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Green and sustainable solvents for biocatalytic oxidations

Roger A. Sheldon^{1,2}, Moira L. Bode¹ and Nompumelelo Mathebula¹

Abstract

The discovery that enzymes could function efficiently in organic solvents revolutionized their use in industry but represented a change from the natural “green” solvent, water, to a host of environmentally undesirable solvents. Considerable effort is being devoted to making such processes greener again. Bio-based solvents, derived from waste biomass, possess the desirable attributes of traditional organic solvents but are more conducive to a circular bio-based economy. Although biocatalytic oxidations have only been tested in bio-based ether solvents, there is considerable scope for expanding this to include bio-based ester solvents. Alternatively, both ionic liquids and deep eutectic solvents, with tunable properties, are proving very interesting solvents for biocatalytic oxidations. In particular, oxidative depolymerization of lignin, catalyzed by laccases, has been extensively investigated. Finally, designer amphiphiles can facilitate the formation of micelles that act as hydrophobic nanoreactors for performing biocatalytic oxidation processes while surrounded by aqueous buffer as solvent.

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Biocatalytic oxidations, Green solvents, Bio-based solvents, Ionic liquids, Deep eutectic solvents, Micellar catalysis.

Introduction: biocatalysis in a changing industrial economy

Biocatalysis is a sustainable technology [1] that we expect to play an increasingly important role in the shift from hydrocarbons to carbohydrates and carbon dioxide as feedstocks in a bio-based circular economy [2]. Biocatalytic oxidations fall into two mechanistic categories:

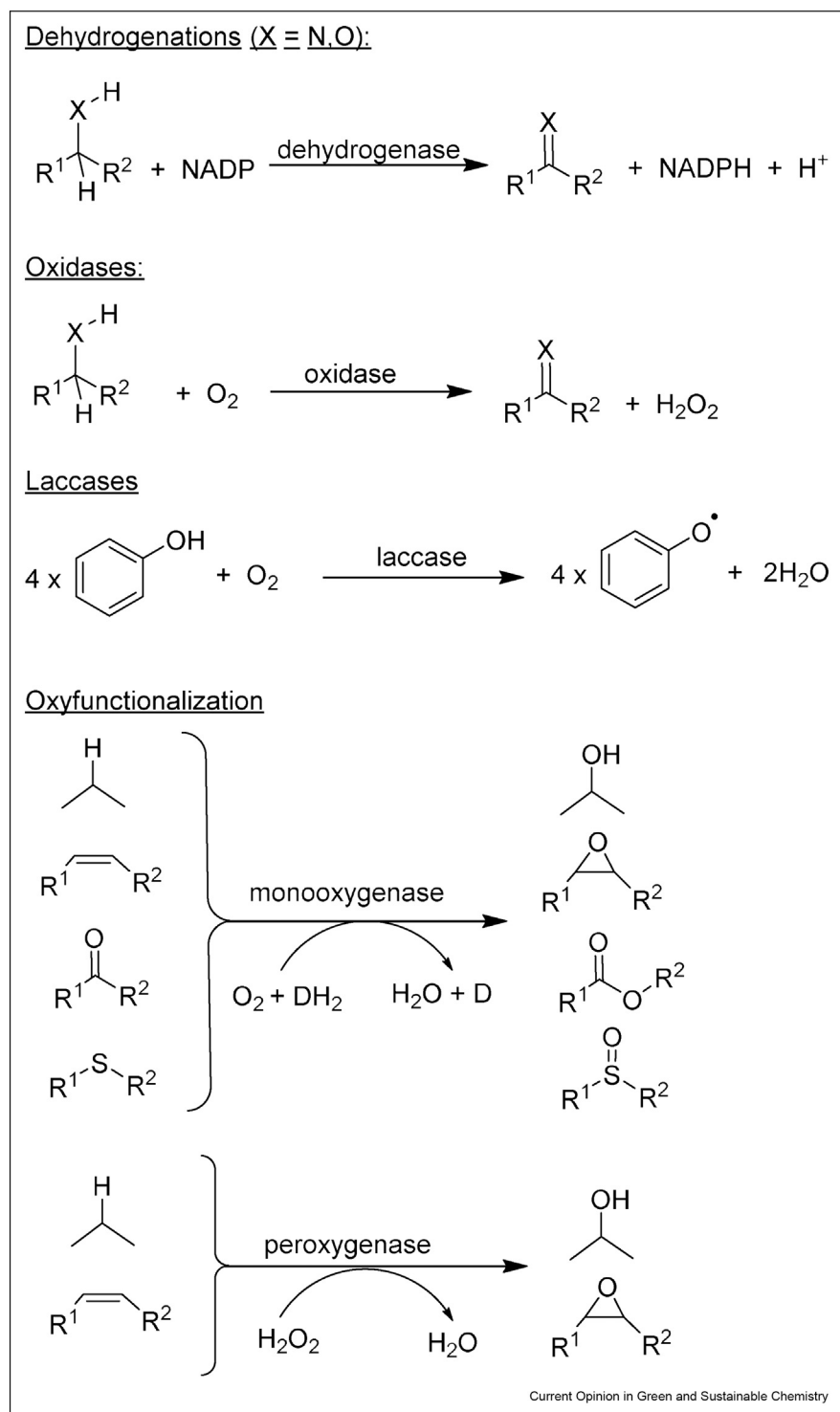
dehydrogenation and oxyfunctionalization [3]. For example, copper- and flavin-dependent alcohol and amine oxidases catalyze the oxidative dehydrogenation of alcohols and amines to aldehydes and ketones or imines, respectively, using dioxygen as the terminal oxidant (Figure 1) [4]. Alternatively, NAD(P)-dependent alcohol and amine dehydrogenases can be used in conjunction with cofactor regeneration with dioxygen catalyzed by NAD(P)H oxidase [5]. Laccases are copper-dependent oxidases that can use a molecule of dioxygen to perform four single-electron transfers from four substrate molecules to give the corresponding radicals together with two molecules of water (Figure 1) [6]. These enzymes are of particular interest in connection with the oxidative depolymerization of lignin [7,8]. In combination with mediators, such as the stable radical, tetramethylpiperidinoxyl (TEMPO), they catalyze the selective aerobic oxidation of alcohols [9].

