Process Chemistry at the Interface of Chemistry and Biology

Andreas Liese

Nordic-Irish Process Chemistry Forum 2023, Belfast, 6.-8.6.2023

Outline

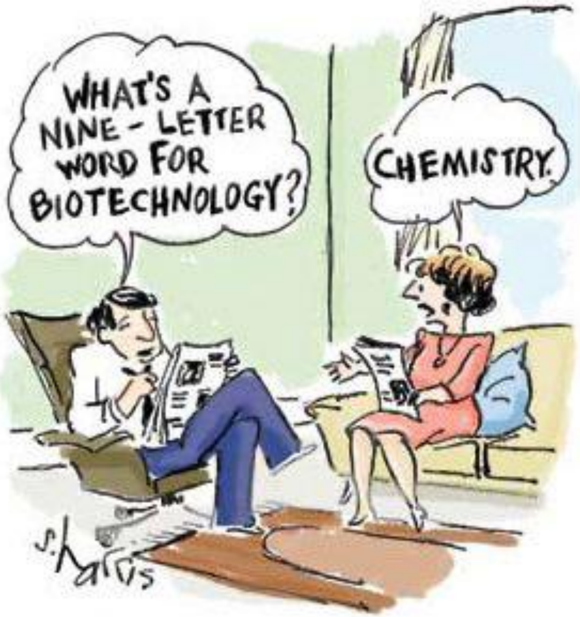
- introduction

- batch processes - moving from aqueous to solvent free synthesis

- intensification of airation

- continuous processes
  of reaction sequences

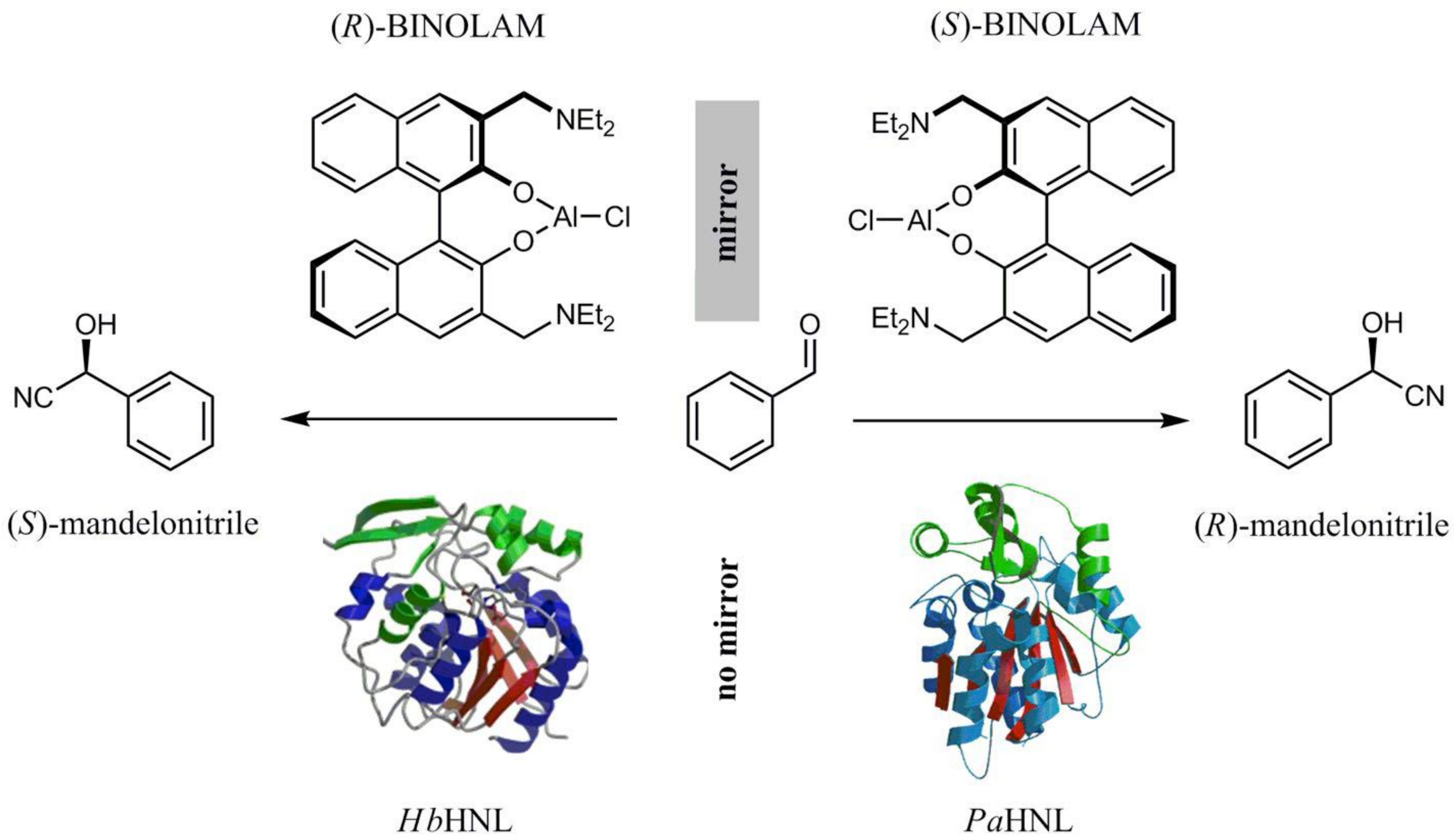
- take home message



TECHNICAL BIOCATALYSIS TUHH

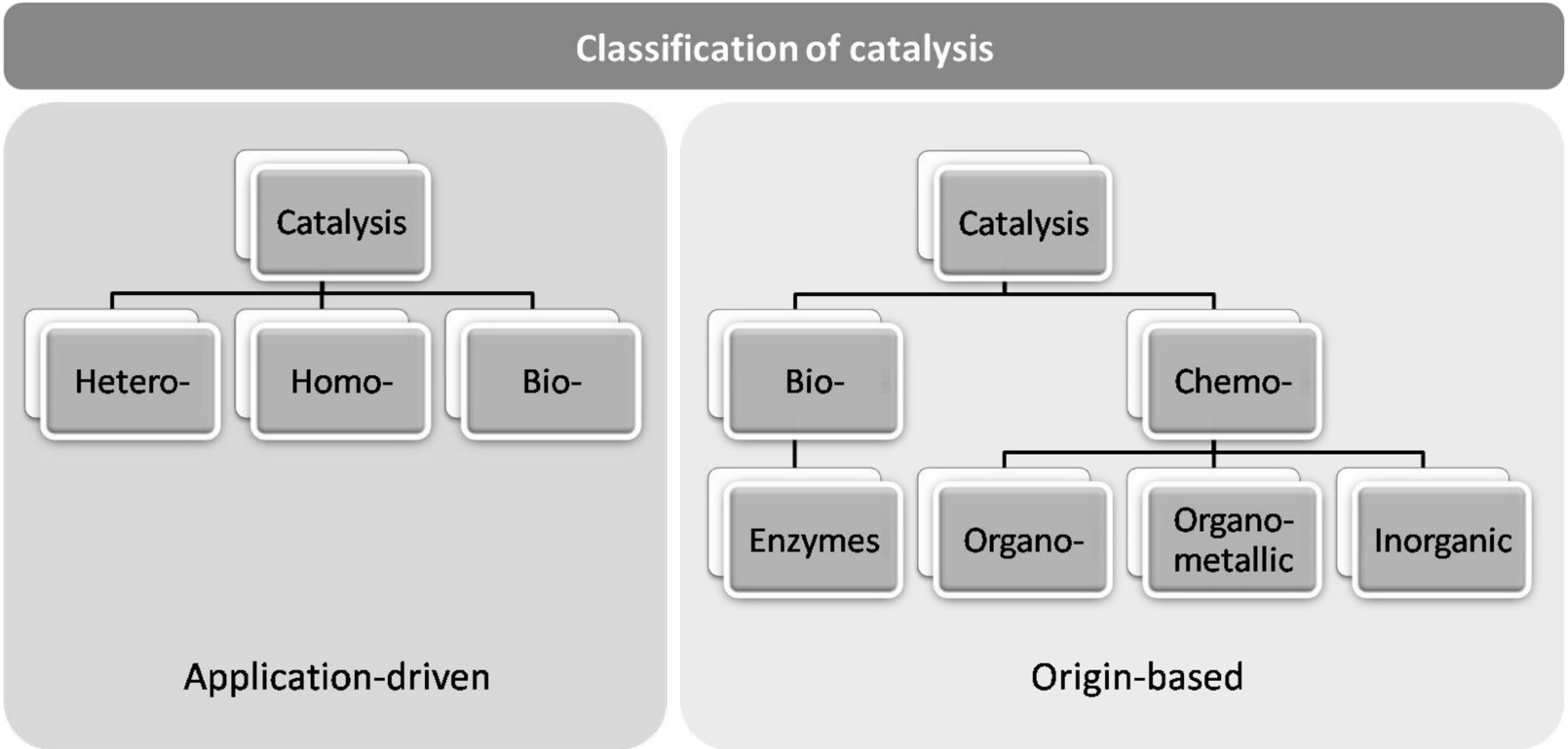
https://acswebcontent.acs.org/biotech/sanfrancisco2006.html

Differences: Chemocatalysis - Biocatalysis



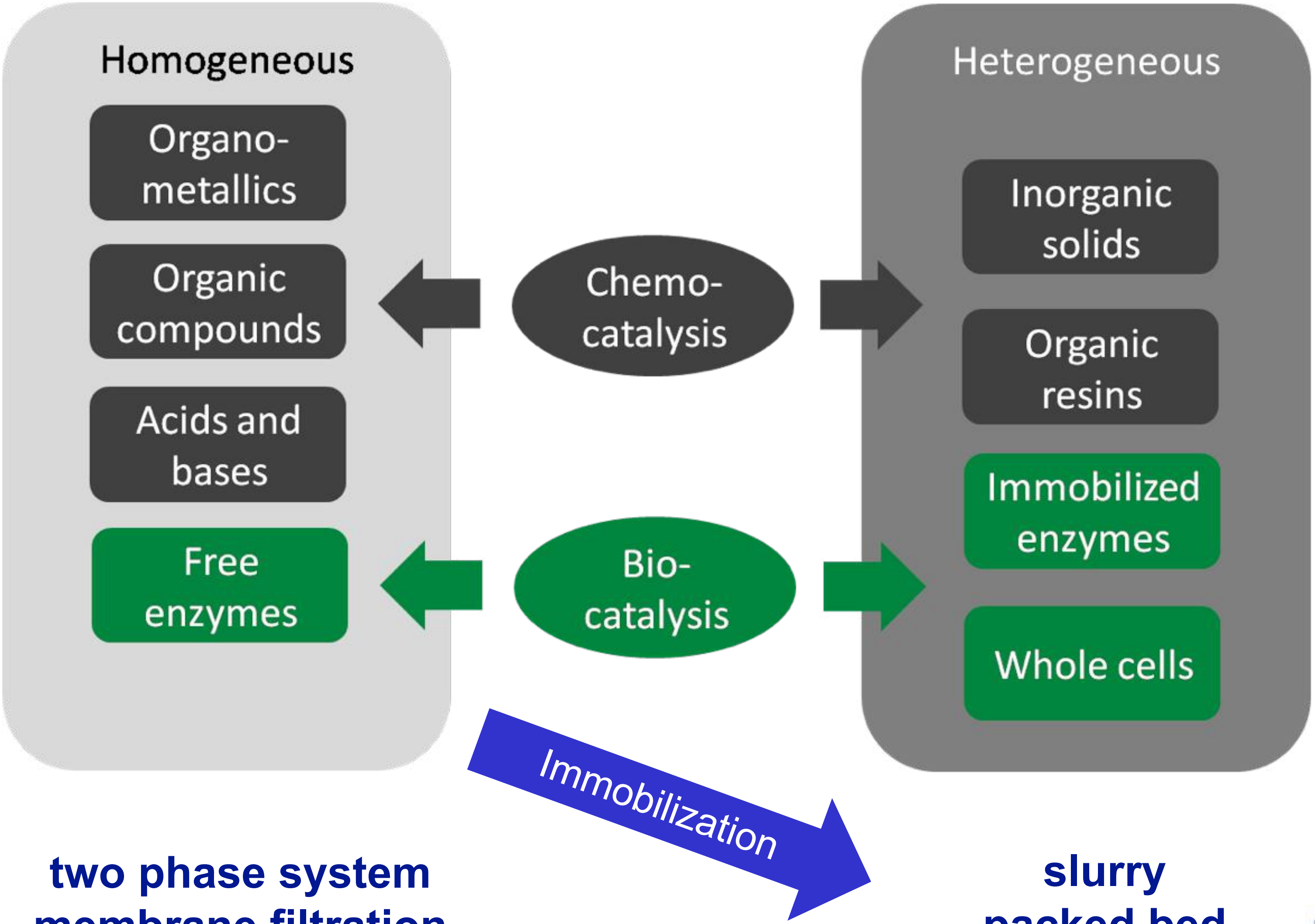
R. Yuryev, A. Liese, *ChemCatChem* 2 (1) (**2010**) 103-107

Classification of Catalysis



R. Yuryev, A. Liese, *ChemCatChem* 2 (1) (**2010**) 103-107

Similarities: Catalyst Preparation

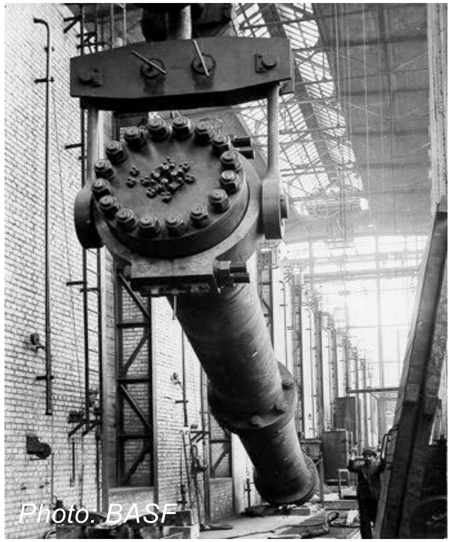


reactor module:	**two phase system** **membrane filtration**	**slurry** **packed bed**

R. Yuryev, A. Liese, *ChemCatChem* 2 (1) (**2010**) 103-107

Missing Technology Developments

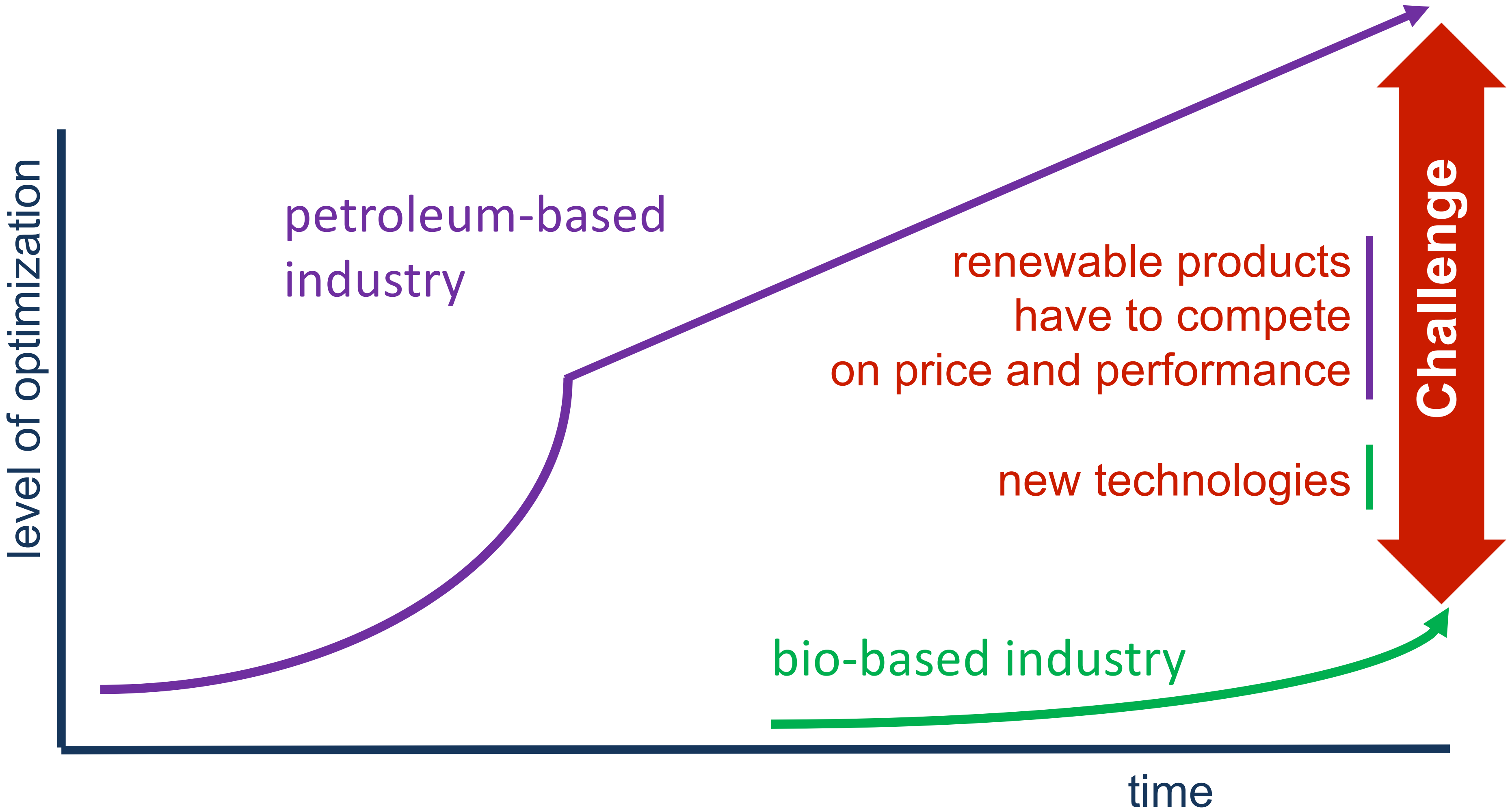
passenger airplane | **chemical reactor**



100 years ago

today

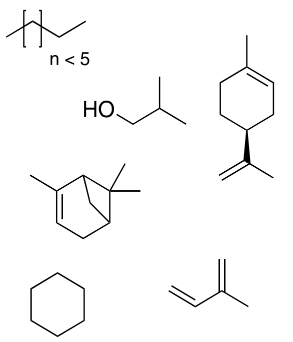
Technology Gap as Challenge



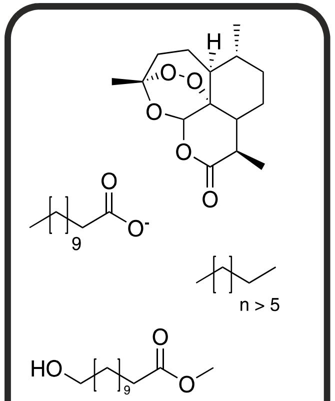
TECHNICAL BIOCATALYSIS TUHH

Change of Raw Material Base

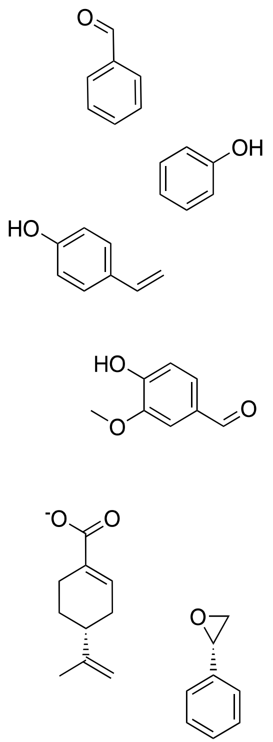
Technical



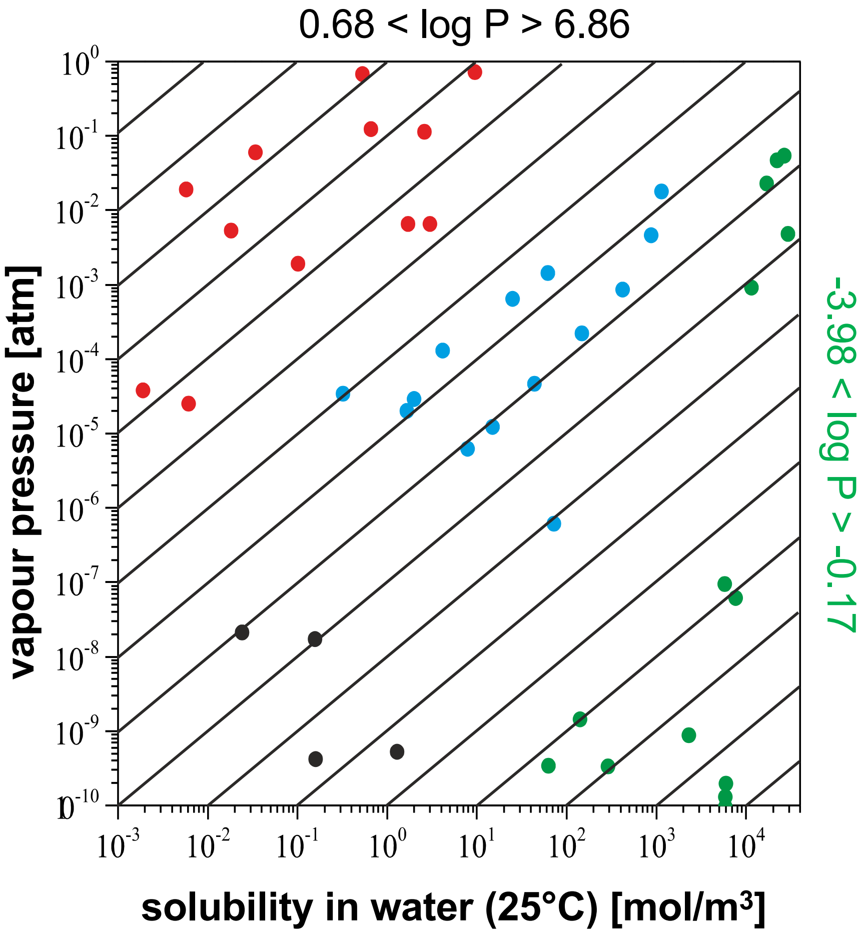
volatile



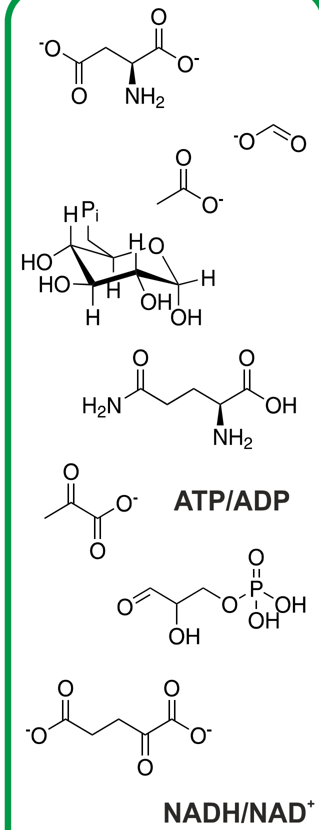
low solubility and low volatility



intermediate solubility/volatility, but often toxic



Natural



central microbial intermediates

Willrodt C, Karande R, Schmid A, Julsing M, *Curr Opin Biotechnol* 31, **2015**, 52–62

TECHNICAL BIOCATALYSIS TUHH

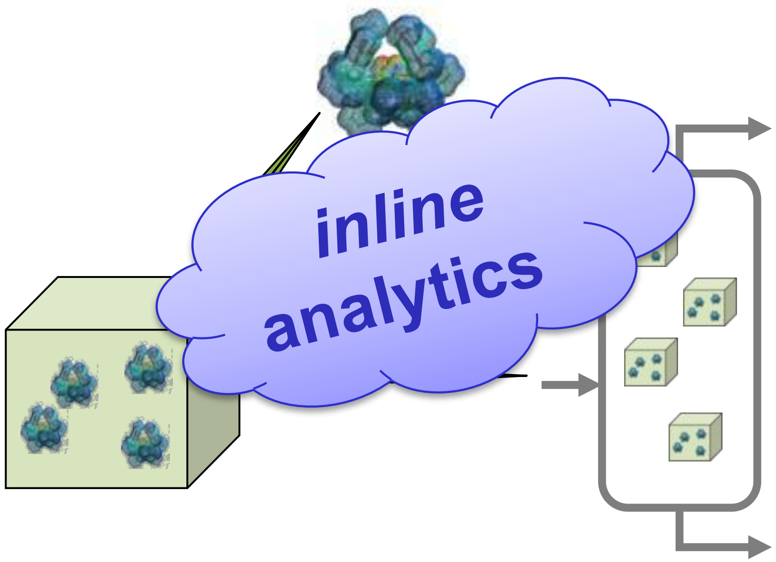
Process Engineering and Integrated Biotechnology

fundamental understanding of the interaction of
biotechnology, **chemistry** and **process engineering**
to realize
chemical / biological processes

A development & adjustment of catalytic steps
- new bio- /chemocatalysts
- kinetics

B directed adjustment to microenvironment

C process engineering integration of catalytic steps and separation processes in a process sequence
- mutliphase mass transport



TECHNICAL BIOCATALYSIS TUHH

A. Liese, K. Seelbach, C. Wandrey: Industrial Biotransformations, Wiley-VCH 2nd , **2006**
B. O. Burek, A. W. H. Dawood, F. Hollmann, A. Liese, D. Holtmann, Front. Cat 2 (**2022**) DOI: 10.3389/fctls.2022.858706

