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Review

Deep eutectic solvents for redox biocatalysis

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ABSTRACT

Deep eutectic solvents (DES) are a class of neoteric solvents used in multiple applications amongst which biocatalytic processes. Due to its simple preparation, low cost and inherent biodegradable properties, its use as a non-volatile biocompatible co-solvent with both whole cells and isolated enzymes has displayed increased enzyme activity and stability translating to higher product conversions and a surprising higher enantioselectivity in a range of biotransformations. This review lays out the latest updates on the use of DES in redox biocatalytic reactions. With that purpose, a clear division has been made to summarize the application of DES in bioreduction reactions using whole cells and purified alcohol dehydrogenases, oxidations involving alcohol dehydrogenases, heme-dependent enzymes, peroxidases, laccases and catalases, ozonolysis reactions, and finally mention the application of lipase in the mediation of chemoenzymatic epoxidation and Baeyer-Villiger reactions.

1. Introduction

The use of non-conventional media has been attracting great interest in biocatalysis, providing alternative solutions for challenges such as substrate and product solubility, enzyme stability and activity (Huang et al., 2018). Traditional co-solvents have been widely used to improve biocatalytic processes while for the past two decades ionic liquids (ILs) have been implemented (Gorke et al., 2010; van Rantwijk and Sheldon, 2007). More recently, deep eutectic solvents (DES) have surged as a new class of solvents (Abbott et al., 2004), and more recently natural DES (NADES) (Liu et al., 2018; Paiva et al., 2014). DES had already attracted considerable attention in chemical synthesis for their simple preparation, tuneable properties, applicability and sustainability. As greener chemistry has become an important parameter to consider when developing chemical processes, the use of these non-volatile, biocompatible and even biodegradable solvents has surged as an ideal alternative (Domínguez de María and Hollmann, 2015; Sheldon, 2017), with a shift in interest from ILs to DES in view of achieving the least toxic solvents possible.

A DES is typically a mixture of a quaternary ammonium salt, the hydrogen bond acceptor (HBA), and a hydrogen bond donor (HBD, Table 1). The structural unit of a DES depends on the intermolecular interactions among its components. The nature of its hydrogen-bonding lowers the overall melting point, forming a eutectic mixture, and as a result of mixing a HBA and a HBD to form a liquid, further purification is avoided, giving this waste-free process a significant advantage in comparison with the preparation of traditional ILs (Zhang et al., 2012). Depending on the selection of HBA and HBD and their molar ratio with respect to each other, the freezing point can be tuned. For instance, choline chloride (ChlCl, Table 1) with urea, ChCl:U, with a molar ratio of 1:2, gives a freezing point of 12 °C, whereas ChCl:glycerol (Gly) (molar ratio 1:2) displays a freezing point of -40 °C. Other advantages of DES include lower cost of production due to the use of inexpensive bulk raw and renewable materials with exceptional atom efficiency.

In terms of toxicity and sustainability, previous biocompatibility screenings on ILs were performed (Paul et al., 2012a; Rebros et al., 2009), which can be applied to DES. Studies have shown overall less toxicity for DES with respect to ILs (Radošević et al., 2015). Regarding the ChCl-based DES with glucose, Gly and oxalic acid as HBD (Table 1), ChCl:Glc and ChCl:Gly exhibited low cytotoxicity, whereas ChCl:Ox

Abbreviations: ADH(s), alcohol dehydrogenase(s); BY, Baker's yeast; CAL-B, *Candida antarctica* lipase type B; CHBE, (*S*)-4-chloro-3-hydroxybutyrate; COBE, ethyl 4-chloro-3-oxobutanoate; cyt c, cytochrome c; DES, deep eutectic solvent(s); *ee*, enantiomeric excess; ChCl:Gly, glyceline; HRP, horseradish peroxidase; IL(s), ionic liquid(s); MOPE, 1-(4-methoxyphenyl)ethanol; NADES, natural deep eutectic solvent(s); NAD(P)H, β-nicotinamide adenine dinucleotide (phosphate), reduced form; *n*Pr, propyl; *Ras*ADH, alcohol dehydrogenase from *Ralstonia* species; ChCl:U, reline; TEA, trimethylamine; v/v, ratio volume/volume; w/w, ratio weight/weight; HBAs, Hydrogen bond acceptors; ChAc, choline acetate; ChCl, choline chloride; EACl, ethylammonium chloride; HBDs, Hydrogen bond donors; Ac, acetate; Acet, acetamide; DEG, diethylene glycol; EG, ethylene glycol; Gal, galactose; Glc, glucose; Gly, glycerol; Im, imidazole; LA, levulinic acid; MA, malonic acid; Man, mannose; Ox, oxalic acid; TEG, triethylene glycol; U, urea; Xyl, xylose; Xylol, xylitol

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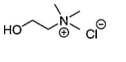
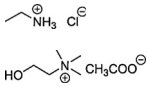
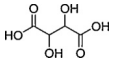
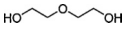
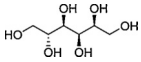
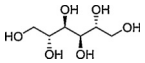
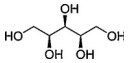
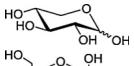
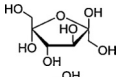
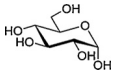
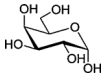
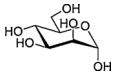
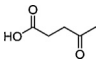
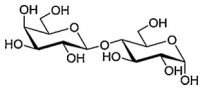
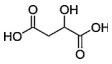
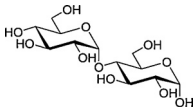
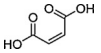
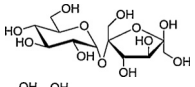
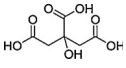
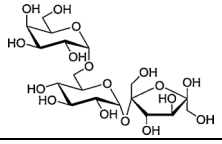
Table 1
Overview of DES mentioned in this review: hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs), their names and corresponding abbreviations.

HBAs	name (abbreviation)	HBAs	name (abbreviation)
	Choline chloride (ChCl)		Ethylammonium chloride (EACl) Choline acetate (ChAc)

HBDs	name (abbreviation)	HBDs	name (abbreviation)
	Glycerol (Gly)		Lactic acid
	Ethylene glycol (EG)		Tartaric acid
	Diethylene glycol (DEG)		Sorbitol
	Propylene glycol		Mannitol
	Urea (U)		Xylitol (Xylol)
	Acetamide (Acet)		Xylose (Xyl)
	Imidazole (Im)		Fructose (Fru)
	Betaine		Glucose (Glc)
	Oxalic acid (Ox)		Galactose (Gal)
	Malonic acid (MA)		Mannose (Man)
	Levulinic acid (LA)		Lactose
	Malic acid		Maltose
	Maleic acid		Sucrose
	Citric acid		Raffinose

displayed moderate cytotoxicity. ChCl-based DES are less toxic than phosphonium-based DES (Bubalo et al., 2015; Hayyan et al., 2013a, b; Hayyan et al., 2016).

