UNIVERSITY OF LEEDS

This is a repository copy of *Highly Productive Oxidative Biocatalysis in Continuous Flow by Enhancing the Aqueous Equilibrium Solubility of Oxygen*.

White Rose Research Online URL for this paper:
http://eprints.whiterose.ac.uk/153125/

Version: Accepted Version

Article:

Chapman, MR, Cosgrove, SC, Turner, NJ et al. (2 more authors) (2018) Highly Productive Oxidative Biocatalysis in Continuous Flow by Enhancing the Aqueous Equilibrium Solubility of Oxygen. Angewandte Chemie, 130 (33). pp. 10695-10699. ISSN 0044-8249

https://doi.org/10.1002/ange.201803675

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

White Rose
university consortium
Universities of Leeds, Sheffield & York

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

Highly Productive Oxidative Biocatalysis in Continuous-Flow by Surpassing the Aqueous Equilibrium Solubility of Oxygen

Michael R. Chapman,[a] Sebastian C. Cosgrove,[c] Nicholas J. Turner,*[c] Nikil Kapur*[b] and A. John Blacker*[a]

Abstract: We report a simple, mild and synthetically clean approach to accelerate the rate of enzymatic oxidation reactions by up to a factor of 100 when compared to conventional batch gas/liquid systems. Biocatalytic decomposition of H_2O_2 is used to produce a soluble source of O_2 directly in reaction media, enabling the ambient solubility of aqueous O_2 to be increased under safe and practical conditions. To best exploit the method, a novel flow reactor was developed to maximize productivity (g product/L/h). This scalable benchtop protocol provides a distinct advantage over conventional bio-oxidation, in that no pressurized gas or specialist equipment is employed. The method is general across different oxidase enzymes and compatible with a variety of functional groups. These results culminate in record space-time-yields for bio-oxidation.

Oxidation reactions represent the second largest process after polymerization in the bulk chemical industry, contributing ~ 30% of total production.[1,2] The pharmaceutical and fine chemical industries generally conduct oxidation steps using metal catalysts, organoperoxides, sulfoxides or amine oxides.[3–5] However, these methods are often accompanied by harsh reaction conditions, poor chemical selectivity and generate significant quantities of waste.[6,7] In fact, a recent publication by the Green Chemistry Institute (GCI), alongside six global pharmaceutical corporations, highlighted oxidation as a reaction type which “companies employ but would strongly prefer better reagents” – ranking 3rd of 7 priority areas.[8] One option to address this problem involves use of oxidative enzymes as natural biocatalysts, which operate under mild, aqueous process conditions and, in principle, produce less waste or product contamination than their chemocatalytic counterparts.[9] The use of water as solvent also allows safer handling of air or pure O_2 as an oxidant when compared with organic solvents, and equally fits well within the remit of “green chemistry”.[10] However, O_2 is poorly soluble in water (~ 8 mgL^{-1} from air), representing a fundamental bottleneck for large scale implementation of enzymatic oxidation. This low driving force for $O_{2(g)} \rightarrow O_{2(aq)}$ results in low biocatalytic turnover rates and places an upper limit on volumetric productivity, as governed by the mass transfer capability of selected equipment (e.g. an industrial scale bioreactor has a maximum k_La ~ 500 h^{-1} for O_2 transfer).[11]

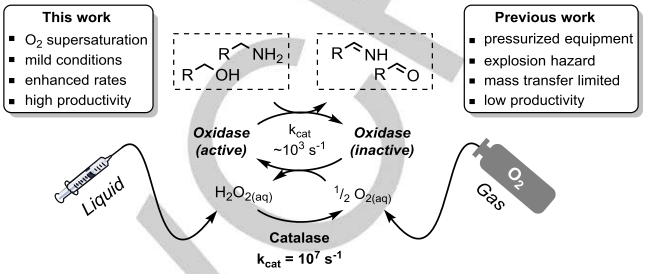


Figure 1. Generating soluble O_2 in situ to overcome mass transfer restrictions.

