

More efficient redox biocatalysis by utilising 1,4-butanediol as a 'smart cosubstrate'[†]Cite this: *Green Chem.*, 2013, **15**, 330Received 12th November 2012,
Accepted 19th December 2012

DOI: 10.1039/c2gc36797a

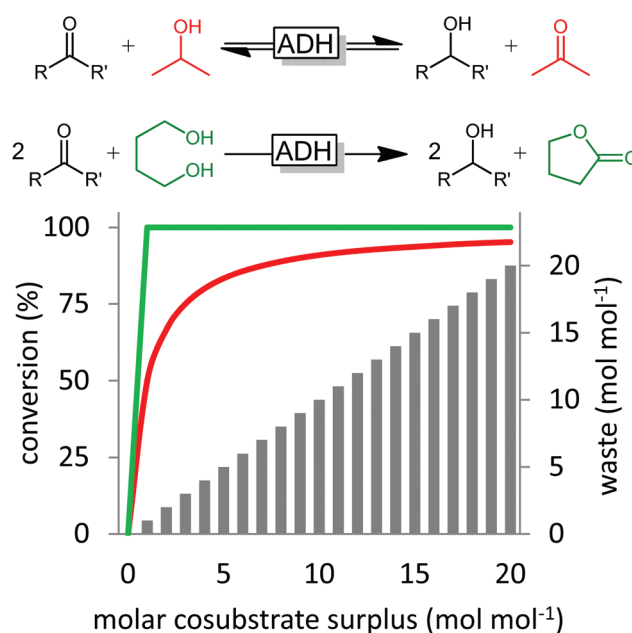
www.rsc.org/greenchem

Selin Kara,^a Dominik Spickermann,^b Joerg H. Schrittwieser,^a Christian Leggewie,^b Willem J. H. van Berkel,^c Isabel W. C. E. Arends^a and Frank Hollmann^{*a}

1,4-Butanediol is shown to be an efficient cosubstrate to promote NAD(P)H-dependent redox biocatalysis. The thermodynamically and kinetically inert lactone coproduct makes the regeneration reaction irreversible. Thereby not only the molar surplus of cosubstrate is dramatically reduced but also faster reaction rates are obtained.

Enzymes are amongst the catalysts of choice if selectivity is desired. This is particularly true for oxidoreductases catalysing preparatively important reactions ranging from reduction of C=O, C=C and other functional groups to specific oxyfunctionalisation reactions such as hydroxylation, epoxidation or Baeyer–Villiger reactions.¹ Most of these reactions depend on the supply with reducing equivalents, delivered to the enzymes through the nicotinamide cofactors (NAD(P)H). For economic and practical reasons, NAD(P)H has to be applied in catalytic amounts combined with a suitable *in situ* regeneration system.^{1,2} After more than 2 decades of intensive research³ this 'cofactor challenge' is generally considered to be solved. Amongst a variety of different regeneration systems, ADH-mediated oxidation of simple alcohols such as ethanol or isopropanol represents one of the most common NAD(P)H regeneration systems (Scheme 1). This approach is most elegant if the regenerating ADH is also the production enzyme mediating an (enantio)selective reduction reaction.⁴ This 'substrate coupled' approach represents a biocatalytic version of the well-known Meerwein–Ponndorf–Verley (MPV) reduction.⁵

However, as is common amongst all MPV reductions, the reversibility and poor thermodynamic driving force of the reaction necessitates (unless elaborate coproduct removal is



Scheme 1 Comparison of the 'classical' biocatalytic MPV-reduction e.g. using isopropanol and the proposed 'smart cosubstrate' approach using 1,4-butanediol. The lactone coproduct renders the regeneration reaction irreversible. The lower part shows the equilibrium conversions as calculated from the law of mass action (lines) and the waste generated (bars).

applied) significant molar surpluses of the cosubstrate. From an environmental point of view this is not desirable as the significant waste generated (Scheme 1) has to be dealt with.

Inspired by recent work of Lavandera *et al.*⁶ we hypothesised that thermodynamically stable coproducts may represent a facile way to shift the equilibrium of ADH-catalysed MPV reductions and thereby reduce the molar surplus of the cosubstrate used. In that respect α,ω-diols such as 1,4-butanediol (1,4-BD) appeared most promising. First, 1,4-BD can be oxidised twice thereby doubling the yield of reducing equivalents liberated from the cosubstrate and, secondly, the resulting γ-butyrolactone (GBL) represents a thermodynamically stable and kinetically inert coproduct (Scheme 1). Hence, the

^aDepartment of Biotechnology, Delft University of Technology, Julianalaan 136, 2628BL Delft, The Netherlands. E-mail: f.hollmann@tudelft.nl;

