Enzyme Catalysis at Elevated Pressure

TUHH
Hamburg University of Technology

Marlene Schmalle[1], Paul Bubenheim[1], Andreas Liese[1], Miriam Aßmann[2], Jonas Andrich[2], Jürgen Kuballa[2]

[1] Hamburg University of Technology, Institute of Technical Biocatalysis, Hamburg, Germany
[2] GALAB Laboratories GmbH, Hamburg, Germany

Pressure Induced Optimization of Biocatalysts

- Industrial processes apply high pressure over 400 MPa to deactivate unwanted enzymes[1]
- Below 200 MPa several enzymes show a **pressure induced change** of their performance in terms of **activity, stability and selectivity**[2,3]
- Stability of enzymes as function of thermal deactivation is sometimes increased by high pressure[4,5]

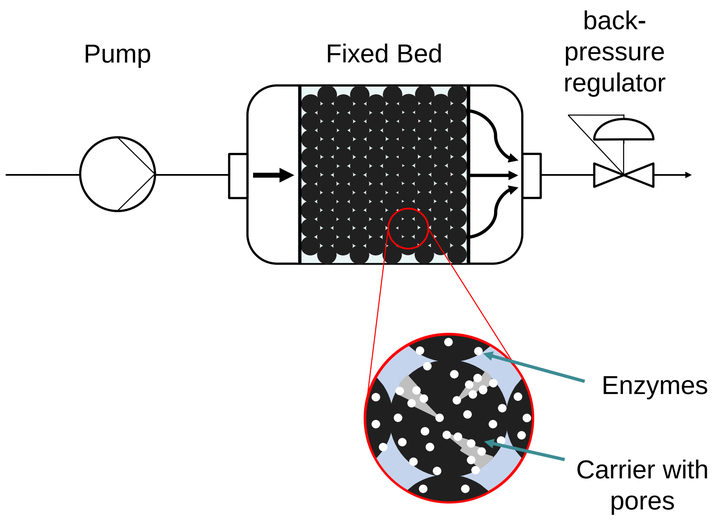
Aim: Conceptualization of a suitable reactor to determine **enzyme kinetics** and increase **enzyme performance** under high pressure

Determination of enzyme kinetics → **Determination of enzyme class characteristics** → **Increase of enzyme performance**

High Pressure Reactor

The high pressure reactor is generated as continuously operated plug flow system **Fig. 1**

- Enzymes are immobilized on the surface of porous carries
- System pressure is generated by a back pressure regulator (BPR)



Specifications

Pump
- Volumetric flow: 0.0001 to 10 ml·min^{-1}
- Pressure up to 1300 bar

Enzymatic fixed bed reactor
- Temperature range: 10 to 100°C
- Dimensions: dxl∅ 4x100 mm (variable)
- Particle size: 300 to 1000 µm

Figure 1: Reactor setup for high pressure determination of enzyme kinetics

→ highly flexible system, which provides the opportunity to analyse different reaction systems and different enzymes

Transesterification

- Effects of high pressure on the stability, activity and selectivity will be investigated on the transesterification of vinyl acetate and 1-phenyl-2-propanol to 1-phenyl-2-propanyl-acetate catalyzed by *Candida rugosa* lipase (EC 3.1.1.3) in **Fig. 2**
- Enzyme kinetics under different temperatures and pressures are determined

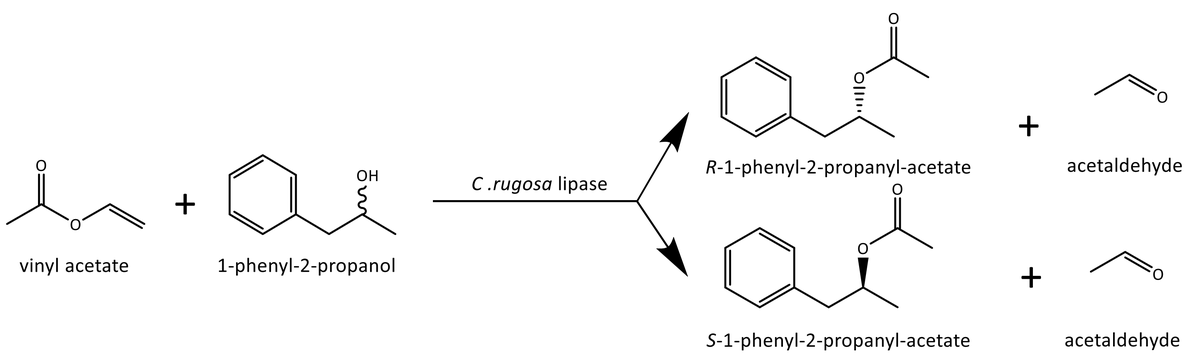


Figure 2: Transesterification reaction catalyzed by *Candida rugosa* lipase (CRL)