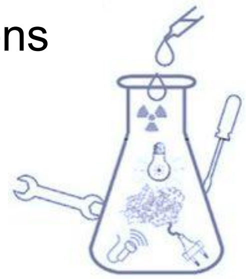
Bioprocess Intensification Challenges

enzyme engineering

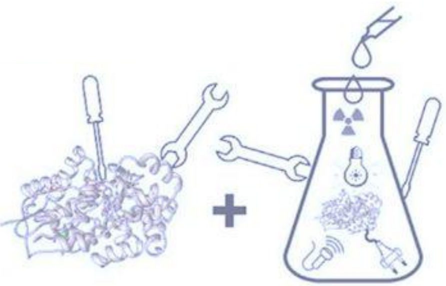
- limited specific activity
- unfavorable kinetics
- limited biocatalyst stability

process engineering

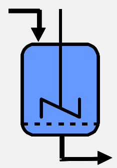
- unfavorable reaction thermodynamics
- selectivity in multistep reactions
- challenging reactants:
  poor water solubility,
  viscosity, volatility, foaming



Σ solution: combined approaches



- **complementary: *substrate inhibition***
  ➔ improve biocatalyst tolerance to the substrate and



- **complementary: *product inhibition***
  ➔ improve biocatalyst tolerance to the product and *in situ* product removal
- **synergistic: *thermodynamic limitation***
  ➔ improve biocatalyst tolerance to substrate
  to enable shift of equilibrium

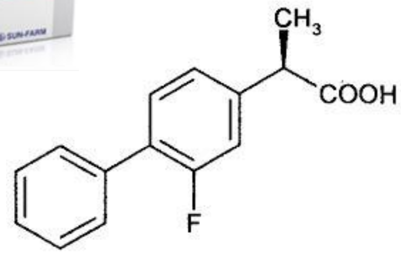
TECHNICAL BIOCATALYSIS TUHH

B. O. Burek, A. W. H. Dawood, F. Hollmann, A. Liese, D. Holtmann,
Front. Cat. (**2022**) DOI: 10.3389/fctls.2022.858706

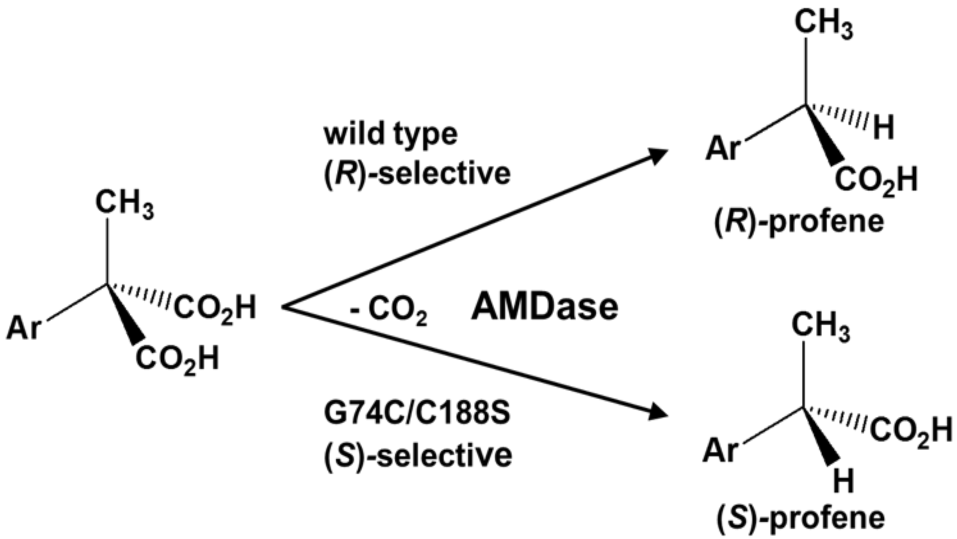
Profen Synthesis by Arylmalonate Decarboxylase

Profenes

- α-arylpropionic acid derivates
- chirale,(*S*)-enantiomer is active
- **50 % yield by chemical synthesis**



Non-steroidal anti inflammatory drugs (NSAID)

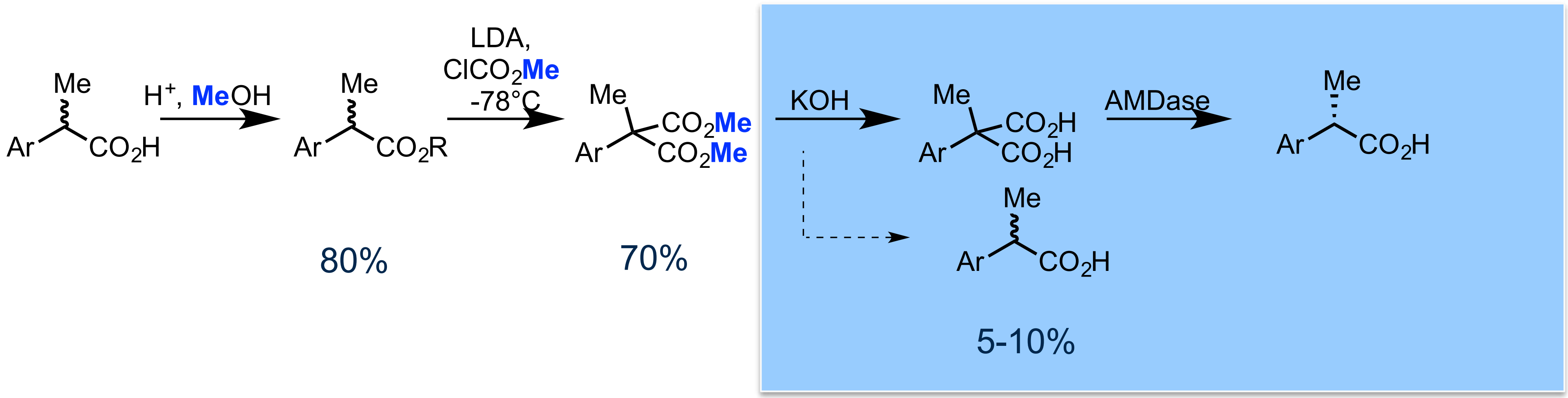


Asymmetric Biocatalysis

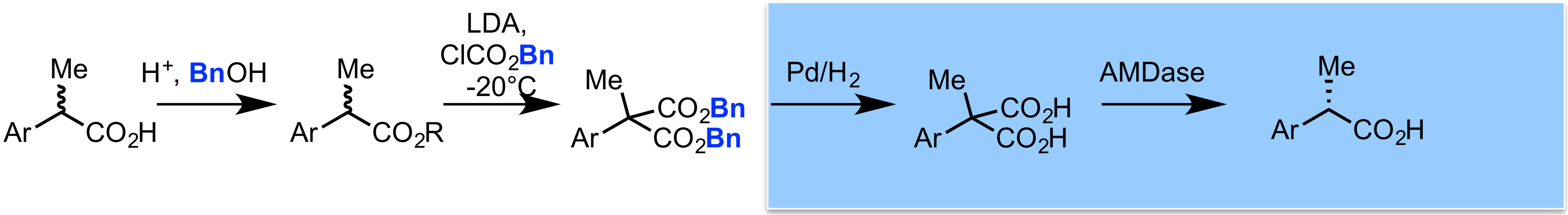
- **up to 100 % yield**
- > 99.5% *ee*
- fewer reaction steps
- mild reaction conditions
- environmentally friendly

Access to Malonates: Optimization of Synthesis

Saponification: The arylmalonic acid undergoes spontaneous decarboxylation



Hydrogenolysis: The side reaction can be suppressed!

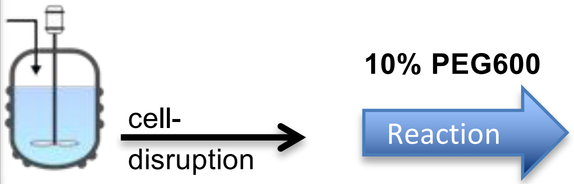


- **less recrystallization required**
- **improved scalability**

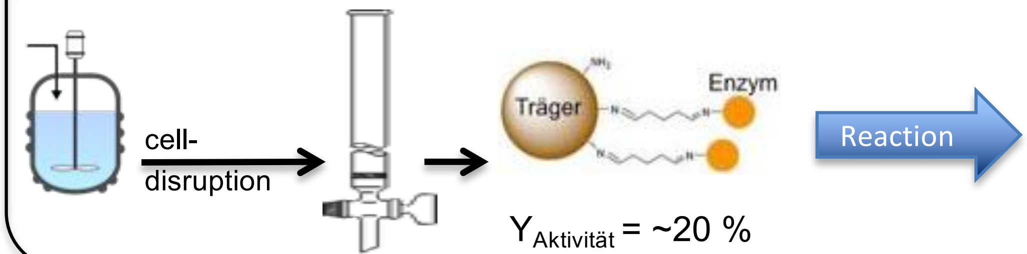
TECHNICAL BIOCATALYSIS TUHH

Biocatalyst Preparation

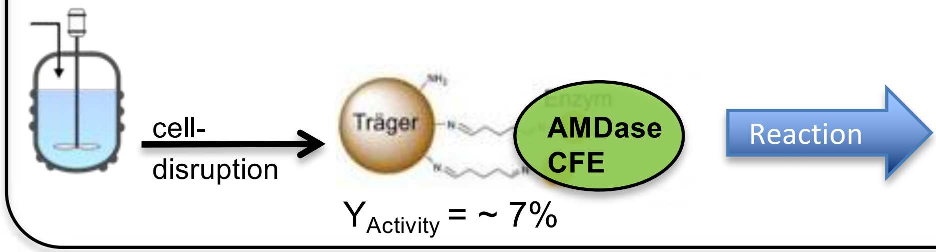
Cell-lysate



Purified enzyme immobilised on Amino C2 Acrylate



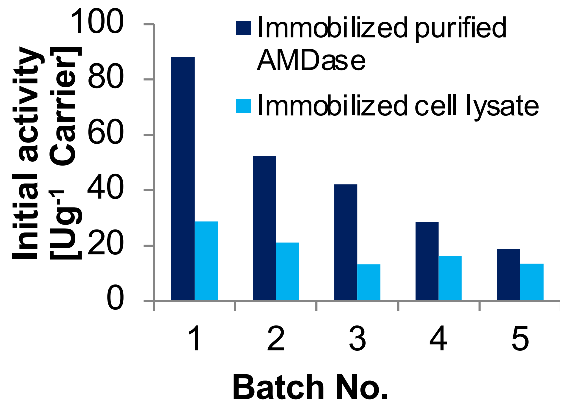
Cell-lysate immobilised on Amino C2 Acrylate



Increase of stability

$t_{1/2\ (free\ enzyme)}$ = 1.5 h

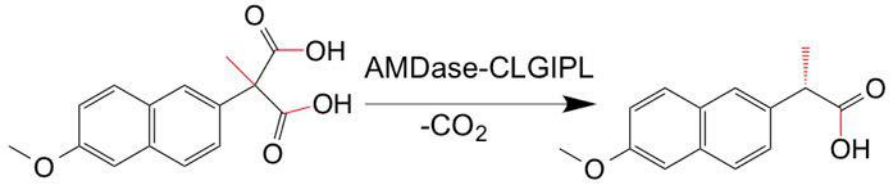
$t_{1/2\ (imm.)}$ = 226 h
(174-fold increase)



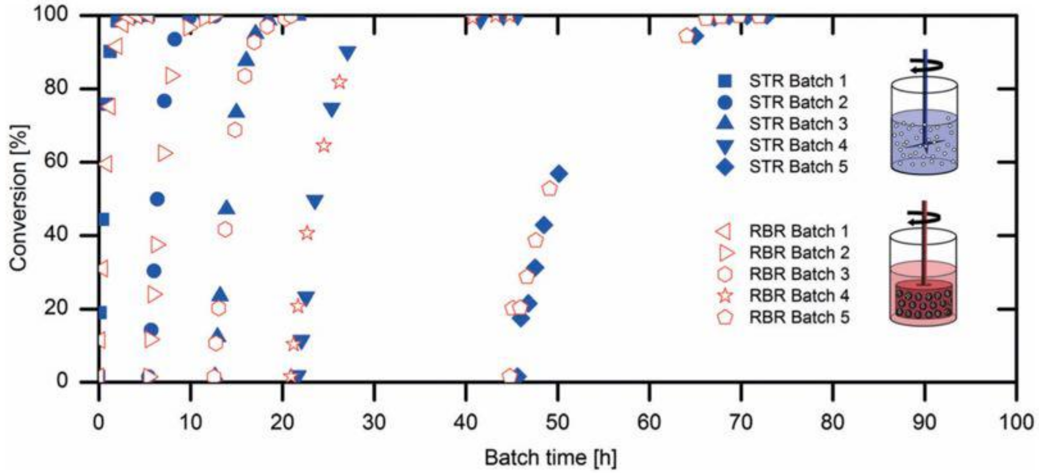
TECHNICAL BIOCATALYSIS TUHH

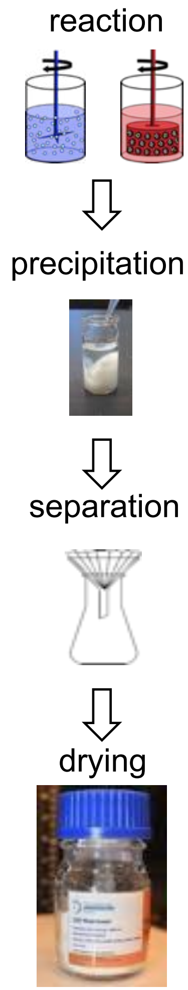
Conditions:Test cell-lysate cell-lysate: 20 mM phenylmalonic acid, 200 mg Amino C2 Acrylate,30°C, 500 rpm,, pH 8, 50 mL
Test PEG600: 20 mM phenylmalonic acid, 9,96 g/L Amino C2 Acrylate, 30°C, 400 rpm,, pH 8, 50 mL, with and w/o10 %(v/v) PEG600.

(*S*)-Naproxen Biosynthesis



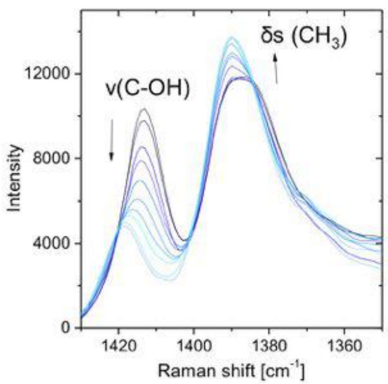
- isolated yield: 92%
- 99% *ee* (*S*)-Naproxen

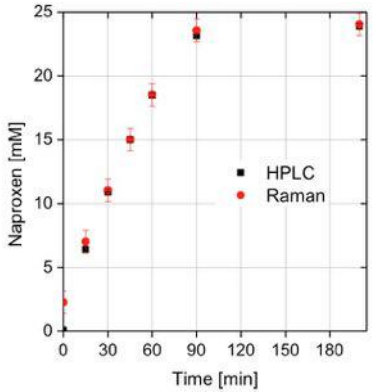




100 mL reaction volume, pH 8, 700 rpm, 30 °C, 45 g/L immobilised AMDase-CLGIPL on Amino C2 Acrylat, 48.6 mM Naproxen malonic acid, 8.6 $mg_{AMDase\ Mutant}/g_{carrier}$

inline Analytics





PLS modeling:
RMSEP: 0.8 mM

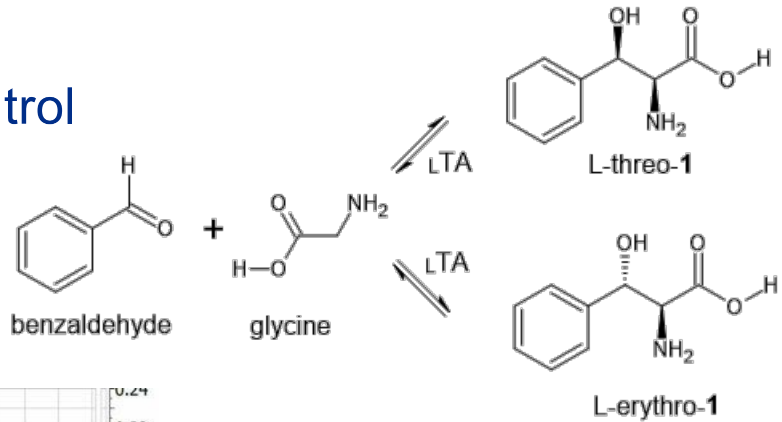
TECHNICAL BIOCATALYSIS TUHH

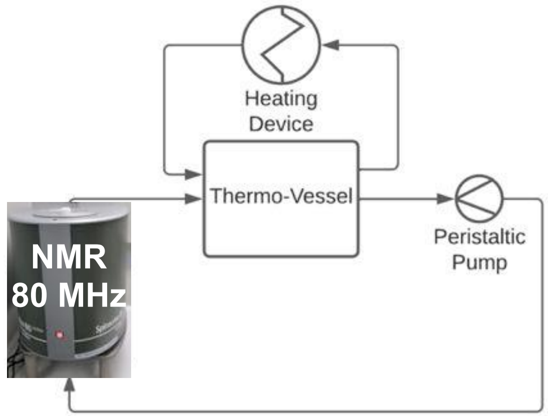
M. Aßmann, A. Stöbener, C. Mügge, S. K. Gaßmeyer, L. Hilterhaus, R. Kourist, A. Liese, S. Kara, *React. Chem. Eng.* **2017**, DOI: 10.1039/c7re00043j

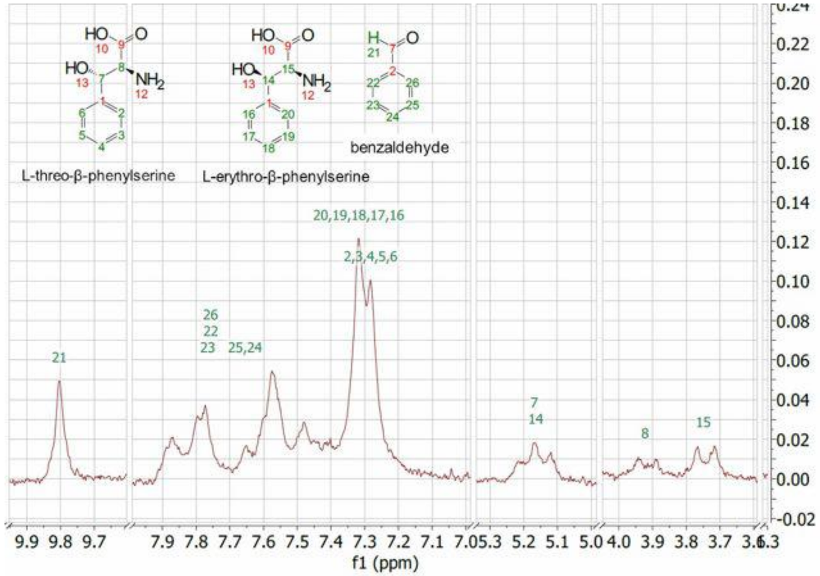
inline Analytics of Diastereomers in Biocatalysis

Threonine Aldolase (TA)

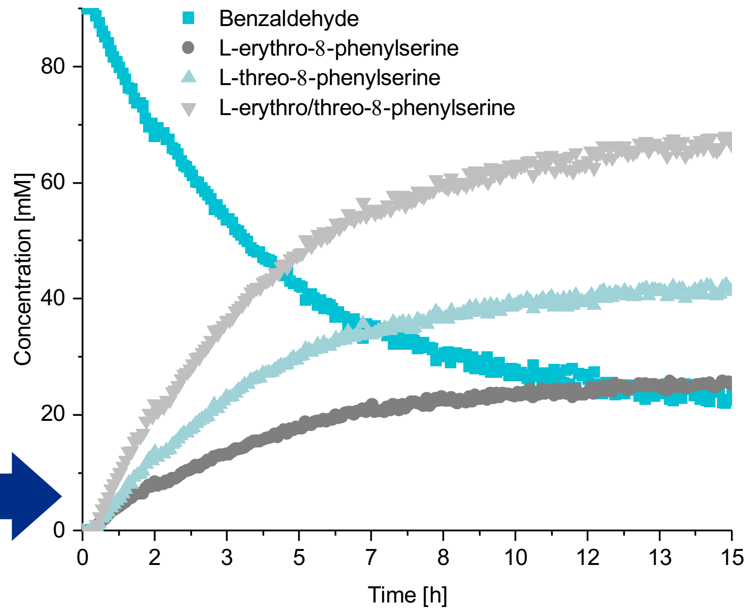
- kinetic/thermodynamic control
- high selectivity at α-C
- low selectivity at β-C







chemometrics



Universität Bielefeld

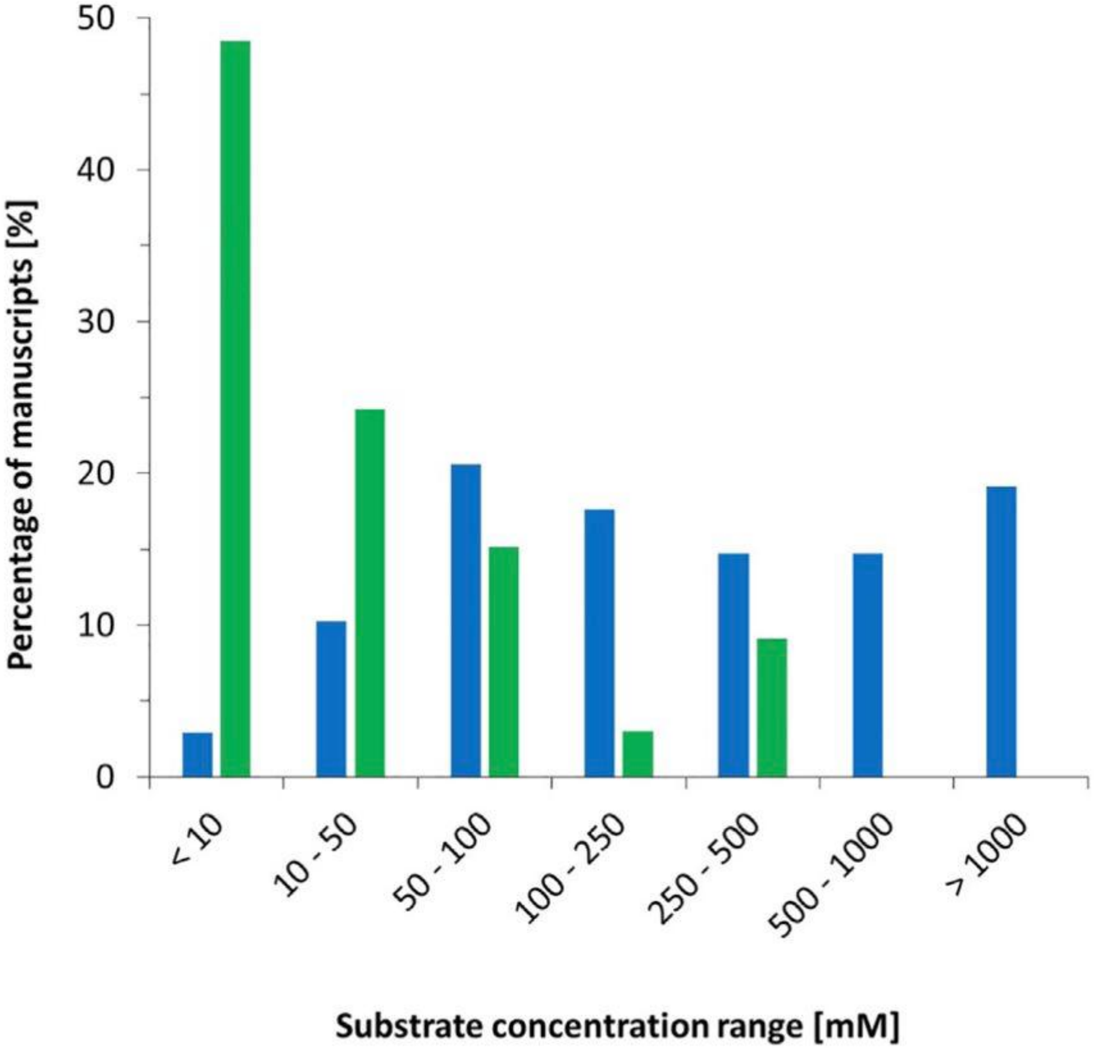
Prof. Harald Gröger

TECHNICAL BIOCATALYSIS TUHH

L. Schmidt, L. Jolly, L. Hennecke, H. Gröger, A. Liese (**2023**) submitted; Steinreiber J, Fesko K, Reisinger C, Schürmann M, van Assema F, Wolberg M, Mink D, Griengl H, Tetrah. 63(4) **2007,** 918ff

Solvents as Key Drivers

for Intensified Biocatalysis



comparison of typical starting material concentrations used in

- **chemocatalysis**
- **biocatalysis**

➔ data from publications in *ChemCatChem* vol. 12 (2020), issues 1–12

B. O. Burek, A. W. H. Dawood, F. Hollmann, A. Liese, D. Holtmann, Front. Cat. (**2022**) DOI: 10.3389/fctls.2022.858706

TECHNICAL BIOCATALYSIS TUHH

Neat Conditions Where Reactants Become Solvents

Levenspiel

- **change of kinetics**
  - high rate
  - prone to inhibition
  - effect on determination of kinetics
- **reactants** themselves become **solvents**
- **shift from concentrations** to
  - weight % (mol %)
  - thermodynamic activities