Fax: (+31) 152 781415; Tel: (+31) 152 781957

^bevocatol GmbH, Merowingerplatz 1A, 40225 Düsseldorf, Germany

^cLaboratory of Biochemistry, Wageningen University, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands

[†]Electronic supplementary information (ESI) available: Detailed description of the experimental and analytical procedures and further experimental data. See DOI: 10.1039/c2gc36797a

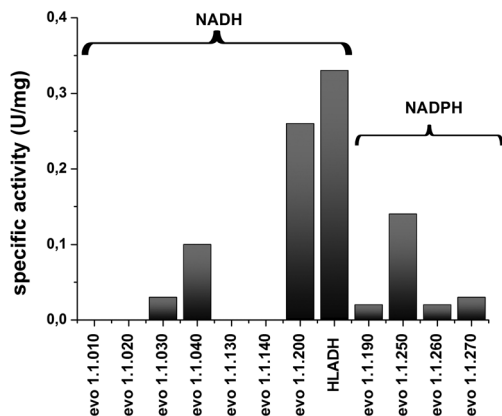


Fig. 1 Screening of ADHs for the oxidation of 1,4-BD. Reaction conditions: $c(1,4\text{-BD}) = 0\text{--}4200\text{ mM}$, $c(\text{NAD}^+) = 0.5\text{ mM}$, $c(\text{NADP}^+) = 0.4\text{ mM}$, $c(\text{ADH}) = 0.12\text{--}0.36\text{ g L}^{-1}$, buffer: Tris-HCl (50 mM, pH 7.0), $T = 30\text{ }^\circ\text{C}$.

waste generated for >95% conversion would be reduced approximately 40-fold (see ESI† for further details). Overall, a ‘smart cosubstrate’ approach solving the above-mentioned limitations was envisaged.

In a first set of experiments we screened a range of commercially available ADHs for activity towards 1,4-BD. Out of the 12 ADHs evaluated 8 showed significant activity (Fig. 1). Both NAD- and NADP-dependent ADHs showed significant activity towards 1,4-BD (Fig. 1). Amongst them, the well-known ADH from horse liver (HLADH) showed the highest activity and therefore was chosen as a biocatalyst for further investigation. Interestingly, HLADH has been known for decades to accept a broad range of 1,4-diols yielding enantiopure lactones but a ‘smart cosubstrate’ application of this reaction has not been proposed yet.⁷

The kinetic parameters of 1,4-BD (Fig. S1†) as well as EtOH and *i*PrOH were determined (Table S1†) showing that HLADH exhibits a reasonable apparent K_M value of 23 mM towards 1,4-BD (together with a mild substrate inhibition, $K_{i,\text{app}} = 1.3\text{ M}$).

Next we compared the performance of 1,4-BD to isopropanol (*i*PrOH) as a sacrificial electron donor in the HLADH-driven reduction of cinnamaldehyde (Fig. 2). HLADH has been reported as a suitable enzyme for cinnamyl alcohol/cinnamaldehyde substrate/product coupling among the other enzyme preparations.⁸

Even when a 5-fold molar excess of *i*PrOH was applied, initial rate and maximal conversion fell back significantly behind the results obtained with 0.5 equiv. of 1,4-BD. Similar observations were also made using ethanol as a cosubstrate and/or using further substrates.†

It is worth mentioning that using 1,4-BD as a cosubstrate always gave GBL in the expected 1:2 molar ratio to the product formed; the intermediate hydroxyaldehyde or its corresponding hemiacetal was not observed.

Unfortunately, cinnamaldehyde proved to be a poor model substrate due to significant product inhibition.† Already in the presence of 0.2 equiv. of alcohol (approx. corresponding to 20% conversion), the initial reduction rate decreased by more

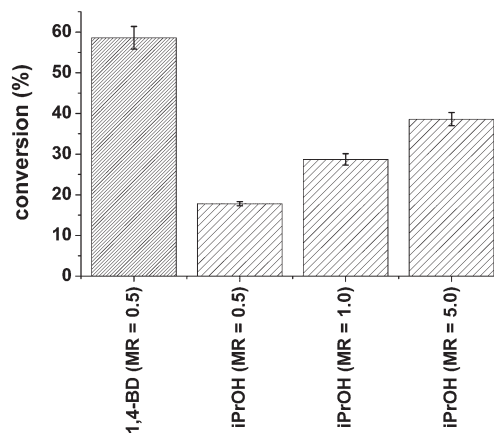


Fig. 2 Equilibrium conversion values of HLADH-catalysed reduction of cinnamaldehyde in the presence of 0.5 equiv. of 1,4-BD, 0.5–5 equiv. of *i*PrOH. Conditions: $c(\text{cinnamaldehyde}) = 5\text{ mM}$, $c(\text{NAD}^+) = 0.1\text{ mM}$, $c(\text{HLADH}) = 1\text{ g L}^{-1}$, buffer: Tris-HCl (50 mM, pH 7.0), $T = 30\text{ }^\circ\text{C}$, time = 72 h.

