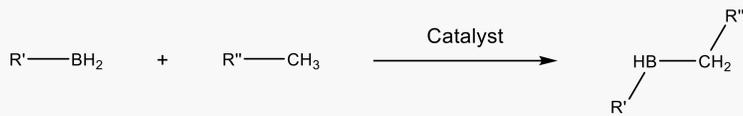


Reaction Engineering Development of Biocatalytic Borylation using Cytochrome c

Introduction

Organoboron compounds:

- organic reactant
 - boron derivative
- C-B hetero-atomic bond



Properties: 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:

- Cytochrome c (Cyt c) driven whole cell biotransformation [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
- Cytochrome c is sensitive to oxygen
- Carbene binding to haem c can cause deactivation

Objectives

Enzymatic characterization: Identification of influencing parameters and collection of experimental data

Build up of a kinetic model: Parameter fitting and reaction simulation

Reaction engineering: 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]

Biocatalytic Organoborane Synthesis

Biocatalytic borylation model reaction:

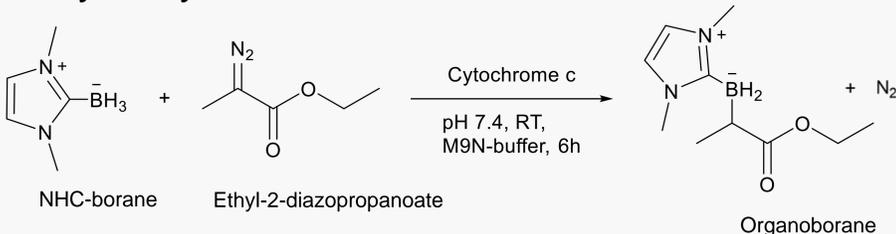
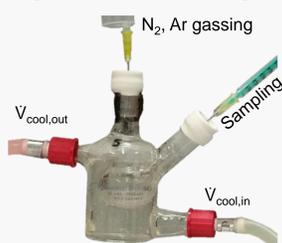


Fig. 1: Reaction scheme of organoborane synthesis in *E. coli* BOR^{R1}. NHC-borane (NHCb) and Ethyl-2-diazopropanoate (DAC) are used as substrates to form the organoborane (OB).

Experimental setup:



- Thermostated reactor vessel ($V_{\text{reactor}} = 10 \text{ mL}$)
- Enables fast and accurate sampling
- Simultaneous operation of 3 reactors

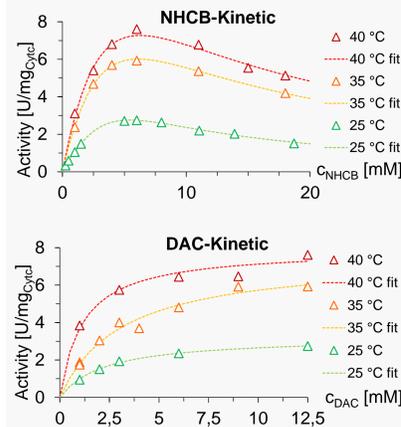
Fig. 2: Improved experimental thermostated reactor-based setup.

References:

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

Kinetic Characterization

Recording and fitting of Michaelis-Menten kinetics:

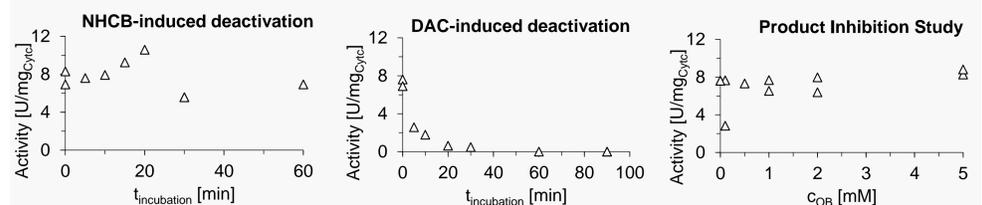


- Non-competitive substrate inhibition by NHCb observed
- No inhibition by DAC observed
- Highest activity at 40 °C

Tab. 1: Numerically fitted kinetic Michaelis-Menten parameter based on experimental data.

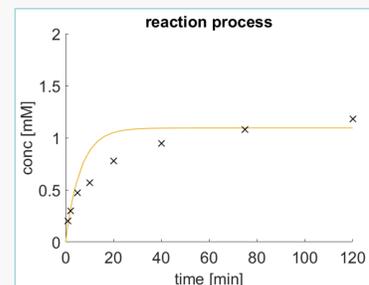
| Parameter | 25 °C | 35 °C | 40 °C |
|--------------------------|-------------|-------------|-------------|
| V_{max} [U/mg] | 26.4 ± 20.0 | 23.8 ± 15.4 | 24.1 ± 15.6 |
| $K_{\text{m,NHCb}}$ [mM] | 19.4 ± 16.2 | 6.6 ± 5.8 | 6.4 ± 5.80 |
| $K_{\text{m,DAC}}$ [mM] | 2.2 ± 0.3 | 3.1 ± 0.8 | 1.1 ± 0.5 |
| $K_{\text{i,NHCb}}$ [mM] | 1.5 ± 1.3 | 5.7 ± 5.1 | 6.2 ± 5.6 |

Fig. 3: Michaelis-Menten curves and their numerically fitted progressions for NHCb (top) and DAC (bottom) for biocatalytic borylation, catalysed by *Rma cyt c* BOR^{R1} harboured in *E. coli* BL21 DE3.



- No NHCb-induced deactivation
- DAC-induced deactivation: $K_{\text{dea}} = 0.18 \text{ min}^{-1}$
- No product inhibition

Model-based simulation of organoborane synthesis:

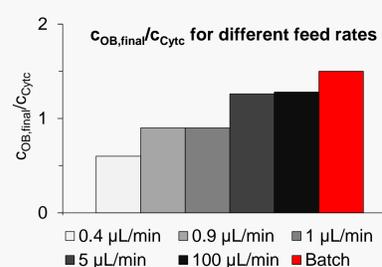


- Overshoot during initial reaction phase
- Underestimating the final OB concentration

$$v = c_{\text{enz}} e^{-K_{\text{dea}} t} * v_{\text{max}} * \frac{[\text{NHCb}]}{K_{\text{m,NHCb}} + [\text{NHCb}] * \left(1 + \frac{[\text{NHCb}]}{K_{\text{i}}}\right)} * \frac{[\text{DAC}]}{K_{\text{m,DAC}} + [\text{DAC}]}$$

Fig. 5: Simulation of OB-synthesis based on developed model.

OB-synthesis in fed-batch mode:



- Lowered feed reduces OB-synthesis
- Minimized reaction rate due to lower substrate concentration

Fig. 6: Investigation of different feed rates on Cytc-specific OB synthesis.

Summary

- Expression of *Rma cyt c* BOR^{R1} in *E. coli* BL21 DE3
- Numerical fitting of kinetic constants V_{max} , $K_{\text{m,NHCb}}$, $K_{\text{m,DAC}}$, $K_{\text{i,NHCb}}$
- Simulation of reaction progress based on kinetic model
- Initial fed-batch experiments carried out



Fig. 7: Enzymatically synthesized organoborane product.

Outlook

- Further investigation of fed-batch and continuous operation modes
- Evaluation of different reactor types
- Immobilization of Cytc on different carriers

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