Natural deep eutectic solvents as performance additives for biocatalysis

Erol, Özlem; Hollmann, Frank

Citation (APA)
Erol, Ö., & Hollmann, F. (2021). Natural deep eutectic solvents as performance additives for biocatalysis. In R. Verpoorte, G.-J. Witkamp, & Y. H. Choi (Eds.), *Eutectic Solvents and Stress in Plants* (Vol. 97, pp. 95-132). (Advances in Botanical Research; Vol. 97). Academic Press. https://doi.org/10.1016/bs.abr.2020.09.004

Important note
To cite this publication, please use the final published version (if applicable).
Please check the document version above.

Running title: Biocatalysis in NADES

Title: Natural Deep Eutectic solvents as performance additives for biocatalysis

Authors: Özlem Erol,[1] Frank Hollmann[2]

Affiliations: [1] Plant Sciences & Natural Products, Institute of Biology, University of Leiden, The Netherlands, o.erol@biology.leidenuniv.nl; [2] Department of Biotechnology, Delft University of Technology, The Netherlands, f.hollmann@tudelft.nl

Keywords: Deep Eutectic Solvents; Biocatalysis; Enzymes

Abstract:

Following ionic liquids, (natural) deep eutectic solvents ((NA)DES) are receiving significant attention as performance additives for biocatalytic reactions.

(NA)DES are increasingly evaluated as solvents to replace water in hydrolase-catalysed esterification reactions thereby shifting the reaction equilibrium. They also frequently outperform water in terms of solubility properties of hydrophobic reagents and thereby enable higher space-time yields. Furthermore, (NA)DES frequently exceed stabilising effects on enzymes and thereby enable more robust (and therefore economically more attractive) biocatalytic syntheses.

In this contribution, we will summarise and critically evaluate the recent literature on (NA)DES-supported biocatalysis.

Manuscript text:

1. Introduction

Cells, microbial and those of higher organisms, are roughly composed of 70% water while the remaining 30% share out to proteins (15%), DNA (1%), RNA (6%), (Phospo)lipids (2%) polysaccharides (2%) and small molecules (4%).(anonymous, 2014) Therefore, microbial cells are broadly seen as aqueous solutions of these components. Even though this model may be too simplistic and the interior of a cell should be more seen as a gel rather than a dilute aqueous solution, biocatalysis is traditionally performed in aqueous media. This approach, however, severely limits the broad applicability of biocatalysis for the synthesis of useful chemicals as many of them are rather hydrophobic and therefore poorly soluble in aqueous media. Dilute product mixtures of a few grams per litre reaction broth, however, are not attractive neither from an economic, not environmental point-of-view. Therefore, limitation to aqueous reaction media poses a severe limitation *en route* to a broad applicability of biocatalysis for chemical synthesis.

Pioneering works by Klibanov and coworkers(Dordick, Marletta, & Klibanov, 1986; Zaks & Klibanov, 1984, 1985) that demonstrated that enzymes can be active under non-aqueous conditions first received mostly academic interest. In recent years, however, the interest in neoteric solvents for biocatalysis has been increasing steadily.

Following the, now fallen from grace, ionic liquids, deep eutectic solvents are enjoying a rapidly increasing popularity in the biocatalysis community as biobased and non-toxic alternatives.(Durand, Lecomte, & Villeneuve, 2013; Gotor-Fernández & Paul, 2019; Ibn Majdoub Hassani, Amzazi, & Lavandera, 2019; Kourist & González-Sabín, 2020; María, Guajardo, & Kara, 2020; Mbous et al., 2017; M. Pätzold et al., 2019; Perna, Vitale, & Capriati, 2020; Tan & Dou, 2020; Xu, Zheng, Zong, Li, & Lou, 2017) The aim of this chapter is to critically summarise the current efforts on establishing, understanding and applying DES as neoteric solvents for biocatalysis.

Kazlauskas and coworkers probably were the first ones to use DES as solvents for biocatalytic reactions,(Gorke, Srienc, & Kazlauskas, 2008) demonstrating that several lipases catalyse the transesterification of ethyl valerate to butyl valerate. These authors, however, also pointed out one possible (undesired) side reaction in this reaction, i.e. the participation of the solvent (e.g. ethylene glycol- or glycerol-based DES) in the lipase-catalysed reaction. Interestingly, probably to thermodynamic stabilisation in the DES-typical H-bond network, DES components such as ethylene glycol or glycerol were significantly less reactive than expected.

Ever since these pioneering works, the number of reports on biocatalysis in DES has been increasing steadily (Figure 1).
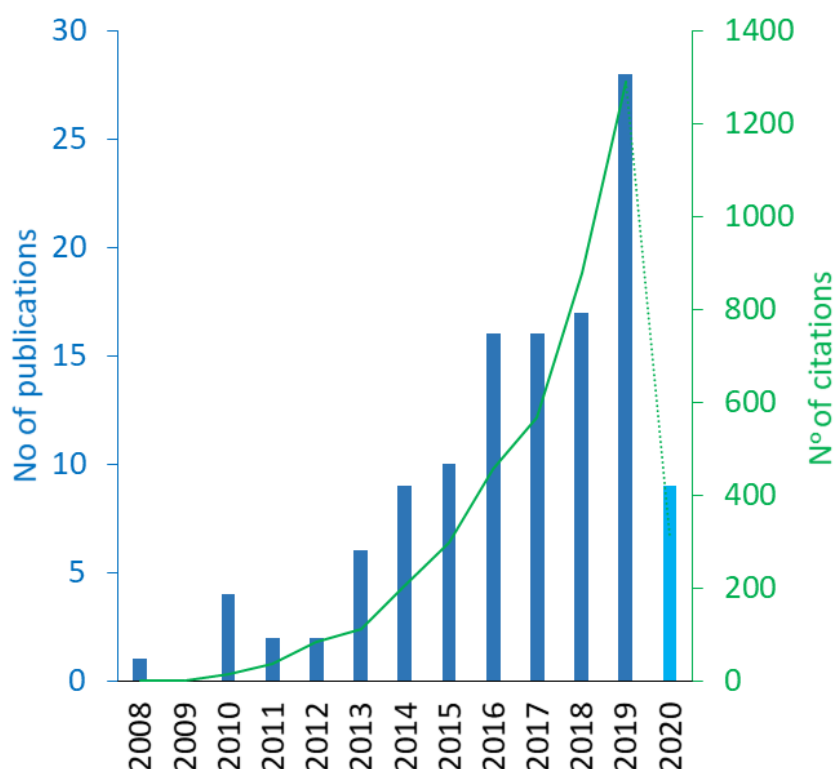

Figure 1. Publications (blue bars) and their citations (green line) found within web of knowledge using the search terms 'Deep Eutectic Solvent' and 'Biocatalysis', accessed on 29.03.2020.

2. Natural Deep Eutectic Solvents in Biocatalysis

Table 1 gives a representative, yet incomplete overview over the manifold applications of DES as solvents for biocatalytic reactions.

DES are rarely used as 'neat solvents' and often water (buffer)/DES mixtures give best results as compared to 'anhydrous' conditions. On the one hand, this may be ascribed to enzymes needing a certain amount of (non-bulk) water to maintain activity (water as lubricant).(María et al., 2020) On the other hand, the high viscosity of many DES necessitates dilution (with water) to attain acceptable viscosities for practical application. Above approximately 50% (v/v) of water as the reaction medium DES are more characterised as 'performance additives' rather than as co-solvent.

In the following, we therefore focus on aspects of DESs with a clear advantage over existing reaction systems.

Clearly, one exciting aspect of DESs is their tuneable solvent properties (hydrophilicity, hydrophobicity). Carbohydrates, for example, are best soluble in aqueous media. If, however, the esterification of carbohydrates with carboxylic acids is the desired reaction, water is a very unfavourable (co-)solvent due to the unfavourable equilibrium of esterifications in aqueous media. In this respect, carbohydrate-based DES (in which the carbohydrates are liquefied in the absence of water) are very promising alternative solvents for the synthesis of glycolipids e.g. as surfactants (Scheme 1).(Siebenhaller et al., 2018; Siebenhaller et al., 2016)
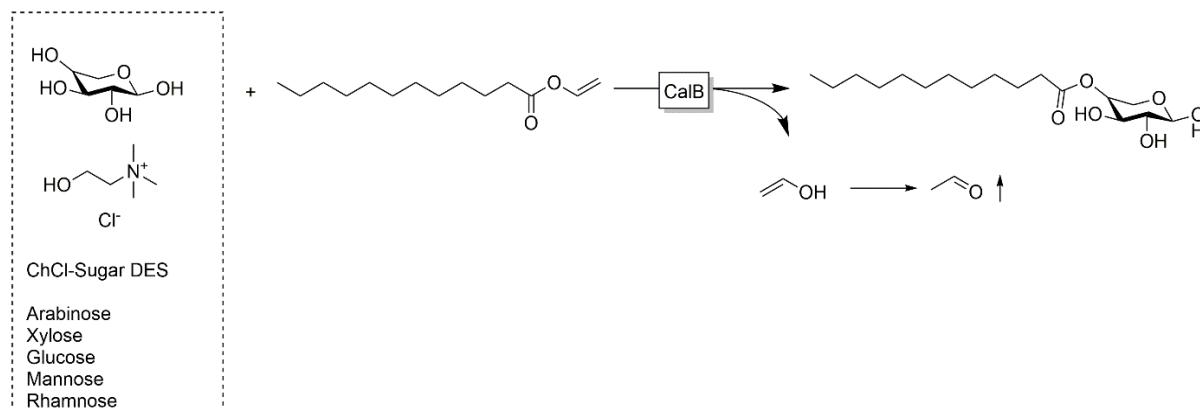
Scheme 1. Using carbohydrate-based DES as solvent for the synthesis of glycolipids.