Table 1 HLADH-catalysed RDKR of 2-phenyl-1-propanal using various cosubstrates

Cosubstrate	MR ^a [mol mol ⁻¹]	Conversion [%]	ee (S) [%]
<i>i</i> PrOH	0.5	14	>99
EtOH	0.5	24	>99
EtOH ^b	1000	99.4	66
1,4-BD ^c	0.5	99	56
1,4-BD	0.5	98	95

Conditions: $c(2\text{-phenyl-1-propanal}) = 5\text{ mM}$, $c(\text{cosubstrate}) = 2.5\text{ mM}$, $c(\text{NAD}^+) = 0.1\text{ mM}$, $c(\text{HLADH}) = 0.1\text{ g L}^{-1}$, buffer: Tris-HCl (50 mM, pH 7.5, 1% v/v MeCN), $T = 30\text{ }^\circ\text{C}$, reaction time: 24 h. ^aMR: molar ratio of cosubstrate to substrate. ^bValues taken from ref. 8. ^c $c(\text{HLADH}) = 1\text{ g L}^{-1}$.

than 75% (Fig. S2†). Nevertheless, 1,4-BD enabled significantly higher conversions than *i*PrOH or EtOH, which we attribute to the higher thermodynamic driving force exerted by the irreversible regeneration half-reaction.

To circumvent inhibition issues, we drew our attention to the reduction of α -arylpropionaldehydes (Profen aldehydes) enabling full conversion of the racemic starting material in a reductive dynamic kinetic resolution approach (RDKR).⁸ Obviously, product inhibition in this case is less pronounced. We therefore evaluated the ‘smart cosubstrate’ approach to promote the synthesis of enantiopure Profen alcohols. Gratifyingly, we found indeed that only 0.5 equiv. of 1,4-BD as a ‘smart cosubstrate’ was necessary to achieve complete conversion of the racemic starting material (Table 1). With comparable

amounts of EtOH or *i*-PrOH, conversions of 24 and 14% were observed, respectively. These numbers are significantly lower than the expected equilibrium conversion assuming an equilibrium constant of 1. Most probably, thermodynamic reasons account for this as an MPV reduction between aldehydes and secondary alcohols should be overall thermodynamically uphill.⁹ This probably also is the reason for the huge molar excess of cosubstrate utilised in previous studies (Table 1).⁸

Interestingly, the optical purity of the final product decreased with increasing enzyme concentration (Table 1). In the presence of 10 times more biocatalyst the optical purity of the product dropped from 95% ee to 56% ee. We attribute this to the comparably slow *in situ* racemisation rate of the starting material under the given reaction conditions combined with an imperfect enantiodiscrimination of HLADH.¹⁰ Hence, at high HLADH concentrations, the enzymatic reduction activity outperforms the racemisation rate leading to decreased optical purity of the product.

Overall, the 'smart cosubstrate' approach appears to be a promising alternative to the established methods of substrate coupled biocatalytic reduction of alcohols. Further exploration of the scope is currently ongoing in our laboratories.

Encouraged by these promising results we also became interested in whether the 'smart cosubstrate' method might be generally applicable to promote NADH-dependent redox reactions such as (1) an enoate reductase-catalysed reduction of conjugated C=C-double bonds and (2) a monooxygenase-catalysed oxyfunctionalisation reaction. It is worth mentioning that these reactions are thermodynamically favoured and hence irreversible. As a model reaction for the reduction of conjugated C=C-double bonds we chose the enantioselective reduction of ketoisophorone using the enoate reductase from *Thermus scotoductus* SA-01 (TsER, Fig. 3).¹¹

Compared to EtOH as a cosubstrate 1,4-BD gave almost 3 times higher initial rates thus comparing well with the initial rates obtained for the biocatalytic MPV reduction. The product (*R*)-levodione was produced in high optical purity (ee > 95%) in both cases but slowly racemised over time;¹² after 24 h the ee-value had dropped to 86% underlining the necessity for fast reaction kinetics as achieved with the 'smart cosubstrate' approach. Hence, we conclude that the 'smart cosubstrate' approach is advantageous in terms of rates and amount of waste product even for a thermodynamically favourable reaction such as the alcohol-oxidation promoted reduction of conjugated C=C-bonds.¹³

