# Multienzymatic cascades for environmentally sustainable processes Marina Guillén<sup>1</sup>; Kírian Bonet-Ragel <sup>1</sup>; Gregorio Álvaro<sup>1</sup>; Glòria González<sup>1</sup>; Glòria Caminal<sup>2</sup>; Antoni Casablancas<sup>1</sup>; Óscar Romero<sup>1</sup> <sup>1</sup>Department of Chemical, Biological and Environmental Engineering, Universitat Autònoma de Barcelona, Barcelona, Spain <sup>2</sup>Institute of Advanced Chemistry of Catalonia, IAQC-CSIC, Barcelona, Spain. marina.guillen@uab.cat

# **INTRODUCTION**

The design and implementation of sustainable processes, which are not dependent on fossil fuels, represents a significant challenge in the coming years [1]. However, biocatalytic processes based on one enzymatic step limit the potential range of molecules that can be produced. A clear trend is emerging towards the development of new and sophisticated biocatalytic cascades, which will replace single-step enzymatic transformations, allowing a cost-effective production of complex molecules while expanding the range of compounds that can be enzymatically obtained. However, further progress is required to industrially implement these systems such as improvements in process metrics, robustness and flexibility. In addition, a reduction in the biocatalysts production costs is also required to enhance the techno-economic feasibility of the processes.

The group of **Bioprocess Engineering and Applied Biocatalysis** aims to address these challenges through the economic and sustainability-oriented design, preparation, and implementation of biocatalysts. This goal is achieved through the implementation of an multidisciplinary approach, involving production, the integrated and the purification/immobilization of enzymes in a single step [2], as well as the design of selfsufficient biocatalysts for their use in enzymatic reactors.

### **Enzyme Reactor** Production **Biocatalyst Design & Characterization Design & Optimization & Purification** High cell density culture • Use the binding tags for Reaction and optimization engineering

#### **Integral Perspective from a Bioprocess Engineering**

 Antibiotic-free expression system **Optimization of conditions** One step purificaction & immobilization

Iron&stee

industry

immobilization Rational design of immobilized biocatalyst

(medium engineering, enzyme ratio, air supply) **Reaction design and Intensification** 

MEPLATCO

**Biodiesel** 



Multi-enzymatic Carbon Capture and Utilization (CCU)

- CCU technologies play a crucial role in industries where limited alternatives exist to mitigate the combustion of fossil fuels, being a promising alternative to produce **high-value chemicals from CO**<sub>2</sub>.
- The multi-enzymatic system, based on the circular economy principles, will allow coupling a CCU strategy to a

waste valorization, producing two compounds of industrial interest (DHA and formate) from CO<sub>2</sub> and glycerol.

- The optimized multi-enzymatic system achieved an impressive 92.3% CO, conversion, facilitated by efficient NADH regeneration.
- Furthermore, the system was validated under industrial relevant environment [3], using a synthetic gas mixture mimicking the composition of the **iron&steel** industry off-gases and a **crude glycerol**.

# Synthesis of acetoin from Bioethanol in a one-pot reactor

- Acetoin (3-hydroxy-2-butanone) is designated as one of top 30 sugar-derived chemicals building block and used as **flavor** in food and beverage industry [4].
- Novel multi-enzymatic synthesis, more environmentally friendly compared to conventional chemical synthesis. In addition, acetoin produces could be **labelled as natural**.
- **Excellent metrics,** obtaining a yield of 87.9% with a productivity of 261.2 mg<sub>acetoin</sub>·L<sup>-1</sup>·h<sup>-1</sup>
- **Intensification** of the multi-enzymatic and further **optimization** is currently ongoing.

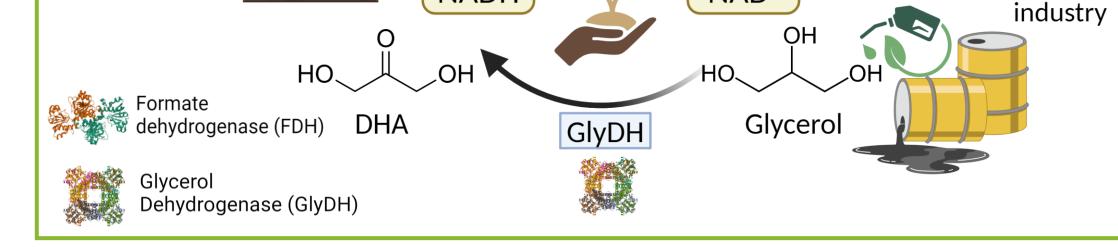
## Intensification of the producction of furandicarboxilic acid (FDCA) for new biobased plastics

- HMF has been identified as a versatile chemical platform from renewable resources, and can be transformed into FDCA, a key building block for renewable materials such as PEF and specialty polymers.
- **HMF oxidase (HMFO)** from *Methylovorus sp*. is able to catalyse the three oxidative steps [5].
- Intensification by one-step purification&immobilization strategy into cellulose support was carried out using a CBM-fused HMFO, obtained high immobilization yield (98%) and protein loading (59mg<sub>protein</sub>/g<sub>support</sub>).
- **Optimization** of the biocatalyst and the **reaction condition** has allowed to increase the **yield** and **productivity** up to 66% and 40.1 mg<sub>FDCA</sub>·L<sup>-1</sup>·h<sup>-1</sup>, respectively.

