Enzyme Systems in Ultra-High Pressure Reactors

TECHNICAL BIOCATALYSIS TUHH

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Introduction

- Until now the pressure is mainly utilized to destroy enzymes and not to control/enhance enzymatic processes [1]
- For **reactions with a reduction in volume**, the pressure should, in theory, shift the equilibrium in favor of the product (see principle of Le Chatelier)
- This should hold true for any two-to-one-reaction (as applied in this work)
- Different groups used the pressure to influence the enantiomeric excess [2]

[1] Eisenmenger, M.; Reyes-De-Corcuera, J., Enzyme Microb. Technol., 2009, 5, 331-347.
[2] Kara, S.; Diss. TUHH, 2012.

Aim

- Setting up a reactor that allows the study of enzyme kinetics under **ultra-high pressure conditions (up to 1300 bar)**
- Study of kinetics, shift of equilibrium and calculation and modelling of pressure dependency
- Goal is to enable pressure as a mean to control an enzymatic reactor

Reaction System

- As a model system the production of **N-Acetyl-Neuraminic Acid** (Neu5Ac) is used, since the second step in the reaction is a **two-to-one-reaction** (N-Acetyl-D-mannosamine (ManNAc) and Pyruvate becoming Neu5Ac)
- Neu5Ac can be used to produce human milk oligosaccharides like Sialyllactose
- The reaction will be carried out using **immobilized enzyme**

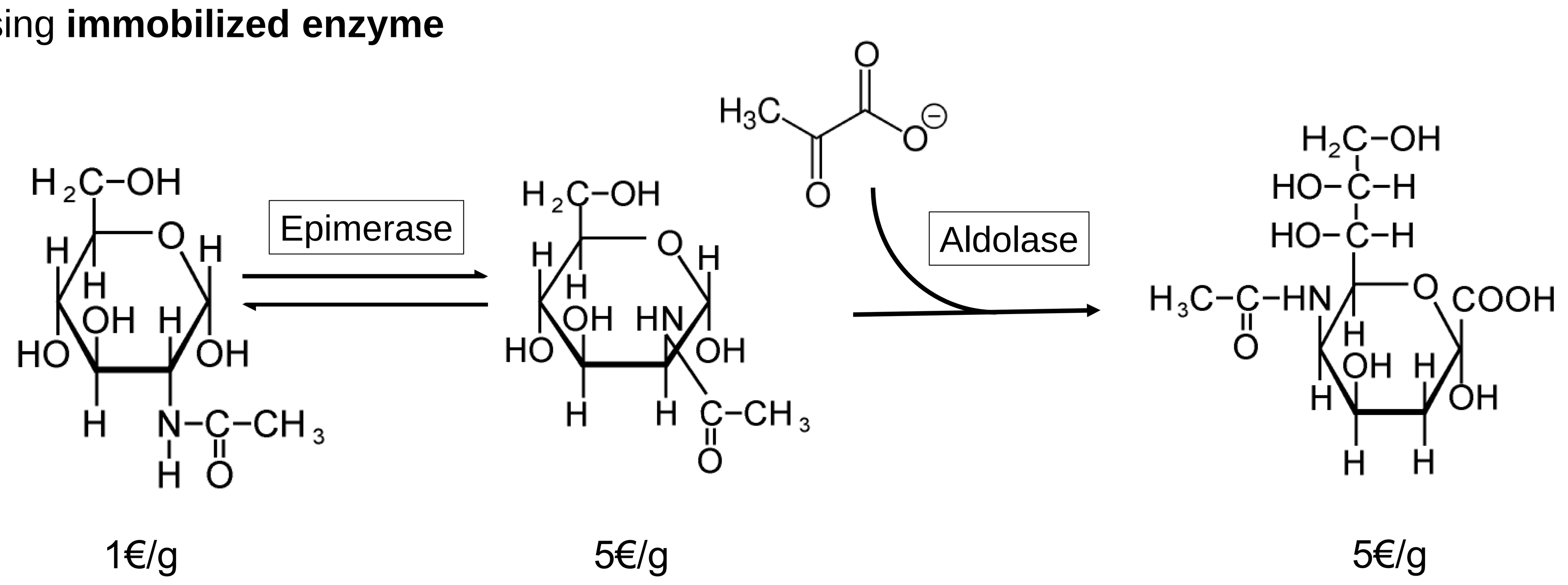

Fig 1: Reaction scheme. Shown are N-Acetyl-D-Glucosamine (GlcNAc), ManNAc, Pyruvate and Neu5Ac [3]

[3] Biochemical pathways by F. Hoffmann-La Roche Ltd, 2018

Practical - Reactor Setup

- Packing a fixed bed with small particles results in a pressure drop in the range of 1000 bar
- By using an UHPLC-pump fluid can be fed though fixed beds that exhibit high pressure drops
- **Pressure-stable carriers** will be investigated