The second model reaction chosen was the regioselective hydroxylation of 3-hydroxybenzoate yielding 2,5-dihydroxybenzoate as catalysed by 3-hydroxybenzoate-6-hydroxylase (3HB6H) from *Rhodococcus jostii* RHA1 (Fig. 4).¹⁴

Again, 1,4-BD proved to be the most efficient cosubstrate enabling initial product formation rates 7.5 and 24.5 times higher than with ethanol and isopropanol, respectively. As a result, full conversion of the starting material was achieved in the time frame of the experiment only using 1,4-BD. Interestingly, conversions obtained using EtOH were somewhat higher than the expected maximal 50%. Possibly, an endogenous

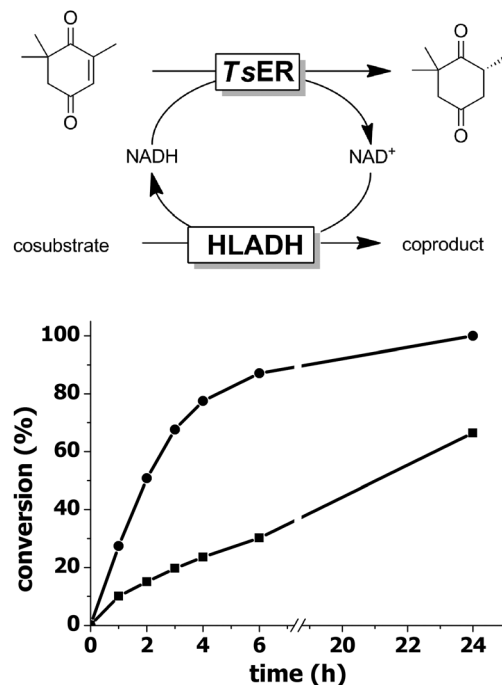


Fig. 3 TsER-catalysed reduction of ketoisophorone to (*R*)-levodione using 1,4-BD (●) or EtOH (■) as a cosubstrate. Reaction conditions: $c(\text{ketoisophorone}) = 10 \text{ mM}$, $c(\text{cosubstrate}) = 5 \text{ mM}$, $c(\text{NAD}^+) = 1 \text{ mM}$, $c(\text{TsER}) = 0.25 \text{ g L}^{-1}$, $c(\text{HLADH}) = 1.0 \text{ g L}^{-1}$, buffer: MOPS (50 mM, pH 7.0, 5 mM CaCl_2 , 1% v/v MeCN), $T = 30^\circ\text{C}$.

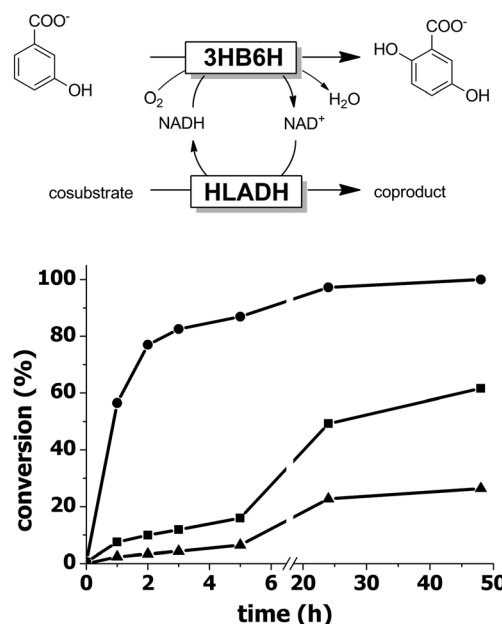


Fig. 4 Hydroxylation of 3-hydroxybenzoate to 2,5-dihydroxybenzoate using *i*-PrOH (▲), EtOH (■) or 1,4-BD (●). Reaction conditions: $c(3\text{-hydroxybenzoate}) = 5 \text{ mM}$, $c(\text{cosubstrate}) = 2.5 \text{ mM}$, $c(\text{NAD}^+) = 1 \text{ mM}$, $c(3\text{HB6H}) = 0.25 \text{ g L}^{-1}$, $c(\text{HLADH}) = 1 \text{ g L}^{-1}$, buffer: Tris- SO_4 (20 mM, pH 8.0), $T = 30^\circ\text{C}$.

