



# Deep eutectic solvents for biocatalytic transformations: focused lipase-catalyzed organic reactions

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## Abstract

Biocatalysis is a green and sustainable technology for which the ideal solvent should be nontoxic, biocompatible, biodegradable, and sustainable, in addition to supporting high enzyme activity and stability. Deep eutectic solvents (DESs), a novel class of green solvents, have recently emerged as excellent alternatives for use in various biocatalytic reactions and, in particular, in lipase-catalyzed reactions with enzymes. This review discusses the achievements that have been made so far in the use of DESs as reaction media for lipase-catalyzed reactions. In addition, the application of DESs in esterification, transesterification, and amidation reactions with isolated or immobilized biocatalysts, toward enabling the synthesis of biodiesels, sugar esters, phenolipids, and fatty acyl ethanolamides, is summarized, while advances in lipase-catalyzed chemoenzymatic epoxidation reactions, C–C bond-forming Aldol reactions, and hydrolysis reactions in DESs are also discussed. This review also summarizes some remaining questions concerning the use of DESs, including the intriguing role of water as a cosolvent in biocatalytic reactions carried out in DESs, and the relationship between the nature of the DESs and their influence on the enzyme stability and activity at the molecular level.

**Keywords** Green chemistry · Deep eutectic solvent · Lipase · Biocatalysis · Biocompatibility

## Introduction

Biocatalysis is a green and sustainable technology in terms of the metrics and principles of green chemistry (Sheldon and Woodley 2018). In manufacturing processes, including biocatalytic transformations, solvent use is generally an important consideration. Traditionally, biocatalysis is performed in volatile organic solvents such as acetone, hexane, or methanol, which are flammable, toxic, volatile, and harmful to the environment (Sheldon and Woodley 2018; Kumar et al. 2016; Dominguez de Maria and Hollmann 2015; Sheldon 2016). As alternatives to organic solvents, ionic liquids (ILs) are widely acknowledged as green solvents for nonaqueous biocatalytic reactions (Itoh 2017), and their use has been widely reported to enhance enzyme stabilities, selectivities, and activities (Sheldon 2016; Dwivedee et al. 2018; Xu et al. 2015;

Muhammad et al. 2015). However, ILs based on non-sustainable feedstocks are expensive, and due to their high ecotoxicity and low biodegradability, they cannot be classed as being truly green solvents (Thamke et al. 2019; Gomes et al. 2019; Perales et al. 2018).

More recently, deep eutectic solvents (DESs) have emerged as alternate solvents for biocatalysis (Sheldon and Woodley 2018; Sheldon 2016; Gotor-Fernández and Paul 2019; Juneidi et al. 2018). DESs have been shown to exhibit similar properties to ILs, in that they are nonflammable, have a low volatility, and exhibit high thermal and chemical stabilities (Guajardo and Domínguez de María 2019; Guajardo et al. 2016; Gu and Jerome 2013; Perez-Sanchez et al. 2013; Durand et al. 2013b; Alonso et al. 2016; Abbott et al. 2003; Smith et al. 2014; Zhang et al. 2012). Furthermore, DESs hold certain benefits, as they can be simply prepared from cheap and renewable starting materials, and they are relatively biodegradable. In light of such advantages, DESs have been successfully used as solvents in catalytic and biocatalytic organic reactions, in biomass refining (Soh and Eckelman 2016), and in materials science (Tomé et al. 2018), as electrolytes for electrodeposition and electrochemistry (Cruz et al. 2017), and as solvents for extracting natural bioactive compounds (Huang et al. 2019; Zainal-Abidin et al.

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2017; Shang et al. 2018; Zeng et al. 2019; Shang et al. 2019). The synthesis of DESs typically involves the mixing of a quaternary ammonium salt and alcohols, amides, carboxylic acids, and polyols as the hydrogen bond donors (HBDs). When both the hydrogen bond acceptor (HBA) and the HBD are natural occurring primary metabolites (i.e., amino acids, choline, sugars, alcohols, or amino and organic acids), the resulting DES is referred to as a natural deep eutectic solvent (NADES) (Fig. 1) (Dai et al. 2013).

Compared to volatile organic solvents and ILs, the benign characteristics of DESs have propelled their use across a range of biochemical applications, and over the past decade, enormous advances have been made in terms of their application in biocatalysis. They have also been discussed in several DES reviews (Sheldon and Woodley 2018; Sheldon 2016; Gotor-Fernández and Paul 2019; Juneidi et al. 2018; Durand et al. 2013b; Pätzold et al. 2019b). To distinguish from previously published reviews, this contribution is intended as an overview of the achievements reported to date in the use of DESs as reaction media for lipase-catalyzed reactions, in addition to discussing the proof-of-concept that revealed the high stabilities and activities of lipases in aqueous DESs containing high substrate loads. This review therefore focuses on the advances made in the use of DESs in various types of lipase-catalyzed reactions (Tables 1 and 2) to provide insight into the use of DESs as valuable tools for improving enzyme-based catalytic processes.

## Lipase-catalyzed reactions in DESs

### Esterification, hydrolysis, and transesterification reactions in DESs

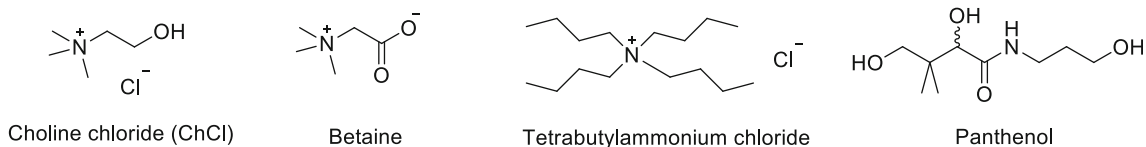
Esterification, hydrolysis, and transesterification reactions are some of the most fundamental chemical transformations. Previously, these reactions have been carried out under biocatalytic conditions to achieve control of various reaction parameters, such as the selectivity, conversion, and by-product formation. Recently, there has been a significant increase in the use of DESs for biotransformations involving esterification, hydrolysis, and transesterification reactions (Fig. 2).

#### Esterification

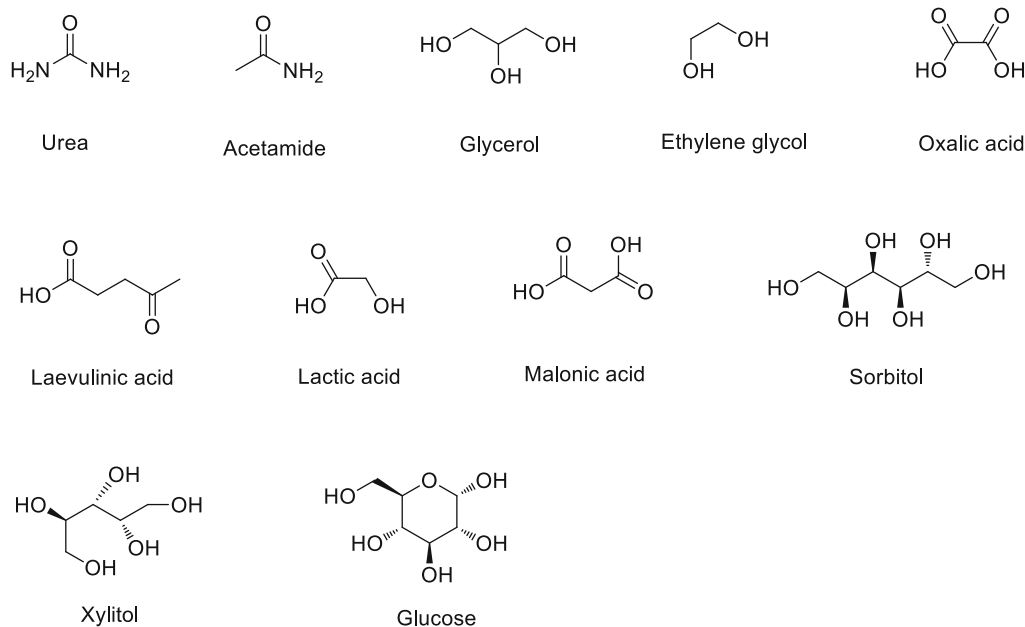
Gorke et al. (2008) were the first to employ DESs in biotransformations involving the esterification reaction, and subsequently Bubalo et al. (2015) systematically studied the advantages and limitations of ChCl-based DESs as green solvents for the immobilized *Candida antarctica* lipase B-catalyzed esterification reaction. ChCl/ethylene glycol was found to be the most promising combination,

resulting in an esterification yield of 80%, which was higher than that obtained in heptane. Similarly, a DES has been used as both a substrate and a solvent in a Novozym 435-catalyzed esterification reaction to synthesize 1,3-diacylglycerols (1,3-DAGs) (Fig. 2a). Furthermore, the addition of ChCl was found to improve the enzyme selectivity, thereby allowing the rapid and efficient synthesis of 1,3-DAG. Under these conditions, 42.9 mol% of 1,3-DAG was prepared over 1 h using ChCl/glycerol as the solvent (Zeng et al. 2015). Moreover, the use of DESs consisting of ChCl and urea for the lipase-catalyzed esterification of glycerol with n-3 polyunsaturated fatty acids (n-3 PUFA) was investigated, and a 1.2-fold increase of the n-3 PUFA triacylglycerol yield was obtained (i.e., 55%) compared to that achieved using the solvent-free system. This enhancement can be attributed to the esterification reaction equilibrium benefiting from the adsorption of the produced water by the DES (Xu et al. 2017). The Novozym 435-catalyzed esterification of benzoic acid and glycerol proceeds well in a ChCl/glycerol–water mixture, and a high conversion of 99%  $\alpha$ -monobenzoate glycerol was obtained (Fig. 2a) (Guajardo et al. 2016). In addition, low viscosity ChCl/glycerol–water blends have also been used in the continuous biocatalysis of the esterification of glycerol with benzoic acid (Guajardo et al. 2019). These reactions were carried out in a batch-fed bioreactor and at a substrate flow rate of 0.01 mL/min, with a maximum conversion of 90% being obtained. Importantly, after 10 days, only a 2% decrease was observed in the stability of the biocatalyst in continuous mode. A proof-of-concept reaction has also been reported, namely, the *Candida rugosa* lipase type VII (CRL)-catalyzed esterification of (–)-menthol fatty acid esters in a DES. In biphasic reaction systems, 10 wt.% water was added to the DES phase to increase the conversions of octanoic, decanoic, and dodecanoic acids to 50, 83, and 71%, respectively (Fig. 2b) (Hümmer et al. 2018). Panthenol, also known as provitamin B5, is a bioactive molecule that has important applications in both the pharmaceutical and cosmetics industries (Pavlačková et al. 2019), and through a combination of enzyme catalysis and DES technologies, a green synthetic method for the selective synthesis of panthenyl monoacyl esters for cosmetics has been reported. More specifically, the biocatalytic reaction of panthenol and free fatty acids in DESs to produce panthenyl monolaurate proceeds in up to 83% conversion and 98% selectivity, thereby highlighting the excellent compatibility of DESs for this conversion (Fig. 2c). Furthermore, the enzymatic activity of Novozym 435 in biocatalytic reactions has been reported to remain unchanged after reuse of the catalyst over seven consecutive cycles (Lozano et al. 2019). Moreover, ethyl lactate is an important bio-based solvent that is normally