→ **multidisciplinarity**

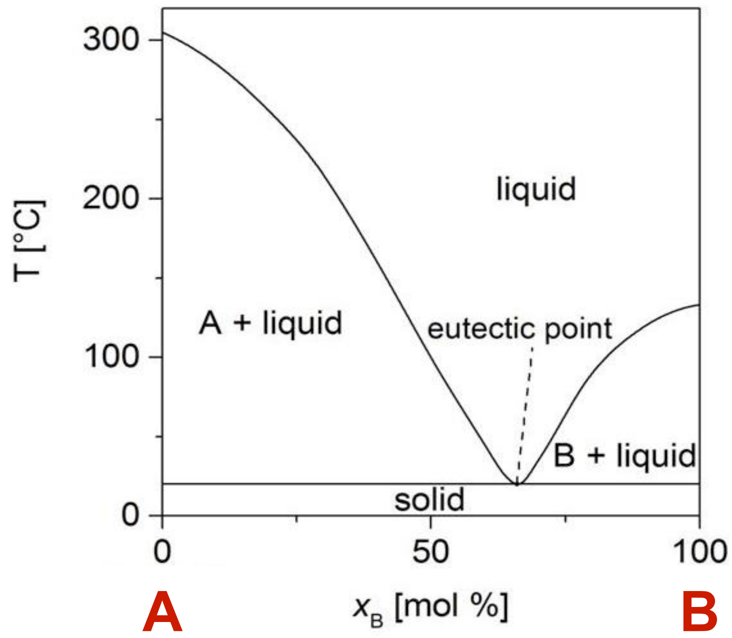
TECHNICAL BIOCATALYSIS TUHH

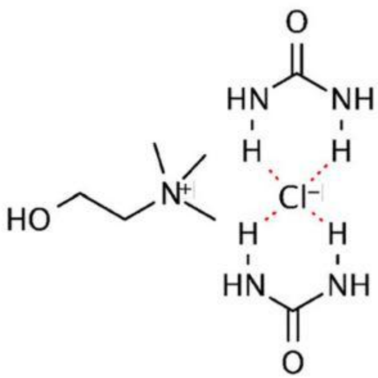
Changing Reaction Media: Deep Eutectic Solvent (DES)

H-bond donor + H-bond acceptor = DES

- Certain **molar ratio** required
- Prepared by **heating** and **stirring**

Viscous liquid due to strong **H-bond network**





choline chloride / urea

+ **physicochemical properties** are **tuneable**

+ **natural precursors → biodegradability**

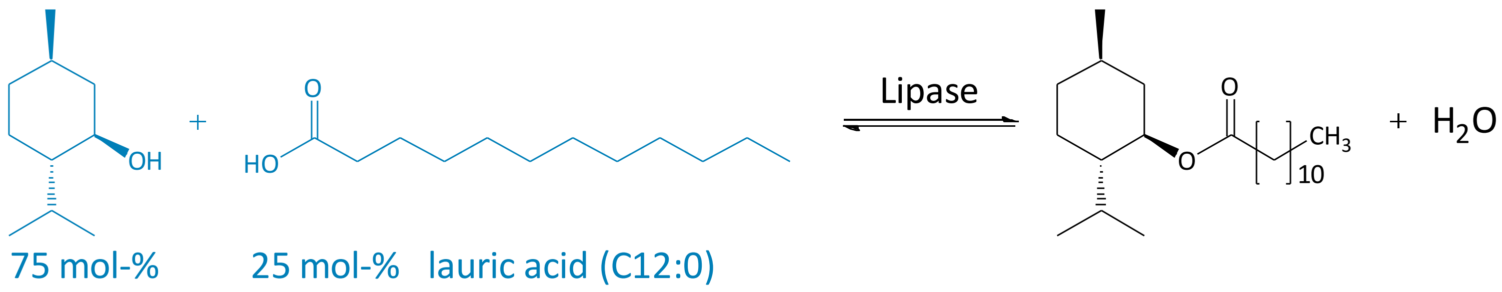
+ **synthesis** is **straightforward** and **cheap**

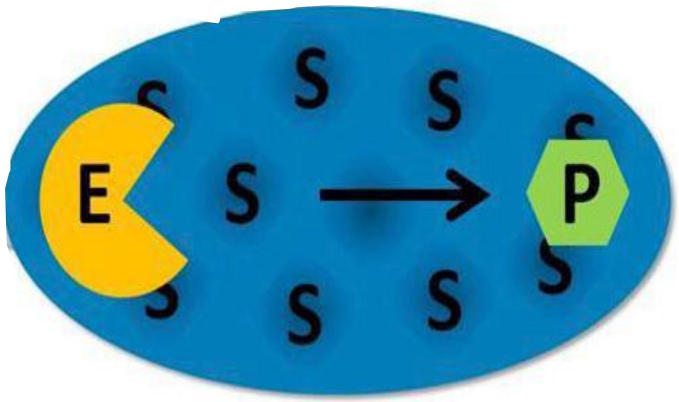
M. Pätzold, S. Siebenhaller, S. Kara, A. Liese, C. Syldatk, D. Holtmann, *Trends in Biotechnology 37(9) (**2019**) 942-959*

KIT
TECHNICAL BIOCATALYSIS TUHH

Prof. Dirk Holtmann

Application Principles of DES in Biocatalysis

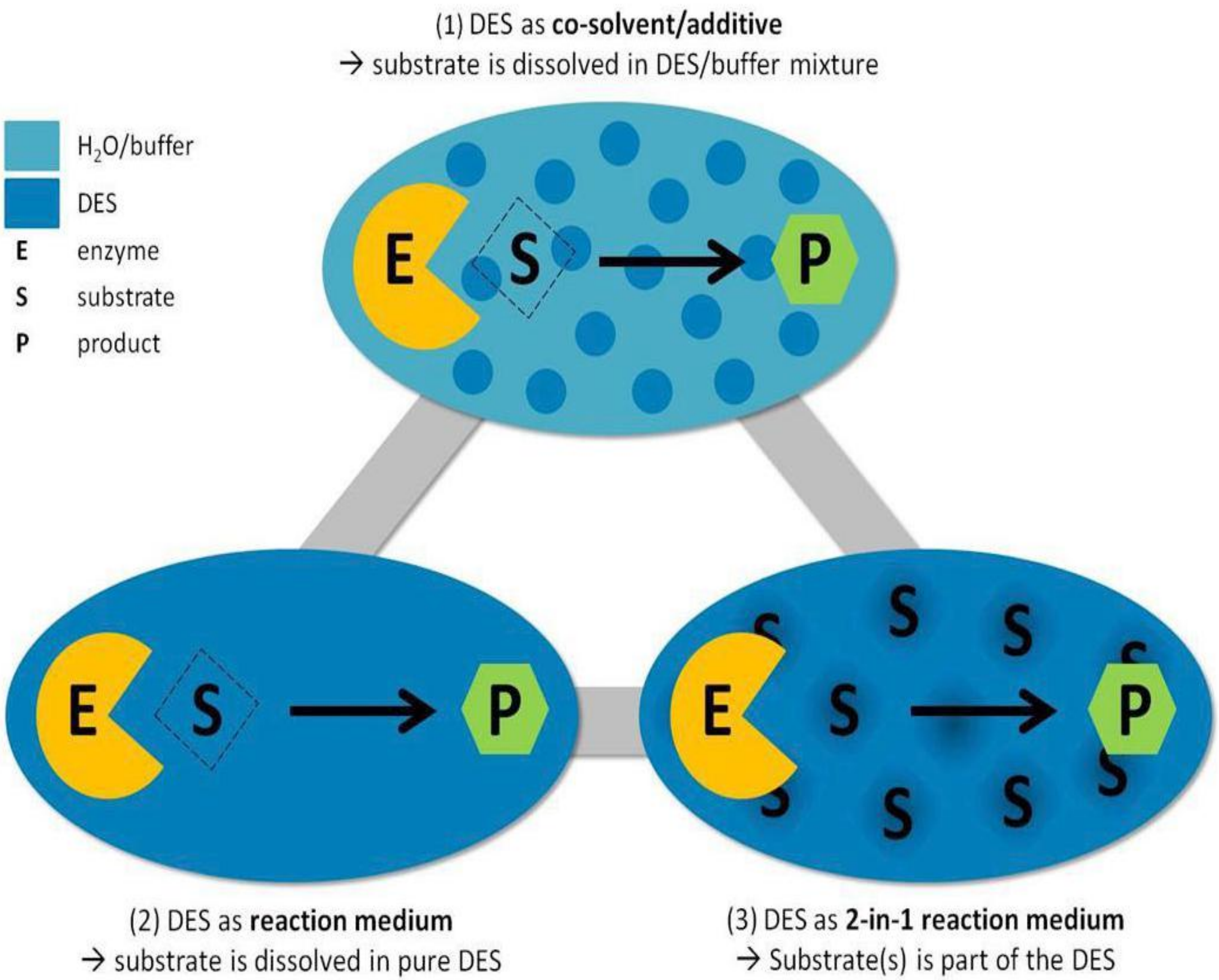




(3) DES as **2-in-1 reaction medium**
→ Substrate(s) is part of the DES

M. Pätzold, S. Siebenhaller, S. Kara, A. Liese, C. Syldatk, D. Holtmann,
*Trends in Biotechnology 37(9) (**2019**) 942-959*

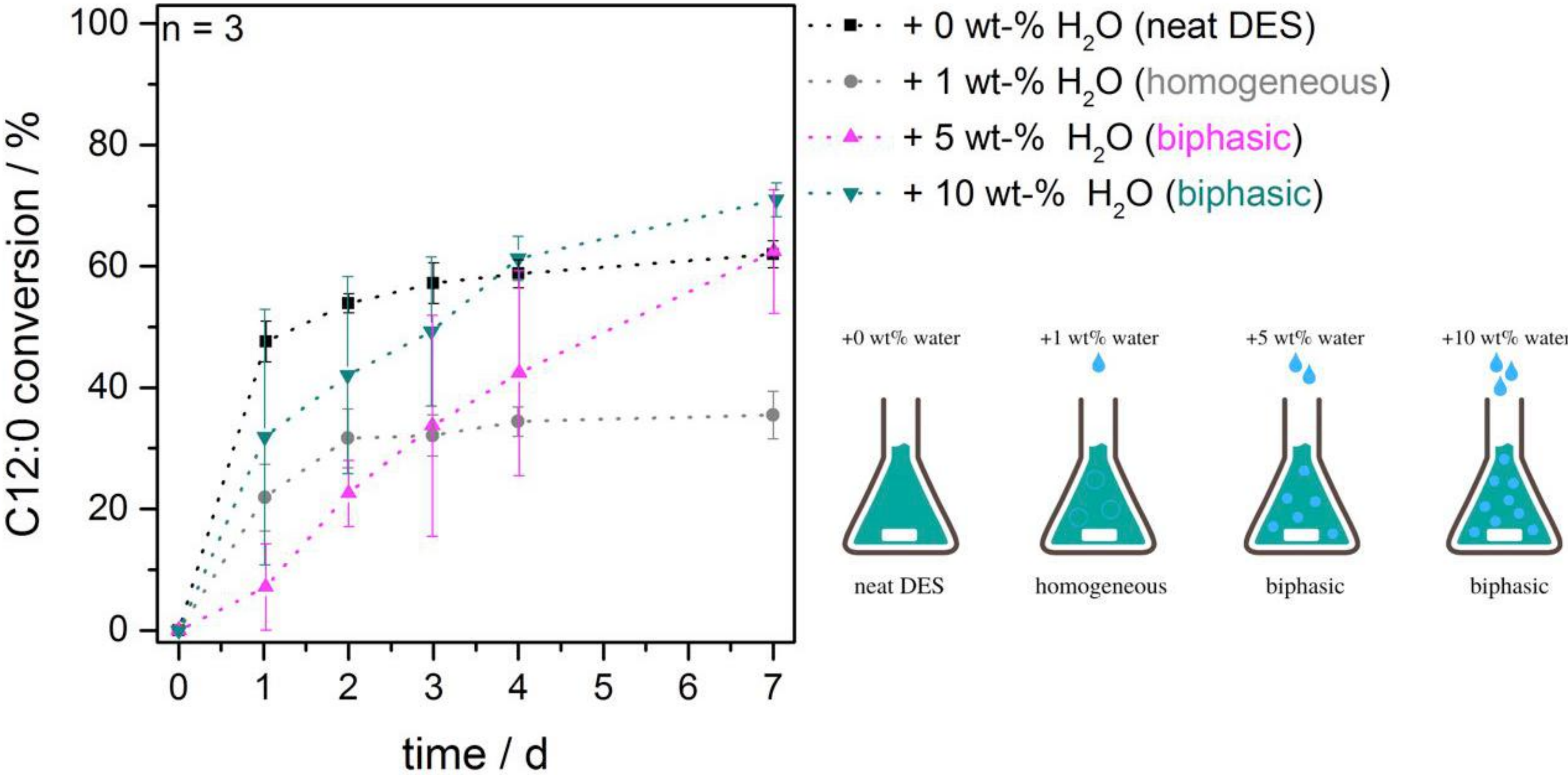
Application Principles of DES in Biocatalysis



M. Pätzold, S. Siebenhaller, S. Kara, A. Liese, C. Syldatk, D. Holtmann,
*Trends in Biotechnology 37(9) (**2019**) 942-959*

TECHNICAL BIOCATALYSIS TUHH

Influence of Water on DES Esterification



- **biphasic reaction systems**
  → saturated with water
  → formed water in equilibrium with aqu. phase

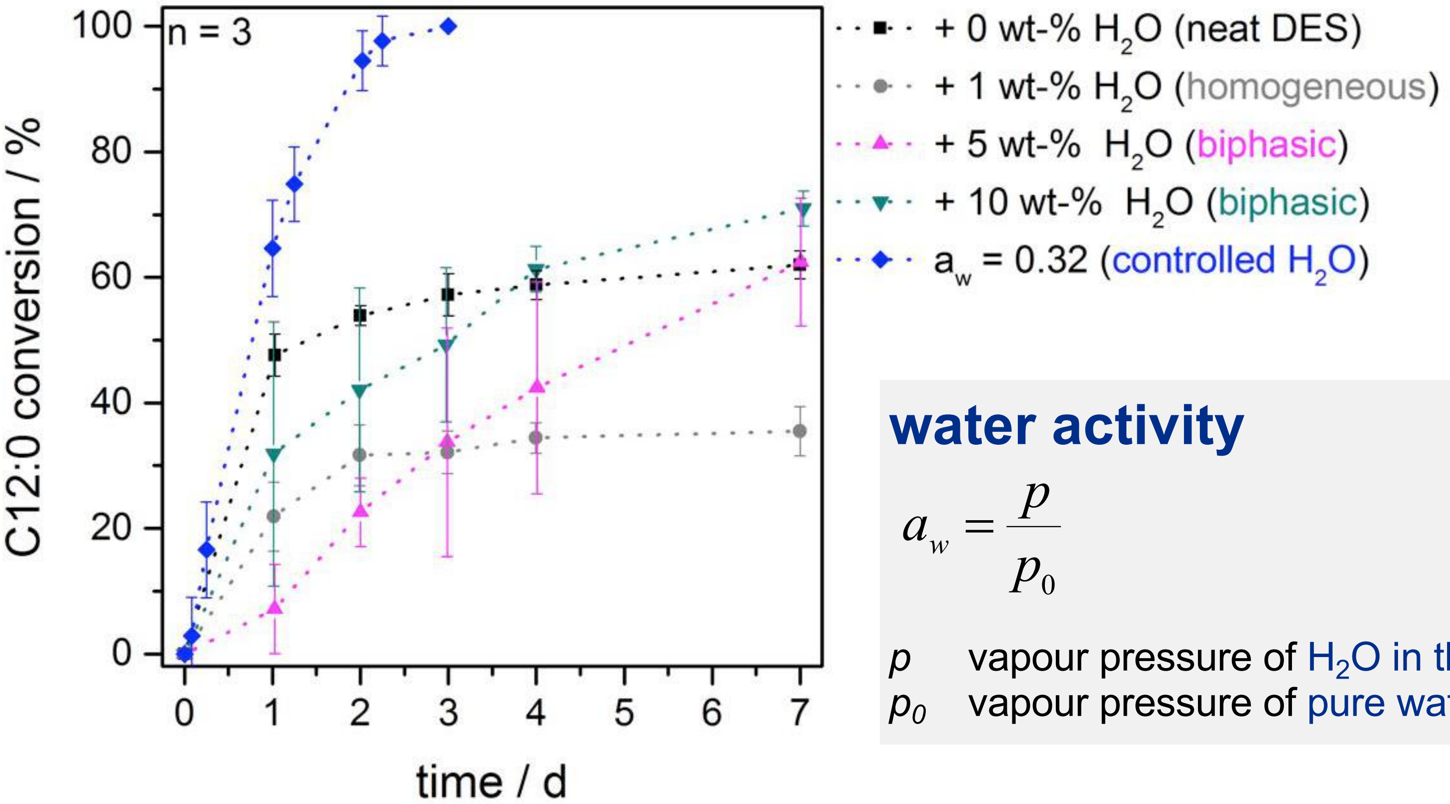
- **neat DES:** water accumulates

(–)-menthol:lauric acid 3:1 mol/mol, m_{DES} = 0.5 g, 5 mg CRL, 700 rpm, T = 35°C, n = 3

M. Hümmer, S. Kara, A. Liese, I. Huth, J. Schrader, D. Holtmann, Mol. Cat. 458 (**2018**) 67-72
M. Pätzold, A. Weimer, A. Liese, D. Holtmann, Biotechnol. Reports 24 (**2019**) DOI: 10.1016/j.btre.2019.e00333

TECHNICAL BIOCATALYSIS TUHH

Effect of Water Activity Controlled Esterification



water activity

$$a_w = \frac{p}{p_0}$$

p vapour pressure of H_2O in the matrix

p_0 vapour pressure of pure water

→ **water content** of the DES is controlled **during reaction**

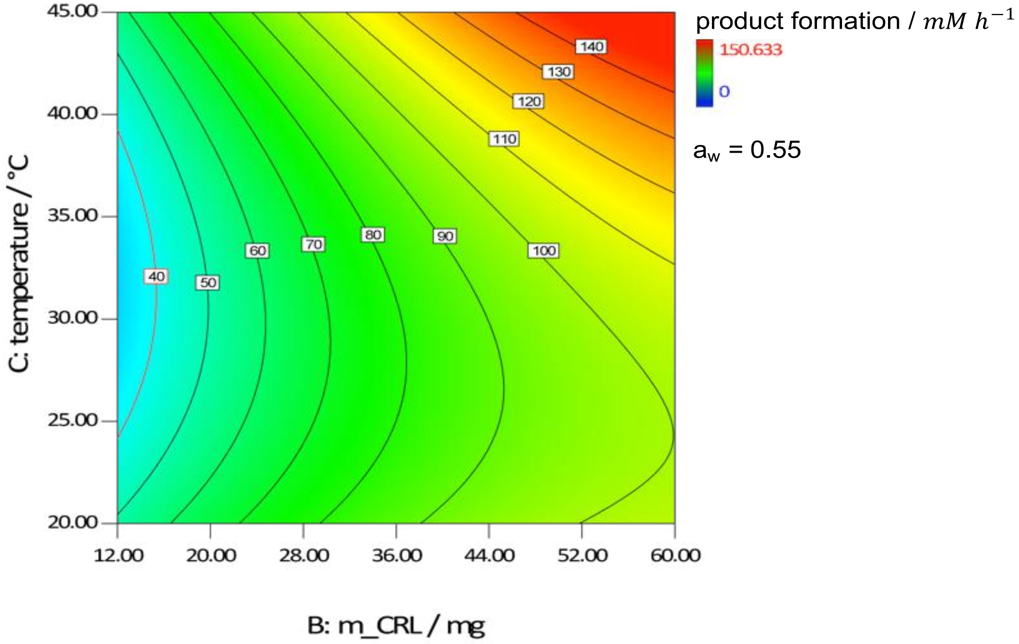
→ **optimal water levels** generated with a_w = 0.22 - 0.55

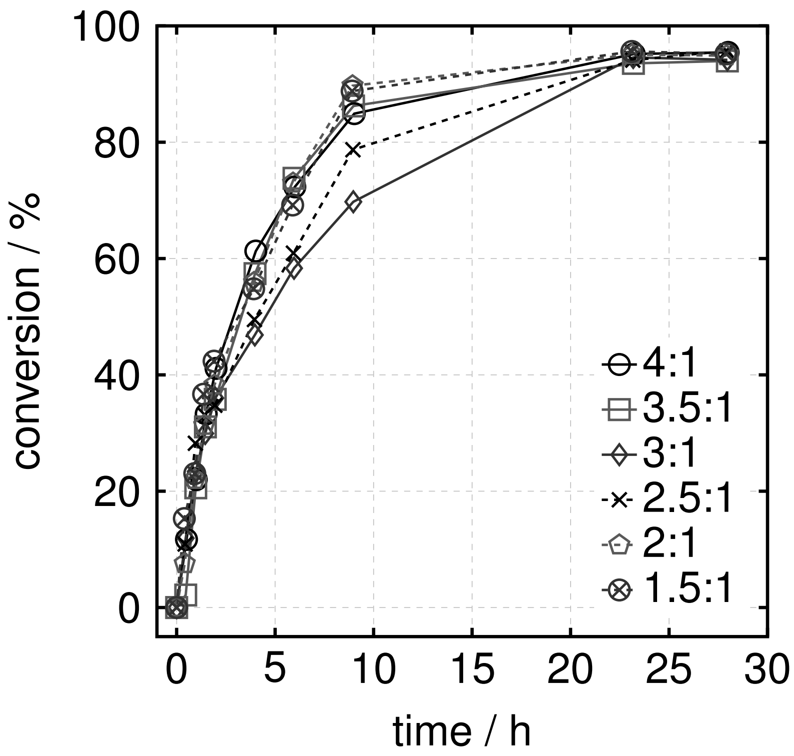
→ **full conversion** with a_w = 0.32 after 3 d

M. Hümmer, S. Kara, A. Liese, I. Huth, J. Schrader, D. Holtmann, Mol. Cat. 458 (**2018**) 67-72
M. Pätzold, A. Weimer, A. Liese, D. Holtmann, Biotechnol. Reports 24 (**2019**) DOI: 10.1016/j.btre.2019.e00333

DoE Based Process Optimization

Design of Experiment (DoE)





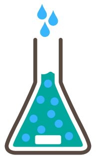
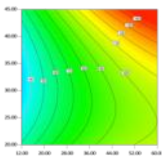
model calculates conditions:

- **a_w = 0.55**
- **m_{CRL} = 60 mg**
- **T = 45 °C**

→ **95 % conversion** (23 h)
for all compositions

Pre-incubation (DES): 3 d, 45 °C, NaBr
Reaction: 3 mmol lauric acid, 60 mg CRL (10 wt% $m_{CRL}/m_{C12:0}$), 900 rpm, 45°C, $a_{w,NaBr}$ = 0.52 (n = 1)

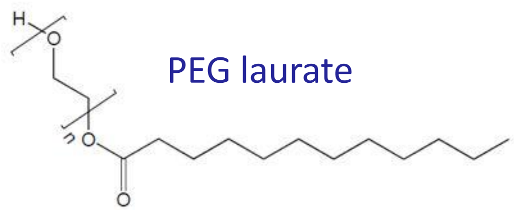
Comparison of Reaction Systems

Reaction system		Productivity $g\ L^{-1}\ d^{-1}$	E-factor
	DES	205	2.2
	biphasic DES + water	133	1.8
	**controlled water activity**	**443**	**1.1**

$$\text{E-factor} = \frac{m_{waste}}{m_{product}}$$

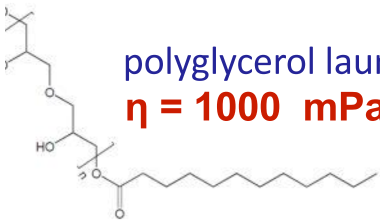
bulk	< 1 – 5
fine	up to 50
pharma	up to 100

Fatty Acid Esters – NEAT Synthesis

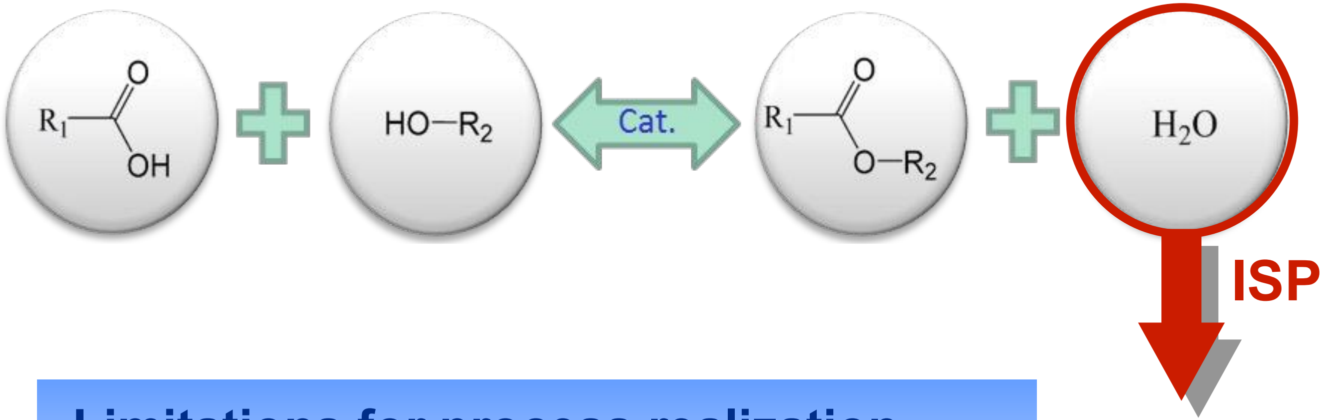


PEG laurate

myristyl myristate
η = 6 mPa*s



polyglycerol laurate
η = 1000 mPa*s



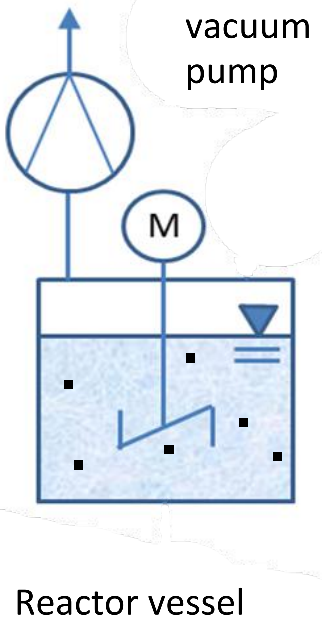
Limitations for process realization

- equilibrium of reaction
- difference in polarity
- viscosity → **high temperature**

