Enzymatic cascade to produce (*R*)-citronellal using an Old Yellow Enzyme and a Short-Chain Dehydrogenase

Claudia Ferrer-Carbonell,[a] Rocio Villa,[b] Caroline E. Paul[a]

[a] Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft, The Netherlands

[b] Department of Biochemistry, University of Murcia, Murcia, Spain

C.FerrerCarbonell@tudelft.nl

(*R*)-Citronellal, a terpene prised for its distinct lemon, rose, and citronella odours, serves as a precursor for (–)-menthol.[1] This chiral monoterpene alcohol is highly valued in the chemical sector for its cooling properties and minty fragrance.[2] Nowadays, the production pathways for (–)-menthol involve (*R*)-citronellal as an intermediate, together with metal catalysts such as Ru and Pt.[3]

In contrast, flavin mononucleotide (FMN)-containing ene-reductases, specifically from the Old Yellow Enzyme family (OYE; EC 1.6.99.1), have proven to be effective biocatalysts for the reduction reactions of citral, demonstrating asymmetric bioreduction capabilities for a broad range of α,β-unsaturated compounds.[4] Notably, the OYE NCR from *Zymomonas mobilis* was engineered to alter its stereoselective reduction of citral, to obtain (*R*)-citronellal instead of the (*S*)-enantiomer, albeit with 88% *ee*.[5] Alternatively, OYE2 from *Saccharomyces cerevisiae* has shown the ability to selectively catalyse the reduction of geranial to (*R*)-citronellal with 95.5% *ee*.[6]

This study focuses on the development of a bienzymatic cascade with two consecutive reactions for the (*R*)-citronellal formation from the inexpensive geraniol (Figure 1). The cascade involves a short-chain dehydrogenase (SDR) catalysing the oxidation of geraniol to geranial, preventing the formation of neral, followed by OYE2 catalysing the reduction to yield (*R*)-citronellal. Due to potential cross-reactions with the SDR leading to the formation of (*R*)-citronellol, this cascade requires a two-step biphasic system to provide >95% *ee* (*R*)-citronellal.

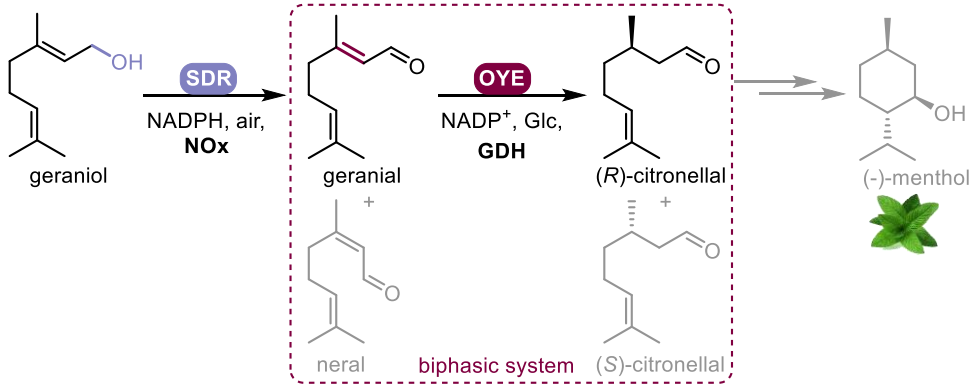

Figure 1. Enzymatic cascade for the production of (*R*)-citronellal from geraniol.

[1] M. Guentert, *Flavours and Fragrances* **2007**, Springer Berlin, Heidelberg.

[2] G. P. Kamatou, I. Vermaak, A. M. Viljoen, B. M. Lawrence, *Phytochemistry* **2013**, *96*, 15-25.

[3] M. Azkaar, P. Mäki-Arvela, Z. Vajglová, *et al. React. Chem. Eng*. **2019**, *4*, 2156-2169.

[4] Scholtissek, A.; Tischler, D.; Westphal, A. H.; Willem J. H. van Berkel, W. J. H.; Paul, C. E. *Catalysts* **2017**, *7*, 130.

[5] Kress, N.; Rapp, J.; Hauer, B. *ChemBioChem* **2017**, *18*, 694-823

[6] Ribeaucourt, D.; Höfler, G. T.; Yemloul, M.; Bissaro, B.; Lambert, F.; Berrin, J-G.; Lafond, M.; Paul, C. E. *ACS Catal.* **2022**, *12*, 1111-1116.