Enzyme Systems in Ultra-High Pressure Reactors

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]

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

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

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

![Fig 2: Currently planned reactor setup. First fixed bed contains immobilized enzymes](image)

![Fig 3: Scheme of the change in volume due to a reaction](image)

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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