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Enzyme-catalyzed selective oxidation of 5-hydroxymethylfurfural (HMF) and separation of HMF and 2,5-diformylfuran using deep eutectic solvents

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Abstract: An enzyme toolbox was developed for the synthesis of 2,5-diformylfuran (DFF), 5-hydroxymethyl-2-furancarboxylic acid (HMFCA), 5-formyl-2-furancarboxylic acid (FFCA) and 2,5-furandicarboxylic acid (FDCA) with good yields from 5-hydroxymethylfurfural (HMF) via selective oxidation. In addition, a proof-of-concept based on deep eutectic solvents (DES) was provided for the efficient separation of HMF and DFF. The DFF purity was improved to 97% from 76% after extraction by choline-based DES.

With depletion of fossil resources and increasing concerns about global warming in recent years, the production of biofuels and platform chemicals has been attracting growing interest from the carbon-neutral and renewable biomass.[1-4] 5-Hydroxymethylfurfural (HMF) that could be synthesized via the dehydration of carbohydrates is among the U.S. Department of Energy (DOE) "Top 10 + 4" list of bio-based chemicals.[5, 6] HMF have two active groups that could be subjected to various chemical modifications to prepare the useful intermediates.[6, 7] For example, the selective oxidation of HMF can afford many interesting C-6 compounds such as 2,5-diformylfuran (DFF), 5-hydroxymethyl-2-furancarboxylic acid (HMFCA) and 2,5-furandicarboxylic acid (FDCA), which are shown in Scheme 1. Like HMF, these oxidized derivatives are also versatile building blocks, and have a promising application potential in fuels, polymers and drugs, etc. For example, DFF can be used as the precursor for the synthesis of pharmaceuticals,[8, 9] furan-containing polymers,[10] and functional materials.[11] HMFCA is a starting material in the synthesis of various polyesters[12] as well as an interleukin inhibitor.[13] FDCA is considered as a highly promising bio-based alternative to terephthalic acid and isophthalic acid for the production of polyesters.[14, 15] Like HMF, FDCA is also listed as one of the DOE "Top 10 + 4" bio-based

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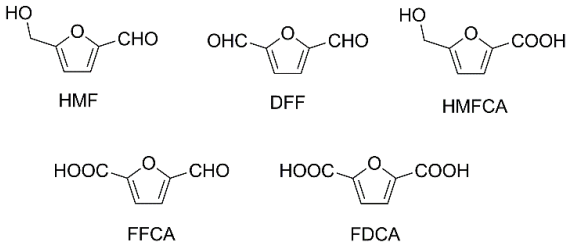
chemicals.[6]



Scheme 1 HMF and its derivatives

A number of chemical methods have been reported for the selective oxidation of HMF based on stoichiometric oxidants and metal catalysts.[6, 7] As compared to chemical methods, biocatalysis appears to be more preferable for the transformation of bio-based chemicals,[16, 17] particularly HMF with two active functional group, due to a lot of advantages such as mild reaction conditions, exquisite selectivity and being environmentally friendly. However, there are limited reports on biocatalytic oxidation of HMF in the literature. The first example of biocatalytic oxidation of HMF was reported by Sheldon and coworkers, where chloroperoxidase-catalyzed oxidation of HMF afforded DFF with the selectivity of 60-74% and HMFCA as the main byproduct.[18] Lipase was used for the oxidation of HMF, thus furnished HMFCA in the yield of approximately 80%, together with about 20% of HMFCA acetate.[19] Recently, an enzymatic tandem approach involving a fungal aryl-alcohol oxidase and an unspecific peroxygenase has been applied for the synthesis of FDCA from HMF.[20] Interestingly, Fraaije and coworkers identified a flavoprotein oxidase that enables to produce FDCA from HMF directly with a yield of 95%.[21] In addition to oxidases, whole-cell biocatalytic approaches were developed for the production of various derivatives of HMF as well.[22] For example, growing cells of recombinant *Pseudomonas putida* were capable of producing 30 g/L FDCA with a 97% yield from HMF in a fed-batch process.[23]

In the present work, a new enzyme toolbox was developed for the selective oxidation of HMF to

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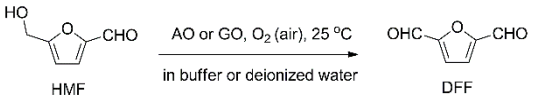
DFF, HMFCA, 5-formyl-2-furancarboxylic acid (FFCA) and FDCA. In addition, highly efficient separation of HMF and DFF was described by using deep eutectic solvents (DES).

