Hydrogen-driven cofactor regeneration for sustainable whole-cell biotransformations in *Cupriavidus necator*

Javier García Navarro[1,2], Stéphane Guillouet[3], Robert Kourist[1]

[1] *Graz University of Technology, Institute for Molecular Biotechnology*
[2] *ACIB GmbH – Austrian Centre of Industrial Biotechnology*
[3] *INSA Toulouse, Toulouse Biotechnology Institute*

ConCO₂rde

Funded by the European Union

Abstract

Oxidoreductases comprise a large number of industrially relevant enzymes catalyzing the transfer of electrons from an electron donor to an electron acceptor molecule. Enzymatic redox reactions in the reductive direction often require the presence of reduced redox cofactors, commonly nicotinamide adenine dinucleotide (NADH) or its phosphorylated form (NADPH). Since the addition of such reducing equivalents in stochiometric amounts is economically prohibitive, the implementation of efficient cofactor regeneration systems is pivotal to achieve large-scale biocatalytic applications [1]. To this end, the most widespread cofactor recycling strategies rely on the partial oxidation of auxiliary organic substrates resulting in poor atom efficiencies, unwanted by-products and increased downstream processing costs [2].

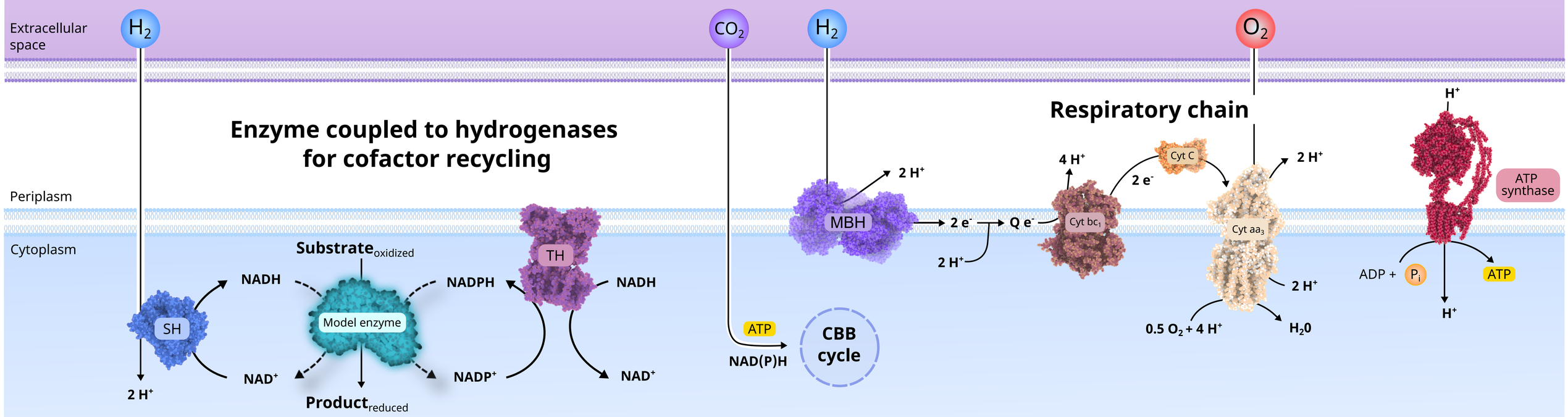
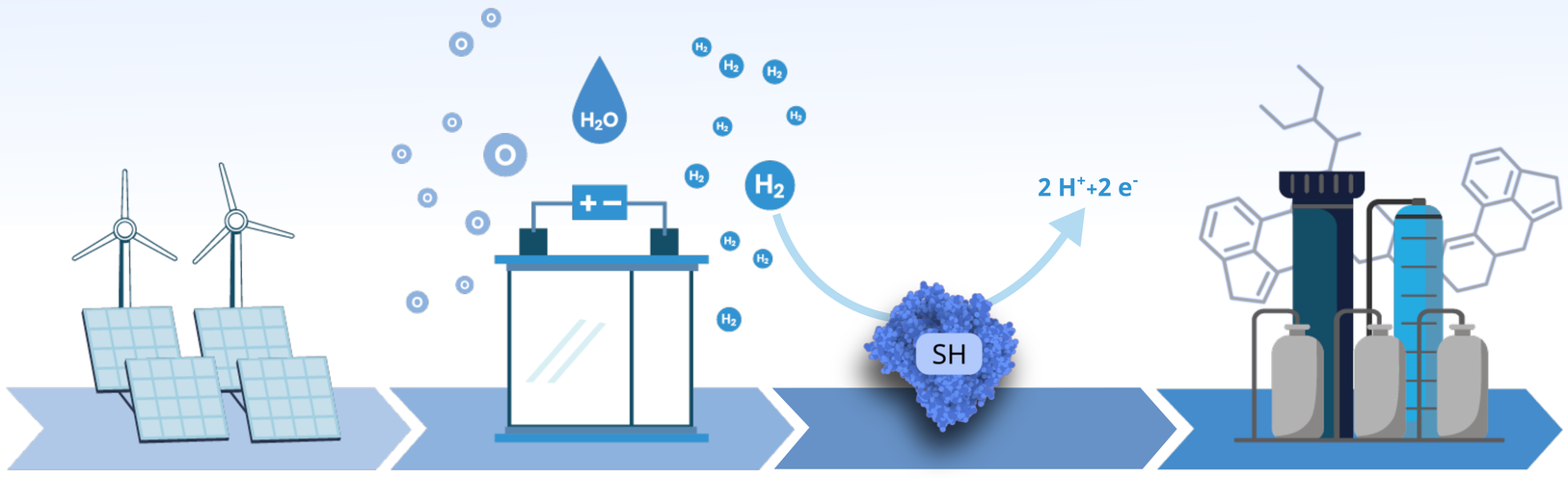