Oxyfunctionalizations involving the insertion of an oxygen atom in, for example, hydrocarbons, are catalyzed by oxygenases via reductive activation of dioxygen or by peroxygenases using hydrogen peroxide as a reduced form of dioxygen. Flavin-dependent oxidases and monooxygenases utilize enzyme-bound flavin adenine nucleotide (FAD) as a hydride acceptor and cofactor regeneration with dioxygen.

Biocatalysis in aqueous, organic, and biphasic media

Enzymes function optimally in aqueous media, but the limited solubility of most organic substrates in water is a serious limitation. Hence, the demonstration, by Klivanov and Zaks [10] in 1984, that lipases function admirably in hydrophobic organic solvents at elevated temperatures, marking the advent of non-aqueous enzymology, led to the widespread application of enzymes in organic solvents and the adoption of biocatalysis as a mainstream synthetic tool with broad industrial scope. The toxicity and/or flammability of many organic solvents, in particular the formation of explosive mixtures with dioxygen in the gas phase, is a serious disadvantage when compared to aqueous reactions. In terms of oxidation reactions, in particular, the low solubility of dioxygen in water (ca. 0.25 mM at ambient temperature) [11] is a distinct disadvantage compared to the solubility of oxygen in organic solvents [12,13]. One approach to solving this problem is to use water-miscible aprotic solvents, such as DMF and

Figure 1



General scheme of different types of catalytic oxidation.

DMSO. However, many of these solvents are currently in disfavor owing to serious health and environmental concerns.