Synthesis of DFF

Table 1 Oxidase-mediated synthesis of DFF



Entry	Enzymes	Conditions[a]	Oxidase dosage (U)	Time (h)	DFF yield (%)
1	AO from *Candida boidinii* + catalase	A	6	72	41
2	AO from *Hansenula* sp. + catalase	A	6	72	16
3	AO from *Pichia pastoris* + catalase	A	6	72	16
4	GO	B	4	72	2
5	GO + catalase	C	4	72	23
6	GO + HRP	D	4	72	46
7	GO + catalase + HRP	E	4	72	28
8	Catalase + HRP	F	0	72	-[b]
9	GO+ catalase + HRP	G	4	72	56
10	GO+ catalase + HRP	G	6	120	81
11	GO+ catalase + HRP	G	8	96	92
12	GO + catalase + HRP	G	10	96	91

[a] Conditions A: 2 mL phosphate buffer (50 mM, pH 7.5), 30 mM HMF, 6 U AO, 1.1 mg catalase, air bubbling for 5 min each day, 25 ºC, 150 rpm. Conditions B: 2 mL phosphate buffer (50 mM, pH 7.0), 30 mM HMF, 4 U GO, air bubbling for 5 min each day, 25 ºC, 150 rpm. Conditions C: 2 mL phosphate buffer (50 mM, pH 7.0), 30 mM HMF, 4 U GO, 1.1 mg catalase, air bubbling for 5 min each day, 25 ºC, 150 rpm. Conditions D: 2 mL phosphate buffer (50 mM, pH 7.0), 30 mM HMF, 4 U GO, 1.3 mg HRP, air bubbling for 5 min each day, 25 ºC, 150 rpm. Conditions E: 2 mL phosphate buffer (50 mM, pH 7.0), 30 mM HMF, 4 U GO, 1.1 mg catalase, 1.3 mg HRP, air bubbling for 5 min each day, 25 ºC, 150 rpm. Conditions F: 2 mL phosphate buffer (50 mM, pH 7.0), 30 mM HMF, 1.1 mg catalase, 1.3 mg HRP, air bubbling for 5 min each day, 25 ºC, 150 rpm. Conditions G: 2 mL deionized water, 30 mM HMF, GO(4-10 U), 1.1 mg catalase, 1.3 mg HRP, air

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bubbling for 5 min each day, 25 ºC, 150 rpm.

[b] No reactions occurred.

Four oxidases including alcohol oxidases (AOs) and galactose oxidase (GO) from *Dactylium dendroides* were tested for the transformation of HMF with air as the oxidant (Table 1). Oxidase-catalyzed oxidation of alcohols and aldehydes would produce H_2O_2 as the byproduct, which can result in significant inactivation of the enzymes. Therefore, catalase was added to transform H_2O_2 into water and O_2, the latter of which could again act as substrate of the AOs. In contrast to the previous results,[24, 25] we found that the three AOs had the ability to oxidize HMF (Table 1, entries 1-3), resulting in the formation of DFF as the sole product (Fig. S1, ESI†). Of the AOs tested, AO from *Candida boidinii* showed the highest activity in the oxidation of HMF, and DFF was obtained with a yield of 41% after 72 h under non-optimized conditions. Recently, Kalum et al. reported that the commercially available GO from *D. dendroides* could not catalyze the oxidation of HMF.[25] However, we found that GO was a good biocatalyst for the synthesis of DFF after careful optimization (Table 1, entries 4-12). As shown in Table 1, similar to the previous report,[25] almost no reactions took place when only GO was added into the reaction mixture (Entry 4). On the contrary, GO-mediated oxidation afforded the desired product with a 23% yield in the presence of catalase (Table 1, entry 5). The improved yield may be attributed to the rapid removal of the harmful H_2O_2 by catalase, instead of the catalytic activity of catalase or horseradish peroxidase (HRP), as no reactions occurred in the control (Table 1, entry 8). In addition, no any products were detected in the presence of HRP and H_2O_2 (data not shown). Although GO could accept HMF as substrate in the presence of catalase, its catalytic activity was not satisfactory. Therefore, various strategies were applied to improve the synthesis of DFF. Inspired by the work