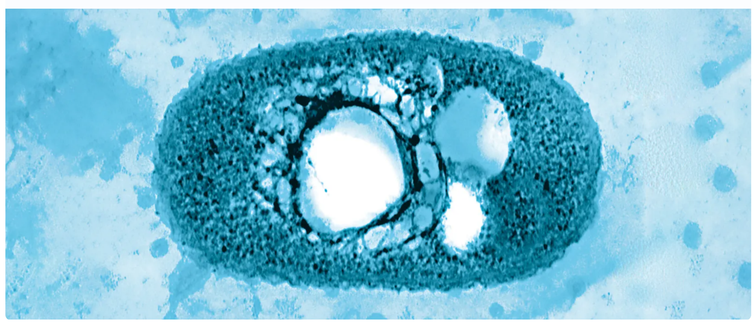
Fig.1 Schematic representation of the chemolitoautotrophic metabolism in *Cupriavidus necator* coupled to heterogously expressed model enzyme for hydrogen-driven cofactor regeneration. Note that the above representation of the respiratory electron transfer chain complexes is not meant to imply that such complexes are necessarily in 1:1 ratio. Likewise, enzyme morphology is intended to be merely descriptive and does not correspond to the real tertiary or quaternary structures.*This image was adapted from the textbook Brock Biology of Microorganisms and created using 3D Protein Imaging[4].*

Hydrogenases: an interface between renewable energy and biotechnology



Cupriavidus necator as a microbial cell factory

- CO_2 Sequestration
- PHAs producer
- Improved genetic amenability
- Versatile metabolism



Main challenges

Reaction rate optimization

The reaction rates obtained when *C. necator* was fed with fructose for cofactor recycling outperform those conducted with hydrogen [3]. What will increase the rate best? Providing more H_2 or improving the intracellular concentration of enzyme?