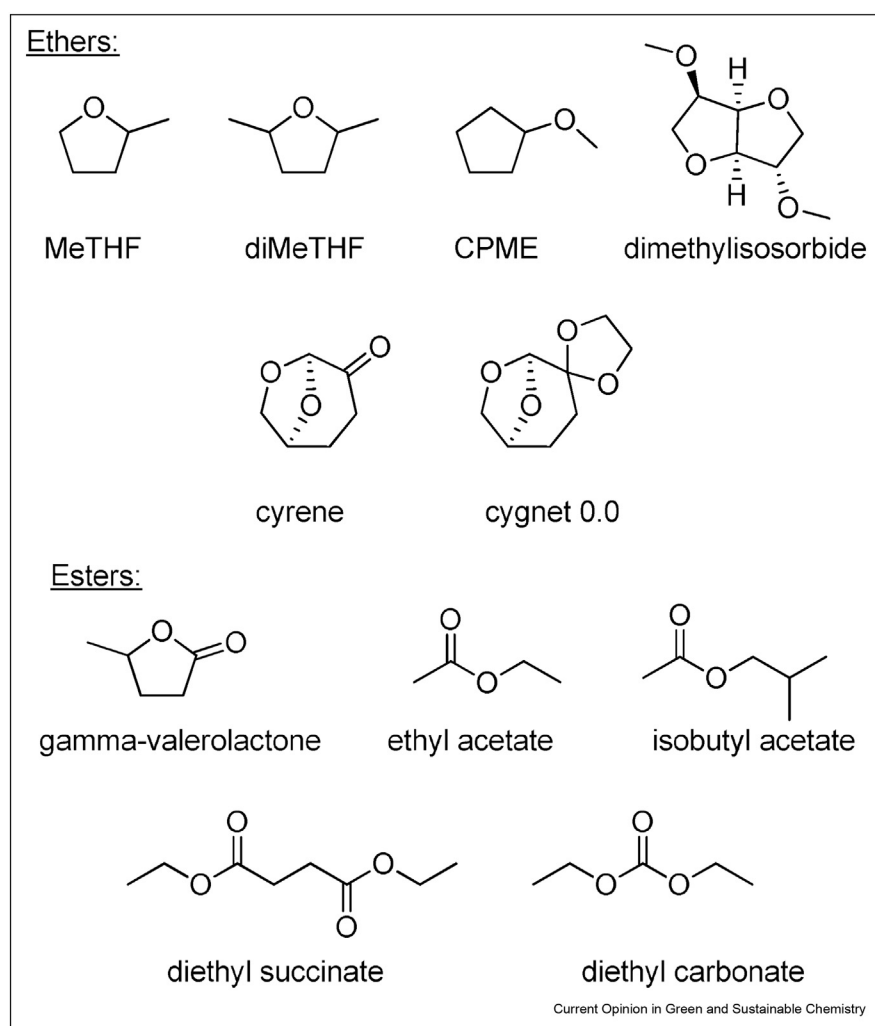
Biocatalytic processes in water often involve product separation by solvent extraction. Alternatively, product isolation can be back integrated by performing the reaction in an aqueous–organic biphasic mixture and using phase separation to separate the product and recycle the aqueous phase containing the catalyst.

Bio-based solvents

In support of the shift to greener industrial processes, there is a need to replace traditional organic solvents with more environmentally benign organic solvents while still maintaining their inherent advantages. Solvents derived from (waste) biomass, so-called bio-based solvents (Figure 2) [14], constitute a real alternative to

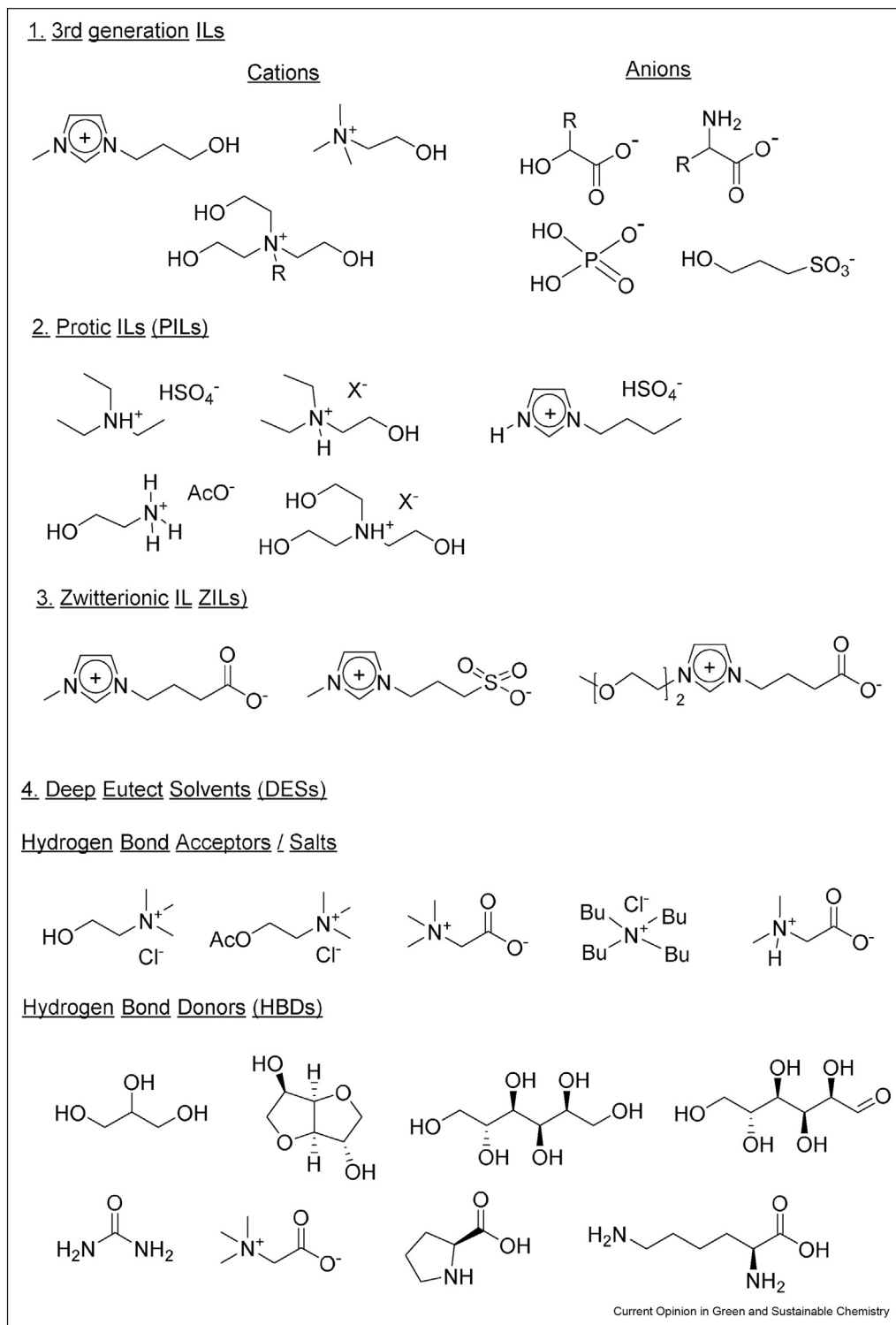
traditional solvents and are finding widespread applications in biocatalysis. When considering solvents for biocatalytic oxidations, particularly those using molecular oxygen, the choice is limited to relatively unreactive and non-volatile organic solvents. Examples of bio-based solvents that have been most widely used in biotransformations to date include the ethers cyclopentyl methyl ether (CPME) [15] and 2-methyltetrahydrofuran (2-MeTHF) [16]. Although most of these examples relate to hydrolases, these two solvents have been used in reactions catalyzed by oxidoreductases [17]. In one example, a combination of a mono-oxygenase and alcohol dehydrogenase was used for the preparation of γ -butyrolactone from cyclobutanone using 5% CPME as co-solvent [18]. However, it must be borne in mind that the use of bio-based ethers such as 2-MeTHF and, to a lesser extent, CPME as solvents for aerobic oxidations involves the

