Non-Conventional Media as Strategy to Overcome the Solvent Dilemma in Chemoenzymatic Tandem Catalysis

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The amazing potential of multi-catalytic cascade reactions to reduce the number of reaction steps and to solve synthetic problems brings the challenge of an increasing complexity that must be controlled. Particularly chemo-enzymatic reactions are prone to conflicts regarding different optimal reaction conditions for chemical and biological catalysts. The preference of many chemical catalysts for hydrophobic (and often water-free) solvent and of many enzymes for water poses a solvent dilemma. Recently, non-conventional solvents have been very successful to provide suitable reaction media for catalysts that otherwise require very different solvents, and to alleviate fundamental problems such as the low solubility of many substrates in aqueous solvents. In the last few years, several examples underlined the considerable potential of ionic liquids and deep eutectic solvents for the engineering of cascade reactions. This mini-review showcases the recent developments on the implementation of the so-called non-conventional media in such processes.

1. Introduction

Chemoenzymatic cascade reactions face a general solvent dilemma: While the majority of enzymes prefer aqueous systems, chemical catalysts have been mostly developed for organic solvents. Many metal catalysts dissolved in water are sensitive against oxidation. Moreover, the solubility of many substrates in water is very low. While the number of examples for chemo-enzymatic catalysis is rapidly increasing,[1] these are intrinsic challenges for all tandem reactions and other multi-catalytic one pot cascades. Enzyme classes such as lipases and proteases with outstanding stability in organic solvents were successfully applied together with metal catalysts (such as the Shvo-catalyst) already in the 1990s. As few enzyme classes have high tolerance towards organic solvents, it took several years to expand the concept of chemoenzymatic one pot reactions towards other enzyme classes. Nowadays, the pool of metallic catalysts able to operate in aqueous media has expanded significantly and provides novel opportunities for combination with enzymes in aqueous media. Yet, a large number of catalysts still requires (co)-solvents or even water-free systems, and the low solubility of many hydrophobic substrates in water limits space-time yields. Biphase systems can alleviate these issues to some extent, but are complex and difficult to use in continuous systems. In parallel, the irruption and quick development of tools such as compartmentalization (natural and synthetic) or enzyme-metal nanohybrids enables to control the microenvironment of catalysts with the aim to set up truly simultaneous chemoenzymatic cascades. Compartmentalization encompasses carrier-free techniques such as encapsulation,[2] enzyme cross-linking using cross-linked enzyme aggregates or, quite recently, autocatalytic bioconjugation,[3] and classical carrier-based enzyme immobilization.[4] The field of enzyme immobilization and compartmentalization is vast and covered by several recent reviews.[5] Here it should suffice to say that the application of these enzymes to cascades is challenging, but that great progress has been made[6] that they offer the possibility to channel reaction intermediates of cascades;[7] that, in addition to stabilizing the biocatalysts, they can influence the reactivity of enzymes;[8] that they offer potential additional advantages such as the perspective to exploit the selectivity of natural transport molecules[9] and, that the interaction with formulation and medium is crucial for a successful solvent engineering.

In this context, the staging of non-conventional media has brought new paradigms in many research fields, including catalysis. The term ‘non-conventional media’ compiles the following non-aqueous environments:[10] Solvent-free processes, supercritical fluids (SCFs), biomass-derived solvents, fluorinated solvents, ionic liquids (ILs) and deep eutectic solvents (DESs) (Figure 1). In particular ILs, which are salts with low melting point composed of an organic cation and an organic or inorganic anion, have been widely investigated as non-conventional media in biocatalysis for two decades.[11] Many reports have demonstrated the ability of ILs to stabilize different kinds of enzymes through the microenvironment generated and acting as such a liquid support.[12] More recently, DESs have emerged as a new family of green solvents which find widespread applications in a variety of areas including biocatalysis and metal catalyzed organic reactions. DESs are mixtures of