An attractive approach to both improve mass transfer and productivity is to translate bio-oxidation into continuous-flow mode, whereby the interfacial surface-to-volume ratio may be maximized, whilst overall reaction time may be minimized. This was well-demonstrated by Kragl and Löb, who developed a continuous falling-film microreactor (FFMR) which reaches an O_2 saturation of 8.9 mgL^{-1} in < 6 s, equating to a k_La value of ~ 20590 h^{-1} (170 $gO_2L^{-1}h^{-1}$) – 40 × faster than observed in batch mode.[12] When applied to the O_2-dependent galactose oxidase (GOase) catalyzed oxidation of glucose to gluconic acid, space-time-yield (STY) was increased 300-fold in the FFMR compared with an equivalent bubble column bioreactor. More recently, Woodley has employed an agitated cell reactor (ACR) for the same transformation.[13] The ACR was shown to double overall reaction rate solely due to improved O_2 mass transfer rates. Likewise, Gasparini and Jones have independently shown the continuous ACR model to facilitate the rate of biocatalytic oxidation reactions,[14,15] whilst Ludwig and Berger have separately developed aerated-membrane flow reactors for the same purpose.[16,17] Sadowski has also shown that co-solvents can be used in biological media to increase the rate of enzyme catalyzed reactions.[18,19] Despite these recent contributions, no whole-cell or enzyme flow oxidation process has been demonstrated for commercial chemical manufacture. In every example described, the enzyme underperforms due to O_2 deprivation.

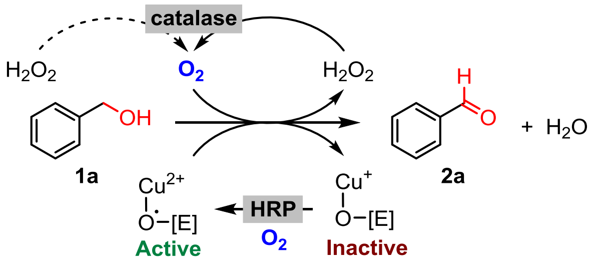
We envisioned that a different mode of access to soluble O_2 is necessary which does not rely on interfacial mass transfer. Bowers and Hofstetter have previously shown that the iodide-catalyzed decomposition of aqueous H_2O_2 results in a maximum O_2 concentration of 0.12M, or ~100 times the equilibrium solubility.[20] We hypothesized that prior to O_2 evaporation, there is an opportunity for an oxidative biocatalyst to function under 1 bar external pressure yet akin to conditions of a solution saturated under 100 bar pressure. In doing so, the rate of bio-oxidation may be increased significantly under mild, safe and general conditions, avoiding specialist pressurized equipment (Figure 1). By accelerating biocatalysis in this manner, it becomes feasible to consider new continuous-flow routes for large-scale oxidation which would be inoperable in batch mode.

---

[a] Dr M. R. Chapman, Prof A. J. Blacker
School of Chemistry and School of Chemical and Process Engineering, Institute of Process Research and Development, University of Leeds, Woodhouse Lane, Leeds, LS2 9JT, UK
E-mail: j.blacker@leeds.ac.uk
[b] Prof N. Kapur
School of Mechanical Engineering, University of Leeds
Woodhouse Lane, Leeds, LS2 9JT, UK
E-mail: n.kapur@leeds.ac.uk
[c] Dr S. C. Cosgrove, Prof N. J. Turner
Manchester Institute of Biotechnology
131 Princess Street, Manchester, M1 7DN, UK

Supporting information for this article is given via a link at the end of the document.

For the purpose of our study, catalase was selected as an enzyme capable of catalyzing the decomposition of H_2O_2 at near-diffusive rate (k_{cat} ~ 10^7 s^{-1}), for in situ generation of O_2 equivalents.[21] The irreversible oxidation of benzyl alcohol to benzaldehyde via GOase $M_{3\text{-}5}$, a mutant capable of oxidizing benzylic alcohols, was chosen as benchmark reaction for study.[22] This choice was deliberate as benzyl alcohol represents a volatile, poorly soluble and non-natural substrate for the enzyme, which under traditional aerated conditions is notoriously slow to oxidize (for time-point experiments, see ESI). The catalyst was deployed in the form of the cell-free extract (CFE) throughout the investigation, to maximize industrial relevance; consequently, a Cu salt was also added to load the metal into the apoenzyme. Horseradish peroxidase (HRP), a well-known GOase activator enzyme, was used to maximize catalyst activity (Scheme 1). [23,24]



Scheme 1. Model bio-oxidation reaction for study. [E] = enzyme.