Figure 2



Examples of bio-based ethers and esters.

Figure 3



Examples of ionic liquids and deep eutectic solvents.

risk of peroxide formation. For example, the extent of peroxide formation in MeTHF was roughly the same as that observed in THF [19]. In contrast, peroxide formation in CPME was very low and comparable to methyl tert-butyl ether (MTBE), which lacks reactive C–H bonds, and much lower than with THF and diisopropyl ether (which was the worst)[20].

The undisputed suitability of esters (including lactones) as solvents for oxidation reactions means that although there are not yet examples in the literature, in our opinion, it is only a matter of time before biocatalytic oxidations are also carried out in bio-based esters, for example, those shown in Figure 2. Indeed, γ -valerolactone is particularly attractive since it has solvent properties very similar to those of polar aprotic solvents, such as DMF[21].

As has been stated before, the best solvent is no solvent [22], and there are a number of examples of biocatalytic oxidation reactions that have been carried out under neat conditions. For example, immobilized recombinant unspecific peroxygenase (UPO) from *Agrocysteis aegerita* was used in the oxidation of ethylbenzene [23] and a range of styrene derivatives [24] under neat conditions using *tert*-butyl hydroperoxide as oxidant. The use of neat reaction conditions, although desirable from the perspective of waste minimization, is probably limited in scope.

Neoteric solvents: ionic liquids and deep eutectic solvents

The ongoing transition to carbon-neutral, bio-based chemicals manufacture largely involves water-insoluble polysaccharides, in particular, lignocellulose, as feedstocks and water-soluble mono- and di-saccharides instead of hydrocarbons as basic chemicals. Hence, biocompatible ionic liquids (ILs) [25,26] and deep eutectic solvents (DESs) [27–29], based on their tunable properties and accompanying advantages as green reaction media, have been used extensively as solvents in biocatalytic transformations (see Figure 3 for structures). ILs are essentially salts that are liquid at room temperature, and, depending on their structure, they may be water miscible [24]. DESs, on the other hand, are formed by gently heating and stirring a stoichiometric mixture of a hydrogen bond acceptor (HBA), usually a salt, and a hydrogen bond donor (HBD), in a certain ratio, until a liquid is formed [30,31]. DESs are generally hydrophilic and are not suitable for use where aqueous biphasic conditions are required [25].

