Enzymes in a Continuous Packed Bed Reactor at Variable Pressure up to 1300 bar

TECHNICAL BIOCATALYSIS TUHH

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Introduction

- **High hydrostatic pressure** caught interest for enhancement of reaction in biotechnology [1,2]
- Pressure as a **parameter to improve performance** of enzymes

Aim

- Investigate **kinetics** and **equilibrium of enzymatically catalysed reaction with fixed bed** at **high pressure**
- A continuous production while utilizing high pressure

Fixed Bed – Reaction Rate

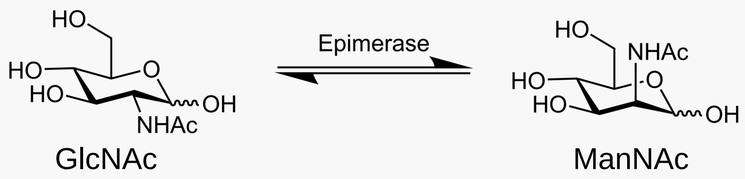


Fig. 1: Reaction scheme epimerase

- Short residence time allows for kinetic measurements
- **Pressure drop of capillaries** utilized to generate high pressure in fixed bed

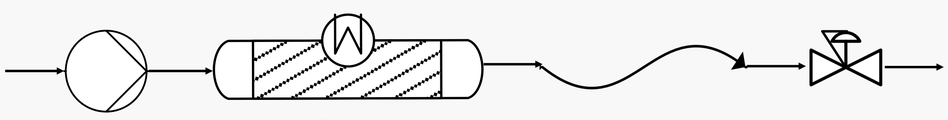
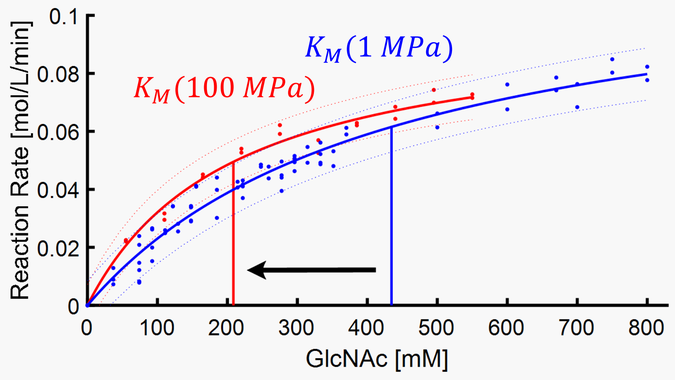


Fig 2: Scheme of the fixed bed reactor with UHPLC pump, packed bed, capillaries and back pressure regulator

Fig 3: 39 °C, 10 mM KPi buffer 7.50. flow rate 2 mL/min resulting in 6 s contact time, c_E=55.8 mg_{Immo}/0.21 mL. At least 45 s were waited for the system to be in steady state
Thin lines: 95% confidence



	1 MPa	100 MPa
K_M [mmol/L]	434 ± 69	209 ± 54
v_{max} [mol/L/min]	0.123 ± 0.010	0.099 ± 0.010

Tab 1: Determined kinetic parameters at low and high pressure. Conditions as Fig. 4. ± 95 % confidence

Advantages

- Sampling at ambient pressure
- Reaction at high pressure
- Pressure can be easily adjusted by $\dot{V}$, back pressure regulator or more/ fewer capillaries
- Setup can easily be changed from continuous to batch production
- High flow rates in circular reactor minimize the risk of film diffusion effects and result in high exchange of vessel volume
- Pressure can be changed for an ongoing experiment

Summary

- Changes in pressure can change the position of an equilibrium
- Volumetric changes are small ⇒ high pressure needed
- Reactor concept for continuously working high pressure with sampling at ambient pressure
- Influence of pressure on kinetics and reaction equilibrium found

Circular Reactor - Equilibrium

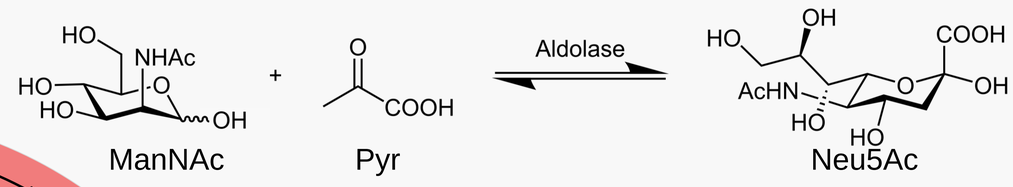


Fig. 3: Reaction scheme aldolase

- To reach equilibrium long residence times are necessary
  - Pressure built up at small flow rate challenging
  - Solution: circular reactor allowing arbitrary flow rates (Fig. 4)
- Position of equilibrium is measured via **equilibrium constant K** (independent of molar ratio of starting material)

$$K = \frac{a_P}{a_{S1} a_{S2}} \approx \frac{c_P}{c_{S1} c_{S2}} \cdot 1 \frac{mol}{L}$$

a_i: activity
c_i: concentration

- Reaction in equilibrium: change in pressure let to change in equilibrium position and constant