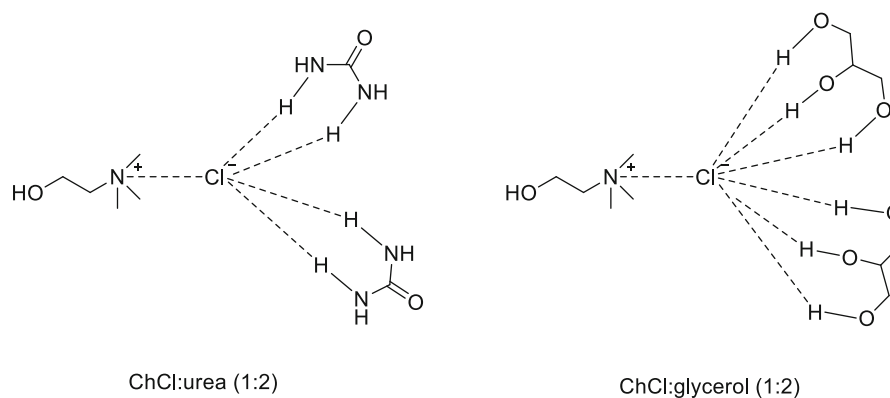
### Hydrogen Bond Acceptors



### Hydrogen Bond Donors



### Hydrogen bond interactions between ChCl:urea and ChCl:glycerol



**Fig. 1** Chemical structures of the HBAs, HBDs, and hydrogen bond interactions between ChCl/urea and ChCl/glycerol

produced via the esterification of lactic acid with ethanol. Arikaya et al. first studied the use of DESs as an alternative reaction medium for the Novozym 435-catalyzed production of ethyl lactate. A maximum 28.7% yield of ethyl

lactate was obtained using ChCl/glycerol (1:2) as the solvent (Fig. 2d), and a kinetic model indicated that the reaction follows the Ping-Pong Bi-Bi mechanism (Arikaya et al. 2019).

**Table 1** Examples of lipase-catalyzed esterification and hydrolysis reactions carried out in DESs

Reaction	Lipase	Solvents	Comments	Refs
Esterification of benzoic acid or fatty acid with glycerol	Novozym 435	ChCl/urea, ChCl/glycerol	High conversion of glyceride was obtained	Zeng et al. 2015, Xu et al. 2017, Guajardo et al. 2016
Esterification of glucose with fatty acids	Novozym 435	ChCl/hydrolysate from beechwood	An enzymatic approach for the synthesis of sugar esters was developed, based solely on lignocellulosic biomass	Siebenhaller et al. 2017
Esterification of (–)-menthol with fatty acid	CRL	(–)-menthol/fatty acid	(–)-menthol act both as a substrate and a component of DES; vacuum distillation was used for the separation of (–)-methyl dodecanoate ester from (–)-menthol	Hümmer et al. 2018, Pätzold et al. 2019a
Esterification of panthenol with free fatty acids	Novozym 435	Panthenol/free fatty acids	Panthenyl monolaurate proceeds in up to 83% conversion and 98% selectivity	Lozano et al. 2019
Hydrolysis of <i>para</i> -nitrophenyl palmitate	CRL, BCL	ChCl/ethylene glycol, ChCl/glycerol	ChCl/ethylene glycol (1:2) and ChCl/glycerol (1:2) were the most promising solvent	Juneidi et al. 2017, Kim et al. 2016
Hydrolysis of dimethyl-3-phenylglutarate	Novozym 435	ChCl/urea/phosphate buffer 50% (v/v)	The <i>R</i> -monoester was produced with a high degree of purity (99% ee) compared to that with 100% phosphate buffer	Fredes et al. 2019

## Hydrolysis

To date, lipase-catalyzed hydrolysis reactions in DESs have rarely been reported in the literature. As an example, Kim et al. systematically evaluated the influence of 13 types of DESs on the activity and stability of lipase by studying the *Candida rugosa* lipase (CRL)-catalyzed hydrolysis of *para*-nitrophenyl palmitate as a model reaction (Fig. 2e). The composition of the HBDs was found to play an important role in the stability of the lipase. Furthermore, glycerol-containing DESs have been found to be superior compared to formamide-containing DESs for promoting lipase activity (Kim et al. 2016). Both pure and aqueous DESs were employed as the solvents for the hydrolysis of *para*-nitrophenyl palmitate using *Burkholderia cepacia* lipase (BCL), and the obtained results showed that the ChCl/ethylene glycol (1:2) combination was the most promising solvent system among the tested DESs. In addition, DESs containing 4% water were superior for promoting the catalytic activity, and in this system, the lipase activity was found to be 2.6 times higher than in phosphate buffer, 1.5 times higher than in 1-ethyl-3-methylimidazoliumtetrafluoroborate, and 14 times higher than in methanol (Juneidi et al. 2017). Fredes et al. recently studied the effects of solvent and substrate concentrations on the lipase-catalyzed asymmetric hydrolysis of dimethyl 2,3-phenylglutarate (Fredes et al. 2019), where an excellent enantioselectivity was achieved for a high substrate concentration (i.e., 18 mmol/mL) in ChCl/urea/phosphate buffer (50% (v/v)) (Fig. 2f). Under the optimized conditions, the *R*-monoester was produced in a high degree of purity (99% ee). These results therefore indicate that Novozym 435 is more stable in a DES-based buffer than in 100% phosphate

buffer. Indeed, Novozym 435 retained half of its activity after 27 h of incubation at 40 °C, and its higher reactivity led to an improved selectivity.

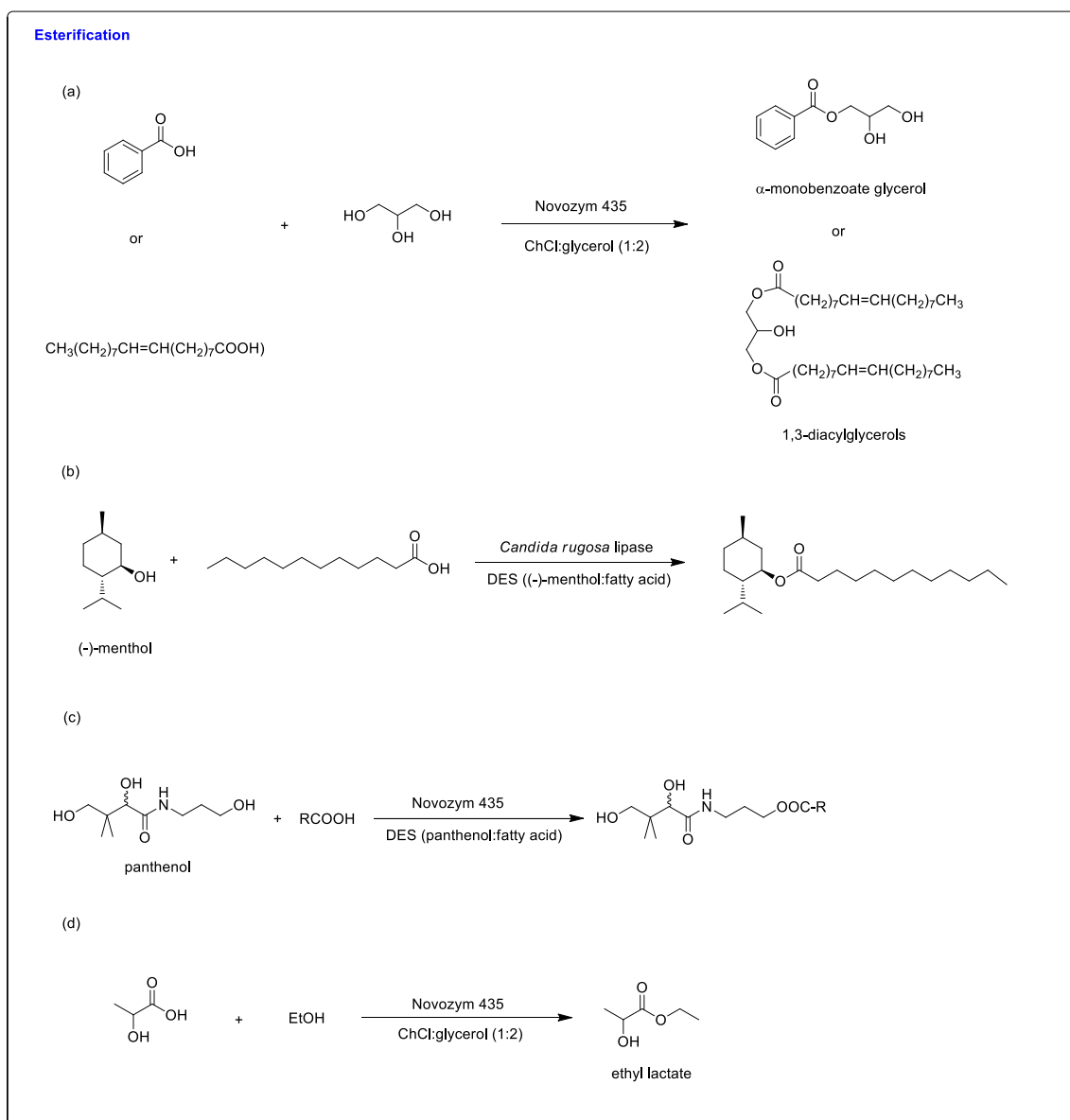
## Transesterification

Durand et al. (2012) systematically investigated the influence of DESs on lipase-catalyzed transesterification reactions involving vinyl laurate and alcohols of varying chain lengths. In their study, they showed that DESs such as ChCl/glycerol and ChCl/urea exhibit high activities and selectivities, highlighting their promise for use as solvents in lipase-catalyzed reactions. However, in the case of dicarboxylic acid- or ethylene glycol-based DESs, side reactions were observed, thereby restricting their use in biocatalysis.

