Enzymatic Cascade Reactions in Non-Conventional Media

10

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Abstract

The amazing challenge of orchestrate enzymatic cascades (multi-enzymatic or chemo-enzymatic) usually faces numerous issues to be addressed. Together with the advances in synthetic biology, materials science and protein engineering, the discovery of new reaction media represents a valuable tool in the search towards cost-efficient and sustainable enzymatic cascades in an industrial setting. This chapter showcases the recent developments on the implementation of the so-called non--conventional media in such processes.

Keywords

Non-conventional media · Deep eutectic solvent · Ionic liquid · Chemo-enzymatic cascade · Sustainable media · Biocatalysis

10.1 Introduction

Challenges coped by synthetic chemists when trying to access highly-functionalised molecules are evolving rapidly due to the new environmental and economic needs imposed by modern society [1]. As stated in the 'Twelve Principles of Green Chemistry' established by Anastas and Warner, a new awareness arose to produce chemicals and materials in a safer and more sustainable manner [2]. This chapter sets up the guidelines of what would make a greener chemical process:

- Be safe for the human being and the environment,
- Take place under mild reaction conditions,
- Be catalytic [3],
- Be performed using inexpensive and sustainable solvents [4].

At this point, the inherently green enzyme catalysis fits ten of the previous 12 principles and it has experimented a spectacular growth for the synthesis of chiral molecules [5, 6]. Nature's catalysts are usually non-toxic, biodegradable and work under physiological conditions, thus achieving numerous safety and economic benefits.

Deepening in the 12 Principles, a special emphasis is placed in '*Safer Solvents and Auxiliaries*' with the prospect of replacing the classical petroleum-based *volatile organic solvents (VOCs)*, which suffer from toxic/carcinogenic risks, with alternative greener solvents. While water (considered the ideal solvent) is the physiological medium of enzymes, a concomitant challenge for biocatalysis is the low solubility of hydrophobic substrates and products which limits the volumetric productivity demanded at industrial settings [7]. In this context, the staging of

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non-conventional media as biorenewable reaction media has brought new paradigms in many research fields. The term '*non-conventional media*' highlighted in the title of the present chapter compiles the following non-aqueous environments [8]:

- Solvent-free processes
- Supercritical fluids (SCFs)
- Biomass-derived solvents
- Fluorinated solvents
- Ionic liquids (ILs)
- Deep eutectic solvents (DESs)

A shallow analysis reveals attractive advantages but also limitations for all of them: Technology for CO_2 and water in supercritical conditions involves high cost; fluorinated solvents are non-toxic but very expensive and prevalent in the atmosphere; biomass-derived solvents are cheap, safe and biorenewable although non-susceptible to being tuned; ILs offer tunability but suffer from toxicity and low biodegradability; the more recent DESs, intimately related to ILs, meet most of the pursued properties.

Defined as salts with low melting point ($<100\ ^\circ C$), ILs are composed of an organic cation and an organic or inorganic anion (Fig. 10.1). Interestingly, the appropriate combination of such components allows modifying the physical properties (viscosity, hydrophobicity, polarity and hydrogen bond basicity). Ultimately, the so-called *task specific ionic liquids (TSILs)* go beyond a mere solvent to display key roles in a myriad of areas such as catalysis, electrochemistry, spectroscopy or material science [9]. ILs were the first non-conventional medium exploited in biocatalysis, the last two decades witnessing a plethora of biotransformations [10]. ILs stabilise some kinds of enzymes through the microenvironment generated and acting such a liquid support (interactions between enzyme and IL). Thus, although lipases and proteases were the most extensively used enzymes, C-C bond formation and redox reactions have also been conducted in such reaction media.

DESs are mixtures of low-cost biodegradable components such as hydrogen bond acceptors (HBA; ammonium salts) and uncharged hydrogen bond donors (HBD; urea, carboxylic acids or polyols) with a lower melting point than either of their components (Fig. 10.2). Compared to ILs, DESs are cheaper, readily available, do not require further purification and are considered to be less toxic given the nature of its components [11, 12]. In particular, those DES consisting of primary metabolites, namely, amino acids, organic acids, sugars or choline derivatives, are defined as *natural deep eutectic solvents (NADESs)* [13], and their use is allowed in food and pharmaceutical formulations [14]. Although research on DESs runs still in its infancy, the amazing properties of these neoteric solvents have permitted the flourishment of applications in research fields such as electrochemistry and metal processing, material chemistry, nanotechnology, photosynthesis and energy technology, and separation processes. With regard to synthetic applications, DESs have provided examples of improved activity and selectivity in: (1) organometallic-mediated stoichiometric transformations and (2) metal- and organo-catalysed reactions. Likewise, they offer an ideal reaction medium for both isolated enzymes (lipases, proteases, epoxide hydrolases, peroxidases, ketoreductases (KREDs), lyases and transaminases (TAs)) and whole cells [15, 16].

The preceding chapters have evidenced fundamental principles and impressive advances in multi-enzymatic processes and their monitoring during the last decade, enabling the construction of enantiopure high added-value chemicals under mild reaction conditions. To increase the sustainability of chemical manufacturing, these systems emulate the biosynthetic pathways where the living cells work as a perfect machinery of enzymatic networks. Nevertheless, when mimicking Nature things are more complicated than it seems. Once planned a synthetic cascade, a number of concerns must be circumvented to reach an efficient implementation of such a process. Incompatibility of catalysts and their preferred reaction profiles are commonly found as well as problems arising from inhibition and instability.