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Figure 1. Non-conventional media for sustainable chemical processes.
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. With regards to ILs, DESs are cheaper, more readily available and less toxic given the nature of its components.\cite{11,12,13} For instance, NADES (natural deep eutectic solvents) contain primary metabolites such amino acids, organic acids, sugars, or choline derivatives and can be used in food and pharmaceutical formulations.\cite{11} Very recently, DESs have unexpectedly opened the floodgates to new perspectives and applications in polar organometallic chemistry (organolithium and Grignard reagents), thereby contributing to build new bridges between main group chemistry and metal-, bio-, and organocatalysis.\cite{14}

The purpose of this review is to showcase recent progress in non-conventional media as a valuable tool to interface enzymes and chemocatalysts and tackle some of the pending gaps detected in organic solvents and aqueous media. Together with impressive advances in synthetic biology, materials science as well as protein engineering (in particular for the improvement of enzymes in non-conventional solvents),\cite{11} a simpler technique like medium engineering can be a powerful solution for an efficient implementation of chemoenzymatic cascade networks. Last but not least, the growing relevance of non-conventional media in cascade processes will be revealed throughout recent examples for the valorization of bioresources. We hope that this overview will be helpful for those researchers interested not only in chemoenzymatic cascades but also in sustainable chemical processes in general, and encourages future research and deepening in this area. Seeing as today’s world claims for a more sustainable chemical industry, we anticipate that the role of non-conventional media in catalysis will continue increasing in the immediate future.

2. Chemoenzymatic synthetic cascades

Biphasic systems and encapsulation are proficient strategies to overcome the solvent dilemma.\cite{12,13} Bacterial phenolic acid decarboxylase (PAD) converts bio-based hydroxycinnamic acids such as coumaric or ferulic acid to the corresponding p-hydroxy styrenes.\cite{15} This enzyme is a typical example for an enzyme not active in pure organic solvent whose substrates are poorly soluble in water. As the enzyme produces bio-based hydroxystyrene derivatives, combination with chemical reactions for the conversion of the olefin group appeared to be straightforward. In particular, combination with olefin-metathesis would allow the preparation of symmetric 4,4'-dihydroxy stilbenes with potent antioxidant activity from readily available bio-based precursors (Scheme 1a). The tendency of the intermediate 4-hydroxystyryls to undergo spontaneous polymerization complicates their work-up and isolation and makes a cascade the reaction of choice. While the olefin metathesis of styrene is straightforward, few commercially available catalysts showed satisfactory activity towards the four hydroxystyrol derivatives investigated. In organic solvent, however, the metathesis reaction was successfully established. The cascade thus presented a typical solvent dilemma, with a biocatalyst requiring aqueous media, and a metal catalyst requiring organic solvents. Encapsulation of PAD in polyvinyl alcohol cryogels allowed conducting the reaction in tert-butyl methyl ether. After separation of the beads, the chemical catalyst was added, resulting in a facile one-pot-two-steps cascade with quantitative conversion and high isolated yields of the final products.\cite{15} Kara et al. developed a similar solution for the same enzyme.\cite{16} They investigated the coupling of the previous decarboxylation to hydrogenation of the hydroxystyrols by Pd on charcoal to produce the flavor compound 4-ethylguaiacol (Scheme 1b). Once the enzymatic decarboxylation was accomplished in a two-liquid phase system (2LPS), namely buffer/hexane, and after continuous extraction with hexane in a two-phase reactor, the reduction was performed in such organic solvent. These two examples are representative for the potential of biphasic systems to overcome solvent incompatibilities of chemoenzymatic tandem reactions. Yet, biphasic systems have an intrinsic complexity which restricts them rather to batch reactions and is a serious obstacle in view of conducting tandem catalysis in continuous systems.