Glycolipids are structurally complex biomolecules, and their chemical syntheses are highly challenging. While several such approaches to this class of molecules have been reported, enzymatic routes present a number of advantages. Furthermore, DESs have been shown to be promising solvents for the synthesis of glycolipids using free or immobilized enzymes. Sugars extracted from the lignocellulose of beechwood have been used as starting materials in the enzyme-catalyzed production of surface-active sugar esters (Fig. 2g), and it was found that the sugars were more soluble in a DES–water system than in other anhydrous solvents. Under optimal conditions, the synthesis of sugar esters in DESs was successful. However, the transformation proceeded with a conversion of < 5%, which is a significantly lower yield than that obtained for sugar ester synthesis with alcohols exhibiting a low solubility in the hydrophilic phase, e.g., *tert*-butanol (Siebenhaller et al. 2017). Furthermore, according to

**Table 2** Examples of lipase-catalyzed non-natural reactions carried out in DESs

Reaction	Lipase	Solvents	Comments	Refs
Transesterification of vinyl laurate with alcohols	Novozym 435	ChCl/urea, ChCl/glycerol	ChCl/urea and ChCl/glycerol exhibited high activities and selectivities in lipase-catalyzed reactions	Durand et al. 2012
Transesterification of glucose or fructose with methyl or vinyl fatty acid ester	Novozym 435	ChCl/beechnwood cellulose fiber hydrolysates, ChCl/agave syrup and honey	Sugar component act both as a substrate and a component of DES	Siebenhaller et al. 2018a, Siebenhaller et al. 2018b
Transesterification of methyl <i>p</i> -coumarate and methyl ferulate with octanol	Novozym 435	ChCl/urea (1:2) [10% water (w/w)]	Phenolipids were obtained in high conversions	Durand et al. 2014, Shi et al. 2019
Transesterification of methyl caffeate with glycerol	Novozym 435	ChCl/urea containing 10% (v/v) water	Under continuous microflow conditions, delivering a total yield of 96.5%	Liu et al. 2019
Acylation of DMY	ANL@PD-MNP	ChCl/glycerol–DMSO (1:3, v/v)	The catalyst was reused for five runs without any pronounced loss in its catalytic activity	Cao et al. 2017
Transesterification of tyrosol	lipase B from <i>Pseudozyma antarctica</i> on nanoflowers	ChCl/urea	The solid catalyst preserved almost 100% of activity after eight successive catalytic cycles	Fotiadou et al. 2019
Amidation reaction				
Amidation of DHA and EPA with acyl ethanolamides	Novozym 435	ChCl/glucose, 5:2) [8.5% water (w/w)]	96.8% for EPEA and 90.1% for DHEA were obtained	Liang et al. 2018
<i>N</i> -arylation of nitrobenzene	PPL	ChCl/urea (1:2)	Products in yields ranging from 76 to 98%	Pant & Shankarling 2017
Oxidation reaction				
Epoxidation of glyceryl trioleate	Lipase G	ChCl/xylol (1:1)	Lipase G mitigates the formation of the hydrolytic by-products observed with CALB	Zhou et al. 2017a
Epoxidation of alkenes	CALB	ChCl/sorbitol (1:1)	Epoxides were obtained in good to excellent yields	Zhou et al. 2017b
Baeyer–Villiger oxidation of ketones	Ser105Ala mutant of CALB	ChCl/sorbitol (1:1)	“Perhydrolyase-only” reactions furnish Baeyer–Villiger products only	Wang et al. 2017
Epoxidations of 3-carene, limonene, and $\alpha$ -pinene	CALB	ChCl/U/H <sub>2</sub> O <sub>2</sub>	ChCl/U/H <sub>2</sub> O <sub>2</sub> exhibited better results than conventional DESs; complete conversions were achieved within 2–3 h	Ranganathan et al. 2017
Aldol reaction				
Aldol reaction of 4-nitrobenzaldehyde	PPL	ChCl/glycerol (1:2)	High yields and selectivities were afforded compared to those in toluene	González-Martínez et al. 2016
Aldol reaction of 4-nitrobenzaldehyde	PPL	TBACl/4-nitrobenzaldehyde (2.2:1.5)	A 4-nitrobenzaldehyde-containing DES was used, and this system exhibited the highest solubility for the substrate and furnished a final yield of 1296.5 mmol/mL	Milker et al. 2019
Henry and aza-Henry reaction of 4-nitrobenzaldehyde and <i>N</i> -Ts imines	Lipase AS	ChCl/glycerol (1:2) [30% water (v/v)]	An excellent yield was achieved in 4 h; water plays a key role in stabilizing the enzyme	Zheng et al. 2016



**Fig. 2** Examples of lipase-mediated esterification, hydrolysis, and transesterification reactions that can be carried out in DESs

Kleiner's results, alcohols presenting higher solubilities in DESs are more difficult to incorporate into esters (Kleiner and Schörken 2014). Moreover, Zhao and co-workers reported that the synthesis of sugar esters is less efficient in DESs than in the ILs-2M2B (2-methyl-2-butanol) bisolvent mixture (Zhao et al. 2016). Since lignocellulosic biomass is a natural source of sugar, an eco-friendly enzymatic approach that allows the direct synthesis of sugar esters from lignocellulosic biomass was investigated. More specifically, beechwood cellulose fiber hydrolysates can be used in biocatalysis as part of a DES reaction system, as a sugar component, or as a carbon source for the oleaginous yeast *Cryptococcus curvatus*-cultivated synthesis of fatty acids. In addition, *Candida antarctica* lipase B (iCALB), an immobilized biocatalyst, has been used for the esterification of in situ-generated sugars with fatty

acids in DESs (Siebenhaller et al. 2018b). Similarly, agave syrup and honey have been employed as sugar sources to produce sugar esters in an iCALB-catalyzed transesterification process in DESs (Siebenhaller et al. 2018a).

The enzymatic transesterification of triglycerides derived from the combination of fatty acids from either vegetable oils or animal fat waste with methanol or ethanol is a widely used approach for biodiesel production. Recently, Zhao et al. (2013) demonstrated that this can be achieved using DESs as solvents. The methanolysis of soybean oil catalyzed by Novozym 435 has also been shown to afford up to 88% conversion of triglycerides in 24 h. Glycerol, a by-product of biodiesel production, can act as an HBD-forming DES, and its presence affords additional benefits as a result of the ChCl/glycerol DES possessing a lower viscosity compared to ChCl/urea. Using this

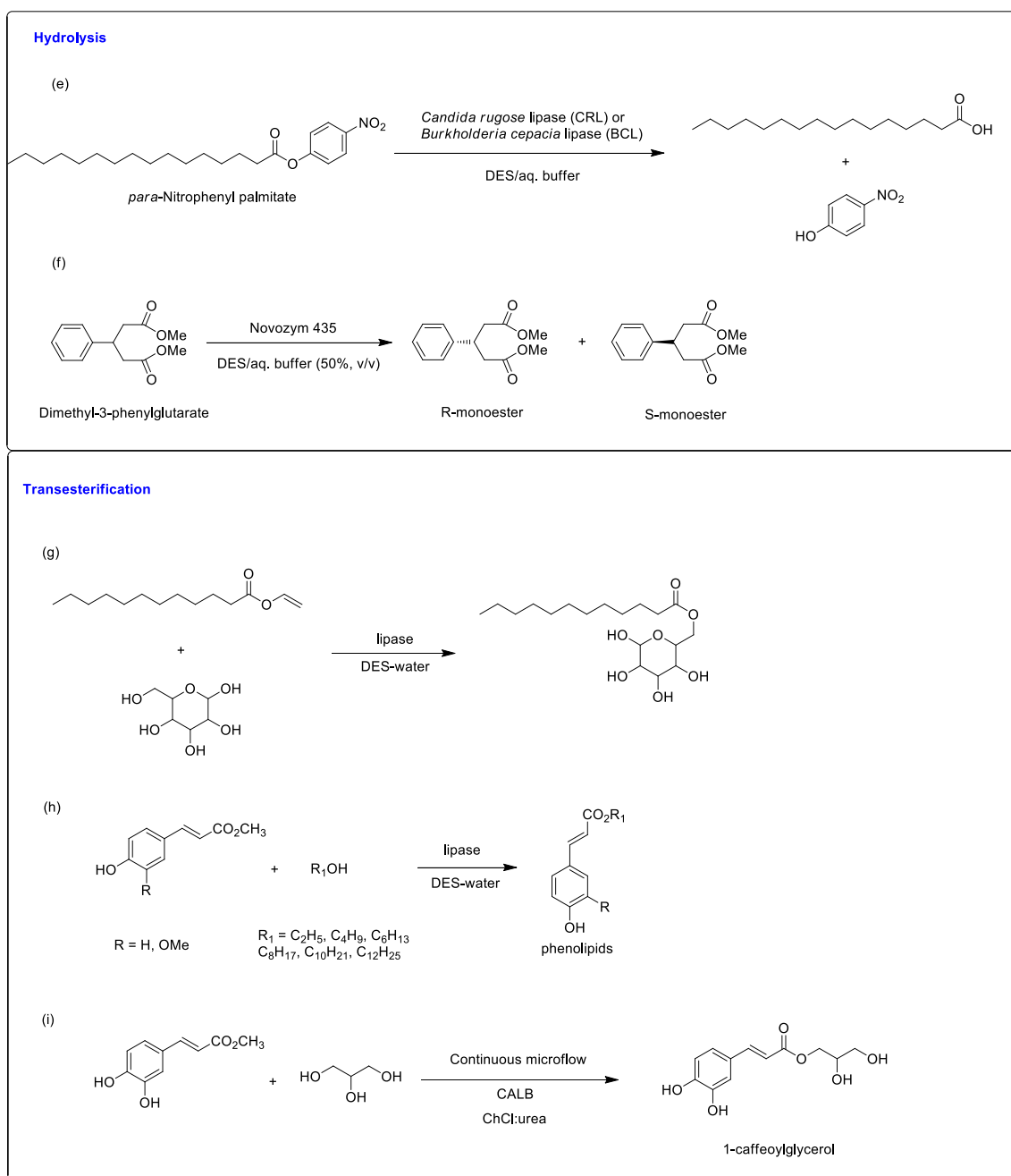


Fig. 2 (continued)

approach, Huang et al. (2014) investigated the synthesis of biodiesel from the oil of inedible *Milletia pinnata* seeds using ChOAc/glycerol as a medium and Novozym 435 as the catalyst. More recently, there has been a report into the enzymatic transesterification of rapeseed or cooking oil with ethanol via a two-step, one-pot reaction (Kleiner et al. 2016). The two steps involved in these transesterification processes are as follows: (1) complete conversion of 3–4% free fatty acid-containing triglycerides in the presence of lipase derived from *Thermomyces lanuginosus* and 2) the use of CALB in ChCl/urea or ChCl/glycerol to convert the remaining free fatty acids

into ethyl esters. In addition, DESs have been successfully implemented as solvents in enzymatic biodiesel production from waste oils, and the results have been compared with those obtained in ILs (Merza et al. 2018). Notably, biodiesel production in ChCl/glycerol (1:2) containing 4 wt.% water furnished a 71.4% yield compared to the 23% achieved in [bmim][PF<sub>6</sub>]; the solid Novozym 435 catalyst used in this reaction could be recycled up to five times without any significant loss in activity.

