Enzymatic Reactions at High Pressures: Synthesis of Neuraminic Acid

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Introduction

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

Aim

- Setting up a reactor that allows the study of enzyme kinetics under **high pressure conditions (up to 1300 bar) in a flow reactor**
- Study of kinetics and thermodynamics
- Calculation and modelling of pressure dependency
- Enable pressure as a novel parameter to improve reaction performance thereby improving enzymatic reactors

Reaction System

- As a model system the enzymatic reaction sequence leading to ***N*-acetyl-neuraminic acid** (Neu5Ac) is used
- in a first reaction step *N*-acetyl-D-glucosamine (GlcNAc) is epimerized to *N*-acetyl-D-mannosamine (ManNAc) which is then coupled with Pyruvate (Pyr) to Neu5Ac (2:1-reaction) (Fig. 1)
- Kinetic parameters are being determined via progress curve analysis using a numerical solver for differential equations (Fig. 2)
- For this, published and validated rate expressions are being used [3]
- Neu5Ac can be used to produce human milk oligosaccharides like Sialyllactose
- The reaction will be carried out by using **immobilized enzymes**

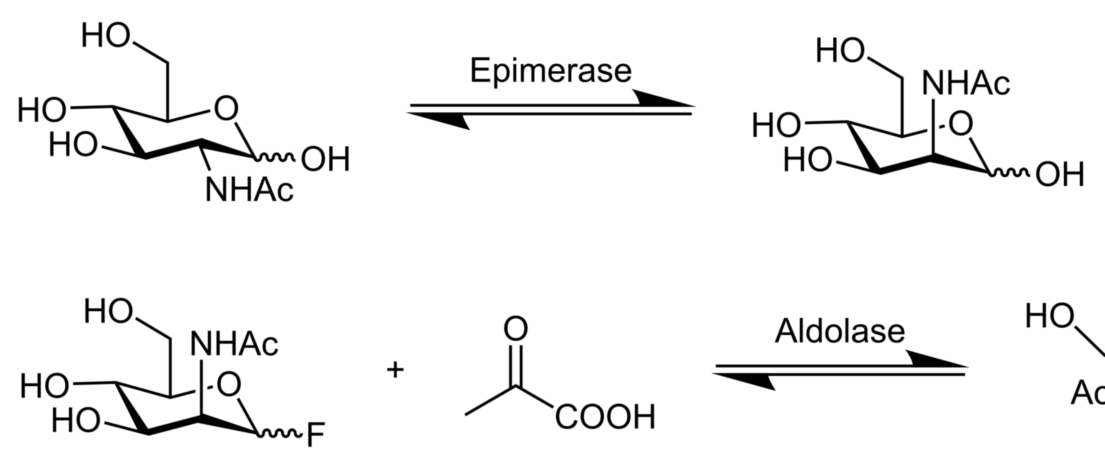

Fig. 1: Reaction scheme

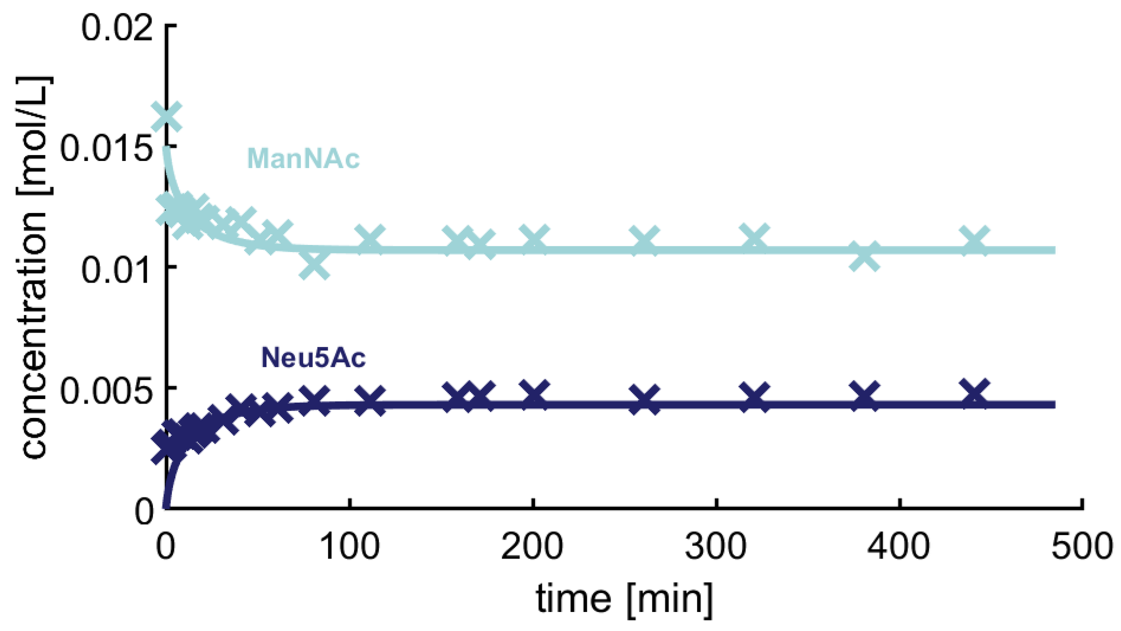

Fig. 2: Progress curve analysis to determine kinetic parameters; 37 °C, 700 rpm, pH 8 (potassium phosphate buffer), Aldolase: 1 g/L, volume: 1000 µL, starting concentration: 15 mM ManNAc, 25 mM Pyr.

