

Supporting Information

A multi-enzyme cascade for the production of high value aromatic compounds

Claudia Engelmann¹, Jens Johannsen², Thomas Waluga², Georg Fieg², Andreas Liese¹ and Paul Bubenheim^{*1}

¹ Institute of Technical Biocatalysis, Hamburg University of Technology, Denickestr. 15, 21073 Hamburg

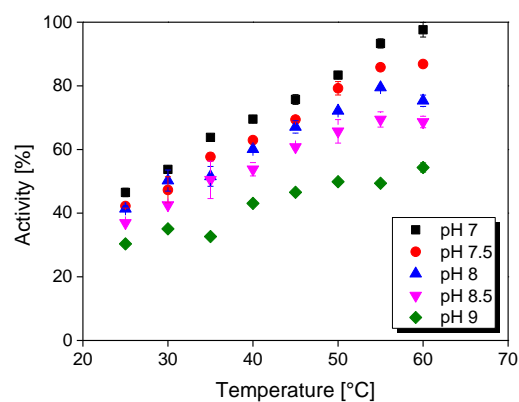
² Institute of Process and Plant Engineering, Hamburg University of Technology, Am Schwarzenberg-Campus 4, 21073 Hamburg

* Correspondence: paul.bubenheim@tuhh.de

Enzyme choice

The activity of the different ADHs and the pH and temperature screening were determined *via* initial reaction rate measurements.

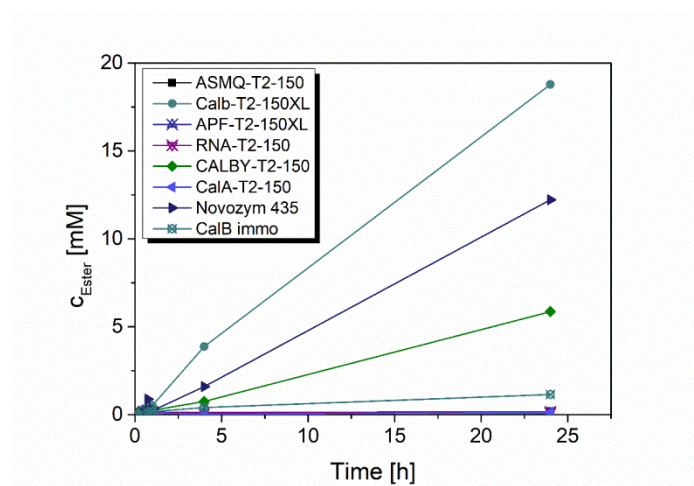
No.	Enzyme	Activity _{Aldehyde}	Activity _{Alcohol}
1	ADH-39L	0.616 U/mg	0.021 U/mg
2	ADH-80	/	/
3	ADH-99L	/	/
4	ADH-125	/	/
5	ADH-168	0.624 U/mg	0.082 U/mg
6	ADH-97L	0.953 U/mg	0.030 U/mg



Cond.: Left: $c = 20$ mM (5% DMSO), Tris/HCl buffer (pH 8, 0.1 M), $c_{\text{NAD}^+} = 0.05$, $\lambda = 340$ nm, $T = 30$ °C.

Right: Tris/HCl buffer (pH 8, 0.1 M), $c = 5$ mM, $c_{\text{NADH}} = 0.05$ mM, $\lambda = 340$ nm, $T = 30$ °C.