Phenolic compounds, such as phenolic acids and flavonoids, are important secondary plant metabolites. However, the high polarity of phenolics often hinders their penetration of the lipid

layers of cell membranes. To address this issue, novel multi-functional molecules with an improved hydrophilic–lipophilic balance, i.e., phenolipids, have been synthesized through the covalent grafting of hydrophobic alkyl moieties to the phenolics (Durand et al. 2017). Durand et al. (2013a) reported the first example of the lipase-catalyzed lipophilization of phenolic acids in the presence of octanol in DESs. However, regardless of the choice of DES, only a < 2% conversion was observed. In contrast, conversions of 98 and 90% were achieved in the Novozym 435-catalyzed transesterification reaction of methyl *p*-coumarate and methyl ferulate in the presence of octanol in ChCl/urea solution (10% water, w/w) (Fig. 2h) (Durand et al. 2014). Similarly, Novozym 435 was used to catalyze the alcoholysis of methyl ferulate with fatty alcohols, to afford ferulic acid alkyl esters in good to excellent yields (78–90%) in a ternary DES (ChCl/urea/glycerol, 1:1:1) containing 10% (w/w) water (Shi et al. 2019). Similarly, a ChCl/urea DES containing 10% (v/v) water was found to be an excellent solvent for the Novozym 435-catalyzed synthesis of 1-caffeoylglycerol under continuous microflow conditions, delivering a total yield of 96.5% (Fig. 2i) (Liu et al. 2019). Along similar lines, a novel DES – DMSO cosolvent system was used for the dihydromyricetin (DMY) acylation reaction catalyzed by an immobilized lipase derived from *Aspergillus niger* (ANL). DMY is a natural flavanone, which displays an extensive bioactivity (Hou et al. 2015). Finding a suitable reaction medium in which DMY is highly soluble and exhibits a suitable enzyme activity is therefore critical for its use in enzymatic acylations. With this in mind, a ChCl/glycerol–DMSO (1:3, v/v) system was developed for the ANL@PD-MNP-(ANL immobilized on magnetic nanoparticles)-catalyzed acylation of DMY, which afforded up to 90% conversion (Cao et al. 2017).

## Process aspects

### DES properties necessary for enzyme activation and stabilization

To render DESs suitable solvents for enzyme-catalyzed biotransformations, the effect of the added water content was examined. For example, Durand et al. found that the initial enzyme reaction rate in aqueous ChCl/urea was 67 times higher than in the pure DES in the presence of 6% (w/w) water. No phenolipids were obtained without the addition of water even after extending the reaction time to 72 h, while the yield of the desired products increased to 20% upon the addition of 2% water, increasing further up to 100% with 10–20% water (Durand et al. 2013a). Another study into the lipase-catalyzed hydrolysis reaction of *para*-nitrophenyl palmitate showed that the enzyme was mostly deactivated in pure DESs. In addition, the half-life of lipase at 40 °C in an aqueous solution of ChCl/glycerol was enhanced by 9.2 times (Kim

et al. 2016). Again, Novozym 435 was found to catalyze the production of  $\alpha$ -monobenzoate glycerol, whereby the yield increased from 40 to 100% upon the addition of 20% water (v/v) to the DES mixture (Guajardo et al. 2017). Generally, DESs possess higher viscosities than conventional solvents due to the presence of hydrogen bonding, van der Waals forces, and electrostatic interactions between the components, which restrict their application in biotransformation due to slow mass transfer and insufficient interactions with the enzyme. The addition of water alters the viscosity and polarity of the DESs, thereby allowing their properties to be tailored to render them suitable solvents for application in biotransformations. To better understand the role of water and the molecular interactions taking place between lipase and the DES, molecular dynamics simulations were carried out between CALB and the ChCl/glycerol–water binary system. The COM RDFs (radial distribution function) results indicated that a greater number of ChCl/glycerol components (i.e., choline cations, glycerol, and chloride ions) diffused into the CALB active sites upon the addition of an optimal volume of water. The presence of greater numbers of chloride ions will strengthen the hydrogen bonding interactions with the CALB active site, thereby increasing the enzyme activity and stability (Nian et al. 2019).

It was therefore found that irrespective of the reaction and enzyme type, similar conclusions were reached in terms of the superior effects of glycerol and urea-based DESs over other tested DESs. Considering that urea is a well-known potent protein denaturant, these results are quite astonishing. Thus, molecular dynamics simulations of CALB in urea and ChCl/urea (10 M urea) were carried out to better understand this result (Monhemi et al. 2014). When the enzymes are present in urea alone, the enzyme is denatured via urea diffusion to the protein core, which destroys the intramolecular hydrogen bonds. However, the chloride ions and urea present in the ChCl/urea DES form strong hydrogen bonds, thereby preventing the diffusion of urea into the protein core. Moreover, an increased enzyme stability can be achieved via hydrogen bond formation between the surface amino acid residues and the chloride ions, choline, and urea.

Lee et al. systematically evaluated the relationship between the solvatochromic parameters of DESs and the activities and stabilities of the lipases present in these solvents (Oh et al. 2019). In this context, the  $\beta$  value, a solvatochromic parameter, provides a meaningful correlation with the lipase activity. Furthermore, the lipase activity in amide- and hydroxyl-based DESs was found to be related to the  $\beta$  value, resulting in correlation coefficients ( $r$ ) of 0.81 and 0.76, respectively. Moreover, the stability of lipase has been found to be related to the  $\alpha$  values of DESs, with the thermal stability of lipase increasing at higher  $\alpha$  values.



## Immobilized enzymes

Immobilization is a highly effective approach that can be used to increase enzyme activities and extend their utilization in industrial applications. As such, a range of techniques (e.g., physical adsorption, entrapment, cross-linking, and covalent attachment) have been developed for the immobilization of lipase and ameliorate in esterification/transesterification reactions (Sheldon and van Pelt 2013; Barbosa et al. 2019). The most frequently used immobilized enzyme is Novozym 435, a commercially available lipase adsorbed on acrylic resin beads. Novozym 435 has been shown to exhibit a good catalytic activity and recyclability. Furthermore, it was found that macrostructured and nanostructured hierarchical materials can stabilize lipase B derived from *Candida antarctica*. Even after exposure to ChCl/urea at 60 °C for 48 h, the immobilized lipase microparticles still exhibited a significant activity and outperformed the commercially available immobilized lipase Novozym 435 in the synthesis of glucose esters (Andler et al. 2017). In addition, *Aspergillus niger* immobilized on magnetic nanoparticles was successfully used in lipase-catalyzed acylations of DMY. Importantly, the solid catalyst was found to be stable and robust under the chosen reaction conditions and was reused for five runs without any pronounced loss in its catalytic activity (Cao et al. 2017). Furthermore, the immobilization of lipase B from *Pseudozyma antarctica* on nanoflowers enriched with carbon and magnetic nanomaterials was reported by Fotiadou et al. (2019). The immobilized lipase preserved almost 100% of its initial activity after eight successive catalytic cycles in the transesterification of tyrosol, indicating that these immobilized enzymes can be successfully applied in nanobiocatalytic systems. Cross-linked enzyme aggregates (CLEA) have also been successfully implemented for the immobilization of *Candida antarctica* lipase B. This solid catalyst is stable for at least 14 days without any loss in activity. Overall, CLEA-CALB was found to outperform Novozym 435, giving 30% higher conversions (Guajardo et al. 2018).

## Downstream processing

Despite the exploration of enzyme immobilization techniques for facilitating separation of the catalyst from the DES-based reaction media, few examples exist regarding downstream processing, especially for systems where the DES acts as both the reaction medium and the substrate. A combination of water with ethyl acetate was used for purification of the terpene epoxide from a lipase-mediated epoxidation process in a DES, and isolated yields of 87.2, 77.0, and 84.6% were obtained for 3-carene, limonene, and  $\alpha$ -pinene epoxide, respectively (Ranganathan et al. 2017). In addition, Kleiner et al. (2016) used a single distillation method for the downstream processing of the *Pseudozyma antarctica* lipase B-catalyzed synthesis

of biodiesel in ChCl/glycerol. Glycerol was obtained in a higher purity at the low feeding rate and high wiper speed distillation process, and the ChCl/glycerol residue was successfully reused in the transesterification process. Furthermore, following the *Candida rugosa* lipase-catalyzed esterification of (–)-menthol with dodecanoic acid, vacuum distillation was used for the separation of the (–)-methyl dodecanoate ester from (–)-menthol due to their different boiling points. Thus, the desired ester was obtained in 94% purity, and the recovered (–)-menthol could be used as the HBD for DED formation, with almost full conversion being obtained after 3 days (Pätzold et al. 2019a).

