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Utilising hardly water soluble substrates as second phase enables straightforward syntheses of chiral alcohols

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Experimental

Alcohol dehydrogenase from *Lactobacillus brevis* (*Lb*ADH) and glucose dehydrogenase from *Bacillus spec.* (GDH) are available from X-Zyme GmbH (Düsseldorf, Germany). $NADP^+$ was provided by Carl Roth GmbH (Karlsruhe, Germany) and N-methyl-N-trimethylsilyl-trifluoracetamide (N-MSTFA) from CS-Chromatographie Service GmbH (Langerwehe, Germany). All other reagents were purchased from Sigma Aldrich (Schnelldorf, Germany), and were at least of analytical grade. Batch experiments were performed in 10mL glass vials with screw caps and teflonated sealings (Th. Geyer, Germany).

Potassium buffer (100 mmol, pH 7) was used throughout and prepared by dissolving K_2HPO_4 (12.63 g, 72.5 mmol) and KH_2PO_4 (3.76 g, 27.6 mmol) in 1000 mL water and $MgCl_2$ (238 mg, 2.5 mmol) was added.

For the batch experiments glucose (238 mg, 1.20 mmol or 301 mg, 1.52 mmol), LbADH (0.5 mg lyophilisate, 0.079 mg protein) and GDH (5 mg lyophilisate, 1.36 mg protein) were dissolved in 7.9 mL buffer. To maintain constant pH $CaCO_3$ (128 mg, 1.28 mmol or 256 mg, 2.56 mmol) was added. The ketones were added by weight. With the addition of $NADP^+$ (0.63 mg, 0.8 µmol in 100 µL buffer) the reaction was started. Reactions were carried out at 30°C and shaken at 150 rpm (Thermomixer, Eppendorf). Analytical samples of typically 200µL were withdrawn , extracted with hexane for quantitative recovery and analysed via GC (column: Chirasil-Dex (25 m x 0.25 mm ID) from Varian GC Capillary Columns, carrier gas: H_2, 0.4 bar).

Determination of reaction progresses:

3-octanone/3-octanol: 90 °C (2 min), 5 °C min^{-1} to 130 °C (2 min), 40°*C* min^{-1} to 180°*C* (2 min). Retention times: 3-octanone (6.2 min), 3-octanol (10.3 min), internal standard 1-octanol (10.3 min).
2-octanone/2-octanol: 80°*C* (3 min), 10°*C* min^{-1} to 120°*C* (6 min), 40°*C* min^{-1} to 180°*C* (2 min). Retention times: 2-octanone (7.3 min), 2-octanol (9.4 min), internal standard 1-octanol (11.8 min).
2-nonanon/2-nonanol: 110°*C* (0 min), 2°*C* min^{-1} to 120°*C* (0 min), 40°*C* min^{-1} to 180°*C* (2 min). Retention times: 2-nonanone (5.5 min), 2-nonanol (6.4 min), internal standard 1-octanol (6.3 min).
2-decanon/2-decanol: 120°*C* (2 min), 3°*C* min^{-1} to 150°*C* (0 min), 40°*C* min^{-1} to 180°*C* (2 min). Retention times: 2-decanone (6.7 min), 2-decanol (8.7 min), internal standard 1-octanol (5.9 min).

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Determination of enantiomeric excess:

Samples were treated as follows: the organic sample (250 µL) was mixed with N-MSTFA (50 µL) and heated to 80°*C* for 30 min.
(R)-/(S)-3-octanol*:* 60°*C* (51 min), 40°*C* min^{-1} to 180°*C* (2 min). Retention times: (*R*)-3-octanol (47.1 min), (*S*)-3-octanol (48.4 min).
(R)-/(S)-2-octanol: 80°*C* (3 min), 1°*C* min^{-1} to 100°*C* (5 min), 40°*C* min^{-1} to 180°*C* (2 min). Retention times: (*R*)-2-octanol (14.9 min), (*S*)-2-octanol (15.2 min).
(R)-/(S)-2-nonanol: 75°*C* (60 min), 40°*C* min^{-1} to 180°*C* (2 min). Retention times: (*R*)-2-nonanol (55.0 min), (S)-2-nonanol (56.6 min).
(*R*)-/(S)-2-decanol: 80°*C* (92 min), 40°*C* min^{-1} to 180°*C* (2 min). Retention times: (*R*)-2-decanol (86.0 min), (*S*)-2-decanol (87.7 min).