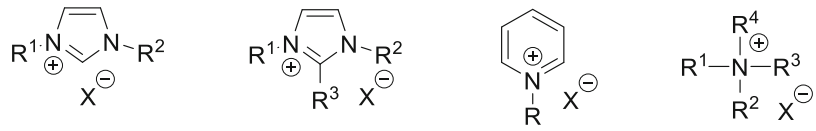


Fig. 10.1 Chemical structure of commonly used ILs

As a result, chemists have developed a battery of ingenious approaches aimed at circumventing the multiple drawbacks inherent to these processes [17]. The object of this chapter is to showcase non-conventional media as a new platform to perform enzyme cascade reactions (multi-enzymatic or chemo-enzymatic) in a more efficient manner. Along with advances in synthetic biology, materials science or protein engineering, medium engineering can be a practical solution for filling remaining gaps in some examples disseminated in the book as well as a valuable tool for future biocatalytic processes.

10.2 Chemo-enzymatic Cascades in Non-conventional Media

As reflected in Chap. 5, the wide interest in chemo- and biocatalysis has spurred smart methodologies to merge the synthetic potential of these pivotal areas of catalysis [18]. Historically, chemists combined reactions belonging to a single type of catalysis. Metal-catalysed reactions usually rely on the use of VOCs to avoid catalyst degradation in water, and are accomplished at high loadings of substrate. Conversely, enzyme catalysis is performed in aqueous medium and suffers from low substrate concentrations due to the poor water solubility of the reaction partners. Likewise, most biocatalysts are readily inactivated in organic solvents. Bearing all these facts in mind, the implementation of hybrid catalytic systems demands a compatibility window where both catalysts coexist and exert their activity. Interestingly, the pool of metal catalysts operating in water has expanded enormously in the last years, and the advances in protein engineering and immobilisation have boosted the combination of several metal-catalysed reactions (Pd-catalysed C−C coupling, Wacker oxidation, and C−H activation, Cu(I)-catalysed click chemistry, Ru-catalysed olefin metathesis or Au-catalysed cyclisations) and

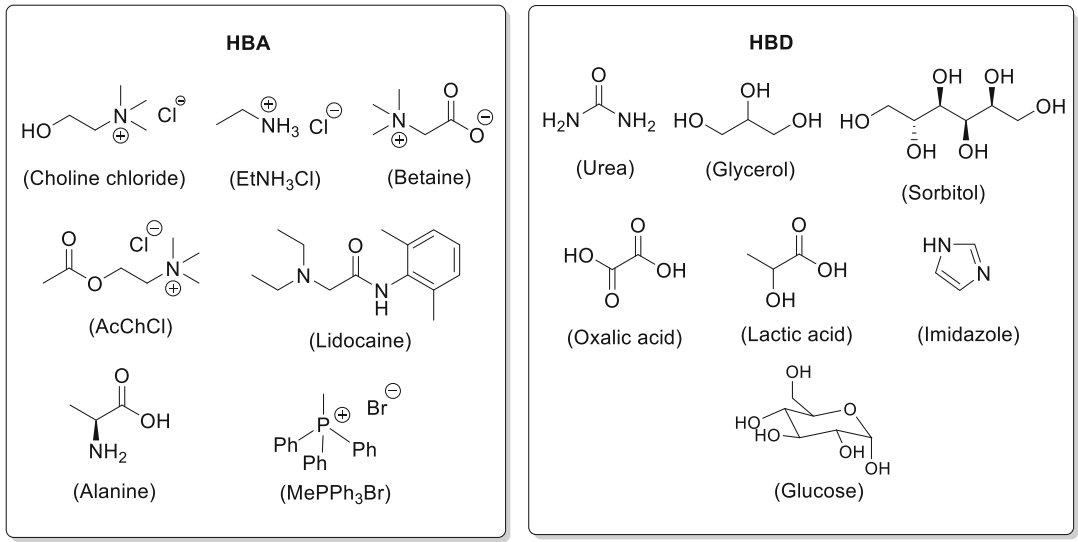


Fig. 10.2 Typical HBA and HBD components in DES mixtures; the formed DES is named as HBA:HBD (mol:mol)

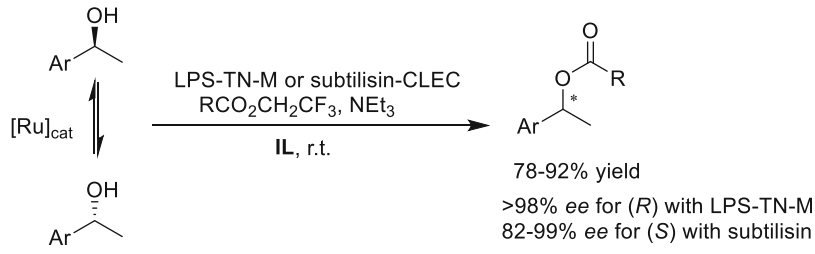
biotransformations such as reductions, halogenations, hydrolyses or epoxidations [19]. The emergence of ILs and DESs, bordering with organic solvents and water, offers a new scenario to interface metal and enzymes in a common reaction medium.

The case for combining enzymes and metal catalysts in a one-pot fashion reached relevance in the 1990s throughout *dynamic kinetic resolution (DKR)* processes mediated by a lipase and a Pd or Rh racemisation catalyst [20, 21]. These early studies were conducted in organic solvent and overcame the inherent 50% maximum yield of classical kinetic resolutions. In 2004, Kim and Park practiced the same process, namely a DKR of secondary alcohols by a combo metal-enzyme catalytic system, in a neat ionic liquid (Scheme 10.1) [22]. Together with the avoidance of VOCs, the metal catalyst turned out to be much more active in ILs and allowed the reaction to proceed at room temperature. The combination of a cymene-ruthenium complex either with LPS-TN-M lipase and subtilisin rendered (*R*)- or (*S*)-alcohols, respectively, in high yield and optical purity.