When considering alternative solvents, such as neoteric solvents, for aerobic oxidations, an important factor is the oxygen transfer rate (OTR) from the gaseous phase to the liquid phase. The OTR of many DESs has been tested and found to be considerably lower than that of water. This could, however, be improved by the addition

of water or by increasing the temperature to reduce the overall viscosity of the medium [32]. Similarly, increasing the temperature of ILs resulted in a significant increase in oxygen solubility and diffusivity[33].

Ionic liquids

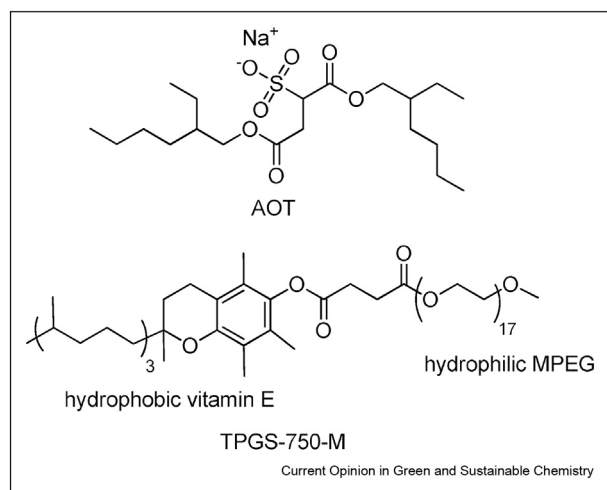
Third-generation ILs, based on biocompatible cations and anions, are derived from inexpensive, readily available, and renewable raw materials, including carbohydrates and amino acids (Figure 3). Certain examples of these ILs are particularly suitable for use in aqueous biphasic systems [34] and some show excellent biocompatibility [35]. However, a significant disadvantage of all quaternary ammonium ILs is that their synthesis requires an atom-inefficient quaternization step. Protic ILs (PILs), in contrast, with their simple and atom-efficient synthesis, better biodegradability, and lower toxicity, can overcome this problem. Moreover, they have H-donating properties that stabilize enzymes, and they are self-buffering [25]. One promising approach to lignocellulose pretreatment involves the use of enzymes and mixtures of water with inexpensive PILs [36]. However, some simple, inexpensive ILs and PILs are not biocompatible and hence not suitable for further conversion after pretreatment. In order to address this deficiency, zwitterionic liquids (ZILs) have been developed that not only dissolve lignocellulose but are also biocompatible, making them suitable for further biocatalytic processing[37].

Laccase is proving to be an extremely important enzyme in the economically viable oxidative bioconversion of the intractable lignin component of lignocellulose, resulting in depolymerization and the formation of useful monomers [38]. Laccase substrates are extremely hydrophobic, and solubility in aqueous media is incomplete. It is not surprising, therefore, that the highly modifiable ILs and DESs have been tested as solvents in laccase-catalyzed reactions. The activity of laccases in ILs was found to be either suppressed or enhanced, depending on the exact structure of the IL, but the outcome was difficult to predict [39].

Deep eutectic solvents

Biocompatible DESs have also been investigated as possible solvents for biomass pretreatment and further conversion to fuels and chemicals. Results have proved to be extremely promising, with laccase activity preserved and even enhanced by certain DESs [27]. An interesting example of a biocatalytic oxidation reaction where a choline chloride-based DES was used as both co-solvent (with buffer) and co-substrate was demonstrated by Hollmann and Wang and co-workers [40]. The previously discussed peroxygenase-catalyzed hydroxylation of ethylbenzene and epoxidation of styrene derivatives was performed successfully in a DES, in the presence of a second enzyme, choline oxidase, that converted choline

Figure 4



Structures of surfactants.

into betaine using molecular oxygen and generating the hydrogen peroxide required for the peroxxygenase-catalyzed reaction. Other biocatalytic oxidations investigated in DESs include those catalyzed by alcohol dehydrogenase, horseradish peroxidase, and catalase[41].

If the components of a DES are natural products, such as choline chloride as the HBA and a sugar, amino acid, or alcohol as the HBD, they are referred to as natural deep eutectic solvents (NADES). Both increased thermostability and enhanced conversions at higher substrate concentrations were observed for 5-hydroxymethylfurfural oxidase (HMFO) in the presence of NADES containing glucose or fructose [42]. Similarly, the kinetic resolution of racemic 1-indanol by both wild-type and a thermostable variant of HMFO with improved enantioselectivities was observed in 60%v/v carbohydrate-based NADES as co-solvents and molecular oxygen as the primary oxidant[43].