Applications of DES are extremely diverse due to their tuneable properties such as conductivity, polarity, viscosity, density, surface tension, etc. (Smith et al., 2014). Examples of applications are their use as reaction media in organic synthesis (Alonso et al., 2016; García-Álvarez, 2015; Massolo et al., 2016; Zhang et al., 2012), including biocatalytic reactions discussed in this review (Domínguez de María and Maugeri, 2011; Guajardo et al., 2016; Xu et al., 2017), extraction processes (Krystof et al., 2013; Maugeri et al., 2012; Pena-Pereira and Namieśnik, 2014), or for the production of materials (Wagle et al., 2014), among others. Other relevant and emerging applications of DES include their use in organometallic chemistry reactions (García-Álvarez, 2015; García-Álvarez et al., 2018), photosynthesis (Milano et al., 2017), and energy technology (Boldrini et al., 2018)

One of the reasons DES are attractive for biocatalytic reactions is the idea that they reproduce the environment in cells, mimicking metabolites and forming a different type of liquid besides water and lipids (Choi et al., 2011). In this medium, the protein structure may be better preserved in the absence of water than in other organic solvents (Choi et al., 2011). The use of DES with lipases has been well developed (Durand et al., 2013) whereas only a couple of examples with lyases are available (Donnelly et al., 2015; Maugeri and Domínguez de María, 2014a) and recently more processes using oxidoreductases are being introduced. To distinguish from previously published book chapters (Gotor-Fernández and Paul, 2018; Paul and Gotor-Fernández, 2016), other extensive reports on biotransformations in DES (Juneidi et al., 2018) and to update from the latest review (Xu et al., 2017), this contribution is meant as an overview of the biocatalytic processes carried out with DES exclusively focusing on redox reactions, whether using whole cells or isolated enzymes. We have divided the sections of this review according to reaction type catalysed by different enzymes. In the following sections we will discuss the use of DES in redox biocatalytic processes with oxidoreductases and whole cell biocatalysts, followed by chemoenzymatic oxygenation reactions mediated by hydrolases through chemoenzymatic cascades.

2. Reduction reactions catalysed by oxidoreductases

2.1. Bioreductions with ketoreductases in whole cells

The use of whole cells to catalyse reactions can give several advantages over isolated purified enzymes, such as a readily available cofactor recycling system, preservation of the enzyme environment when using non-conventional solvents, lower costs without purification or cofactor needed (Kratzer et al., 2015). Typically, the use of DES enables higher substrate loadings thus avoiding inhibition. Combining both *E. coli* whole cells and DES-buffer water mixtures showed that the cells could retain their integrity. Nevertheless, one drawback that arises with whole cells is the presence of other enzymes with potentially overlapping substrate scope and opposite enantioselectivity. In this section the use of DES:water mixtures for reduction reactions catalysed by ketoreductases in whole cells will be described.

2.1.1. Stereoselectivity effect of DES

The group of Domínguez de María was pioneer in reporting the use of Baker's yeast (BY) *Saccharomyces cerevisiae* in mixtures of water and DES for the stereoselective bioreduction of ethyl acetoacetate (Maugeri and Domínguez de María, 2014b). When using BY in DES-water mixtures they found that BY could retain activity for reaction times of > 200 h. Interestingly, when increasing the amount of DES to > 80% in water, there was a clear stereoinversion for the reduction of the ketone in ethyl acetoacetate with ChCl:Gly (molar ratio 1:2), affording 95% *ee* of the (*R*)-ethyl 3-hydroxybutyrate, compared to 95% *ee* of the (*S*)-alcohol product in water only (Fig. 1). With a DES:water mixture of 30:70, racemic product was obtained. The reason for this

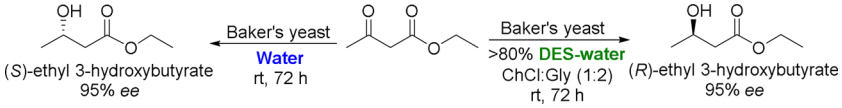


Fig. 1. Bioreduction of ethyl acetoacetate catalysed by Baker's yeast in DES:water mixtures or water.

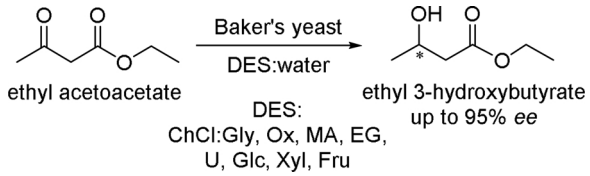


Fig. 2. Baker's yeast-catalysed reduction of ethyl acetoacetate in DES.

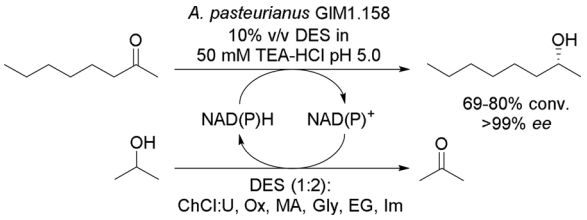


Fig. 4. Bioreduction of 2-octanone catalysed by *Acetobacter pasteurianus* GIM1.158 cells in 10% v/v DES:buffer.

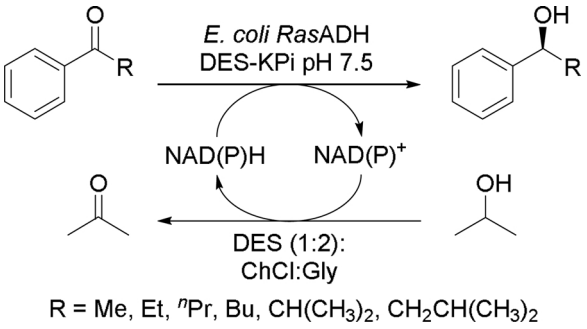


Fig. 5. Bioreduction of aromatic ketones to chiral alcohols catalysed by whole cells *E. coli Ras*ADH.

stereoinversion was ascribed to the potential inhibitory effect of DES on (*S*)-oxidoreductases present in BY. The competition between (*R*)- and (*S*)-oxidoreductases in the bioreduction of prochiral ketones was demonstrated to take place in pure water as well, albeit with longer reaction time (Vitale et al., 2017).

The group of Redovnioković also investigated the bioreduction of ethyl acetoacetate catalysed by BY in DES:water mixtures (Bubalo et al., 2015). A wider range of ChCl-based DES were tested with HBDs ChCl:Gly (1:2), EG (1:2), Glc (2:1), Fru (3:2), Xyl (2:1), MA (1:1), Ox (1:1) and U (1:2), with different ratios of buffer (Fig. 2). The conversions to the ethyl 3-hydroxybutyrate product obtained were affected by the type of HBD in the DES and the ratio of DES:water. The conversions with the reaction ran in mixtures of 50% w/w water:sugar and alcohol DES were comparable to those in phosphate buffer (> 93%). HBDs that potentially lowered the pH of the medium, acid and amide-based DES, gave lower conversions (< 49%). Two factors were found to influence the stereoselectivity: (i) the DES:water ratio, as previously observed by Domínguez de María; (ii) the type of DES, *e.g.* ChCl:Fru induced stereoinversion. Sugar DES, ChCl:Glc and Xyl, were observed to be the most biocompatible with BY cells as a non-conventional reaction medium for biotransformations.

A similar observation was later reported regarding the inhibition of (*S*)-selective ketoreductases present in BY caused by the use of DES as co-solvents (Vitale et al., 2017). Various ChCl-based DES were screened in combination with water for the bioreduction of arylpropanones (Fig. 3), observing the stereoinversion from (*S*)- to (*R*)-alcohol products at higher DES contents, and the structure of the DES played a significant role in the enantioselectivity. The DES ChCl:Gly (1:2) mixed with 10% (w/w) of water gave the (*R*)-enantiomer with up to 96% *ee* compared to 96% *ee* of its antipode with water as unique solvent.