One of the pioneering examples combining metals and enzymes in aqueous medium consisted of a Suzuki cross-coupling of halogenated acetophenone followed by an alcohol dehydrogenase (ADH)-mediated reduction of the transiently formed ketone. The original report in 2008 described the coupling step at 33 mM loading of substrate and 70 °C and the bioreduction at 25 mM (4.9 g/L) and room temperature [23]. Later, the employment of water-soluble palladium catalysts enabled the first step at room temperature although the loading of substrate remained challenging (40 mM) [24]. Owing to the good tolerance exhibited by palladium catalysts and ketoreductases towards ILs, the employment of IL-buffer mixtures as biphasic media was envisaged to tackle the solubility hurdles (Scheme 10.2) [25]. Accordingly, the Suzuki-coupling was catalysed by $Pd(PPh_3)_4$ (1.2 mol%) in IL:H_2O (IL: [bmim][NTf_2]), at 100 °C and 210 mM (41 g/L). For the second-stage enzymatic reduction the reaction mixture was diluted to 125 mM with a buffer solution containing *E. coli*/ADH-A cells, NADH as cofactor and *i*-PrOH for cofactor recycling. The biphasic medium enabled to recycle both catalytic species four times. On the one hand, the supernatant aqueous phase harbouring all the components of the biotransformation was directly separated and ready for re-use. On the other hand, once extracted the target biaryl alcohol from the lower IL-phase with an organic solvent, the palladium (Pd) catalyst remained active in the IL. The process exhibited broad substrate scope and a variety of enantiomerically pure (*S*)-biaryl alcohols were obtained in high yields, being in fact the first example of chemoenzymatic cascade in IL-buffer biphasic system.

Some years later, the burgeoning interest in DESs motivated the study of the above cascade in these media (Scheme 10.3) [26]. Indeed, both Pd-catalysed coupling reactions (Suzuki–Miyaura, Sonogashira and Heck couplings) and bioreductions had been efficiently established in neat DESs and DES–buffer mixtures [27]. Through parametrisation, the Suzuki-coupling was set up by the water-soluble system $PdCl_2$/TPPTS (1 mol%/3 mol%) in DES–buffer 4:1 [DES = choline chloride (ChCl)/glycerol (Gly) 1:2 mol:mol] at 100 °C. The reaction was highly influenced by both aryl halide and boronic acid, with a limiting substrate concentration of 200 mM. Then, the reaction mixture was diluted to 75 mM (DES–buffer ~1:1 v/v) with a solution containing *i*-PrOH, KRED and cofactor. The general applicability of the process was demonstrated with unsubstituted, fluorinated and pyridyl derivatives, and the employment of stereocomplementary ADHs from *Lactobacillus kefir* ($NADP^+$ dependent) [28] and *Rhodococcus ruber* (NAD^+ dependent) [29, 30] gave access to (*R*)- and (*S*)-enantiopure biaryl alcohols, respectively.

The first cascade involving organocatalysts and enzymes in DESs was described in 2014 and consisted of the enzymatic production of acetaldehyde and its further cross-aldol reaction on aromatic aldehydes (Scheme 10.4) [31]. Regarding such enzymatic reaction, a previous study on lipase-catalysed transesterification of alcohols ascertained that, under some reaction

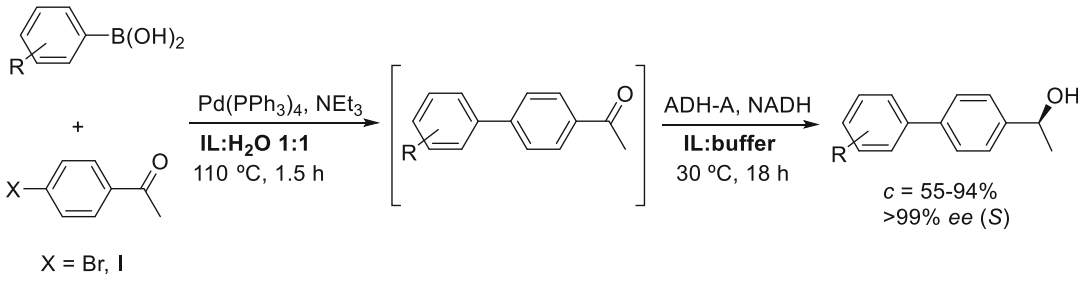


Scheme 10.1 DKR of secondary alcohols by tandem chemoenzymatic catalysis

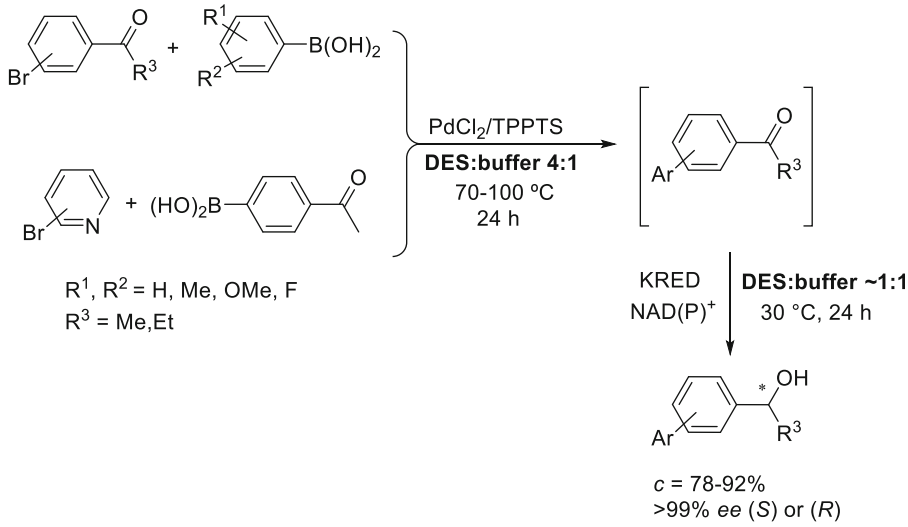
conditions, the hydrolysis of vinyl acetate predominates over the expected acylation. As a result, acetaldehyde and acetic acid are released at the expense of water consumption. This side reaction also produces hemiacetal derivatives which are responsible for disappointing enantioselectivities [32, 33]. This concept was exploited to couple the enzymatic acetaldehyde production (CAL-B catalysed transesterification of vinyl acetate with *i*-PrOH) with an enantioselective C-C aldol reaction mediated by proline-based organocatalysts. A DES turned out to be the optimal reaction medium, namely ChCl/Gly (1:2 mol:mol), and some aromatic 1,3-diols were obtained in good yield and enantioselectivity. Interestingly, both catalysts and DES could be reused for several cycles.

