DECHEMA FORSCHUNGSINSTITUT

Chemical Technology

Cofactor monitoring during enzymatic reactions by means of Fluorescence Spectroscopy

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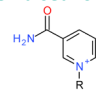
Motivation and project aims

The nicotine amide dinucleotide cofactors NADP+/NADPH and NAD+/NADH are of paramount importance for the industrial utilisation of oxidoreductases. For the rational use a knowledge gap persists between predictions based on initial rate measurements and their application in processes. The goal of the project is the inline quantification of cofactors under conditions as close as possible to process conditions by application of fluorescence spectrometry allowing monitoring at µmol^{-1} concentrations which are common in their application.

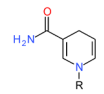
Nicotinamide cofactors

Oxidised form

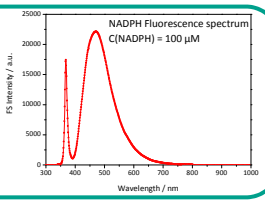


NAD(P)$^+$
No absorption at 340 nm

Reduced form



NAD(P)H
Absorption at 340 nm (Emission/ Fluorescence) at 460 nm)

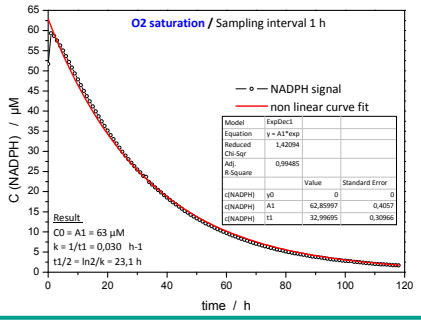


NADPH stability under UV irradiation

NADPH half life experiments were performed in a batch set-up with the possibility to saturate the NADPH/Buffer solution with either N_2 or O_2.

The instrument
tec5 Spectrometer with Helma®
Fluorescence Probe
100 % lamp intensity
120 ms irradiation with 1s, 300s or 3600s sampling interval

Experimental conditions
T = 25°C / V = 100 ml
100 mM ADA Buffer (N-(2-Acetamido)-iminodiacetic acid)
20 mM $MgCl_2$



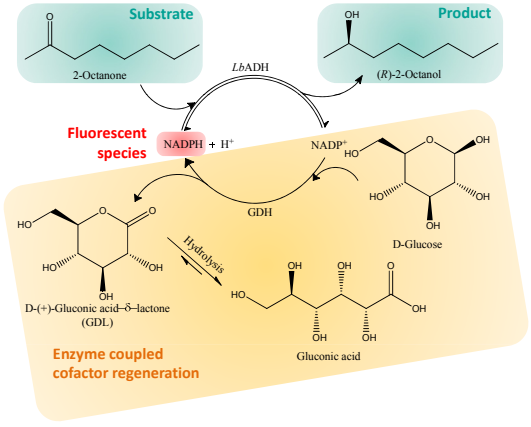
NADPH half life / h

Sampling interval	N_2 saturation	O_2 saturation
1 s	16.4	14.4
300 s (5 min)	23.9	23.9
3600 s (1 h)	23.7	23.1

- irradiation frequency is the main factor for NADPH degradation
- presence of O_2 does not seem to have an effect on degradation
- sampling interval of 300s has no adverse effects

Enzymatic process

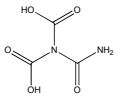
Model reaction: Continuous enzymatic synthesis of (R)-2-octanol from 2-octanone with enzyme coupled cofactor regeneration



Enzymes

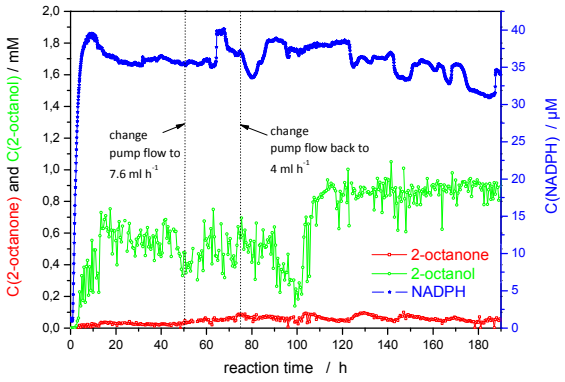
- alcohol dehydrogenase from *Lactobacillus brevis* (*Lb*ADH)
- glucose dehydrogenase (GDH)

Reaction medium

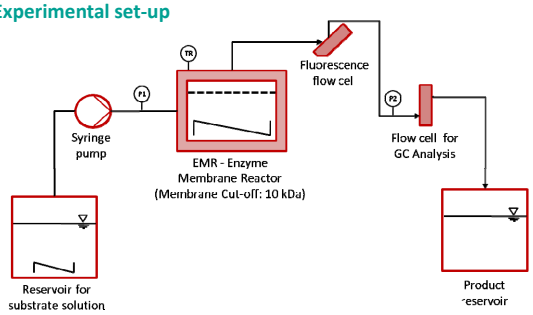


ADA-buffer

Concentration profiles



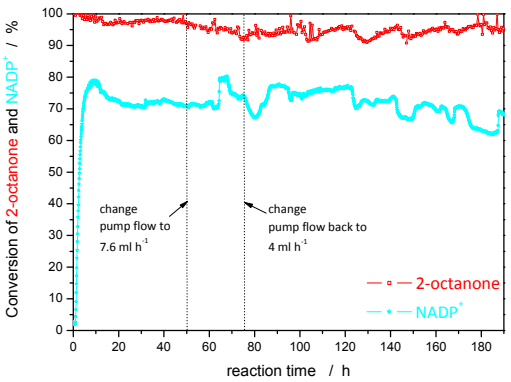
Experimental set-up



Reaction figures (after 190 h run time)

$X_{2\text{-octanone}}$ / %	95
TON_{LbADH} / 10^5	1.9
TON_{GDH} / 10^4	1.8
STY / mg $L^{-1}d^{-1}$	15

Conversion plot



Experimental conditions

C(2-octanone) = 1 mM
C (ADA) = 100 mM
C ($MgCl_2$) = 10 mM
C($NADP^+$) = 0.050 mM
C(glucose) = 100 mM
C (*Lb*ADH) = 33 mg L^{-1}
C (*GDH*) = 113 mg L^{-1}
V (EMR) = 15 mL
dV/dt = 4 ml h^{-1}
τ= 3,75 h
pH = 7
T = 25°C

Summary

- based on batch experiments for NADPH stability, the optimal conditions for its detection via a fluorescence probe have been identified
- „in-line" monitoring of µM NADPH concentrations under process conditions has been demonstrated for up to 200 hours

Outlook

- verification of NADPH concentration in regular time intervals via „off-line" measurement with a Fluoresence Multiplate Reader
- optimisation of reaction conditions for run times of up to 1000 hours
- test of a different enzymatic system relying on $NADP^+$/NADPH as a cofactor pair