E. coli aldehyde dehydrogenase present in the commercial enzyme preparation may further oxidise the acetaldehyde coproduct thereby providing more NADH equivalents.¹⁵

Encouraged by these results, we further scaled up the reaction to the semi-preparative scale (7 g L⁻¹ with full conversion into 2,5-dihydroxybenzoate within 8 h, Fig. S3†). It should be mentioned here that the catalytic performance of the nicotinamide cofactor under the reaction conditions chosen here still is far from economic feasibility. However, we are convinced that after further optimization and upscaling, high total turnover numbers for the nicotinamide cofactors can be achieved.

Conclusions

The applicability of the 'smart cosubstrate' approach was demonstrated on a range of NAD(P)H-dependent redox reactions ranging from enantioselective reduction of carbonyl- or C=C-bonds to specific aromatic hydroxylation. 1,4-BD can be oxidised twice forming a thermodynamically and kinetically stable lactone coproduct. Especially compared to the 'traditional' substrate-coupled regeneration approach, the smart cosubstrate concept excels by a significant reduction in cosubstrate needed to achieve quantitative conversion, thereby significantly reducing waste product formation. Furthermore, the coproduct (γ -butyrolactone) serves as an activated precursor for the synthesis of biodegradable polyesters.¹⁶

Of course a broad range of NAD(P)H regeneration systems is known to promote enoate reductase- and monooxygenase-reactions (Table 2).^{1,2,17–22} Being intrinsically favourable reactions, the equilibrium issue here, if existing at all, is less pronounced. Nevertheless, apart from the electrochemical methods and hydrogenations, the 'smart cosubstrate' approach ranges amongst the least waste-intensive methods shown in Table 2.

Table 2 Comparison of the waste generated by various NAD(P)H regeneration systems

Cosubstrate	Coproduct	Catalyst(s)	Waste ^a [g mol ⁻¹ product]	Ref.
Cathode	—	Hase Rh	0 ^b	17
H ₂	—	Hase	0	18
HCO ₂ H	CO ₂	FDH Rh	44	19
ⁱ Prop	Acetone	ADH	58	4
EtOH	Acetaldehyde	ADH	44	
EtOH	Acetic acid	ADH/AldDH	30 ^c	20
H ₃ PO ₃	H ₃ PO ₄	PDH Rh	98 ^d	21
Glucose	Gluconic acid	GDH	196	22
1,4-BD	GBL	HLADH	43 ^e	^e

Hase: hydrogenase; Rh:[Cp*Rh(bpy)(H₂O)]²⁺; FDH: formate dehydrogenase; ADH: alcohol dehydrogenase; AldDH: aldehyde dehydrogenase; PDH: phosphite dehydrogenase; GDH: glucose dehydrogenase. ^aTheoretical value according to the reaction equation. ^bDepending on the way the electrical current was generated. ^cDouble oxidation of the cosubstrate. ^dA phosphite buffer is transformed into a phosphate buffer. ^eThis study.

We believe that the 'smart cosubstrate' approach is a robust and versatile concept to promote a broad range of NAD(P)H-dependent reactions. Ongoing research in our laboratories focuses on broadening the 'smart cosubstrate' scope (e.g. yielding enantiopure lactone products) and optimisation and application of this approach.

Experimental

HLADH, isoform E, recombinantly expressed in *Escherichia coli* is commercially available from evocat GmbH (Düsseldorf, Germany). TsER was produced according to a literature procedure^{11d} by recombinant expression in *E. coli* followed by heat-purification. 3HB6H was produced by recombinant expression in *E. coli* following a literature procedure.¹⁴ All chemicals were purchased from Sigma Aldrich (Zwijndrecht, The Netherlands) in the highest purity available and used as received. A detailed description of the experimental procedures as well as the analytical protocols can be obtained from the ESI.†

The authors thank Deutsche Bundesstiftung Umwelt (DBU) for financial support of the project (AZ 13261). Serena Gariglio, Remco van Oosten and Maarten Gorseling are acknowledged for fruitful discussions and technical support.