Another class of reactions that is severely hampered by the presence of water is the amidation of

carboxylic acid esters as catalysed by amidases. In this reaction, the amine group of the starting

amine nucleophilically attacks the carboxylate group of the acid ester, eventually substituting the

ester alcohol and forming the desired amide. Water, however, competes as nucleophile yielding the

free carboxylic acid, yielding thermodynamically stable and kinetically inert carboxylate salts of the

starting amine (Scheme 2).
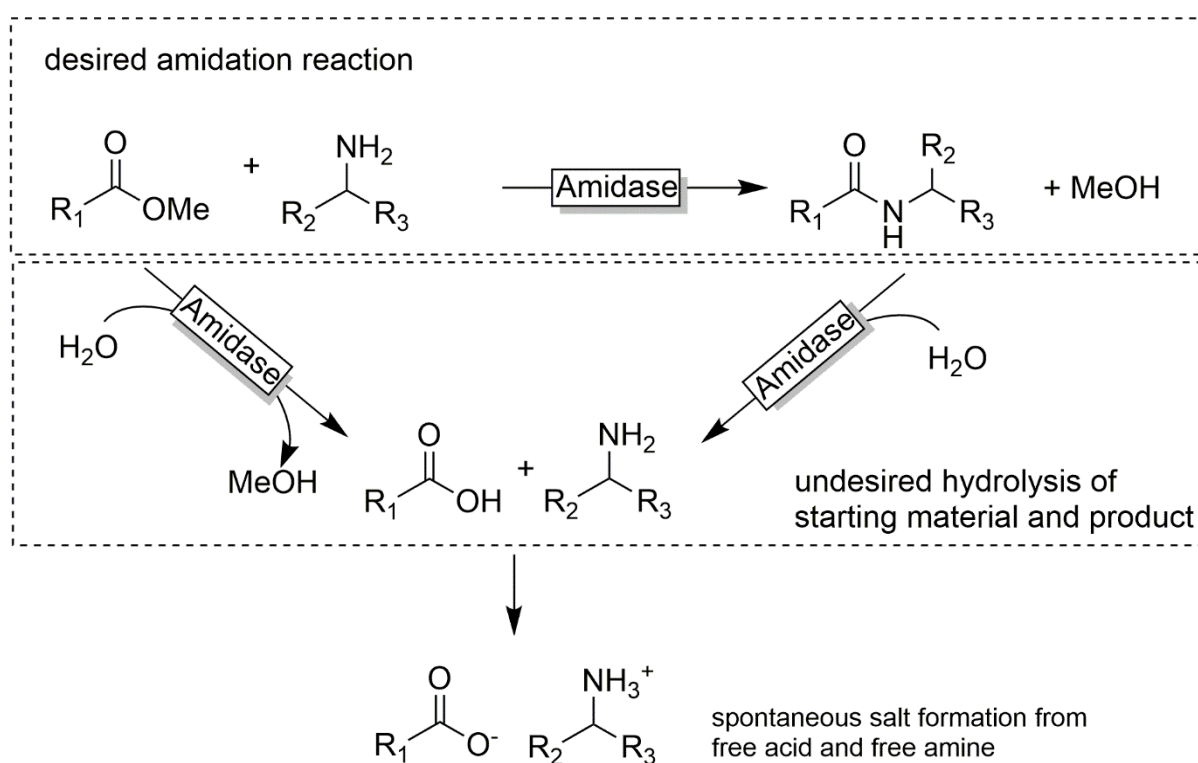
Scheme 2. Amidase-catalysed acylation of amines using carboxylic acids and amines. In aqueous media also hydrolysis of
the starting ester and the desired amide occur yielding the free acid and amine, which spontaneously form
thermodynamically and kinetically inert salts.

Performing enzymatic amidation reactions in DES offers the possibility of significantly reduced water

contents, leading to increased yields of the desired amide products. This has been successfully

exploited in the Chymotrypsin-(Zaira Maugeri, Leitner, & Domínguez de María, 2013) or Papain-(Cao,

Xu, Li, Lou, & Zong, 2015) catalysed synthesis of dipeptides or in the penicillin acylase catalysed

synthesis of the antibiotic Cefaclor.(X. Wu et al., 2019)

The aforementioned tuneable solvent properties of DES can be exploited to increase the substrate

loadings (and eventually the product titres). Rutin, for example, is practically insoluble in aqueous

media while its solubility in aconitic acid-choline chloride is more than 80 g $kg^{-1}_{solvent}$.(Choi et al.,

2011)

The Gotor-Fernández group, for example, established ChCl-Gly-based DES as solvent for

benzaldehyde and other hydrophobic ketones to perform aldol reactions with molar concentrations

of these reagents (Scheme 3).(González-Martínez, Gotor, & Gotor-Fernández, 2016)

EWG: o-, m-, p-NO$_2$,
p-CN, p-CF$_3$
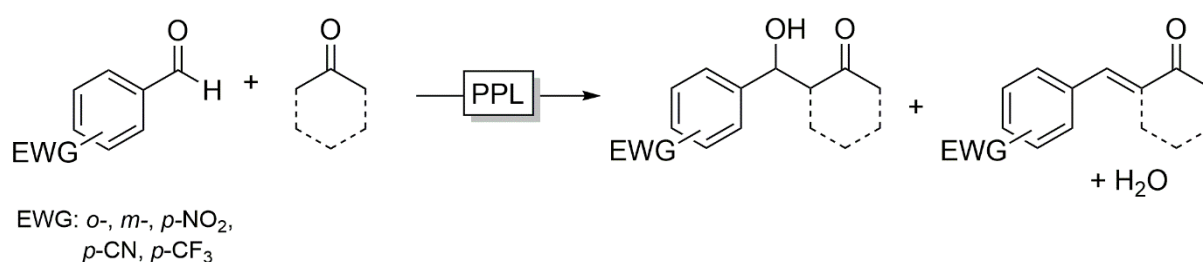
Scheme 3. Aldol reaction (condensation) catalysed by porcine pancreatic lipase (PPL) in ChCl-Gly-DES.

These results were confirmed later by Holtmann and coworkers. (Milker, Pätzold, Bloh, & Holtmann,

2019) These authors, however also found that the highest productivities were found in the absence

of DES and using acetone (one of the reagents) as solvent.

Further examples wherein DES enable higher substrate loadings are shown in Table 1.

The broad variability of components of which DES can be formed from also opens up a remarkable

extension of DES beyond the mere solvent application: DES in a dual function as solvent and starting

material for the (biocatalytic) reaction.

Holtmann and coworkers, for example, reported an esterification reaction of menthol and various

fatty acids, forming a DES and hence providing a suitable medium for the solvent-free synthesis of

menthol fatty acid esters (Scheme 4).(Hümmer et al., 2018) Addition of water increased the catalytic

activity of the biocatalyst, which was attributed to the formation of a two liquid phase system and

the resulting activity increase of the lipase due to interfacial activation. Under optimised conditions,

full conversion of e.g. lauric acid and molar concentrations of the desired product have been

achieved.(Pätzold, Weimer, Liese, & Holtmann, 2019)
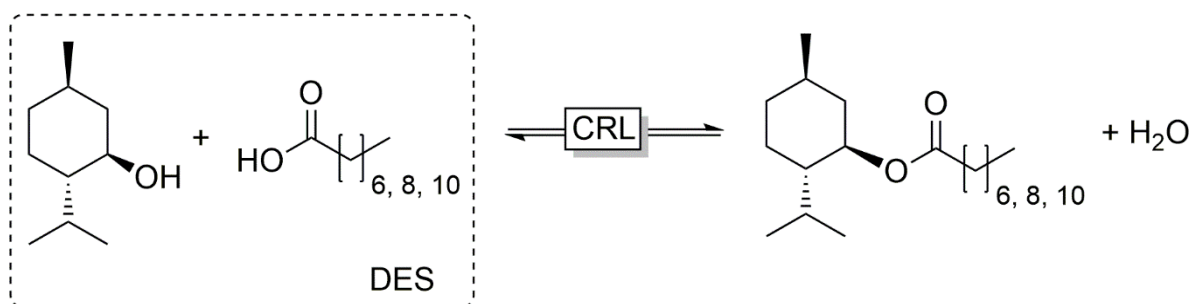
Scheme 4. Using a DES formed by menthol and fatty acids for the lipase (from *Candida rugosa*, CRL)-catalysed synthesis of menthol esters in a solvent-free approach.

The original work used enantiomerically pure (-) menthol, which was later extended in work by Paiva and coworkers to the kinetic resolution of *rac*-menthol.(Craveiro et al., 2019)

This two-in-one approach has also been used with sugar-based DES for the synthesis of fatty acid esters of carbohydrates,(M. Pätzold et al., 2019; Pöhnlein et al., 2015; Siebenhaller et al., 2018; Siebenhaller et al., 2016) structured lipids,(Zeng, Qi, Xin, Yang, & Wang, 2015) or benzoate glycerides.(Nadia Guajardo et al., 2017)

In addition to forming part of the desired product, DESs can also be used to promote cofactor- or cosubstrate-dependent biocatalytic reactions. A very interesting application of glucose-based DESs to promote alcohol dehydrogenase (ADH)-catalysed stereospecific reduction reactions of prochiral ketones was reported by Lavandera, Gotor and coworkers (Scheme 5).(Mourelle-Insua, Lavandera, & Gotor-Fernández, 2019)
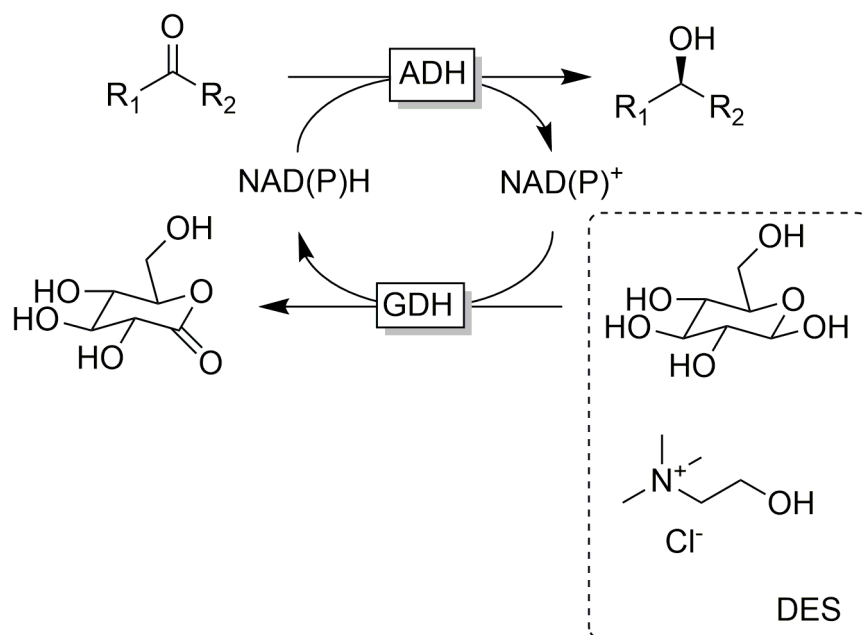
Scheme 5. Double use of a Glu-ChCl-DES as solvent and cosubstrate in ADH-catalysed stereospecific carbonyl reduction reactions. *(Mourelle-Insua et al., 2019)* Glu-ChCl, on the one hand, serves as cosubstrate to promote the glucose-dehydrogenase (GDH)-catalysed regeneration of NAD(P)H and as cosolvent to enable higher substrate loadings.

These dual-function 'designer' NADES enabled stereoselective reductions of a range of ketones with various ADHs. On the one hand, the NADES served as cosubstrate enabling *in situ* regeneration of the reduced nicotinamide cofactors (NAD(P)H). On the other hand, the NADES also enabled significantly higher substrate concentrations than in aqueous reaction media.

Choline-based DESs have recently been reported as dual-purpose solvents to also serve as stoichiometric electron donors for the reductive activation of molecular oxygen.(Y. Li et al., 2020; Ma et al., 2019; Ma et al., 2020) The resulting H_2O_2 can be used as oxidant to promote lipase-initiated chemoenzymatic epoxidation reactions(Ma et al., 2019) or peroxygenase-catalysed oxyfunctionalisation reactions (Scheme 6).

