan (1977) [20], [b]Data measured with a humidity sensor.

Table 2

Solvent	*i*PrOH [10% (v/v)]	Benzoin [g/L]	Solubility	Remarks/Observations
Toluene	-	20	+	Completely soluble
2-MeTHF	-	20	+	Completely soluble
CPME	-	20	+	Completely soluble
1,3-Dioxolane	-	20	+	Completely soluble
EAC:Gly (1:1.5)[a]	+	5	-	Many crystals visible
ChCl:Gly (1:2)	+	5	-	Many crystals visible
ChCl:U (1:2)	+	5	-	Many crystals visible
ChCl:Iso (1:2)	-	5	+	Very few crystals visible
ChCl:Iso (1:2)	-	10	-	Many crystals visible
ChCl:Iso (1:2)	+	10	+	Completely soluble
ChCl:Iso (1:2)	-	20	-	Many crystals visible
ChCl:Iso (1:2)	+	20	-	Many crystals visible
ChCl:Ox (1:2)	-	5	-	Highly-viscous mixture
ChCl:LA (1:2)	-	20	+	Completely soluble

[a] Molar ratio (MR) of ammonium salt : hydrogen bond donor.

Table 3

DES	Benzoin [g/L]	Enzyme form	Activity [U/$g_{immoLipTL}$]	Activity [U/$g_{protein}$]
ChCl:Gly:*i*PrOH	2.5	free	-	n.d.
EAC:Gly:*i*PrOH	2.5	free	-	n.d.
ChCl:Iso	5	free	-	28.5
ChCl:Iso:*i*PrOH	10	free	-	14.0
ChCl:LA	20	free	-	n.d.
ChCl:Iso	5	immobilized	0.30	12.0
ChCl:Gly:*i*PrOH	2.5	*f*. immobilized	n.d.	n.d.
EAC:Gly:*i*PrOH	2.5	*f*. immobilized	n.d	n.d.
ChCl:Iso	5	*f*. immobilized	1.94	77.3
ChCl:Iso:*i*PrOH	10	*f*. immobilized	0.30	12.0
ChCl:LA	20	*f*. immobilized	n.d.	n.d.

Reaction conditions: various *rac*-benzoin concentrations, *c*(vinyl butyrate) = 6 equiv., *c*(immoLipTL) = 20 g/L, at 1100 rpm and 50 °C. Activity of immoLipTL in U/$g_{protein}$ was determined with a carrier loading of 25.1 $mg_{protein}/g_{immoLipTL}$. *f*. = fragmented.

Table 4

Solvent	Conversion [%][a]	*ee* [%][a]
CPME	98.2	99.0
Toluene	98.4[17]	98.5[17]
2-MeTHF	96.9[17]	97.0[17]

[a] after 5 h. Reaction: *c*(benzoin) = 94 mM, *c*(vinyl butyrate) = 6 equiv., *c*(immoLipTL) = 20 g/L, *c*(Zr-TUD-1) = 40 g/L at 1100 rpm and 50 °C.

Scheme Caption

Scheme 1. Lipase TL-catalyzed dynamic kinetic resolution of racemic benzoin using vinyl butyrate as an acyl donor and the chemo-catalyst Zr-TUD-1 for *in situ* racemization of (*R*)-benzoin.