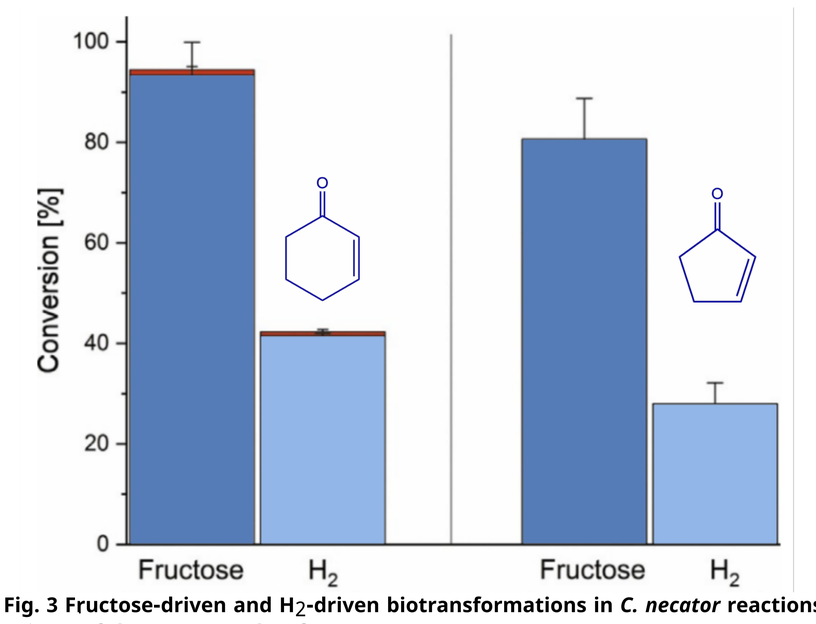


Fig. 3 Fructose-driven and H_2-driven biotransformations in *C. necator* reactions using cyclohexanone and cyclopentanone.

Transport limitation

Autotrophic microorganisms might have less membrane transport proteins and are prone for transport limitation.

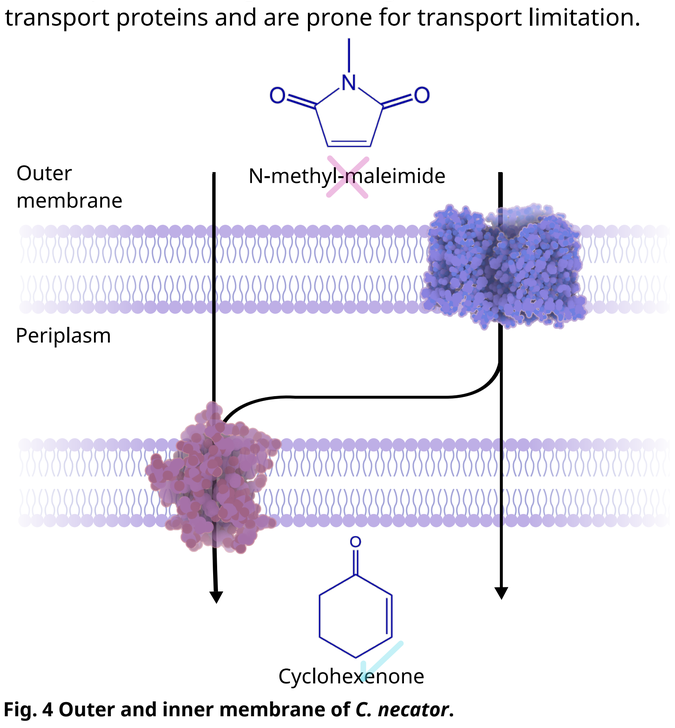


Fig. 4 Outer and inner membrane of *C. necator*.

Methodology

- Identification of model enzymes
- Feasibility test supplying fructose
- Stirred-tank H_2-biotransformations
- Transport engineering
- Bioreactor up-scaling
- Process intensification

Outlook

Despite the numerous advantages that *C. necator* offers as a biocatalytic platform, its space-time-yields still require further improvement to industrially compete with other established heterotrophic chassis. Accordingly, the optimisation of the operating conditions combined with strain engineering is expected to leverage the power of the hydrogen-oxidation energy module, paving the way towards a more sustainable organic synthesis.

References

[1] X. Wang, T. Saba, H. H. P. Yiu, R. F. Howe, J. A. Anderson, J. Shi, **Chem**, 2017, 2, 621–654

[2] Y. Ni, D. Holtmann, F. Hollmann, **ChemCatChem** 2014, 6, 930–943.

[3] L. Assil-Companioni, S. Schmidt, P. Heidinger, H. Schwab, R. Kourist, **ChemSusChem** 2019, 12, 2361–2365.

[4] G. Tomasello, I. Armenia, G. Molla, **Bioinformatics** 2020, 36, 2909–2911.

TU Graz

acib

INSA TOULOUSE

This project has received funding from the **European Union's Horizon 2020** research and innovationprogramme under the **Marie Skłodowska-Curie grant agreement No 955740.** Any statements herein reflect only the author's views. The European Union is not responsible for any use that may be made of the information it contains.