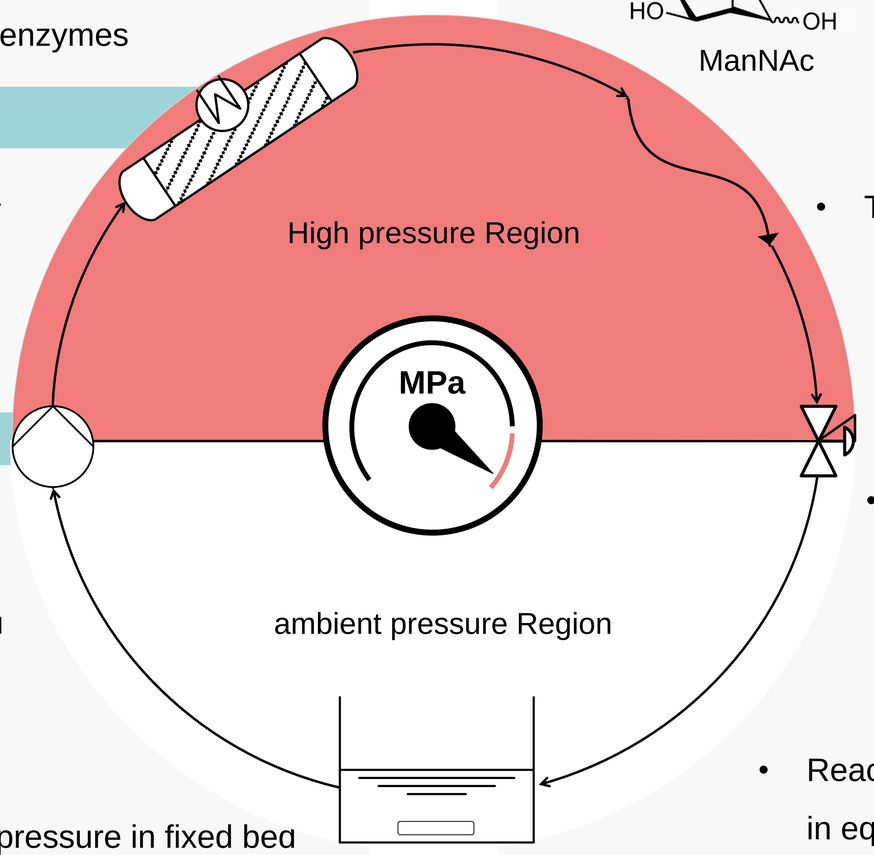


Fig 4: Circular High Pressure Reactor consisting of UHPLC-pump (west), fixed bed reactor (northwest), HPLC oven (northwest), capillaries (northeast), back pressure regulator (east) and vessel (south)

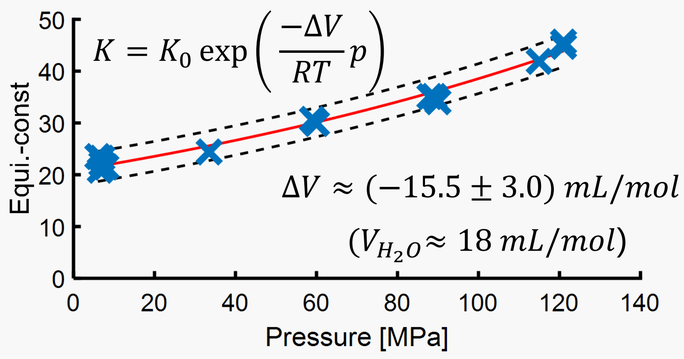


Fig 5: Equilibrium constant at different pressures. 39-40 °C, ManNAc / Pyr: 100 / 250 mM or 100 / 100 mM, 10 mM KPi buffer 8.00. 272.3 mg_{Immo} / 0.71 mL, Sampling after 16 h or when no changes occurred within 1 h, dashed lines and ±: 95 % confidence

- By applying pressure, equilibrium conversion was increased from 70 % to 88 %
- Pressure is constant over the experimental time (median average distance: 0.1 MPa)

Setup Information

- UHPLC pump Shimadzu Nexera X2
- 50 µm Capillary (length: 30 cm)
- Back pressure regulator (up to 30 MPa)
- 2 mL in vessel
- pump rate: 1.5 mL/min ⇒ exchange times of less then 2 min
- Similar residence time distributions for packed beds ⇒ beds are similar
- Sample analysis using HPLC with a Refractive Index Detector

Fig 6: Resulting bed from sedimentation in a syringe

Outlook

- Both enzymes to be investigated e.g. epimerase at equilibrium and reaction rate of aldolase, reverse reaction of the epimerase
- Combination of both enzymes in the same reactor

References
[1] Eisenmenger, M.; Reyes-De-Corcuera, J., Enzyme Microb. Technol., 2009, 5, 331-347.
[2] Kara, S., Long W., Berheide, M., Peper, S., Niemeyer, B., Liese, A.; J. Biotechnol., 2011, 152(3), 87-92.

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