DECHEMA FORSCHUNGSINSTITUT

Chemical Technology

# Continuous, enzymatic production of hardly water-soluble, enantiopure alcohols: Two opposed approaches combined

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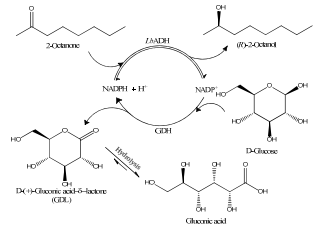
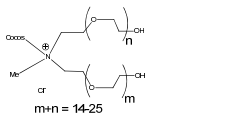
## Reaction System

### Synthesis of (*R*)-2-Octanol in a Cascade of 2 EMR



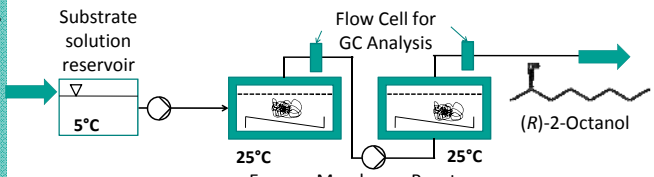

Reaction System used for the production of (*R*)-2-alcohols

ADA-buffer



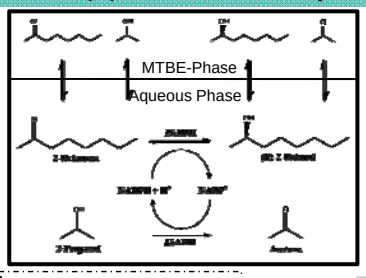

Ionic Liquid TEGO IL K5 as solubiliser

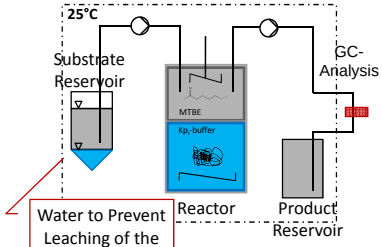
## Reaction Setup




Enzyme Membrane Reactor (Membrane Cut-off: 10 kDa)

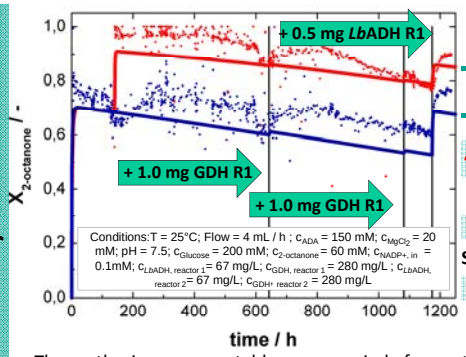
### Synthesis of (*R*)-2-Octanol in a Biphasic Reactor




[5]

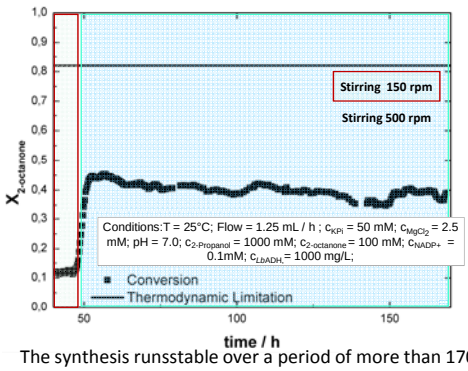
## Synthesis




Blue= Conversion reactor 1;
Red = conversion reactor 2;

| Results (1170 h) | R1 | R2 |
|---|---|---|
| $X_{2\text{-octanone}}$ / - | 75.8 | 96.9 |
| $TON_{LbADH}$ / $10^6$ | 45.6 | 12.84 |
| $TON_{GDH}$ / $10^6$ | 7.7 | 2.93 |
| $TON_{NADP+}$ | 411 | 539 |
| STY / mmol $L^{-1}$ $d^{-1}$ | 291 | 124 |
| *ee* | 99.9 | 99.9 |

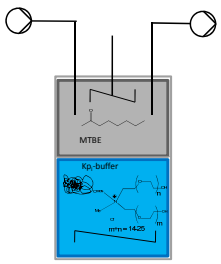
The synthesis runs very stable over a period of more than 1200 h. Turnover numbers ($mol_{product}/mol_{catalyst}$) for both enzymes are extremely high.