The first chemoenzymatic cascade in DESs–buffer mixtures connected a metal-catalysed isomerisation reaction of allylic alcohols with an enantioselective bioreduction promoted by KREDs (Scheme 10.5) [34]. The process had been previously set up in aqueous medium in both sequential and concurrent mode, establishing a practical approach to convert a racemic mixture of allylic alcohols into saturated enantiopure alcohols without isolation/purification steps [35]. The overall transformation occurred through three steps, namely reduction of the allylic C–C double bond, oxidation of the secondary carbinol moiety and asymmetric bioreduction of the generated prochiral ketone.

The reported process in aqueous medium was robust and operationally simple in the sequential fashion (one-pot two steps); once the isomerisation was complete at 50 °C, the only adjustment before adding the pair enzyme/cofactor consisted of a slight decrease on temperature. The implementation of DESs resulted in a beneficial effect on the enantioselectivity exerted by the KRED, particularly in the case of substrates unresponsive to be selectively reduced in aqueous medium. ChCl/Gly (1:2 mol:mol)-buffer and ChCl/sorbitol (Sorb, 1:1 mol:mol)-buffer mixtures led to substantial enhancement of the optical purity of the resulting alcohol at high percents of DES. With regard to the concurrent process (one-pot one-step), an open issue was the stability of the KRED in aqueous medium. In fact, the enzyme underwent rapid deactivation in the buffer which impacted negatively in the overall conversion when starting from allylic alcohols with slow isomerisation rate. With these premises, ChCl/Gly (1:2 mol:mol)-buffer 4:1 (w/w) was tested for a set of allylic alcohols combining a commercial KRED (KRED-P2-



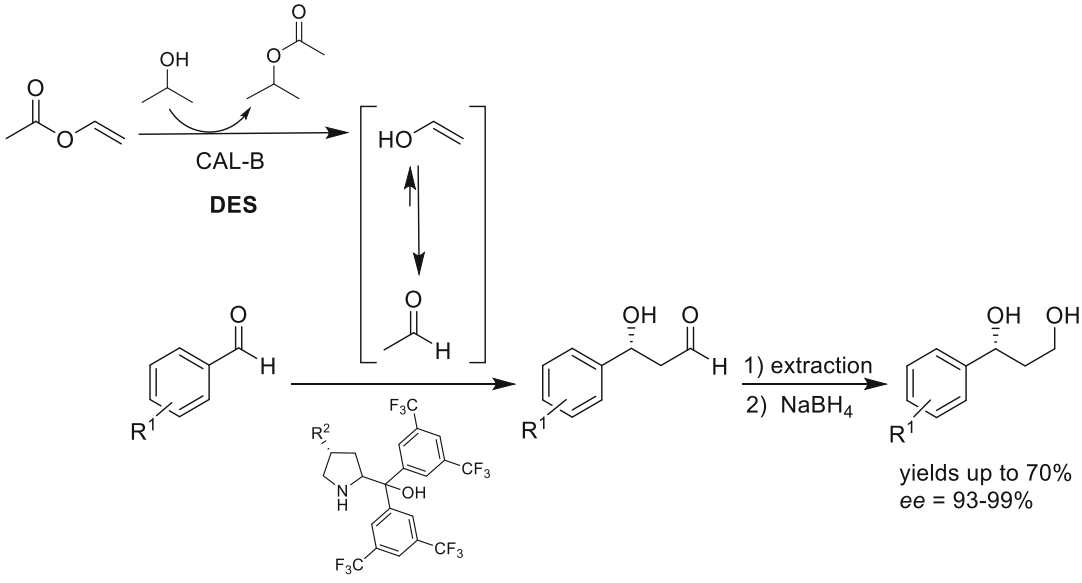
Scheme 10.2 Cascade synthesis of enantiomerically pure biaryl alcohols in IL-buffer mixtures



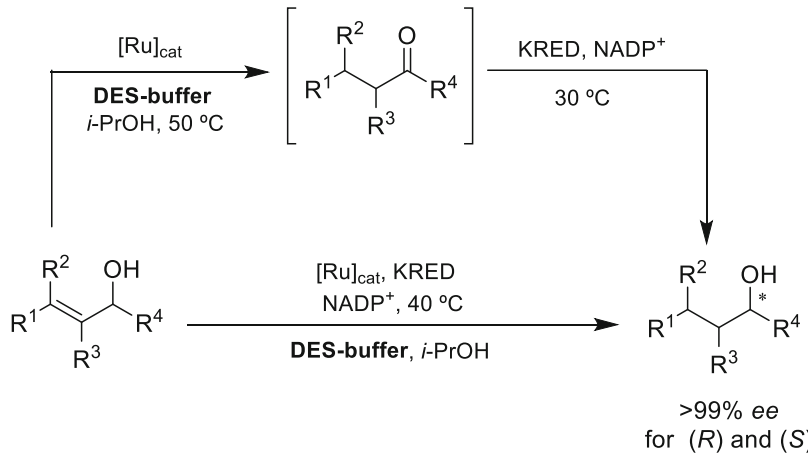
Scheme 10.3 Cascade synthesis of enantiomerically pure biaryl alcohols in DES–buffer mixtures

C11) and a ruthenium complex. Working at 40 °C and 10% mol of catalyst loading, the saturated chiral alcohols were obtained in enantiopure form with overall conversions ranging from 68 to 96% [(*R*)-enantiomer]. In particular, 1-(4-bromophenyl)prop-2-en-1-ol rendered the saturated analogue in 96% overall conversion, the biggest so far.

