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Synthetic pathway and process engineering for terminal oxy- and aminofunctionalization via multistep biocatalysis in living microbial cells

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Summary

Biocatalysis shows high potential for the functionalization of unactivated C-H bonds at ambient conditions with absolute chemo-, regio-, and stereoselectivities being unrivalled by any chemical approach. In order to efficiently exploit nature's toolbox towards an industrial implementation, engineering targets on the reaction as well as the catalyst level need to be identified and tackled in a concerted way.

In this thesis, recombinant microbial cells containing the alkane monooxygenase AlkBGT from *Pseudomonas putida* GPo1 were applied for the ω-functionalization of renewable fatty acid methyl esters (FAMEs). The resulting bifunctional products serve as building blocks for polymer syntheses. AlkBGT-containing *Escherichia coli* were shown to convert FAMEs with 5 to 12 carbon atoms in the alkyl chain giving the highest oxyfunctionalization activities (104 U g_{CDW}^{-1}) for nonanoic acid methyl ester. Kinetic studies revealed that AlkBGT catalyzes a three-step oxidation, yielding ω-alcohols, aldehydes and acids as products. In order to achieve ω-amino-functionalization, AlkBGT-based aldehyde formation was successfully coupled in recombinant *E. coli* with ω-transaminase catalysis (CV2025 from *Chromobacterium violaceum*), enabling the conversion of dodecanoic acid methyl ester (DAME) to 12-aminododecanoic acid methyl ester. Substrate uptake was identified as key factor limiting the conversion of the large and hydrophobic substrate DAME (1.4 U g_{CDW}^{-1}; 0.1 g L^{-1} h^{-1}). Co-expression of the gene encoding the outer membrane protein AlkL relieved the uptake limitation and boosted the activities 62-fold to 87 U g_{CDW}^{-1} allowing productivities of 4-8 g L^{-1} h^{-1} in two-liquid phase biotransformations. Furthermore, the latter reaction engineering concept enabled to control and govern product formation during AlkBGT-based multistep whole-cell biocatalysis. Overoxidation was prevented providing excess of substrate, yielding ω-alcohol as predominant product (up to 52 mM), whereas pronounced substrate limitation using bis(2-ethylhexyl)phthalate as organic carrier solvent resulted in almost exclusive acid accumulation (up to 93 mM). Introduction of the NAD(P)H-independent alcohol dehydrogenase AlkJ, which was shown to catalyze irreversible alcohol oxidation, into *E. coli* containing AlkBGT and AlkL enabled a shift towards the formation of overoxidized compounds in two-liquid phase biotransformations of DAME. This allowed the formation of the aldehyde as predominant product (up to 20 mM).

Via catalyst and reaction engineering, this study sets the stage for the industrial implementation of recombinant microbial cells for terminal FAME functionalizations.