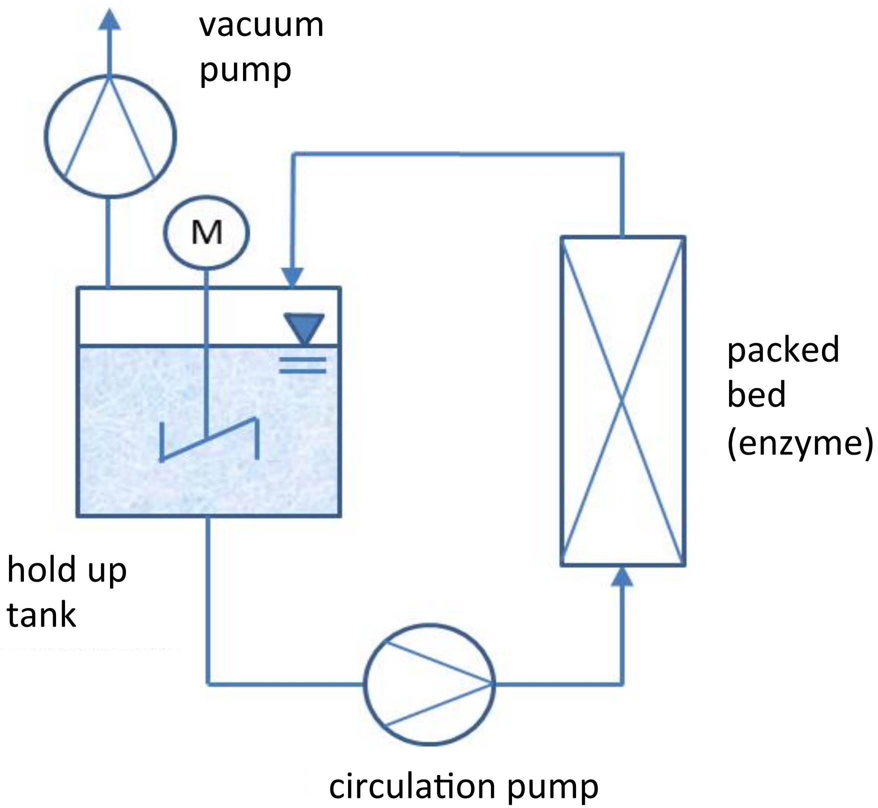
L. Hilterhaus, O. Thum, A. Liese, Org. Proc. Res. Dev. 12 (4) (**2008**) 618
K. Goldberg, K. Edegger, W. Kroutil, A. Liese, Biotechnol. Bioeng., 95 (**2006**) 192-198

Multiphase Reactor Types

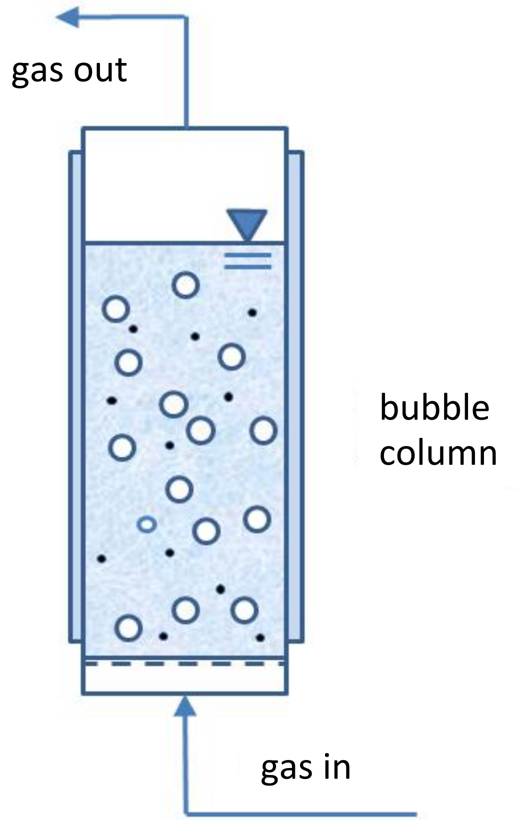
STR



packed bed reactor



bubble column reactor



simultaneous **water removal & reaction**

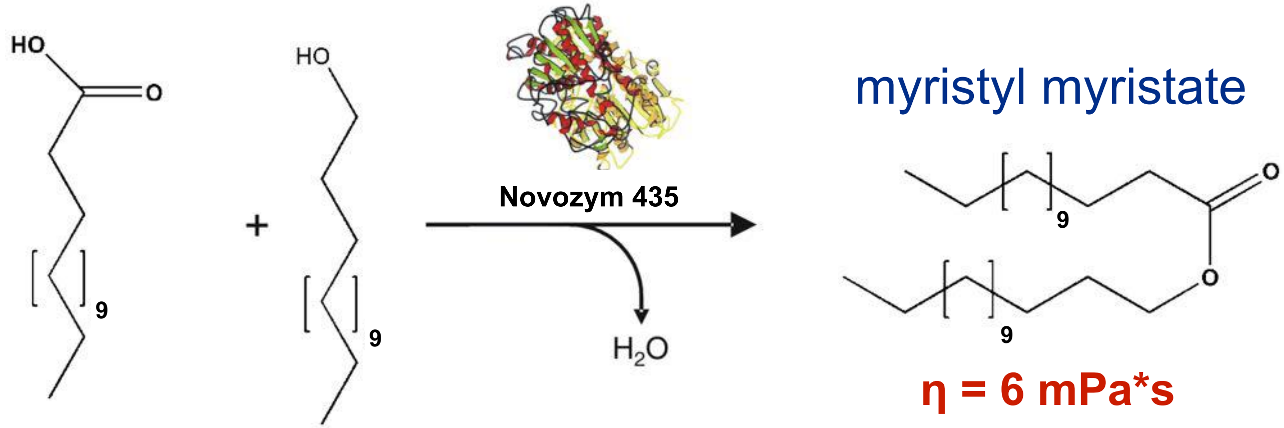
separated **water removal & reaction**

simultaneous **water removal & reaction**

TECHNICAL BIOCATALYSIS TUHH

L. Hilterhaus, O. Thum, A. Liese, Org. Proc. Res. Dev. 12 (4) (**2008**) 618-625
J.J. Müller, M. Neumann, P. Scholl, L. Hilterhaus, M. Eckstein, O. Thum, A. Liese, Anal. Chem. (**2010**) 82 (14) 6008

Comparison of Reactor Concepts



η = 6 mPa*s

nonpolar substrates & product

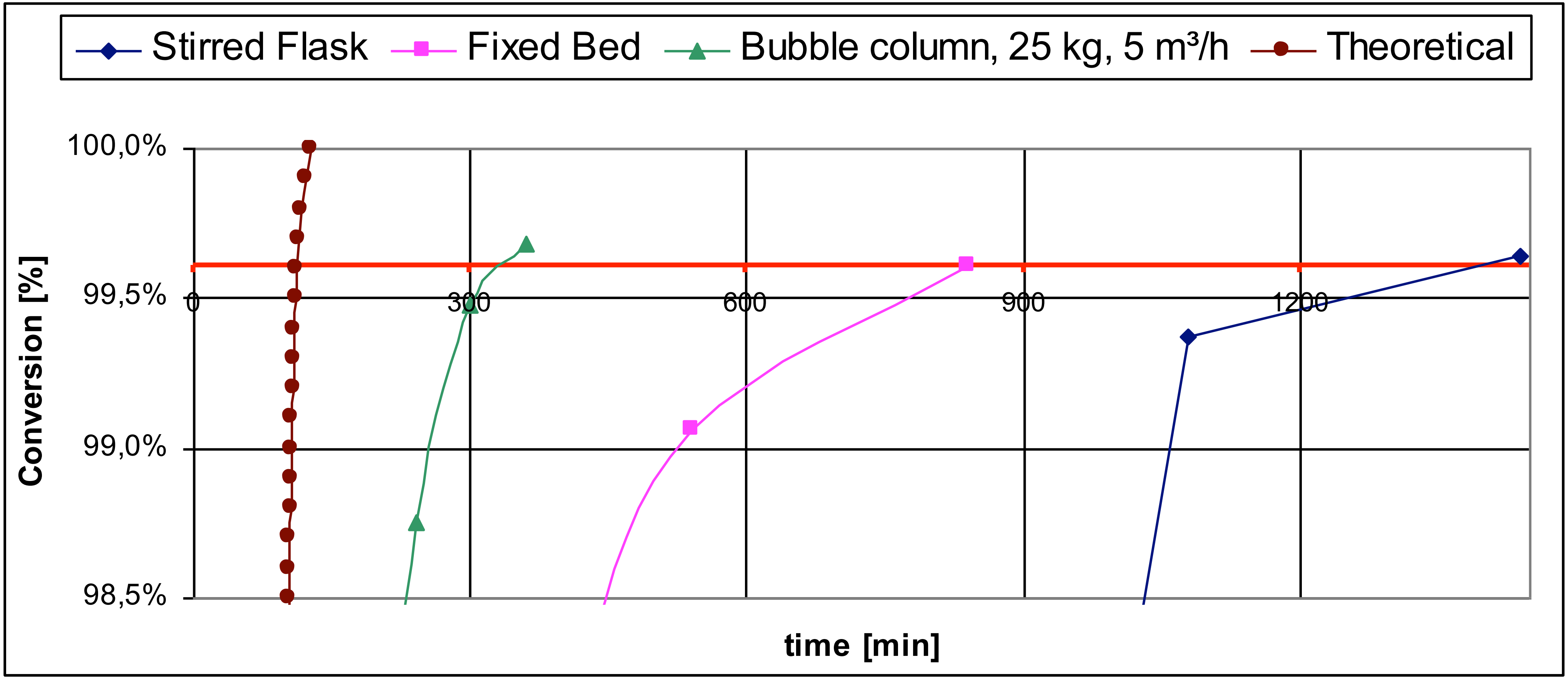
- all reactions: 0.4 weight-% Novo 435
- “stirred tank”: 1 L stirred flask, 60°C, 5 mbar
- “fixed bed”: fresh enzyme
- “bubble column”: pilot plant scale
- ”theoretical”: integrated Michaelis Menten equation:

$$TR_n = \frac{[S_0]-[S]}{v_{\max}} + \frac{K_M}{v_{\max}} \cdot \ln \frac{[S_0]}{[S]}$$

TECHNICAL BIOCATALYSIS TUHH

L. Hilterhaus, O. Thum, A. Liese, Org. Proc. Res. Dev. 12 (4) (**2008**) 618-625

Comparison of Reactor Concepts

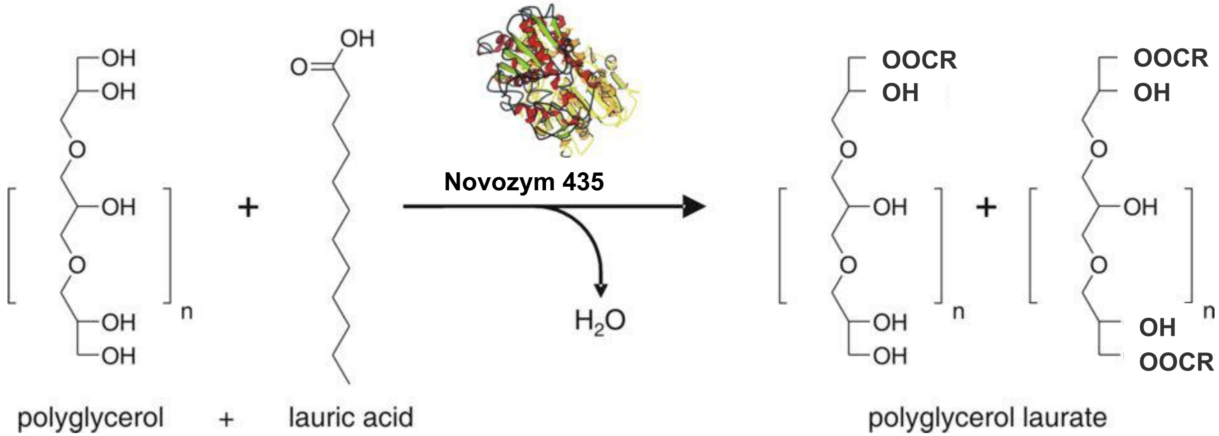


- **bubble column in pilot scale cuts reaction time by half**

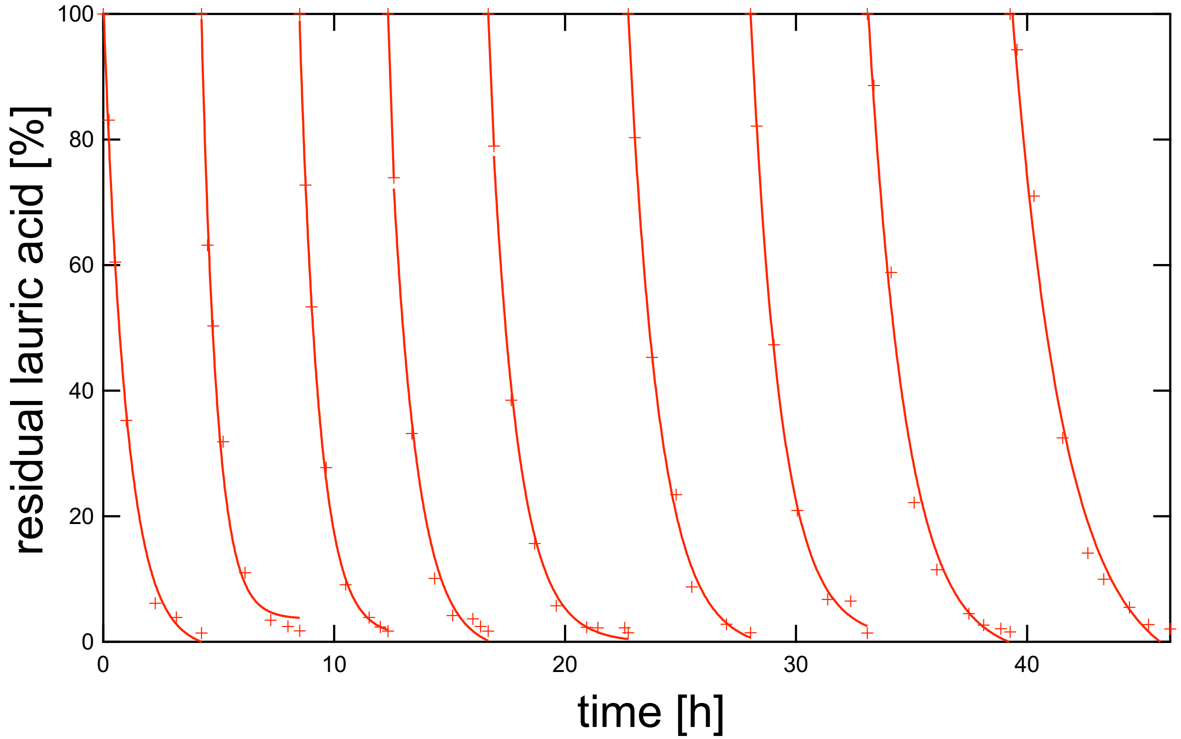
TECHNICAL BIOCATALYSIS TUHH

L. Hilterhaus, O. Thum, A. Liese, Org. Proc. Res. Dev. 12 (4) (**2008**) 618-625
J.J. Müller, S Baum, L. Hilterhaus, M. Eckstein, O. Thum, A. Liese, Anal Chem. 83 (24) (**2011**) 9321

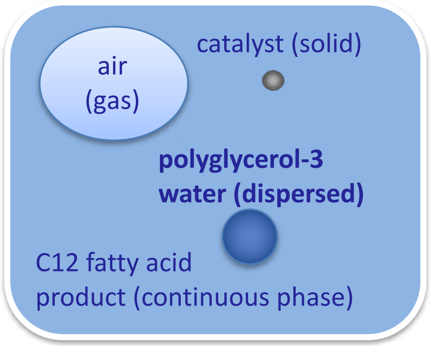
Repetitive Batch @ High Viscosity @ 75 °C



η = 1000 mPa*s



- **polar & nonpolar substrates**
- **4-phase system**



- C12 fatty acid (nonpolar)
- polyglycerol-3 (polar)
- polyglycerol-3-laurate (polar/nonpolar)
- water (polar)

TECHNICAL BIOCATALYSIS TUHH

L. Hilterhaus, O. Thum, A. Liese, Org. Proc. Res. Dev. 12 (4) (**2008**) 618-625

J.J. Müller, S Baum, L. Hilterhaus, M. Eckstein, O. Thum, A. Liese, Anal Chem. 83 (24) (**2011**) 9321

Projects and Products: Example

- **Bubble Column Reactors for Enzymatic Esterification**

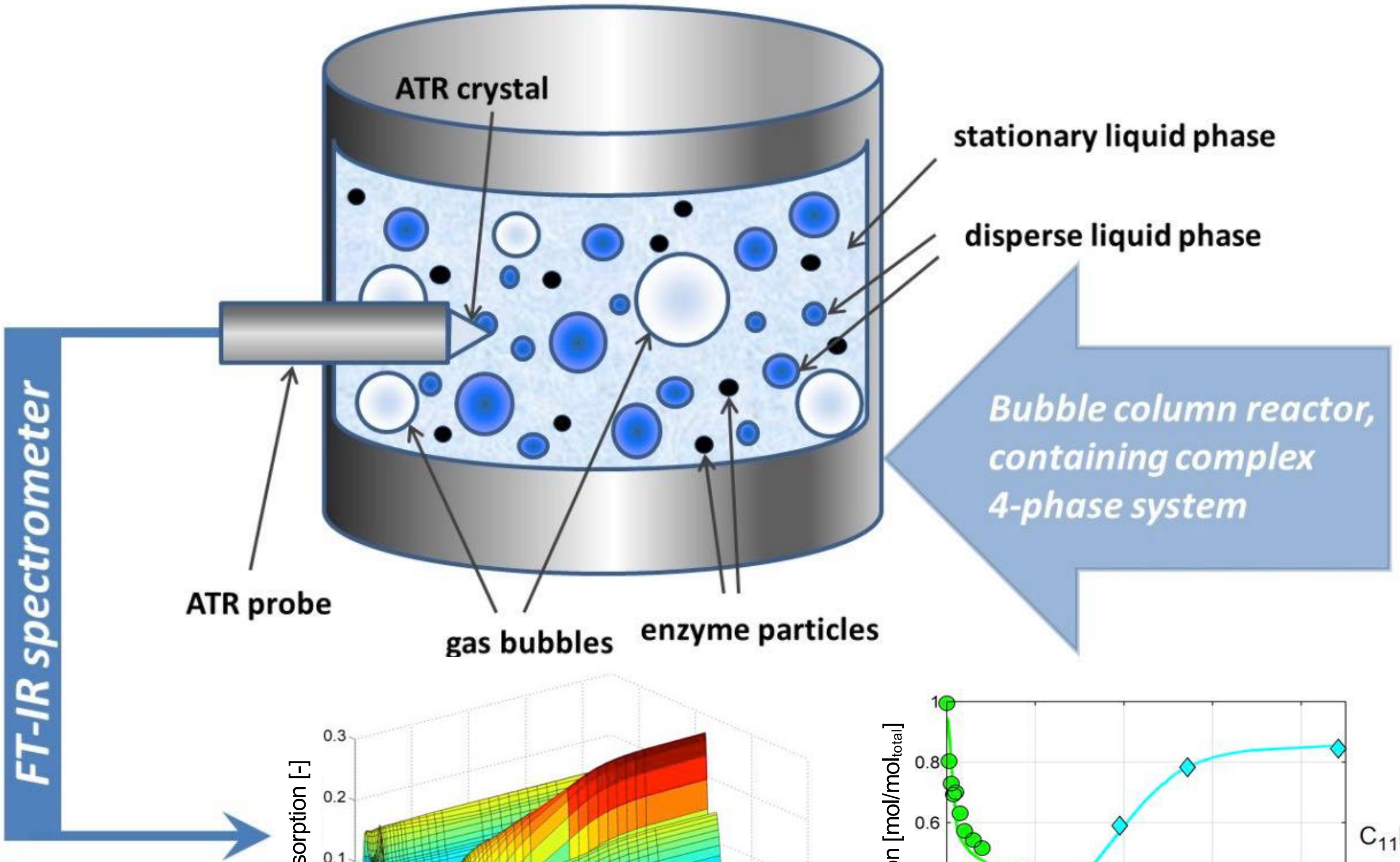
Lab Scale (1-2 kg)

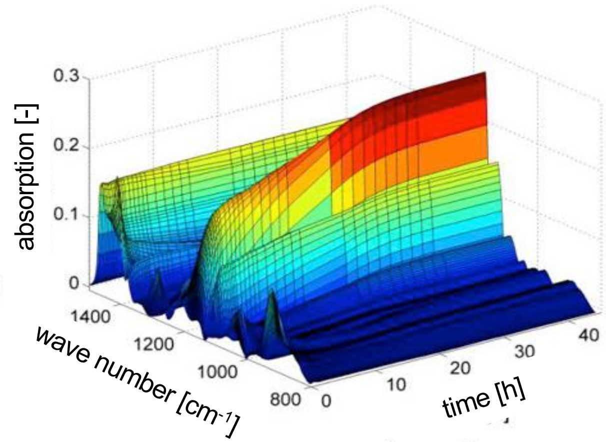
Pilot trial

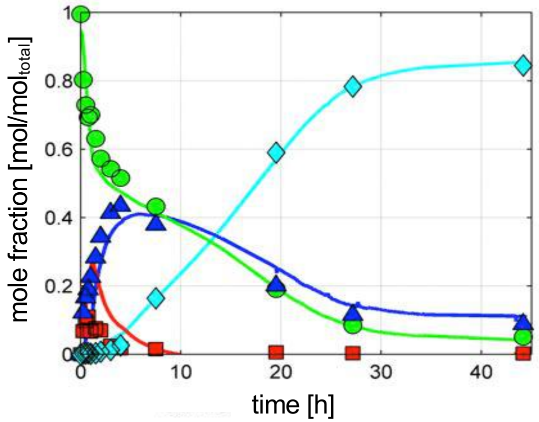
- Efficient mixing of highly viscous mixture (solvent free).
- Avoids high mechanical stress on enzyme carriers and facilitates product water removal.
- Pilot reactor put into operation in 2009.