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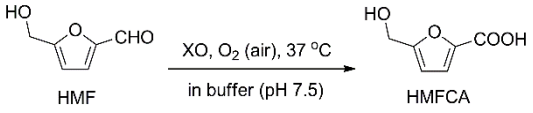
of Parikka and Tenkanen,[26] the addition of HRP was attempted (Table 1, entries 6 and 7). Interestingly, it was found that the DFF yield (46%) improved significantly when the combination of GO and HRP was used (Table 1, entry 6), indicating that the significant activation effect of HRP toward GO. Unexpectedly, the addition of catalase into the combination of GO and HRP resulted in a negative effect on the synthesis of DFF (Table 1, entry 7). However, the yield could be improved significantly when the oxidation mediated by the combination of the aforementioned three enzymes was conducted in deionized water rather than in buffer (Table 1, entry 9 *vs.* entry 7). Other groups reported similar phenomenon in the enzymatic oxidations.[26, 27] To uncover the reason, the enzyme performance in various buffer systems was compared for oxidation of HMF (Table S1, ESI†). As shown in Table S1, the lowest yields were obtained in phosphate buffer (Entries 1 and 4). As we know, GO is a copper-containing, free radical enzyme.[28] The precipitant of $Cu_3(PO_4)_2$ might be formed when GO was incubated in phosphate buffer,[29] thus leading to the lower activities. As expected, the yields were improved significantly using deionized water and sodium acetate buffer to replace phosphate buffer (Table S1, entries 2, 3 and 5). The DFF yield was further improved upon the optimization of enzyme dosage. An almost quantitative yield (92%) was obtained with 8 U GO after the reaction of 96 h in deionized water (Table 1, entry 11).

Synthesis of HMFCA



Scheme 2 Enzymatic synthesis of HMFCA by XO

To our knowledge, there is only one report on biocatalytic synthesis of HMFCA, in which

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peracids formed *in situ* were used for the oxidation of HMF to HMFCA.[19] As mentioned above, approximately 20% of HMFCA acetate was produced as the byproduct. Xanthine oxidase (XO) from *Escherichia coli*, a molybdenum-dependent enzyme,[30] has been known as the therapeutic target in the prevention and treatment of diseases.[31, 32] However, XO was seldom used in the organic synthesis.[33] Herein, it was applied for the selective oxidation of the formyl group in HMF (Scheme 2). As depicted in Fig. 1, the oxidation of HMF catalyzed by XO could proceed smoothly. HMFCA was afforded with the yield of 94% after 7 h. The high reaction rate might be relevant to the formation of the highly active superoxide anion radicals by XO.[34, 35] In addition, no other oxidized products were formed (Fig. S2, ESI†). As compared to lipase-catalyzed oxidation of HMF,[19] the XO-mediated process reported in this work has a lot of advantages: 1) using air as the oxidant, instead of toxic H_2O_2; 2) no use of organic solvents; 3) excellent selectivity; 4) no byproducts; 5) shorter reaction time. The oxidation of DFF was conducted by using this oxidase as well. Unfortunately, DFF seemed not to be a good substrate for XO, and FFCA was obtained with a pretty low yield (approximately 2%, Fig. S3, ESI†).

