Asymmetric reduction of ketones with recombinant *E. coli* whole cells in neat substrates

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Biocatalysis is receiving increasing attention as a powerful synthetic tool providing environmentally-friendly reaction conditions with high regio- and stereoselectivities. However, a broader extension of industrial biocatalysis is still hampered due to several reasons, e.g. restricted enzyme availabilities, insufficient productivities and stabilities, time- and material-intensive work-ups in aqueous media—often leading to waste production—as well as costs related to catalysts and cofactors. Low product concentrations resulting from a limited water solubility of many organic compounds can be, to some extent, enhanced by reaction engineering using organic co-solvents or water-organic biphasic systems. On the other hand, process stability of biocatalysts can be increased and costs for catalysts and cofactors can be reduced by the use of whole cells rather than isolated enzymes.

For the industrial performance of the asymmetric reduction of prochiral ketones by alcohol dehydrogenases the demand for expensive nicotinamide cofactors plays a particular role in cell-free systems. Hence, the use of whole cells exhibits as a major advantage that cofactors can be intrinsically recycled via the coupling of a second substrate or by a second enzyme. Few reduction reactions have been reported which work in water-organic mixtures showing exceedingly high substrate loadings and no or only little demand for external cofactors. However, in such water-based systems emulsions may be formed, decreasing isolated yields and requiring tedious product separation. Moreover, the general applicability of the latter system is restricted when substrates and products with low stability in aqueous environments are involved.

**COMMUNICATION**

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reaction rate on the volumetric substrate:co-substrate ratio, indicating the underlying equilibrium conditions (Fig. 2).

Nevertheless, the conversion of acetophenone was always driven to completion, when acetone was intermittently removed from the reaction, and the enantioselectivity was always at maximum (ee > 99%).20,21 Obviously, with a highly optimized process development these full conversions could be achieved in much shorter reaction times. Remarkably, after 14 days of operation in the system cells still displayed activity rendering this biocatalyst extremely stable.22 This is consistent with the reported stable operation of E. coli cells harboring an alcohol dehydrogenase at high concentrations of isopropanol in aqueous media,23 and can mainly be attributed to the enzyme preserving microenvironment of the host cell.5 Inhibition or inactivation of the catalyst at elevated co-substrate concentrations could not be observed. Consequently, with this system it was possible to produce up to 500 g L\(^{-1}\) optically pure (S)-phenylethanol. Other substrates like 4-chloroacetophenone or aliphatic 2-butanone were converted in analogous systems as well.

The role of the water on the activity of the biocatalyst was analyzed in depth, since it is widely accepted that water plays a crucial role in biocatalysis in non-conventional media.26 In lyophilized whole cells in gas–solid reactor systems,27,28 and in whole cell biocatalysis in transesterification and trans-gluco-sylation reactions.29,30 Lyophilized cells were equilibrated to different \(a_w\) values ranging from dry \((a_w = 0)\) to wet \((a_w = 1)\) and used in neat substrates (Fig. 3).24,31 For low \(a_w\) values biocatalytic activity is increasing with water activity, which is in agreement with former findings.27,28 The optimum water activity is around an \(a_w\) value of 0.84 (Fig. 3). Additionally, non-equilibrated wet cells were active but rapidly deactivated within few hours, whereas lyophilized cells remained stable for several days (data not shown).

As indicated previously, application of the herein described concept may be particularly beneficial for the asymmetric reduction of compounds exhibiting low stabilities in aqueous environments. For instance, propargylic ketone 3-butyn-2-one is unstable in aqueous media, shows thermal decomposition and, moreover, has been reported to deactivate enzymes and cells.32–34 Notably, the corresponding (S)-3-butyn-2-ol is an important building block for anti-asthma drugs like 5-lipoxygenase inhibitors,34 the potent \(\beta_2\)-adrenergic stimulant broxaterol35 or the protease inhibiting hydroxyethylene dipeptide isosteres.36 In virtue of this importance, the enzymatic production of (S)-3-butyn-2-ol has already been attempted via the asymmetric reduction of 3-butyn-2-one with alcohol dehydrogenases or via the enantioselective hydrolysis of the corresponding esters. To overcome substrate and product instability in aqueous media, either biphasic systems were applied, or the derivatized trimethylsilane-based compound was used as a substrate, albeit at the cost of adding further synthetic steps.33–35,37

So far, alcohol dehydrogenases displayed low-to-moderate enantioselectivities in monophase systems (5–86%), Whereas the hydrolase-catalyzed ester hydrolysis led to high ee’s, but with limited conversions at 50%.38,39 To date, there is only one multi gram-scale preparation of (S)-3-butyn-2-ol using an alcohol dehydrogenase from Pseudomonas aeruginosa in a biphasic system with ee > 99%, though at low conversion (45.5%).32

In the herein developed solvent-free whole-cell system high enantioselectivities were achieved (ee = 99.9%; Fig. 4).19 Data are at odds with the value reported by Schubert et al. for CPCR for 3-butyn-2-one (ee = 49%).34 However, in the mentioned work, CPCR was used not in the recombinant form but as a crude extract from the Candida host. Hence, discrepancies might be explained by the acceptance of 3-butyn-2-one by yeast oxidoreductases other than CPCR which in turn may lower the ee, as it has been reported for other yeast systems like baker’s yeast.40

![](https://example.com/image1)

**Fig. 2** Conversion of acetophenone by E. coli whole cells with overexpressed CPCR in neat substrates. Different volumetric ratios of isopropanol to acetophenone were set (50 : 50 ▲, 70 : 30 ◦). Closed symbols: daily removed acetone; open symbol: no acetone removal.

**Fig. 3** Initial reaction rate of acetophenone in neat substrates employing E. coli whole cells with overexpressed CPCR. Dried cells and reaction mixtures were previously equilibrated to different water activities.24,25

![](https://example.com/image2)

**Fig. 4** Biocatalytic reduction of 3-butyn-2-one using lyophilized E. coli cells with overexpressed CPCR in the developed solvent-free system with isopropanol-coupled cofactor regeneration.
According to the above-described findings that increasing the co-substrate concentration favors the product formation, a volumetric ratio 90:10 (isopropanol to 3-butyln-2-one) at a minimum $a_{w}$ was set up in this case. As proof-of-concept 67.6% conversion was achieved in 24 h, corresponding to 57.4 g L$^{-1}$ of optically pure (S)-3-butyln-2-ol. This product concentration is already 2-fold higher than the so far reported maximum value (24.7 g L$^{-1}$). Without acetone removal (hence, under non-optimized conditions), full conversion could be obtained within 120 h, yielding a product concentration of 87 g L$^{-1}$. This example notably points out that enzymatic reductions in neat substrates, while lacking bulk water, is very powerful in accessing optically pure alcohols, even if substrates and/or products are unstable in aqueous solutions. It can be expected that this will open new biocatalytic routes to the production of so far non- or hardly biocatalytically accessible building blocks.

In summary, lyophilized E. coli whole-cells with overexpressed carbonyl reductases perform enantioselective ketone reductions in neat substrates, producing enantiomerically pure alcohols in large amounts. The activity is characterized by high cost-effectiveness (high added-value, no cofactor addition, simple work-up) and by an environmentally-friendly operation mode (largely diminished waste production, bio-based catalysis). A further notable point is the use of compounds in the reaction system that are unstable in aqueous environments. Together with inherent advantages of biocatalysis (high selectivity, high activity), the approach may represent a competitive alternative with inherent advantages of biocatalysis (high selectivity, high activity), the approach may represent a competitive alternative with inherent advantages of biocatalysis.

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**Notes and references**