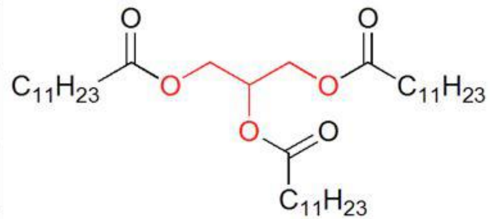
O. Thum, L. Hilterhaus, A. Liese, **2008**, Ger. Pat. Appl. 102008004726.0, DE 2008P00002
O. Thum, L. Hilterhaus, A. Liese, **2008**, Ger. Pat. Appl. 102008004725.2, DE 2008P00003

FTIR Inline Analysis in 4-Phase System





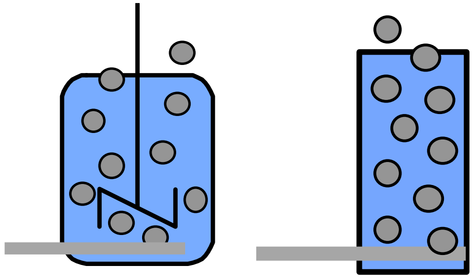




TECHNICAL BIOCATALYSIS TUHH

J. Müller, M. Neumann, P. Scholl, L. Hilterhaus, M. Eckstein, O. Thum, A. Liese, Anal. Chem. 82 (**2010**) 6008
J. Müller, S Baum, L. Hilterhaus, M. Eckstein, O. Thum, A. Liese, Anal Chem. 83 (24) (**2011**) 9321

Intensification of Airation in Biocatalysis

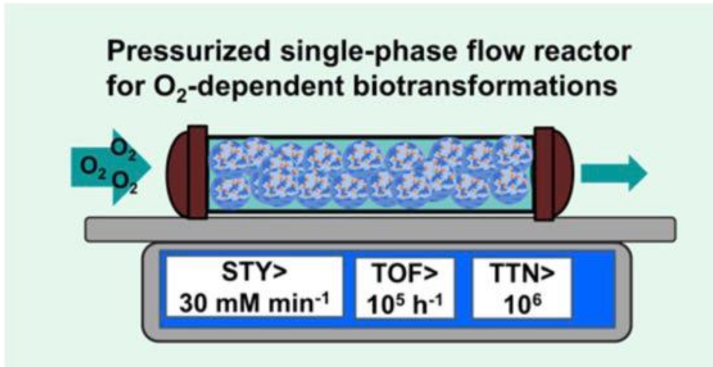


Challenges

- mass transport limitation
- foaming
- high gas consumption
- biocatalyst deactivaton

Solutions in Literature

- bubbling with macrobubbles
- diffusive airation
- *in situ* generation of gas reactant
- overpressure



TECHNICAL BIOCATALYSIS TUHH

J. Bolivar, A. Mannsberger, M. S. Thomsen, G. Tekautz, B. Nidetzky, Biotechnol. Bioeng. 116(3) **2019** 503-514

Intensification of Aeration

diameter ≤ 100 µm - fine bubbles

used in various fields

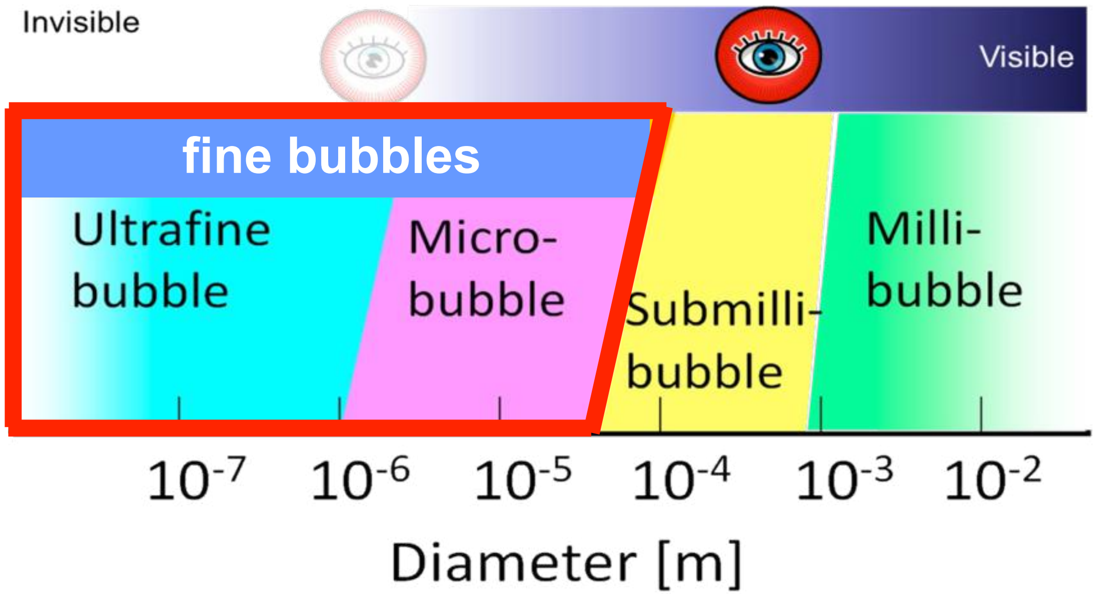
- — aquaculture
- — agriculture
- — water treatment purposes

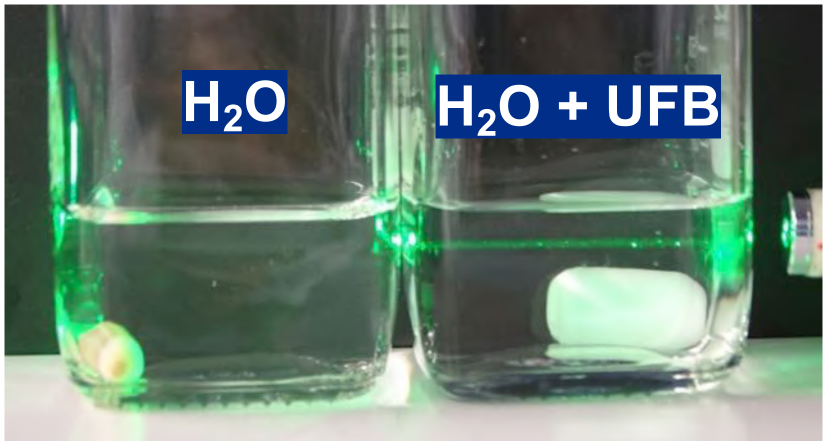
TUHH Institute of ims Multiphase Flows

Prof. Schlüter

Keio University

Prof. Terasaka

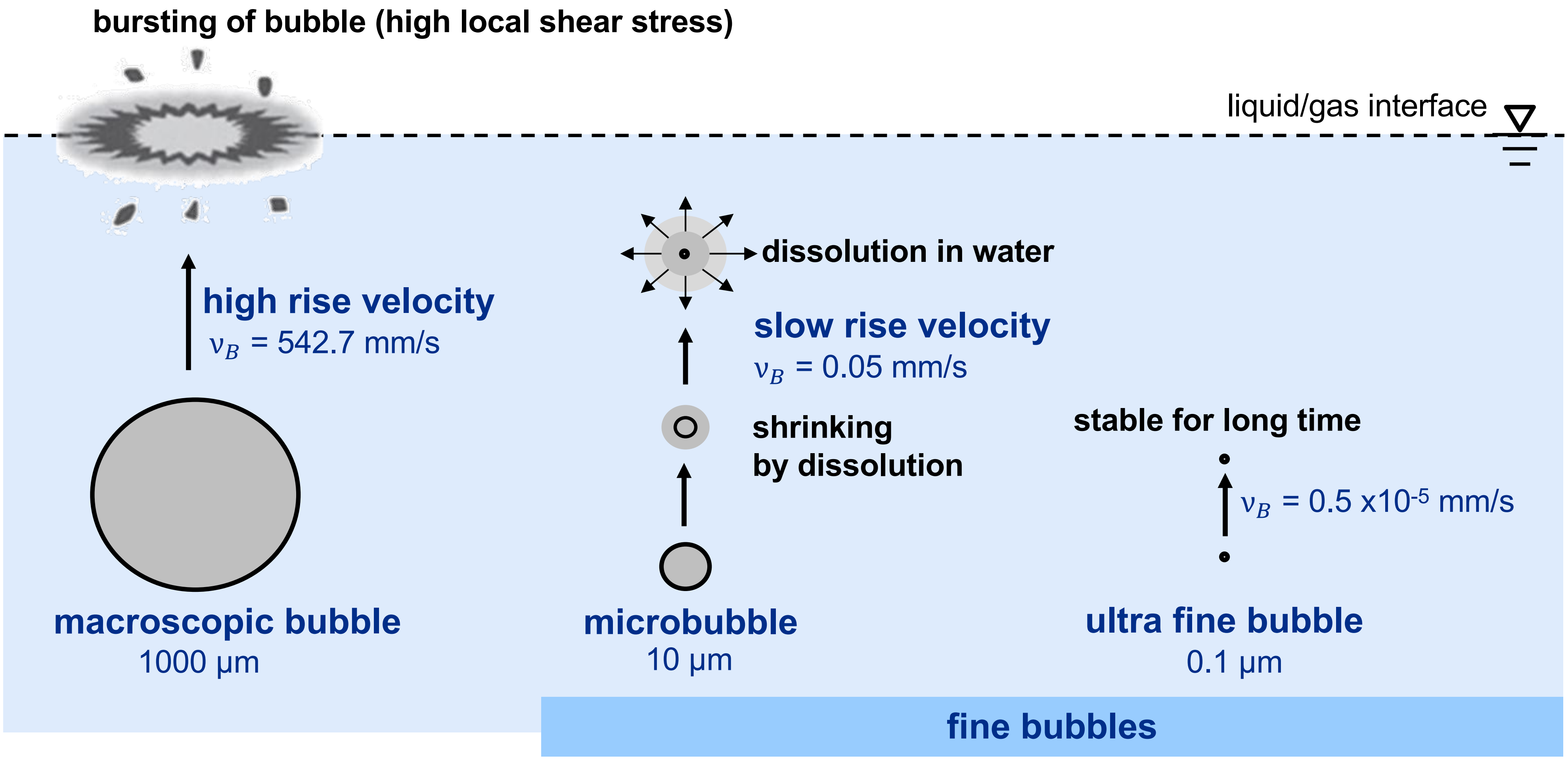




TECHNICAL BIOCATALYSIS TUHH

K. Terasaka, A. Hirabayashi, T. Nishino, S. Fujioka, D. Kobayashi, *Chem. Eng. Sci.* **2011**, *66*, 3172.

Comparison of Bubble Properties

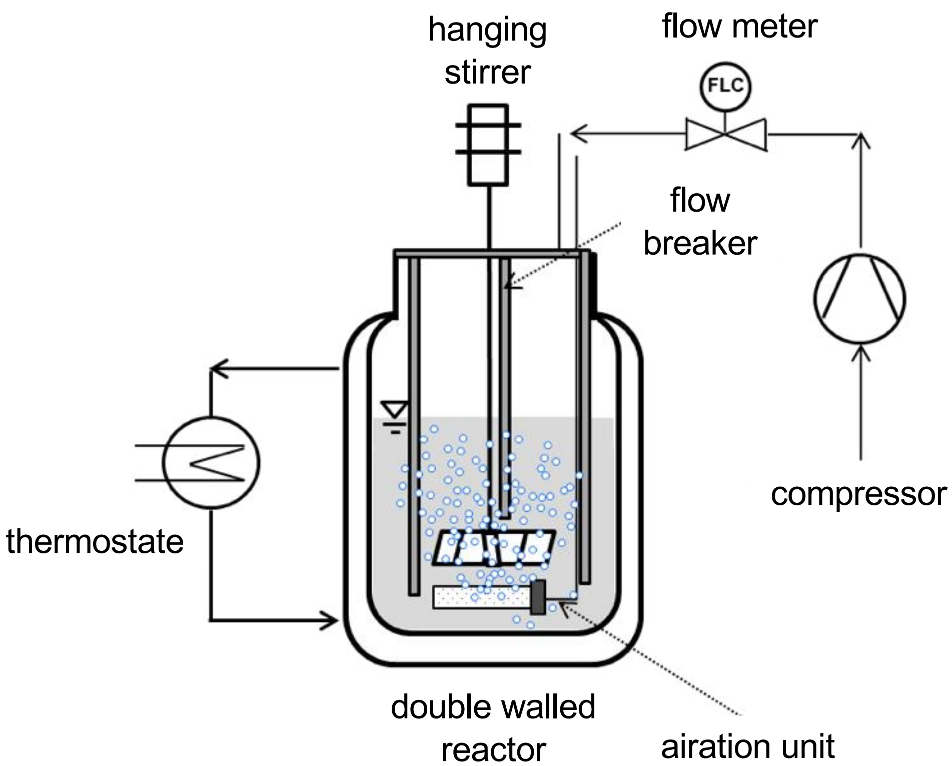


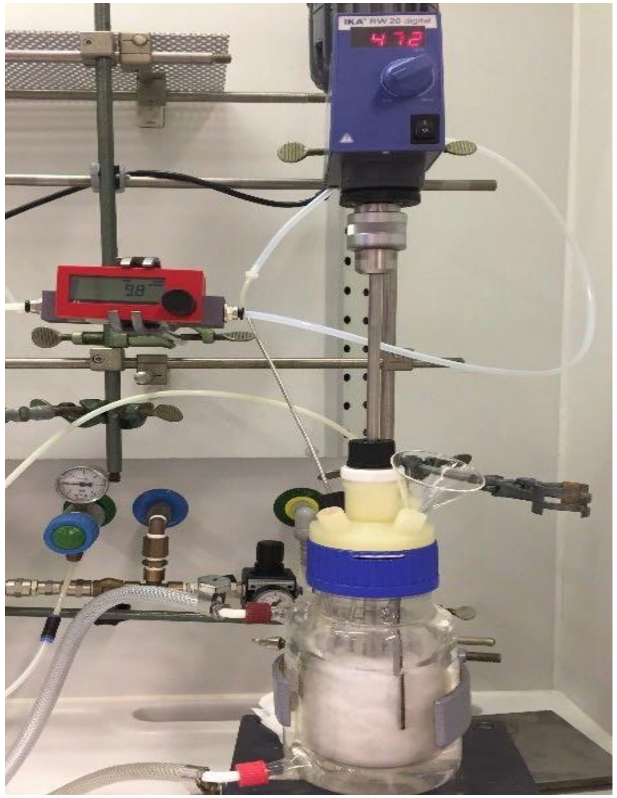
- high residence time
- high specific interfacial area
- low foaming / reactant evaporation

TECHNICAL BIOCATALYSIS TUHH

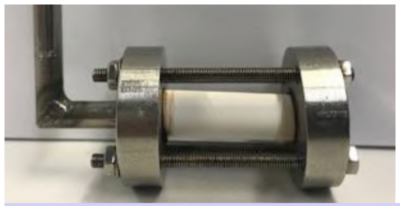
B. Thomas, D. Ohde, S. Matthes, C. Engelmann, P. Bubenheim, K. Terasaka, M. Schlüter, A. Liese, Biotechnol. Bioeng. 118 (1) (**2021**) 130-141, DOI: 10.1002/bit.27556

Variation of Power Input: Aerators





1 µm SPG membrane

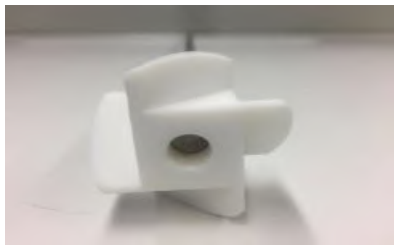


2 µm sintered frit

fine bubbles

6 mm open pipe

pitched blade impeller



D. Ohde, B. Thomas, S. Matthes, S. Tanaka, P. Bubenheim, K. Terasaka, M. Schlüter, A. Liese,
RSC Adv. 11 (7) (**2021**) 4087-4096

SPG = Shirazu-porous glas (Shiras = volcanic ash)

TECHNICAL BIOCATALYSIS TUHH

Oxygen Mass Transfer Performance

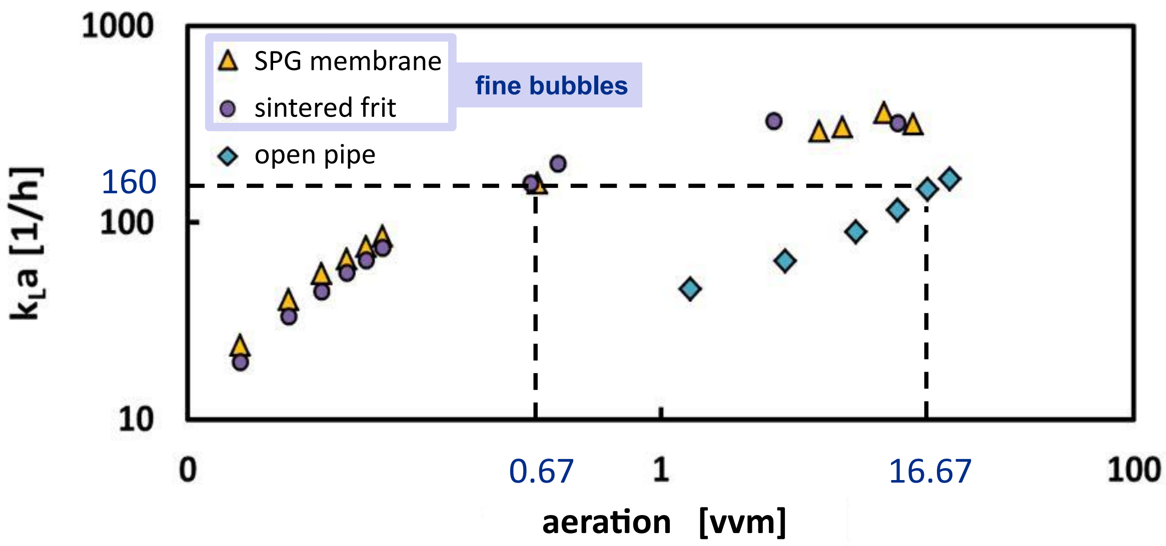
$$OTR = k_L a \left(c^*_{O_2 sat} - c_{O_2}(t) \right)$$

$$A + O_2 \rightarrow C + D$$

$$\text{reaction rate} = \frac{V_{max} \cdot c_A \cdot c_{O_2}}{K_{m,A} \cdot c_{O_2} + K_{m,O_2} \cdot c_A + c_A \cdot c_{O_2}}$$

- the **lower the O_2 mass transfer rate**, the **slower the reaction rate** occurs

no reaction:

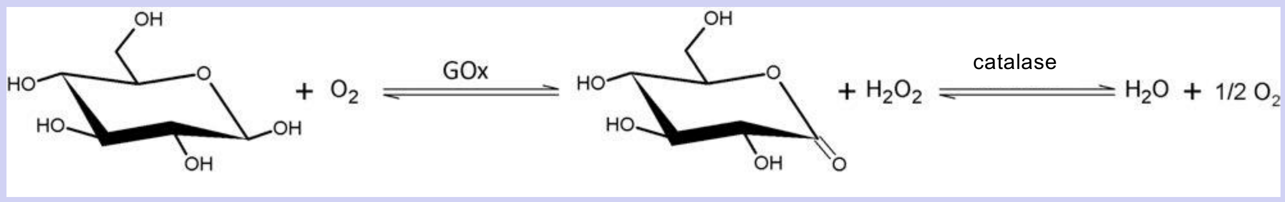


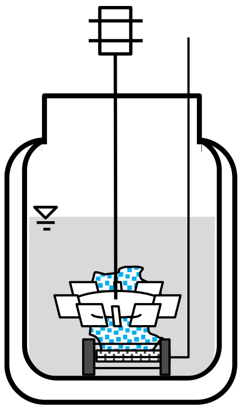
- at 160 h^{-1} **25 times higher gas utilization**

T = 25°C, V = 300 ml, 500 ml glass double walled reactor, 86 mg/l BSA, 10 mM Na-acetate buffer pH = 5.3, 400 rpm pitch blade turbine

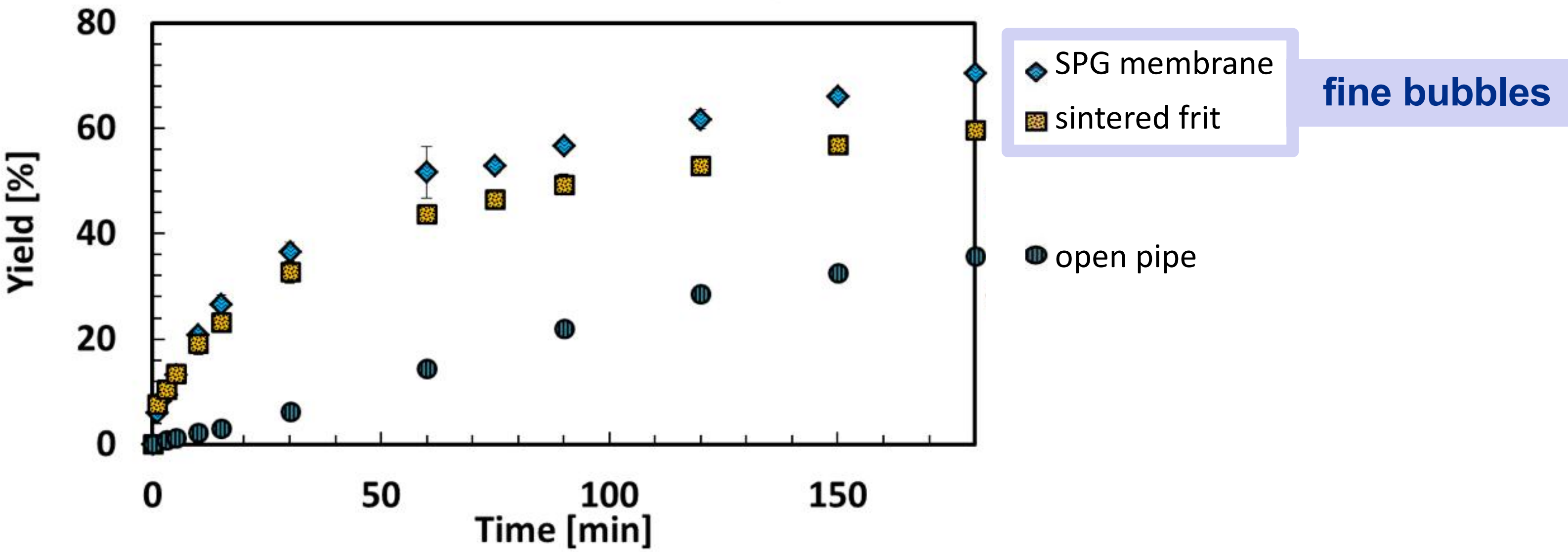
TECHNICAL BIOCATALYSIS TUHH

Comparison of Macro / Fine Bubble Aeration





k_La of 160 h^{-1}



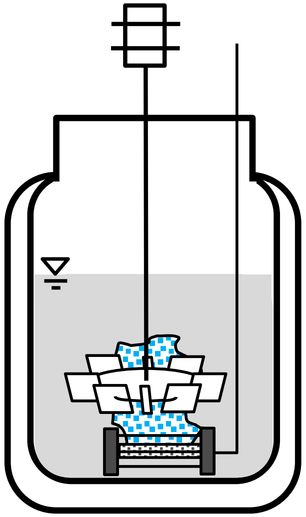
- microbubble aeration with fine bubbles :

equal k_La performance results in higher yield of 75% after 3 h

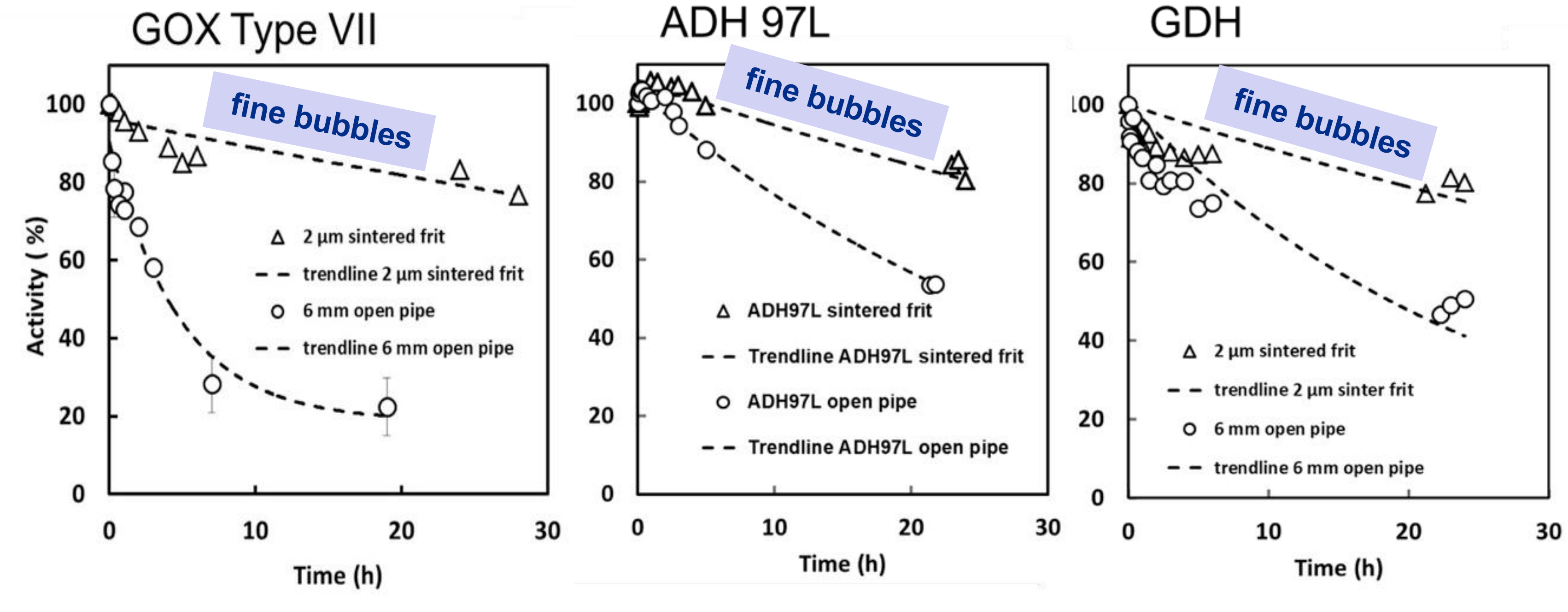
T = 35°C, V = 300 ml, 500 ml glass double walled reactor, 0.04 mol glucose, 25 mg GOx, 0.5 mg catalase, pH stat. titration (1 M KOH), 400 rpm Rushton turbine

B. Thomas, D. Ohde, S. Matthes, C. Engelmann, P. Bubenheim, K. Terasaka, M. Schlüter, A. Liese, Biotechnol. Bioeng. 118 (1) (**2021**) 130-141, DOI: 10.1002/bit.27556

