Integrated electrosynthesis and biosynthesis for the production of adipic acid from lignin-derived phenols

Alexander Franz[a], Micjel Chávez Morejón[b], Falk Harnisch[b], Rohan Karande[a]
[a] Interfaculty Centre for Bioactive Matter b-ACT Matter, Leipzig University; [b] Helmholtz-Centre for Environmental Research GmbH – UFZ
Johannisallee 21-23, 04103 Leipzig, Germany
alexander.franz@uni-leipzig.de

Biobased processes are considered to be an sustainable alternative to established petro-chemical processes for the production of Nylon-based monomers. Nylon (polyamides) together with polyesters, make 80 % of the global production of synthetic fibers [1]. The important advantages of biocatalytic processes are ambient conditions and fewer process steps, which shall reduce energy consumption and production costs [2]. In the previous study, a recombinant *Pseudomonas taiwanensis* VLB120 was constructed that efficiently converted cyclohexane to adipic acid using a 6-step enzyme cascade at a specific activity of 48.6 U g_{CDW}^{-1} [3]. Although the biotransformation was scaled to a litre scale, resulting in 10.2 g L^{-1} of adipic acid, the specific product yield was very low (9%) due to the volatile nature of cyclohexane. To maximise product yield, the first intermediate compound of the cascade was chosen instead, to perform a 5-step biotransformation with the less volatile cyclohexanol.

In a biobased economy, fossil cyclohexanol needs to be replaced as feed for *P. taiwanensis* by renewables for which here electrochemically upgraded phenol derivates from lignin depolymerization were investigated [5]. Liquid fractions from lignin depolymerization are rich in monomeric and oligomeric phenols (e.g., phenol, catechol, syringol, and guaiacol). These unsaturated compounds were electrochemically hydrogenated into their corresponding aliphatic counterparts, i.e., substituted cyclohexane derivatives, which are further transformed into adipic acid using microbial biotransformation. The suitability of phosphate buffer as a supporting electrolyte, making the electrochemical conversion compatible with the later biotransformation process.

P. taiwanensis VLB120 cells were grown in batch mode in the bubble column reactor up to 0.5-0.8 g_{CDW} L^{-1}, and biotransformation was started by continuously feeding 41.5 mM cyclohexanol at a rate of 41.3 µL min^{-1} over 5 hours. In previous studies, cyclohexanol has been shown to inhibit cyclohexanone monooxygenase, accumulating intermediate products and poor whole-cell catalytic performance [3, 4]. Therefore, we selected a substrate fed-batch approach to perform biotransformation under substrate-limited conditions for minimising cyclohexanol inhibitory effects. As a result, under strictly substrate-limited conditions, a productivity of 0.02 g L^{-1} h^{-1} and an improved 60.8 % molar yield of adipic acid on cyclohexanol were obtained. Regarding both process steps combined, the overall yield for the conversion of phenol to adipic acid was 40 %, and for the conversion of an aromatic mixture to adipic acid, the yield was 57 %.

---

[1] G. Bellussi and C. Perego, CATTECH, **2000**, 4, 4–16.
[2] Bretschneider, L., Heuschkel, I., Bühler, K., Karande, R., et al., Rational orthologous pathway and biochemical process engineering for adipic acid production using Pseudomonas taiwanensis VLB120. Metab. Eng. **2022**, 70, 206–217.
[3] Bretschneider, L., Heuschkel, I., Ahmed, A., Bühler, K., et al., Characterization of different biocatalyst formats for BVMO-catalyzed cyclohexanone oxidation. Biotechnol. Bioeng. **2021**, 118, 2719–2733.
[4] Gundekari, S., Karmee, S.K., Selective Synthesis of Cyclohexanol Intermediates from Lignin-Based Phenolics and Diaryl Ethers using Hydrogen over Supported Metal Catalysts: A Critical Review. Catal. Surv. Asia **2021**, 25, 1–26.
[5] Chávez Morejón, M., Franz, A., Karande, R., Harnisch, F., Integrated electrosynthesis and biosynthesis for the production of adipic acid from lignin-derived phenols. Green Chem. **2023**, 25 (12), 4662–4666.