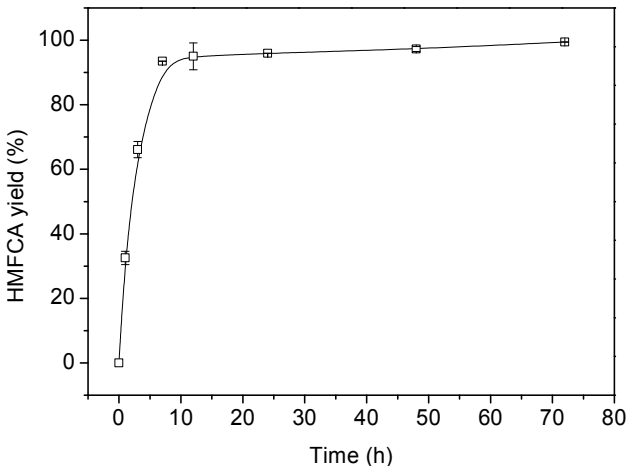

Fig. 1 XO-catalyzed synthesis of HMFCA

Reaction conditions: 2.25 mL phosphate buffer (50 mM, pH 7.5), 26 mM HMF, 5.6 U *E. coli* XO, 1.1 mg catalase, air bubbling for 5 min each day, 37 ºC, 150 rpm

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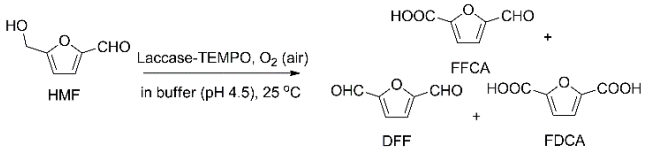
Synthesis of FFCA

Three laccases were tested for the oxidation of HMF to FFCA, with 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) as the mediator (Table 2). It was found that the three laccases could accept HMF as substrate. At the initial stage, DFF was formed as the dominant intermediate, which was further transformed into FFCA (Figs. S4 and S5, ESI†). However, the oxidation efficiency of FFCA into FDCA was poor, likely due to the low degree of hydration for this aldehyde.[21] The chemoenzymatic oxidation of HMF resulted in the mixtures, in which FFCA was dominate. Of the laccases examined, *Panus conchatus* laccase provided the highest FFCA yield (82%). In addition to the laccase-mediator system, AO- and GO-catalyzed oxidation of HMFCA into FFCA were attempted also. Unfortunately, poor results (<20% yields) were obtained with 5 mM HMFCA (data not shown), partially due to the significant pH decrease in the presence of the acidic substrate.

Table 2 Laccase-catalyzed synthesis of FFCA with TEMPO as the mediator



Enzymes	Time (h)	FFCA yield (%)	DFF yield (%)	FDCA yield (%)
Trametes versicolor laccase	48	68	6	5
Panus conchatus laccase	96	82	4	10
Flammulina velutipes laccase	72	70	6	6

[a]: Reaction conditions: 2 mL sodium acetate buffer (50 mM, pH 4.5), 30 mM HMF, 20 mol%

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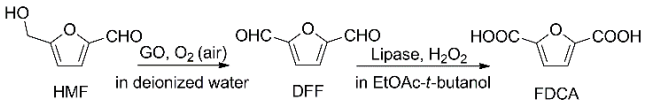
TEMPO, 5.2 mg laccase, air bubbling for 5 min each day, 25 ºC, 150 rpm

Synthesis of FDCA via tandem reactions



Scheme 3 Enzymatic synthesis of FDCA via tandem oxidations

In general, one-step oxidation of HFM to FDCA by a single biocatalystis rare as it involves three consecutive oxidations.[21] Therefore, tandem oxidations involving GO and lipase were designed for the conversion of HFM to FDCA (Scheme 3). First, HMF was transformed into DFF by GO under the optimized conditions. After 48 h, the conversion of HMF reached approximately 75%. Then, DFF and HMF was extracted from the reaction mixture using ethyl acetate (EtOAc) for 3 times. The organic phase was merged and concentrated to approximately 1 mL, and *t*-butanol of the same volume was added. Immobilized lipase B from *Candida antarctica* (CAL-B, Novozym 435) and H_2O_2 were added to initialize the oxidation of DFF (Fig. 2). As shown in Fig. 2, the reaction proceed quickly, and DFF was used up within 7 h. At the beginning, FFCA was the major product, and this intermediate was completely transformed into FDCA with the elongation of reaction time. FDCA was obtained with a yield of 88% after 24 h. Although CAL-B has also the ability to oxidize the residual HMF, the reaction rate is very low. HMF was consumed completely after 48 h; nonetheless, HMFCA was obtained with the yield of approximately 18%, and an unidentified product was obtained as a major product (Fig. S6, ESI†).