The potential and versatility of DESs were featured by means of an enzymatic cascade to convert limonene from orange peel wastes into epoxide derivatives (Scheme 10.6) [36]. Typically, the enzymatic version of the Prilezhaev reaction (epoxidation of an alkene with peracid to give an oxirane) is performed by a hydrolase which catalyses the in situ formation of peracid from an acid and H_2O_2. In an innovative concept,



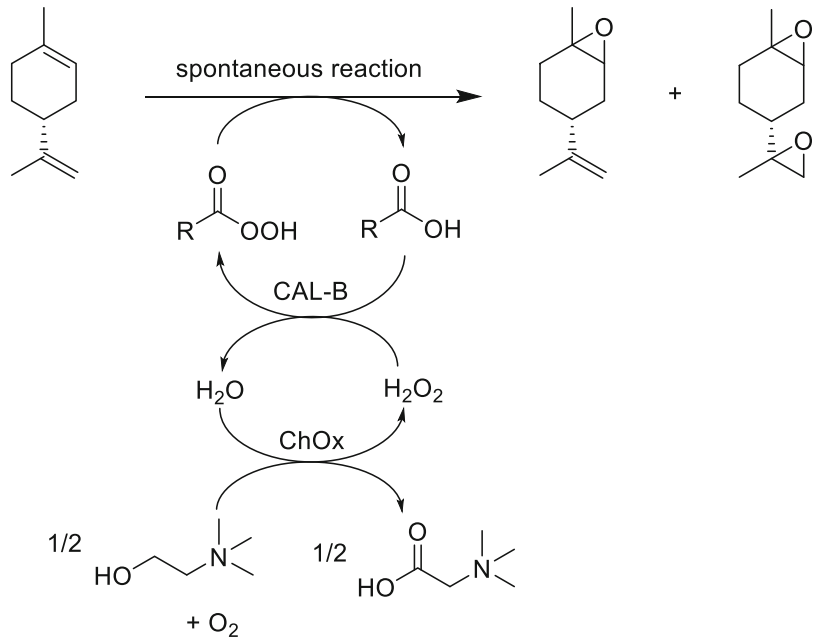
Scheme 10.4 Tandem catalysis of enzymes and organocatalysts in DES towards optically active 1,3-diols



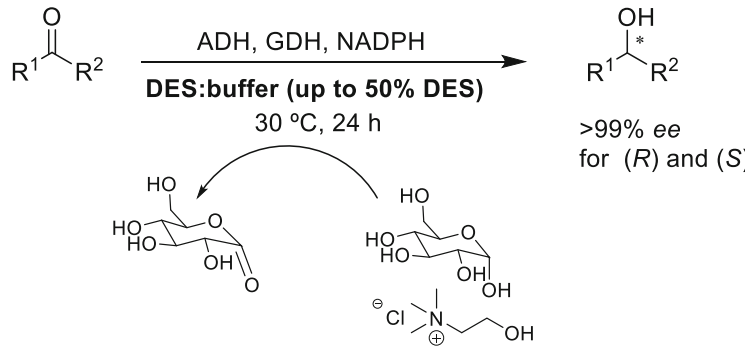
Scheme 10.5 Cascade synthesis of optically active alcohols by Ru-catalysed isomerisation/enzymatic reduction in DES–buffer medium in both sequential and concurrent mode

a DES displayed a triple role as extracting solvent, reaction medium for the biotransformation and reagent for cofactor recycling. In first instance, two DESs, namely ChCl/propane-1,2-diol/H_2O (1:1:1 mol:mol:mol) and ChCl:ethylene glycol (EG, 1:1 mol:mol) showed comparable efficacy to ILs and organic solvents to recover limonene from the agricultural waste. On the other hand, the choline oxidase (ChOx) from *Arthrobacter nicotianae* was able to produce H_2O_2 from the previous ChCl-based DESs. Meeting both facts, the coupled catalytic system consisted of orange peels, octanoic acid, hydrolase Novozym 435 (CAL-B) and ChOx in a mixture DES:buffer 1:1. The reaction mixture was heated at 40 °C and 500 rpm with an O_2 atmosphere. The overall yield was 33%, for a mixture mono/diepoxide (70:30).

Similarly, a rationally designed NADES served as the reaction medium for the bioreduction of prochiral ketones and acted as co-substrate for the recycling of the required cofactor (Scheme 10.7) [37]. The ADHs considered in the study operate with the assistance of



Scheme 10.6 Enzymatic cascade epoxidation of limonene employing DES as multifunctional solvent



Scheme 10.7 Bioreduction of prochiral ketones in a rationally designed DES exerting a dual function as solvent and co-substrate for cofactor recycling