Reactor Setup

- Packing a fixed bed with particles (diameter about 1 µm) results in a pressure drop in the range of 1000 bar
- By using an UHPLC-pump fluid can be fed through fixed beds that exhibit high pressure drops
- An enzymatic reactor is placed in the pressurized region between the pump and the fixed bed to carry out flow experiments (Fig. 3)
- **Pressure-stable enzyme carriers** will be characterized by monitoring changes in size distribution

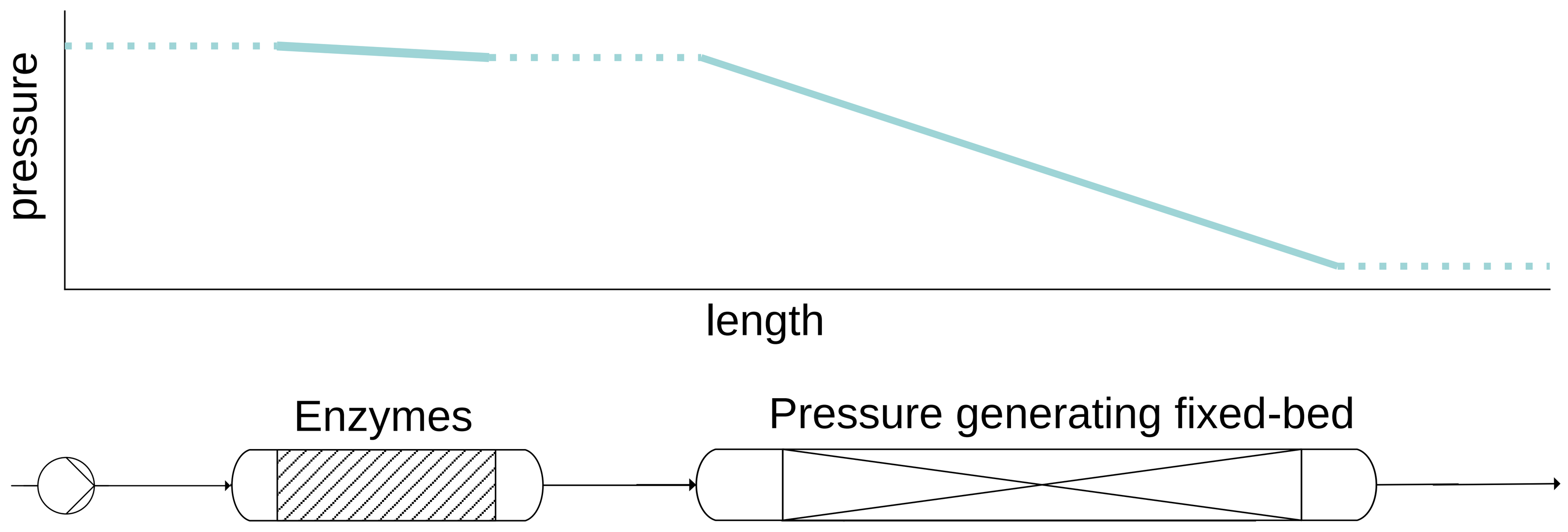

Fig 3: Current reactor setup. First fixed bed contains immobilized enzymes

Kinetic Studies

- First, the general **effect of pressure on rate constants** was investigated
- From transition state theory the effect of pressure on the rate constants was derived (Eq. (1) and (2) and Fig. 4) [4]
- 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

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

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

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

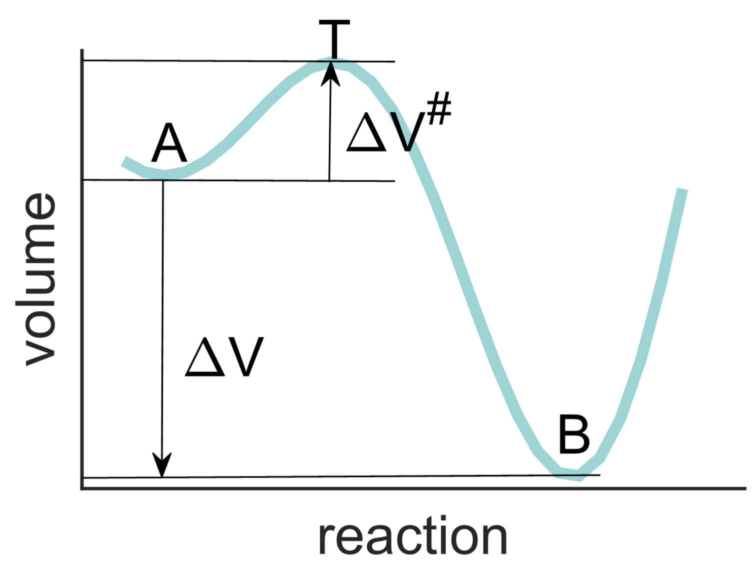

Fig 4: Scheme of the change in volume due to a reaction

Summary and Outlook

- By using the steady state theory, equations describing the expected **effect of pressure on the reaction rate** where derived
- Since changes in volume are small, **very high pressures** will be required and applied in the reactor setup
- **Kinetic parameters** will be calculated at different pressure levels

References

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