It is noteworthy that fed-batch addition of H_2O_2 to the biocatalytic reaction mixture led to a rapid increase in $[O_2]$ (up to 40 mg.L^{-1}). Under this condition, the rate of production of **2a** was at its highest (see ESI, Figure S7), though challenging to maintain in fed-batch mode as all concentrations were diluted over time. Using a miniaturized cascade CSTR recently developed in our laboratory,[25] we began optimizing the proposed biocatalytic cascade reaction with emphasis on the development of a scalable continuous-flow protocol. An aqueous solution of GOase, HRP, catalase and $CuSO_4$ was flowed into the CSTR to meet a solution of **1a** (30 mM) and H_2O_2 – initiating production of both O_2 and **2a** (Scheme 1, Table 1). Due to gas liberation, a back-pressure regulator (40 psi) was used to balance flow-rate and an antifoam agent employed to prevent blockage (0.01% $^{w}/_{w}$).

It is important to first note that despite a complex oxidation mixture, omission of any component led to a reduction in reaction rate, with no conversion when the GOase CFE was absent. Initial optimization involved employment of 0.5 mg.mL^{-1} oxidase CFE (1.9 U.mL^{-1}), keeping all other enzyme concentrations fixed. Equal flow-rate of a substrate stream containing 2 and 4 equivalents H_2O_2 gave 5 and 9% conversion after 11 minutes residence time (τ_{res}), respectively (entries 1 and 2). Increasing catalyst loading 13-fold allowed 40% conversion of **1a** under identical conditions (entry 3). At this point, supplying more H_2O_2 (8 equiv.) or doubling τ_{res} (22 min) did not show a notable improvement in conversion (43%, entry 4). Based on these observations, it was deemed likely that much of the oxidation is occurring at the initial mixing zone of the reactor (i.e. injection point), prior to O_2 evaporation.

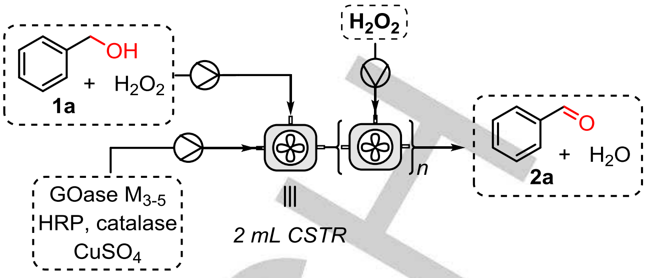


Table 1. Optimization of GOase-catalyzed bio-oxidation in continuous-flow.

Entry	GOase CFE (mg.mL^{-1})[a]	$[H_2O_2]$[b] (# feeds)	CSTRs (n)	τ_{res} (min)	Conv. (%)[c]/ STY (g.$L^{-1}d^{-1}$)
1	0.5	60 (1)	1	11	5
2	0.5	120 (1)	1	11	9
3	6.5	120 (1)	1	11	40
4	6.5	240 (1)	1	22	43
5	6.5	120 (2)	2	11	64
6	6.5	300 (3)	3	13	84
7	6.5	300 (4)	4	26	95/41
8[d]	15	300 (4)	4	13	92/168
9	6.5	0(0)	4	26	18
10	0	300 (4)	4	26	0

[a] Specific activity of CFE = 3.8 U.mg^{-1}. [b] Total H_2O_2 concentration (mM) relative to **1a**. [c] Determined by HPLC analysis at steady-state. [d] [**1a**] = 60 mM. τ_{res} = residence time, conv. = conversion.

By cascading a second CSTR in series, a sequential H_2O_2 feed was added to the geometry. Delivering 4 equivalents H_2O_2 over 2 feeds, 64% conversion of alcohol was observed in equivalent τ_{res} (entry 5). Extending τ_{res} to 26 minutes, a 10-fold excess of H_2O_2 was supplied across 4 sequential reactor inlets, affording **2a** in 95% conversion (41 g.L^{-1}.d^{-1}, entry 7). By increasing catalyst loading further, a 60 mM substrate feed was converted to 92% in half the τ_{res} (entry 8), demonstrating a space-time-yield of 168 g.L^{-1}.d^{-1} (for further optimization details, see ESI).