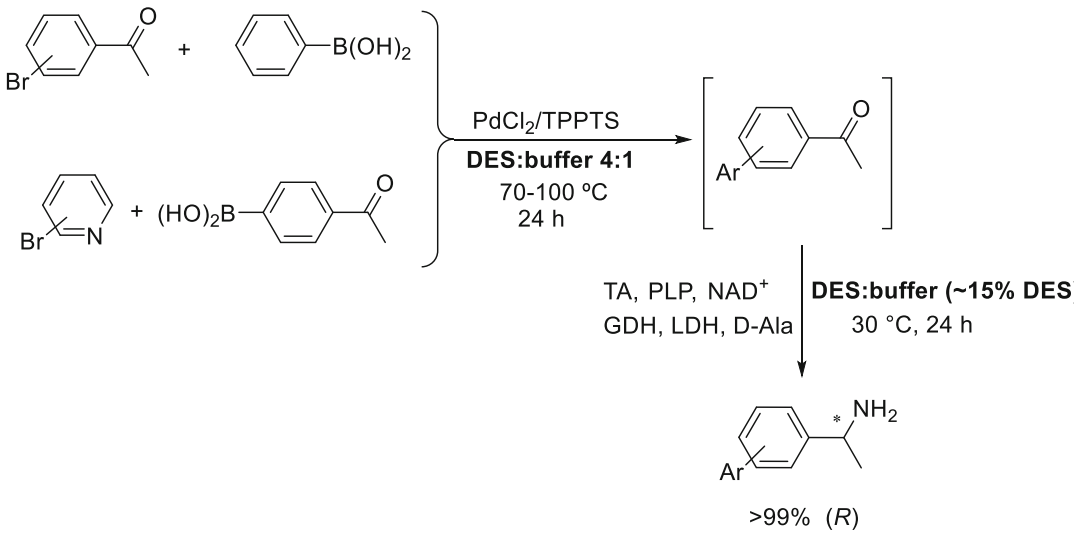
NADPH which is typically recycled by a parallel enzymatic reaction mediated by GDH or FDH. Seeing as GDH takes advantage of glucose as sacrificial co-substrate, the authors conceived a DES containing such sugar to perform that role. Thus, the mixtures of ChCl:glucose (Glu, 1.5:1 mol:mol) and an aqueous buffer with up to 50% (v/v) of DES were ideal media for the five overexpressed ADHs, namely *Lactobacillus brevis* (LbADH) [38], *Thermoanaerobacter ethanolicus* (TeSADH) [39], *Thermoanaerobacter* sp. (ADH-T) [40], *Sphingobium yanoikuyae* (SyADH) [41] and *Ralstonia* sp. [42]. Interestingly, since D-gluconic acid is released as by-product, the presence of a buffered aqueous solution was necessary to avoid a drastic drop of pH which would damage the enzyme. As a result, the enantioselective reduction of several ketones was accomplished in very high enantiomeric excess and yields, enabling higher loadings of substrate than those reported by the solubilising effect of the DES.

Very recently the good tolerance of transaminases towards DESs was unveiled, and by extension the asymmetric bioamination of ketones within these solvents [43]. Remarkably, some TAs turned out to be stable in DES–buffer mixtures containing up to 75% (w/w) of DES. Among the biocatalysts studied was included the (*R*)-selective transaminase *from Exophiala xenobiotica* (EX-ωTA), a biocatalyst found by data mining which is able to accept differently bulky biaryl ketones [44]. While EX-ωTA only accepted 15% (w/w) of neoteric solvent, it was the least harmful co-solvent of those essayed.

On the other hand, the discovery of EX-ωTA in parallel to that of TA from *Aspergillus fumigatus* (4CHI-TA) paved the way to establish a chemoenzymatic cascade toward chiral biaryl amines by combining a Suzuki-coupling with an enantioselective bioamination. In first instance, Bornscheuer's group developed such a sequential process in aqueous medium employing 4CHI-TA at low substrate concentration, namely 2 mM and 1 mM for each step [45]. Soon after, an identical cascade with EX-ωTA was reported in a reaction medium consisting of a DES–buffer mixture (Scheme 10.8). As a result, the metal-catalysed step was accomplished at 200 mM loading of substrate and the subsequent biotransformation at 25 mM. It is worth noting that ChCl/Gly (1:2, mol:mol) emerged as the only co-solvent compatible for both steps since DMSO inhibited the Pd catalyst and THF and *i*-PrOH were harmful for the TA. The methodology was extended to *meta*- and *para*-biaryl ketones and pyridylphenyl ketones as well, rendering the corresponding (*R*)-biaryl amines with >99% *ee*.

10.3 Enzymatic Cascades in Non-conventional Media

The inherent biocompatibility and solubilising properties of DESs crystallised in the application for one-pot biomass processing (Fig. 10.3) [46]. First, ChCl-based DESs turned out to be suitable solvents for the pretreatment step of crude biomass, the levels of degradation products such as furfural (polysaccharides) and ferulic acid (lignin) being low enough for the growth of yeast



Scheme 10.8 Cascade synthesis of enantiomerically pure biaryl amines in DES–buffer mixtures

Saccharomyces cerevisiae. Likewise, DESs were also biocompatible with the hydrolytic enzymes involved in the process. As a result, saccharification and fermentation steps were effectively established in one-pot fashion resulting in an ethanol production of 77.5% theoretical yield in ChCl/Gly (1:2 mol:mol) pH 5.8 (10 wt% aqueous solution). Compared to previous approaches relying on ILs, the implementation of DESs avoided any pH adjustment and solid/liquid separation steps throughout the above process.

Similarly, DESs served as the reaction medium for a one-pot two-step enzymatic process to obtain biodiesel from waste cooking oils as feedstock (Fig. 10.4) [47]. A first enzymatic esterification in aqueous medium and 30 °C catalysed by *Thermomyces lanuginosus* lipase was selective on triglycerides; further sequential addition of *Pseudozyma antarctica* lipase B and ChCl/Gly (1:2 mol:mol) completed the transesterification of the remaining glycerides and fatty acids at 45 °C. The resulting two-phase system delivered lipids to the upper phase and a glycerol-DES mixture to the lower one. Conventional alkaline refinement delivered the product with an ethyl ester content of 97.6% and free of glycerol and acid, which meets the requirements for Biodiesel standard EN 14214 in Europe. An extra benefit of using DESs lies in the easy purification of glycerol from the hydrophilic phase by

