Multi-enzyme/whole cell catalytic production of short-medium chain terminal alkane diols and diacids

Frederik Vig Benfeldt,[a] Zheng Guo[a], Bekir Engin Eser[a]

[a] Aarhus University, Department of Biological- and Chemical Engineering

Gustav Wieds vej 10A, Aarhus C, 8000, Denmark

fvb@bce.au.dk

The rising issue of plastic pollution and the limited motivation for mechanical recycling urges the academia and industry to develop the technologies that enable close- or open- loop recycling and upcycling. To address this, the ACTPAC project seeks to develop a practical method to transform chemically inert C-C backboned plastic waste, specifically polyethylene (PE), into high-value monomers and biochemicals. This method combines chemical and biological processes to convert polyethylene (PE) through a series of steps: from PE to alkanes, then to terminally functionalized monomers, and finally back into polymers, establishing a complete production cycle (**Figure 1**).

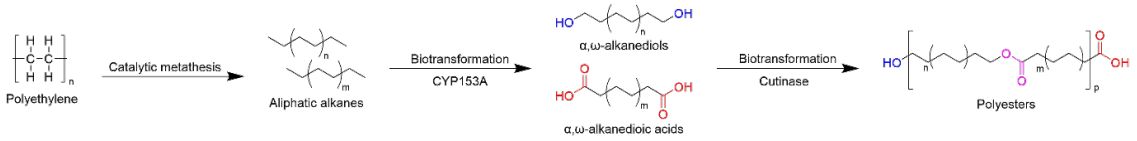

Figure 1: Processing path from waste polyethylene plastics to oxy-functionalized alkanes and new polyester plastics.

One of the pivotal challenges in this transformation is the biotransformation of alkanes into α,ω-alkandiols and diacids, particularly due to the difficulty in directing C-H oxy-functionalization at the least reactive terminal positions. The ACTPAC project aims to utilize the unique capabilities of Cytochrome P450 (CYP450) enzymes to overcome this challenge. Specifically, Cytochrome P153A (CYP153A) enzymes have garnered attention lately due to their ability to catalyze the selective terminal α,ω-hydroxylation of medium to long-chain alkanes and fatty acids [1]. Furthermore, some CYP153A orthologs have been proven to catalyze the full terminal oxidation of alkanes into their corresponding alkanedioc acids [2]. However, utilization of CYP153A enzymes is still challenged by low conversions, requirement for cofactor regeneration and overoxidation of products. That's why this work aims to develop a biotransformation platform for the efficient terminal regioselective oxidation of medium to long-chain alkanes into their corresponding α,ω-alkandiols and diacids. Our strategy involves screening and characterization of promising CYP153A orthologs capable of hydroxylating and oxidizing medium length alkanes into α, ω-diols and diacids. Engineering of selected CYP153A enzymes to fine-tune their specificity and activity towards targeted substrates through computational methods and machine learning algorithms. Lastly, scale-up is conducted in large-scale bioreactors, with a focus on enhancing efficiency, cofactor regeneration, and fine-tuning reaction conditions to maximize yields. Through collaboration with industrial partners, we seek to bring this technology to a level where it can make a significant impact on PE plastic waste management and recycling practices, contributing to a more sustainable circular economy.

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[1] Wang, L.; ...; Li, Y. Mol Biol Rep 2023, 50 (8), 6955–6961.

[2] Funhoff, E. G.; …; van Beilen, J. B., J Bacteriol 2006, 188 (14), 5220–5227.