Wang et al.\cite{16} demonstrated that the addition of DESs to a biphasic (hydrophobic/aqueous) system resulted in stable microemulsions. As a result, the rate of the chemoenzymatic epoxidation of soybean oil could be enhanced more than six-

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fold due to a lower interfacial surface tension (Scheme 2). On the one hand, the lipase from *Penicillium camemberti* (PCL) catalyzed a perhydrolysis reaction from acids and hydrogen peroxide to peracids in the aqueous phase. On the other hand, the migration of such peracids to the organic phase consisting of soybean oil enabled the spontaneous Prilezhaev epoxidation. Remarkably, the addition of other traditional surfactants had no effect. The authors ascribe the beneficial effect of DES to the higher activity of interfacially activated lipases and higher partitioning of reagents in a medium with a reduced surface tension.

Flow systems have been receiving increasing attention in biocatalysis since they offer the perspective of a continuous production with facile separation of catalyst and product. In particular, setting up continuous reactors in environmentally-friendly solvents such as neoteric solvents (ILs and DESs) or biogenic solvents (p-cymene, limonene, anisole, 2-Me-THF or γ-valerolactone) is especially appealing due to the ecological footprint of these media. The first examples of continuous bioprocesses in neoteric solvents consisted of packed-bed bioreactors for the lipase-catalyzed transesterification of alkyl caffeate with 2-phenylethanol or glycerol in IL or DES medium respectively, and the lipase-catalyzed esterification of glycerol acting simultaneously as substrate and constituent of the DES medium.

For chemo-enzymatic tandem reactions, flow systems allow to conduct several steps at different temperatures, which is often a crucial advantage. Sieber et al. demonstrated the feasibility with the combination of a chemo-catalyzed oxidation with an enzymatic dehydration in aqueous system for the synthesis of 2-ketoacids from sugars (Scheme 3a). Both steps had different requirements regarding pH and temperature. In particular, the thermostable dehydratase required temperatures higher than 50 °C, while the chemical step was hampered by unwanted isomerization and degradation reactions under these conditions. A combination of a batch reactor for the gold catalyst coupled via a cross-flow filtration (and intermediary catalase treatment) to a column reactor with immobilized biocatalyst provided optimal reaction conditions for both steps and allowed the synthesis of several sugar acids at substrate loads of 40 mM and yields from 69–91%. This example underlines the practicability of continuous systems to alleviate conflicting reaction conditions for chemical and biological catalysts.

Yet, the limited solubility of many hydrophobic compounds in water on one hand and the oxidation sensitivity of many chemical catalysts on the other hand often exclude aqueous solvents for tandem reactions. Organic solvents have been used successfully with lipases, but few other enzymes are active in them. The viscosity of DES significantly decreases with the addition of water, which greatly facilitates application in flow. Yet, it has been reported that DES/water mixtures maintain the DES character up to a water content of approx. 50% (v/v), above which the solvent has the properties of an aqueous solution of the DES components. Recently, the use of mixtures of DES and water for a chemoenzymatic cascade in continuous flow was demonstrated. A combination of phenolic acid decarboxylase (PAD)-catalyzed decarboxylation of bio-based hydroxycinnamic acids with the Heck-cross coupling reaction would give access to asymmetrical substituted stilbene-derivatives such as the well-known anti-oxidant resveratrol (Scheme 3b). The low solubility of the phenolic acids in water limited the substrate concentration to 10–20 mM. The addition of 30% cosolvent ethanol for the Heck reaction further diluted the reaction solution. PAD from *Bacillus subtilis* shows very good activity in a mixture of choline chloride: glycerol (1:1) with 50% (v/v) water, which allowed to increase the substrate solubility dramatically. Due to the high viscosity of pure DES-solutions, a 50% mixture with water appeared to be highly suitable for a continuous approach. In a first, enzymatic step, a 70 mM solution of coumaric acid was decarboxylated by PAD encapsulated in alginate beads at 30 °C. Addition of a solution of an aryl halide dissolved in ethanol and
water led to a solution of DES:buffer:ethanol:water 1:1:1:1 for the Heck cross-coupling at 80 °C. While the reaction was hampered by side-product formation in the chemical steps, a continuous yield of 25% over 24 h shows the practicability. The cascade eliminates unit operations for the isolation of the (unstable) intermediate hydroxyxystere, uses bio-based, inexpensive starting material and allows the reuse of the expensive Pd-catalyst. While the choice of solvents for continuous tandem reactions has been limited to aqueous solvents, organic solvents and biphasic systems, DES provides a new dimension of homogeneous solvents for continuous chemoenzymatic cascades.

