

Green Chemistry

Accepted Manuscript



This article can be cited before page numbers have been issued, to do this please use: S. McKenna, P. Mines, P. Law, K. Kovacs-Schreiner, W. Birmingham, N. J. Turner, S. Leimkühler and A. Carnell, *Green Chem.*, 2017, DOI: 10.1039/C7GC01696D.



This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the [author guidelines](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the ethical guidelines, outlined in our [author and reviewer resource centre](#), still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.



Green Chemistry

COMMUNICATION

The continuous oxidation of HMF to FDCA and the immobilisation and stabilisation of periplasmic aldehyde oxidase (PaoABC)

S. M. McKenna,^a P. Mines,^b P. Law,^b K. Kovacs-Schreiner,^b W. R. Birmingham,^c N.J. Turner^c,
S. Leimkühler,^d A. J. Carnell^{*a}

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

By manipulating the reaction conditions, furandicarboxylic acid (FDCA) was prepared by biooxidation of hydroxymethyl furfural (HMF) in a continuous one-pot reaction using galactose oxidase M₃₋₅, periplasmic aldehyde oxidase (PaoABC), catalase and horseradish peroxidase. In addition, PaoABC was successfully entrapped in a SiO₂ hydrogel and recycled 14 times without loss of activity. The catalyst was able to tolerate up to 200mM DFF concentration giving FDCA in full conversion with very promising TOF and TON values.

With the rapid growth of the world population and increasing consumption of fossil fuels, there is an urgent need to produce chemicals from renewable sources.¹ Biomass is cheap and abundant and can provide a potential substitute for chemicals production.² 75% of biomass is composed of carbohydrates, so developing efficient approaches to transform biomass derived carbohydrates into value-added chemicals is a high priority.³

2,5-Furandicarboxylic acid (FDCA) **5** is one of 12 identified important platform chemicals derived from biomass and a highly efficient, sustainable and cost effective integrated process to achieve the conversion is in demand.⁴ FDCA can serve as a polymer building block for the production of biopolymers such as polyamides and polyesters.^{5,6} The polyethylene terephthalate (PET) packaging market was worth \$48.1 billion in 2014 and this is expected to increase to \$60 billion in 2019.⁷

^a Department of Chemistry, University of Liverpool
Crown Street, Liverpool (UK). Fax: +44 1517943500; Tel: +44 151
7943534; E-mail: acarnell@liv.ac.uk

^b Biome Bioplastics, North Road, Marchwood, Southampton, SO40 4BL
(UK).

^c School of Chemistry, Manchester Institute of Biotechnology, University of
Manchester, 131 Princess Street, Manchester, M1 7DN, (UK). Fax: +44 161
2751311; Tel: +44 161 3065173; E-mail: nicholas.turner@manchester.ac.uk

^d Institute of Biochemistry and Biology, University of Potsdam
D-14476 Potsdam, Germany. Fax: +44 331/977-5128; Tel: +49 331/977-
5603; E-mail: sleim@uni-potsdam.de

† Electronic Supplementary Information (ESI) available:
[Supporting information for this article including HPLC data
and NMR spectra is available XX]. See DOI: 10.1039/b000000x/

Bio-based **5** could replace terephthalate in polyethylene furanoate (PEF) and related bioplastics which have improved performance compared to their terephthalate counterparts. Notably BASF and Avantium have recently developed a partnership 'Synvina' to manufacture biobased FDCA using a chemocatalytic approach for PEF production.⁸

5-hydroxymethylfurfural (HMF) **1** which is obtained from the dehydration of lignocellulose⁹⁻¹¹ can be converted to FDCA **5** via intermediates DFF **2**, HMFA **3** and FFCA **4** (Figure 1).