Notes and references

- (a) K. Faber, *Biotransformations in Organic Chemistry*, Springer-Verlag, Berlin, Heidelberg, 2011; (b) K. Drauz, H. Groeger and O. May, *Enzyme Catalysis in Organic Synthesis*, Wiley-VCH, Weinheim, 2012; (c) F. Hollmann, I. W. C. E. Arends and D. Holtmann, *Green Chem.*, 2011, **13**, 2285–2313; (d) F. Hollmann, I. W. C. E. Arends, K. Buehler, A. Schallmey and B. Bühler, *Green Chem.*, 2011, **13**, 226–265; (e) U. Kragl, M. Mueller, M. Wolberg, T. Schubert and W. Hummel, in *Technology Transfer in Biotechnology*, Springer-Verlag, Berlin Heidelberg, 2005, vol. 92, p. 261; (f) S. Wenda, S. Illner, A. Mell and U. Kragl, *Green Chem.*, 2011, **13**, 3007–3047.
- (a) A. Weckbecker, H. Groger and W. Hummel, *Adv. Biochem. Eng./Biotechnol.*, 2010, **120**, 195–242; (b) W. Kroutil, H. Mang, K. Edegger and K. Faber, *Curr. Opin. Chem. Biol.*, 2004, **8**, 120–126; (c) F. Hollmann, I. W. C. E. Arends and K. Buehler, *ChemCatChem*, 2010, **2**, 762–782; (d) R. Wichmann and D. Vasic-Racki, in *Technology Transfer in Biotechnology*, Springer-Verlag, Berlin, Heidelberg, 2005, p. 225.
- H. Chenault and G. Whitesides, *Appl. Biochem. Biotechnol.*, 1987, **14**, 147–197.
- (a) M. Wolberg, M. V. Filho, S. Bode, P. Geilenkirchen, R. Feldmann, A. Liese, W. Hummel and M. Muller, *Bioprocess Biosyst. Eng.*, 2008, **31**, 183–191; (b) M. Villela Filho, T. Stillger, M. Muller, A. Liese and C. Wandrey, *Angew. Chem., Int. Ed.*, 2003, **42**, 2993–2996; (c) T. Daussmann, T. C. Rosen and P. Dünkemann, *Eng. Life Sci.*, 2006, **6**,