Supported ionic liquid phase/supercritical CO\(_2\) systems (SILP/scCO\(_2\)) represent another non-conventional media suitable for the requirements for sustainable bioprocesses. These systems, which are well suited for working in flow conditions, enable the easy separation of products and the recovery of the active IL-phase since the IL is immobilized in a given support. Lozano and Iborra developed a biphasic SILP/scCO\(_2\) continuous flow system for the dynamic kinetic resolution (DKR) of a racemic alcohol (Scheme 4). An immobilized lipase (Novozym 435°) catalyzed the acylation of the preferred (R)-enantiomer of the alcohol meanwhile an acidic zeolite catalyst mediated the racemization of the remaining (S)-counterpart. The reaction parameters involved 50 °C and 100 bar and the bed reactor was packed with both immobilized lipase and zeolite particles coated with ILs. As a result, (R)-phenylethyl propionate was produced with >97% ee and without loss of activity after 14 days of reaction. An important feature of this hybrid reaction medium is the protective effect exerted by ILs on biocatalysts, which avoids deactivation by high temperature, CO\(_2\), or acidic pH.

The chemoenzymatic catalytic system combining a Suzuki-Miyaura cross-coupling with an asymmetric reductive biotransformation could be taken as the paradigm of the topic discussed in this review. From the original report dated in 2008, successive developments in both medium and protein engineering have enabled to develop this chemical transformation towards high robustness, wide scope and applicability. In the pioneering approach, Gröger et al. demonstrated the feasibility to sequentially combine a Suzuki cross-coupling of halogenated acetophenone with an ADH-catalyzed bioreduction of the resulting ketone (Table 1, entry 1). The first step was accomplished in water at 70 °C but the poor solubility of the reactants limited the substrate loading to 33 mM. After pH adjustment, the bioreduction took place at room temperature and 25 mM in water (25% v/v of IPA for cofactor recycling). Four years later, the same authors took advantage of water-soluble palladium catalysts to address the overall process at room temperature (entry 2). However, in the absence of heating the maximum substrate concentration was limited to 40 mM despite a high percent of IPA (50% v/v) as co-solvent.

In between those reports, Kroutil et al. set up the cascade in IL-buffer mixtures as biphasic media (entry 3). The Suzuki-coupling occurred in (bmim)[NTf\(_2\)]; H\(_2\)O at 110 °C and 210 mM and the subsequent enzymatic reduction at 30 °C and 125 mM previous dilution of the mixture with a buffer containing \(E. coli\) ADH-A cells, NADH and IPA (15% v/v). Interestingly, both catalytic species and the IL could be efficiently recycled. On the one hand, enzyme and cofactor remained in the upper aqueous phase meanwhile the lower IL-phase harbored the metal catalyst and the resulting biaryl alcohol. Once extracted the target product with organic solvent, the Pd catalyst preserved the activity and was ready to use in the IL. Seeing as the excellent outcome displayed by IL-based media, DESs, a related class of those, were also tested for such a cascade (Entry 4). Additionally, both enzymatic reductions and palladium-catalyzed coupling reactions have been recently reported in neat DESs and DES-buffer mixtures. The employment of 1ChCl/2Gly-buffer 4:1 at 100 °C enabled the coupling step at 200 mM. Then, further dilution to 75 mM with a buffer containing IPA, KRED and cofactor was necessary to set up an efficient bioreduction of the transiently formed ketone. The processes reported in entries 3 and 4 (IL-buffer and DES-buffer mixtures) were demonstrated with a broad selection of substrates covering unsubstituted, fluorinated and pyridyl derivatives, and both (R)- and (S)-enantiopure biaryl alcohols were accessible by using stereocomplementary ADHs from \(Lactobacillus kefir\) and \(Rhodococcus rhodochrous\).