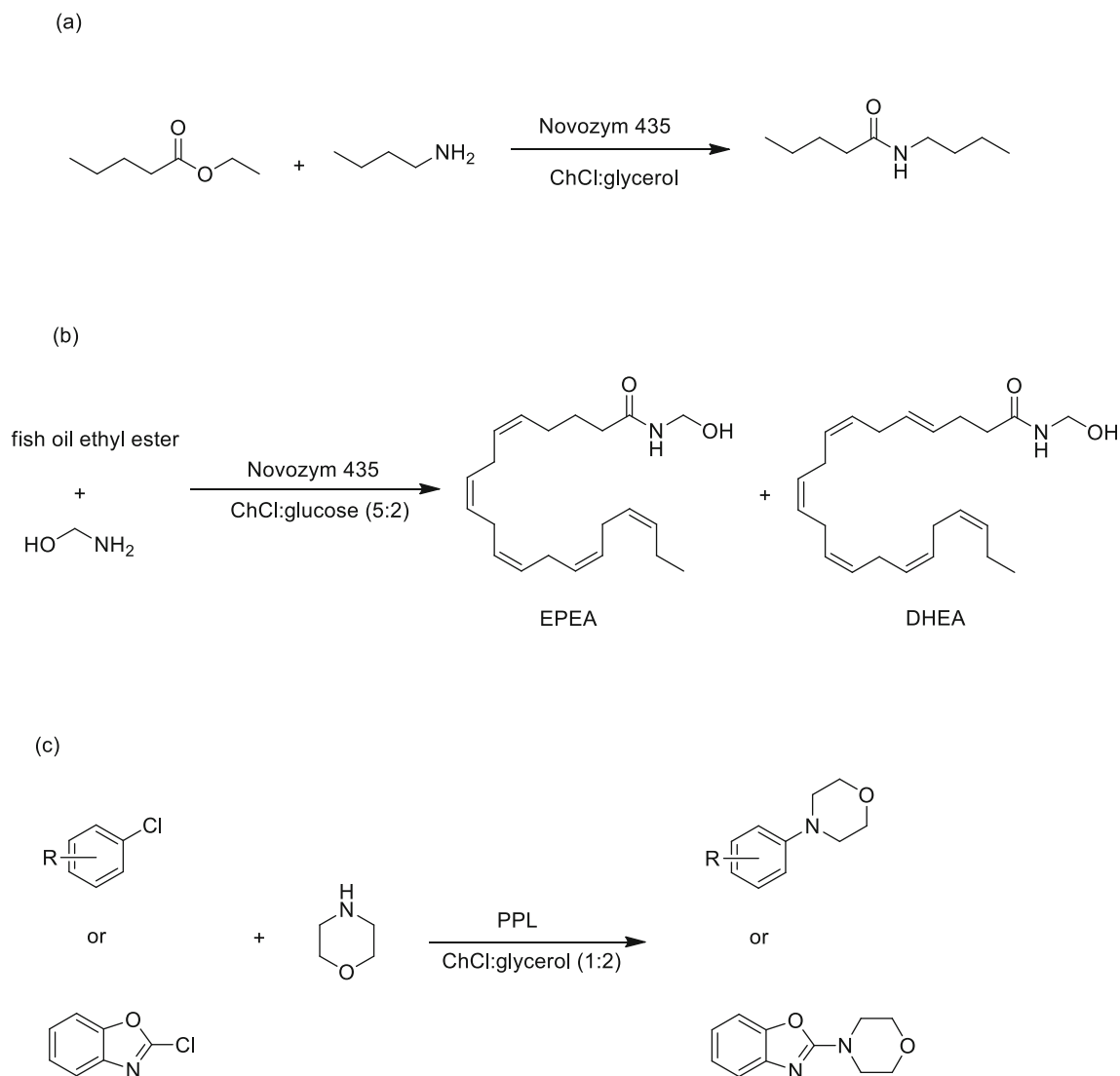
## Amidation reactions in DESs

DESs have also been successfully used as solvents in amidation reactions. The catalyzed amidation of ethyl valerate with 1-butylamine using Novozym 435 as a catalyst in ChCl-based DESs such as ChCl/urea (1:2) and ChCl/glycerol (1:2) has been reported, where the conversions achieved are comparable to those reported in toluene (Gorke et al. 2008) (Fig. 3a). Recently, fatty acid acyl ethanolamides with long alkyl chains, such as docosahexaenoyl ethanolamide (DHEA) and eicosapentaenoyl ethanolamide (EPEA) have been efficiently produced via enzymatic processes by employing ChCl-based natural DESs as the reaction media (Fig. 3b). Approximate yields of 96.8% for EPEA and 90.1% for DHEA were achieved in the aminolysis of fish oil ethyl esters in a DES (i.e., ChCl/glucose, 5:2) containing 8.5% (w/w) water catalyzed by Novozym 435 (Liang et al. 2018). Moreover, Pant et al. reported the catalyzed *N*-arylation of nitrobenzene in ChCl/urea (1:2) using metal and ligand-free *porcine pancreas* lipase (PPL) (morpholine), which proceeded under mild conditions and afforded the products in yields ranging from 76 to 98% (Pant & Shankarling 2017) (Fig. 3c).

## Lipase-mediated oxidation reactions in DESs

Gorke et al. (2010) reported the first example of the CALB-catalyzed perhydrolysis of cyclohexene in an aqueous DES solution in the presence of H<sub>2</sub>O<sub>2</sub>. The reaction proceeded well in the DES (ChCl/glycerol, 1:2) and afforded the desired product in 22% yield. However, the reaction delivered a higher yield in acetonitrile. The CALB-catalyzed epoxidation of alkenes in various ChCl-based DESs in the presence of H<sub>2</sub>O<sub>2</sub> (30%) and octanoic acid was later described by Zhou et al. (Fig. 4a). After screening nine DESs along with 100% phosphate buffer, a ChCl/sorbitol (1:1) DES was found to be the optimal solvent for the reaction, giving the desired epoxides in good to excellent conversions with yields of 72–97% (Zhou et al. 2017b).

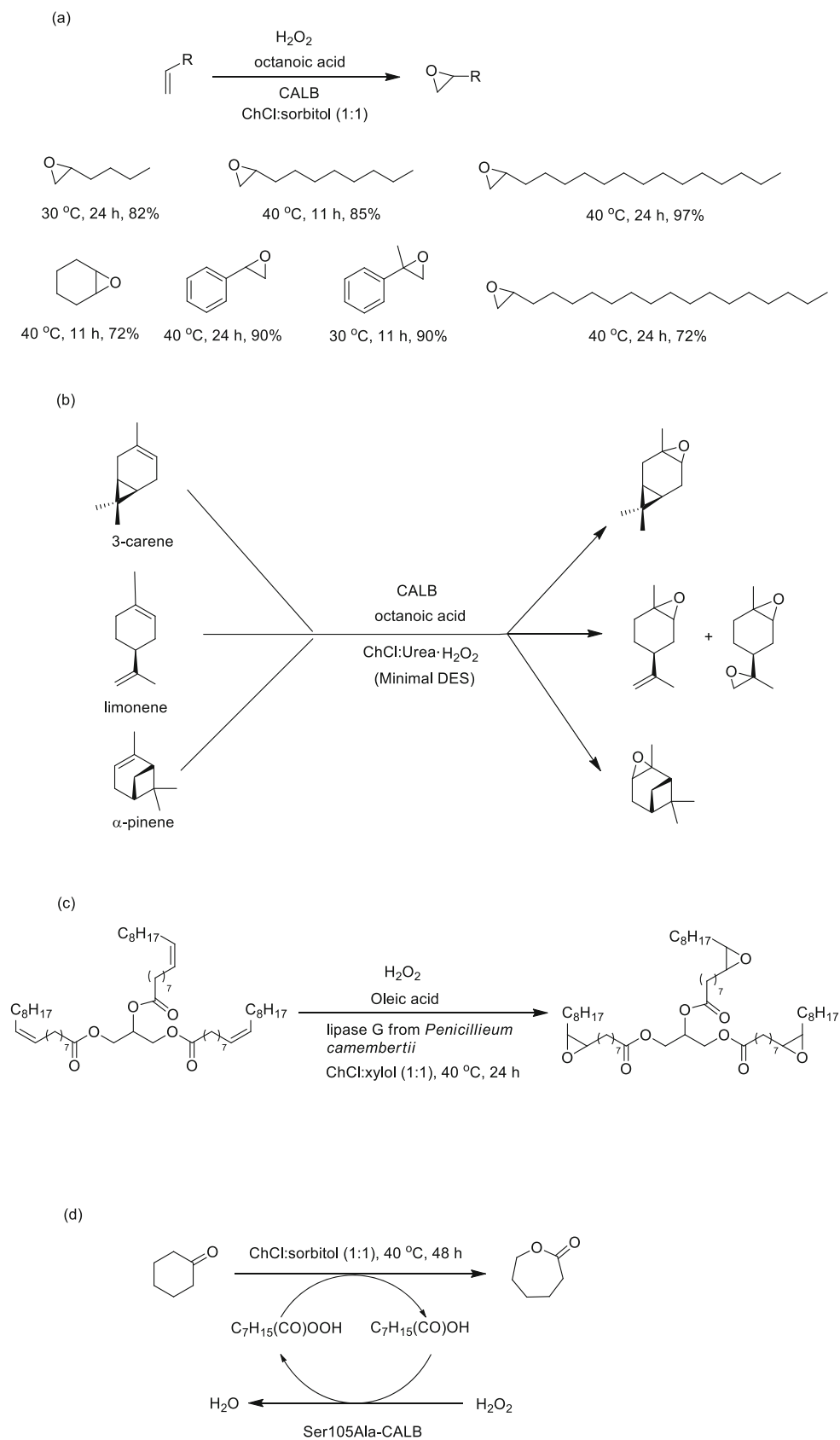
Terpenes, which are abundant by-products of the fruit, paper, and pulp industries, are nontoxic and can be used as



**Fig. 3** Examples of lipase-mediated amidation reactions that can be carried out in DESs

renewable feedstocks. Terpene epoxidation is a key biorefining process that can be used to produce useful precursors for the polymer industry, including alcohols, diols, ketones, and monomers (Mewalal et al. 2017). Thus, Sieber and co-workers developed a novel DES mixture, known as minimal DES, which acts as both the solvent and the substrate to enable the fast and efficient CALB-catalyzed epoxidation reactions of 3-carene, limonene, and  $\alpha$ -pinene (Fig. 4b) (Ranganathan et al. 2017). Two conventional DES systems, namely, ChCl/glycerol and ChCl/sorbitol, were found to be the optimal, affording the complete conversion of the terpenes within 8 h. However, ester by-products were unavoidably produced under these conditions, and so various other DES systems were evaluated, and a DES comprising urea- $H_2O_2$  (U- $H_2O_2$ ) and ChCl was found to give superior results, with complete conversion being achieved within 2–3 h. The improved reactivity was thought to be due to the in situ generation of the

DES, which resulted in the improved dissolution of  $H_2O_2$ , the adducts, and the peroxy acid generator. In addition, Hollmann and Wang showcased the multifunctional use of DESs (Ma et al. 2019), wherein they employed ChCl-based DESs for three different purposes in a biocatalysis process, namely, as extraction solvents for limonene from waste orange peel, as reaction solvents for chemoenzymatic epoxidations, and as sacrificial electron donors for the in situ generation of  $H_2O_2$  to promote chemoenzymatic epoxidation reactions. Furthermore, a ChCl/1,2-propanediol/ $H_2O$  (molar ratio 1:1:1) system, i.e., a polyhydric alcohol-based DES, yielded the highest extraction efficiencies for limonene and gave a 17.8 mg/g extracting yield of limonene. A sequential enzymatic reaction system was then employed to evaluate the efficacy of different hydrolases as catalysts for the in situ generation of peracids from limonene in a DES solution, and Novozym 435 was found to display an excellent activity



**Fig. 4** Examples of lipase-mediated oxidation reactions that can be carried out in DESs

compared to other hydrolases. Notably, 8.72 mM of limonene epoxides were obtained after 2 days. A mechanistic study revealed that the overall epoxidation rate increased with an increase in the ChOx concentration, which suggests that the reaction involving the formation of the H<sub>2</sub>O<sub>2</sub>-dependent peracid is the rate-determining step.

