

The **EnzOx2** Project is a Research & Innovation Action funded by the Bio-based Industries Joint Undertaking, a Public -Private Partnership between the EU (under H2020 framework programme) and the Bio-based Industries Consortium. Twelve participants from five European countries will join efforts for the sustainable production of chemical building blocks and other added value products from plant biomass using enzymatic technologies.

PROJECT CONCEPT



The overall aim of the **EnzOx2** project is to develop new **bio-chemical technologies** based on the use of oxidative enzymes, largely unexplored at the industrial level, to provide innovative solutions in the production of some **added value** compounds from **biomass** components to substitute others of petrochemical origin. The potential of **oxidative enzymes** in such biotransformations has been shown by some of the **EnzOx2** partners in previous projects, including several **oxidation** and **oxygen functionalization** reactions catalyzed by different types of fungal oxidoreductases (such as oxidases and peroxigenases).

In this context, **EnzOx2** plans to develop a 100% enzymatic conversion of **bio-based** 5-hydroxymethylfurfural (**HMF**) or 5-methoxymethylfurfural (**MMF**) into diformylfuran, a platform chemical, and 2,5-furandicarboxylic acid (**FDCA**), a plastic building-block to be used in substitution of terephthalic acid. On the other hand, highly selective hydroxylation of **plant lipids** (such as fatty acids, terpenes and steroids) will be optimized for cost-effective production of flavour and fragrance (**F&F**) ingredients, active pharmaceutical ingredients (**APIs**) and others.

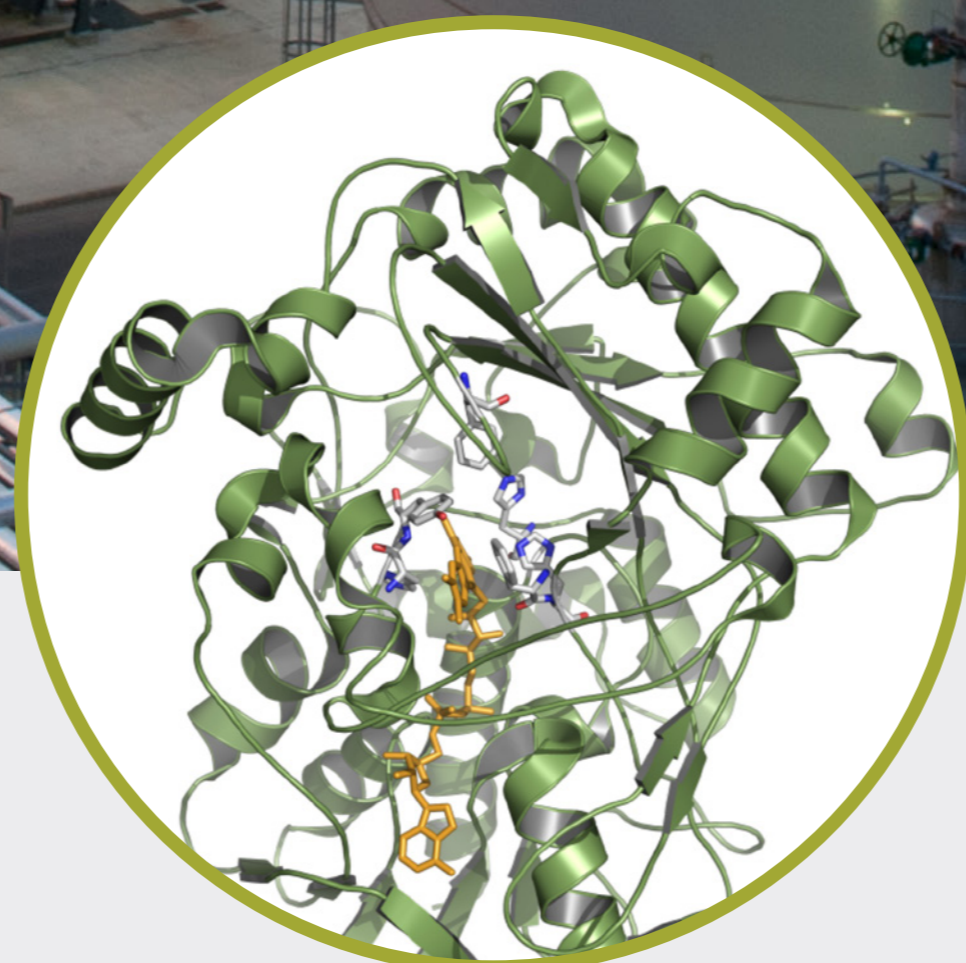
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PROJECT WORKFLOW



To attain these goals, **EnzOx2** will take advantage from the largely unexploited diversity of **oxidoreductases** in sequenced **microbial genomes** to obtain new enzymes of interest. Moreover, the catalytic performance, selectivity and/or stability of the best candidates will be **adapted**, when needed, to the required reaction conditions using **protein engineering** tools. Several concepts such as substrate loading, co-factor addition, biocatalyst stability and downstream processing, among others, will also be considered to further **optimize** the enzymatic reactions. Finally, life cycle assessment (**LCA**) analyses of the enzymatic processes, compared with chemical processes for the production of the same or similar compounds will be conducted in order to evaluate their **technical, economic** and **environmental feasibility**.

The **EnzOx2** consortium brings together three highly-specialized SMEs in the areas of special (basidiomycete) enzymes (**JenaBios**), HMF production (**AVA Biochem**) and chiral chemicals and APIs (**Chiracon**); two world-leading companies in the sectors of industrial enzymes (**Novozymes**) and F&F ingredients (**Firmenich**); one technological centre dedicated to the plastics sector (**AIMPLAS**); and six research/academic partners with high expertise in oxidoreductase structure-function and engineering (**CIB** and **ICP**), their application in different biotransformations (**TUDresden** and **IRNAS**), LCA analysis and biocatalyst immobilization (**USC**) and the optimization of enzyme-based bioprocesses (**TU Delft**).

OBJECTIVES

- 1 To provide biomass-derived sugar and lipid substrates for the enzymatic technologies proposed in the subsequent objectives, and evaluate some separation/pre-treatment technologies.
- 2 To provide the most adequate enzymatic biocatalysts (new or engineered oxidative enzymes) for development of the bio-chemical technologies proposed in the subsequent objectives.
- 3 To develop an enzymatic technology for the production of FDCA as a renewable polymer building block or other chemicals derived from HMF or MMF.
- 4 To develop new enzymatic technologies for the selective hydroxylation of biomass-derived lipid components in the manufacture of F&F ingredients, APIs and other added value products.
- 5 To optimize the above enzymatic (or chemo-enzymatic) technologies in terms of biocatalyst stability, substrate concentration, reaction medium, co-substrate supply (when required) and downstream processing.
- 6 To evaluate the technical, economic and environmental feasibility (LCA analysis) of the enzymatic processes, compared with chemical processes for the production of the same or similar compounds.