## Chemoenzymatic epoxidation of waste-derived limonene
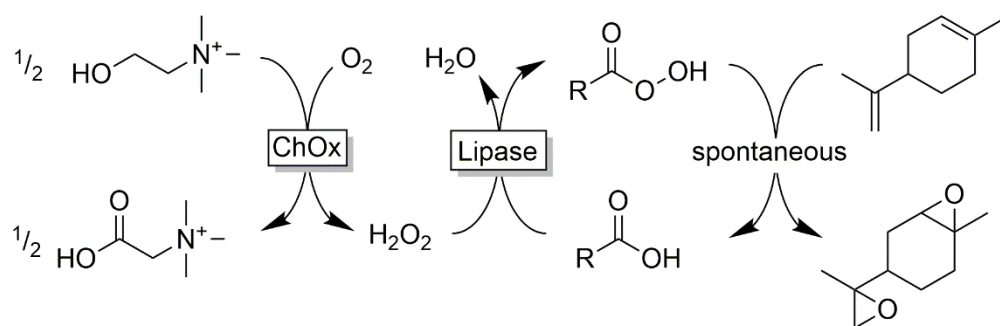
In situ H₂O₂ generation to promote peroxygenase-catalysed oxyfunctionalisations
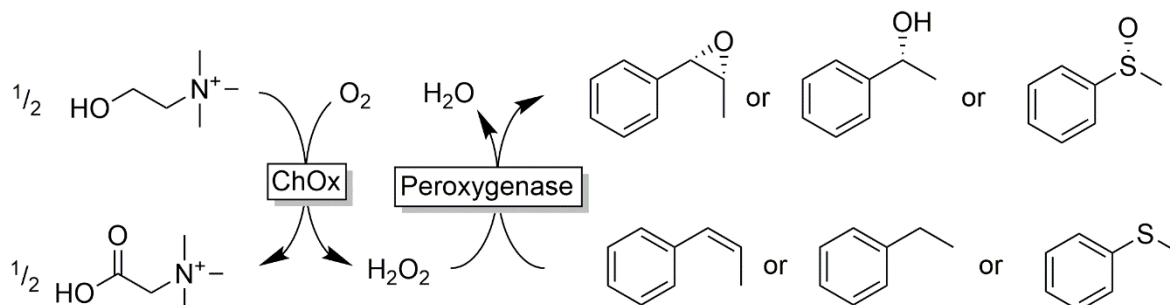
Scheme 6. Dual use of Choline-based DES for the *in situ* generation of H_2O_2 catalysed by a choline oxidase (ChOx) and the use of the H_2O_2 to promote chemoenzymatic Prilezhaev-type epoxidations and peroxygenase-catalysed oxyfunctionalisation reactions.

In case of the chemoenzymatic epoxidation of limonene, the DES (Ch-Pro) was also used to extract the terpene starting material from waste lemon peels thereby representing a triple-use (as extraction solvent, reaction solvent and sacrificial cosubstrate) for the overall process.(Ma et al., 2019)

A fascinating influence of ChCl-Gly on the enantioselectivity of the Bakers' yeast-catalysed stereospecific reduction of ketones was reported by Domínguez de María and co-workers (Scheme 7).(Zaira Maugeri & Domínguez de María, 2014) Depending on the water content of the ChCl-glycerol DES used as solvent, a marked switch of the overall enantioselectivity of the reaction was observed. Puzzling at first sight, this observation may be explained by (de)activation of enantiocomplementary ADHs within the *S. cerevisiae* cell.
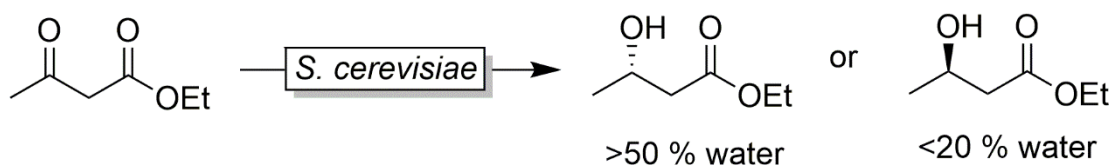
Scheme 7. Baker's yeast (*Saccharomyces cerevisiae*)-catalysed reduction of acetoacetate in ChCl-glycerol/water mixtures. At water contents below 20% (v/v) the reaction was (*R*)-selective whereas at water contents above 50% (v/v) high (*S*)-selectivity was observed.

Similar effects have also been observed by Capriati and coworkers(Vitale et al., 2017) and Redovnikovic and coworkers(Cvjetko Bubalo, Mazur, Radošević, & Radojčić Redovniković, 2015; Panic, Delac, Roje, Redovnikovic, & Bubalo, 2019) in the Baker's yeast-catalysed reduction of acetophenone derivates in ChCl-Gly.

Several authors have observed accelerating effects of DESs on the rate of whole cell-biotransformations. For example, the *Lysinibacillus fusiformis*-mediated transformation of isoeugenol into vanillin was markedly accelerated in the presence of various (NA)DES (Scheme 8).(T.-X. Yang et al., 2017)
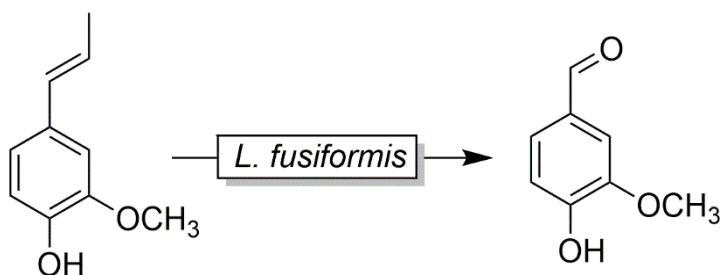
Scheme 8. *Lysinibacillus fusiformis*-catalysed conversion of isoeugenol to vanillin.

Generally, this is ascribed to cell wall/membrane permeabilisation resulting in facilitated diffusion of the reagents into the (biocatalyst-containing) whole cells.(Zhang et al., 2020)

Frequently, a stabilising effect of DES on the biocatalysts is mentioned. Lipases have been the preferred study object for the influence of a myriad of DESs on their activity and stability.(Bernardo Dias, Lucas de Carvalho, Maria Alice Zarur, & Isabel, 2019; Kim et al., 2016; Nian, Cao, & Liu, 2020; Oh

et al., 2019) This vast amount of data, however, is blurred by differences in the experimental design and analysis and interpretation of the data. As a result, the sometimes contradicting findings are difficult to structure and interpret. Trends observed with one enzyme cannot be transferred easily to another.(Z. L. Huang, Wu, Wen, Yang, & Yang, 2014; B. P. Wu, Wen, Xu, & Yang, 2014) Furthermore, a given DES may influence the enzyme and assay conditions in various ways, sometimes not directly obvious to the experimenter. Viscosity is frequently mentioned as a factor influencing activity assays. Some DESs, however, also exhibit emulsifying properties, which in two-liquid-phase-systems (as commonly used in lipase-catalysed transformations) can influence the surface area of the two liquids and thereby influence the reaction rate.(Lan, Wang, Zhou, Hollmann, & Wang, 2017)

Activity and stability data are also available for oxidoreductases such as horse liver alcohol dehydrogenase,(L. Huang, Bittner, Domínguez de María, Jakobtorweihen, & Kara, 2020) the peroxidase from horseradish,(B. P. Wu et al., 2014) versatile peroxidase,(Mamashli et al., 2019) catalase,(Harifi-Mood, Ghobadi, & Divsalar, 2017) laccase(Toledo et al., 2019) or haloalkane dehalogenases.(Stepankova, Vanacek, Damborsky, & Chaloupkova, 2014)

DES have been demonstrated exhibiting a stabilising effect on the chemoenzymatic epoxidation of C=C-double bonds (Scheme 9).(Lan et al., 2017; Ranganathan, Zeitlhofer, & Sieber, 2017; Zhou, Wang, Yang, Hollmann, & Wang, 2017; Zhou, Wang, Zeng, et al., 2017)
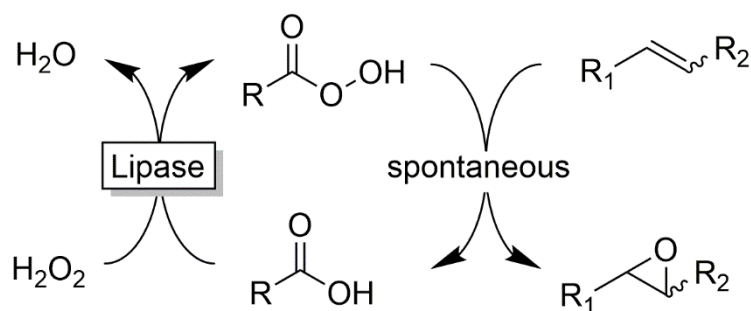
Products
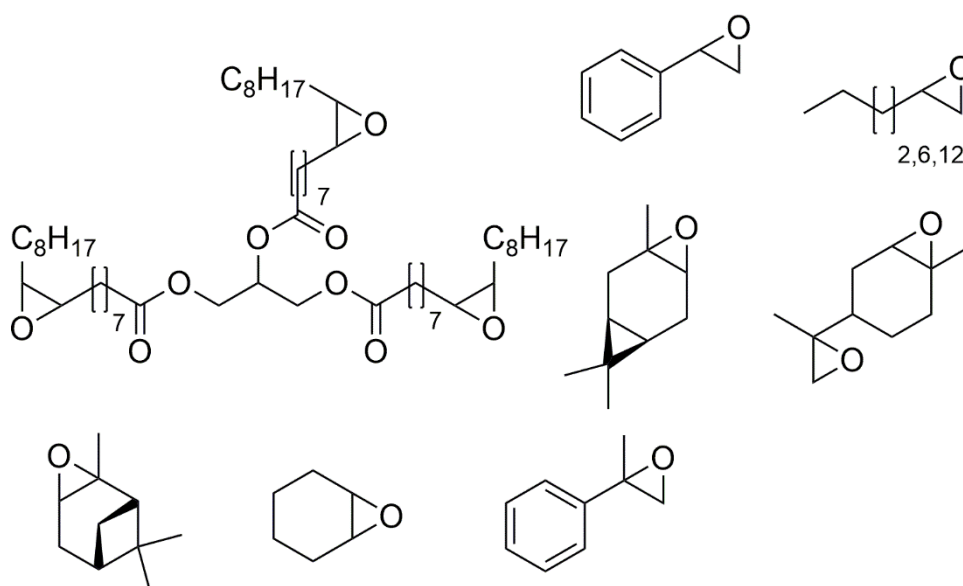
Scheme 9. Chemoenzymatic epoxidation of (non)-natural alkenes in DES.

The well-known perohydrolase activity of lipases (Björkling, Godtfredsen, & Kirk, 1990; Warwel & Klaas, 1995) allows them to accept H_2O_2 *in lieu* of water as nucleophile to hydrolyse the enzyme-acyl intermediate. The resulting peracids the mediate the Prilezhaev-type epoxidation of a broad range of C=C-double bonds. Unfortunately, the high K_M values of most lipases for H_2O_2 in water-containing media necessitate high H_2O_2-concentrations, which in turn can be detrimental to the robustness of the biocatalyst. Interestingly, DES seems to alleviate this inactivation, possibly by stabilising the free H_2O_2 through additional H-bonding.

