sustainable chemistry

MDPI

Communication

Biocatalyzed Sulfoxidation in Presence of Deep Eutectic Solvents

Gonzalo de Gonzalo

Department of Organic Chemistry, University of Seville, 41012 Seville, Spain; gdegonzalo@us.es

Received: 29 September 2020; Accepted: 6 November 2020; Published: 12 November 2020

Abstract: The flavin-containing monooxygenase from *Methylophaga* sp. strain SK1 (*m*FMO) is a valuable biocatalyst for the preparation of optically active sulfoxides, among other valuable compounds. In this study, we explored to benefits of using Natural Deep Eutectic Solvents (NADESs) when doing oxidation with this biocatalyst, fused to phosphite dehydrogenase for cofactor regeneration (PTDH-*m*FMO). It was found that optically active sulfoxides could be obtained with slightly higher conversions in 10% *v/v* NADES when working at substrate concentrations of 50–200 mM, whereas there was no loss in the enantioselectivity. With these results, it is demonstrated for the first time that flavin-containing monooxygenases can be employed as biocatalysts in presence of NADESs.

Keywords: deep eutectic solvents; biocatalysis; flavin-containing monooxygenases; sulfoxides

1. Introduction

Flavin-containing monooxygenases (FMOs, E.C. 1.14.13.8) are a class of oxidative enzymes which catalyze the oxidation of various heteroatoms including nitrogen and sulfur [1–3] employing NADPH as electron source and dioxygen as oxidant. FMOs play an important role in drug and xenobiotics metabolism, but in the last years these enzymes have been also applied in biotransformations [4]. The flavin-containing monooxygenase from *Methylophaga sp.* strain SK1 (*m*FMO) is a bacterial FMO [5], and can be overexpressed as a self-sufficient monooxygenase by covalent coupling the monooxygenase to an NADPH-regenerating phosphite dehydrogenase (PTDH) from *Pseudomonas stuzteri* [6]. This bifunctional biocatalyst is able to catalyze the oxidation of indole and indole derivatives in order to obtain the corresponding indigo compounds, which represent valuable compounds as dyes and with pharmaceutical applications. *m*FMO has also been employed as biocatalyst in the enantioselective oxidation of prochiral sulfides. It allows production of the corresponding optically active sulfoxides with good conversions and enantiomeric excesses [7]. These conversions merely require molecular oxygen as oxidant and can be operated at mild reaction conditions, a great advantage of biocatalytic oxidations [8]. Chiral sulfoxides have a wide range of applications, as they can be employed as chiral auxiliaries in organic synthesis, whereas the sulfoxide moiety takes part of several biologically active compounds [9,10]. Oxidations catalyzed by *m*FMOs are typically performed in aqueous solution, but the use of water as solvent in biocatalytic sulfide oxidations can present some drawbacks such as the possibility of undesired side reactions or solubility issues. For this reason, novel and environmentally friendly non-conventional media are often considered [11,12].

Deep Eutectic Solvents are neoteric solvents formed by the combination of a hydrogen bond acceptor (HBA) with a hydrogen bond donor (HBD) at a fixed molar ratio at high temperature. These compounds present several advantages, as they can be obtained in easy and waste-free procedures, are biodegradable, non-volatile and show a very low toxicity [13,14]. In addition, the structure of these solvents can be modified, allowing a dedicated design of DESs for specific purposes. When the DESs are formed by compounds such as amino acids, organic acids, polyols, urea or sugars obtained from natural renewable sources, they can be called natural DESs (NADESs). In view of their low

ecological footprint, NADESs have been widely applied in different fields, including extractions of organic compounds [15], solar energy [16], electrochemical applications [17], and in catalytic processes. Thus, DESs have been employed as solvents, cosolvents, additives or as supports to anchor catalysts and/or solvents [18–20]. Several reviews have demonstrated the applicability of DES as solvents or cosolvents in reactions catalyzed by biological systems. The huge majority of these examples cover the application of NADESs when employing hydrolases, but also oxidoreductases have been applied in presence of these neoteric solvents, especially when carrying out selective bioreductions of carbonyl compounds [21,22]. Some oxidative enzymes, including oxidases [23] and peroxygenases [24,25], have been studied in aqueous media containing NADESs, but until now, no examples have been shown where flavin-dependent monooxygenases were employed for biocatalytic purposes.