Obtaining chiral biaryl amines could be feasible following a similar chemoenzymatic scheme consisting of a cross-coupling reaction followed by an enzymatic transamination. However, combination of Suzuki-Miyaura cross-coupling with enzymes poses two main challenges. Firstly, a sequential mode requires that the second catalyst should be compatible with the remaining first catalyst and its reagents (Scheme 5). Even after removal of an immobilized catalyst, traces from catalyst leaking might be present in the solvent. Secondly, the substrate spectrum of the second catalyst should include the intermediary product of the first step. Unlike ADHs, ATAs have a narrow and more restrictive active site for bulky substituents. Consequently, biaryl ketones remained as elusive substrates for ATAs from commercially available kits (Scheme 5, A). Switching the order of the reactions is, in theory, possible since a large number of ATAs have demonstrated to accept haloaryl ketones (Scheme 5, B). Yet, the Pd-catalyst of the coupling step is very sensitive for inactivation by protein traces or chelating free amines (excess of amino donor and produced chiral amine)
protein traces even in a sequential mode. Therefore, all reports so far have reagents of the metal-catalyzed reaction. enzymatic step first poses less requirements for the substrate scope of the cross-coupling with stereoselective enzymatic reduction. Conducting the selective ATA from this tandem reaction. In 2016, a rationally engineered (Scheme 5). Therefore, the identification of ATAs defines the requirements for the substrate scope of the considers dictate the order of reactions. In turn, this example for a cascade-specific problem, in which compatibility to the corresponding biaryl amine.

Combination of Suzuki-Miyaura cross-coupling with asymmetric reductive biotransformations.

![Scheme 5. Possible reaction modes for the combination of Suzuki-Miyaura cross-coupling with stereoselective enzymatic reduction. Conducting the enzymatic step first poses less requirements for the substrate scope of the enzyme (below), but suffers from the poisoning of the metal-catalyst by protein traces even in a sequential mode. Therefore, all reports so far have focused on conducting the cross-coupling prior to the enzymatic reduction.](image)

tus (4CHI-TA), catalyzed efficiently the amination of meta- and para-biaryl ketones. Further structure-guided protein engineering resulted in broader substrate scope and improved stability which paved the way for the straightforward approach A in Scheme 5. Accordingly, Bornscheuer et al. reported such a sequential process in aqueous medium employing 4CHI-TA (2 mM substrate concentration for the coupling step (DMF, 50% v/v) and 1 mM for the bioamination (DMF, 30% v/v)), several (R)-meta- and para-biaryl and pyridylphenyl amines being obtained with >99% ee (Table 1, Entry 5). Further, the cascade was implemented in a flow continuous system by means of the enzyme immobilization with a metal affinity resin (EZiG) employing identical reaction media for each step that in the batch reaction setup (Entry 6). Soon after, an identical cascade with EX-oTA was reported in a reaction medium consisting of a DES:buffer mixture (Entry 7). In fact, this was the first report of ATAs in these neoteric solvents and 1ChCl/2Gly turned out to be the only co-solvent compatible for both steps due to inhibition of Pd and EX-oTA catalysts. As a result, the metal catalyzed step was accomplished at 200 mM of substrate loading in a DES: buffer 4:1 mixture, and the further bioamination at 25 mM previous dilution to a buffer containing 10% w/w DES due to stability issues of the biocatalyst. The methodology was extended to meta- and para-biaryl ketones and pyridylphenyl ketones as well, rendering the corresponding (R)-biaryl amines with >99% ee.