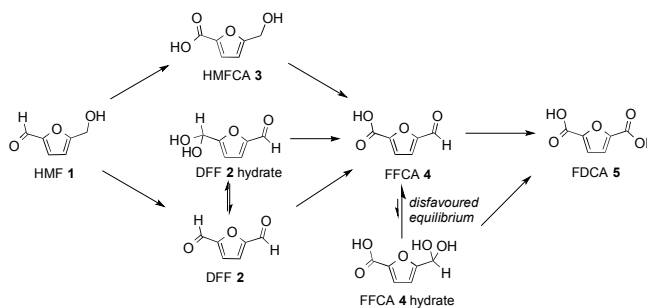


Figure 1 Intermediates in the oxidation of HMF **1** to FDCA **5**

Chemocatalytic approaches¹² used for HMF oxidation include Au–C modified with Pd,¹³ Au–hydrotalicite,¹⁴ Pt–C,¹⁵ Co/Mn/Br¹⁶ and MnO_x–CeO₂.¹⁷ However, these reactions require high pressure/temperature and additives and due to the instability of **1**, side products are often produced. Biocatalysts have emerged as a potential solution to this problem since they operate under mild reaction conditions such as ambient temperature/pressure and physiological pH and often exhibit excellent regio/chemo selectivity, decreasing the formation of side products.¹⁸ The first biocatalytic approach for HMF oxidation used chloroperoxidase which gave mixtures of oxidation products.¹⁹ A whole-cell fed batch process using recombinant *P. putida* showed promise, although product isolation was challenging.²⁰ More recently biocatalysts such as hydroxymethyl furfural oxidase (HMFO)²¹ and fungal aryl alcohol oxidase (AAO) plus unspecific peroxygenase (UPO)²² have been employed to carry out the

oxidation of **1** to **5** under mild reaction conditions in good conversion, albeit at low substrate concentration (4mM). Although inherently green, a limitation of the HMFO is that it has been shown to require the hydrated form of 2,5-diformylfuran (DFF) **2** and of 5-formylfuran-2-carboxylic acid (FFCA) **4**. Fraaije and coworkers have shown that although **2** is well hydrated, the degree of hydration of **4** at pH 7.0 is low (1.8%) and that this correlates with the low activity of the biocatalyst. A HMFO double mutant showed a promising increase in activity, although overall the activity remained modest.^{21b} Laccase-mediator systems also appear to have the same requirement, giving predominantly FFCA from HMF.²³ FDCA **5** was obtained by an initial oxidation of HMF by galactose oxidase (GOase) followed by CAL-B/peroxide oxidation of the intermediate 2,5-diformylfuran (DFF).²³ However, the second step required a change in solvent and the slow addition of hydrogen peroxide. In addition, residual HMF from the first step was converted to 5-hydroxymethyl-2-furan carboxylic acid (HMFCa, **3**), which is not converted to FDCA by the lipase/peroxide system.

We have previously reported the step-wise bio-oxidation of **1** using two isolated oxidases, galactose oxidase M_{3,5} variant (GOaseM_{3,5}) and periplasmic aldehyde oxidase ABC (PaoABC) with the addition of catalase for both enzymes to destroy the hydrogen peroxide produced (Figure 2a).²⁴ The advantage of this methodology is that PaoABC catalyses the full double oxidation of DFF to FDCA and does not require the hydrated form of FFCA **4**. In addition, PaoABC uses oxygen from air rather than hydrogen peroxide used by the peroxygenase.²² This resulted in a much higher rate of conversion and in turn higher substrate concentration being tolerated (>100 mM). However, stepwise addition of the GOaseM_{3,5} and PaoABC was required because the rate of HMF oxidation by GOase M_{3,5} could not compete with fast oxidation of **1** by PaoABC to give HMFCa **3** (see Figure 1), itself a poor substrate for GOaseM_{3,5}, thus creating a bottleneck. Herein, we describe the continuous oxidation of **1** to **5** using GOaseM_{3,5}, PaoABC and catalase with the addition of horse radish peroxidase (HRP) (Figure 2b). In addition, immobilisation of PaoABC was explored to facilitate future scale up of FDCA production.

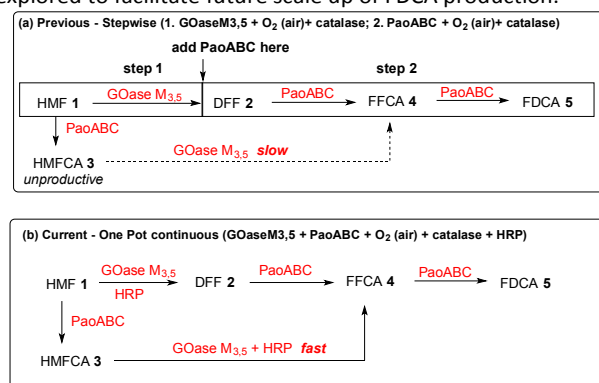


Figure 2 (a) Original stepwise and (b) new continuous one-pot conversion for HMF to FDCA using HRP. For compound structures see Figure 1.

