DECHEMA FORSCHUNGSINSTITUT

Chemical Technology

Continuous Biocatalytic Synthesis of Enantiopure Alcohols with Integrated Product Separation

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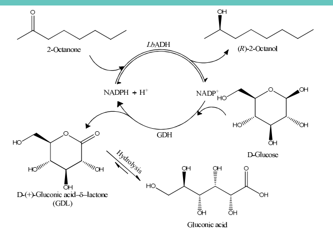
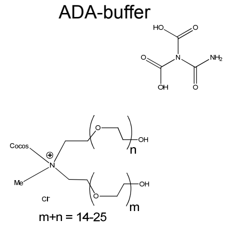
Introduction

- The enantioselective reduction of hardly water-soluble ketones is carried out with an alcohol dehydrogenase from *Lactobacillus brevis* (*Lb*ADH).
- Cofactor Regeneration is performed with a Glucose dehydrogenase (GDH).
- To overcome solubility restrictions of long-chain ketones (2-octanone and 2-nonanone), the ionic liquid (TEGO IL K5) is used as solubiliser.
- The continuous synthesis of the corresponding (*R*)-2-alcohols is carried out in an enzyme membrane reactor (EMR) reactor cascade (two EMR).
- The down-stream-processing (DSP) of the ketone and alcohol is feasible *via* solid phase extraction (SPE) and subsequent elution with *n*-heptane.
- The reaction mixture can be recycled and is re-used as substrate solution.
- Modelling, based on initial rate experiments and a detailed (cost -)analysis of the synthesis was used for further (cost-)optimisation



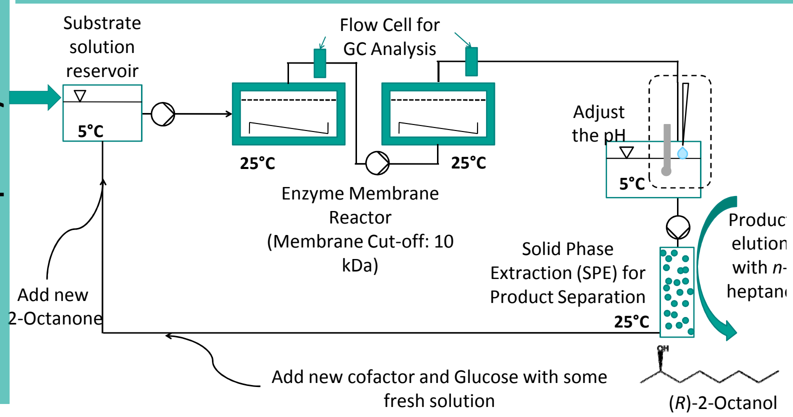
Reaction System used for the production of (*R*)-2-alcohols



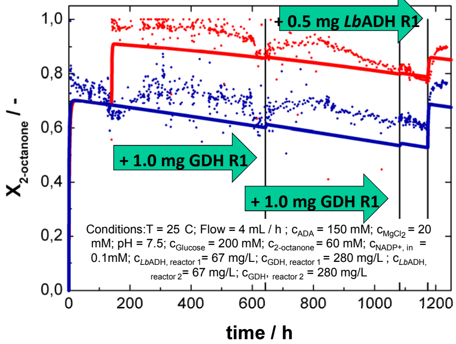
Ionic Liquid TEGO IL K5

Reaction Setup and Synthesis

Reaction Setup



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



Blue= Conversion reactor 1; red = conversion reactor 2; solid lines:prediction from initial rate experiments

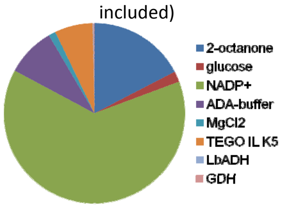
Results (1170 h)	R1	R2
$X_{2\text{-octanone}}$ / -	75.8	96.9
TON_{LbADH} / 10^6	45.6	12.84
TON_{GDH} / 10^6	7.7	2.93
TON_{NADP+}	411	539
STY / mmol L^{-1} d^{-1}	124	291
ee	99.9	99.9

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.