Further bioreductions of ketones were reported such as the asymmetric reduction of 2-octanone catalysed by *Acetobacter pasteurianus* GIM1.158 cells using a biphasic system with DES and water-immiscible ILs in 10% v/v (Fig. 4), with a series of choline chloride-based DES (Xu et al., 2016). HBDs used included either U, Gly, EG, Ox, MA or Im. The DES ChCl:EG was shown to be biocompatible and increase cell membrane permeability, displaying the best results. ChCl:EG increased the optimal substrate concentration from 40 mM to 60 mM and afforded > 99% *ee* of (*R*)-2-octanol. In terms of stability during the reaction, the addition of DES led to good operational stability. The DES ChCl:EG (1:2) and an imidazole-based IL (1-butyl-3-methylimidazolium hexafluorophosphate) were found to be ideal co-solvents for the efficient biocatalytic reduction of 2-octanone.

The group of Domínguez de María investigated the bioreduction of aromatic ketones with DES using whole cells of *E. coli* with recombinantly expressed ADHs such as horse liver ADH (HLADH) and *Ralstonia* sp. (*Ras*ADH) (Müller et al., 2015). The ADHs were shown to remain active with up to 80% v/v DES-water (Fig. 5). The DES used ChCl:Gly, ChCl:U, ChCl:EG in increasing amount resulted in increasing *ee* from < 20% *ee* with 10% DES-buffer to > 90% *ee* with 80% DES-buffer.

The bioreduction of 1-(3,4-dimethylphenyl)ethanone using carrot root at 25 °C with DES was also recently reported (Panic et al., 2018), observing an inversion of the enantioselectivity by the addition of increasing amounts of DES. Mixtures of ChCl with Glc (1:1) EG (1:2), Gly (1:2), Xylol (5:2) and Xyl (2:1) were tested in combination with pure water (Fig. 6). On the one hand, starting from a 91% conversion in pure water a dramatic decrease in activity was observed at higher DES contents (11–50% conversion, Glc > Xyl > Gly > Xylol > EG), while on the other hand the stereopreference was inverted from the formation of the (*S*)-alcohol when water was used as solvent, to the formation of the (*R*)-alcohol (96% *ee*), which was the major enantiomer in all cases when only 30% of water was employed (33–75% *ee*). The use of lower percentages of water led to conversion values below 10% due to high viscosity of the DES that obstruct the mass transfer.

Very recently Capriati et al. have described the use of BY resting cells in the bioreduction of acetophenone and 3-acetylphenyl ethyl (methyl)carbamate (Vitale et al., 2018). Interestingly, low activity was found in tap water (entries 1 and 2, Table 2), while the substrate was recovered unaltered using ChCl:Gly as reaction media (entry 3). The use of both media allowed to obtain slightly higher conversions when a significant amount of water was added (entries 4 and 5). This system was also applied to the bioreduction of an acetophenone bearing a carbamate substitution in the C-3 position, which led to the corresponding alcohol precursor of (*S*)-rivastigmine, a drug used in the treatment of dementia diseases (entries 6 and 7). Better results were later found when using the *Lactobacillus reuteri* DSM 20016 resting cells, although the use of DES was not reported in these reactions.

Recently, the stereoselective bioreduction of α-acetylbutyrolactone for the production of *anti*-stereoisomers of α'-1′-hydroxyethyl-γ-butyrolactone has been described in both growing and resting culture of seven yeast strains (*Yarrowia lypolytica* AM71, *Yarrowia lypolytica*

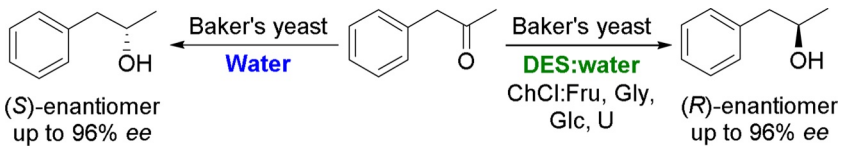


Fig. 3. Bioreduction of phenylpropanone catalysed by Baker's yeast in DES:water mixtures or water.

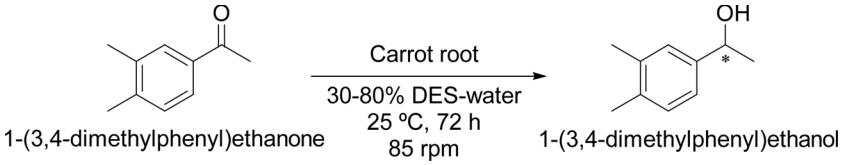
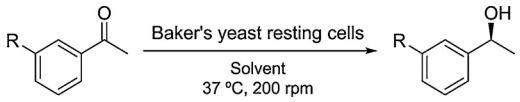


Fig. 6. Bioreduction of 1-(3,4-dimethylphenyl)ethanone using carrot root.

Table 2
Bioreduction of acetophenone and 3-acetylphenyl ethyl(methyl)carbamate using DES and Baker's yeast.



Entry	R	Solvent	Time (h)	(*S*)-Alcohol (%)[a]	Alcohol *ee* (%)
1	H	Water	24	8	Not determined
2	H	Water	120	27	98
3	H	ChCl:Gly 2:1	120	0	–
4	H	ChCl:Gly 2:1 + 20% water (w/w)	120	8	> 98
5	H	ChCl:Gly 2:1 + 40% water (w/w)	120	32	> 98
6	Et(Me)HCOO	Water	24	12	> 98
7	Et(Me)HCOO	ChCl:Gly 2:1 + 20% water (w/w)	120	5	95

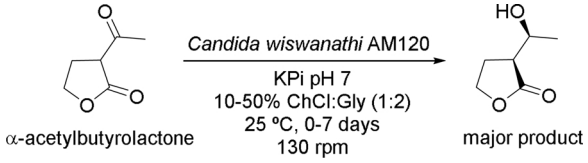


Fig. 7. Bioreduction of α-acetylbutyrolactone using *Candida viswanathi* AM120.

AM72, *Yarrowia lypolytica* P26 A, *Candida viswanathi* AM120, *Hansenula anomala* C2, *Saccharomyces cerevisiae* K1 and *Saccharomyces pombe* C1, Fig. 7). Organic solvents such as ethanol, glycerol, hexane or isopropanol, or alternatively ChCl:Gly (1:2) were used as co-solvents in some cases (Mączka et al., 2018). Particularly, the DES was used in 10, 25 or 50% v/v concentration in comparison with phosphate buffer pH 7.0 for the reaction using *Candida viswanathi* AM120. The benefit of the application of DES resides in the decrease of the reaction time, improving the enantio- and diastereoselectivity when 10 and 25% v/v ratio were applied.

For the biocatalytic reduction of the ketoester substrate ethyl 4-chloro-3-oxobutanoate (COBE) into ethyl (*S*)-4-chloro-3-hydroxybutyrate (CHBE), whole cells of *E. coli* CCZU-T15 were used in combination with ChCl:Gly (1:2) (Fig. 8). The reaction conditions were optimised through the addition of L-glutamine or D-ribose for the synthesis of cofactors, overcoming the need to use NAD^+, the product (*S*)-CHBE was obtained with > 99% *ee* starting from a 2 M COBE solution containing L-glutamine (150 mM) and 12.5% v/v ChCl:Gly-water at pH 6.5 (Dai et al., 2017). The cell membrane was shown to be more permeable in the presence of DES ChCl:Gly and a surfactant such as Tween-80.