Table 1. Combination of Suzuki-Miyaura cross-coupling with asymmetric reductive biotransformations.

| Entry | Reaction concept (year) | Coupling Step | Biotransformation | Conversion[a] Ref. |
|-------|------------------------|---------------|-------------------|-------------------|
| 1     | Sequential combination of cross-coupling with selective ADH[b] in water (2008) | Water, 33 mM substrate, 70°C, 17 h | Water:IPA 3:1, 25 mM substrate, RT | >95 31 |
| 2     | Sequential combination of cross-coupling with selective ADH in buffer; use of water-soluble Pd-catalysts (2012) | Buffer:IPA 1:1, 40 mM substrate, RT, 24 h | Buffer: IPA 1:1, 40 mM substrate RT, 24 h | 97 32 |
| 3     | Sequential combination of cross-coupling with selective ADH in ionic liquids (2010) | Ionic liquid:water 1:1, 210 mM substrate, 110°C, 1.5 h | Buffer:ionic liquid 2.5:1,15% IPA, 125 mM substrate, 30°C, 18 h | 55–94 33 |
| 4     | Sequential combination of cross-coupling with selective ADH in DES/buffer mixture (2018) | DES (1ChCl/2Gly):buffer 4:1, 200 mM substrate, 100°C, 24 h | DES (1ChCl/2Gly):buffer 1:1, 10% IPA, 75 mM substrate, 30°C, 24 h | 78–90 34 |
| 5     | Sequential combination of cross-coupling with selective ATA[c] in buffer /DMF solution (2018) | Buffer:DMF 1:1, 2 mM substrate, 30°C, 20 h | Buffer: DMF 1:1, 2 mM substrate, 30°C, 20 h | 87 38 |
| 6     | Cross-coupling followed by selective immobilized ATA in flow in buffer /DMF solution (2018) | Buffer:DMF 1:1, 2 mM substrate, 30°C, 20 h | Buffer: DMF 1:1, 2 mM substrate, 30°C, 20 h | 43 38 |
| 7     | Sequential combination of cross-coupling with selective ATA in DES/buffer mixture (2019) | Buffer:DES 2:5, 15% IPA, 210 mM substrate, 125 mM substrate, 30°C, 18 h | Buffer (15% DES), 25 mM substrate, 30°C, 24 h | up to >99 39 |

[a] Conversion regarding the overall two-steps process, [b] ADH: Alcohol dehydrogenase, [c] IPA: Propan-2-ol, [d] ATA: Amine transaminase.
Domínguez de María et al.\textsuperscript{[40]} described in 2014 the first tandem combination of organo- and biocatalysts in DESs. The enzyme, namely the lipase B from \textit{Pseudozyma} \textit{antartica} (CAL-B) was responsible for producing acetaldehyde from the transesterification of vinyl acetate with propan-2-ol. Immediately, a proline-based organocatalyst catalyzed the enantioselective cross aldol reaction of the acetaldehyde with a selected benzaldehyde derivative (Scheme 6). The authors took advantage of the affinity between DES and the organocatalyst to reuse six cycles, once extracted the target product with organic solvent, both the reaction medium and the catalytic species. In a further report, the structure of the organocatalyst was tailored by the introduction of HBD groups to enhance the interactions with DES and optimize the cycles of recycling without loss of catalytic activity.\textsuperscript{[41]}

