DECHEMA
Karl-Winnacker-Institut

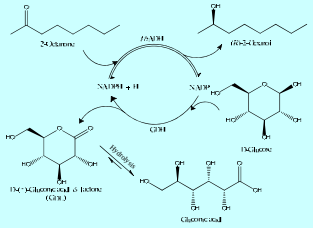
Chemical Technology

Continuous Reduction of Hardly Water-Soluble Ketones with an Ionic Liquid as Solubiliser

S. Leuchs, L. Greiner
e-mail: leuchs@dechema.de
Funded by: BMWi via AIF (ZIM)
Period: 01.10.2010 – 31.12.2011

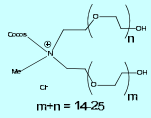
Introduction

1. The enantioselective reduction of hardly water-soluble ketones is carried out with an alcohol dehydrogenase from *Lactobacillus brevis* (*Lb*ADH).
2. Cofactor Regeneration is performed with a Glucose dehydrogenase (GDH).
3. To overcome solubility restrictions of long-chain ketones (2-octanone and 2-nonanone), the ionic liquid (TEGO IL K5) is used as solubiliser.
4. The continuous synthesis of the corresponding (*R*)-2-alcohols is carried out in an enzyme membrane reactor (EMR) and in a reactor cascade (two EMR).
5. The isolation of the ketone and alcohol is feasible *via* solid phase extraction (SPE) and subsequent elution with *n*-heptane.
6. The reaction mixture can be recycled and is re-used as substrate solution.



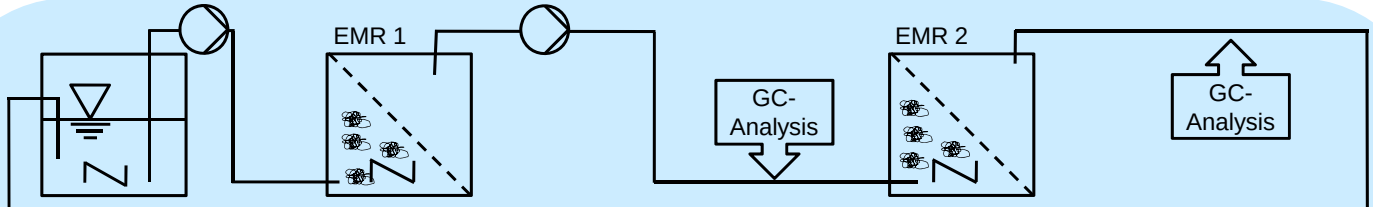
Reaction System used for the production of (*R*)-2-alcohols

ADA-buffer



Ionic Liquid TEGO IL K5

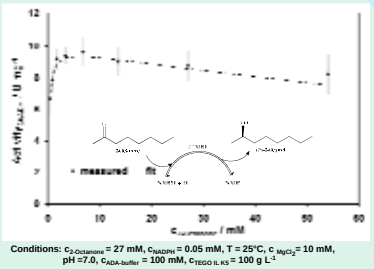
Continuous Synthesis of (*R*)-2-alcohols



Kinetic modelling

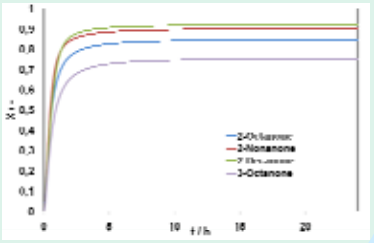
Kinetic measurements in a Multiplate-reader

Extended Michaelis-Menten-Model



Conditions: $c_{2\text{-Octanone}}$ = 27 mM, c_{NADPH} = 0.05 mM, T = 25°C, c_{MgCl_2} = 10 mM, pH =7.0, $c_{ADA\text{-buffer}}$ = 100 mM, $c_{TEGO\ IL\ K5}$ = 100 g L^{-1}

Prediction for four linear, aliphatic ketones as substrates with c_{Ketone} = 30 mM.



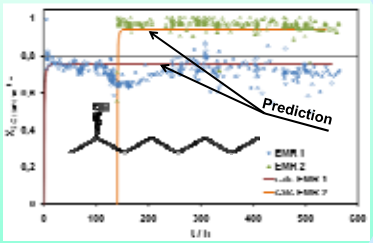
Conditions:
m_{LbADH} = 1.0 mg, m_{GDH} = 4.2 mg, c_{NADP+} = 0.1 mM; $c_{Glucose}$=200 mM; $c_{2\text{-Ketone}}$ =30 mM; $c_{ADA\text{-buffer}}$=150 mM; $c_{TEGO\ IL\ K5}$=100 gL^{-1}; c_{MgCl_2} =10 mM; pH=7.5, τ=3.75 h; dV/dt= 4.0 mL/h