2.1.2. DES for immobilised whole cells

To improve the effectiveness of whole cells for biotransformations, *Acetobacter* sp. CCTCC M209061 was immobilised on polyvinyl alcohol-sodium sulphate for higher stability to reduce 3-chloropropiophenone to (*S*)-3-chloro-1-phenylpropanol (Fig. 9) (Xu et al., 2015b). One of the DES used ChCl:U (1:2) among other HBD (Ox, MA, Gly, EG, Im), displayed the best results in terms of biocompatibility and flow cytometry studies. In the presence of DES, the cell membrane permeability was slightly increased, affording 1.37 mM/h of the enantiopure (*S*)-3-chloro-1-phenylpropanol at 30 °C after 6 h. This biocatalytic process was upscaled to 500 mL to demonstrate its applicability.

2.2. Bioreductions with isolated ketoreductases

Isolated alcohol dehydrogenases (ADHs) have been used with ILs (Paul et al., 2014), and very recently the first example with DES has been demonstrated for the asymmetric bioreduction of propiophenone and derivatives using commercially available ADHs from a Codexis kit (Cicco et al., 2018). Mixtures of DES-buffer were used, increasing the percentage of DES to 80%. The best results were obtained with the DES ChCl:Gly (molar ratio 1:2) and ChCl:sorbitol (1:1), affording > 99% conversion and up to > 99% *ee* with a DES:buffer of 50:50 and even 80:20 (Fig. 10). Taking advantage of the excellent activities displayed by purified ADHs, a chemoenzymatic cascade process was developed using a ruthenium catalyst. The later was used for the isomerisation of racemic allylic alcohols to the corresponding ketones, which were then selectively reduced with an ADH. This cascade process was performed sequentially (94– > 99% of alcohol saturated formation) and concurrently (68–96%) for 4 α-vinylbenzyl substrates (R = H, 4-Me, 4-OMe and 4-Br), leading to the enantiopure alcohols with selected enzymes.

3. Oxidation reactions catalysed by oxidoreductases

The use of oxidoreductases for enzymatic reductive transformations has received considerable attention, as described in the previous section, since the development of stereoselective transformations is highly accessible through the reduction of carbonyl groups to form the

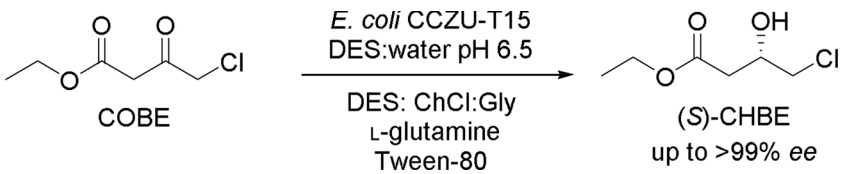


Fig. 8. Reduction of COBE to (*S*)-CHBE catalysed by *E. coli* CCZU-T15 whole cells.

corresponding optically active alcohols (Hollmann et al., 2011b). The reverse oxidation reaction can also be catalysed by oxidoreductases through simple process engineering (Hollmann et al., 2011a). In the next two sections, we will describe the oxidation and oxyfunctionalisation of organic molecules by oxidoreductase-catalysed reactions and lipase-mediated chemoenzymatic processes. The latter implying the enzyme is responsible for the perhydrolysis reactions that further allow the formation of active peroxides able to introduce oxygen atoms in alkenes and cyclic ketones to produce non chiral epoxides and lactones, respectively.

3.1. Whole-cell ADH-catalysed oxidation reactions

In this section, the development of enzymatic oxidation reactions using DES as reaction medium is reported for ketoreductases and more specifically ADHs. The application of these enzymes is well known especially for the reverse reaction, dehydrogenation, which is a common strategy for the bioreduction of prochiral or racemic ketones serving for the introduction of chirality in the target molecule. The oxidation of alcohols has been also reported, and several examples are reported using aqueous medium for the formation of the corresponding carbonyl products, although the use of DES is a premature stage.

Enzymatic functionalisation of steroids is an attractive approach due to the lack of chemical general strategies for the selective functionalisation of this type of molecules. The microbial oxidation of 1,2-dehydrogenation of cortisone acetate to prednisone acetate employing *Arthrobacter simplex* has been studied using three different ChCl-based DES as co-solvents to improve the bioconversion efficiency (Mao et al., 2016). Gly, EG and U were used as HBD, finding that the use of ChCl:U has a clear benefit in the conversion in comparison with the reaction in the absence of DES (Fig. 11). Meanwhile in water around 70% conversion was obtained. Interestingly a 93% conversion was attained working at a 5 g/L substrate concentration when using immobilised cells of *Arthrobacter simplex* on calcium alginate (4 g/L) and 6% of ChCl:U (1:2) as co-solvent. The advantage of using this particular DES resides in the better solubility of the cortisone acetate in the reaction medium, which was much higher when compared with water and even when using the same amount of ethanol as co-solvent. Another advantage of the method is the possibility to reuse the enzyme, which was demonstrated after recovery of the immobilised cells and application in 5 cycles, leading to a 82–93% conversion values.

Stereoselective oxidation of secondary alcohols has been also reported when using DES as co-solvents. For instance, whole cells of *Acetobacter* sp. CCTCC M209061 were applied in the resolution of racemic 1-(4-methoxyphenyl)ethanol (MOPE) through asymmetric oxidation (Fig. 12). After testing six DES, the best resolution was achieved with 20% v/v ChCl:Gly (1:2) that led to 49.4% conversion, recovering the substrate in excellent optical purity (98.7% *ee*). The DES concentration exhibited also a significant influence on the reaction, with the optimal content being 5% v/v for a 50% conversion in a completely selective oxidation (Xu et al., 2015a). In the ChCl:Gly-buffer mixture, the substrate concentration was significantly increased (55 mM *vs* 30 mM) as compared with the used of pure buffer, while the substrate enantiomeric excess was kept higher than 99%. The good biocompatibility of ChCl:Gly with cells and the improved cell membrane permeability in the ChCl:Gly-buffer mixture could partly account for the clearly enhanced reaction efficiency.

This oxidative resolution was further investigated in the presence of an IL and DES in a biphasic system (Fig. 13) by the same group (Wei et al., 2016). The IL 1-butyl-3-methylimidazolium hexafluorophosphate gave good results in combination with the buffer (97.8 μmol/min initial rate, 50.5% conversion and > 99% *ee* after 10 h), obtaining a significant improvement when the ChCl:Gly was also added as 10% v/v to this system (124.0 μmol/min initial rate, 51.3% conversion and > 99% *ee* after 7 h). In addition, the substrate concentration was enhanced from 50 mM until 80 mM. Remarkably, the reusability was also nicely addressed when using these immobilised cells as 72% of their activity was retained after 9 cycles in the IL/DES-buffer mixture, and the reaction could be upscaled to a total volume of 500 mL.