Analysis and possible Improvements

Cost analysis of the process*

Cost distribution for all substances/ %(recycling of substrate solution included, NADP⁺-recycling not included)



Substance	contribution to overall cost / %
2-octanone	17,4
glucose	1,9
NADP⁺	63,5
ADA-buffer	8,8
$MgCl_2$	1,3
TEGO IL K5	6,8
LbADH	0,1
GDH	0,2

A detailed cost analysis reveals: recycling of the substrate solution reduces the costs of glucose, ADA-buffer, MgCl2, and TEGO IL ;overall cost is reduced by ~37% (data not shown); cost analysis of the process including recycling shows: the cofactor contributes with > 60 % to the overall costs

*In collaboration with Joana Lima-Ramos; Naweed Al-Haque; Pär Tufvesson; John M Woodley, DTU Chemical Engineering, Lyngby, Denmark

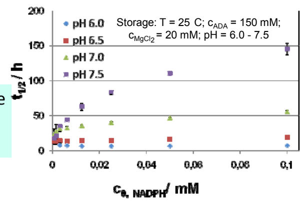
Possible improvements

Recycle cofactor, but the stability of the reduced cofactor is restricted.

Improvement of cofactor utilisation by modelling based on initial rate experiments

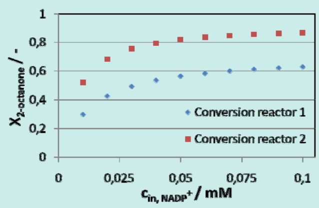
Increase the ratio of substrate/cofactor by decreasing the cofactor inlet concentration

Measurements of Cofactor Half-life



The higher the pH, the higher is the stability of the reduced cofactor. Therefore, the suggested reaction setup is favourable. The pH is adjusted to the initial value immediately after the solution has left reactor 2.

Modelling of the expected conversion using results from initial rate experiments



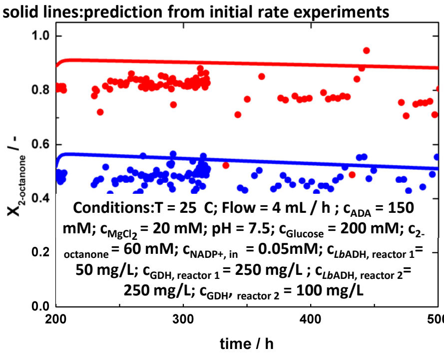
A decrease of the cofactor inlet concentration from 0.1 mM to 0.05 mM is possible at a nearly constant conversion. Reducing the cofactor-concentration further will lead to a reduced conversion (and STY).

Conditions assumed:T = 25 C; Flow = 4 mL / h; c_{ADA} = 150 mM; c_{MgCl_2} = 20 mM; pH = 7.5; $c_{Glucose}$ = 200 mM; $c_{2\text{-octanone}}$ = 60 mM; $c_{NADP+, in}$ = 0.01 – 0.1 mM; $c_{LbADH, reactor 1}$= 50 mg/L; $c_{GDH, reactor 1}$ = 250 mg/L ; $c_{LbADH, reactor 2}$= 250 mg/L; $c_{GDH, reactor 2}$ = 50 mg/L

Improvement

Improved process

Blue= Conversion reactor 1; red = conversion reactor 2; solid lines:prediction from initial rate experiments



Using 0.05 mM of NADP+ in the inlet concentration also leads to a stable process and halves the cofactor cost. Due to the higher ratio *Lb*ADH/GDH in reactor 2, an average of 0.025 mM of the cofactor can be used for recycling at a TON_{NADP+} of 940 per cycle. In total, the cofactor utilisation can be increased ~4fold as revealed by cofactor stability tests and kinetic modelling.

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Summary & Outlook

- ✓ *Lb*ADH and GDH are stable under process conditions, high conversion and TON
- ✓ Possible to recycle the reaction mixture and though decrease the E-factor (kg_{waste} / $kg_{product}$) by a factor of 5 from 123 to 27 and to reduce the overall costs by 37%
- ✓ Cost analysis and kinetic modelling aid to improve the process.
- ➢ Run the process with reduced cofactor concentration for > 1000 h
- ➢ Transfer the concept to different substrates

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 aliphatic ketones:Enabling technology for the enzymatic continuous reduction, 2012, in preparation