Micellar catalysis in aqueous media

Another approach to the use of enzymes in organic solvents, dating back to the 1970s, involves the use of surfactants, such as sodium 1,2-bis(2-ethylhexyloxycarbonyl)-1-ethane sulfonate (AOT) (Figure 4), that form reverse micelles: nanosized aggregations of surfactant molecules in which the lipophilic chains are exposed to the hydrophobic organic solvent [44]. The polar head groups are directed toward the interior of the micelles forming a novel water-containing nanoreactor in which the dissolution of the enzyme enables aqueous biocatalysis in hydrocarbon solvents.

However, organic solvents are currently out of fashion, and water is beautiful, so attention has shifted to the use

of oil-in-water micelles, in which the hydrophilic head groups of a surfactant are in contact with the water solvent, and the lipophilic tails form a microenvironment that acts as a nanoreactor for performing biocatalysis with hydrophobic substrates. This concept was taken to a new level of sophistication by Lipshutz and co-workers, who developed designer amphiphiles, such as the commercially available non-ionic TPGS-750-M (Figure 4), that facilitate biocatalysis, for example, with an alcohol dehydrogenase in a reduction mode [45] in aqueous buffer at 2 wt% loading. An added benefit was the overall increase in rate which was attributed to a “reservoir effect” in which nanomicelles act as an alternative “solvent” for the otherwise insoluble product, thus mitigating enzyme inhibition resulting from the accumulation of water-insoluble product at the entrance to the active site. This portends a bright future for micellar biocatalysis in aqueous media [46]. We note, however, that product isolation generally involves extraction with an organic solvent, but this could be an environmentally attractive solvent.

Closing remarks

Hopefully, we have shown that there is potentially an abundance of greener solvents available for use in biocatalytic oxidation processes. Bio-based ethers, such as 2-MeTHF and CPME, have already become well established as highly suitable solvents for biocatalytic reactions in general, and their potential for biocatalytic oxidation reactions has been demonstrated. Bio-based esters, such as diethyl carbonate, are potentially interesting on the basis of their excellent oxidation stability, and γ -valerolactone is particularly attractive because of its interesting solvent properties. The use of innovative ILs and DESs that are both biocompatible and tunable presents an opportunity for designing solvents with exceptional properties through a judicious choice of the different components. However, water is still the solvent of choice and whether or not a solvent is water miscible will be an important selection criterion. Alternatively, designer amphiphiles can induce the formation of micelles that act as hydrophobic nanoreactors within an overall aqueous environment. In short, there is much-unexplored potential for designing greener solvents for biocatalytic oxidation processes to address the need for environmentally attractive bio-based chemicals manufacture in a circular economy.

In the words of E. F. Schumacher, “wisdom demands a new orientation of science and technology toward the organic, the gentle, the elegant and beautiful.”

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data were used for the research described in the article.

References

Papers of particular interest, published within the period of review, have been highlighted as:

- * of special interest
- ** of outstanding interest

1. Sheldon RA, Woodley JM: **Role of biocatalysis in sustainable chemistry**. *Chem Rev* 2018, **118**:801–838.
2. Sheldon RA, Basso A, Brady D: **New frontiers in enzyme immobilisation: robust biocatalysts for a circular bio-based economy**. *Chem Soc Rev* 2021, **50**:5850–5862.
3. Dong J, Fernandez-Fueyo E, Hollmann F, Paul CE, Pesic M, Schmidt S, Wang Y, Younes S, Zhang W: **Biocatalytic oxidation reactions: a chemist's perspective**. *Angew Chem Int Ed* 2018, **57**:9238–9261.
4. Puetz H, Puchlova E, Vrankova K, Hollmann F: **Biocatalytic oxidation of alcohols**. *Catalysts* 2020, **10**:952.
5. Wang J, Woodley JM: **In situ cofactor regeneration using NAD(P)H oxidase: enzyme stability in a bubble column**. *ChemCatChem* 2022, **14**, e202200255.
6. Riva S: **Laccases: blue enzymes for green chemistry**. *Trends Biotechnol* 2006, **24**:219–226.
7. Cardullo N, Muccilli V, Tringali C: **Laccase-mediated synthesis of bioactive natural products and their analogues**. *RSC Chem Biol* 2022. <https://doi.org/10.1039/d1cb00259g>.
8. Itoh T, Tagaki Y: **Laccase-catalyzed reactions in ionic liquids for green sustainable chemistry**. *ACS Sustainable Chem Eng* 2021, **9**:1443–1458.
9. Gross J, Tauber K, Fuchs M, Schmidt NG, Rajagopalan A, Faber K, Fabian WMF, Pfeffer J, Haas T, Kroutil W: **Aerobic oxidation of isosorbide and isomannide employing TEMPO/laccase**. *Green Chem* 2014, **16**:2117–2121.
10. Zaks A, Klibanov AM: **Enzymatic catalysis in organic media at 100 degrees C**. *Science* 1984, **224**:1249–1251.
11. Pederse AT, Birmingham WR, Rehn G, Charnoc SJ, Turner NJ, Woodley JM: **Process requirements of galactose oxidase catalyzed oxidation of alcohols**. *Org Process Res Dev* 2015, **19**:1580–1589.
12. Sato T, Hamada Y, Sumikawa M, Araki S, Yamamoto H: **Solubility of oxygen in organic solvents and calculation of the hansen solubility parameters of oxygen**. *Ind Eng Chem Res* 2014, **53**:19331–19337.
13. Ramesh H, Mayr T, Hobisch M, Borisov S, Klimant I, Krühne U, Woodley JM: **Measurement of oxygen transfer from air into organic solvents**. *J Chem Technol Biotechnol* 2016, **91**: 832–836.
14. Clark JH, Farmer TJ, Hunt AJ, Sherwood J: **Opportunities for bio-based solvents created as petrochemical and fuel products transition towards renewable resources**. *Int J Mol Sci* 2015, **16**:17101–17159.
15. de Gonzalo G, Alcántara AR, de María PD: **Cyclopentyl methyl ether (CPME): a versatile eco-friendly solvent for applications in biotechnology and biorefineries**. *ChemSusChem* 2019, **12**: 2083–2097.
16. Alcántara AR, de María PD: **Recent advances on the use of 2-methyltetrahydrofuran (2-MeTHF) in biotransformations**. *Current Green Chemistry* 2018, **5**:86–103.
17. Aranda C, de Gonzalo G: **Biocatalyzed redox processes employing green reaction media**. *Molecules* 2020, **25**:3016.
18. Huang L, Aalbers FS, Tang W, Röhlig R, Fraaije MW, Kara S: **Convergent cascade catalyzed by monooxygenase–alcohol dehydrogenase fusion applied in organic media**. *Chem-biochem* 2019, **20**:1653–1658.
19. Bijoy R, Agarwala P, Roy L, Thorat BN: **Unconventional ethereal solvents in organic chemistry: a perspective on applications of 2-methyltetrahydrofuran, cyclopentyl methyl ether, and 4-methyltetrahydropyran**. *Org Process Res Dev* 2022, **26**:480–492.
20. Watanabe K, Yamagiwa M, Torisawa Y: **Cyclopentyl methyl ether as a new and alternative process solvent**. *Org Process Res Dev* 2007, **11**:251–258.
21. Kerker F, Markiewicz M, Stolte S, Müller E, Kunz W: **The green platform molecule gamma-valerolactone- ecotoxicity, biodegradability, solvent properties, and potential applications**. *Green Chem* 2021, **23**:2962–2976.
22. Sheldon RA: **Biocatalysis, solvents, and green metrics in sustainable chemistry**. In *Chapter 1 in biocatalysis in green solvents*. Edited by Lozano P, Ed, Amsterdam: Elsevier; 2022.
23. Fernández-Fueyo E, Ni Y, Baraibar AG, Alcalde M, van Langen LM, Hollmann F: **Towards preparative peroxxygenase-catalyzed oxyfunctionalization reactions in organic media**. *J Mol Catal B Enzym* 2016, **134**:347–352.
24. Rauch MCR, Tieves F, Paul CE, Arends IWCE, Alcalde M, Hollmann F: **Peroxygenase-catalysed epoxidation of styrene derivatives in neat reaction media**. *ChemCatChem* 2019, **11**: 4519–4523.
25. Sheldon RA: **Biocatalysis in ionic liquids: state-of-the-union**. *Green Chem* 2021, **23**:8406–8427.
26. Lozano P, Alvarez E, Bernal JM, Nieto S, Gómez C, Sanchez-Gomez G: **Ionic liquids for clean biocatalytic processes**. *Current Green Chemistry* 2017, **4**:116–129.
27. Toledo ML, Pereira MM, Freire MG, Silva JPA, Coutinho JAP, Tavares APM: **Laccase activation in deep eutectic solvents**. *ACS Sustainable Chem Eng* 2019, **7**:11806–11814.
28. Xu P, Zheng G-W, Zong M-H, Li N, Lou W-Y: **Recent progress on deep eutectic solvents in biocatalysis**. *Bioresour. Bio-process.* 2017, **4**:34.
29. Tan J-N, Dou Y: **Deep eutectic solvents for biocatalytic transformations: focused Lipase catalyzed organic reactions**. *Appl Microbiol Biotechnol* 2020, **104**:1481–1496.
30. Pätzold M, Siebenhaller S, Kara S, Liese A, Syltatk C, Holtmann D: **Deep eutectic solvents as efficient solvents in biocatalysis**. *Trends Biotechnol* 2019, **37**:943–959.
31. Hassani FZIM, Amzazi S, Lavandera I: **The versatile applications of DES and their influence on oxidoreductase-mediated transformations**. *Molecules* 2019, **24**:2190. <https://doi.org/10.3390/molecules24112190>.
32. Zhang N, Steininger F, Meyer L-E, Koren K, Kara S: **Can deep eutectic solvents sustain oxygen-dependent bioprocesses? Measurements of oxygen transfer rates**. *ACS Sustainable Chem Eng* 2021, **9**:8347–8353.