Intensified Airation: Enhanced Biocatalyst Stability



constant k_La
160 h^{-1}



- aeration with fine bubbles

  significant increase in enzyme stability

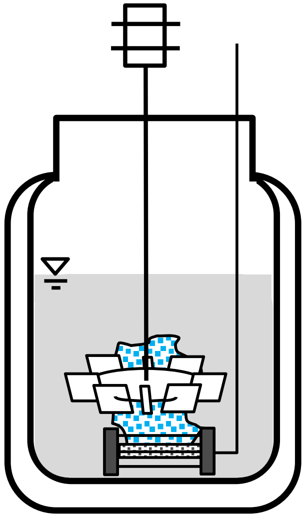
  & gas utilization

B. Thomas, D. Ohde, S. Matthes, P. Bubenheim, K. Terasaka, M. Schlüter, A. Liese, Cat. Sci. Technol. (**2023**) 13, 1098

GOx = glucose oxidase, ADH = alcohol dehydrogenase, GDH = glucose dehydrogenase

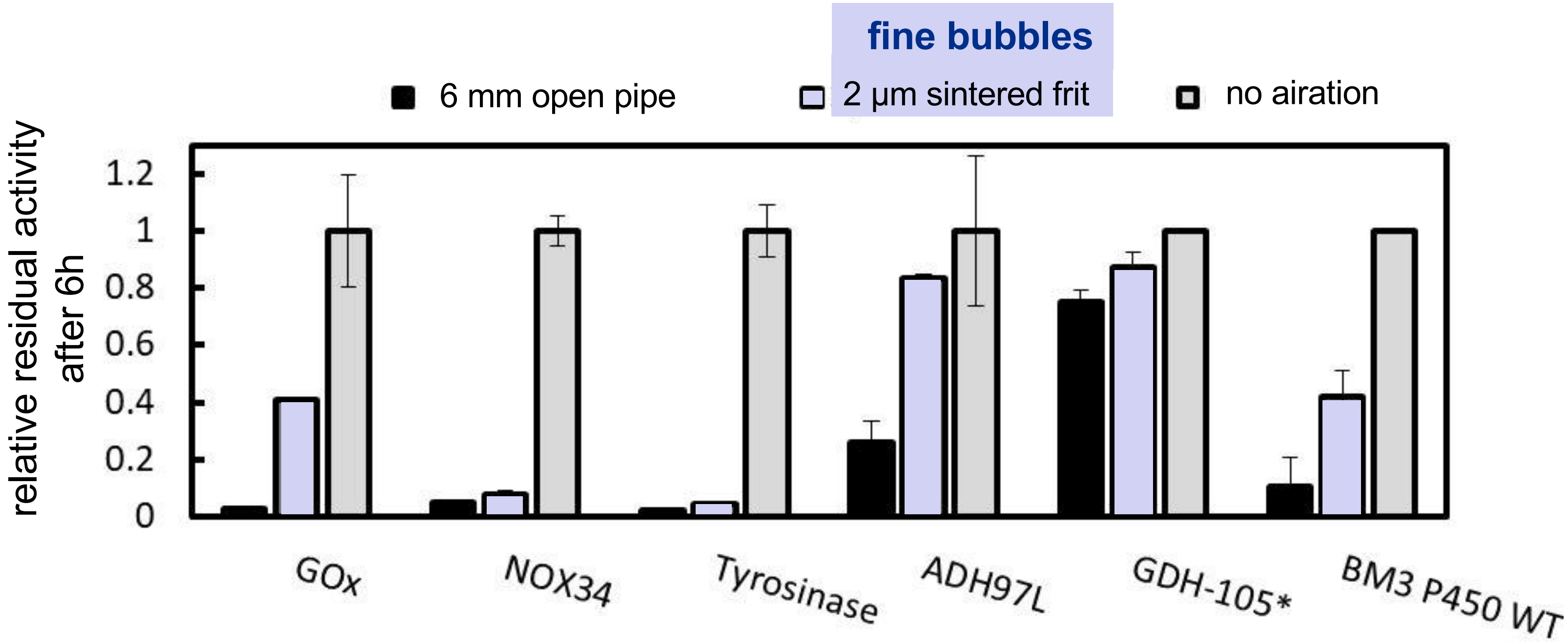
TECHNICAL BIOCATALYSIS TUHH

Intensified Airation: Enhanced Biocatalyst Stability



constant k_La

160 h^{-1}



- aeration with fine bubbles

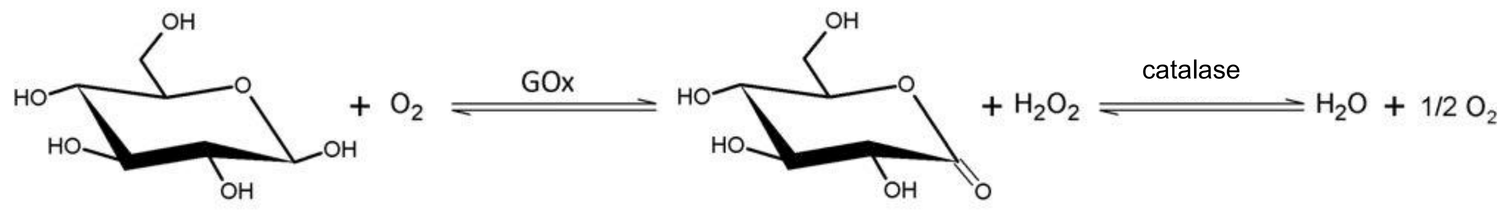
  significant increase in enzyme stability

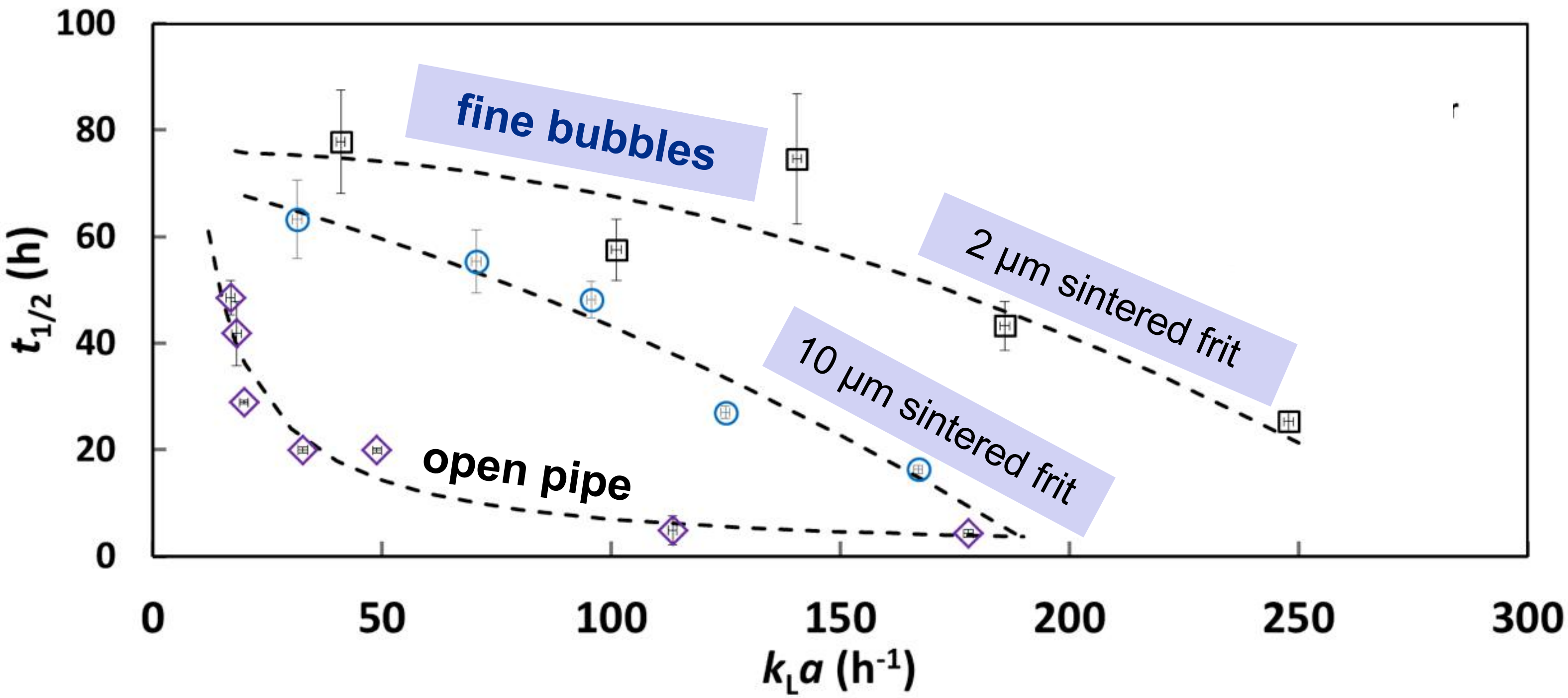
  & gas utilization

TECHNICAL BIOCATALYSIS TUHH

B. Thomas, D. Ohde, S. Matthes, P. Bubenheim, K. Terasaka, M. Schlüter, A. Liese,
Cat. Sci. Technol. (**2023**) 13, 1098

Enzyme Stability as Function of $k_L a$





optimizing enzyme stability based on $k_L a$ and type of airation

… to be complemented by enzyme engineering

… and immobilization

TECHNICAL BIOCATALYSIS TUHH

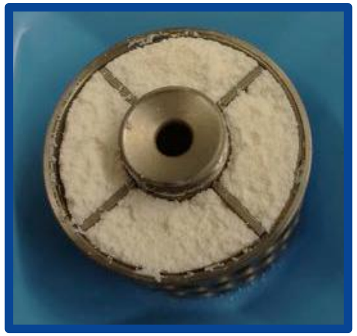
B. Thomas, D. Ohde, S. Matthes, P. Bubenheim, K. Terasaka, M. Schlüter, A. Liese,
Cat. Sci. Technol. (**2023**) 13, 1098

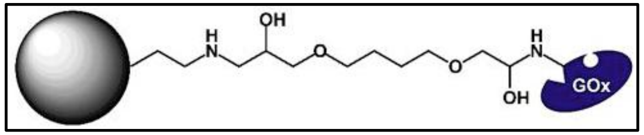
GOx: T = 35°C, 83,3 mg/l GOx (~12000 U), 0,02 mg/ml catalase (~12000 U), 10 mM Na-acetate buffer pH = 5,3, 430 rpm

Establishment of Repetitive Batch

RBR

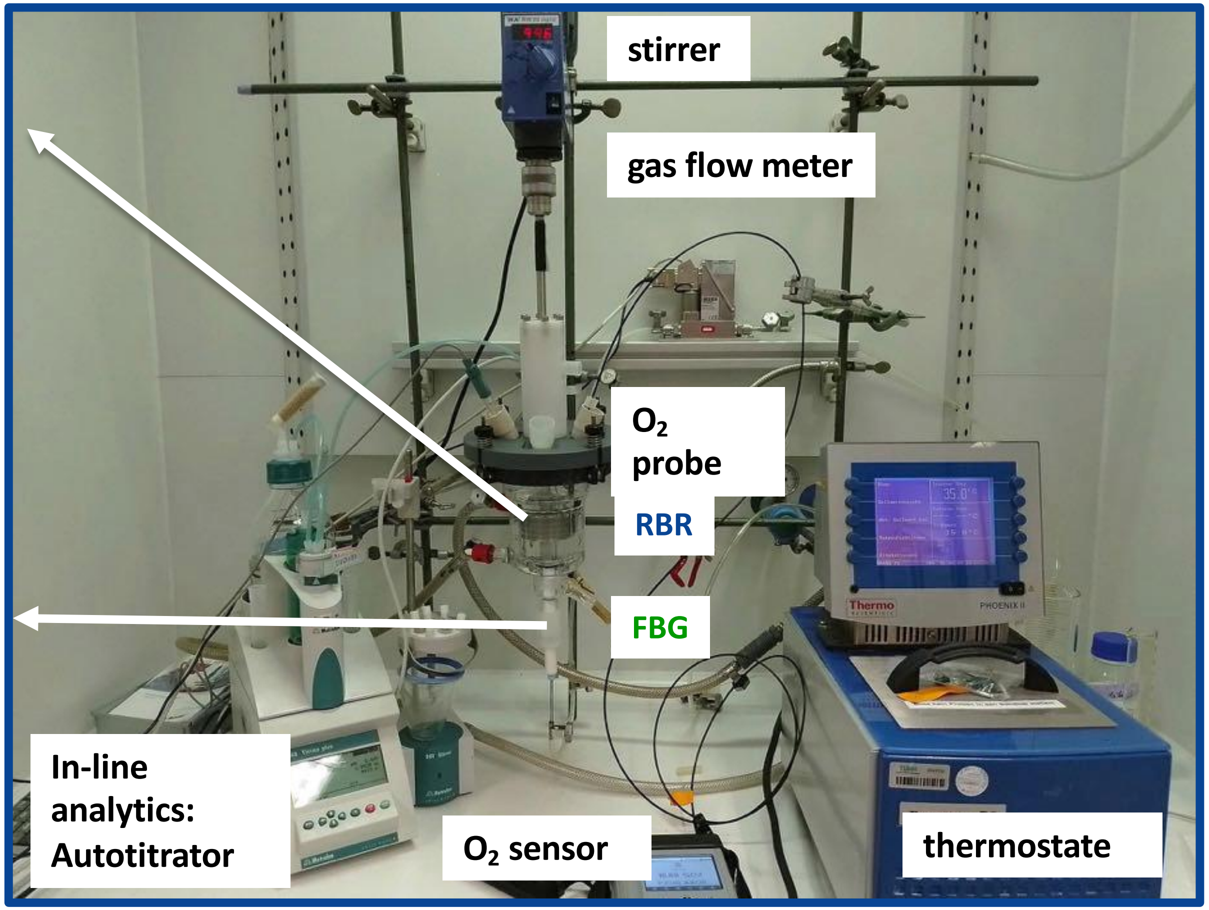
Rotating packed bed reactor is created.





FBG

pore size 2 µm



immobilized GOx 8.2 g (Resindion ReliZyme™, 200-500 µm, pore diameter of Resindion ReliZyme™: 40 - 60 nm), 35°C, catalase: 20 U/L, *1000 rpm, 10 mM Na-acetate buffer pH 5.3, glucose 600 mM, V 200 mL,* 1 vvm, saturation level of O_2 21%

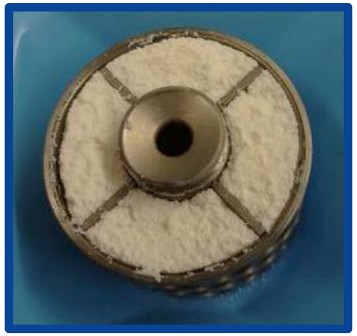
Z. Percin, L. Kursula, P. Bubenheim, M. Schlüter, A. Liese, (**2023**) unpublished

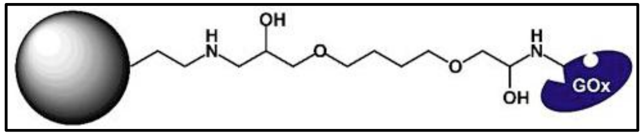
TECHNICAL BIOCATALYSIS TUHH

Applicability of FB with Packed Bed in RBR

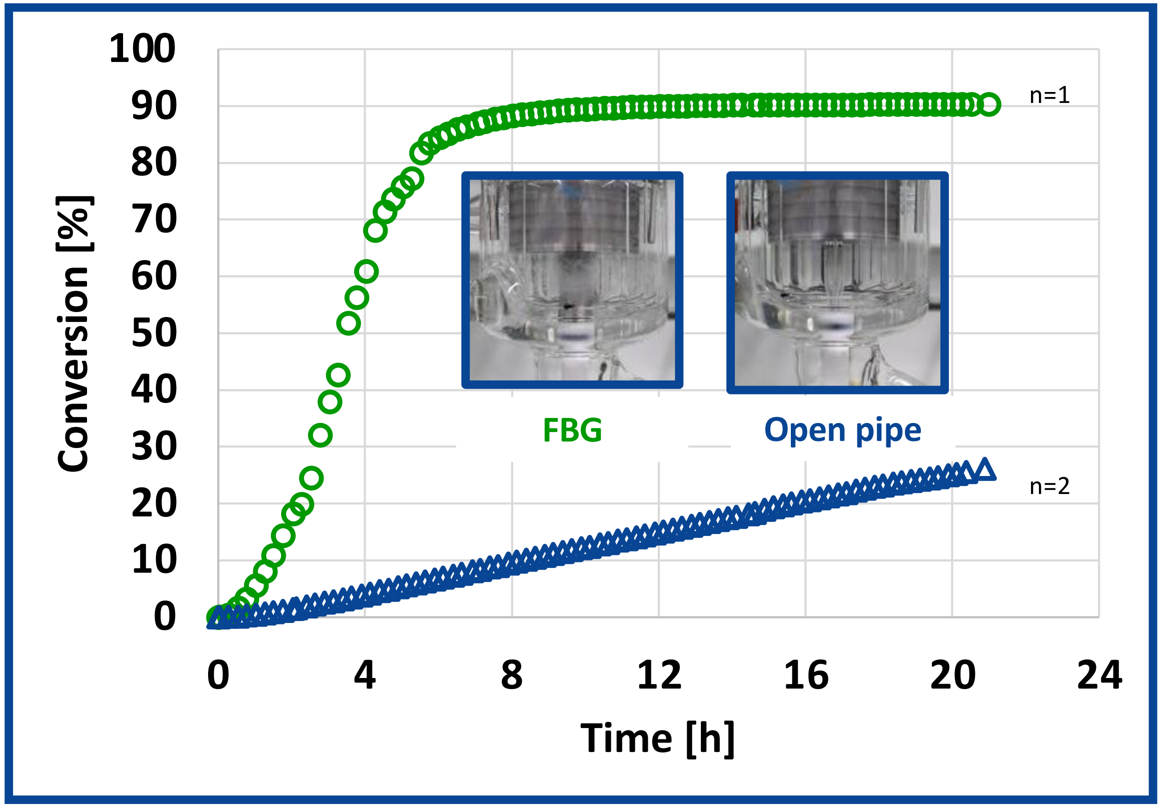
RBR

Rotating packed bed reactor is created.





FBG
pore size 2 µm



- **reaction rate: increased 14.5 fold with fine bubble aeration**
- **significant increase in productivity**

immobilized GOx 8.2 g (Resindion ReliZyme™, 200-500 µm, pore diameter of Resindion ReliZyme™: 40 - 60 nm), 35°C, catalase: 20 U/L, *1000 rpm, 10 mM Na-acetate buffer pH 5.3, glucose 600 mM, V 200 mL,* 1 vvm, saturation level of O_2 21%

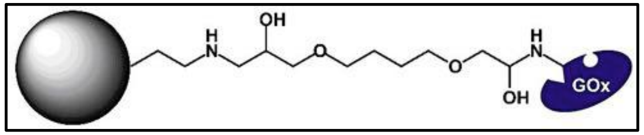
Z. Percin, L. Kursula, P. Bubenheim, M. Schlüter, A. Liese, (**2023**) unpublished

TECHNICAL BIOCATALYSIS TUHH

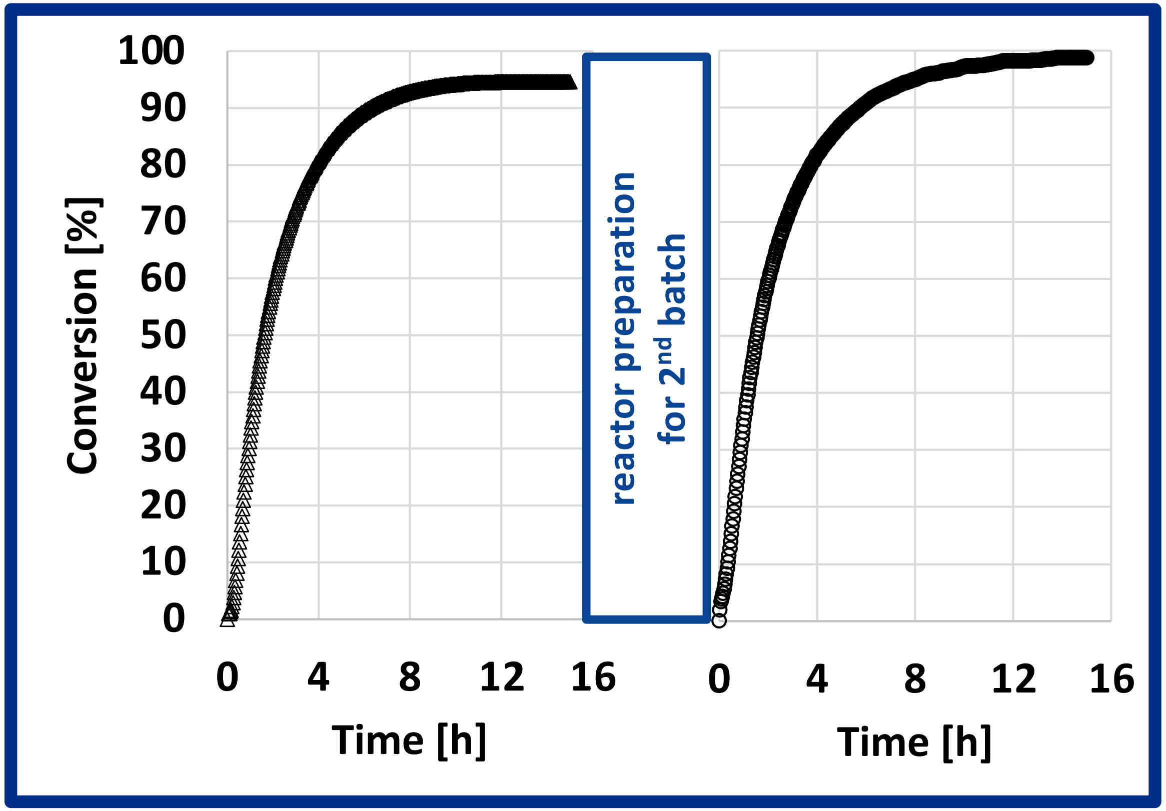
Validation in Repetitive Batch

RBR

Rotating packed bed reactor is created.



FBG
pore size 2 µm

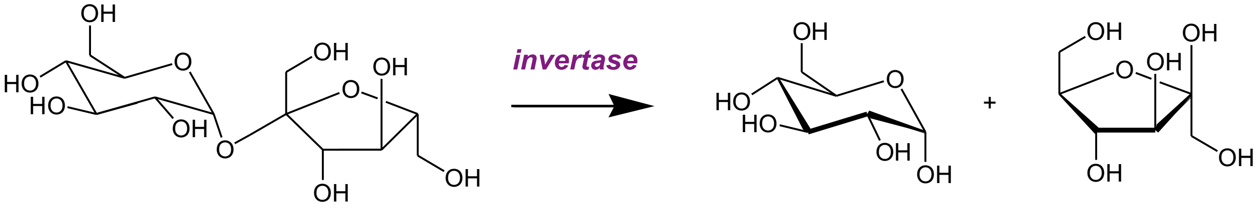


- **fine bubble airation** leads to **less** gas **consumption / foaming**
- significant increase in **enzyme stability by fine bubbles**
- opens up **new operation windows** for biotransformations

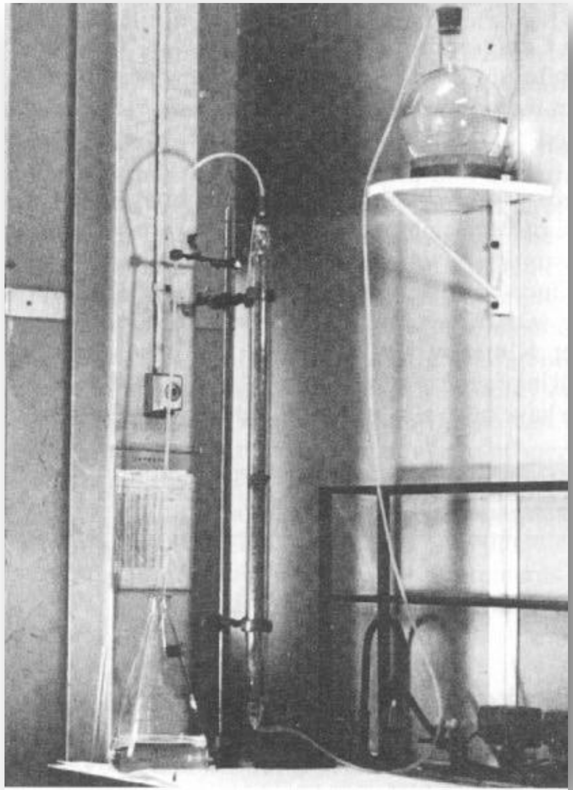
Z. Perçin, L. Kursula, P. Bubenheim, M. Schlüter, A. Liese, (**2023**) unpublished

TECHNICAL BIOCATALYSIS TUHH

Flow Chemistry in Biocatalysis – A New Old Topic ?!