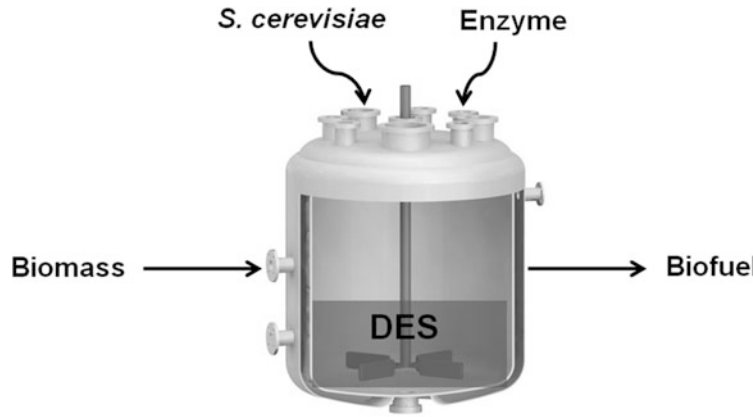


Fig. 10.3 Enzymatic one-pot production of bioethanol in DESs

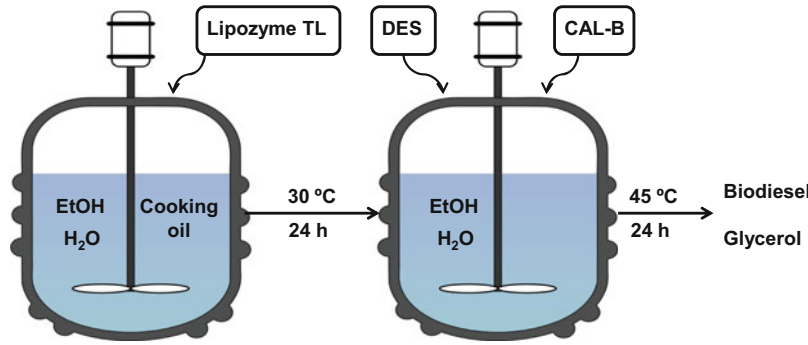


Fig. 10.4 One-pot-two-step enzymatic production of biodiesel from used cooking oil in a DES–water medium

distillation of the DES component. Likewise, Lipozyme CAL-B was recovered from such phase and reused in five cycles.

The capture, storage and processing of carbon dioxide (CO_2) represent a capital challenge today due to the threat of greenhouse effect. As a sustainable solution, the enzymatic conversion of CO_2 into methanol in the presence of ILs was investigated (Fig. 10.5) [48]. This multi-enzymatic cascade process had been described in aqueous medium, though the production of methanol reached a yield of 44% due to unfavourable kinetics in the first step, namely the conversion of CO_2 into formic acid mediated by formate dehydrogenase (FDH) [49]. The incorporation as co-solvent of biocompatible ILs based on ChCl and amino acids enabled a higher solubilisation of CO_2 and also had a stabilising effect on FDH. The four enzymes involved in the biotransformation (FDH, FaldDH, ADH and GDH) were immobilised in a cellulose membrane and a separation system platform enabled the recycling of biocatalysts and the removal of methanol. Under the optimised reaction conditions, an aqueous solution containing 20% of [choline][L-glutamic acid], the production of methanol was improved fivefold with regard to the aqueous medium used as control.

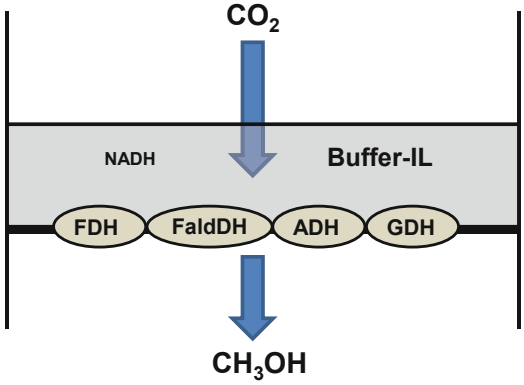


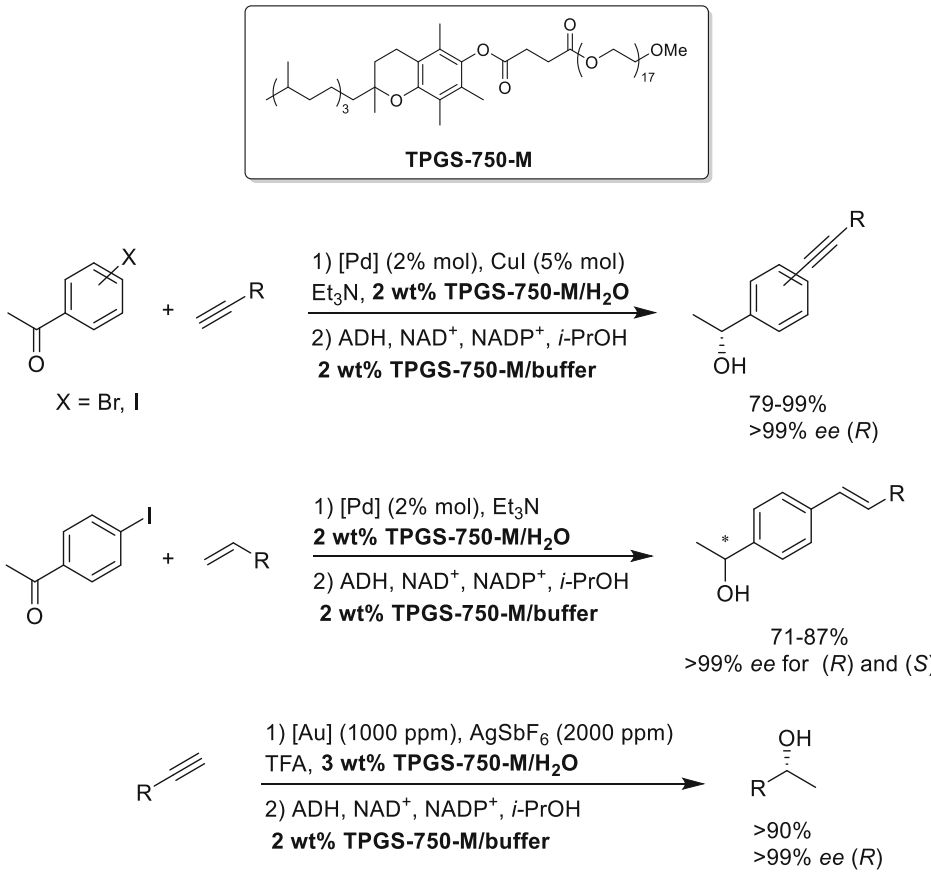
Fig. 10.5 Enzymatic cascade conversion of carbon dioxide to methanol in buffer-IL mixtures

