Biocatalytic Oxyfunctionalization of Butane in a Bubble Column Reactor

F. Perz[1], S. Bormann[2],H.-G. Hennemann[3], F. Nissen[3], F. Hollmann[4], D. Holtmann[5], P. Bubenheim[1], A. Liese[1]

[1]Institute of Technical Biocatalysis, Hamburg University of Technology, Hamburg, Germany
[2]Industrial Biotechnology, DECHEMA-Forschungsinstitut, Frankfurt am Main, Germany
[3]Evonik Creavis GmbH, Marl, Germany
[4]Department of Biotechnology, Delft University of Technology, Delft, Netherlands
[5]Institute of Bioprocess Engineering and Pharmaceutical Technology, University of Applied Sciences Mittelhessen, Giessen, Germany

Introduction & Project Aim

- **Short chain alkanes** are a low value and abundant resource. Chemical activation is difficult, energy demanding and environmentally unfriendly.[1]
- In comparison, **selective biocatalytic activation** is an appealing alternative to chemical oxyfunctionalization as various biocatalysts can convert alkanes to different organic compounds under mild reaction condition.

⇨ Project aim: Investigation and comparison of a whole cell (alkBGT in *E. coli*) and a free enzyme (rAaeUPO) approach for the hydroxylation of butane.

Whole Cell

- Hydroxylation of butane to 1-butanol and overoxidation to butyric acid by membrane bound **alkBGT**-system from *Pseudomonas putida* GPo1 expressed in *E. coli.*
- Mixed gas (butane-air) and glucose feed for internal regeneration of reducing equivalents (NADH)
- Single parameter investigation shown previously[2] in 2 L bubble column reactor (glass, DN 80, H/D ≈ 6)

C**hallenge:** Mediation between reaction performance, mass transport limitation, and the need of the whole cell.

- Design of Experiment for multivariable analysis of the parameters: butane content, gassing rate and overpressure in a face centered composite design

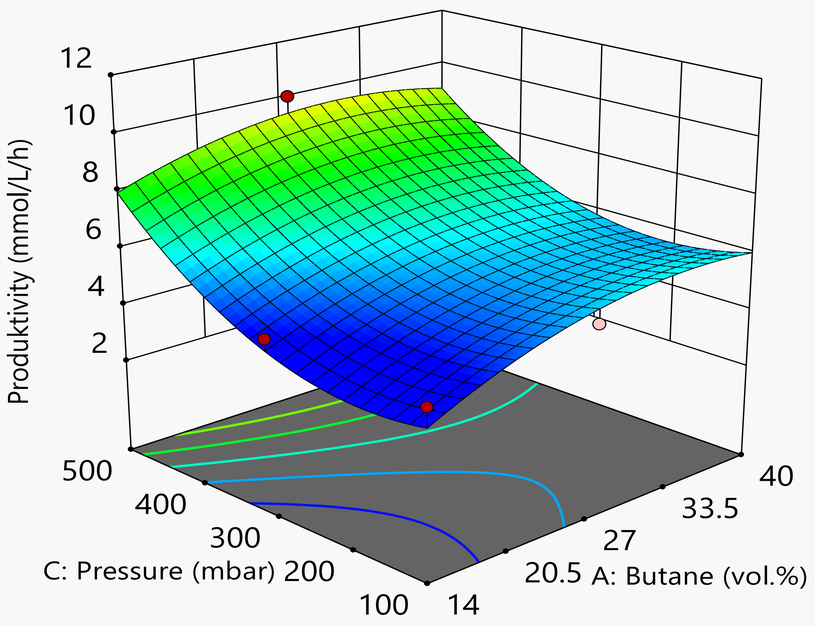

Fig. 1: DoE response: Interaction of butane content and overpressure on volumetric productivity for a gassing rate of 1.1 L/min. Design space: overpressure 100-500 mbar, gassing rate 0.7-1.5 L/min, butane content 14-40 vol.%

- High butane content in feed gas can lead to oxygen limitation
- Pressure optimum outside of design space, limited by reactor material and maximum pressure from butane bottle

Opportunities for improvement

- Addition of mass transfer vectors for improved butane transfer
- Change of reactor setup or configuration, maintaining a explosion-safe setup e.g. minimum of moving parts