A unified theory rationalising the effects of DES on enzymes is urgently needed!
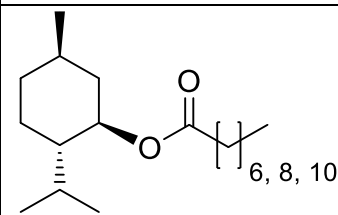
Finally, also the emerging field of chemoenzymatic synthesis in DES is worth mentioning. Particularly

popular are cascade reactions combining typical transition-metal catalysed, but not known in enzyme

catalysis, reactions such as cross-coupling or metathesis reactions with stereospecific enzyme-

catalysed reactions (such as the stereoselective reduction of ketones) (Scheme 10).(Cicco et al., 2018;

Grabner, Schweiger, Gavric, Kourist, & Gruber-Woelfler, 2020; Paris, Ríos-Lombardía, Morís, Gröger,

& González-Sabín, 2018)
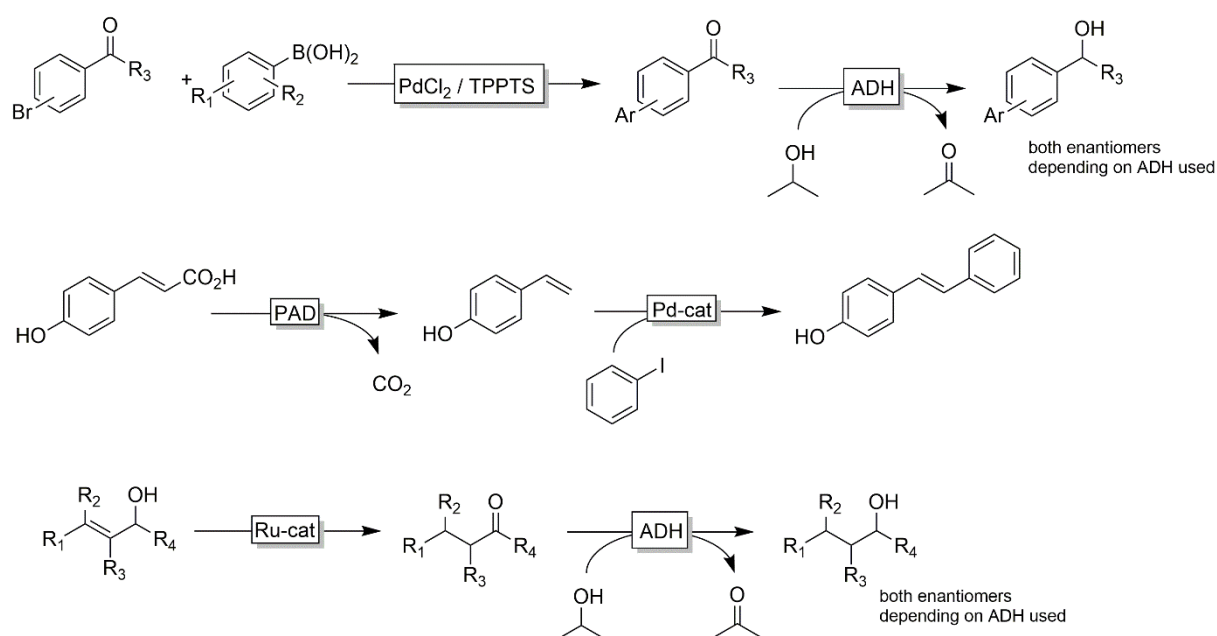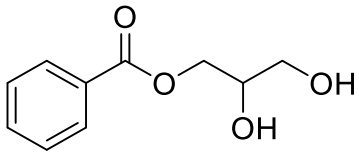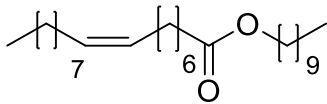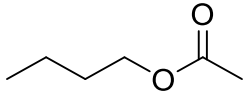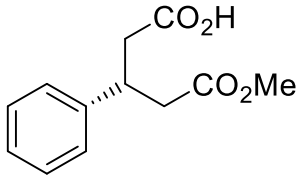
Scheme 10. Examples for chemoenzymatic cascade reactions performed in DES. *(Cicco et al., 2018; Grabner et al., 2020; Paris et al., 2018)*
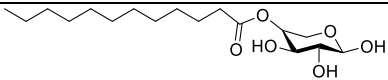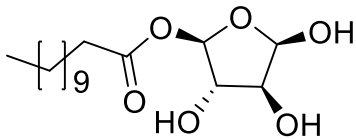
Issues of incompatibility of the two catalysis worlds, such as different requirements for the reaction

conditions or mutual inactivation, are generally solved by spatial of temporal separation of the

chemical and the enzymatic step.
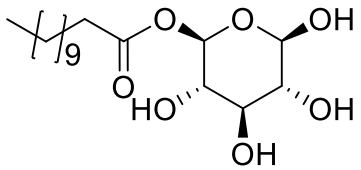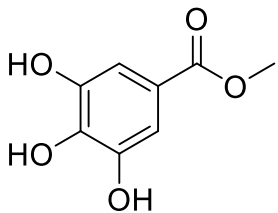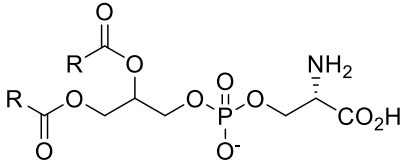
Table 1. Biocatalytic reactions performed in DES.

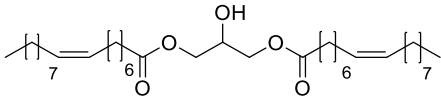| Product	Enzyme	DES used	Remarks	Reference
Esterifications				
	CRL N435 CALB BCL PCL PFL	Men:OA (55:45) Men:DA (63:35) Men:DDA (75:25) H_2O-content: 0, 1, 5 or 10 wt-%	DES as solvent and reagent, addition of water improves the enzymatic reaction significant, BCL,PCL and PFL showed no activity in DES,best results obtained with CRL with 10% water	(Hümmer et al., 2018)
	CRL	Men:La (9:1 to 1:1)	As above, kinetic resolution of rac-menthol	(Craveiro et al., 2019)
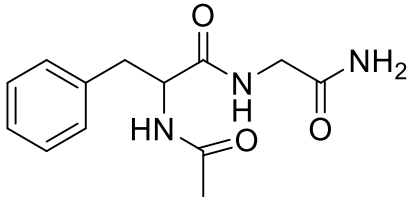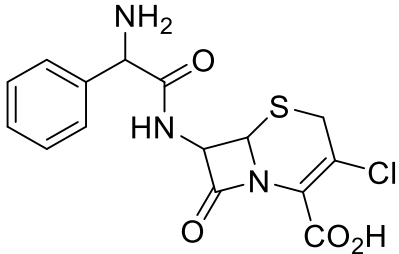
	CalB immobilized	ChCl:Gly : Phosphate buffer	DES as solvent and reagent, positive influence of water on the reaction	(N. Guajardo, Ahumada, & de Maria, 2020)
	N435 RMIM TLIM Lipase G50 F-AP	B:Gly ChCl:Gly H_2O-content: 1-4%	Selectivity depends on DES	(Zeng et al., 2015) (L. Xu et al., 2017)
	CalB immoblised	CHCL:G (1:1) CHCl:U (1:2) H_2O-content: 0-10%	in ChCl:Gly DES high enzyme stability (1200h), -ChCl:U DES advantage is ester production free of by product	(Kleiner & Schorken, 2015)
	CalB	CHCL:G (1:2) CHCL:EG (1:2) CHCl:U (1:2) H_2O-content: 0.5-2.5 mol-%	Higher esterification yield 80% (higher than reference solvent n- heptan (50%)	(Bubalo et al., 2015)
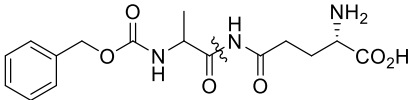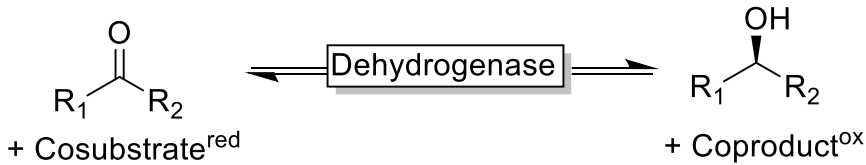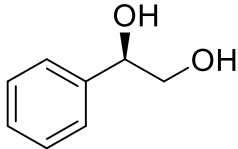
Ester hydrolysis

CO$_2$H / CO$_2$Me (phenyl glutaric acid monoester structure)	CalB	CHCL:U (1:2) H$_2$O-content: 50%	Desymmetrisation reaction, increased enantioselectivity in DES	(Fredes, Chamorro, & Cabrera, 2019)
Free fatty acids from hydrolysis of natural oils Pine nut oil	Amino Lipase PS	ChCl:U (1:2) ChCl:Gly (1:2) ChCl:EG (1:2) ChCl:1,2-PG (1:2 – 1:3) ChCl:CA (1:3) ChCl:LA (1:1) H$_2$O-content: 38%	Best reaction conditions reached in ChCl:U with 38% of water	(G. L. Yang, Tong, Yang, Liu, & Wang, 2019)
Palmitic acid hydrolysis of para-nitrophenyl palmitate	BCL	ChCl:U (1:2) ChCl:Gly (1:2) ChCl:EG (1:2)	enzyme activity enhanced by up to 230%	(Juneidi, Hayyan,

16
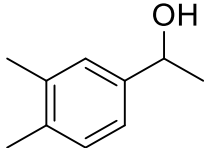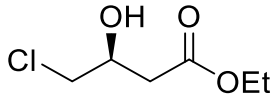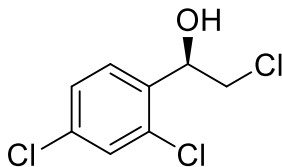
		ChCl:DEG (1:2)		Hashim, &
		EAC:Gly (1:2)		Hayyan, 2017)
		EAC:EG (1:2)		
		EAC:TEG (1:2)		
		H_2O-content: varable		

Transesterifications

	CalB	ChCl:carbohydrate (71.6% Glu & 16.6% Xyl)	DES as solvent and reagent, Carbohydrate obtained from beech wood	(Siebenhaller et al., 2018)
	CalB	ChCl:Ara (1:1) ChCl:Glu (1:1) ChCl:Xyl (1:1) ChCl:Man (1:1) ChCl:Rha (1:1) ChCl:Lev (1:1)		(Siebenhaller et al., 2016)
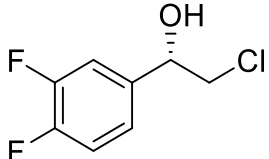
	N435	ChCl:U	Increased stability of the biocatalyst	(Andler, Wang, Rotello, & Goddard, 2017)
	CalB	ChCl:Gly H_2O-content: varable	Optimised water content for maximised activity and minimised hydrolysis	(Ulger & Takac, 2017)
	PLD	ChCl:U (1:2) ChCl:A (1:2) ChCL:EG (1:2) ChCL:Gly (1:2) ChCl:1,4-Bu (1:4) ChCl:TEG (1:4) ChCL:X (1:1) ChCL:OA (1:1) ChCl:LA (1:2) ChCL:MAA (1:1)	ChCl:EG DES best t for Phosphatidylserine synthesis	(S.-L. Yang & Duan, 2016)
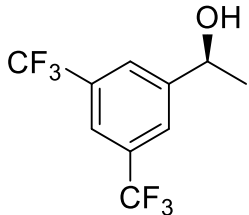
		ChCL:MA (1:1) ChCl:CA (1:1)		
Biodiesel	Various lipases	ChCl:Gly (1:2) CHAc:Gly (1:1.5) MeOH: 20-50% (v/v)	best conditions were ChCl:Gly (1:1) in the presence of 30% MeOH	(Zhao, Zhang, & Crittle, 2013)
biodiesel	Lipozym TL CALB L	ChCl:U (1:2) ChCl:Gly (1:2)		(Kleiner, Fleischer, & Schorken, 2016)
biodiesel	PEL N435	ChCl:U (1:1 – 2:1) ChCl:A (1:1 – 2:1) ChCl:Gly (1:1 – 2:1) ChCl:EG (1:1 – 2:1) ChAc:U (1:1 – 2:1) ChAc:A (1:1 – 2:1)		(Z. L. Huang et al., 2014)
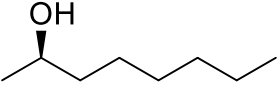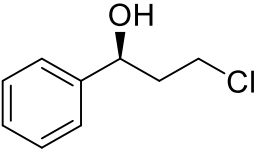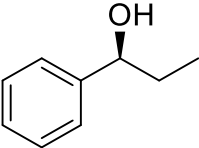
		ChAc:Gly (1:1 – 2:1)		
		ChCl:EG (1:1 – 2:1)		