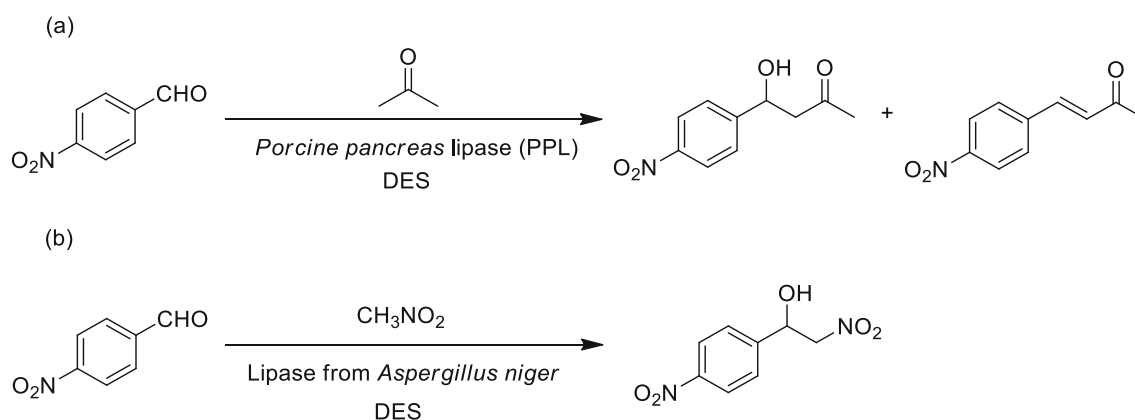
The chemoenzymatic epoxidation of glyceryl trioleate in a ChCl/xylitol DES system using lipase G derived from *Penicillium camemberti* (PCL) has been shown to deliver superior performances and higher yields in epoxidation reactions than when CALB is employed (Fig. 4c) (Zhou et al. 2017a). As estimated from the <sup>1</sup>H NMR spectra of the epoxidation products from the lipase G-catalyzed reaction, the signals at 5.3 ppm, which represent the CH=CH protons in the glyceryl trioleate, almost disappeared, indicating that the majority of the substrate was transformed into the epoxidation products; in contrast, these signals remained in the CALB-catalyzed chemoenzymatic system. Furthermore, the use of lipase G prevents the formation of the hydrolytic side products observed when CALB is used. The optimal conditions for this transformation were determined to be 3 eq. of H<sub>2</sub>O<sub>2</sub> in ChCl/xylitol (1:1) with oleic acid as the peracid precursor at 40 °C. Notably, the addition of oleic acid as a starting material was not required, since it is already present in vegetable oils.

The oxidation of cyclohexanone to  $\epsilon$ -caprolactone via a chemoenzymatic Baeyer–Villiger reaction has also been successfully carried out in a DES using a Ser105Ala mutant of lipase CALB (Fig. 4d) (Wang et al. 2017). These “perhydrolase-only” reactions exhibit a high selectivity in ChCl/sorbitol (1:1), furnishing significant amounts of product in all cases. Interestingly, DESs improved the overall ketone conversions compared to the use of a water/*n*-hexane (1:2) mixture.

### Aldol reactions in DESs

In their first report on the use of DESs in lipase-catalyzed Aldol reactions, Gotor-Fernández et al. carried out a PPL-catalyzed

bio-Aldol reaction using 4-nitrobenzaldehyde to prepare the corresponding Aldol adducts in a ChCl/glycerol (1:2) DES (González-Martínez et al. 2016). Under their optimized conditions, the reaction afforded high yields and selectivities compared to those carried out in toluene, and the developed system was found to be compatible with a series of ketone substrates, including acetone, cyclohexanone, and cyclopentanone. However, the aldehyde substrate scope was limited to compounds containing strongly electron-withdrawing groups. Milker et al. (2019) also investigated the Aldol reaction of 4-nitrobenzaldehyde with acetone in other DESs, and their results for the PPL-catalyzed Aldol reaction indicated that the composition of the product is directly related to the hydrophobicity of the DES (Fig. 5a). Furthermore, the initial reaction rate was found to be higher in ChCl/glycerol. However, the poor solubility of 4-nitrobenzaldehyde in this hydrophilic DES hinders the reaction. Moreover, the addition of a hydrophobic quaternary ammonium salt (tetrabutylammonium chloride, TBACl) as an HBA alongside a 4-nitrobenzaldehyde-containing DES gave the highest solubility of 4-nitrobenzaldehyde, thereby resulting in the fastest reaction and a final yield of 1296.5 mmol/mL, corresponding to a 71% yield and 82% conversion after 32 h when acetone was employed as the cosolvent. Moreover, Zheng et al. developed a biocatalyzed nitro Aldol reaction (Henry reaction) (Zheng et al. 2016), where the excellent catalytic activity in the reaction between aromatic aldehydes and nitromethane in a ChCl/glycerol (1:2) system was observed using a lipase derived from *Aspergillus niger* (lipase AS) as the catalyst (Fig. 5b). It is also noteworthy that water plays a key role in improving enzyme activity of the above reaction, with fluorescence analysis suggesting that the addition of water enhances the reaction rate through the synergistic effects of the extensive hydrogen bonding network. Indeed, a 92% yield was achieved within 4 h. Although an aza-Henry reaction has been carried out using the same methodology, no enantioselectivity was observed in the transformation.



**Fig. 5** Examples of lipase-catalyzed Aldol reactions that can be carried out in DESs

## Conclusions

To date, many lipase-catalyzed reactions have been carried out in deep eutectic solvents (DESs), and these novel solvent systems are now regarded as alternate reaction media for various enzyme classes in the development of more sustainable processes. Additionally, they are particularly useful for application in the bioconversion of substrates with limited solubilities in volatile organic solvents or in water alone. Optimal reaction yields were obtained in these systems when the DESs were formed using the substrates of the enzymatic reaction. In addition, proof-of-concept studies have revealed the high stabilities and activities of lipases in aqueous DESs containing high substrate loads, thereby indicating their suitability as valuable solvents for use in the production of bioactive and sustainable chemicals and products.

However, many questions remain concerning the use of DESs that warrant further investigation. For example, the intriguing role of water as a cosolvent in biocatalytic reactions carried out in DESs merits further investigation since the addition of water has been found to significantly improve reaction yields. Furthermore, the relationship between the nature of the DESs and their influence on the enzyme stability and activity must be studied at the molecular level to gain insight into the interactions taking place between the enzymes and the DESs. The underlying mechanisms that allow the facile separation of the products from DES reaction media and the efficient reuse of any unconverted substrates also require further investigation. Importantly, other perspectives, including the rational design of immobilized lipases and their use in industrial applications for the development of biocatalytic process in DESs must be examined further to expand the scope and use of DESs in enabling biocatalysis.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical statement** This work does not contain any studies with human participants or animals performed by any of the authors.

## References

- Abbott AP, Capper G, Davies DL, Rasheed RK, Tambyrajah V (2003) Novel solvent properties of choline chloride/urea mixtures. *Chem Commun* 49:70–71. <https://doi.org/10.1039/b210714g>
- Alonso DA, Baeza A, Chinchilla R, Guillena G, Pastor IM, Ramón DJ (2016) Deep eutectic solvents: the organic reaction medium of the century. *Eur J Org Chem* 2016:612–632. <https://doi.org/10.1002/ejoc.201501197>
- Andler SM, Wang L-S, Rotello VM, Goddard JM (2017) Influence of hierarchical interfacial assembly on lipase stability and performance in deep eutectic solvent. *J Agr Food Chem* 65:1907–1914. <https://doi.org/10.1021/acs.jafc.6b05372>
- Ankaya A, Ünlü AE, Takaç S (2019) Use of deep eutectic solvents in the enzyme catalysed production of ethyl lactate. *Process Biochem* 84: 53–59. <https://doi.org/10.1016/j.procbio.2019.06.003>
- Barbosa O, Torres R, Ortiz C, Berenguer-Murcia Á, Rodrigues RC, Fernandez-Lafuente R (2019) Heterofunctional supports in enzyme immobilization: from traditional immobilization protocols to opportunities in tuning enzyme properties. *Biomacromolecules* 14(8): 2433–2462. <https://doi.org/10.1021/bm400762h>
- Bubalo CM, Tušek AJ, Vinkovič L, Radošević K, SrLek VG, Redovnikovič IR (2015) Cholinium-based deep eutectic solvents and ionic liquids for lipase-catalyzed synthesis of butyl acetate. *J Mol Catal B-Enzym* 122:188–198. <https://doi.org/10.1016/j.molcatb.2015.09.005>
- Cao S-L, Deng X, Xu P, Huang Z-X, Zhou J, Li X-H, Zong M-H, Lou W-Y (2017) Highly efficient enzymatic acylation of dihydromyricetin by the immobilized lipase with deep eutectic solvents as cosolvent. *J Agr Food Chem* 65:2084–2088. <https://doi.org/10.1021/acs.jafc.7b00011>
- Cruz H, Jordão N, Branco LC (2017) Deep eutectic solvents (DESs) as low-cost and green electrolytes for electrochromic devices. *Green Chem* 19:1653–1658. <https://doi.org/10.1039/c7gc00347a>
- Dai Y, Witkamp G-J, Verpoorte R, Choi YH (2013) Natural deep eutectic solvents as a new extraction media for phenolic metabolites in *Carthamus tinctorius* L. *Anal Chem* 85:6272–6278. <https://doi.org/10.1021/ac400432p>
- Dominguez de Maria P, Hollmann F (2015) On the (un) greenness of biocatalysis: some challenging figures and some promising options. *Front Microbiol* 6:1257. <https://doi.org/10.3389/fmicb.2015.01257>
- Durand E, Lecomte J, Baréa B, Piombo G, Dubreucq E, Villeneuve P (2012) Evaluation of deep eutectic solvents as new media for *Candida antarctica* B lipase catalyzed reactions. *Process Biochem* 47:2081–2089. <https://doi.org/10.1016/j.procbio.2012.07.027>
- Durand E, Lecomte J, Baréa B, Dubreucq E, Lortie R, Villeneuve P (2013a) Evaluation of deep eutectic solvent–water binary mixtures for lipase-catalyzed lipophilization of phenolic acids. *Green Chem* 15:2275. <https://doi.org/10.1039/c3gc40899j>
- Durand E, Lecomte J, Villeneuve P (2013b) Deep eutectic solvents: synthesis, application, and focus on lipase-catalyzed reactions. *Eur J Lipid Sci Tech* 115:379–385. <https://doi.org/10.1002/ejlt.201200416>
- Durand E, Lecomte J, Baréa B, Villeneuve P (2014) Towards a better understanding of how to improve lipase-catalyzed reactions using deep eutectic solvents based on choline chloride. *Eur J Lipid Sci Tech* 116(1):16–23. <https://doi.org/10.1002/ejlt.201300246>
- Durand E, Lecomte J, Villeneuve P (2017) The biological and antimicrobial activities of phenolipids. *Lipid Technol* 29:67–70. <https://doi.org/10.1002/lite.201700019>
- Dwivedee BP, Soni S, Sharma M, Bhaumik J, Laha JK, Banerjee UC (2018) Promiscuity of lipase-catalyzed reactions for organic synthesis: a recent update. *ChemistrySelect* 3:2441–2466. <https://doi.org/10.1002/slct.201702954>
- Fotiadou R, Patila M, Hammami MA, Enotiadis A, Moschovas D, Tsiarka K, Spyrou K, Giannelis EP, Avgeropoulos A, Paipetis A, Gournis D, Stamatis H (2019) Development of effective lipase-hybrid nanoflowers enriched with carbon and magnetic nanomaterials for biocatalytic transformations. *Nanomaterials* 9(6). <https://doi.org/10.3390/nano9060808>