10.4 Recent Developments

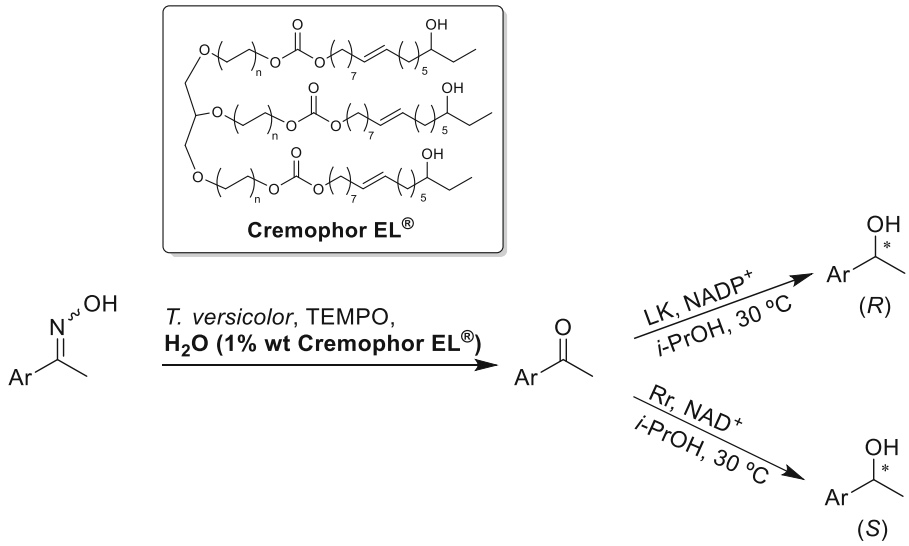
Although outside the established classification of non-conventional media, recently it has appeared an innovative reaction medium based on aqueous micellar solutions. The concept was originally conceived with the aim of making synthetic chemistry in water upon the assistance of designer surfactants [50]. Now, these would act not as mere solubilisers of catalysts and reagents but as key co-solvents forming such smart nanoreactors for specific transformations. After many successful examples in which the chemical reaction occurs in the inner hydrophobic core of the micelles, Lipshutz and co-workers went one step further and envisaged to meet enzymes and metal catalysts through this so-called micellar catalysis. Most logically, the enzymes would remain in the aqueous solution and the micelle host both organic substrates and chemo-catalysts to minimise the expected metal-enzyme inhibition. Accordingly, TPGS-750-M, a surfactant bearing a vitamin E as hydrophobic moiety, was introduced with the prospect of suit apolar substrates and metal species. Once demonstrated the perfect tolerance of alcohol dehydrogenases

towards TPGS-750-M, this pioneering report showed the versatility of the methodology through several hybrid catalytic systems combining metal-catalysed processes such as Sonogashira and Heck couplings (Pd catalysts) or alkyne hydrations (Au and Ag catalysts) with a further bioreduction (Scheme 10.9) [51]. On the one hand, the micellar medium impacted significantly on the enzyme activity, leading to improved productivities. On the other hand, once suppressed the inhibition of the metal catalyst on the enzyme the chemoenzymatic one-pot sequential processes were successfully accomplished leading to products in high overall yield and enantiomeric excess.

Similarly, the beneficial impact of surfactants on enzymatic activity was exploited in an enzymatic cascade in aqueous medium to convert prochiral ketoximes into optically active alcohols by sequential laccase-catalysed deoximation and further bioreduction of the triggered ketone (Scheme 10.10) [52]. Owing to the employment of exclusively 1% (w/w) of Cremophor®, a polyethoxylated castor oil typically used as a formulation vehicle for poorly-water soluble drugs, a high enzymatic performance was achieved. Thus, both laccases and ketoreductases showed perfect tolerance towards the surfactant and such medium free of organic co-solvents enabled to increase the substrate concentration up to 200 mM in the initial biodeoximation and 100 mM in the later bioreduction. As a result, and depending of the KRED employed, the (*R*)-or (*S*)-enantiomer of the corresponding alcohol was isolated in good overall yield and >99% *ee*.



Scheme 10.9 Representative examples of chemoenzymatic cascades performed in micellar aqueous medium



Scheme 10.10 One-pot sequential enzymatic transformation of ketoximes into optically active alcohols in an aqueous medium supplemented with surfactant

10.5 Conclusions and Future Prospects

Enzyme-enabled cascade processes have boosted incredibly in the recent years. Together with the combination between a series of enzymes, the assembly of biocatalysis with other disciplines such as metal- or organo-catalysis has endowed these processes with great synthetic potential. In this context, recent research in medium engineering has brought non-conventional media to the forefront of organic synthesis. As highlighted along this chapter, these reaction media have gone from mere spectators to display key roles in the synthetic transformation. Some of the disclosed examples revealed a critical impact on enantioselectivity, loading of substrate or yield of different cascades processes. Likewise, the implementation of non-conventional media in some selected processes enabled to alleviate the inhibition between metals and enzymes. Once the first seeds have been planted and seeing as today's world claims for a more sustainable chemical industry, we anticipate that the necessary symbiosis between enzymatic cascades and non-conventional media will result in astonishing breakthroughs in a near future.

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