Optimization of Enzyme Performance

- Application of enzymes in industrial processes might be limited by their sensitivity to extreme reaction conditions such as temperature, pH-value and aggressive chemical[4]
- Various **methods to enhance enzyme performance** (activity and stability): genetic engineering, immobilization and operation in non-aqueous media[1]

New approach→ performing **enzyme catalyzed reactions under pressure**

- Determination of enzyme kinetics under different temperatures and pressures will lead to a better understanding of enzyme properties and its optimal reaction conditions

→ a suitable combination of different methods to enhance enzyme performance will lead to optimal reaction conditions

Activity Increase by High Pressure

- Transesterification reaction catalyzed by CRL was performed at different pressures: 0, 200, 400 bar
- **Activity increased** by 338% from 1.9 $\frac{\text{unit}}{mg_{enzyme}}$ at 0 bar to 6.3 $\frac{\text{unit}}{mg_{enzyme}}$ at 400 bar

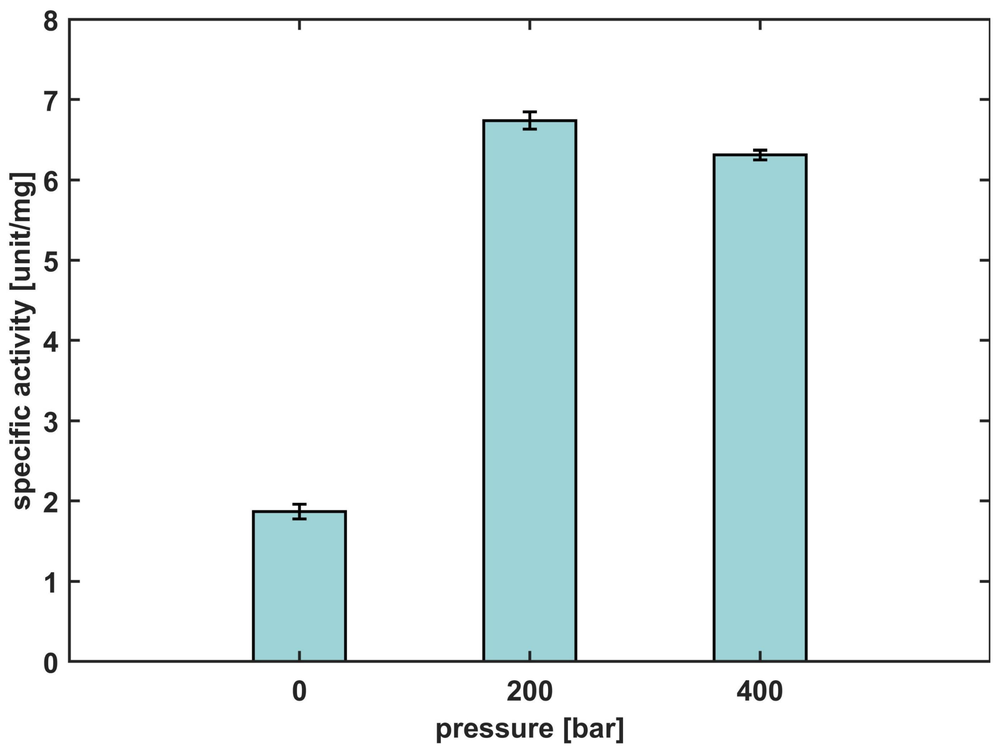


Figure 3: Specific activity of CRL in dependence of pressure, 0.1 g Purolite® ECR 1090 with immobilized CRL, 10 mM 1-phenyl-2-propanol in heptane/vinylacetate (75/25) vol.-%, 35°C, $\dot{V} = 0.1 \frac{\text{ml}}{\text{min}}$, Series of repeated experiments

Outlook

Enzyme kinetics will be determined in a novel conceptualized **high pressure reactor** to understand pressure induced **changes in enzyme performance**. This knowledge can be used to determine **enzyme class specific pressure effects** to identify **optimal reaction conditions** and to **improve enzymatic reaction systems**.

References
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Contact
Marlene Schmalle
Institute of Technical Biocatalysis
Hamburg University of Technology
Marlene.schmalle@tuhh.de
+49-40-42878-4265