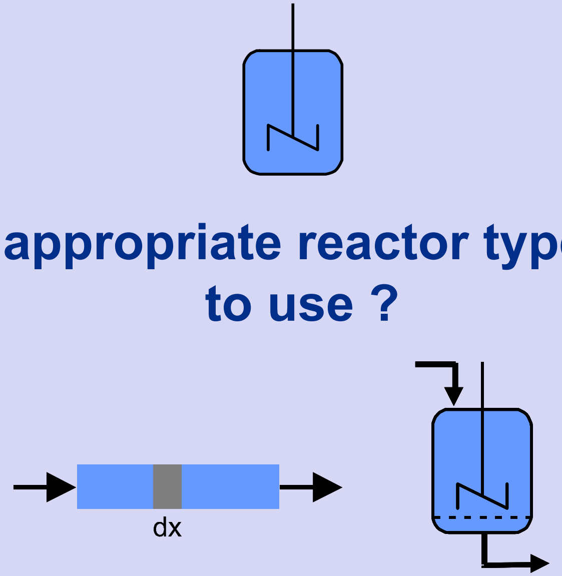


sucrose α-D-glucose β-D-fructose



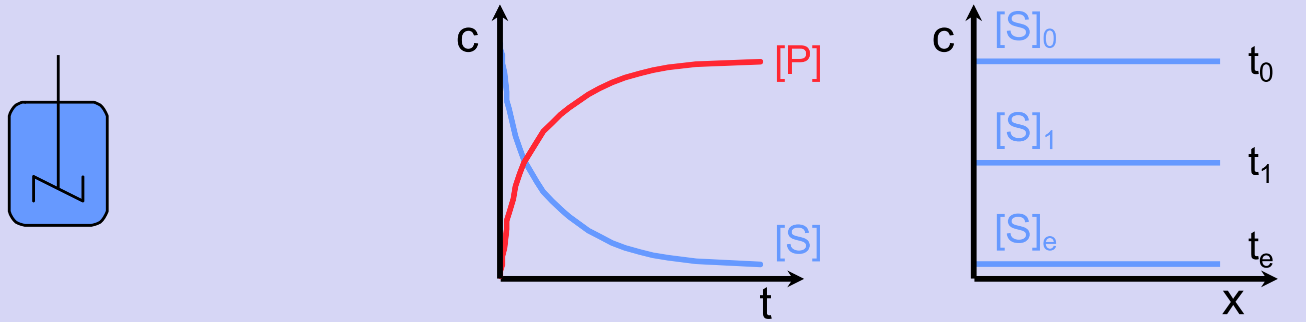
Immobilization of invertase on charcoal demonstrated with retention of activity

(***Nelson and Griffin, 1916***)

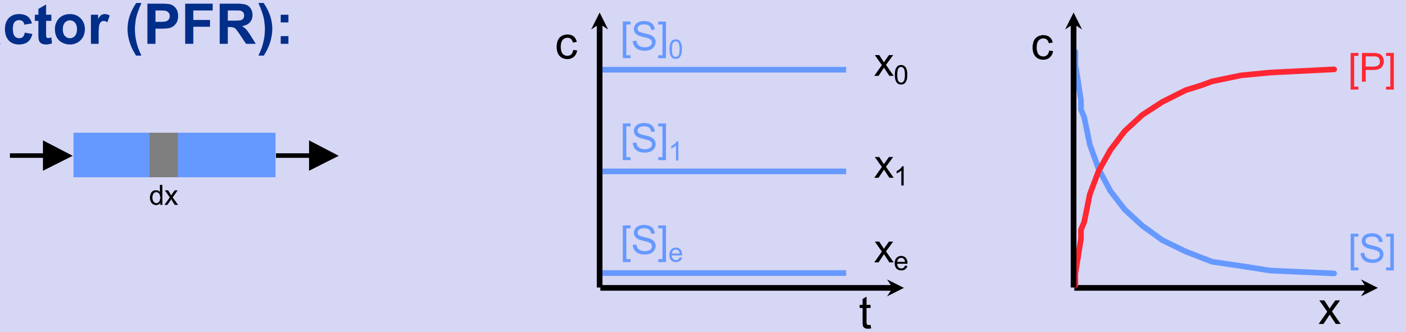


Characterization of Fundamental Reactor Types

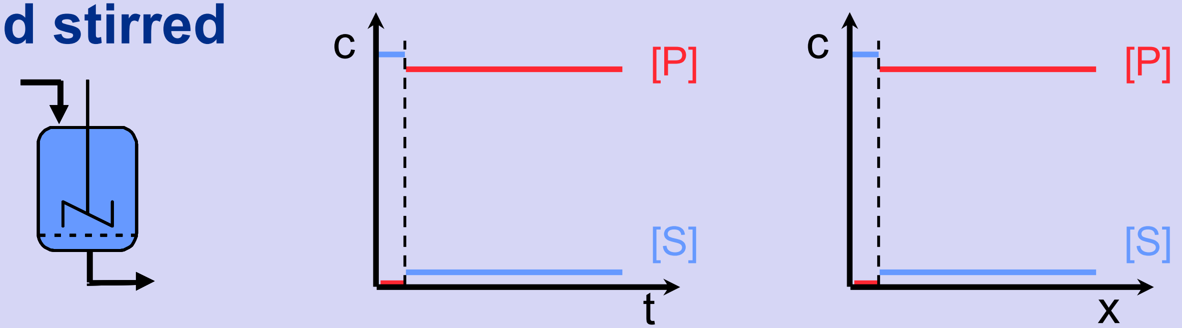
batch reactor:



plug flow reactor (PFR):



continuously operated stirred tank reactor (CSTR):

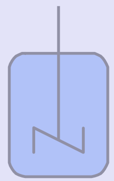


S. Kühn, A. Liese, *Enzymreaktoren und Prozessführung,* in: Einführung in die Enzymtechnologie Springer Berlin Heidelberg (**2018**) 77-101

TECHNICAL BIOCATALYSIS TUHH

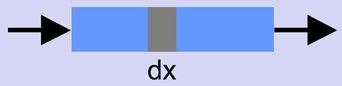
Characterization of Fundamental Reactor Types

batch reactor:



Learning Points

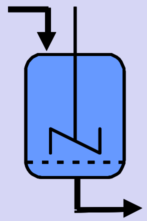
plug flow reactor (PFR):



performance = batch

continuous version of batch

continuously operated stirred tank reactor (CSTR):



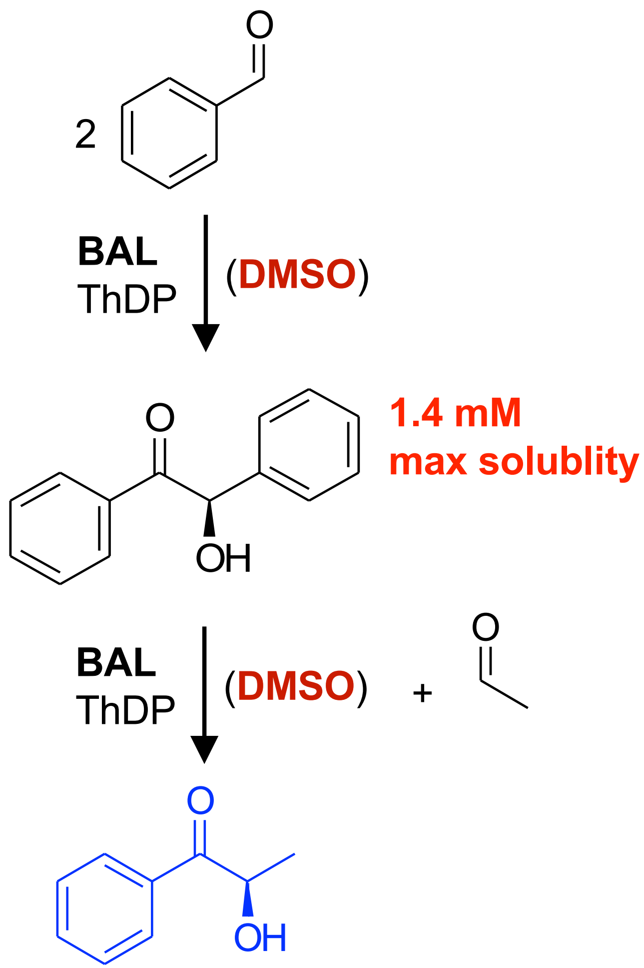
performance =/= batch

outflow conditions

TECHNICAL BIOCATALYSIS TUHH

S. Kühn, A. Liese, *Enzymreaktoren und Prozessführung,* in: Einführung in die Enzymtechnologie Springer Berlin Heidelberg (**2018**) 77-101

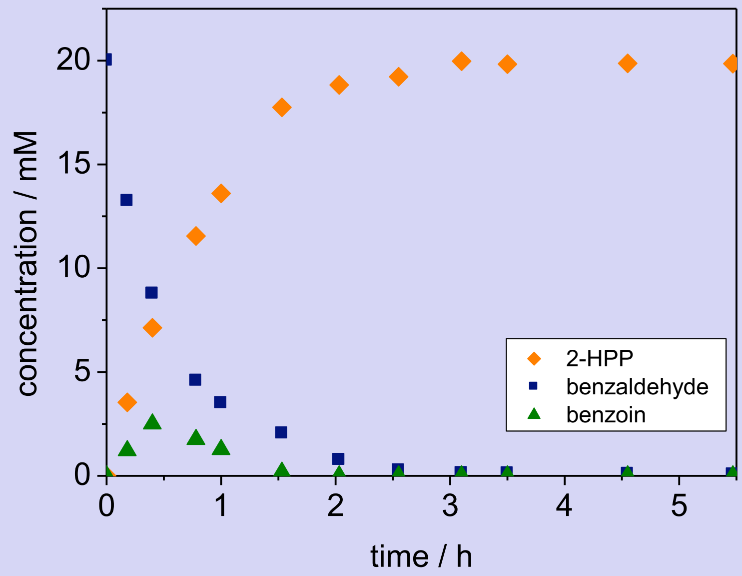
Appropriate Reactor For Continuous Synthesis



(*R*)-2-Hydroxy-1-phenylpropanon

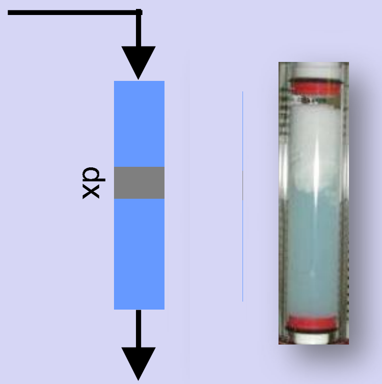
ee > 99%

batch



20 mM benzaldehyde,
60 mM acetaldehyde,
30 vol% DMSO,
100 mg BAL on 11 mL NiNTA,
35 mM TEA (pH 8.0),
0.5 mM ThDP/$MgSO_4$

continuous (PFR)

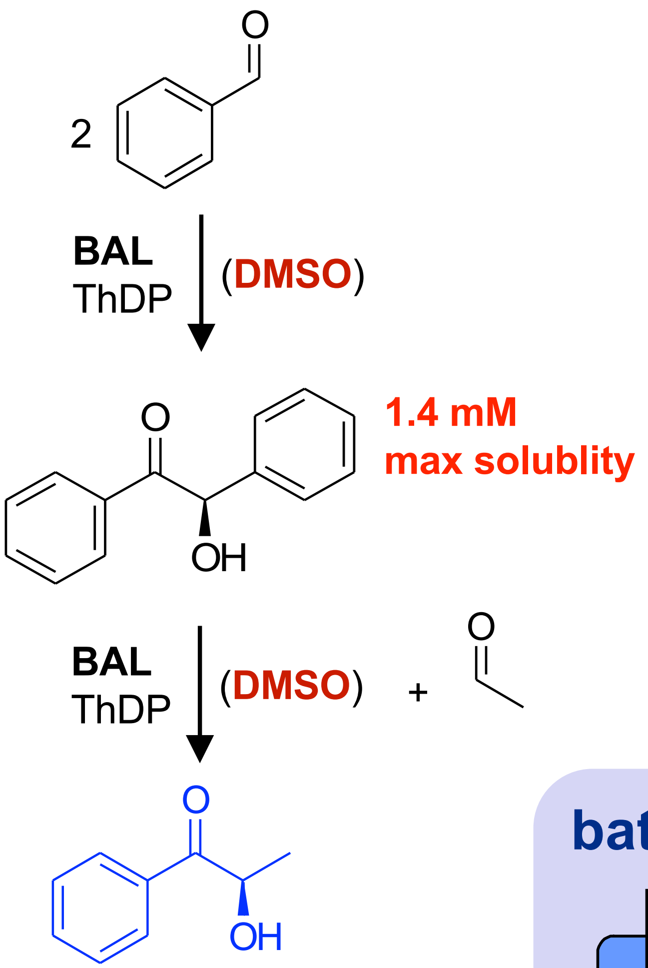


TECHNICAL BIOCATALYSIS TUHH

T. Stillger, M. Pohl, C. Wandrey, A. Liese, Org. Proc. Res. Dev. 10 (6) (**2006**) 1172-1177

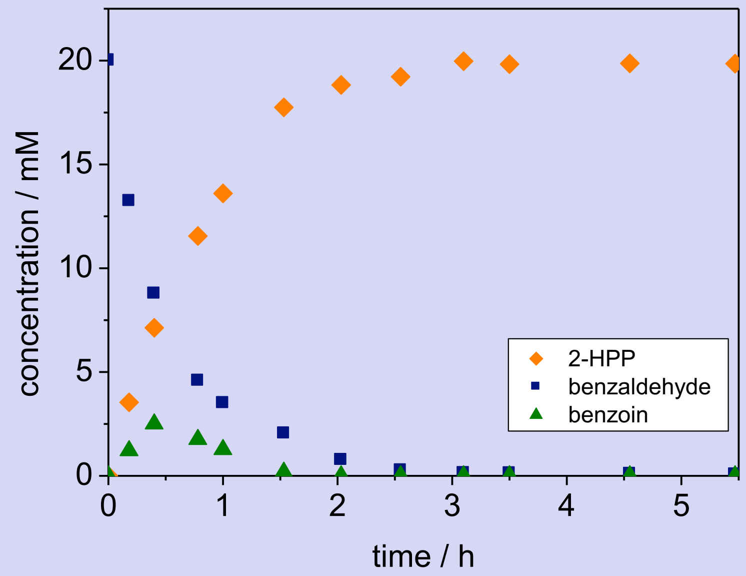
BAL = benzaldehyde lyase

Appropriate Reactor For Continuous Synthesis

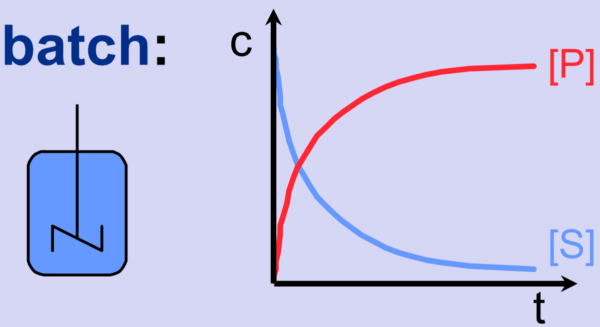


(*R*)-2-Hydroxy-1-phenylpropanon
ee > 99%

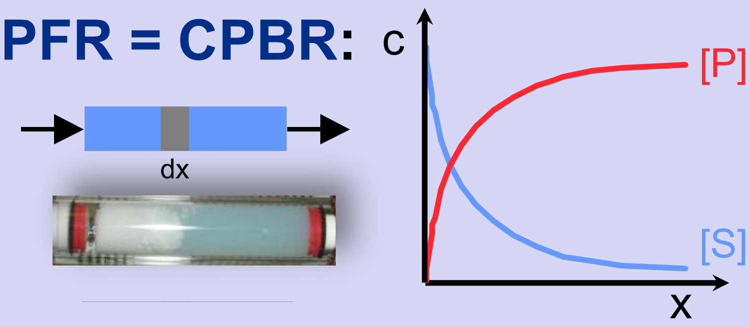
batch



batch:

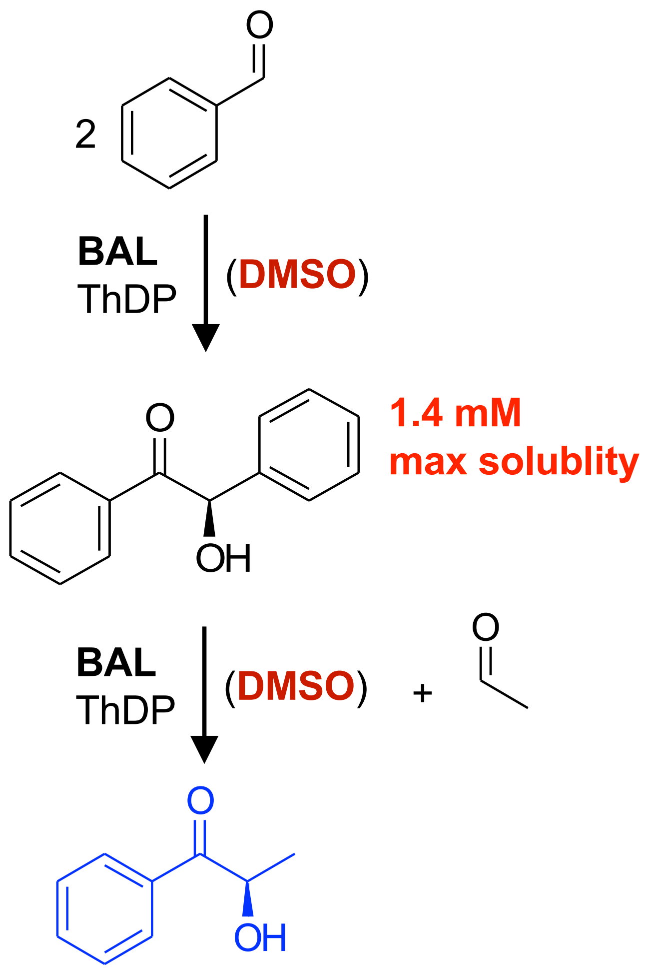


PFR = CPBR:



T. Stillger, M. Pohl, C. Wandrey, A. Liese, Org. Proc. Res. Dev. 10 (6) (**2006**) 1172-1177
BAL = benzaldehyde lyase

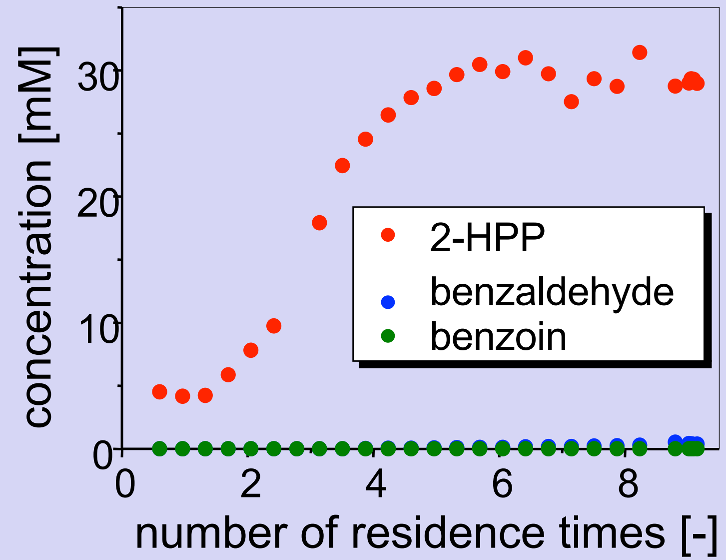
Redesign of Reactor Operation PFR -> CSTR



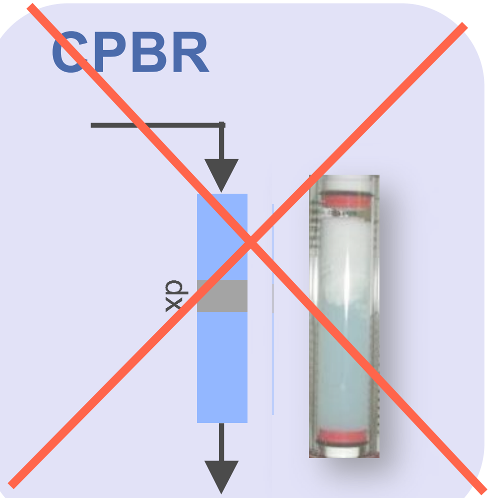
(*R*)-2-Hydroxy-1-phenylpropanon

ee > 99%

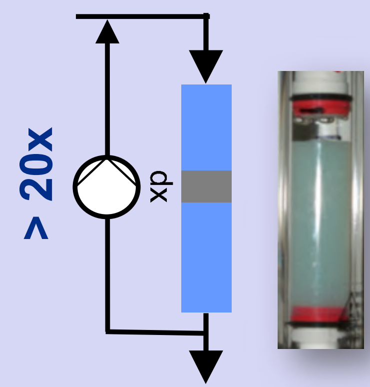
CSTR



CPBR



CSTR mode

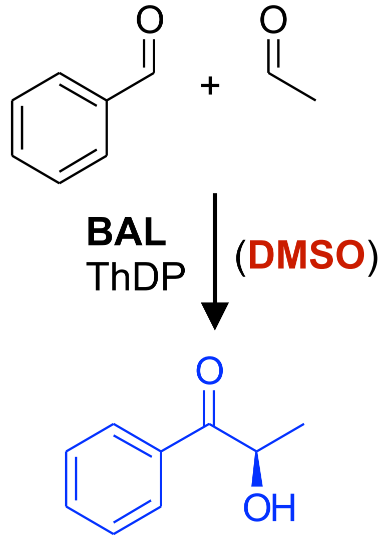


30 mM benzaldehyde, 80 mM acetaldehyde, 30 vol% DMSO, 100 mg BAL on 11 mL NiNTA, 50 mM TEA, 0.5 mM ThDP/$MgSO_4$

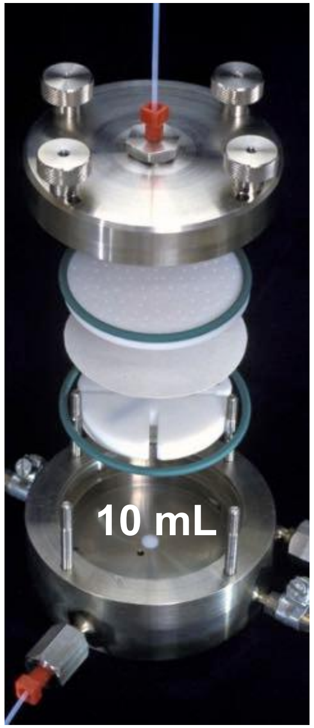
BAL = benzaldehyde lyase

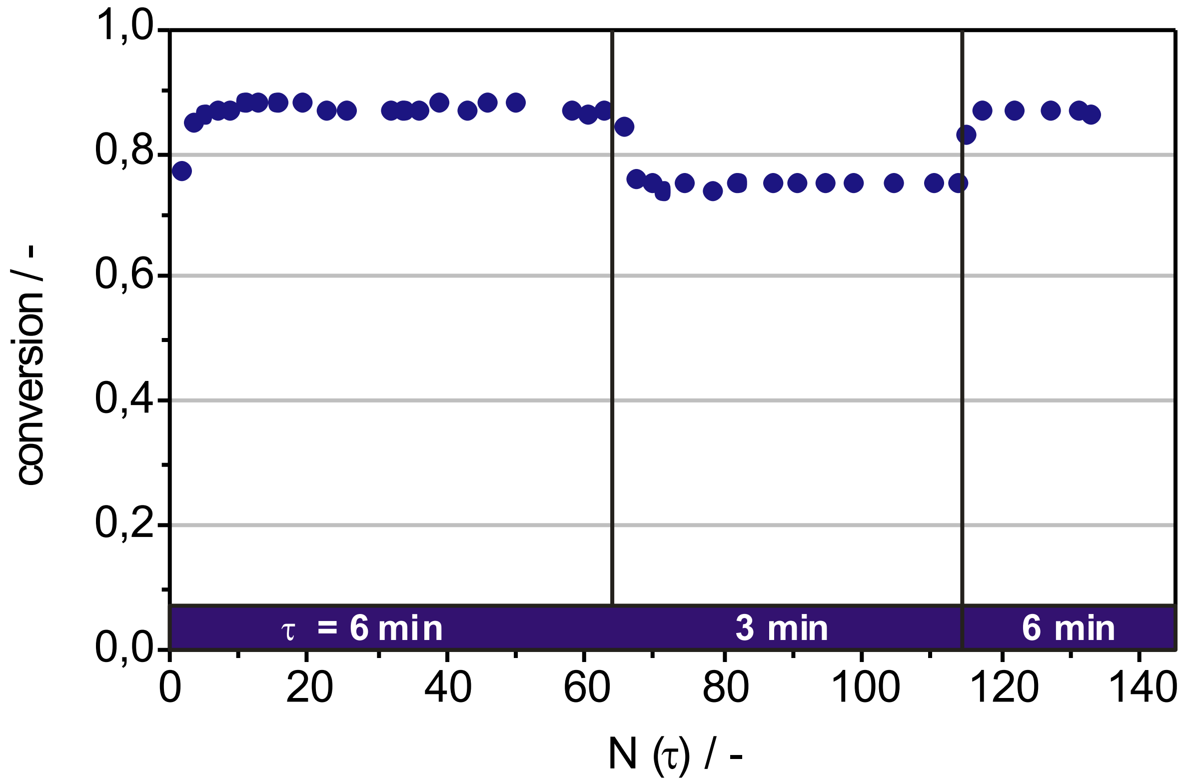
TECHNICAL BIOCATALYSIS TUHH

Flow Chemistry: (*R*)-2-HPP Synthesis



(*R*)-2-Hydroxy-1-phenylpropanon
ee > 99%





space-time yield

τ = 6 min : 650 g L^{-1} d^{-1}

τ = 3 min : 1120 g L^{-1} d^{-1}

20	mM	benzaldehyde
80	mM	acetaldehyde
35	mM	TEA buffer (pH 8.0)
0.35	mM	ThDP
0.35	mM	$MgSO_4$
30	vol%	DMSO
285	U/mL	BAL