Synthesis

Synthesis of (*R*)-2-Octanol in a cascade of two EMR

Results	EMR 1	EMR 2
TON_{LbADH} / 10^6	22.9	6.10
TON_{GDH} / 10^6	5.20	1.39
TON_{NADP+}	435	144
STY/mmol·L^{-1}·d^{-1}	272	97
ee	> 99.9	

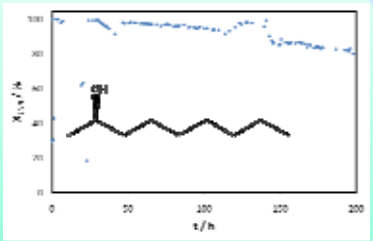
Conditions:
m_{LbADH} = 1.0 mg/per reactor, m_{GDH} = 4.2 mg/per reactor, c_{NADP+}= 0.1 mM; $c_{Glucose}$=200 mM; $c_{2\text{-Octanone}}$=60mM; $c_{ADA\text{-buffer}}$=150 mM; $c_{TEGO\ IL\ K5}$=100 gL^{-1}; c_{MgCl_2}=20 mM; pH=7.5; τ=3.75 h; dV/dt= 4 mL/h



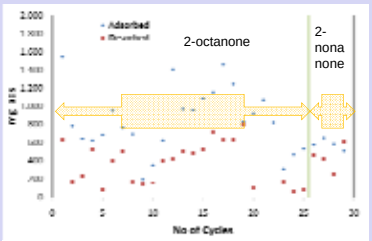
Synthesis of (*R*)-2-Nonanol in a single EMR

Results	
TON_{LbADH} / 10^6	3.6
TON_{GDH} / 10^6	0.82
TON_{NADP+}	552
STY/mmol·L^{-1}·d^{-1}	350
ee	> 99.9

Conditions:
m_{LbADH} = 1.0 mg, m_{GDH} = 4.2 mg, c_{NADP+}= 0.1 mM; $c_{Glucose}$=200 mM; $c_{2\text{-Nonanone}}$=53 mM; $c_{ADA\text{-buffer}}$=150 mM; $c_{TEGO\ IL\ K5}$=100 gL^{-1}; c_{MgCl_2}=10 mM; pH=7.5; τ=5.5 h; dV/dt= 2.7 mL/h



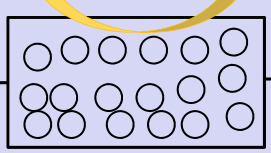
Down Stream Processing & Recycling



The product solution is pumped through the column filled with SPE-material. The product adsorbs on the material and is subsequently eluted with *n*-Heptane.

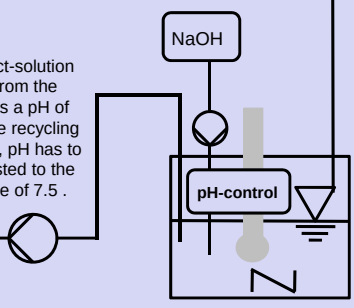
n-Heptane

n-Heptane



Stainless steel column filled with 4.4 g SPE-material HR-P (Macherey-Nagel)

The product-solution coming from the reactor has a pH of ~6.5. Before recycling the solution, pH has to be re-adjusted to the initial value of 7.5 .



Add fresh ketone, $NADP^+$ and Glucose

GC-Analysis

Conclusions

- ✓An ionic solubiliser (TEGO IL K5, Evonik Goldschmidt) is available to increase the ketone-solubility
- ✓Both enzymes are stable under process conditions, high conversions and TON were achieved.
- ✓The SPE-material is stable for more than 25 cycles with different substrate/product-pairs.
- ✓It is possible to recycle the reaction mixture and though decrease the E-factor (kg_{waste} / $kg_{product}$) by a factor of 10.from 130 to 13
- ✓The use of a reactor cascade is favorable compared to a single reactor
- ✓A Michaelis-Menten based model is available to describe the EMR

Outlook

- ➢Automate the whole setup
- ➢Transfer the concept to different substrates
- ➢Establish the product elution with supercritical CO_2

Acknowledgement: Prof. Dr. Marcel Liauw (RWTH Aachen), Macherey Nagel for the supply of the SPE-material, Kerstin Hell and Dr. Peter Schwab (Evonik Goldschmidt) for providing TEGO IL K5, and X-Zyme GmbH for providing *Lb*ADH and GDH

Literature:
General:
[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
Information concerning SPE:
[2] E. Thurman, M. Mills, Solid-Phase Extraction - Principles and Practice, John Wiley, 1998
[3] Solid Phase Extraction Application Guide, Macherey Nagel, 2009

Related work:
[4] 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
[5] 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