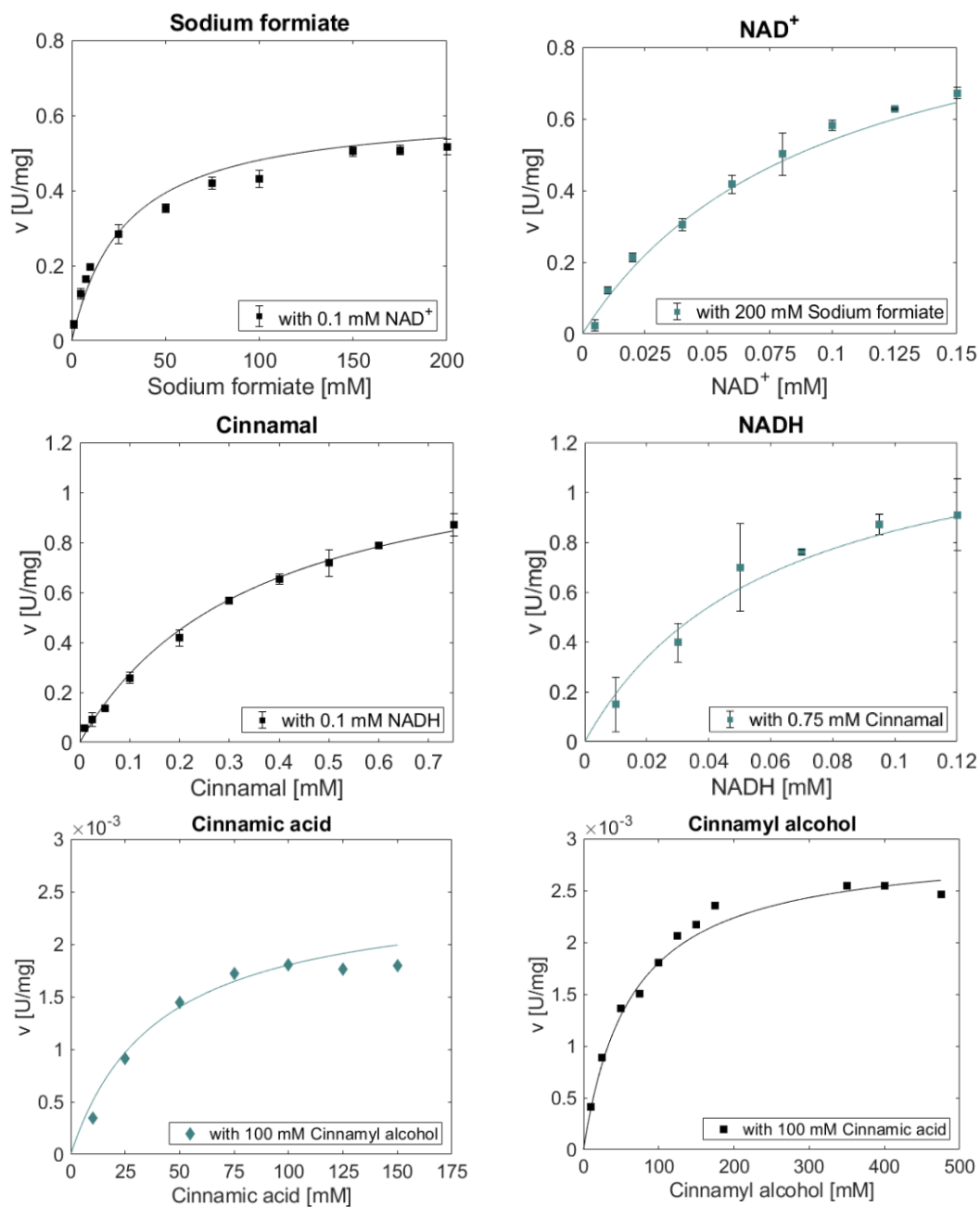
The different lipases from were screened batchwise in 1 mL in a tempered shaker and the reaction solution was analyzed via gas chromatography.



Cond.: $V_{\text{CPME}} = 1$ mL, $c_{\text{Acid/Alcohol}} = 100$ mM, $m_{\text{Lipase}} = 5$ mg, $T = 50$ °C.

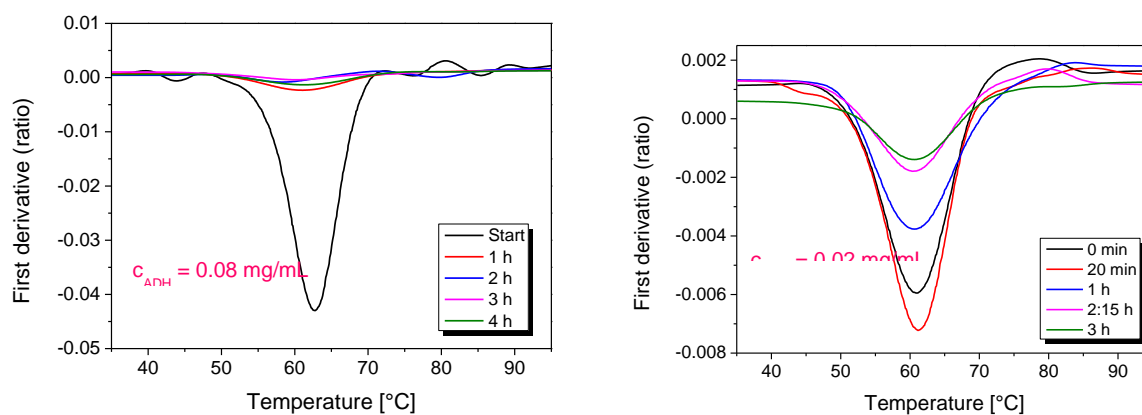
Kinetics

The kinetics were investigated as described in the manuscript.



Differential scanning fluorimetry

Measurement of protein stability by differential scanning fluorimetry as described in the manuscript. The plots show the reaction progress of the in situ co-factor regeneration depending on the ADH concentration.



Cond.: Potassium phosphate buffer pH 8, 0.1 M, $V = 25 \text{ mL}$, $c_{\text{Aldehyde}} = 7.5 \text{ mM}$, $c_{\text{NADH}} = 0.15 \text{ mM}$, $m_{\text{ADH}} = 0.08/0.02 \text{ mg}$, $m_{\text{FDH}} = 5 \text{ mg}$, $c_{\text{sodium formate}} = 100 \text{ mM}$, $30 \text{ }^{\circ}\text{C}$.