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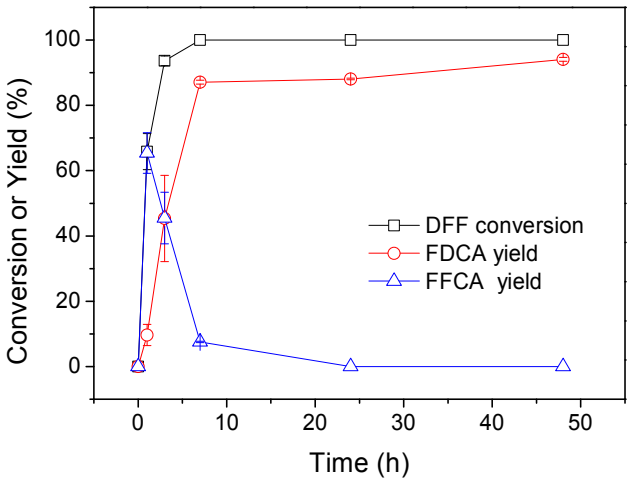

Fig. 2 Lipase-mediated oxidation of DFF

Reaction conditions: 2 mL *t*-butanol-EtOAc (1:1, v/v), approximately 22.5 mM DFF, 9.6 mg CAL-B, addition of 1.6 equiv. aqueous H_2O_2 (30%, v/v) each hour for 6 times, 40 ºC, 150 rpm. After 24 h, 1.6 equiv. H_2O_2 was added.

Separation of HMF and DFF using DES

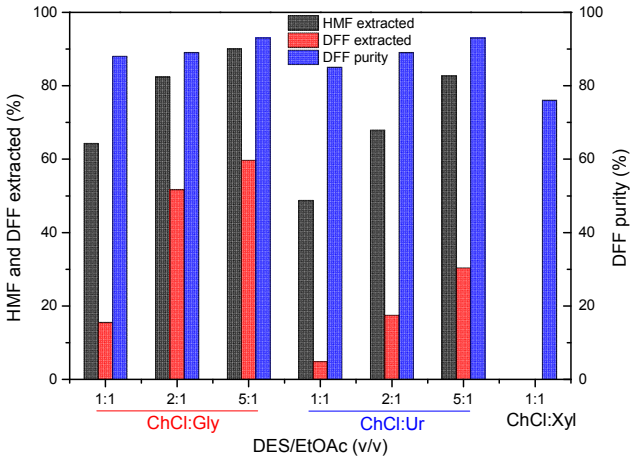

Fig. 3 Separation of HMF and DFF using DES. The original composition of the mixture is 25% HMF (0.67 mg/mL) and 75% DFF (2.09 mg/mL) in EtOAc. DES: ChCl:Gly = choline chloride:glycerol (1:2, mol/mol); ChCl:Ur = choline chloride:urea (1:2, mol/mol); ChCl:Xyl = choline chloride:xylitol (1:1, mol/mol)

As described above, the oxidase-mediated synthesis of DFF reached the equilibrium with the best yield of 92%. Therefore, the separation of the residual HMF and the formed DFF is required. However, the separation of HMF and DFF using the traditional organic solvent/water biphasic systems such as EtOAc/water was not conducted successfully. DES generally composed of a

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quaternary ammonium salt and a hydrogen bond donor (HBD) have emerged as a promising kind of solvents, since they not only exhibit similar physicochemical properties to traditional imidazolium ILs, but also their preparation is 100% atom-economic with no requirement of purification.[36, 37] It has been reported that DES show higher affinity toward HBD molecules (*e.g.*, alcohols and phenols), resulting in HBD dissolution in DES, whereas other non-HBD molecules (*e.g.*, esters) remain as a second phase.[38-41] Based on this property, the separation of HMF and DFF was conducted using DES (Fig. 3). Herein, three DES including ChCl:Gly, ChCl:Ur and ChCl:Xyl were used to extract HMF from EtOAc containing DFF and HMF. As shown in Fig. 3, ChCl:Gly and ChCl:Ur enabled to selectively extract HMF from the mixture. After extraction using DES, the DFF purity was improved to 88-93%. Inspired by the results, the extraction of HMF using ChCl:Gly and ChCl:Ur of the equal volume was conducted for 3 times (Table S2, ESI †). It could be found that the DFF purity significantly increased to 97% from the original purity of 76% after extraction using ChCl:Gly for 3 times. Although the recovery of DFF (approximately 55%) is still unsatisfactory, the proof-of-concept based on DES for the separation of HMF and DFF has been demonstrated. It is presumable that the DFF recovery may be improved markedly upon careful optimization of DES composition.