From our optimization study, we recognized that generating soluble O_2 at multiple positions across the length of the reactor is essential for rapid oxidation of substrate. To do so more efficiently, a multi-point injection flow reactor (MPIR) was designed and constructed, capable of sequentially dosing H_2O_2 across the full reactor manifold (Figure 2). Our original model consisted of a winding PTFE flow channel (660 × 2 × 2 mm) positioned between two Perspex blocks (100 × 100 × 20 mm). Eleven subchannels (8 × 0.1 × 2 mm each) are connected between the master channel and two distributor channels milled directly into the front-face of the reactor. Each distributor channel acts as a reservoir for H_2O_2, allowing fresh peroxide to be injected through multiple points of the main reaction channel, maximizing the availability of soluble O_2 for the biocatalyst as a function of reactor position (for computational fluid dynamic analysis, see ESI).

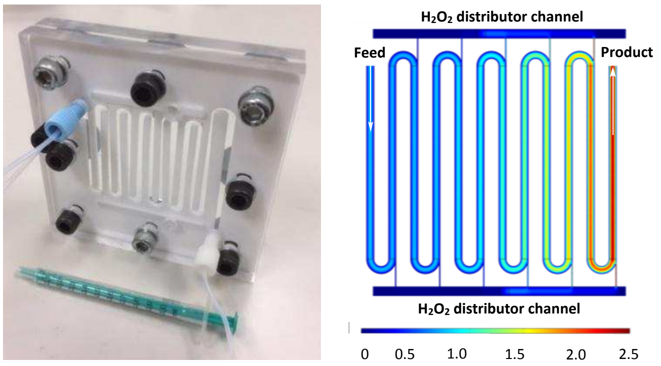
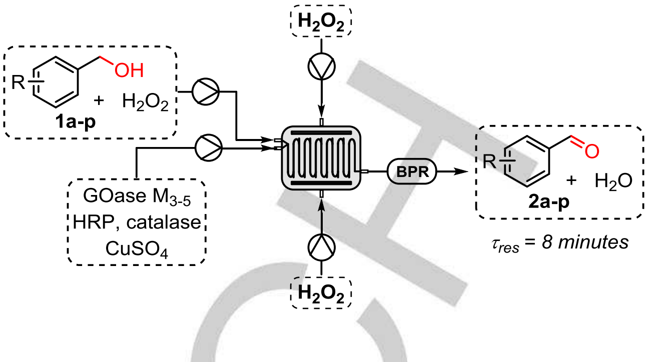


Figure 2. Left: multi-point injection flow reactor (MPIR), 1 mL syringe for scale. Right: flow velocities through channel (mm/s), generating O_2 at 11 addition ports.

The total volume of the reactor is 2.6 mL, with a pressure capacity of 5.2 bar. To our knowledge, no flow reactor of this type has been reported before.

Experimental evaluation of the MPIR showed significant improvement in performance over the CSTR, as demonstrated by quantitative conversion of **1a** (30 mM) in a τ_{res} of 8 minutes (Table S7, entry 3). Under these unoptimized conditions, reducing [H_2O_2] 3-fold showed no significant loss in conversion (97%, entry 7). Alternatively, a 3-fold increase in [**1a**] (90 mM) allowed 95% conversion in a τ_{res} of 12 minutes (entry 14). Following this productivity study, [**1a**] was increased 5-fold (150 mM) and oxidized to 90% conversion (τ_{res} = 15 min), affording a space-time-yield of 344 g.L^{-1}.d^{-1} which is twice the maximum theoretical value calculated by Woodley using a mass transfer coefficient for this system.[11] It is of note however, that the CFE loading required for this level of reactivity leads to increased viscosity of enzyme feed, which may become problematic in flow mode. With this in mind, and to maintain a practical biocatalyst yield (g product/g enzyme), the conditions of entry 7 (Table S7) were carried forward for further investigations.