3.2. Heme-dependent enzymatic oxidation reactions

3.2.1. Biooxidation by horseradish and cytochrome c peroxidases

Peroxidases (EC 1.11.1.X) contain a heme cofactor in their active site and typically use hydrogen peroxide as a substrate. These include horseradish and cytochrome c peroxidases. The stability and activity of the horseradish peroxidase (HRP) were experimentally and structurally studied with a series of DES (Wu et al., 2014). Various DES were produced with two choline salts (ChCl and ChAc) and four HBDs (U, Gly, Acet and EG) at three different molar ratios (1:2, 1:1 and 2:1). Thus, 24 DES were studied with HRP, observing various trends. The ChAc-based DES lowered the activity of HRP when compared to ChCl-based DES. When comparing the different molar ratios, the DES with higher concentrations of choline chloride salt (2:1 > 1:1 > 1:2) increased the peroxidase activity. With structural studies using circular dichroism and fluorescence, the DES that could bring the HRP to a higher α-helix content and a slightly more relaxed tertiary structure were reported to facilitate the HRP activity. In conclusion, the 24 choline-based DES were found to stabilize the HRP, but did not significantly increase its activity.

Stamatis et al. investigated the peroxidase activity of both HRP and cytochrome c (cyt c), using an activity assay through the oxidation of guaiacol in DES with either ChCl or EACl based-salts with the HBDs U, Gly and EG at molar ratios of 1:1.5 and 1:2 (Papadopoulou et al., 2016). The oxidation of guaiacol catalysed by each enzyme was measured according to the colour change from yellow to red at 470 nm (Fig. 14).

The peroxidase activity was found to be dependent on (i) the choline salt, either ChCl or EACl; (ii) the HBD (U, Gly, EG); (iii) the amount of DES used in the water mixture (0–90%). Cyt c activity increased with DES-water mixtures compared to buffer only, which could be explained by changes in the microenvironment of the heme cofactor. Cyt c displayed higher stability with EACl-based compared to ChCl-based DES, with an increase of 8-fold with 30% v/v ChCl:U-buffer, compared to that in pure buffer, and 100-fold with EACl:U-buffer. No significant increase in activity was observed for HRP. Cyt c mostly retained its activity in DES-buffer, whereas there was a 35% loss after 24 h in buffer. Cyt c stability was enhanced with ChCl- and EACl-based DES depending on the HBD, from the highest stability U > Gly > EG. Urea as a HBD also had a stabilising effect on HRP. The lowest stability observed was with ChCl:Gly and ChCl:EG, correlating with conformational changes of the heme prosthetic group. Higher activity of cyt c with DES was shown for the decolourization of a pinacyanol chloride dye, of industrial relevance, noting a 3.3-fold increase with 50% v/v EACl:U and EACl:EG in buffer. The DES could also be reused through 4 cycles retaining the same enzyme activity.

Free-radical polymerization reactions with acrylamide can also be promoted by HRP in DES ChCl:U and ChCl:Gly with almost no water (Sánchez-Leija et al., 2016). HRP activity and thermal stability were lower at high DES concentration when compared with phosphate buffer at pH 7. Nevertheless, the low freezing point of ChCl:Gly (-40 °C) enabled to carry out the production of polyacrylamide at 4 °C in 80% v/v DES. No polymerization was observed in buffer at 4 °C, therefore the use of DES offer further possibilities for the synthesis of materials through enzyme-mediated cryo-polymerization reactions.

3.2.2. Biooxidation by catalases

Catalases are heme enzymes that catalyse the disproportionation of hydrogen peroxide, typically forming water and oxygen. Bovine liver catalase was used with mixtures of DES:buffer, observing the conversion of hydrogen peroxide to water (Harifi-Mood et al., 2017). The DES used were mixtures of ChCl:U (1:2) and ChCl:Gly (1:2). Hydrogen peroxide displayed an increasing binding affinity to the catalase with

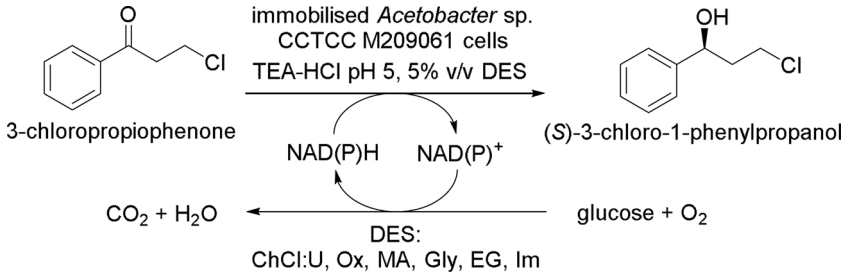


Fig. 9. Bioreduction of 3-chloropropiophenone catalysed with immobilised cells of *Acetobacter* sp. CCTCC M209061.

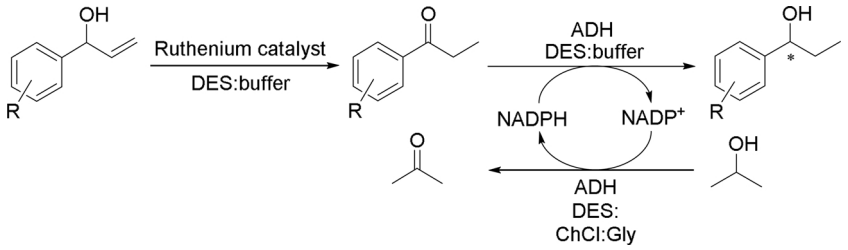


Fig. 10. Ruthenium-catalysed isomerisation of racemic vinyl alcohols coupled with ADH-catalysed bioreduction of the corresponding propiophenones in different DES-buffer media through sequential or concurrent approaches.

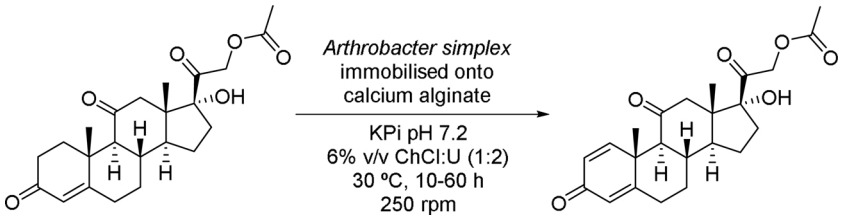


Fig. 11. Microbial 1,2-dehydrogenation of cortisone acetate for the synthesis of prednisone acetate using immobilised whole cells of *Arthrobacter simplex* and DES as co-solvents.

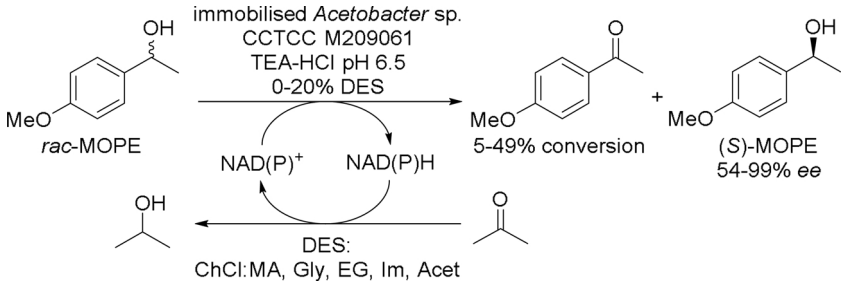


Fig. 12. Kinetic resolution of racemic 1-(4-methoxyphenyl)ethanol *via* oxidation with immobilised *Acetobacter* sp. CCTCC M209061 cells.