In conclusions, we have developed successfully a versatile enzyme toolbox for the synthesis of various valuable C-6 compounds from HMF via selective oxidation. The desired products could be readily obtained with good yields and excellent selectivity by choosing the suitable enzymes or their combination. The enzymatic approaches appear to be green, because of mild reaction conditions and use of environmentally friendly catalysts as well as using water and air as the

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solvent and oxidant, respectively. In addition, the efficient separation of HMF and DFF was realized by using DES, a novel kind of ecofriendly solvents, which would further make a considerable contribution to the sustainability in the production of DFF.

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DOI: 10.1039/C5GC00788G

References

1. V. Menon and M. Rao, *Prog. Energy Combust. Sci.*, 2012, **38**, 522-550.
2. R. A. Sheldon, *Catal. Today*, 2011, **167**, 3-13.
3. P. Gallezot, *Chem. Soc. Rev.*, 2012, **41**, 1538-1558.
4. F. Cherubini, *Energy Convers. Manage.*, 2010, **51**, 1412-1421.
5. J. J. Bozell and G. R. Petersen, *Green Chem.*, 2010, **12**, 539-554.
6. R.-J. van Putten, J. C. van der Waal, E. de Jong, C. B. Rasrendra, H. J. Heeres and J. G. de Vries, *Chem. Rev.*, 2013, **113**, 1499-1597.
7. A. A. Rosatella, S. P. Simeonov, R. F. Frade and C. A. Afonso, *Green Chem.*, 2011, **13**, 754-793.
8. M. Del Poeta, W. A. Schell, C. C. Dykstra, S. Jones, R. R. Tidwell, A. Czarny, M. Bajic, M. Bajic, A. Kumar and D. Boykin, *Antimicrob Agents Chemother*, 1998, **42**, 2495-2502.
9. K. T. Hopkins, W. D. Wilson, B. C. Bender, D. R. McCurdy, J. E. Hall, R. R. Tidwell, A. Kumar, M. Bajic and D. W. Boykin, *J. Med. Chem.*, 1998, **41**, 3872-3878.
10. A. S. Amarasekara, D. Green and L. D. Williams, *Eur. Polym. J.*, 2009, **45**, 595-598.
11. J. Ma, Z. Du, J. Xu, Q. Chu and Y. Pang, *ChemSusChem*, 2011, **4**, 51-54.
12. H. Hirai, *J. Macromol. Sci., Part A: Chem.*, 1984, **21**, 1165-1179.
13. A. C. Braisted, J. D. Oslob, W. L. Delano, J. Hyde, R. S. McDowell, N. Waal, C. Yu, M. R. Arkin and B. C. Raimundo, *J. Am. Chem. Soc.*, 2003, **125**, 3714-3715.
14. J. Zhu, J. Cai, W. Xie, P.-H. Chen, M. Gazzano, M. Scandola and R. A. Gross, *Macromolecules*, 2013, **46**, 796-804.
15. C. Vilela, A. F. Sousa, A. C. Fonseca, A. C. Serra, J. F. J. Coelho, C. S. R. Freire and A. J. D.

View Article Online
DOI: 10.1039/C5GC00788G

Silvestre, *Polym. Chem.*, 2014, **5**, 3119-3141.

16. R. De Regil and G. Sandoval, *Biomolecules*, 2013, **3**, 812-847.

17. A. J. Straathof, *Chem. Rev.*, 2013, **114**, 1871-1908.

18. M. van Deurzen, F. van Rantwijk and R. Sheldon, *J. Carbohydr. Chem.*, 1997, **16**, 299-309.

19. M. Krystof, M. Perez-Sanchez and P. Dominguez de Maria, *ChemSusChem*, 2013, **6**, 826-830.

20. J. Carro, P. Ferreira, L. Rodriguez, A. Prieto, A. Serrano, B. Balcells, A. Arda, J. Jimenez - Barbero, A. Gutierrez and R. Ullrich, *FEBS J*, 2015, DOI: 10.1111/febs.13177.