Scheme 1

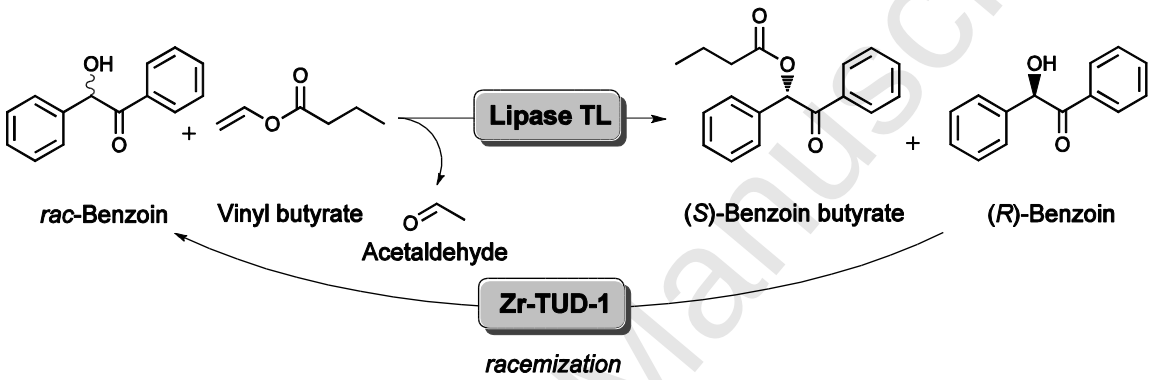


Figure Captions

Figure 1. Effect of water activity on the Lipase TL-catalyzed kinetic resolution of *rac*-benzoin running in (A) toluene and (B) 2-MeTHF. Reaction conditions: *c*(benzoin) = 20 g/L (94 mM), *c*(vinyl butyrate) = 6 equiv., *c*(immoLipTL) = 20 g/L, at 1100 rpm and 50 °C.

Figure 2 Effect of water activity on process stability of immobilized Lipase TL for kinetic resolution of *rac*-benzoin running in toluene and 2-MeTHF. Reaction conditions: *c*(benzoin) = 10 g/L (47 mM), *c*(vinyl butyrate) = 3 equiv., *c*(immoLipTL) = 8.3 g/L (50 mg immoLipTL), at 500 rpm, 0.5–1 mL/min flow rate and 50 °C.

Figure 3. Effect of water activity (a_w) on catalytic performance of Zr-TUD-1 for racemization of (*R*)-benzoin in (a) toluene and (b) 2-MeTHF. Reaction conditions: *c*((*R*)-benzoin) = 20 g/L (94 mM), *c*(TUD-1) = 40 g/L, at 1100 rpm and 50 °C.

Figure 4. Activity and half-life time of immobilized Lipase TL in different dry solvents. Reaction conditions: for activity measurement: *c*(benzoin) = 20 g/L (94 mM), *c*(vinyl butyrate) = 6 equiv., *c*(immoLipTL) = 20 g/L, at 1100 rpm and 50 °C; for process stability measurement: *c*(benzoin) = 10 g/L (47 mM), *c*(vinyl butyrate) = 3 equiv., *c*(immoLipTL) = 8.3 g/L (50 mg immoLipTL), at 500 rpm, 0.5–1 mL/min flow rate and 50 °C.

Figure 5. Catalytical performance of Zr-TUD-1 for racemization of (*R*)-benzoin in dry solvents: CPME and 1,3-dioxolane compared to toluene and 2-MeTHF. Reaction conditions: *c*((*R*)-benzoin) = 20 g/L (94 mM), *c*(Zr-TUD-1) = 40 g/L, at 1100 rpm and 50 °C.

Figure 6. Continuous DKR of *rac*-benzoin catalyzed by immobilized Lipase TL and Zr-TUD-1 in dry CPME. Reaction conditions: *c*(benzoin) = 10 g/L (47 mM), *c*(vinyl butyrate) = 3 equiv., *c*(immoLipTL) = 16.7 g/L (100 mg immoLipTL), *c*(Zr-TUD-1) = 33.3 g/L (200 mg Zr-TUD-1) at 500 rpm, 0.5–1 mL/min flow rate and 50 °C.