Continuous oxidation of HMF to FDCA

Under continuous conditions using galactose oxidase GOaseM_{3,5} and aldehyde oxidase PaoABC, two oxidation products can be formed initially. The alcohol oxidation product, diformylfuran (DFF) **2** and aldehyde oxidation product, 5-hydroxymethylfuran-2-carboxylic acid (HMFCa) **3**. However, we have previously shown that **3** is a poor substrate for GOaseM_{3,5} at high substrate concentrations in cascade conditions (>20mM).²⁴ Therefore, stepwise addition of the biocatalysts was required to circumvent the unwanted formation of **3**.

To allow the continuous oxidation of **1** to **5**, further directed evolution of GOaseM_{3,5} may well improve the activity of GOase variants for HMFCa at higher substrate concentrations. Although directed evolution has delivered excellent biocatalysts, these strategies are often labour intensive. Therefore, we chose to look at the reaction kinetics of PaoABC to determine if we could manipulate reaction conditions to furnish **5** in a continuous manner and minimise the formation of HMFCa. The apparent K_m and V_{max} values for PaoABC with HMF **1** as substrate are 0.13mM and 0.92 μmol/min/mg respectively (ESI Section 2.5). In addition, uncompetitive inhibition was observed at higher HMF concentration. For DFF **2**, the apparent V_{max} is similar (0.93 μmol/min/mg) and the apparent K_m is slightly higher 0.73mM. However, DFF readily forms a hydrate when compared with HMF.²¹ This suggests that DFF has a more electrophilic carbonyl which would undergo attack by the nucleophilic molybdenum-OH centre at the active site of PaoABC. Thus it may be possible to preferentially oxidise DFF in the presence of HMF. To achieve this, the rate at which GOaseM_{3,5} oxidises HMF to DFF needs to be fast enough to provide a sufficiently high concentration of DFF for PaoABC.

Although the precise mechanism of activation is unclear, horse radish peroxidase (HRP) has been long known to activate GOase,^{23,25,26} and addition of HRP to GOase catalysed oxidation contributes to significantly increased activity and yield.²⁶ The inclusion of HRP did indeed increase the rate of oxidation of HMF **1** to give greater conversion to DFF **2** in a short, 1 hour reaction (Table 1, cf. entries 1&2). Varying pH showed that pH 7.0 was the optimum for the oxidation of HMF (Table 1 entry 3). Recently, it was reported that WT GOase performs well in unbuffered systems.²³ This would decrease costs associated with the scale up of this process, however, the M_{3,5} variant performed poorly in unbuffered water (Table 1, entry 5). In addition, KPi was previously shown to be a poor buffer for the wild type enzyme. It was postulated that a precipitate of Cu₃(PO₄)₂ might be formed when the copper-dependent GOase is incubated in phosphate buffer, thus leading to copper deficiency and lower activities.²³ However, the M_{3,5} variant performed better in KPi, even at 100mM **1** (Table 1, Entry 7). Strangely, we identified that HMFCa **3** is an excellent substrate of GOaseM_{3,5}, with and without HRP (Table S1). This contradicts our previous cascade results in which we noted that **3** was not sufficiently oxidized under cascade conditions. The likely reason for this is that **5** produced in the dual enzyme

system may partially inhibit GOaseM_{3,5} oxidation of HMFA 3. This suggests future work should include in-situ product removal of 5 to allow optimal activity of GOaseM_{3,5}.

With improved GOaseM_{3,5}-HRP conditions in hand, we turned our attention to PaoABC. By varying the concentration of PaoABC in the reaction we would be able to reduce the rate of the off target oxidation of 1 and limit HMFA formation. We were delighted to observe that at each concentration tested we were able to produce the desired 5 in a continuous rather than stepwise manner (Table 2, Entries 1-3).

