Orthogonal pathway design for eco-efficient biocatalytic monomer production from cyclohexane

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Plastics are found in every aspect of human life and have applications in packaging, electronics, transport, construction, and textile manufacturing. Polyesters such as polycaprolactone and polyamides like nylons are the most important synthetic fibers. However, the production of the monomers, for instance, ε-caprolactone (ε-CL), 6-hydroxyhexanoic acid (6HA), 6-aminohexanoic acid (6AHA), and adipic acid (AA), are associated with severe environmental concerns. Their production from the crude oil derived cyclohexane comprises several steps at elevated temperature and pressure and produces hazardous by-products such as the greenhouse gas nitrous oxide and heavy metals. Consequently, there is a demand for greener and more efficient production routes. Biocatalytic processes are operated under mild conditions and can be engineered as cascade reactions enabling one-pot processes. Therefore, they offer enormous potential to save time, energy, and waste, allowing for ecological and economical solutions. In this study, recombinant *Pseudomonas taiwanensis* VLB120 strains harboring different *in vivo* cascades to produce the monomers ε-CL, 6HA, 6AHA, and AA were generated, optimized, and evaluated under process conditions.

The synthetic pathway to produce ε-CL was composed of a P450 monooxygenase (Cyp), an alcohol dehydrogenase (CDH), and a Baeyer-Villiger monooxygenase (CHMO) all originating from the bacterium *Acidovorax* CHX100. Cyp-catalyzed cyclohexane oxidation was identified as rate-limiting step and was optimized for a whole-cell activity to 55 and a 3-step cascade activity of 43 U g_{CDW}^{-1} [1,2]. The characterization of the CHMO in different biocatalyst formats, i.e., isolated enzyme, suspended whole cells, and biofilms demonstrated its broad substrate spectrum and favorable properties of suspended whole cells [3]. The efficient regeneration of cofactors via the central carbon metabolism makes this format highly promising from a kinetics perspective. The cascade's amendment with the *Acidovorax* lactonase improved the yield of the less toxic, but equally attractive monomer to 6HA 100% with similar whole-cell activities [4].

For the synthesis of the nylon 6 monomer 6AHA from cyclohexane, the cascade was amended by two additional enzymes catalyzing further oxidation and subsequent amination [5]. High metabolic burden and expression issues of the six enzymes in favored a mixed-species approach. Engineered *P. taiwanensis* to convert cyclohexane to ε-CL, serving as appropriate shuttle molecule, and its further conversion to 6AHA by recombinant *Escherichia coli* strains turned out most feasible.

For AA production, the introduction and expression fine tuning of alcohol and aldehyde dehydrogenases in the 6HA-producing *P. taiwanensis* enabled high stability and activity (49 U g_{CDW}^{-1}) in a stirred-tank reactor for an average productivity of1.3 g L^{-1} h^{-1} and final AA titer 10.2 g L^{-1} [6]. Proof of concept DSP enabled the isolation of 3.4 g AA at a purity of 96%.

This work demonstrates and exploits the potential of whole-cell catalysis for cascade reactions allowing one-pot conversions with high activities and superior conversions. It thereby contributes to the development of eco-efficient monomer production routes.

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