2. Materials and Methods

2.1. Materials

Recombinant PTDH-fused *m*FMO was acquired from GECCO-Biotech (Groningen, The Netherlands). All other chemicals and analytical grade solvents were obtained from Acros Organics (Geel, Belgium), Sigma-Aldrich (Steinheim, Germany) and TCI Europe (Zwijndrecht, Belgium), being used without further purification. NADESs were prepared by mixing the equimolar amounts of the components at 80 ºC for at least two hours. When the reactions were cooled down to room temperature, the NADESs were formed.

Flash chromatography was performed using Merck silica gel 60 (230–400 mesh). ^{1}H-NMR spectra were recorded with TMS (tetramethylsilane) as the internal standard, on a Bruker AC-300-DPX (^{1}H: 300.13 MHz) spectrometer. GC/MS analyses were performed with a GC Hewlett Packard 7890 Series II equipped with a Hewlett Packard 5973 chromatograph MS (Agilent Technologies) using a HP-5MS cross-linked methyl siloxane column (30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2). To monitor levels of conversion, substrates and products were quantified by use of calibration curves. HPLC analyses were performed on a Thermo-Fischer UltiMate chromatograph equipped with a Thermo UltiMate detector using a Chiralcel OD column (Daicel, 0.46 cm × 25 cm). Sulfoxide configurations were established by comparing the retention times of the chiral sulfoxides obtained with those described in the bibliography [26].

2.2. General Procedure for the Biocatalyzed Sulfoxidations Catalyzed by mFMO in Buffer Containing NADES

Unless otherwise stated, sulfides 1-6a (10–200 mM) were dissolved in the corresponding mixtures of Tris/HCl buffer 50 mM pH 9.0/NADES at different concentrations (1.0 mL), containing sodium phosphite (10–200 mM), NADPH (0.2 mM) and *m*FMO (1 μM). Reactions were shaken at 220 rpm and 28 °C for the times indicated. Once finished, the crude reactions were extracted with EtOAc (3 × 500 μL). The organic phases were dried onto Na_2SO_4 and analyzed directly by GC/MS in order to determine the conversion of the biocatalyzed sulfoxidations of compounds 1-6a, as well as by HPLC to determine the optical purities of sulfoxides 1-6b (see Supplementary Materials).

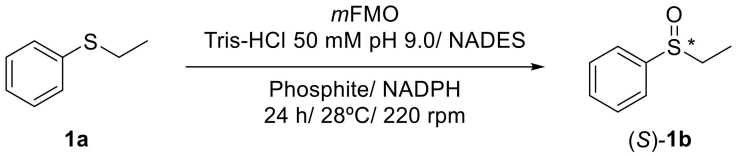
3. Results

3.1. Effect of NADESs in the Biocatalyzed Sulfoxidation of Ethyl Phenyl Sulfide

Initial efforts were devoted to analyze the sulfoxidation of ethyl phenyl sulfide (1a) to (*S*)-ethyl phenyl sulfoxide (1b) in absence and presence of different NADESs. When this compound was oxidized by PTDH-*m*FMO in Tris/HCl 50 mM buffer pH 9.0 at 28 °C, (*S*)-1b was recovered with good conversion (73.5%) and optical purity (75.0%) after 24 h (Table 1, entry 1). This sulfide was considered as a good starting material for analyzing the effect of NADESs in the enzymatic activity and selectivity, as shown in Table 1. Next, the oxidation of 1a was performed in presence of NADESs presenting different structure, carrying out the reactions with increasing amounts of the cosolvent. As can be observed in