Amide synthesis

	Chymotrypsin	ChCL:U (1:2)	productivities of approx. 20 gL^{-1} h^{-1}, presence of water absolutely crudial	(Zaira Maugeri et al., 2013)
		ChCL:Gly (1:2)		
		ChCL:X (1:1)		
		ChCL:Is (1:2)		
		H_2O-content: 4-50% (v/v)		
	Penicillin acylase	ChCl: CA (1:1)	Higher solubility of 7-ACCA in DESthan in purely aqueous buffer	(X. Wu et al., 2019)
		ChCl:OA (1:1)		
		ChCl:TA (1:1)		
		ChCl:MA (1:1)		
		ChCl:p-toluene		
		ChCL:X (1:1)		
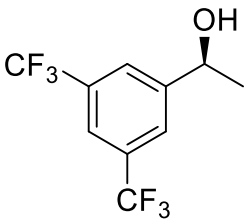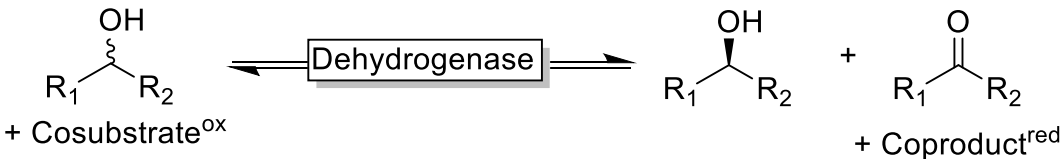
		ChCL:Gly (1:2)		
		ChCl:PG (1:1)		
		ChCL:BG (1:4)		
		ChCL:Gl (1:2)		
		ChCL:I (1:2)		
		ChCl:U (1:2)		
		Buffer		
	Papain	ChCl:U (1:2) H$_2$O-content: variable		(Cao et al., 2015)

Reduction reactions

	Kurthia gibsonii SC031	ChCl:1,4-Bu (1:4) ChCl:U (1:2) ChCl:Gly 1:2)	Increased activity due to cell permeabilisation,	(Fei Peng et al., 2020)
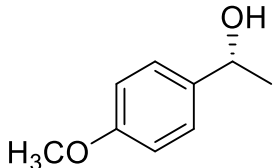
		ChCl:E (1:2) ChCL:TEG (1:4)	DESs on whole-cell catalytic properties	
	Carrot root	ChCl:G (1:1) ChCl:Xyl (2:1) ChCl:X (5:2) ChCl:G (1:2) ChCl:EG 1:2) H_2O-content 30-80%	Stereoselectivity depends on the DES used and the HBD used in DES	(Panić, Elenkov, Roje, Bubalo, & Redovniković, 2018)
	E. coli CCZU-T15	ChCL:U (1:2) ChCl:Gly (1:2) ChCL:EG (1:2) H_2O-content: variable	DES better than toluene-water solvent	(Dai, Huan, Zhang, & He, 2017)
  Several examples	*Lb*ADH, ADH-A, TeSADH	ChCl:Gly (1:2) H_2O-content: 50-80%	increased substrate concentration (up to 400 mM) in 20% v/v of DES, preparative scale,	(Ibn Majdoub Hassani, Amzazi, Kreit, &
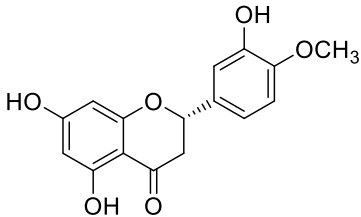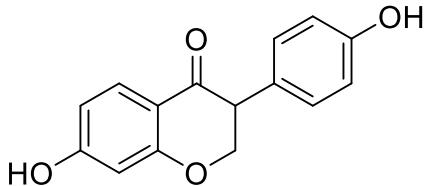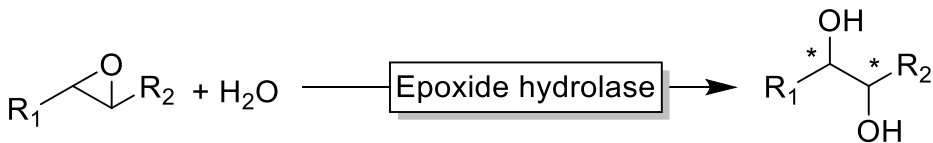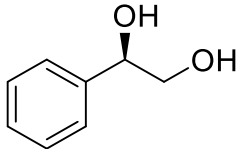
				Lavandera, 2020)
	recombinant *E. coli*	ChCl :GLy (1:1)	ChAc/Lys improves cofactor	(He, Huang, &
		ChCl:Lys (1:1)	regeneration,	Wang, 2020)
		ChCl:GSH (1:1)	Increased cell membrane	
		ChCl:Glu (1:1)	permeability	
		ChCl:Trp (1:1)		
		ChCl:Ala (1:1)		
		ChAc:Gly (1:1)		
		ChAc:Lys (1:1)		
		ChAc:GSH (1:1)		
		ChaC:Glu (1:1)		
		ChAc:Trp (1:1)		
		ChAc:Ala (1:1)		
		H_2O-content: <1%		
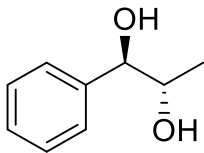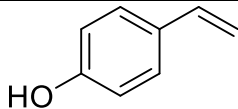
	Rhodococcus erythropolis XS1012	ChCl:Ala (1:1) ChCl:Cys (1:1) ChCl:EG (1:1) ChCl:Glu (1:1) ChCl:GlY (1:1) ChCl:GSH (1:1) ChCl:IPA (1:1) ChCl:Lys (1:1) ChCl:Trp (1:1) ChCL:Tyr (1:1) ChCl:U (1:1) ChCl:U (1:2) ChCl:U (2:1) H_2O-content: 99%	Increased activity due to cell membrane permeabilisation	(Chen, Qian, Lin, Chen, & Wang, 2020)
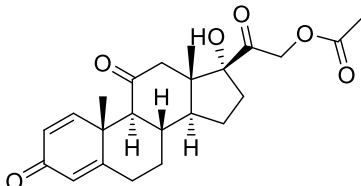
OH (structure)	*Acetobacter* pasteurianus GIM1.158	ChCl:U (1:2) ChCl:Gly (1:2) ChCl:EG (1:2) CHCL:OA (1:2) ChCl:MA (1:2) ChCL:I (1:2) H_2O-content: 90%	DES increased initial rate	(Xu, Du, Zong, Li, & Lou, 2016)
OH (structure with Cl)	*Acetobacter* sp. CCTCC M209061	ChCl:U (1:2) ChCl:Gly (1:2) ChCl:EG (1:2) CHCL:OA (1:2) ChCl:MA (1:2) ChCL:I (1:2) H_2O-content: 95%	Best results obtained with ChCl:U which also increased cell permeability, combining DES with ILs improved the reduction of CPE (85.2 v 93.3)	(Xu, Xu, et al., 2015)
OH (structure)	*Te*SADH HLADH	ChCl:Gly (1:2) H_2O-content: 20%	Significant influence of DES on enantoselectivity	(Müller, Lavandera,
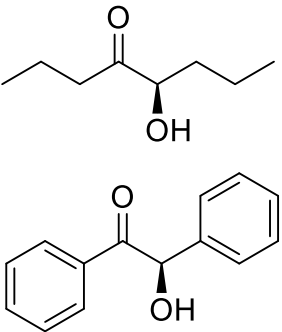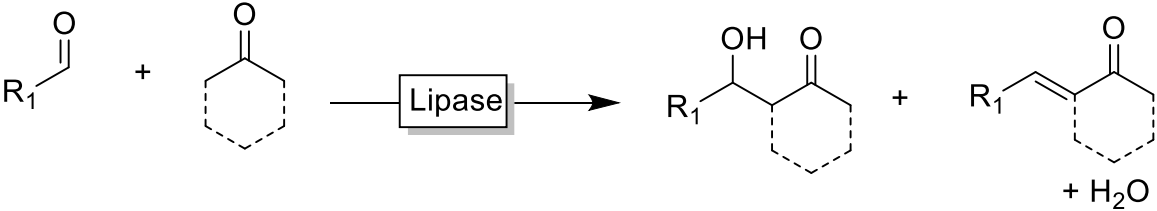
	*Ras*ADH RasADH			Gotor-Fernández, & Domínguez de María, 2015)
	T. asperellum ZJPH0810 *Candida tropicalis* 104 *Candida parapsilosis* ZJPH1305	ChCl:GSH (1:1 – 1:2)) ChCL:Glu (1:1 – 1:2) CHCl:Cys (1:1 – 1:2) ChCL:G (1:1 – 1:2) H_2O-content: <1%		(J. Li, Wang, He, Zhu, & Huang, 2019)

Oxidative kinetic resolution
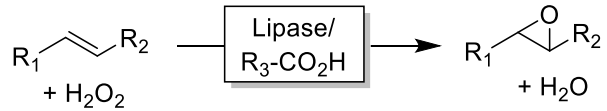
	Acetobacter sp. CCTCC M209061	ChCL:Gly: [C$_4$MIM]PF$_6$ Biphasic system with buffer	Oxidative kinetic resolution, A combination of ChCl:Gly with [C$_4$MIM]PF$_6$ gave best results in terms of substrate solubility and rate	(Wei, Liang, Cheng, Zong, & Lou, 2016; Xu, Cheng, Lou, & Zong, 2015)

(De)glycosylation reactions

isoquercitin to rutin	*E.coli* BL21-pET21a-rhaB1	ChCl:U (1:2) ChCl:Gly (1:2) ChCl:MA (1:1) ChCl:EG (1:2) ChCl:A (1:2) H$_2$O content: 99%		(Zhang et al., 2020)

	Acremonium sp. DSM24697	ChCl:U (1:2) ChCL:EG (1:2) ChCl:Gly (1:2)		(Weiz, Braun, Lopez, de María, & Breccia, 2016)
	D-glucosidase	ChCl:PEG (1:1 – 1:3) ChCl:Glu (1:1 – 1:3) ChCl:Gly (1:1 – 1:3) ChCl:EG (1:1 – 1:3) ChCl:U (1:1 – 1:3) H_2O content: 80%		(Cheng & Zhang, 2017)

