

Cite this: *Green Chem.*, 2011, **13**, 3093

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## Utilising hardly-water soluble substrates as a second phase enables the straightforward synthesis of chiral alcohols†

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Received 10th July 2011, Accepted 19th August 2011

DOI: 10.1039/c1gc15823f

So far, the alcohol dehydrogenase-catalysed conversion of longer chain aliphatic substrates has been challenging due to their low solubility in aqueous solution. However, by utilising the ketone directly as a second organic phase, the straightforward synthesis of long chain aliphatic chiral alcohols is enabled.

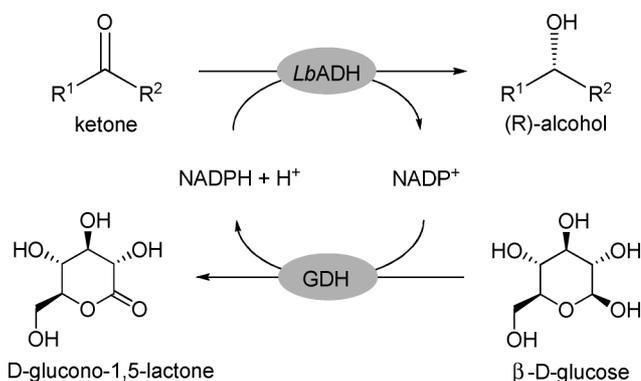
Chiral compounds play an important role in chemical and pharmaceutical industries, as they are building blocks for various pharmaceuticals, flavours, agrochemicals, food additives and chemical catalysts. It is supposed that 40% of all chiral centres in drugs are secondary or tertiary hydroxyl groups.<sup>1</sup> Hence, enantiopure alcohols are of special importance among the available building blocks. Therefore, various methods for the synthesis of these alcohols exist.<sup>2</sup> Firstly, classical methods like the chemical resolution of racemates, chromatography or chiral pool syntheses can be used. Secondly, asymmetric chemical methods like CBS reduction<sup>3</sup> or transfer hydrogenation with chiral catalysts<sup>4</sup> can be applied. Finally, enzymes or whole cells can also be suitable catalysts.<sup>5</sup>

Due to superior regio- and enantioselectivity, along with higher product purity, more and more biocatalytic processes are being established in industry. Prevalent alcohol dehydrogenases (ADH) are used to catalyse the synthesis of chiral alcohols *via* the reduction of prochiral ketones.<sup>6</sup> Unfortunately, without strategies to overcome the frequently poor solubility of industrially attractive substrates, the application of these biocatalysts is limited due to the dependency on water. On a technical scale, almost insoluble and non-water soluble ketones are preferably converted into chiral alcohols *via* chemical methods that either involve transition metal catalysts, high pressures and high temperatures or hazardous borane and chiral oxazaborolidine.<sup>3,4</sup> Since, biocatalysts work under mild reaction conditions (*e.g.* room temperature and ambient pressure), their application

for the conversion of hardly-water soluble substrates could enable sustainable and competitive synthesis routes.

As a consequence, the potential of aqueous organic one-phase and two-phase systems has been explored in several examples to overcome solubility problems.<sup>7</sup> In both cases, the selection of an adequate organic solvent is challenging. While in one-phase systems direct influences on the biocatalyst performance and cofactor stability have to be taken into account, in two-phase systems sufficient partitioning of the substrate and product in dependence of enzyme kinetics needs to be achieved to avoid mass transfer limitations. However, two-phase systems are of particular interest, since they may aid the overcoming of inhibitions, facilitate product separation, as well as catalyst and cofactor recycling.<sup>8</sup>

An interesting strategy is the application of a pure substrate as the second phase, since the use of a non-miscible solvent as an additional organic component can be avoided, which, in consequence, further simplifies product separation and reduces the amount of waste. Therefore this concept was investigated for the *Lactobacillus brevis* alcohol dehydrogenase (*LbADH*)<sup>9</sup>-catalysed reductions of hardly-water soluble ketones (3-octanone, 2-octanone, 2-nonanone and 2-decanone) to their corresponding (*R*)-alcohols. Regeneration of the cofactor NADPH was carried out by a glucose dehydrogenase (GDH) from *Bacillus sp.* (Fig. 1). The tested substrates showed only limited solubility in aqueous phosphate buffer (7.4 mmol L<sup>-1</sup> for 3-octanone, 7.9 mmol L<sup>-1</sup> for 2-octanone, 2.1 mmol L<sup>-1</sup> for



**Fig. 1** *LbADH*-catalysed reduction of prochiral ketones with GDH-catalysed cofactor regeneration (for details of R<sup>1</sup> and R<sup>2</sup>, see text).

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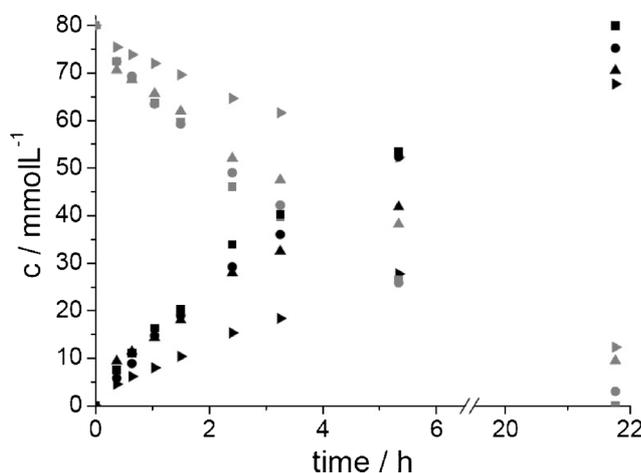
† Electronic supplementary information (ESI) available: Experimental and analysis details. See DOI: 10.1039/c1gc15823f

