

Accepted Manuscript

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PII: S0141-0229(17)30176-X
DOI: <http://dx.doi.org/10.1016/j.enzmictec.2017.09.003>
Reference: EMT 9128

To appear in: *Enzyme and Microbial Technology*

Received date: 12-6-2017
Revised date: 22-8-2017
Accepted date: 10-9-2017

Please cite this article as: Kohl Anna, Srinivasamurthy Vishnu, Böttcher Dominique, Kabisch Johannes, Bornscheuer Uwe T. Co-expression of an alcohol dehydrogenase and a cyclohexanone monooxygenase for cascade reactions facilitates the regeneration of the NADPH cofactor. *Enzyme and Microbial Technology* <http://dx.doi.org/10.1016/j.enzmictec.2017.09.003>

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Co-expression of an alcohol dehydrogenase and a cyclohexanone monooxygenase for cascade reactions facilitates the regeneration of the NADPH cofactor

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Highlights:

- A cyclohexanone monooxygenase (CHMO) and an alcohol dehydrogenase (ADH), were successfully cloned into the vector pRSFDuet.
- Expression of CHMO and ADH in *E. coli* cells using this Duet vector enabled complete substrate conversion without the necessity of cosubstrates for cofactor recycling.
- Ribosomal binding site engineering (RBS) enabled balanced expression levels of CHMO and ADH.
- In whole-cell biocatalysis, a RBS mutant of ADH together with the CHMO exhibited faster substrate conversion and self-sufficient cofactor recycling of NADPH was confirmed.

Abstract

The introduction of a three-enzyme cascade (comprising a cyclohexanone monooxygenase (CHMO), an alcohol dehydrogenase (ADH) and a lipase (CAL-A)) for the production of oligo- ϵ -caprolactone provided self-sufficiency with respect to NADPH-cofactor regeneration and reduced inhibiting effects on the central CHMO enzyme. For further optimization of cofactor regeneration, now a co-expression of CHMO and ADH in *E. coli* using a DuetTM vector was performed. This led to higher conversion values of the substrate cyclohexanol in whole-cell biocatalysis compared to an expression of both enzymes from two separate

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