- 125–129; (d) K. Baer, M. Krausser, E. Burda, W. Hummel, A. Berkessel and H. Groger, *Angew. Chem., Int. Ed.*, 2009, **48**, 9355–9358; (e) T. Schubert, W. Hummel and M. Mueller, *Angew. Chem., Int. Ed.*, 2002, **41**, 634–635; (f) M. Wolberg, W. Hummel, C. Wandrey and M. Mueller, *Angew. Chem., Int. Ed.*, 2000, **39**, 4306–4308; (g) W. Stampfer, K. Edegger, B. Kosjek, K. Faber and W. Kroutil, *Adv. Synth. Catal.*, 2004, **346**, 57–62; (h) D. de Gonzalo, I. Lavandera, K. Faber and W. Kroutil, *Org. Lett.*, 2007, **9**, 2163–2166; (i) M. Amidjojo and D. Weuster-Botz, *Tetrahedron: Asymmetry*, 2005, **16**, 899–901; (j) K. Schroer, E. Tacha, S. Bringer-Meyer, W. Hummel, T. Daussmann, R. Pfaller and S. Lutz, *J. Biotechnol.*, 2007, **131**, 95–96; (k) A. Jakoblinert, R. Mladenov, A. Paul, F. Sibilla, U. Schwaneberg, M. B. Ansorge-Schumacher and P. D. de Maria, *Chem. Commun.*, 2011, **47**, 12230–12232; (l) L. J. Wang, C. X. Li, Y. Ni, J. Zhang, X. Liu and J. H. Xu, *Bioresour. Technol.*, 2011, **102**, 7023–7028; (m) J. Liang, E. Mundorff, R. Voladri, S. Jenne, L. Gilson, A. Conway, A. Krebber, J. Wong, G. Huisman, S. Truesdell and L. James, *Org. Process Res. Dev.*, 2010, **14**, 188–192; (n) J. Liang, J. Lalonde, B. Borup, V. Mitchell, E. Mundorff, N. Trinh, D. A. Kochrekar, R. N. Cherat and G. G. Pai, *Org. Process Res. Dev.*, 2010, **14**, 193–198.
- 5 (a) C. F. Degraauw, J. A. Peters, H. van Bekkum and J. Huskens, *Synthesis*, 1994, 1007–1017; (b) H. Meerwein and R. Schmidt, *Justus Liebigs Ann. Chem.*, 1925, **444**, 221–238; (c) W. Ponndorf, *Angew. Chem.*, 1926, **39**, 138–143; (d) M. U. Raja, R. Ramesh and K. H. Ahn, *Tetrahedron Lett.*, 2009, **50**, 7014–7017.
- 6 I. Lavandera, A. Kern, V. Resch, B. Ferreira-Silva, A. Glieder, W. M. Fabian, S. de Wildeman and W. Kroutil, *Org. Lett.*, 2008, **10**, 2155–2158.
- 7 (a) A. J. Irwin and J. B. Jones, *J. Am. Chem. Soc.*, 1977, **99**, 556–561; (b) A. J. Irwin and J. B. Jones, *J. Am. Chem. Soc.*, 1977, **99**, 1625–1630; (c) A. J. Irwin, K. P. Lok, K. W.-C. Huang and J. B. Jones, *J. Chem. Soc., Perkin Trans. 1*, 1978, 1636–1642; (d) K. P. Lok, I. J. Jakovac and J. B. Jones, *J. Am. Chem. Soc.*, 1985, **107**, 2521–2526; (e) I. J. Jakovac, H. B. Goodbrand, K. P. Lok and J. B. Jones, *J. Am. Chem. Soc.*, 1982, **104**, 4659–4665; (f) A. J. Bridges, P. S. Raman, G. S. Y. Ng and J. B. Jones, *J. Am. Chem. Soc.*, 1984, **106**, 1461–1467; (g) S. Gargiulo, I. W. C. E. Arends and F. Hollmann, *ChemCatChem*, 2011, **3**, 338–342; (h) F. Boratyński, G. Kielbowicz and C. Wawrzęczyk, *J. Mol. Catal. B: Enzym.*, 2010, **65**, 30–36; (i) G. Hilt, B. Lewall, G. Montero, J. H. P. Utley and E. Steckhan, *Liebigs Ann./Rec.*, 1997, 2289–2296; (j) I. Schröder, E. Steckhan and A. Liese, *J. Electroanal. Chem.*, 2003, **541**, 109–115.
- 8 (a) P. Galletti, E. Emer, G. Gucciardo, A. Quintavalla, M. Pori and D. Giacomini, *Org. Biomol. Chem.*, 2010, **8**, 4117–4123; (b) D. Giacomini, P. Galletti, A. Quintavalla, G. Gucciardo and F. Paradisi, *Chem. Commun.*, 2007, 4038–4040; (c) T. Kawamoto, A. Aoki, K. Sonomoto and A. Tanaka, *J. Ferment. Bioeng.*, 1989, **67**, 361–362.
- 9 M. L. Mavrovouniotis, *Biotechnol. Bioeng.*, 1990, **36**, 1070–1082.
- 10 P. Könst, H. Merckens, S. Kara, S. Kochius, A. Vogel, R. Zuhse, D. Holtmann, I. W. Arends and F. Hollmann, *Angew. Chem., Int. Ed.*, 2012, **51**, 9914–9917.
- 11 (a) S. Gargiulo, D. J. Opperman, U. Hanefeld, I. W. Arends and F. Hollmann, *Chem. Commun.*, 2012, **48**, 6630–6632; (b) J. Bernard, E. van Heerden, I. W. C. E. Arends, D. J. Opperman and F. Hollmann, *ChemCatChem*, 2012, **4**, 196–199; (c) D. J. Opperman, B. T. Sewell, D. Litthauer, M. N. Isupov, J. A. Littlechild and E. van Heerden, *Biochem. Biophys. Res. Commun.*, 2010, **393**, 426–431; (d) D. J. Opperman, L. A. Piater and E. van Heerden, *J. Bacteriol.*, 2008, **190**, 3076–3082.
- 12 A. Fryszkowska, H. Toogood, M. Sakuma, J. M. Gardiner, G. M. Stephens and N. S. Scrutton, *Adv. Synth. Catal.*, 2009, **351**, 2976–2990.
- 13 K. Tauber, M. Hall, W. Kroutil, W. M. F. Fabian, K. Faber and S. M. Glueck, *Biotechnol. Bioeng.*, 2011, **108**, 1462–1467.
- 14 S. Montersino and W. J. van Berkel, *Biochim. Biophys. Acta*, 2012, **1824**, 433–442.
- 15 A. Chang, M. Scheer, A. Grote, I. Schomburg and D. Schomburg, *Nucleic Acids Res.*, 2009, **37**, 588–592.
- 16 T. Moore, R. Adhikari and P. Gunatillake, *Biomaterials*, 2005, **26**, 3771–3782.
- 17 (a) F. Hollmann, A. Schmid and E. Steckhan, *Angew. Chem., Int. Ed.*, 2001, **40**, 169–171; (b) K. Delecouls-Servat, A. Bergel and R. Basseguy, *Bioprocess Biosyst. Eng.*, 2004, **26**, 205–215; (c) F. Hildebrand and S. Lütz, *Tetrahedron: Asymmetry*, 2007, **18**, 1187–1193; (d) R. Ruppert, S. Herrmann and E. Steckhan, *Tetrahedron Lett.*, 1987, **28**, 6583–6586; (e) J. Cantet, A. Bergel and M. Comtat, *Bioelectrochem. Bioenerg.*, 1992, **27**, 475–486.
- 18 (a) J. Ratzka, L. Lauterbach, O. Lenz and M. B. Ansorge-Schumacher, *Biocatal. Biotransform.*, 2011, **29**, 246–252; (b) R. Mertens, L. Greiner, E. C. D. van den Ban, H. Haaker and A. Liese, *J. Mol. Catal. B: Enzym.*, 2003, **24–25**, 39–52; (c) Y. Ni, P.-L. Hagedoorn, J.-H. Xu, I. W. C. E. Arends and F. Hollmann, *Chem. Commun.*, 2012, **48**, 12056–12058.
- 19 (a) K. Hofstetter, J. Lutz, I. Lang, B. Witholt and A. Schmid, *Angew. Chem., Int. Ed.*, 2004, **43**, 2163–2166; (b) C. N. Jensen, J. Cartwright, J. Ward, S. Hart, J. P. Turkenburg, S. T. Ali, M. J. Allen and G. Grogan, *ChemBioChem*, 2012, **13**, 872–878; (c) K. Kuehnel, S. C. Maurer, Y. Galeyeva, W. Frey, S. Laschat and V. B. Urlacher, *Adv. Synth. Catal.*, 2007, **349**, 1451–1461; (d) K. Durchschein, S. Wallner, P. Macheroux, W. Schwab, T. Winkler, W. Kreis and K. Faber, *Eur. J. Org. Chem.*, 2012, **2012**, 4963–4968; (e) M. Hall, C. Stückler, B. Hauer, R. Stürmer, T. Friedrich, M. Breuer, W. Kroutil and K. Faber, *Eur. J. Org. Chem.*, 2008, 1511–1516; (f) M. Hall, C. Stückler, H. Ehammer, E. Pointner, G. Oberdorfer, K. Gruber, B. Hauer, R. Stürmer, W. Kroutil, P. Macheroux and K. Faber, *Adv. Synth. Catal.*, 2008, **350**, 411–418; (g) M. Hall, C. Stueckler, W. Kroutil, P. Macheroux and K. Faber, *Angew. Chem., Int.*