Table 1 Optimisation of HMF 1 to DFF 2 conversion by GOase M_{3,5}.

| Entry ^a | [HMF] 1 (mM) | pH | Buffer | % DFF 2 |
|--------------------|--------------|-----|--------|---------|
| 1 ^b | 50 | 7.5 | KPi | 73 |
| 2 | " | " | " | 88 |
| 3 | " | 7.0 | " | 91 |
| 4 | " | 6.5 | " | 77 |
| 5 | " | - | Water | 51 |
| 6 | " | 7.0 | NaOAc | 60 |
| 7 | 100 | " | KPi | 80 |

[a] Reaction conditions: Final volume 0.3 mL, 33 μ L catalase (3.3 mg/mL), 70 μ L HRP (1.0 mg/mL), 37 $^{\circ}$ C, 103 μ L GOaseM_{3,5} (3mg/mL), 1 hr. Conversion calculated by RP-HPLC and adjusted with a 1:1:1 standard of HMF:DFF:FFCA. [b] No HRP

Time course analysis (Figure 3) indicated that at lower PaoABC concentration (Table 2, entry 1) HMFA remained low at all times. At higher PaoABC concentrations (entries 2&3), increasing amounts of HMFA is formed as an intermediate (Cf Figures S3-S5), although with HRP present, complete conversion to FDCA is still observed over the same time period (3h.).

Table 2 Optimisation of PaoABC in the continuous oxidation of HMF to FDCA

| Entry | 1 (mM) | GOase (3 mg/ml) (μ L) | PaoABC (28.9 mg/ml) (μ L) | % FDCA 5 |
|-------|--------|----------------------------|--------------------------------|-----------|
| 1 | 50 | 103 | 1 | 100 |
| 2 | " | " | 2.5 | 100 |
| 3 | " | " | 5 | 100 |
| 4 | 100 | " | 2.5 | 55 (6hr) |
| 5 | 100 | 130 | 1 | 100 (6hr) |

Reaction conditions: Final volume 0.3 mL, 70 μ L HRP (1 mg/mL), 33 μ L catalase (3.3 mg/mL), 0.2 mM KPi pH 7.0, 37 $^{\circ}$ C, 3 hr, pH adjusted with 2M NaOH.

This supports our initial observation that in the presence of HMF and DFF, PaoABC will oxidise DFF preferentially. Upon increasing the substrate HMF concentration to 100 mM, incomplete conversion to FDCA (55%) was observed and HMFA 3 was present as 45% of the reaction mixture (Table 2, entry 4). Again, lowering the amount of PaoABC decreased the aldehyde group oxidation in 1, resulting in lower conversion to 3 and in 5 being obtained in 100% conversion (Table 2, entry 5).

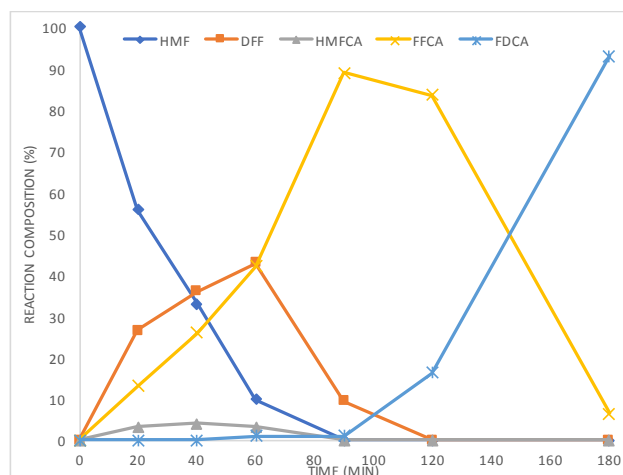


Figure 3 Time course analysis of the continuous oxidation of HMF to FDCA (Table 2, Entry 1) Reaction conditions: Final volume 0.3 mL, 33 μ L catalase (3.3 mg/mL), 70 μ L HRP (1.0 mg/mL), 103 μ L GOaseM_{3,5} (3.0 mg/mL), 1 μ L PaoABC (28.9 mg/mL) 0.2 M KPi pH 7.0, 50 mM HMF, 37 $^{\circ}$ C.

Immobilisation and use of PaoABC

A substantial effort has been devoted to developing effective immobilisation methods to increase the operational stability of enzymes and to facilitate their recovery and recyclability.²⁷

Combined-cross linked enzyme aggregates (combi-CLEAS) have emerged as a multi purpose, novel and versatile carrier free immobilised biocatalyst formulation for co-immobilisation of two or more enzymes, and are capable of combining multi-step cascade and non-cascade biotransformations into one catalytic pot.^{28,29} Combi-CLEAs have been used to co-immobilise glucose oxidase and catalase with great success.³⁰ We attempted the formation of a combi-CLEA with PaoABC and catalase but were unsuccessful (ESI 4) Although both enzymes retained activity after aggregation, cross-linking appeared to remove all activity. PaoABC contains many lysine residues around the active site³¹ and we believe that the bi-functional glutaraldehyde may cross-link these residues, altering the active site and leading to inactivity.