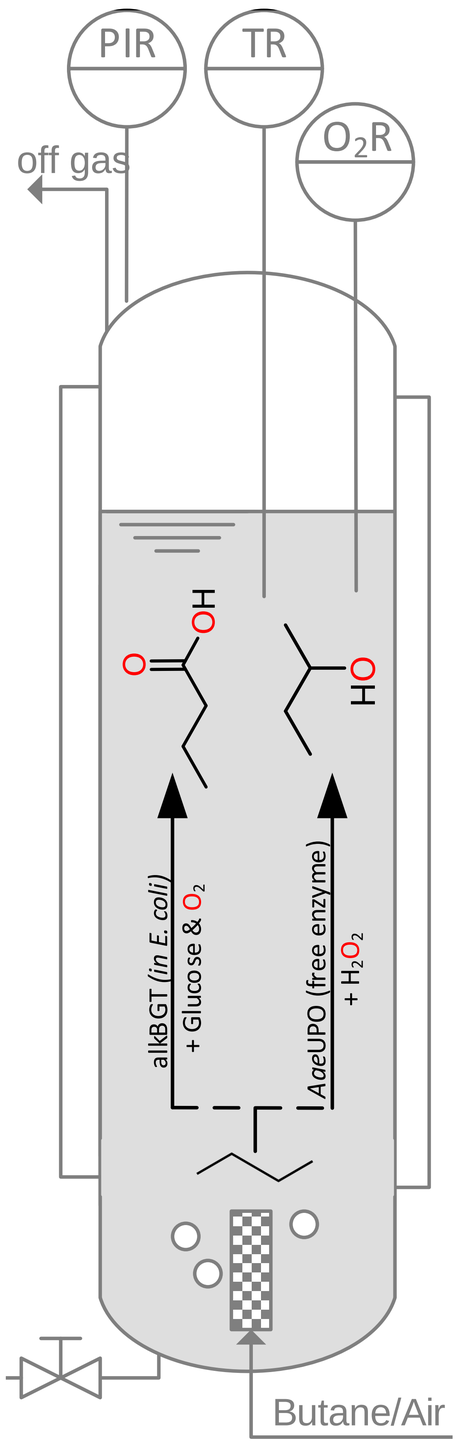

Fig. 2: Simplified scheme of the experimental setup and the investigated reaction system. Adjustment of the feed gas is done with a gas mixing station. A arbitrary mixture of butane with air or nitrogen is possible.

Free Enzyme

- Hydroxylation of butane to 2-butanol by recombinant expressed unspecific peroxygenases from *Agrocybe aegerita: "**rAae**UPO"*
- Butane (pure) and hydrogen peroxide feed as substrates
- First experiments outside of analytical scale: 0.2 L bubble column and scale up to 2 L with ISPR[3]

Challenges: Mediation between reaction rate and stability of the enzyme under process conditions

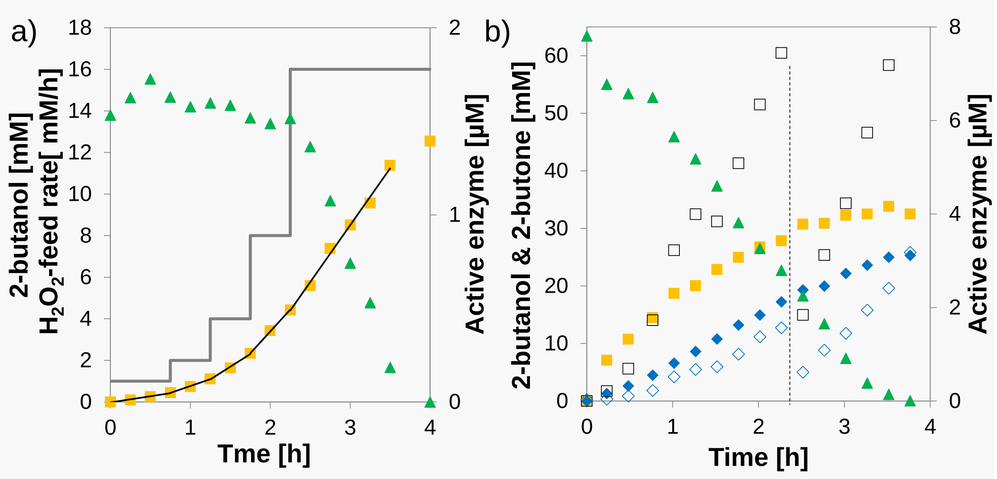

Fig. 3: Reaction progress of r*Aae*UPO catalyzed butane hydroxylation. Concentration of: Active enzyme (▲) and 2-butanol (■) in a) 0.2 L bubble column reactor with increasing hydrogen peroxide feed (-) and b) in 2 L bubble column setup with ISPR in a 0.2 L extraction column, overoxidation to 2-butanone (♦) and concentrations in the extractant, n-dodecane (complete exchange of solvent indicated by dashed line): 2-butanol (□), 2-butanone (◊).[2]

- Only minor enzyme deactivation by gassing of butane
- Total turnover numbers of up to 16000
- Despite ISPR, overoxidation of the target product (2-butanol) pronounced in 2 L scale

⇨ Mass transport limitation, butane to aqueous reaction media and 2-butanol to organic phase

Opportunities for improvement

- Kinetic investigation of the system for modeling and optimization
- *In situ* generation and measurement of hydrogen peroxide concentration
- Improvement of ISPR for the reduction of over oxidation, use of a mobile organic phase and/or increased power input

Summary & Outlook

- Oxidation of short chain alkanes by whole cells (alkBGT) and free enzyme (UPO) in a multiphase reactor
- Determination of process windows for these systems
- Screening for promising mass transfer vectors
- Optimization of enzyme usage through kinetic investigations and determination of optimal process conditions

References:
[1] van Beilen, J.B., Funhoff, E.G., 2005, DOI 10.1016/j.copbio.2005.04.005
[2] Sluyter, G., Kleber, J., Perz, F., Grund, B., Leuchs, S., Sieberz, S., Bubenheim, P., Thum, O., Liese, A., 2020, 10.1016/j.bej.2020.107486
[3] Perz F., Bormann S., Ulber R., Alcalde M., Bubenheim P., Hollmann F., Holtmann D., Liese A., 2020, DOI 10.1002/cctc.202000431

Acknowledgement:
We are grateful to Evonik Industries for intellectual, technical and financial support.

Frederic Perz
Institute of Technical Biocatalysis
Denickestr. 15, 21073 Hamburg

Tel. +49 40-42878-2400
E-Mail: frederic.perz@tuhh.de