Table 1, D-glucose (Glu) or D-fructose (Fru)-based DES as Glu:Fru:H_2O (1:1:6) or ChCl:Glu:H_2O (5:2:5) (entries 2 and 3, respectively), had a negative impact, as a decrease in the conversion of sulfoxide (*S*)-1b was observed. These solvents did not affect the optical purity of the final compound. Only at higher concentrations of these NADESs (up to 20% v/v) a decrease in the optical purity of (*S*)-1b was observed (see Supplementary Materials). A similar behavior was observed for xylitol (Xyl) choline chloride DES (ChCl:Xyl; 1:1), as 10% *v/v* of this cosolvent afforded (*S*)-1b with 28% conversion and 61% *ee* (Table 1, entry 1). The use of ChCl:Urea (1:1) has a very negative impact on both the conversion and selectivity of the biocatalyst (Table 1, entry 5). Even at 5% *v/v* of this cosolvent caused a marked decrease in conversion while little enantiomeric excess of (*S*)-1b was observed: only 15.5% sulfoxide was recovered with 49.5% *ee*. No reaction was observed when employing higher contents of this cosolvent. The best performance for this biocatalyst was found with NADESs composed of choline chloride and polyols such as ethylene glycol (EG) or glycerol (Gly). Thus, the use of ChCl:EG (1:2) or ChCl:Gly (1:2) up to 10% *v/v* led to (*S*)-1b with similar results when compared with the reaction in buffer. Especially in the case of ChCl:Gly (1:2): the use of 10% *v/v* of this NADES yielded 1b with 72.5% conversion and 74.6% enantiomeric excess (entry 11). Higher NADESs amounts have a negative impact in both the biocatalyst activity and selectivity, especially when using more than 20% *v/v* of both eutectic solvents, as shown in entries 9 and 13.

Table 1. Effect of different NADESs in the enzymatic sulfoxidation of ethyl phenyl sulfide catalyzed by fused *m*FMO.



Entry	DES	% DES	Conv. (%) [1]	*ee* (%) [2]
1	None	—	73.3 ± 1.2	75.0 ± 1.4
2	Glu:Fru:H_2O (1:1:6)	5	44.5 ± 0.7	71.5 ± 2.1
3	ChCl:Glu:H_2O (5:2:5)	5	51.5 ± 2.1	72.0 ± 1.4
4	ChCl:Xyl (1:1)	5	37.5 ± 0.7	71.5 ± 2.1
5	ChCl:Urea (1:1)	5	15.5 ± 0.7	49.5 ± 0.7
6	ChCl:EG (1:2)	5	71.5 ± 0.7	73.5 ± 0.7
7	ChCl:EG (1:2)	10	68.3 ± 1.6	73.2 ± 2.2
8	ChCl:EG (1:2)	20	61.0 ± 1.4	47.0 ± 1.3
9	ChCl:EG (1:2)	40	12.5 ± 0.7	15.5 ± 0.7
10	ChCl:Gly (1:2)	5	72.2 ± 1.3	75.3 ± 1.6
11	ChCl:Gly (1:2)	10	72.5 ± 2.1	74.6 ± 1.5
12	ChCl:Gly (1:2)	20	59.0 ± 1.4	50.0 ± 1.4
13	ChCl:Gly (1:2)	40	7.5 ± 0.7	13.5 ± 0.7

[1] Conversion was determined by GC/MS. [2] Optical purity of (*S*)-1b was measured by HPLC. Average values of two or more experiments.