Classical immobilisation on solid supports is an alternative to CLEA formation due to the many commercially available resins available.³⁰ Co-immobilisation of PaoABC with catalase on Eupergit-CM showed good conversion after one cycle. However, enzymatic deactivation was seen in subsequent cycles (ESI, Table S8). We identified that catalase was denaturing on the resin since the reaction could be rescued by

COMMUNICATION

Journal Name

adding an additional portion of catalase in subsequent reactions. We then created a CLEA of catalase (CAT-CLEA)³⁰ which resulted in this biocatalyst being able to be recycled 20 times without any loss in conversion in the test reaction (Figure S9). With this modified procedure, the PaoABC immobilized on Eupergit-CM in combination with the CAT-CLEA could be recycled 5 times (Figure S8). However, the activity of Eupergit-CM immobilised PaoABC was low when compared with soluble PaoABC (Figure 4). This poor activity may result from reaction of surface epoxy groups on the resin with lysine residues near the enzyme active site or from interference with the enzyme's quaternary structure.³¹

Physical entrapment of cells/isolated enzymes in polymer networks or organic/inorganic materials has been widely used in industrial applications.^{32,33} Proteins have been stabilised by sol-gel encapsulation in hydrated silicas, including flavoprotein oxidases.³⁴ These enzymes have been co-immobilised in sol-gels with counter-charged polymers that form electrostatic adducts with the enzyme (based on their isoelectric point) to aid stabilization during the sol process.

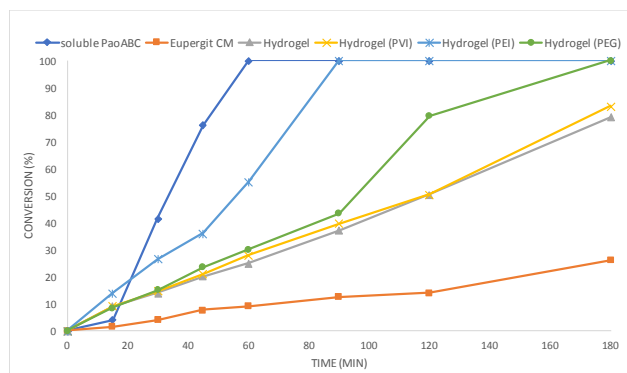


Figure 4. Comparison of immobilised forms of PaoABC for oxidation of DFF 2 to FDCA 5. Reactions include catalase-CLEA (ESI 4.2.4, 4.4.2). Reaction conditions: Final volume 0.3 mL, 5 mg catalase-CLEA, 0.065 mg soluble PaoABC or 50 mg PaoABC immobilised hydrogel, 0.1 mM KPi pH 7.0, 0.2M DFF, 37°C, pH adjusted with saturated solution of bicarbonate.

PaoABC was co-encapsulated in a tetramethyl orthosilicate (TMOS) based sol-gel with stabilisers polyethylene imine (PEI), polyvinyl imidazole (PVI) or polyethylene glycol and these catalysts tested in conjunction with CAT-CLEA for oxidation of 50 mM DFF 2 to determine the activity of the hydrogel-encapsulated enzyme (Figure 4, Figures S10-13). Co-encapsulating PaoABC with PVI made little difference to the activity of the catalyst compared with the hydrogel formulation without a stabiliser. PEG showed some stabilisation of the protein. However, the PEI-stabilised PaoABC solgel gave full conversion to 5 after 90 minutes which is almost comparable to that of the soluble PaoABC (Figure 4). The isoelectric points of PaoABC subunits are 5.5, 9.0 and 5.9 respectively³⁵ and so more basic PEI (polycationic at neutral pH) effectively binds to the polyanionic Pao A and C subunits. This can be seen by a precipitation of the enzyme on addition of PEI to soluble enzyme. PVI on the other hand, is less basic

and presumably insufficient electrostatic interactions occur with the enzyme.