The first example of efficient integration of metallic and biological catalysis in DESs-buffer mixtures was not reported until 2018 (little earlier than the Suzuki coupling-bioreduction biological catalysis in DESs-buffer mixtures was not reported without loss of catalytic activity.\textsuperscript{[42]}) and was based on a ruthenium-catalyzed isomerization reaction of allylic alcohols to an \(\alpha,\beta\)-saturated ketone followed by enantioselective bioreduction promoted by KREDs (Scheme 7). Despite this catalytic system had been successfully implemented in aqueous media in sequential and concurrent mode (up to 86% yield and > 99% ee),\textsuperscript{[43]} the motivation to extend the study to DES-based media arose to solve pending issues in such processes. With regards to the sequential approach (one-pot two-steps), the only adjustment once completed the starting isomerization step involved a slight decrease on temperature (from 50 °C to 30 °C) and the addition of the biocatalyst with its cofactor. The presence of DES, in good agreement with previous reports,\textsuperscript{[44]} impacted notably on the enantioselectivity exhibited by the KREDs which led to optimized enantioselectivities. The most remarkable results were achieved with some challenging substrates by the employment of ChCl/Gly and ChCl/sorbitol at high percentages in the DES-buffer mixture. In the case of the concurrent process (one-pot one-step) with both catalysts coexisting from the beginning, the low stability of the enzyme in the aqueous buffer resulted in incomplete processes (mixture of ketone and saturated alcohol) for those allylic alcohols undergoing slow isomerization. Albeit the KRED exhibited comparable stability in buffer and DES-buffer mixtures, the transformation of a set of allylic alcohols was efficiently accomplished combining a KRED and a Ru complex (10 % mol) at 40 °C in 1 ChCl/Gly-buffer 4:1. Interestingly, one of the substrates, namely 1-(4-bromophenyl) prop-2-en-1-ol rendered the saturated analogue in 96% overall conversion, the biggest so far.

Designer surfactants based on aqueous micellar solutions have recently been demonstrated as innovative reaction media for chemo-enzymatic cascades.\textsuperscript{[45]} While they were initially designed for synthetic chemistry in water,\textsuperscript{[46]} Lipshutz et al. showed that ketoreductases are active in the aqueous part, and can be combined \textit{in situ} with metal-catalyzed reactions. In the so-called micellar catalysis, the enzyme remains in the aqueous solution, while the micelle acts as a solvent and a reservoir for substrate and product. The separation of both reactions reduces the risk of mutual inhibition by both catalysts and their reaction conditions. The surfactant TPGS-750-M bears vitamin E as hydrophobic moiety and forms micelles of 50 nm size in average. Several alcohol dehydrogenases showed excellent tolerance towards the detergent, and 24 h did not lead to loss of activity. The detergent did not exert any measurable effect on the enzyme structure, as stated by circular dichroism and NMR studies. Similarly, the micelle remained unaltered according to light scattering analysis. The micelles were applied in several sequential two-steps, one-pot processes, where the enzyme was used for the second reaction. Combining Sonogashira and Heck couplings (Pd catalysts), alkyne hydrations [Au and silver (Ag) catalysts], or 1,4-additions (Rh catalysts) with a further bioreduction, enabled the formation of secondary alcohols in high yield and excellent optical purity (Scheme 8, A, B and C pathways). Moreover, acting as a reservoir for substrates and products, the micelles limited the enzyme saturation and the activity of the ketoreductases towards lipophilic substrates increased.

Similarly, the beneficial impact of surfactants on enzymatic activity was exploited for the conversion of poorly-water soluble drugs in a two-enzyme cascade reaction.\textsuperscript{[47]} A sequential cascade consisting of laccase/TEMPO-catalyzed deoximation of prochiral ketoximes followed by bioreduction or bioamination...
of the intermediate ketone was used for the synthesis of optically active alcohols and amines (Scheme 9). Cremophor® is a polyethoxylated castor oil typically used as a formulation vehicle for poorly-water soluble drugs. Addition of 1% (w/w) improved substrate solubility and enhanced the enzymatic performance, the enzymes (laccases, KREDs and ATAs) and the mediator TEMPO being perfectly tolerated by the surfactant. Without use of organic co-solvents, substrate concentration of up to 200 mM for the laccase-catalyzed oxidation and 100 mM in the following ketoreduction were achieved, leading to the formation of the (S)- and (R)-enantiomers of the chiral alcohols with high yield and excellent optically purity (>99% ee).