3.2. Effect of Substrate Concentration

Once having identified ChCl:EG (1:2) and ChCl:Gly (1:2) as the best NADESs for the biooxidation of ethyl phenyl sulfide, the effect of the sulfide concentration in the activity and selectivity of the system was analyzed when carrying out the reaction in both buffer Tris/HCl pH 9.0 or buffer containing 5 or 10% v/v of these polyol-based NADESs. In order to compare the sulfoxidations performed at different reaction times, the reaction rate has been defined as the mmoles of sulfide 1a consumed per liter and per hour (mmol/L h). Initial experiments aimed at analyzing the effect of the sulfide concentration on the biocatalyst properites when working in buffer alone, as shown in Table 2. The highest reaction rate was achieved at 10 mM (30.6 mmoles/L h, entry 1). Higher substrate concentrations led to a decrease in the reaction rate, but PTDH-mFMO was still able to catalyze the bioxidation of 1a at concentrations

of 200 mM with a reaction rate of 9.4 mmoles/L h (entry 5). When the oxidations were carried out in presence of 5 or 10% v/v of ChCl:EG (1:2), higher sulfide concentrations (20 and 50 mM) led to some loss in the enzyme activity, as indicated in entries 7, 8, 10 and 11. Thus, when working at 50 mM substrate, the reaction rates are around 11–14 mmol/L h, similar values to the ones obtained in buffer alone when working at 100 mM, indicating a worse performance of this biocatalyst in presence of ChCl:EG (1:2) when increasing 1a concentration.

Table 2. Effect of ethyl phenyl sulfide concentration in the activity and selectivity of the biocatalyzed sulfoxidation of ethyl phenyl sulfide in presence of fused *m*FMO.

Entry	DES	% DES	[1a] (mM)	Time (h)	Conv. (%) [1]	*ee* (%) [2]	Rate (mmol/L h)
1	None	—	10	24	73.3 ± 1.2	75.0 ± 1.4	30.6 ± 0.9
2	None	—	20	46	52.0 ± 1.4	73.5 ± 2.1	22.6 ± 1.7
3	None	—	50	46	19.0 ± 0.7	74.5 ± 0.7	20.7 ± 0.9
4	None	—	100	96	12.0 ± 1.4	72.5 ± 2.1	12.5 ± 0.5
5	None	—	200	96	4.5 ± 0.7	72.0 ± 1.4	9.4 ± 0.7
6	ChCl:EG (1:2)	5	10	24	71.5 ± 0.7	73.5 ± 0.7	29.8 ± 0.8
7	ChCl:EG (1:2)	5	20	46	47.0 ± 1.4	73.5 ± 2.1	20.4 ± 1.4
8	ChCl:EG (1:2)	5	50	46	12.5 ± 1.7	74.0 ± 1.4	13.6 ± 1.3
9	ChCl:EG (1:2)	10	10	24	68.3 ± 1.6	73.2 ± 2.2	28.5 ± 1.4
10	ChCl:Gly (1:2)	10	20	46	34.5 ± 0.7	75.5 ± 2.1	15.0 ± 0.8
11	ChCl:Gly (1:2)	10	50	46	10.0 ± 1.4	73.0 ± 2.1	10.9 ± 1.3

[1] Conversion was determined by GC/MS. [2] Optical purity of (*S*)-1b was measured by HPLC. Average values of two or more experiments.

The effect of the substrate concentration was also analyzed when performing the oxidations in the glycerol-based eutectic solvent, as shown in Figure 1. When employing substrate concentrations from 10 to 50 mM, similar reaction rates were obtained by the addition of 5 or 10% v/v ChCl:Gly (1:2). Yet, at higher 1a concentrations there is a different behaviour, as can be observed in Figure 1. Thus, when working at 100 mM, the reaction rate in only buffer is 12.5 mmol/L h, whereas this value incresed to 15.0 in presence of 5% v/v NADES and to 15.4 when increasing the NADES to 10% v/v. This trend is also observed at 200 mM, obtaining again the best result in 10% ChCl:Gly (1:2) (12.6 mmol/L h), indicating a better performance of the biocatalyst in this reaction medium. Similar as for the rest of the biooxidations performed, the increase in the substrate concentration for both 5 or 10% v/v of the starting material has no impact on the biocatalyst selectivity, with recovering the (S)-sulfoxide with optical purities around 73%.