We next looked at the formulation of PaoABC (Figure 5, S14-16) with catalase and to determine the recyclability of the immobilised enzyme. Enzyme immobilisation often leads to increased stability of enzymes to denaturing conditions so we first tested to determine if PaoABC-gel could withstand reaction conditions (H₂O₂) without addition of catalase. This severely decreased the reaction rate (Figure 5, blue bars). When soluble catalase was co-entrapped with PaoABC (grey bars), the formulation could be recycled three times, maintaining conversion above 70%. Interestingly the second recycle (91%) gave better conversion than the first (78%). This could be due to swelling of the gel in the buffer which allows better mass transfer of the substrate into the catalyst. We have shown that soluble catalase is unstable under the reaction conditions and so we opted to test the highly stable CAT-CLEA encapsulated within the hydrogel formulation. However, this proved detrimental to the activity of the biocatalyst (Figure 5, yellow bars). However, when the CAT-CLEA was combined with the already encapsulated PaoABC, the biocatalysts could be recycled up to 14 times without loss in yield (Figure 5, orange bars). Subsequent recycles resulted in lower conversion to 5.

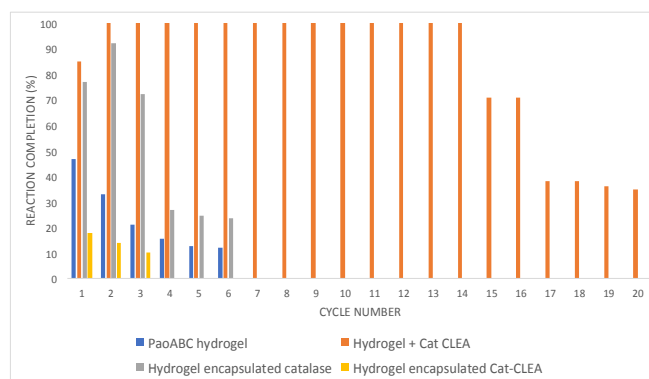
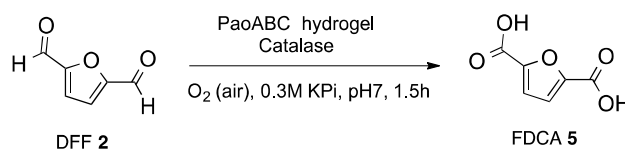


Figure 5. Recycling of different formulations of PaoABC-gel. Blue – PaoABC hydrogel with no catalase; Orange – PaoABC hydrogel with separate catalase-CLEA; Grey – PaoABC hydrogel co-encapsulated with catalase; Yellow – PaoABC hydrogel co-encapsulated with pre-made catalase-CLEA; 300 μ L of 0.3 M KPi pH 7.0, hydrogel (50 mg containing 0.065 mg PaoABC), 50 mM DFF, catalase (CLEA), 5 mg; reaction time 90 min.

PaoABC-Gel/CAT-CLEA was able to tolerate substrate concentrations up to 200 mM (Figure S24). Soluble PaoABC actually performed better at this higher substrate level (Figure S23) which may be a result of insufficient mass transfer with

the heterogeneous biocatalyst on a small scale. We also identified the optimum temperature (ESI 4.4.6) for the oxidation of DFF **2** as 35°C, although this and higher temperatures resulted in lower TOF when using the hydrogel (Table 3), which could be a result of hydrolysis of the hydrogel. Nevertheless, the PaoABC hydrogel did show additional thermostability at 45°C compared to the soluble enzyme (Figures S29, S30). The turnover numbers (TON) and frequencies (TOF) achieved with the biocatalyst are shown in Table 3.

Table 3 TON and TOF of PaoABC solgel and soluble PaoABC.

| Catalyst | Temperature (°C) | TON [mol mol ⁻¹] | TOF [hr ⁻¹] | TOF [s ⁻¹] |
|----------|------------------|------------------------------|-------------------------|------------------------|
| Soluble | 25 | 62305 | 8900 | 2.4 |
| Hydrogel | 25 | " | 7788 | 2.1 |
| Soluble | 35 | " | 20768 | 5.76 |
| Hydrogel | 35 | " | 10384 | 2.88 |

TON = moles of product divided by moles of catalyst used [b] TOF = TON divided by reaction time

