

Introduction

Organoboron compounds:

- organic reactant
 - boron derivative
- C-B heteroatomic bond



Advantages: H⁺-donor, strong electrophile → versatile platform chemical

Applications: polymerization, catalysts antioxidants, fuel additives, antibiotics

Chemical synthesis: hydroboration, borylation, grignard reaction

Limitations of conventional synthesis:

- low regioselectivity
- catalysts total turnover number (TTN) < 100
- environmentally offensive synthesis

Novel approach: Biocatalytic Organoborane Synthesis:

- Enzyme driven whole cell biotransformation (Cytochrome C) [1]
- Sustainable and reusable [2]
- Higher TTN compared to chemo catalysts [1]
- Highly enantioselective [1]

Challenges of the biocatalytic reaction system:

- Undetermined kinetic reaction parameters
- Only approved in analytic and milligram scale
- Cytochromes c is sensible to oxygen
- Carbene binding to haem c can cause inactivation

Objective

Enzymatic characterization: Determination of kinetic reaction parameters of biocatalytic borylation

Simulation based screening: Identification of optimal reaction conditions

Transfer to preparative scale: Reactor type and operation mode selection

Improvement of enzymatic performance from an reaction engineering point of view to bring biocatalytic borylation to an **industrial relevant alternative**. [3]

Cultivation of Biocatalysts

Molecular biological preparations:

- Set up of a strain and plasmid security collection
- Construction of production strain *E. coli* BOR^{R1}
- Expression and purification of cytochrome

Fermentation cascade:



Fig. 1: LB-Agar plate with 20 mg/L chloramphenicol and 100 mg/L ampicillin

Fig. 2: LB medium overnight culture, 160 rpm and 37 °C

Fig. 3: *E. coli* BOR^{R1} cultures in HB medium with induction (red) and without (yellow)

Fig. 4: M9N buffer cell suspension for organoborane synthesis

Organoborane Synthesis

Biocatalytic borylation reaction mixture:

- E. coli* BOR^{R1} cells expressing cytochrome c
- 10 mM ethyl-2-diazopropanoate
- 10 mM N-heterocyclic carbene-borane
- 5 % (v/v) MeCN

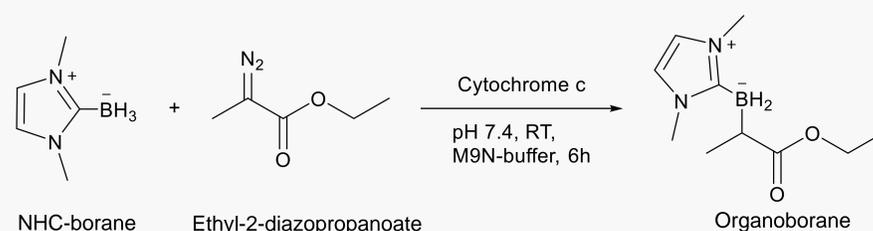


Fig. 5: Reaction scheme of organoborane synthesis in *E. coli* BOR^{R1}. NHC-borane and Me-EDA are used as substrates to form the organoborane product.

Preliminary results

- Chemical synthesis of NHC-borane substrate
- Enzymatical synthesis of organoborane product in whole cells

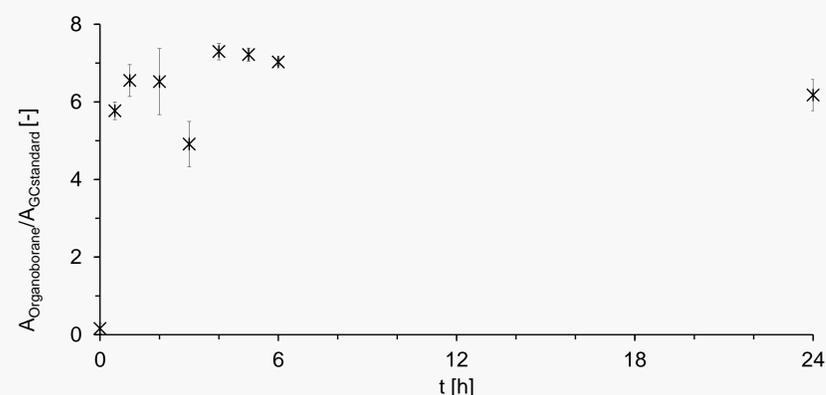


Fig. 6: Organoborane synthesis with whole cells. Concentration of organoborane referred to intern standard 1,2,3-trimethoxybenzene. Synthesis was performed at 25 °C and pH 7.4.

- Whole cells yield highest concentration of organoborane product
- Cell lysate and purified cytochrome c achieve only low product amounts
- Enzymatic synthesis of organoborane product of 53 mg with a selectivity of 97 % (e.r.)

Summary

- Construction of production strain *E. coli* BOR^{R1}
- Successful organoborane synthesis
- Biocatalytic borylation most efficient in whole cells
- Organoborane synthesis in milligram scale

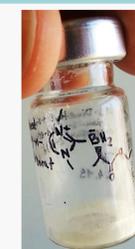


Fig. 7: Enzymatic synthesized organoborane product

Outlook

- Establishing of 10 mL thermostated vessel reactor for enzyme characterization
- Evaluating influence of temperature, pH value and substrate concentration
- Kinetic characterization in terms of K_m and K_i, v_{max} values
- Implementation of kinetic model based on experimental data

References:

- [1] J. Kan, X. Huang, Y. Gumulya, K. Chen, F.H. Arnold: Nature, No.552, 132-136, 2017.
- [2] L. Hilterhaus, A. Liese, U. Ketting, G. Antranikian: Wiley-VCH, 2016, 464 S., ISBN 978-3-527-33669-2.
- [3] A. Liese, L. Hilterhaus: Chemical Society Reviews, No. 42, 6236-6249, 2013.

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Contact: Jens Hennig

Institute of Technical Biocatalysis
Hamburg University of Technology
Denickestr. 15; 21073 Hamburg
Tel: +49-40-42878-2205
Email: jens.hennig@tuhh.de