A reaction scope survey indicated that the method is general across a range of electronically differentiated aryl alcohols. Literature precedent suggests that electron-poor benzyl alcohols are necessary for complete oxidation, whilst electron-rich versions rarely reach > 50% conversion.[26] Directly translating the optimized flow conditions towards various 4-substituted benzyl alcohols (**1b – 1f**, **1i**, **1k**), we observed ≥ 88% conversion for all examples, with no apparent electronic requirement on the aromatic ring with respect to rate of oxidation. Supporting this, doubly activated aryl **1l** was oxidized under the same conditions to give aldehyde **2l** in excellent yield. A broad range of functional groups were well tolerated, highlighting the exceptionally mild reaction conditions. Aromatic halides **1g – 1j** underwent clean oxidation to their aldehydes, showing no signs of dehalogenation to represent an advantage over metal-catalyzed routes. Furthermore, the method was extended towards N- and S-heteroaryl alcohols **1n** and **1o**, producing carboxaldehydes **2n** and **2o** in good yield. To exemplify industrial relevance, 5-hydroxymethylfurfural (**1p**) was converted to 2,5-diformylfuran (**2p**), a key platform chemical in the polymer and pharmaceutical industries, in 86% at steady-state with full selectivity.



Scheme 2. Flow process diagram for bio-oxidation using MPIR. BPR = 40 psi.

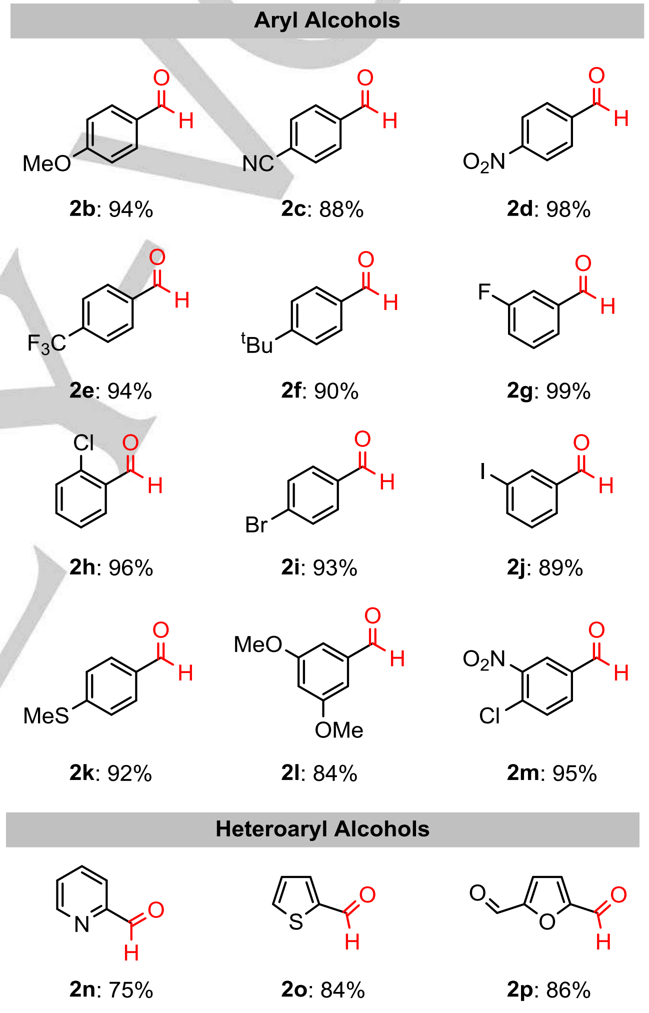


Figure 3. Aldehydes produced from substrate scope investigations. General conditions are as follows. Enzyme feed: GOase M_{3-5} (6.5 mgmL^{-1} CFE), HRP (0.1 mgmL^{-1}), catalase (0.13 mgmL^{-1}), $CuSO_4$ (0.13 mgmL^{-1}). Substrate feed: alcohol substrate (30 mM), H_2O_2 (1 equiv.), antifoam 204 (0.01 wt. %). Peroxide injection feeds (linked to the same pot): H_2O_2 (2 equiv.), antifoam 204 (0.01 wt. %). All solutions are made up of aqueous NaPi buffer at pH 7.4 at rt.

This symbolizes a productivity of ~ 144 g.L^{-1}.d^{-1}, which is > 5-fold greater than the optimized preparative batch version.[27] Importantly, over-oxidation to the acid was not detected in any case.

