Enzymatic Reactions at High Pressures: Synthesis of Neuraminic Acid

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

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

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

\[ k_{\text{A,T}} = k_{\text{A,exp}} \left( \frac{-\Delta V P}{RT} \right) \]  
\[ k_{\text{A,B}} = k_{\text{A,exp}} \left( \frac{-\Delta V P}{RT} \right) \]

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