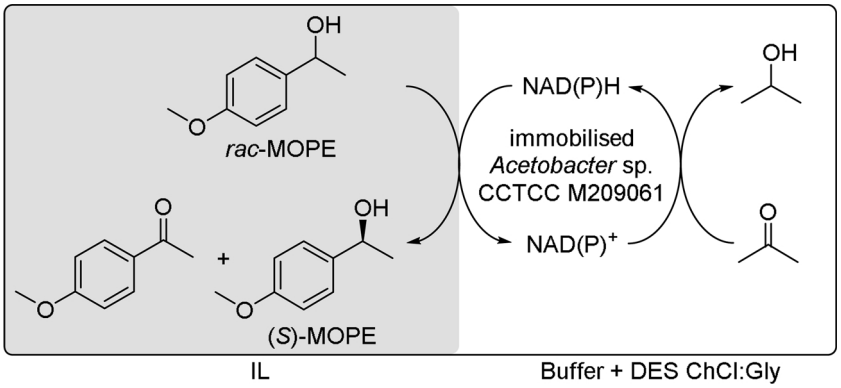


Fig. 13. Oxidative kinetic resolution of racemic 1-(4-methoxyphenyl)ethanol using immobilised *Acetobacter* sp. CCTCC M209061 cells in a biphasic system of IL (grey) and buffer with DES (white).

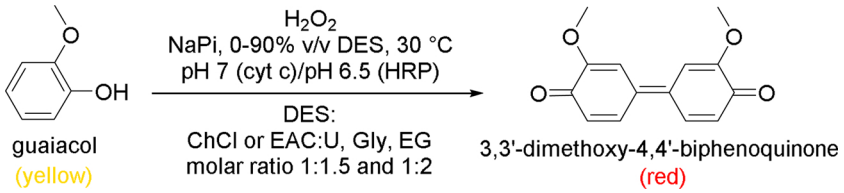


Fig. 14. Peroxidase activity assay for HRP and cyt c, determined by the colour change at 470 nm of guaiacol (yellow) to 3,3′-dimethoxy-4,4′-biphenoquinone (red) through oxidation by hydrogen peroxide (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

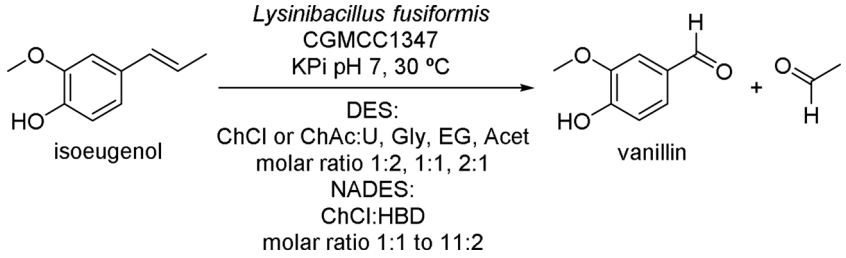


Fig. 15. Whole-cell catalysed oxidative cleavage of isoeugenol to vanillin.

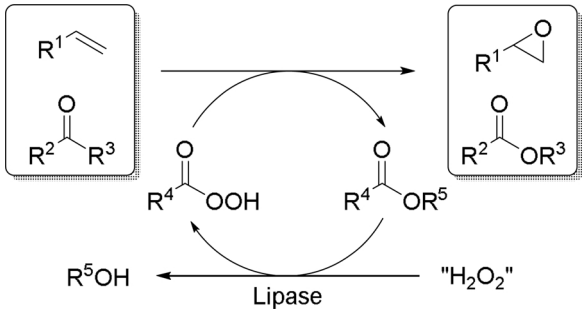


Fig. 16. General scheme for the lipase-mediated redox reactions *via* formation of peracid intermediates.

100 mM ChCl:Gly, which was reported to influence the structure of the catalyse. K_M and k_{cat} values changed upon addition of the DES. Catalase activity was retained when used in both 70% ChCl:Gly and 80% ChCl:U.

3.3. Biooxidations mediated by laccases

Laccases (EC 1.10.3.2) are copper-containing oxidases. The activity, thermal stability and tertiary structure of a laccase from *Bacillus* HR03 were studied in betaine- and choline-based DES (Khodaverdian et al., 2018). The use of choline-based DES led to lower activity and stability, whereas betaine-based DES exhibited better laccase stability when compared with buffer and ChCl DES and an increased activity with 20% v/v Gly:betaine (2:1). The highest laccase stability results were achieved with a mixture of sorbitol:betaine:water (1:1:1) and Gly:betaine (2:1) at 80 and 90 °C. Conformational changes due to DES were observed using fluorescence.

3.4. Other oxidation reactions catalysed by oxidoreductases in whole-cells

Enzymatic alkene oxidative-cleavage is highly desirable to replace traditional methods such as ozonolysis (Paul et al., 2012b; Rajagopalan et al., 2013). *Lysinibacillus fusiformis* cells CGMCC1347 were found to catalyse such a reaction, converting isoeugenol to vanillin (Fig. 15). Yang et al. studied this biocatalytic alkene oxidative-cleavage reaction with 24 DES and 21 NADES (Yang et al., 2017).

The enzymes catalysing the reaction have not yet been identified, although they are thought to be monooxygenases based on a previous study (Hua et al., 2007). The DES used were based on choline salts, either ChCl or ChAc, with four HBDs (Gly, U, EG and Acet) at molar ratios of 1:2, 1:1 or 2:1. The NADES were based on ChCl as HBA and HBDs MA, Ox, lactic acid, maleic acid, malic acid, citric acid, tartaric acid, EG, Gly, propylene glycol, Xylol, sorbitol, Xyl, Glc, Fru, Man, Gal, sucrose, maltose, lactose or raffinose, at molar ratios ranging from 1:1 to 11:2 (Yang et al., 2017). The DES and NADES used led to increased conversion from isoeugenol to vanillin by a margin of 142% for DES and 132% for NADES when compared to the use of buffer only. Interestingly, ChAc-based DES offered higher conversions with respect to the

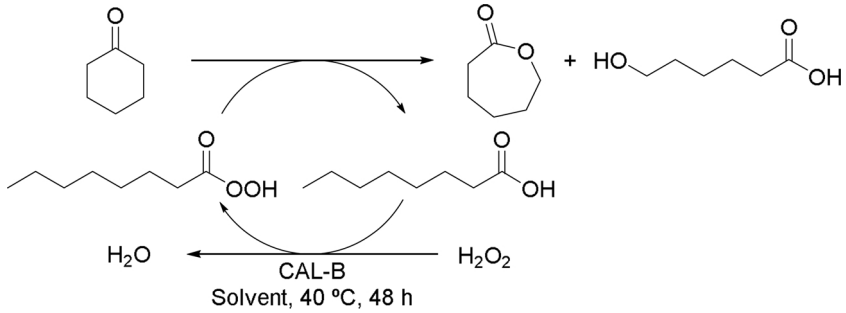


Fig. 17. Lipase-mediated oxidation of cyclohexanone with CAL-B using octanoic acid and hydrogen peroxide.

Table 3
Baeyer-Villiger oxidation of a series of ketones using 2 equivalents of H_2O_2. CAL-B wild type and its Ser105Ala mutant were used in ChCl:sorbitol (1:1) after 48 h at 40 °C.

Entry	Ketone	CAL-B wild type		CAL-B Ser105Ala mutant	
		Conv. (%)	Selectivity (%)[a]	Conv. (%)	Selectivity (%)[a]
1	Cyclobutanone	99	93	99	100
2	Cyclopentanone	95	48	51	97
3	Cyclohexanone	92	46	47	99
4	4-Heptanone	79	35	38	96

[a] The selectivity displays the percentage between the lactone (or ester product) *vs* the hydrolysis side reaction.