With a reliable flow protocol in hand, we turned our attention toward other oxygen dependent biocatalysts. Monoamine oxidases (MAO-N from Aspergillus niger) are emerging as a popular class of biocatalyst for the deracemization of racemic amines, via enantioselective amine oxidation to produce achiral imine intermediates. These intermediates may then be intercepted by a source of nucleophile,[28] or non-selectively reduced back to the racemate for further rounds of enantiopurification.[29-31] However, all preparative scale employment of these biocatalysts involve reaction times in the order of days due to slow oxidation of the covalently-bound FAD cofactor, which is essential to the catalytic cycle.

We questioned whether our flow method could be useful in accelerating the rate of oxidation of cyclic amines, a common substructure to many bioactive molecules. As test candidate, tetrahydroisoquinoline **3a** (THIQ) was chosen to be oxidized to the corresponding dihydroisoquinoline (DHIQ), **4a**, via the D9 mutant of MAO-N. Administering the enzyme in the form of whole cells in the MPIR, 23% conversion to DHIQ was observed in τ_{res} = 8 minutes (Table 2, entry 1). It was recognized that diffusion of substrate/product into/out of the cells may impede the overall rate of oxidation. Consequently, glass beads (2 mm dia.) were packed into the flow channel, reducing the reactor volume by 1 mL to facilitate mixing (see ESI). With these in place, conversion to DHIQ doubled under otherwise uniform conditions (49%, entry 3). Optimum conditions were identified maintaining 3 equivalents H_2O_2 and τ_{res} = 12 minutes, producing DHIQ in 97% conversion at steady-state (entry 7), with a complete mass balance.

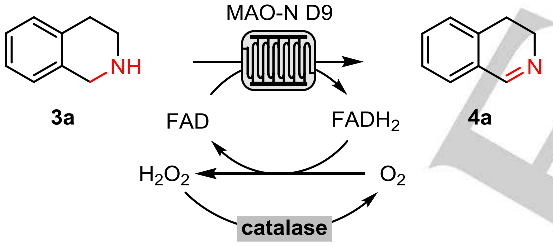


Table 2. Benchmark MAO-catalyzed bio-oxidation in continuous-flow for study.

Entry	MAO-D9 (mgmL^{-1})[a]	[H_2O_2][b]	τ_{res} (min)	Conv.[c] (%)
1	20	30	8	23
2	20	60	8	38
3[d]	20	30	8	49
4[d]	20	60	8	61
5[d]	40	30	8	83
6[d]	60	30	8	94
7[d]	80	30	12	97
8[d]	80	0	12	19

[a] Used in the form of whole cells. [b] Total H_2O_2 concentration (mM) relative to **3a** (10 mM input concentration). [c] Determined by HPLC analysis at steady-state. [d] Glass beads employed. τ_{res} = residence time, conv. = conversion.

In conclusion, we have exploited a biocatalytic cascade reaction which utilizes catalase-mediated decomposition of H_2O_2 to increase the natural aqueous equilibrium solubility of O_2 under ambient conditions. Whilst under this regime, we have shown the rate of enzymatic oxidation reactions to be enhanced significantly without the requirement of pressurized gas. To capitalize on this, a novel multi-point injection flow reactor was constructed which enables in situ generation of O_2 across the entire reaction space. Using this reactor geometry, various alcohols and amines have been oxidized to produce a range of aldehydes and imines, respectively, in excellent yield with unprecedented productivities. This methodology could be expanded to many O_2-dependent enzymatic reactions, thus unlocking continuous-flow bio-oxidation more generally.

Acknowledgements

We would like to thank the ACS GCI Pharmaceutical Roundtable for Green Chemistry Award (M.R.C., N.K., N.J.T., A.J.B.). N.K. thanks GSK and RAEng for funding his research chair. The authors would also like to thank Prozomix Ltd. for their generous donation of biocatalysts.

Keywords: biocatalysis • oxidation • flow reactor • continuous-flow • space-time-yield

[1] A. M. Thayer, Chem. Eng. News **1992**, 70, 27–49.