**Table 1** Key figures for the syntheses of different (*R*)-alcohols

	( <i>R</i> )-3-Octanol	( <i>R</i> )-2-Octanol	( <i>R</i> )-2-Nonanol	( <i>R</i> )-2-Decanol
STY/mmol L <sup>-1</sup> d <sup>-1</sup>	74.6	77.8	88.1	82.9
STY/g L <sup>-1</sup> d <sup>-1</sup>	9.72	10.1	12.7	13.0
TON <sub>ADH</sub> /10 <sup>3</sup>	713	743	842	792
TON <sub>GDH</sub> /10 <sup>3</sup>	16.3	17.0	19.3	18.1
TON <sub>NADP<sup>+</sup></sub>	677	705	799	752
<i>E</i> factor	117	112	89	87

2-nonanone and 0.6 mmol L<sup>-1</sup> for 2-decanone) and were therefore suitable for application as a non-reactive phase. The product alcohols could be easily separated from the aqueous phase by decantation. For analysis, all small-scale samples were extracted with hexane to allow quantitative recovery.

Initial batch experiments were carried out with substrate amounts corresponding to a concentration of 80 mmol L<sup>-1</sup> (assuming the substrate would be dissolved in the overall reaction volume) and compared on the basis of space time yields (STY = amount of product produced per litre of reaction volume per day), turnover numbers (TON = amount of product per amount of catalyst or cofactor, respectively) and environmental factors (*E* factor = kg waste per kg product) (see Table 1). The time course of the reactions is presented in Fig. 2.



**Fig. 2** Reaction progress for the *LbADH*-catalysed syntheses of chiral alcohols utilizing the substrate as the second phase (3-octanone  $\blacktriangle$ , (*R*)-3-octanol  $\blacktriangle$ , 2-octanone  $\blacktriangle$ , (*R*)-2-octanol  $\blacktriangle$ , 2-nonanone  $\blacksquare$ , (*R*)-2-nonanone  $\blacksquare$ , 2-decanone  $\bullet$  and (*R*)-2-decanol  $\bullet$ ).

Even though *LbADH* showed product inhibitions for all the alcohols,<sup>10</sup> it was possible to achieve conversions of 84.6% for 3-octanone, 88.2% for 2-octanone, 99.9% for 2-nonanone and 94.0% for 2-decanone. Notably, only for 2-octanone was a substrate surplus inhibition found.<sup>10</sup> This is most probably the reason for the low conversions compared to the other 2-ketones. Within all experiments, STYs of 75 to 83 mmol L<sup>-1</sup> d<sup>-1</sup>, respectively, and enantioselectivities (enantiomeric excess, ee) of > 99.9% were reached. Remarkably, the achieved STYs are similar to those obtained for syntheses with comparable TONs of about 90 mmol L<sup>-1</sup> d<sup>-1</sup>, where a biocompatible ionic liquid was applied as a solubiliser.<sup>10</sup> However, when applying the substrate as a second phase, an additional organic component can be avoided; hence, facilitating downstream processing and thereby

**Table 2** Key figures for the synthesis of (*R*)-2-nonanone with increased substrate and 10-fold increased biocatalyst concentrations

<i>c</i> <sub>2-nonanone</sub> /mmol L <sup>-1</sup>	80	100	150
STY/mmol L <sup>-1</sup> d <sup>-1</sup>	881	889	814
STY/g L <sup>-1</sup> d <sup>-1</sup>	127	128	117
TON <sub>ADH</sub> /10 <sup>3</sup>	83.8	105	157
TON <sub>GDH</sub> /10 <sup>3</sup>	1.92	2.40	3.60
TON <sub>NADP<sup>+</sup></sub>	795	994	1 490
<i>E</i> factor	101	81	54

leading to reduced amounts of waste. When comparing the obtained *E* factors of 87 to 117 to those found in industry, a high potential for the development of an environmentally friendly process is apparent.<sup>11</sup>

Subsequently, for the synthesis of (*R*)-2-nonanone, reactions with 10-times increased biocatalyst concentrations and higher substrate concentrations (80, 100 and 150 mmol L<sup>-1</sup>) were carried out (Table 2). Within these batch experiments, conversions of at least 99.4% and ee values of >99.9% were achieved. Due to increased biocatalyst concentration, reduced TONs for both enzymes were found; nevertheless, the achieved values are still promising. Moreover, it was possible to improve the STY and TON<sub>NADP<sup>+</sup></sub> to reach industrially relevant values. Also, the *E* factors could be improved; by increasing the substrate concentration to 150 mmol L<sup>-1</sup>, an *E* factor of 54 was possible, which is within an acceptable range for fine chemical production.<sup>10</sup>

## Conclusions

In conclusion, the application of the substrate itself as a second phase for biocatalytic reactions represents a straightforward method to enable environmentally friendly conversions of hardly-water soluble substrates. Applying this strategy to the *LbADH*-catalysed syntheses of chiral aliphatic alcohols led to promising STYs, *E* factors and TONs for both the biocatalyst and the nicotinamide cofactor. Reaction engineering could further improve those syntheses.

## Acknowledgements

The authors thank the Federal Ministry of Economics and Technology *via* ZIM and DECHEMA for funding, as well as Prof. W. Leitner (RWTH Aachen University) for fruitful discussions.

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