3.3. Synthesis of Other Sulfoxides

In view of the results obtained by using higher sulfide concentrations in combination with the glycerol-based NADES, sulfoxidations carried out in buffer pH 9.0 containing 10% v/v ChCl:Gly (1:2) were extended to other aromatic sulfides at 100 mM concentration. As can be observed in Figure 2, for some of the substrates tested, a slight increase in the conversion was achieved when employing a 10% v/v of the neoteric solvent, whereas for other starting materials (substrates 2a and 5a) no effect was achieved at these conditions. It was gratifying to observe that the enantiomeric excesses of the final sulfoxides were not affected for neither of the starting materials when carrying out the oxidations in presence of the NADES.

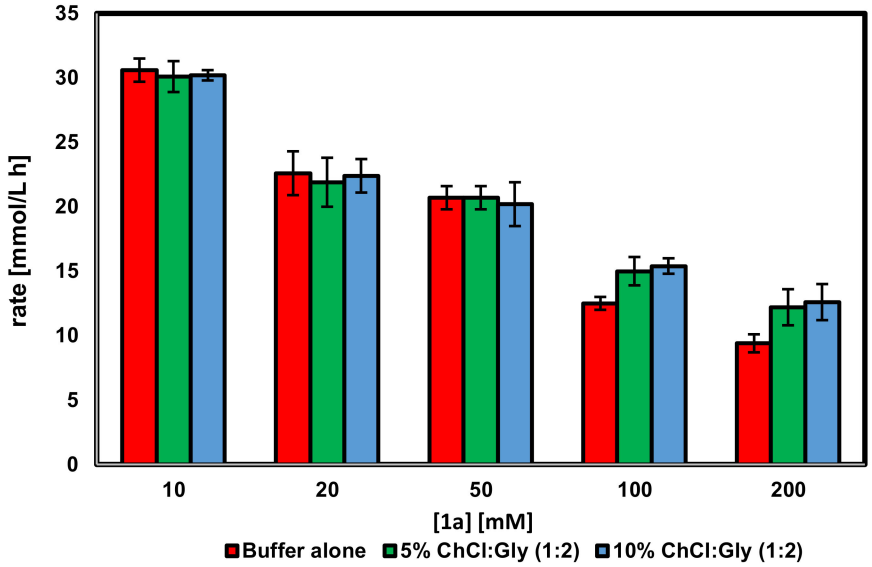


Figure 1. Effect of substrate concentration in the reaction rate in the *m*FMO-catalyzed sulfoxidation of ethyl phenyl sulfide when carrying out the biotransformations in buffer alone (red), in buffer containing 5% *v/v* ChCl:Gly (1:2) (green) and in buffer containing 10% *v/v* ChCl:Gly (1:2) (blue).