- Fredes Y, Chamorro L, Cabrera Z (2019) Increased selectivity of Novozym 435 in the asymmetric hydrolysis of a substrate with high hydrophobicity through the use of deep eutectic solvents and high substrate concentrations. *Molecules* 24:doi:<https://doi.org/10.3390/molecules24040792>. <https://doi.org/10.3390/molecules24040792>
- Gomes JM, Silva SS, Reis RL (2019) Biocompatible ionic liquids: fundamental behaviours and applications. *Chem Soc Rev* 48:4317–4335. <https://doi.org/10.1039/c9cs00016j>
- González-Martínez D, Gotor V, Gotor-Fernández V (2016) Application of deep eutectic solvents in promiscuous lipase-catalysed Aldol reactions. *Eur J Org Chem* 2016:1513–1519. <https://doi.org/10.1002/ejoc.201501553>
- Gorke JT, Srienc F, Kazlauskas RJ (2008) Hydrolase-catalyzed biotransformations in deep eutectic solvents. *Chem Commun*:1235–1237. <https://doi.org/10.1039/b716317g>
- Gorke JT, Srienc F, Kazlauskas RJ (2010) Deep eutectic solvents for *Candida Antarctica* lipase B-catalyzed reactions. *Pharmaceuticals, Therapeutics, and Biotechnology, Ionic Liquid Applications*, pp 169–180. <https://doi.org/10.1021/bk-2010-1038.ch014>
- Gotor-Fernández V, Paul CE (2019) Deep eutectic solvents for redox biocatalysis. *J Biotechnol* 293:24–35. <https://doi.org/10.1016/j.jbiotec.2018.12.018>
- Gu Y, Jerome F (2013) Bio-based solvents: an emerging generation of fluids for the design of eco-efficient processes in catalysis and organic chemistry. *Chem Soc Rev* 42(24):9550–9570. <https://doi.org/10.1039/c3cs60241a>
- Guajardo N, Domínguez de María P (2019) Continuous biocatalysis in environmentally-friendly media: a triple synergy for future sustainable processes. *ChemCatChem* 11(14):3128–3137. <https://doi.org/10.1002/cctc.201900773>
- Guajardo N, Müller CR, Schrebler R, Carlesi C, Domínguez de María P (2016) Deep eutectic solvents for organocatalysis, biotransformations, and multistep organocatalyst/enzyme combinations. *ChemCatChem* 8(6):1020–1027. <https://doi.org/10.1002/cctc.201501133>
- Guajardo N, Domínguez de María P, Ahumada K, Schrebler RA, Ramírez-Tagle R, Crespo FA, Carlesi C (2017) Water as cosolvent: nonviscous deep eutectic solvents for efficient lipase-catalyzed esterifications. *ChemCatChem* 9(8):1393–1396. <https://doi.org/10.1002/cctc.201601575>
- Guajardo N, Ahumada K, Domínguez de María P, Schrebler RA (2018) Remarkable stability of *Candida antarctica* lipase B immobilized via cross-linking aggregates (CLEA) in deep eutectic solvents. *Biocatal Biotransfor* 37(2):106–114. <https://doi.org/10.1080/10242422.2018.1492567>
- Guajardo N, Schrebler RA, Domínguez de María P (2019) From batch to fed-batch and to continuous packed-bed reactors: lipase-catalyzed esterifications in low viscous deep-eutectic-solvents with buffer as cosolvent. *Bioresour Technol* 273:320–325. <https://doi.org/10.1016/j.biortech.2018.11.026>
- Hou XL, Tong Q, Wang WQ, Shi CY, Xiong W, Chen J, Liu X, Fang JG (2015) Suppression of inflammatory responses by dihydromyricetin, a flavonoid from *Ampelopsis grossedentata*, via inhibiting the activation of NF-kappa B and MAPK signaling pathways. *J Nat Prod* 78:1689–1696. <https://doi.org/10.1021/acs.jnatprod.5b00275>
- Huang Z-L, Wu B-P, Wen Q, Yang T-X, Yang Z (2014) Deep eutectic solvents can be viable enzyme activators and stabilizers. *J Chem Technol Biotechnol* 89:1975–1981. <https://doi.org/10.1002/jctb.4285>
- Huang J, Guo X, Xu T, Fan L, Zhou X, Wu S (2019) Ionic deep eutectic solvents for the extraction and separation of natural products. *J Chromatogr A* 1598:1–19. <https://doi.org/10.1016/j.chroma.2019.03.046>
- Hümmer M, Kara S, Liese A, Huth I, Schrader J, Holtmann D (2018) Synthesis of (–)-menthol fatty acid esters in and from (–)-menthol and fatty acids—novel concept for lipase catalyzed esterification based on eutectic solvents. *Mol Catal* 458:67–72. <https://doi.org/10.1016/j.mcat.2018.08.003>
- Itoh T (2017) Ionic liquids as tool to improve enzymatic organic synthesis. *Chem Rev* 117:10567–10607
- Juneidi I, Hayyan M, Hashim MA, Hayyan A (2017) Pure and aqueous deep eutectic solvents for a lipase-catalysed hydrolysis reaction. *Biochem Eng J* 117:129–138. <https://doi.org/10.1016/j.bej.2016.10.003>
- Juneidi I, Hayyan M, Hashim MA (2018) Intensification of biotransformations using deep eutectic solvents: overview and outlook. *Process Biochem* 66:33–60. <https://doi.org/10.1016/j.procbio.2017.12.003>
- Kim SH, Park S, Yu H, Kim JH, Kim HJ, Yang Y-H, Kim YH, Kim KJ, Kan E, Lee SH (2016) Effect of deep eutectic solvent mixtures on lipase activity and stability. *J Mol Catal B-Enzym* 128:65–72. <https://doi.org/10.1016/j.molcatb.2016.03.012>
- Kleiner B, Schörken U (2014) Native lipase dissolved in hydrophilic green solvents: a versatile 2-phase reaction system for high yield ester synthesis. *Eur J Lipid Sci Technol* 117:167–177. <https://doi.org/10.1002/ejlt.201400494>
- Kleiner B, Fleischer P, Schörken U (2016) Biocatalytic synthesis of biodiesel utilizing deep eutectic solvents: a two-step-one-pot approach with free lipases suitable for acidic and used oil processing. *Process Biochem* 51:1808–1816. <https://doi.org/10.1016/j.procbio.2015.10.016>
- Kumar A, Dhar K, Kanwar SS, Arora PK (2016) Lipase catalysis in organic solvents: advantages and applications. *Biol Proced Online* 18:1–11. <https://doi.org/10.1186/s12575-016-0033-2>
- Liang H, Qin X, Tan CP, Li D, Wang Y (2018) Choline-chloride-based eutectic solvent for the efficient production of docosahexaenoyl and eicosapentaenoyl ethanolamides via an enzymatic process. *J Agr Food Chem* 66:12361–12367. <https://doi.org/10.1021/acs.jafc.8b04804>
- Liu X, Meng X-Y, Xu Y, Dong T, Zhang D-Y, Guan H-X, Zhuang Y, Wang J (2019) Enzymatic synthesis of 1-caffeoylglycerol with deep eutectic solvent under continuous microflow conditions. *Biochem Eng J* 142:41–49. <https://doi.org/10.1016/j.bej.2018.11.007>
- Lozano P, Alvarez E, Nieto S, Villa R, Bernal JM, Donaire A (2019) Biocatalytic synthesis of panthenyl monoacyl esters in ionic liquids and deep eutectic solvents. *Green Chem* 21:3353–3361. <https://doi.org/10.1039/c9gc01076a>
- Ma Y, Li P, Li Y, Willot SJP, Zhang W, Ribitsch D, Choi YH, Verpoorte R, Zhang T, Hollmann F, Wang Y (2019) Natural deep eutectic solvents as multifunctional media for the valorization of agricultural wastes. *ChemSusChem* 12:1310–1315. <https://doi.org/10.1002/cssc.201900043>
- Merza F, Fawzy A, AlNashef I, Al-Zuhair S, Taher H (2018) Effectiveness of using deep eutectic solvents as an alternative to conventional solvents in enzymatic biodiesel production from waste oils. *Energy Rep* 4:77–83. <https://doi.org/10.1016/j.egy.2018.01.005>
- Mewalal R, Rai DK, Kainer D, Chen F, Kulheim C, Peter GF, Tuskan GA (2017) Plant-derived terpenes: a feedstock for specialty biofuels. *Trends Biotechnol* 35:227–240. <https://doi.org/10.1016/j.tibtech.2016.08.003>
- Milker S, Pätzold M, Bloh JZ, Holtmann D (2019) Comparison of deep eutectic solvents and solvent-free reaction conditions for aldol production. *Mol Catal* 466:70–74. <https://doi.org/10.1016/j.mcat.2019.01.012>

