

## Bienzymatic cascades for the sustainable synthesis of citral-based fragrances

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In recent years, the flavour and fragrance industry is working to develop greener and more sustainable solutions for the production of its ingredients. This could be achieved through three main strategies: the use of renewable feedstocks, such as essential oils and terpenes, as raw materials; the selection of synthetic strategies with high chemo-, regio-, and stereoselectivity and mild reaction conditions; the development of potent fragrances with high odour-to-carbon ratio to minimize the amount of ingredients required [1]. The implementation of biocatalysis in such processes could help achieve all these goals, as enzymes are inherently renewable, highly selective, and work in aqueous environment with limited energy consumption [2]. Of particular interest is the application of more than one biocatalysed reaction in the same synthetic strategy, to gain the maximum advantage from telescoping subsequent steps through the exploitation of multiple enzymes catalysing specific reactions in a one-pot system [3].

In this work, a bienzymatic cascade involving a first step of oxidation of an allylic alcohol with alcohol dehydrogenases (ADHs) and a subsequent step of reduction of the C=C double bond conjugated to the carbonyl group with ene-reductases (ERs) was explored for the synthesis of citral-based fragrances of commercial interest (Figure 1) [4]. The enzymes employed were overproduced as recombinant proteins in competent *E. coli* cells and used as crude lysate for the reactions. For each step of the cascade, the library of enzymes obtained was screened in homogeneous phase, biphasic systems, and/ with entrapment of the aqueous phase in polyacrylate to explore which catalyst gave the highest conversion and, if applicable, the highest enantiomeric excess for the stereoisomer of interest.

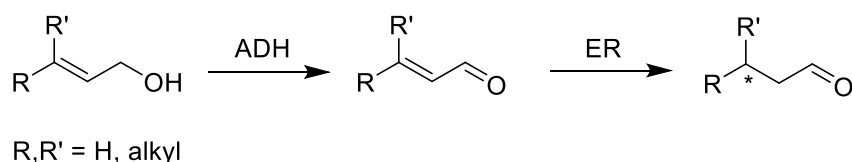


Figure 1. Enzymatic cascade of oxidation with ADH and reduction with ER.

[1] Lecourt, M., & Antoniotti, S., *ChemSusChem* **2020**, 13(21), 5600-5610.

[2] Michailidou, F., *ChemBioChem* **2023**, 24(19), e202300309.

[3] Brenna, E et al., *J. Mol. Catal. B: Enzym.* **2015**, 114, 37-41; Tentori, F. et al. *Catalysts* **2020**, 10(2), 260; Venturi, S et al., *J. Org. Chem* **2022**, 87(9), 6499-6503.

[4] Parmeggiani, F. et al. *ChemBioChem* **2022**, 23(1), e202100445.