This paper discusses biocatalytic redox processes in all types of media including bio-based solvents, ILs and DES.

Oxygen transfer rates were evaluated in a range of DESs

33. Song T, Morales-Collazo O, Brennecke JF: **Solubility and diffusivity of oxygen in ionic liquids**. *J Chem Eng* 2019, **64**: 4956–4967. Data.

Oxygen solubility in different ionic liquids was measured and reported

34. Tian H, Berton P, Rogers RD: **Phase behavior of aqueous biphasic systems with choline alkanoate ionic liquids and phosphate solutions: the influence of pH**. *Fluid Phase Equil* 2019, **502**, 112258.
35. MacFarlane DR, Chong AL, Forsyth M, Kar M, Vijayaraghavan R, Somers A, Pringle JM: **New dimensions in salt–solvent mixtures: a 4th evolution of ionic liquids**. *Faraday Discuss* 2018, **206**:9–28.

36. Nakasu PYS, Barbara PV, Firth AEJ, Hallett JP: **Pretreatment of biomass with protic ionic liquids**. *Trends Chem* 2022, **4**:175–178.
 37. Kuroda K, Satria H, Miyamura K, Tsuge Y, Ninomiya K, Takahashi K: **Design of wall destructive but membrane compatible solvent**. *J Am Chem Soc* 2017, **139**:16052–16055.
 38. Itoh T, Takagi Y: **Laccase-catalyzed reactions in ionic liquids for green sustainable chemistry**. *ACS Sustainable Chem Eng* 2021, **9**:1443–1458.
- An excellent review of laccase catalyzed reactions in ILs.
39. Liu H, Wu X, Sun J, Chen S: **Stimulation of laccase biocatalysis in ionic liquids: a review on recent progress**. *Curr Protein Pept Sci* 2018, **19**:100–111.
 40. Ma Y, Li Y, Ali S, Li P, Zhang W, Rauch MCR, Willot SJ-P, Ribitsch D, Choi YH, Alcalde M, Hollmann F, Wang Y: **Natural deep eutectic solvents as performance additives for perox-ygenase catalysis**. *ChemCatChem* 2020, **12**:989–994.
 41. Gotor-Fernandez V, Paul CE: **Deep eutectic solvents for redox biocatalysis**. *J Biotechnol* 2019, **293**:24–35.
- This paper includes many examples of biocatalytic redox reactions performed in DESs
42. de Gonzalo G, Martin C, Fraaije MW: **Positive impact of natural deep eutectic solvents on the biocatalytic performance of 5-hydroxymethyl-furfural oxidase**. *Catalysts* 2020, **10**:447.
 43. de Gonzalo G, Lončar N, Fraaije M: **Kinetic resolution of racemic benzofused alcohols catalysed by HMFO variants in presence of natural deep eutectic solvents**. *Biocatal Bio-transform* 2022. <https://doi.org/10.1080/10242422.2022.2038582>.
 44. Luisis PL: **Enzymes hosted in reverse micelles in hydrocarbon solution**. *Angew Chem, Int Ed Engl* 1985, **24**:439–528.
 45. Cortes-Clerget M, Akporji N, Zhou J, Gao F, Guo P, Parmentier M, Gallou F, Berthon JY, Lipshutz BH: **Bridging the gap between transition metal catalysis and bio-catalysis via aqueous micellar catalysis**. *Nat Commun* 2019, **10**:2169.
- This paper describes the use of designer surfactants to form micelles in aqueous buffer in which a biocatalytic transformation using an alcohol dehydrogenase takes place.
- [46]. Groger H, Gallou F, Lipshutz BH: *Chem Rev* 2022. <https://doi.org/10.1021/acs.chemrev.2c00416>.