Figure 1

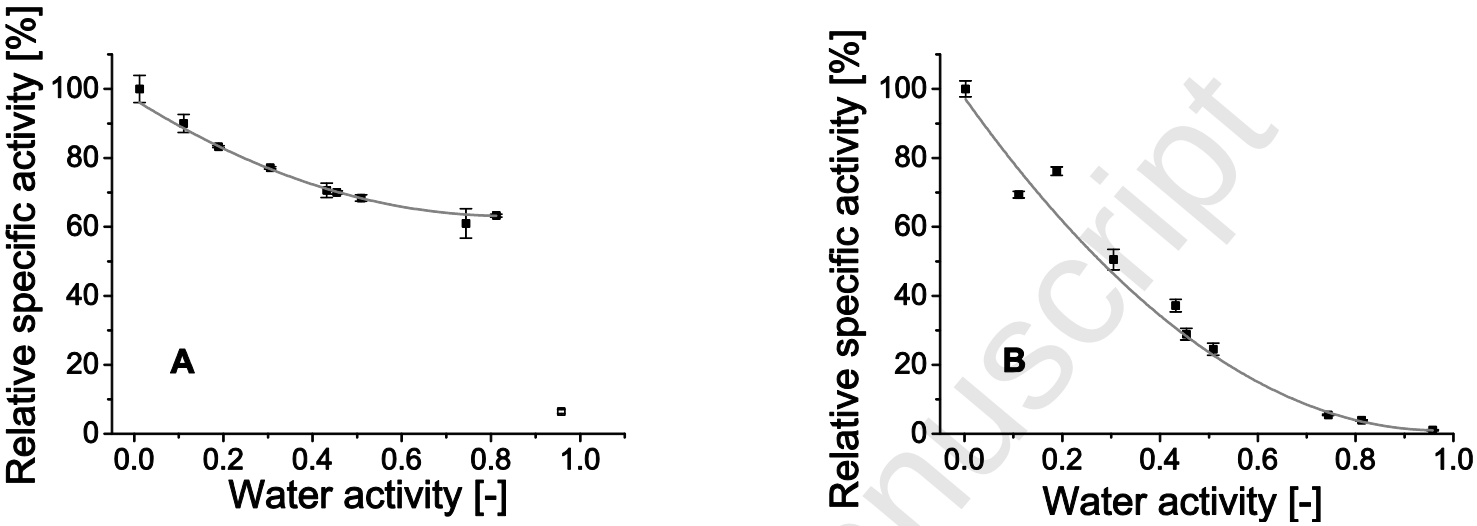


Figure 2

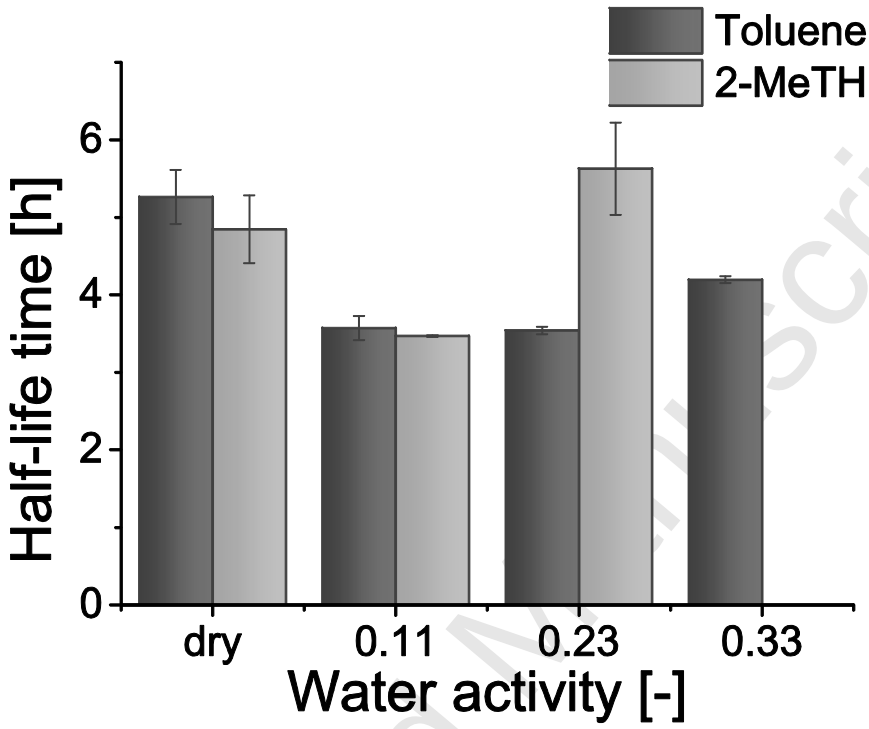


Figure 3

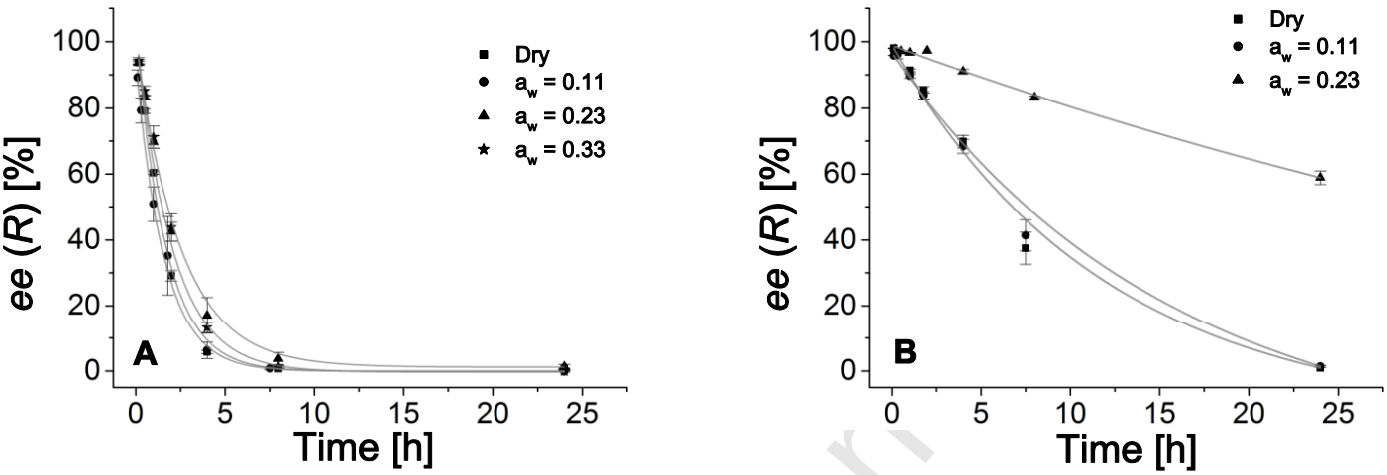


Figure 4

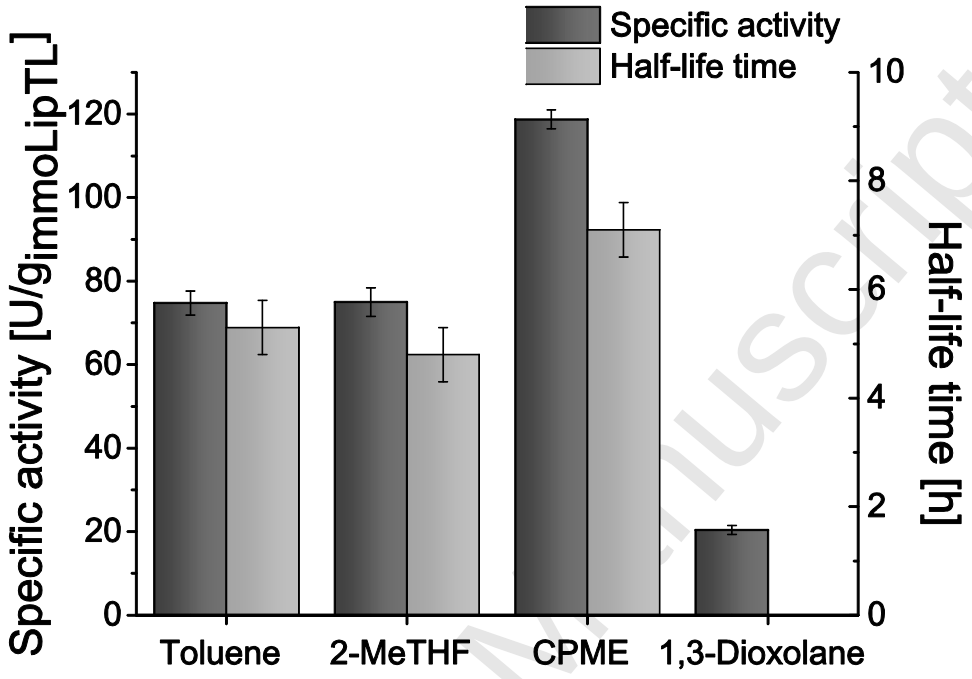


Figure 5

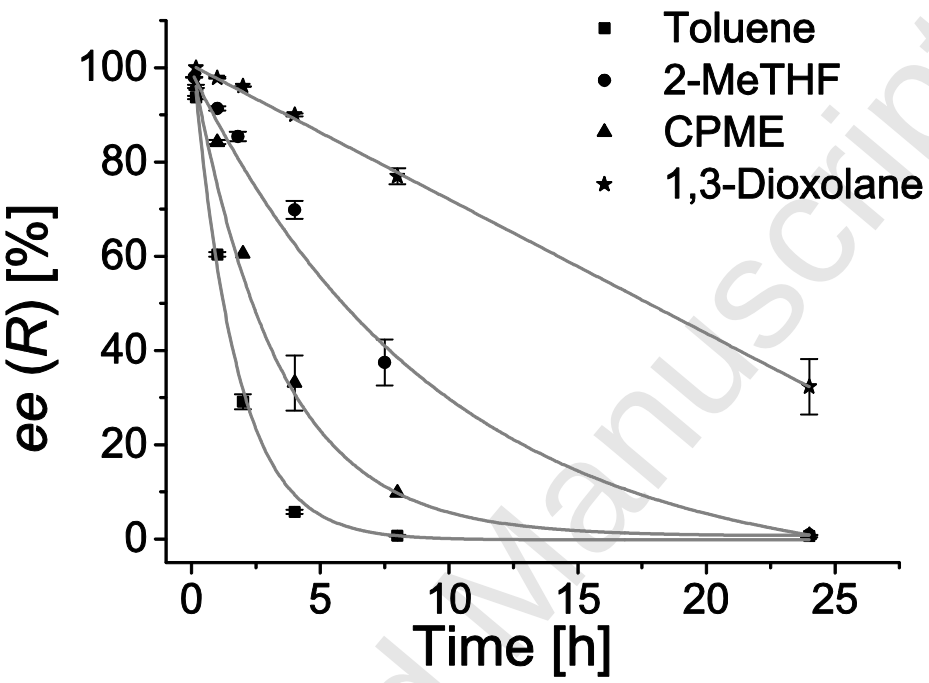
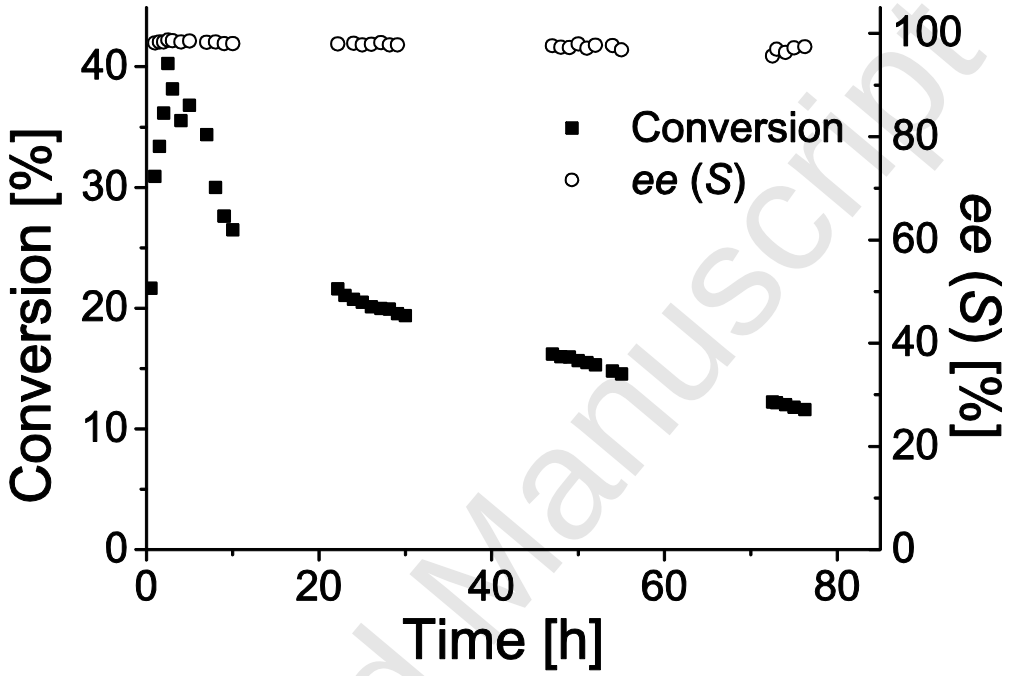


Figure 6



Abstract: The effect of the reaction parameters water activity and reaction solvent was investigated for the dynamic kinetic resolution (DKR) of rac-benzoin with immobilized Lipase TL as biocatalyst for transesterification and the heterogeneous chemo-catalyst Zr-TUD-1 (Si/Zr=25) for racemization. Overall dry reaction conditions led to the best results for both catalysts. The immobilized lipase in a more environmentally benign solvent like cyclopentyl methyl ether (CPME) exhibited a 1.6-fold higher activity and an up to 1.5-fold higher half-life time than in the standard solvents such as toluene and 2-methyltetrahydrofuran (2-MeTHF). Among a variety of deep eutectic solvents (DESs) choline chloride:isosorbide (ChCl:Iso) was found to be suitable for the reaction system. The activity was lower than in the aforementioned solvents, but the very low solubility of the product (S)-benzoin butyrate in ChCl:Iso compared to the investigated organic solvents possesses great potential with respect to downstream processing. Optimized reaction parameters (dry CPME) were applied for DKR in batch and continuous mode yielding comparable or slightly better results than in toluene or 2-MeTHF.
Keywords: Lipases; Dynamic kinetic resolution; Process stability; Water activity; Organic solvents; Deep eutectic solvents; Continuous operation