21. W. P. Dijkman, D. E. Groothuis and M. W. Fraaije, *Angew. Chem. Int. Ed.*, 2014, **53**, 6515-6518.

22. N. Wierckx, T. D. E. Schuurman, L. M. Blank and H. J. Ruijssenaars, in *Microorganisms in Biorefineries*, ed. B. Kamm, Springer, Berlin Heidelberg, 2015, pp. 207-223.

23. F. Koopman, N. Wierckx, J. H. de Winde and H. J. Ruijssenaars, *Bioresour. Technol.*, 2010, **101**, 6291-6296.

24. *U.S. Pat.,* 8183020, 2012.

25. *WO Pat.,* 2014015256-A2, 2014.

26. K. Parikka and M. Tenkanen, *Carbohydr. Res.*, 2009, **344**, 14-20.

27. P. Galletti, M. Pori, F. Funiciello, R. Soldati, A. Ballardini and D. Giacomini, *ChemSusChem*, 2014, **7**, 2684-2689.

28. A. Siebum, A. van Wijk, R. Schoevaart and T. Kieboom, *J. Mol. Catal. B: Enzym.*, 2006, **41**, 141-145.

29. J. Ge, J. Lei and R. N. Zare, *Nat. Nanotechnol.*, 2012, **7**, 428-432.

30. C. D. Brondino, M. J. Romao, I. Moura and J. J. G. Moura, *Curr. Opin. Chem. Biol.*, 2006, **10**,

View Article Online
DOI: 10.1039/C5GC00788G

109-114.

31. K.-H. Yu, *Recent Pat. Inflammation Allergy Drug Discovery*, 2007, **1**, 69-75.

32. J. Dawson and M. Walters, *Br. J. Clin. Pharmacol.*, 2006, **62**, 633-644.

33. B. Bechi, S. Herter, S. McKenna, C. Riley, S. Leimkuhler, N. J. Turner and A. J. Carnell, *Green Chem.*, 2014, **16**, 4524-4529.

34. L. Selloum, S. Reichl, M. Müller, L. Sebihi and J. Arnhold, *Arch. Biochem. Biophys.*, 2001, **395**, 49-56.

35. I. Fridovich, *J. Biol. Chem.*, 1970, **245**, 4053-4057.

36. A. P. Abbott, D. Boothby, G. Capper, D. L. Davies and R. K. Rasheed, *J. Am. Chem. Soc.*, 2004, **126**, 9142-9147.

37. Q. Zhang, K. D. O. Vigier, S. Royer and F. Jerome, *Chem. Soc. Rev.*, 2012.

38. Z. Maugeri, W. Leitner and P. Dominguez de Maria, *Tetrahedron Lett.*, 2012, **53**, 6968-6971.

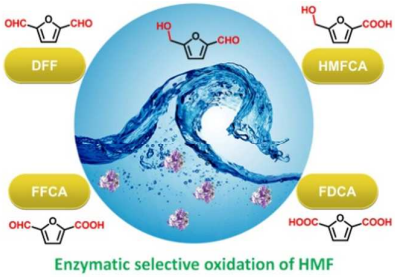
39. A. P. Abbott, P. M. Cullis, M. J. Gibson, R. C. Harris and E. Raven, *Green Chem.*, 2007, **9**, 868-872.

40. M. Krystof, M. Perez‐Sanchez and P. Dominguez de Maria, *ChemSusChem*, 2013, **6**, 630-634.

41. K. Pang, Y. Hou, W. Wu, W. Guo, W. Peng and K. N. Marsh, *Green Chem.*, 2012, **14**, 2398-2401.

View Article Online
DOI: 10.1039/C5GC00788G

Graphical Abstract



An enzyme toolbox was developed for the synthesis of valuable C-6 compounds via selective oxidation of HMF. A proof-of-concept based on DES was provided for separation of HMF and DFF