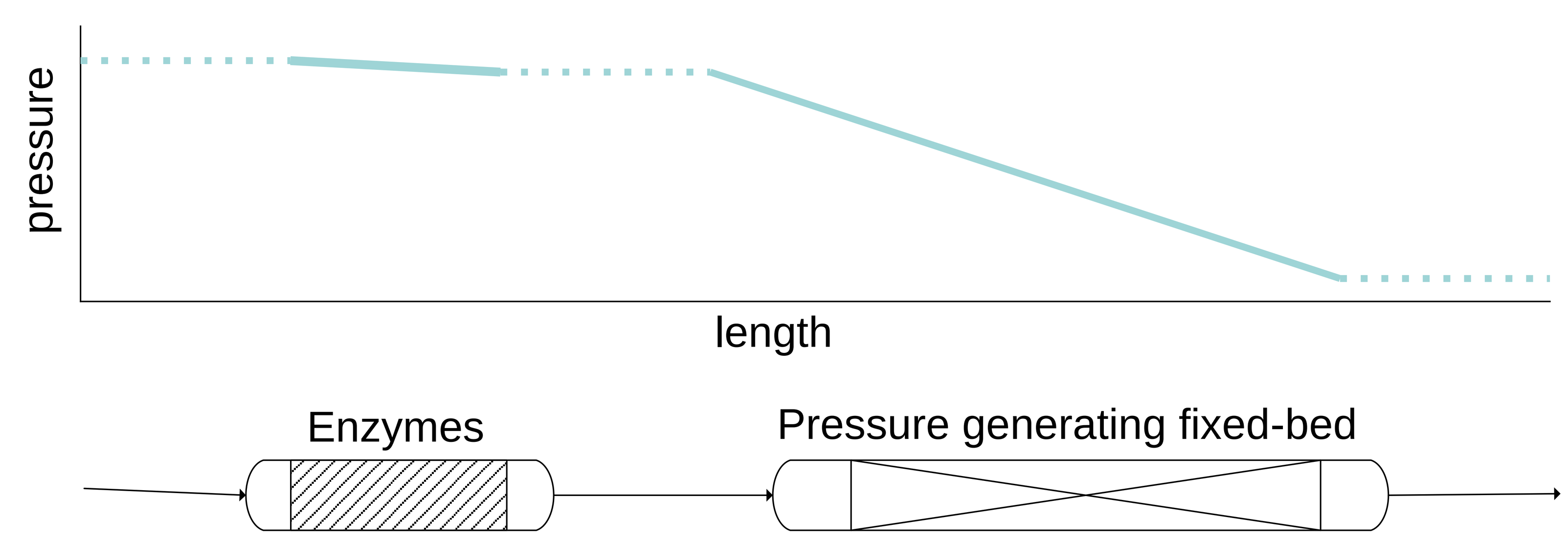

Fig 2: Currently planned reactor setup. First fixed bed contains immobilized enzymes

Theoretical - Kinetic Studies

- First the general **effect of pressure on rate constants** was investigated
- Afterwards this dependency was applied in rate expressions for different mechanisms such as Michaelis-Menten-Kinetics and **Ordered-Bi-Uni-Reactions**
- From Eq. (1) follows that a negative change in volume is needed to increase the reaction rate with increasing pressure

$$K^{A-T} = K_0 \exp\left\{\frac{-\Delta V^{\#} P}{RT}\right\} \quad (1)$$

$$k^{A-B} = \frac{k_B T}{h} K^{A-T} \quad (2)$$

$$k^{A-B} = k_0 \exp\left\{\frac{-\Delta V^{\#} P}{RT}\right\} \quad (3)$$

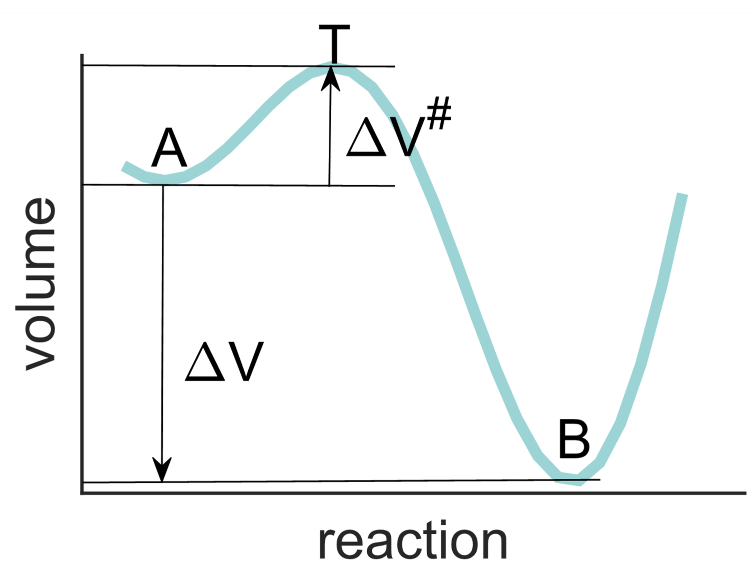

Fig 3: Scheme of the change in volume due to a reaction

Summary and Outlook

- By using the Steady State Theory equations where found to describe the **effect of pressure on reaction rate**
- Since changes in volume are small, **very high pressures** will be needed and applied in a reactor setup
- **Kinetic parameters** will be calculated from high pressure experiments

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