Epoxide hydrolysis

	mEH	ChCl:U (1:2) ChCl:EG (1:2) ChCl:Gly (1:2)		(F. Peng, Zhao, Li, Zong, & Lou, 2018)

28

		ChCl:1,4-Bu (1:4)	Improvement of enatiopurity	
		ChCl:TEG (1:4)	achieved wit 10-20% of DES	
		ChCl:OA (1:1)	ChCl:TEG in phosphate buffer	
		ChCl: LA (1:2)		
		ChCl:MA (1:1)		
		ChCl:MA (1:1)		
		ChCl:CA (1:1)		
	StEH1	ChCl:E (1:2) ChCl:Gly (1:2) ChCL:U (1:2) H_2O content: 40-80%	DES enabled higher substrate concentrations	(Lindberg, Revenga, & Widersten, 2010)

Decarboxylation reactions

| | BsPAD | ChCl:Gly (1:2) ChCl:S (1:1) ChCl:U (1:2) | Significantly increased substrate solubility | (Schweiger et al., 2019) |

| | | | | DES:Water

H_2O content: 50% | | |

Desaturation reactions

R$_2$ — (O) — R$_1$ →[Desaturase]→ R$_2$ — (O) — R$_1$
+ Cosubstrate[ox]   + Coproduct[red]

| | *Arthrobacter simplex* | ChCl:U (1:2)

ChCl:EG (1:2)

ChCl:Gly (1:2)

H_2O content: 94% | Higher substrate solubility and

increased rate due to

permeabilisation | (Mao et al.,

2018; Mao, Yu,

Ji, Liu, & Lu,

2016) |

C-C-bond formation

R$_1$ — (O) + R$_2$ — (O) →[Lyase]→ R$_1$ — (O) — R$_2$ with OH

	BAL	ChCl:Gly (1:2)		(Z. Maugeri &
		ChCl:U (1:1)		de Maria, 2014)
		ChCl:Xyl (1:1)		
		H_2O content: 40%		
	PPL	ChCl:Gly (1:1.5,1:2)	ChCl-Gly-based DES as solvent for	(González-
	Alcalase-CLEA	H_2O content: 0-20%	benzaldehyde and other	Martínez et al.,
	CalB		hydrophobic ketones to perform	2016)
			aldol reactions with molar	
			concentrations	
	PPL	ChCl:Gly (1:1.5)	Best results obtained in the co-	(Milker et al.,
		TOABr:EG (1:3)	solvent acetone	2019)

		TOABr:1,5PD (1:3)		
		TOABr:4-NBA (2.2:1.5)		
		H_2O		
		Acetone content (up to 20% (v/v))		

Epoxidation reactions

	CalB	ChCl:VA (1:2)	DES system ChCl:U with H_2O_2	(Ranganathan
		ChCl:L (1:2)	achieved the fastest total	et al., 2017)
		ChCl:4-HPA (1:2)	conversion of reactants,	
		ChCl:MAA (1:1)	DES have a stabilising effect on	
		ChCl:TA (2:1)	the chemoenzymatic epoxidation	
		ChCl:GA (1:2)	of C=C-double bonds	

		ChCl:Gly (1:2)		
		ChCl:EG (1:2)		
		ChCl:U (1:2)		
		ChCl:F (1:2)		
		ChCl:Glu (1:2)		
		ChCl:X (1:1)		
		ChCl:S (1:1)		
	CalB	ChCl:U (1:2)		(Zhou, Wang, Yang, et al., 2017)
		ChCl:EG (1:2)		
		ChCl:A (1:2)		
		ChCl:Gly (1:1)		
		ChCl:X (1:1)		
		ChCl:S (1:1)		
		ChCl:Xyl:H_2O (5:2:5)		
		ChCl:Glu:H2O (5:2:5)		
		ChCl:Su:H_2O (5:2:5)		

	Lipase G	ChCl:U (1:2)	DES increased the enzyme	(Zhou, Wang,
		ChCl:Gly (1:2)	stability against H_2O_2	Zeng, et al.,
		ChCl:X (1:1)		2017)
		ChCL:EG (1:2)		
		B:Gly (1:2)		

	PCL	ChCl:U (1:2)	two liquid phase (2LP) approach,	(Lan et al.,
		ChCl:Gly (1:1)	DES lower the surface tension of	2017)
		ChCl:X (1:1)	hydrophobic organic phases in	http://dx.doi.org/
		ChCl:S (1:1)	aqueous reaction media and	10.1039/C7RA067
			thereby enable more efficient	55K
			biphasic biocatalytic reactions	

Candida rugosa lipase type VII (CRL), Amano lipase PS from *Burkholderia cepacia* (BCL), lipase from *Pseudomonas cepacia* PCL, Amano lipase *Pseudomonas fluorescence* (PFL), Candida Antarctica lipase B (CALB), immobilized *Thermomyces lanuginosus* lipase (TLIM), immobilized *Rhizomucor miehei* lipase (RMIM), *Penicillium camemberti* lipase (G50), *Rhizopus oryzae* lipase (F-AP), Amano lipase PS (free enzyme）from *Burkholderia cepacia* (BCL), *Candida antarctica* lipase B (CV-CALBY), Phospholipase D from *Streptomyces chromofuscus* (PLD), lipase from *T. lanuginosus* (Lipozyme TL), lipase from *Penicillium expansum* (PEL), alcohol dehydrogenase from *Lactobacillus brevis* (LBADH), alcohol dehydrogenase from *Thermoanaerobacter ethanolicus* (TeSADH),), alcohol dehydrogenase from *Thermoanaerobacter* sp, (ADH-A),), alcohol dehydrogenase from *Ralstonia* sp (RasADH), Horse liver ADH (HLADH), potato epoxide hydrolase (StEH1), Phenolic acid decarboxylase from *Bacillus subtilis* (BsPAD), mung bean epoxide hydrolases (mEH), benzaldehyde lyase from *P. fluorescens* (BAL), Porcine pancreas lipase (PPL), protease from *Bacillus licheniformis* (Alcalase-CLEA)

Choline chloride (ChCl), Choline Acetate (ChAc), Ethanediol (E), Glycol (Gl), Imidazole (I), Propylene ethylene glycol (PEG), Butyl glycol (BG), Propylene glycol (PG), 1,2-Propylene glycol (1,2-PG), Tartaric acid (TA), Isosorbide (Is), Citric acid (CA), Malic acid (MA), Malonic acid (MAA), Lactic acid (LA), Carbolic acid (CA), Levulinic acid (LA), Glutamic acid (GA), Valeric acid (VA), 4-Hydroxy phenyl acetic acid (4-HPA), Cystein (Cys), Glutamine (Gln), Lysin (Lys), Alanine (Ala), Tryptophan ((Trp), Octanoic acid (OCA), Decanoic acid (DA), Dodecenoic acid (DDA), Sucrose (Su), Oxalic acid (OA),Lauric acid (LA), Xylitol (X), 1,4-Butanediol (1,4-Bu), 1,5-Pentadiol (1,5-PD), Acetamide (A), Levoglucosan (Lev), Sorbitol (S), Rhamnose (Rha), Mannose (Man), Xylose (Xyl), Arabinose (Ara), Glucose (Glu), Frucose (F),N,N-diethyl ethanol ammonium chloride (EAC), Triethylene glycol (TEG), Diethylene glycol (DEG), Ethylene glycol (EG), Urea (U), Betaine (B), Glycerol (Gly), 4-nitrobenzaledehyde (4-NBA), Tetraoctylammonium bromide (TOABr), Glutathione (GSH) ,Menthol (Men)

3. Conclusions

Undoubtedly, (NA)DES represent an upcoming class of alternatives to established solvents in biocatalysis. Reactions, where too high water activities negatively influence the yield or selectivity of a reaction can benefit from using DES as (co)solvents. Also, DES can enable higher regent solubilities than water and thereby substitute volatile, non-renewable organic solvents. Particularly interesting are those applications where the DES not only serves as solvent or enzyme stabiliser but also actively influences the reaction outcome or serves as (co)substrate itself. We are convinced that the near future will bring about many more exciting applications of (NA)DES for biocatalysis.

There are, however, certain risks we have identified: From many contributions, the specific reason behind choosing a DES as (co-)solvent is not evident and one gets the impression that many contributions are just 'surfing the (NA)DES wave' without particular reason for this choice. Frequently, terms such as 'non-toxic', 'biobased' and 'biodegradable' are used in a prayer-wheel like fashion to underline the greenness of (NA)DES. We believe that limiting the evaluation of greenness to such terms is not sufficient. As shown in this contribution, DES have a (de)stablising effect on proteins. Therefore, unless a broader empiric basis exists, claiming non-toxicity appears premature. Furthermore, as pointed out by Holtmann and coworkers(M. Pätzold et al., 2019) the sometimes very high viscosity of DES-based reaction mixtures also implies higher energy demands for pumping and stirring. As a result, unless the energy used for these processes is obtained from entirely renewable sources, also increased CO_2 emissions due to stirring and pumping can be expected.

4. References

Andler, S. M., Wang, L. S., Rotello, V. M., & Goddard, J. M. (2017). Influence of Hierarchical Interfacial Assembly on Lipase Stability and Performance in Deep Eutectic Solvent. *Journal of Agricultural and Food Chemistry, 65*(9), 1907-1914.

anonymous. (2014). What is a Cell?   Retrieved 29.03.2020, 2020, from https://www.nature.com/scitable/topicpage/what-is-a-cell-14023083/

Bernardo Dias, R., Lucas de Carvalho, I., Maria Alice Zarur, C., & Isabel, M. M. (2019). Influence of Betaine- and Choline-based Eutectic Solvents on Lipase Activity. *Current Biochemical Engineering, 5*(1), 57-68.

Björkling, F., Godtfredsen, S. E., & Kirk, O. (1990). LIPASE-MEDIATED FORMATION OF PEROXYCARBOXYLIC ACIDS USED IN CATALYTIC EPOXIDATION OF ALKENES. *J. Chem. Soc. Chem. Commun.*(19), 1301-1303.

Bubalo, M. C., Tusek, A. J., Vinkovic, M., Radosevic, K., Srcek, V. G., & Redovnikovic, I. R. (2015). Cholinium-based deep eutectic solvents and ionic liquids for lipase-catalyzed synthesis of butyl acetate. *Journal of Molecular Catalysis B-Enzymatic, 122*, 188-198.

Cao, S.-L., Xu, H., Li, X.-H., Lou, W.-Y., & Zong, M.-H. (2015). Papain@Magnetic Nanocrystalline Cellulose Nanobiocatalyst: A Highly Efficient Biocatalyst for Dipeptide Biosynthesis in Deep Eutectic Solvents. *Acs Sustainable Chemistry & Engineering, 3*(7), 1589-1599.

Chen, H., Qian, F., Lin, H., Chen, W., & Wang, P. (2020). Using Choline Chloride-Based DESs as Co-Solvent for 3,5-Bis(trifluoromethyl) Acetophenone Bioreduction with Rhodococcus erythropolis XS1012. *Catalysts, 10*, 30.