[2] (a) Z. Guo, B. Liu, Q. Zhang, W. Deng, Y. Wang, Y. Yang, H. Bengaard, B. Hammer, Z. Sljivancanin, M. Mavrikakis, Y. Xu, S. Dahl, C. J. H. Jacobsen, N. Nilius, H. J. Freund, H. Hagen, C. J. Kiely, G. J. Hutchings, Chem. Soc. Rev. **2014**, 43, 3480–3524; (b) W. Kroutil, H. Mang, K. Edegger, K. Faber, Adv. Synth. Catal. **2004**, 346, 125–142; (c) F. Hollmann, I. W. C. E. Arends, K. Bühler, A. Schallmey, B. Bühler, Green Chem. **2011**, 13, 226–228.

[3] G. P. Chiusoli, P. M. Maitlis in Metal-catalysis in industrial organic processes, Vol. 1 (Eds.: G. Paolo Chiusoli, P. M. Maitlis), RSC Publishing, Great Britain, **2008**.

[4] J. D. Blackmore, R. H. Crabtree, G. W. Brudvig, Chem. Rev. **2015**, 115, 12974–13005.

[5] Y. Sawama, M. Takubo, S. Mori, Y. Monguchi, H. Sajiki, Eur. J. Org. Chem. **2011**, 3361–3367.

[6] J. A. Labinger, J. Mol. Catal. A Chem. **2004**, 220, 27–35.

[7] B. L. Conley, W. J. Tenn, K. J. H. Young, S. K. Ganesh, S. K. Meier, V. R. Ziatdinov, O. Mironov, J. Oxgaard, J. Gonzales, W. A. Goddard, R. A. Periana, J. Mol. Catal. A Chem. **2006**, 251, 8–23.

[8] D. J. C. Constable, P. J. Dunn, J. D. Hayler, G. R. Humphrey, J. L. Leazer, Jr., R. J. Linderman, K. Lorenz, J. Manley, B. A. Pearlman, A. Wells, A. Zaks, T. Y. Zhang, P. J. Sheeran, C. E. Silverman, R. E. Waltermire, C. C. Wood, Green Chem. **2007**, 9, 411–420.

[9] J.-P. Mahy, J.-D. Maréchal, R. Ricoux, N. Doucet, A. Lledós, J. D. Marechal, A. Urvoas, J. P. Mahy, R. Ricoux, G. J. Davies, R. Mondiere, A. Taglieber, Chem. Commun. **2015**, 51, 2476–2494.

[10] C.-J. Li, B. M. Trost, Proc. Natl. Acad. Sci. U. S. A. **2008**, 105, 13197–13202.

[11] (a) A. Toftgaard Pedersen, W. R. Birmingham, G. Rehn, S. J. Charnock, N J. Turner, J. M. Woodley, Org. Process Res. Dev. **2015**, 19, 1580–1589; (b) R. H. Ringborg, A. Toftgaard Pedersen, J. M Woodley, ChemCatChem **2017**, 9, 3285–3288.

[12] S. Illner, C. Hofmann, P. Löb, U. Kragl, ChemCatChem **2014**, 6, 1748–1754.

[13] A. Toftgaard Pedersen, T. M. de Carvalho, E. Sutherland, G. Rehn, R. Ashe, J. M. Woodley, Biotechnol. Bioeng. **2017**, 114, 1222–1230.

[14] G. Gasparini, I. Archer, E. Jones and R. Ashe, Org. Process Res. Dev. **2012**, 16, 1013–1016.

[15] E. Jones, K. McClean, S. Housden, G. Gasparini, I. Archer, Chem. Eng. Res. Des. **2012**, 90, 726–731.

[16] W. Van Hecke, D. Haltrich, B. Frahm, H. Brod, J. Dewulf, H. Van Langenhove, R. Ludwig, Journal Mol. Catal. B, Enzym. **2010**, 68, 154–161.

[17] J. Onken, R. G. Berger, Appl. Microbiol. Biotechnol. **1999**, 51, 158–63.

[18] A. Wangler, R. Canales, C. Held, T. Q. Luong, R. Winter, D. H. Zaitsau, S. P. Verevkin, G. Sadowski, Phys. Chem. Chem. Phys. **2018**, 20, 11317-11326.

[19] C. Held, G. Sadowski, Annual Review of Chemical and Biomolecular Engineering, **2016**, 7, 395-414.

[20] P. G. Bowers, C. Hofstetter, C. R. Letter, R. T. Toomey, J. Phys. Chem. **1995**, 99, 9632–9637.