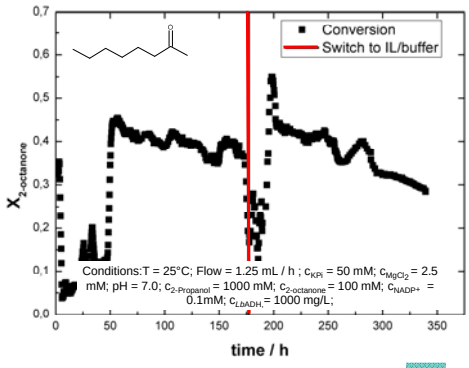

| Results (175 h) | |
|---|---|
| $X_{2\text{-octanone}}$ / - | 40 |
| $TON_{LbADH}$ / $10^3$ | 143 |
| $TON_{NADP+}$ / $10^3$ | 13.8 |
| STY / mmol $L^{-1}$ $d^{-1}$ | 122 |
| *ee* | 99.9 |

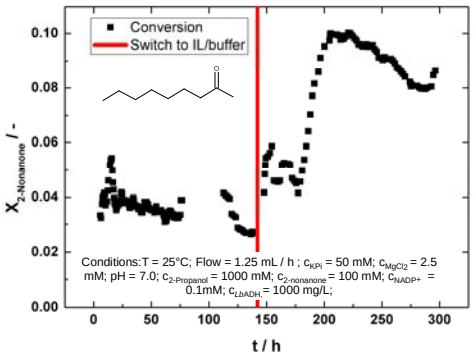
The synthesis runsstable over a period of more than 170 h. The turnover numbers ($mol_{product}/mol_{catalyst}$) for the cofactor $NADP^+$ is very high.

## Combined Approach




Reactor
Ionic Liquid TEGO IL K5 as solubiliser in aq phase




| | 2-Octanone | | 2-Nonanone | |
|---|---|---|---|---|
| | no IL | 100g/L IL | no IL | 100g/L IL |
| Runtime / h | 105 | 105 | 50 | 50 |
| STY mmol/$L^{-1}d^{-1}$ | 122 | 123 | 10,8 | 28,5 |
| $TON_{LbADH}$ / $10^3$ | 110 | 109 | 4610 | 12350 |
| $TON_{NADP+}$ / $10^3$ | 10.6 | 10.5 | 440 | 1190 |
| Deact / % $h^{-1}$ | 0.06 | 0.06 | 0,02 | 0,01 |
| Conversion / % | 40.5 | 41.0 | 3,6 | 9,6 |

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## Summary & Outlook

✓Monophasic and biphasic synthesis of (*R*)-2-alcohols is possible with *Lb*ADH
✓A combination of both approaches has no effect on the synthesis of (*R*)-2-octanol, but improves the synthesis of (*R*)-2-nonanol
➢The biphasic synthesis of longer chain alcohols, for example (R)-2-decanol will be investigated

**See also:**
[1] S. Leuchs,L. Greiner, Alcohol Dehydrogenase from Lactobacillus brevis: A Versatile Robust Catalyst for Enantioselective Transformations Chemical & Biochemical Engineering Quarterly, 2011, 25, 267-281
[2] C. Kohlmann, S. Leuchs, L. Greiner, W. Leitner, Continuous Biocatalytic Synthesis of (R)-2-Octanol with Integrated Product Separation, Green Chemistry, 2011, 13, 1430-1436
[3] C. Kohlmann, N. Robertz, S. Leuchs; Z. Dogan, S. Lütz, S. Na'amnieh, L. Greiner, Ionic liquid facilitates biocatalytic conversion of hardly water soluble ketones, Journal of Molecular Catalysis B: Enzymatic, 2011, 68, 147-153
[4] S. Leuchs, L. Greiner, Enantioselective reduction of sparingly water-soluble ketones: Continuous process and recycle of the aqueous buffer system, 2013, *Green Chemistry*, 2013, *15*, 167-176
[5] A. van den Wittenboer et al., Biphasic mini-reactor for characterization of biocatalyst performance *Biotech. J.* 2009, *4*, 44-50