Cheng, Q. B., & Zhang, L. W. (2017). Highly Efficient Enzymatic Preparation of Daidzein in Deep Eutectic Solvents. *Molecules, 22*(1), 15.

Choi, Y. H., van Spronsen, J., Dai, Y., Verberne, M., Hollmann, F., Arends, I. W. C. E., et al. (2011). Are Natural Deep Eutectic Solvents the missing link in understanding cellular metabolism and physiology? *Plant Physiology, 156*, 1701-1705.

Cicco, L., Ríos-Lombardía, N., Rodríguez-Álvarez, M. J., Morís, F., Perna, F. M., Capriati, V., et al. (2018). Programming cascade reactions interfacing biocatalysis with transition-metal catalysis in Deep Eutectic Solvents as biorenewable reaction media. [10.1039/C8GC00861B]. *Green Chemistry, 20*(15), 3468-3475.

Craveiro, R., Meneses, L., Durazzo, L., Rocha, A., Silva, J. M., Reis, R. L., et al. (2019). Deep Eutectic Solvents for Enzymatic Esterification of Racemic Menthol. *Acs Sustainable Chemistry & Engineering, 7*(24), 19943-19950.

Cvjetko Bubalo, M., Mazur, M., Radošević, K., & Radojčić Redovniković, I. (2015). Baker's yeast-mediated asymmetric reduction of ethyl 3-oxobutanoate in deep eutectic solvents. *Process Biochemistry, 50*(11), 1788-1792.

Dai, Y., Huan, B., Zhang, H. S., & He, Y. C. (2017). Effective Biotransformation of Ethyl 4-Chloro-3-Oxobutanoate into Ethyl (S)-4-Chloro-3-Hydroxybutanoate by Recombinant E-coli CCZU-T15 Whole Cells in ChCl Gly -Water Media. *Applied Biochemistry and Biotechnology, 181*(4), 1347-1359.

Dordick, J. S., Marletta, M. A., & Klibanov, A. M. (1986). Peroxidase Depolymerize Lignin in Organic Media but not in Water. *Proceedings of the National Academy of Sciences of the United States of America, 83*(17), 6255-6257.

Durand, E., Lecomte, J., & Villeneuve, P. (2013). Deep eutectic solvents: Synthesis, application, and focus on lipase-catalyzed reactions. *European Journal of Lipid Science and Technology, 115*(4), 379-385.

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*(4), 9.

González-Martínez, D., Gotor, V., & Gotor-Fernández, V. (2016). Application of Deep Eutectic Solvents in Promiscuous Lipase-Catalysed Aldol Reactions. *European Journal of Organic Chemistry, 2016*(8), 1513-1519.

Gorke, J. T., Srienc, F., & Kazlauskas, R. J. (2008). Hydrolase-catalyzed biotransformations in deep eutectic solvents. [10.1039/B716317G]. *Chemical Communications*(10), 1235-1237.

Gotor-Fernández, V., & Paul, C. E. (2019). Deep eutectic solvents for redox biocatalysis. *Journal of Biotechnology, 293*, 24-35.

Grabner, B., Schweiger, A. K., Gavric, K., Kourist, R., & Gruber-Woelfler, H. (2020). A chemo-enzymatic tandem reaction in a mixture of deep eutectic solvent and water in continuous flow. [10.1039/C9RE00467J]. *Reaction Chemistry & Engineering, 5*(2), 263-269.

Guajardo, N., Ahumada, K., & de Maria, P. D. (2020). Immobilized lipase-CLEA aggregates encapsulated in lentikats (R) as robust biocatalysts for continuous processes in deep eutectic solvents. *Journal of Biotechnology, 310*, 97-102.

Guajardo, N., Domínguez de María, P., Ahumada, K., Schrebler, R. A., Ramírez-Tagle, R., Crespo, F. A., et al. (2017). Water as Cosolvent: Nonviscous Deep Eutectic Solvents for Efficient Lipase-Catalyzed Esterifications. *ChemCatChem, 9*(8), 1393-1396.

Harifi-Mood, A. R., Ghobadi, R., & Divsalar, A. (2017). The effect of deep eutectic solvents on catalytic function and structure of bovine liver catalase. *International Journal of Biological Macromolecules, 95*, 115-120.

He, Y., Huang, Q., & Wang, P. (2020). Design and evaluation of novel bio-based deep eutectic solvents for highly efficient bioproduction of chiral aryl alcohol. *Journal of Chemical Technology & Biotechnology, DOI*: 10.1002/jctb.6386

Huang, L., Bittner, J. P., Domínguez de María, P., Jakobtorweihen, S., & Kara, S. (2020). Modeling Alcohol Dehydrogenase Catalysis in Deep Eutectic Solvent/Water Mixtures. *ChemBioChem, 21*(6), 811-817.

Huang, Z. L., Wu, B. P., Wen, Q., Yang, T. X., & Yang, Z. (2014). Deep eutectic solvents can be viable enzyme activators and stabilizers. *Journal of Chemical Technology and Biotechnology, 89*(12), 1975-1981.

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. *Molecular Catalysis, 458*, 67-72.

Ibn Majdoub Hassani, F. Z., Amzazi, S., Kreit, J., & Lavandera, I. (2020). Deep Eutectic Solvents as Media in Alcohol Dehydrogenase-Catalyzed Reductions of Halogenated Ketones. *ChemCatChem, 12*(3), 832-836.

Ibn Majdoub Hassani, F. Z., Amzazi, S., & Lavandera, I. (2019). The Versatile Applications of DES and Their Influence on Oxidoreductase-Mediated Transformations. *Molecules, 24*(11), 2190.

Juneidi, I., Hayyan, M., Hashim, M. A., & Hayyan, A. (2017). Pure and aqueous deep eutectic solvents for a lipase-catalysed hydrolysis reaction. *Biochemical Engineering Journal, 117*, 129-138.

Kim, S. H., Park, S., Yu, H., Kim, J. H., Kim, H. J., Yang, Y.-H., et al. (2016). Effect of deep eutectic solvent mixtures on lipase activity and stability. *Journal of Molecular Catalysis B: Enzymatic, 128*, 65-72.

Kleiner, B., Fleischer, P., & Schorken, 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 Biochemistry, 51*(11), 1808-1816.

Kleiner, B., & Schorken, U. (2015). Native lipase dissolved in hydrophilic green solvents: A versatile 2-phase reaction system for high yield ester synthesis. *European Journal of Lipid Science and Technology, 117*(2), 167-177.

Kourist, R., & González-Sabín, J. (2020). Non-Conventional Media as Strategy to Overcome the Solvent Dilemma in Chemoenzymatic Tandem Catalysis. *ChemCatChem, n/a*(n/a).

Lan, D., Wang, X., Zhou, P., Hollmann, F., & Wang, Y. (2017). Deep eutectic solvents as performance additives in biphasic reactions. [10.1039/C7RA06755K]. *RSC Advances, 7*(64), 40367-40370.

Li, J., Wang, P., He, Y.-S., Zhu, Z.-R., & Huang, J. (2019). Toward Designing a Novel Oligopeptide-Based Deep Eutectic Solvent: Applied in Biocatalytic Reduction. *ACS Sustainable Chemistry & Engineering, 7*(1), 1318-1326.

Li, Y., Ma, Y., Li, P., Zhang, X., Ribitsch, D., Alcalde, M., et al. (2020). Enantioselective Sulfoxidation of Thioanisole by Cascading a Choline Oxidase and a Peroxygenase in the presence of Natural Deep Eutectic Solvents. *ChemPlusChem, 85*, 254-257.

Lindberg, D., Revenga, M. D., & Widersten, M. (2010). Deep eutectic solvents (DESs) are viable cosolvents for enzyme-catalyzed epoxide hydrolysis. *Journal of Biotechnology, 147*(3-4), 169-171.

Ma, Y., Li, P., Willot, S. J.-P., Zhang, W., Ribitsch, D., Choi, Y. H., et al. (2019). Natural deep eutectic solvents as multifunctional media for the valorisation of agricultural wastes. *ChemSusChem, 12*, 1310-1315.

Ma, Y., Li, Y., Ali, S., Li, P., Zhang, W., Rauch, M., et al. (2020). Natural Deep Eutectic Solvents as Performance Additives for Peroxygenase Catalysis. *ChemCatChem, 20*, 989-994.

Mamashli, F., Badraghi, J., Delavari, B., Sabbaghian, M., Hosseini, M., & Saboury, A. A. (2019). Evaluation of Versatile Peroxidase's Activity and Conformation in the Presence of a Hydrated Urea Based Deep Eutectic Solvent. *Journal of Solution Chemistry, 48*(5), 689-701.

Mao, S., Li, K., Hou, Y., Liu, Y., Ji, S., Qin, H., et al. (2018). Synergistic effects of components in deep eutectic solvents relieve toxicity and improve the performance of steroid biotransformation catalyzed by Arthrobacter simplex. *Journal of Chemical Technology & Biotechnology, 93*(9), 2729-2736.

Mao, S., Yu, L., Ji, S., Liu, X., & Lu, F. (2016). Evaluation of deep eutectic solvents as co-solvent for steroids 1-en-dehydrogenation biotransformation by Arthrobacter simplex. *Journal of Chemical Technology & Biotechnology, 91*(4), 1099-1104.

María, P. D. d., Guajardo, N., & Kara, S. (2020). Enzyme Catalysis: In DES, with DES, and in the Presence of DES *Deep Eutectic Solvents* (pp. 257-271).

Maugeri, Z., & de Maria, P. D. (2014). Benzaldehyde lyase (BAL)-catalyzed enantioselective C-C bond formation in deep-eutectic-solvents-buffer mixtures. *Journal of Molecular Catalysis B-Enzymatic, 107*, 120-123.

Maugeri, Z., & Domínguez de María, P. (2014). Whole-Cell Biocatalysis in Deep-Eutectic-Solvents/Aqueous Mixtures. *ChemCatChem, 6*(6), 1535-1537.

Maugeri, Z., Leitner, W., & Domínguez de María, P. (2013). Chymotrypsin-Catalyzed Peptide Synthesis in Deep Eutectic Solvents. *European Journal of Organic Chemistry, 2013*(20), 4223-4228.

Mbous, Y. P., Hayyan, M., Hayyan, A., Wong, W. F., Hashim, M. A., & Looi, C. Y. (2017). Applications of deep eutectic solvents in biotechnology and bioengineering—Promises and challenges. *Biotechnology Advances, 35*(2), 105-134.

Milker, S., Pätzold, M., Bloh, J. Z., & Holtmann, D. (2019). Comparison of deep eutectic solvents and solvent-free reaction conditions for aldol production. *Molecular Catalysis, 466*, 70-74.

Mourelle-Insua, Á., Lavandera, I., & Gotor-Fernández, V. (2019). A designer natural deep eutectic solvent to recycle the cofactor in alcohol dehydrogenase-catalysed processes. [10.1039/C9GC00318E]. *Green Chemistry, 21*(11), 2946-2951.

Müller, C. R., Lavandera, I., Gotor-Fernández, V., & Domínguez de María, P. (2015). Performance of Recombinant-Whole-Cell-Catalyzed Reductions in Deep-Eutectic-Solvent–Aqueous-Media Mixtures. *ChemCatChem, 7*(17), 2654-2659.