- Monhemi H, Housaindokht MR, Moosavi-Movahedi AA, Bozorgmehr MR (2014) How a protein can remain stable in a solvent with high content of urea: insights from molecular dynamics simulation of *Candida antarctica* lipase B in urea:choline chloride deep eutectic solvent. *Phys Chem Chem Phys* 16:14882–14893. <https://doi.org/10.1039/c4cp00503a>
- Muhammad N, Elsheikh YA, Mutalib MIA, Bazmi AA, Khan RA, Khan H, Rafiq S, Man Z, Khan I (2015) An overview of the role of ionic liquids in biodiesel reactions. *J Ind Eng Chem* 21:1–10. doi:<https://doi.org/10.1016/j.jiec.2014.01.046>
- Nian B, Cao C, Liu Y (2019) Activation and stabilization of *Candida antarctica* lipase B in choline chloride-glycerol-water binary system via tailoring the hydrogen-bonding interaction. *Int J Biol Macromol* 136:1086–1095. <https://doi.org/10.1016/j.ijbiomac.2019.06.150>
- Oh Y, Park S, Yoo E, Jo S, Hong J, Kim HJ, Kim KJ, Oh KK, Lee SH (2019) Dihydrogen-bonding deep eutectic solvents as reaction media for lipase-catalyzed transesterification. *Biochem Eng J* 142:34–40. <https://doi.org/10.1016/j.bej.2018.11.010>
- Pant PL, Shankarling GS (2017) Deep eutectic solvent/lipase: two environmentally benign and recyclable Media for Efficient Synthesis of *N*-aryl amines. *Catal Lett* 147:1371–1378. <https://doi.org/10.1007/s10562-017-2046-0>
- Pätzold M, Burek BO, Liese A, Bloh JZ, Holtmann D (2019a) Product recovery of an enzymatically synthesized (–)-menthol ester in a deep eutectic solvent. *Bioprocess Biosyst Eng* 42(8):1385–1389. <https://doi.org/10.1007/s00449-019-02125-6>
- Pätzold M, Siebenhaller S, Kara S, Liese A, Syltatk C, Holtmann D (2019b) Deep eutectic solvents as efficient solvents in biocatalysis. *Trends Biotechnol* 37(9):943–959. <https://doi.org/10.1016/j.tibtech.2019.03.007>
- Pavlačková J, Egner P, Sedláček T, Mokrejš P, Sedlářiková J, Polášková J (2019) In vivo efficacy and properties of semisolid formulations containing panthenol. *J Cosmet Dermatol* 18:346–354. <https://doi.org/10.1111/jocd.12527>
- Perales E, Lomba L, García-Escudero M, Sarasa E, Lafuente CE, Giner B (2018) Toxicological study of some ionic liquids. *Green Process Synth* 7:287–295. <https://doi.org/10.1515/gps-2017-0031>
- Perez-Sanchez M, Sandoval M, Hernaiz MJ, Domínguez de María P (2013) Biocatalysis in biomass-derived solvents: the quest for fully sustainable chemical processes. *Curr Org Chem* 17:1188–1199. <https://doi.org/10.2174/1385272811317110006>
- Ranganathan S, Zeithofer S, Sieber V (2017) Development of a lipase-mediated epoxidation process for monoterpenes in choline chloride-based deep eutectic solvents. *Green Chem* 19:2576–2586. <https://doi.org/10.1039/c7gc01127j>
- Shang X, Tan J-N, Du Y, Liu X, Zhang Z (2018) Environmentally-friendly extraction of flavonoids from *Cyclocarya paliurus* (Batal.) Iljinskaja leaves with deep eutectic solvents and evaluation of their antioxidant activities. *Molecules* 23:2110. <https://doi.org/10.3390/molecules23092110>
- Shang X, Dou Y, Zhang Y, Tan J-N, Liu X, Zhang Z (2019) Tailor-made natural deep eutectic solvents for green extraction of isoflavones from chickpea (*Cicer arietinum* L.) sprouts. *Ind crop prod* 140:111724. <https://doi.org/10.1016/j.indcrop.2019.111724>
- Sheldon RA (2016) Biocatalysis and biomass conversion in alternative reaction media. *Chem Eur J* 22:12984–12999. <https://doi.org/10.1002/chem.201601940>
- Sheldon RA, van Pelt S (2013) Enzyme immobilisation in biocatalysis: why, what and how. *Chem Soc Rev* 42(15):6223–6235. <https://doi.org/10.1039/c3cs60075k>
- Sheldon RA, Woodley JM (2018) Role of biocatalysis in sustainable chemistry. *Chem Rev* 118:801–838. <https://doi.org/10.1021/acs.chemrev.7b00203>
- Shi Y-G, Bian L-Q, Zhu Y-J, Zhang R-R, Shao S-Y, Wu Y, Chen Y-W, Dang Y-L, Ding Y, Sun H (2019) Multifunctional alkyl ferulate esters as potential food additives: antibacterial activity and mode of action against *Listeria monocytogenes* and its application on American sturgeon caviar preservation. *Food Control* 96:390–402. <https://doi.org/10.1016/j.foodcont.2018.09.030>
- Siebenhaller S, Hajek T, Muhle-Goll C, Himmelsbach M, Luy B, Kirschhöfer F, Brenner-Weiß G, Hahn T, Zibek S, Syltatk C (2017) Beechwood carbohydrates for enzymatic synthesis of sustainable glycolipids. *Bioresour Bioprocess* 4:1–9. <https://doi.org/10.1186/s40643-017-0155-7>
- Siebenhaller S, Gentes J, Infantes A, Muhle-Goll C, Kirschhöfer F, Brenner-Weiß G, Ochsenreither K, Syltatk C (2018a) Lipase-catalyzed synthesis of sugar esters in honey and Agave syrup. *Front Chem* 6:24. <https://doi.org/10.3389/fchem.2018.00024>
- Siebenhaller S, Kirchhoff J, Kirschhöfer F, Brenner-Weiß G, Muhle-Goll C, Luy B, Haitz F, Hahn T, Zibek S, Syltatk C, Ochsenreither K (2018b) Integrated process for the enzymatic production of fatty acid sugar esters completely based on lignocellulosic substrates. *Front Chem* 6:21. <https://doi.org/10.3389/fchem.2018.00421>
- Smith EL, Abbott AP, Ryder KS (2014) Deep eutectic solvents (DESS) and their applications. *Chem Rev* 114:11060–11082. <https://doi.org/10.1021/cr300162p>
- Soh L, Eckelman MJ (2016) Green solvents in biomass processing. *ACS Sustain Chem Eng* 4:5821–5837. <https://doi.org/10.1021/acssuschemeng.6b01635>
- Thamke VR, Chaudhari AU, Tapase SR, Paul D, Kodam KM (2019) In vitro toxicological evaluation of ionic liquids and development of effective bioremediation process for their removal. *Environ Pollut* 250:567–577. <https://doi.org/10.1016/j.envpol.2019.04.043>
- Tomé LIN, Baião V, da Silva W, Brett CMA (2018) Deep eutectic solvents for the production and application of new materials. *Appl Mater Today* 10:30–50. <https://doi.org/10.1016/j.apmt.2017.11.005>
- Wang X-P, Zhou P-F, Li Z-G, Yang B, Hollmann F, Wang Y-H (2017) Engineering a lipase B from *Candida antarctica* with efficient perhydrolysis performance by eliminating its hydrolase activity. *Sci Rep* 7:44599. <https://doi.org/10.1038/srep44599>
- Xu P, Zheng G-W, Du P-X, Zong M-H, Lou W-Y (2015) Whole-cell biocatalytic processes with ionic liquids. *ACS Sustain Chem Eng* 4:371–386. <https://doi.org/10.1021/acssuschemeng.5b00965>
- Xu L, Zhang L, Li D, Liu P, Tan C-P, Wang W, Liu X, Yang B, Lan D, Wang Y (2017) Deep eutectic solvents enable the enhanced production of n-3 PUFA-enriched triacylglycerols. *Eur J Lipid Sci Tech* 119(12). <https://doi.org/10.1002/ejlt.201700300>
- Zainal-Abidin MH, Hayyan M, Hayyan A, Jayakumar NS (2017) New horizons in the extraction of bioactive compounds using deep eutectic solvents: a review. *Anal Chim Acta* 979:1–23. <https://doi.org/10.1016/j.aca.2017.05.012>
- Zeng C-X, Qi S-J, Xin R-P, Yang B, Wang Y-H (2015) Enzymatic selective synthesis of 1,3-DAG based on deep eutectic solvent acting as substrate and solvent. *Bioprocess Biosyst Eng* 38:2053–2061. <https://doi.org/10.1007/s00449-015-1445-0>
- Zeng J, Dou Y, Yan N, Li N, Zhang H, Tan J-N (2019) Optimizing ultrasound-assisted deep eutectic solvent extraction of bioactive compounds from Chinese wild rice. *Molecules* 24:2718. <https://doi.org/10.3390/molecules24152718>
- Zhang Q, De Oliveira VK, Royer S, Jerome R (2012) Deep eutectic solvents: syntheses, properties and applications. *Chem Soc Rev* 41:7108–7146. <https://doi.org/10.1039/c2cs35178a>
- Zhao H, Zhang C, Crittle TD (2013) Choline-based deep eutectic solvents for enzymatic preparation of biodiesel from soybean oil. *J Mol Catal B-Enzym* 85:243–247. <https://doi.org/10.1016/j.molcatb.2012.09.003>

- Zhao K-H, Cai Y-Z, Lin X-S, Xiong J, Halling PJ, Zhen Y (2016) Enzymatic synthesis of glucose-based fatty acid esters in bisolvent systems containing ionic liquids or deep eutectic solvents. *Molecules* 21:1294. <https://doi.org/10.3390/molecules21101294>
- Zheng L, Zhang S, Tian X (2016) Enzyme-catalyzed Henry reaction in choline chloride-based deep eutectic solvents. *J Microbiol Biotechnol* 26:80–88. <https://doi.org/10.4014/jmb.1506.06075>
- Zhou P, Wang X, Zeng C, Wang W, Yang B, Hollmann F, Wang Y (2017a) Deep eutectic solvents enable more robust chemoenzymatic epoxidation reactions. *ChemCatChem* 9:934–936. <https://doi.org/10.1002/cctc.201601483>
- Zhou P, Wang X, Yang B, Hollmann F, Wang Y (2017b) Chemoenzymatic epoxidation of alkenes with *Candida antarctica* lipase B and hydrogen peroxide in deep eutectic solvents. *RSC Adv* 7:12518–12523. <https://doi.org/10.1039/c7ra00805h>

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