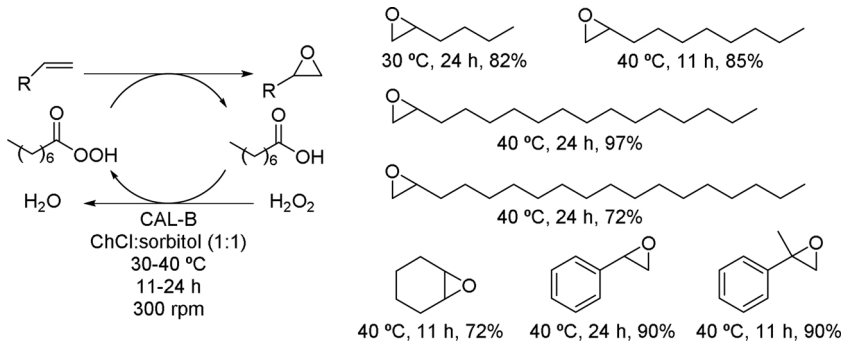


Fig. 18. CAL-B-mediated epoxidation of alkenes using octanoic acid and hydrogen peroxide.

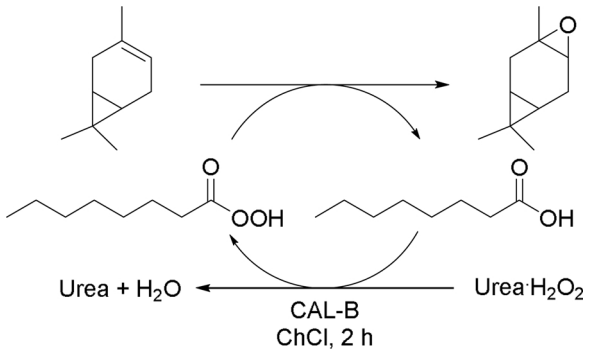


Fig. 19. CAL-B mediated epoxidation of 3-carene in minimal DES.

ChCl-based solvents. This effect was explained in part by ChAc having more influence on cellular membranes than ChCl. With 20% v/v of NADES, the yields were significantly improved, especially with ChCl:lactose (4:1) and ChCl:raffinose (11:2), affording 132% and 131% higher conversions when compared to buffer only. The source of HBD showed an effect on the yields as well, with the order sugars > alcohols > organic acids. The latter tend to lower the pH of the medium, and hence led to lower yields, such as with ChCl:Ox (1:1). This effect was also observed with in other whole-cell-catalysed processes (Bubalo et al., 2015; Xu et al., 2016, 2015b). Immobilisation of the cells on poly(vinyl alcohol)-alginate beads increased conversion with DES and NADES. Best results were achieved using ChAc-based DES, with activity retained for at least 13 cycles, showing good operational stability. Therefore, oxidative alkene cleavage catalysed by whole cells benefitted from the addition of (NA)DES for increased conversions in the production of vanillin.

4. Oxidation reactions mediated by hydrolases

Hydrolases are versatile enzymes for the organic chemists since they are able to catalyse a wide set of transformations in aqueous media but also in organic and neoteric solvents (Bornscheuer and Kazlauskas, 2006). In this manner, the traditional use of hydrolases in hydrolytic reactions have been complemented with their action in transesterification, aminolysis and ammonolysis reactions among others. The inclusion of this section is motivated for the ability of hydrolases, and mainly lipases, to catalyse perhydrolysis reactions when using carboxylic acid or esters as starting materials. Their reaction with hydrogen peroxide (H_2O_2) or other safer H_2O_2 precursors such as the urea-hydrogen peroxide complex allow the formation of peracid stable species, which are the real responsible for the chemoenzymatic oxidation

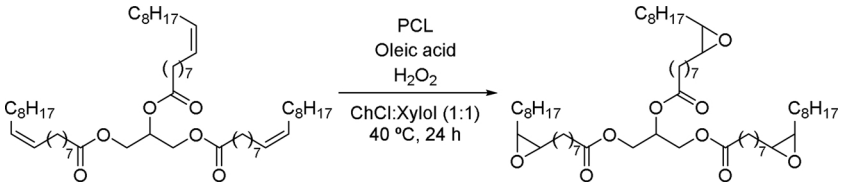


Fig. 20. Epoxidation of glyceryl triolate using the lipase G from *Penicillium camembertii*.

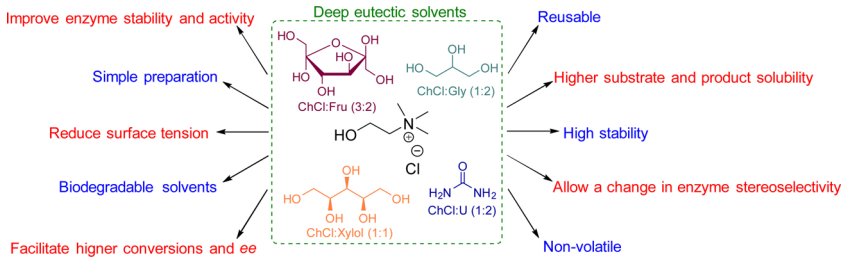


Fig. 21. Overview of the general benefits of DES in redox biocatalysis described in Table 4 (in blue: DES properties; in red: effect on enzymes) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

(Fig. 16). In this manner, hydrolases have mediated several redox processes such as Baeyer-Villiger or epoxidation reactions. Satisfyingly, DES have been used as proper solvents for these lipase-mediated transformations, and these examples are briefly explained below.

Recently, the use of *Candida antarctica* lipase type (CAL-B) has been explored in the perhydrolysis reaction of cyclobutanone, cyclopentanone, cyclohexanone and 4-pentanone, comparing its activity with the one displayed by the engineered mutant Ser105Ala (Wang et al., 2017). As source of peracid precursor, octanoic acid was employed, which is converted into peroctanoic by reaction with an aqueous solution of hydrogen peroxide (30% H_2O_2). Interestingly, in the model reaction with cyclohexanone in hexane-water (2:1 v/v), the mutant favoured the formation of the ε-caprolactone while the wild-type led predominantly to the formation of the hydrolysis product (Fig. 17). Therefore, evolution of CAL-B towards the Ser105Ala variant enables the discovery of a perhydrolase enzyme removing the hydrolytic activity displayed by the native enzyme.

Higher selectivities were found when the reactions were carried out in a DES such as ChCl:sorbitol (1:1), favouring the formation of the Baeyer-Villiger products (Table 3). In this manner, DES has a beneficial effect in the chemoselectivity, observing significant amounts of the Baeyer-Villiger products in all cases.

Epoxidation reactions have attracted considerable attention when using lipases as biocatalytic tools for the formation of peracid intermediates, the use of DES considerably reported towards this aim in recent years. Wang et al. have described the epoxidation of aliphatic alkenes and styrenes using equimolar amounts of H_2O_2 (30%) and octatonic acid (Zhou et al., 2017a). ChCl:sorbitol (1:1) was found to best solvent after screening nine eutectic mixtures, yielding the corresponding epoxides in good to excellent conversions (72–97%) after short reaction times (11–24 h) and mild temperatures (30 or 40 °C, Fig. 18).

Sieber et al. described the epoxidation of monoterpenes including 3-carene, limonene, α-pinene and camphene using also CAL-B and octanoic acid (Ranganathan et al., 2017). Several DES were employed, and in this case the authors proposed the successful use of DES mixtures prepared *in situ* by mixing ChCl and two equivalents of urea-hydrogen peroxide (minimal DES, Fig. 19). This new method provided cleaner protocols, obtaining the desired epoxides in quantitative conversions after short reaction times (2–3 h) and good to high yields and a simple extraction protocol with water and ethyl acetate.

