Reaction Engineering Development of Biocatalytic Borylation using Cytochrome c

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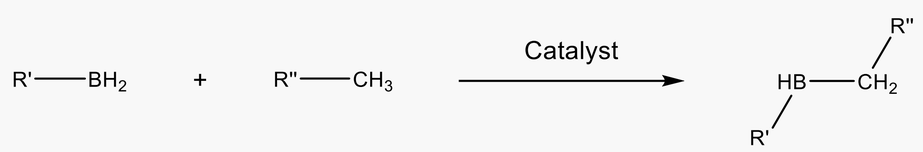
Introduction

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 (Cytc) 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

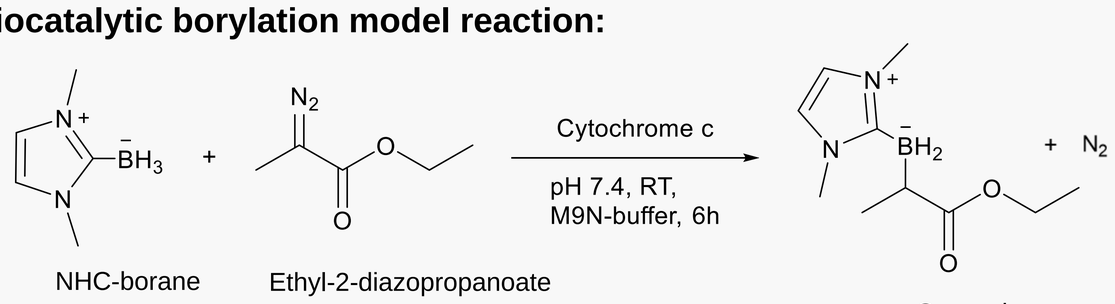
Build up of a kinetic model: Parameter fitting and reaction simulation

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

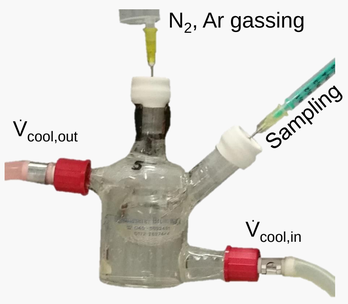
Biocatalytic borylation model reaction:



NHC-borane — Ethyl-2-diazopropanoate — Organoborane

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:

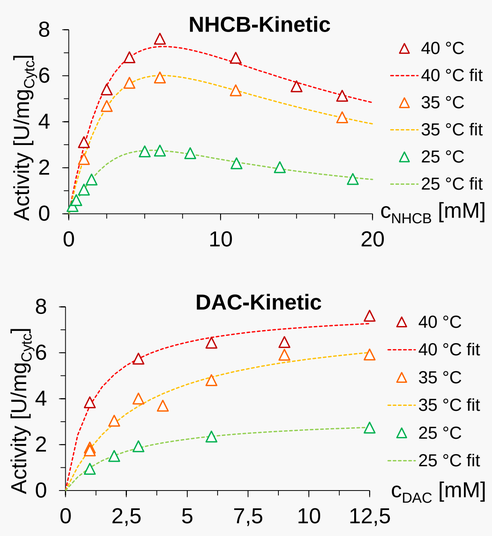


- Thermostated reactor vessel ($V_{reactor}$ = 10 mL)
- Enables fast and accurate sampling
- Simultaneous operation of 3 reactors

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

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
$K_{m,NHCB}$ [mM]	19.4	± 16.2	6.6	± 5.8	6.4	± 5.80
$K_{m,DAC}$ [mM]	2.2	± 0.3	3.1	± 0.8	1.1	± 0.5
$K_{i,NHCB}$ [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* cytc BOR^{R1} harboured in *E. coli* BL21 DE3.

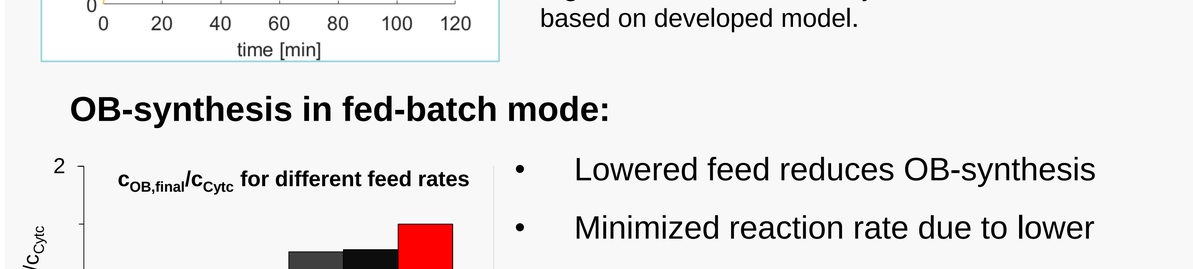
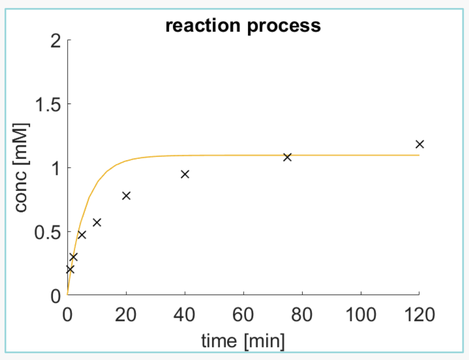


Fig. 4: Cytc activities for different OB concentrations and various incubation times with DAC and NHCB, to identify possible irreversible inhibitions.

- No NHCB-induced deactivation
- DAC-induced deactivation: K_{dea} = 0.18 min^{-1}
- No product inhibition

Model-based simulation of organoborane synthesis:

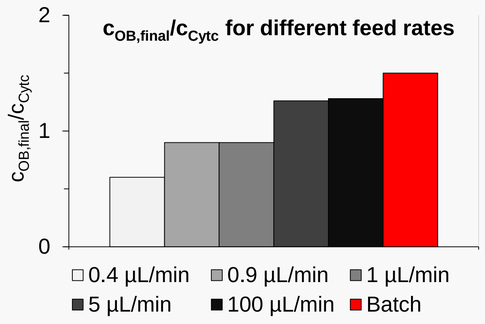


- Overshoot during initial reaction phase
- Underestimating the final OB concentration

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

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}, $K_{m,NHCB}$, $K_{m,DAC}$, $K_{i,NHCB}$
- Simulation of reaction progress based on kinetic model
- Initial fed-batch experiments carried out

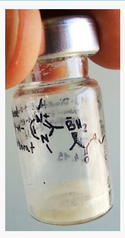


Fig. 7: Enzymatically synthesized organoborane product.

Outlook

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

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. Kettling, 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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