Conclusions

The ability to run cascades of enzyme bioconversions in a single reaction process will be a major driving force in future exploitation of enzymes in industrial biocatalysis. Until now, efforts to use isolated enzymes either as single biocatalysts (HMFO) or as combinations for HMF to FDCA conversion have often faltered at the aldehyde acid stage (FFCA), because the alcohol oxidases used require the hydrate form of the substrate which is disfavoured in the aldehyde-hydrate equilibrium. We have used *E. coli* periplasmic oxidase (PaoABC) to overcome this issue since it can directly oxidise the aldehyde group of FFCA and does not require the hydrate. In addition we have shown that through incorporation of horse radish peroxidase, galactose oxidase M_{3,5} can be used in combination for the one-pot continuous bioconversion of HMF to FDCA to give full conversion at a HMF concentration of 100mM. In the presence of HRP, GOase M_{3,5} was sufficiently active to speed up oxidation of HMF to DFF (and HMFC **3** to FFCA prior to FDCA formation) and this can be balanced with lower amounts of PaoABC to limit the formation of HMFC **3** from HMF. PaoABC could be entrapped as a solgel and re-used 14 times without loss of activity, in conjunction with catalase CLEA for DFF **2** to FDCA **5** conversion at 200mM substrate concentration. We have previously shown that FDCA can be precipitated by acidification of the reaction media following removal of the enzymes.²⁴ This augurs well for combination with immobilised GOaseM_{3,5} and HRP for scale up of the HMF to FDCA bioconversion and also for product isolation.

Acknowledgements

The research leading to these results received support from the Biotechnology and Biological Sciences Research Council and (BBSRC BB/M028631/1 to SMM and AJC), Innovate UK (Biome Bioplastics), The Royal Society Wolfson Merit Award (to NJT).

Notes and references

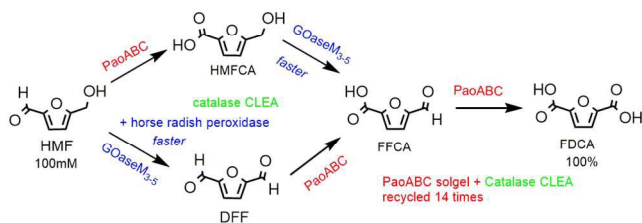
- 1 A. Corma, S. Iborra, A. Velty, *Chem. Rev.* **2007**, *107*, 2411–2502.
- 2 C. Zhou, X. Xia, C. Lin, D. Tonga and J. Beltramini *Chem. Soc. Rev.*, **2011**, *40*, 5588–5617.
- 3 L. Hu, G. Zhao, W. Hao, X. Tang, Y. Sun, L. Lin and S. Liub *RSC Adv.*, **2012**, *2*, 11184–11206.
- 4 (a) A. Boisen, T. B. Christensen, W. Fu, Y. Y. Gorbanev, T. S. Hansen, J. S. Jensen, S. K. Klitgaard, S. Pedersen, A. Riisager, T. Ståhlberg and J. M. Woodley, *Chemical Engineering Research and Design*, **2009**, *87*, 1318–1327.
- 5 N. Jacquet, R. Saint-Loup, J. Pascault, A. Rousseau and F. Fenouillot, *Polymer* **2015**, *59*, 234–242.
- 6 C. H. R. Wilsens, N. J. M. Wullems, E. Gubbels, T. F. Yao, S. Rastogi, B. A. Noordover, *Polym. Chem.* **2015**, *6*, 2707–2716
- 7 <http://www.smitherspira.com/news/2014/april/demand-for-pet-packaging-material-in-2019>
- 8 <https://www.basf.com/en/company/news-and-media/news-releases/2016/10/p-16-322.html>.
- 9 C. M. Cai, N. Nagane, R. Kumara and C. E. Wyman, *Green Chem.*, **2014**, *16*, 3819–3829.
- 10 B. Seemala, V. Haritos, and A. Tanksale, *ChemCatChem*, **2016**, *8*, 640–647.
- 11 P. Bhaumik and P. L. Dhepe, *Catalysis Reviews*, **2016**, *58*, 36–112.
- 12 (a) J. Zhang, J. Li, Y. Tang, L. Lin, M. Long, *Carbohydr. Polym.* **2015**, *130*, 420–428; (b) Z. Zheng, K. Deng, *ACS Catalysis*, **2015**, *5*, 6529–6544.
- 13 L. Prati, A. Villa, M. Schiavoni, S. Campisi and G. M. Veith, *ChemSusChem*, **2013**, *6*, 609–612.
- 14 N. K. Gupta, S. Nishimura, A. Takagaki and K. Ebitani, *Green Chem.*, **2011**, *4*, 824.
- 15 S. E. Davis, B. N. Zope and R. J. Davis, *Green Chem.*, **2012**, *1*, 143.
- 16 X. Zuo, P. Venkatasubramanian, D. H. Busch and B. Subramanian, *ACS Sustainable Chem. Eng.*, **2016**, *4*, 3659–3668.
- 17 X. Han, C. Li, X. Liu, Q. Xia, Y. Wang, *Green Chem.*, **2017**, *19*, 996–1004.
- 18 B. M. Nestl, S. C. Hammer, B. A. Nebel and B. Hauer, *Angew. Chem., Int. Ed.*, **2014**, *53*, 3070–3095.
- 19 M. van Deurzan, F. van Rantwijk and R. Sheldon, *J. Carbohydr. Chem.*, **1997**, *16*, 299–309.
- 20 F. Koopman, N. Wierckx, J. H. de Winde and H. J. Ruijsseenaars, *Bioresour. Technol.*, **2010**, *101*, 6291–6296.
- 21 (a) W. P. Dijkman, D. E. Groothuis, and M. W. Fraaije, *Angew. Chem., Int. Ed.*, **2014**, *53*, 1–5; (b) W. P. Dijkman, C. Binda, M. W. Fraaije, A. Mattevi, *ACS Catalysis*, **2015**, *5*, 1833–1839.
- 22 J. Carro, P. Ferreira, L. Rodríguez, A. Prieto, A. Serrano, B. Balcells, A. Ardá, J. Jiménez-Barbero, A. Gutiérrez, R. Ullrich, M. Hofrichter A. T. Martínez, *FEBS*, **2015**, *16*, 3218.
- 23 Y. Qin, Y. Li, M. Zong, H. Wu and N. Li *Green Chem.*, **2015**, *17*, 3718–3722.
- 24 S. M. McKenna, S. Leimkühler, S. Herter, N. J. Turner and A. J. Carnell, *Green Chem.*, **2015**, *17*, 3271–3275.