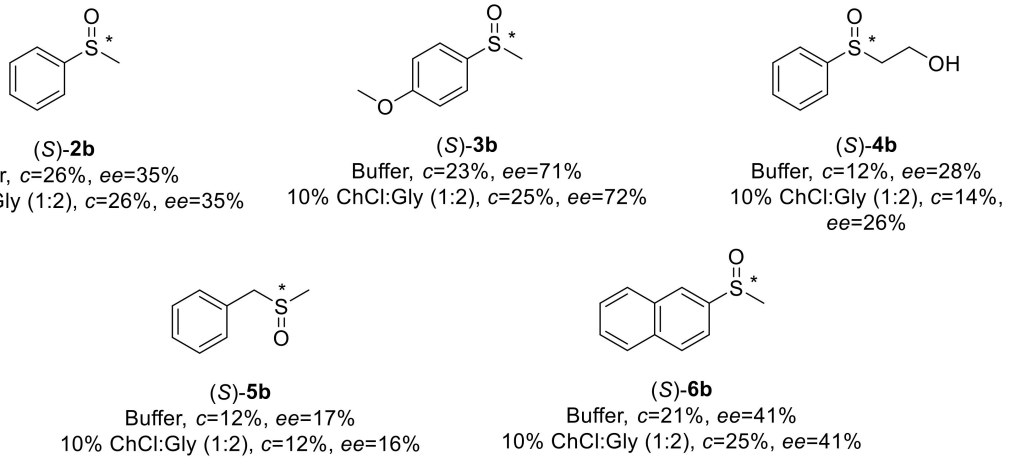


Figure 2. PTDH-*m*FMO-biocatalyzed sulfoxidations in buffer alone and in buffer containing 10% *v/v* ChCl:Gly (1:2).

4. Discussion

NADESs have shown to have an important effect as cosolvents in different biocatalytic procedures employing oxidoreductases, by affecting activity and/or the selectivity of enzyme systems. The data presented in this paper show for the first time that flavin-containing monooxygenases are able to carry out their oxidative activity in presence of these neoteric solvents. These type of enzymes rely on nicotinamide cofactors for carrying out their activity, as well as a cosubstrate for the cofactor regeneration, so the presence of the neoteric solvent can potentially have an impact on the complete enzymatic system. One of the main advantages of NADESs is that they can be easily tuned by modifying the nature of the HBA and/or the HBD components. The experiments have shown that PTDH-*m*FMO does not tolerate sugar-based NADESs, as very small amounts of these compounds have a negative effect on the activity. The NADESs formed by urea and choline chloride seem also to have a strong negative impact, with a decrease in biocatalyst activity and selectivity at 5% *v/v*. Opposite, this enzyme is more tolerant to those NADESs formed by choline chloride and polyols as ethylene glycol or glycerol, allowing the use of 10% *v/v* of these compounds with no effect on the

conversion neither the enantiomeric excess of the final sulfoxide. It has to be mentioned that the best results are obtained at 5–10% *v/v* of the polyol-based NADESs and at these concentrations, most of the hydrogen-bond network between the starting components will be broken [27], appearing to have in solution the individual components of the eutectic solvents in a great extent.

The effect of the sulfide concentration in the biooxidations catalyzed by PTDH-*m*FMO was analyzed which revealed that this biocatalyst is very sensitive to this parameter. From 10 mM sulfide concentrations, the reaction rates decrease, but it was still possible to perform conversions at 200 mM substrate concentration. ChCl:Gly (1:2) at 5% *v/v*, and especially at 10% *v/v* have a positive effect on the biocatalyst performance at 100 and 200 mM substrate concentrations, as at this range the sulfoxidations are faster than in buffer alone. This increase can be due to a higher solubility of the starting material in the reaction mixture, so the result obtained can be promising for developing biotransformations with sulfides which are poorly soluble in aqueous systems. The best results [100 mM, 10% *v/v* ChCl:Gly (1:2)] were extended to the sulfoxidation of other aromatic sulfides. This resulted in a similar observation that the use of NADES can be beneficial in some case.

5. Conclusions

The selective sulfoxidation of ethyl phenyl sulfide catalyzed by the bifunctional biocatalyst PTDH-*m*FMO can be performed in the presence of polyol-choline chloride based deep eutectic solvents at 5 or 10% *v/v* with no significant effect on the biocatalytic properties of the system. The use of ChCl:Gly (1:2) seems to have a positive effect in the biocatalytic oxidation when working at high substrate concentrations (100 or 200 mM). Apart from demonstrating for the first time that monooxygenases containing flavin as prosthetic group can carry out their activity in presence of these neoteric solvents, the results obtained are promising for a further development of these biocatalyzed oxidations, being possible to develop more effective catalytic procedures while using mild and environmentally friendly conditions.

Supplementary Materials: The following are available online at http://www.mdpi.com/2673-4079/1/3/19/s1, Table S1: Biocatalyzed oxidations of ethyl phenyl sulfide in buffer containing NADESs, Table S2: GC/MS conditions to determine the conversion of the biocatalyzed oxidations, Table S3: HPLC conditions to measure the optical purity of the enantioenriched sulfoxides 1-6b.