- Ed.*, 2007, **46**, 3934–3937; (*h*) V. Köhler, Y. M. Wilson, M. Durrenberger, D. Ghislieri, E. Churakova, T. Quinto, L. Knorr, D. Haussinger, F. Hollmann, N. J. Turner and T. R. Ward, *Nat. Chem.*, 2013, DOI: 10.1038/nchem.1498; (*i*) D. Westerhausen, S. Herrmann, W. Hummel and E. Steckhan, *Angew. Chem., Int. Ed.*, 1992, **31**, 1529–1531; (*j*) R. Ruppert, S. Herrmann and E. Steckhan, *J. Chem. Soc., Chem. Commun.*, 1988, 1150–1151.
- 20 S. Broussy, R. W. Cheloha and D. B. Berkowitz, *Org. Lett.*, 2009, **11**, 305–308.
- 21 (*a*) B. Kosjek, F. J. Fleitz, P. G. Dormer, J. T. Kuethe and P. N. Devine, *Tetrahedron: Asymmetry*, 2008, **19**, 1403–1406; (*b*) C. Rodriguez, G. de Gonzalo and V. Gotor, *J. Mol. Catal. B: Enzym.*, 2011, **74**, 138–143; (*c*) D. E. Torres Pazmiño, A. Riebel, J. D. Lange, F. Rudroff, M. D. Mihovilovic and M. W. Fraaije, *ChemBioChem*, 2009, **10**, 2595–2598; (*d*) D. E. Torres Pazmiño, R. Snajdrova, B.-J. Baas, M. Ghobrial, M. D. Mihovilovic and M. W. Fraaije, *Angew. Chem., Int. Ed.*, 2008, **47**, 2307–2310.
- 22 (*a*) J. D. Zhang, A. T. Li, H. L. Yu, T. Imanaka and J. H. Xu, *J. Ind. Microbiol. Biotechnol.*, 2011, **38**, 633–641; (*b*) Y. Lu and L. H. Mei, *J. Ind. Microbiol. Biotechnol.*, 2007, **34**, 247–253; (*c*) H. J. Park, J. Jung, H. Choi, K. N. Uhm and H. K. Kim, *J. Microbiol. Biotechnol.*, 2010, **20**, 1300–1306; (*d*) J. F. Chaparro-Riggers, T. A. Rogers, E. Vazquez-Figueroa, K. M. Polizzi and A. S. Bommaris, *Adv. Synth. Catal.*, 2007, **349**, 1521–1531; (*e*) H. S. Toogood, J. M. Gardiner and N. S. Scrutton, *ChemCatChem*, 2010, **2**, 892–914; (*f*) D. J. Bougioukou, A. Z. Walton and J. D. Stewart, *Chem. Commun.*, 2010, **46**, 8558–8560.