3. Enzymatic cascades for the valorization of bioresources

The burgeoning interest in non-conventional media has triggered a plethora of applications in many research areas. As a platform for cascades processes, the utility is not restricted to smart synthetic cascades facing chemocatalysis and biocatalysis as those depicted above. Besides, the valorization of bioresources towards high added value chemicals represents another topic with immense potential for the neoteric solvents. In this regard, a major issue of biomass relies on the poor solubility in classical solvents, which hinders their pretreatment and conversion. The inherent biocompatibility of DESs and their excellent solubilizing properties open new possibilities for revisiting established processes for valorization of biosourced substrates. In the context of this revision, some of these processes for valorization of natural feedstock occur throughout cascade processes wherein the implementation of non-conventional media provides remarkable advantages.

As a first example, the transformation of waste cooking oils into biodiesel by means of a one-pot two-steps enzymatic process was established in DESs (Figure 2). First, Thermomyces lanuginosus lipase catalyzed selectively the esterification of triglycerides in aqueous medium and 30°C. Then, and without intermediate purification, a second biocatalyst, namely CAL-B, and a DES as cosolvent (ChCl/Gly 1:2) were sequentially added to accomplish the effective transesterification of the remaining glycerides and fatty acids at 45°C. The role of the eutectic solvent was reflected on the partition of the mixture into a two-phase system where lipids are hosted in the upper phase and a glycerol-DES mixture makes up the lower one. Additionally, DESs facilitated the recovery of glycerol from the hydrophilic phase by distillation of the DES counterpart.

Similarly, and taking advantage of the inherent biocompatibility of DESs, these solvents were tested as the medium for the production of biofuel from lignocellulosic biomass (Figure 3). First, Saccharomyces cerevisiae yeast growth proved effective in a 5 wt% DES aqueous solution (ChCl/Gly 1:2). Pretreatment step of crude biomass revealed levels of degradation products (furfural and ferulic acid) low enough to ensure the growth of the yeast. Likewise, DESs were also biocompatible with the hydrolytic enzymes involved in the process. Then, the integration of saccharification and fermentation steps in a one-pot approach resulted in an ethanol production of 77.5% theoretical yield in 10 wt% ChCl/Gly 1:2 aqueous solution. Remarkably, the implementation of DESs avoided any pH adjustment and solid/liquid separation steps throughout the above process.
A sustainable solution to cope with the emission of CO\textsubscript{2} to the atmosphere has been recently proposed by means of the enzymatic conversion of CO\textsubscript{2} into methanol in the presence of ILs (Figure 4).\textsuperscript{[13]} Previously the analogue enzymatic process in aqueous medium had rendered low production of methanol (44\%) due to unfavorable kinetics in the starting reduction of CO\textsubscript{2} into formic acid mediated by formate dehydrogenase (FDH). The presence of a biocompatible IL such as [choline][L-glutamic acid] enabled both a higher solubilization of CO\textsubscript{2} and a stabilizing effect on FDH. The three reductive enzymes involved in the biotransformation [FDH, formaldehyde dehydrogenase (FaldDH) and alcohol dehydrogenase (ADH)] were immobilized in a cellulose membrane and a separation system platform enabled the recycling of biocatalysts and the removal of methanol. The optimized reaction conditions involving a 20 wt\% IL aqueous solution led to a 5-fold higher production of methanol with regards to the aqueous medium.

4. Conclusions

The amazing challenge to orchestrate enzymatic cascades (multi-enzymatic or chemo-enzymatic) usually faces numerous issues to be addressed. As it is often unclear whether optimization should focus either on the molecular properties of the catalysts or on the process conditions, a wide application will require closer interaction between bioscience, chemistry and process engineering. 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. Despite a few very encouraging examples in the last years, the potential of non-conventional media for the reduction of reaction steps by establishment of new cascade reactions is still far from being exploited. Together with techniques such as compartmentalization (that are of high academic interest but so far have somewhat resisted industrial implementation) or enzyme-metal hybrid catalysts, non-conventional solvents greatly expand the available toolbox for cascade development and can be expected to make a positive contribution to the environmental improvement of the chemical and pharmaceutical industries.

Conflict of Interest

The authors declare no conflict of interest.

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