[21] J. H. Northrop, J. Gen. Physiol. **1925**, 7**,** 373–387.

[22] F. Escalettes, N. J. Turner, ChemBioChem **2008**, 9, 857–860.

[23] P. J. Kersten, Proc. Natl. Acad. Sci. USA **1990**, 87, 2936–2940.

[24] L. D. Kwiatkowski, D. J. Kosman, Biochem. Biophys. Res. Commun. **1973**, 53, 715–721.

[25] M. R. Chapman, M. H. Kwan, G. King, K. E. Jolley, M. Hussain, S. Hussain, I. E. Salama, C. González Nino, L. A. Thompson, M. E. Bayana, A. Clayton, B. N. Nguyen, N. J. Turner, N. Kapur, A. J. Blacker, Org. Process Res. Dev. **2017**, 21, 1294–1301.

[26] B. Bechi, S. Herter, S. McKenna, C. Riley, S. Leimkühler, N. J. Turner, A. J. Carnell, J. Clayden, F. Hollmann, N. J. Turner, Green Chem. **2014**, 16, 4524–4529.

[27] S. M. McKenna, S. Leimkühler, S. Herter, N. J. Turner, A. J. Carnell, D. P. Heuts, D. B. Janssen, M. W. Fraaije, A. T. Martinez, R. Ullrich, M. Hofrichter and A. T. Martínez, Green Chem. **2015**, 17, 3271–3275.

[28] V. Köhler, K. R. Bailey, A. Znabet, J. Raftery, M. Helliwell, N. J. Turner, Angew. Chemie Int. Ed. **2010**, 49, 2182–2184.

[29] J. H. Schrittwieser, B. Groenendaal, V. Resch, D. Ghislieri, S. Wallner, E.-M. Fischereder, E. Fuchs, B. Grischek, J. H. Sattler, P. Macheroux, N. J. Turner, W. Kroutil, Angew. Chem. Int. Ed. Engl. **2014**, 53, 3731–3734.

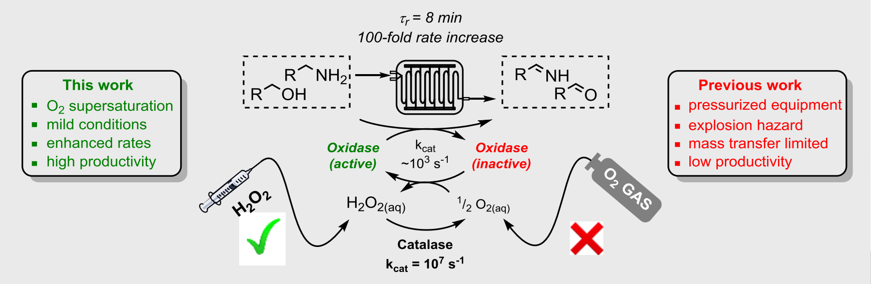
[30] D. Ghislieri, A. P. Green, M. Pontini, S. C. Willies, I. Rowles, A. Frank, G. Grogan, N. J. Turner, J. Am. Chem. Soc. **2013**, 135, 10863–10869.

[31] J. M. Foulkes, K. J. Malone, V. S. Coker, N. J. Turner, J. R. Lloyd, ACS Catal. **2011**, 1, 1589–1594.

COMMUNICATION

Layout 2:

COMMUNICATION


τ_r = 8 min
100-fold rate increase
This work
O2 supersaturation
mild conditions
enhanced rates
high productivity
R NH2
R OH
R NH
R O
Oxidase (active)
kcat ~10^3 s^-1
Oxidase (inactive)
H2O2(aq)
1/2 O2(aq)
Catalase
kcat = 10^7 s^-1
H2O2
O2 GAS
Previous work
pressurized equipment
explosion hazard
mass transfer limited
low productivity

Dr Michael R. Chapman, Dr Sebastian C. Cosgrove, Prof Nicholas J. Turner,* Prof Nikil Kapur,* Prof A. John Blacker*

Page No. – Page No.

Highly Productive Oxidative Biocatalysis in Continuous-Flow by Surpassing the Aqueous Equilibrium Solubility of Oxygen

Text for Table of Contents