T. Stillger, M. Pohl, C. Wandrey, A. Liese, Org. Proc. Res. Dev. 10 (6) (**2006**) 1172-1177

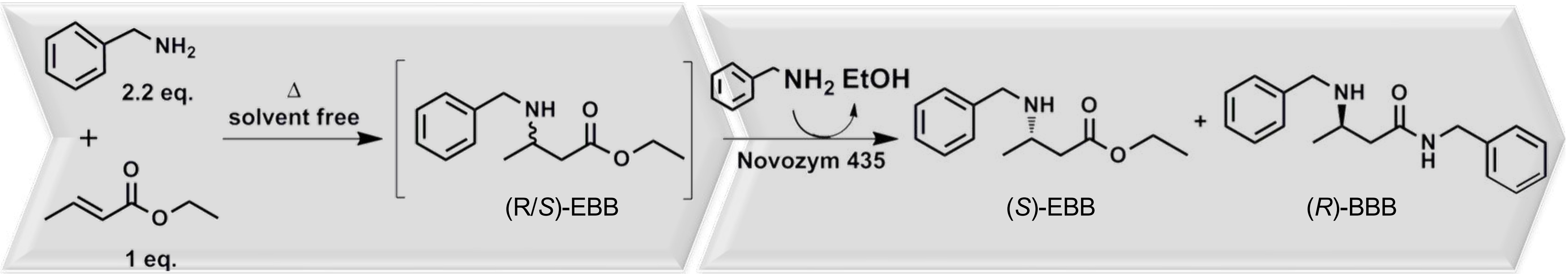
One-pot Chemoenzymatic Reaction Sequence

cooperation with Dr. M. Weiß, Prof. H. Gröger

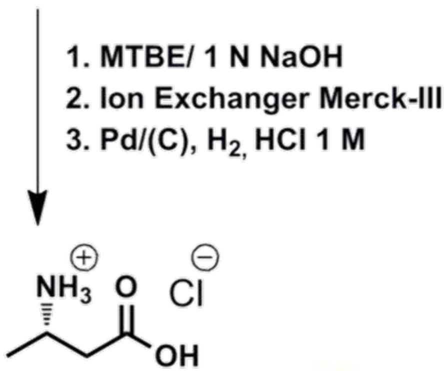
Universität Bielefeld

1. aza-Michael addition

2. selective aminolysis



3. work-up



(*S*)-β-amino acid

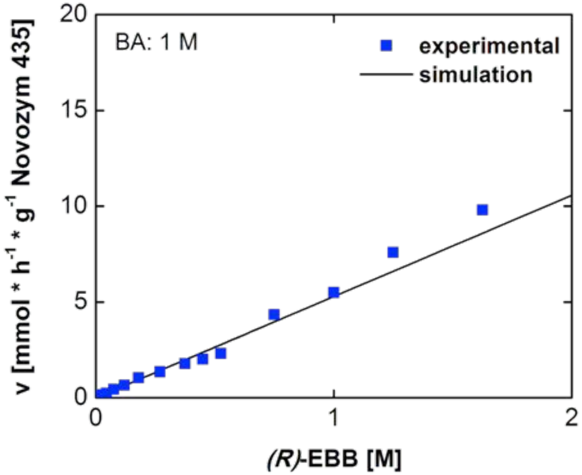
- **solvent free**
- high enantiomeric excess (99%)
- **aim: setup of continuous process**

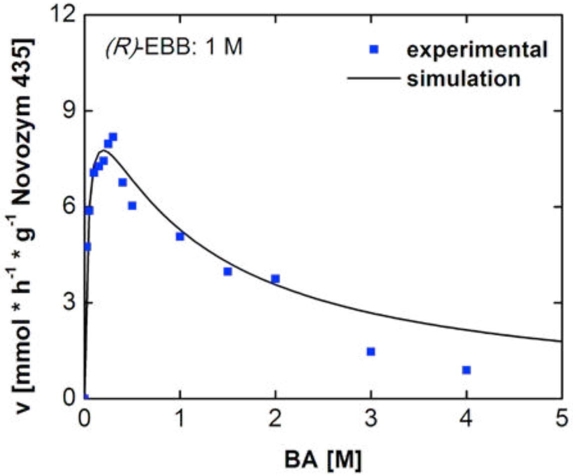
TECHNICAL BIOCATALYSIS TUHH

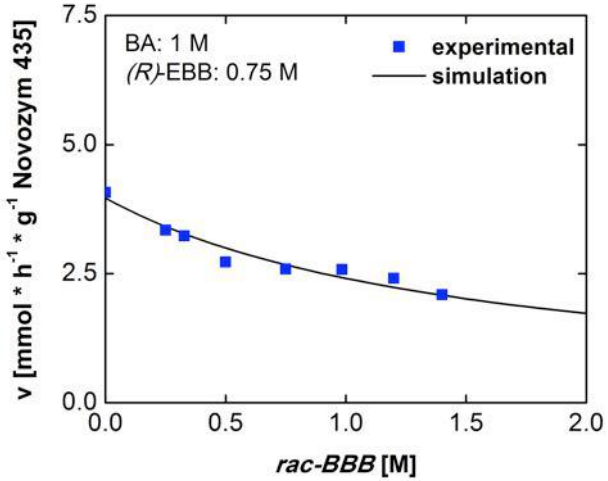
Weiß, M., Gröger, H. Synlett **2009**, 8, 1251
Weiß, M., Brinkmann, T., Gröger, H. Green Chem. **2010**, 12, 1580

Transfer from Solvent to Neat Kinetics (Aminolysis)

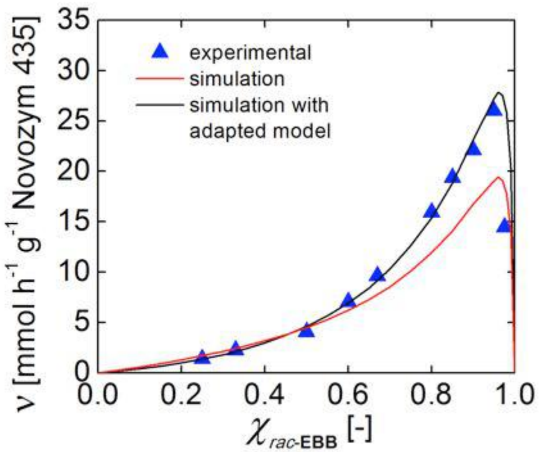
THF: Kinetics "as usual"

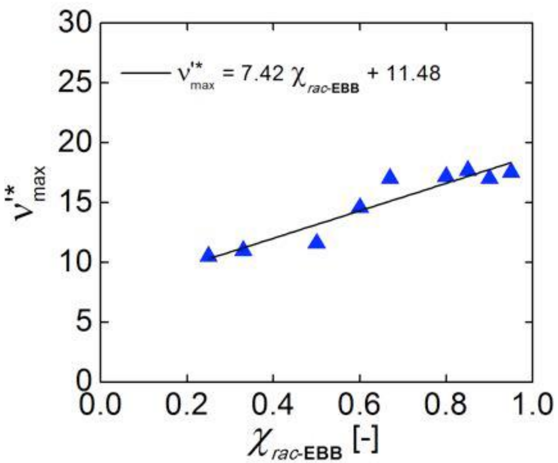






- **Neat: Kinetic parameters affected by solvent effects of reactants**





➔ neat substrates = solvents

S. Strompen, M. Weiss, T. Ingram, I. Smirnova, H. Gröger, L. Hilterhaus, A. Liese, Biotechnol. Bioeng. 109 (6) (**2012**) 1479–1489

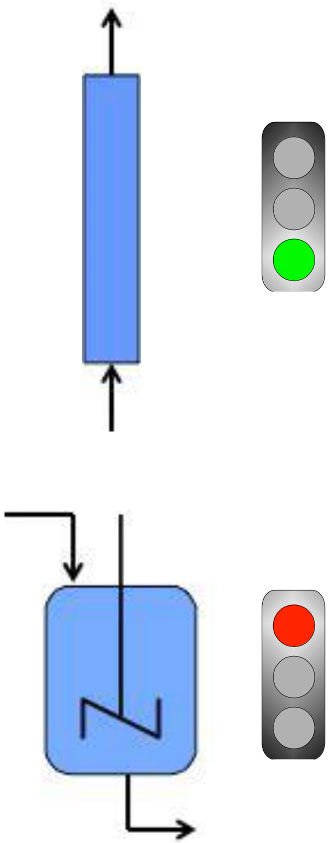
TECHNICAL BIOCATALYSIS TUHH

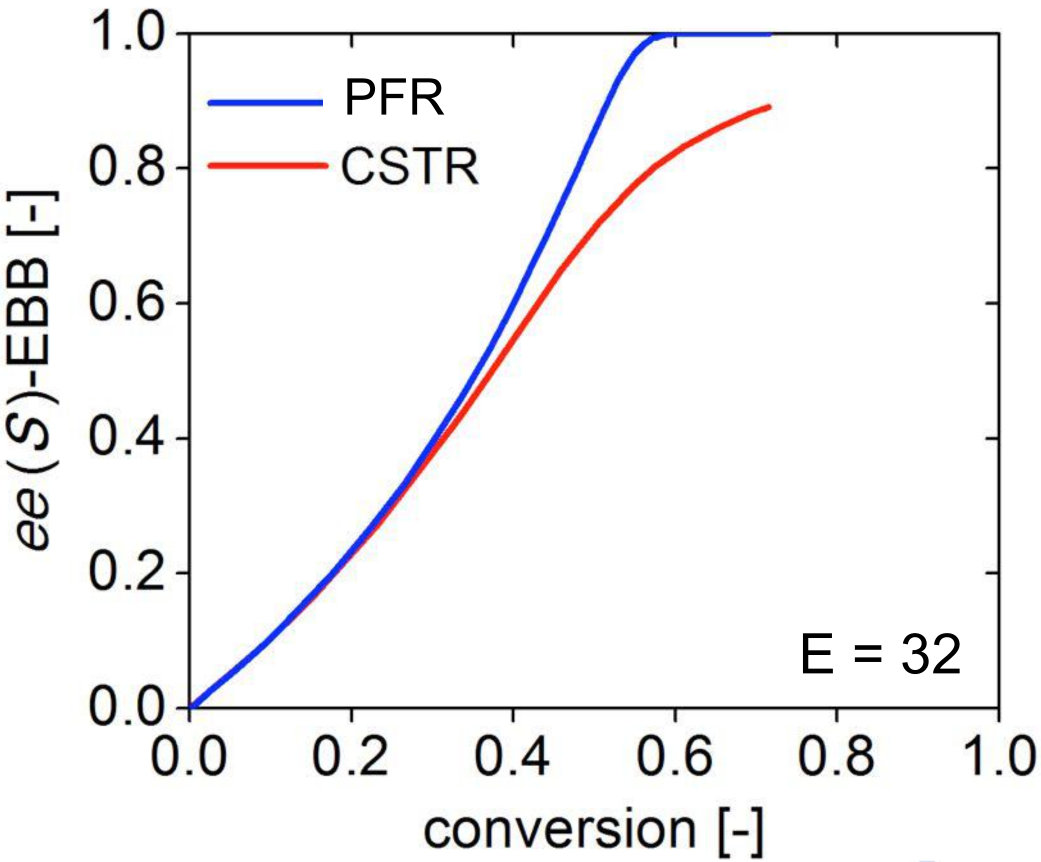
Selection of Continuously Operated Reactor Type

Application of kinetic model for prediction of enantiomeric excess

PFR

CSTR



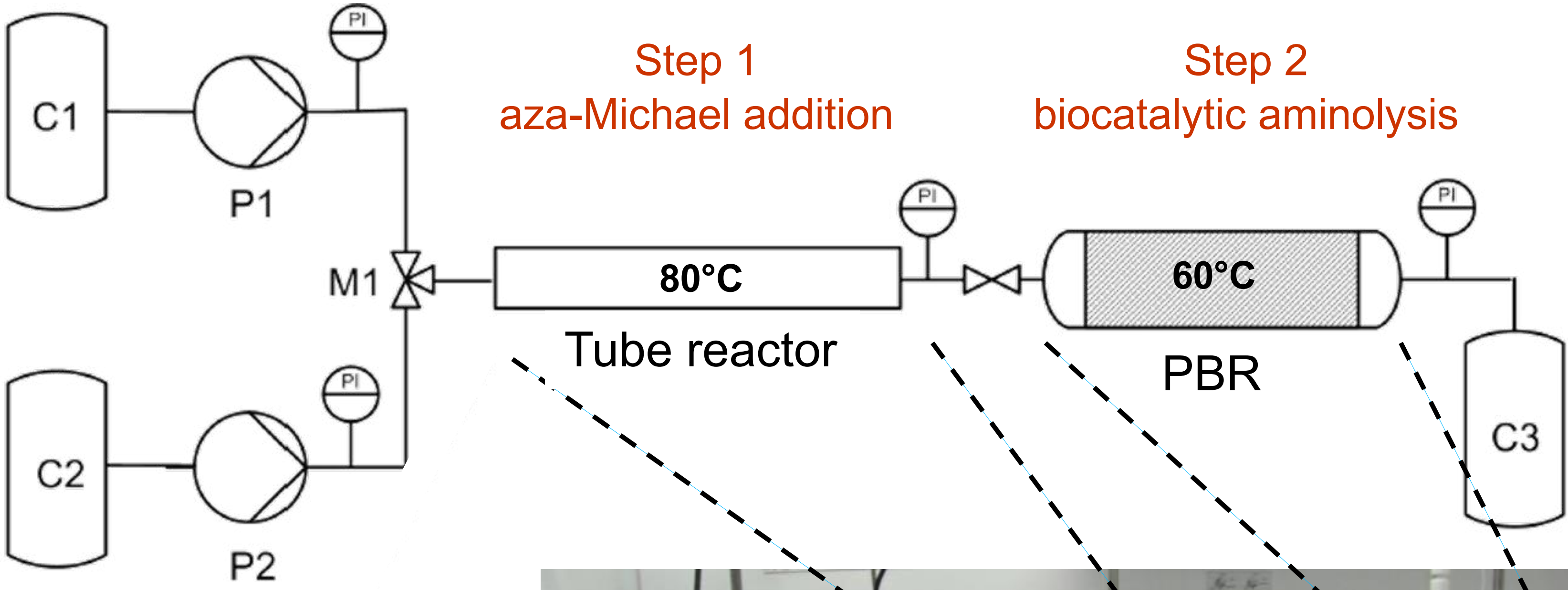


CSTR (Continuous Stirred Tank Reactor)
PFR (Plug Flow Reactor)

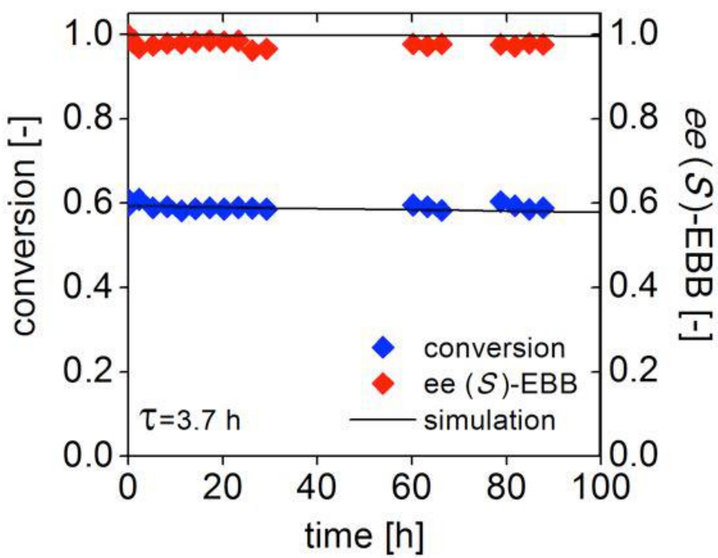
S. Strompen, M. Weiss, H. Gröger, L. Hilterhaus, A. Liese, Adv. Synth. Cat. 355 (**2013**) 2391
U. Kragl, A. Liese, Chem. Ing. Techn. 85(6) (**2013**) 826

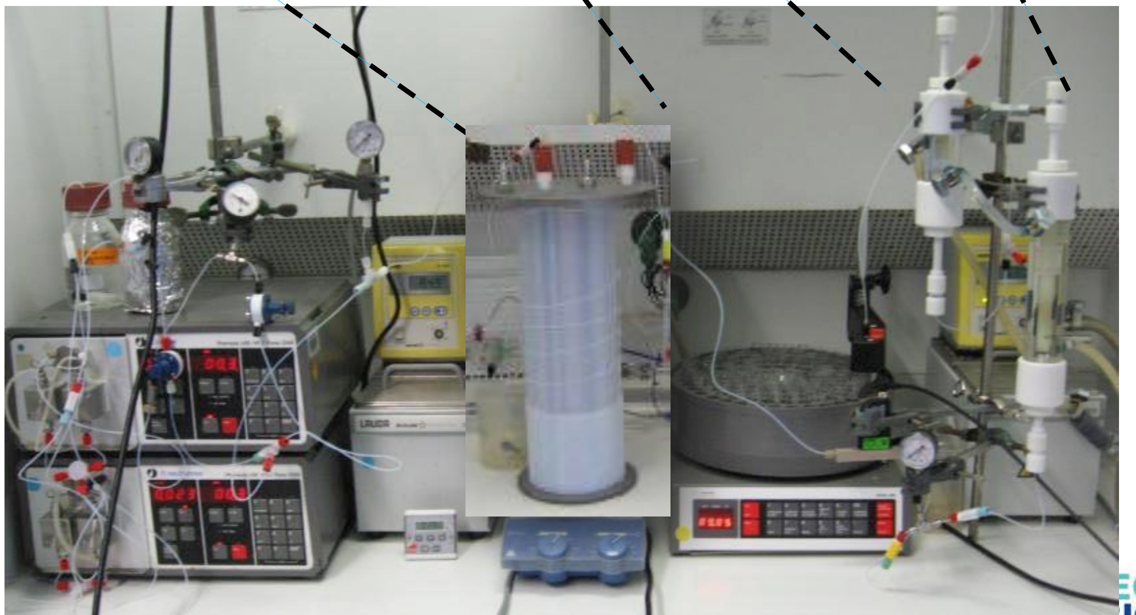
TECHNICAL BIOCATALYSIS TUHH

Reactor Setup: Continuous Operation



sty
> 1 kg/L/d

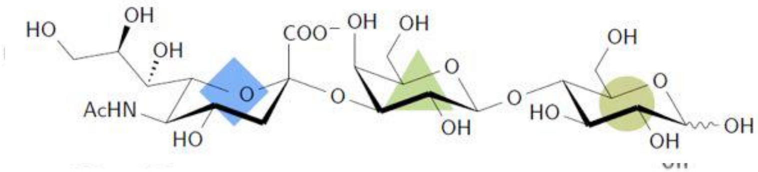




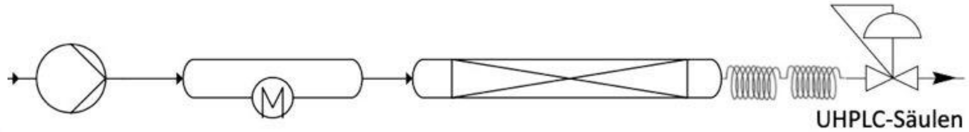
S. Strompen, M. Weiss, H. Gröger, L. Hilterhaus, A. Liese, Adv. Synth. Cat. 355 (**2013**) 2391
U. Kragl, A. Liese, Chem. Ing. Techn. 85(6) (**2013**) 826

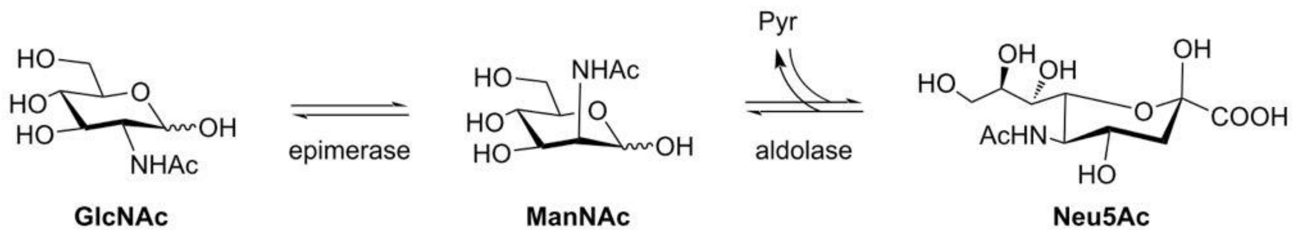
Overcoming Thermodynamic Limitations

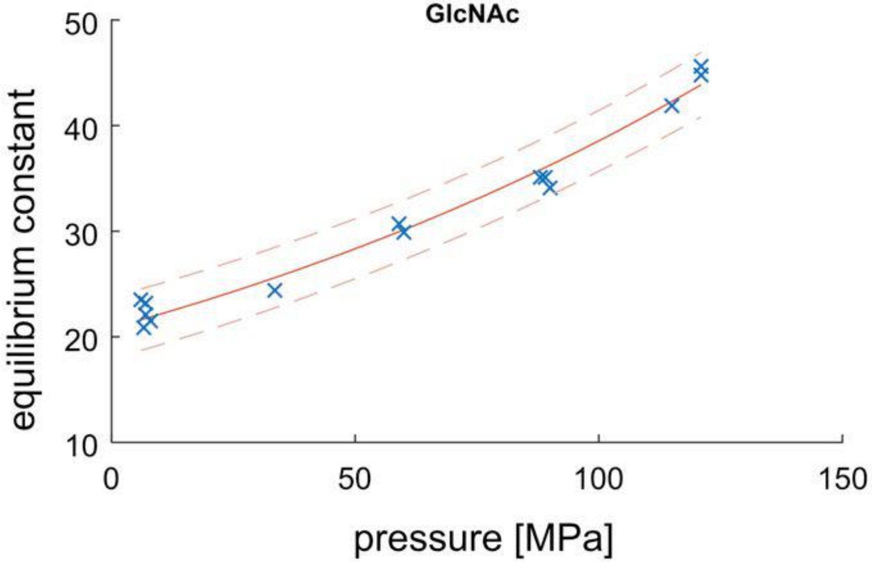
Continuous Processes @ High Pressure (120 MPa)

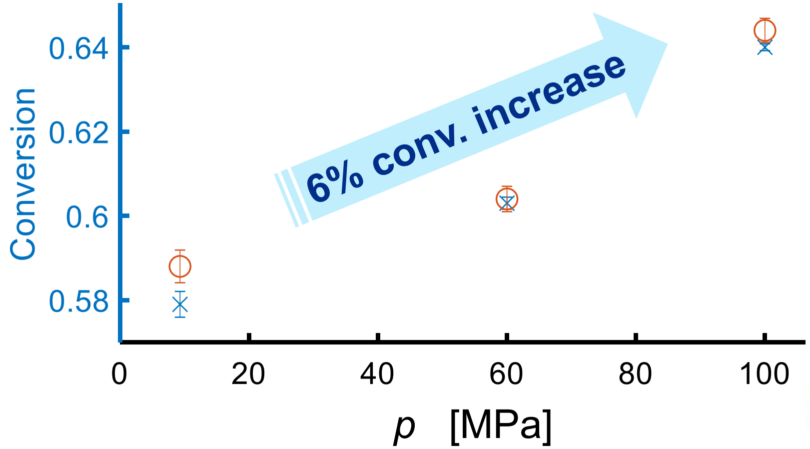


2,3-Sialyllactose (oder 2,6-Sialyllactose)





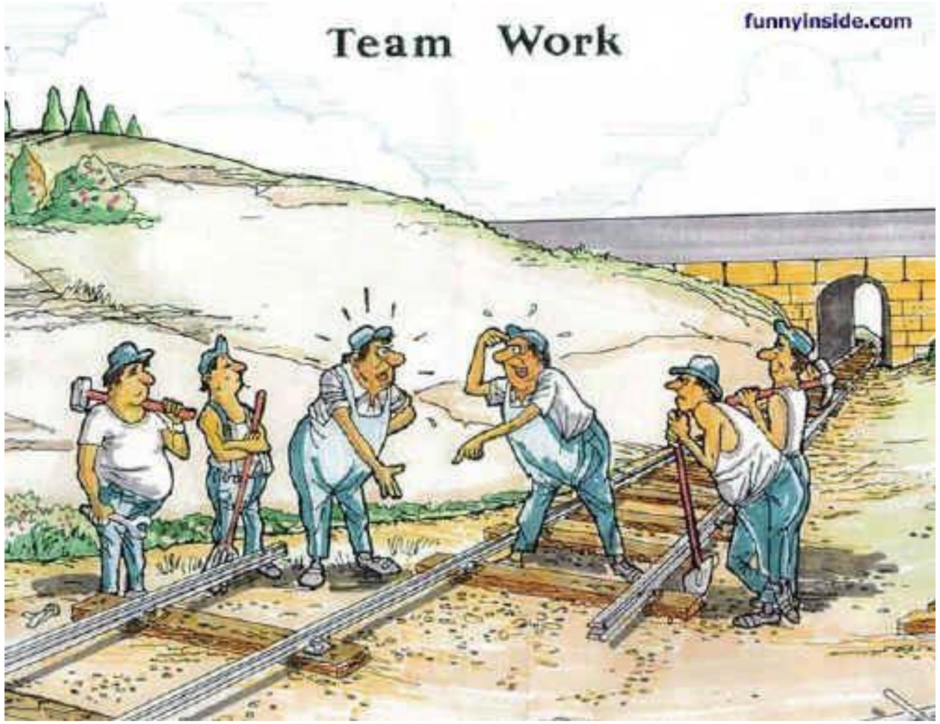




J. A. Reich, M. Aßmann, K. Hölting, P. Bubenheim, J. Kuballa, A. Liese,
Beil. J. Org. Chem. 18 (**2022**) 567-579

Process Intensification Challenges

Communication Challenges



multidisciplinarity

requires interdisciplinary understanding

TECHNICAL BIOCATALYSIS TUHH

Inspiring Youths Ages 8-18+ for STEM

STEM = Science, Technology, Engineering & Mathematics

Nachwuchs campus

13-18 years

School-Company STEM cooperation
educational material & projects
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for at home and/or the classroom
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TECHNICAL BIOCATALYSIS TUHH

Take Home Messages

- ***inline* analytics** is key to **process optimization.**
- Consider biotransformations under **neat conditions.**
- Consider **fine bubble airation** to **enhance stability** and **reduce gas consumtion.**
- **Continuous** version of the **batch** is the **plug flow reactor.**
- Consider **influence of reactor type** on **reaction selectivity**.

→ **Process Engineering Needs to Complement Chemistry and Biomolecular Engineering**

TECHNICAL BIOCATALYSIS TUHH

Acknowledgements

Prof. S. Kara

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Prof. H.K. Trieu