Funding: This work was supported by the Spanish MINECO (CTQ2016- 76908-C2-1-P).

Acknowledgments: GECCO-Biotech (Groningen, the Netherlands) and Prof. Marco W. Fraaije from University of Groningen are acknowledged by their technical support.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Fabara, A.N.; Fraaije, M.W. An overview of microbial indigo-forming enzymes. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 925–933. [CrossRef]
2. Dijkman, W.P.; de Gonzalo, G.; Mattevi, A.; Fraaije, M.W. Flavoprotein oxidases: Classification and applications. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 5177–5188. [CrossRef] [PubMed]
3. Thodberg, S.; Jakobsen Neilson, E.H. The "Green" FMOs: Diversity, functionality and application of plant flavoproteins. *Catalysts* **2020**, *10*, 329. [CrossRef]
4. Riebel, A.; de Gonzalo, G.; Fraaije, M.W. Expanding the biocatalytic toolbox of flavoprotein monooxygenases from *Rhodococcus jostii* RHA1. *J. Mol. Catal. B Enzym.* **2013**, *88*, 20–25. [CrossRef]
5. Choi, H.S.; Kim, J.K.; Cho, E.H.; Kim, Y.C.; Kim, J.I.; Kim, S.W. A novel flavin-containing monooxygenase from *Methylophaga* sp strain SK1 and its indigo synthesis in *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **2003**, *306*, 930–936. [CrossRef]

