Supplementary Material

Towards biocatalytic oxidation of secondary alcohols to carbonyl products of relevance for flavors and fragrances

Eva Puchľová1, Kvetoslava Vranková1, Thomas Hilberath2 Frank Hollmann2\*

1 Axxence Slovakia s.r.o, Mickiewiczova 9, 81107 Bratislava, Slovakia

2 Department of Biotechnology, Delft University of Technology, van der Maasweg 9, 2629 HZ Delft, The Netherlands

# Supplementary Material

DNA Sequence of the native *syadh* gene (yellow background) between the restriction site NdeI and XhoI in pET24b-*syadh*

CATATGACCACGCTTCCGACTGTTCTCATCACCGGCGCCTCCTCCGGTATCGGCGCCACCTACGCCGAGCGCTTCGCCCGCCGCGGCCACGACCTCGTGCTGGTTGCACGCGACAAGGTGCGCCTCGATGCGTTGGCCGCGCGCCTGCGTGACGAAAGCGGTGTGGCCGTCGAAGCGCTGCAGGCCGACCTGACCCGGCCTGCGGATCTGGCCGCGGTCGAGATTCGTCTGCGCGAGGACGCTCGCATAGGCATCCTGATCAACAACGCCGGGATGGCTCAGTCGGGCGGCTTCGTGCAGCAGACCGCTGAGGGCATCGAACGCCTGATCACGCTCAACACCACGGCGCTCACGCGGCTCGCAGCCGCGGTCGCTCCGCGCTTCGTGCAGTCGGGTACCGGCGCAATCGTCAACATCGGCTCGGTGGTGGGTTTCGCGCCCGAGTTCGGCATGTCGATTTACGGCGCCACCAAGGCGTTTGTGCTGTTCCTGTCGCAGGGATTGAACCTCGAGTTGTCGCCCAGCGGCATCTACGTCCAGGCGGTGCTTCCGGCAGCGACCCGCACGGAGATCTGGGGGCGCGCCGGCATCGACGTCAATACGCTTCCCGAGGTGATGGAAGTCGACGAACTGGTTGATGCGGCACTGGTCGGCTTCGATCGCCGCGAACTCGTGACTATCCCGCCGCTGCACGTGGCGGCGCGCTGGGACGCATTGGATGGCGCGCGTCAAGGGCTCATGTCGGACATTCGACAAGCCCAGGCGGCCGACCGCTATCGGCCGGAAGCCTGACTCGAGCACCACCAC

# Supplementary Results

E:\Data 25-3-21\SDS-PAGE\21-03-16_SyADH first analysis\21-03-16_first analysis SyADH.tif

Supplementary Figure 1 : SDS-PAGE for analysis of the production of *Sy*ADH (27.9 kDa) in *E.coli* BL21 (DE3) or C43 (DE3)pEG53-*syadh*. Either whole-cell samples equal to an OD600 of 0.05 or 10 µg of protein sample were loaded on the gel. The picture shows the samples as follows: whole-cell samples before induction (t0) and at x h after induction (tx), crude cell extract (CE). S: Precision Plus Protein All Blue Standard (Biorad).

H:\Desktop\SDS-PAGE\21-6-01_SDS-PAGE SyADH TB AI TsOYE\21-04-08_SDS PAGE SyADH_AI TB medium.tif

Supplementary Figure 2 : SDS-PAGE for analysis of the production of *Sy*ADH (native sequence: 27.9 kDa) in *E.coli* C43(DE3) under IPTG-induction or via autoinduction with lactose. Either whole-cell samples equal to an OD600 of 0.05 or 10 µg of protein sample were loaded on the gel. The picture shows the samples as follows: whole-cell samples before induction (t0) and at x h after induction (tx), crude cell extract (CE). S: Precision Plus Protein All Blue Standard (Biorad).

Supplementary Table 1. Expression optimization of *syadh* in *E. coli* under variation of the host strain (*E. coli* BL21 (DE3) or C43 (DE3)), the expression vector (pEG53-*syadh* or pET24b-*syadh*) and the inductor-concentration (IPTG, Autoinduction medium). Data are derived from two independent cultivations.

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| --- | --- | --- | --- |
| **SyADH expressed in** | **IPTG-concentration [mM]** | **specific activity [U/mg]** | **cell-specific activity [U/gCDW]** |
| ***E. coli* BL21 (DE3) (pEG53)** | 0.5 | 0.8 \*10-3 | 0.47 ± 0.05 |
| ***E. coli* C43 (DE3) (pEG53)** | 0.5 | 3.9 \*10-3 | 1.37 ± 0.33 |
| ***E. coli* C43 (DE3) (pET24b)** | 0.1 | 0.11 ± 0.01 | 44.70 ± 1.69 |
| ***E. coli* C43 (DE3) (pET24b)** | 0.5 | 0.16 ± 0.00 | 47.97 ± 1.37 |
| ***E. coli* C43 (DE3) (pET24b)** | 1.0 | 0.13 ± 0.01 | 46.54 ± 2.59 |
| ***E. coli* C43 (DE3) (pET24b)** | Auto-induction medium | 0.08 ± 0.00 | 35.47 ± 2.37 |