COMMUNICATION

Journal Name

25. K. Parikka and M. Tenkanen *Carbohydr. Res.*, **2009**, 344, 14-20.
26. A. T Pedersen, W. R. Birmingham, G. Rehn, S J. Charnock, N. J. Turner, and J. M. Woodley *Org. Process Res. Dev.*, **2015**, 19, 1580–1589.
27. L. Cao, L. van Langen and R.M. Sheldon, *Curr. Opinion in Biotechnol*, **2003**, 14, 387-394.
28. C. Ning, E. Su, Y. Tian and D. Wei *Journal of Biotechnology* **2014**, 184, 7–10.
29. S. Dalal, M. Kapoor and M. N. Gupta, *Journal of Molecular Catalysis B: Enzymatic*, **2007**, 44, 128–132.
30. Ö. Alptekin, S. S. Tükel, D. Yıldırım and D. Alagöz, *Journal of Molecular Catalysis B: Enzymatic*, **2010**, 64 177-183.
31. M. A. S. Correia, A.R. Otrelo-Cardoso, V. Schwuchow, K. G. V. Sigfridsson Claus, M. Haumann, M. J. Romao, S. Leimkuhler, T. Santos- Silva, *ACS Chemical Biology*, **2016**, 11, 2923-2935.
32. W. Tischer W and V. Kasche V, *Biotechnol* **1999**, 17, 326-335.
33. S. Cantone, V. Ferrario, L. Corici, C. Ebert, D. Fattor, P. Spizzoa and L. Gardossi, *Chem. Soc. Rev.*, **2013**, 42, 6262-6276.
34. Q. Chen, G. L. Kenausis, and A. Heller, *J. Am. Chem. Soc.* **1998**, 120, 4582-4585.
35. A. Badalyan, M. Dierich, K. Stiba, V. Schwuchow, S. Leimkühler and U. Wollenberger *Biosensors*, **2014**, 4, 403-421.

One-pot multienzyme continuous cascade for conversion of HMF to FDCA using HRP to activate GOaseM_{3,5} *in situ*.



Chem Draw 3

124x160mm (300 x 300 DPI)