6. Torres Pazmiño, D.E.; Riebel, A.; de Lange, J.; Rudroff, F.; Mihovilovic, M.D.; Fraaije, M.W. Efficient biooxidations catalyzed by a new generation of self-sufficient Baeyer-Villiger monooxygenases. *ChemBioChem* **2009**, *10*, 2595–2598. [CrossRef]
7. Rioz-Martínez, A.; Kopacz, M.; de Gonzalo, G.; Torres Pazmiño, D.E.; Gotor, V.; Fraaije, M.W. Exploring the biocatalytic scope of a bacterial flavin-containing monooxygenase. *Org. Biomol. Chem.* **2011**, *9*, 1337–1341. [CrossRef]
8. Holtmann, D.; Fraaije, M.W.; Arends, I.W.C.E.; Opperman, D.J.; Hollmann, F. The taming of oxygen: Biocatalytic oxyfunctionalizations. *Chem. Commun.* **2014**, *50*, 13180–13200. [CrossRef]
9. Trost, B.M.; Rao, M. Development of chiral sulfoxide ligands for asymmetric catalysis. *Angew. Chem. Int. Ed.* **2015**, *54*, 5026–5043. [CrossRef]
10. Carreño, M.C.; Hernández-Torres, G.; Ribagorda, M.; Urbano, A. Enantiopure sulfoxides: Recent applications in asymmetric synthesis. *Chem. Commun.* **2009**, 6129–6144. [CrossRef]
11. Aranda, C.; de Gonzalo, G. Biocatalyzed redox processes employing green reaction media. *Molecules* **2020**, *25*, 3016. [CrossRef] [PubMed]
12. Domínguez de María, P.; Hollmann, F. On the (un)greenness of biocatalysis: Some challenging figures andsome pr omising options. *Front. Microbiol.* **2016**, *6*, 1257. [CrossRef]
13. Liu, Y.; Friesen, J.B.; Lankin, J.B.; Chen, S.-N.; Pauli, G.F. Natural deep eutectic solvents: Properties, applications and perspectives. *J. Nat. Prod.* **2018**, *81*, 679–690. [CrossRef] [PubMed]
14. Smith, E.L.; Abbott, A.P.; Ryder, K.S. Deep Eutectic Solvents (DES) and their applications. *Chem. Rev.* **2014**, *114*, 11060–11082. [CrossRef] [PubMed]
15. Florindo, C.; Branco, L.C.; Marrucho, I.M. Quest for green solvents design: From hydrophilic to hydrophobic (deep) eutectic solvents. *ChemSusChem* **2019**, *12*, 1549–1559. [CrossRef] [PubMed]
16. Boldrini, C.L.; Manfredi, N.; Perna, F.M.; Trifiletti, V.; Capriati, V.; Abbotto, A. Dye-sensitized solar cells that use an aqueous choline chloride-based deep eutectic solvent as effective electrolyte solution. *Energy Technol.* **2017**, *5*, 345–353. [CrossRef]
17. Milia, L.; Dall'Asta, V.; Ferrara, C.; Berbenni, V.; Quartarone, E.; Perna, F.M.; Capriati, V.; Mustarelli, P. Bio-inspired choline chloride-based deep eutectic solvents as electrolytes for lithium-ion batteries. *Solid State Ion.* **2018**, *323*, 44–48. [CrossRef]
18. Alonso, D.A.; Baeza, A.; Chinchilla, R.; Guillena, G.; Pastor, I.M.; Ramón, D.J. Deep eutectic solvents: The organic reaction medium of the century. *Eur. J. Org. Chem.* **2016**, 612–632. [CrossRef]
19. Guajardo, N.; Müller, C.R.; Schrebler, R.; Carlesi, C.; Domínguez de Maria, P. Deep eutectic solvents for organocatalysis, biotransformations, and multistep organocatalyst/enzyme combinations. *ChemCatChem* **2016**, *8*, 1020–1027. [CrossRef]
20. Perna, F.M.; Vitale, P.; Capriati, V. Deep eutectic solvents and their applications as green solvents. *Curr. Opin. Green Sust. Chem.* **2020**, *21*, 27–33. [CrossRef]
21. Gotor-Fernández, V.; Paul, C.E. Deep eutectic solvents for redox biocatalysis. *J. Biotechnol.* **2019**, *293*, 24–35. [CrossRef] [PubMed]
22. Hasani, F.Z.I.M.; Amzazi, S.; Lavandera, I. The versatile applications of DES and their influence on oxidoreductase-mediated transformations. *Molecules* **2019**, *24*, 2190. [CrossRef]
23. de Gonzalo, G.; Martin, C.; Fraaije, M.W. Positive impact of natural deep eutectic solvents on the biocatalytic performance of 5-hydroxymethyl-furfural oxidase. *Catalysts* **2020**, *10*, 447. [CrossRef]
24. Li, Y.; Ma, Y.; Li, P.; Zhang, X.; Ribitsch, D.; Alcalde, M.; Hollmann, F.; Wang, Y. Enantioselective sulfoxidation of thioanisole by cascading a choline oxidase and a peroxygenase in the presence of natural deep eutectic solvents. *ChemPlusChem* **2020**, *85*, 254–257. [CrossRef] [PubMed]
25. Ma, Y.; Li, Y.; Ali, S.; Li, P.; Rauch, M.C.R.; Willot, S.J.-P.; Ribitsch, D.; Choi, Y.H.; Alcaide, M.; Hollman, F.; et al. Natural deep eutectic solvents as performance additives for peroxygenases catalysis. *ChemCatChem* **2019**, *11*, 1–7. [CrossRef]

26. de Gonzalo, G.; Fürst, M.J.L.J.; Fraaije, M.W. Polycyclic Ketone Monooxygenase (PockeMO): A robust biocatalyst for the synthesis of optically active sulfoxides. *Catalysts* **2017**, *7*, 288. [CrossRef]
27. Hammond, O.S.; Bowrom, D.T.; Edler, K.J. The effect of water upon Deep Eutectic Solvent nanostructure: An unusual transition from ionic mixture to aqueous solution. *Angew. Chem. Int. Ed.* **2017**, *56*, 9782–9785. [CrossRef]

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2020 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).