Nian, B., Cao, C., & Liu, Y. (2020). How Candida antarctica lipase B can be activated in natural deep eutectic solvents: experimental and molecular dynamics studies. *Journal of Chemical Technology & Biotechnology, 95*(1), 86-93.

Oh, Y., Park, S., Yoo, E., Jo, S., Hong, J., Kim, H. J., et al. (2019). Dihydrogen-bonding deep eutectic solvents as reaction media for lipase-catalyzed transesterification. *Biochemical Engineering Journal, 142*, 34-40.

Panic, M., Delac, D., Roje, M., Redovnikovic, I. R., & Bubalo, M. C. (2019). Green asymmetric reduction of acetophenone derivatives: Saccharomyces cerevisiae and aqueous natural deep eutectic solvent. *Biotechnology Letters, 41*(2), 253-262.

Panić, M., Elenkov, M. M., Roje, M., Bubalo, M. C., & Redovniković, I. R. (2018). Plant-mediated stereoselective biotransformations in natural deep eutectic solvents. *Process Biochemistry, 66*, 133-139.

Paris, J., Ríos-Lombardía, N., Morís, F., Gröger, H., & González-Sabín, J. (2018). Novel Insights into the Combination of Metal- and Biocatalysis: Cascade One-Pot Synthesis of Enantiomerically Pure Biaryl Alcohols in Deep Eutectic Solvents. *ChemCatChem, 10*(19), 4417-4423.

Pätzold, M., Siebenhaller, S., Kara, S., Liese, A., Syldatk, C., & Holtmann, D. (2019). Deep Eutectic Solvents as Efficient Solvents in Biocatalysis. *Trends Biotechnol., 10.1016/j.tibtech.2019.03.007*.

Pätzold, M., Weimer, A., Liese, A., & Holtmann, D. (2019). Optimization of solvent-free enzymatic esterification in eutectic substrate reaction mixture. *Biotechnology Reports, 22*, e00333.

Peng, F., Chen, Q.-S., Li, F.-Z., Ou, X.-Y., Zong, M.-H., & Lou, W.-Y. (2020). Using deep eutectic solvents to improve the biocatalytic reduction of 2-hydroxyacetophenone to (R)-1-phenyl-1,2-ethanediol by Kurthia gibsonii SC0312. *Molecular Catalysis, 484*, 110773.

Peng, F., Zhao, Y., Li, F. Z., Zong, M. H., & Lou, W. Y. (2018). The effect of deep eutectic solvents on the asymmetric hydrolysis of styrene oxide by mung bean epoxide hydrolases. *Bioresources and Bioprocessing, 5*, 6.

Perna, F. M., Vitale, P., & Capriati, V. (2020). Deep eutectic solvents and their applications as green solvents. *Current Opinion in Green and Sustainable Chemistry, 21*, 27-33.

Pöhnlein, M., Ulrich, J., Kirschhöfer, F., Nusser, M., Muhle-Goll, C., Kannengiesser, B., et al. (2015). Lipase-catalyzed synthesis of glucose-6-O-hexanoate in deep eutectic solvents. *European Journal of Lipid Science and Technology, 117*(2), 161-166.

Ranganathan, S., Zeitlhofer, S., & Sieber, V. (2017). Development of a lipase-mediated epoxidation process for monoterpenes in choline chloride-based deep eutectic solvents. [10.1039/C7GC01127J]. *Green Chemistry, 19*(11), 2576-2586.

Schweiger, A. K., Ríos-Lombardía, N., Winkler, C. K., Schmidt, S., Morís, F., Kroutil, W., et al. (2019). Using Deep Eutectic Solvents to Overcome Limited Substrate Solubility in the Enzymatic Decarboxylation of Bio-Based Phenolic Acids. *ACS Sustainable Chemistry & Engineering, 7*(19), 16364-16370.

Siebenhaller, S., Kirchhoff, J., Kirschhöfer, F., Brenner-Weiß, G., Muhle-Goll, C., Luy, B., et al. (2018). Integrated Process for the Enzymatic Production of Fatty Acid Sugar Esters Completely Based on Lignocellulosic Substrates. [Original Research]. *Frontiers in Chemistry, 6*(421).

Siebenhaller, S., Muhle-Goll, C., Luy, B., Kirschhöfer, F., Brenner-Weiss, G., Hiller, E., et al. (2016). Sustainable enzymatic synthesis of glycolipids in a deep eutectic solvent system. *Journal of Molecular Catalysis B: Enzymatic, 133*, S281-S287.

Stepankova, V., Vanacek, P., Damborsky, J., & Chaloupkova, R. (2014). Comparison of catalysis by haloalkane dehalogenases in aqueous solutions of deep eutectic and organic solvents. *Green Chemistry, 16*(5), 2754-2761.

Tan, J.-N., & Dou, Y. (2020). Deep eutectic solvents for biocatalytic transformations: focused lipase-catalyzed organic reactions. *Applied Microbiology and Biotechnology*.

Toledo, M. L., Pereira, M. M., Freire, M. G., Silva, J. P. A., Coutinho, J. A. P., & Tavares, A. P. M. (2019). Laccase Activation in Deep Eutectic Solvents. *ACS Sustainable Chemistry & Engineering, 7*(13), 11806-11814.

Ulger, C., & Takac, S. (2017). Kinetics of lipase-catalysed methyl gallate production in the presence of deep eutectic solvent. *Biocatalysis and Biotransformation, 35*(6), 407-416.

Vitale, P., Abbinante, V. M., Perna, F. M., Salomone, A., Cardellicchio, C., & Capriati, V. (2017). Unveiling the Hidden Performance of Whole Cells in the Asymmetric Bioreduction of Aryl-containing Ketones in Aqueous Deep Eutectic Solvents. *Advanced Synthesis & Catalysis, 359*(6), 1049-1057.

Warwel, S., & Klaas, M. R. G. (1995). Chemo-enzymatic epoxidation of unsaturated carboxylic acids. *Journal of Molecular Catalysis B-Enzymatic, 1*(1), 29-35.

Wei, P., Liang, J., Cheng, J., Zong, M. H., & Lou, W. Y. (2016). Markedly improving asymmetric oxidation of 1-(4-methoxyphenyl) ethanol with Acetobacter sp CCTCC M209061 cells by adding deep eutectic solvent in a two-phase system. *Microbial Cell Factories, 15*, 11.

Weiz, G., Braun, L., Lopez, R., de María, P. D., & Breccia, J. D. (2016). Enzymatic deglycosylation of flavonoids in deep eutectic solvents-aqueous mixtures: paving the way for sustainable flavonoid chemistry. *Journal of Molecular Catalysis B: Enzymatic, 130*, 70-73.

Wu, B. P., Wen, Q., Xu, H., & Yang, Z. (2014). Insights into the impact of deep eutectic solvents on horseradish peroxidase: Activity, stability and structure. *Journal of Molecular Catalysis B-Enzymatic, 101*, 101-107.

Wu, X., Xiong, J., Huang, Z., Cao, S., Zong, M., & Lou, W. (2019). Improving biocatalysis of cefaclor with penicillin acylase immobilized on magnetic nanocrystalline cellulose in deep eutectic solvent based co-solvent. *Bioresource Technology, 288*, 121548.

Xu, L., Zhang, L., Li, D. M., Liu, P. Z., Tan, C. P., Wang, W. F., et al. (2017). Deep Eutectic Solvents Enable the Enhanced Production of n-3 PUFA-Enriched Triacylglycerols. *European Journal of Lipid Science and Technology, 119*(12), 6.

Xu, P., Cheng, J., Lou, W.-Y., & Zong, M.-H. (2015). Using deep eutectic solvents to improve the resolution of racemic 1-(4-methoxyphenyl)ethanol through Acetobacter sp. CCTCC M209061 cell-mediated asymmetric oxidation. [10.1039/C4RA12905A]. *RSC Advances, 5*(9), 6357-6364.

Xu, P., Du, P.-X., Zong, M.-H., Li, N., & Lou, W.-Y. (2016). Combination of deep eutectic solvent and ionic liquid to improve biocatalytic reduction of 2-octanone with Acetobacter pasteurianus GIM1.158 cell. *Scientific Reports, 6*(1), 26158.

Xu, P., Xu, Y., Li, X.-F., Zhao, B.-Y., Zong, M.-H., & Lou, W.-Y. (2015). Enhancing Asymmetric Reduction of 3-Chloropropiophenone with Immobilized Acetobacter sp. CCTCC M209061 Cells by Using Deep Eutectic Solvents as Cosolvents. *ACS Sustainable Chemistry & Engineering, 3*(4), 718-724.

Xu, P., Zheng, G.-W., Zong, M.-H., Li, N., & Lou, W.-Y. (2017). Recent progress on deep eutectic solvents in biocatalysis. *Bioresources and Bioprocessing, 4*(1), 34.

Yang, G. L., Tong, T., Yang, Y. Y., Liu, W., & Wang, X. D. (2019). Amano Lipase PS-catalyzed Hydrolysis of Pine Nut Oil for the Fatty Acids Production Using Deep Eutectic Solvent as Co-solvent. *Journal of Oleo Science, 68*(10), 977-988.

Yang, S.-L., & Duan, Z.-Q. (2016). Insight into enzymatic synthesis of phosphatidylserine in deep eutectic solvents. *Catalysis Communications, 82*, 16-19.

Yang, T.-X., Zhao, L.-Q., Wang, J., Song, G.-L., Liu, H.-M., Cheng, H., et al. (2017). Improving Whole-Cell Biocatalysis by Addition of Deep Eutectic Solvents and Natural Deep Eutectic Solvents. *ACS Sustainable Chemistry & Engineering, 5*(7), 5713-5722.

Zaks, A., & Klibanov, A. M. (1984). ENZYMATIC CATALYSIS IN ORGANIC MEDIA AT 100-DEGREES-C. *Science, 224*(4654), 1249-1251.

Zaks, A., & Klibanov, A. M. (1985). ENZYME-CATALYZED PROCESSES IN ORGANIC-SOLVENTS. *Proceedings of the National Academy of Sciences of the United States of America, 82*(10), 3192-3196.

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 and Biosystems Engineering, 38*(11), 2053-2061.

Zhang, F., Zhu, C.-T., Peng, Q.-M., Wang, F.-Q., Sheng, S., Wu, Q.-Y., et al. (2020). Enhanced permeability of recombinant E. coli cells with deep eutectic solvent for transformation of rutin. *Journal of Chemical Technology & Biotechnology, 95*(2), 384-393.

Zhao, H., Zhang, C., & Crittle, T. D. (2013). Choline-based deep eutectic solvents for enzymatic preparation of biodiesel from soybean oil. *Journal of Molecular Catalysis B-Enzymatic, 85-86*, 243-247.

Zhou, P., Wang, X., Yang, B., Hollmann, F., & Wang, Y. (2017). Chemoenzymatic epoxidation of alkenes with Candida antarctica lipase B and hydrogen peroxide in deep eutectic solvents. [10.1039/C7RA00805H]. *RSC Advances, 7*(21), 12518-12523.

Zhou, P., Wang, X., Zeng, C., Wang, W., Yang, B., Hollmann, F., et al. (2017). Deep eutectic solvents enable more robust chemoenzymatic epoxidation reactions. *ChemCatChem, 9*, 934–936.