Other lipases have also been applied in the epoxidation of alkenes such as the lipase G from *Penicillium camembertii* (PCL), which displayed even better results than CAL-B in the oxidation of glyceryl trioleate (Zhou et al., 2017b). In fact, it was seen that the use of PCL avoids the formation of side hydrolytic products observed with CAL-B (Fig. 20). The best conditions were found using 3 equivalents of hydrogen peroxide, 40 °C with and the ChCl:Xylol (1:1). Remarkably, oleic acid was used as peracid precursor without requiring its external addition as it was present in the vegetable oil samples used as starting materials. The same research group has also recently reported a similar reaction using the PCL but in this cased the soybean oil as source of triglycerides, finding the best results in a biphasic system composed by the oil and water, ChCl:sorbitol (1:1) serving to form a micro emulsion that lowers the surface tension of hydrophobic organic phases in aqueous medium (Lan et al., 2017).

5. Conclusions

The use of DES in biocatalytic processes is advancing to previously established systems with ILs and other co-solvents. DES are emerging as an alternative media for various enzyme classes to develop more sustainable processes. These neoteric solvents are simple and cost-effective to produce, retain low volatility and are more biocompatible.

Fig. 21 and Table 4 give an overview of the redox biocatalytic systems used and discussed in this review, with the type of reaction and the main beneficial effects of using DES as co-solvents or even in few cases as unique solvents. Redox reactions catalysed by whole cells containing ketoreductases in the presence of DES were shown to afford higher conversions and last longer periods of times, although these results are highly dependent on the DES nature and percentage. Including with some biocatalytic system such as with Baker's yeast, a stereoinversion can be achieved with high DES contents in comparison with the results with water as unique solvent. Other whole-cell catalysed reactions showed good biocompatibility with DES and increased cell permeability. Higher enantioselectivity was also noted with higher DES content. Isolated enzymes also performed well, being able in some cases to work in an orchestral manner in combination with metal catalysts,

Further investigation would be interesting to fully understand the effects of DES on ketoreductases, such as inhibition and conformational changes. For heme-dependent enzymes, such as haloperoxidases or peroxygenases, it would be interesting to use DES to aid with the typical low substrate solubility of these enzymes in buffer. In short, DES are already on the way to very promising outcomes for redox biocatalysis, their application in lipase-catalysed reaction being well demonstrated. Nevertheless, the use of DES with other enzymes such as lyases, transferases, isomerases and ligases remains unexplored. Current efforts are mainly focused in the study of enzyme activity and the influence of DES in the selectivity displayed by enzymes. Additional efforts must be made in the near future to present DES as compatible media for the enzymes, providing additional data about their potential with synthetic

Table 4
Overview of the DES used for redox biocatalysis.

Enzyme/whole cell	DES (molar ratio)	Reaction	Impact of DES	References
Baker's yeast	ChCl:Gly (1:2)	Bioreduction of ethyl acetoacetate	Stereoinversion (*S*) to (*R*) with > 80% DES	Maugeri and Domínguez de María (2014b)
Baker's yeast	ChCl:Fru (3:2)	Bioreduction of ethyl acetoacetate	Stereoinversion; sugar DES more biocompatible	Bubalo et al. (2015)
Baker's yeast	ChCl:Gly (1:2)	Bioreduction of arylpropanones	Stereoinversion with > 90% DES	Vitale et al. (2017)
Carrot root	ChCl:Glc, Xyl, Gly, Xylol, EG	Bioreduction of 1-(3,4-dimethylphenyl)ethanone	Lower activity due to viscosity; stereoinversion with > 70% DES	Panic et al. (2018)
Baker's yeast	ChCl:Gly (2:1)	Bioreduction of acetophenone and 3-acetylphenyl ethyl(methyl) carbamate	Higher conversions	Vitale et al. (2018)
Candida wiswanathi AM120	ChCl:Gly (1:2)	Bioreduction of α-acetylbutyrolactone	Shorter reaction time, higher enantio- and diastereoselectivity	Mączka et al. (2018)
*E. coli Ras*ADH	ChCl:Gly (1:2)	Bioreduction of ketones to chiral alcohols	Increased *ee* with 80% DES	Müller et al. (2015)
A. pasteurianus GIM1.158	ChCl:EG (1:2)	Asymmetric reduction of 2-octanone	Good biocompatibility, increased substrate solubility, good stability and cell permeability	Xu et al. (2016)
E. coli CCZU-T15	ChCl:Gly (1:2)	Reduction of COBE to (*S*)-CHBE	Improved cell permeability	Dai et al. (2017)
immobilised *Acetobacter* sp. CCTCC M209061	ChCl:U (1:2)	Bioreduction of 3-chloropropiophenone	Good biocompatibility; improved cell permeability; demonstrated preparative scale	Xu et al. (2015b)
Isolated ADH	ChCl:Gly (1:2)	Bioreduction of propiophenone and derivatives	Higher conversions	Cicco et al. (2018)
immobilised *Arthrobacter simplex*	ChCl:U (1:2)	Microbial 1,2-dehydrogenation of cortisone acetate	Better substrate solubility; recyclable enzyme	Mao et al. (2016)
immobilised *Acetobacter* sp. CCTCC M209061	ChCl:Gly (1:2)	Oxidative kinetic resolution of racemic MOPE; also in a biphasic system (ILs:buffer/DES)	Good biocompatibility; improved cell permeability; higher conversions and substrate concentration	Xu et al. (2015a) and Wei et al. (2016)
HRP or cyt c	ChCl:U (1:2)	Peroxidase activity assay for HRP and cyt c, from guaiacol (yellow) to 3,3'-dimethoxy-4,4'-biphenoquinone (red)	Beneficial for stability of HRP and cyt c, and for activity of cyt c	Papadopoulou et al. (2016)
Bovine liver catalase	ChCl:Gly (1:2)	Disproportionation of hydrogen peroxide to water	Increased binding affinity of H_2O_2 to catalase	Harifi-Mood et al. (2017)
Lysinibacillus fusiformis CGMCC1347	ChAc:U, EG, ChCl:lactose, raffinose	Alkene oxidative cleavage of isoeugenol to vanillin	Increased conversions; 20% NADES: best HBDs sugars > alcohols > organic acids	Yang et al. (2017)
CAL-B	ChCl:sorbitol (1:1)	Baeyer-Villiger oxidation of cyclohexanone to caprolactone	Better selectivity for BV products	Wang et al. (2017)
CAL-B	ChCl:sorbitol (1:1)	Epoxidation of alkenes with formed peroctanocid acid	Higher conversions	Zhou et al. (2017a)
CAL-B	Minimal DES ChCl:U formed *in situ*	Epoxidation of 3-carene, limonene, α-pinene, camphene	Higher conversions; *In situ* formation of DES led to better solubility and efficiency	Ranganathan et al. (2017)
Lipase G	ChCl:Xylol (1:1)	Epoxidation of glyceryl triolate	Higher conversions	Zhou et al. (2017b)
PCL	ChCl:Xylol (1:1)	Epoxidation of soybean oil	Reduced surface tension of water	Lan et al. (2017)

purposes including scaling-up experiments, and the role that they display in terms of product isolation and enzyme reusability.

Declarations of interest

None.

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