Transition towards environment-friendly consumer products

DEMUBÍ

**MEPLATCO**<sub>2</sub>



CO

NADH

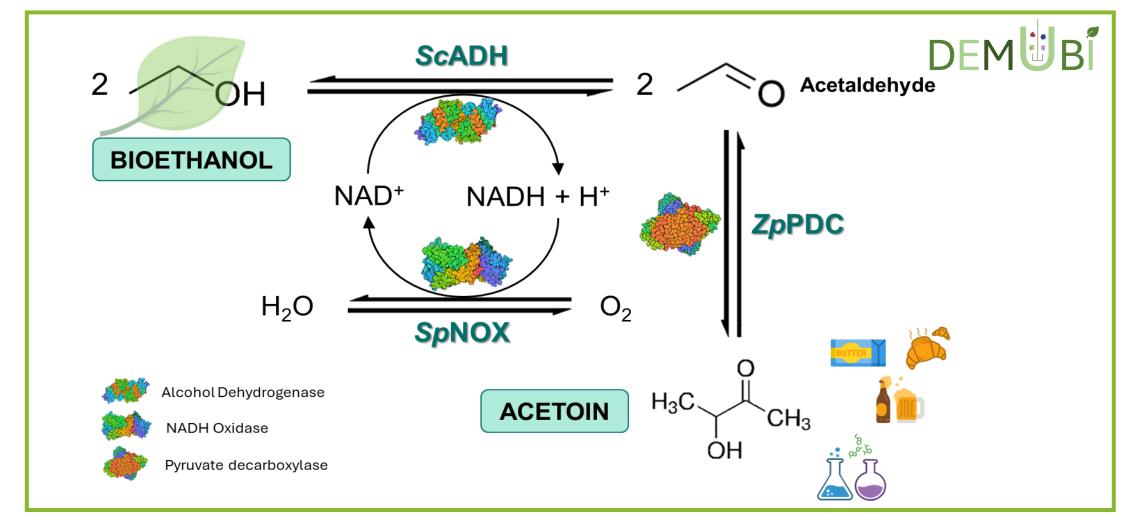
FDH

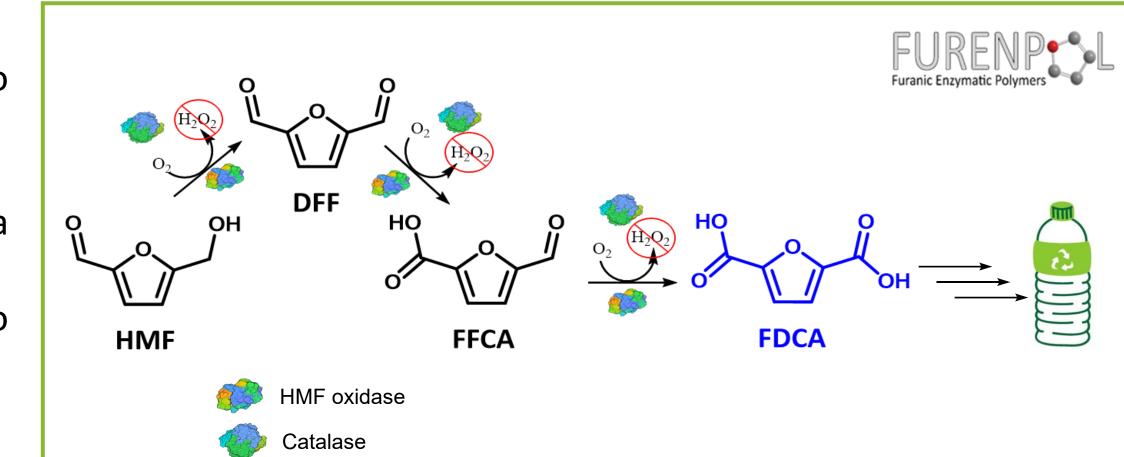
In situ

regeneration

Formate

 $\mathsf{NAD}^+$ 





## by co-creation of an oxidoreductase foundry

- **Carbohydrate oxidases** are used to develop a **circular cotton-textile biobleaching** process for reduction of energy, water and chemical consumption.
- Carbohydrate rich wastewaters from previous steps of cotton processing are using as feedstock to generate H<sub>2</sub>O<sub>2</sub> for the bleaching process.

# **TO TAKE HOME**

- Design and implementation of green, sustainable and fossil-fuel independent processes is crucial to face the challenges and goals that the next decades will bring.
- **Biocatalysis** will contribute to **boost key technologies** and solutions underpinning policies and sustainable development goals.
- **Economically** & green driven biocatalyst design and preparation.
- **Integral** & **multidisciplinary** approach for process development



PLEC2021-007690 funded MICIU/AEI/10.13039/501100011033 and by the European **Union NextGenerationEU/PRTR** 

This project has received funding from the European Union's Horizon TED2021-129732A-I00 **2020** research and innovation programme under grant agreement No "NextGenerationEU"/PRTR" 101000607



 $\overline{\mathbf{A}}$ 

funded MICIU/AEI/10.13039/501100011033 and the EU

Project PID2022-139725OA-I00 funded by Generalitat de Catalunya (2021 SGR 00143) MICIU /AEI/10.13039/501100011033/FEDER, UE

